

Detection of Two Patients, who were Infected by Eye *Thelazia callipaeda* in Vietnam 2017

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Abstract

Parasitic zoonoses are particularly important infections transmitted between animals and humans, distributed over the world including Vietnam. Among these zoonotic parasites, many species have been for the first time reported in Vietnam in recent years. One of these species is *Thelazia callipaeda*, which parasitized in the human eye and was reported the first time in 2012. This paper is a report of two cases, including a child of 7 months old in Dien Bien province and a 50 year old woman in Bac Giang province. Both provinces are in the North-mountainous area in Vietnam. These nematodes have been identified based on morphological characterization and verified by molecular and phylogenetic analysis also as *Thelazia callipaeda* in Thelaziidae family.

Keywords: *Thelazia callipaeda*; Oriental Eyeworm; Eye; Human Case; Vietnam

Introduction

Thelazia callipaeda Railliet and Henry, 1910 (Nematoda; Thelaziidae) is a nematode parasite in the genus *Thelazia* [1]. Thelaziasis caused by this nematodes in the eyes. This nematode was detected from a dog in Pakistan in 1910. The later it was reported in some countries as China, Burma, Korea, Japan, France, Germany, India, Indonesia, Italy, the Netherlands, Russia, Switzerland, Taiwan, Thailand, and Myanmar. This nematode was found from human first in China 1917, and later also from India, Thailand, Korea and others [1-3]. In Japan, it was discovered from the dog in 1956 and from human in 1957, and up to 1981 with a total of 30 reported human cases [1]. In Korea, 39 human cases were reported until 2011, and a total of 146 adult worms were collected from patients [4]. *Thelazia* parasite in definitive hosts, which were mainly dogs and cats, and occasionally in rabbits, monkeys, raccoons, dogs, foxes, wolves and humans [5]. Insects as flies were intermediate hosts of *Thelazia* [6]. It is relatively well recognized that drosophilid flies, other than *Musca domestica*, *Amiota okadai*, *A. magna* and *A. nagatai* are the vector hosts for *T. callipaeda* [7]. When a tear-feeding fly feeds, it ingests the *T. callipaeda* larvae. In fly, the infective larva gradually leaves the genital organs for the head and hide in a space between mouth parts. When the fly licks the tear in the eye of the definitive host, including humans, the larvae from fly enter the conjunctival sac, eyelid, tear glands, or tear ducts, and become adults in one month [1,7]. *Thelazia* infection in the eye is caused some symptoms include conjunctivitis, excessive watering, visual impairment, and ulcers or scarring of the cornea [1]. But in some cases, the only symptom is the presence of worms obscuring the host's vision as a floater [3].

In Vietnam, some cases were reported by the nematode infection in the human eyes as *Dirofilaria repens*, *Angiostrongylus cantonensis*, *Toxocara canis*, the larvae of *Taenia solium* and the first case of *Thelazia callipaeda* (2012) [8-10]. In this study, we report two cases, from which the worms were identified species by morphology and molecular method using the mitochondrial genome, including a child who is the youngest patient of human *T. callipaeda* infection in Vietnam, and an adult case.

Materials and Methods

Description cases: The main symptoms of the patients were described.

Parasite samples: Samples of worms from the eyes of two patients were collected.

Identification species: Identification for species used morphological method and molecular method with cytochrome C oxidase subunit 1 (Cox1) gene.

DNA isolation

For DNA isolation performance, Qiagen kits (DNeasy Blood and Tissue Kit - Qiagen Sciences, Maryland, USA) were used for the extraction of total DNA from *Thelazia callipaeda* in accordance with the manufacturer's protocol. In brief, worms were resuspended in the 100 µl of manufacturer's lysis buffer (ATL [> 8 mM EDTA, $> 0.5\%$ SDS]) containing 20 µl proteinase K and incubate at 56°C for 30 minutes, thereafter add 4 µl RNase and 200 µl AL buffer and treated in accordance with the manufacturer's protocol (for a microfuge scale preparation).

PCR amplification of the Cox1 of mitochondrial genome

PCR for amplification of the 664 nucleotides of the Cox1 of *Thelazia* was performed in a 50 µl volume. PCR reactions were performed in 10 mM Tris-HCl, pH 8.4, 50 mM KCl; 3.0 mM MgCl₂, 250 µM each of dATP, dCTP, dGTP and dTTP; 50 pmol of each primer with 1 U Taq polymerase (Promega). The following primers were used in separate reaction mixes: NTF (5'- TGATTGGTGGTTTTGGTAA -3') and NTR (5'- ATAAGTACGAGTATCAATATC -3') (Hany, *et al.* 2015). Amplification was conducted for 40 cycles using the primer set NTF/NTR. Cycling was performed in a Proflex Cycler (Applied Biosystem) using the following parameters: initial denaturation at 95°C for 15 minutes, followed by 40 cycles of 95°C for 30s (denaturation), 48°C for 1 minute (annealing) and 72°C for 30s (extension), followed by a final extension at 72°C for 5 minutes.

Sanger sequencing

Dideoxy sequencing was performed using BigDye™ Terminator Chemistry v. 3.1 (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions, as described previously. Forward and reverse primers for *Thelazia callipaeda* were used as sequencing primers (section 2.3) using the ABI 3130 Bioanalyzer (Applied Biosystems).

Phylogenetic analysis

DNA sequences were processed and compared amino acid and nucleotide homology by ATGC software versions 7.0.2 and Clustal W. The phylogeny was constructed using MEGA software with the neighbor-joining toolbar and calculating distance evolution by bootstrapping.

Results

Case 1

The first patient was a 7 month old male residing in the Hoi Len commune, Muong Cha district, Dien Bien Province of mountainous North Vietnam. In May 2017, he felt a bulge-sticking pain in his left eye and his mother collected five small worms but did not keep them. Then his mother took him to the Dien Bien Hospital and a medical doctor collected two more worms.

Case 2

The second patient was a 50 year old female residing in the Tan Thanh commune, Bac Quang district, Ha Giang Province of mountainous North Vietnam. In November 2017, she felt a bulge-sticking pain in her right eye and her son collected eight small worms.

The worms from two patients were identified by morphology and confirmed by molecular method. The male worm was thin, long, and cylindrical with milk-white color with 12 mm in length and 0.4 mm in width, with a curled tail end (Figure 1); and the female worms were similar morphology but longer than the male (15 mm in length and 0.5 mm in width) and a straight tail end (Figure 2). The buccal cavity was 0.06 mm in length and 0.05 in width; the esophagus was 1.1 mm in length. Comparison with the figure described as *Thelazia callipaeda* in the "Book of Helminthic Zoonoses" by Miyazaki [1] showed that these worms were *Thelazia callipaeda* (Nematoda; Thelaziidae).

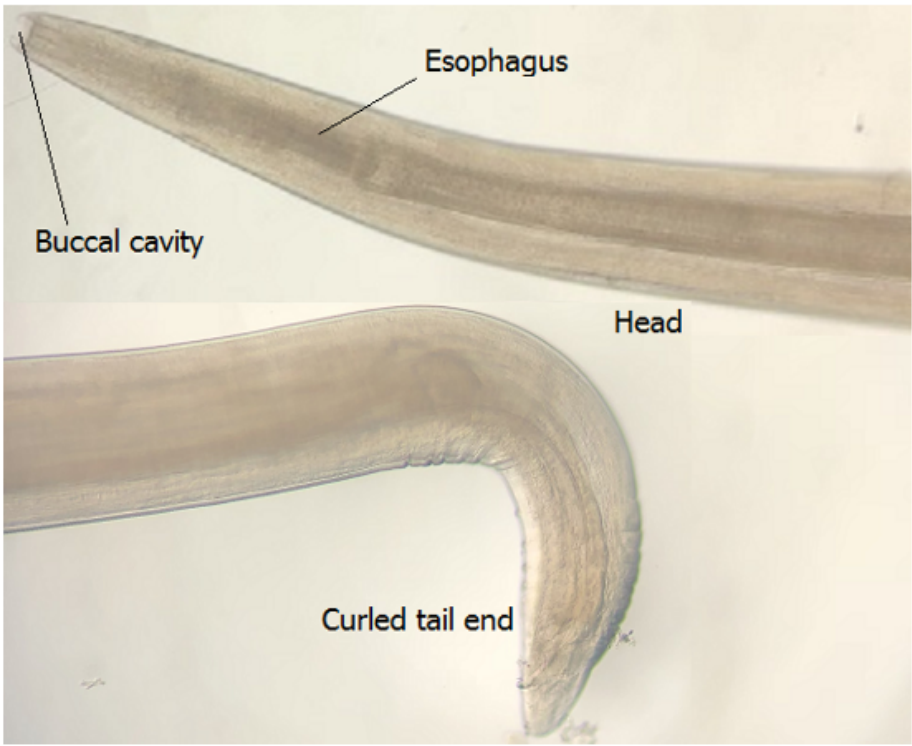


Figure 1: Morphology of the *Thelazia callipaeda* male from patients

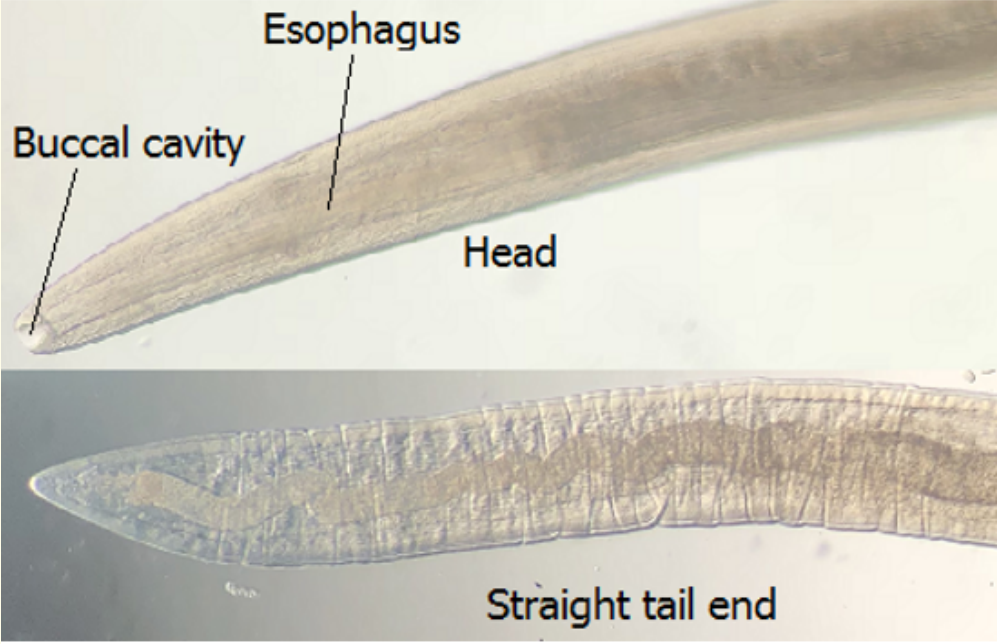


Figure 2: Morphology of the *Thelazia callipaeda* female from patients.

These worms were identified as *Thelazia callipaeda* by molecular method using Cox1 with 99% homology compared with *Thelazia callipaeda* in GenBank (Table 1, 2 and Figure 3). Phylogenetic tree of *Thelazia callipaeda* Vietnam and other strains from part of Cox1 nucleotide sequence estimated by Neighbor-Joining (NJ) using MEGA5.1 (Tamura, et al. 2004) showed that strain of Vietnamese *Thelazia callipaeda* in one group with strains of *Thelazia callipaeda* in GenBank (Figure 4).

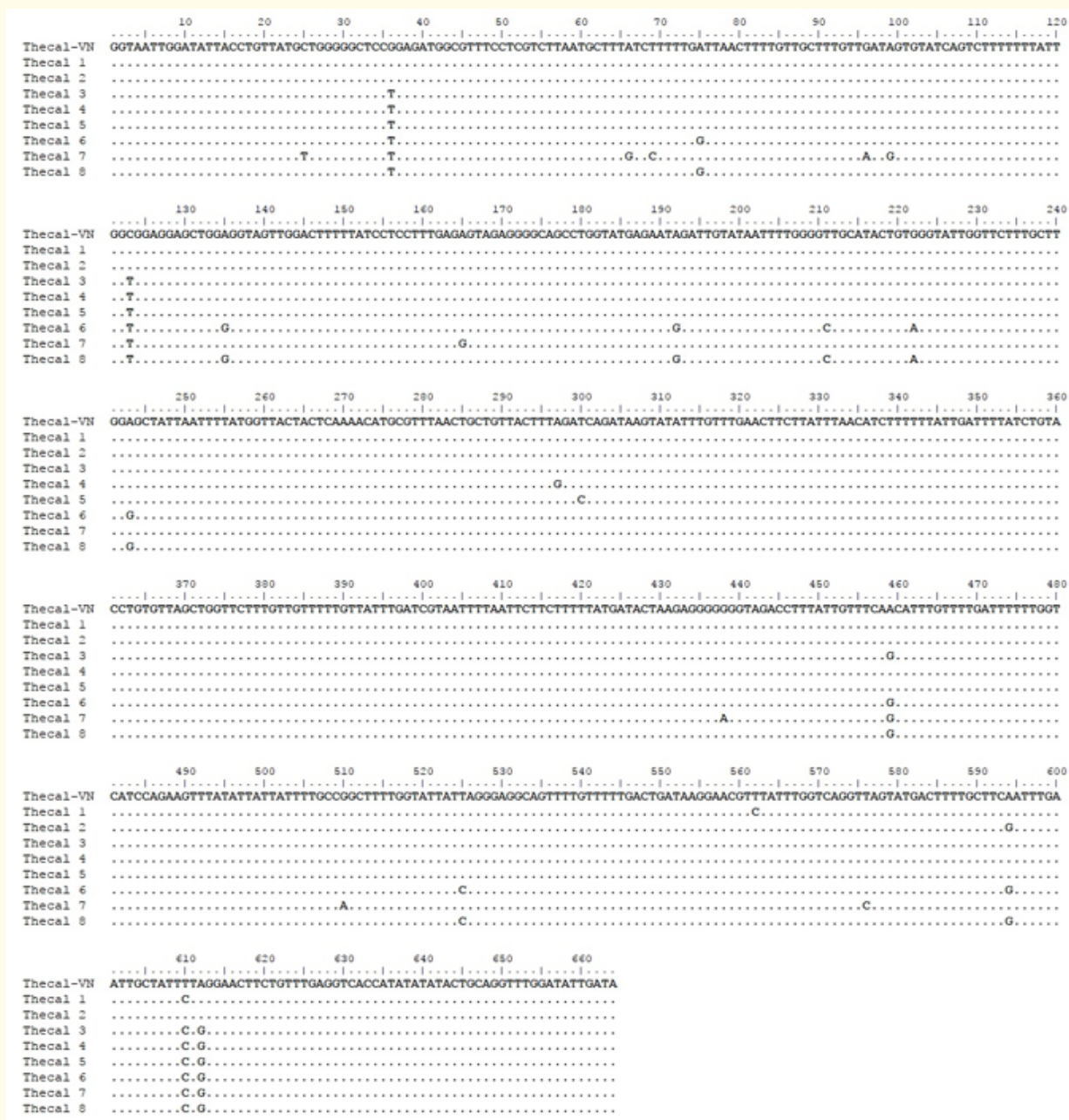


Figure 3: Comparison of 664 nucleotide of portion cytochrome C oxidase subunit 1 (cox1) gene between Vietnamese *Thelazia callipaeda* and other species of *Thelazia* family Morphology of *Thelazia callipaeda*.

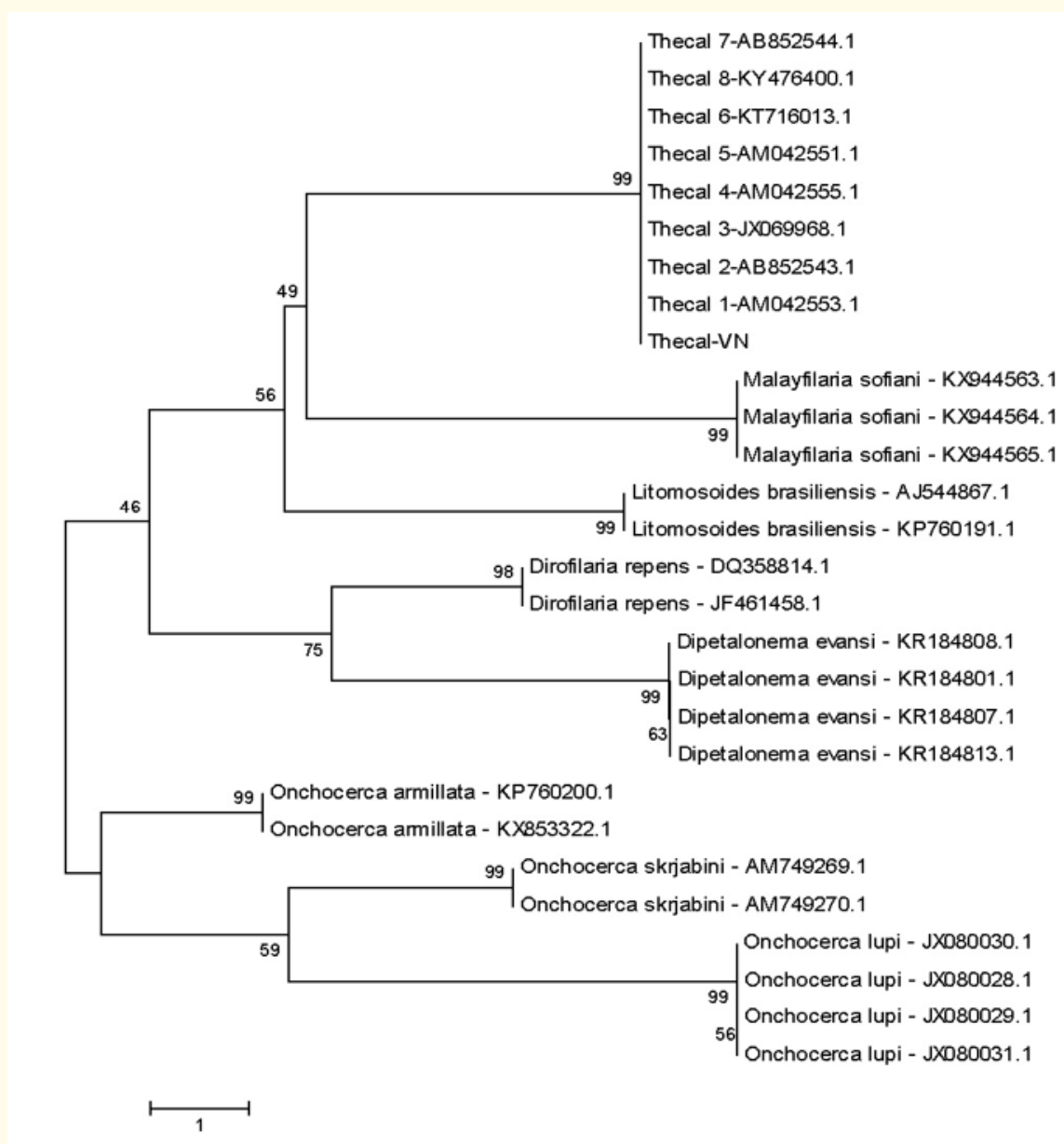


Figure 4: Phylogenetic tree of *Thelazia callipaeda* Vietnam and other strains from *cox1* gene nucleotide sequence estimated by Neighbor-Joining (NJ) using MEGA5.4 (Tamura et al., 2004). Note: Thecal-VN = Vietnamese *Thelazia*; other *T. callipaeda* (GenBank number: AM042553.1, AB852543.1, JX069968.1, AM042555.1, AM042551.1, KT716013.1, AB852544.1, and KY476400.1 respectively). *Malayfilaria sofiani* are from Malaysia (GenBank number: KX944563.1, KX944564.1, and KX944565.1), *Litomosoides brasiliensis* are from Brazil (GenBank number: AJ544867.1 and KP760191.1). *Dirofilaria repens* (GenBank number: DQ358814.1 and JF461458.1). *Dipetalonema evansi* are from Iran (GenBank number: KR184801.1, KR184807.1, KR184808.1, and KR184813.1). *Onchocerca armillata* (GenBank number: KP760200.1 and KX853322.1). *Onchocerca skrjabini* are from Japan (GenBank number: AM749269.1 and AM749270.1). *Onchocerca lupi* are from USA (GenBank number: JX080028.1, JX080029.1, JX080030.1, and JX080031.1).

Notation	Origin	Host	Length	Species	GeneBank	Author
Thecal-VN	VietNam	Human	664 bp	<i>Thelazia callipaeda</i>		This study
Thecal 1	China	-	664 bp	<i>Thelazia callipaeda</i>	AM042553	Otranto, <i>et al.</i> 2016
Thecal 2	Japan	Canis	664 bp	<i>Thelazia callipaeda</i>	AB852543	Yoshikawa, <i>et al.</i> 2013
Thecal 3	China	Dog	664 bp	<i>Thelazia callipaeda</i>	JX069968	Liu., <i>et al.</i> 2013
Thecal 4	China	-	664 bp	<i>Thelazia callipaeda</i>	AM042555	Otranto, <i>et al.</i> 2016
Thecal 5	China	-	664 bp	<i>Thelazia callipaeda</i>	AM042551	Otranto, <i>et al.</i> 2016
Thecal 6	Rumania	Canis	664 bp	<i>Thelazia callipaeda</i>	KT716013	Ionita., <i>et al.</i> 2016
Thecal 7	Japan	Canis	664 bp	<i>Thelazia callipaeda</i>	AB852544	Yoshikawa, <i>et al.</i> 2013
Thecal 8	Slovakia	Canis	664 bp	<i>Thelazia callipaeda</i>	KY476400	Kabanova., <i>et al.</i> 2017

Table 1 : Sequencing of the portion cytochrome C oxidase subunit 1 (*cox1*) of different *Thelazia* species from GenBank compared with *Thelazia callipaeda** in Vietnam.

Note: *Result after study

	ThecalVN	Thecal I	Thecal 2	Thecal 3	Thecal 4	Thecal 5	Thecal 6	Thecal 7	Thecal 8
ThecalVN	99	99	99	99	99	99	99	99	99
Thecal 1	99	99	99	99	99	99	99	99	99
Thecal 2	99	99	99	99	99	99	99	99	99
Thecal 3	99	99	99	99	99	99	99	99	99
Thecal 4	99	99	99	99	99	99	99	99	99
Thecal 5	99	99	99	98	99	99	99	99	99
Thecal 6	99	99	99	99	99	99	99	99	99
Thecal 7	98	98	98	98	98	98	98	98	98
Thecal 8	98	98	98	98	98	98	98	98	98

Table 2 : Percentage of identity of nucleotide of *cox1* sequences of Vietnamese *Thelazia callipaeda* and others *Thelazia* in GenBank.

Note: ThecalVN is Vietnamese *Thelazia*; Thecal1, Thecal3, Thecal4, Thecal5 are China *Thelazia callipaeda* (GenBank number: AM042553, JX069968, AM042555 and AM042551); Thecal2, Thecal7 are from Japan (GeneBank number: AB852543 and AB852544), Thecal6 is from Rumania (GeneBank number: KT716013), Thecal8 is from Slovakia (GeneBank number: KY476400).

Discussion

The first human case of thelaziasis was reported in Vietnam in 2012 [8] with a 26-year-old man in the mountainous area of Northern Vietnam. This report included two cases in 2017, who also from the mountainous area of the country. This report, the first case was a 7 months old child and second case was 50 years old woman, and collected 7 and 8 small worms, respectively. The worms were parasitic in the conjunctival sac of an eye of two these cases similar to a 26-year-old man reported in 2012. The clinical symptoms of these case also similar as felt a bulge-sticking pain in their left or right eye and they cried or felt uncomfortable all day. The total number of worms collected from the patients was morphologically identified as *T. callipaeda*. With molecular method by sequencing of the portion cytochrome C oxidase subunit 1 (*cox1*) of different *Thelazia callipaeda* species from GenBank compared with *Thelazia callipaeda* in Vietnam were done. The nucleotide sequences of the *cox1* gene of our Vietnamese worms were compared with those of 8 isolates of *T. callipaeda* reported in GenBank (4 from China, 2 from Japan, 1 from Rumania and 1 from Slovakia) (Table 1). The results showed that there were 1 - 14 places of nucleotide differences in this study isolate with others. Thus, the nucleotide homology between the Vietnamese isolate and 8 other isolates in the world was 98% - 99%. Therefore, the worms from two cases were identified by morphology and molecular methods as *Thelazia callipaeda*. Although the flies as intermediate hosts of *T. callipaeda* in the Drosophilidae are very common in Vietnam, *T. callipaeda* is very rare in the country (there were three cases up to now). However, this information is less important for communities, but may be of more importance to treating doctors and epidemiologists [11].

Conclusion

In conclusion, two cases were infected by eye *Thelazia callipaeda* in 2017, which were identified by morphology and molecular method.

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