First report of Taenia laticollis and a new species, “Taenia sp. Eurasian lynx” from the Eurasian lynx (Lynx lynx) in Northwestern China

Gang Liu
Shihezi University
Shanshan Zhao
Shihezi University
Sándor Hornok
University of Veterinary
Xueling Chen
Shihezi University
Suwen Wang
Shihezi University
Wurelihazi Hazihan
Shihezi University
Xinli Gu
Shihezi University
Yuan-Zhi Wang (wangyuanzhi621@126.com)
Shihezi University

Short report

Keywords: Cestoda, tapeworms, Cyclophyllidea, Taeniidae, cox1

Posted Date: May 28th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-520054/v1

Abstract

Background

The Eurasian lynx (*Lynx lynx*) is a medium-sized wild cat species distributed throughout Eurasia, from Europe to the Far East. There has been no report on *Taenia* species (Cestoda: Cyclophyllidea) infecting this felid in China.

Methods

In this study, 24 tapeworms were found in two Eurasian lynxes (#1 and #2) in Xinjiang, northwestern China. The tapeworms were identified based on two mitochondrial genetic markers, the cytochrome *c* oxidase subunit 1 (*cox1*) and *16S* r*DNA*. This was followed by detailed morphologic characterization.

Results

Molecular and phylogenetic analyses of the *cox1* gene revealed that *i*) a single tapeworm from lynx #2 shared 100% identity with *Taenia laticollis* genotype C detected in Eurasian lynx in Finland, and *ii*) the remaining 23 tapeworms, provisionally named here as "*Taenia* sp. Eurasian lynx", had two nucleotide substitutions but phylogenetically clustered together. The latter showed only 93.25% sequence identity to *T. hydatigena* from sheep (*Ovis aries*) in Slovakia. The scolex morphology is characteristic enough to distinguish "*Taenia* sp. Eurasian lynx" from other species of its genus.

Conclusions

"*Taenia* sp. Eurasian lynx" is a novel tapeworm species found in Eurasian lynx. In addition, *T. laticollis* was found in this wild felid for the first time in China. The intermediate hosts of *T. laticollis* and "*Taenia* sp. Eurasian lynx" should be explored in the near future.

Background

The Eurasian lynx (*Lynx lynx*) is a very widespread species, with a distribution range covering much of Europe and Asia. It has five subspecies (northern lynx, Balkan lynx, Carpathian lynx, Caucasian lynx, Siberian lynx and Central Asian lynx). The Central Asian lynx inhabits Afghanistan, Bhutan, China, India, Kazakhstan, Kyrgyzstan, Nepal, Pakistan, Tajikistan, Turkmenistan and Uzbekistan [1].

Adult taeniid tapeworms typically occur in the small intestine of carnivorous definitive hosts, and their cystic larvae (metacestodes) develop in tissues or body cavities of herbivorous or omnivorous intermediate hosts [2]. The genera *Taenia* and *Echinococcus* have a global socioeconomic impact by causing morbidity in humans, domestic livestock and wildlife [3]. To date, there are at least 15 valid *Taenia* species infecting felids [4, 5]. Thus, the Eurasian lynx (*Lynx lynx*) was previously reported to harbor *Taenia pisiformis*, *Taenia laticollis*, *Taenia hydatigena*, *Taenia taeniaeformis*, *Taenia*

lynciscapreoli, *Taenia krabbei*, *Echinococcusmultilocularis*, *Diphyllobothrium latum*, *Mesocestoides lineatus*, *Mesocestoides* spp. and *Spirometra* sp. [6-9].

In China, the Central Asian lynx is mainly distributed in Xinjiang, Qinghai, Gansu, Inner Mongolia and Tibet Province. The aim of the present study was to identify tapeworms in the Eurasian lynx (*Lynx lynx*) from Xinjiang, northwestern China.

Methods

Sample collection

Two Eurasian lynxes were found during our field investigation on ticks and fleas in the West Junggar Mountains, Xinjiang, northwestern China. 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 (Supplementary **Fig. 1**). All tapeworms were washed in physiological saline prior to DNA extraction or morphological identification.

DNA extraction and molecular-phylogenetic analyses

DNA was extracted from a small part of the strobila 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-long fragment of the cytochrome *c* oxidase subunit I (*cox1*) gene and a 526-bp-long fragment of the 16S rDNA (*16S* r*DNA*) gene as reported previously [10, 11]. 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*: MW846305, MW846313 and MW843568; *16S rRNA*: MW854635, MW854636 and MW843496). A phylogenic tree was constructed using the Neighbor-Joining method in MEGA 7.0.

Morphological identification

The staining procedure was performed as reported [12]. 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. The scolex, neck, strobila (mature proglottid, gravid proglottid) of tapeworms were observed by microscopy.

Ethical approval

This study was approved by the Animal Ethics Committee of Shihezi University (Approval No. A2018-143-01).

Results

Molecular identification

Molecular and phylogenic analysis of *cox1* sequences showed that *i*) a single tapeworm from lynx #2 shared 100% sequence identity with *T. laticollis* genotype C (JX860623) found in Eurasian lynx in Finland, *ii*)

the remaining 23 tapeworms, provisionally named here as "*Taenia* sp. Eurasian lynx", had two nucleotide substitutions but clustered together in the phylogenetic tree. These showed only 93.25% (400/444 bp) sequence identity to *T. hydatigena* (MW336935) from sheep (*Ovis aries*) reported in Slovakia (**Fig. 1**). Analysis of the COX1 protein amino acid sequences showed that "*Taenia* sp. Eurasian lynx" differs from *T. hydatigena*, *Taeniaregis* and *T. lynciscapreoli* with 2-4 amino acids (Supplementary **Fig. 2**).

Morphological description

The tapeworm, identified here as *T. laticollis*, measured 8 cm in length and 0.25 cm in width, similarly to *T. laticollis* from lynx reported in Alaska and Canada [13]. Out of the remaining 23 tapeworms, tentatively referred to as "*Taenia* sp. Eurasian lynx", three specimens were measured and further characterized (**Fig. 2**). Their lengths were 60.8 cm, 67.9 cm and 62.8 cm, whereas their widths were 0.51 cm, 0.60 cm and 0.64 cm. The diameter of their scolex, rostellum and sucker were 1474-1730 mm, 641-657 mm and 384-448 mm, respectively. Further measurements (ratio of total length to total width, posterior length, anterior length and guard length about large and small rostellar hooks) are shown in Supplementary **Table 1.**

Discussion

Here we report a novel *Taenia* species, provisionally named as "*Taenia* sp. Eurasian lynx", from the Eurasian lynx. Some common herbivorous prey items of this wild felid, such as the common hare (*Lepus capensis*) and the ibex (*Capra ibex*) are the most likely candidates to act as intermediate hosts of this novel species, which should be investigated further. In addition, another tapeworm species, *T. laticollis* was found in Eurasian lynx for the first time in China.

"*Taenia* sp. Eurasian lynx", 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 [4, 5, 13-15]. Analysis of the COX1 protein amino acid sequences showed that in comparison with *T. hydatigena*, *T. regis* and *T. lynciscapreoli*, "*Taenia* sp. Eurasian lynx" has 2-4 amino acids substitutions (Supplementary **Fig. 2**). These findings confirm "*Taenia* sp. Eurasian lynx" as a novel *Taenia* species, which is also supported by the morphological characteristics of the scolex and rostellar hooks showing differences from those of other *Taenia* spp. that are known to infect lynxes [4, 15].

As reported previously, the definitive hosts of *T. laticollis* include the Eurasian lynx, the Canada lynx (*Lynx canadensis*), the timber wolf (*Canis lupus lycaon*) and the coyote (*Canis latrans*) [16-19]. Here *T. laticollis*

haplotype C was also found in Eurasian lynx in China. This finding suggests that the host specificity of *T. laticollis* in lynxes and wolves should be evaluated further according to haplotypes.

The definitive hosts of *T. hydatigena*, the species closest related to "*Taenia* sp. Eurasian lynx", include the coyote (*Canis latrans*), the timber wolf (*Canis lupus lycaon*), the black-backed jackal (*Lupulellamesomelas*), the Eurasian lynx (*Lynx lynx*), the Canada lynx (*Lynx canadensis*), the brown bear (*Ursus arctos*), whereas its intermediate hosts are the black-tailed jack rabbit (*Lepus californicus*), the European ground squirrel (*Spermophilus citellus*), domesticated ruminants, the springbok (*Antidorcas marsupialis*), the black wildebeest (*Connochaetus gnou*), the impala (*Aepyceros melampus*), the hartebeest (*Alcelaphus buselaphus*), the blue wildebeest (*Connochaetus taurinus*), the blesbuck (*Damaliscus pygargus phillipsi*) and the tsessebe (*Damaliscus lunatus*) [5, 7, 13, 17, 18, 20-22]. The West Junggar Mountains, between the Tianshan and Altai mountain belts, are located on the western rim of the Junggar Basin in northwestern China. Their landscape can be characterized as discontinuous treeless hills, with an altitude range from 2000-3000 m above sea level. In this region, the wildlife fauna relevant to the life cycles of cyclophyllidean cestodes of the family Taeniidae include the Pallas's cat (*Otocolobus manul*), the Eurasian lynx (*Lynx lynx*), the snow leopard (*Panthera uncia*), the grey wolf (*Canis lupus*), the red fox (*Vulpes vulpes*), the corsac fox (*Vulpes corsac*) and the Cape hare (*Lepus capensis*), all of which are sporadically distributed [23]. Since the number of Eurasian lynxes is limited in the West Junggar Mountains, other felids, as well as canids and lagomorphs should be investigated to systematically explore the definitive and intermediate hosts of *T. laticollis* and "*Taenia* sp. Eurasian lynx" in the near future.

Declarations

Ethical approval and consent to participate

This study was approved by the Animal Ethics Committee of Shihezi

University (Approval No. A2018-143-01).

Consent for publication

Not applicable.

Availability of data and materials

The sequences obtained and analyzed during the present study are deposited in the GenBank database, under the following accession numbers: MW846305, MW846313 and MW843568 (*cox1*); MW854635, MW854636 and MW843496 (*16S rRNA*).

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by the National Natural Science Foundation of China (81960379 and 31960709), International Scientific and Technological Cooperation in Bingtuan (2020BC008) and Non-profit Central Research Institute Fund of Chinese Academy of Medical Sciences (2020-PT330-003).

Authors' contributions

GL, SZ and YW conceived and designed the study, and wrote the manuscript. GL, SZ, CX, SW, XG and YW performed the experiments, analyzed the data. SH contributed to study design and edited the manuscript. All authors read and approved the final manuscript.

Acknowledgments

The authors thank the contributions by the staff at the School of Medicine and School of Animal Science and Technology, Shihezi University.

Author details

[1]Department of Basic Medicine, School of Medicine, Shihezi University, Shihezi, Xinjiang Uygur Autonomous Region, China. [2]Department of Parasitology and Zoology, University of Veterinary Medicine, Budapest, Hungary. [3]Department of Veterinary Medicine, College of Animal & Science, Shihezi University, Shihezi, Xinjiang Uygur Autonomous Region, China.

References

1. Castelló JR. Felids and hyenas of the world: Wildcats, Panthers, Lynx, Pumas, Ocelots, Caracals, and Relatives[M]. Princeton University Press, Princeton and Oxford. 2020.
2. Abuladze KI. "Principles of Cestodology, edited by K. I. Skryabin. Vol. IV. Taeniata-cestodes of animals and man and the diseases caused by them." (1964).
3. Hoberg EP. Taenia tapeworms: their biology, evolution and socioeconomic significance. Microbes Infect. 2002;4:859-66.
4. Lavikainen A, Haukisalmi V, Deksne G, Holmala K, Lejeune M, Isomursu M, et al. Molecular identification of *Taenia* in the Eurasian lynx (*Lynx lynx*) from Finland. Parasitology. 2013;140:653-62.
5. Loos-Frank B. An up-date of Verster's (1969) 'Taxonomic revision of the genus *Taenia* Linnaeus' (Cestoda) in table format. Syst Parasitol. 2000;45:155-83.
6. Sağlam YS, Oruç E, Avcıoğlu H, Altun S. Diagnostic exercise: gastroenteritis and pneumonia due to *Mesocestoides* and *Toxascaris leonina* in a Eurasian lynx (*Lynx lynx*). Pakistan Vet J. 2014;34:143-5.
7. Valdmann H, Moks E, Talvik H. Helminth fauna of Eurasian lynx (*Lynx lynx*) in Estonia. J Wildl Dis. 2004;40:356-60.

8. Avcioglu H, Guven E, Balkaya I, Kirman R. *Echinococcus multilocularis* in a Eurasian lynx (*Lynx lynx*) in Turkey. Parasitology. 2018;145:1147-50.
9. Kołodziej-Sobocińska M, Yakovlev Y, Schmidt K, Hurníková Z, Ruczyńska I, Bednarski M, et al. Update of the helminth fauna in Eurasian lynx (*Lynx lynx*) in Poland. Parasitol Res. 2018;117:2613-21.
10. Liu GH, Lin RQ, Li MW, Liu W, Liu Y, Yuan ZG, et al. The complete mitochondrial genomes of three cestode species of *Taenia* infecting animals and humans. Mol Biol Rep. 2011;38:2249-56.
11. Ali I, Panni MK, Iqbal A, Munir I, Ahmad S, Ali A. Molecular characterization of *Echinococcus* species in Khyber Pakhtunkhwa, Pakistan. Acta Scientiae Veterinariae. 2015;43:1277.
12. Li XJ, Yang YM. Identification of Taenia cestodes in Xianggelila, Yunnan by molecular and morphological methods. J Pathogen Bio 2009; 4:196-7.
13. Verster A. A toxonomic revision of the genus *Taenia* Linnaeus, 1758 S. str. Onderstepoort J Vet Res. 1969;36:3-58.
14. Myczka AW, Jeżewski W, Filip-Hutsch KJ, Pyziel AM, Kowal J, Demiaszkiewicz AW, et al. The morphological and molecular identification of the tapeworm, *Taenia lynciscapreoli*, in intermediate and definitive hosts in Poland. Int J Parasitol Parasites Wildl. 2020;11:213-20.
15. Haukisalmi V, Konyaev S, Lavikainen A, Isomursu M, Nakao M. Description and life-cycle of *Taenia lynciscapreoli* n. (Cestoda, Cyclophyllidea). Zookeys. 2016;584:1-23.
16. Skinker MS. Two new species of tapeworms from carnivores and a redescription of *Taenia laticollis* Rudolphi, 1819. Proceedings of the United States National Museum, 1935;83:211-20.
17. Grundmann AW. Cestodes of mammals from the Great Salt Lake desert region of Utah. J Parasitol. 1958;44:425-9.
18. Freeman RS, Adorjan A, Pimlott DH. Cestodes of wolves, coyotes, and coyote-dog hybrids in Ontario. Canadian J Zool, 1961;39:527-532.
19. Smith JD, Ad Dison EM, Joachim DG, Smith LM, Quinn NWS. Helminth parasites of Canada lynx (*Felis canadensis*) from northern Ontario. Canadian Journal of Zoology. 1986;64:358-364.
20. Bridger KE. A comparative study of the dietary habits and helminth fauna of Canada lynx (*Lynx canadensis*), Red fox (*Vulpes vulpes*) and Eastern coyote (*Canis latrans*) on insular Newfoundland. Masters thesis, 2005.
21. Nguyen MT, Gabriël S, Abatih EN, Dorny P. A systematic review on the global occurrence of *Taenia hydatigena* in pigs and cattle. Vet Parasitol. 2016;226:97-103.
22. Ouchenekhelifi NA, Ouchene N. Infestation and biochemical content of *Cysticercus tenuicollis* cysts (*Taenia hydatigena* cysts) in sheep and goats. J Entomol and Zoo Studies 2017; 5: 822-5
23. Ablimiti AQ. Classification and distribution of mammals in Xinjiang[M]. Science Press, Beijing. 2013.

Figures

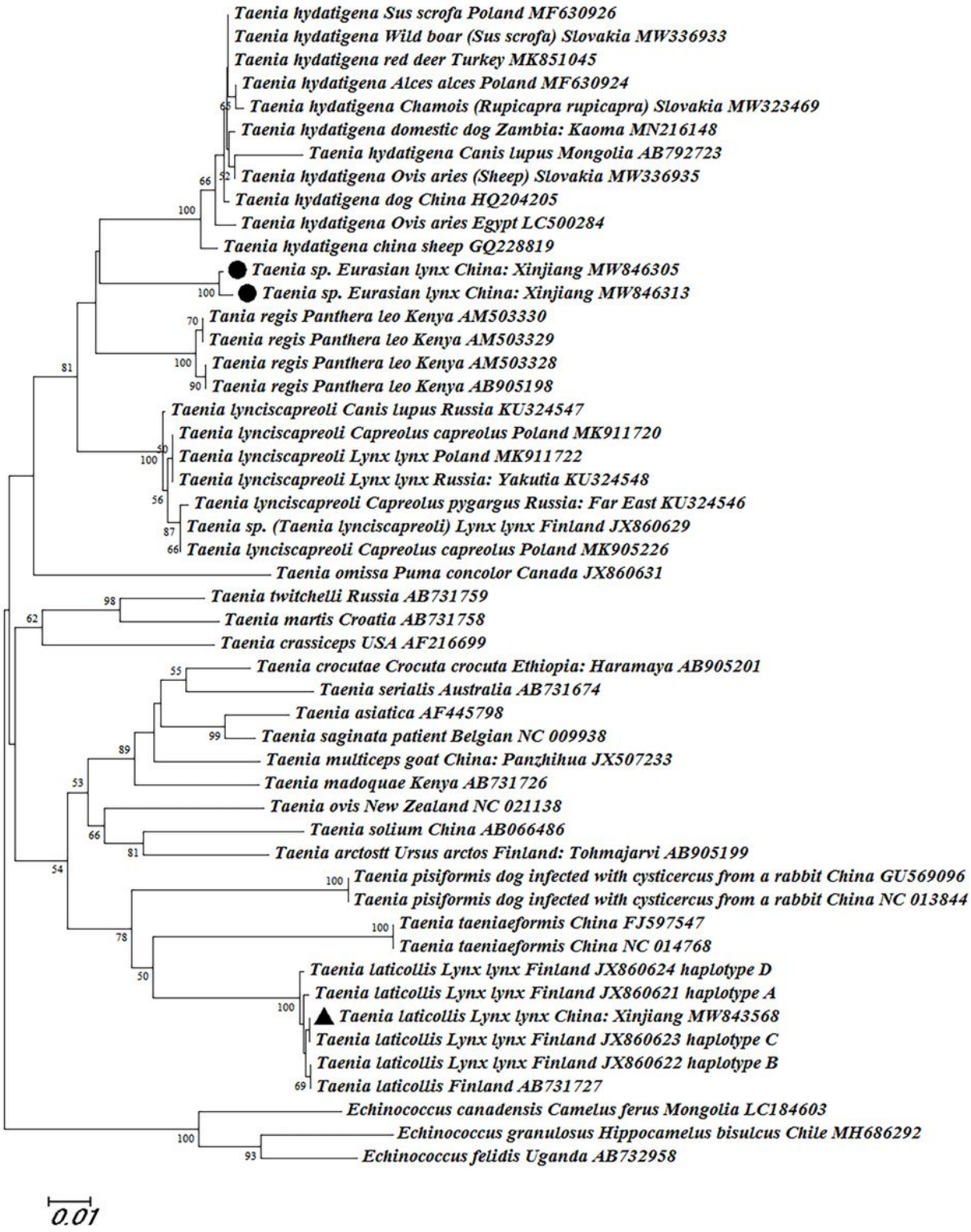
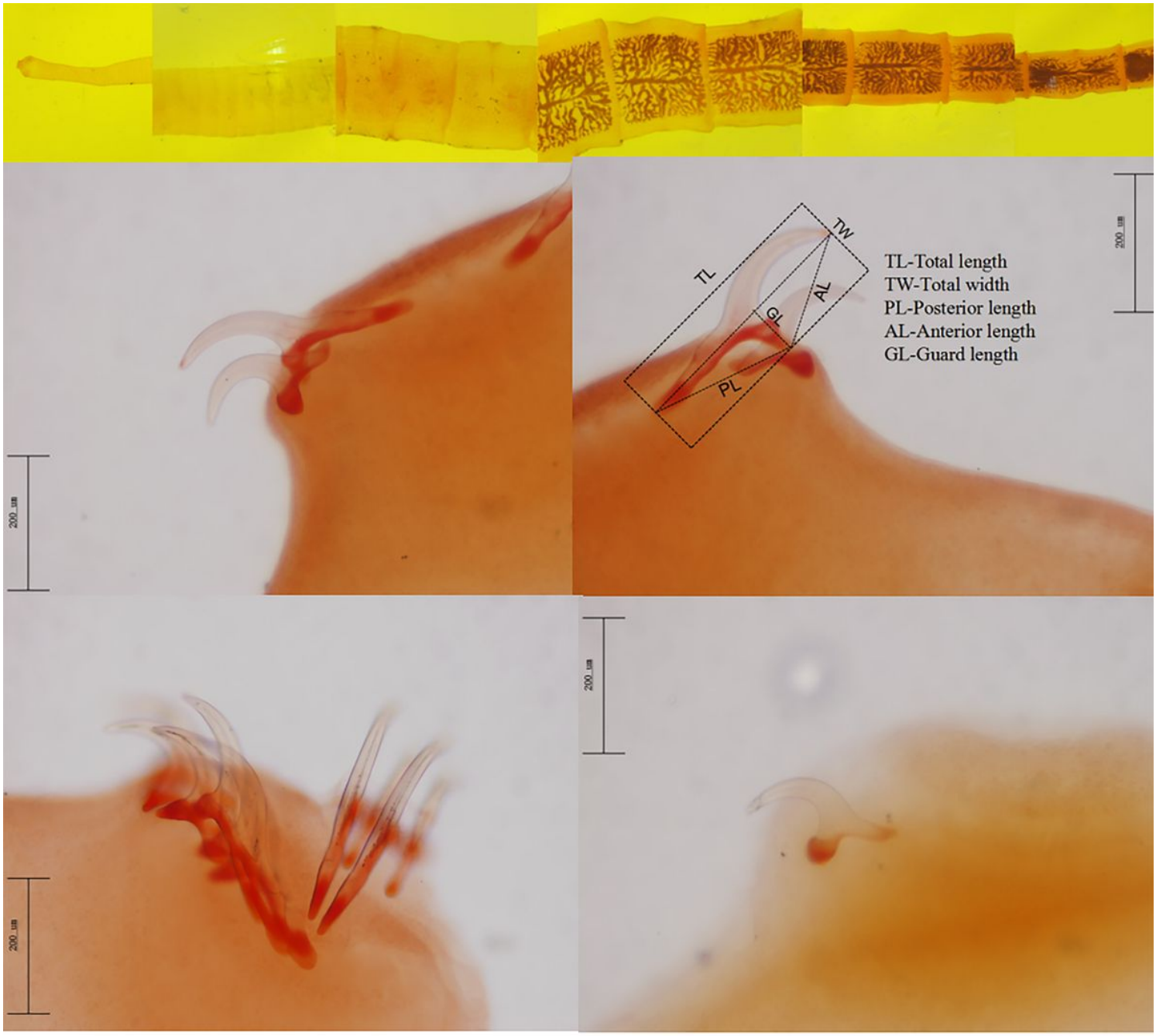


Figure 1

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



Taenia sp. Eurasian lynx (adult)

Figure 2

Morphological characteristics of “Taenia sp. Eurasian lynx”.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- GraphicalAbstract.tif
- SupplementaryFig.1.tif

- SupplementaryFig.2.tif
- SupplementaryTable1.doc