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Mitochondrial DNA diversity in the acanthocephalan *Prosthenorchis elegans* in Colombia based on cytochrome c oxidase I (COI) gene sequence

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ABSTRACT

Prosthenorchis elegans is a member of the Phylum Acanthocephala and is an important parasite affecting New World Primates in the wild in South America and in captivity around the world. It is of significant management concern due to its pathogenicity and mode of transmission through intermediate hosts. Current diagnosis of *P. elegans* is based on the detection of eggs by coprological examination. However, this technique lacks both specificity and sensitivity, since eggs of most members of the genus are morphologically indistinguishable and shed intermittently, making differential diagnosis difficult, and coprological examinations are often negative in animals severely infected at death. We examined sequence variation in 633 bp of mitochondrial DNA (mtDNA) cytochrome c oxidase I (COI) sequence in 37 isolates of *P. elegans* from New World monkeys (*Saguinus leucopus* and *Cebus albifrons*) in Colombia held in rescue centers and from the wild. Intraspecific divergence ranged from 0.0 to 1.6% and was comparable with corresponding values within other species of acanthocephalans. Furthermore, comparisons of patterns of sequence divergence within the Acanthocephala suggest that *Prosthenorchis* represents a separate genus within the Oligacanthorhynchida. Six distinct haplotypes were identified within *P. elegans* which grouped into one of two well-supported mtDNA haplogroups. No association between haplogroup/haplotype, holding facility and species was found. This information will help pave the way to the development of molecular-based diagnostic tools for the detection of *P. elegans* as well as furthering research into the life cycle, intermediate hosts and epidemiological aspects of the species.

1. Introduction

The genus *Prosthenorchis* belongs to the Acanthocephala phylum (i.e. the thorny-headed worms), endoparasites living part of their life in arthropods and part in the digestive tract of vertebrates (Baker, 2008). Two species have been recognized for nonhuman primates on the basis of morphological differences: *Prosthenorchis elegans* and *Prosthenorchis spirula* (Pissinatti et al., 2007). Differentiation of the two species is based mainly on differences in the number and arrangement of hooks on the proboscis (Stunkard, 1965; Müller, 2007), and the presence of a collar between the proboscis and the body in *P. elegans* (Machado Filho, 1950).

The species *Prosthenorchis elegans* has been reported in the wild in New World primates and some carnivores of South America and in captivity around the world, affecting several species of primates in zoos, rescue centers and laboratories (Schoeb, 1989; Perez et al., 2007). It is an important cause of mortality in captive New World primates, especially in callitrichid species (Martin, 1978; Potkay, 1992; Garber and Kitron, 1997), where mortality in animals results from secondary bacterial infection due to lesions caused by the adult parasite in the intestinal tract of the definitive host (Takos and Thomas, 1958; Toft, 1982). Currently, the diagnosis of *P. elegans* is based on the detection of eggs (by microscopic observation in fecal samples). This technique lacks both sensitivity and specificity, since eggs of most members of the genus are morphologically indistinguishable and shed intermittently (Machado Filho, 1950).

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Where infection is identified, treatments employed have not proved completely effective (Nielsen, 1980; Jimenez, 2009; Johnson-Delaney, 2009) with surgical removal being the best option.

The application of DNA based methods has had a major impact in many areas of parasitology, where genetic information has provided important insights into taxonomic relationships and the history of evolution of parasite species and their variants (Criscione et al., 2005; Gasser, 2006; Varcasia et al., 2006; Huyse and Littlewood, 2007; Steinauer at al., 2007). Such approaches are also useful in parasite diagnosis, where they can potentially contribute to the accurate detection of species (Gasser, 2006; Al-Sabi and Kapel, 2011). Molecular based DNA approaches for the differential diagnosis of parasites have been developed for numerous parasite species (Ndao, 2009): polymerase chain reaction (PCR) based assays, using oligonucleotide primers derived from species-specific sequences, providing the greatest sensitivity (Gasser, 2006). While numerous studies have published sequence data for acanthocephalans (Westram et al., 2011; García-Varela et al., 2013; Pinacho-Pinacho et al., 2014), so far, no sequence data has been published for the acanthocephalan *P. elegans* (nor any other *Prosthenorchis* species), thus its phylogenetic relationship with other Acanthocephala or genetic variation within the species is unknown. In addition, the absence of genetic information for *Prosthenorchis* presents an obstacle to the development of molecular-based diagnostic tools for the genus.

The present study aimed to provide the first analysis of mitochondrial DNA (mtDNA) variation in *P. elegans*. The primary objectives were to: 1) obtain DNA sequence of the mitochondrial cytochrome oxidase subunit I (COI) gene of *P. elegans* and 2) provide the first description of intraspecific diversity among different isolates obtained from primate hosts in Colombia. This information will help pave the way to the development of molecular-based diagnostic tools for the detection of *P. elegans* as well as furthering research into the life cycle, intermediate hosts and epidemiological aspects of the species.

2. Materials and methods

2.1. Sample collection and morphological description

A total of 37 adult parasites (Table 1) were collected opportunistically from the intestines of primates. Twenty-two were obtained from four wild captive-held individuals (taken previously from the wild) of *Saguinus leucopus* and 13 collected from a single wild captive-held individual of *Cebus albifrons* held within wildlife rescue facilities in Colombia. In addition, two adult parasites were obtained from two wild-caught individuals of *S. leucopus* collected by the Wildlife Conservation Society – Colombia. Adult parasites were either obtained during post-mortem examination or surgical intervention carried out by qualified veterinarians following ethical practices and according to the surgical procedure described in Perez et al. (2008). All post-mortem examinations were carried out on individuals that died of natural causes. Adult parasites were stored in either 10% formaldehyde (10 samples) or absolute ethanol (27 samples). Samples received in formaldehyde were washed three times in saline solution and placed in absolute ethanol for long-term storage. The parasites were stored at −20 °C until processing. Individual parasites were identified by conventional morphological criteria following Machado Filho (1950) using a stereo and an electron microscope.

2.2. Amplification and sequencing of DNA

Total DNA was isolated from adult parasites using protocols of proteinase K digestion and silica/guanidinium thiocyanate extraction or standard phenol-chloroform extraction and ethanol precipitation. For silica DNA extraction, samples were digested overnight at 55 °C using 2.0 mg/ml proteinase K in lysis buffer (EDTA 0.5 M, Tris 10 mM, NaCl 100 mM, SDS buffer 2% and Triton X-100 0.5%). Binding buffer (GuSCN 5 M, NaCl 25 mM and Tris 50 mM) and silica suspension (Sigma–Aldrich, 0.5 to 10 microns particle size) were added, incubated for 3 h at room temperature and spun at 15,000 rpm for 2 min to pellet the silica. The supernatant was removed and the silica pellet was washed twice using wash buffer (ethanol 50% v/v, NaCl 125 mM, EDTA 1 mM, and Tris 10 mM). The silica pellet was dried at room temperature and DNA was recovered using ultra-pure water.

Part (703 bp) of the mitochondrial cytochrome oxidase subunit I (COI) gene was amplified using the forward primer 5′-CTAATCATAARGRTATYGG-3′ and reverse primer 5′-TAAACYTCAGGRTGACCAAARAAYCA-3′ modified from Folmer et al. (1994). M13 sequence (M13REV or M13[-21]) was added to the 5′ end of each primer in order to facilitate sequencing. Polymerase chain reactions (PCRs) were carried out in a final reaction volume of 35 μL containing 1× PCR reaction buffer [75 mM Tris–HCl, 20 mM $(NH_4)_2SO_4$, 0.01% (v/v) Tween 20; Fermentas], 2.0 mM $MgCl_2$, 0.2 mM of each dNTP, 0.875 U recombinant *Taq* polymerase (Fermentas) and 0.5 μM of each primer. After an initial denaturation step of 2 min at 94 °C, 34 cycles of 30 s at 94 °C, 30 s at 48 °C and 45 s at 72 °C were followed by a final extension of 72 °C for 1 min. PCR products were purified by agarose gel extraction using spin columns (MinElute Gel Extraction Kit – Qiagen; Zymoclean Gel DNA Recovery Kit – Zymo Research) and sequenced using Big-Dye (Applied Biosystems) cycle sequencing reactions with the M13REV or M13(-21) sequencing primers. Sequencing products were run on an ABI 3500 Genetic Analyzer automated sequencer (Applied Biosystems). Resulting sequence traces were checked and edited using the program CodonCode Aligner ver. 4.2. (CodonCode Corporation; www.codoncode.com). Six hundred and thirty-three bases of reliable COI gene sequence were obtained from all individuals analyzed.

Table 1
List of *Prosthenorchis elegans* specimens used in the present study.

Samples	Host	Locality[a]	Source	Date of collection	Collection	Storage[b]
9	*Saguinus leucopus* 1	URRAS-Bogotá	Rescue centre	<2011	Unknown	Absolute ethanol
13	*Cebus albifrons*	URRAS-Bogotá	Rescue centre	2011	Necropsy	Absolute ethanol
7	*Saguinus leucopus* 2	AMVA-Medellín	Rescue centre	2011	Surgery	10% formaldehyde
1	*Saguinus leucopus* 3	AMVA-Medellín	Rescue centre	2010	Surgery	10% formaldehyde
5	*Saguinus leucopus* 5	URRAS-Bogotá	Rescue centre	2010	Surgery	Absolute ethanol
2	*Saguinus leucopus* 6	WCS- Puerto Berrío	Wild	2013	Necropsy	10% formaldehyde

[a] URRAS: Unidad de Rescate y Rehabilitación de Animales Silvestres, Universidad Nacional de Colombia; AMVA: Area Metropolitana del Valle de Aburrá; WCS: Wildlife Conservation Society-Colombia.
[b] Sample storage prior to receipt.

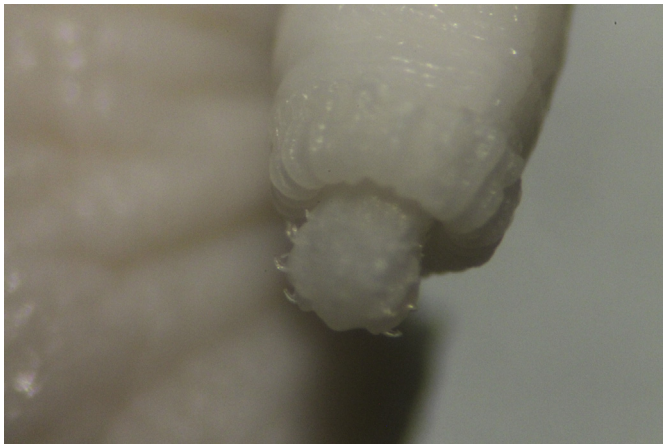

Fig. 1. Photo showing the characteristic external morphology of *Prosthenorchis elegans*.

2.3. Alignment and phylogenetic analysis

The COI sequences of isolates of *P. elegans* were aligned using "Clustal W" (Thompson et al., 1994) as implemented within the program BioEdit ver. 7.0 (Hall, 1999). Homologous COI sequences from other Oligacanthorhynchida available in GenBank were included as outgroup taxa (accession numbers: *Oncicola luehe*; JN710452: *Oncicola* sp; AF417000: *Macracanthorhynchus ingens*; AF416997: *Macracanthorhynchus hirudinaceus*; FR856886: *Oligacanthorhynchus tortuos*; AF416999). Sequences were translated into amino acid sequences using the program MEGA ver. 6 (Tamura et al., 2013) to check for the presence of premature stop codons. There were no premature stop codons, insertions, or deletions, and therefore pseudogenes were not suspected. Genetic divergence between species and among isolates was determined using the uncorrected (p-distance) method as performed in MEGA ver. 6 (Tamura et al., 2013). Phylogenetic relationships among sequences were estimated using Bayesian Inference (BI) and Maximum Likelihood (ML) methods. BI analyses were performed using MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001). Two Markov Chain Monte Carlo (MCMC) samplers were run in parallel (one cold and three incrementally heated chains), starting from random tree, for 5×10^6 generations (with trees sampled every 100 generations). The model parameters were treated as unknown variables (with uniform priors) to be estimated in each Bayesian analysis. The first 5000 samples of each run were discarded to ensure that stationary status had been reached (assessed from the standard deviation of the split frequencies from the two runs). Clade reliability was estimated using posterior probabilities. ML analyses were performed using MEGA ver. 6 (Tamura et al., 2013), with branch support values estimated using bootstrap with 1000 replications. Finally, intraspecific relationships within *P. elegans* were assessed using an unrooted parsimony network. Parsimony networks were estimated using the program TCS ver. 1.21 (Clement et al., 2000). This procedure estimates the maximum number of substitutions to connect two haplotypes with 95% confidence under parsimony and performs better when there are few variable sites (Templeton et al., 1992; Crandall, 1994).

3. Results

3.1. Morphological description

All specimens examined by stereoscopy were confirmed as *P. elegans* based on morphological characteristics: cylindrical worms (pinkish, yellowish white and some dark worms) with striations and wrinkles in the cuticle along its body length, and a proboscis armed with hooks. The hooks were arranged in 6 oblique rows with 5–6 hooks per row for a total of 30–36 hooks. At the union of body and the proboscis, a single cuticular collar composed of 18–20 cuticular folds was present in all the parasites examined, coinciding with the species description by Machado Filho (1950) (Fig. 1). Morphological detail and hooks (present on the proboscis) were clearly visible using scanning electron microscopy (Fig. 2).

3.2. Phylogenetic analysis and genetic diversity

Sequences of COI of 37 isolates of *P. elegans* from primates from Colombia were aligned with homologous COI sequence from other Oligacanthorhynchidae available in GenBank. The final alignment contained 633 nucleotides. Eleven variable sites (all parsimony informative) were found within *P. elegans*, resulting in six distinct haplotypes (Table 2, Fig. 3) (see later). Haplotype sequences have been added to GenBank under accession numbers KT818499-KT818504 (see Table 2). Three variable sites (49, 274 and 293) resulted in changes in amino acid. A single site (position 357, not shown in Table 2) exhibited heteroplasmy in five parasites from the same individual resulting in a variant of haplotype C. This provided the only case of ambiguity and was removed from analyses.

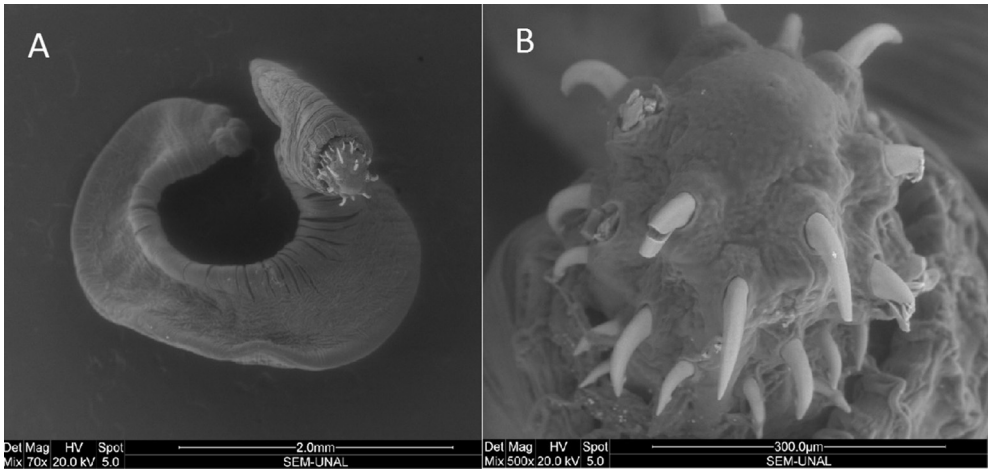

Fig. 2. External morphology of *Prosthenorchis elegans* via scanning electron microscopy (SEM). A. View of entire body of parasite. B. Proboscis armed with hooks.

Table 2
Prosthenorchis elegans haplotypes based on 633 bp of COI mitochondrial DNA gene sequence. Three variable sites (49, 274 and 293) resulted in changes in amino acid.

Haplotype	freq (N)	GenBank accession numbers	Position									
			49	90	108	156	186	225	274	293	345	447	561
A	13	KT818499	A	C	G	G	A	T	C	C	C	A	G
B	3	KT818500	.	.	.	.	.	.	.	.	T	.	.
C	12	KT818501	G	T	C	T	.	C	A	T	.	G	A
D	1	KT818502	G	T	C	T	.	.	A	T	.	G	A
E	5	KT818503	G	.	C	T	.	C	A	T	.	G	A
F	3	KT818504	G	.	C	T	G	C	A	T	.	G	A

Uncorrected sequence divergence was 0.0–1.6% within *P. elegans*. Mean uncorrected sequence divergence between genera was considerably higher and ranged from 18.3% between *Oncicola* and *Prosthenorchis*, 27.6% between *Macracanthorhynchus* and *Prosthenorchis* and 27.9% between *Macracanthorhynchus* and *Oncicola*, up to 30.9% between *Oligacanthorhynchus* and *Prosthenorchis*, 31.6% between *Oligacanthorhynchus* and *Macracanthorhynchus* and 32.1% between *Oligacanthorhynchus* and *Oncicola*.

Both BI and ML trees had the same topology, with *P. elegans* isolates forming a well-supported monophyletic group sister to the genus *Oncicola* (Fig. 4). The unrooted parsimony network revealed more detailed information on relationships among *P. elegans* haplotypes by recovering two well-supported haplogroups: haplogroup I was formed by haplotypes C, D, E, F and haplogroup II by haplotypes A and B (Fig. 3). The two haplogroups are separated by at least eight mutational steps, with differentiation generally low among the remaining haplotypes within each haplogroup (maximum three mutational steps between neighboring haplotypes). The three variable sites (49, 274 and 293) resulting in changes in amino acid corresponded to fixed differences between the two haplogroups.

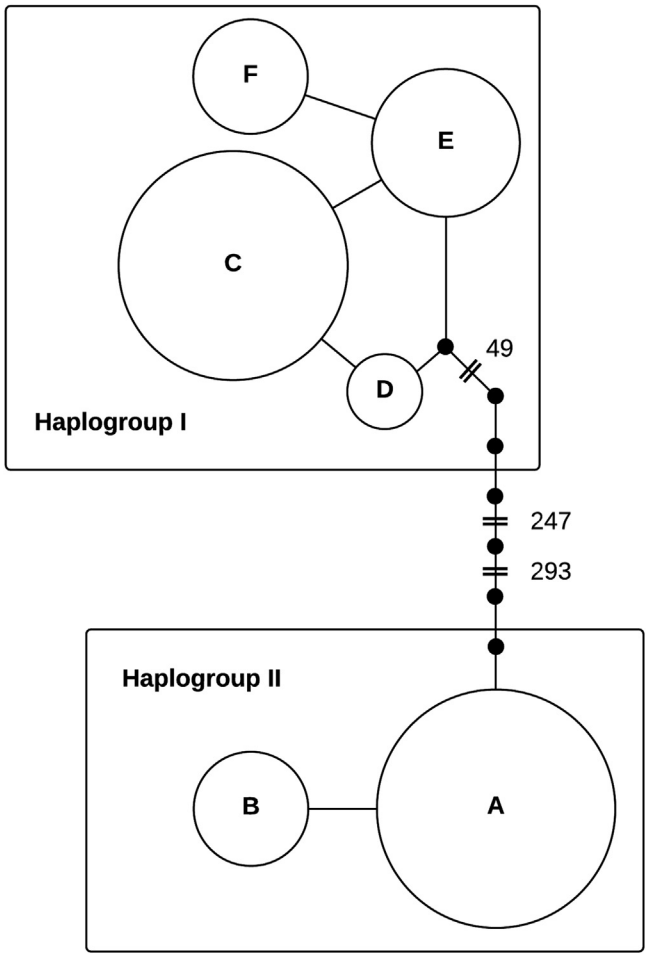

Fig. 3. Haplotype network of *Prosthenorchis elegans*. Network shows relationships among *P. elegans* haplotypes (A–F) recovered from *Saguinus leucopus* and *Cebus albifrons* based on 633 bp of COI. All branches are of unit length (one mutational step). Labeled open circles represent observed haplotypes; areas of circles are proportional to the number observed for each haplotype. Filled circles indicate inferred haplotypes not found among sampled individuals. Double lines indicate variable sites (49, 274 and 293) resulting in changes in amino acid. Haplogroups are identified.

No association was found between haplogroup, species or holding facility (Fig. 5). Haplogroup I was detected in all holding facilities and both species (with the exception of a single individual of *S. leucopus* [#3] from AMVA). Haplogroup II was detected in both species (with the exception of a single individual of *S. leucopus* [#1] from URRAS-Bogotá, and a single individual of *S. leucopus* [#6] from Puerto Berrío-Antioquia). Haplogroup II was predominant in AMVA (being found in five of seven adult parasites sampled) and haplogroup I was predominant in URRAS (being found in 17 of 27 adult parasites sampled). Haplogroup I was the most abundant haplogroup, being found in 21 of the 37 adult parasites sampled.

No association was found between holding facility and haplotype, with individual haplotypes being shared between individuals from distinct rescue centers (with the exception of haplotype B and E which were only found in URRAS). Haplotype A and C were most abundant. Furthermore, even distinct haplogroups and individual haplotypes were found to be shared between species: with the exception of haplotype D which was only detected in a single parasite from a single wild-caught *S. leucopus*. Haplotype D was not detected in *C. albifrons*. All haplotypes were present in *S. leucopus*. In AMVA, haplotype A was predominant, with haplotypes C and F having frequencies of one. In URRAS, haplotype C was the predominant haplotype ($n = 10$) followed by haplotype A ($n = 7$), haplotype E ($n = 5$), and haplotypes B ($n = 3$) and F ($n = 2$). Haplotype diversity was not the same among individuals, with some individuals containing five haplotypes (*S. leucopus* #5) and others containing two (*S. leucopus* #1), despite the number of parasites analyzed being greater in the latter (nine parasites compared to five parasites). Furthermore, both haplogroups were detected within the same individual (for example, *S. leucopus* #5 contained three parasites from haplogroup I and two parasites from haplogroup II).

4. Discussion

This study provides the first genetic analysis for a member of the genus *Prosthenorchis*. Levels of sequence divergence (0.0–1.6%) found among *P. elegans* isolates are similar to intraspecific divergences described in other Acanthocephala species: 0.05–1.5% in *Hexaglandula corynosoma* (Guillén-Hernández et al., 2008) and 0.0–1.0% in *Southwellina hispida* (García-Varela et al., 2012). A study on related Acanthocephala from the family Polymorphidae

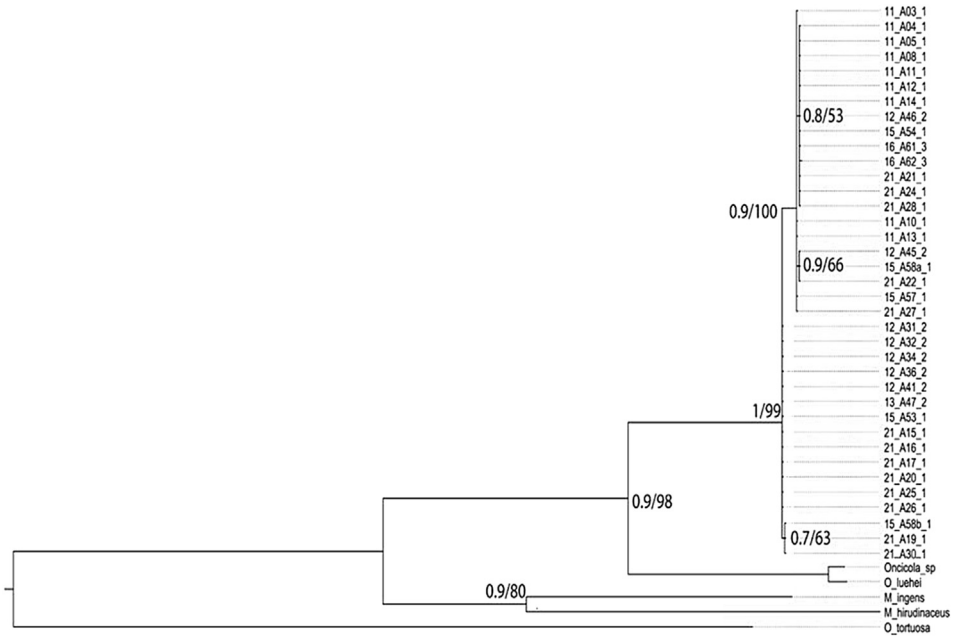

Fig. 4. Phylogenetic tree obtained using Bayesian Inference (BI) and Maximum Likelihood (ML); node supports are provided for BI\ML. Outgroup taxa: Oncicola sp, AF417000; O. luehe = *Oncicola luehe*, JN710452; M. ingens = *Macracanthorhynchus ingens*, AF416997; M. hirudinaceus = *Macracanthorhynchus hirudinaceus*, FR856886; O. tortuous = *Oligacanthorhynchus tortuous*, AF416999.

provides estimates of intergeneric, interspecific and intraspecific patterns of sequence divergence (Garcia-Varela and Pérez-Ponce de León, 2008), with sequence divergences of 1.0–5.0% within species, 11.0–21.0% among species and 22.0–30.0% among genera. Sequence divergences among genera from the family Oligacanthorhynchidae (including *Prosthenorchis*) were generally similar to those reported by Garcia-Varela and de Ponce de León (2008), with the exception of the comparison between *Oncicola* and *Prosthenorchis* which was slightly lower (18.3%) and similar to among-species divergence: sequence divergences from comparisons among other genera in the family Oligacanthorhynchidae ranged from 27.6% between *Macracanthorhynchus* and *Prosthenorchis* and 32.1% between *Oligacanthorhynchus* and *Oncicola*. The pattern of sequence divergence reported by Garcia-Varela and de Ponce de León (2008) for members of the family Polymorphidae and those reported here for members of the family Oligacanthorhynchidae (including *Prosthenorchis*) suggest that all *P. elegans* isolates belong to the same evolutionary lineage and a single species, with

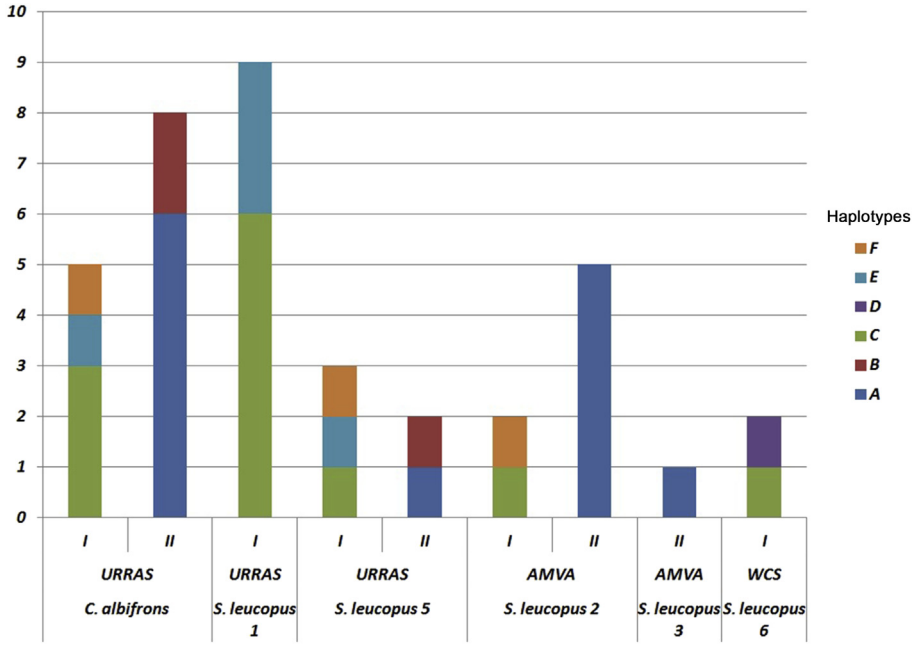

Fig. 5. Distribution and frequency of *Prosthenorchis elegans* haplotypes (A–F) and haplogroups (I–II) by locality and individual. URRAS: Unidad de Rescate y Rehabilitación de Animales Silvestres, Universidad Nacional de Colombia; AMVA: Area Metropolitana del Valle de Aburrá; WCS: Wildlife Conservation Society-Colombia.

Prosthenorchis representing a separate genus (closely related to the genus *Oncicola*) within the family Oligacanthorhynchidae.

One of the most surprising results from this study is that *P. elegans* contains at least two well-supported mtDNA lineages (corresponding to haplogroups I and II). One possible explanation for the presence of distinct mtDNA lineages in *P. elegans* is the physical subdivision of *P. elegans* populations resulting in isolation and genetic differentiation. The physical subdivision of parasite populations may arise at many levels, including at the geographical, host species and host individual level (Criscione et al., 2005; Huyse et al., 2005). Further subdivision may also arise from the intermediate host species used by the parasite. Host specificity in the definitive host seems an unlikely explanation because *P. elegans* has been shown to exhibit wide host tolerance (at least in captivity) where it can readily adapt to new definitive host species (Stunkard, 1965). In *P. elegans* the alimentary habits of the definitive host and the presence of the intermediate host as part of the diet are fundamental to acquire infection. Thus subdivision of parasite populations due to the intermediate host is possible. However, indirect life cycles are common among parasites and the extent to which parasites use different means of transmission (e.g. single or multiple intermediate hosts) is rarely fully known, as in the case of *P. elegans*. While physical subdivision is likely to play an important role in the differentiation observed in *P. elegans* an absence of information relating to definitive and intermediate hosts as well as possible reservoir hosts (a host in which a parasite is able to survive and reproduce, but not considered to be the normal host) in the wild limits assessment of possible explanations. Furthermore, the possibility of an underlying selective component cannot be ruled out. Of the eight nucleotide differences separating the two mtDNA lineages, three resulted in amino acid changes, which could be suggestive of possible selection effects due to specific microhabitat differences in different tissues or body locations in the host species (leading to reproductive isolating mechanisms between groups of parasites). Whatever the cause of genetic differentiation, the presence of two different mtDNA lineages in *P. elegans* is of interest and worthy of further study. Clearly, the analysis of parasites from wild-caught definitive and intermediate hosts is required if actual patterns of distribution of genetic diversity within *P. elegans* as well as possible causes are to be understood.

Another interesting finding from this study was the occurrence together of both *P. elegans* mtDNA lineages in several captive individuals. Whether or not this also occurs in the wild is difficult to assess based on the current data. Parasites analyzed from the only wild-caught individual (*S. leucopus* #6) grouped within the same mtDNA lineage. Although this is suggestive of possible restrictions on the occurrence of *P. elegans* mtDNA lineages in host individuals, the lack of sampling of individuals from the wild in the present study makes this difficult to assess. Assuming *P. elegans* mtDNA lineages are separated in the wild, then the occurrence in the same individual of haplotypes from both mtDNA lineages in captivity most likely results from mixing individuals as primates carrying different haplotypes/haplogroups come into contact in the same holding facility. Under this scenario, the intermediate host in holding facilities acts as a reservoir for transmission in captivity, where the occurrence in the same individual of different haplotypes/haplogroups is most likely related to the presence of different haplotypes/haplogroups in the intermediate host (Gesy et al., 2014). Alternatively, the presence of distinct lineages in the same individual could also be related to the consumption of different intermediate hosts in the wild and during the pet trade. As suggested by Gesy *et al.* (2014), analysis of haplotypes from definitive and intermediate hosts from the same locality could help to find similarities between haplotypes and to detect the rank of action for each haplotype.

The illegal trade of wildlife is an important problem at both local and international level, because pathogens may be transmitted between regions and species. The risk posed to wildlife through the movement of pathogens depends in part on their pathogenicity (the potential capacity of the pathogen to cause a disease) and probability of persistence in a new environment (Gomez and Aguirre, 2008). Where trade in wildlife results in multiple and repeated contacts among wildlife (as might occur in rescue centers), even in cases where the probability of establishment is low, the possibility of cross-species pathogen exchange should be considered an important risk. The alimentary habits of the definitive host and the presence of the intermediate host as part of the diet are fundamental to acquire *P. elegans* infection and as such to its persistence the environment. However, gaps in our knowledge relating to definitive and intermediate hosts (as well as possible reservoir hosts) limits evaluation of the risk posed to wildlife through the movement of *P. elegans* and the probability of persistence in new environments. Furthermore, although *P. elegans* is a parasite found in wildlife and in captivity, mortality has only been reported in captivity, where increased stress seems to be relevant to the negative effects of the parasite on the host (Jimenez, 2009). Under this scenario, increased stress results from inappropriate methods of capture, transport, housing and feeding during illegal trade. When animals arrive at rescue centers they have already suffered elevated levels of stress having shared spaces with humans, pets and other animals.

In the present study, most of the sequences were obtained from parasites from URRAS located in Bogotá, with three of the haplotypes (A, C and F) detected in Bogotá accounting for the haplotypes recovered from parasites analyzed from AMVA Area Metropolitana del Valle de Aburrá. Since *S. leucopus* is the most trafficked primate species in Colombia, and most of the *P. elegans* parasites were obtained from this species, it is important to better understand the distribution of genetic diversity of *P. elegans* in this species throughout its natural distribution (which includes the departments of Tolima, Caldas, Antioquia and Bolivar). It is noteworthy that Bogota is not within the distribution of *S. leuocopus*. Thus all individuals present in Bogotá are the consequence of illegal trade in this species. The white-fronted capuchin (*C. albifrons*) has a broad distribution in Colombia and Latin America, coexisting in some areas with *S. leucopus*. The white-fronted capuchin individual included in this study is an old female that has lived a long time in captivity, and was rejected by its conspecifics. We suggest that this individual most likely acquired the parasite at the rescue center rather than in the wild. Although further information is required to corroborate this idea.

It is presently unknown if *P. elegans* is a cause of mortality in the wild, however, the presence of *P. elegans* in a threatened species like *S. leucopus* is clearly of importance. Throughout the species' distribution there has been a severe decline in available habitat, and habitat that is available is severely fragmented, increasing population densities (Roncancio et al., 2013). Increasing population densities in habitat fragments could act as an environmental trigger leading to increased stress which in turn could result in mortality in wild populations as consequence of *P. elegans* infection.

If the occurrence in the same individual of parasites from different mtDNA lineages is shown to be the result of bringing together animals in captivity then this could have severe consequences for management practices. This primate species has been involved in several release or reintroduction programs possibly creating even more interaction between the parasites and hosts both in captivity and in the wild. The distribution of the two parasite lineages may need to be taken into account when considering liberations. Each lineage could represent the delicate balance between host–parasite interactions that could be

disrupted if released animals carry a parasite lineage previously not present in the natural population.

Finally, the availability of *P. elegans* COI sequence represents an important step for the development of molecular tools that will allow a better understanding of the life cycle, hosts and intermediate hosts and epidemiological aspects of the species, as well as the development of non-invasive molecular diagnostic tools for more rapid and accurate diagnosis. For *P. elegans*, the potential uses of this tool are still in its early stages, however, the results of the present study will clearly provide the basis for further research on this poorly understand but important parasite species.

Conflicts of interest

None declared.

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