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Human ocular thelaziasis with genetic analysis in Niigata Prefecture, Japan: A case report on an emerging zoonosis

Tianxiang Huang [a,b], Takenori Inomata [a,b,c,d,e,*], Jaemyoung Sung [a], Naoko Yoshida [f], Gaku Ishida [a,g], Hitomi Ohara [g], Masahiro Yamaguchi [a], Yasutsugu Akasaki [a,b], Yuichi Okumura [a,b,d], Ken Nagino [a,b,c,d], Kunihiko Hirosawa [a,b], Toshihiro Mita [f], Shintaro Nakao [a], Nobuo Ishida [g]

[a] Juntendo University Graduate School of Medicine, Department of Ophthalmology, Tokyo, Japan
[b] Juntendo University Graduate School of Medicine, Department of Digital Medicine, Tokyo, Japan
[c] Juntendo University Graduate School of Medicine, Department of Hospital Administration, Tokyo, Japan
[d] Juntendo University Graduate School of Medicine, Department of Telemedicine and Mobile Health, Tokyo, Japan
[e] Juntendo University Graduate School of Medicine, AI Incubation Farm, Tokyo, Japan
[f] Juntendo University School of Medicine, Department of Tropical Medicine and Parasitology, Tokyo, Japan
[g] Ishida Eye Clinic, Niigata, Japan

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ABSTRACT

Purpose: We report the clinical findings and molecular identification of ocular *Thelazia callipaeda* from Niigata Prefecture in the Hokuriku area of Japan during winter.

Observations: A 77-year-old male visited an ophthalmology clinic in Niigata Prefecture in January 2022 after a 2-week-duration of a conjunctival injection in the left eye and foreign body sensation. Slit-lamp microscopy revealed 11 active nematodes in the left conjunctival sac. Morphological characteristics included longer female body length than male, buccal cavity lacking teeth and lips, and serrated striations along the body surface. The specimens were determined to be *T. callipaeda*. Genetic analysis of the mitochondrial cytochrome *c* oxidase subunit 1 (*cox1*) gene revealed an h9 haplotype.

Conclusions and Importance: T. callipaeda infection, especially the h9 haplotype, commonly occurs in western Japan owing to its higher incidence in warmer climates, suggesting the origin of the case. Here, we report a human case of *Thelaziasis* diagnosed in a cold region of Japan (the Hokuriku area) during winter. This human case of *T. callipaeda* infection from a cold, previously unassociated region, raises concerns about the potential geographical widening of its distribution, and further investigation may be warranted to prevent its spread.

1. Introduction

Thelazia callipaeda is a parasitic nematode that frequently infests the conjunctival sac of human hosts and uses Drosophilid flies as intermediate hosts. More common definitive hosts for *T. callipaeda* are dogs, cats, foxes, rabbits, and other mammals. These nematodes are zoonotic and may also infect the human eye, leading to conjunctivitis, keratitis, corneal ulcers, and various other ocular manifestations.[1–4]

The first known case of thelaziasis occurred in a dog in 1910, followed by a human case in 1917 from China.[5] *T. callipaeda* is largely distributed around southeast Asia–spanning from China to Indonesia–and India, likely contributing to its other moniker, the "oriental eye worm".[4,6] However, in the last three decades, since the early 1990s, cases of *T. callipaeda* infections have been found in European countries,[7–9] which may indicate a geographical widening of *T. callipaeda* distribution. The first case of thelaziasis in Japan was reported in 1957 in Kumamoto Prefecture in the Kyushu area.[10] Since then, there have been an increasing number of human and animal cases of thelaziasis in West Japan, particularly in the Kyushu region,[11,12] where the climate tends to be warmer than in the remaining areas of the country.

The current standard for diagnosing thelaziasis is two-fold: the morphology of the parasite of interest is first visualized under a microscope, followed by molecular identification of the inciting parasite

* Corresponding author. Juntendo University Graduate School of Medicine, Department of Ophthalmology, Hongo, Bunkyo-ku, Tokyo, 113, Japan.
E-mail address: tinoma@juntendo.ac.jp (T. Inomata).

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through polymerase chain reaction (PCR)-based techniques.[3,4,9,11–18] Interestingly, detailed PCR analysis and comparison of *T. callipaeda* from different regions, based on variants of the mitochondrial cytochrome *c* oxidase subunit 1 (*cox1*) gene, revealed a pattern in the observed *cox1* variants based on geography. Haplotype 1 (h1) was found in Italy, Germany, and the Netherlands, h2–5 were found in China, and h5–8 were identified in South Korea.[11] In Japan, there have been three cases with two variants (h9 and h10) locally identified through PCR at the time of this report: one case of the h9 variant from Okayama Prefecture by Kumase et al.,[12] and two cases of h10 variants from Oita and Kumamoto Prefectures by Ishibe et al.[16] From an epidemiologic perspective, haplotype determination during thelaziasis diagnosis may not only help with specifying a definitive diagnosis but may provide crucial information on infection routes and changes in their distribution.

In this case report, we discuss a case of human thelaziasis infection from Niigata Prefecture in the Hokuriku area of Japan, along with its haplotype, identified through molecular analysis.

2. Case report

A 77-year-old male arrived at an ophthalmology clinic located in Jōetsu-shi, Niigata Prefecture of the Hokuriku area of Japan in January 2022 after a 2-week-duration of foreign body sensation in the left eye. The patient had no medical history, family history, tobacco use or known allergies. The patient reported an average drinking pattern of approximately 180 ml per day (one "gō"). His primary occupation was agricultural work during summer and unemployment during winter. He resided with five other family members and a pet parrot; no other pet history was shared. The patient denied having hiking as a hobby. He had no history of foreign travel. An in-clinic examination revealed 20/13 best-corrected visual acuity in both eyes and intraocular pressure measurements of 12 and 15 mmHg in the right and left eyes, respectively.

Slit-lamp microscopy of the anterior segment of the left eye revealed conjunctival injection, and retraction of the left lower eyelid revealed multiple white nematodes with active peristaltic movement in the conjunctival sac (Fig. 1A). The peristaltic movements of the isolated nematodes were better visualized using the blue light-free filter of a slit-lamp microscope (Fig. 1B). Due to the high activity of the parasite and concern for further penetration through the conjunctival sac, topical administration of 4% Xylocaine Ophthalmic Solution (Lidocaine HCl eye drops, Sandoz Pharmaceutical, Switzerland) was first performed to decrease the activity, with subsequent extraction of 11 nematodes using ophthalmic forceps under microscopy. Three samples of isolated parasites were sent to the Department of Tropical Medicine and Parasitology, Juntendo University Graduate School of Medicine, Tokyo, Japan. For morphological analysis, two samples were preserved in 10% formalin solution, and for genetic analysis, one sample was preserved in 99% ethanol. After parasite extraction, the patient's left eye was treated with a 4-times-a-day administration of 1.5% Cravit Ophthalmic Solution (levofloxacin hydrate; Santen Pharmaceutical Co., Ltd., Osaka, Japan) for one week. At one-week follow up, the patient reported a reduction in foreign body sensation and improved conjunctival injection. No new parasites were detected during the patient's most recent exam at one-year follow up.

2.1. Morphological identification

The first of the two morphologically analyzed specimens was a male with a body length of 12 mm, and the second specimen was a female with a body length of 15 mm (Fig. 2A). A detailed examination of the female specimen using light microscopy revealed a rectangular oral cavity lacking lips and teeth (Fig. 2B). Additionally, the vulva was located anterior to the esophagointestinal junction (Fig. 2C), and its body surface was covered with serrated annulations (Fig. 2D). In the male *T. callipaeda*, a long left spicule (Fig. 2E) and a short right spicule were observed in the caudal region of the body. In contrast, the female sample had a straight anatomy at the tail end (Fig. 2F). The specimens were determined to be *T. callipaeda* based on the observed morphological characteristics of the extracted specimens, which were consistent with those reported previously.[12,16]

2.2. Sequence and phylogenetic analyses

One of the 11 extracted nematodes was sent for genetic analysis to obtain its *cox1* gene sequence. DNA extracted from the *T. callipaeda* isolated from the patient was used as a template for the PCR amplification of *cox1* genes. The DNA was extracted using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany). Primers for *T. callipaeda* (TcF: 5′-AGCTGGTTTAGGTGGGTCTG-3′) and (TcR: 5′-AAACTGTAAGTTCAA-CACAACAAAGG-3′) were designed based on the genomic sequences of reference strains of *T. callipaeda* (NC018363) isolated from a dog. The following cycling parameters were used: initial denaturation at 95 °C for 5 min; 35 cycles of 95 °C for 10 s, 60 °C for 30 s, and 68 °C for 1 min; and a final extension at 68 °C for 5 min. The obtained PCR products were directly sequenced using an ABI Prism BigDye Terminator v3.1 cycle sequencing ready reaction kit in an ABI Prism 3500 Genetic Analyzer (Applied Biosystems, California, USA). Multiple alignments and phylogenetic analysis of the *cox1* gene sequences of *T. callipaeda* were performed using the ClustalW and Neighbor-Joining (NJ) method,[19] respectively, in the MEGA version X software.[20] The NJ tree was derived using the Kimura 2-parameter model.[21] The tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method[22] and are in the units of the number of base substitutions per site. Statistical significance was evaluated using bootstrapping with 1000 replicates.

The sequence of DNA isolated from the sample was deposited in DDBJ/EMBL/GenBank under accession number LC790039. A 960-bp portion (102–1061) of the *cox1* gene (total length:1647 bp) was compared to the *T. callipaeda* mitochondrial DNA sequence released by the National Institutes of Health (NIH),[13] which revealed 12 nucleotide

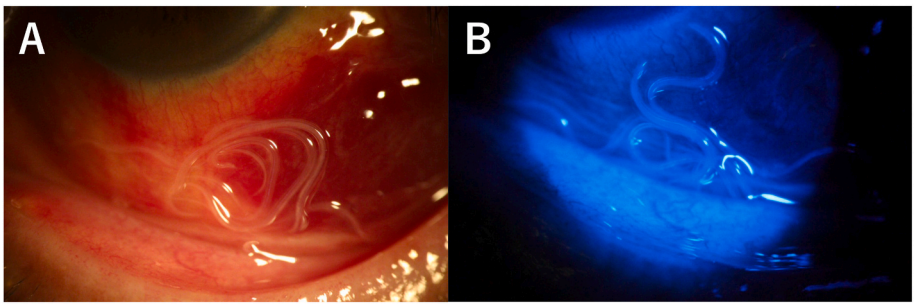

Fig. 1. Slit-lamp microscopy showing a representative photograph of the worm. A. Actively peristaltic white linear worm in inferior tarsal conjunctiva in the left eye (magnification, × 6). B. Slit-lamp microscope with blue filter showing the worm in inferior tarsal conjunctiva in the left eye (magnification, × 6).

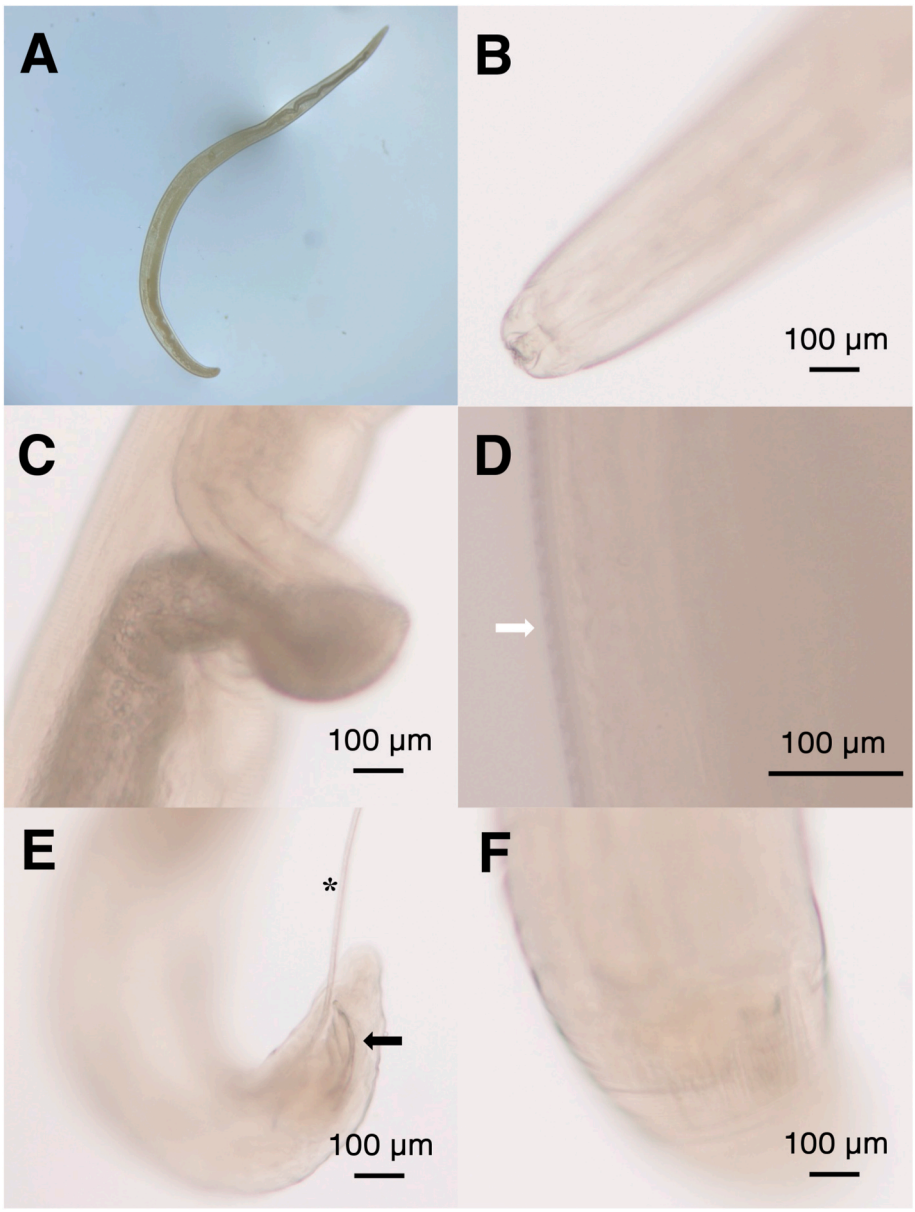

Fig. 2. Microscopic examination showing a representative photograph of *Thelazia callipaeda.* A. Photograph of a female worm body of *T. callipaeda*. B. Light microscopy image showing the anterior region of a female *T. callipaeda* with a rectangular oral cavity, which lacks teeth and lips (scale bar, 100 μm). C. Vulva located anterior to the esophago-intestinal junction in a female *T. callipaeda* (scale bar, 100 μm). D. Marked fine striations (white arrow) are seen on the body surface and serrated annulation at the edges (scale bar, 100 μm). E. Caudal region of the body of a male *T. callipaeda* presenting a long left spicule (*) and short right spicule (black arrow) (scale bar, 100 μm). F. Caudal region of a female *T. callipaeda* showing straight tail end (scale bar, 100 μm).

differences (1.33%) between them, and a match rate of 98.7%. Generally, less than 6% difference in the *cox1* gene sequence is seen in an intraspecies comparison, whereas a congeneric comparison yields a sequence difference exceeding 10%.[11] The extracted specimen was determined to be *T. callipaeda* based on genetic analysis. Notably, a 583-bp portion (309–891) of the analyzed 960-bp sequence for our specimen matched a previously reported sequence of *T. callipaeda cox1* registered in the NIH GenBank by Kumase et al.[12] in 2010 (Fig. 3, Accession number: AB538283.1). According to a subsequent analysis of this sequence by Zhang et al.,[15] it corresponds to h9 haplotype of *T. callipaeda*. Hence, it was determined that the specimen obtained from Niigata Prefecture was also an h9 haplotype.

Fig. 4 shows the phylogenetic analysis of the isolates (Tc: LC790039) and *T. callipaeda* species (Haplotypes 1–10) according to the *cox1* sequences using the NJ method[19] that were based on the partial *cox1* gene nucleotide sequences of *T. callipaeda*. The results indicated that our sample belonged to h9. Fig. S1 shows the phylogenetic analysis, in addition to that presented in Fig. 4, with an outgroup.

3. Discussion

Thelaziasis, specifically that caused by *T. callipaeda*, is a zoonotic disease frequently reported in warmer climates, in which parasites infect the conjunctival sac of the host. In this case study, we report a human thelaziasis incident in Niigata Prefecture, Hokuriku area, during the winter season. Further molecular analysis revealed the inciting parasite to be h9 *T. callipaeda*. Determination of the h9 haplotype suggested that the thelaziasis infection found in Niigata Prefecture may have originated in western Japan. Considering that thelaziasis was previously associated with warmer climates, this case raises concerns about the ongoing national distribution of *T. callipaeda*, including that in the colder regions of the country.

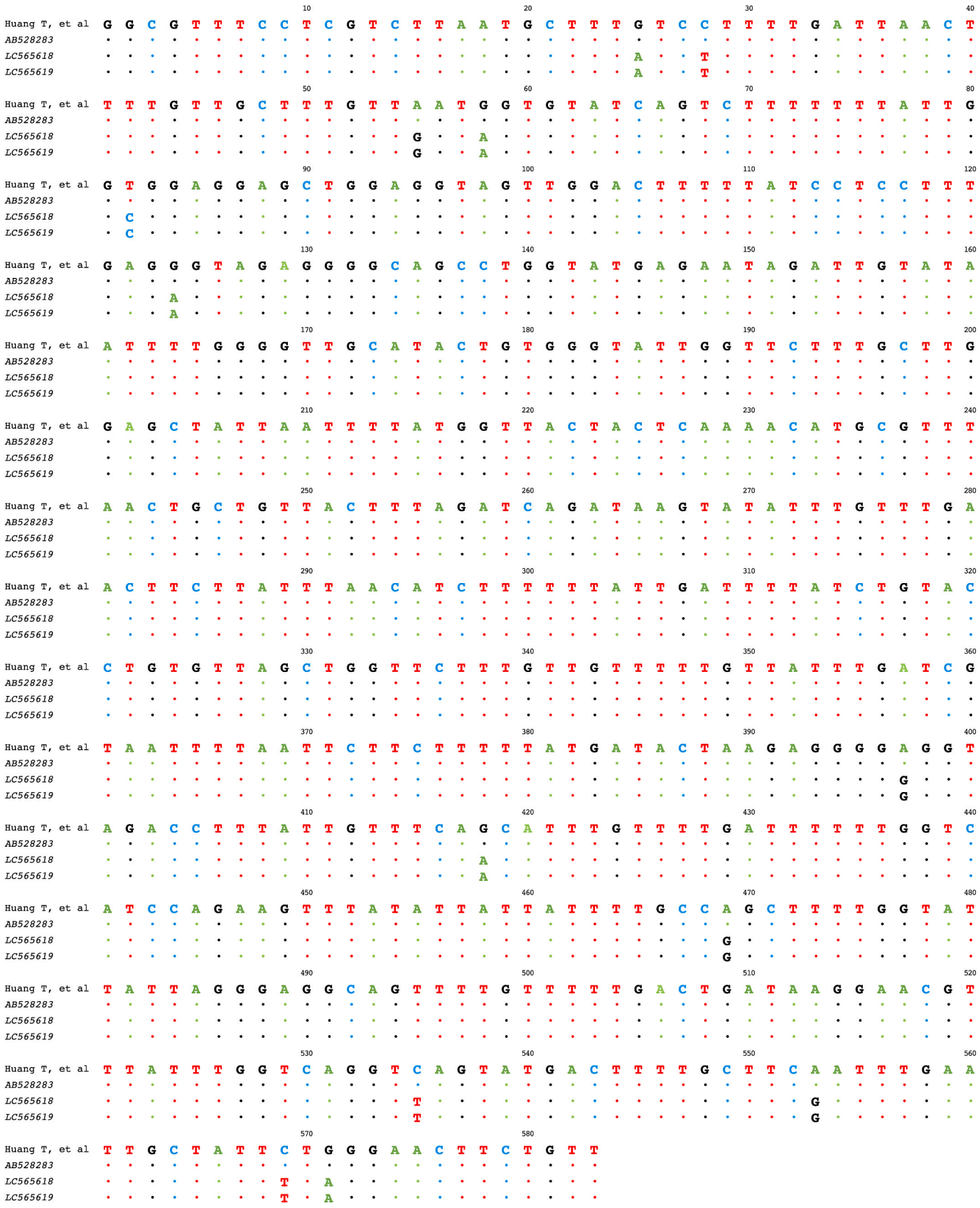

Fig. 3. Mitochondrial cytochrome C oxidase subunit 1 (*cox1*) gene sequence analysis. A 960-bp portion (102–1061) of the entire *cox1* gene (total length: 1647 bp) was sequenced. A 583-bp portion (309–891) of the analyzed 960-bp sequence from our specimen fully matched a previously reported sequence of *T. callipaeda cox1* registered in the NIH GenBank by Kumase et al.[12] in 2010 (Accession number: AB538283.1). The collected *cox1* gene sequence matched that described by Kumase et al.,[12] which was determined to be an h9 specimen. The two specimens from Ishibe et al.[16] were h10 specimens.

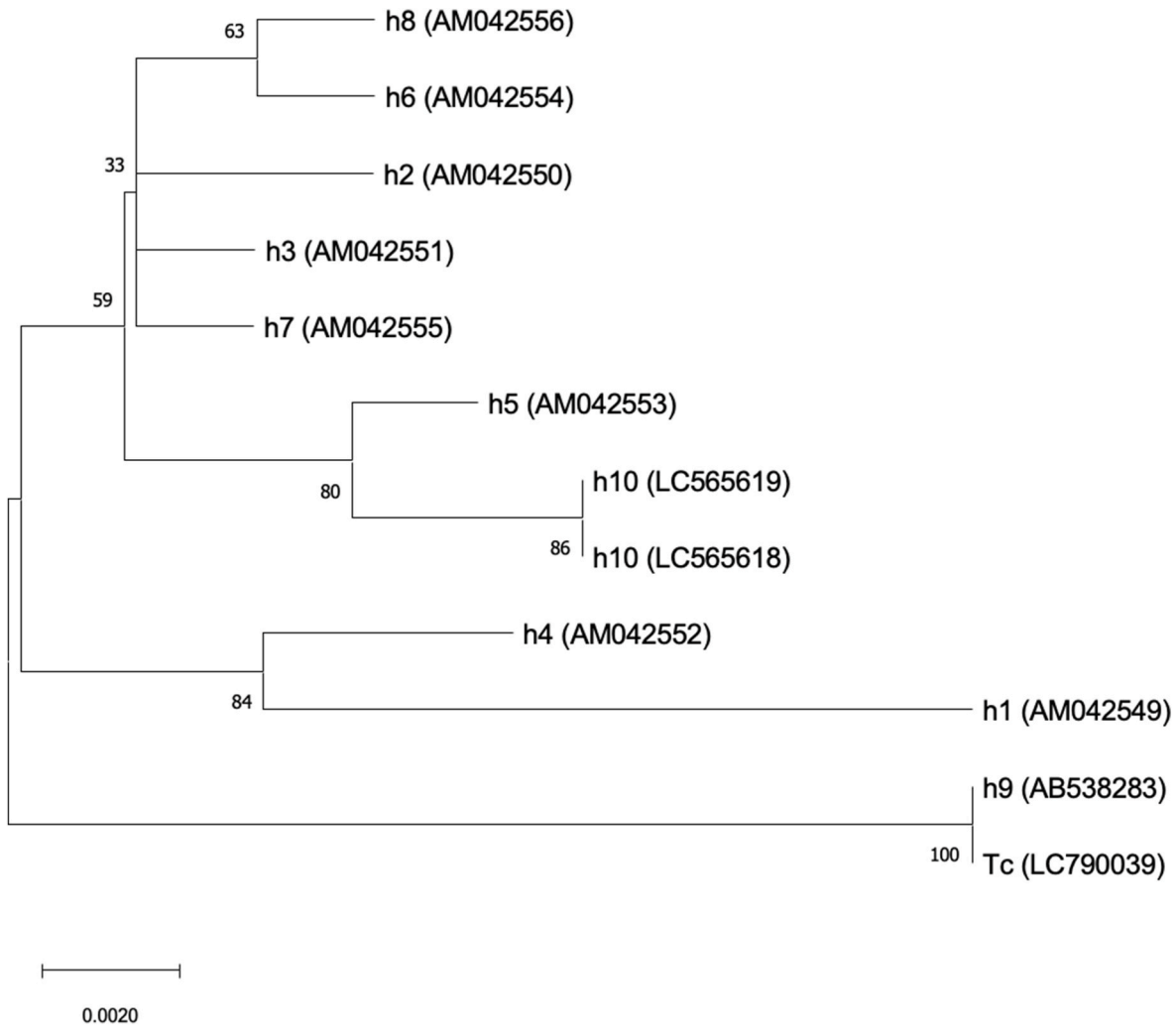

Fig. 4. Phylogenetic analysis of a partial sequence of the mitochondrial cytochrome oxidase subunit 1 (*cox1*) gene of *Thelazia callipaeda*. Phylogenetic analysis of the isolates (Tc: LC790039) and *T. callipaeda* species (Haplotype 1–10) based on *cox1* sequences. An NJ tree was derived using the Kimura-2 parameter model. Significant bootstrap support (>500) from 1000 replicates is indicated on the left of the supported nodes. The scale bar represents the evolutionary distance based on the number of changes per site. Numbers in parentheses represent GenBank accession numbers.

Phortica variegata, the intermediate host for *T. callipaeda*, exhibits high activity within the 20–25 °C temperature range,[23] which likely contributes to the association between a warm climate and thelaziasis.[24] According to the Japan Meteorological Agency,[25] the average temperatures in Okayama Prefecture during October 2008,[12] in Kumamoto Prefecture during April 2020, and in Oita Prefecture during June 2020[16] were 19.1, 14.1, and 24.0 °C, respectively; the three cases of thelaziasis were reported from these regions in the mentioned month and year (Table 1). The incident in this study was diagnosed during winter (January 2022) in Niigata Prefecture of the Hokuriku area. The annual average temperature of Niigata Prefecture was 2.5 °C in January 2022,[25] when this particular incident was diagnosed. Therefore, considering the substantially colder temperatures than those commonly associated with thelaziasis, this case study suggests that *T. callipaeda* infections can occur beyond warm seasons and locations. Additionally, the geographical distribution of *T. callipaeda* may encroach on eastern Japan, including the Hokuriku area.

Considering the h9 haplotype of *T. callipaeda* determined in this case study through PCR analysis, the likely origin of the parasite was western Japan, known for its warmer climate, which spread to the Hokuriku area, with an appreciably lower average annual temperature. The mitochondrial genome of *T. callipaeda* is circular in form, 13,668 bp in length, and contains 12 protein-coding genes, 22 transfer RNA genes, and two ribosomal RNA genes[13]; *cox1* is the most well conserved of the

Table 1
Characteristics of reported cases with haplotype identification in human ocular *Thelazia callipaeda* in Japan.

Author	Year of publication	Age, years	Sex	Residential district, prefecture	Date of onset	Average of monthly temperature, °C	No. Of worms	Haplotype
This study	2023	77	Male	Niigata	Jan. 2022	2.5	11	h9
Kumase Y. et al.[12]	2010	89	Male	Okayama	Oct. 2008	19.1	9	h9
Ishibe T. et al.[16]	2021	78	Male	Kumamoto	Apr. 2020	14.1	6	h10
Ishibe T. et al.[16]	2021	80	Male	Oita	Jun. 2020	24.0	2	h10

Abbreviation: h, haplotype.

contained genes, which is the basis for its use in *T. callipaeda* identification and haplotype determination.[13] A comprehensive population analysis of *T. callipaeda* haplotypes and their distribution by Zhang et al.[14] revealed two major classes of *T. callipaeda* populations in Japan (h10 vs. h9, 11, and 12), based on phylogenetic diversion. Currently, the majority of research on *T. callipaeda* haplotype analysis is performed in Europe and China. In contrast, there have been three known reports of *T. callipaeda* infections in Japan, followed by haplotype determination by Kumase et al.[12] (one case) and Ishibe et al.[16] (two cases). The *T. callipaeda* described by Kumase et al.[12] from Okayama Prefecture in the Chūgoku area was an h9 haplotype. Similarly, the two specimens described by Ishibe et al.[16] from Oita and Kumamoto Prefectures in the Kyushu area were both h10 haplotypes. In this case study of a patient presenting in Niigata Prefecture in the Hokuriku area with a colder climate, the parasite was determined to be the h9 haplotype, matching the haplotype reported in Okayama Prefecture. This result may indicate that the geographic origin of the inciting parasite may be western Japan, where the climate tends to be warmer than that of the rest of the country, ultimately spreading to the colder Hokuriku area.

This case report had several limitations. This study primarily focused on human infections of *T. callipaeda,* and animal cases were not considered in the discussion. However, due to the zoonotic nature of the disease, further analysis of the epidemiology of animal thelaziasis may elucidate the route of infection. Additionally, no conclusion was reached regarding how the parasite reached the conjunctival sac. However, this case warrants investigation of the current state of thelaziasis spread in both humans and animals, and we encourage researchers and clinicians to share clinical findings of thelaziasis and the characteristics of *T. callipaeda* from known cases to increase awareness and prevent the extensive spread of the disease.

4. Conclusions

In this report, we describe the clinical findings of a case of ocular *T. callipaeda* infection in the Hokuriku area, which is known for its cold climate. Molecular analysis through PCR of the specimen suggested that it originated from west Japan, which has a warm climate, raising concerns about the widening distribution of *T. callipaeda* in Japan.

5. Patient consent

We obtained written informed consent from the patient, and can provide this consent upon request.

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Authorship

All authors attest that they meet the current ICMJE criteria for Authorship.

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Intellectual property

We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property.

Research ethics

Written consent to publish potentially identifying information, such as details or the case and photographs, was obtained from the patient(s) or their legal guardian(s).

CRediT authorship contribution statement

Tianxiang Huang: Writing – review & editing, Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Takenori Inomata:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Jaemyoung Sung:** Writing – review & editing, Writing – original draft. **Naoko Yoshida:** Writing – review & editing, Resources, Methodology, Formal analysis. **Gaku Ishida:** Writing – review & editing, Writing – original draft, Data curation. **Hitomi Ohara:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation. **Masahiro Yamaguchi:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation. **Yasutsugu Akasaki:** Writing – review & editing, Writing – original draft. **Yuichi Okumura:** Writing – review & editing, Writing – original draft. **Ken Nagino:** Writing – review & editing, Writing – original draft. **Kunihiko Hirosawa:** Writing – review & editing, Writing – original draft. **Toshihiro Mita:** Writing – review & editing, Writing – original draft, Supervision. **Shintaro Nakao:** Writing – review & editing, Writing – original draft, Supervision. **Nobuo Ishida:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial 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.ajoc.2024.102030.

References

1. Otranto D, Lia RP, Buono V, Traversa D, Giangaspero A. Biology of Thelazia callipaeda (Spirurida, Thelaziidae) eyeworms in naturally infected definitive hosts. *Parasitology*. 2004;129:627–633.
2. Yagi T, Sasoh M, Kawano T, Ito K, Uji Y, Ando K. Removal of Thelazia callipaeda from the subconjunctival space. *Eur J Ophthalmol*. 2007;17:266–268.
3. Otranto D, Dutto M. Human thelaziasis, Europe. *Emerg Infect Dis*. 2008;14:647–649.
4. Martínez-Sánchez MI, Bolívar-de-Miguel G, Cuadros-González J, Rubio González JM. Ocular thelaziosis: a case report of an emerging zoonosis. *Am J Ophthalmol Case Rep*. 2021;22, 101045.
5. Stuckey EJ. CIRCUMOCULAR FILARIASIS. *Br J Ophthalmol*. 1917;1:542–546.
6. Otranto D, Dantas-Torres F. Transmission of the eyeworm Thelazia callipaeda: between fantasy and reality. *Parasites Vectors*. 2015;8:273.
7. Otranto D, Dantas-Torres F, Brianti E, et al. Vector-borne helminths of dogs and humans in Europe. *Parasites Vectors*. 2013;6:16.
8. Pendones Ulerio J, Redero Cascón M, Parra Morales AM, Muñoz Bellido JL, Ávila Alonso A. Ocular thelaziosis: an emerging human parasitosis in Europe. *Eur J Clin Microbiol Infect Dis*. 2023;42:793–796.
9. Colella V, Kirkova Z, Fok É, et al. Increase in eyeworm infections in eastern Europe. *Emerg Infect Dis*. 2016;22:1513–1515.

10. Hagiwara T, Kusumoto T, Murakami K, Matsusita F, Utida K. Two cases of nematodes excised from human conjunctiva. *J Kumamoto Med Soc*. 1957;31:179–183.
11. Otranto D, Testini G, De Luca F, Hu M, Shamsi S, Gasser RB. 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*. 2005;19:306–313.
12. Kumase Y, Kato M, Takabatake M, Nakayama T, Ogihara N, Hasegawa H. A case of infestation of *Thelazia callipaeda* in the conjunctival sac with nine worms in one eye. *Jpn J Ophthalmol*. 2010;64:1747–1750.
13. Liu GH, Gasser RB, Otranto D, et al. Mitochondrial genome of the eyeworm, Thelazia callipaeda (Nematoda: spirurida), as the first representative from the family Thelaziidae. *PLoS Neglected Trop Dis*. 2013;7, e2029.
14. Zhang X, Shi YL, Wang ZQ, et al. Morphological and mitochondrial genomic characterization of eyeworms (Thelazia callipaeda) from clinical cases in Central China. *Front Microbiol*. 2017;8:1335.
15. Zhang X, Shi YL, Han LL, et al. Population structure analysis of the neglected parasite Thelazia callipaeda revealed high genetic diversity in Eastern Asia isolates. *PLoS Neglected Trop Dis*. 2018;12, e0006165.
16. Ishibe T, Hino S, Kishi D, et al. Haplotype analysis of two cases of conjunctival infestation with *Thelazia callipaeda*. *Jpn J Ophthalmol*. 2021;75:1026–1030.
17. Juhász H, Thury G, Szécsényi M, et al. Human thelaziosis caused by Thelazia callipaeda eyeworm, Hungary. *Emerg Infect Dis*. 2022;28:2559–2561.
18. Morgado ACT, do Vale B, Ribeiro P, et al. First report of human Thelazia callipaeda infection in Portugal. *Acta Trop*. 2022;231, 106436.
19. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol*. 1987;4:406–425.
20. Kumar S, Stecher G, Li M, Knyaz C, Tamura K, Mega X. Molecular evolutionary genetics analysis across computing platforms. *Mol Biol Evol*. 2018;35:1547–1549.
21. Kimura M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol*. 1980;16:111–120.
22. Tamura K, Nei M, Kumar S. Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proc Natl Acad Sci U S A*. 2004;101:11030–11035.
23. Otranto D, Brianti E, Cantacessi C, Lia RP, Máca J. The zoophilic fruitfly Phortica variegata: morphology, ecology and biological niche. *Med Vet Entomol*. 2006;20:358–364.
24. Une K, Iwamoto K, Shiraki K, Teramoto-Kimiata I. A case of *Thelazia callipaeda* found with foreign body sensation. *Folia Ophthalmol Jpn*. 2013;6:630–632.
25. Japan Meteorological Agency. https://www.data.jma.go.jp/gmd/risk/obsdl/.