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Molecular and morphological evidence of *Taenia omissa* in pumas (*Puma concolor*) in the Peruvian Highlands

Evidência molecular e morfológica de *Taenia omissa* em onça-pardas (*Puma concolor*) dos Andes Peruanos

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Abstract

A total of 41 cestodes were collected during necropsy examination on 2 pumas (*Puma concolor*) that were found in 2 communities in Canchis province, Cuzco region, Peru, at 4500 meters above sea level (Peruvian Andes). The cestodes were evaluated morphologically and molecularly. A fragment of the mitochondrial cytochrome c oxidase subunit 1 gene (*cox1*) was used as a genetic marker. All the cestodes were identified as *Taenia omissa*. In the present report, we give a brief description by molecular and morphological diagnosis of the cestodes and compare nucleotide sequences with previous isolates from GenBank. Upon comparison, the sequences showed a difference in the *cox1* gene of 5.1 to 5.3% with other teniids sequences. This finding constitutes the first report of *T. omissa* in Peru and expands the geographic distribution of this parasite.

Keywords: *Taenia omissa*, cestode, taeniid, puma, *Puma concolor*, cytochrome C oxidase subunit 1 gene.

Resumo

Um total de quarenta e um cestóides foram coletados durante a necropsia de duas onça-pardas (*Puma concolor*) encontradas em duas comunidades na província de Canchis, em Cuzco, a 4500 metros acima do nível do mar, nos Andes peruanos. Os cestóides foram avaliados morfologicamente e molecularmente. Um fragmento do gene citocromo C oxidase subunidade 1 (*cox1*) foi utilizado como marcador genético. Todos os cestóides foram identificados como *Taenia omissa*. No presente relato, dá-se uma breve descrição dos cestóides e compara-se sequências de nucleotídeos com isolados anteriores presentes no GenBank. Após a comparação, as sequências mostraram uma diferença de 5,1-5,3% entre o gene *cox1* e outras sequências de tênias. Esse achado constitui o primeiro relato de *T. omissa* no Peru e amplia a informação sobre a distribuição geográfica deste parasita.

Palavras-chave: *Taenia omissa*, cestoide, tênia, onça-parda, *Puma concolor*, gene citocromo C oxidase subunidade 1.

The genus *Taenia* Linnaeus, 1758 (Cestoda: Taeniidae) includes approximately 45 established species (HOBERG, 2006; LAVIKAINEN et al., 2008; ROSSIN et al., 2010; HAUKISALMI et al., 2011). Adult stages of these tapeworms develop in the small intestine of carnivorous mammals, and their metacestodes develop in different tissues of herbivorous or omnivorous mammals (ABULADZE, 1964). Many species of felids can act as definitive hosts for at least 14 species of *Taenia* (LOOS-FRANK, 2000).

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The puma (*Puma concolor* Linnaeus, 1771), also called the mountain lion or cougar, is a large wild felid whose range extends from northern British Columbia in Canada to southern Chile and Argentina. In Peru, pumas are distributed from the rainforest to the Andes mountains, and can be found at altitudes as high as 5800 meters above sea level (LÓPEZ-GONZÁLEZ & GONZÁLEZ-ROMERO, 1998). Many studies about parasites in pumas have now been published (RAUSCH et al., 1983; WAID & PENCE, 1988; RICKARD & FOREYT, 1992; FOSTER et al., 2006; DARE & WATKINS, 2012). However, most of these studies were conducted in North America (RAUSCH et al., 1983; WAID & PENCE, 1988; RICKARD & FOREYT, 1992; FOSTER et al., 2006; DARE & WATKINS, 2012). Approximately 9 species of

tapeworms have been found in pumas to date (SCHMIDT & MARTIN, 1978; D'ALESSANDRO et al., 1981; RAUSCH et al., 1983; FOSTER et al., 2006; VIEIRA et al., 2008), of which only four species have been found in South America: *Echinococcus oligarthrus* and *Hydatigera taeniaeformis* in Brazil (VIEIRA et al., 2008); and *Taenia omissa* and *Spirometra* sp. in Brazil and Paraguay (SCHMIDT & MARTIN, 1978; VIEIRA et al., 2008).

The present study confirms morphologically and molecularly the occurrences of *T. omissa* parasitizing two adult male pumas in Cuzco, Peru. This finding represents the first report of *T. omissa* in Peru.

In May and September of 2013, 2 adult male pumas were found dead, apparently killed by poachers, on two farms that were operated as alpaca production systems, located in the Pumaconca (14°18'51.73''S, 71°09'07.41''W) and Abra La Raya (14°28'43.03''S, 71°01'41.34''W) communities, in the province of Canchis, Cuzco region in Peru, at 4500 meters above sea level. The carcasses of the two pumas were donated for necropsy by the Technical Administration for Forestry and Wildlife of Peru to the veterinary research center IVITA-Marangani, at the Universidad Nacional Mayor de San Marcos. During the necropsy, twenty-eight and thirteen complete cestodes were collected from the small intestines, respectively. The parasites were fixed in 4% formaldehyde and then preserved in 70% ethanol. Some gravid proglottids were preserved in absolute ethanol for molecular studies.

Scoleces were mounted in Berlese's medium to facilitate observation and measurement of rostellar hooks. The rostellar hooks were measured in accordance with the parameters described by Haukisalmi et al. (2011). Both mature and gravid proglottids were stained with Semichon's acetocarmine stain and were dehydrated in an ascending alcohol series up to absolute ethanol. Subsequently, the samples were cleared in clove oil and terpineol, and then mounted in Canada balsam.

Photographs were taken using a Carl Zeiss microscope (Axioskop 40). Measurements were made using image analysis software (Leica IM50, version 4.0 R117). The measurements are reported in micrometers unless otherwise stated. Measured characteristics are given as range, with averages and standard error (SE) values in parentheses. The parasite taxonomic nomenclature used in this study follows Loos-Frank (2000) and Rausch (1994). The host taxonomic nomenclature follows Currier (1983).

Total DNA was extracted from three tapeworms from the puma from Pumaconca (To1, To2 and To3) using Chelex100, in accordance with the methodology described by Gadau (2009) with a minor modification. Tissue samples from gravid proglottids of approximately 1-3 mm^3 were put into 0.2 mL plastic vials and were kept at 37 °C for 30 minutes. The tissue was then completely dried, and 100 µl of 5% Chelex solution and 1 µl of proteinase K (20 mg/mL) were added into the vials. The vials were then incubated in a thermocycler using the following program: 1 hour at 57 °C, 10 minutes at 95 °C, 1 minute at 37 °C, 10 minutes at 95 °C, and finally 15 minutes at 15 °C. The DNA samples were then stored at –70 °C until use.

PCR was used to amplify an approximately 400-bp fragment of the mitochondrial cytochrome c oxidase subunit 1 gene (*cox1*) using the primers JB3 and JB4.5 (BOWLES & MCMANUS, 1994). The PCR solution was prepared in a 50 µl volume containing 4 µl of template DNA, 0.25 µM of each primer and GoTaq® Green Master Mix, 2X (Promega, Madison, WI, USA). A three-step thermal process consisting of 94 °C for 30 seconds, 55 °C for 30 seconds and 72 °C for 30 seconds was repeated 36 times to amplify the short fragment of *cox1* (LIU et al., 2011). The PCR products were analyzed by means of electrophoresis on 1.5% agarose gel with ethidium bromide staining. The PCR products were then sequenced using the Big Dye™ Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA, USA) and an ABI 3100 automated sequencer (Applied Biosystems). The sequences were assembled using the ChromasPro 1.7.6 software (TECHNELYSIUM, 2015). All the sequences were compared with a reference sequence in GenBank using ClustalX (CLUSTAL, 2015). A phylogenetic tree was constructed by means of the neighbor-joining method with the Kimura two-parameter distance, using the MEGA6 software (MEGA, 2015) (TAMURA et al., 2004; TAMURA et al., 2013). Unique nucleotide sequences of the partial *cox1* gene of *T. omissa* from these pumas were deposited in the GenBank database under accession numbers KR095312, KR095313 and KR095314.

All the cestodes studied (41) were identified as *T. omissa*. The puma from Pumaconca was infected with a total of 28 cestodes (11 mature and 17 immature tapeworms). The puma from Abra La Raya was infected with a total of 13 tapeworms (9 mature and 4 immature tapeworms).

The immature tapeworms were 2.6-7.6 (4.4; SE: 0.3) cm in total length and 2.1-5.0 (2.9; SE: 0.2) mm in maximum width. All immature tapeworms did not show internal organs. The mature tapeworms had a strobila of 19.7-39.5 (29.7; SE: 2.5) cm in length, and 7.5-9.0 (8.2; SE: 0.2) mm in maximum width. The scoleces measured 977-1312 (1106; SE: 77.2) µm in diameter. Each scolex had four muscular suckers measuring 319-581 (467; SE: 43.2) µm in diameter. Each scolex had a rostellum armed with two rows of hooks (21 to 23 hooks in each row). The rostella measured 457-552 (508; SE: 20.1) µm in diameter. Large hooks measured 243-289 (266; SE: 4.4) µm in length and small hooks 186-229 (208; SE: 3.8) µm (Figure 1A-C). Additional morphological characteristics of the hooks are shown in Table 1.

The width of the mature proglottid was greater than its length (Figure 1D). The mature proglottid was 4.8-5.6 (5.3; SE: 60.1) mm long by 1.8-2.8 (2.3; SE 75.9) mm wide. Each mature proglottid had one set of genital organs. The genital pores alternated irregularly, and were positioned in the middle of the lateral margins of the proglottids. The genital atrium measured 210-239 (227; SE: 5.7) µm wide. The number of testes ranged from 315 to 378, with testes distributed anteriorly and laterally to the ovary between longitudinal osmoregulatory canals. The testes were subspherical and measured 43-74 (56; SE: 1.2) µm in diameter. The cirrus sacs were elongate and measured 357-472 (415; SE: 24.3) µm long by 122-155 (142; SE: 7.2) µm wide, extending across the longitudinal ventral canal. The vagina opened posteriorly to the cirrus sac. The ovaries were bilobate and measured 837-1137 (941; SE: 28.1) µm long by 285-640 (410; SE: 33.7) µm wide, and were distributed in the anterior half of the proglottid. The vitellarium was situated posteriorly to the ovary and measured 522-724 (600; SE: 20.0) µm long by 64-104 (83; SE: 4.2) µm wide. Mehlis' gland was oval

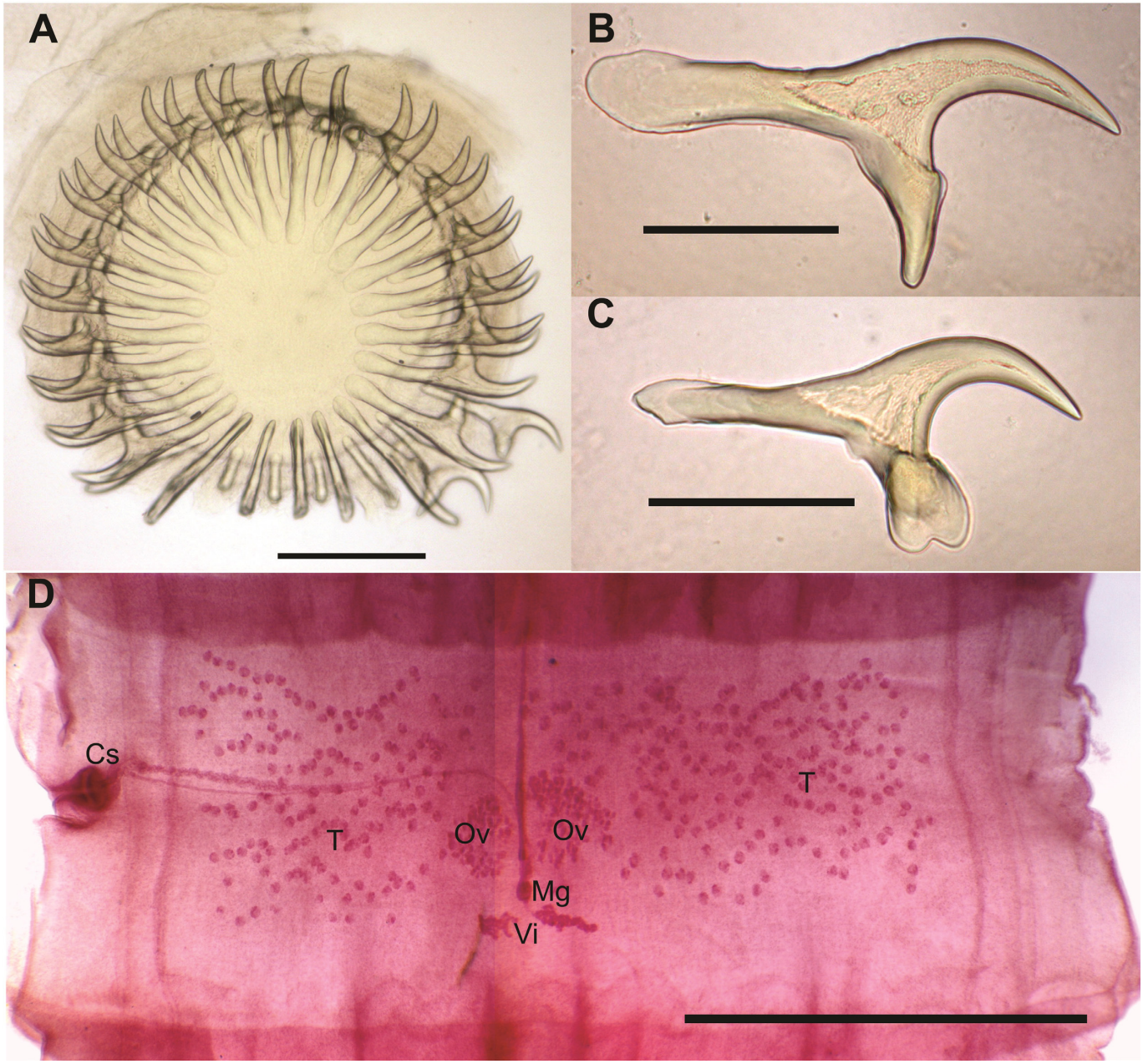

Figure 1. (A) Rostellum of a specimen of *Taenia omissa* collected from *Puma concolor* in Peru. Scale bar = 200 µm; (B) Large hook. Scale bar = 100 µm; (C) Small hook. Scale bar = 100 µm; (D) Mature proglottid. Ov = ovarium; T = testes; Cs = cirrus sac; Vi = vitellarium and Mg = Mehlis' gland. Scale bar = 2.0 mm.

or subspherical and measured 89-123 (109; SE: 5.2) µm long by 80-116 (96; SE: 4.8) µm wide.

Nucleotide sequences of the *cox1* gene were generated for PCR-positive isolates from three *T. omissa* (To1, To2 and To3). The first two were from the Pumaconca puma, and the third was from the Abra La Raya puma. The genetic identity of *T. omissa* was calculated from the alignments of the nucleotide sequences of the *cox1* gene (Figure 2 and 3). Sequences of *T. omissa* from this study (GenBank Nos. KR095312 (To1), KR095313 (To2) and KR095314 (To3)) had single nucleotide differences of 0.7% between each other. However, the sequences for the To1 and To3 isolates were identical. All the sequences were compared with a sequence previously published for this species of tapeworm that had been collected from a puma in Canada (GenBank No. JX860631) (LAVIKAINEN et al., 2013). The isolate from Canada differed with regard to *cox1* by 5.1 – 5.3% from the sequences in this study.

Different species of *Taenia* have been reported in pumas, including *Taenia multiceps*, *Taenia hydatigena*, *Taenia ovis*, *Taenia krabbei*, *T. omissa* and *H. taeniaeformis* (Syn. *Taenia taeniaeformis* see Nakao et al., 2013 and Haukisalmi, 2016) (LUHE, 1910; RAUSCH et al., 1983; VIEIRA et al., 2008; WAID & PENCE, 1988). The main criteria for the morphological diagnosis of taeniid cestodes are measurements and numbers of rostellar hooks (Table 2) (RISER, 1956; ABULADZE, 1964; LOOS-FRANK,

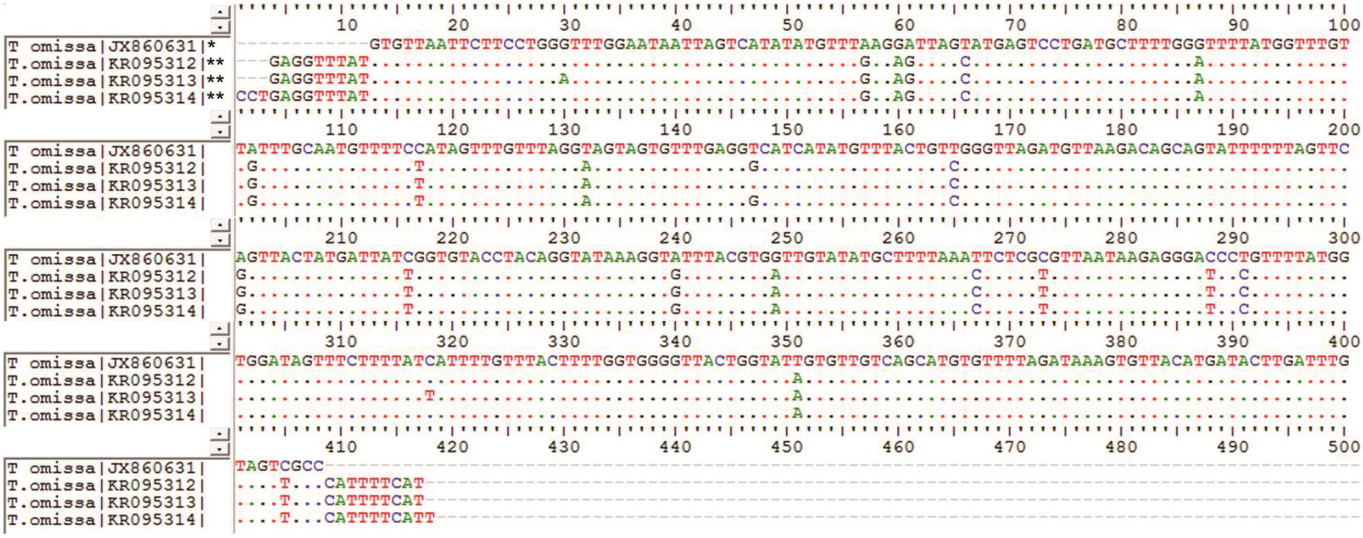

Figure 2. Genetic diversity in *Taenia omissa* sequences of the *cox1* gene in pumas. Dots (.) denote nucleotides identical to those of the *T. omissa* control (JX860631). Sequences from pumas from Pumaconca (KR095312 and KR095313) and from Abra La Raya (KR095314)

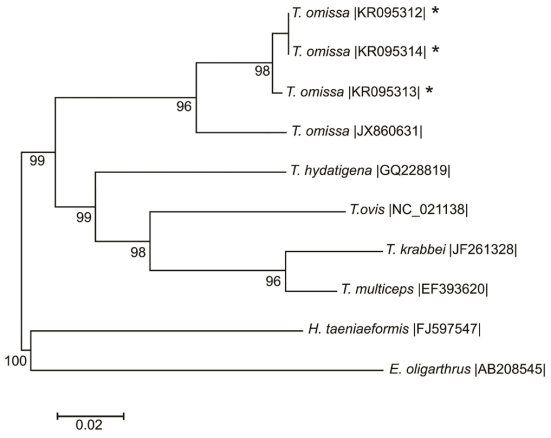

Figure 3. Phylogenetic relationship for *Taenia* species, based on *cox1* gene. Neighbor-joining tree inferred by means of Kimura two-parameter distances from the partial *cox1* gene. Sequences from GenBank were used as references. Sequences marked (*) were obtained in this study. The scale bars are proportional to the number of substitutions per site.

Table 1. Hook measurements of *Taenia omissa* (in μm) isolated from pumas in the Andes mountain range of Peru. Selected parameters were based on the system used by Haukisalmi et al. (2011).

	Taenia omissa		
	N	**Mean**	**Range**
Large hooks		21-23 hooks	
Total length (TL)	60	266	243-289
Total width (TW)	20	122	105-136
Basal length (BL)	20	190	173-207
Apical length (AL)	20	125	108-148
Guard length (GL)	20	52	38-63
Guard width (GW)	20	35	29-40
Blade curvature (BC)	20	33	30-36
Handle width (HW)	20	37	28-48
Small hooks		21-23 hooks	
Total length (TL)	60	208	186-229
Total width (TW)	20	102	88-113
Basal length (BL)	20	149	135-162
Apical length (AL)	20	96	79-114
Guard length (GL)	20	39	28-49
Blade curvature (BC)	20	29	28-30

N = number.

2000). Based on the morphological and molecular characteristics of the specimens in this study, which coincided with data reported by other authors (LUHE, 1910; RISER, 1956; JONG, 1966; RAUSCH, 1981; RAUSCH et al., 1983; LOOS-FRANK, 2000; DARE & WATKINS, 2012; LAVIKAINEN et al., 2013), we conclude that the species isolated in this study corresponded to *T. omissa* (Table 3). This tapeworm has been reported in pumas in Canada, the USA, Paraguay and Brazil (RISER, 1956; SCHMIDT & MARTIN, 1978; VIEIRA et al., 2008; DARE & WATKINS, 2012). The current study demonstrates additional geographic distribution for this parasite.

Morphologically, *T. omissa* is characterized by proglottids that are wider than they are long, as was demonstrated in this study (Figure 1D). This is also a morphological characteristic of *H. taeniaeformis*, a common cestode in felids, including pumas (ABULADZE, 1964). However, *T. omissa* differs from *H. taeniaeformis* in some morphological characteristics such as the numbers (42-46 vs. 34-36, respectively) and larger rostellar hooks in *H. taeniaeformis* (Table 2) (RISER, 1956; ABULADZE, 1964; LOOS-FRANK, 2000).

Nucleotide sequences of the *cox1* gene showed intraspecific variation of 0.7-5.3% between all the *T. omissa* isolates, including the isolate from Canada (JX860631). Similar variations have been demonstrated in other taeniid cestodes. Lavikainen et al. (2008) performed molecular phylogeny on various taeniid cestodes and

Table 2. Charactetristics of rostellar hooks of *Taenia* species reported in *Puma concolor*.

	T. omissa[a]	*T. ovis*[b]	*T. krabbei*[b]	*T. hydatigena*[b]	*T. multiceps*[b]	*H. taeniaeformis*[b]
Number of hooks	42-46	30-34	26-36	28-36	22-30	34-36
Larger hook[c]	243-289	170-191	137-195	191-218	157-177	370-402
Small hook[c]	186-229	111-127	85-141	118-143	98-136	210-261

[a] Present study. [b] Loos-Frank (2000). [c] Length in micrometers.

Table 3. Reported characteristics of rostellar hooks of *Taenia omissa*.

	Present study	Dare & Watkins (2012)	Loos-Frank (2000)	Rausch et al. (1983)	Rausch (1981)	Jong (1966)	Riser (1956)	Luhe (1910)
Number of hooks	42-46	–	38-44	–	–	–	–	40
Larger hook[a]	266 (243-289)	246.5-276.1	270-297	223-273	232-254	253	240-280	270-290
Small hook[a]	208 (186-229)	193.7-221.8	192-223	165-223	183-210	203	180-210	190-220

[a] Length in micrometers.

found an intraspecific variation of 0.0-3.4% in *Taenia mustelae*, 0.0-6.8% in *Taenia polyacantha* and 1.3-9.8% in *H. taeniaeformis* (LAVIKAINEN et al., 2008).

The life cycle of *T. omissa* has been described by Forrester & Rausch (1990), they identified metacestodes collected from white-tailed deer (*Odocoileus virginianus*) in southern Florida, and morphological analysis showed the metacestodes to be *T. omissa* cysticerci (FORRESTER & RAUSCH, 1990). Currently, two species of deer are known to be involved in the life cycle of *T. omissa*, i.e. *Odocoileus hemionus* and *O. virginianus* (JENSEN et al., 1982; FORRESTER & RAUSCH, 1990; PYBUS, 1990). In Peru, *O. virginianus* has wide geographic distribution that includes the Andes mountain range and overlaps with the geographic distribution of the puma (SMITH, 1991). Therefore, we predict that *O. virginianus* is involved in the life cycle of *T. omissa* in Peru. Futures research will be needed to identify the animal species involved as an intermediate host in the life cycle of *T. omissa* in the Peruvian highlands.

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