Large lungworms (Nematoda: Dictyocaulidae) recovered from the European bison may represent a new nematode subspecies

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A B S T R A C T

Although the Dictyocaulus lungworm, the agent of dictyocaulosis, is one of parasitological threats to European bison, its systematic position remains unclear. The aim of the present study was to evaluate the morphological features of the lungworm and the pathological lesions it induces, and to analyse mitochondrial (mt) genetic markers for systematic and molecular epidemiological studies. The morphological findings indicate that Dictyocaulus lungworms of European bison can be distinguished from those of cattle on the basis of differences in buccal capsule wall length, total body length, and spicules length in males, all of which were significantly longer in those of European bison. Nucleotide diversity calculated from pairwise sequence alignments of partial cytochrome oxidase subunit 1 (cox1), cytochrome B (cytB) and NADH dehydrogenase subunit 5 (nad5) of specimens from cattle and European bison varied from 1.7% for nad5, 2.1% for cytB, to 3.7% for cox1 gene. Thus, among the lungworms of European bison and cattle, nad5 and cytB were the most conserved proteins, whereas cox1 was the most diverse. The mt cytB marker gene may be a suitable candidate for distinguishing between the two genotypes, as nad5 demonstrated the greatest within-genus sequence variation. The lung tissue of infected European bison manifests signs of verminous pneumonia characterized by interstitial pneumonia, bronchitis and bronchiolitis. Therefore, it appears that European bison and cattle are infected with slightly diverged, morphologically-different, genotypes of D. viviparus, indicating they belong to two separate worm populations. We propose, therefore, that the lungworm of European bison should be classified as D. viviparus subsp. bisontis.

1. Introduction

Free-roaming populations of wild ruminants enrich the biodiversity of heavily-urbanized landscapes of European countries. A unique bovid inhabiting Poland is the European bison, Bison bonasus: the largest terrestrial mammal in Europe; however, it is considered vulnerable to extinction by the International Union for Conservation of Nature - Red List of Threatened Species (IUCN, 2020). The European bison also
suffers from a loss of genetic variation, and is hence more vulnerable to pathogens due to inbreeding depression and the depletion of variation in the genes responsible for immunity against parasites (Keller and Waller, 2002; Radwan et al., 2010). Dictyocaulus is a condition affecting various ungulate hosts, characterized by symptoms of bronchitis, with infection leading to fatal outcomes in heavily-infected individuals (Demińska-Woźniak et al., 2009; Pyziel et al., 2018a). In order to understand the risk for cross-transmission between free-roaming European bison and cattle, and to control the spread of dictyocaulosis, it is essential to have the potential to correctly identify lungworm species. However, although some initial studies on European bison-derived lungworms have been published, their systematic position remains unclear (Pyziel, 2014, 2018; Pyziel et al., 2017).

The small subunit (SSU) rDNA sequence of *D. viviparus* from European bison has been found to be generally identical to those from cattle; however, the two groups were found to diverge at the internal transcribed spacer (ITS2) region of the ribosomal RNA-array (Pyziel, 2014). Additionally, the mt cytochrome c oxidase subunit 1 (cox1) nucleotide sequence demonstrates high intraspecific diversity between the two strains of *D. viviparus* (Pyziel et al., 2017). However, the mt cytochrome c oxidase subunit 3 (cox3) gene was found as an inefficient mt marker for systematic study on *Dictyocaulus* spp. (Pyziel et al., 2018b).

The present study examines the morphological features of European bison lungworm, evaluates its impact on the lung tissue of the host, and identifies the most efficient mt genetic markers for systematic and molecular epidemiological studies.

### 2. Materials and methods

#### 2.1. Specimen collection

A total of 30 male and 35 female adult *Dictyocaulus* lungworms were isolated from the respiratory tract of 15 free-roaming European bison. All European bison had been euthanized for various health reasons in All European bison had been euthanized for various health reasons in the years 2017–2019. Lethal control is an element of health monitoring conducted annually. Individuals of weak condition with general or specific signs of disease (e.g. necrotic inflammation of the prepuce) were selected. The formal aspects of conducted annual lethal control was described in Klich et al. (2020). The respiratory tracts were taken exclusively from animals infected by *Dictyocaulus* sp. The trachea, bronchi, and bronchioles were cut open and immersed into beakers filled with tap water, the sediment was investigated under a microscope and worms were recovered with the use of dissecting needles (Pyziel et al., 2017). Lungworms from each animal were stored separately, preserved in 70% ethanol and then transported to the Swedish University of Agricultural Sciences for morphological and molecular investigations.

#### 2.2. Morphological study

Specimens were cleared in lactophenol and mounted in glycerin jelly on slides following Pyziel et al. (2017). Selected morphological features were measured: body length, buccal capsule (BC), buccal capsule wall (BCW), head, cephalic vesicle, esophagus of males and females; copulatory bursa, gubernaculum, spicules of male reproductive system; vestibules, anterior spinner and infundibulum, posterior spinner and infundibulum, mature and immature eggs in the uterus, tail and phas- moids of the female reproductive system. Additionally, location of nerve ring and excretory pore from anterior extremity were evaluated, as well as location of vulva opening from tail-tip and body width at vulva opening in females. Photomicrographs of the features were taken using an Opta-Tech Lab40 light microscope (× 40 - × 1000 magnifications), and the OptaView IS-PL Opta-Tech software package (Opta-Tech, Warsaw, Poland). Specimens intended for scanning electron microscopy (SEM) were dehydrated in increasing concentrations of ethanol and stored in acetone (Eisenback, 1985). The dehydrated specimens were then subjected to critical-point drying with liquid CO2, their proximal endings were cut and mounted on a SEM mounting stub with double-coated tape, sputter-coated with gold, and examined with the use of a LEO 1430VP scanning electron microscope.

### 2.3. DNA extraction, amplification and sequencing

Depending on individual intensity of infection, 1–5 male worms from each examined European bison were used for molecular analyses. Genomic DNA was extracted individually from 48 adult male lungworms (preserved in 70% ethanol) using a Nucleospin tissue DNA extraction kit ( Macherey-Nagel, Düren, Germany) according to the manufacturer’s protocol. Partial regions of mitochondrial (mt) cox1, NDAD dehydrogenase subunit 5 (nad5), and cytochrome B (cytb) of the worms were amplified by PCR using the following sets of primers: LCO1490 (5′-GCT CAA CAA CAT ATA AAG ATA TG-3′) + HC02198 (5′-TAA ACT ATG GAG TGA CCA AAA AAT CA-3′) for cox1 (Folmer et al., 1994); OP24 (5′-ATG ACT ATG CTT TGT GGA GA-3′) + OP425 (5′-TAA ACT TCA GGG TGA CCA AAA AAT CA-3′) for nad5 (Højlund et al., 2006); cytB_F (5′-TGA AAT GGA TAT GAT TGG GAC-3′) + cytB_R (5′-TTA GGA GAT GCA GCC AAA ATA GC-3′) for cytB (present study). The primers were designed with the use of FastPCR software, version 5.4 (Primer Digital, Helsinki, Finland).

PCR was performed in a 2720 thermal cycler (Applied Biosystems, Foster City, California) in a volume of 50 μl. Each 50 μl PCR reaction contained 20 μl of Molecular Biology Reagent Water (Sigma-Aldrich, USA), 25 μl Quant-Bio’s AccuStart™ II PCR ToughMix® (× 2 concentration) (Quantabio, Beverly, USA), 1 μl GelTrack Loading Dye (× 50 concentration) (Quantabio, Beverly, USA), 1 μl forward primer (20 mM), 1 μl reverse primer (20 mM), and 2 μl of template DNA. The conditions for PCR were as follows: 94 °C for 2 min to denature the DNA, with 35 cycles at 94 °C for 40 s (cox1 and nad5) or for 45 s (cytB), 57 °C for 45 s (cox1 and nad5) or 56 °C for 60 s (cytB), and 72 °C for 30 s (cox1, nad5) or for 45 s (cytB); with a final extension of 5 min (cox1, nad5) or 10 min (cytB) at 72 °C to ensure complete amplifications. The PCR product was purified with the use of the Nucleospin Gel and PCR Clean-up Kit ( Macherey-Nagel, Germany), eluted with 30 μl of Molecular Biology Reagent Water (Sigma-Aldrich, USA) and sequenced in both directions by Macrogen Europe (Amsterdam, the Netherlands) with the use of primers used for amplification (5 mM). The sequences were then assembled into contigs using CodonCode Aligner version 8.0 (CodonCode Corporation, Massachusetts, USA). The generated sequences were then compared with those from the same regions in *D. viviparus* of cattle, and those downloaded from GenBank (see below). The accession numbers are listed in Table 1.

### Table 1

| Species                  | Host          | cox1 GenBank | cytB GenBank | nad5 GenBank |
|--------------------------|---------------|--------------|--------------|--------------|
| Dictyocaulus viviparus   | Bos taurus    | JX519460.1   | JX519460.1   | JX519460.1   |
| Dictyocaulus viviparus   | Bos taurus    | AP017683.1   | AP017683.1   | AP017683.1   |
| Dictyocaulus viviparus   | Bison bonus   | KM359417.1   | KM359417.1   | KM359417.1   |
| Dictyocaulus viviparus   | Bison bonus   | MN566991.1   | MN566991.1   | MN566991.1   |
| Dicyocaulus eckerti      | Capra hircus  | NC_019809.1  | NC_019809.1  | NC_019809.1  |
| Angiostrongylus cantonensis| Rattus norvegicus | AP017672.1 | AP017672.1   | AP017672.1   |
2.4. Phylogenetic analyses

Phylogenetic analysis of Dictyocaulus spp. taxa was based on aligned amino acid sequences of the combined partial cox1 (654 bp), cytB (714 bp) and nad5 (351 bp) nucleotide sequences with the use of the GTR + G (cox1 and cytB) and GTR + I (nad5) models of evolution (Table 1). The GTR model was chosen on the basis of JModelTest 2.1.10 (Guindon and Gascuel, 2003; Darriba et al., 2012) using AIC criterion. Phylogenetic tree was constructed using Bayesian inference (BI) analysis with MrBayes version 3.2. (Huelsenbeck and Ronquist, 2001).

2.5. Histopathological examination of the lungs

The lungs were initially checked for the lungworms directly during dissection conducted under field conditions. Fifteen lungs with parasitic infection were macroscopically examined, packed into plastic bags and transported immediately to the laboratory.

Samples of pulmonary tissue surrounding parasites were fixed in 10% buffered formalin and submitted for histopathological investigation as described in Pyziel et al. (2018a). The samples were dehydrated through graded series of ethanol and cleared in xylene, then embedded in paraffin wax, cut into sections (4 μm), stained with hematoxylin and eosin (HE), and processed for light microscope examination (Olympus BX43; Olympus, Japan).

2.6. Statistical analysis

The measurements for total body length, BCW length, BCW width, and spicule length of European bison-derived lungworms taken during this study were compared with those of D. viviparus of cattle presented in Divina et al. (2000) with regard to their mean, standard deviation and number of samples. The females and males were separated into groups and Welch t-test was used to compare those from the European bison with those from the cattle. It was assumed that the data from the cattle-derived lungworms obtained by Divina et al. (2000) met the assumptions for the t-test, as they had been analyzed using a general linear model. The assumptions regarding the data for Dictyocaulus lungworm isolated from European bison were also checked. Probability values less than 0.05 (p < 0.05) were considered statistically significant.

3. Results

3.1. Morphological study

Buccal capsule wall (BCW) of investigated European bison-derived specimens was oval, flattened dorsoventrally with an elongated oval oral opening (Fig. 1A). European bison-derived BCWs were thin (Fig. 1B). A single ring of four symmetrical submedian cephalic papillae and two lateral amphids were observed in the head region (Fig. 1C). Cervical papillae were absent, and a nerve ring was present (Fig. 1A and B). European bison-derived lungworms were longer than the cattle-derived D. viviparus (Table 2), with the difference being statistically significant for both sexes (p < 0.001 for males and p = 0.029 for females). In addition, the BCW was significantly longer in both the male and female European bison lungworm (p < 0.001) (Table 3); however, while the BCWs of males isolated from European bison were significantly narrower than those from cattle (p < 0.001), no such statistically significant difference was observed for the females (p = 0.176) (Table 3). The European bison and cattle-derived lungworms differed with regard to head width; specifically, the European bison lungworms presented higher minimum and maximum values (Table 5). The female reproductive system of the European bison lungworm was didelphic-amphidelphic (Fig. 2A). As the anterior/posterior sphincter and infundibulum were only slightly separated from each other (Fig. 2B), their measurements are given together (Table 4). The female body ended in a tail with two phasmids at its mid-length (Fig. 2C). The male body ended in a bell-shaped copulatory bursa (Fig. 3A and B). A pair of dark brown spicules and gubernaculum was observed in each male (Fig. 3C). A small transparent membrane around the tip of each spicule was clearly visible (Fig. 3C). The spicules of European bison-derived lungworms were significantly longer (p < 0.001) than those isolated from cattle (Table 5).

3.2. DNA sequences

Partial nucleotide sequences of mt cox1, nad5, and cytB genes of European bison-derived lungworms were obtained from 15 animals in the Białowieża Primeval and Borecka Forests. Beside one nucleotide difference in a partial sequence of mt nad5, no diversity was found between the lungworm sequences obtained from these geographical regions: the remaining sequences of the mt genes were identical. Therefore, only single nucleotide sequences of the cox1 and cytB genes of European bison worms was submitted from both locations; in contrast, three diverse sequences of nad5 were submitted to GenBank.

Nucleotide diversity was calculated from pairwise sequence alignments of partial cox1, cytB and nad5 of D. viviparus from cattle (GenBank: JX519460.1; AP017683.1) and from European bison (GenBank: KM359417.1 + KM503300.1 + KM359431.1; MN656991.1 + MNS53301.1 + MT157226.1; MN656991.1 + MNS53301.1 + MN656992.1, respectively). These results varied from 1.7% for nad5 (6 nucleotides of difference), and 2.1% for cytB (15 nucleotides of
difference) to 3.7% for the cox1 marker gene (24 nucleotides of difference) (Table 6). Thus, cox1 demonstrated the greatest sequence variation, whereas cytB and nad5 were more conserved. At the same time, nad5 was the most diverse gene within European bison-derived worms (0.6%), with up to two nucleotides of difference (Table 6). Additionally, in the analysis of mt sequence variation between D. viviparus (GenBank: JX519460.1; AP017683) and D. sp. cf. eckerti (NC_019809.1) the highest level of diversity (18.2%–18.5%) was found for the nad5 region, while the cox1 region being more conserved (11.3%–11.6%) (Table 6).

3.3. Phylogenetic reconstruction

Bayesian analysis (BI) of mt sequence data (cox1 + nad5 + cytB) with Angiostrongylus cantonensis (GenBank: AP017672.1) as an outgroup revealed two strongly supported clades (Fig. 4). One cluster of Dictyocaulus lungworms comprised two subclades: one including taxa of European bison from Białowieża Primeval Forest (GenBank: KM359417.1 + KM359431.1 + MN503300.1) with their sister taxa from Borecka Forest - Borki (GenBank: MN656991.1 + MN656992.1/ MT157226.1 + MN503301.1). Another clade included D. viviparus of cattle (GenBank: JX519460.1; AP017683.1). A third clade included D. sp. cf. eckerti of red deer (GenBank: NC_019809.1).

3.4. Macroscopic examination of the lungs

In all cases the lungs were pale pink, airy, pasty and soft. In two cases the lungs were also focally congested. Lungworms were found in bronchi and bronchioles of each examined lung, mostly in caudal and cranial lung lobes. They were covered with mucus. The range of infection was 14–76 adult lungworms per host.
3.5. Histopathology of the lung tissue

The histopathological sections of European bison lung tissue revealed various lung pathologies associated with *Dictyocaulus* infection, including numerous cross-sections of mature adult or young adult worms in the lumen of the bronchi and bronchioles (Fig. 5A). Moreover, sections of nematode larvae were detected multifocally in pulmonary alveoli (Fig. 5B). Alveolar walls were infiltrated by lymphocytes, plasma cells, macrophages and eosinophils, whereas the bronchi, bronchioles and blood vessels were surrounded by mononuclear cells (Fig. 5A, C). Histopathological examination revealed lesions consisted with interstitial pneumonia with hyperplasia of the lymphoid follicle (Fig. 5D). At the same time hyperplasia of smooth muscle and bronchial epithelial cells was observed, accompanied by the thickening of alveolar septa and the blood vessel walls (Fig. 5C). Other pulmonary lesions included emphysema and congestion.

4. Conclusion

Investigated European bison were infected exclusively with morphologically-different genotype of *Dictyocaulus viviparus* than cattle. No mixed infections of bison and cattle-derived lungworms was noted in investigated European bison. We propose, therefore, that the lungworm of European bison should be classified as *D. viviparus* subspp. *bisontis*.
4.1. Taxonomic summary

The specific epithet: D. viviparus subsp. bisontis.

Type and only known host: European bison, Bison bonasus (Linnaeus, 1758) (Artiodactyla: Bovidae)

Site of infection: Trachea, bronchi, bronchioli

Type locality: Biało wieża Primeval Forest, podlaskie voivodship, Poland (52°29′N - 52°57′N, 23°31′E – 24°21′E), Borecka Forest, warmińsko-mazurskie voivodship, Poland (54°08′01″, 9850′01″N, 22°05′39″, 1214′21″E)

Specimens deposited: Holotype (male) and allotype (female): MNHW 1353 (Museum of Natural History, Wroclaw University, Poland)

Sequences deposited: GenBank: KC771250.1, KF007338.1 – KF007341.1, KM359411.1 - KM359435.1, KT581635.1 - KT581637.1, MT157226.1, MN503300.1, MN503301.1, MN656991.1, MN656992.1.

Etymology: The specific epithet is derived from host genus.

Table 5

Dimensional characterization of morphological features of male reproductive system of Dictyocaulus viviparus derived from European bison in comparison with cattle-derived lungworm data following Divina et al. (2000).

| FEATURE                                      | HOST               |
|----------------------------------------------|--------------------|
|                                              | Cattle             | European bison    | Cattle vs. European bison |
|                                              | Range              | 265.7–415         | –                        |
| Length of copulatory bursa                   | Sample size (n)    | –                 | 25                       |
|                                              | Mean ± standard deviation (X ± SD) | 310.5 ± 59.1    | –                        |
| Width of gubernaculum                        | Sample size (n)    | 26.7–44           | –                        |
|                                              | Mean ± standard deviation (X ± SD) | 33.4 ± 4.6       | –                        |
| Length of spicules                           | Range              | 100.1–286.9       | 271.5–385                |
|                                              | Sample size (n)    | 64                | 64                       |
|                                              | Mean ± standard deviation (X ± SD) | 209.7 ± 15.4     | 310.8 ± 18.8             |

a All dimensions are given in μm.

1214°E)

Specimens deposited: Holotype (male) and allotype (female): MNHW 1353 (Museum of Natural History, Wroclaw University, Poland)

Sequences deposited: GenBank: KC771250.1, KF007338.1 – KF007341.1, KM359411.1 - KM359435.1, KT581635.1 - KT581637.1, MT157226.1, MN503300.1, MN503301.1, MN656991.1, MN656992.1.

Etymology: The specific epithet is derived from host genus name.

Table 6

Pairwise comparison of sequence alignment (1719 bp) consisted of cox1 (654 bp), cytB (714 bp) and nad5 (351 bp) mt DNA nucleotide sequence and inferred amino acid sequence (alignment: 573; cox1: 218; cytB: 238; nad5: 117) variability among Dictyocaulus eckerti and isolates of D. viviparus of cattle and European bison. Above diagonal = Number of variable sites in the 1719 base pair (bp). Below diagonal = Number of variable sites in the 573 amino acid. Total number of variable sites between 2 species/isolates is given prior to parenthesis, whereas number of variable sites regarding particular marker genes (cox1, cytB and nad5, respectively) are given in parenthesis.

| Species and hosta | 1   | 2   | 3   | 4   | 5   | 6   |
|------------------|-----|-----|-----|-----|-----|-----|
| 1. D. eckerti, red deer | 258 (76; 115; 68) | 259 (76; 115; 67) | 258 (76; 115; 67) | 256 (74; 117; 65) | 258 (76; 118; 64) |
| 2. D. viviparus, European bison | 51 (5; 29; 17) | 1 (0; 0; 1) | 0 | 45 (22; 15; 7) | 46 (24; 15; 7) |
| 3. D. viviparus, European bison | 51 (5; 29; 17) | 0 | 1 (0; 0; 1) | 44 (22; 15; 7) | 45 (24; 15; 6) |
| 4. D. viviparus, European bison | 51 (5; 29; 17) | 0 | 0 | 45 (22; 15; 8) | 46 (24; 15; 7) |
| 5. D. viviparus, cattle | 52 (6; 28; 18) | 6 (1; 4; 1) | 6 (1; 4; 1) | 6 (1; 4; 1) | 6 (1; 4; 1) |
| 6. D. viviparus, cattle | 52 (6; 28; 18) | 6 (1; 4; 1) | 6 (1; 4; 1) | 6 (1; 4; 1) | 6 (1; 4; 1) |

GenBank: 1 = NC.019809.1; 2 = KM359417.1 + KM503300.1 + KM359431.1; 3 = MN656991.1 + MN503301.1 + MT157226.1; 4 = MN656991.1 + MN503301.1 + MN656992.1; 5 = JX519460.1; 6 = AP017683.1.
Fig. 4. Phylogenetic tree of *Dictyocaulus* spp. based on *cox1*, *cytB* and *nad5* partial sequences, constructed with the use of Bayesian inference (BI) analysis using MrBayes version 3.2. The GTR + G (*cox1*, *cytB*) and GTR + I (*nad5*) model was chosen based on jModelTest version 2.1.4 using Akaike Information Criterion. The analysis was run for 2,000,000 generations, with 500,000 generations discarded as ‘burn-in’. Hosts (for *Dictyocaulus viviparus*) and GenBank accession numbers of origin are shown. Nodal support is indicated as Bayesian posterior probabilities. Sequence from *Angiostrongylus cantoniensis* (AP017672.1) was used as an outgroup.

Fig. 5. Histopathological lesions in the lungs of European bison infected with *Dictyocaulus viviparus*. (A) The cross-section of a nematode in the lumen of bronchiole (arrow); mononuclear cells infiltration in the mucous membrane of the bronchiole. H-E stain (× 40 magnification). (B) Multiple cross-sections of nematodes scattered within alveoli and bronchiole (arrows). H-E stain (× 40 magnification). (C) Interstitial pneumonia, diffuse, lymphoplasmacytic, eosinophilic with the hyperplasia of the vascular wall (arrows). H-E stain (× 40 magnification). Inset: Perivascular, peribronchiolar lymphocytic and eosinophilic infiltration; on the left side: the tunica media of vessels is thickened (hyperplastic). H-E stain (× 200 magnification). (D) Prominent peribronchiolar hyperplasia of the lymphoid follicle (arrow); pulmonary tissue showed interstitial pneumonia pattern. H-E stain (× 200 magnification).
5. Discussion

This study provides a detailed morphological description of European bison-derived *Dictyocaulus* lungworms, and assess the phylogenetic position of lungworms among closely-related hosts: European bison and domestic cattle. According to Divina et al. (2000), the most important morphological diagnostic characteristics for *Dictyocaulus* spp. are BCW thickness and length, while spicular length does not differ between cattle, roe deer and moose-derived lungworms. In the present study, the specimens from the European bison were found to have longer BCW than those isolated from cattle: this different was almost three-fold in males and more than four-fold in females; in addition, the BCW was narrower in the males of European bison than in those diagnosed in cattle. In addition, like the majority of cattle-derived lungworms, the BCW of European bison lungworm was classified as thin (Divina et al., 2000). Furthermore, the spicules of *Dictyocaulus* were significantly longer in European bison than those of cattle (Divina et al., 2000). Divina et al. (2000) also report that the thickness and length of BCW were positively correlated with the total body length of the lungworm. Our morphological findings suggest that European bison host a distinct *Dictyocaulus* species; however, it is well known that morphological differences (morphotypes) can be induced by the host species (Nadler, 2002). Nevertheless, although our present results suggest that *Dictyocaulus* lungworms of European bison and cattle can be morphologically distinguished on the basis of the BCW length, the total body length, and spicule lengths in males, their phylogenetic position suggests that cattle lungworm can infect both hosts. This suggestion is supported by previous findings involving rDNA datasets, showing that the SSU sequence of European bison lungworms (GenBank: KC771250) was homologous with those from cattle (GenBank: AY168856) (Pyziel, 2014); additionaly, both isolates diverged only slightly in the ITS2 region (GenBank: KC771250) was homologous to European bison-derived lungworms in the present study, which found cox1 to capture the least within-genus sequence variation and nad5 the greatest. Similarly, cox1 was found to demonstrate the least within-species variation of mtDNA genes among populations of *D. viviparus* isolated from cattle (Höglund et al., 2006; Gasser et al., 2012). These findings contrast with those obtained from cattle and European bison-derived lungworms in the present study, which found nad5 and cytb to be more conserved than cox1. In addition, the worms from both hosts demonstrated comparable levels of sequence diversity for the same gene regions to those noted previously between various *D. viviparus* isolated from cattle; this diversity did not reach 4.0% (Hu et al., 2002; Höglund et al., 2006; Gasser et al., 2012). In contrast, mt sequence variation has been found to range from 10 to 20% between closely-related species of nematodes and from 1 to 3% between individuals of inbreeding population (Blouin, 1998).

Our findings also indicate that lungworm infection can cause interstitial pneumonia, bronchitis and bronchiolitis. Similar histopathological lesions have been observed in the lung tissue of cattle and buffaloes (Schnider et al., 1991; Mahmood et al., 2014) exhibiting parasitic pneumonia with extensive mononuclear infiltration in the lung parenchyma, bronchial mucosa, and bronchial and alveolar bronchiolitis. Additionally, the histopathological picture of European bison dictyocaulosis revealed the presence of similar lesions to those noted previously in red deer dictyocaulosis (Pyziel et al., 2018a).

To summarise, it appears that *D. viviparus* infecting cattle and European bison are characterized by both morphological differences and slightly diverging genotypes. We therefore propose that the lungworm of European bison should be classified as *D. viviparus* subsp. *bisonis*. An additional recommendation is that mt cytb marker gene should be used to distinguish between the genotypes of the lungworms infecting cattle and bison.

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