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Unveiling patterns of genetic variation in parasite–host associations: an example with pinworms and Neotropical primates

Brenda Solórzano García[1,2], Amanda D. Melin[3,4,5], Filippo Aureli[6,7] and Gerardo Pérez Ponce de León[1]

[1]Departamento de Zoología, Instituto de Biología, Universidad Nacional Autónoma de México, Mexico City, Mexico; [2]Posgrado en Ciencias Biológicas, UNAM, Mexico City, Mexico; [3]Department of Anthropology and Archaeology, University of Calgary, Calgary, Canada; [4]Department of Medical Genetics, University of Calgary, Calgary, Canada; [5]Alberta Children's Hospital Research Institute, University of Calgary, Calgary, Canada; [6]Instituto de Neuroetología, Universidad Veracruzana, C.P. 91190, Xalapa, Veracruz, Mexico and [7]Research Centre in Evolutionary Anthropology and Palaeoecology, Liverpool John Moores University, Liverpool, UK

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Author for correspondence:
Brenda Solórzano García, E-mail: brenda_solorzano@yahoo.com.mx

Abstract

Patterns of genetic variation among populations can reveal the evolutionary history of species. Pinworm parasites are highly host specific and form strong co-evolutionary associations with their primate hosts. Here, we describe the genetic variation observed in four *Trypanoxyuris* species infecting different howler and spider monkey subspecies in Central America to determine if historical dispersal processes and speciation in the host could explain the genetic patterns observed in the parasites. Mitochondrial (*cox1*) and ribosomal (*28S*) DNA were analysed to assess genetic divergence and phylogenetic history of these parasites. Sequences of the *28S* gene were identical within pinworms species regardless of host subspecies. However, phylogenetic analyses, haplotype relationships and genetic divergence with *cox1* showed differentiation between pinworm populations according to host subspecies in three of the four *Trypanoxyuris* species analysed. Haplotype separation between host subspecies was not observed in *Trypanoxyuris minutus,* nor in *Trypanoxyuris atelis* from *Ateles geoffoyi vellerosus* and *Ateles geoffoyi yucatanensis.* Levels of genetic diversity and divergence in these parasites relate with such estimates reported for their hosts. This study shows how genetic patterns uncovered in parasitic organisms can reflect the host phylogenetic and biogeographic histories.

Introduction

Patterns of genetic variation among populations can reveal the evolutionary history of species. Population genetic differentiation and structure across a species' distribution range is generally associated with the migration rates and its dispersal capability, but it could also be a product of demographic changes that occurred during the species colonization process into new habitats, such as population expansions or bottlenecks, which in turn mediate the effects of genetic drift and inbreeding (Carmichael *et al.*, 2007; Latch *et al.*, 2014; Ngeve *et al.*, 2017). The combined action of genetic drift, selection and limited migration could result in local genetic variation, leading to genetic structure among populations (Hey and Machado, 2003; Bradburd *et al.*, 2013). In parasites, the population genetic composition is shaped not only by the parasite's intrinsic characteristics, such as transmission mode, number of hosts involved in the life cycle, and type of reproduction but also by certain host' traits like population density, vagility, social structure and its particular population genetic makeup; each of these factors highly influence the microevolutionary processes of parasites (Nadler, 1995; Huyse *et al.*, 2005; Barrett *et al.*, 2008; Blasco-Costa and Poulin, 2013). Since many of the genetic attributes observed in parasite populations are driven by their host's demographic and dispersal histories, host and parasite phylogeographic patterns are expected to be intertwined, especially in highly host-specific parasites.

Pinworms are parasitic nematodes, directly transmitted and commonly found in primates (Hugot *et al.*, 1996). These nematodes are highly host specific, with one genus of pinworms parasitizing each major group of primates (*Enterobius* in Catarrhini; *Lemuricola* in Strepsirrhini; and *Trypanoxyuris* in Platyrrhini) and one to two species of pinworms specific to each host species, forming tight co-evolutionary associations with their primate hosts (Hugot, 1999). *Trypanoxyuris* is a genus of pinworms that infect Neotropical non-human primates; this genus currently includes 22 described species (Solórzano-García *et al.*, 2016).

Mantled howler monkeys (*Alouatta palliata*) and Central American spider monkeys (*Ateles geoffroyi*) inhabit tropical forests across Middle America, from the western coast of northern Peru, Ecuador and north-western Colombia to south-eastern Mexico (Rylands *et al.*, 2006). Morphological and genetic variations of these primates are found along their range, with 5 recognized subspecies of *Al. palliata* (*Alouatta palliata mexicana; Alouatta palliata palliata; Alouatta palliata aequatorialis; Alouatta palliata coibensis; Alouatta palliata trabeata*) and

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allegedly 7 subspecies of *At. geoffroyi* (*Ateles geoffroyi vellerosus; Ateles geoffroyi yucatanensis; Ateles geoffroyi geoffroyi; Ateles geoffroyi frontatus; Ateles geoffroyi ornatus; Ateles geoffroyi grisescens; Ateles geoffroyi azuerensis*) (Rylands *et al.*, 2006); all of them inhabiting different biogeographic areas across Mexico and Central America (Ford, 2006) (distribution shown in Supplementary Material S1). A recent assessment of the phylogenetic relationships among Mesoamerican spider monkeys showed that the recognized subspecies of *At. geoffroyi* were not monophyletic, suggesting the need for a taxonomic revision of this group (Morales-Jimenez *et al.*, 2015).

Both species of primates are parasitized by two species of *Trypanoxyuris; Trypanoxyuris minutus* and *Trypanoxyuris multilabiatus* infect *Al. palliata;* while *Trypanoxyuris atelis* and *Trypanoxyuris atelophora* are found in *At. geoffroyi* (Solórzano-García *et al.*, 2015, 2016). A study of the population genetics of *T. minutus* and *T. atelis* from primate populations inhabiting forest fragments across south-eastern Mexico showed genetic panmixia in both pinworm species despite habitat loss and fragmentation. This indicates that host isolation in time and space due to relatively recent changes in landscape configuration has not promoted genetic differentiation and structure among local parasite populations. The large population sizes of parasites could additionally be delaying the effects of genetic drift (Solórzano-García *et al.*, 2017).

In this study, we expand the scale of the analysis by exploring a broader geographic range; pinworm specimens were sampled from Central American primate populations in Nicaragua and Costa Rica. Based on the genetic patterns observed in the parasite populations in light of the host phylogeography, we examined whether parasites from Central America represent the same evolutionary significant unit as the ones previously sampled in Mexico and whether biogeographic history and speciation of the primates explain the genetic variation of these parasites. To test our hypotheses, we compared the genetic divergence and phylogenetic history of four *Trypanoxyuris* species infecting different subspecies of howler and spider monkeys inhabiting tropical forests in Mexico, Nicaragua and Costa Rica. We followed the traditional classification for *At. geoffroyi* considering *At. g. vellerosus* and *At. g. yucatanensis* as separate subspecies, in order to test if parasite molecular data could serve as additional information to clarify the taxonomic status of these subspecies.

Materials and methods

Collection of specimens

Trypanoxyuris specimens were collected from free-living populations of two subspecies of howler monkeys and three subspecies of spider monkeys. *Alouatta palliata mexicana* was sampled in four different locations in south-eastern Mexico, and *Al. p. palliata* was sampled in Apoyo, Nicaragua and Sector Santa Rosa, Guanacaste, Costa Rica (Fig. 1). Samples from *At. geoffroyi vellerosus* and *At. g. yucatanensis* were obtained in four and two south-eastern Mexico locations, respectively, and samples from *At. g. frontatus* were collected at Sector Santa Rosa, Guanacaste, Costa Rica (Fig. 1). Adult pinworms were recovered from faeces of these primates *in situ* and fixed either in 100% alcohol for DNA extraction, or 4% formalin for morphological examinations. Faecal samples were also collected and preserved at −4 °C to be examined for additional adult pinworms in the laboratory following the procedure suggested by Hasegawa (2009). For morphological examination, worms were cleared with an alcohol-glycerol solution, and observed using an Olympus BX51 light microscope equipped with differential interference contrast. En face observations were made following the technique proposed by Hasegawa

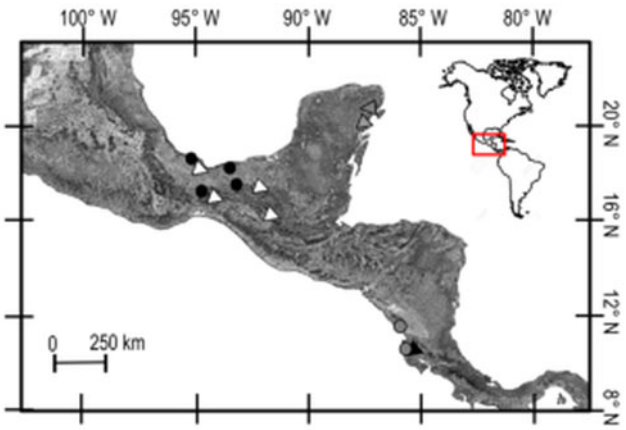

Fig. 1. Collection sites of *Trypanoxyuris* spp. from different host subspecies. Triangles = *Ateles geoffroyi*; black: *At. g. frontatus;* white: *At. g. vellerosus;* gray: *At. g. yucatanensis.* Circles = *Alouatta palliata*; black: *Al. p. mexicana*; gray: *Al. p. palliata*.

et al. (2004). Finally, some specimens were processed for scanning electron microscopy (SEM) (Solórzano-García *et al.*, 2015, 2016).

DNA extraction and sequencing

Individual pinworms fixed in ethanol were digested overnight at 56 °C in a solution containing 10 mM Tris-HCl (pH 7.6), 20 mM NaCl, 100 mM EDTA (pH 8.0), 1% Sarkosyl and 0.1 mg mL^{-1} proteinase K. DNA was extracted from the supernatant using the DNAzol® reagent (Molecular Research Center, Cincinnati, OH) according to the manufacturer's instructions. A fragment of the mitochondrial cytochrome c oxidase subunit 1 gene (*cox1*), and a region of the large subunit of the nuclear ribosomal gene (*28S*) were amplified by polymerase chain reaction (PCR), using the procedure and primers specified in Solórzano-García *et al.* (2016, 2017). Contig sequences of *cox1* were aligned using CLUSTAL W and MESQUITE v. 2.75; two final sets of *cox1* alignments were employed, one including all *Trypanoxyuris* species with available molecular information, consisting of 61 sequences with 707 bp, with 4–12 sequences per *Trypanoxyuris* species per host subspecies; and a species-specific alignment for each *Trypanoxyuris* species including all the previously published sequences of pinworms from primates in Mexico (Solórzano-García *et al.*, 2017). The *28S* contig sequences were aligned using MUSCLE and consisted of 35 sequences of 1149 bp, with 2–5 sequences per *Trypanoxyuris* species per host subspecies. All sequences obtained in this study were deposited in GenBank (28S: MH733397-MH733410; cox 1: MH733411–MH733440).

Phylogenetic analyses

Phylogenetic analyses were conducted through Maximum likelihood (ML) and Bayesian Inference (BI) separately for each gene. To add phylogenetic context, we included DNA sequences available in GenBank from other pinworm species from primates. Phylogenetic analyses for the *cox1* alignments where conducted independently for each *Trypanoxyuris* species to corroborate the distinction between pinworm populations from different host subspecies. MrModeltest v.2.3 (Nylander, 2004) was used to select the best model of evolution for each gene using the AIC, selecting the GTR + I + G substitution model as the best model for both genes. ML trees were inferred using the program RaxML v.8 (Stamatakis, 2014) as implemented in the CIPRES Science Gateway (Miller *et al.*, 2010), using 1000 replications of bootstrap resampling to assess clade support. BI analyses were performed

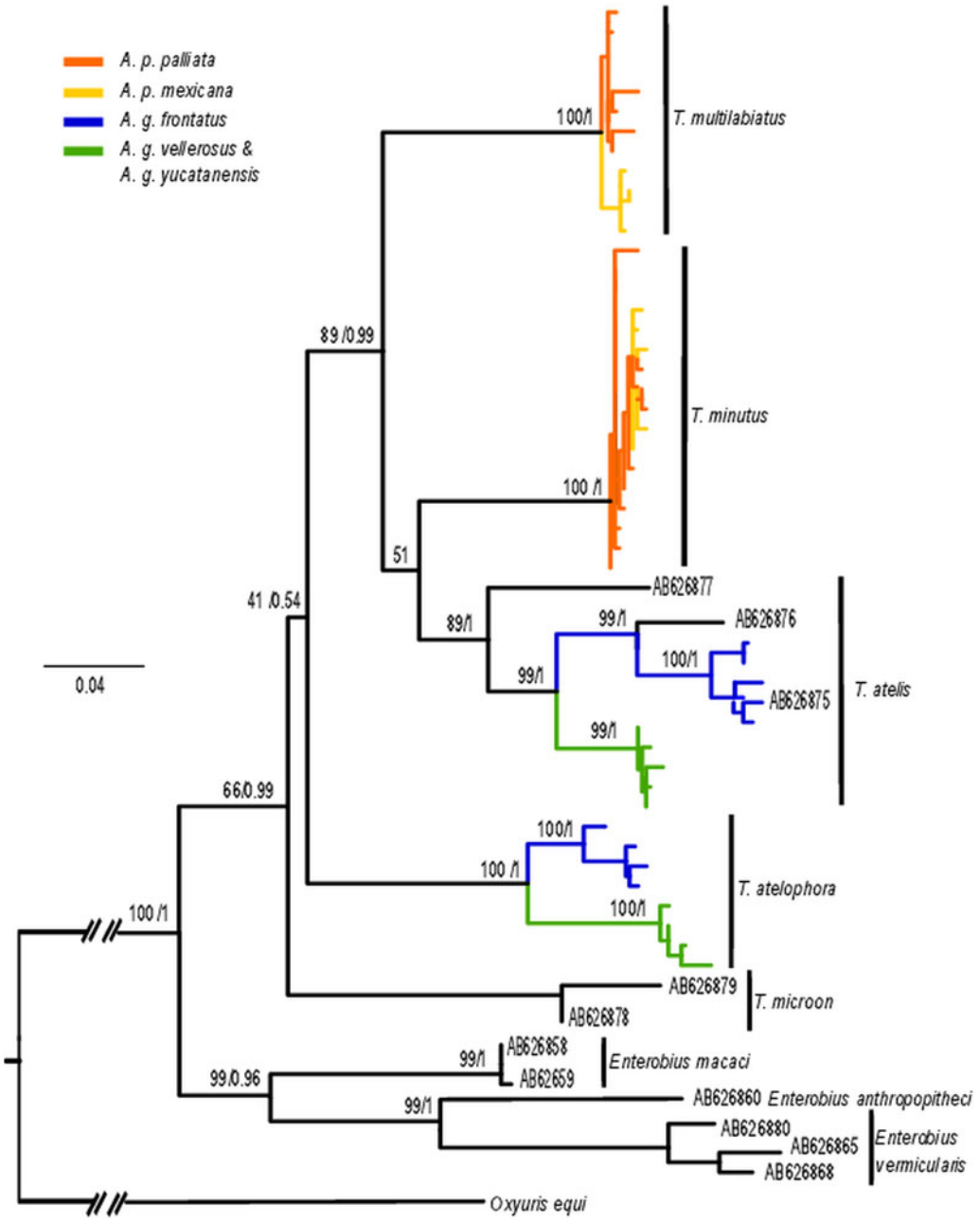

Fig. 2. Maximum likelihood cox1 phylogenetic tree of *Trypanoxyuris* spp. Numbers at the nodes represent ML bootstrap percentage, followed by posterior probabilities from Bayesian Inference. *Trypanoxyuris* species are indicated by bars at the right extreme of the tree. *Trypanoxyuris* from different host subspecies are marked as indicated at the top left of the figure. GenBank accession numbers indicate pinworm sequences acquired from different hosts: AB626877 *Lagothrix lagotricha;* AB626976 *Ateles belzebuth;* AB626875 *Ateles geoffroyi;* AB626878–79 *Aotus azarae;* AB626858–59 *Macaca fuscata*; AB626860, AB626880 *Pan troglodytes*; AB626865, AB626868 *Homo sapiens*.

using MrBayes v.3.2.2 (Ronquist and Huelsenbeck, 2003) and the CIPRES Science Gateway. Bayesian analyses included two simultaneous runs of MCMC, each for four million generations, sampling trees every 4000 generations, a heating parameter value of 0.2, and a 'burn-in' of 25%. A 50% majority-rule consensus tree was constructed from the post-burn-in trees.

Analyses of genetic variation

Median-joining *cox1* haplotype networks were constructed using Network v.5.000 (Bandelt *et al.*, 1999) independently for each *Trypanoxyuris* species to show the evolutionary relationships among haplotypes from pinworms infecting different primate subspecies. In the cases where the resulting networks were too complex, involving multiple alternative linkages, we used the Maximum Parsimony calculation post-processing option to visualize the most parsimonious tree (Polzin and Dabeschmand, 2003).

Genetic divergence in *cox1* (*P*-distance) was calculated using MEGA v.6 (Tamura *et al.*, 2013); standard error of the distances was estimated by bootstrap resampling with 500 replications. Molecular diversity indices including the number of segregating sites (S), number of haplotypes (h), haplotype diversity (H_d), nucleotide diversity (π), and average number of nucleotide differences (k) were derived for *cox1* using DnaSP v.5 (Rozas *et al.*, 2003) for each species of *Trypanoxyuris*.

Results

A pattern of genetic differentiation in concordance with host subspecies was evident in three of the four *Trypanoxyuris* species analysed (Fig. 2). Both the combined and the species-specific *cox1*

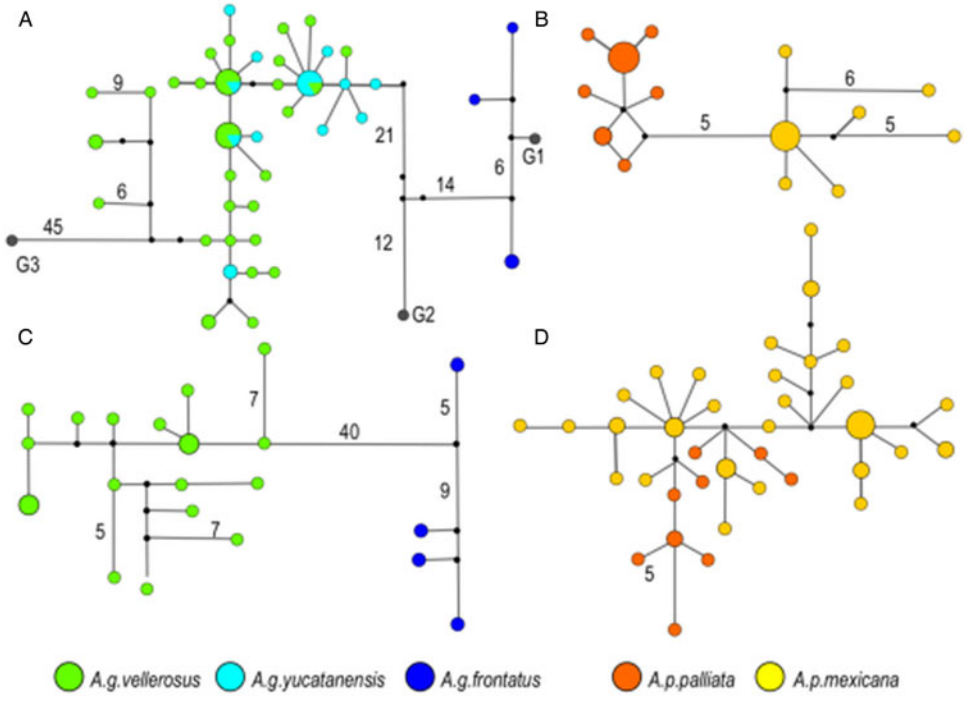

Fig. 3. Median-joining haplotype network based on cox 1 for *Trypanoxyuris* spp. Haplotype frequency is represented by the diameter of the circle. Numbers indicate mutational steps larger than 3 between haplotypes. (A) *Trypanoxyuris atelis*; (B) *T. multilabiatus*; (C) *T. atelophora*; (D) *T. minutus. Trypanoxyuris* from different host subspecies are marked as indicated at the bottom of the figure.

phylogenetic analyses showed that *T. multilabiatus* from the two *Al. palliata* subspecies (*Al. p. mexicana* and *Al. p. palliata*) formed separated clades; however, in *T. minutus* no pattern of genetic variation related to host subspecies was observed (Fig. 2). Similarly, *cox1* sequences showed a pattern of differentiation between pinworms infecting different *Ateles geoffroyi* subspecies. *Trypanoxyuris atelis* and *T. atelophora* from *At. g. frontatus* sampled in Costa Rica grouped in clades separated from those pinworms collected from *At. g. vellerosus* and *At. g. yucatanensis* from Mexico (Fig. 2).

This pattern of genetic differentiation between host subspecies was also supported by the haplotype networks. In *T. multilabiatus, T. atelis* and *T. atelophora* median-joining networks showed separation of several mutations between pinworm haplotypes found in host subspecies from Mexico and Central America (Fig. 3). Haplotype separation between host subspecies was not observed in *T. minutus* (Fig. 3D), nor in *T. atelis* from *At. g. vellerosus* and *At. g. yucatanensis* (Fig. 3A). No shared haplotypes were found between pinworm populations infecting different host subspecies except in *T. atelis* from *At. g. vellerosus* and *At. g. yucatanensis.* Also, an ancestral haplotype from which the rest of the haplotypes are derived was not evident in any network (Fig. 3).

The genetic divergence estimated for *cox1* among *Trypanoxyuris* parasitizing different primate subspecies ranged from 0.8% to 7.4%; the divergence among pinworm species ranged from 9.2% to 13.4% and from 14.2% to 16.2% between pinworm genera (Table 1). Within clades, the genetic divergence was very low and ranged from 0.8% in *T. minutus* to 1.9% in *T. atelophora* (Table 1). Genetic distances found among and within *Trypanoxyuris* clades from *A. palliata* are lower than the genetic distances found among and within *Trypanoxyuris* species sampled from *A. geoffroyi* (Table 1). All four *Trypanoxyuris* species are highly genetically diverse for mitochondrial DNA (mtDNA); however, pinworm populations infecting *At. geoffroyi* showed higher genetic diversity than pinworm populations from *A. palliata* (Table 2). In contrast, *28S* rDNA sequences were identical within *Trypanoxyuris* species regardless of host subspecies.

Despite the high levels of mitochondrial genetic variation observed between pinworms infecting different *At. geoffroyi* subspecies, no morphological differences were identified among them, either in males, females or in eggs, with diagnostic traits in agreement with the descriptions of *T. atelis* and *T. atelophora* (Hasegawa *et al.*, 2004; Solórzano-García *et al.*, 2015).

Discussion

Pinworms are highly host specific parasites that form strong co-evolutionary associations with their primate hosts (Hugot, 1999). In this study, we evaluated the population genetics of four *Trypanoxyuris* species infecting different howler and spider monkey subspecies distributed across Middle America and found genetic variation concordant with parasite–host association patterns.

As expected, morphological and molecular evidence indicate that the same species of *Trypanoxyuris* previously reported in *Al. palliata* and *At. geoffroyi* in Mexico (Solórzano-García *et al.*, 2015, 2016) are also found parasitizing these primate species across its distribution in Central America, supporting the notion of the presence of two pinworm species per host species (Conga *et al.*, 2016; Solórzano-García *et al.*, 2016). Sequences of the *28S* gene from primate populations from Central America and southern Mexico were identical within pinworms species regardless host subspecies, and no differences were observed through the morphological examination of the specimens with light and SEM. Nonetheless, phylogenetic analyses, haplotype relationships and genetic divergence in the mitochondrial gene *cox1* showed some level of segregation of pinworm populations according to host subspecies for three of the four *Trypanoxyuris* species studied. The number of mutational steps estimated between haplogroups and the absence of shared haplotypes between *Trypanoxyuris* from different host subspecies suggest that local selective pressures related to each host subspecies along with restricted gene flow and the action of local genetic drift processes could have contributed to the differentiation of pinworm populations.

Table 1. *Cox1* genetic divergence among pinworms from different host subspecies

Ateles geoffroyi			*Alouatta palliata*		
Parasite species	Host subspecies	*P*-distance	Parasite species	Host subspecies	*P*-distance
T. atelis	**vell**	**0.4 (0.002)**	*T. minutus*	**mex**	**0.6 (0.002)**
	yuc	**0.2 (0.001)**		**pall**	**0.8 (0.002)**
	fron	**1.9 (0.004)**		mex – pall	0.9 (0.002)
	vell – yuc[a]	0.8 (0.003)	*T. minutus* – *T. microon*	mex	12.5 (0.013)
	vell – fron	7.4 (0.011)		pall	12.1 (0.013)
	vell – G2	6.4 (0.010)	*T. minutus* – *Enterobius* spp.	mex	14.7 (0.013)
	fron – G2	5.6 (0.010)		pall	14.4 (0.012)
	vell – G3	8.1 (0.011)	*T. multilabiatus*	**mex**	**0.4 (0.002)**
	fron – G3	9.3 (0.011)		**pall**	**0.5 (0.002)**
T. atelis – *T. microon*	vell	12.2 (0.013)		mex – pall	1.4 (0.004)
	front	13.7 (0.013)	*T. multilabiatus* – *T. microon*	mex	12.8 (0.013)
T. atelis – *Enterobius* spp.	vell	14.6 (0.012)		pall	12.6 (0.013)
	fron	16.2 (0.013)	*T. multilabiatus* – *Enterobius* spp.	mex	14.6 (0.012)
T. atelophora	**vell**	**1.0 (0.003)**		pall	14.2 (0.012)
	fron	**1.8 (0.004)**			
	vell – fron	7.2 (0.010)			
T. atelophora – *T. microon*	vell	12.1 (0.013)			
	fron	12.2 (0.014)			
T. atelophora – *Enterobius* spp.	vell	14.9 (0.012)			
	fron	15.6 (0.012)			

vell, *vellerosus*; yuc, *yucatanensis*; fron, *fontatus*; mex, *mexicana*; pall, *palliata*; G2, *T. atelis* from *At. belzebuth* (GenBank AB626876); G3, *T. atelis* from *Lagothrix lagotricha* (GenBank AB626877). *P* distance expressed as percentage (± S.E.). Bold values indicate within clade distances.
[a]The genetic divergence between pinworms from yuc and the rest of the populations is not shown given the level of relatedness with those from vell, in order to avoid redundancy.

Table 2. Estimates of genetic diversity of the mitochondrial *cox1* gene for each species of *Trypanoxyuris* collected from each primate host

Host	Parasite	*n*	*S*	*h*	H_d (S.D.)	π (S.D.)	*k*
Ateles geoffroyi	*T. atelis*	59	106	43	0.98 (0.009)	0.02225 (0.00460)	13.3
	T. atelophora	24	92	21	0.99 (0.018)	0.02509 (0.00597)	20.4
Alouatta palliate	*T. minutus*	50	44	37	0.98 (0.009)	0.00579 (0.00047)	4.4
	T. multilabiatus	25	41	15	0.91 (0.040)	0.01019 (0.00161)	7.1

n, number of sequences; *S*, segregating sites; *h*, number of haplotypes; H_d, haplotype diversity; π, nucleotide diversity; *k*, average number of nucleotide differences.

It is possible to speculate that the biogeographic history of these primates may have influenced the evolutionary dynamics among pinworm populations. Strong evidence suggests multiple independent waves of colonization of Central America by primates from northern South America throughout a series of founder events (Ellsworth and Hoelzer, 2006; Ford, 2006). These independent colonization events and demographic fluctuations caused the intraspecific patterns of genetic and phenotypic variation currently observed across *Al. palliata* and *At. geoffroyi* range that justifies the designation of different populations as distinct subspecies. The patterns of genetic variation found among pinworm populations from different host subspecies are well explained by these historical events resulting in the genetic structure among howler and spider monkeys populations as well as their pinworms.

Both the genetic diversity and the level of genetic divergence found in *Trypanoxyuris* infecting spider monkeys (*At. geoffroyi*) was higher than the values observed in *Trypanoxyuris* from howler monkeys. The same genetic patterns of the parasites have been also observed in their hosts, with howler monkeys, *Al. palliata* tending to be less genetically diverse across its geographical range than the spider monkeys, *At. geoffroyi* (Jasso-del Toro *et al.*, 2016; Ruiz-García *et al.*, 2016, 2017). Moreover, the genetic distinctiveness of *Al. palliata* subspecies uncovered through mtDNA is relatively minor (0.5%) (Cortés-Ortiz *et al.*, 2003), while that reported for *At. geoffroyi* subspecies is ∼5% (Collins and Dubach, 2000).

The two pinworm species found in *Al. palliata* showed contrasting results in the population genetics and phylogenetic analyses; the genetic differentiation of *T. multilabiatus* was closely tied with howler monkey subspecies, while the species *T. minutus* formed one single clade with no structure in terms of host subspecies. This disparity on the genetic configuration could be explained by the population size of each pinworm species, and by the strength of the evolutionary ties between host and parasite given by the level of host specificity. *Trypanoxyuris multilabiatus* has only been reported in *Al. palliata* (Solórzano-García *et al.*, 2016), whereas *T. minutus* is a common and abundant pinworm

reported in several species of howler monkeys along their distributional range in the Neotropical biogeographic region; i.e. *Al. guariba, Al. seniculus* and *Al. cayara* (Solórzano-García and Pérez-Ponce de León, 2018). Hence, *T. minutus* can be regarded as a more generalist parasite species, and perhaps less susceptible than *T. multilabiatus* to subtle changes in the physiology, genetics and demography linked to host subspecies. Furthermore, *T. multilabiatus* showed the lowest haplotype diversity, probably as a consequence of a small population size that could be related to the series of population reductions experienced by *A. palliata* during its dispersal through Central America up to south-eastern Mexico (Cortés-Ortiz *et al.*, 2003; Ford, 2006), whereas a broader host distribution and hence, larger population sizes could have allowed the other species, *T. minutus* to cope with such demographic changes in the host.

Both pinworm species found in spider monkeys, *T. atelis* and *T. atelophora,* showed genetic differences associated with host subspecies, the two alleged subspecies occurring in Mexico (*At. g. vellerosus* and *At. g. yucatanensis*) and the subspecies from Central America (*At. g. frontatus*). However, no separation was observed between *T. atelis* from *At. g. vellerosus* and *At. g. yucatanensis.* Molecular phylogenetic analyses of Central American spider monkeys through a multilocus approach of mtDNA have shown a lack of distinction between *At. g. vellerosus* and *At. g. yucatanensis*, and thus they should be considered as the same subspecies, *At. g. vellerosus* (Morales-Jimenez *et al.*, 2015). The resulting genetic patterns of the parasites unveiled in this study constitute additional evidence supporting this proposal.

Our results showed substantial intraspecific genetic differences among *cox1* sequences from *T. atelis* and *T. atelophora* collected from *At. g. vellerosus* and *At. g. frontatus* (7.4% and 7.2%, respectively). MtDNA is characterized as rapidly evolving among nematodes (Blouin, 1998); however, the genetic divergence observed between *Trypanoxyuris* populations from these two spider monkey subspecies is noticeably high considering the intraspecific genetic distances reported in pinworms. The mean intraspecific genetic divergence, for the same molecular marker, among species of *Trypanoxyuris* occurring in Mexico, varied between 0.5% and 1.1% (Solórzano-García *et al.*, 2016), while the maximum intraspecific genetic distance in *cox1* reported for pinworms is 6.5% in *Enterobius vermicularis* (Nakano *et al.*, 2006). The time at which *At. g. vellerosus* and *At. g. frontatus* diverged from the common ancestor has been estimated ~1.5 Ma (Morales-Jimenez *et al.*, 2015), and this might be considered a relatively recent date for the accumulation of the observed genetic differences in these pinworm populations. Moreover, specimens of *T. atelis* have been previously obtained and sequenced from different primate species of the family Atelidae, all from captive populations in Japan, including the spider monkey, *At. geoffroyi* (unknown subspecies), the yellow-bellied spider monkey, *At. belzebuth*, and the brown woolly monkey, *Lagothrix lagotricha* (Hasegawa *et al.*, 2012) (Fig. 2). The ability of *T. atelis* to infect different host species in addition to the magnitude of the genetic variation among populations of this pinworm imply that the species of *Trypanoxyuris* in spider monkeys exhibit a high evolutionary potential. This would facilitate adaptation to the specific selective pressures of each host, resulting in the presence of different genetic configurations, but could also indicate the potential existence of cryptic species (Nadler and Pérez-Ponce de León, 2011) of *Trypanoxyuris* parasitizing spider monkeys.

The results of this study show how genetic patterns uncovered in parasitic organisms may reflect host phylogeny and biogeography (Whiteman and Parker, 2005; Criscione, 2008). Moreover, because of the intimate association between pinworms and primates, genetic data of the parasite could shed light on host evolutionary history, helping us to better understand primate phylogenetic relationships and vice versa. These additional data aid the construction of a more accurate taxonomy. Still, denser taxon sampling and sequencing work are required to test the strength of the co-evolutionary processes between pinworms and primates.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0031182018001749.

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Conflict of interest. None.

Ethical standards. Not applicable.

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