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Lack of genetic structure in pinworm populations from New World primates in forest fragments ☆

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ABSTRACT

Microevolutionary processes in parasites are driven by factors related to parasite biology, host abundance and dispersal, and environmental conditions. Here, we test the prediction that isolation of host populations results in reduced genetic diversity and high differentiation among parasite populations. We conducted a population genetic analysis of two pinworms, *Trypanoxyuris minutus* and *Trypanoxyuris atelis*, commonly found parasitizing howler and spider monkeys in tropical rainforests across south-eastern Mexico, whose populations are currently isolated due to anthropogenic habitat loss and fragmentation. Mitochondrial DNA was employed to assess parasite genetic patterns, as well as to analyse their demography and population history. Both pinworm species showed high haplotype diversity but, unexpectedly, lower nucleotide diversity than that reported for other parasites. No genetic differentiation or population structure was detected in either pinworm species despite habitat loss, fragmentation and host isolation. Several scenarios are discussed that could help to explain the genetic panmixia found in both pinworm species, including higher than expected primate inter-fragment dispersal movements, and passive dispersal facilitating gene flow between parasite populations. The results suggest that large population sizes of parasites could be helping them to cope with the isolation and fragmentation of populations, delaying the effects of genetic drift. The present study highlights the complexity of the drivers that intervene in the evolutionary processes of parasites. Detailed genetic studies are needed, both in host and parasite populations, to assess the effects that habitat perturbation and environmental changes could have on the evolutionary dynamics of pinworms and primates.

1. Introduction

Microevolution in parasites is mediated by many factors related to the biology of each parasite and that of its host, as well as to environmental conditions intervening in their subsistence. Parasite life history traits such as life cycle complexity, reproductive mode and population sizes, together with host abundance and dispersal, regulate some of the most important aspects of parasite population genetics (Nadler, 1995; Criscione et al., 2005; Blasco-Costa and Poulin, 2013; Lagrue et al., 2016). General patterns of parasite genetic structure have not been investigated in great detail (Blasco-Costa and Poulin, 2013), however, sexually reproducing parasites which are capable of infecting multiple host species, and with high dispersal capabilities and long-lived definitive hosts, are expected to be highly diverse and poorly differentiated. In contrast, strong host specificity, autogenic life cycles, and an aggregated distribution of host populations (either through behavioural, environmental or geographical factors) will most likely promote genetic structure among parasite populations (Nadler, 1995; Criscione and Blouin, 2004; Barrett et al., 2008; Blasco-Costa et al., 2012).

In addition, processes of parasite local adaptation, speciation and coevolutionary dynamics will be affected by host and parasite gene flow (Criscione et al., 2005; Lagrue et al., 2016). Genetic interchange among parasite populations is believed to be strongly correlated with host dispersal ability (Blouin et al., 1995; Prugnolle et al., 2005; Louhi et al., 2010); however, host vagility is mediated not only by its dispersal capability but also by landscape properties such as habitat extent and arrangement, connectivity and matrix configuration (Tischendorf et al., 2003). Habitat loss and fragmentation are considered among the most important threats for

☆ Note: Nucleotide sequence data reported in this paper are available in the GenBank™ database under the accession numbers MF379058 to MF379260.

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biodiversity (Fischer and Lindenmayer, 2007). The expansion of human settlements and the associated changes in landscape configuration have reduced native vegetation to patches within a matrix of anthropogenic vegetation, harbouring isolated wildlife populations. The severity of the effects that these processes could have on the persistence of any organism are species-specific and depend on the species' ecology and life history requirements (Betts et al., 2014).

Oxyurid nematodes of the genus *Trypanoxyuris* are commonly found in New World primates (Hugot et al., 1996). Pinworms of primates are highly host-specific and show interesting patterns of host-parasite coevolution (Hugot, 1999). These nematodes are characterized by having a direct life cycle with no free-living stages. They present a haplodiploid reproduction mode where males are haploid and derived from unfertilized eggs, while females are diploid and derived from fertilized eggs (Adamson, 1989). Transmission occurs by the ingestion of eggs which are passed to the external environment with host faeces and deposited in clusters; contact with infected conspecifics, autoinfection and retroinfection are thus common transmission modes (Cook, 1994; Felt and White, 2005; Gónzalez-Hernández et al., 2014).

Trypanoxyuris minutus and *Trypanoxyuris atelis* parasitize howler monkeys (*Alouatta* spp.) and spider monkeys (*Ateles geoffroyi*), respectively (Solórzano-García et al., 2015, Solórzano-García et al., 2016). In Mexico, these primates are considered endangered, mainly due to habitat loss and fragmentation (Rodríguez-Luna et al., 2009; SEMARNAT, 2010), leaving isolated primate populations in what used to be a continuous tropical rainforest (Rodríguez-Luna et al., 2009; Solórzano-García et al., 2012). Both primate species can be considered as specialist dispersers since the probability of dispersal between forest fragments declines with increasing habitat loss; specifically by imposing a higher risk of mortality while crossing the matrix (Estrada and Coates-Estrada, 1996; Mandujano et al., 2004; Pozo-Montuy and Serio-Silva, 2007).

In this study, we evaluated the population genetic patterns of *T. minutus* and *T. atelis* occurring in isolated howler and spider monkey populations in south-eastern Mexico. First, we assessed the amount and geographic distribution of genetic diversity among parasitic pinworms in fragmented tropical forests. Second, we tested whether the processes of habitat loss and fragmentation, and the consequent isolation of host populations, have resulted in the genetic isolation and genetic structure of pinworm populations. Third, we investigated the demographic history of parasites based on the genetic data to reveal the genetic consequences of habitat fragmentation for the pinworm populations. In order to make regional and local inferences for both pinworm species, several primate populations were sampled across their distribution range in Mexico. Mitochondrial DNA (mtDNA) was used to assess parasite genetic patterns among and within fragments and geographic regions, as well as to analyse their demographic and population history. The life history properties of pinworms, the fragmented condition of the habitat, the limited ability of primates to cross the matrix and move across forest patches (Mandujano and Estrada, 2005), and the tight parasite-host association between pinworms and primates, fit the conditions for low diversity and strong genetic structure in parasite populations. Our unexpected results are discussed in light of their implications for primate ecology and conservation, in addition to contributing to our growing understanding of parasite evolution.

2. Materials and methods

2.1. Collection of pinworm specimens

Trypanoxyuris minutus specimens were collected from free-ranging howler monkey troops inhabiting 16 isolated forest fragments assigned to four geographic regions across their distribution range in south-eastern Mexico (Fig. 1). Distances between

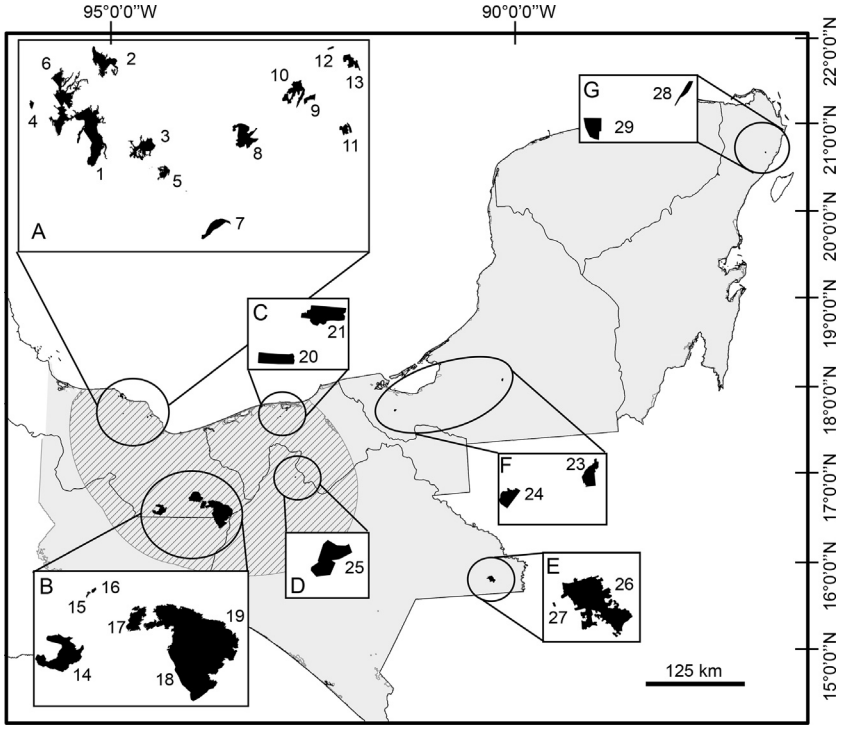

Fig. 1. Collection sites for pinworms across the distributional ranges of mantled howler monkeys (dashed area) and spider monkeys (grey area) in Mexico. Circles and ellipses correspond to geographic regions: (A) Los Tuxtlas, (B) Uxpanapa, (C) Tabasco, (D) Chiapas for mantled howler monkeys, (E) Chiapas for spider monkeys, (F) Campeche, (G) Quintana Roo. Black polygons are the tropical forest fragments where sampling was performed; numbers correspond to forest fragment IDs in Tables 1 and 2.

centroids of forest fragments in each geographic region ranged from 2 km to 77 km, while distances between centroids of regions ranged from 79 km to 226 km. Similarly, *T. atelis* specimens were collected from spider monkey troops inhabiting 15 forest fragments distributed in six regions (Fig. 1). Distances between centroids of forest fragments in each geographic region ranged from 2 km to 142 km, while that between centroids of regions ranged from 130 km to 870 km. All spider monkey samples were collected from free-ranging populations, except those in Villahermosa, Tabasco which came from spider monkeys reared in a zoo. These samples were included to test whether we could recover a particular genetic makeup in parasite populations from captive hosts compared with parasite populations in free-ranging hosts.

Non-invasive sampling techniques were employed to obtain adult pinworms from primate faeces. Faecal samples were collected immediately after defecation and placed on ice until transported to the laboratory where those were preserved at −20 °C. Whenever possible, adult pinworms were recovered in situ before storing the faecal sample. Searching for additional adult pinworms was done following the procedure proposed by Hasegawa (2009) using the frozen faecal samples. All recovered specimens were fixed in 100% ethanol for DNA extraction. An average of 10 pinworms per host species per forest fragment was sampled, preferably from distinct host individuals. Approximately two pinworm specimens per faecal sample were sequenced, with the exception of two localities for *T. minutus* (Playa and El Fortuño) and one for *T. atelis* (Playa) where four pinworms were obtained per sample (Tables 1 and 2).

2.2. DNA extraction, amplification and sequencing

Individual pinworms 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 of proteinase K. DNA was extracted from the supernatant using DNAzol® reagent (Molecular Research Center, Cincinnati, OH, USA) according to the manufacturer's instructions. mtDNA has been shown to be an excellent molecular marker for population genetic studies in nematodes, given its high substitution rate (Blouin et al., 1998); thus a section of the cytochrome c oxidase subunit 1 gene (*cox*1) was amplified using the primers TrycoxF, 5′-TGGTTGGCAGGTCTTTATC-3′ (forward) and TryCoxR, 5′-AACCAACTAAAAACCTTAATMC-3′ (reverse) (Solórzano-García et al., 2015). The PCR conditions were: initial denaturation at 94 °C for 1 min, followed by 30 cycles at 94 °C for 1 min, 54 °C for 1 min, 72 °C for 2 min, and a post-amplification extension for 7 min at 72 °C. PCR products were treated with Exo-SAP (Thermo Scientific, USA), according to the manufacturer's instructions. Sequences were assembled and base-calling differences were resolved using Geneious v.5.1.7 (Biomatters, Auckland, New Zealand). Sequences were aligned using Clustal W and MESQUITE v.2.75 (Maddison, W., Maddison, D., 2011. Mesquite: a modular system for evolutionary analysis.), and checked for accuracy using the translated amino acid sequences based on the invertebrate mitochondrial genetic code. A total of 140 sequences of 841 bp were obtained for *T. minutus* (GenBank accession Nos. MF379131 to MF379260), while 98 sequences of 844 bp were obtained for *T. atelis* (GenBank accession Nos. MF379058 to MF379130).

2.3. Analysis of genetic variation and population differentiation

Molecular diversity indices including the number of segregating sites (S), number of haplotypes (h), haplotype diversity (Hd), nucleotide diversity (π), and average number of nucleotide differences (k) were derived using DnaSP v.5 (Rozas et al., 2003) for each forest fragment, and each geographic region, for each pinworm species. Genetic diversity parameters were not estimated for populations with less than five sampled parasite individuals, but these populations were included in the regional and total estimations. To assess the amount of genetic differentiation among fragments and regions, pairwise Fst based on pairwise genetic differences were estimated using Arlequin v.3.5 (Excoffier et al., 2005). We also included the nearly unbiased Gst estimator using DnaSP v.5. Gst is based on haplotype frequency, and it is corrected by the product of the number of localities multiplied by the harmonic mean of the sample sizes per locality (Hudson et al., 1992); the unbiased Gst is

Table 1
Genetic diversity and neutrality tests for *Trypanoxyuris minutus* from howler monkeys in Mexico. Bold indicates total values per region.

Region	Fragment	ID[a]	pps	*n*	S	h	Hd (S.D.)	π (S.D.)	k	Fu's Fs
Los Tuxtlas North	R.Huber	F1	2.4 (2–3)	12	13	9	0.955(0.047)	0.00467(0.00071)	3.92	−3.01
	Montepío	F2	1.6 (1–2)	11	14	10	0.982(0.046)	0.00493(0.00061)	4.15	−5.30[b]
	P. escondida	F3	2.0 (1–3)	10	12	6	0.778(0.137)	0.00357(0.00098)	3.00	−0.82
	Organos II	F4	1.4 (1–2)	10	12	9	0.978(0.054)	0.00380(0.00054)	3.20	−5.26[c]
	Jicacal	F5	1.5 (1–2)	3						
	2 Abril	F6	1.4 (1–2)	7	7	5	0.857(0.137)	0.00328(0.00074)	2.76	−0.87
	Subtotal		**1.7**	**53**	**33**	**34**	**0.943(0.024)**	**0.00421(0.00034)**	**3.54**	**−32.8[c]**
Los Tuxtlas Centre	Agaltepec	F7	**1.3 (1–2)**	**10**	**15**	**8**	**0.933(0.077)**	**0.00510(0.00082)**	**4.29**	**−2.42**
Los Tuxtlas South	Magallanes	F9	2.8 (2–3)	11	12	10	0.982(0.046)	0.00454(0.00071)	3.82	−5.66[b]
	M. Pilapa	F11	2.0 (1–3)	10	10	7	0.867(0.107)	0.00402(0.00055)	3.38	−1.73
	Playa	F12	4.0 (4)	8	10	8	1 (0.063)	0.00539(0.00079)	4.54	−4.21[b]
	Subtotal		**2.6**	**29**	**19**	**22**	**0.973(0.018)**	**0.00477(0.00043)**	**4.02**	**−16.7[c]**
	Total		**1.8**	**92**	**42**	**53**	**0.957(0.012)**	**0.00450(0.00026)**	**3.79**	**−61.9[c]**
Uxpanapa	El Fortuño	F14	4.0 (4)	4						
	M.Vidal	F15	2.5 (2–3)	5	9	5	1 (0.126)	0.00428(0.00087)	3.6	−1.90[b]
	Liberales	F17	1.7 (1–3)	5	8	4	0.900(0.161)	0.00428(0.00108)	3.6	−0.04
	Total		**2.3**	**14**	**17**	**11**	**0.967(0.037)**	**0.00512(0.00058)**	**4.31**	**−4.52[b]**
Tabasco	H. la Luz	F20	1.7 (1–3)	10	12	9	0.978(0.054)	0.00407(0.00053)	3.42	−4.99[c]
	Comalcalco	F21	1.4 (1–2)	10	15	9	0.978(0.054)	0.00526(0.00068)	4.42	−4.01[b]
	Total		**1.5**	**20**	**20**	**17**	**0.979(0.024)**	**0.00469(0.00044)**	**3.94**	**−12.58[c]**
Chiapas	Pichucalco	F25	**1.8 (1–2)**	**14**	**16**	**13**	**0.989(0.031)**	**0.00431(0.00076)**	**3.63**	**−9.80[c]**
Total			**1.8**	**140**	**62**	**83**	**0.971(0.007)**	**0.00471(0.00020)**	**3.96**	**−124.72[c]**

Pps, number of sequenced pinworms per faecal sample (and range); *n*, number of sequences; S, segregating sites; h, number of haplotypes, Hd, haplotype diversity; π, nucleotide diversity; k, average number of nucleotide differences.

[a] Fragment ID numbers correspond to those in Fig. 1.

[b] $P \leq 0.05$ were considered significant.

[c] $P \leq 0.001$ were considered significant.

thought to be less susceptible to standard error when taking multiple samples from a single population (Meirmans and Hedrick, 2011). To assign the genetic variation among or within populations, we performed a hierarchical analysis of molecular variance (AMOVA) as implemented in Arlequin using pairwise differences, and 1000 bootstrap replicates to evaluate significance. Also, a median-joining haplotype network was constructed using Network v.5.000 (Bandelt et al., 1999) to show the evolutionary relationships between haplotypes from different fragments and regions. Because 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).

2.4. Analysis of population structure

We used two methods to evaluate the population structures of both pinworm species. A spatially explicit Bayesian clustering method was implemented in the program BAPS v.6 (Cheng et al., 2013). BAPS defines the neighbourhood of each individual based on Voronoi tessellation, with neighbouring individuals more likely to be co-assigned to a cluster than individuals who are far apart; also the correlation between clusters decreases with the distance between sites (Corander et al., 2008). We tested a maximum number of clusters (K) of $K = 5$, $K = 10$, $K = 15$, running 10 replicates for each value of K, using the spatial clustering of groups in the population mixture analysis.

Also, a discriminant analysis of principal components (DAPC) (Jombart et al., 2010) was performed using the adegenet v.3.1.9. package (Jombart, 2008) for R (R Development Core Team 2010) to attempt to differentiate pinworm populations based on fragments and regions. DAPC does not make assumptions regarding population genetic models, and the optimal number of population clusters is established through the Bayesian information criterion (BIC) using the find.clusters function in adegenet v.3.1.9.

Finally, because primate movements between forest fragments decrease with greater distances between those, we evaluated whether pinworm populations presented a pattern of isolation by distance (IBD) by performing a Mantel test (Mantel, 1967) to correlate Edward's genetic distance (Edwards, 1971) with geographic distance, using the R console. Geographical distances between forest fragments were calculated using the raster v.2.5.8. package (Hijmans, R., van Etten, J., Cheng, J., Mattiuzzi, M., Sumner, M., Greenberg, J.A., Lamigueiro, O., Bevan, A., Racine, E., Shortridge, A., 2016. Raster: Geographic data analysis and modeling. R package version 2.5-8). Edward's distance was estimated using the adegenet v.3.1.9 package. Mantel tests were run for each pinworm species with the ade4 v.1.7.4 (Dray and Dufour, 2007) package, with 999 repetitions.

2.5. Demographic and population history

To investigate the population histories and demographies of *T. minutus* and *T. atelis*, three methods were used. First we calculated Fu's Fs neutrality test using DnaSp v.5. This test evaluates whether populations are evolving at equilibrium between mutation and genetic drift or if some non-random process is happening such as natural selection or population expansion or decline. A negative value of Fu's Fs indicates a larger number of alleles than expected given the observed level of genetic diversity, consistent with population expansion, while a positive value suggests deficiency of alleles as would be expected in a declining population. Second, we constructed a Bayesian Skyline Plot (BSP) using the software BEAST v.1.7.5 (Drummond et al., 2005) to infer changes in the population sizes of both pinworms over time. The BSP is a coalescent method that uses a Markov chain Monte Carlo (MCMC) procedure to estimate a posterior distribution of effective population sizes over time directly from a sample of gene sequences, given a specified nucleotide substitution model (Drummond et al., 2005). For both pinworm species the appropriate model of nucleotide evolution was HKY + I + G, determined using the AIC criterion in MrModeltest v.2.3 (Nylander, J.A.A., 2004. MrModeltest v.2). BSP analyses were run with the strict molecular clock option and a mutation rate of 1.57×10^{-7} substitutions per site per generation, which was estimated from *Caenorhabditis elegans* mtDNA (Denver et al., 2000). One hundred million iterations were performed, sampling model parameters every 20,000 iterations with 10% burn-in. Plots and the performance of the MCMC process were visualized in Tracer v 1.5. (Rambaut, A., Suchard, M., Drummond, A., 2013. Tracer.). Third, we used Lamarc v.2.1.3 software (Kuhner, 2006) to estimate the demographic parameters Θ_0 and the population growth rate (g) of the expression $\Theta t = \Theta_0 \exp(-gt\mu)$. For mtDNA, Θ_0 equals $2\mu N_e$, where μ is the mutation rate of 1.57×10^{-7} substitutions per site per generation, and N_e is the effective population size of females. Positive values of g indicate that the population has been growing, and negative values indicate that it has been shrinking, while a value of zero indicates no change in population size. The analyses used the F84 model with the substitution rates corrected in order to adjust for the previously determined HKY+G+I model. We used the Bayesian search with five independent runs, 10 initial chains and five final chains each using 10,000,000 steps with a burn-in period of 10,000 steps. For both species of pinworms the growth priors ranged from -500 to 10,000 in order to ensure that the search included growth, decline and no population change.

3. Results

3.1. Genetic diversity and population differentiation

Values of average molecular diversity within fragments and regions are shown in Tables 1 and 2. Populations of *T. minutus* and *T. atelis* were highly genetically diverse for mtDNA, showing a haplotype diversity per fragment or region ranging from 0.778 to 1.0 and a nucleotide diversity ranging from 0.0023 to 0.0126. Both haplotype and nucleotide diversities were higher in *T. atelis* than in *T. minutus*.

Pairwise Fst ranged from low to high differentiation in both pinworm species. For *T. minutus*, no significant Fst values were observed between populations from the same region. Notably, the southernmost populations showed the highest population differentiation compared with the rest of the populations, however it was not a uniform pattern (Table 3, Supplementary Table S1). In *T. atelis*, significant Fst values were observed between certain populations in forest fragments belonging to the same region (Supplementary Table S2); at the regional level, Campeche and Quintana Roo showed the highest Fst values, suggesting a moderate genetic differentiation between *T. atelis* populations in these regions and the rest (Table 3). On the other hand, Gst values were considerably lower than Fst values in both pinworm species (Table 4). Moreover, pinworms from captive and free-living hosts behaved genetically as one population.

Fst values calculated by AMOVA also indicated low to moderate differentiation between populations of both pinworm species, with most of the variation distributed within populations rather than among populations or among regions (Table 5). These findings were supported by the haplotype networks. In both pinworm species, median-joining networks showed no clear distribution of haplotypes according to geographical location (Fig. 2). Additionally, an ancestral haplotype was not evident in either network. The most common haplotype in *T. minutus* was recovered from 17 specimens (15 from Los Tuxtlas and two from Uxpanapa); nine haplotypes

Table 2
Genetic diversity and neutrality tests for *Trypanoxyuris atelis* from spider monkeys in Mexico. Bold indicates total values per region.

Region	Fragment	ID[a]	pps	n	S	h	Hd (S.D.)	π (S.D.)	k	Fu's Fs
Los Tuxtlas	Guadalupe	F8	1.2 (1–2)	7	14	7	1(0.076)	0.00609(0.00088)	5.14	−2.94[b]
	Magallanes	F10	2.0 (2)	2						
	Playa	F13	2.5 (1–4)	10	8	5	0.8(0.1)	0.00232(0.00073)	1.96	−0.65
	subtotal		**1.7**	**19**	**19**	**12**	**0.924(0.042)**	**0.00405(0.00068)**	**3.42**	**−4.83[b]**
Uxpanapa	El Fortuño	F14	1.8 (1–2)	7	13	7	1(0.076)	0.00621(0.00092)	5.24	−2.89[b]
	Murillo Vidal	F16	1.5 (1–2)	3						
	Liberales	F17	1.0 (1)	1						
	El Jaguar	F18	1.7 (1–2)	10	14	9	0.978(0.054)	0.00529(0.00064)	4.47	−3.98[b]
	El Desengaño	F19	2.0 (1–3)	10	16	10	1(0.045)	0.00670(0.00064)	4.44	−6.42[c]
	subtotal		**1.7**	**31**	**32**	**28**	**0.994(0.010)**	**0.00625(0.00047)**	**5.28**	**−26.4[c]**
Tabasco	**Villahermosa**	**F22**	**2.5 (1–3)**	**10**	**12**	**10**	**1(0.045)**	**0.00469(0.00066)**	**3.96**	**−6.94[c]**
Campeche	La Libertad	F23	1.2 (1–2)	11	30	10	0.982(0.046)	0.01267(0.00198)	10.7	−2.05
	El Zapote	F24	3.0 (3)	3						
	Subtotal		**1.4**	**14**	**33**	**13**	**0.989(0.031)**	**0.01178(0.00183)**	**9.95**	**−4.26[b]**
Chiapas	R. Agraria	F26	2.0 (2)	4	12	4	1(0.177)	0.0077(0.00186)	6.50	
	Guacamayas	F27	2.7 (2–3)	8	18	8	1(0.063)	0.00711(0.00246)	6.00	−3.40[b]
	Subtotal		**2.4**	**12**	**21**	**11**	**0.985(0.040)**	**0.00707(0.00180)**	**5.97**	**−4.74[b]**
Quintana Roo	P. Morelos	F28	1.3 (1–2)	10	17	10	1(0.045)	0.00685(0.00078)	5.78	−5.33[b]
	San Joaquín	F29	1.0 (1)	2						
	Subtotal		**1.2**	**12**	**12**	**11**	**0.985(0.040)**	**0.00632(0.00072)**	**5.33**	**−5.23[b]**
Total			**1.7**	**98**	**66**	**73**	**0.988(0.005)**	**0.00724(0.00059)**	**6.11**	**−91.9[c]**

Pps, number of sequenced pinworms per faecal sample (and range); *n*, number of sequences; S, segregating sites; h, number of haplotypes; Hd, haplotype diversity; π, nucleotide diversity; k, average number of nucleotide differences.
[a] Fragment ID number correspond to those in Fig. 1.
[b] $P \leq 0.05$ were considered significant.
[c] $P \leq 0.001$ were considered significant.

Table 3
Pairwise Fst values between pinworm populations from different regions in southern Mexico. *Trypanoxyuris minutus* data is shown under the diagonal, *Trypanoxyuris atelis* data is shown above the diagonal. Bold shaded values are significantly different from zero. Dashes indicate unsampled regions.

	TN	TS	Tuxtlas	UXP	TAB	CHI	CMP	QRoo
TS	0.002		—	—	—	—	—	—
Tuxtlas	NA	NA		0.012	**0.056**	0.034	**0.226**	**0.201**
UXP	-0.015	-0.019	-0.019		0.047	0.029	**0.175**	**0.101**
TAB	**0.103**	**0.094**	**0.089**	**0.055**		0.049	**0.191**	**0.239**
CHI	**0.106**	**0.088**	**0.087**	0.056	0.009		**0.133**	**0.143**
CMP	—	—	—	—	—	—		**0.092**
QRoo	—	—	—	—	—	—	—	

NA, not applicable; TN, Tuxtlas North; TS, Tuxtlas South; UXP, Uxpanapa; TAB, Tabasco; CHI, Chiapas; CMP, Campeche; QRoo, Quintana Roo.

Table 4
Pairwise Gst values between pinworm populations from different regions in southern Mexico. *Trypanoxyuris minutus* data is shown under the diagonal, *Trypanoxyuris atelis* data is shown above the diagonal. Bold shaded cells show highest values. Dashes indicate unsampled regions.

	TN	TS	Tuxtlas	UXP	TAB	CHI	CMP	QRoo
TS	0.0006		------	------	------	------	------	------
Tuxtlas	NA	NA		**0.0129**	**0.0116**	0.0026	**0.0225**	**0.0240**
UXP	0.0058	-0.001	0.0094		0.0062	0.0056	0.0055	**0.0099**
TAB	**0.0178**	**0.0117**	**0.0146**	**0.0107**		0.0000	0.0000	0.0000
CHI	**0.0154**	0.0084	**0.0143**	0.0060	0.0053		0.0038	0.0042
CMP	------	------	------	------	------	------		0.0038
QRoo	------	------	------	------	------	------	------	

NA, not applicable; TN, Tuxtlas North; TS, Tuxtlas South; UXP, Uxpanapa; TAB, Tabasco; CHI, Chiapas; CMP, Campeche; Qroo, Quintana Roo.

were shared between regions, with only one haplotype present in the four regions. For *T. atelis,* the most common haplotype was recovered from seven specimens (four from Los Tuxtlas, one from Uxpanapa and two from Chiapas); five haplotypes were shared among regions, nevertheless no haplotype was present in all six regions.

3.2. Population structure

The optimal number of groups obtained from BAPS analysis was $K = 1$ for *T. minutus* and $K = 2$ for *T. atelis* regardless of the maximum value of K tested. Similarly, the DAPC was not able to discriminate population clusters in either pinworm species (Supplementary Fig. S1). These analyses in conjunction with the AMOVA and Gst results indicated a lack of correlation between genetic structure and the geographical distribution of the different fragments and regions. Patterns of IBD were tested for both pinworm species. Mantel tests showed a modest relationship between genetic distances and geographical distances ($R^2 = 0.24$, $P = 0.028$) for *T. minutus*. In contrast, no IBD was observed in *T. atelis* populations ($R^2 = 0.16$, $P = 0.113$) (Supplementary Fig. S2).

3.3. Demography and population history

Overall, Fu's Fs values indicated a population expansion in both *T. minutus* and *T. atelis;* at a local level, Fu's Fs were high and negative in most of the fragments, especially in *T. minutus* populations (Tables 1 and 2). The demographic expansion scenario was also supported by the other two methods employed to evaluate population history. BSP showed population expansions in both pinworm species that occurred approximately 12,000 generations ago (Fig. 3). Lamarc analyses also indicated a rapid population growth with $g = 3{,}111$ (2,040–4,924, 95% PPD) for *T. minutus* and $g = 811$ (492–972, 95% PPD) for *T. atelis*. A value of $g = 200$ has been suggested as indicative of fast population growth, i.e. that the population has grown hugely in a relatively short period of time (Kuhner, 2006). Thus the values obtained for *T. minutus* and *T. atelis* support the idea of large population sizes in both species of pinworms.

4. Discussion

Here, we present a population genetic analysis of two highly host-specific parasite species, with haplodiploid reproduction,

Table 5
Analysis of molecular variance (AMOVA) of *Trypanoxyuris minutus* and *Trypanoxyuris atelis* populations from southern Mexico based on cytochrome c oxidase subunit 1 gene (*cox*1).

Source of variation	d.f	Sum of squares	Variance components	Percentage of variation	Statistics	*P* value
Trypanoxyuris minutus						
Among regions	3	14.17	0.0867	4.28	Fct = 0.043	0.023
Among fragments within regions	12	29.99	0.0751	3.71	Fsc = 0.039	0.019
Within fragments	124	231.23	1.8648	92.01	Fst = 0.079	<0.0001
Trypanoxyuris atelis						
Among regions	5	41.70	0.2242	7.19	Fct = 0.088	0.089
Among fragments within regions	8	33.04	0.2555	8.20	Fsc = 0.072	0.014
Within fragments	84	221.46	2.6365	84.61	Fst = 0.154	<0.0001

d.f, degrees of freedom.

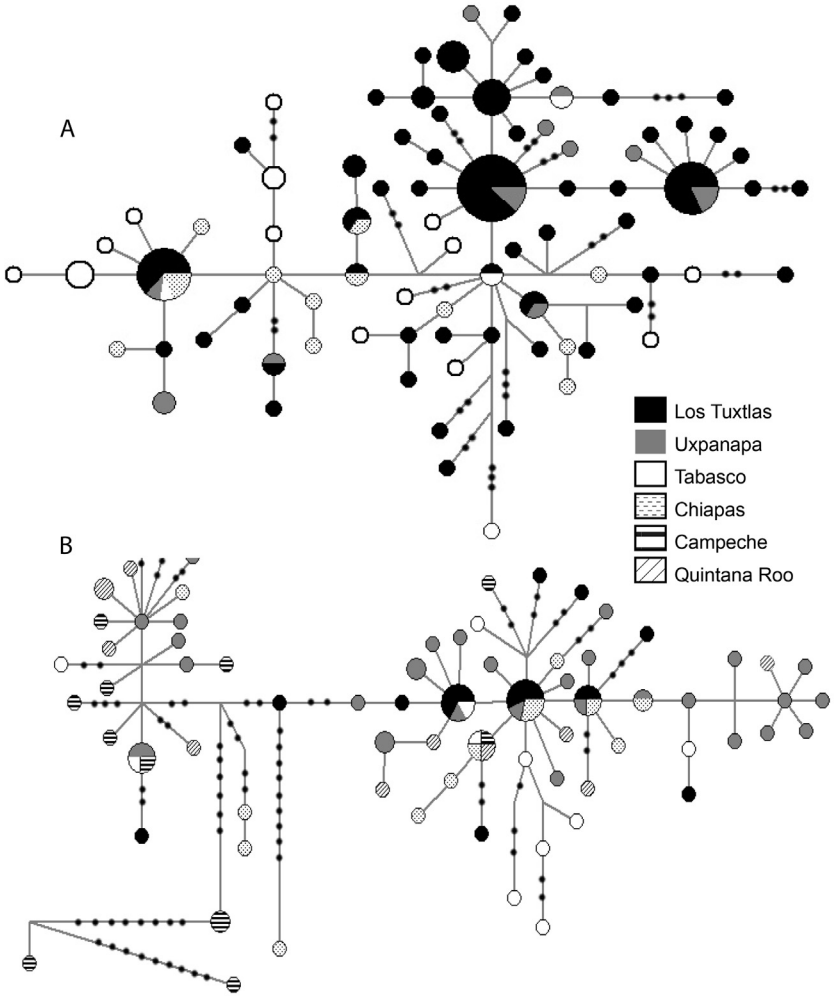

Fig. 2. Median-Joining haplotype network for *Trypanoxyuris minutus* (A) and *Trypanoxyuris atelis* (B) based on the cytochrome c oxidase subunit 1 gene (*cox1*). Haplotype frequency is represented by the diameter of the circle.

direct life cycle, and a transmission mode that commonly involves autoinfection and retroinfection; both pinworms occur in host populations living in forest fragments. The life history traits of these parasites together with the current isolation of their host populations due to habitat loss and fragmentation, lead to predictions of reduced genetic diversity and high differentiation between parasite populations (Criscione et al., 2005; Huyse et al., 2005; Barrett et al., 2008). Nevertheless, our results showed genetic patterns that were inconsistent with these predictions, giving new insights into parasite population history and host ecology. In both parasites we found high haplotype diversity, low nucleotide diversity, a null population structure and signals of large population sizes characteristic of population expansion. Nadler (1995) postulated that both parasite and host traits are responsible for the genetic make-up of parasite populations. The interplay of these factors in shaping the observed genetic patterns of *Trypanoxyuris* spp. in conjunction with landscape features is further discussed below, together with the possible scenarios that could yield the lack of population structure.

A transmission mode where offspring constantly reinfect their natal individual host and a haplodiploid sex determination system

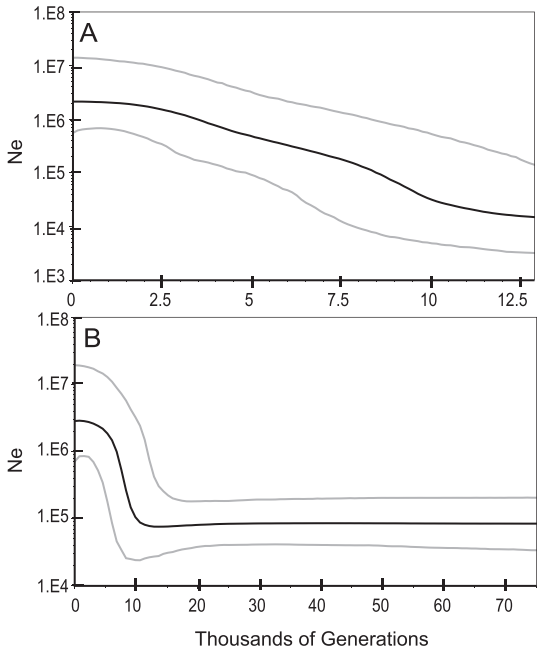

Fig. 3. Bayesian skyline plots of the change in effective population size (N_e) of *Trypanoxyuris minutus* (A) and *Trypanoxyuris atelis* (B). The black line is the median estimate of population size, and the grey lines indicate the 95% highest and lowest confidence intervals. A continuous but high population growth is observed for *T. minutus*, while a more sudden expansion is observed in *T. atelis*.

may both increase inbreeding and limit gene flow between populations, reducing parasite genetic diversity (Adamson, 1989; Nadler, 1995). However, both pinworm species showed high haplotype diversity in all sampled populations, and nucleotide diversity similar to that found in free-living animals (Goodall-Copestake et al., 2012). Notably, the levels of nucleotide diversity found in these nematodes were considerably lower than those reported for *cox*1 in other parasites with direct life cycles (π = 0.012–0.021 (Miranda et al., 2008; Archie and Ezenwa, 2011; Haynes et al., 2014; Ács et al., 2016)), and instead resemble those of parasites with asexual reproduction (π = 0.006 (Keeney et al., 2009; Marigo et al., 2015)). The levels of nucleotide diversity found in the two species of *Trypanoxyuris* could be explained by their haplodiploid condition and transmission mode; however, other pinworm species and even *Enterobius vermicularis*, the sister genus of *Trypanoxyuris* and also a pinworm that parasitizes primates, show higher levels of nucleotide diversity (π = 0.014–0.049 (Falk and Perkins, 2013; Rodriguez-Ferrero et al., 2013)). We believe that the relationship found between haplotype and nucleotide diversity in the two pinworm species herein studied is probably a consequence of the parasites' large population sizes and reproductive mode, and the tight co-evolutionary associations between these parasites and their hosts.

Little is known about the genetic diversity of primates occurring across Mexico; nevertheless, a few local studies with howler monkeys (*Alouatta palliata*) have found lower genetic diversity in these populations compared with others along the species distributional range (Baiz, M.D., 2013. Intragroup genetic relatedness in two howler monkey species (*Alouatta pigra* and *A. palliata*): implications for understanding social systems and dispersal. Master Thesis. University of Michigan, USA; Dunn et al., 2014; Alcocer-Rodriguez, M., 2015. Evolución demográfica de *Alouatta palliata mexicana* en fragmentos de selva: migración, parentesto y características del hábitat que determinan su persistencia. Ph.D. Thesis. Universitat de Barcelona, Spain; Jasso-del Toro et al., 2016). Howler and spider monkeys occurring in Mexico represent the northernmost populations in these species' geographical range (Rylands et al., 2006). The colonization of Mexican tropical rainforests is relatively recent, with the primates moving from South to North America, apparently after the emergence of the Panama land bridge (Cortes-Ortiz et al., 2003; Lynch Alfaro et al., 2015). The low genetic diversity in Mexican primates is thought to be a consequence of population bottlenecks suffered by these species during their expansion through central America (Dunn et al., 2014). A reduction in host populations also could have resulted in a reduction in their pinworm populations, with current levels of nucleotide diversity reflecting the host-parasite biogeographical histories.

High levels of host specificity have been proposed to increase parasite genetic structure since parasites with narrow host ranges are more likely to experience processes of local extinction (Barrett et al., 2008). Also, parasites with direct life cycles and no free living stages (such as pinworms) are expected to strongly rely on their host movements to disperse (Nadler, 1995; Criscione et al., 2005). In these cases, parasite gene flow mainly depends upon the potential of their hosts to disperse parasites between geographically isolated populations (Blasco-Costa et al., 2012). In spite of the high host specificity of pinworms and the isolation of host populations between forest fragments and regions, we found no genetic structure in either *Trypanoxyuris* sp.

At a local scale, pairwise Fst and Gst values show no differentiation between pinworm populations inhabiting forest fragments in the same region, with the exception of only a couple of *T. atelis* populations where moderate to high genetic differentiation was detected; however, this differentiation seemed random and did not obey any geographical or spatial pattern, and could be an artefact of the relatively small sample sizes per fragment (Criscione et al., 2005). Moreover, since no differentiation was observed between other populations in the same region separated by greater distances, we believe that factors other than geographical distance and host movement are responsible for the observed differentiation patterns.

At a broader scale, Gst and Fst values suggest the emergence of population differentiation patterns, yet not enough to induce a clear genetic population structure. In *T. minutus*, we observed a tendency of the southernmost regions to differentiate from the rest. This is supported not only by the AMOVA results showing a slight but significant variation among regions, but also by the existence of a rather week IBD pattern. Contrary to *T. minutus*, no clear geographic pattern was observed in *T. atelis* by either Gst, Fst, nor IBD. However, the appearance of genetic differentiation between regions may be associated with environmental factors such as habitat type, since primate habitat in the regions of Campeche and Quintana Roo, which showed the highest Fst values, consists mainly of semi-deciduous low canopy tropical forest, while the rest of the regions are dominated by tall evergreen tropical rainforest. Environmental and climatic regimes characteristic of each forest type, together with differences in the feeding ecology of spider monkeys in each habitat, could be promoting the observed Fst values between regions in *T. atelis* populations. Nonetheless, this pattern was not observed in the Gst results. The magnitude of Gst values is dependent on the amount of genetic variation (Meirmans and Hedrick, 2011), thus the observed small values among *Trypanoxyuris* populations could be a consequence of the high variability of mtDNA in nematodes (Blouin et al., 1998). In addition, since this estimator is corrected by the sample size, the disagreement between Gst and Fst could be indicative of some sampling bias.

Regardless of the limited gene flow between certain forest fragments and regions, neither of the different clustering methods applied could discriminate between pinworm populations, preventing us from detecting any genetic structure in these parasites from host populations in Mexico. This apparent panmixia suggests

at least three possible scenarios. Below, we provide further discussion on these scenarios, presented in increasing order of likelihood.

First, *Trypanoxyuris* eggs could be transported by wind or water to different forest fragments. Dissemination of infective eggs by air currents has been reported in *Enterobius vermicularis* (Nolan and Reardon, 1939) in humans, however no information is available for *Trypanoxyuris*, and in addition to that, the chances of these eggs being ingested by their primate hosts seem to be somewhat low, since monkeys rarely come down the trees to drink water from rivers (Campbell et al., 2005). Furthermore, pinworm eggs are very sensitive to low humidity and survive only a few days outside the host (Adamson, 1989; Nadler, 1995); this is particularly true for *Trypanoxyuris* in forest fragments, where edge effects impose hostile conditions for egg survival (Escorcia-Quintana, M., 2014. Efecto del borde selva-pastizal sobre el desarrollo del parásito gastrointestinal *Trypanoxyuris* sp. del mono aullador de manto (*Alouatta palliata mexicana*) en Los Tuxtlas, Veracruz, México. Master Thesis. Universidad Veracruzana, Mexico.), making gene flow through wind rather unlikely.

Second, in spite of the fragmented condition of the study sites, and the low probability of primates crossing the matrix (Mandujano et al., 2004), movement of primates between forest fragments may be more frequent than expected. Even though the few available population genetic analyses of howler monkeys show limited gene flow between forest fragments in Los Tuxtlas and Uxpanapa (Dunn et al., 2014; Alcocer-Rodriguez, M., 2015. Evolución demográfica de *Alouatta palliata mexicana* en fragmentos de selva: migración, parentesto y características del hábitat que determinan su persistencia. Ph.D. Thesis. Universitat de Barcelona, Spain), gene flow among parasite populations does not require reproductive success of the host, only the dispersal of individuals. Thus, monkeys could be using different fragments for feeding or be transient visitors carrying their parasites with them. Moreover, the longevity of parasite eggs also plays a key role in the success of this mechanism. It has been argued that long-lived eggs favour greater gene flow than short-lived eggs, reducing genetic structure (Nadler, 1995). Hence, parasite eggs that can persist longer in the environment would have greater opportunities to colonise new hosts when dispersed by host movement. Little is known about the capacity of monkeys to move between forest fragments; a study of howler monkeys in Mexico reports a threshold distance between 60 m and 200 m depending on landscape connectivity (Mandujano and Estrada, 2005). However, hazardous movements have been observed in both howler and spider monkeys (Chaves and Stoner, 2010; Herrera et al., 2015) to reach forest fragments. The lack of genetic structure in the two species of parasites suggests that monkeys could probably overcome the adversities imposed by the non-suitable matrix more often than previously thought. The relatively frequent movement of primates between forest fragments has important implications for tropical forest regeneration (Link and Di Fiore, 2006; Chaves et al., 2011; Arroyo-Rodríguez et al., 2015), enhancing the conservation value of these organisms.

Finally, the third scenario assumes the absence of gene flow between pinworm populations, with the relatively recent tropical forest fragmentation and the large population sizes of the parasites slowing the effects of genetic drift, delaying the appearance of genetic structure. We believe this is the more plausible scenario. Massive tropical forest fragmentation in Mexico only began around 1940–1960, primary motivated by policies encouraging deforestation for farming purposes and human settlement expansions (Gonzalez-Montagut, 1999; Merino-Perez and Segura-Warnholtz, 2007). In terms of evolutionary time, the isolation of primate populations as a consequence of habitat loss and fragmentation is quite recent. Variations in the genetic structure of populations due to landscape changes are not instantly evident; there is a time lag between the occurrence of the landscape change and the genetic response (Anderson et al., 2010). Moreover, the different demographic tests applied in the present study indicated large population sizes of both *Trypanoxyuris* spp., together with population expansions and high population growth rates over time. Longer times are needed for genetic drift to be reflected in the allelic frequencies of larger parasite populations (Nadler, 1995). All these factors could be preventing us from detecting population structure in the parasites despite the absence of gene flow.

Other studies using mtDNA to assess population genetic patterns in parasites with direct life cycles have also found a lack of genetic structure (e.g., Braisher et al., 2004; Archie and Ezenwa, 2011; Haynes et al., 2014; Ács et al., 2016). Furthermore, a tendency of parasite populations to be less genetically differentiated than their hosts has been observed, with host dispersal being a poor predictor of parasite genetic patterns (Mazé-Guilmo et al., 2016). Our results concur with these previous observations, indicating that large population sizes in parasites can help them cope with the isolation and fragmentation of populations imposed by limited host movement. They are also consistent with passive dispersion of parasites (through wind, water or other sources), as unlikely as it seems, occurring at higher rates than expected, facilitating gene flow between parasite populations regardless of host vagility; this phenomenon requires confirmation, however.

Forest loss and fragmentation, rather than decreasing population sizes, could be facilitating population growth in these parasites. Some studies have shown higher parasite prevalence in primates living in forest fragments due to an increase in host density and immune-suppression caused by the stress related to habitat perturbation and competition for resources (Gillespie and Chapman, 2006; Arroyo-Rodríguez and Dias, 2010). Furthermore, spider monkey latrines are found to be closer to each other in forest fragments compared with continuous forest (González-Zamora et al., 2012). Thus, habitat loss and fragmentation could be promoting the transmission of pinworms by favouring contact between host individuals, but also by intensifying exposure to contaminated areas. This could explain why pinworm populations in these isolated host populations continue to grow despite habitat loss and fragmentation.

Howler and spider monkeys inhabiting the forest fragments sampled during the present study showed high prevalences of *Trypanoxyuris* infections (Solórzano-García and Pérez-Ponce de León, 2017). Even though pinworms of primates are not highly injurious parasites (Adamson, 1989), a howler monkey death caused by a severe *T. minutus* infection has been reported in Brazil (Amato et al., 2002). Parasites are important components of any ecosystem, and should not be neglected in efforts towards biodiversity conservation (Gómez and Nichols, 2013); however, the health hazards imposed by dense parasite populations, together with the threats that habitat loss and fragmentation impose on monkey populations (Estrada et al., 2017), are jeopardizing not only host subsistence but also parasite survival.

The results presented here provide a snapshot of pinworm population genetics from isolated host populations, and highlight the complexity of the factors that intervene in the evolutionary processes of parasites. Further studies expanding sampling efforts to include populations across the complete distribution ranges of these parasites, incorporating different molecular markers (Galtier et al., 2009) and tackling the role of environmental factors such as climatic features, topology and landscape connectivity, are essential to determine which mechanisms are generating this lack of genetic differentiation between pinworm populations in forest fragments. Population structure in parasites with a direct life cycle and high host specificity is expected to parallel that of their hosts (Huyse et al., 2005). Detailed genetic assessment of non-human primate populations in Mexico remains essential to make the

proper comparisons between pinworms and primates, in order to determine whether the genetic patterns that we found in parasites are also shown by their hosts, and to understand how habitat perturbation and environmental changes affect the evolutionary dynamics between these parasites and their hosts.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijpara.2017.06.008.

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