Parasitology International 114 (2026) 103255

Contents lists available at ScienceDirect

Parasitology International

journal homepage: www.elsevier.com/locate/parint

Research paper

Molecular and morphological characterization of *Oxyspirura conjunctivalis* (Spirurida: Thelaziidae) in a captive gibbon with evidence of cockroach intermediate hosts

Kazuki Kiuno [a], Koichi Murata [b], Masae Tamaru [c], Rino Takaba [c], Masashi Sakurai [a,b], Hiroshi Shimoda [a,b], Daisuke Hayasaka [a,b,*], Hiroshi Sato [a,b,*]

[a] *Joint Graduate School of Veterinary Medicine, Yamaguchi University, 1677-1 Yoshida, Yamaguchi 753-8515, Japan*
[b] *Joint Faculty of Veterinary Medicine, Yamaguchi University, 1677-1 Yoshida, Yamaguchi 753-8515, Japan*
[c] *Ube City Tokiwa Zoo, 3-4-1 Norisada, Ube, Yamaguchi 755-0003, Japan*

ARTICLE INFO

Keywords:
Cockroach
cox1
Eyeworm
Oxyspirura conjunctivalis
ITS1
Primate
Phylogeny
rDNA
Thelaziidae

ABSTRACT

Eyeworms of the genus *Oxyspirura* infect a wide range of avian hosts worldwide, with more than 50 species described. However, *Oxyspirura conjunctivalis* (syn. *O. youngi*) and the recently described *O. tamarina* have been reported exclusively from captive primates in zoological gardens in Europe and North America. In this study, we report the first record of *Oxyspirura* infection in a captive Bornean white-bearded gibbon (*Hylobates albibarbis*) in Japan. Five adult worms recovered from the conjunctival sac measured 7.8–11.1 mm in length and 0.29–0.35 mm in width and exhibited a bipartite buccal capsule, pointed posterior ends in both sexes, markedly unequal spicules with a gubernaculum in male worms, and a vulval opening near the anus in oviparous female worms, indicating their classification in the genus *Oxyspirura*. Morphological features and the available ribosomal RNA gene (rDNA) sequence of the present specimens were identical to those of *O. conjunctivalis* previously collected from captive primates at the Moscow Zoo and *O. tamarina* from captive primates in the Czech Republic. Parasitological examination of smokybrown cockroaches (*Periplaneta fuliginosa*) collected from the gibbon enclosure revealed encysted larvae in their fat bodies. The mitochondrial cytochrome *c* oxidase subunit I (*cox1*) sequence of these larvae was identical to that of the newly collected adult worm, indicating the establishment of a transmission cycle for *O. conjunctivalis* within the zoo. Furthermore, the small- and large-subunit rDNA and *cox1* sequences, together with morphological features, demonstrated that *O. conjunctivalis* and *O. tamarina* were almost identical, indicating their conspecific relationship.

1. Introduction

Eyeworms of the genera *Thelazia* and *Oxyspirura* (Nematoda: Spirurida: Thelaziidae) parasitize the orbital tissues of mammals and birds, including conjunctiva, nictitating membrane, and Lacrimal glands and ducts [1]. Species of *Thelazia* in the subgenus *Thelazia* (17 species) infect a wide range of mammals [2], including many species of veterinary importance, such as *T. callipaeda* in caniids and lagomorphs, *T. californiensis* in dogs, *T. gulosa, T. rhodesi,* and *T. skrjabini* in cattle, *T. lacrymalis* in equids, and *T. leesei* in camels [1,2]. Human infection with *T. callipaeda* is a well-recognized ophthalmic zoonosis in Asia, which has recently spread across Europe [3–7]. Zoonotic infections with *T. californiensis* and *T. gulosa* have occasionally been reported in North America [8–11]. In contrast, *Thelazia* species of the subgenus *Thelaziella* (seven species), and most *Oxyspirura* spp. (50 species) are recorded almost exclusively from wild birds and domestic poultry [2,12–14]. An exception is *Oxyspirura conjunctivalis* (syn. *O. youngi*), which has been recorded from captive primates in zoological gardens in Germany (Berlin), the United States of America (USA) (Florida), and Russia (Moscow) [15–18]. More recently, *Oxyspirura tamarina* was described from captive New World monkeys (three species of tamarins) in the Czech Republic [19], based on the asymmetric arrangement of precloacal caudal papillae, uncertainty regarding the presence of a gubernaculum in males, and molecular divergence from previously reported *O. conjunctivalis* in captive primates.

In the present study, we recovered thread-like eyeworms from a

* Corresponding authors.
E-mail addresses: dhaya@yamaguchi-u.ac.jp (D. Hayasaka), sato7dp4@yamaguchi-u.ac.jp (H. Sato).

https://doi.org/10.1016/j.parint.2026.103255
Received 13 January 2026; Received in revised form 11 February 2026; Accepted 24 February 2026
Available online 25 February 2026

captive Bornean white-bearded gibbon (*Hylobates albibarbis*) in Japan. We compared the morphological features and molecular markers of these specimens with those of *O. conjunctivalis* and *O. tamarina*, two rarely reported *Oxyspirura* species from captive primates in Europe and North America.

2. Materials and methods

2.1. Parasite collection

A female Bornean white-bearded gibbon, estimated to be over 44 years old (exact birth year unknown), had been housed at Tokiwa Zoo in Ube City, Yamaguchi Prefecture, Japan, since its arrival in 1980. The gibbon cohabited with a male from 2012 until the male's death in 2019 and was re-paired with another male in 2023. On April 16, 2024, the female gibbon died of weakness caused by anesthesia and traumatic injuries inflicted by the male roommate. The carcass was submitted for necropsy, during which five thread-like eyeworms were collected from the conjunctival sac of the left eye. The worms were fixed, and preserved in 70% ethanol.

2.2. Morphological observation

Two male and two female eyeworms preserved in 70% ethanol were immersed in lactophenol for optimal cleaning prior to morphological examination using a BX60 microscope (OLYMPUS Co., Hachioji, Tokyo, Japan) equipped with differential interference contrast optics. All measurements are expressed as means with ranges in parentheses. The specimens collected in this study were deposited in the Meguro Parasitological Museum, Tokyo, Japan, under collection no. 25371.

2.3. Fecal examination

Nine primates, including the deceased female gibbon, were housed in a backyard enclosure with six chambers. Fecal samples were collected in mid-July 2024 from the remaining eight primates (the male housed with the deceased gibbon, one female Bornean white-bearded gibbon, four male and one female white-handed gibbon (*Hylobates lar* (Linnaeus)), and one unspecified gibbon (*Hylobates* sp.). Samples were examined microscopically following standard processing using the egg flotation technique with a saturated sugar solution and sedimentation technique [20]. The fecal mass during repeated examinations exceeded 10 g per primate. Tokiwa Zoo contained 15 primate species, with 123 individuals maintained in small or big groups (Supplementary Table S1).

2.4. Collection of encysted larvae in cockroaches

After the removal of primates from the enclosure where oxyspirurosis was detected, cockroaches present in the surrounding corridors were killed using a commercial insecticide and collected in mid-July 2024. The collected cockroaches were identified morphologically to species level and dissected to collect encysted eyeworm larvae within the viscera. The viscera were compressed between two glass plates, examined under a dissection microscope, and encysted larvae were collected in a plastic dish containing physiological saline. Remaining cockroach tissues were digested in 500 mL of physiological saline containing 0.6% (*w/v*) pepsin and 0.6% (*v*/v) HCl and incubated at 37 °C on a magnetic stirrer. After one hour of artificial digestion, sediments were examined under a dissecting microscope.

2.5. Molecular analysis

Genomic DNA was extracted from the middle third of the body of an adult female worm recovered from a gibbon and from three larvae collected from two cockroaches. Each sample preserved in 70% ethanol was placed in a clean 1.5 mL plastic tube and washed three times with pure distilled water. Genomic DNA was extracted separately using the DNeasy Blood & Tissue Kit (Qiagen GmbH, Hilden, North Rhine-Westphalia, Germany) according to the manufacturer's instructions.

Polymerase chain reaction (PCR) amplification of overlapping fragments of the ribosomal RNA gene (rDNA), spanning the small subunit (SSU) to the large subunit (LSU), was performed as previously described [21]. When direct sequencing was unsatisfactory, the purified PCR products were cloned into the plasmid vector, pTA2 (Target Clone™; TOYOBO, Kita-ku, Osaka, Japan), and transformed into *Escherichia coli* JM109 (TOYOBO) according to the manufacturer's instructions. After propagation, the plasmid DNA was extracted using the FastGene™ Plasmid Mini Kit (NIPPON Genetics Co., Tokyo, Japan), and inserts from at least three independent clones were sequenced using universal M13 forward and reverse primers. Overlapping fragments of the mitochondrial cytochrome *c* oxidase subunit 1 (*cox1*) were also amplified using PCR and sequenced as previously described [22], with an additional primer pair COIintF (5′-TGA TTG GTG GTT TTG GTA A-3′) and COIintR (5′-ATA AGT ACG AGT ATC AAT ATC-3′) according to Casiraghi et al. [23]. PCR amplification was performed in a thermal cycler using the following cycling protocol: 5 min at 95 °C, followed by 40 cycles at 94 °C for 30 s, 52 °C for 30 s, and 72 °C for 1 min, then a final extension at 72 °C for 10 min. The PCR products were electrophoresed on a 2% agarose gel, stained with ethidium bromide, and visualized under ultraviolet illumination.

Amplified fragments were purified using the MinElute® PCR Purification Kit (QIAGEN) or a High Pure PCR Cleanup Micro Kit (Roche Diagnostics GmbH, Mannheim, Baden-Württemberg, Germany) according to the manufacturers' instructions. Purified fragments were sequenced by commercial services (Eurofins Genomics, Keihin-jima, Tokyo, Japan; or FASMAC, Atsugi, Kanagawa, Japan), and sequence similarity was determined using the Basic Local Alignment Search Tool (BLAST) on the National Center for Biotechnology Information (NCBI) website (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Newly generated nucleotide sequences were deposited in the GenBank database under the accession numbers LC885620 (SSU rDNA–5.8S rDNA), LC885621 (LSU rDNA), LC885622 (adult worm *cox1*), and LC885623 (larva *cox1*).

2.6. Phylogenetic analysis

Fragments of the newly obtained rDNA sequences and partial *cox1* sequences were subjected to the BLAST analysis to identify closely related nucleotide sequences. The newly generated sequences, together with homologous sequences of related species within the suborder Spirurina retrieved from the DDBJ/EMBL/GenBank databases, were used for phylogenetic analyses. Twenty-nine SSU rDNA sequences of 28 species, and 40 *cox1* sequences of 34 species were aligned using CLUSTALW in MEGA11 [24] with subsequent manual adjustment. Regions that were poorly aligned and characters with gaps in any sequence were manually excluded from the analysis. After trimming, the SSU rDNA alignment comprised 1452 characters, of which 187 were variable (Supplementary Table S2), and the *cox1* alignment comprised 573 characters, of which 281 were variable (Supplementary Table S3). Phylogenetic trees were separately reconstructed for each gene using the maximum likelihood (ML) method with the HKY85 substitution model implemented in PhyML [25,26] via the 'phylogeny.fr' website (http://www.phylogeny.fr/). The probability of inferred branches was assessed using the approximate likelihood ratio test, an alternative to the nonparametric bootstrap estimation of branch support [27]. Graphical representation and edition of the phylogenetic tree were performed with TreeDyn (v198.3) implemented in PhyML, and cleaned illustrations were prepared using Adobe® Photoshop® Elements 2024 (Adobe Systems, San Jose, CA, USA).

3. Results

At necropsy, five thread-like nematodes were recovered from the

conjunctival sac of the left eye. No gross lesions were observed in the eyeball or conjunctiva, aside from conjunctival pallor. Histopathological examination revealed mild lymphocytic infiltration in the palpebral conjunctiva. The five eyeworms were fully matured, including two adult males and three adult females.

3.1. Morphology of adult eyeworms

Two male worms measured 7.97 (7.83 and 8.11) mm in length and 0.28 mm in maximum width and two female worms 10.70 (10.26 and 11.14) mm in length and 0.34 (0.33 and 0.35) mm in maximum width. They possessed a bipartite buccal capsule, pointed posterior ends in both sexes, distinctly unequal spicules with a gubernaculum in males, and a vulval opening near the anus in oviparous females, indicating their classification within the genus *Oxyspirura* (Fig. 1A–F). In males, the thin left spicules measured 1.39 (1.26 and 1.52) mm in length, and the thick right spicules measured 0.18 (0.17 and 0.19) mm in length. The small, helmet-shaped gubernaculum was 0.067 (0.056 and 0.078) mm long. Male worms had three pairs of precloacal, one pair of adcloacal, and two pairs of postcloacal caudal papillae, and a pointed tail, 0.35 (0.30 and 0.40) mm in length. The female worms had pointed tails (0.33 mm long) and vulval openings near the anus located 0.85 mm from the posterior end. The intrauterine eggs were oval and larvated, measuring 0.043 (0.040–0.046) mm × 0.028 (0.025–0.030) mm (n = 30).

Detailed measurements and morphometric comparisons of the newly-collected specimens with *Oxyspirura* eyeworms from primates reported in previous studies are shown in Table 1. Except for the esophageal length, the eyeworms collected from a female Bornean

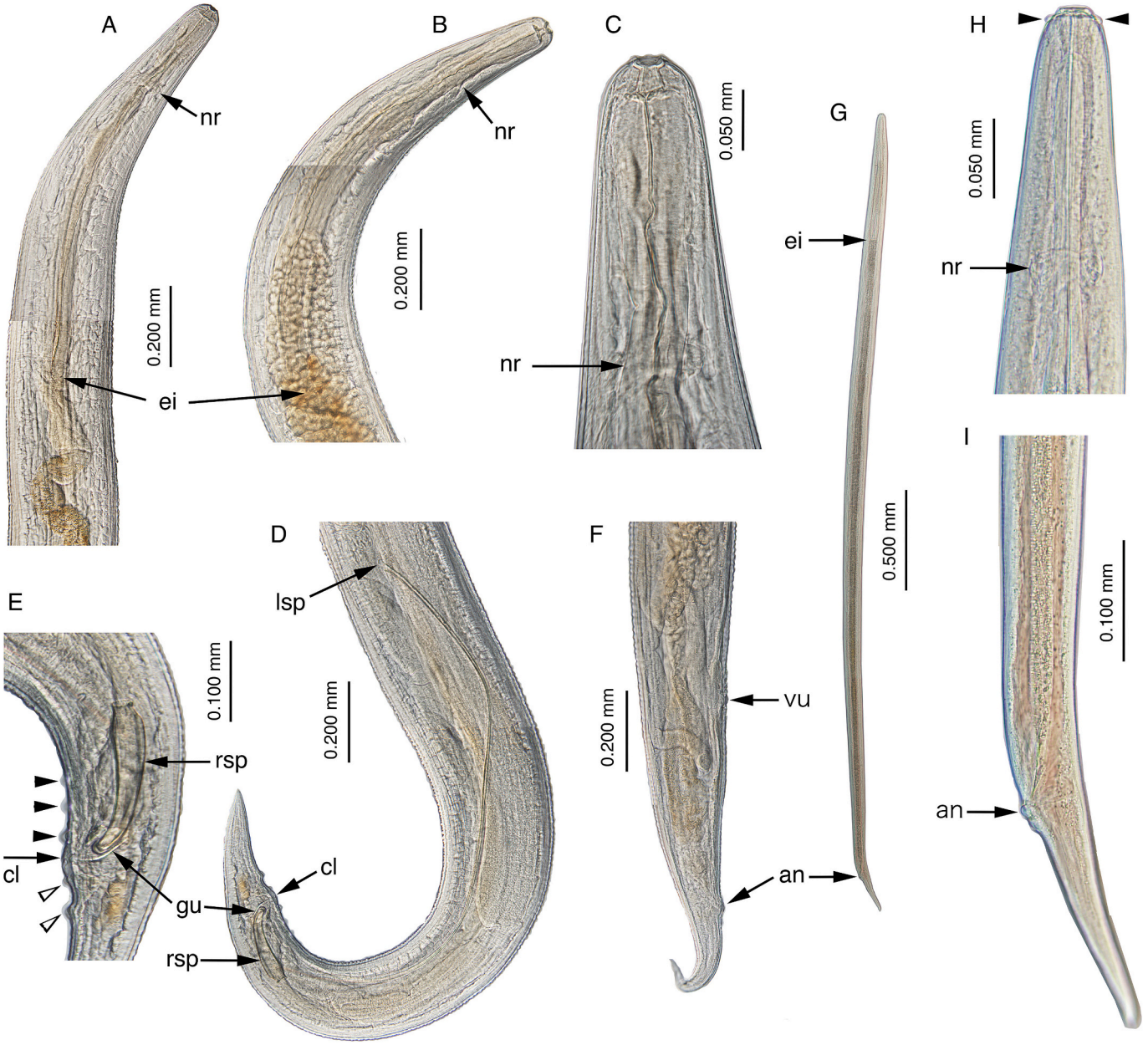

Fig. 1. *Oxyspirura conjunctivalis* adult eyeworms from a captive Bornean white-bearded gibbon (**A**–**F**) and an encysted larva from a smokybrown cockroach (**G**–**I**). (**A**, **C**) Anterior end of a male worm. (**B**) Anterior end of a female worm. (**D**, **E**) Posterior end of a male worm. (**F**) Posterior end of a female worm. Closed arrowheads in (**E**) indicate three preanal caudal papillae, and open arrowheads indicate two postanal papillae on one side. (**G**) Whole body of an encysted larva. (**H**) Anterior end of an encysted larva. Arrowheads indicate button-like cephalic papillae. (**I**) Posterior end of an encysted larva. Abbreviations: an, anus; cl, cloaca; ei, border between esophagus and intestine; gu, gubernaculum; lsp, right spicule; nr, nerve ring; rsp, right spicule; and vu, vulva.

Table 1A
Morphometrical comparison of adult *Oxyspirura* worms from captive primates (in μm unless otherwise stated)[a].

Original parasite species name	*O. conjunctivalis*	*O. conjunctivalis*	*O. conjunctivalis*	*O. conjunctivalis*	*O. youngi*	*O. tamarina*
Host species	*Hylobates lar*	*Nycticebus coucang*	*Microcebus murinus*	*Loris tardigradus*	*Erythrocebus patas*	*Saguinus oedipus*, *Leontopithecus chrysomelas*
Locality	Tokiwa Zoo, Japan	Moscow Zoo, Russia	Berlin Zoo, Germany	Berlin Zoo, Germany	Jacksonville Zoo in Florida, USA	A private breeder and a zoological garden in Czech Republic
Reference	Present study	[18]	[18]	[18]	[17]	[19]
Male	(*n* = 2)	(*n* = 12)	(*n* = 3)	(*n* = 4)	(*n* = 9)	(n = 3)[b]
Worm length (mm)	7.97 (7.83–8.11)	7.23 (5.80–8.21)	6.02 (5.90–6.75)	6.01 (5.75–6.75)	7.6 (6.3–9.4)	8.45 (6.77–7.75)
Max. worm width	286 (286)	220 (180–270)	183 (170–210)	195 (180–210)	282 (260–310)	250 (212–250)
Buccal capsule						
Anterior portion length	11 (8–14)	—	—	—	12.2 (10–15)	9 (9)
Anterior portion width	26 (23–30)	—	—	—	20.5 (18–22)	22 (10–19)
Posterior portion length	21 (17–26)	— 34 (27–45)	— 26 (23–30)	— 30 (29.5–30)	20.8 (18–27)	17 (11–19)
Posterior portion width	23 (22–23)	—	—	—	18.7 (14–22)	12 (9–12)
Esophagus length	1086 (938–1233)	664 (589–698)	568 (540–615)	585 (575–595)	670 (565–740)	775 (663–714)
Nerve ring from the anterior end	229 (213–244)	188 (150–270)	191 (180–205)	184 (155–205)	194 (180–205)	234 (198–224)
Excretory pore from the anterior end	—	244 (170–312)	270 (230–300)	272 (230–310)	265 (195–295)	326 (326)
Deirids from the anterior end	—	204 (160–288)	228 (210–270)	241 (205–280)	249 (200–295)	— (261)
Right spicule						
Length	181 (172–189)	182 (125–251)	192 (162–250)	152 (155–160)	170 (145–195)	199 (141–159)
Max. width	41 (39–43)	—	—	—	28 (19–35)	—
Left spicule						
Length	1386 (1256–1515)	1282 (1078–1433)	1192 (1000–1415)	1,093 (1000–1180)	1290 (1125–1400)	1372 (886–1295)
Width at capitulum	19 (15–22)	—	—	—	19.5 (15–25)	—
Width near posterior end	10 (8–13)	—	—	—	6.9 (6–8)	—
Gubernaculum length	67 (56–78)	– (40–120)	53 (42–85)	78 (48–100)	104 (95–113)	32 (absent or 47)
No. of preanal caudal papillae	3 pairs	3 pairs	3 pairs	3 pairs	3 pairs	5+3 (3–4 pairs)
No. of adanal caudal papillae	1 pair	1 pair	1 pair	1 pair	1 pair	1 pair
No. of postanal caudal papillae	2 pairs	2 pairs	2 pairs	2 pairs	2–3 pairs	2 pairs
Tail length	349 (300–397)	271 (214–323)	258 (190–308)	222 (190–250)	301 (265–335)	357 (306–367)

[a]Measurements are expressed as average and range in parentheses, except for specimens of *O. tamarina* [19]. "—", no available data.
[b]Measurements of the holotype specimen from *S. oedipus*, and ranges of two paratype specimens (one each from *L. chrysomelas* and *S. oedipus*) in parentheses.

white-bearded gibbon were identical to *O. conjunctivalis* previously reported from captive primates in Berlin, Moscow, and Jacksonville zoos (Table 1). Similarly, the newly collected eyeworms in the present study were morphologically similar to *O. tamarina* reported from captive primates in the Czech Republic, differing only in esophageal length and variation in the arrangement of precloacal caudal papillae and the uncertainty of gubernaculum presence in male worms.

3.2. Fecal examination

Fecal examination of eight cohabiting primates, including a male gibbon housed with the infected female, conducted 3 months after the death of the infected female gibbon in the backyard house, revealed no *Oxyspirura* eggs by either egg flotation or sedimentation techniques.

3.3. Morphology of encysted larvae

At the time of fecal sample collection, 38 smokybrown cockroaches (*Periplaneta fuliginosa* Serville), comprising 36 adults and two nymphs, were collected from the backyard primate house. Three encysted larvae were collected from the fat bodies of two adult cockroaches under a dissection microscope (Fig. 1G, H). No additional larvae were found after artificial digestion of the remaining cockroach tissues.

Three larvae encysted in the fat body of two cockroaches measured 3.11 (2.81–3.39) mm in length and 0.091 (0.081–0.105) mm in maximum width after artificial excystation. The oral opening was surrounded by six protruding lips with labial papillae, and four button-like cephalic papillae were visible around the labial region (Fig. 1H). The mouth opened into a short pharynx measuring 0.022 (0.019–0.026) mm in length, followed by an esophagus 0.53 (0.46–0.60) mm in length, composed of a muscular portion, 0.21 (0.20–0.23) mm long, and a glandular portion, 0.32 (0.26–0.37) mm long. The nerve ring was situated 0.161 (0.156–0.164) mm from the anterior end. The tail was conical, 0.197 (0.163–0.280) mm in length, and ended in a constricted blunt extremity. These morphological features and measurements of the encysted larvae were consistent with a previous description of *O. conjunctivalis* larvae collected from cockroach intermediate hosts at the Moscow Zoo [18].

Table 1B
Morphometrical comparison of adult *Oxyspirura* worms from captive primates (in μm unless otherwise stated)[a].

Original parasite species name	*O. conjunctivalis*	*O. conjunctivalis*	*O. conjunctivalis*	*O. conjunctivalis*	*O. youngi*	*O. tamarina*
Host species	*Hylobates lar*	*Nycticebus coucang*	*Microcebus murinus*	*Loris tardigradus*	*Erythrocebus patas*	*Saguinus oedipus*, *Leontopithecus chrysomelas*
Locality	Tokiwa Zoo, Japan	Moscow Zoo, Russia	Berlin Zoo, Germany	Berlin Zoo, Germany	Jacksonville Zoo in Florida, USA	A private breeder and a zoological garden in Czech Republic
Reference	Present study	[18]	[18]	[18]	[17]	[19]
Female worm	(*n* = 2)	(*n* = 10)	(*n* = 5)	(*n* = 9)	(*n* = 9)	(n = 9)[b]
Worm length (mm)	10.70 (10.26–11.14)	10.90 (6.20–12.80)	9.94 (8.65–11.00)	8.31 (5.00–10.70)	10.45 (8.57–11.80)	10.45 (8.57–11.80)
Max. worm width	341 (331–351)	266 (206–308)	298 (280–315)	278 (250–310)	325 (237–325)	325 (237–325)
Buccal capsule						
Anterior portion length	15 (15)	—	—	—	13.6 (10–18)	— (9–14)
Anterior portion width	30 (30)	—	—	—	24 (21–27)	— (19–26)
Posterior portion length	21 (17–26)	— 36 (30–42)	— 28 (25–32)	— 33 (29–35)	21.4 (16–29)	19 (19–27)
Posterior portion width	26 (23–28)	—	—	—	19.9 (11–26)	14 (7–14)
Esophagus length	1285 (1178–1392)	767 (686–863)	635 (560–730)	705 (630–810)	800 (710–930)	795 (714–826)
Nerve ring from the anterior end	237 (225–249)	191 (175–210)	195 (160–270)	206 (200–230)	214 (188–235)	204 (193–255)
Excretory pore from the anterior end	—	281 (231–320)	284 (245–320)	311 (280–340)	332 (280–390)	346 (257–387)
Deirids from the anterior end	—	212 (190–250)	247 (247)	264(205–280)	270 (240–330)	— (244–285)
Vulva from the posterior end	846 (844–848)	774 (660–904)	660 (450–1,200)	664 (600–800)	809 (680–1,020)	700 (700–820)
Tail length	330 (328–331)	254 (210–304)	245 (200–280)	257 (240–280)	329 (250–400)	265 (285–346)
Egg	(*n* = 30)	(*n* = ?)	—	—	(*n* = ?)	(*n* = ?)
Length	43 (40–46)	46 (43–50)	—	—	44.8 (40–50)	42–47 (34–44)
Width	28 (25–30)	26 (24–30)	—	—	29.8 (28–32)	24–27 (19–29)

[a]Measurements are expressed as average and range in parentheses, except for specimens of *O. tamarina* [19]. "—", no available data.
[b]Measurements of the allotype specimen from *S. oedipus*, and ranges of eight paratype specimens (four each from L. *chrysomelas* and *S. oedipus*) in parentheses.

3.4. Molecular characterization

Two rDNA contigs were obtained from the adult female eyeworm. The first contig was composed of nearly complete SSU rDNA (1784 bp), internal transcribed spacer 1 (ITS1) (615 bp), and partial 5.8S rDNA (36 bp), while the second contig consisted of a partial LSU rDNA (3162 bp) (GenBank accession nos. LC885620 and LC885621, respectively). The ITS sequence of the present eyeworm isolate (LC885620; 615 bp) was highly identical to that of *O. conjunctivalis* collected from the Sunda slow loris (*Nycticebus coucang* Boddaert) and the intermediate host cockroach in the Moscow Zoo (accession no. EF417873; 610 bp) with 99.51% (605/608) identity and 14 nucleotide insertion/deletion sites (indels). Most indels were located in places with single-nucleotide repeats (Table 2A). The deposited nucleotide sequence of *O. conjunctivalis* is limited to a single ITS1 sequence (EF417873). In contrast, the ITS1 sequences of *O. conjunctivalis* were clearly distinct from those of *Oxyspirura petrowi* (KF110799, KF110800, and KF306222) collected from the Northern bobwhite (*Colinus virginianus* (Linnaeus)) in the Rolling Plains Ecoregion of Texas, USA, and *Oxyspirura mansoni* (LC538186) from domestic chickens in Bangladesh. Notably, intraspecific nucleotide changes within the ITS region of *O. petrowi* were minimal (99.75% identity; 409/410), despite the presence of 26 indels (Table 2B), as observed in *O. conjunctivalis*.

The ITS1 sequence of *O. tamarina* collected from the captive cotton-top tamarin (*Saguinus oedipus* (Linnaeus)) in a zoological garden in the Czech Republic (accession no. PV704038; 579 bp) showed a high similarity with those of *O. conjunctivalis* across its 84.8% areas (initial 366 bp from the 5′-terminus, and 125 bp from the 3′-terminus of the partially characterized gene of *O. tamarina*; PV704038) as shown in Table 2. The remaining 15.2%, corresponding to a partial gene fragment from the 402nd to the 490th nucleotide of the ITS1 of *O. conjunctivalis*, characterized in this study (LC885620), showed remarkable nucleotide variations between *O. conjunctivalis* Moscow and Japan isolates and *O. tamarina,* with 67.82% (59/87) identity.

A BLAST search using the SSU rDNA sequence (GenBank accession no. LC885620) showed the highest identity with *O. tamarina* from a

Table 2A
Comparison of the ITS1 nucleotide sequences of *Oxyspirura conjunctivalis* from different origins.

O. conjunctivalis isolate	GenBank accession no.	Nucleotide position in the ITS1 region[a]							
		9–20	52–54	104–106	228–240	372–380	436	495–508	605
Japan isolate	LC885620	TTTTTTTTTCT	AAA	AAA–	AAAAAAAAAAAAA	TTTTTTTTT–	C	GAAAAAAAAAAAA	A
Moscow isolate	EF417873	TTTTTTTTTTT	GA–	AAAA	AAAAAAAA———	TTTTTTTTTT	A	GAAAAAAAAAAA–	A
Czech isolate	PV704038		GA–	AAAA	AAAAAAAA———	TTTTTTTTT–	C	AAAAAAAAAAAAA	T

[a] Nucleotide position relative to the 5′-terminus of the ITS sequence of *O. conjunctivalis* Japan isolate (LC885620). Blank means no data.

Table 2B
Comparison of the ITS1 nucleotide sequences of *Oxyspirura petrowi* from different origins.

O. petrowi isolate	GenBank accession no.	Nucleotide position in the ITS1 region[a]		
		50	55–68	421–437
USNPC 106874	KF306222	T	TTTTTTTTTTTTTT	AAAAAAAAAAAAAAA
EW_rRNA_type1	KF110799	A	TTTTTTTTTTT——	AAAAAAAAA———–
EW_rRNA_type2	KF110800	A	————————	AAAAAGAAA———–

[a] Nucleotide position relative to the 5′-terminus of the ITS1 sequence of *O. petrowi* USNPC_106,874 isolate (KF306222).

captive cotton-top tamarin in the Czech Republic (accession no. PV661113) with 99.82% (1709/1712) identity. This was followed by *Oxyspirura* sp. (MJY-2023 isolate; accession no. OQ474909) from a barred owl (*Strix varia* Barton) in California, USA, with 98.55% (1700/1725) identity and five indels, *O. petrowi* (KF110799, KF110800) with 97.55% (1714/1757) identity and seven indels, and various filarial species of the superfamily Filarioidea (Spirurida: Spiruridea) such as *Dirofilaria repens* (AB973229), *Loa loa* (XR_002251421, DQ094173), *Dipetalonema gracile* (MZ727043), *Mansonella perstans* (MN432520), and *Mansonella ozzardi* (MN432519) with 96.78 (1714/1771)–97.06 (1682/1733) identity and six to eight indels.

A BLAST search using an 800-bp fragment of the LSU rDNA sequence (GenBank accession no. LC885621) showed higher identity with various nematodes of Spiruroidea and Filarioidea, but all showed less than 89% identity with multiple indels. The partial LSU rDNA sequence of *O. tamarina*, available 295 bp near the 5′-terminus of the gene (PV704038), was completely identical with the LSU rDNA sequence of *O. conjunctivalis* collected in the present study (the initial 295 bp long nucleotide sequence of LC885621). A 683-bp fragment of the *O. tamarina* LSU rDNA sequence (PV661114) showed 98.75% (237/240) identity with the 240-bp-long 3′-terminal sequence of the *O. conjunctivalis* partial LSU rDNA sequence (LC885621: 3162 bp in length).

Two *cox1* sequences were obtained, one from an adult eyeworm (845 bp; GenBank accession no. LC885622) and another from encysted larvae from cockroaches (632 bp; LC885623). These sequences were completely identical across overlapping regions, indicating that the larva and adult represent different stages of the same species, *O. conjunctivalis*. The trimmed *cox1* sequence of *O. tamarina* (595 bp; PV655167 after removing primer annealing 12-bp fragment) showed the highest similarity (99.83%; 594/595) to the newly sequenced *cox1* sequence of *O. conjunctivalis* (LC885622 and LC885623). Apart from these primate-derived *Oxyspirura cox1* sequences, a BLAST search using the latter *cox1* sequence (LC885623) revealed higher similarities to various nematodes of Spiruroidea (including *O. petrowi* [PV203592]) and Filarioidea, but all showed less than 88% identity.

In the ML phylogenetic tree based on SSU rDNA sequences (Fig. 2), the Japanese isolate of *O. conjunctivalis* (accession no. LC885620) and the Czech isolate of *O. tamarina* (PV661113) formed a well-supported clade with *Oxyspirura* sp. MJY-2003 from the barred owl (OQ474909)

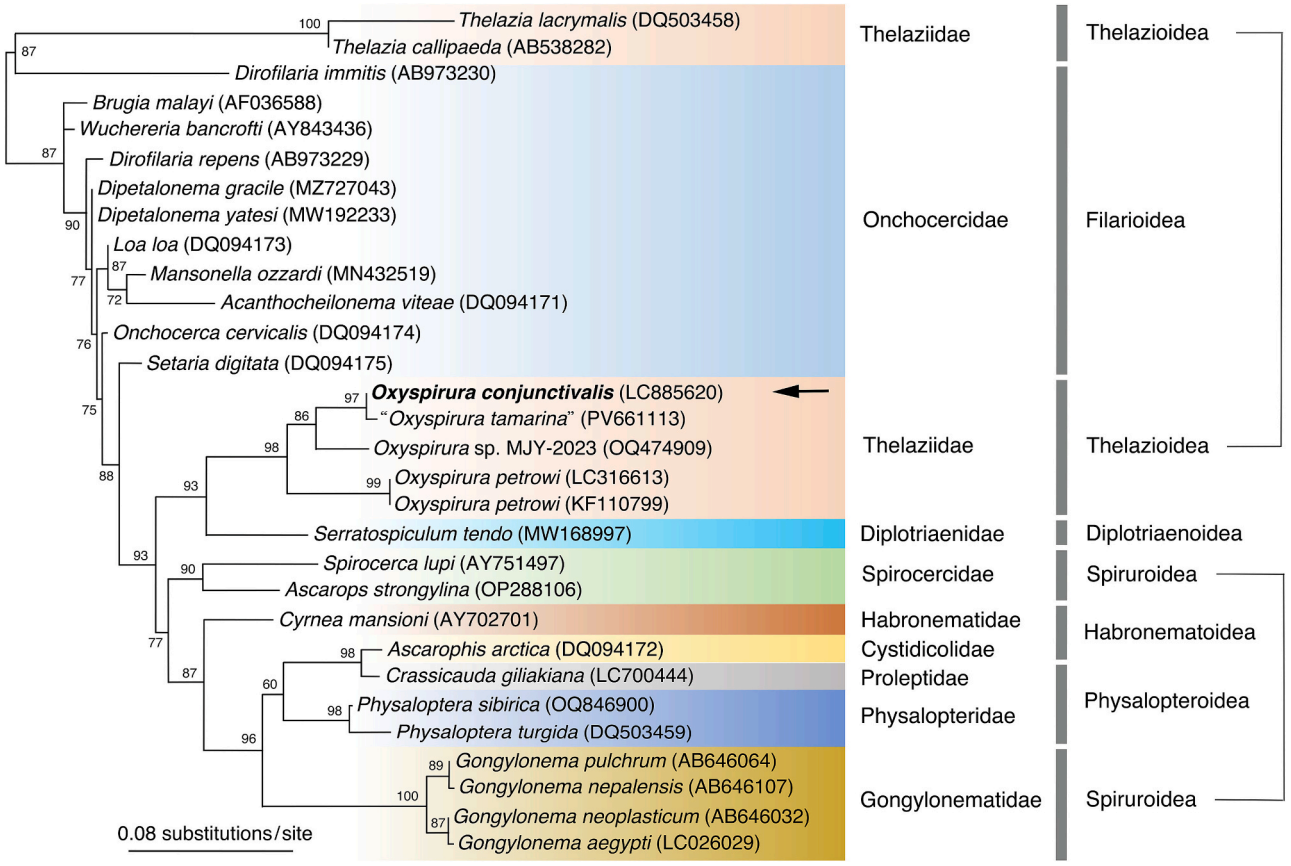

Fig. 2. ML phylogenetic tree based on SSU rDNA sequences (1452 characters) of representative species of the suborder Spirurina. Species names are followed by the GenBank accession number in parentheses. The newly obtained sequence is marked in bold. Family and superfamily classification of nematodes in different branches are indicated on the right side.

and *O. petrowi* from the Northern bobwhite (KF110799, and LC316613). This clade was sister to *Serratospiculum tendo* (Diplotriaenoidea: Diplotriaenidae; accession no. MW168997). The clade of *Thelazia* spp. (LC538282 for *T. callipaeda* and DQ503458 for *T. lacrymalis*) was located distantly from the clade of *Oxyspirura* spp. mentioned above in the ML phylogenetic tree based on the SSU rDNA sequences. In the ML phylogenetic tree constructed based on *cox1* (Fig. 3), the Japanese isolate of *O. conjunctivalis* (accession no. LC885622) and the Czech isolate of *O. tamarina* (PV655167) formed a well-supported clade with *O. petrowi* from the Northern bobwhite (PV203592). This clade was distinct from that of various *Thelazia* spp., and various filarial worms of the Onchocercidae (Filarioidea) formed another independent clade from the thelaziid eyeworms of genera *Oxyspirura* or *Thelazia*.

4. Discussion

In the present study, we collected mature adult eyeworms of *O. conjunctivalis* from a captive gibbon housed in a zoological garden in western Japan, where parasite transmission appears to be maintained through cockroaches acting as intermediate hosts. This epidemiological setting is similar to previous reports of *O. conjunctivalis* in captive primates at the Berlin Zoo (Germany), Moscow Zoo (Russia), and Jacksonville Zoo in Florida (USA) [17,18]. To our knowledge, this represents the fourth documented record of *O. conjunctivalis* infection in primates worldwide. Species identification based on morphology was confirmed by molecular characterization of the ITS1 sequence, which showed a few nucleotide variations when compared with the sequence of the Moscow isolate of *O. conjunctivalis* [18]. The two currently available ITS1 sequences of the primate eyeworms (EF417873 and LC885620) showed 99.51% (605/608) identity and 14 indels, all in repeat units of a single nucleotide (see Table 2). Given that ITS1 sequencing requires gene cloning owing to sequencing uncertainties caused by occasional single-nucleotide repeats, intraspecific and intraindividual minimal variations of the gene are suspected. In contrast, the newly generated SSU and LSU

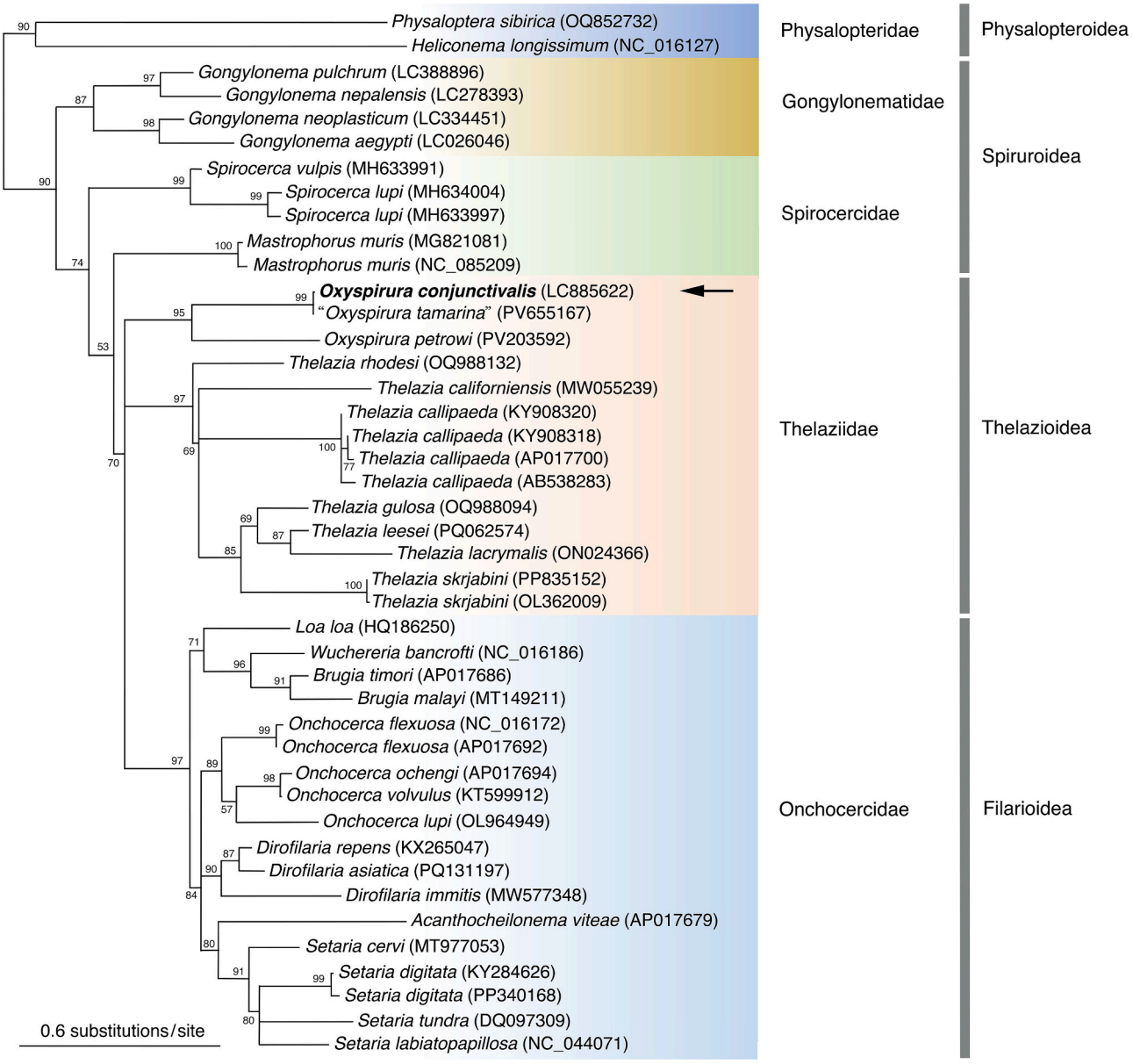

Fig. 3. ML phylogenetic tree based on mitochondrial *cox1* sequences (573 characters) of representative species of the suborder Spirurina. Species names are followed by the GenBank accession number in parentheses. The newly obtained sequence is marked in bold. Family and superfamily classification of nematodes in different branches are indicated on the right side.

Table 3
Primates recorded as hosts for *Oxyspirura conjunctivalis* [16–19].

Scientific name	Common name	Endemic area	Locality in captivity
Suborder Strepsirrhini			
Infraorder Lemuriformes			
Superfamily Lorisoidea Gray 1821			
Family Lorisidae Gray, 1821			
Loris gracilis É. Geoffroy, 1796	Slim-bodied lemur	India, and Sri Lanka	Berlin Zoo
Nycticebus coucang (Boddaert, 1785)	Greater slow loris	Indonesia, West Malaysia, southern Thailand, and Singapore	Moscow Zoo
Perodicticus potto (Müller, 1766)	West African potto	Tropical West Africa	Moscow Zoo
Xanthonycticebus pygmaeus (Bonhote, 1907)[a]	Pygmy slow loris	Vietnam, Laos, and eastern Cambodia	Moscow Zoo
Family Galagidae Gray, 1825			
Galago senegalensis É. Geoffroy, 1796	Senegal galago	Africa south of the Sahara	Moscow Zoo
Otolemur crassicaudatus É Geoffroy, 1812	Brown greater galago	Angola, Tanzania, and southern Kenya	Moscow Zoo
Superfamily Lemuroidea Gray 1821			
Family Cheirogaleidae Gray, 1873			
Cheirogaleus medius É. Geoffroy, 1812	Fat-tailed dwarf lemur	Madagascar	Moscow Zoo
Microcebus murinus (Miller, 1777)	Gray mouse lemur	Madagascar	Berlin Zoo; Moscow Zoo
Suborder Haplorhini			
Infraorder Simiiformes			
Superfamily Cercopithecoidea Gray, 1821			
Family Cercopithecidae Gray, 1821			
Erythrocebus patas (Schreber, 1775)[b]	Common patas monkey	Semi-arid areas of West Africa, and into East Africa	Jacksonville Zoo
Superfamily Hominoidea Gray, 1825			
Family Hylobatidae Gray, 1870			
***Hylobates albibarbis* Lyon, 1911**	**Bornean white-bearded gibbon**	**Southern Borneo**	**Tokiwa Zoo**
Superfamily Ceboidea			
Family Callitrichidae Thomas, 1903			
Leontocebus fuscicollis (Spix, 1823)[c]	Brown-mantled tamarin	Bolivia, Brazil, and Peru	Moscow Zoo
Leontopithecus chrysomelas (Kuhl, 1820)[d]	Golden-headed lion tamarin	Brazil	Czechia
Saguinus midas (Linnaeus, 1758)[d]	Golden-handed tamarin	Brazil, Guyana, French Guiana, Suriname, and Venezuela	Czechia
Saguinus oedipus (Linnaeus, 1758)[d]	Cotton-top tamarin	Northwest Colombia	Czechia
Family Cebidae Bonaparte, 1831			
Cebuella pygmaea (Spix, 1823)[e]	Western pygmy marmoset	Northwestern Amazon rainforest in Brazil, Colombia, Ecuador, and Peru	Moscow Zoo

[a] Syn. *Nycticebus pygmaeus* Bonhote, 1907
[b] Eyeworms from the common patas monkey were originally named as *Oxyspirura youngi* [17], presently a junior synonym of *O. conjunctivalis* [18].
[c] Syn. *Saguinus fuscicollis* Spix, 1823
[d] Eyeworms from these three tamarins were originally named as *Oxyspirura tamarina* [19], presently a junior synonym of *O. conjunctivalis* [Present study].
[e] Syn. *Callithrix (Cebuella) pygmaea* Spix, 1823

rDNA and *cox1* sequences of *O. conjunctivalis* in this study provide a more reliable and practical molecular approach for the specific identification of this rarely reported eyeworm.

Addison et al. [17] recovered *Oxyspirura* eyeworms from two captive common patas monkeys born in the Jacksonville Zoo, Florida, and distinguished *O. youngi* from *O. conjunctivalis* solely on the basis of a longer gubernaculum (95–113 μm vs. 58 μm). However, an intensive morphological assessment of the four nematode isolates recovered from diverse primates at different zoos (Berlin, Jacksonville, and Moscow) by Ivanova et al. [18] demonstrated that the gubernaculum length is a reliable feature for distinguishing these primate *Oxyspirura* eyeworms and provisionally synonymized *O. youngi* with *O. conjunctivalis*. More recently, Macá and González-Solís [19] described *O. tamarina* from three New World monkey species (golden-headed lion tamarin, golden-handed tamarin, and cotton-top tamarin) captive in a private breeder and a zoological garden in the Czech Republic, based on variation in the number and arrangement of precloacal caudal papillae (asymmetric arrangement of three or four pairs, or unpaired arrangement of eight papillae vs. three pairs in *O. conjunctivalis*) and the occasional absence of gubernaculum in male worms. Aside from these, other morphological features of *O. conjunctivalis* and *O. tamarina* were similar, including the distribution of bosses in the vulval region. As demonstrated in the present study, the high genetic similarities between these two species based on SSU rDNA (99.82%), partial LSU rDNA (100%), and *cox1* sequences (99.83%) strongly suggest their conspecific relationship. Furthermore, the majority of the ITS1 region (84.8% of the area) also showed high sequence similarity between these two primate *Oxyspirura* spp.

In avian hosts, *Oxyspirura* eyeworms deposit eggs in lacrimal secretions, which are transported via the lacrimal ducts to the buccal cavity, swallowed, and subsequently secreted in feces [1,28,29]. Based on this, fecal examinations were conducted on eight primates housed with the infected gibbon in the same backyard enclosure; however, no *Oxyspirura* eggs were detected. Notably, Kistler et al. [28] reported very low fecal egg counts (1.2–3.2 eggs/g; 2.2 in average) at 52 days or later after experimental infection of Northern bobwhites with encysted larvae of *O. petrowi* in the plains lubber grasshopper (*Brachystola magna* (Girard)), even when the average mass of feces used for fecal egg flotation analysis was 3.7 g/day/bobwhite. Therefore, it would be inappropriate to conclude that no additional primates were infected.

In the present study, encysted larvae of *O. conjunctivalis* were detected in the fat bodies of two smokybrown cockroaches, confirming their role as important intermediate hosts for sympatric primates. In a previous report on the transmission cycle of *O. conjunctivalis* in the Moscow Zoo [18], four cockroach species (the American cockroach, *Periplaneta americana* Linnaeus; Madagascar hissing cockroach, *Gromphadorrhina portentosa* Schaum; German cockroach, *Blattella germanica* Linnaeus; and speckled cockroach, *Nauphoeta cinerea* (Olivier)) were examined from primate enclosures. Of these, 20 of 33 examined speckled cockroaches harbored encapsulated *O. conjunctivalis* larvae in the fat bodies, whereas no eyeworm larvae were found in American ($n = 21$), Madagascar hissing ($n = 10$), and German ($n = 43$) cockroaches. The number of *O. conjunctivalis* capsules in the infected speckled cockroaches ranged from 2 to 99.

At the Moscow Zoo, oxyspirurosis had been recorded since 1995 in

several Old World monkeys of the infraorder Lemuriformes, as well as in some Neotropical monkeys of the superfamily Ceboidea (Table 3). Similarly, in the Berlin Zoo, oxyspirurosis was reported in two species of Lemuriformes, whereas in the Jacksonville Zoo and Tokiwa Zoo, it was reported in Old World monkeys of the infraorder Simiiformes. In the Czech Republic, oxyspirurosis was detected in captive New World monkeys of the family Callitrichidae. These observations indicate a broad host range for *O. conjunctivalis*, and the parasite's host preference is not consistent [18]. However, Ivanova et al. [18] traced the origin of the *O. conjunctivalis* outbreak at the Moscow Zoo to the arrival of *Xanthonycticebus pygmaeus* (Bonhote) (syn. *Nycticebus pygmaeus* Bonhote) from Vietnam in 1995. The rapid proliferation of cockroaches in zoo facilities and the insectivorous habits of monkeys facilitate the spread of oxyspirurosis across a wide range of monkeys species [18].

Tokiwa Zoo currently maintains eight primate species in the family Cercopithecidae, two in the family Hylobatidae, two in the family Cheirogaleidae, and three in the family Cebidae (Supplementary Table S1), within a total area of 1.9 ha. Five species in the family Cercopithecidae and two species in the family Hylobatidae are distributed in India, Sri Lanka, and Southeast Asia, which Ivanova et al. [18] considered a natural endemic area of *O. conjunctivalis*. Over the past decade, numerous primates from other domestic zoos have been frequently introduced, including ring-tailed lemurs *(Lemur catta* Linnaeus), black-and-white ruffed lemurs (*Varecia variegata* Kerr), tufted capuchins (*Sapajus apella* (Linnaeus)), Guianan squirrel monkeys (*Saimiri sciureus* Linnaeus), De Brazza's monkeys (*Cercopithecus neglectus* Schlegel), common patas monkeys (*Erythrocebus patas* (Schreber)), Celebes crested macaques (*Macaca nigra* (Desmarest)), toque macaque (*Macaca sinica* (Linnaeus)), and white-handed gibbons (*Hylobates lar*). A male white-handed gibbon, introduced from another zoo in 2019, cohabited with a female individual. Although the precise origin of *O. conjunctivalis* at the Tokiwa Zoo is uncertain, it is likely that the life cycle is maintained by latent infection in captive monkeys. Such low-level, asymptomatic infections persisting within the zoo may explain the absence of diagnosed cases of oxyspirurosis in this zoological garden in the past. This situation may be similar to that observed in other zoological gardens worldwide. To date, no specifications for natural hosts or natural endemic areas for *O. conjunctivalis* have been identified.

Phylogenetic analyses based on SSU rDNA and *cox1* sequences revealed polyphyletic relationships between eyeworms of the genera *Oxyspirura* and *Thelazia*, both classified in the family Thelaziidae, although the congeners of both genera were separately monophyletic, as suggested by previous studies [19,29–32]. According to Ivanova et al. [18], the apparent genetic distance between *Oxyspirura* and *Thelazia* suggests that the parasitism of the orbital cavities by both is due to convergence. Apart from this systematic problem, SSU/LSU rDNA or *cox1* sequences may serve as reliable molecular markers for species-level identification of *Oxyspirura* eyeworms, although currently available nucleotide sequence data remain limited. As shown in the present study, the use of ITS sequences is laborious due to frequent, uncertain repeats of a single nucleotide that necessitate gene cloning. To clarify intraspecific relationships, the accumulation of genetic information on more *Oxyspirura* spp. is necessary to reveal the evolutionary origin of the only mammal-parasitizing species, *O. conjunctivalis*.

In conclusion, the present study demonstrated a latent infection of *Hylobates albibarbis* in captivity; however, no clinical infection was observed in captive primates at the same zoological garden. Nonetheless, the recovery of encysted *O. conjunctivalis* larvae from cockroaches suggests the maintenance of a transmission cycle with a low-level, asymptomatic infection in primates within the zoo. Given the frequent movement of primates between zoos in Japan, similar conditions may exist in many zoological settings. However, infection is not always subtle, as it can occasionally elicit serious diseases, such as complete destruction of the eyeball, as reported in *N. coucang* imported from Vietnam [18]. To mitigate the threat of diseases in captive primates, active cockroach control and routine epidemiological surveillance in zoological gardens should be considered. Cockroaches play an important role as intermediate hosts for not only *O. conjunctivalis* but also *Gongylonema pulchrum* (family Gongylonematidae), *Pterygodermatites nycticebi* (Rictulariidae), and *Prosthenorchis elegans* (Oligacanthorhynchidae) [33–38].

CRediT authorship contribution statement

Kazuki Kiuno: Writing – original draft, Validation, Methodology, Investigation, Data curation, Conceptualization. **Koichi Murata:** Methodology, Investigation, Data curation. **Masae Tamaru:** Resources, Methodology, Investigation, Data curation. **Rino Takaba:** Resources, Methodology, Investigation, Data curation. **Masashi Sakurai:** Methodology, Investigation, Data curation. **Hiroshi Shimoda:** Writing – review & editing, Validation, Methodology, Investigation, Conceptualization. **Daisuke Hayasaka:** Writing – review & editing, Supervision. **Hiroshi Sato:** Writing – review & editing, Methodology, Investigation, Conceptualization.

Ethical standards

Not applicable.

Financial support

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

Acknowledgments

We thank the staff members of Tokiwa Zoo for their support during sampling.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.parint.2026.103255.

References

[1] R.C. Anderson, Nematode Parasites of Vertebrates. Their Development and Transmission, 2nd ed., CABI Publishing, Wallingford, UK, 2000,. ISBN 0–85199–421–0.
[2] M. Hodda, Phylum Nematoda: a classification, catalogue and index of valid genera, with a census of valid species, Zootaxa 5114 (2022) 1–289, https://doi.org/10.11646/zootaxa.5114.1.1.
[3] D. Otranto, G. Testini, F. De Luca, M. Hu, S. Shamsi, R.B. Gasser, Analysis of genetic variability within *Thelazia callipaeda* (Nematoda: Thelazioidea) from Europe and Asia by sequencing and mutation scanning of the mitochondrial cytochrome *c* oxidase subunit 1 gene, Mol. Cell. Probes 19 (2005) 306–313, https://doi.org/10.1016/j.mcp.2005.05.001.
[4] V. Čabanová, M. Miterpáková, M. Oravec, Z. Hurníková, S. Jerg, G. Nemčíková, M. B. Červenská, Nematode *Thelazia callipaeda* is spreading across Europe. The first survey of red foxes from Slovakia, Acta Parasitol. 63 (2018) 160–166, https://doi.org/10.1515/ap-2018-0018.
[5] B. do Vale, A.P. Lopes, M. da Conceição Fontes, M. Silvestre, L. Cardoso, A. C. Coelho, Systematic review on infection and disease caused by *Thelazia callipaeda* in Europe: 2001–2020, Parasite 27 (2020) 52, https://doi.org/10.1051/parasite/2020048.
[6] S. Roston, J.E. Kulenkamp, P. Ferrieri, S. Strul, Rare *Thelazia californiensis* infant ocular infestation, Am. J. Ophthalmol. Case Rep. 30 (2023) 101813, https://doi.org/10.1016/j.ajoc.2023.101813.
[7] A.F. Lopes, M. Ribeiro Ferreira, B. do Vale, M. Santos, I. Silveira, S. Claudino, M. Martins, T. Brida, L. Figueira, L. Cardoso, A.P. Lopes, A.C. Coelho, M. Matos, A. C. Matos, Update on infections with *Thelazia callipaeda* in European wildlife and a report in a red fox, *Vulpes vulpes*, in Portugal, Curr. Res. Parasitol. Vector Borne Dis. 6 (2024) 100211, https://doi.org/10.1016/j.crpvbd.2024.100211.

[8] R. Knierim, M.K. Jack, Conjunctivitis due to *Thelazia californiensis*, Arch. Ophthalmol. 93 (1975) 522–523, https://doi.org/10.1001/archopht.1975.01010020538011.
[9] B.I. Kirschner, J.P. Dunn, H.B. Ostler, Conjunctivitis caused by *Thelazia californiensis*, Am. J. Ophthalmol. 110 (1990) 573–574, https://doi.org/10.1016/s0002-9394(14)77889-4.
[10] R.S. Bradbury, K.V. Breen, E.M. Bonura, J.W. Hoyt, H.S. Bishop, Case report: conjunctival infestation with *Thelazia gulosa*: a novel agent of human thelaziasis in the United States, Am. J. Trop. Med. Hyg. 98 (2018) 1171–1174, https://doi.org/10.4269/ajtmh.17-0870.
[11] R.S. Bradbury, D.T. Gustafson, S.G.H. Sapp, M. Fox, M. de Almeida, M. Boyce, P. Iwen, V. Herrera, M. Ndubuisi, H.S. Bishop, A second case of human conjunctival infestation with *Thelazia gulosa* and a review of T. gulosa in North America. Clin. Infect. Dis. 70 (2020) 518–520, doi:10.1093/cid/ciz469, Erratum in: Clin. Infect. Dis. 70 (2020) 2459, https://doi.org/10.1093/cid/ciz1128.
[12] E.M. Addison, R.C. Anderson, A review of the eye worms of the genus *Oxyspirura* (nematode: Spiruroidea), Wildl. Dis. 55 (1969) 1–58.
[13] A. Kalyanasundaram, M.Z. Brym, K.R. Blanchard, C. Henry, K. Skinner, B.J. Henry, J. Herzog, A. Hay, R.J. Kendall, Life-cycle of *Oxyspirura petrowi* (Spirurida: Thelaziidae), an eyeworm of the northern bobwhite quail (*Colinus virginianus*), Parasit. Vectors 12 (2019) 555, https://doi.org/10.1186/s13071-019-3802-3.
[14] R. Fagundes-Moreira, M.A. Bezerra-Santos, R.P. Lia, C. Daudt, J.T. Wagatsuma, E. C.O. de Carmo, L. Berger, F.R. Chaves da Silva, J.F. Soares, D. Otranto, Eyeworms of wild birds and new record of *Thelazia* (*Thelaziella*) *aquilina* (Nematoda: Spirurida), Int. J. Parasitol. Parasites Wildl. 23 (2024) 100910, https://doi.org/10.1016/j.ijppaw.2024.100910.
[15] O. Linstow, Nematoden aus dem Königlichen Zoologischen Museum zu Berlin, Mitt. Zool. Mus. Berl. 3 (1907) 251–259.
[16] J.G. Baer, Etude de quelques helminthes de Lémuriens, Rev. Suisse Zool. 42 (1935) 275–291.
[17] E.M. Addison, D.J. Forrester, R.D. Whitley, M.M. Curtis, *Oxyspirura youngi sp. n. (Nematoda: Thelaziidae) from the patas monkey, Erythrocebus patas*, Proc. Helminthol. Soc. Wash. 53 (1986) 89–93.
[18] E. Ivanova, S. Spiridonov, O. Bain, Ocular oxyspirurosis of primates in zoos: intermediate host, worm morphology, and probable origin of the infection in the Moscow zoo, Parasite 14 (2007) 287–298, https://doi.org/10.1051/parasite/2007144287.
[19] O. Máca, D. González-Solís, A new *Oxyspirura* (Nematoda, Thelaziidae) in three captive non-human primate species, Front. Vet. Sci. 12 (2025) 1650452, https://doi.org/10.3389/fvets.2025.1650452.
[20] L.S. Garcia, Diagnostic Medical Parasitology, 5th ed., ASM Press, Washington, DC, USA, 2007. ISBN: 978–1–683-67408-5.
[21] P. Makouloutou, A. Setsuda, M. Yokoyama, T. Tsuji, E. Saita, H. Torii, Y. Kaneshiro, M. Sasaki, K. Maeda, Y. Une, H. Hasegawa, H. Sato, Genetic variation of *Gongylonema pulchrum* from wild animals and cattle in Japan based on ribosomal RNA and mitochondrial cytochrome *c* oxidase subunit I genes, J. Helminthol. 87 (2013) 326–335, https://doi.org/10.1017/S0022149X12000442.
[22] A. Setsuda, A. Ribas, K. Chaisiri, S. Morand, M. Chou, F. Malbas, M. Yunus, H. Sato, Molecular genetic diversity of *Gongylonema neoplasticum* (Fibiger & Ditlevsen, 1914) (Spirurida: Gongylonematidae) from rodents in Southeast Asia, Syst. Parasitol. 95 (2018) 235–247, https://doi.org/10.1007/s11230-018-9778-0.
[23] M. Casiraghi, T.J. Anderson, C. Bandi, C. Bazzocchi, C. Genchi, A phylogenetic analysis of filarial nematodes: comparison with the phylogeny of *Wolbachia* endosymbionts, Parasitology 122 (2001) 93–103, https://doi.org/10.1017/s0031182000007149.
[24] K. Tamura, G. Stecher, S. Kumar, MEGA11: molecular evolutionary genetics analysis version 11, Mol. Biol. Evol. 38 (2021) 3022–3027, https://doi.org/10.1093/molbev/msab120.
[25] S. Guindon, O. Gascuel, A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood, Syst. Biol. 52 (2003) 696–704, https://doi.org/10.1080/10635150390235520.
[26] A. Dereeper, V. Guignon, G. Blanc, S. Audic, S. Buffet, F. Chevenet, J.F. Dufayard, S. Guindon, V. Lefort, M. Lescot, J.M. Claverie, O. Gascuel, Phylogeny.fr: robust phylogenetic analysis for the non-specialist, Nucleic Acids Res 36 (Web Server issue) (2008) W465–W469, https://doi.org/10.1093/nar/gkn180.
[27] M. Anisimova, O. Gascuel, Approximate likelihood-ratio test for branches: a fast, accurate, and powerful alternative, Syst. Biol. 55 (2006) 539–552, https://doi.org/10.1080/10635150600755453.
[28] W.M. Kistler, S. Hock, B. Hernout, E. Brake, N. Williams, C. Downing, N. Dunham, N. Kumar, U. Turaga, J.A. Parlos, R.J. Kendall, Plains lubber grasshopper (*Brachystola magna*) as a potential intermediate host for *Oxyspirura petrowi* in northern bobwhites (*Colinus virginianus*), Parasitol. Open 2 (2016) e7, https://doi.org/10.1017/pao.2016.5.
[29] A. Kalyanasundaram, K.R. Blanchard, C. Henry, M.Z. Brym, R.J. Kendall, Phylogenetic analysis of eyeworm (*Oxyspirura petrowi*) in northern bobwhite (*Colinus virginianus*) based on the nuclear 18S rDNA and mitochondrial cytochrome oxidase 1 gene (COX1), Parasitol. Open 4 (2018) e7, https://doi.org/10.1017/pao.2018.2.
[30] C. Henry, A. Kalyanasundaram, M.Z. Brym, R.J. Kendall, Molecular identification of *Oxyspirura petrowi* intermediate hosts by nested PCR using internal transcribed spacer 1 (ITS1), J. Parasitol. 106 (2020) 46–52.
[31] K.D. Niedringhaus, J.P. Dumbacher, F. Dunker, S. Medina, B. Lawson, H.M. A. Fenton, J.M. Higley, E. Haynes, M.J. Yabsley, Apparent prevalence, diversity, and associated lesions of periorbital nematodes in a population of barred owls (*Strix varia*) from northern California, USA, J. Wildl. Dis. 59 (2023) 299–309, https://doi.org/10.7589/JWD-D-21-00186.
[32] C.M. Gherman, A.M. Ionică, K.A. Hołówka, V.D. Cotuțiu, C.A. Culda, G.I. Lupu, A. D. Mihalca, *Oxyspirura petrowi* causing ocular parasitism in a free-ranging common buzzard (*Buteo buteo*) in Romania and a review of the potential zoonotic implications as cutaneous larval migrans, Animals (Basel) 15 (2025) 1606, https://doi.org/10.3390/ani15111606.
[33] Y. Ikeda, A. Fujisaki, K. Murata, H. Hasegawa, Redescription of *Pterygodermatites* (*Mesopectines*) *nycticebi* (Mönnig, 1920) (Nematoda: Rictulariidae), a parasite of slow loris *Nycticebus coucang* (Mammalia: Primates), Folia Parasitol. (Praha) 50 (2003) 115–120, https://doi.org/10.14411/fp.2003.020.
[34] H. Sato, Y. Une, M. Takada, High incidence of the gullet worm, *Gongylonema pulchrum*, in a squirrel monkey colony in a zoological garden in Japan, Vet. Parasitol. 127 (2005) 131–137, https://doi.org/10.1016/j.vetpar.2004.10.005.
[35] M.J. Adkesson, J.N. Langan, A. Paul, Evaluation of control and treatment of *Gongylonema* spp. infections in callitrichids, J. Zoo Wildl. Med. 38 (2007) 27–31, https://doi.org/10.1638/06-005.1.
[36] L.S. Catenacci, A.C. Colosio, L.C. Oliveira, K.M. De Vleeschouwer, A.D. Munhoz, S. L. Deem, J.M. Pinto, Occurrence of *Prosthenorchis elegans* in free-living primates from the Atlantic forest of southern Bahia, Brazil, J. Wildl. Dis. 52 (2016) 364–368, https://doi.org/10.7589/2015-06-163.
[37] A. Balsiger, K. Federer, F. Grimm, P. Deplazes, Transmission of *Pterygodermatites nycticebi* in a colony of goeldi's monkeys (*Callimico goeldii*) and evaluation of treatment and control, J. Zoo Wildl. Med. 49 (2018) 893–901, https://doi.org/10.1638/2017-0177.1.
[38] L.M.R. Silva, I. Voelker, C. Geiger, N. Schauerte, J. Hirzmann, C. Bauer, A. Taubert, C. Hermosilla, *Pterygodermatites nycticebi infections in golden lion tamarins (Leontopithecus rosalia rosalia) and aye-ayes (Daubentonia madagascariensis) from a German zoo*, Zoo Biol. 40 (2021) 59–64, https://doi.org/10.1002/zoo.21578.