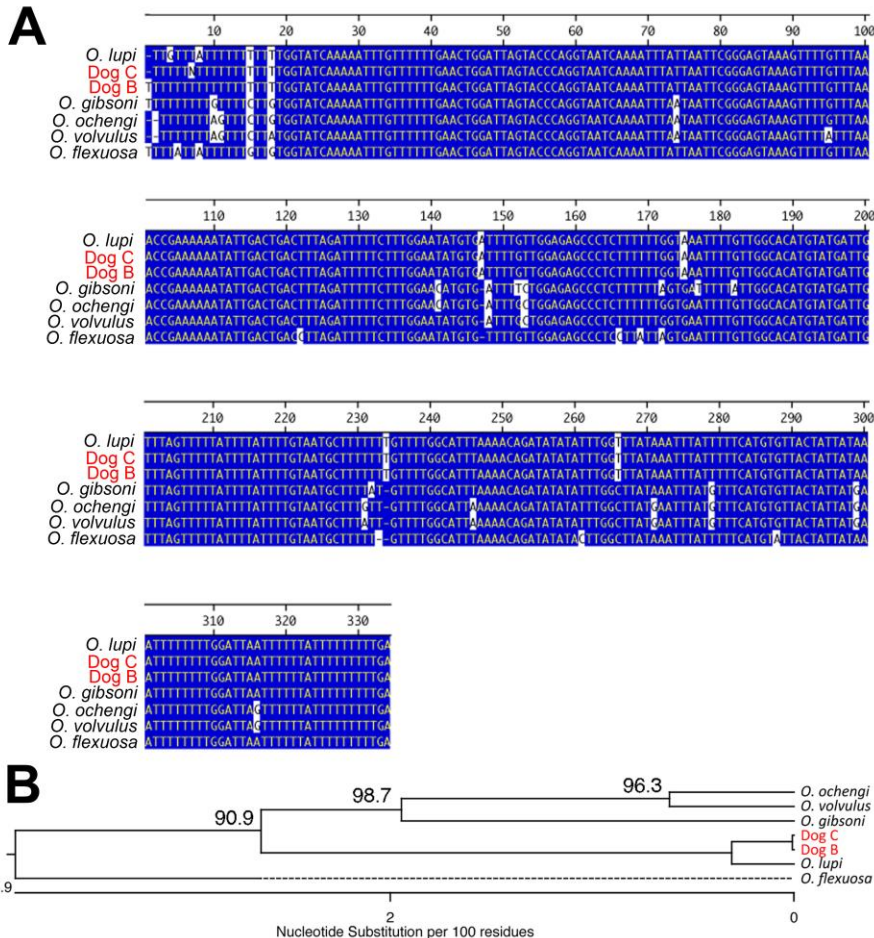


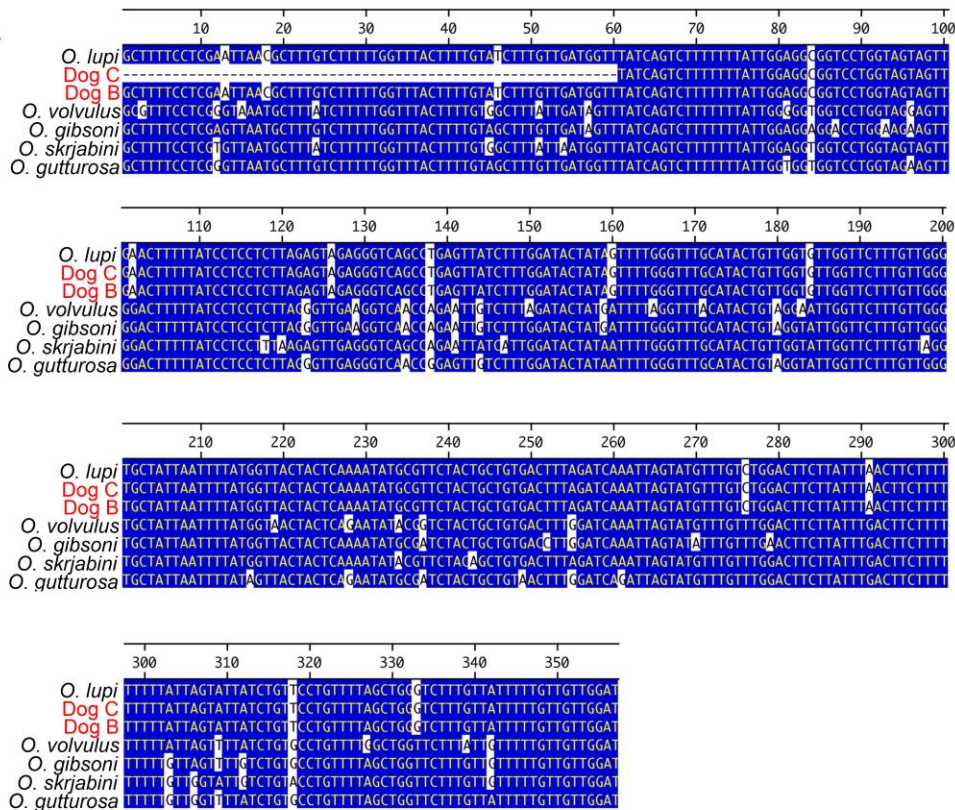
Isolation of *Onchocerca lupi* in Dogs and Black Flies, California, USA

Technical Appendix

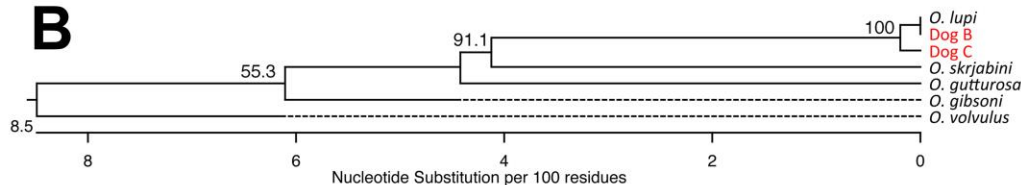


Technical Appendix Figure 1. Molecular identification of parasites collected from dogs by using sequences derived from the mitochondrial 12S rRNA gene. A) Multiple sequence alignment of 12S rRNA sequences from the 3 dogs described in the text and sequences from various *Onchocerca* parasites. B) Unrooted phylogeny of the sequences shown in panel A. Numbers refer to the percentage of times that the grouping distal to the number was supported in a bootstrap analysis of 1,000 replicate datasets. GenBank accession numbers for the sequences used in the alignment and phylogeny were as follows: *O. lupi*, GU365879; dog C, KC763783; dog B, KC763784; *O. gibsoni*, AY462913; *O. ochengi*, DQ523741; *O. volvulus*, DQ523741; *O. flexuosa*, HQ214004.

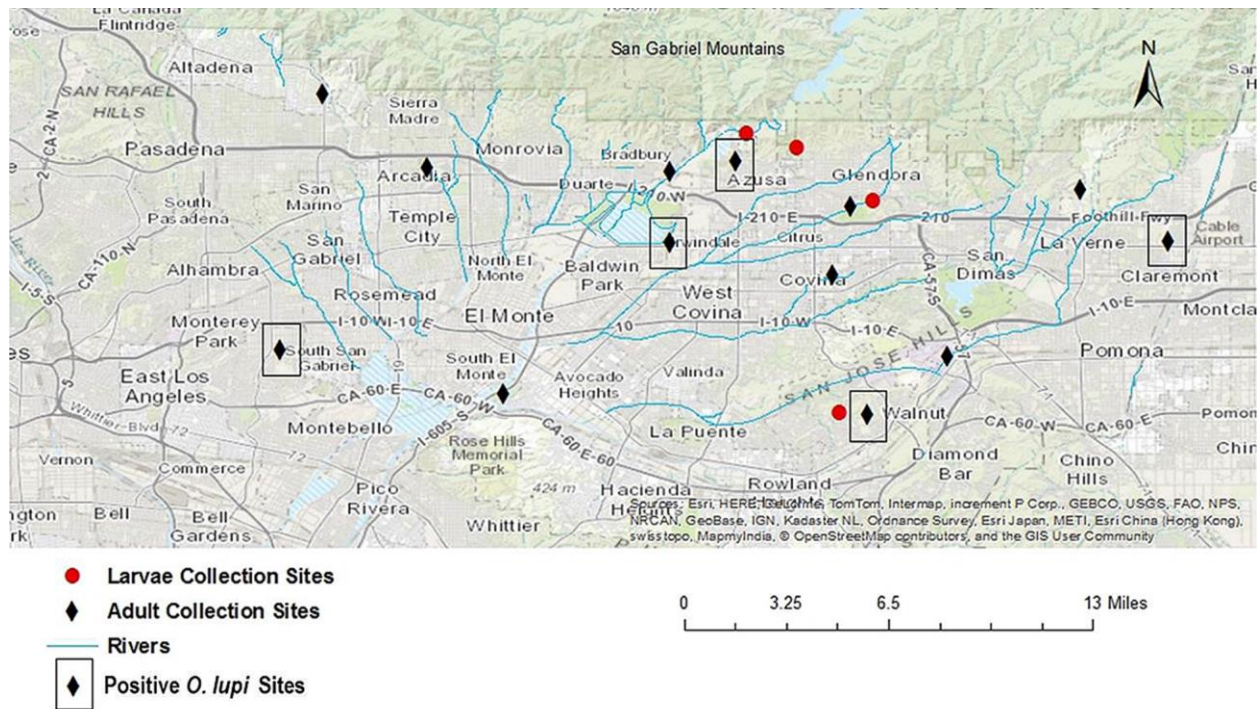
A



B



Technical Appendix Figure 2. Molecular identification of parasites collected from dogs by using sequences derived from the cytochrome oxidase subunit 1 (CO1) gene. A) Multiple sequence alignment of CO1 sequences from the 3 dogs described in the text and sequences from various *Onchocerca* parasites. Blue shading indicates areas of sequence identity. B) Unrooted phylogeny of the sequences shown in panel A. Numbers refer to the percentage of times the grouping distal to the number was supported in a bootstrap analysis of 1,000 replicate datasets. GenBank accession numbers for the sequences used in the alignment and phylogeny were as follows: *O. lupi*, JX080031; dog C, KC763785; dog B, KC763786; *O. volvulus*, AF0151931; *O. skrjabini*, AM749271; *O. gutturosa*, AJ271617.



Technical Appendix Figure 3. Map of San Gabriel Valley in Los Angeles County, California, showing locations where adult black flies (black circles) and black fly larvae (black diamonds) were collected during 2012. Boxes indicate sites where flies were positive for *Onchocerca lupi*.

		10	20	30	40	50			
<i>S. tribulatum</i> A	1	CCGCAGTATT	TTGACTGTGC	AAAGGTAGCA	TAATCATFAG	TCTTTAAATT	50		
<i>S. tribulatum</i> B	1	CCGCAGTATT	TTGACTGTGC	AAAGGTAGCA	TAATCATFAG	TCTTTAAATT	50		
		60	70	80	90	100			
<i>S. tribulatum</i> A	51	GAAGGCTGGT	ATGAATGGTT	GGATGAGGTA	CAAGCTGTGT	CATAAAAATT	100		
<i>S. tribulatum</i> B	51	GAAGGCTGGT	ATGAATGGTT	GGATGAGGTA	CAAGCTGTGT	CATAAAAATT	100		
		110	120	130	140	150			
<i>S. tribulatum</i> A	101	AATATTTGAA	TTTAACTTTT	TAGTCAAAG	GCTAAAATGT	AATTTAAAGA	150		
<i>S. tribulatum</i> B	101	AATAATTGAA	TTTAACTTTT	TAGTCAAAG	GCTAAAATTT	AATTTAAAGA	150		
		160	170	180	190	200			
<i>S. tribulatum</i> A	151	CGAGAAGACC	CTATAGAGCT	TTATATAGTT	GATATTTAAT	TTATTAAGAT	200		
<i>S. tribulatum</i> B	151	CGAGAAGACC	CTATAGAGCT	TTATAT	TACA	AATATTTAAT	TTATTAAGAT	200	
		210	220	230	240	250			
<i>S. tribulatum</i> A	201	TTATTTAAAT	TAATTATTTT	ACTGTATTTT	GTTGGGGTGA	CAATAAAATT	250		
<i>S. tribulatum</i> B	201	TTATTTAAAT	TAATTATTTT	GT	TTTATTTT	GTTGGGGTGA	CAATAAAATT	250	
		260	270	280	290	300			
<i>S. tribulatum</i> A	251	TATAAACTT	TTATTATTAT	TTAACATTTA	TTTATGGTTA	TATGATCCAG	300		
<i>S. tribulatum</i> B	251	TATAAACTT	TTATTAA	TAT	TTAACATTTA	TTTATG	TTA	TATGATCCAG	300
		310	320	330	340	350			
<i>S. tribulatum</i> A	301	TTTTATTGAT	TATAAATTTA	AGTTACCTTA	GGGATAACAG	CGTAATTTTT	350		
<i>S. tribulatum</i> B	301	TTTTATTGAT	TATAAATTTA	AGTTACCTTA	GGGATAACAG	CGTAATTTTT	350		
		360	370	380	390	400			
<i>S. tribulatum</i> A	351	TTTGAGAGTT	CATATCGACA	AAAAAGATTG	CGACCTCGAT	GTTGGATTAA	400		
<i>S. tribulatum</i> B	351	TTTGAGAGTT	CATATCGACA	AAAAAGATTG	CGACCTCGAT	GTTGGATTAA	400		
		410	420	430	440	450			
<i>S. tribulatum</i> A	401	GAGTAATTTT	GGGTGTAGAA	GTTCAAAGTT	TAAGTCTGTT	CGACTTTTAA	450		
<i>S. tribulatum</i> B	401	GAGTAATTTT	GGGTGTAGAA	GTTCAAAGTT	TAAGTCTGTT	CGACTTTTAA	450		
		460	470	480	490	500			
<i>S. tribulatum</i> A	451	ATTCTTACAT	500		
<i>S. tribulatum</i> B	451	ATTCTTACAT	500		

Technical Appendix Figure 4. Sequence of the 2 alleles obtained from amplification of a portion of the mitochondrial 16s rRNA gene from *Simulium tribulatum* infected with *Onchocerca lupi*.