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The first finding of *Dictyocaulus cervi* and *Dictyocaulus skrjabini* (Nematoda) in feral fallow deer (*Dama dama*) in Australia

Keira Brown [a,b], David J. Jenkins [a,b], Alexander W. Gofton [c], Ina Smith [c], Nidhish Francis [a,b], Shokoofeh Shamsi [a,b], Diane P. Barton [a,b,*]

[a] School of Agricultural, Environmental and Veterinary Sciences, Charles Sturt University, Wagga Wagga, New South Wales, Australia
[b] Gulbali Institute, Charles Sturt University, Wagga Wagga, New South Wales, Australia
[c] CSIRO, Health and Biosecurity, Canberra, Australian Capital Territory, Australia

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ABSTRACT

Feral deer are widespread throughout Australia with the capacity to impact livestock production *via* transmission of parasites. Samples of *Dama dama* (fallow deer), *Rusa unicolor* (sambar deer), *Cervus elaphus* (red deer) and an unidentified deer were sourced from various locations in south-eastern Australia for examination for parasites. Adult nematodes were collected from the lungs of all deer species across four separate geographical locations. The nematodes were identified as species of *Dictyocaulus* through both morphological and molecular means. Species identification based on morphological features was difficult, with many measurements from described species overlapping. Molecular analyses targeting three markers, namely 18S rRNA, ITS2, and cox1 revealed the presence of two distinct species: *Dictyocaulus cervi* and *Dictyocaulus skrjabini*. These are the first genetically confirmed reports of species of *Dictyocaulus* in feral deer in Australia, and although cross-transmission of species of *Dictyocaulus* with livestock has not yet been reported, it cannot be completely discounted without further research.

1. Introduction

There are 55 species of deer found around the world; one or more of these species are native to every continent, except Antarctica, and throughout Oceania (Mennecart et al., 2017). Six cervid species were introduced to Australia in the late 1800s for recreational hunting and farming (Moriarty, 2004; Davis et al., 2016; Cripps et al., 2018; National Feral Deer Action Plan, 2023): *Dama dama* (fallow deer), *Cervus elaphus* (red deer), *Rusa timorensis* (rusa deer), *Rusa unicolor* (sambar deer), *Axis porcinus* (hog deer), and *Axis axis* (chital deer). Since the 1990s, feral deer populations increased and subsequently almost doubled their geographic range, with current population estimates of 1–2 million spread across Australia (National Feral Deer Action Plan, 2023). One, or more, of these species are now found in every State and Territory of Australia, with their habitats ranging from arid woodlands, to temperate and tropical rainforest, tropical savanna, and grasslands (Davis et al., 2016). Along with a variety of environmental and economic impacts, feral deer also carry diseases and parasites that may be transmitted to livestock (Jenkins et al., 2020; Lamb et al., 2021; Huaman et al., 2023; National Feral Deer Action Plan, 2023).

Research on the parasites of feral deer in Australia has been recently reviewed, with Huaman et al. (2023) and Shamsi et al. (2024) listing known protozoan and helminth species. Within the helminths, the liver fluke, *Fasciola hepatica*, had the widest distribution from Queensland to Victoria (McKenzie et al., 1985; Jenkins et al., 2020; Lamb et al., 2021). A variety of gastro-intestinal parasites, mostly from unpublished theses, were also grouped together (Huaman et al., 2023). Low levels of infection with lungworms, all reported as *Dictyocaulus viviparus*, have been reported in red deer in Queensland (McKenzie et al., 1985) and in fallow deer in Victoria (Presidente, 1979), Tasmania (Presidente, 1984) and New South Wales (Mylrea et al., 1991).

The genus *Dictyocaulus* (Nematoda: Dictyocaulidae) are found in the small and large airways of the host, potentially causing parasitic bronchitis (dictyocaulosis), which can be fatal, especially in cattle, sheep and farmed red deer (Cafiso et al., 2023). Prior to the advent of molecular identification, all infections of lungworms from deer were identified as *D. viviparus* (as above); however, research has shown that species within deer are separate from those in cattle (Gasser et al., 2012; Pyziel et al.,

* Corresponding author. School of Agricultural, Environmental and Veterinary Sciences, Charles Sturt University, Wagga Wagga, New South Wales, Australia.
E-mail address: dibarton@csu.edu.au (D.P. Barton).

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2023). Levels of infection with lungworms in free-ranging deer, and their potential impacts, including with respect to their interaction with livestock, remains largely unknown (Pyziel et al., 2015; Cafiso et al., 2023). The full host range and epidemiology of *Dictyocaulus* spp. in cervids world-wide still needs to be elucidated, with the potential of cross-transmission events between cervids and livestock possible (Halvarsson et al., 2022; Cafiso et al., 2023), although cross-transmission has not yet been described (Bangoura et al., 2021).

This manuscript reports on the molecular characterisation of species of *Dictyocaulus* collected from feral deer in south-eastern Australia providing the opportunity to confirm species identification.

2. Material and methods

Deer samples were collected in November 2020–February 2021 and January–April 2022 from south-eastern Australia (Fig. 1): Mudgee, Narrengullen, Kybeyan, Bega and Braidwood (southern New South Wales) and Tallangatta Valley (northern Victoria). Samples were collected following routine culling operations by licensed pest control officers from the NSW Department of Primary Industries (DPI), NSW Local Land Services (LLS), and licensed land holders. Deer were necropsied in the field, with lungs collected, bagged and frozen. At the time of examination at the Veterinary Diagnostic Laboratory (VDL) at Charles Sturt University (CSU) in Wagga Wagga, lungs were defrosted, then visually inspected and palpated prior to being processed as per the methodology of Barton et al. (2020): lung tissue was cut into smaller pieces, mashed and sieved. The contents of the sieve were backwashed into a container and the contents examined under a dissecting microscope to detect parasites. Any parasites found were preserved in 70% v/v ethanol.

Nematode specimens were aseptically sampled for molecular analysis and the anterior and posterior sections placed in lactophenol on slides for morphological analysis. Nematodes were measured with an eye-piece micrometer. Photographs were taken with an eyepiece camera (AMScope MU900). Specimens were identified to genus using the keys of Durette-Desset (1983).

DNA extraction was conducted using the DNeasy Blood & Tissue Kits (Qiagen) following the manufacturers' protocol. PCR amplification of *Dictylocaulus* 18S ribosomal RNA (18SrRNA), Internal Transcribed Spacer 2 ribosomal DNA (ITS2), and Cytochrome *c* oxidase subunit 1 (cox1) gene sequences were performed with primers as listed in Table 1 and PCR thermal cycling as published previously (Pyziel et al., 2017), with exception of the annealing temperature for primers NF50 and BNR1, which was performed at 54 °C. PCR amplicons were examined in a 1–2% w/v agarose gel, stained with GelRed™ before being photographed using transillumination.

Positive samples were Sanger sequenced at the Australian Genome Research Facility (AGRF) using the same primers used in PCR. Resulting forward and reverse chromatograms were quality checked in Sequence Scanner Software 2 (Applied Biosystems/Thermo Fisher). High quality sequences were aligned with MUSCLE (Edgar, 2004) with other *Dictylocaulus* spp. sequences retrieved from GenBank using MEGA v11 (Tamura et al., 2021). This alignment was used for maximum likelihood phylogenetic inference performed using IQ-TREE 2 (Minh et al., 2020) with model selection (Kalyaanamoorthy et al., 2017) and 1000 bootstrap approximations (Hoang et al., 2018).

3. Results

A total of 52 deer across three species (42 fallow deer, 9 sambar deer, 1 red deer) were examined; an additional deer specimen did not have a label (but was most likely a fallow deer) and was considered an

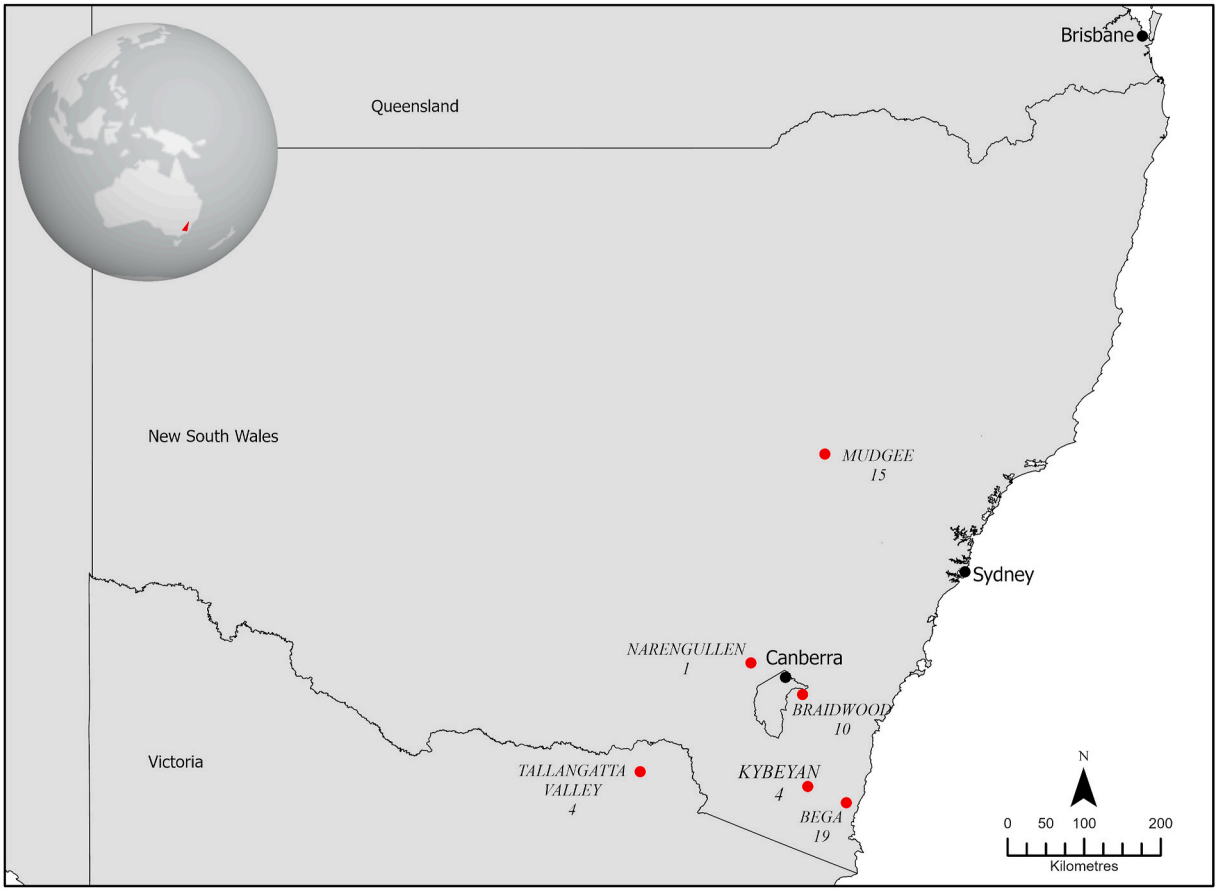

Fig. 1. Map of collection locations for feral deer examined in this study.

Table 1
Primer sequences used for the amplification of *Dictylocaulus* gene sequences.

Gene target	PCR primer pairs	Sequence (5′-3′)	Reference
cox1	JB3	TTTTTTGGGCATCCTGAGGTTTAT	Hu et al. (2002)
	JB4.5	TAAAGAAAGAACATAATGAAAATG	
	Cox1F	TGTAGATCTATTTCTTTGGARCATAT	Pyziel et al. (2017)
	Cox1R	CAGCMCCCAAACTTAAAACA	
ITS2	NC1	ACGTCTGGTTCAGGGTTGTT	Johnson et al. (2004)
	NC2	TTAGTTTCTTTTCCTCCGCT	
	ITS2F	ACGTCTGGTTCAGGGTTGTT	Pyziel et al. (2017)
	BD3R	TATGCTTAAGTTCAGCGGGT	
18S rRNA	18S1A-F	GGCGATCGAAAAGATTAAGCCATGCA	Carreno et al. (2009)
	136-R	TGATCCTTCTGCAGGTTCACCTAC	
	NF50	TGAAATGGGAACGGCTCAT	Pyziel et al. (2017)
	BNR1	ACCTACAGATACCTTGTTACGAC	

unidentified species for this study. All deer species were infected with nematodes in the lung tissues: 54.8% of fallow deer, 66.7% of sambar deer and both the red deer and the unidentified deer were infected with at least one nematode (Table 2). Due to the collection technique, complete nematodes were not always collected so the calculation of intensity of infection was not possible; however, infections were light (<10). No obvious pathology of the bronchi or lung tissue was observed in the deer at the time of dissection.

Presence of nematode infections differed between collection locations (Table 2): 100% of deer (all sambar) from Tallangatta Valley and Kybeban (3 fallow and 1 red), 84% of deer (all fallow) from Bega and 70% of deer (4 fallow, 2 sambar and 1 unidentified) from Braidwood were infected; no deer from Mudgee and Narengullen were infected.

All nematodes were identified as representatives of *Dictyocaulus* through morphological examination and their location in the respiratory system of ungulates (Durette-Desset, 1983; Gibbons and Khalil, 1988; Gibbons and Höglund, 2002; Pyziel et al., 2017, 2023). Morphologically, male nematodes had a small rounded caudal bursa with the dorsal ray divided at the base, and thick, stout spicules (Fig. 2; Table 3).

Molecular results found that two species of *Dictyocaulus* were present in fallow deer: *D. cervi* and *D. skrjabini* (Fig. 3). Of the 31 individual nematodes sequenced, 19 were determined as *D. cervi* and 12 were determined as *D. skrjabini*. Two fallow deer were found to have a mixed infection. No nematodes collected from the other deer species were successfully sequenced.

Table 2
Prevalence of infection with *Dictyocaulus* spp. in deer examined from south-eastern Australia. Data is presented as a percentage of infected deer for each location, with the total number of examined deer in brackets. NSW, New South Wales; VIC, Victoria.

State	Location	Fallow deer	Sambar deer	Red deer	Unidentified species
		Prevalence	Prevalence	Prevalence	Prevalence
NSW	Mudgee	0% (15)			
	Narengullen		0% (1)		
	Braidwood	80% (5)	50% (4)		100% (1)
	Bega	84% (19)			
	Kybeyan	100% (3)		100% (1)	
VIC	Tallangatta Valley		100% (4)		
	TOTAL	55% (42)	67% (9)	100% (1)	100% (1)

Phylogenetic reconstructions based on the 18S rRNA and the ITS showed similar patterns in the distribution of the sequences within the trees, but the cox1 phylogenetic tree formed a different pattern between the clades (Fig. 3). Sequences for *D. skrjabini* always formed a well-supported clade with the first set of sequences generated in this study. In both the 18S rRNA and ITS trees, the *D. skrjabini* clade was at the base of the tree; in the cox1 tree, the *D. skrjabini* clade was a sister clade to *D. viviparus*, rooted by *D. capreolus*.

The remaining sequences from this study grouped in a clade of *D. cervi/D. eckerti* sequences, across the ITS and 18S phylogenetic trees (Fig. 3). The results for the cox1 tree were different, with the sequences from this study grouping with sequences of *D. eckerti* and sequences determined as *D. cervi* falling into a separate clade.

4. Discussion

This is the first report of species of *Dictyocaulus* – *D. cervi* and *D. skrjabini* – infecting feral deer in Australia confirmed through molecular sequences. Identification to genus is relatively easy, however morphological differentiation of species within the genus is problematic (Gibbons and Khalil, 1988) with significant skill required for an exact identification (Cafiso et al., 2023), such as measurement of the thickness and length of the buccal capsule wall (Divina et al., 2000).

Of the nine species recognised within the genus, four are reported to infect deer: *D. eckerti*, *D. capreolus*, *D. cervi* and *D. skrjabini*. *Dictyocaulus eckerti* was originally named *D. noerneri* but this name was determined to be invalid and subsequently changed to *D. eckerti* by Skrjabin in 1931 (in Gibbons and Khalil, 1988). The remaining species in the genus have been reported in cattle (*D. viviparus*), sheep and goats (*D. filaria*), donkeys and horses (*D. arnfeldi*), camels (*D. cameli*) and African artiodactylids (*D. africanus*). There are also subspecies, or possible new species, of *D. viviparus* within both European (Pyziel et al., 2020) and North American (Danks et al., 2022) bison.

Dictyocaulus cervi was first described from red deer collected in Poland by Pyziel et al. (2017) and subsequently reported in red deer in Hungary (Ács et al., 2016) and the Italian Alps (Cafiso et al., 2023), moose (*Alces alces*) in Poland (Filip-Hutsch et al., 2020), and rocky mountain elk (*Cervus canadensis nelsoni*) in the USA (Bangoura et al., 2021). This study now reports *D. cervi* in a new host species, fallow deer, and in a new geographical location, Australia. The results of this study also outlines that previous reports of *D. eckerti* from red deer in New Zealand (Johnson et al., 2004; Gasser et al., 2012) and other hosts in Europe (Epe et al., 1997; Höglund et al., 1999) are potentially also *D. cervi*. Levels of genetic variation, however, remains high, although not apparently related to host species or geographical location. Thus, more research into the molecular characterisation of *Dictyocaulus* species within deer is required in case further species remain to be discovered. Additionally, specimens morphologically identified as *D. eckerti* require sequencing to confirm levels of genetic variability between these species.

Dictyocaulus skrjabini was described from specimens collected from red deer in Poland by Pyziel et al. (2023). It was first determined as a distinct species by molecular sequences which matched with molecular sequences generated from a *Dictyocaulus* sp. that had been previously collected from fallow and red deer in Sweden by Höglund et al. (2003). Cafiso et al. (2023) also reported *Dictyocaulus* sp. in red deer in the Italian Alps which matched the sequences for *D. skrjabini*. This study now reports *D. skrjabini* in fallow deer in Australia for the first time.

Genetic results have determined a clear separation between the *Dictyocaulus* species that infect deer (Gibbons and Höglund, 2002; Pyziel et al., 2017, 2023; Cafiso et al., 2023). Within the *D. skrjabini* clade (Fig. 3), sequences labelled as *Dictyocaulus* sp. (OP628627-OP628632; OP617690-OP617692), collected from red deer in the Italian Alps (Cafiso et al., 2023), were not included in the original trees produced during the description of *D. skrjabini* by Pyziel et al. (2023). However,

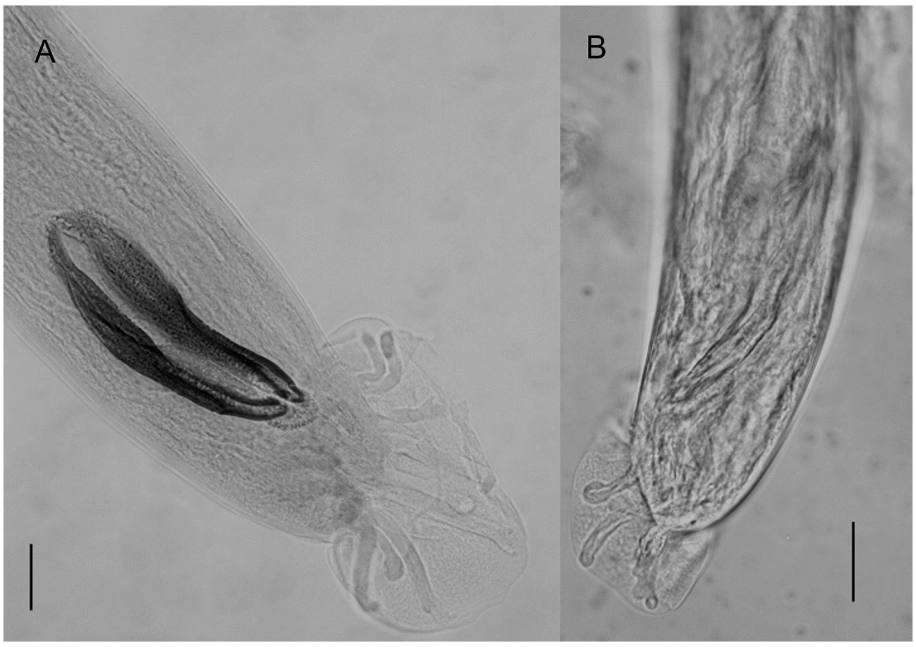

Fig. 2. A. Posterior end of a male specimen of *Dictyocaulus cervi* collected from a fallow deer from Bega (NSW), ventral view. B. Posterior end of a male specimen of *Dictyocaulus skjrabini* collected from a fallow deer from Bega (NSW), lateral view. Scale bar: 50 μm.

Table 3
Measurements of male specimens of species of *Dictyocaulus* collected in this study in comparison to measurements of *D. cervi* and *D. skrjabini* from the literature.

	Dictyocaulus cervi	D. cervi	Dictyocaulus skrjabini	Dictyocaulus skrjabini
Host	*Dama dama*	*Cervus elaphus*	*Dama dama*	*Cervus elaphus, Dama dama*
Geographical location	Australia	Poland	Australia	Poland
Reference	This study	Pyziel et al. (2017)	This study	Pyziel et al. (2023)
Body length	14169 (12375-17500)[a]	25600-56700	5808 (5175–6950)[a]	8200–46600
Body width	155.4 (87.5–200)		75 (–)	–
Head width	132.5 (90–150)	77.9–113.6	63.3 (55–70)	91–144
Cephalic vesicle length	161-227 (135–400)	110.5–232.6	92.5–105 (60–150)	126–207
Nerve ring to anterior end		297.9–423.3		348–491
Excretory pore to anterior end	385 (365–405)	326.5–544.1	280 (–)	–
Oesophagous length	836.3 (680–990)	824.5–1309.7	483.3 (475–495)	901–1449
Spicule length	209.1 (180–225)	208.9–302.9	135.7 (125–152.5)	195–306
Gubernaculum length	45.7 (32.5–57.5)	43.1–73.4	39.2 (35–47.5)	46–71

[a] Total body length estimated for specimens that had been cut for molecular analysis.

their consistent placement within the *D. skrjabini* clade across the three phylogenetic trees would suggest that these Italian sequences belong to *D. skrjabini*.

The majority of sequences available in GenBank designated as *D. eckerti* had been collected prior to the description of *D. cervi* in 2017 and were designated as *D. eckerti* based on morphology alone, if examined at all. For example, the specimens collected from fallow deer in Germany by Epe et al. (1997) (U37716) were identified as *D. eckerti* as they "showed the morphological characteristics described for *D. eckerti*" (p17), despite high levels of sequence variation between samples. Höglund et al. (1999, 2003) determined the identification of sequences of nematodes collected from moose (AY168857), musk ox (AY168863), red deer (AY168858) and reindeer (AY168864) to be the same as the sequences generated by Epe et al. (1997), that is, *D. eckerti*. Subsequently, Johnson et al. (2004) (AJ580764-AJ 580766) and Gasser et al. (2012) (NC019809), who used the same nematodes originally collected from red deer in New Zealand, found similar levels of sequence variation to Epe et al. (1997) but labelled the sequences as a unique genotype (Johnson et al., 2004) eventually called *Dictyocaulus* cf. *eckerti* (Gasser et al., 2012); Gasser et al. (2012) noted that the sequences were genetically distinct from the recognised *D. eckerti* and suggested that this could be a different species rather than just a population variant. Subsequently, both Ács et al. (2016) (KT438070) and Cafiso et al. (2023) (OP628626, OP617687, OP617688) determined the identification of their sequences for nematodes collected from Hungary and Italy, respectively, through comparison against the New Zealand "*D. eckerti*". Interestingly, Cafiso et al. (2023) had noted that despite the fact all nematodes collected from red deer in the Italian Alps morphologically identified as *D. cervi*, molecular results found that 3 of the worms (the VdA subgroup) grouped with the New Zealand *D. eckerti* sequences. Thus, based on the results in the 18S and ITS trees (Fig. 3), given that all of these sequences have grouped with morphologically and genetically identified *D. cervi* (KM374673-KM374678), they all should now be identified as *D. cervi*. The results presented in the cox1 tree are more problematic, with *D. cervi* and *D. eckerti* apparently forming distinct clades, although with less than the nominal 10% threshold required for species differentiation (Cafiso et al., 2023). A lack of morphologically verified *D. eckerti* specimens with corresponding sequences, however, prevents a clear cut answer on the validity of the identification of sequences as *D. eckerti*.

Morphological measurements of the male nematodes collected in this study did not match the descriptions of either *D. cervi* or *D. skrjabini*. This

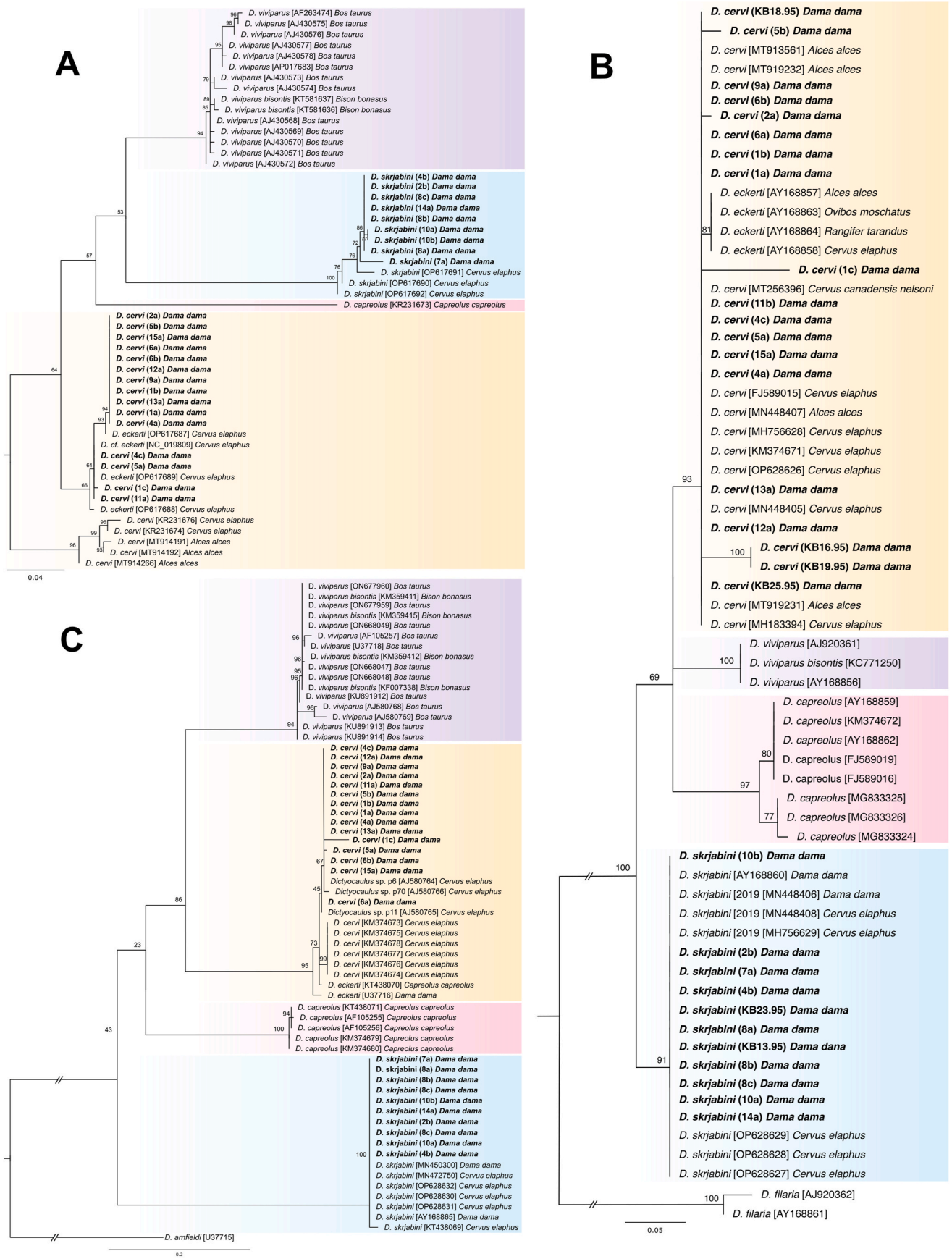

Fig. 3. Maximum likelihood phylogenetic trees of cox1 (A), 18S rRNA (B), and ITS2 (C) *Dictyocaulus* sequences. Sequences from this study are indicated in bold. Square brackets indicate GenBank accessions. Coloured boxes indicate putative *Dictyocaulus* species groups.

could be due to several reasons including the reproductive maturity of the parasite as well as the collection method causing damage to specimens, making measurements difficult. In the description of *D. skrjabini*, Pyziel et al. (2023) describes the copulatory spicules as dark brown (ie, heavily chitinised) and 195–306 μm long. In the specimens of *D. skrjabini* collected in this study, the spicules were not heavily chitinised and were generally under 150 μm in length, suggesting reproductive immaturity. However, the measurements presented by Pyziel et al. (2023) were for the male "holotype" but consisted of a range of measurements, not an individual measurement, and to complicate the situation, *D. skrjabini* was described from two different host species, fallow and red deer, which may have an influence on overall measurements due to different host sizes. Measurements for spicule length for *D. cervi* collected in this study matched more closely with measurements from *D. cervi* collected from red deer in Poland by Pyziel et al. (2017), although overall body length for the Australian specimens was much shorter. However, the Australian specimens were collected from fallow deer, which is significantly smaller than red deer, so there may also be a host-induced effect on overall body size for this species. These issues highlight the problems of relying solely on morphological characteristics for identification for species of *Dictyocaulus* (Bangoura et al., 2021; Cafiso et al., 2023).

Infection levels reported by Pyziel et al. (2023) found 6.1–9.6% of sampled deer infected with *D. skrjabini*, with intensities up to 98 worms, although the mean intensities were below 20 worms per infected host. Infections with *D. cervi*, in the same study had infections ranging from 21.7 to 57.9% across different locations, with intensities up to 78 worms, although infections were much lower, with 1–6 worms, in Pyziel et al. (2017). Prevalence of infection with nematodes in this study were variable, although with the opportunistic sampling methodology, combined with lack of molecular identification for nematodes from other deer species, it is difficult to draw any conclusions regarding infection dynamics. All infections, however, were light, with less than 10 nematodes per host. No mixed infections for *D. cervi* and *D. skrjabini* have previously been reported (Pyziel et al., 2023); two deer were found to have a mixed infection in this study.

As the species found in the fallow deer in this study are known to infect a wide range of ungulates around the world, it is likely that they are found in many of the deer species in Australia. It is unknown if infections with either of the species of *Dictyocaulus* are impacting the health of deer, but *Dictyocaulus* spp. are known to cause significant pathology, and *D. cervi* has been found to be the cause of death in an elk (Bangoura et al., 2021) and repiratory illness, due to lungowrm infection, in a farmed sambar deer in New Zealand (Semiadi et al., 1994). Deer are still farmed across Australia and the potential for feral deer to transmit parasites to domestic deer needs to be considered. Although cross-species transmission of *Dictyocaulus* between deer and other hosts has not yet been proven, it should not be discounted as a possibility (Semiadi et al., 1994; Bangoura et al., 2021; Halvarsson et al., 2022; Cafiso et al., 2023). Further research needs to be undertaken on the epidemiology of *Dictyocaulus* spp. infections in deer, both feral and farmed, across Australia to determine the full host and geographical distribution of the parasite species.

Ethics declaration

This study was approved by the Charles Sturt University Animal Care and Ethics Committee Approval number A22402.

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Conflict of interest

The authors declare no conflicts of interest.

CRediT authorship contribution statement

Keira Brown: Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **David J. Jenkins:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Conceptualization. **Alexander W. Gofton:** Writing – review & editing, Resources, Methodology, Formal analysis, Data curation. **Ina Smith:** Writing – review & editing, Resources, Methodology. **Nidhish Francis:** Writing – review & editing, Supervision, Resources, Methodology, Formal analysis, Data curation. **Shokoofeh Shamsi:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **Diane P. Barton:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Conceptualization.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors did not utilise any generative AI or AI-assisted technologies in the writing process of this manuscript.

Declaration of competing interest

The authors have no affiliation with any organisation with a direct or indirect financial interest in the subject matter discussed in the manuscript.

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