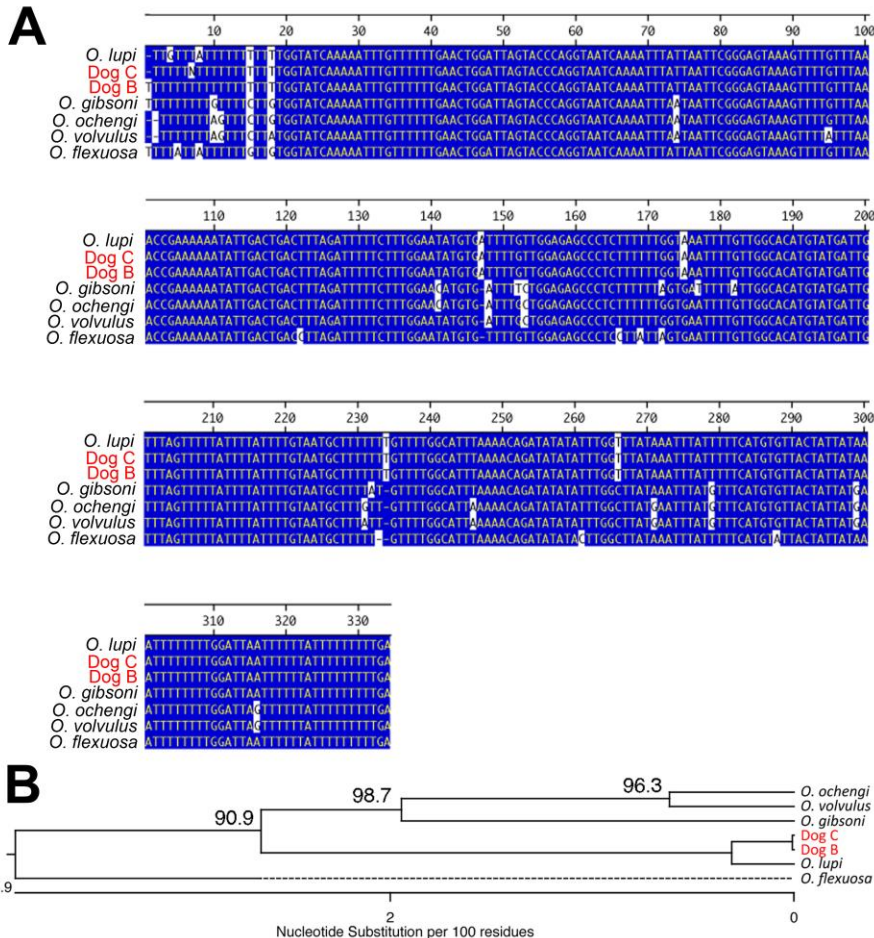


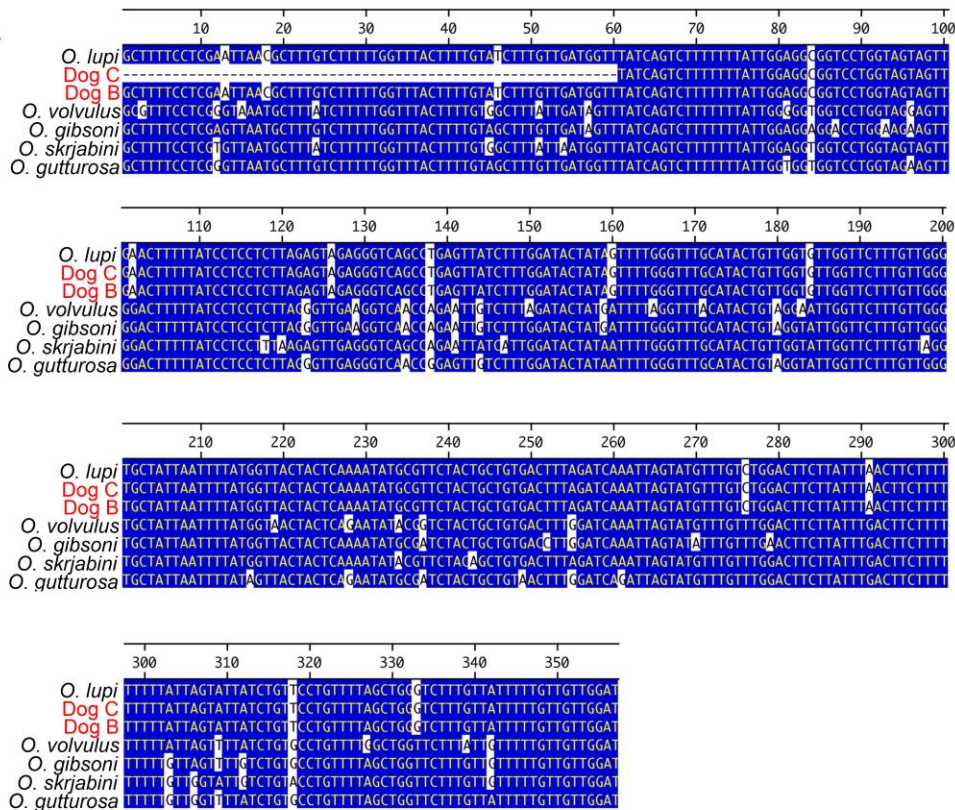
Isolation of *Onchocerca lupi* in Dogs and Black Flies, California, USA

Technical Appendix

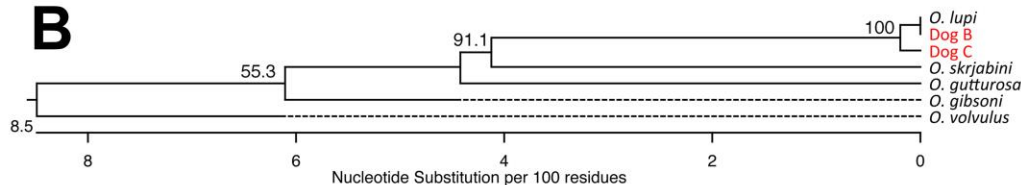


Technical Appendix Figure 1. Molecular identification of parasites collected from dogs by using sequences derived from the mitochondrial 12S rRNA gene. A) Multiple sequence alignment of 12S rRNA sequences from the 3 dogs described in the text and sequences from various *Onchocerca* parasites. B) Unrooted 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.

| | | | | | | | | | |
|------------------------|-----|------------|------------|------------|------------|------------|------------|------------|-----|
| | | 10 | 20 | 30 | 40 | 50 | | | |
| <i>S. tribulatum</i> A | 1 | CCGCAGTATT | TTGACTGTGC | AAAGGTAGCA | TAATCATFAG | TCTTTTAATT | 50 | | |
| <i>S. tribulatum</i> B | 1 | CCGCAGTATT | TTGACTGTGC | AAAGGTAGCA | TAATCATFAG | TCTTTTAATT | 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 | TAGTCAAAAG | GCTAAAATGT | AATTTAAAGA | 150 | | |
| <i>S. tribulatum</i> B | 101 | AATAATTGAA | TTTAACTTTT | TAGTCAAAAG | 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 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 | AATAAAAGA | 150 |
| <i>S. tribulatum</i> B | 101 | AATAATTGAA | TTTAACTTTT | TAGTCAAAG | GCTAAAATTT | AATAAAAGA | 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 | TTATATACATA | AATATTTAAT | TTATTAAGAT | 200 |
| | | 210 | 220 | 230 | 240 | 250 | |
| <i>S. tribulatum</i> A | 201 | TTATTAATTT | TAATTATTTT | ACTGTATTTT | GTTGGGGTGA | CAATAAAATT | 250 |
| <i>S. tribulatum</i> B | 201 | TTATTAATTT | 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 | TTATTAAATAT | TTAACATTTA | TTTATGATTA | 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*.