# Opening a can of lungworms: Molecular characterization of *Dictyocaulus* (Nematoda: Dictyocaulidae) infecting North American bison (*Bison bison*)

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A B S T R A C T

*Dictyocaulus* is a globally distributed genus of lungworms of domestic and wild ungulates. *Dictyocaulus* adults inhabit the bronchi, frequently causing subclinical and clinical disease, and that impacts animal health and production. North American bison (*Bison bison*) and cattle (*Bos taurus*) share various parasitic nematode species, particularly in areas where co-grazing occurs. The current assumption is that North American bison share the lungworm *D. viviparus* with cattle, but this has not been confirmed on a molecular basis. The aim of this study was to molecularly characterize *Dictyocaulus* lungworm isolates from North American plains bison (*Bison bison bison*). Fecal samples were collected from 5 wild conservation bison herds located in Iowa, North Dakota, Oklahoma, Colorado, and Montana in 2019 and 2020, and from ranched and feedlot bison from 2 herds in Oklahoma and Texas. First-stage lungworm larvae (L1) were isolated via Baermann technique. Genomic DNA was extracted from L1s of up to 3 samples per herd and followed by PCR and sequencing targeting the internal transcribed spacer 2 (ITS2) region of the nuclear ribosomal DNA and the partial cytochrome oxidase c subunit 1 (*cox1*) of mitochondrial DNA. Phylogenetic analyses were performed in MEGA X 10.1. Sequences of North American plains bison *Dictyocaulus* belong to a single, uncharacterized species, clustering in well-supported clades (100% and 100% bootstrap support for ITS2 and *cox1*, respectively), differing from *D. viviparus* of cattle in North America and Europe, and European bison (*Bison bonasus*). Our results contradict previous assumptions regarding parasite identity, highlighting the need for characterization of this species through morphological and molecular methods, elucidating its biology and host range, and potential impact on host health. Further investigation into the biodiversity of *Dictyocaulus* species infecting bovids and cervids in North America is warranted.

## 1. Introduction

*Dictyocaulus* Railliet and Henry, 1907 (Nematoda: Dictyocaulidae) is a globally distributed genus of lungworm parasites of domestic and wild ungulates, including an array of bovid and cervid hosts (Gibbons and Khalil, 1988; Anderson, 2000). *Dictyocaulus* adults inhabit the bronchi and have a direct life cycle. Briefly, the first-stage larvae (L1) are passed in feces and will develop to third-stage larvae (L3) in 1–4 weeks in the environment. Susceptible ungulate species are infected by ingestion of L3s on pasture, after which larvae migrate to the lungs. In the lungs, larvae develop into adult males and females, which produce eggs after sexual reproduction. In many *Dictyocaulus* species associated with

ruminant hosts, the eggs hatch and the resulting L1 larvae are coughed up, swallowed, and deposited in the feces, completing the transmission to the environment (Anderson, 2000; Panuska, 2006). The pre-patent period is 3–4 weeks, however, some *Dictyocaulus* species, specifically *Dictyocaulus viviparus* (Bloch, 1782) of cattle, may undergo hypobiosis in the case of overwintering which can increase the pre-patent period to 150 days.

*Dictyocaulus* infection has been shown to cause subclinical and clinical disease in domestic and wild ungulate hosts (Panuska, 2006; Kutz et al., 2012). For example, previous studies with *D. viviparus* in cattle have revealed a negative impact on animal health and decreased milk production (Panuska, 2006; Dank et al., 2015; May et al., 2018). In

high intensity infections, *Dictyocaulus* leads to verminous pneumonia, associated with inflammation, obstruction of bronchioles, emphysema, and pneumonia resulting in clinical signs including coughing, dyspnea, nasal discharge, or occasionally mortality (Panuska, 2006). Information on the impact of *Dictyocaulus* infection in wild ungulates remains scarce.

The North American bison (*Bison bison* (Linnaeus, 1758)) is the largest terrestrial mammal of North America, and is listed as "near threatened" in the International Union for Conservation of Nature (IUCN) Red List of Threatened Species (Aune et al., 2017). Bison were driven to near extinction in the late 1800s, resulting in a genetic bottleneck (Hedrick, 2009), and possibly including the parasites they carry. Today, an estimated number of 11,000 to 13,000 wild individuals remain in severely fragmented populations (Aune et al., 2017). In addition, the North American bison has been kept and bred as livestock, with an estimated slaughter of 66,000 bison in the US in 2020, with an increasing trend in recent years (United States Department of Agriculture, 2020). Previous studies have suggested that bison grazed on range more likely to have been previously occupied by cattle have different parasite communities than bison grazing ranges without shared cattle history (Avramenko et al., 2018). The current paradigm is that North American bison share the lungworm *D. viviparus* with cattle, likely due to range overlap, but this has not been confirmed using molecular methods (Dikmans, 1936; Frick 1951; Locker, 1953; Boddicker and Hugghins, 1969; Wade et al., 1979). However, *Dictyocaulus hadweni* Chapin (1925) was historically described as a species in North American bison from western Canada (Chapin, 1925; Roudabush 1936). The validity of this species was later questioned by Dikmans (1936), who considered it a junior synonym of *D. viviparus*, influencing subsequent reports.

The aim of this study was to molecularly characterize *Dictyocaulus* lungworm isolates from plains bison across the United States and understand its phylogenetic relationships with other species within the genus. This study highlights the need to elaborate on the uncharacterized species of *Dictyocaulus* present in North American bison and investigate other lungworm infections in North American ungulates.

## 2. Materials and methods

### 2.1. Collection

Bison fecal samples were collected from five US Fish and Wildlife Service National Wildlife Refuge herds and 2 ranched herds across the US in 2019–2020: Wichita Mountains Wildlife Refuge (Oklahoma), National Bison Range (Montana), White Horse Hill National Game Preserve (North Dakota), Neal Smith National Wildlife Refuge (Iowa), Rocky Mountain Arsenal National Wildlife Refuge (Colorado), Cheyenne and Arapaho tribes (Oklahoma), and a privately-owned Texas feedlot (containing a herd originally from Oklahoma) (Table 1). Wild bison samples were collected from each refuge herd by US Fish and Wildlife Service personnel using techniques to approach and collect samples from the herd in a short period of time to reduce the potential for duplicate sample collection from any individual animal. Ranched and feedlot bison samples were collected either during routine handling and vaccination operations of from the field. In addition, a bovine calf (*Bos taurus* Linnaeus, 1758) fecal sample originating from Marion County, northeastern Texas, was included for comparison. The samples were refrigerated in either 50 mL plastic tubes or plastic bags for no more than 12 days prior to shipment in insulated containers with coolant packs to the Texas A&M University Parasitology Diagnostic Laboratory via overnight courier service (Fig. 1).

### 2.2. Baermann technique

The Baermann technique was used to isolate *Dictyocaulus* lungworm larvae from bison and cattle fecal samples of bison or cattle using a modification of a previously protocols (Zajac and Conboy, 2012; Verocai et al., 2020). Briefly, we utilized a plastic 50 mL conical tube and 5 g of

**Table 1**
Description of bovid herds samples, and larvae included in PCR, sequencing, and alignment.

| Herd | State | Animals Sampled | Sequenced L1 (ITS2) | Sequenced L1 (*cox1*) |
|---|---|---|---|---|
| **Bovine** | Texas | 1 | 2/3 | 1/3 |
| **Cheyenne and Arapaho** | Oklahoma | 4 | 10/10 | 8/9 |
| **National Bison Range** | Montana | 3 | 8/8 | 8/8 |
| **Neal Smith National Wildlife Refuge** | Iowa | 3 | 9/9 | 9/9 |
| **Privately owned feedlot** | Texas | 3 | 8/8 | 8/8 |
| **Rocky Mountain Arsenal National Wildlife Refuge** | Colorado | 3 | 9/9 | 9/9 |
| **White Horse Hill National Game Preserve** | North Dakota | 6 | 17/18 | 16/17 |
| **Wichita Mountains Wildlife Refuge** | Oklahoma | 3 | 9/9 | 9/9 |
| **Total** | | 25 bison, 1 cattle | 70/71 bison, 2/3 cattle | 67/69 bison, 1/3 cattle |

feces wrapped in cheesecloth and a Kimwipe submerged in water for 24 h. The following day, after removal of the wrapped feces, the bottom 1 mL was collected and microscopically examined at 4× magnification on a glass slide. Any Baermann filtrate containing *Dictyocaulus* L1 was collected and stored in cryogenic vials at −80 °C for subsequent processing. Archived samples were thawed to room temperature and *Dictyocaulus* larvae were isolated with the aid of micropipettes under a compound microscope at 4× magnification.

### 2.3. DNA extraction and PCR

We have extracted DNA of *Dictyocaulus* L1 recovered from a total of 25 bison fecal samples were analyzed from 7 herds, including up to 4 larvae from each sample per year (Table 1). DNA extraction of single larva was performed using DirectPCR® lysis reagent (Cell) (Viagen Biotech, Inc., Los Angeles, CA, USA) with Proteinase K (QIAGEN®, Hilden, Germany) (0.5 mg/mL) as per manufacturer's instructions. The extracted DNA was kept frozen at −20 °C. To amplify the internal transcribed spacer 2 (ITS2) region of the nuclear ribosomal DNA, NC1 and BD3R primers were utilized as stated in Höglund et al. (1999) and Pyziel et al. (2017), respectively (Table 2). Additionally, to amplify the partial cytochrome oxidase c subunit 1 (*cox1*) region of mitochondrial DNA, LCO1490 and HCO2198 were utilized according to Folmer et al. (1994). Reactions were carried out in a volume of 25 μL containing 1x GoTaq® Green Master Mix (Promega Corporation, Madison, WI, USA), 0.625 μL of each primer at 0.25 μM concentration and 1 μL of DNA template. Extracted DNA of *Pseudostertagia bullosa* (Ransom and Hall, 1912) was included as a positive control and water as the negative control. Cycling conditions for ITS2 amplification were an initial denaturation at 95 °C for 2 min, followed by 40 cycles of 95 °C for 30 s, 51 °C for 45 s, and 72 °C for 1 min, and a final extension step at by 72 °C for 5 min. Cycling conditions for *cox1* were identical except for the annealing temperature, which was lowered to 50 °C. PCR products were visualized utilizing gel electrophoresis and purified using E.Z.N.A. Cycle Pure Kit (Omega Bio-tek, Norcross, GA, USA) according to the manufacturer's protocol. The purified DNA products were sequenced in both directions using the original PCR primers in 3730xl DNA Analyzer at Eurofins Genomics (Louisville, KY, USA).
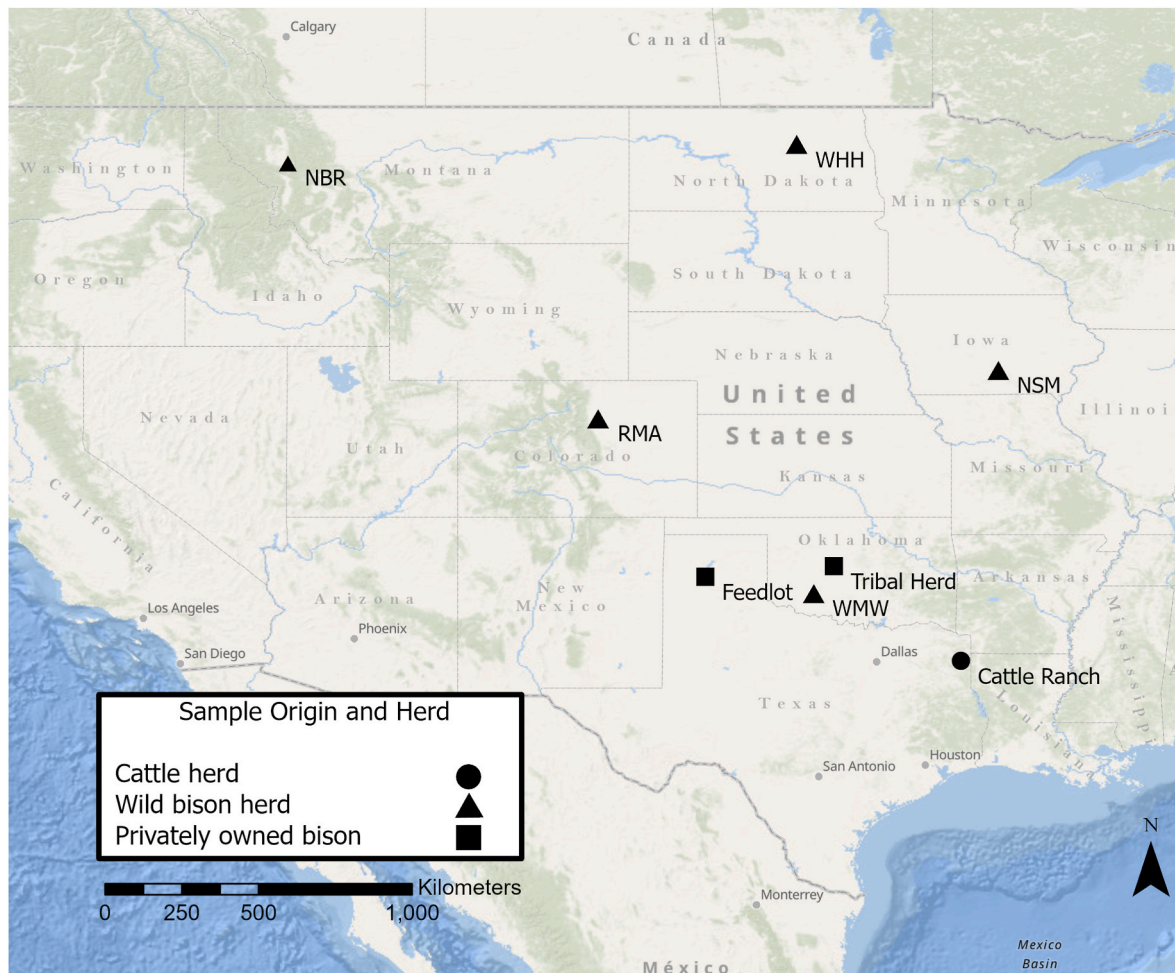
**Fig. 1.** Sites of bison fecal collections; WHH: White Horse Hill National Game Preserve; RMA: Rocky Mountain Arsenal National Wildlife Refuge; NBR: National Bison Range; NSM: Neal Smith National Wildlife Refuge; WMW: Wichita Mountains Wildlife Refuge.

**Table 2**

Primers used for polymerase chain reaction amplification and sequencing of the internal transcribed spacer 2 (ITS2) region of the nuclear ribosomal DNA and cytochrome *c* oxidase subunit 1 (*cox1*) of mitochondrial DNA in *Dictyocaulus* isolates.

| Gene | Primer | Forward/Reverse | Sequence | Reference |
|------|--------|-----------------|----------|-----------|
| **ITS2** | NC1 | Forward | 5′-ACGTCTGGTTCAGGGTTGTT-3′ | Höglund et al. (1999) |
| | BD3R | Reverse | 5′-TATGCTTAAGTTCAGCGGGT-3′ | Pyziel et al. (2017) |
| ***cox1*** | LCO1490 | Forward | 5′-GGTCAACAAATCATAAAGATATTGG-3′ | Folmer et al. (1994) |
| | HCO2198 | Reverse | 5′-TAAACTTCAGGGTGACCAAAAAATCA-3′ | Folmer et al. (1994) |

### 2.4. Phylogenetic analyses

Sequencing data were analyzed using SnapGene software (GSL Biotech LLC, San Diego, CA, USA) as chromatograms to verify sequence quality. Initially, *Dictyocaulus* isolate sequences were compared with other *Dictyocaulus* sequences available in the GenBank database through BLAST searches. Sequences were aligned and analyzed using MEGA X 10.1 (Kumar et al., 2018). Gaps and point mutations were verified referring to the chromatograms. Forward and reverse sequences were analyzed to create a composite sequence. A maximum composite likelihood model was utilized to generate pairwise distances. ITS2 was run with partial deletion penalties while *cox1* was run with complete deletion penalties based on the degree to which gaps persisted in the alignment. Maximum likelihood phylogenetic trees were generated in MEGA X 10.1 (Kumar et al., 2018) using 1,000 bootstrap replicates. The best fit DNA/protein models for ITS2 and *cox1* analyses were Tamura (1992) with invariant sites, and Tamura-Nei (Tamura and Nei, 1993)

with gamma distribution, respectively. *Angiostrongylus vasorum* (Baillet, 1866) was included as an outgroup for both analyses.

### 3. Results

#### 3.1. Samples analyzed

Representative pooled L1 from each wild bison herd were deposited at the Division of Parasites of the Museum of Southwestern Biology, University of New Mexico, under the accession numbers: MSB: Para:32452–7 (arctos.database.museum).

For the ITS2 region of the nuclear ribosomal DNA, 86.6% (71/82) of larvae were successfully amplified by PCR, with 98.6% (70/71) of those successfully sequenced and included in the phylogenetic analysis. A total of 70 *Dictyocaulus* ITS-2 sequences (229–235 base pairs) of individual L1 from North American plains bison (Accession Numbers: OK575977-OK576046) and 2 from cattle (OM417137-OM417138) were produced

and accessioned in GenBank.

For *cox1* of mitochondrial DNA, 84.1% (69/82) of the larvae obtained were successfully amplified by PCR, with 97% (67/69) of those successfully sequenced for subsequent phylogenetic analysis. A total of 67 *Dictyocaulus* partial *cox1* sequences (621 base pairs) from North American plains bison (Accession Numbers: OK562219-OK562285) and 1 from cattle (OM368333) were generated and accessioned in GenBank.

### 3.2. Pairwise distance analysis

Maximum identity within and between species for the ITS2 region can be found in Table 3. The *Dictyocaulus* from North American plains bison shared an average of 99.9% identity among each other. *Dictyocaulus viviparus* from our North American cattle isolate, European cattle, and European bison averaged 88.9%, 90.6%, and 90.7% shared identity with *Dictyocaulus* of North American plains bison, respectively. The lowest shared identity with North American plains bison *Dictyocaulus* was 62.9% with *Dictyocaulus capreolus Gibbons and Höglund, 2002*.

Calculated maximum identities were obtained from *cox1* sequence data between species of *Dictyocaulus* (Table 4). The species of *Dictyocaulus* found in the present study from North American plains bison had an average of 99.9% shared identity. When compared to *D. viviparus* of our North American cattle isolate, the *Dictyocaulus* samples of our plains bison had an average of 88.8% shared identity. The other *Dictyocaulus* species assessed averaged a shared identity with *Dictyocaulus* of our plains bison ranging between 85.5% (*D. capreolus*) and 88.9% (*D. viviparus*).

### 3.3. Phylogenetic analysis

Maximum likelihood analysis of ITS2 sequence data demonstrated that *Dictyocaulus* of North American plains bison demonstrated distinction from other *Dictyocaulus* species. A well-supported clade (83% bootstrap support) containing North American *Dictyocaulus* isolates and European *D. viviparus* was further divided into two equally well-supported subclades (100% and 99% bootstrap support respectively), one containing only North American plains bison *Dictyocaulus* isolates, and one including *D. viviparus* of cattle and European bison (Fig. 2). Within *D. viviparus*, there were two well-supported subclades, one containing North American cattle isolates and another of European bison (*D. viviparus bisontis*) and cattle. Basal to this bovine *Dictyocaulus* clade, was a clade containing *D. eckerti Skrjabin, 1931* of moose in Sweden. Basal was a clade containing *Dictyocaulus cervi Pyziel, Laskowski, Demiaszkiewicz and Höglund, 2017* and *D. eckerti* of fallow deer and red deer of European origin. Basal to the above clades were *D. capreolus* of roe deer and red deer also with a European origin.

Maximum likelihood analysis of *cox1* sequence data further

demonstrated the reciprocal monophyly of isolates of *Dictyocaulus* of North American plains bison with 100% bootstrap support (Fig. 3). The North American plains bison isolates clustered with *D. viviparus* and *D. capreolus*, but with moderate (72%) bootstrap support. However, the subclade containing *D. viviparus* from North American cattle, European cattle, European bison, and *D. capreolus* from European roe deer had 91% bootstrap support. Within *D. viviparus*, cattle and European bison isolates also formed well-supported clusters. A separate clade contained *Dictyocaulus* spp. of red deer from New Zealand and Europe, roe deer from Europe, and fallow deer from Europe.

## 4. Discussion

Since 1936, the species of *Dictyocaulus* in North American bison has been presumed to be *D. viviparus* (Dikmans, 1936). The results of our study contradict this long-held assertion with strong support from pairwise distance and phylogenetic analyses of both a nuclear and mitochondrial gene. Instead, both wild and ranched North American plains bison across the United States are parasitized by a genetically distinct *Dictyocaulus* species. Notably, we found this same uncharacterized species across diverse geographies with minimal variation between genetic sequences. This finding may be explained by the historic genetic bottleneck experienced by wild bison in the last century, with modern wild and ranched herds established from small remnant herds gathered in the late 1800s (Hartway et al., 2020). Furthermore, the broad geographic distribution of those small remnant herds across the United States suggests that this genetically distinct *Dictyocaulus* species may have been historically widespread prior to the introduction of European cattle, occurring commonly enough to survive the bottleneck with their bison host. Although our study revealed surprising homology across bison herds under different management models across different geographic areas, the history of bison translocations among wild herds in North America is well documented and can be utilized for further study of genetic structure within this uncharacterized *Dictyocaulus* species. Bison interactions with other North American ungulates, including a larger sampling of domestic cattle, as well as cervids such as white-tailed deer, elk, and moose, should be further investigated to assess if this newly identified *Dictyocaulus* species infects other sympatric hosts. While these isolates are distinct from other *Dictyocaulus* species and it is the only species found in all of the North American plains bison herds sampled in our study, its phylogenetic relationships among other *Dictyocaulus* species warrants further investigation.

### 4.1. Phylogenetic relationships

While variation in clades identified in our analysis are minimal, most notably, *cox1* of mitochondrial DNA clusters *D. capreolus* with

**Table 3**

Pairwise identity among *Dictyocaulus* species based on sequences of the internal transcribed spacer 2 (ITS2) region of the nuclear ribosomal DNA.

| *Dictyocaulus* species | *Dictyocaulus* sp., US bison | *D. viviparus*, US cattle | *D. viviparus*, European cattle | *D. viviparus*, European bison | *D. cervi* | *D. capreolus* | *D. eckerti* |
|---|---|---|---|---|---|---|---|
| *Dictyocaulus* sp., US bison | 99.0–100 (99.9 ± 0.20) | – | – | – | – | – | – |
| *D. viviparus*, US cattle | 88.3–89.0 (88.87 ± 0.21) | 100 | – | – | – | – | – |
| *D. viviparus*, European cattle | 90.0–90.7 (90.6 ± 0.21) | 98.9–99.0 (99.0 ± 0) | 100[a] | – | – | – | – |
| *D. viviparus*, European bison | 90.1–90.8 (90.7 ± 0.2) | 99.0 | 100 | 100 | – | – | – |
| *D. cervi* European red deer | 74.5–75.2 (74.5 ± 17) | 74.6 | 73.8 | 74 | 100[a] | – | – |
| *D. capreolus* European red deer European roe deer | 62.9–62.9 (62.9 ± 0) | 56.4 | 53.5 | 53.9 | 59.6 | 100 | – |
| *D. eckerti* European fallow deer | 81.7–82.4 (81.7 ± 0.16) | 81.2 | 79.0 | 79.1 | 91.5 | 69.5 | 100[a] |

[a] Single sequence.

**Table 4**

Pairwise identity among *Dictyocaulus* species based on sequences of the partial cytochrome oxidase c subunit 1 (*cox1*) of the mitochondrial DNA.

| *Dictyocaulus* species | *Dictyocaulus* sp., US bison | *D. viviparus*, US cattle | *D. viviparus*, European cattle | *D. viviparus*, European bison | *D. cervi* | *D. capreolus* | *D. eckerti* |
|---|---|---|---|---|---|---|---|
| *Dictyocaulus* sp., US bison | 99.2–100 (99.9 ± 0.12) | – | – | – | – | – | – |
| *D. viviparus*, US cattle | 87.9–88.9 (88.8 ± 0.12) | 100[a] | – | – | – | – | – |
| *D. viviparus*, European cattle | 87.7–89.1 (88.9 ± 0.23) | 99.83 | 99.7–100 (99.8 ± 0.20) | – | – | – | – |
| *D. viviparus*, European bison | 87.9–88.8 (88.8 ± 0.12) | 96.3 | 96.1–96.5 (96.4 ± 0.15) | 100 | – | – | – |
| *D. cervi* European red deer, European fallow deer, European roe deer | 85.8–88.0 (87.2 ± 0.38) | 83.5–85.1 (84.4 ± 0.35) | 83.2–85.3 (84.4 ± 0.41) | 85.5–87.3 (86.28 ± 0.45) | 96.5–99.8 (98.3 ± 0.70) | – | – |
| *D. capreolus* European roe deer | 84.0–85.9 (85.5 ± 0.30) | 86.8–87.5 (87.2 ± 0.27) | 86.6–87.7 (87.3 ± 0.34) | 87.0–87.7 (87.3 ± 0.25) | 82.4–85.9 (84.1 ± 0.62) | 97.9–100 (99.1 ± 0.50) | – |
| *D. eckerti* European red deer | 86.9–87.6 (87.6 ± 0.10) | 85.3 | 85.1–85.5 (85.4 ± 0.26) | 85.4 | 89.4–90.7 (90.0 ± 0.34) | 83.4–84.8 (84.3 ± 0.40) | 100[a] |

[a] Single sequence.
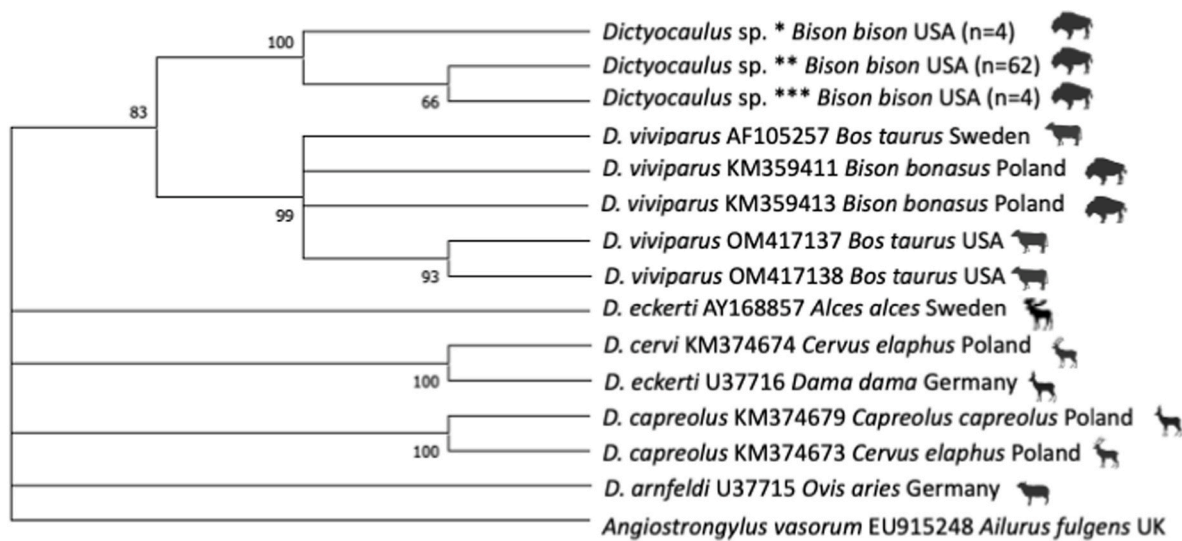


**Fig. 2.** Maximum likelihood analysis of internal transcribed spacer 2 (ITS2) sequence data of *Dictyocaulus* spp. Analysis was run with T92 as best nucleotide substitution model and 1,000 bootstraps. *Angiostrongylus vasorum* = outgroup.

*D. viviparus*, while it is represented by a separate clade under ITS2 of the nuclear ribosomal DNA. Additional sequencing of other genetic markers is required to further elucidate phylogenetic relationships among the North American plains bison *Dictyocaulus* isolate and other species within the genus.

### 4.2. Novel species or Dictyocaulus hadweni?

The lack of adult *Dictyocaulus* specimens isolated from North American plains bison for detailed morphologic assessment hampers direct comparison with the description of *D. hadweni*, a species that has been considered invalid by Dikmans (1936), following reassessment of its type-specimens. The original description of *D. hadweni* is relatively poor, contains confusing if not erroneous morphological comparisons with other *Dictyocaulus* species, and male specimens were considered virtually indistinguishable from those of *D. viviparus* from cattle. The most recent revision of the genus *Dictyocaulus* also failed to recognize *D. hadweni* as a valid species (Gibbons and Khalil, 1988). Nevertheless, the morphological differentiation among adult male and female can be rather challenging, and recent studies integrating classical and molecular approaches have recognized two new species in Eurasian cervids,

namely *D. capreolus* and *D. cervi*, and a proposed susbspecies infecting the European bison, *D. v. viviparus* (Gibbons and Khalil, 1988; Gibbons and Höglund, 2002; Pyziel et al., 2017, 2020). Overall, these recent advances around the biodiversity of the genus *Dictyocaulus* suggest that cryptic species may yet to be recognized, especially since our analysis identified a previously uncharacterized species genetically distinct from both *D. viviparus* and the proposed subspecies *D. v. bisontis* described in European bison (Pyziel et al., 2020).

Our laboratory has begun acquiring adult nematode specimens from North American plains bison for a detailed morphological characterization in tandem with the confirmation of the species identity based on our ITS2 and *cox1* data. Future studies should include experimental infections with *Dictyocaulus* of cattle to elucidate transmissibility as well as additional fecal samples from cattle which co graze with bison.

### 4.3. Host-specificity of Dictyocaulus

Recent studies have shown that some cervid and bovid-associated *Dictyocaulus* may not be host-specific, especially in areas of sympatry of susceptible hosts (Pyziel et al., 2017). For instance, through molecular characterization, *D. eckerti* has been reported from various cervids
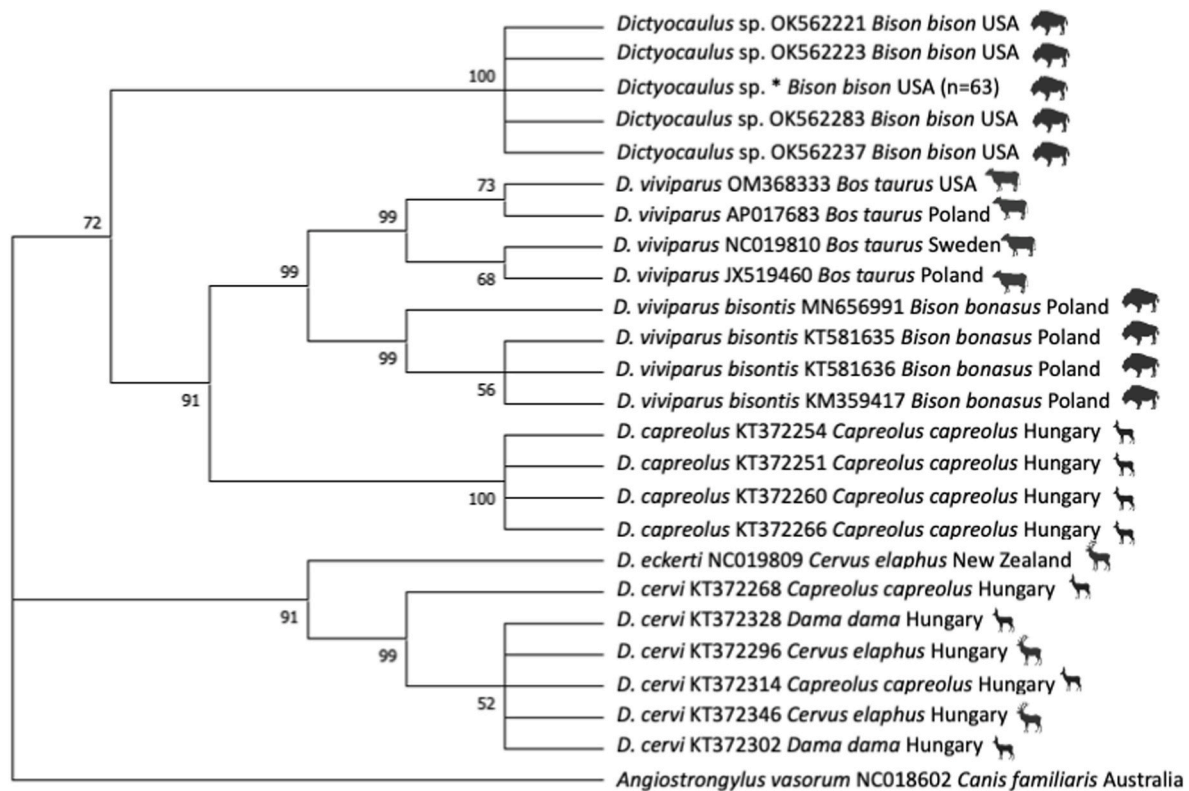
**Fig. 3.** Maximum likelihood analysis of cytochrome oxidase c subunit 1 (*cox1*) sequence data of *Dictyocaulus* spp. Analysis was run with TN93 + G as best nucleotide substitution model and 1,000 bootstraps. *Angiostrongylus vasorum* = outgroup.

including red deer (*Cervus elaphus* Linnaeus, 1758), Eurasian moose (*Alces alces* (Linnaeus, 1758)), and roe deer (*Dama dama* (Linnaeus, 1758)); *D. capreolus* in roe deer and moose (Gibbons and Khalil, 1988; Gibbons and Höglund, 2002; Pyziel et al., 2017), and *D. viviparus* in cattle and European bison (Höglund et al., 2003; Pyziel, 2014). In North America, reports of *Dictyocaulus in* various wild ungulates were identified or assumed to be associated with *D. eckerti* (revised in Kutz et al., 2012). Currently, it is unknown if the North American plains bison may be infected by *D. viviparus*, and if the bison *Dictyocaulus* isolate we have characterized can infect cattle or other ungulate hosts. Further molecular screening of *Dictyocaulus* in different hosts, along with cross transmission studies should be conducted to further elucidate host-specificity. While it is unknown if the bison *Dictyocaulus* identified in our study may infect cervids, Chapin (1925) reported that *D. hadweni* of bison was morphologically indistinguishable from isolates from moose (*Alces americanus* (Linnaeus, 1758)) and elk or wapiti (*Cervus canadensis* (Erxleben, 1777)) from North America. Elucidating the host range of *Dictyocaulus* associated with the North American plains bison will have implications for future management practices, specifically where bison co-graze with other wild ungulates and domestic cattle.

### 4.4. Potential impacts of Dictyocaulus infection in bison

Studies in cattle show that subclinical *D. viviparus* infections can cause significant losses in milk production, with a reduction ranging from 1.01 to 1.68 kg/cow/day of milk (Dank et al., 2015). Dank et al. (2015) also demonstrated that cows with subclinical *D. viviparus* infections produced milk with 0.14% less milk fat, potentially reducing growth in calves. Dictyocaulosis has been also associated with reduction of yearly calf survival and weakening of individuals in cattle (Panuska, 2006; David, 1999). Currently, the impact of *Dictyocaulus* infection in North American plains bison is unknown, although previous reviews suggest that subclinical or clinical disease may develop in infected

individuals managed under high density production models (Haigh et al., 2002; Berezowski et al., 2018). While dictyocaulosis could cause direct economic impact to the bison industry, the effects on wild herds managed at low densities on natural range are likely minimal, and especially if our newly identified species of *Dictyocaulus* evolved in North American plains bison. In contrast with many production management models, wild bison health is determined by the resilience and sustainability of this species to native pathogens and parasites (Stephen, 2014; Jones et al., 2020).

### 4.5. Future explorations of the historical biogeography of Dictyocaulus in North American bison

The substantial genetic divergence of North American plains bison *Dictyocaulus* isolates in relation to those found in cattle and European bison allows us to infer on its historical biogeography. Assuming *B. bison bison* is the primary host for this species, one would expect a deeper association with this wild bovine species – the larger remnant bovine in the Nearctic fauna. The genus *Bison* arrived in North America through the Bering Land Bridge in two waves: one 195-135kybp (thousand years before present) and another 45-21kybp in the Pleistocene Epoch, likely as *Bison priscus*. This species later evolved into *Bison latifrons*, and later as *Bison antiquus* and *Bison occidentalis* with the latter existing until 1730ybp (Froese et al., 2017; Zver et al., 2021). According to fossil records, *B. bison* only appeared around 10kybp within the Holocene, and is currently subdivided into two subspecies, the plains bison, *B. b. bison*, and the wood bison, *B. b. athabascae* (Heintzman et al., 2016; Geist, 1991). To explore beyond our identification of this distinct *Dictyocaulus* species in North American plains bison, sampling of wood bison and additional plains bison herds may further elucidate the geographic distribution of this lungworm. Moreover, a deeper understanding of the phylogeography of this newly identified *Dictyocaulus* may be attained by means of comparative mitogenome and whole genome sequencing and

molecular clock analysis (Gasser et al., 2012; McNulty et al., 2016).

## 5. Conclusions

Our recent discovery of a previously uncharacterized *Dictyocaulus* isolate contradicts previous assumptions regarding parasite identity, necessitating further research of *Dictyocaulus* infecting wild bovids and cervids in North America. Investigations should include studies into host health impact and how to preserve this unique host-parasite assemblage, especially in systems likely to have co-evolved over long periods of time. Thought-provoking studies have stated the importance of parasite conservation and how vital it is to keeping ecosystems in balance. In addition, taxonomic description of parasites is crucial to enhance monitoring and quantifying of named species, especially in the face of climate change and other anthropogenic processes (Carlson et al., 2020). This study adds to the knowledge of *Dictyocaulus* and thereby contributes to overall parasite conservation, including in the fragile ecosystems in which wild North American plains bison exist.

## Declaration of competing interest

The authors declare no conflict of interest.

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