



RESEARCH NOTE

Haemonchus placei and *Haemonchus contortus* are Distinct Species Based on mtDNA Evidence

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Abstract—Blouin M. S., Yowell C. A., Courtney C. H. & Dame J. B. 1997. *Haemonchus placei* and *Haemonchus contortus* are distinct species based on mtDNA evidence. *International Journal for Parasitology* 27: 1383–1387. Debates continue over the extent to which the parasitic trichostrongylids *Haemonchus placei* and *Haemonchus contortus* hybridise in nature, and whether they deserve species status. Mitochondrial ND4 gene sequences from individuals of each putative species collected from populations around the United States indicate that the two species are highly differentiated at the mtDNA level. Furthermore, there was no evidence of introgressive hybridisation occurring in wild populations. © 1997 Australian Society for Parasitology. Published by Elsevier Science Ltd.

Key words: *Haemonchus placei*; *Haemonchus contortus*; mtDNA; ND4 gene.

The “large stomach worms”, *Haemonchus placei* and *Haemonchus contortus*, are economically important parasites of domestic ruminants worldwide. *H. placei* infects primarily cattle, while *H. contortus* is mainly a parasite of sheep. However, both are found in the other’s main host, and in other domestic and wild ruminants (Lichtenfels *et al.*, 1994). The average values of several morphological traits differ between populations of *Haemonchus* from cattle and sheep, but phenotypic distributions often overlap, requiring measurements on multiple characters in order to classify individual nematodes (Lichtenfels *et al.*, 1988, 1994). *Haemonchus placei* and *H. contortus* will hybridise under experimental conditions, but there is partial or complete sterility in the F1 or F2 generations (depending on which reciprocal cross is used; Le Jambre, 1979), so the extent of introgression possible is unclear. Given that the two species are sympatric

over most of their current range, it is not clear how much gene flow may be occurring between them, and debates continue over whether they even deserve species status (e.g., Gibbons, 1979; Stevenson *et al.*, 1995; R. Lichtenfels, personal communication). There are practical reasons for resolving this issue. First, if the species are very distinct at the genetic level, then drugs designed to control one species may not necessarily work well in the other. Second, if there is high gene flow between the species, then anthelmintic resistance genes selected in one species could easily spread into the other.

Recent molecular evidence suggests that *H. placei* and *H. contortus* are indeed distinct species. First, several diagnostic probes have been found that differentiate between particular isolates of the two species (rDNA external-spacer region: Zarlenga *et al.*, 1994; random genomic repetitive DNA clone: Christensen *et al.*, 1994). Second, Stevenson *et al.* (1995) sequenced ribosomal internal transcribed spacer 2 (ITS-2) from samples of *H. placei* from three locales in Australia, and from samples of *H. contortus* from Switzerland,

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the U.K., China and Australia. The ITS-2 region was 321-bp long in both species. Although there was no variation in *H. placei* and one variable nucleotide site in their samples of *H. contortus*, the two species showed a fixed difference at three nucleotide sites. Third, Jacquet *et al.* (1995) scored RAPD band sharing patterns among individual nematodes of *H. placei*, *H. contortus* and *H. longistipes* from a locale in Mauritania. They generated phenograms based on a matrix of pairwise bandsharing distances among individuals. These phenograms clustered individuals of the putative species into three monophyletic clusters.

Here we present mitochondrial ND4 gene sequence data from individuals of *H. placei* and *H. contortus* collected from around the United States. These data show that *H. placei* and *H. contortus* are well differentiated at the mtDNA level, and that there is no evidence of gene flow between the two species in domestic populations in the United States.

Blouin *et al.* (1995) studied mtDNA variation among individuals of each species sampled from around the United States (*H. placei* collected from cattle in Florida, Georgia, Louisiana and North Carolina, and *H. contortus* collected from sheep in Ohio, Tennessee, Virginia and Wyoming). That study was designed to compare the population genetic structures of five species of trichostrongylid nematodes (genetic diversity within populations, and rates of gene flow among populations of each species; see also Blouin *et al.*, 1992), but did not compare phylogenetic relationships among the species. Here we use the *H. placei* and *H. contortus* sequences from that study, plus sequences from an additional population of *H. contortus* (Boerne, TX; courtesy of T. Craig), to describe the relationship between *H. placei* and *H. contortus*. Ten individual nematodes were sampled from each population, giving a total of 40 individual *H. placei* and 50 individual *H. contortus*. Four-hundred and sixty-three base pairs of the 3' end of the mitochondrial ND4 gene were sequenced from each individual as described in Blouin *et al.* (1995). A matrix of all pairwise distances among the 90 haplotypes was created using the maximum likelihood distance in the DNADIST module of PHYLIP (J. Felsenstein, 1993. Phylogeny Inferences Package version 3.5p. Department of Genetics, University of Washington, Seattle). This distance was chosen because it allows one to input the transition/transversion (TS/TV) ratio, because it accounts for skewed base composition (there is a strong A + T bias in ND4 and in nematode mtDNA in general; e.g., Okimoto *et al.*, 1992), and because PHYLIP allows you to assign different expected rates of substitution to first, second and third position sites in codons. We used a TS/TV ratio of 7, and used 3:1:18 as the relative

expected rates of substitution at first, second and third codon positions (7 is an average TS/TV ratio obtained by counting substitutions observed at 4-fold degenerate sites in intraspecific comparisons using sequences from *H. placei*, *H. contortus*, *Mazamastrongylus odo-coilei* and *Teladorsagia circumcincta*; the 3:1:18 estimate was obtained by counting all substitutions in first, second and third position sites in the same datasets; unpublished data). The 90 individual sequences were clustered using neighbour-joining and UPGMA. Phenograms were rooted by a sequence from *Ostertagia ostertagi* (from the *O. ostertagi* mtDNA clone described in Tarrant *et al.*, 1992).

The UPGMA phenogram in Fig. 1 shows that mtDNA haplotypes of individuals from cattle and sheep separate into two very distinct clusters, within which there is much less variation than between. The neighbour joining tree gave the same result, and we also obtain the same results if we use simple per cent difference as the distance measure, instead of the maximum likelihood distance (not shown). The average per cent sequence difference (uncorrected) between individuals of the same species equals 2.6% for *H. contortus* and 2.0% for *H. placei*, while the average difference between individuals of different species equals 15.6% (over 40% after correction for multiple hits). The distinctness of the two groups is also apparent from the large number of fixed differences that separate them (34 sites out of 463, 10 of which result in a fixed amino acid difference; Fig. 2). Thus, these two lineages have clearly had separate evolutionary histories, which is consistent with the assertion that *H. contortus* and *H. placei* are distinct species. In addition, in no instance did an individual having the morphology and preferred host of one species contain mtDNA typical of the other species. Thus we see no evidence of introgression of mtDNA, as would be expected given frequent gene flow between the two species. These data and the molecular data cited above show that *H. contortus* and *H. placei* from around the world are clearly distinct at the DNA level. In conjunction with Le Jambre's (1979) data on cytogenetics and hybrid sterility, these molecular studies should finally put to rest the notion that *H. placei* and *H. contortus* are the same species.

Finally, the large number of fixed differences separating the two species in the ND4 gene suggests that this might be a good region in which to design species-specific PCR primers. Several probes based on nuclear DNA are now available that discriminate between the two species (see references cited above). One based on mtDNA would be useful if one wanted to search large numbers of individuals for evidence of introgression, or to study asymmetries in the direction of hybridisation (e.g., Lamb & Avise, 1986).

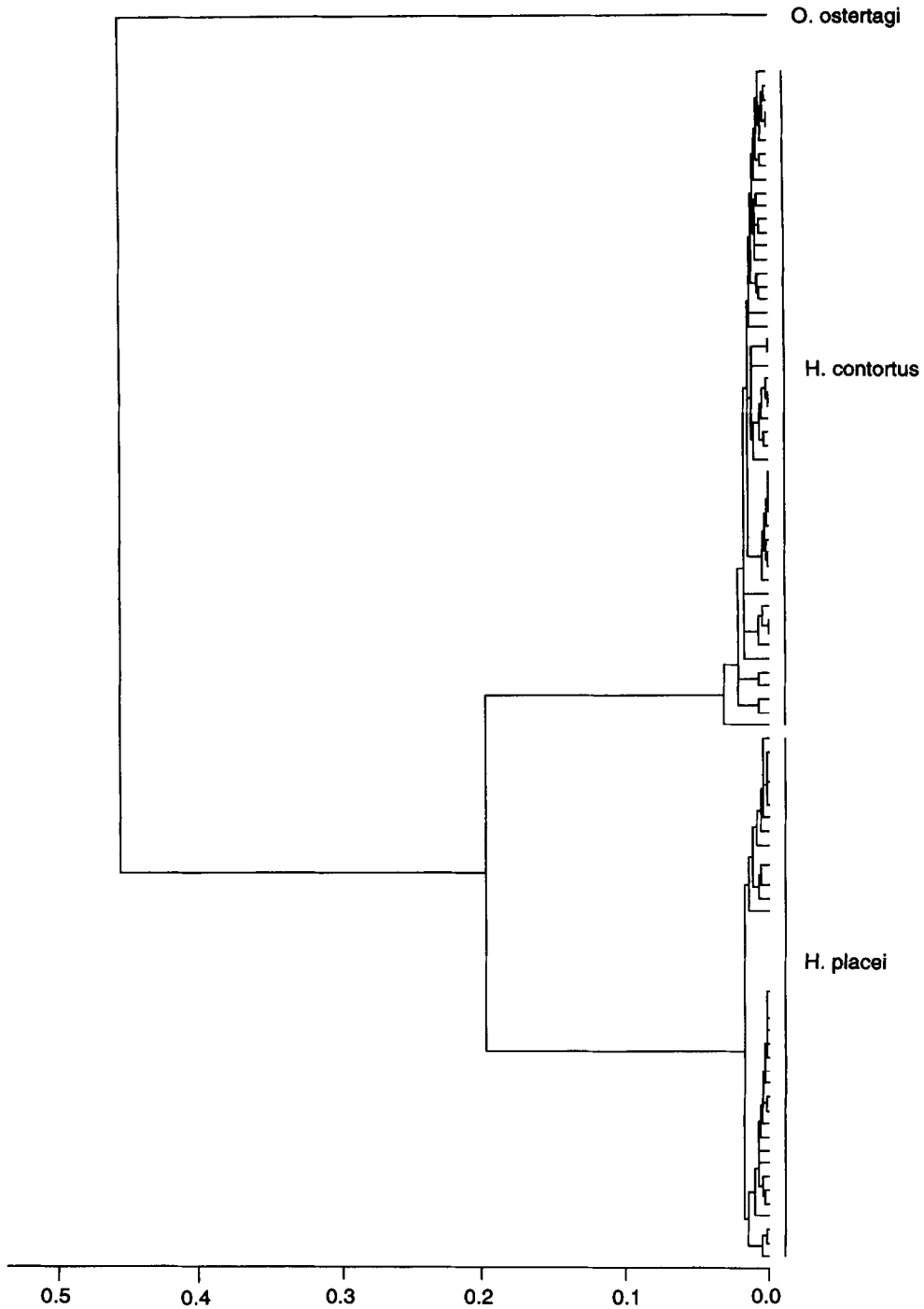


Fig. 1. UPGMA phenogram showing relationships among the 90 individuals sequenced in this study, rooted by a sequence from *Ostertagia ostertagi*. Scale on the bottom shows estimated average number of nucleotide substitutions per site between branches (maximum likelihood estimate corrected for multiple substitutions per site, as discussed in text). Individuals in the branch labelled *H. contortus* were collected from sheep abomasa, and were identified as *H. contortus* by the morphological criteria of Lichtenfels *et al.* (1988). Individuals in the branch labelled *H. placei* were collected from cattle abomasa, and were identified by the same morphological criteria. Note that the *H. contortus* and *H. placei* individuals form two monophyletic groups.

Hp	F	L	L	L	S	L	I	L	M	M	M	S	S	K	N	S	A	L	M	M	M	L	A	H	G	Y				
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Hc	F	L	L	L	S	L	I	F	M	M	M	S	S	K	N	S	A	L	M	M	M	L	A	H	G	Y				
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1	ATTTTGYTW	TTATCATTA	TTT A AATAAT	AATAAGAAGA	AARAACAGTG	CGYTTATRAT	RATATTRGCW	CAYGGYTATA	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*				
	RDTTTTATR	TTRTSTYTAA	TTTT T ATRAT	RATAAGCAGR	AAAAATAGDG	CTTTRATRAT	AATATTGGCY	CAYGGYTAYA	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*				
	T	S	T	L	M	F	Y	V	I	G	E	F	Y	H	S	S	S	S	R	M	I	Y	F	M	N	S	F			
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	CATCYACATT	AATGTTTTAT	GTAAT T GGRG	AATTTTATCA	TTCTAGATYT	TCRCGTATAA	TTTATTTTAT	AAAYAGATTT	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*			
	CRTRACATT	RATRITYTAY	GT T ATTGGDG	ARTTTTAYCA	YWCTAGRTCY	ACACGTATAA	TTTATTTTAT	RAATAGATTT	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*			
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	M	N	S	S	M	I	F	S	I	M	F	A	M	I	F	L	S	N	S	G	M	P	P	S	L	S	F			
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	ATRAATCTA	GTATAATTTT	HAGTR T GTG	TTTGCTYTTA	TTTTTTTTRTC	TAATAGYGGT	ATACCHCCTT	CMTTATCRTT	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*		
	ATAAATCTA	GTATAATTTT	TAGTAT T ATG	TTTGYTRTRA	TTTTTTTATC	WAAYAGVGGT	ATRCVCCCYT	CMTTATCGTT	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*		
	L	S	E	F	I	I	I	V	N	S	M	M	L	S	K	I	L	F	F	F	I	F	L	Y	F	M				
	-	-	-	-	-	-	-	*	-	-	-	-	-	-	*	-	-	-	-	-	-	-	*	-	-	-	-			
	L	S	E	F	I	I	I	T	N	S	M	M	L	N	K	I	L	F	F	F	V	F	V	Y	F	M				
241	3123123123	1231231231	231 2 312312	3123123123	1231231231	2312312312	3123123123	1231231231	2312312312	3123123123	1231231231	2312312312	3123123123	1231231231	2312312312	3123123123	1231231231	2312312312	3123123123	1231231231	2312312312	3123123123	1231231231	2312312312	3123123123	1231231231	2312312312	3123123123		
	TTTATCRGAA	TTTATYATTA	TT G TYAACAG	AATRRRTGTR	AGRAAAATTT	TATTTTTTTT	TRTTTTYTTA	TATTTTTRTA	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*		
	TTTRTCNGAR	TTTRTWATTA	TY A CTAAYAG	TATRDTRTTR	WATAAAATTT	TGKTTTYTTT	TGTATTY G TR	TATTTTHATRA	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*		
	I	S	F	Y	Y	S	L	F	L	I	V	N	S	F	A	G	K	Y	Y	L	K	F	N	N	N	N	F			
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*	-	*	*	*	-	-	-	-	-			
	I	S	F	Y	Y	S	L	F	L	I	V	N	S	F	A	G	K	V	Y	I	N	Y	N	N	N	N	F			
321	2312312312	3123123123	1231231231	2312312312	3123123123	1231231231	2312312312	3123123123	1231231231	2312312312	3123123123	1231231231	2312312312	3123123123	1231231231	2312312312	3123123123	1231231231	2312312312	3123123123	1231231231	2312312312	3123123123	1231231231	2312312312	3123123123	1231231231	2312312312	3123123123	
	TTTCGTTYTA	TTATTCATTR	TT T CTTATYG	TWAATTCCTT	TGYYGTAAG	WATTATTTRA	AAT T TAATAA	TAATAATTTT	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	
	TTYCRTTYTA	TTAYCTTTR	TTTT T AATTG	TTAATTCATT	HGCAGGHAAR	RTTTATAWTR	AHT A YAYAA	TAATAATTTY	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	
	G	V	T	L	F	L	M	M	M	M	Y	N	V	F	W	L	S	Y	F	S	ter									
	-	-	-	*	-	-	-	*	-	-	-	-	-	-	-	-	-	-	-	-	*									
	G	I	M	M	F	L	M	V	M	M	Y	N	I	F	W	L	S	Y	F	T	ter									
401	1231231231	2312312312	3123123123	1231231231	2312312312	3123123123	1231231231	2312312312	3123123123	1231231231	2312312312	3123123123	1231231231	2312312312	3123123123	1231231231	2312312312	3123123123	1231231231	2312312312	3123123123	1231231231	2312312312	3123123123	1231231231	2312312312	3123123123	1231231231	2312312312	3123123123
	GGTRTAAC T	TATTTTTRAT	AATAATA A TR	TATAAYR K TT	TTTGAT T RTC	RTATTT T CA	TAA	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	
	GGTATHAY R A	TATTTTTRAT	R G TDATR A TR	TATAATR T TT	TYTGACT H TC	ATATTT T ACA	TAA	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	-----*	

Fig. 2. Nucleotide and amino acid differences between *H. placei* (top) and *H. contortus* (bottom). Standard ambiguity codes mark nucleotide sites that are polymorphic within each species (Y=C or T, R=G or A, S=C or G, W=A or T, M=A or C, K=G or T, H=not G, B=not A, V=not T, D=not C, N=G, A, C or T). Consensus amino acid sequence is shown. Asterisks denote sites at which the two species do not share a nucleotide or amino acid (each species is fixed for a different nucleotide, or one species is fixed for one nucleotide and the other species is segregating for different nucleotides). Numbers above the nucleotide sequence indicate the position of each nucleotide within codons (first, second, or third position). Bold face indicates fixed nucleotide substitutions that result in amino acid substitutions between the species (i.e. each species is fixed for a single amino acid that is different between the two species).

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