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The morphological and molecular identification of the tapeworm, *Taenia lynciscapreoli*, in intermediate and definitive hosts in Poland

Anna W. Myczka[a,*], Witold Jeżewski[a], Katarzyna J. Filip – Hutsch[a], Anna M. Pyziel[b], Jerzy Kowal[c], Aleksander W. Demiaszkiewicz[a], Zdzisław Laskowski[a]

[a] Witold Stefański Institute of Parasitology Polish Academy of Sciences, Twarda 51/55, 00-818, Warsaw, Poland
[b] Department of Food Hygiene and Public Health Protection, Institute of Veterinary Medicine, Warsaw University of Life Sciences-SGGW, Nowoursynowska 166, 02-787, Warsaw, Poland
[c] Department of Zoology and Ecology, Faculty of Animal Breeding and Biology, Agricultural University in Cracow, Adama Mickiewicza 24/28, 30-059, Cracow, Poland

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ABSTRACT

The tapeworm *Taenia lynciscapreoli* is a new species of the genus *Taenia* described in 2016, and which remains poorly understood. The aim of the present study is to extend current knowledge regarding its, morphology and genome. Biological material was analysed from three species of wild animals: Eurasian lynx (*Lynx lynx*), roe deer (*Capreolus capreolus*) and moose (*Alces alces*). Twenty-four adult tapeworms and four larvae were obtained from Eurasian lynx and roe deer respectively; none were detected in the studied moose. On the basis of morphometric (hooks measurements) and molecular analysis (partial 780 bp *cox* 1 gene sequences), the analysed tapeworm was identified as *Taenia lynciscapreoli* species. The phylogenetic analysis of the obtained sequences identified two haplotypes. The obtained findings can be used to supplement the species description. To our knowledge this is the first morphological and molecular identification of *T. lynciscapreoli* in roe deer, intermediate host, in Poland.

1. Introduction

Taenia lynciscapreoli (Haukisalmi et al., 2016) was described for the first time in 2016 (Haukisalmi et al., 2016); however, it had appeared previously in scientific reports as a tapeworm assigned to the genus *Taenia* (Lavikainen et al., 2013). Little is known about biology and life cycle of *T. lynciscapreoli* so far. Eurasian lynx (*Lynx lynx*) (Kerr, 1792) and roe deer (*Capreolus capreolus*) (Linnaeus, 1758) are typical definitive and intermediate host respectively, according to Haukisalmi et al. (2016), the occurrence of the parasite in the environment strictly depends on the presence of both typical hosts in the area. However the tapeworm was found also in small intestine of wolf (*Canis lupus*) (Linnaeus, 1758) as definitive host and moose (*Alces alces*) (Linnaeus, 1758) as intermediate one (Haukisalmi et al., 2016) The larvae (metacestode) of *T. lynciscapreoli* typically encyst on the lungs of intermediate host and no other location was reported so far. According to published work on *T. lynciscapreoli*, this tapeworm occurs in Europe in the south and west parts of Finland, in the north-east of Poland (Haukisalmi et al., 2016; Kołodziej – Sobocińska et al., 2018) and in Asia in the Altai Krai and Yakutia in Russia (Haukisalmi et al., 2016). So far, three intermediate hosts have been identified: roe deer, Siberian roe deer (*Capreolus pygargus*) (Pallas, 1771) and moose and two definitive hosts: lynx and wolf (Haukisalmi et al., 2016).

In Poland, the first and only record of *T. lynciscapreoli* was obtained in 2018 (Kołodziej – Sobocińska et al., 2018) in a supplementary study about parasites in Eurasian lynx. There is no other data about prevalence of this tapeworm in Poland, possibly due to high habitat fragmentation, resulting in small and very dispersed lynx population (Kowalczyk et al., 2015). Despite numerous populations of roe deer (Central Statistical Office; www.stat.gov.pl, access 23.01.2019 and Data Bank about Forests in Poland data bases; www.bdl.lasy.gov.pl, access 23.01.2019), studies of *T. lynciscapreoli* remains limited as occurrence of lynx in the environment is crucial to the transmission of the tapeworm. Additionally, research on *T. lynciscapreoli* is difficult to perform in Poland, because lynx are subject to strict protection while intermediate hosts such as roe deer and moose, are also subject to seasonal or all-season protection, respectively by Ministry of Environment (Regulation of the Minister of the Environment dated 6 October 2014 on the Protection of Animal Species; Ministry of the Environment website www.gov.pl/web/environment, access 13.01.2019).

Lynx is common in eastern and northern Europe (e.g.: Czech Republic, Estonia, Finland, Sweden, Ukraine) and in northern and central Asia (e.g.: Russia, China, Mongolia, Kazakhstan) (IUCN Red List of Threatened Species; www.iucnredlist.org, accessed 10.01.2019). There are two populations of lynx in Poland: Baltic and Carpathian, both of which are regarded as stable (Kaczensky et al., 2012; Mysłajek

* Corresponding author.
E-mail address: annamyczka@twarda.pan.pl (A.W. Myczka).

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Fig. 1. Map of Poland with marked places (approximate locations) of the material collection.

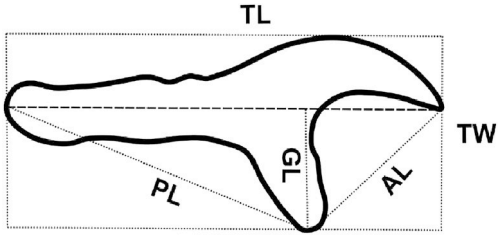

Fig. 2. Measurement scheme used to measure rostellar hooks.

et al., 2019). The Polish Central Statistical Office estimates that 432 lynxes were present in the country in 2017 (Rozkrut et al., 2018). Roe deer is a game species whose population is estimated to range from 922 000 to 946 000 individuals nationwide (Central Statistical Office; www.stat.gov.pl, access 23.01.2019 and Data Bank about Forests in Poland data bases; www.bdl.lasy.gov.pl, access 23.01.2019). Moose was also regarded as a game animal until relatively recently; however, a ban on moose hunting (suspension of hunting/moratorium) imposed in Poland in 2001 resulted in a significant expansion in the moose population. The Ministry of the Environment recently estimated the nationwide moose population to be approximately 28 000 individuals (Wawrzyniak, 2016). Nevertheless, material for parasitological studies is still hard to collect.

The life cycle of *T. lynciscapreoli* parasite can proceed fully in Poland due to the presence of both intermediate and final hosts. Despite still small population of lynx, roe deer displays an upward trend and might be crucial in transmission the tapeworm on other wild hosts, e.g. wolves. As a game animal, roe deer might also play an important role in spreading the tapeworm to hunting dogs and transfer *T. lynciscapreoli* into a domestic life cycle. This makes studies of *T. lynciscapreoli* in Poland an important and emerging matter.

The aims of this study are to supplement the species description of the newly discovered tapeworm and the first morphological and molecular identification of *T. lynciscapreoli* in Poland in an intermediate host.

Table 1
Organism name, GenBank accession number and region of origin, nucleotide sequences of the *cox1* partial gene of tapeworms used to create the phylogenetic tree.

Organism	Sequences	Region of Origin
Taenia lynciscapreoli	MK905226.1	POLAND
	MK805225.1	
	MK911720.1	
	MK911721.1	
	MK911722.1	
	MK911723.1	
	MK911724.1	
	MN117889.1	
Taenia regis	NC_024589.1	KENYA
Taenia hydatigena	JN831308.1	CHINA
	GQ228819.1	
	MF630924.1	POLAND
	MF630925.1	
Taenia saginata	AB533173.1	THAILAND
Taenia asiatica	AB597275.1	JAPAN
Taenia crocutae	AB905201.1	ETHIOPIA
Taenia multiceps	GQ228818.1	CHINA
Taenia madoquae	AB731726.1	KENYA
Taenia arctos	NC_024590.1	FINLAND
Taenia ovis	NC_021138.1	NEW ZEALAND
Taenia pisiformis	NC_013844.1	CHINA
Taenia twitchelli	AB731759.1	RUSSIA
Taenia martis	AB731758.1	CROATIA
Taenia crassiceps	AF216699.1	USA
Echinococcus multilocularis	AB461413.1	FRANCE
Echinococcus granulosus s.s.	AB688619.1	CHINA

2. Materials and methods

2.1. Materials

Biological material was obtained from seven locations in Poland, divided between the north-east, south-east and central regions. All tapeworms isolated from wild animals were preserved in ethanol (70%).

In November 2017, one male lynx (about 18 months old) was killed in a

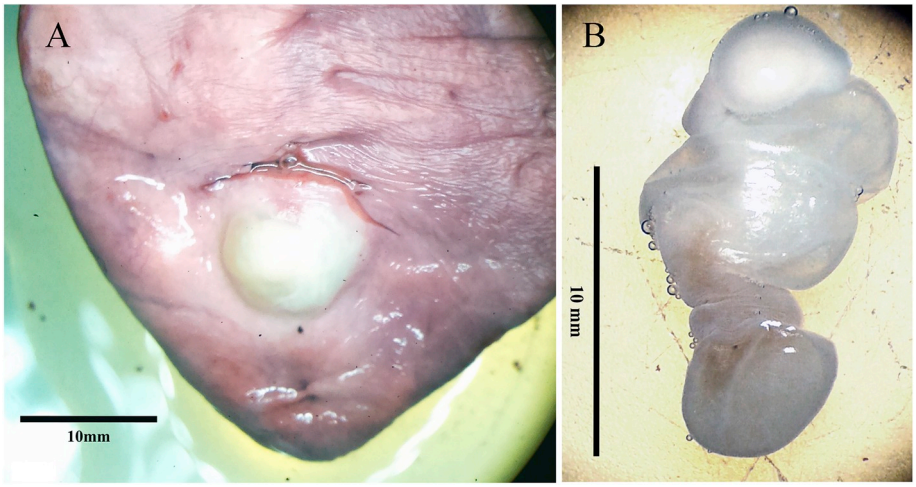

Fig. 3. A – lung of roe deer with cyst of tapeworm *T. lynciscapreoli*; B – Cysticercoid of *T. lynciscapreoli*.

Fig. 4. The crown of *T. lynciscapreoli*; examples of empty spaces, from where hooks have been lost are indicated (arrows).

road accident in Wisłok Wielki in the Bieszczady Mountains (Podkarpackie Province). In addition, 71 roe deer (lungs) from: Strzałowo Forest District in Pisz Forest (Warmia and Mazury Province), Sanok area (Podkarpackie Province), the Niepołomice Forest (Małopolskie Province) and Białowieża Forest (Podlaskie Province). All roe deer were culled during the hunting season 2017/2018. In the period 2018 to 2019, six moose, found dead or killed in road accidents, were necropsied in Kampinos Forest (Mazovia Province) and West Polesie (Lubelskie Province) (Fig. 1).

2.2. Methods

The morphology of the intact cysticerci and rostellar hooks of the metacestodes and adult tapeworms was examined. The hooks crowns were localized and identified by mounting the cysticerci with introverted scolices in Faure's fluid (Brown, 1997), without staining. To analyses the rostellar hooks in the all tapeworms, the crowns were mechanically cut from the scolices, and then was temporarily mounted in Faure's fluid, for photography and study. For morphological identification, the rostellar hooks were liberated from the crowns using an aqueous solution of 1% pepsin and 0.4% HCl. The hooks were then rinsed in water and dehydrated in ethanol. Some of the hooks (from 10 adult and 1 larva) were mounted in Faure's fluid as a temporary slide, and others (14 adult and 3 larvae) in Canada balm as a permanent slide. Only hooks aligned well in the horizontal plane were used for morphometric analysis and photography. The rostellar hooks were examined according to Haukisalmi et al. (2011), using an Olympus BX50 light microscope with a Cell D digital image analysis (Fig. 2).

DNA was extracted from all collected metacestodes (roe deer) and five individual adult tapeworms (lynx) with different numbers of rostellar hooks and of varying sizes (Table 5). The extraction was performed using DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. PCR reactions were then performed with primers Thg452F (5′TGCATTTAGCTGGTGCGTCAAGTA-3′) and Thg1326R (5′ACA AACACGCCGGGGTAACC-3′) (Filip et al., 2019) to obtain a 874 bp fragment of the mitochondrial cytochrome c oxidase subunit 1 gene (*cox*1). The reactions were conducted in a 40 µl reaction mixture containing 2.0 µl of DNA template, 0.2 µl (1U) of Color Taq DNA Polymerase (EURx), 1 µl of dNTPs mix (10 mM), 0.5 µl of each primer (20 mM), 5 µl of 10 × Polymerase buffer (pH 8.6, 25 mM $MgCl_2$) and 30.8 µl of MiliQ water. A negative

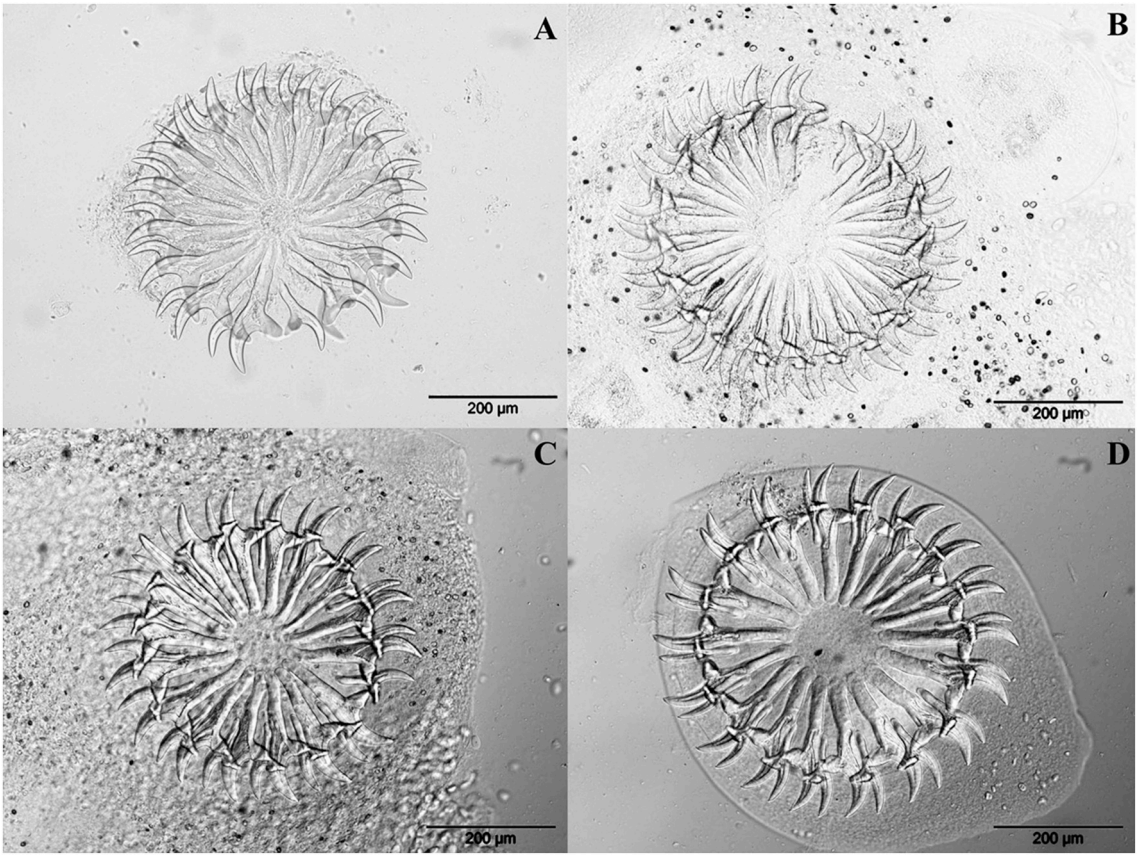

Fig. 5. Four crowns of *T. lynciscapreoli*, A – C larvae, D – adult; A – AM1 (32 hooks), B – APS3 (38 hooks), C – APS2 (34 hooks), D – R17 (36 hooks).

Table 2
Measurements (µm) of large rostellar hooks (TL - Total length; TW - total width; PL - posterior length; AL - anterior length; GL guard length) The table presents the arithmetic mean of measurements, with the minimum and maximum rostellar hooks measurements given in brackets.

Hosts/Isolate	No.	TL	TW	PL	AL	GL
L. lynx R1 – R24	57	223.8 (213.0–235.3)	95.6 (89.0–101.7)	160.5 (146.3–170.6)	90.5 (84.0–96.9)	53.9 (44.2–60.7)
C. capreolus AM1	15	219.5 (215.1–223.2)	88.8 (85.4–90.2)	152.8 (145.6–157.0)	89.3 (86.5–92.0)	49.4 (44.0–51.3)
C. capreolus APS1-APS3	49	219.9 (208.8–226.6)	90.7 (88.0–94.6)	147.9 (133.3–153.2)	92.2 (88.4–96.3)	46.0 (42.5–49.6)

Table 3
Measurements (µm) of small rostellar hooks (TL - Total length; TW - total width; PL - posterior length; AL - anterior length; GL guard length).

Hosts/Isolate	No.	TL	TW	PL	AL	GL
L. lynx R1 – R24	40	137.0 (124.4–147.7)	72.3 (65.9–76.8)	96.9 (84.7–113.0)	68.9 (65.0–73.6)	45.6 (38.8–53.1)
C. capreolus AM1	14	127.2 (122.8–131.1)	66.1 (63.2–68.6)	84.7 (79.5–87.8)	64.6 (63.2–67.4)	38.6 (36.0–41.9)
C. capreolus APS1-APS3	43	132.2 (125.7–142.2)	71.0 (68.4–73.6)	88.4 (83.1–98.3)	70.6 (65.7–73.2)	42.2 (40.5–49.8)

control - consisting nuclease-free water was also added to the PCR mix instead of the tested DNA.

DNA amplification was performed using the DNA Engine T100 Thermal Cycler (BioRad) according to the following program: denaturation at 95 °C for 1 min, followed by 35 cycles of denaturation at 95 °C for 20 s, annealing at 56 °C for 20 s and extension at 72 °C for 40 s, with a final extension performed at 72 °C for 5 min. The PCR products were visualized on a 1.2% agarose gel (Promega) stained with SimplySafe (EURx). Visualization was performed using ChemiDoc, MP Lab software (Imagine, BioRad). The obtained PCR products were purified with QIAquick Gel Extraction Kit (Qiagen, Germany). The purified products were sequenced directly using ABI BigDye™ chemistry (Applied Biosystems, USA) on an ABI Prism 373xl or an ABI Prism 3100™ automated sequencer. The results were compared with relevant sequences of *Taenia lynciscapreoli* from lynxes, Siberian roe and wolf from the GenBank database. Phylogenetic analysis was performed based on partial (780bp) sequences of the mitochondrial cytochrome c oxidase subunit 1 gene using the newly generated sequences of *Taenia lynciscapreoli* and matching sequences of the representatives of other *Taenia* species available in GenBank (Table 1). Forward and reverse sequences were assembled using ContigExpress (Vector NTI Advance 11, Invitrogen) software. Contiguous sequences were submitted to GenBank (Table 1). *Cox 1* sequences were aligned using AlignX implemented in Vector NTI Advance 11 (Invitrogen). The alignment was trimmed to the length of the shortest sequence. Phylogenetic trees were constructed using Bayesian inference (BI) as implemented in the MrBayes version 3.2.0 software (Huelsenbeck and Ronquist, 2001). The HKY + G + I model was chosen as the best-fitting

Table 4
Variable sites in *cox* 1 partial gene sequences from *T. lynciscapreoli* aligned with all available sequences derived from GeneBank. Nucleotide position numbering is based on the *T. lynciscapreoli cox* 1 gene sequence, GenBank Accession no. MK905226.1. New sequences obtained in this study are in bold.

Origin	GeneBank Accession Number	Host	Nucleotide position						
			21	84	282	378	420	612	795
Poland	**MK905226**	***Capreolus capreolus***	**C**	**T**	**C**	**A**	**C**	**G**	**A**
Finland	JX860629	*Lynx lynx*	-	-	C	A	C	G	-
Poland	**MK911720-24, MK905225, MN117889**	***L. lynx, C. capreolus***	**T**	**A**	**C**	**A**	**T**	**C**	**G**
Poland	MK033479	*L. lynx*	T	A	C	A	T	C	G
Russia	KU324548	*L. lynx*	-	-	C	A	T	C	-
Russia	KU324547	*Canis lupus*	-	-	T	A	T	C	-
Russia	KU324546	*Capreolus pygargus*	-	-	C	G	C	G	-

nucleotide substitution model using JModelTest version 2.1.10 software (Guindon and Gascuel, 2003; Darriba et al., 2012). Sequences of *Echinococcus granulosus sensu stricto* GenBank accession number AB688619 and *Echinococcus multilocularis* GenBank accession number AB461413 were used as the outgroup. Analysis was run for 1,000,000 generations, with 250,000 generations discarded as burn-in. Phylogenetic trees were visualized using the TreeView software.

3. Results

3.1. Collection of biological materials

The biological material for the study was taken from three animal species: one Eurasian lynx, 71 roe deer and six moose. Twenty-four individual adult tapeworms were recovered from the small intestine of the lynx. A significant number of the adult tapeworms were damaged, but the longest strobila had 50 cm length. In addition, a total of four metacestodes were obtained from the lungs (on the surface of lung) of two of the analysed roe deer from: Pisz Forest (one metacestode; Warmia and Mazury Province), and Białowieża Forest (three metacestodes; Podlaskie Province) (2/71; 2,82%). The tapeworm cyst in the roe deer lung had the following dimensions before cutting: 13 mm in length, 12 mm in width. However, these were found to be 17 mm (16,8–17,3 mm) long, and 7 mm (6–7,5 mm) wide after being cut form lung (Fig. 3). No *T. lynciscapreoli* larvae were found in the moose.

3.2. Morphological analysis

All isolated tapeworms: adults (n = 24) and larvae (n = 4) had crowns with rostellar hooks. Scolex measurements (μm) based on ten adult tapeworm samples temporarily mounted in Faure's fluid. Scolex width of 728.7–921,1 μm. Suckers 272.3 μm (251.4–298.9 μm) in length and 249.5 μm (221.1–277.8 μm) in width (n = 36). Average of measurements (minimum and maximum measurements). Although some of the *Taenia* specimens had lost some of the hooks from the crown, precise microscopic analysis was able to reveal the empty spaces left in the crown before it was damaged (Fig. 4). The total numbers of small and large hooks in the crowns ranged from 32 to 38 (Fig. 5).

Measurements of rostellar hooks of *Taenia lynciscapreoli* are shown in Table 2 and Table 3.

3.3. Molecular analysis

Although the cestodes obtained from the definitive host, the *L. lynx* from the southern part of Poland, differed with regard to their rostellar hook numbers, their sequences were found to be identical to each other and to the cestodes derived from the intermediate host (*C. capreolus* from Białowieża Forest). A homologous 396 bp section of the DNA sequences from the lynx (Wisłok Wielki) and roe deer (Białowieża Forest) were to that obtained from a lynx from Russia (KU324548.1). The sequence of roe deer (Strzałowo Forest) in this section (396 bp) is identical to the sequence obtained from a lynx from Finland (GeneBank accession number JX860629.1) (Table 4).

Sequence data was obtained from a total of nine isolated cestodes: five adult individuals taken from the lynx and four metacestodes from the roe deer. Upon trimming to the length of the shortest sequence, the *cox*1 gene alignment was found to be 780 bp length. Following Bayesian phylogenetic analysis, a phylogenetic tree containing a three of strongly supported clades was generated (Fig. 6). Isolates of *T. lynciscapreoli*, *T. hydatigena* and *T. regis* appeared in the tree within a major, 100% supported clade. The remaining *Taenia* species form 2 well-supported (100%) separate clades. Unlike *T. lynciscapreoli*, in *T. hydatigena* sequences we can observe a much greater genetic diversity. Isolates of this species clustered with somewhat low posterior probability (88%).

4. Discussion

The tapeworm *T. lynciscapreoli* was described for the first time by Haukisalmi et al. (2016) on the basis of 14 adults and 11 larvae (n = 25), with the tapeworm crown consisting of two rows of large and small rostellar hooks, ranging in number from 30 to 34. The morphological analysis in the present study, based on 28 individuals: 24 adult tapeworms (from Eurasian lynx) and 4 larvae (from roe deer), showed that the number of rostellar hooks range from 32 to 38. Hence the current description of the *T. lynciscapreoli* species should be modified to indicate that the number of hooks is in the range of 30–38.

Previous scientific reports of *T. lynciscapreoli* assumed that can be clearly distinguished from other species of *Taenia*: *T. arctos*, *T. parenchymatosa*, *T. hydatigena*, *T. ingwei*, *T. pisiformis*, *T. kotlani*, *T. krabbei*, *T. cf. kotlani*; by the shape (blade, guard) of its large hooks (Haukisalmi et al., 2016). This was confirmed by the morphological analysis in the present study (Fig. 7).

The mean length of the large rostellar hooks (TL) in the present study is 221.1 μm (208.8–235.3 μm). However, as these values vary considerably (Table 2), it is not possible to divide the individuals into groups solely by the hook length. The first description of *T. lynciscapreoli* (Haukisalmi et al., 2016) divides the species in two groups based on large hook length: smaller-sized hooks from Finland, mean length (TL) 211,6 μm (168–230 μm) and larger-sized hooks from Siberia and Russia Far East, mean length (TL) 227,1 μm (214–238 μm). Unfortunately, it is impossible to place the

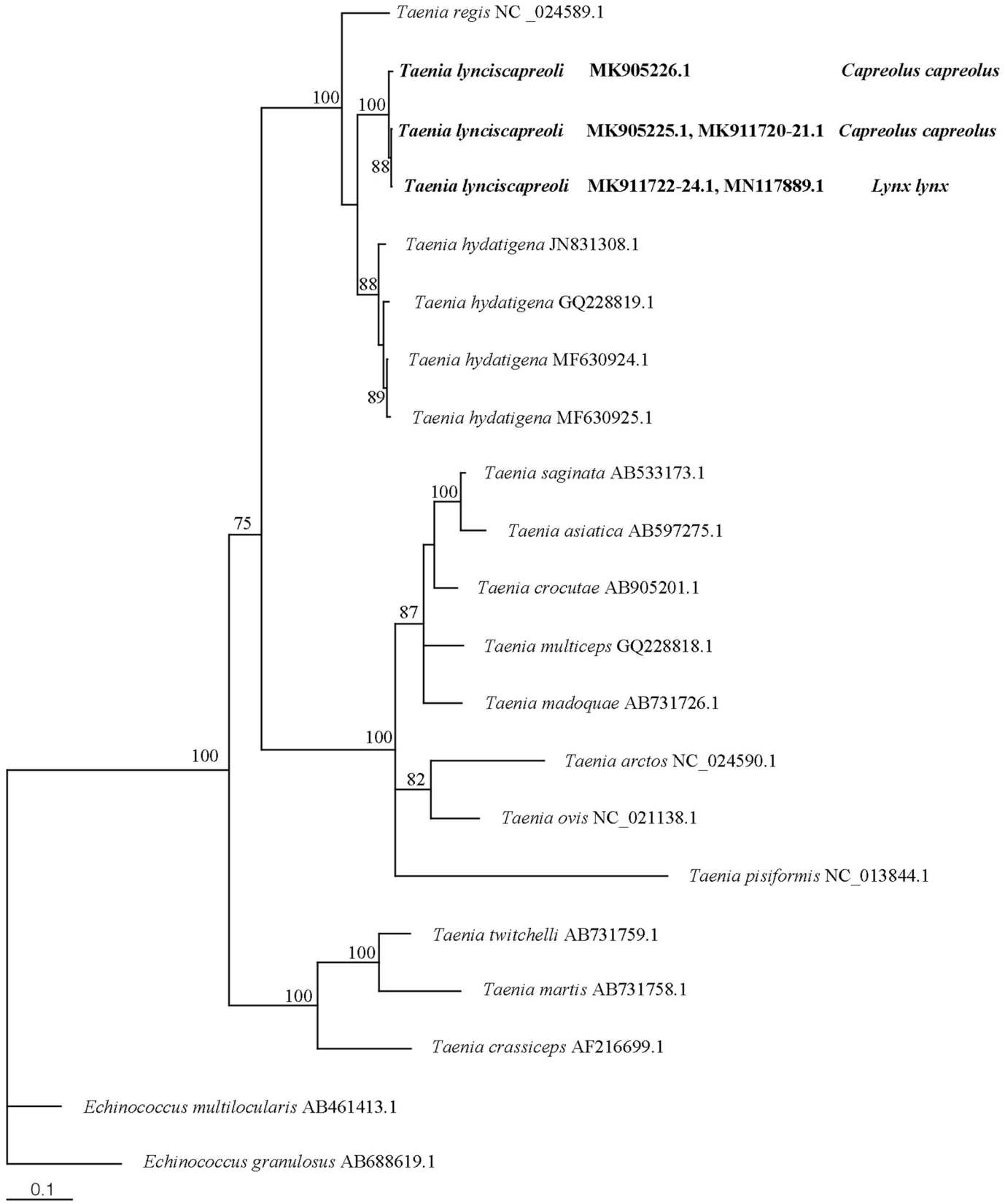

Fig. 6. Phylogenetic tree of *Taenia lynciscapreoli* haplotypes, constructed by Bayesian inference (BI) analysis using MrBayes version 3.2. The HKY + G + I model was chosen as the best-fitting nucleotide substitution model using JModelTest version 2.1.10 software (Guindon and Gascuel, 2003; Darriba et al., 2012). Sequences of *Echinococcus granulosus sensu stricto* GenBank accession number AB688619 and *Echinococcus multilocularis* GenBank accession number AB461413 were used as the outgroup. Analysis was run for 1,000,000 generations, with 250,000 generations discarded as 'burn-in'. Nodal support is indicated as Bayesian posterior probabilities. Sequences generated in this study are shown in bold. The scale bars are proportional to the number of substitutions per site.

Table 5
The haplotypes of *T. lynciscapreoli*. Measurements of rostellar hooks are in micrometers (μm); (TL – total length).

Accession no./Isolate	Hosts	Region	No. hooks in crown	Large hooks (TL)	Small hooks (TL)
MK911722.1/R10	*L. lynx*	Podkarpackie Province	34	225 (222–230)	139 (133–147)
MK033479.1/R16	*L. lynx*	Podkarpackie Province	32	217 (216–221)	132 (128–135)
MN117889.1/R17	*L. lynx*	Podkarpackie Province	36	223 (220–227)	132 (125–137)
MK911723.1/R23	*L. lynx*	Podkarpackie Province	34	216 (211–220)	135 (129–142)
MK911724.1/R24	*L. lynx*	Podkarpackie Province	34	214 (211–216)	141 (135–146)
MK905226.1/AM1	*C. capreolus*	Warmia and Mazury Province	32	215 (211–219)	127 (123–131)
MK905225.1/APS1	*C. capreolus*	Podlaskie Province	34	222 (218–226)	134 (131–138)
MK911720.1/APS2	*C. capreolus*	Podlaskie Province	34	215 (209–222)	130 (126–137)
MK911721.1/APS3	*C. capreolus*	Podlaskie Province	38	222 (217–227)	132 (127–142)

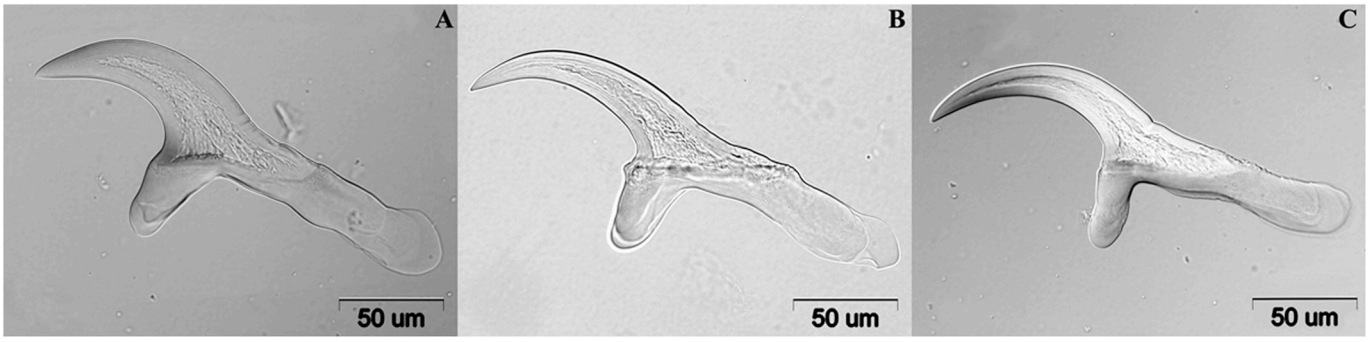

Fig. 7. Large rostellar hooks: A - larvae *T. lynciscapreoli* from roe deer; B – larvae *T. hydatigena* from wild boar, C - larvae *T. hydatigena* from moose.

Table 6
Comparison measurements (μm) of large rostellar hooks form this study and the first description of *T. lynciscapreoli* by Haukisalmi et al. (2016); (TL – total length; TW – total width; PL – posterior length; AL – anterior length; GL – guard length). In bold results from this study, the rest from Haukisalmi et al. (2016).

Hosts, regions	No.	TL	TW	PL	AL	GL
***L. lynx*, Podkarpackie Province**	**57**	**223.8 (213–235.3)**	**95.6 (89–101.7)**	**160.5 (146.3–170.6)**	**90.5 (84–96.9)**	**53.9 (44.2–60.7)**
***C. capreolus*, Warmia and Mazury Province**	**15**	**219.5 (215.1–223.2)**	**88.8 (85.4–90.2)**	**152.8 (145.6–157)**	**89.3 (86.5–92)**	**49.4 (44–51.3)**
***C. capreolus*, Podlaskie Province**	**49**	**219.9 (208.8–226.6)**	**90.7 (88–94.6)**	**147.9 (133.3–153.2)**	**92.2 (88.4–96.3)**	**46.0 (42.5–49.6)**
L. lynx, Finland	11	195.9 (168–228)	84.5 (78–94)	133.8 (144–162)	86.3 (76–97)	47.7 (42–51)
L. lynx, Russia	16	223.4 (214–231)	89.4 (79–96)	152.1 (138–162)	94.9 (87–101)	50.8 (40–59)
C. capreolus, Finland	3	216.5 (213–222)	87.5 (85–92)	144.2 (136–153)	96.9 (95–98)	49.9 (48–56)
C. pygargus, Russia	15	230.7 (215–238)	103.4 (94–109)	162.7 (148–171)	104.3 (92–111)	65.6 (54–88)
A. alces, Finland	7	222.3 (213–230)	90.9 (82–97)	154.8 (145–162)	94.0 (86–100)	52.3 (46–60)

tapeworms identified in the present study clearly into one of these groups as the mean length of the hooks in the present study lie between the two groups (221.1 μm) (Table 6).

Measurements of small rostellar hooks (Table 3) of *T. lynciscapreoli*, in this work, define their dimensions (TL) are of 122.8–147.7 μm. However, in contrast to the analysis of large hooks, the analysis of measurements of small hooks allows to distinguish two groups: smaller length (TL) dimension hooks with a mean size of 127.2 μm and measuring ranges 122.8–131.1 μm (AM1 from Strzałowo Forest) and larger length (TL) dimension hooks with a mean size of 134,6 μm and measuring ranges 124.4–147.7 μm (R1 – R24 from the Bieszczady Mountains) and APS1 – APS2 from Białowieża Forest).

Based on our DNA analysis, to our knowledge, these findings may represent the first molecular identification of *T. lynciscapreoli* form roe deer – intermediate host in Poland.

Our findings indicate the existence of two haplotypes of *T. lynciscapreoli* in Poland. The common 396 bp *cox1* sequences isolated from adult tapeworms (lynx, Wisłok Wielki) and metacestodes (roe deer, Białowieża Forest) are 100% identical with those taken previously from Eurasian lynx (GeneBank accession number: KU324548.1, Russia). The same 396 bp *cox1* sequence obtained from the metacestodes isolated from roe deer (Strzałowo Forest) is 100% identical with that previously isolated from lynx in Finland (GeneBank accession number: JX860629, Finland) (Table 4).

Previous analysis of the 396 bp *cox 1* fragment (Haukisalmi et al., 2016) revealed the occurrence of four haplotypes and two genetic clades within *Taenia lynciscapreoli*. In our present study, two haplotypes were found, each representative of the two clades (Fig. 6). Haplotype 1 found in roe deer from the Strzałowo Forest is located in a clade with tapeworms from Finland (lynx, roe deer and moose) and Russia (Siberian roe deer, wolf, lynx) (Haukisalmi et al., 2016) (Table 4); haplotype 2 found in roe deer in Białowieża and lynx in the Bieszczady Mountains is identical to material obtained from lynx (Altai Krai) is located in a second clad with tapeworms obtained from wolf and lynx respectively from Altai Krai and Yakutia in Russia (Haukisalmi et al., 2016) (Table 4). Our analysis of *cox* 1 sequences (780 bp) confirmed division of *T. lynciscapreoli* into two genetic lines (Fig. 6). The occurrence of individual genetic forms of *T. lynciscapreoli* in *L.*

lynx populations in Europe and Asia requires additional research.

A review of our present morphometric and molecular findings allows the determination of two groups (haplotypes) of *T. lynciscapreoli*. Haplotype 1, comprising one larva obtained from a roe deer (AM1, Strzałowo Forest) display clearly shorter small hooks (Table 3) and form a separate clade according to phylogenetic analyses (Fig. 6). Haplotype 2, comprising all lynx adults tapeworms (R1 - R24, Wisłok Wielki) and three larvae from one roe deer (APS1 - APS3, Białowieża Forest) is characterized by clearly longer small hooks (Table 3) and form a second clade (Fig. 6).

Previous studies suggest that *T. lynciscapreoli* is closely related to *T. hydatigena* and *T. regis* (Haukisalmi et al., 2016; Filip et al., 2019); a relationship confirmed by our present phylogenetic analysis. These tapeworm species possess similar life cycles, with the intermediate hosts being wild and farmed ungulates (cervids and bovids) and the final hosts being carnivores from the felids and canine family (Zhang et al., 2007; Kołodziej - Sobocińska et al., 2018; Filip et al., 2019). The similarities in life cycle, hosts and *cox1* DNA sequence suggests that they may share a common ancestor (Hoberg et al., 2000). In addition, the larvae forms resemble a bladder-like cyst (Fig. 3) in the spaces of the peritoneal cavity, either located inside the peritoneum proper or in the organs within (Hoberg et al., 2000; Zhang et al., 2007). Although there is no data about the impact of *T. lynciscapreoli* on infected animals, cysticercoids caused by similar species from the genus *Taenia* could be the cause of traumatic hepatitis of infected intermediate host and thus economic loses in livestock production (Carlos et al., 2006). Therefore, potentially negative impact of *T. lynciscapreoli* infection on ruminants should not be excluded, despite lack of yet reported evidence.

In addition to *T. lynciscapreoli* and *T. hydatigena* possessing similar life cycles in terms of hosts and are found in similar geographical regions (Sgroi et al., 2019), they may also be confused with each other on macroscopic examination, for example during an animal section. In this case it should be noted that the two species can be distinguished by morphometric analysis of the rostellar hooks in the crown. In both cases, the large rostellar hooks have similar long, thick, straight handles and prominent, slightly pointed guards; however, significant differences can be observed in the construction of the blade: the large hooks of *T. lynciscapreoli* present blades that are shorter, wider and more strongly curved than those of *T. hydatigena* (Fig. 7) (Filip et al., 2019).

In conclusion, the results of the morphometric and DNA analyses described in the present study indicate that our examined metacestodes and adult tapeworms can be classified as *T. lynciscapreoli*. In addition, as our samples presented 32 to 38 hooks in the crown, the official description of *T. lynciscapreoli* should be modified accordingly, i.e. to include 30 to 38 rostellar hooks. Based on our analysis, to our knowledge, these findings may represent the first morphological and molecular identification of *T. lynciscapreoli* form roe deer – intermediate host in Poland.

As *Taenia lynciscapreoli* is a comparatively recent discovery, further studies are needed to present a better understanding of this tapeworm. In the future, distribution range and the prevalence of *T. lynciscapreoli* in a typical definitive and intermediate host in Poland should be examined, possibly using also analysis of faecal samples (from definitive hosts) to increase the range of the studies. It is also necessary to determine what other species of wild animals might be parasite hosts and to evaluate the risk of infection for domestic carnivores and livestock. It is crucial to confirm if the occurrence of the tapeworm is really so strictly dependent from the presence of both typical hosts in the environment and to determine what is the possible pathogenicity of *T. lynciscapreoli*.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Brown, P.A., 1997. A review of techniques used in the preparation, curation and conservation of microscope slides at the natural history museum, London. Biol. Curator 10 (Supplement), 1–4.

Carlos, E., de Balogh, K., Lubroth, J., Amanfu, W., Speedy, A., Battagila, D., Domenech, J., 2006. Veterinary public health activities at FAO: cysticercosis and echinococcosis. Parasitol. Int. 55, 305–308.

Darriba, D., Taboada, G.L., Doallo, R., Posada, D., 2012. jModelTest 2: more models, new heuristics and parallel computing. Nat. Methods 9 (8), 772.

Filip, K.J., Pyziel, A.M., Jeżewski, W., Myczka, A.W., Demiaszkiewicz, A.W., Laskowski, Z., 2019. First molecular identification of *Taenia hydatigena* in wild ungulates in Poland. EcoHealth 16 (1), 161–170.

Guindon, S., Gascuel, O.A., 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst. Biol. 52, 696–704.

Haukisalmi, V., Lavikainen, A., Laaksonen, S., Meri, S., 2011. *Taenia arctos* n. sp. (Cestoda: cyclophyllidea: Taeniidae) from its definitive (brown bear *Ursus arctos* Linnaeus) and intermediate (moose/elk *Alces* spp.) hosts. Syst. Parasitol. 80, 217–230.

Haukisalmi, V., Konyaev, S., Lavikainen, A., Isomursu, M., Minoru, N., 2016. Description and life – cycle of *Taenia lynciscapreoli* sp. n. (Cestoda, Cyclophyllidea). ZooKeys 584, 1–23.

Hoberg, E.P., Jones, A., Rausch, R.L., Eom, K.S., Gardner, S.L., 2000. A phylogenetic hypothesis for species of the genus *Taenia* (Eucestoda: taeniidae). J. Parasitol. 86, 89–98.

Huelsenbeck, J.P., Ronquist, F., 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics Aug 17 (8), 754–755.

Kaczensky, P., Chapron, G., von Arx, M., Huber, D., Andren, H., Linnell, J., 2012. Status, Management and Distribution of Large Carnivores – Bear, lynx, Wolf and Wolverine – in Europe. Status of Large Carnivores in Europe – Update 2012.

Kołodziej – Sobocińska, M., Yakovlev, Y., Schmidt, K., Hurnikova, Z., Ruczyńska, I., Bednarski, M., Tokarska, M., 2018. Update of the helminth fauna in Eurasian lynx (*Lynx lynx*) in Poland. Parasitol. Res. 117 (8), 2613–2621.

Lavikainen, A., Haukisalmi, V., Deksne, G., Holmala, K., Lejeune, M., Isomursu, M., Jokelainen, P., Näreaho, A., Laakkonen, J., Hoberg, E.P., Sukura, A., 2013. Molecular identification of *Taenia* spp. in the Eurasian lynx (*Lynx lynx*). Parasitology 140 (5), 1–10.

Mysłajek, R.W., Kwiatkowska, I., Diserens, T.A., Haidit, A., Nowak, S., 2019. Occurrence of Eurasian lynx in western Poland after two decades of strict protection. Cat. News 69, 12–13.

Rozkrut, D., Czermak, G., Głowacka – Smolis, K., Kozera, A., Kucharska – Singh, P., Lipowska, E., Marikin, M., Nowak, T., Pilaszek, K., Rybak – Nguyen, E., Szydłowska, G., Wojtko, A., Bielak, R., 2018. Concise Statistical Yearbook of Poland, Warsaw 2018. pp. 44.

Sgroi, G., Varcasia, A., Dessi, G., D'Alessio, N., Pacifico, L., Buono, F., Neola, B., Fusco, G., Santoro, M., Toscano, V., Fioretti, A., Veneziano, V., 2019. Massive *Taenia hydatigena* cysticercosis in wild boar (Sus scrofa) from Italy. Acta Parasitol. https://doi.org/10.2478/s11686-019-00110-3.

Wawrzyniak, P., 2016. Dynamika Liczebności, Jej Wpływ Na Środowisko Bytowania, a Konieczność Zarządzania Populacją Łosia *Alces alces W* Polsce. 2016. Zarządzanie populacjami zwierząt, Warszawa, pp. 17–27 [In Polish]).

Zhang, L., Hu, M., Jones, A., Allsopp, B.A., Beveridge, I., Schindler, A.R., Gasser, R.B., 2007. Characterization of *Taenia madoquae* and *Taenia regis* from carnivores in Kenya using genetic markers in nuclear and mitochondrial DNA, and their relationships with other selected taeniids. Mol. Cell. Probes 21, 379–385.

Websites

Central Statistical Office www.stat.gov.pl.

Forest Data Bank www.bdl.lasy.gov.pl.

IUCN Red List of Threatened Species www.iucnredlist.org.

Ministry of Environment www.gov.pl/web/environment.