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Morphological and molecular description of *Dictyocaulus xanthopygus* sp. nov. (Nematoda: Trichostrongyloidea) from the Manchurian wapiti *Cervus elaphus xanthopygus*

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Abstract *Dictyocaulus xanthopygus* **sp. nov.** (Nematoda: Trichostrongyloidea) was isolated from the lungs of the Manchurian wapiti in Primorsky kray, Russia. The newly described species exhibits morphological characteristics of *Dictyocaulus* but is distinct from congeneric species based on morphological (lengths of body and esophagus, distances from the anterior end to nerve ring and to excretory pore, the thickness of the buccal capsule, etc.) and molecular features. High levels of genetic divergence as well as Bayesian phylogenetic analyses based on 18S rRNA nuclear and *cox1* mitochondrial genes supported the independence of *Dictyocaulus xanthopygus* **sp. nov.** Secondary structures of helix 39 of 18S rRNA were identical, while ES9 adjacent to the helix has a unique conformation for newly described worms. Energy-efficient conformational rearrangements of rRNA secondary structures can be applicable in studies on the pathogenesis, epidemiology, taxonomy and evolutionary biology of parasites. Additionally, bracketed dichotomous keys to six valid species of *Dictyocaulus* were prepared.

Introduction

Parasitic diseases caused by helminths are of high importance for the population dynamics of wild ruminants. These helminths are mainly nematodes from the genera *Ascaris* Linnaeus, 1758, *Dictyocaulus* Railliet & Henry, 1907, *Protostrongylus* Kamensky,

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1905, *Strongyloides* Grassi, 1879, *Trichinella* Railliet, 1895, and *Trichuris* Roederer, 1761. Of them four genera, *Ascaris*, *Dictyocaulus*, *Strongyloides*, and *Trichuris* represent geohelminths, or soil-transmitted helminths, which are of particular interest in ruminant health and husbandry (Callejón et al., 2013; Henker et al., 2017; Pyziel et al., 2018). Species of *Dictyocaulus* (Nematoda: Trichostrongyloidea) are distributed worldwide and affect various ungulate mammals most of which are even-toed ungulates (Cetartiodactyla) – *D. viviparus* (Bloch, 1782), *D. filaria* (Rudolphi, 1809), *D. eckerti* Skrjabin, 1931, *D. murmanensis* Poljanskaja & Tschertkowa, 1964, *D. africanus* Gibbons & Khalil, 1988, *D. capreolus* Gibbons & Höglund, 2002, *D. cervi* Pyziel, Laskowski, Demiaszkiewicz & Höglund, 2017. Some species of *Dictyocaulus* infect odd-toed ungulates (Perissodactyla): suborder Hippomorpha, *Equus* species – *D. arnfieldi* (Cobbold, 1884) Railliet & Henry, 1907, *D. pandionis* Sobolev & Sudikarov, 1939; suborder Tylopoda, camels – *D. cameli* Boev, 1951.

The genus *Dictyocaulus* belongs in the monotypic family Dictyocaulidae and includes 18 nominal species. Of them, five species have been confirmed valid based on molecular genetic data (Pyziel et al., 2017): *D. viviparus*, *D. filaria*, *D. eckerti*, *D. capreolus*, and *D. cervi*. Until the 21st century, due to the morphological similarity most species of the genus *Dictyocaulus* were identified by the host in which the parasite was found. Presumably, such identifications were valid due to the strict host specificity of dictyocaulids. Thus, parasitologists considered *Dictyocaulus eckerti* the only representative of dictyocaulids parasitizing in Cervidae (Pyziel et al., 2015). However, the development of molecular genetic approaches for taxonomic identification forced a reassessment of this assumption. Two species of *Dictyocaulus* from cervids were described, and their taxonomic status was confirmed with the ITS2 region, 18S rRNA, and *cox1* mtDNA genes: *Dictyocaulus capreolus* infects roe deer and moose (Gibbons & Höglund, 2002), and *Dictyocaulus cervi* – a parasite of red deer and moose (Pyziel et al., 2017; Filip-Hutsch et al., 2020). These findings demonstrate a greater species diversity of *Dictyocaulus* infecting Cervidae than previously thought and suggest a detailed examination of the taxonomy of this genus. Therefore, the aim of this study is to determine the species of parasitic nematode found in the lungs of the Manchurian wapiti *Cervus elaphus xanthopygus* (Milne-Edwards), from the Russian Far East by applying morphological analysis combined with modern genetics technics.

Materials and methods

Material collection

Twelve specimens of adult lungworms (five females and seven males) were isolated from the bronchi of the Manchurian wapiti (*Cervus elaphus xanthopygus*) which was caught in the north-eastern part of the Primorsky kray. Four (2 males and 2 females) and eight specimens were preserved in 70% and 96% ethanol for the morphological and molecular genetic analyses, respectively.

Morphological analysis

Nematodes from 70% ethanol were placed in a 1:7 mixture of glycerol and water, after which they were transferred to a glass slide and embedded in a glycerol gelatin medium (Roskin & Levinson, 1957). The preparations were analyzed using a ZEISS Primo Star microscope (Carl Zeiss, Germany). Measurements were made from the whole-mounts using the software ZEISS AxioVision 4.8.1 (Carl Zeiss, Germany) in the Department of Cell Biology and Genetics of Far Eastern Federal University. Four specimens of *Dictyocaulus xanthopygus* Vainutis, Voronova, Andreev, **sp. nov.** isolated from the bronchi of the Manchurian wapiti were deposited to the helminthological collection of the Somov Research Institute of Epidemiology and Microbiology, Vladivostok, Russia, with accession No. FECEN-3.

DNA extraction, amplification and sequencing

The total DNA was extracted from lungworms using the HotSHOT technique (Truett et al., 2000). Partial regions of the 18S rRNA and *cox1* mitochondrial genes were amplified by traditional polymerase chain reaction (PCR) using DreamTaq Green Master Mix (Thermo Scientific, Lithuania) and pairs of primers G18s4F+647R, 652F+136R for 18S rDNA (Callejón et al., 2013) and single pair LCO1490+HCO2198 for *cox1* (Folmer et al., 1994).

Cycling conditions for 18S rDNA consisted of a preliminary denaturation at 94°C for 2 min, followed by 30 cycles of denaturation at 94°C for 2 min, annealing at 50°C for 30 sec, elongation at 72°C for 1 min, and a final product extension at 72°C for 5 min. Cycling conditions for *cox1* mtDNA consisted of a preliminary denaturation at 95°C for 2 min, followed by 35 cycles of denaturation at 95°C for 1 min, annealing at 40°C for 1 min, elongation at 72°C for 1.5 min, and a final product extension at 72°C for 7 min. Each PCR reaction included negative and positive controls, using both primers. PCR products were enzymatically cleaned up with ExoSAP-IT PCR Product Cleanup Reagent from Thermo Scientific and then sequenced on Honor 1616 Genetic Analyzer (SUPERYEARS) using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) (as instructed by the manufacturer). Sequencing primers were the same as those used for PCR plus internal sequencing primers (645R, 648F, 649R, 650F) as described by Callejon et al. in case with 18S rDNA. Contiguous sequences were assembled using Finch TV and MEGA 7.0 (Kumar, 2016) and submitted to GenBank under accession numbers: ON479617 and ON595756–ON595758 for 18S rDNA and ON495328–ON495335 for *cox1* mtDNA.

Alignments and phylogenetic analysis

Sequences of worms obtained in this study and sequences of closely related taxa from GenBank were aligned using Clustal W (Thompson et al., 1994). To clarify the phylogenetic relationships of the new species with the other representatives of the genus *Dictyocaulus*, data matrices compiled of partial sequences of 18S rDNA (1604 bp in length) and *cox1* mtDNA (546 bp in length) were used. Genetic divergence was estimated using genetic p-distance values, which were calculated by including all substitution types (Tajima, 1983; Nei, 1987). Phylogenetic relationships among taxa were reconstructed using Bayesian inference (BI) in MrBayes 3.2.7 software (Huelsenbeck et al., 2001). The MCMC algorithm was performed using 5000000 generations and two independent runs, where 25% of generations were discarded as burn-in. jModeltest v. 2.1.10 software (Posada & Crandall, 1998) was used to select the best nucleotide substitution models HGY+I for the 18S rDNA data matrix and HGY+G for the *cox1* gene data matrix.

Secondary structure prediction

Domain (helix 39 + ES9) of the 18S rRNA gene (following the ES nomenclature of Gerbi (1996)) was chosen for the modeling of secondary structures from primary sequences for seven species of *Dictyocaulus*, including the newly described species, using the MFOLD software version 2.3 (http://mfold.rna.albany.edu). MFOLD is one of the best tools for folding of most RNAs based on the principle of minimizing the free energy (dG) (Zuker, 2003). RNA was folded at a fixed temperature of 37 °C. Individual structure drawings were rendered as postscript and processed in Gravit Designer (https://www.designer.io).

Results

Class Chromadorea Inglis, 1983
   Order Rhabditida Chitwood, 1933
   Family Dictyocaulidae Skrjabin, 1941
   Genus Dictyocaulus Railliet & Henry, 1907

Dictyocaulus xanthopygus Vainutis, Voronova, Andreev, sp. nov. (Fig. 1, Table 1)

Type material: Deposited in the helminthological collection of the Somov Research Institute of Epidemiology and Microbiology, Vladivostok, Russia, 10 January 2022. Holotype ♂, accession no. FECEN 9-1. Paratypes, accession nos. FECEN 9-2, FECEN 9-3, FECEN 9-4 (1♂, 2♀).

Type host: *Cervus elaphus xanthopygus* (H. Milne-Edwards) (Ruminantia: Cervidae), delivered by Pankratov D.V.

Localization: Bronchi, collected by Vainutis K.S. and Andreev M.E.

Biology: The knowledge on life cycle is incomplete.

Type locality: Russia, Primorsky kray, Kavalerovsky district.

Molecular genetic data: 18S rRNA gene – ON479617 and ON595756–ON595758; cox1 mtDNA gene – ON495328–ON495335.
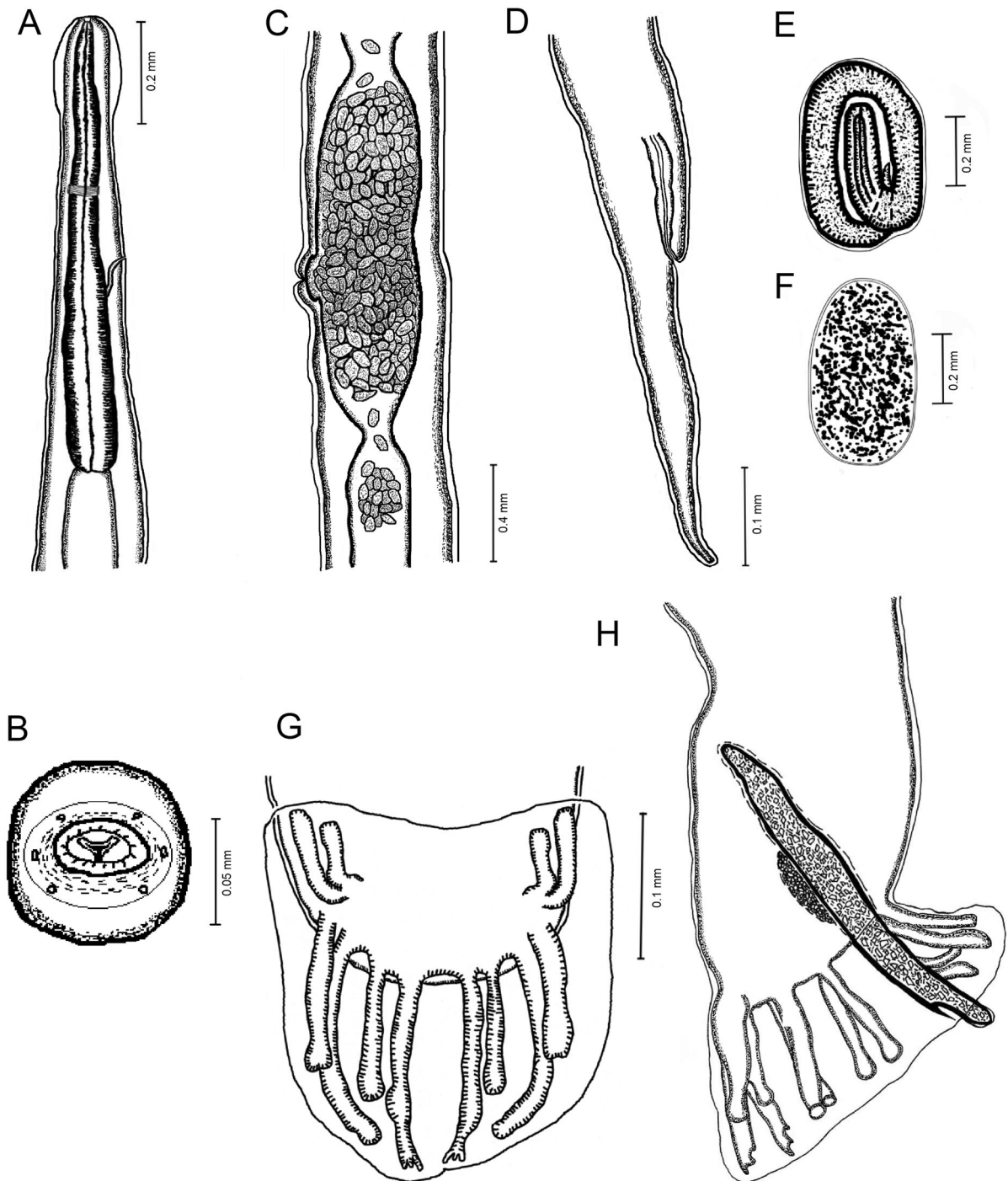
Fig. 1 Line drawings of *Dictyocaulus xanthopygus* sp. nov. A – anterior end; B – apical view of anterior end; C – vulval region; D – posterior end of female; E – immature and mature fertilized eggs; G – bursa of male worm; H – lateral view of male genital sac.

Table 1 Morphometric comparison of seven dictyocaulid species based on the morphology of adult individuals.

Features	Species							
	D. arnfieldi[1]		D. filaria[1]		D. viviparus[1]		D. capreolus[2]	
	male	female	male	female	male	female	male	female
Body length	25–35	43–60	30–80	50–112	26.5–44	29–59	24–62	34–81
Body width	250	400	352–464	528–592	–	–	–	–
Head width					74–91	86–104	60–100	72–176
Buccal capsule, L×W[4]					–	–	32–56 × 32–56	8–20 × 32–68
Buccal capsule wall, L×W					9.9–20.9 × 3.9–15.8	6.8–30.9 × 2–16.6	8.9–23.8 × 7–12	9.1–23.4 × 6–14
Esophagus, L×W			1200–1550 × 144–176	840 × 192–224	808–992 (L)	896–1040 (L)	832–1464 (L)	880–1616 (L)
Anterior to nerve ring					308–398	235–379	276–396	296–400
Anterior to excretory pore					412–526	393–493	352–600	200–576
Copulatory bursa					–		136–312	
Spicules, L×W	250 (L)		448–624 × 48–96		223–259 (L)		224–280 (L)	
Gubernaculum, L×W	50				52–70 (L)		22–72 (L)	
Posterior to vulva opening						13–29.5		12.9–31.6
Posterior to anus		400			160	261–483		336–632
Immature eggs in uterus						46–60 × 74–91		88–104 × 56–64
Mature embryonated eggs, L×W		90			119–135 × 74–91	43–55 × 79–86		76–92 × 40–56

Features	Species					
	D. eckerti[1,3]		D. cervi[3]		D. xanthopygus **sp. nov.**	
	male	female	male	female	male (n=2)	female (n=2)
Body length	19–47(18.88–40.48)	22.35–72.34(31.76–65)	25.6–56.7	30.3–76.8	36.7–49.4	39.9–45.4
Body width	310–550(368–586)	342–763(384–650)	–	–	152–154	195–217
Head width	–	–	77.9–113.6	69.1–128.2	65.7–81.6	54.1–77.6
Buccal capsule, L×W[4]	–	–	11.6–29.2 × 25.1–54.3	14.2–39.2 × 31.4–55	14.7–16.8 × 30.5–39.3	
Buccal capsule wall, L×W	–	–	10.7–33.5 × 5–12.2	14.5–30.7 × 5.3–12.5	19.6–30.4 × 6.7–13.9	
Esophagus, L×W	890–1360(880–1152) × 110–150(120–192)	847–1473(880–1216) × 263–552(336–572)	824.5–1309 × 105.4–172.3	856.5–1453 × 106.5–220.2	880–900 × 112.8–118.3	840–980 × 105.6–120.1
Anterior to nerve ring	(244–400)	(224–400)	297.9–423.3	262.5–466.3	300–410	330–380
Anterior to excretory pore	368–526(332–528)	(336–572)	326.5–544.1	421.3–581.9	460–620	500–570
	–		171.8–282.2		198–302	

Table 1 continued

Features	Species					
	D. eckerti[1, 3]		*D. cervi*[3]		*D. xanthopygus* **sp. nov.**	
	male	female	male	female	male (n=2)	female (n=2)
Copulatory bursa						
Spicules, L×W	230–300(204–260) (L)		208.9–302.9 (L)		243–264 × 30.8–36.2	
Gubernaculum, L×W	50–60 (L)		43.1–73.4 × 27.4–38.9		54.7–60.1 × 10.6–13.5	
Posterior to vulva opening		14.4–29.92		13.3–33		22.3–25.9
Posterior to anus		300–400		383.8–475.5		297.6–339.8
Immature eggs in uterus		–		59.5–87 × 12.5–44.8		
Mature embryonated eggs, L×W		68–92 × 44–50		67.7–101 × 32.8–52.6		51.8–58.2 × 33.4–38.3

The metric values of body length are in millimeters and the rest features are in micrometers

[1]Skrjabin et al. (1954)

[2]Gibbons & Höglund (2002)

[3]Pyziel et al. (2017)

[4]L – length, W – width

Etymology: The specific name "*xanthopygus*" was given after the subspecific name of *Cervus elaphus xanthopygus* — the type host of this nematode.

Description (based on four mature individuals)

General morphology: nematodes filamentous, whitish in color, with body tapering at anterior and posterior ends. Cuticle transversely striated. Mouth opening elongate oval, terminal, surrounded by four sublabia. Cephalic vesicle present. Buccal capsule 30.5–39.3 μm wide and 14.7–16.8 μm long. Buccal capsule wall 6.7–13.9 μm wide and 19.6–30.4 μm long. Esophagus cylindrical in shape, with extension to posterior end.

Male (n=2): Body 36.7–49.4 mm long, esophagus 0.88–0.9 mm long. Anterior to nerve ring and excretory pore 0.30–0.41 mm and 0.46–0.60 mm, respectively. Copulatory bursa 190–302 μm long. Spicules 243–264 μm long and 31–36 μm wide, porous texture, dark brown. Gubernaculum 55–60 μm long and 11–14 μm wide, oval in shape, lighter than spicules. Structure of bursa: dorsal rays longest, divided at base, with three small lobes at distal tip;

externodorsal rays separate from dorsal rays, thickened at apex, shorter than dorsal rays; medio- and posterolateral rays completely fused, long, bent at distal tip; anterolateral rays separate, widened at distal tip; ventral rays stems forming common base.

Female (n=2): Body 39.9–45.4 mm long, esophagus 0.84–0.98 mm long. Anterior to nerve ring and excretory pore 0.33–0.38 mm and 0.50–0.57 mm, respectively. Vulva in anterior half of body, opening 17.6–19.5 mm from anterior end. Eggs oval in shape, measuring 52–58 μm long and 33–38 μm wide.

Diagnosis: *Dictyocaulus xanthopygus* Vainutis, Voronova, Andreev, **sp. nov.** has morphological structures typical of the genus (Fig. 1): four sublabia around the mouth opening on the anterior end of body; cephalic vesicle; elongated cylindrical esophagus with bulbous posterior end; copulatory bursa in males with 12 elongate rays. The presence of three lobes on the dorsal rays and the length of the spicules are similar to those of dictyocaulids from cervids. However, most of the morphometric features of *D. xanthopygus* **sp. nov.** do not correspond to any of the known species of the genus *Dictyocaulus*. The maximum body length of

Table 2 Mean distance in % (S.D. ≤ 0.01) of 18S rDNA sequences between dictyocaulids.

	xanthopygus	cervi	eckerti	capreolus	viviparus
D. xanthopygus **sp. nov.**					
D. cervi	0.45				
D. eckerti	0.51	0.06			
D. capreolus	0.52	0.59	0.65		
D. viviparus	0.64	0.45	0.51	0.85	
D. filaria	3.18	3.31	3.38	3.45	3.50

males is similar to *D. capreolus* and *D. cervi* but exceeds the length of males of *D. eckerti* (49.4 vs. 47 mm). The minimum esophageal length of females coincides with that of *D. eckerti* only, but compared with other dictyocaulids from cervids it is smaller — 0.84 vs. 0.88 and 0.86 of *D. capreolus* and *D. cervi*, respectively). The maximum distance from the anterior end to the nerve ring in males is a little larger than in *D. capreolus* (0.41 vs. 0.4 mm). And the maximum distance from the anterior end to the excretory pore is greater than that of *D. cervi* (0.6 vs. 0.54 mm) (Table 1).

In addition, *D. xanthopygus* **sp. nov.** has significant morphometric differences in relation to *D. noerneri* redescribed by Durette-Desset et al. (1988) from *Capreolus capreolus* in France and by Carrillo-Gonzalez et al. (1994) from *C. capreolus* in Spain. The body length of *D. xanthopygus* **sp. nov.** do not exceed 50 mm while in *D. noerneri* it reaches 71 mm; sizes of eggs in *D. xanthopygus* **sp. nov.** and *D. noerneri* do not overlap: 52–58 μm by 33–38 μm and 80–90 μm by 50–60 μm (Durette-Desset et al., 1988) respectively; maximum size values of spicules and gubernaculum in *D. xanthopygus* **sp. nov.** are nearly two times smaller than that of *D. noerneri* (Carrillo-Gonzalez et al., 1994).

Phylogenetic analysis based on 18S rRNA and *cox1* mtDNA genes

Four and eight new sequences of the 18S rRNA and *cox1* genes of 1738 and 685 bp in length, respectively, were obtained for *D. xanthopygus* **sp. nov.** The 18S rRNA gene sequences of all *D. xanthopygus* **sp. nov.** were identical. When comparing *D. xanthopygus* **sp. nov.** with other species, the smallest and the largest genetic p–distances of 0.4% and 3% were between *D. cervi* and *D. filaria*, respectively (Table 2). Generally, p–distances between studied dictyocaulids were at values 0.6–0.8%, maximum distances were calculated with *filaria* (approximately 3%).

In the *cox1* gene, intraspecific diversity of *D. xanthopygus* **sp. nov.** varied from 0.15% to 0.73%. The smallest and the largest genetic p–distances of approximately 9% and 12% were calculated between *D. xanthopygus* **sp. nov.** and *D. viviparus bisontis* and *D. xanthopygus* **sp. nov.** and *Dictyocaulus* sp. from Hungary, respectively (Table 3). Interspecies p–distances of studied dictyocaulids were at the comparable high values, with two exceptions *bisontis* vs. *viviparus* — 3% and *eckerti* vs. *cervi* — 2%.

In general, phylogenetic trees based on nuclear and mitochondrial markers were in concordance: individuals of *D. xanthopygus* **sp. nov.** were clearly separated from the other representatives of the genus *Dictyocaulus*. 18S–tree demonstrated that *D. filaria* clustered in a distinct clade, occupying basal position; the rest of the nematodes generate five monophyletic subclades strictly according to their species affiliation (Fig. 2). The *cox1* tree topology to some extent differs from the 18S tree topology (Fig. 3). Two clades can be distinguished in the structure of the tree: the first large clade was formed by representatives of *D. eckerti* from New Zealand and Hungary, and the second clade, in turn, divided into two additional subclades, one of which (basal) was formed by *D. xanthopygus* **sp. nov.** The second subclade split into several groups in accordance with species affiliation of studied dictyocaulids.

Secondary structures based on 18S rRNA gene

Reconstructed models contained different numbers of nucleotides (46–72) and had hairpin conformations, where two parts – helix 39 (h39) and expansion segment 9 (ES9) can be identified (Fig. 4). However,

Table 3 Mean distance in % (S.D. ≤ 0.01) of *cox1* mtDNA sequences between dictyocaulids.

	xanthopygus	bisontis	viviparus	capreolus	eckerti	sp.
D. xanthopygus **sp. nov.**						
D. viviparus bisontis	8.92					
D. viviparus	9.11	3.23				
D. capreolus	9.94	9.48	9.91			
D. eckerti	10.11	11.36	12.65	11.63		
D. cervi	10.39	11.72	12.39	11.53	1.841	
Dictyocaulus sp. from Hungary	12.35	12.14	11.4	11.61	13.11	12.51
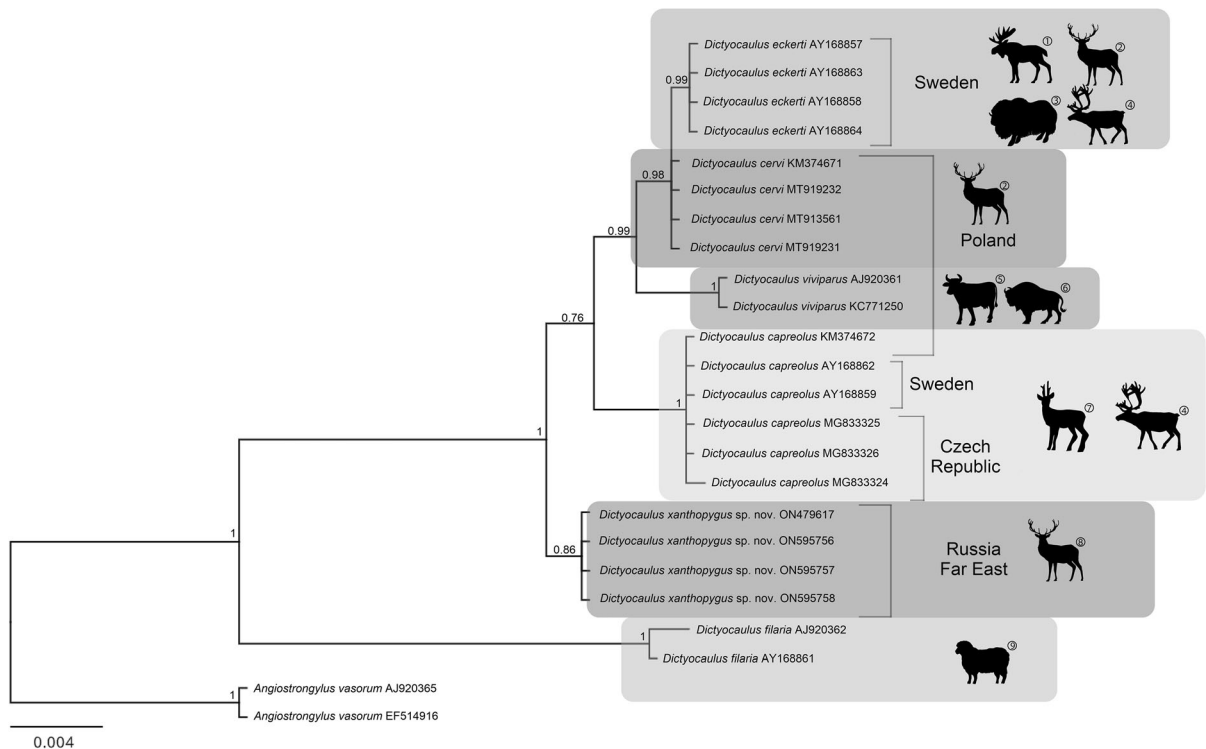
Fig. 2 18S rRNA phylogeny; nodal support shown as posterior probabilities; *Angiostrongylus vasorum* – outgroup; Supporting host information (1 – *Alces Alces* (Moose) ; 2 – *Cervus elaphus* (Red deer); 3 – *Ovibos moschatus* (Muskox); 4 – *Rangifer tarandus* (Reindeer); 5 – *Bos taurus* (Cattle); 6 – *Bison bonasus bonas* (European bison); 7 – *Capreolus capreolus* (Roe deer); 8 – *Cervus elaphus Xantopygus*; 9 – *Ovis aries* (Sheep)) and geographical localities is provided on the trees

helices 39 adjacent to ES9 were identical both in length (40 bp) and nucleotide composition for all studied nematodes. Helix 39 included two single–nucleotide bulge loops and one internal medium–sized loop (8 bp). ES9 varied greatly in nucleotide composition and size (from 6 bp to 32 bp), and was separated from h39 by the internal loop, which localized in its proximal part and composed of 8 or 13 nucleotides depending on species. Two different domain structures were obtained for *D. capreolus*. *D. capreolus* from the Czech Republic has undergone a complete reduction of ES9 leaving only a small external loop in the apical part of the whole domain. The structure for *D. capreolus* from Sweden and Poland was common
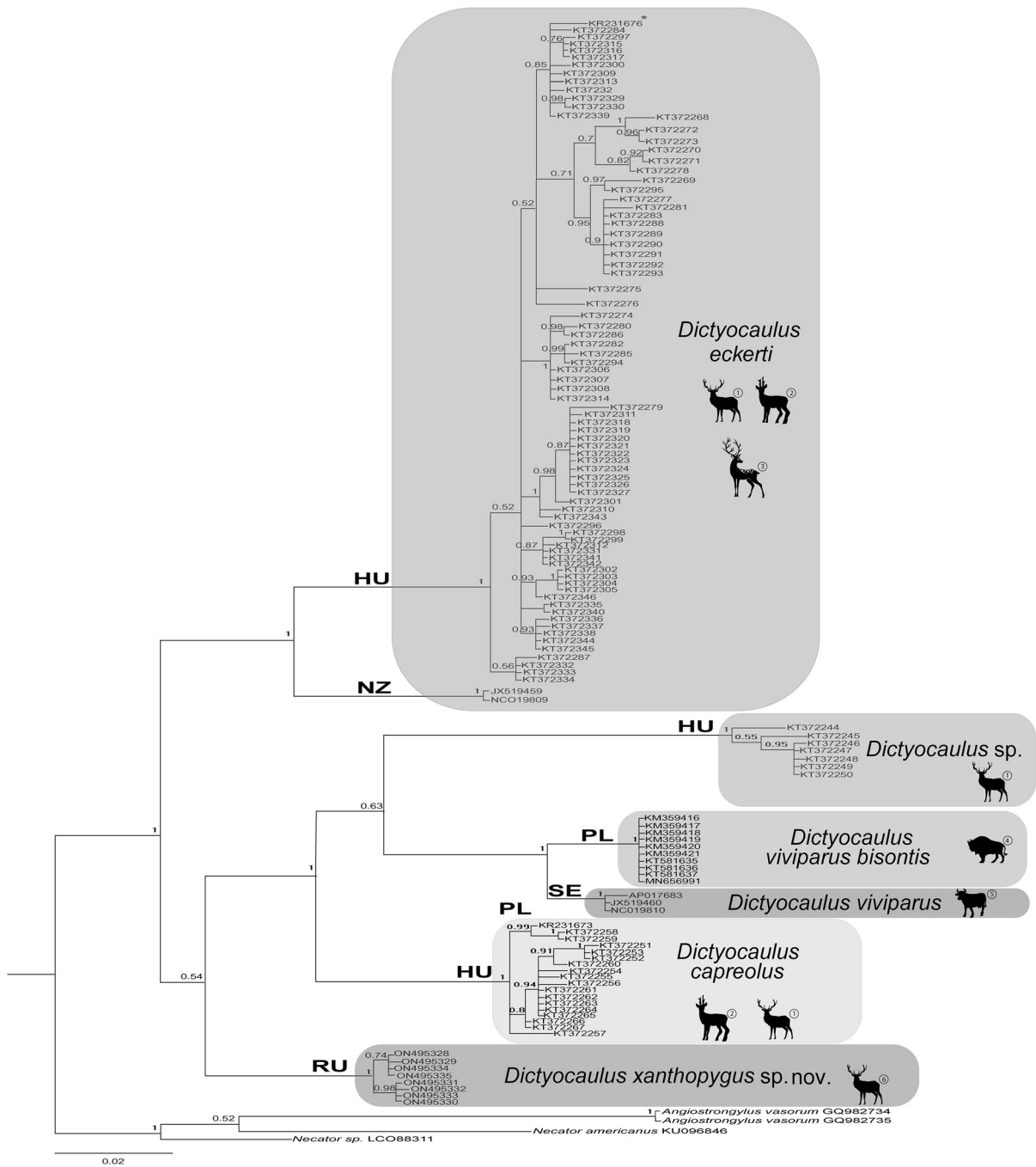
Fig. 3 *Cox1* mtDNA phylogeny; nodal support shown as posterior probabilities; *Angiostrongylus vasorum*, *Necator americanus* and *Necator sp.* comprise the outgroup; Supporting host information (1 – *Cervus elaphus* (Red deer); 2 – *Capreolus capreolus* (Roe deer); 3 – *Cervus nippon* (Sika deer); 4 – *Bison bonasus bonas* (European bison); 5 – *Bos taurus* (Cattle); 6 – *Cervus elaphus Xantopygus*) and geographical localities is provided on the trees.

with that of *D. eckerti*, and *D. cervi* –only a single substitution in the apex of ES9 was observed, which did not affect the resulting folding. We observed gradual elongation of the ES9 stem in the following sequences: (1) *D. xanthopygus* **sp. nov.**, (2) *D. eckerti*,
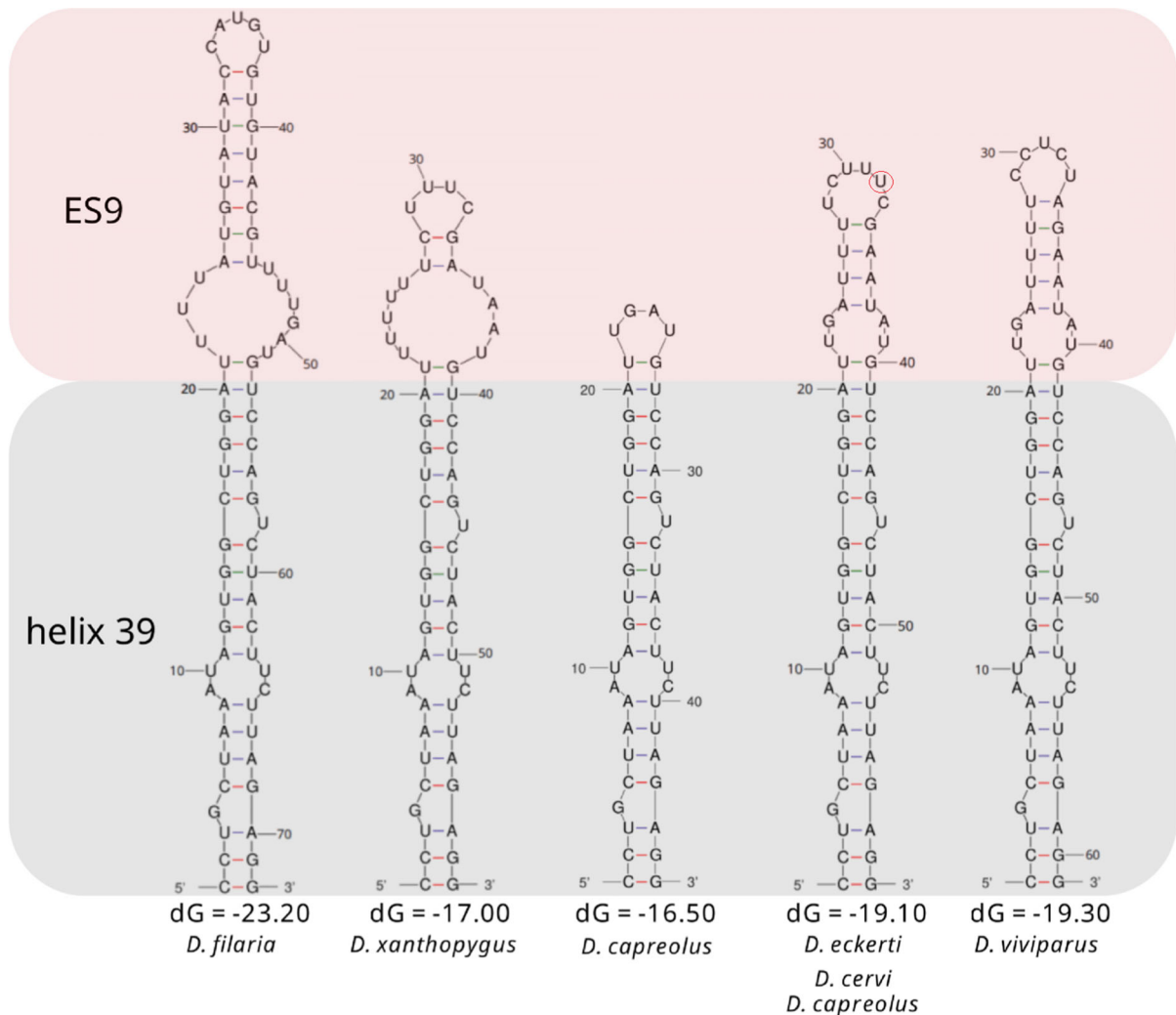
Fig. 4 Secondary structures of Helix 39–ES9 18S rRNA for *Dictyocaulus* spp.; dG – Gibbs free energy.

D. cervi, D. capreolus from Sweden and Poland, (3) *D. viviparus*, (4) *D. filaria*.

Discussion

The first description of dictyocaulus parasitizing deer was presented by Railliet & Henry (1907). Despite the only morphological differences being the length of the spicules, the species was accepted and named *Dictyocaulus noerneri*. In 1925, Chapin found *D. hadweni* in the lungs of moose and deer, previously described from bison. Later, *D. noerneri* and *D. hadweni* were synonymized with *D. viviparus*, which was supposed to infect both cattle and deer. Here we suggest

considering *D. noerneri* the valid species based on the redescriptions of this species from *Capreolus capreolus* of France (Durette-Desset et al., 1988) and Spain (Carrillo Gonzalez et al., 1994). *Dictyocaulus noerneri* is well-distinguished from other valid species by morphometric features (Table 4) and is highly similar to D. eckerti by both general morphology and most overlapping size ranges.

Skrjabin et al. (1954) published a complete morphological description of lung worms from reindeer, and based on the length of the spicules and the unique shape of the end of the dorsal rays, he established *D. eckerti* and proved its independence from *D. viviparus* (Skrjabin et al., 1954). This finding questioned the assumption that *D. viviparus* can infect cervids. At

Table 4 Bracketed dichotomous keys to seven species of *Dictyocaulus*

1a	Parasites of bovids	2
1b	Parasites of cervids	3
2a	Maximum body length reaches 80 mm in males and exceeds 60 mm in females. Spicules length reaches 0.6 mm	*Dictyocaulus filaria* (Rudolphi, 1809)
2b	Maximum body length less than 50 mm in males and 60 mm in females. Spicules length less than 0.3 mm	*Dictyocaulus viviparus* (Bloch, 1782)
3a	Maximum body length less than 50 mm in both males and females. Maximum esophagus length less than 1 mm in both males and females	*Dictyocaulus xanthopygus* **sp. nov.**
3b	Maximum body length exceeds 50 mm in both males and females. Maximum esophagus length exceeds 1 mm in both males and females	4
4a	Two rings of cephalic papillae and two cervical papillae present.	5
4b	Single ring of cephalic papillae present. Cervical papillae absent.	6
5a	Maximum esophagus length reaching 1.6 mm. Maximum length of gubernaculum not exceeding 70 μm.	*Dictyocaulus eckerti* Skrjabin, 1931
5b	Maximum esophagus length reaching 1.82 mm. Maximum length of gubernaculum reaching 100 μm.	*Dictyocaulus noerneri* Railliet et Henri, 1907
6a	Buccal capsule 0.012–0.029 mm long by 0.025–0.054 mm wide in males and 0.014–0.039 mm long by 0.031–0.055 mm wide in females. Buccal capsule wall intermediate.	*Dictyocaulus cervi* Pyziel, Laskowski, Demiaszkiewicz, Höglund, 2017
6b	Buccal capsule 0.008–0.016 mm long by 0.032–0.056 mm wide in males and 0.008–0.020 mm long by 0.032–0.068 mm wide in females. Buccal capsule wall thick.	*Dictyocaulus capreolus* Gibbons & Höglund, 2002

present, there is a tendency to isolate new species from those previously established by using molecular tools. Thus, *D. eckerti* was revised by Gibbons & Höglund (2002) and Pyziel et al. (2017) with description of *D. capreolus* and *D. cervi*. Nematodes from the lungs of the Manchurian wapiti from Primorsky kray which is geographically distant from European countries where most of the dictyocaulids have been described is an interesting species that impacts on the taxonomy of the genus *Dictyocaulus*. The presence of three lobes on the dorsal rays and the length of the spicules unite the new species with other dictyocaulids from cervids and equids (Soliman, 1960; Gibbons & Höglund, 2002; Pyziel et al., 2017). Nevertheless, the unique morphometric characters suggest that these worms are distinct from any of the known species of *Dictyocaulus* and deserve a new name *D. xanthopygus* **sp. nov.** The maximum length of *D. xanthopygus* **sp. nov.** males is similar to *D. capreolus* and *D. cervi* but exceeds the length of males of *D. eckerti* (49.35 vs. 47 mm). The minimum length of the esophagus of *D. xanthopygus* **sp. nov.** females coincides with that only for *D. eckerti*, compared with other dictyocaulids from cervids, it is smaller (0.84 vs. 0.88 and 0.86 for *D.*

capreolus and *D. cervi*, respectively). The maximum distance from the anterior end to the nerve ring in males is a little larger than in *D. capreolus* (0.41 vs. 0.4 mm), and the maximum distance from the anterior end to the excretory pore is greater than that of *D. cervi* (0.6 vs. 0.54 mm) (Pyziel et al., 2017). For example, *D. capreolus* morphometrically differs from *D. eckerti* only in the thickness of the buccal capsule (Gibbons & Höglund, 2002).

The validity of *Dictyocaulus xanthopygus* **sp. nov.** is confirmed by genetic distances from other dictyocaulids — 0.45–0.6% in 18S rDNA. The differences between the accepted species *D. cervi* and *D. eckerti* in the 18S gene were only 0.06% (Pyziel et al., 2017). The *cox1* gene is considered as an important ally to morphology in nematode taxonomy and systematics. In study of ascaridoid nematodes from Iranian cats and dogs interspecies sequence variation among *Toxocara cati*, *T. canis* and *Toxascaris leonina* was 9.5–16.6% (Mikaeili et al., 2015) similar to values calculated for newly described species (9–13%) in this research. Phylogenetic trees also demonstrated the position of *Dictyocaulus xanthopygus* **sp. nov.** in clearly separated monophyletic clades. In addition, the *cox1* tree

included all sequences available in the Genbank and revealed a soft polytomy in the *D. eckerti* clade, which most likely indicates a rapid expansion of the species range, panmixia, and euryxenity, normally lacking for *Dictyocaulus* species.

The efficacy of genetic variation and phylogenetic relationships based on the ribosomal and mitochondrial gene sequences among parasite populations is well proven and has been shown in different studies, but species identification through the secondary structures of marker genes and spacers is also very promising technics (Ghatani et al., 2012; Chaudhary et al., 2014; Voronova & Chelomina, 2020). The secondary structure of ribosomal RNA is largely conserved across all kingdoms of life. Nevertheless, eukaryotes have evolved extra blocks of rRNA sequences, in which the variability is concentrated, called expansion segments (ES). In accordance with the concept proposed by Höglund et al. (2003) we tried to identify *Dictyocaulus* species by the structure of the h39–ES9 domain of the 18S rRNA gene (helix 39–ES9 is equivalent to helix 43 (Wuyts et al., 2002)). Helix 39 turned out to be very conservative and therefore not informative for separating dictyocaulids. Furthermore, in total length of 40 bp, it shares almost 30 identical nucleotides with analogous helix modeled for trematodes (Voronova & Chelomina, 2020), and even 23 nt with ticks' helix (Zhao et al., 2013). As expected, ES9 show remarkable differences among *Dictyocaulus* spp., including *D. xanthopygus.* However, for *D. eckerti*, *D. cervi* and *D. capreolus* from Sweden and Poland, ES9 was identical and cannot be used as a diagnostic tool. The 18S rRNA phylogeny showed the presence of *D. capreolus* from the Czech Republic in the one subclade with other *D. capreolus*, but with different inner branch lengths. It may be that Czech population of *D. capreolus* is on the very initial stage of divergence, but the significant reduction of ES9, accompanied by an increase in the structure's free energy (dG), indirectly indicates a negative effect of such evolutionary trends. Elongation of the ES9 stem is accompanied by a systematic decrease in the folding free energy and probably can be considered as a useful adaptation for the survival of the parasite. We do not have enough data to draw final conclusions, but accurate analysis of secondary structure variation and conformational rearrangements is applicable for studies on the pathogenesis, epidemiology, taxonomy and evolutionary biology of parasites.

According to the molecular genetic study based on the 18S rRNA gene and *cox1* mtDNA we clarified the taxonomic position of six *Dictyocaulus* species as follows: *D. filaria*, *D. viviparus*, *D. xanthopygus* **sp. nov.**, *D. cervi*, *D. eckerti*, *D. capreolus*. We presented keys based on the morphology of the mature worms (Table 4). These species were arranged into two main groups by their definitive hosts as follows: bovids (*D. filaria*, *D. viviparus*) and cervids (*D. xanthopygus* **sp. nov.**, *D. cervi*, *D. eckerti*, *D. capreolus*).

Conclusions

Unique morphometric features, a fully resolved phylogeny, and representative ES9 models, indicate a previously unknown species of parasitic lung nematodes in deer of the North-Eastern territory of the Primorsky kray — *Dictyocaulus xanthopygus* **sp. nov.** The distribution of *D. xanthopygus* seems to dependent on the habitat of its definitive host, *Cervus elaphus xanthopygus*. The latter, Manchurian wapiti, covers the south of the Russian Far East including whole Primorsky kray (Bormotov et al., 2017; Davydov et al., 2019; Tsyndzhapova & Rozlomiy, 2021). ES9 is not universally useful for species identification of the genus *Dictyocaulus* since it can be identical for several species, but given the usefulness and wide applicability of secondary structures, the search for optimal domains should be continued.

Ethical approval   Not applicable.

Consent to participate   Not applicable.

Consent for publication   Not applicable.

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