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A vascular filarial nematode in sika deer (*Cervus nippon*): Morphological and molecular characterization of *Elaeophora* (Nematoda: Onchocercidae) in Japan

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ABSTRACT

Elaeophora (Nematoda: Onchocercidae), a filarial nematode infecting the blood vessels of ruminants and horses, is transmitted by tabanid flies. *Elaeophora elaphi* was previously detected in wild sika deer in Wakayama Prefecture, Japan in 2009; however, detailed information on this species is scarce. In 2023, 26 *Elaeophora* worms were collected from the hepatic vasculature of eight deer in Nara, Mie, Kyoto and Gifu Prefectures of Japan and analyzed. Species identification was performed by morphological and genetic analyses. Additionally, multi-gene analysis of seven genes was performed to determine their taxonomic position within the family Onchocercidae. The specimens were identified as *E. elaphi* based on their morphological characteristics. Analyses of 18S rRNA and cytochrome *c* oxidase subunit 1 genes revealed no variations, indicating that species belonged to the same lineage. Multi-gene analysis revealed that the species belonged to the subfamily Onchocercinae, showing a close relationship with the tick-borne filarial nematodes of the genera *Monanema*, *Acanthocheilonema*, *Litomosoides*, *Cruorifilaria*, *Yatesia*, and *Cercopithifilaria*. This study demonstrated the widespread distribution of *E. elaphi* in Japan and provided insights into its genetic relationship with other onchocercid species. Further research is necessary to determine the ecological and epidemiological implications of this parasite.

1. Introduction

Sika deer (*Cervus nippon*) are native to and are widely distributed throughout Japan, particularly in forests and mountainous regions. Recently, their population has rapidly increased, particularly in rural and suburban areas, owing to the lack of natural predators, changes in land use, and a decline in hunting (Nagata, 2015). Their overpopulation causes various ecological problems, such as forest damage due to overgrazing and crop destruction. Despite extensive efforts, including culling and fencing, balanced conservation and management of this species remains a challenge. The growing deer population also increases the risk of parasitic diseases (Sato et al., 2021; Maruko et al., 2021; Inoue et al., 2022), which could affect both deer health and other species, including livestock and wild ruminants.

Family Onchocercidae Leiper, 1911 consists of a group of filarial nematodes with 91 genera and 737 species (Hodda, 2022). Of these,

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genus *Elaeophora* Railliet and Henry, 1912 is classified under subfamily Onchocercinae Leiper, 1911 and the tribe Dipetalonematini Wehr, 1935 (Wehr, 1935; Hodda, 2022). Members of Onchocercinae exhibit unstable taxonomic characteristics, with the esophagus being externally divided into two parts in some species, caudal alae generally being absent, and tail length varying among species (Wehr, 1935; Chabaud and Bain, 1976). Recently, phylogenetic relationships of filarial nematodes have been investigated via multi-gene analyses (Lefoulon et al., 2015; Bruley and Duron, 2024; Kulpa et al., 2025). However, genetic information of *Elaeophora* remain scarce, and its phylogenetic position remains unclear.

Genus *Elaeophora* comprises nematode species that parasitize various ungulate hosts, including sheep, deer, cattle, and horses. *Elaeophora* species inhabit the arterial and venous systems, often causing pathological changes in the host. To date, seven *Elaeophora* species have been described: *E. poeli*, *E. sagitta*, *E. schneideri*, *E. abramovi*, *E. bohmi*, *E. linglingense*, and *E. elaphi* (Linstow, 1907; Railliet and Henry, 1912; Wehr and Dikmans, 1935; Oshmarin and Belous, 1951; Supperer, 1953; Cheng, 1982; Hernández Rodríguez et al., 1986). Members of the family Cervidae are the definitive host of *E. schneideri* and *E. elaphi*. In the USA, *E. schneideri* has been recorded in mule deer (Cervidae; *Odocoileus hemionus*) and black-tailed deer (Cervidae; *Odocoileus hemionus columbianus*), which serve as its natural definitive hosts (Wehr, 1935; Hibler and Adcock, 1968; Weinmann et al., 1973). Some atypical hosts, including moose (Cervidae; *Alces alces*), white-tailed deer (Cervidae; *Odocoileus virginianus*), elk (Cervidae; *Cervus canadensis*), sika deer, red deer (Cervidae; *Cervus elaphus*), Barbary sheep (Bovidae; *Ammotragus lervia*), Malayan sambar (Cervidae; *Rusa unicolor*), and domestic sheep and goats, have also been reported (Hibler and Adcock, 1968; Robinson et al., 1978; Pence and Gray, 1981; Waid and Warren, 1984; Madden et al., 1991; LeVan et al., 2013; Bernard et al., 2016). Although primarily detected in the carotid artery, this species also infects other vessels, including the brachiocephalic trunk, leptomeningeal vessels, and internal maxillary, pulmonary, and femoral arteries. Infection is subclinical in natural hosts; however, arterial endothelial damage, inflammation, and encephalitis are observed in atypical hosts (Worley et al., 1972; Adcock and Hibler, 1969; Haake et al., 2024). In addition, granulomatous inflammation caused by dead parasites may lead vascular irregularities (Robinson et al., 1978; Hibler and Metzger, 1974). Tabanid flies of the genera *Chrysops*, *Hybomitra*, and *Tabanus* are its intermediate hosts (Hibler and Metzger, 1974; Grunenwald et al., 2018); however, vectors for the other *Elaeophora* species remain unknown. *Elaeophora elaphi* has been reported in red deer in Spain (Hernández Rodríguez et al., 1986; Carrasco et al., 1995; Santin-Durán et al., 2000). Moreover, two *E. elaphi*-infected wild sika deer were reported in Wakayama Prefecture, Japan in 2009 (Omar et al., 2010). The latter identification was based on the morphological characteristics of the specimens obtained from sika deer but has only been recorded in conference abstracts, with no details available. Furthermore, lack of detailed information, including molecular biology data, limits the comprehensive understanding of this species and its potential impacts on wild sika deer populations.

In this study, we detected *Elaeophora* worms in the hepatic vasculature of wild sika deer in Japan. Furthermore, we performed morphological and genetic analyses to determine the species characteristics and clarify their taxonomic identity and phylogenetic relationships to other *Elaeophora* species.

2. Methods

2.1. Specimens

Filarial nematodes were collected from eight sika deer (Cn01–08) between January and May 2023. Of these, six deer were captured via hunting or as part of a pest control program by licensed hunters in Mie ($n = 4$), Gifu ($n = 1$), and Kyoto ($n = 1$) Prefectures (Fig. 1), and the infecting nematodes were collected during meat processing. Other nematodes were collected from two dead deer from Nara Prefecture during necropsy. The recovered nematodes were washed by tap water, preserved in 70% ethanol, and transported to Nippon Veterinary and Life Science University for analysis.

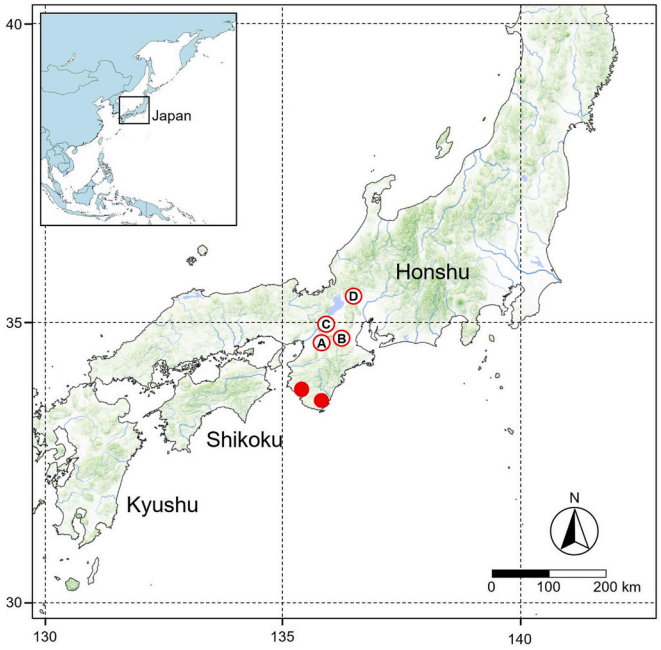

Fig. 1. Geographical locations of *Elaeophora*-infected sika deer in Japan. Red-outlined circles indicate the locations where sika deer were captured in the current study (**A**: Nara, $n = 2$; **B**: Mie, $n = 3$; **C**: Kyoto, $n = 1$; **D**: Gifu, $n = 1$), while red circles indicate the locations reported by Omar et al. (2010).

2.2. Morphological analysis

Worms were mounted in glycerol and observed under the BX53 light microscope (Olympus, Japan) and SZX16 stereomicroscope (Olympus). Photomicrographs were taken with DP27 (Olympus), and images were synthesized via depth synthetic processing using CombineZP software (https://combinezp.software.informer.com/). Measurements were made using the CellSens software (Olympus). Genus and species identification was performed using taxonomic keys, as previously described (Hernández Rodríguez et al., 1986; Hibler and Adcock, 1968; Anderson and Bain, 2009).

2.3. Sequence analysis

For DNA extraction, approximately 4 mm of the midbody of each nematode was dissected under a stereomicroscope using a sterile needle. Genomic DNA was extracted from 19 nematodes (1–5 samples/deer) using the Qiagen DNeasy Blood & Tissue Kit (Qiagen, Germany) according to the manufacturer's standard protocol.

For genetic analysis, partial sequences of the 5′ end of the 18S rRNA gene (*18S*_a) (Blaxter et al., 1998) were examined in all individuals and compared with the *Elaeophora* sequences in the International Nucleotide Sequence Database Collaboration (INSDC). Additionally, partial sequences of the mitochondrial cytochrome *c* oxidase subunit 1 gene (*cox1*) (Casiraghi et al., 2001) were analyzed to determine the intraspecific variations in the identified species. Phylogenetic analysis of two *Elaeophora* worms from two deer (Cn05 and Cn07) was performed using a multi-gene analysis with seven different genes (Lefoulon et al., 2015; Casiraghi et al., 2004): five nuclear genes, 18S (*18S*_b: a different region from the sequence initially mentioned), 28S rRNA (*28S*), myosin heavy chain (*myohc*), RNA polymerase II large subunit (*rbp1*), and 70-kDa heat

shock proteins (*hsp70*), and two mitochondrial genes, 12S rDNA (*12S*) and *cox1*. DNA extract from *Dirofilaria ursi* (Nematoda: Onchocercidae) collected from a wild Japanese black bear (*Ursus thibetanus japonicus*) in Nagano Prefecture, Japan, in December 2021 was used as a control. Subsequently, polymerase chain reaction (PCR) was performed using the TaKaRa Ex Taq polymerase (TaKaRa Bio, Japan) in a 20 μL reaction volume containing 2 μL of 10 × buffer, 1.6 μL of dNTP mix (2.5 mM each), 0.2 μL of Taq polymerase, 0.2 μL of each primer (50 μM), 1 μL of extracted DNA, and 14.9 μL of distilled water. All PCR conditions and primer sequences are presented in Supplementary Table S1. The PCR products were mixed with Midori Green Direct (Nippon Genetics, Japan) and electrophoresed on a 1.5% agarose gel at 100 V. An LED trans-illuminator was used for visualization. PCR products of the expected size were submitted to Macrogen (Japan) for direct sequencing with the same primers used in PCR. The resulting nucleotide sequences were deposited into the GenBank.

The determined *18S*_a and *cox1* sequences of *Elaeophora* were each aligned using the ClustalW (Thompson et al., 1994) implemented in MEGA 12 software (Kumar et al., 2024) and checked for variation. Then, sequence similarity analysis was performed using the Basic Local Alignment Search Tool of the National Center for Biotechnology Information. For phylogenetic analysis of *18S*_a, sequences of *Elaeophora* spp., and those of related species and *Setaria tundra* (Onchocercidae: Setariinae) as the outgroup were aligned using MAFFT (Katoh et al., 2019) with the Q-INS-i strategy setting. For multi-gene analysis, *18S*_b, *28S*, *myohc*, *rbp1*, *hsp70, 12S*, and *cox1* sequences of filarial nematodes reported by Lefoulon et al. (2015) and Kulpa et al. (2025) obtained from INSDC (Supplementary Table S2), with *Protospirura muricola* (Spiruridae) and *Filaria latala* (Filariidae) were selected as outgroups, and the sequences obtained in this study were aligned separately using MAFFT. Then, gene regions were concatenated using the SequenceMatrix v.1.9 (Vaidya et al., 2011), and an incongruence test (Farris et al., 1994) was performed using PAUP* v.4 (Swofford, 2002) to assess the homogeneity between partitions, with no significant incongruence observed ($P > 0.05$). Using the AIC model in the IQ-TREE web version (Trifinopoulos et al., 2016), Kimura 2-parameter (Kimura, 1980) with invariant sites plus gamma-distributed model was identified as the best-fit evolutionary model for *18S*_a, whereas the General Time-Reversible model (Tavaré, 1986) plus gamma distributed model was found to be the best fit for the concatenated alignment data (*18S*_b, *28S*, *myohc*, *rbp1*, *hsp70, 12S*, and *cox1*). Subsequently, phylogenetic trees were constructed using the maximum likelihood method in IQ-TREE, and their reliability was assessed using bootstrap values based on 1500 replicates or SH–aLRT on 1000 replicates. The trees were edited using iTOL v6 (Letunic and Bork, 2024).

3. Results

3.1. Morphological identification

Long, slender, and white worms were observed in the hepatic blood vessels of eight sika deer (Fig. 2, Video). Total number of worms was 26, with a range of 1–6 (Table 1). Of the 26 nematodes recovered, six were male and 18 were female, and the sex of two specimens could not be determined due to body damage. The worms had a small oral opening (Fig. 3A and B) and four pairs of submedian papillae (Fig. 3B) on their cephalic extremities, two dorsal and two ventral, with one pair in each quadrant. The esophagus was divided into muscular and glandular parts. Tails of both sexes did not show protuberances (Fig. 3C and E). In males, three pairs of precloacal papillae (Fig. 3D), two pairs plus one unpaired postcloacal papillae, and transverse cuticular swelling with perpendicular striations (Fig. 3E) were observed. Asymmetrical spicules were also observed (Fig. 3E). Measurements of males and females were similar to those of *E. elaphi* in the original description (Table 2). The worms collected from sika deer were identified as *E. elaphi* (Onchocercidae: Onchocercinae: Dipetalonematini) based on their host, the parasitic site, and morphological characteristics.

Table 1
Collection sites, number of specimens, and genetic analysis of filarial worms detected in wild sika deer from the Kansai region, Japan.

Host ID	Localities	Worm burdens (male/female/unknown)	Number of analyzed specimens for genetic analysis (*18S*_a/*cox1*)
Cn01	Nara, Nara	1 (1/0/0)	1 (0/0)
Cn02	Nara, Nara	1 (1/0/0)	1 (1/1)
Cn03	Iga, Mie	5 (0/4/1)	5 (5/5)
Cn04	Iga, Mie	3 (1/2/0)	3 (3/3)
Cn05	Iga, Mie	3 (1/2/0)	3 (3/3)
Cn06	Iga, Mie	1 (0/1/0)	1 (1/1)
Cn07	Uji, Kyoto	6 (1/5/0)	3 (3/3)
Cn08	Gifu	6 (1/4/1)	3 (3/3)
	Total	26 (6/18/2)	20 (19/19)

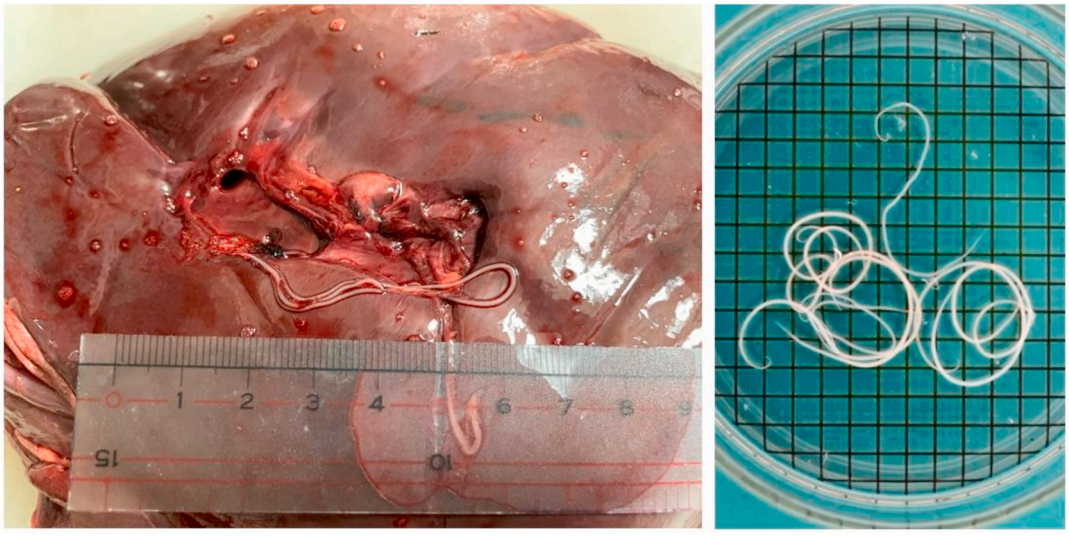

Fig. 2. Macroscopic image of *Elaeophora* parasitizing the liver of a sika deer.

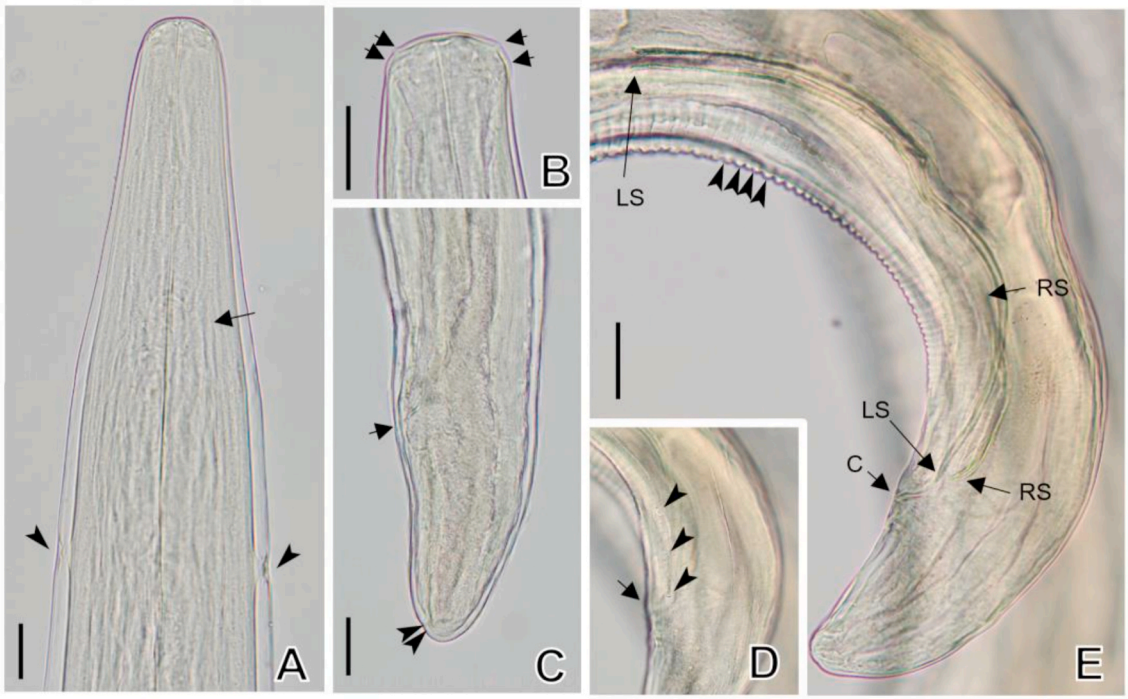

Fig. 3. Light microscopic images of *Elaeophora* collected from the sika deer. **A**: Anterior extremity, female. Nerve ring (arrow) and constriction of the cuticle (arrowheads). **B:** Anterior extremity, female, showing the very small oral opening and cervical papillae (arrows). **C**: Posterior extremity, female. Anus (arrow) and a pair of papillae (arrowhead) that do not protruding from the cuticle. **D**: Lateral view of posterior portion of male, showing three pairs of precloacal papillae (arrowheads) behind the cloaca (arrow). **E**: The posterior extremely, male, showing transverse cuticular swellings with perpendicular striations (arrowheads) on the ventral surface, and left (LS) and right (RS) spicules. Bars = 50 μm.

Table 2
Measurements of *Elaeophora elaphi*. Values (mm) are presented as the minimum–maximum range, with the mean in parentheses.

	Cervus nippon		*Cervus elaphus* (type host)	
	Male (n = 3)	Female (n = 5)	Male (n = 1)	Female (n = 5)
Body L	70–78 (75)	95–101 (99.5)	77	91-109 (102.4)
Body W	0.60–0.65 (0.63)	0.69–0.91 (0.85)	0.55	0.79–1.05 (0.91)
Esophagus L	2.12–2.23 (2.18)	2.20–2.40 (2.30)	2.17	1.93–2.82 (2.21)
Muscular esophagus L	0.56–0.60 (0.58)	0.41–0.46 (0.44)	0.56	0.54–0.64 (0.57)
Distance vulva/ anterior end	–	1.76–2.10 (1.82)	–	1.61–2.01 (1.77)
Tail L	–	0.17–0.20 (1.85)	–	0.15–0.21 (1.77)
Left spicule	0.36–0.38 (0.37)	–	0.36	–
Right spicule	0.13–0.16 (0.14)	–	0.15	–
Reference	Present study		Hernández Rodríguez et al. (1986)	

L: length, W: width.

3.2. Sequence analysis

Partial nucleotide sequences of the *18S*_a and *cox1* genes were successfully obtained from 19 specimens, excluding that from Nara Prefecture (Cn01) (Table 2). The obtained *18S*_a sequences (839-bp) were identical. Sequence similarity search revealed the highest similarity (99.5–100%) with *E. schneideri* (**KT878974** and **KT885226**). A completely identical sequence has been reported in white-tailed deer in Georgia, USA (KT878974). Other sequences showing high similarity included those of *Dipetalonema* spp. (**MZ727043**, **MW192232**, and **MW192233**), with 99.6% identity and *Loa loa* (**DQ094173**) and *Onchocerca cervipedis* (**KT031393**) each with 99.4% identity. However, *18S*_a sequences of other species in genus *Elaeophora*, including those of *E. elaphi,* were unavailable in INSDC. In the phylogenetic tree constructed using the partial *18S*_a sequence (Fig. 4), although highly reliable topologies were not obtained, *E. schneideri* formed a monophyletic group with the isolates from moose, sambar deer, and tabanid fly (*Chrysops* sp.) in the USA, whereas *E. elaphi* detected in this study formed a monophyletic group with the *E. schneideri* from white-tailed deer (**KT878974**). Notably, *cox1* sequences (570-bp) were completely identical. The *cox1* sequences of *Elaeophora* species were unavailable in INSDC.

In the phylogenetic tree constructed using the concatenated dataset (Fig. 5), filarial nematodes of Onchocercidae formed a monophyletic group, well-separated from *Protospirura* (Spiruridae) and *Filaria* (Filariidae). In the clade of Onchocercidae, subfamily Oswaldofilariinae (*Oswaldofilaria*, *Icosiella*, and *Ochoterenella*) diverged first, followed by Setariinae (*Setaria*), whereas Onchocercinae and Splendidofilariinae formed a sister group. In this group, the tree consisted of four clades (A–D), with clade C + D exhibiting a low bootstrap value (67%). Clade A consisted of *Dirofilaria*, *Loxodontofilaria*, and *Onchocerca*, which belonged to Onchocercinae. Clade B comprised *Monanema*, *Acanthocheilonema*, *Litomosoides*, *Cruorifilaria*, *Yatesia*, and *Cercopithifilaria*, which belonged to Onchocercinae. Clade C included *Dipetalonema* in Onchocercinae. Clade D consisted of *Aproctella*, *Breinlia*, *Brugia*, *Foleyella*, *Loa*, *Madathamugadia*, *Mansonella*, and *Pelecitus* in Splendidofilariinae, and *Rumenfilaria* in Onchocercinae. *Elaeophora* was positioned within Clade B and diverged early within this clade. *Dirofilaria ursi* formed a well-supported monophyletic group with other *Dirofilaria* spp.

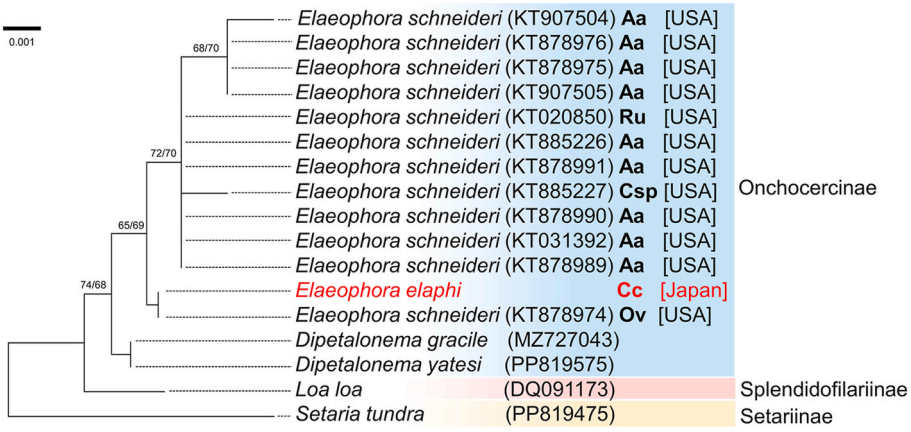

Fig. 4. Phylogenetic tree of filarial nematodes using the partial sequence of *18S* (*18S*_a) sequence. The species name followed by the INSDC accession number, host, and collection site. Nodes are labeled with bootstrap values (left) and SH–aLRT supports (right). Scale bars represent substitutions per site. **Aa** = *Alces alces,* moose (Cevidae: Capreolinae: Alceini); **Cc** = *Cervus nippon,* sika deer (Cevidae: Cervinae: Cervini); **Csp** = *Chrysops* sp. (Diptera: Tabanidae); **Ov** = *Odocoileus virginianus*, white-tailed deer (Cevidae: Capreolinae: Rangiferini); **Ru** = *Rusa unicolor*, sambar (Cevidae: Cervininae: Cervini).

4. Discussion

In the present study, *E. elaphi* was collected from wild sika deer and identified morphologically. Nobably, *18S*_a and *cox1* sequences of 19 worms from seven deer in four prefectures in the central-western part of Honshu, the main island of Japan, were identical to each other, indicating that they belonged to the same lineage of *E. elaphi*. Although the *18S*_a sequences completely matched the *E. schneideri* sequence from white-tailed deer in USA (Grunenwald et al., 2018), genetic diversity was observed among the *18S*_a sequences of *E. schneideri*, and *E. elaphi* from sika deer and *E. schneideri* from white-tailed deer were located in a different clade from *E. schneideri* from moose. This indicates the difficulty of distinguishing between *E. schneideri* and *E. elaphi* based on the *18S*_a region or possibility of misidentification of *Elaeophora* species collected from white-tailed deer. As the adult stages of *E. schneideri* and *E. elaphi* are distinguishable by the presence or absence of caudal protuberances (Hernández Rodríguez et al., 1986; Hibler and Adcock, 1968), worms collected from white-tailed deer must be carefully identified based on their morphological characteristics. Future studies should compare the *E. elaphi* isolates from sika deer in Japan and red deer, the type host for this parasite, in Spain for more insights.

Although previously detected in two wild sika deer in Wakayama Prefecture in 2009 (Omar et al., 2010), *E. elaphi* has not been identified in any other region. In this study, *E. elaphi* was identified for the first time in Mie, Kyoto, Nara, and Gifu Prefectures in the central-western part of Honshu, suggesting that *E. elaphi* may be widely distributed in these areas. The lack of intraspecific variation in *cox1* analysis suggests that this *E. elaphi* population experienced a bottleneck effect or founder event, a pattern often observed in invasive parasites (Sromek et al., 2023; Tokiwa et al., 2012). Höfle et al. (2004) documented *E. elaphi* infection in a red deer imported from Germany to Spain and noted the unintentional colonization associated with the transportation of the animal. Similarly, in Japan, this parasite may have been introduced to Japan along with imported deer in the past and subsequently established a life cycle involving the wild sika deer and native flies. Although the exact period of introduction remains unclear, imports of even-toed ungulates from Europe has not occurred in Japan for over 20 years (Animal Quarantine Service, 2022), suggesting that the introduction may have occurred prior to that period.

Wild deer of the genus *Cervus* are distributed throughout Japan, except some islands (Nagata, 2015). These reports highlight the importance of the continuous investigation of the infection status of *E. elaphi* in Japan. To date, no infection vectors have been reported from Asia, warranting further investigations, including elucidation of their life cycle. Genus *Elaeophora* is classified into seven species, and ruminants and horse are definitive hosts. *Elaeophora elaphi* has been detected in both deer and domestic sheep (Hernández Rodríguez et al., 1986). Therefore, future studies should evaluate its potential to infect domestic ruminants, horses, and the Japanese serow (*Capricornis crispus*), a nationally designated natural treasure.

Multi-gene analysis revealed phylogenetic relationships similar to those reported in previous studies. Onchocercidae was broadly divided into six major clades. Clade B containing *Elaeophora* was clearly distinguishable from clade C consisting *Dipetalonema* spp. Lefoulon et al. (2015) and Bruley and Duron (2024) showed that clades C and B are monophyletic and collectively referred to these groups as ONC4, whereas in our phylogenetic tree, clade C and B exhibited paraphyletic relationships, similar to the topology shown by Mirzaei et al. (2018). Definitive hosts of clade B members were diverse, with *Litomosoides* infecting bats and rodents, *Monanema* infecting rodents, *Achanthocheilonema* infecting insectivores, carnivores, pinnipeds, rodents, and *Cercopithifilaria* infecting carnivores and ruminants (Lefoulon et al., 2015). Their intermediate hosts include ticks (*Achanthocheilonema*, *Cercopithifilaria*, *Cruorifilaria*, *Monanema*, and *Yatesia*) (Ajileye et al., 2025) and mites (*Litomosoides*) (Espinal-Palomino et al., 2025). Many other members of Onchocercidae, including *Dipetalonema*, are transmitted by blood-sucking dipterans, suggesting that the use of tabanid flies as intermediate hosts by *Elaeophora* is a conserved ancestral trait in this group.

5. Conclusion

This study collected *E. elaphi* from wild sika deer in Japan. Multigene analysis revealed that *E. elaphi* is closely related to the tick-borne filarial species of Onchocercidae. Future studies should investigate sika deer infection rate in the same region and determine *E. elaphi* infectivity in other ruminants and its potential vectors.

CRediT authorship contribution statement

Toshihiro Tokiwa: Writing – review & editing, Writing – original draft, Visualization, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Keita Sakashita:** Investigation, Formal analysis. **Saki Miura:** Investigation, Formal analysis. **Hisashi Yoshimura:** Resources, Investigation. **Shiro Matsuo:** Resources, Investigation. **Toshiaki Yamamoto:** Resources, Investigation. **Rie Maruko:** Resources, Investigation, Conceptualization. **Junji Moribe:** Visualization, Resources, Investigation. **Yasuhiro**

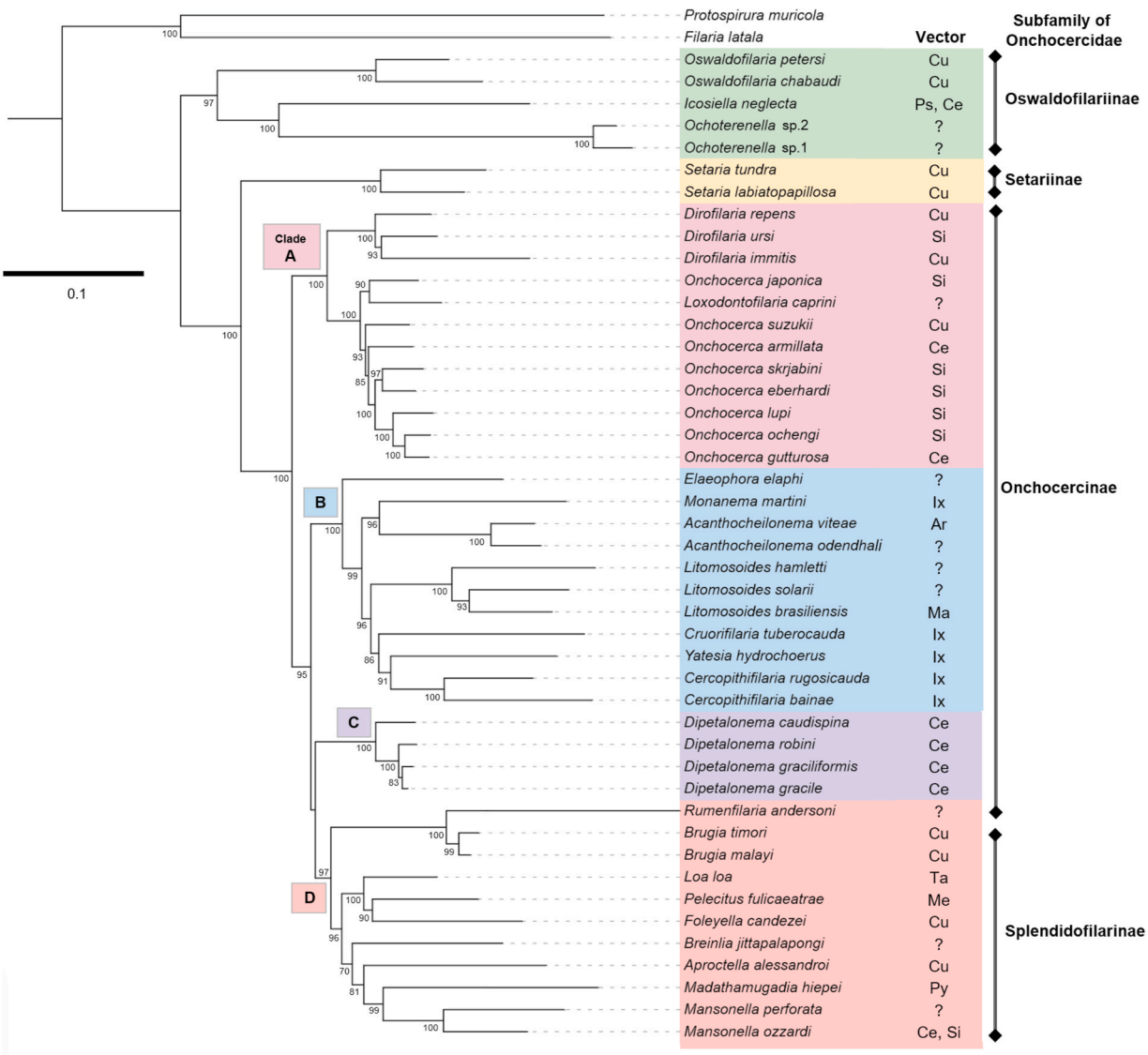

Fig. 5. Phylogenetic tree of Onchocercidae based on concatenated data sets of *18S* (*18S*_b), *28S*, *myohc*, *rbp1*, *hsp70*, *12S*, and *cox1* sequences using maximum likelihood. Nodes are labeled with bootstrap values (≥70). Scale bars indicate the substitutions per site. **Ar** = Argasidae (Arachnida: Ixodida); **Ce** = Ceratopogonidae (Insecta: Diptera); **Cu** = Culicidae (Insecta: Diptera); **Ix** = Ixodidae (Archnida: Ixodida); **Ma** = Macronyssidae (Arachnida: Mesostigmata); **Me** = Menoponidae (Insecta: Psocodea); **Ps** = Psychodidae (Insecta: Diptera); **Si** = Simuliidae (Insecta: Diptera); **Ta** = Tabanidae (Insecta: Diptera).

Takashima: Resources, Investigation. **Ayako Yoshida:** Writing – review & editing, Resources, Investigation, Conceptualization. **Kayoko Matsuo:** Writing – review & editing, Visualization, Supervision, Resources, Investigation, Conceptualization.

6. Note

Nucleotide sequence data reported in this paper are available in the GenBank™, EMBL and DDBJ databases under the accession numbers—*Elaeophora elaphi*: **PV382169** (*18S*_a), **PV382170** (*18S*_b), **PV389591** (*28S*), **PV415125** (*myohc*), **PV415129** (*hsp70*), **PV415127** (*rbp1*), **PV389593** (*12S*), **PV382166** (*cox1*); *Dirofilaria ursi*: **PV382171** (*18S*_b), **PV389592** (*28S*), **PV415126** (*myohc*), **PV415129** (*hsp70*), **PV415128** (*rbp1*), **PV389594** (*12S*).

Data availability statement

The alignment data of the gene sequences used for the phylogenetic analysis are available from Mendeley Data (https://doi.org/10.17632/6jxyrx4rff.1).

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Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijppaw.2025.101068.

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