

Eyeworms of wild birds and new record of *Thelazia* (*Thelaziella*) *aquilina* (Nematoda: Spirurida)

Renata Fagundes-Moreira^{a,b,c}, Marcos Antonio Bezerra-Santos^b, Riccardo Paolo Lia^b,
Cíntia Daudt^d, Juliana Tessália Wagatsuma^d, Elaine Christina Oliveira de Carmo^e,
Laura Berger^a, Flavio Roberto Chaves da Silva^d, João Fabio Soares^a, Domenico Otranto^{b,f,*}

^a Laboratório de Protozoologia e Rickettsioses Vetoriais, Faculty of Veterinary, Federal University of Rio Grande Do Sul (UFRGS), Porto Alegre, Brazil

^b Department of Veterinary Medicine, University of Bari, Valenzano, Italy

^c Department of Public Health, Experimental and Forensic Medicine, University of Pavia, Pavia, Italy

^d Laboratório de Virologia e Parasitologia, Veterinary Medicine Course, Universidade Federal do Acre (UFAC), Rio Branco, Brazil

^e Centro de Triagem de Animais Silvestres (CETAS), IBAMA- Rio Branco, Acre, Brazil

^f Department of Veterinary Clinical Sciences, City University of Hong Kong, Hong Kong

ARTICLE INFO

Keywords:
Eyeworms
Thelaziosis
Thelaziella
Wild birds

ABSTRACT

The eyeworms of the subgenus *Thelaziella* infect orbital cavities, conjunctival sacs and lachrymal ducts of many wild birds, being able to cause conjunctivitis. In Brazil, at least 10 species of *Thelaziella* have been described. Here we present a brief review of cases of *Thelazia* (*Thelaziella*) *aquilina* and describe the infection of this parasite in *Harpia harpyja*, as a new host record. Two nematodes were collected from the surface of the cornea of an adult female *H. harpyja* in the Amazon region (Brazil) and were morphologically identified as two males of *T. (T.) aquilina*. This data demonstrated the need for further studies focused on elucidating the transmission, biological cycle, and phylogenetic positioning of *Thelaziella* as subgenus.

1. Introduction

Eyeworms of the genus *Thelazia* (Family Thelaziidae) are transmitted by non-biting flies that feed on ocular secretions of different animal species, including humans (Otranto and Traversa, 2005; Vale et al., 2019). First-stage larvae (L1) ingested with lachrymal secretions undergo through two development stages becoming infective third-stage larvae (L3), eventually being transmitted by the insects while feeding on ocular secretions of a receptive host. Eyeworm infestations can result in different degrees of ocular symptoms (e.g., irritation, redness, tearing, visual disturbances and even corneal ulceration and blindness) according to the parasitic burden as well as to host susceptibility and the occurrence of concomitant bacterial infection (Otranto and Traversa, 2005; Otranto et al., 2021).

The genus *Thelazia* includes subgenera *Thelazia* and *Thelaziella*, which are differentiated based on the presence of the gubernaculum in *Thelaziella* spp. (Chabaud, 1975). Additionally, while nematode species in the subgenus *Thelaziella* (Travassos, 1918) parasitize the orbital cavities, conjunctival sacs, and lachrymal ducts of wild birds (Chabaud

1975; Barus et al., 1978), those in the *Thelazia* infect the eyes and annexed tissues of many domestic and wild mammalian species, including humans (Otranto and Traversa, 2005; Bezerra-Santos et al., 2022a). *Thelazia* spp. primarily develop in the celomatic cavity of muscid flies (Diptera: Muscidae), or of drosophilids of the subfamily Steganinae, in the case of *Thelazia callipaeda* (Otranto et al., 2006; Bezerra-Santos et al., 2022b). Conversely, no information is available about the life cycle of *Thelaziella*. Species of this eyeworm subgenus infect birds throughout many countries, including Japan (Murata and Asakawa 1999), India (Majumdar and Sarkar, 1989), Russia (Daiya, 1968), and mainly Brazil (Pinto et al., 1996; Rodrigues and Rodrigues, 1970). These parasites have been reported in a range of wild bird species (Table 1), within the families Accipitridae, Ciconiidae, Pandionidae, and Falconiiformes (Anderson and Diaz-Ungria, 1959).

Among the wild birds, the Harpy Eagle (*Harpia harpyja*) is one of the world's largest raptors, with a decreasing population in the South America (Brown and Amadon, 1968). The existence of this bird species is threatened by the anthropogenic activities and illegal hunting (Girald-Amaya et al., 2021; Birdlife International, 2021). In this study, we

* Corresponding author. Department of Veterinary Medicine, University of Bari, Valenzano, Italy.

E-mail address: domenico.otranto@uniba.it (D. Otranto).

<https://doi.org/10.1016/j.ijppaw.2024.100910>

Received 14 January 2024; Received in revised form 25 January 2024; Accepted 25 January 2024

Available online 4 February 2024

2213-2244/© 2024 Published by Elsevier Ltd on behalf of Australian Society for Parasitology. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Table 1
Species of *Thelazia* (*Thelaziella*) spp. reported in wild birds from Brazil.

Species	Wild bird species	Reference
<i>Thelazia</i> (<i>Thelaziella</i>) <i>anobialata</i>	Hyacinth Macaw (<i>Anodorhynchus hyacinthinus</i>), Rusty-margined Guan (<i>Penelope superciliaris</i>), Black-necked Araçari (<i>Pteroglossus aracari</i>), Red-billed Toucan (<i>Ramphastos tucanus</i>), Bare-faced Curassow (<i>Crax fasciolata</i>), Chaco Chachalaca (<i>Ortalis canicollis</i>)	Skrjabin et al. (1967); Rodrigues and Rodrigues (1970)
<i>Thelazia</i> (<i>Thelaziella</i>) <i>anadorhynchi</i>	Hyacinth Macaw (<i>A. hyacinthinus</i>)	Strachan (1957)
<i>Thelazia</i> (<i>Thelaziella</i>) <i>aquilina</i>	Bicolored Hawk (<i>Accipiter bicolor</i>), Great Black Hawk (<i>Buteogallus urubitinga</i>), Yellow-headed Caracara (<i>Milvago chimachima</i>), Southern Caracara (<i>Caracara plancus</i>), Ornate Hawk-eagle (<i>Spizaetus ornatus</i>)	Strachan (1957); Pinto et al. (1994); Rodrigues and Rodrigues (1970, Rodrigues, 1992)
<i>Thelazia</i> (<i>Thelaziella</i>) <i>buteonis</i>	Blue-throated Piping-guan (<i>Pipile cumanensis</i>)	Strachan (1957)
<i>Thelazia</i> (<i>Thelaziella</i>) <i>campanulata</i>	Roadside Hawk (<i>Rupornis magnirostris</i>), Brazilian Tinamou (<i>Crypturellus strigulosus</i>)	Molin (1858); Travassos (1918)
<i>Thelazia</i> (<i>Thelaziella</i>) <i>cholodkowskii</i>	Tropical Screech-owl (<i>Megascops choliba</i>)	Strachan (1957)
<i>Thelazia</i> (<i>Thelaziella</i>) <i>chungkingensis</i>	Alagoas Curassow (<i>Mitu</i>)	Strachan (1957)
<i>Thelazia</i> (<i>Thelaziella</i>) <i>dacelonis</i>	Black-tailed Trogon (<i>Trogon melanurus</i>)	Strachan (1957)
<i>Thelazia</i> (<i>Thelaziella</i>) <i>papillosa</i>	Crane Hawk (<i>Geranoospiza caerulescens</i>)	Molin (1860)
<i>Thelazia</i> (<i>Thelaziella</i>) <i>pittae</i>	Black-and-white Hawk-eagle (<i>Spizastur melanoleucus</i>)	Strachan (1957)
<i>Thelazia</i> (<i>Thelaziella</i>) <i>sicki</i>	Owel (<i>Otus</i> sp.)	Strachan (1957); Rodrigues (1996)
<i>Thelazia</i> (<i>Thelaziella</i>) <i>tonkinensis</i>	Amazonian Umbrellabird (<i>Cephalopterus ornatus</i>)	Strachan (1957)
<i>Thelazia</i> (<i>Thelaziella</i>) sp.	Red-billed Curassow (<i>Crax blumenbachii</i>), Toco Toucan (<i>Ramphastos toco</i>), Roadside Hawk (<i>R. magnirostris</i>), Yellow-headed Caracara (<i>M. chimachima</i>), Chaco Chachalaca (<i>O. canicollis</i>), Grey-headed Kite (<i>Leptodon cayanensis</i>), Grey-lined Hawk (<i>Buteo nitidus</i>), and Turquoise-fronted Amazon (<i>Amazona aestiva</i>)	Travassos et al. (1939); Travassos (1941, 1942); Pinto et al. (1993)

identify *Thelazia* (*Thelaziella*) *aquilina* in a *H. harpyja*, establishing a new host record. Additionally, we review and discuss the occurrence of these eyeworms in wild birds in Brazil.

2. Materials and methods

An adult female Harpy Eagle was rescued after being shot in the municipality of Rio Branco, Acre State, Brazil, in the Amazon region (Latitude 9° 58'26.49"S Longitude 67° 48'35.35"O). During the clinical examination, two nematodes were collected from the corneal surface (Fig. 1; Supplementary Material 1). The nematodes were cleared in lactophenol solution and observed for morphometric examination in optical microscopy (Zeiss Axioscope) with Zen Pro version 3.1 software (Carl Zeiss Microscopy GmH, Germany).

3. Results and discussion

The nematodes were male, whitish with a cylindrical body attenuated at both ends (Fig. 2a and b). The cuticle exhibited transverse striations evenly distributed, forming annulations (Fig. 2c and d). The specimens featured a slightly ventrally curved posterior end with copulatory organs, and spicules that were notably unequal and structurally dissimilar (Fig. 3a and b). The right spicule was thicker and shorter than the left, with the presence of a gubernaculum, which is a peculiar characteristic of the subgenus *Thelaziella* (Fig. 3c). At the caudal end, numerous papillae were located on the ventral side (Fig. 3d), with the posterior end curved ventrally and terminating in a conic tail. Detailed measurements of adults are provided in Table 2 and compared with both male and female specimens of this subgenus.

The description of *T. (T.) aquilina* in the eye of a *H. harpyja* from Brazil represents a new host record for this parasite. Wild birds do not commonly develop ocular lesions, though conjunctivitis was reported in *Ciconia boyciana* (Murata and Asakawa, 1999). Herein, the reported Harpy Eagle did not present any ocular lesion. The taxonomic classification of *Thelaziella* remains unclear. While the Interim Register of Marine and Nonmarine Genera (IRMNG) does not acknowledge it as a



Fig. 1. *Thelazia* (*Thelaziella*) *aquilina* on the cornea of an adult female *Harpia harpyja* in Amazonia region (Brazil).

subgenus (Register of Marine and Nonmarine Genera- IRMNG, 2021), others have included it in their classification as a subgenus (Hodda, 2022). This situation is worsened by the absence of molecular and

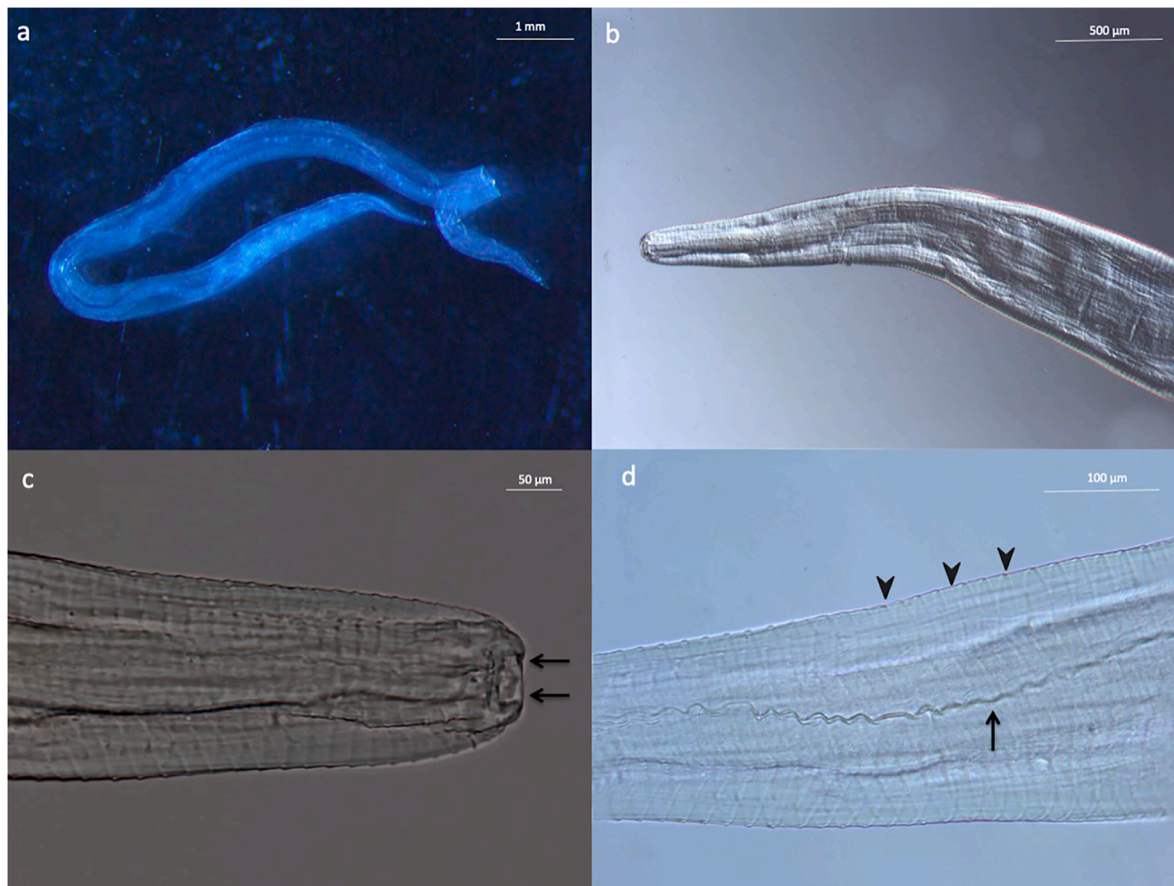


Fig. 2. a) Morphology of *Thelazia (Thelaziella) aquilina* male after extraction from the conjunctival sac; b) Anterior part of male; c) Cephalic extremity, male, lateral view, buccal cavity hexagonal (arrows); d) anterior part in lateral view, portion oesophagus lumen (arrows) and striations (arrowhead) around anterior end.

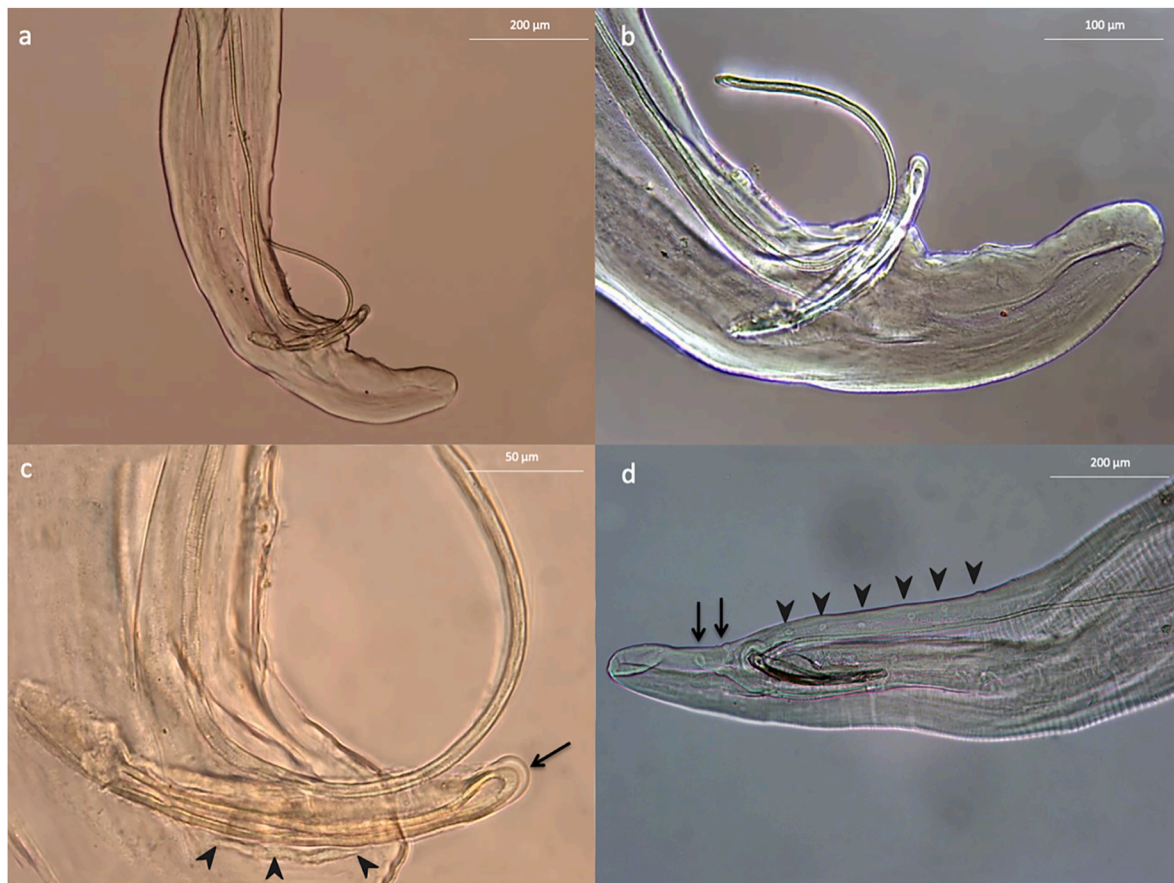


Fig. 3. Morphology of *Thelazia (Thelaziella) aquilina* male: **a)** Posterior end, male, lateral view, spicules; **b)** Posterior end, right spicule and distal portion of the left spicule; **c)** Right spicule and gubernaculum (arrowhead); Distal portion of the right spicule, bulbar expansion with transparent hyaline membrane (arrows); **d)** Posterior end, male, ventral view, spicules and papillae pre (arrowhead) and post-cloacal (arrows).

Table 2

Morphological descriptions of male and female specimens of *Thelazia (Thelaziella) aquilina* specimens in wild birds.

<i>Thelazia (Thelaziella) aquilina</i>											
Host	<i>Harpia harpyja</i>		<i>Spizaetus ornatus</i>		<i>Aquila audax, Haliaeetus leucogaster, and Hieracidea berigora</i>		<i>Ciconia boyciana</i>		Ciconiiformes		
Locality	Brazil		Brazil		Australia		Japan		NA		
Reference	Present study ^a		Strachan (1957)		Baylis (1934)		Murata and Asakawa (1999)		Barus et al. (1978)		
Gender	Male 1	Male 2	Male	Female	Male	Female	Male	Female	Male	Female	
Body length (mm)	13.04	18.63	14–14.5	19.5	12.0–18.0	18.0–19.0	18.1–20.1	20.1–24.8	13.5–18	19.0–20.0	
Body width (μm)	406.4	250.5	333–570	500	390–430	300–370	391–429	489–652	440–470	430–470	
Buccal capsule depth (μm)	18.2	18.0	–	–	16–20	20–24	29–31	29–44	39–39	39	
Buccal capsule width (μm)	34.3	35.2	39–43	47	25–30	34–36	39–47	42–54	20–40	29–40	
Nerve-ring from anterior extremity (μm)	412.3	401.4	310–390	300	310–410	–	382–484	406–490	340	340–360	
Oesophagus length (mm)	0.90	1.160	0.90–1.20	1.20	0.72–1.20	0.94–1.2	0.84–1.00	0.87–1.11	0.93–0.95	0.88–1.11	
Oesophagus width (μm)	65.8	83.3	–	–	–	–	–	–	–	–	
Left spicule length (mm)	2.257	2.447	2.40–2.50	–	1.7–2.3	–	1.90–2.90	–	2.18–2.28	–	
Left spicule width (μm)	6.7	7.1	–	–	–	–	–	–	–	–	
Right spicule length (μm)	210.2	214.4	142–150	–	170–240	–	160–230	–	210	–	
Gubernaculum length (μm)	75.7	77.1	–	–	36–68	–	75–114	–	Present	–	
Pairs of pre-cloacal papillae	8	8	8	–	6–10	–	8–9	–	9	–	
Pairs of post-cloacal papillae	4	–	4	–	4–5	–	3–4	–	5	–	
Tail length (μm)	231.7	220.3	280–300	260	130–220	150–170	201.0	312–429	199–203	–	

^a Female specimens not collected in the present study.

phylogenetic data on these nematodes (Liu et al., 2013), which is, in this case, caused by the preservation in formalin. This emphasizes the necessity of proper preservation in ethanol for nematodes. In addition, the biological cycle and transmission of *Thelaziella* is completely unknown, differently from data available for *Thelazia* spp. (Otranto et al., 2003).

The findings reported here demonstrate the presence of *T. (T.) aquilina* in Brazilian wild birds, even three decades since the last report (Pinto et al., 1994), with *H. harpyja* identified as a new host for this eyeworm. Additional comprehensive studies are clearly warranted to elucidate the transmission, biological cycle, and phylogenetic

relationship of *Thelazieella* species, as well as to clarify its subgenus classification.

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.

Acknowledgements and funding

Fundação de Amparo à Pesquisa do Rio Grande do Sul (FAPERGS) (Finance code:19/2551-0001842-8). Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) (Finance code 001). The coauthors MABS and DO were supported by the EU funding within the MUR PNRR Extended Partnership initiative on Emerging Infectious Diseases (Project no. PE00000007, INF-ACT). The coauthor JFS is funded by CNPq (Grant #312576/2021-8). This article is based on the development of activities carried out by RFM during the Programa Institucional de Internacionalização (CAPES- PRINT) sandwich doctoral period at the Department of Veterinary Medicine, University of Bari, Italy.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ijppaw.2024.100910>.

References

- Anderson, R.C., Diaz Ungria, C., 1959. Revision preliminar de las especies de *Thelazia* Bosc (Spiruroidea, Thelaziidae) parasitas de aves. Mem. Soc. Cienc. Nat. La Salle 19 (52), 37–75.
- Barus, V., Sergeeva, T.P., Sonin, M.D., Ryzhikov, K.M., 1978. Heminths of Fish-Eating Birds of the Palaearctic Region. I. Nemaooda. Academia, Prague.
- Bezerra-Santos, M.A., Moroni, B., Mendoza-Roldan, J.A., Perrucci, S., Cavicchio, P., Cordon, R., Cianfanelli, C., Lia, R.P., Rossi, L., Otranto, D., 2022a. Wild carnivores and *Thelazia callipaeda* zoonotic eyeworms: a focus on wolves. Int J Parasitol Parasites Wildl 17, 239–243.
- Bezerra-Santos, M.A., Bernardini, I., Lia, R.P., Mendoza-Roldan, J.A., Beugnet, F., Pombi, M., Otranto, D., 2022b. *Phortica oldenbergi* (Diptera: drosophilidae): a new potential vector of the zoonotic *Thelazia callipaeda* eyeworm. Acta Trop. 233, 106565.
- BirdLife International, 2021. *Harpia harpyja*. The IUCN Red List of Threatened Species 2021. <https://doi.org/10.2305/IUCN.UK.2021-3.RLTS.T22695998A197957213.en>. Retrieved from.
- Brown, L.H., Amadon, D., 1968. Eagles, Hawks, and Falcons of the World. Hamlyn, England.
- Chabaud, A.G., 1975. CIH Keys to the Nematode Parasites of Vertebrates, third ed. Bureau, England.
- Daiya, G.G., 1968. Nematode fauna of diurnal predatory birds and owls in the Yakutsk region. Tr. Gel'mintol. Lab. 19, 73–82.
- Giraldo-Amaya, M.H., Aguiar-Silva, F.H., Aparicio, U., K, M., 2021. Human persecution of the Harpy Eagle: a widespread threat? J. Raptor Res. 55 (2), 281–286.
- Hodda, M., 2022. Phylum Nematoda: a classification, catalogue and index of valid genera, with a census of valid species. Zootaxa 5114 (1), 1–289.
- Liu, G.H., Gasser, R.B., Otranto, D., Xu, M.J., Shen, J.L., Mohandas, N., Zhu, X.Q., 2013. Mitochondrial genome of the eyeworm, *Thelazia callipaeda* (Nematoda: spirurida), as the first representative from the family Thelaziidae. PLoS Neglected Trop. Dis. 7 (1), e2029.
- Majumdar, N., Sarkar, S.D., 1989. On four new species of avian nematodes from Orissa, India. Rec. zool. Surv. India. 85 (4), 467–480.
- Molin, R., 1858. Versuch einer Monographie der Filarien. Sitzungsberichte der Kaiserlichen Akademie der Wissenschaften. Wien. Mathematisch-Naturwissenschaftliche Classe 28, 365–461.
- Molin, R., 1860. Uma monografia del genere Spiroptera. Sitzungsberichte der Akademie der Wissenschaften. Wien. Mathematisch-Naturwissenschaftliche Classe 28, 911–1005.
- Murata, K., Asakawa, M., 1999. First report of *Thelazia* sp. from a captive Oriental white stork (*Ciconia boyciana*) in Japan. J. Vet. Med. Sci. 61 (1), 93–95.
- Otranto, D., Traversa, D., 2005. *Thelazia* eyeworm: an original endo-and ecto-parasitic nematode. Trends Parasitol. 21 (1), 1–4.
- Otranto, D., Lia, R.P., Buono, V., Traversa, D., Giangaspero, A., 2003. Biology of *Thelazia callipaeda* (Spirurida, Thelaziidae) eyeworms in naturally infected definitive hosts. Parasitology 129, 627–633.
- Otranto, D., Cantacessi, C., Testini, G., Lia, R.P., 2006. *Phortica variegata* as an intermediate host of *Thelazia callipaeda* under natural conditions: evidence for pathogen transmission by a male arthropod vector. Int. J. Parasitol. 36 (10–11), 1167–1173.
- Otranto, D., Mendoza-Roldan, J.A., Dantas-Torres, F., 2021. *Thelazia callipaeda*. Trends Parasitol. 37 (3), 263–264.
- Pinto, R.M., Vicente, J.J., Noronha, D., 1993. Nematode parasites of Brazilian psittacid birds, with emphasis on the genus *Pelecitus* Railliet & Henry, 1910. Mem. Inst. Oswaldo Cruz 88 (2), 279–284.
- Pinto, R.M., Vicente, J.J., Noronha, D., 1994. Nematode parasites of Brazilian accipitrid and falconid birds (Falconiformes). Mem. Inst. Oswaldo Cruz 89, 359–362.
- Pinto, R.M., Vicente, J.J., Noronha, D., 1996. Nematode parasites of Brazilian piciformes birds: a general survey with description of *Procyrnea anterovulvata* n. sp. (Habronematodea, Habronematidae). Mem. Inst. Oswaldo Cruz 91 (4), 479–487.
- Register of Marine, Interim, Nonmarine Genera- IRMNG, 2021. *Thelazieella* Travassos, 1918. Accessed at: <https://www.irmng.org/aphia.php?p=taxdetails&id=1408959>. on 2023-08-09.
- Rodrigues, H.O., 1992. On *Thelazia anolabiata* (Molin, 1860) Railliet & Henry, 1910 (Nematoda, Thelazioidea). A new host record and systematic considerations. Mem. Inst. Oswaldo Cruz 87, 217–222.
- Rodrigues, H.O., 1996. Espécies de Thelaziinae (Skrjabin) Baylis & Daubney (Nematoda, Thelazioidea) referidas para o Brasil. Rev. Bras. Zool. 13, 343–350.
- Rodrigues, H.O., Rodrigues, S.S., 1970. Redescricao de *Thelazia anolabiata* (Molin, 1860) (Nematoda, Spiruroidea), vol. 12. Atas da Sociedade de Biologia do Rio de Janeiro, pp. 29–31.
- Skrjabin, K.I., Sobolev, A.A., Ivashkin, V.M., 1967. Principles of Nematology, vol. IX. Spirurata of animals and man and the disease caused by them. Part 4, 1–54.
- Strachan, A.A., 1957. Eye worms of the family Thelaziidae from Brazilian birds. Can. J. Zool. 35, 179–187.
- Travassos, L., 1918. Contribuição para o conhecimento da fauna helmintológica brasileira. VII. Espécies brasileiras do gênero *Thelazia* Bosc, 1819. Rev. Mus. Paul. 10, 215–230.
- Travassos, L., 1941. Relatório da quarta excursão do Instituto Oswaldo Cruz à zona da Estrada de Ferro Noroeste do Brasil, realizada em agosto de 1940. Mem. Inst. Oswaldo Cruz 35 (4), 697–722.
- Travassos, L., 1942. Relatório da sexta excursão do Instituto Oswaldo Cruz, realizada à zona da Estrada de Ferro Noroeste do Brasil, em novembro de 1941. Mem. Inst. Oswaldo Cruz 37 (3), 259–286.
- Travassos, L., Lent, H., Freitas, J.F.T., 1939. Relatório da excursão científica do Instituto Oswaldo Cruz, realizada na zona da Estrada de Ferro Noroeste do Brasil em outubro de 1938. II. Pesquisas helmintológicas. Boletim Biológico. 4 (2), 221–249.
- Vale, B., Lopes, A.P., da Conceição Fontes, M., Silvestre, M., Cardoso, L., Coelho, A.C., 2019. Thelaziosis due to *Thelazia callipaeda* in Europe in the 21st century—a review. Vet parasitology 275, 108957.