Presence of a Cryptic Onchocerca Species in Black flies of Northern California, USA

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Abstract

Background: Black flies (Diptera: Simuliidae) serve as arthropod vectors for various species of *Onchocerca* (Nematoda: Onchocercidae) that may be associated with disease in humans, domestic animals, and wildlife. The emergence of zoonotic *Onchocerca lupi* in North America and reports of cervid-associated zoonotic onchocerciasis highlight the need for increased entomological surveillance. In addition, there is mounting evidence that *Onchocerca* diversity in North America is far greater than previously thought, currently regarded as *Onchocerca cervipedis* species complex. This study reports new geographic records and black fly vector associations of an uncharacterized *Onchocerca* species.

Methods: To better understand the biodiversity and geographic distribution of *Onchocerca*, 485 female black flies (2015: 150, 2016: 335) were collected using CO_2-baited traps from February to October, 2015-2016 in Lake County, northern California, USA. Individual flies were morphologically identified and pooled (£ 10 individuals) by species, collection date, and trap location. Black fly pools were processed for DNA extraction, and subsequent PCR and sequencing targeting of the NADH dehydrogenase subunit 5 gene.

Results: Among the pools of black flies, there were: 158 individuals of *S. tescorum* (2015: 57, 2016: 101), 302 individuals of *S. vittatum* (s.l.) (2015: 82, 2016: 220), 16 individuals of *S. clarum* "black" phenotype (2015: 5, 2016: 11), and 13 individuals of *S. clarum* "orange" phenotype (2015: 6, 2016: 7). PCR analysis revealed the percentage of positive pools in 2015-16 were 7.50% (n=3) for *S. tescorum*, 3.75% (n=3) for *S. vittatum* (s.l.; likely *S. tribulatum*), 7.69% (n=1) for *S. clarum* "black" phenotype, and no positives for *S. clarum* "orange" phenotype. Genetic distance and phylogenetic analyses suggest that the northern California *Onchocerca* isolates belong to the same species reported in black flies from southern California (average pairwise comparison: 0.32%), and seems closely related to *Onchocerca* isolates of white-tailed deer from upstate New York (average pairwise comparison: 2.31%).

Conclusion: *Onchocerca cervipedis* is part of larger, continentally-distributed species complex rather than a single described species of North America. It is unclear how many isolates of *Onchocerca cervipedis* exists at this time and more data sampling will be required before a scientific consensus can be determined. In addition, there are at least three putative vectors of black flies (*S. clarum*, *S. tescorum*, *S. vittatum*) associated with this cryptic *Onchocerca* species. A comprehensive reassessment of North American *Onchocerca* biodiversity, host, and geographic range is necessary.

Background

Onchocerca Diesing, 1841, a genus of filarial nematodes, is a globally distributed, vector-borne parasite that infects a wide variety of species that includes both animals and humans [1]. Well-known species of *Onchocerca* include *Onchocerca volvulus* (Leuckart, 1893), also known as the agent of river blindness in humans, and the emerging zoonotic parasite *Onchocerca lupi* Rodonaja, 1967, the agent for causing canine ocular onchocerciasis [1]. *Onchocerca* species are transmitted via blood-sucking dipteran vectors, including black flies (Simuliidae) and biting midges (Ceratopogonidae), to definitive mammalian hosts [1].

Despite the zoonotic potential and possible deleterious impacts to host health of most *Onchocerca* species, little is known about the clinical and ecological significance of the ungulate parasite *Onchocerca cervipedis* Wehr & Dikmans, 1935, or what is commonly known as the "foot worm". Described nearly a century ago [2], *O. cervipedis* has an extensive distribution range from areas of Central America to Canada and infects a variety of cervids including: the white-tailed deer *Odocoileus virginianus* (Zimmermann, 1780); mule deer *Odocoileus hemionus* (Rafinesque, 1817); moose *Alces americanus* Clinton, 1822; elk or wapiti *Cervus canadensis* Erxleben, 1777; caribou *Rangifer tarandus* (Linnaeus, 1758.); and also, the antilocaprid pronghorn *Antilocapra americana* (Ord, 1815) [3–15]. *O. cervipedis* has always been assumed to be the only *Onchocerca* sp. to infect these North American ungulates, however there is mounting evidence that suggests otherwise. Molecular characterization of DNA of cryptic *Onchocerca* species were recently discovered in the skin of white-tailed deer in New York [16] and in black fly vectors from southern California [17] are genetically distinct from isolates of moose from northern Canada [14]. Therefore, *O. cervipedis* is currently regarded as a species complex, and all previous reports across the Americas, including ungulate host and vector associations, require a comprehensive reevaluation [14, 16, 17].

In order to shed further light on the cryptic diversity within the *O. cervipedis* species complex, we molecularly screened putative black fly vectors trapped in Lake County, northern California, USA for filarial nematode DNA. We discuss these results in the current context of known cryptic biodiversity and historical biogeography of *Onchocerca* in North America.

Methods

Black Fly Collection

Lake County, California was the designated area targeted for black fly collection. Lake County is located in one of the broad valleys of northern California (122°50' W, 39°00' N) and contains the largest freshwater lake entirely in California, Clear Lake [18]. While Lake County is considered a mild and temperate climate, in 2015, a destructive forest fire burned 76,067 acres of land and forced a third of the 60,000 residents of Lake County to evacuate. This was the third-most destructive Californian fire in history at the time and is considered part of the growing trend of extreme weather seen in North America as climate change becomes more pronounced [19]. Through coordination with the Lake County Vector Control District, female black flies were caught by mosquito traps baited with CO_2 between April 2015 and October 2016. Traps were located around the shores of Clear Lake (Fig. 1). Once collected, the black flies were morphologically identified to species/species-complex level according to taxonomic keys [20], and stored at -80°C until further analysis.

Molecular Screening And Sequencing

Individual flies were morphologically identified and pooled (≤ 10 individuals) by species, collection date, and trap location (Table 1; Fig. 1). DNA extraction of pools of black flies was performed manually using the Qiagen DNeasy© Blood & Tissue kit (Qiagen, Valencia, CA, USA). Briefly, black flies were macerated with sterile plastic pestles within an Eppendorf tube, and homogenized with ATL buffer and proteinase K. Samples were then incubated in a dry heat block for 45 min at 56°C, and then centrifuged for five minutes at 8000 x *g*.

Polymerase chain reactions (PCR) targeting the mitochondrial NADH dehydrogenase subunit 5 (*nad5*) gene of filarioid nematodes, using the primers ND5-Ov5A-F (5'-TTG GTT GCC TAA GGC TAT GG-3') and ND5OvC-R (5'-CCC CTA GTA AAC AAC AAA CCA CA-3') [21]. Cycling conditions consisted of 95°C for 2 min, followed by 35 cycles of 95°C for 30s, 50°C for 45 s, and 72°C for 30s, and a final extension at 72°C for 5 min, following previously published protocols [17].

Potential PCR products were subjected to agarose gel to determine if amplicon was present. A Cycle Pure ENZA kit (Omega Bio-Tek, Norcross, GA, USA) was used to purify DNA using the manufacturer's protocol. Products were then directly sequenced using the same primers using BigDye Terminator Cycle sequencing.

Phylogenetic Analysis

Sequences were aligned and edited through MEGA X software [22]. Phylogenetic trees of the partial *nad5* gene (427 bp) were constructed by the Maximum Likelihood method with 2000 bootstrap replicates. All sequences at the *nad5* gene for *Onchocerca* species available through GenBank were included. *Dirofilaria immitis* (Leidy, 1856) and *Dirofilaria repens* Railliet and Henry, 1911 were used as outgroups within the family Onchocercidae.

Taxonomy of simuliid vectors and mammalian hosts for Onchocerca

The taxonomy of black flies and artiodactyl mammalian hosts followed the most recent and comprehensive literature [20, 23, 24].

Results

Collections

A total of 485 black flies were collected from 27 different collection sites in the Lake County area (Fig. 1). Overall, 150 flies were collected in 2015, and 335 flies in 2016, representing three black fly species. Of these, 158 individuals were identified as *Simulium tescorum* Stone & Boreham, 1965 (2015: 57, 2016: 101), 302 individuals of *Simulium vittatum* Lugger, 1897 (s.l., likely *S. tribulatum*) (2015: 82, 2016: 220), 16 individuals of *Simulium clarum* (Dyar and Shannon, 1927) "black" phenotype (2015: 5, 2016: 11), and 13 individuals of *Simulium clarum* "orange" phenotype (2015: 6, 2016: 7).

Onchocerca **Screening**

Regarding the samples collected in 2015, a total of 2/31 *S. vittatum* pools (6.5%), 1/17 *S. tescorum* pools (5.9%), 1/3 *S. clarum* "black" phenotype pools (33.3%), and 0/6 *S. clarum* "orange" phenotype pools (0.0%) were positive for filarioid DNA and subsequently sequenced for *Onchocerca* DNA (Table 1). In 2016, a total of 1/49 *S. vittatum* pools (2.0%), 2/23 *S. tescorum* pools (8.7%), 0/10 *S. clarum* "black" phenotype pools (0.0%), and 0/7 *S. clarum* "orange" phenotype pools (0.0%) were positive for filarioid DNA and subsequently sequenced for *Onchocerca* DNA (Table 1). All positive *S. vittatum* (n = 3) pools came from Lower Lake, while each of the *S. tescorum* positive pools (n = 3) came from three different locations: Middletown, Lakeport, and Kelseyville. The single positive *S. clarum* "black" phenotype (n = 1) was also found in Kelseyville (Table 1).

Phylogenetic Analysis

All seven generated *nad5* sequences were deposited in the GenBank database (Accession numbers: MZ420192; MZ420193; MZ420194; MZ420195; MZ420196; MZ420197; MZ420198). Phylogenetic analysis showed a strong support that the Lake County *Onchocerca* isolates in northern California are conspecific with the isolates from Los Angeles in southern California (94% bootstrap support), and likely belong to an uncharacterized species (Fig. 2). In addition, the upstate New York *Onchocerca* isolates appear to be closely related to both Californian isolates (92% bootstrap support) (Fig. 2). Other *Onchocerca* isolates or species that have been reported from North American wildlife, namely *O. cervipedis* sensu Verocai et al. [14] of moose from Canada, and *O. lupi*, reported from companion animals, coyotes and humans in North America [25–27], were not included within this clade.

Pairwise distance data (Table 2) also concludes strong support for each of the three isolates to be closely related to each other with the Californian isolates being more similar to each other with a pairwise distance averaging 0.32% (0.00-2.54%). The New York *Onchocerca* isolate had an average pairwise distance of 2.31% (2.12–3.27%) when compared to the Lake County isolates and 2.34 % (2.12–3.27%) when compared to the Los Angeles isolates. On the other hand, when Lake County isolates were compared to *O. cervipedis* sensu Verocai et al. [14] isolates, pairwise distance was a much higher average of 10.04% (9.64–10.64%). These higher numbers are similar to interspecific *Onchocerca* species comparisons like *O. lupi*, 11.75% average (11.24–11.86%), rather than intraspecific comparisons (Table 2; Fig. 3). While no definitive quantitative cutoff exists to delineate interspecific from intraspecific species comparisons (or in this case, inter-isolate vs. intra-isolate), there is an overlapping range where separation occurs. The majority of pairwise comparisons fall outside the range of ~ 3.00–5.00% (Table 2; Fig. 3), indicating a zone of separation that merits intra- versus inter-species differences. Furthermore, removal of the New York and Californian isolate's pairwise comparison will expand this zone to around ~ 2.00–5.00%. While evidence clearly indicates that all Californian isolates are conspecifics (Table 2; Fig. 3), the phylogenetic relationships among the New York and Californian isolates remain ambiguous.

Discussion

Our study further supports the current evidence that *Onchocerca cervipedis* is not a single species, but a widely distributed species complex. We discovered that *Onchocerca* isolates found in Lake County, northern California, black flies belong to the same cryptic *Onchocerca* species previously found in Los Angeles County, southern California, black flies [17]. Corroborating the findings from southern California, *Onchocerca* DNA was detected in two black fly species: *S. vittatum* (s.l.) and *S. tescorum* [17]

(Table 1). In addition, a third species of black fly was shown to carry the same cryptic *Onchocerca* DNA: *S. clarum* belonging to the "black" phenotype (Table 1).

Phylogenetic analyses of the *nad*5 gene demonstrates that the cryptic *Onchocerca* found in southern and northern California black flies (present study; [17]) and the equally cryptic *Onchocerca* isolate found in New York, northeastern USA, [16] represents one individual clade with little genetic divergence (Fig. 2). However, a definitive conclusion on whether the Californian isolates are conspecific with the New York isolates cannot yet be determined (Table 2; Fig. 3). Further studies targeting a multi-locus approach could help shed light on the exact phylogenetic relationships and taxonomic status of these geographically distant isolates. This notion is best exemplified by comparing the *nad5* gene to the *cox-1* gene, which appears to exhibit greater diversity within the cryptic *Onchocerca* isolates [17]. In addition, at this stage, it is not possible to conclude that the cryptic species present in northern California belongs to the originally described *O. cervipedis.* In the original description of the species by Wehr and Dikmans [28], the authors used specimens from two different locations and from at least two different hosts, including *O. virginianus* and *O. hemionus* from Montana, USA and *O. hemionus* from British Columbia, Canada. To further elucidate this taxonomic conundrum, isolates from these hosts and locations should be collected, morphologically re-evaluated, molecularly characterized, and subsequently compared to these many isolates within the *O. cervipedis* complex.

Molecular screening and putative vectors of cryptic Onchocerca isolates

The finding of cryptic *Onchocerca* DNA through molecular screening of arthropod vectors (i.e., xenomonitoring) provides a straightforward approach to understanding more about parasite biodiversity, geographic distribution, and putative vector associations. In addition, vector screening allows researchers to not solely rely on labor-intensive measures such as post-mortem examination of host carcasses for adult worms [28] or an unstandardized technique to obtain microfilariae [29, 30], given the potential variable predilection sites of adults and microfilariae of the species within the *O. cervipedis* complex. For instance, while evidence indicates that microfilariae could be present near the skin of the ear [29, 31–33], the true predilection site of *O. cervipedis* species complex microfilariae does not have clear a consensus [34, 35] and what, if any, variation exists among ungulate host species. Moreover, the utilization of xenomonitoring of North American parasites allows for concurrent monitoring of other similar *Onchocerca* species, such as the zoonotic *O. lupi*, that are of current public health concern [36].

Comparable to Verocai et al. [17], our results showed the positive rate for *Onchocerca* DNA was low in the black fly populations. Indeed, this low prevalence is an expected result of pool sampling, as prior evidence has shown that pool samples are highly sensitive to low prevalence of pathogens in vector species [37]. Furthermore, other closely related filarial nematode studies revealed similarly low positive prevalence rates of *O. lupi* in southern California [36], *O. volvulus* in Africa [38, 39], and *Wuchereria bancrofti* (Cobbold, 1877) in American Samoa and Guinea [40–42].

Our study also provided evidence for an additional species of black fly as a probable vector of this *Onchocerca* species. According to the literature, *S. clarum* has been reported to feed on a variety of mammals (horses, cattle, rabbits, and humans) and birds [43, 44]. The finding of DNA of an *Onchocerca* species likely associated with a cervid host(s) suggest that these mammals may serve as a blood source for this dipteran, similar to that of *S. tescorum* and *S. vittatum*, as suggested by Verocai et al. [17, 20]. However, *S. clarum* is restricted to the Central Valley California region near the present study site of Lake County [20]. Similarly, *S. tescorum* has been reported with a limited range, spanning only California and Arizona [20, 23]. This means that even if these two vectors are competent host for this *Onchocerca* species, they would only contribute to the transmission within their more restricted distribution. In contrast, species within the *S. vittatum* complex which includes *S. tribulatum*, have a widespread distribution across North America, including both California and New York [23]. We postulate that it could play an epidemiologically relevant vector role for this *Onchocerca* isolate found in northern and southern California (present study; [17, 36]) and possibly New York [16].

Definitive hosts of cryptic Onchocerca isolates

The recent discoveries of at least two or more genetic *Onchocerca* isolates in North America likely associated with at least three of the cervid hosts (i.e., mule deer, white-tailed deer, and moose) raise many questions regarding *Onchocerca*-host assemblages. Of these three cervid hosts, only the mule deer's range encompasses southern California, including Los Angeles county [45–47].

Thus, it was reasonably hypothesized that the mule deer could be the putative host to the *Onchocerca* isolate from southern California [17]. Lake County also includes the range of the mule deer [45], however, unlike southern California, Lake County is also home to the Californian Tule elk or *Cervus elaphus nannodes* Merriam, 1905 [48]. This elk subspecies was hunted to near extinction in the late 1800s, and now has a thriving population in California. According to most recent data, about 6,000 Tule elk populate California, including many herds that live near the Lake County region of northern California where black flies were sampled for this current study [48–50]. While there was no blood meal analysis completed, it is possible that these cervids could be a blood meal source for black flies and consequently be a potential host to the *O. cervipedis* species complex [7]. Ideally, adult worms or microfilariae should be sampled from necropsied elk hosts and molecularly analyzed to confirm its definitive host status.

Species within *O. cervipedis* complex have been reported in a variety of locations across North America in the six ungulate hosts: pronghorn from Idaho [8]; moose from Alaska, Alberta, British Columbia, and Northwest Territories [11, 13–15, 35]; elk from Montana [7]; mule deer from Arizona, California, Montana, Utah, Wyoming, and British Columbia [3, 4, 6, 7, 9, 17, 28, 29, 33, 34, 51–56]; white-tailed deer from Arizona, Missouri, Montana, New York, Oregon, Pennsylvania, British Columbia, and also from Costa Rica [4, 5, 7, 12, 16, 28, 29, 31, 32, 52, 57–59]; and caribou from Alaska and British Columbia [10, 14]. Additional records from *Odocoileus* from Colorado, Idaho, and Montana, were reported as “deer”, without species designation [60–64]. Therefore, it can be inferred that sample collection should begin in these reported locations and include all six ungulate hosts when obtaining biological samples. Recovery of nematodes from necropsy, with subsequent morphological and DNA identification, will confirm parasitic infection of a definitive host and aid in interpreting the distribution of cryptic *Onchocerca* isolates.

Evolutionary history and ecological considerations of cryptic Onchocerca isolates

Currently, it is hypothesized that the two, and possibly more, known *Onchocerca* species (i.e., *O. cervipedis* sensu Verocai et al. [14] and the clade comprising the Californian and New York isolates [16, 17]) are the result of independent expansion events from Palearctic ungulates hosts colonizing from across the Bering Landbridge into the Nearctic [65–67]. It is currently unknown if the finding of at least two *Onchocerca* species within the complex is the result of a small, incomplete sampling of a larger species complex or the true representation of diversity in North America. Nevertheless, there is substantial evidence from eastern Asia for prior underestimation of *Onchocerca* species diversity and richness. For instance, *Onchocerca suzukii* Yagi, Bain and Shoho, 1994, *Onchocerca eberhardi* Uni et al., 2007, and *Onchocerca takaokai* Uni, Fukuda and Bain, 2015 have been recently described from wild ungulates of Japan [68–70]. Furthermore, *Onchocerca borneensis* Uni, Mat Udin & Takaoka, 2020 [71], was described in bearded pigs of Borneo with additional molecular evidence suggesting two closely related parasites, *Onchocerca dewittei* Bain, Ramachandran, Petter & Mak, 1977 and *Onchocerca japonica* Uni, Bain and Takaoka, 2001, which were considered subspecies of the former were, in fact, separate species [71]. Indeed, it is feasible that the North American *Onchocerca* species complex, which much is still unknown about, could comprise undescribed *Onchocerca* diversity, similar to the pattern that we have witnessed in Asian suids and ungulates. Moreover, host-parasite biogeography appears to play a critical role in *Onchocerca* diversification. As noted by Uni et al. [71], *O. borneensis* and *O. dewittei* infect *Sus barbatus* Müller and *Sus scrofa vittatus* Boie in the Indomalayan region, but *O. japonica* and *O. dewittei* infect different subspecies of the same host species in the Palearctic and Indomalayan regions. Thus, when re-evaluating *Onchocerca* in the North American landscape, collecting specimens from sympatric and allopatric host ranges may yield more complete information about parasitic diversity.

Conclusion

A cryptic *Onchocerca* species was found in Lake County, California which is likely conspecific to isolates previously characterized from southern California. Putative vectors of this cryptic parasite include *S. tescorum* and *S. vittatum*. In addition, a previously unrecognized black fly vector, *S. clarum*, was discovered to be a potential vector. In order to understand the true biodiversity of the genus *Onchocerca* in North America, a complete continental reevaluation of definitive hosts, vector associations, and geographic distribution is necessary through the integration of classical and molecular methods.

Declarations

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Availability of data and materials

The data supporting the conclusions of this article are included within the article. The sequences have been submitted to the GenBank database under the accession numbers MZ420192-MZ420198 (nad5).

Authors' contributions

MK and GGV drafted manuscript. GGV acquired funding. and BMR, MLK, AAM, and JJS performed blackfly collections and KJN performed identification. GGV performed the molecular genetic study. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1. Summary of positive collected black flies and their location

Black flies were collected in the 2015-16 field season using CO_2 bait traps in the Lake County, California area. Four species of black flies were caught: *S. clarum* (black); *S. clarum* (orange) *S. tescorum*; and *S. vittatum*. However, *S. clarum* (orange) had no positive individuals. Each row denotes the number of black flies examined, the number of pools (n = ≤ 10), the positive black fly pools, coordinates and cities of where the positive was located, and the percentage of positive pools by species.

Table 1 Summary of positive collected black flies and their location

	Number examined	Number of pools	Positive black fly pools	Coordinates	Location	Percentage of positive pools by species
2015						
S. clarum “black”	5	3	1) SCB-15-039	38° 53' 21.9" N, 122° 43' 53.6" W	Kelseyville	33.3%
S. clarum “orange”	6	6	None	-	-	0.0%
S. tescorum	57	17	1) ST-15-010	38° 43' 16.7" N, 122° 37' 12.8" W	Middletown	5.9%
S. vittatum	82	31	1) SV-15-020A	38° 55' 3.8" N, 122° 35' 20.9" W	Lower Lake	6.5%
			2) SV-15-043	38° 55' 3.8" N, 122° 35' 20.9 " W	Lower Lake	
Total (2015)	150	57	4			7.0%
2016						
S. clarum “black”	11	10	None	-	-	-
S. clarum “orange”	7	7	None	-	-	-
S. tescorum	101	23	1) ST-16-011	38° 56' 49.5" N, 122° 54' 14.3" W	Lakeport	8.7%
			2) ST-16-014	38° 55' 10.2" N, 122° 46' 35.5" W	Kelseyville	
S. vittatum	220	49	1) SV-16-030A	38° 55' 19.1" N, 122° 37' 35.0" W	Lower Lake	2.0%
Total (2016)	335	89	3			3.4%
2015-16						
S. clarum “black”	16	13	1	-	-	7.7%
S. clarum “orange”	13	13	0	-	-	0.0%
S. tescorum	158	40	3	-	-	7.5%
S. vittatum	302	80	3	-	-	3.8%
Overall total	485	146	7			4.8%

Table 2. Average pairwise comparison of various Onchocerca sp. or isolates at the nad5 gene level

This table shows average pairwise comparisons, with ranges in parentheses, of *nad5* gene with different *Onchocerca* isolates or species. *Onchocerca* isolates are broken down by region (Lake County, CA; Los Angeles, CA; and Ithaca, NY) or by the species it is from (*O. lupi; Onchocerca* sp.). *O. lupi* was chosen because it is a North American *Onchocerca* species that is not considered part of the *Onchocerca cervipedis* species complex.

Table 2. Average Pairwise Comparison of various Onchocerca sp. or isolates at the nad5 gene level

Onchocerca Isolate	Lake County, CA	Los Angeles, CA	Upstate New York	Onchocerca sp.	Onchocerca lupi	Reference
Lake County, CA	0.24% (0.00%-0.95%)					Present study
Los Angeles, CA	0.32% (0.00%-2.26%)	0.48% (0.00%-2.54%)				Verocai et al. 2018
Upstate New York	2.31% (2.12%-3.27%)	2.34% (2.12%-3.27%)	0.24% (0.00%-0.48%)			McFrederick et al., 2013
Onchocerca sp.	10.04% (9.64%-10.64%)	9.93% (7.65%-11.11%)	9.47% (8.61%-9.77%)	0.12% (0.00%-0.24%)		Verocai et al. 2012
Onchocerca lupi*	11.75% (11.24%-11.86%)	11.82% (11.24%-12.21%)	10.30% (9.18%-10.70%)	10.99% (10.53%-11.13%)	0.61% (0.00%-1.51%)	Various sources

* This is was included for comparative because it has been reported in non-cervid wildlife in North America.

Figures

Figure 1

Black fly collection sites Locations of adult black fly collection sites in Lake County, California. Each collection site is marked with a black dot. Sites denoted with a right staggered red star indicate an Onchocerca positive PCR test in 2015 (n = 4), and sites denoted with a left staggered blue star indicate an Onchocerca positive PCR test in 2016 (n = 3).

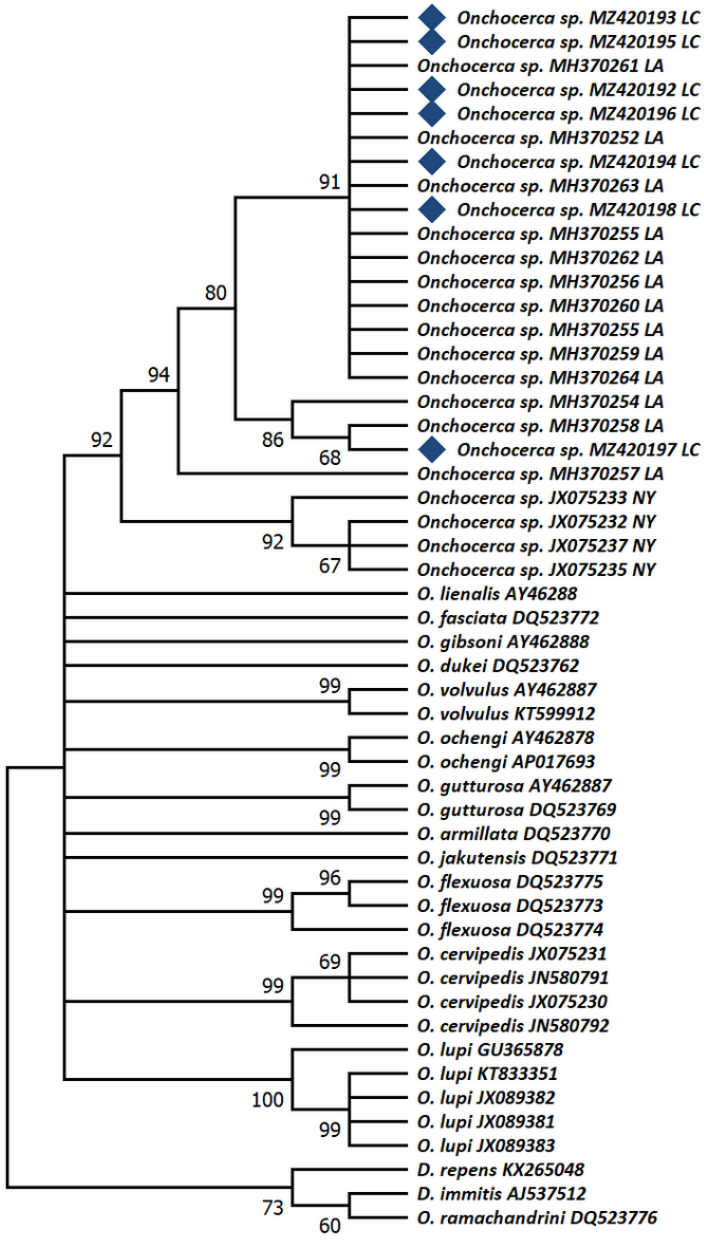

Figure 2

Phylogenetic Tree of Onchocerca sp. and discovered isolates Maximum Likelihood tree depicting phylogenetic relationship of the nad5 gene between species of known Onchocerca and the cryptic Onchocerca DNA found across geographic isolates of Onchocerca in California and New York, USA created with MEGAX. Branches with less than 50% bootstrap were collapsed and bootstrap support shown besides branches indicate 2000 replicates. All cryptic DNA samples obtained from Black Fly from Lake County, California are denoted with a black diamond and have been accessioned in GenBank (MZ420192; MZ420193; MZ420194; MZ420195; MZ420196; MZ420197; MZ420198).

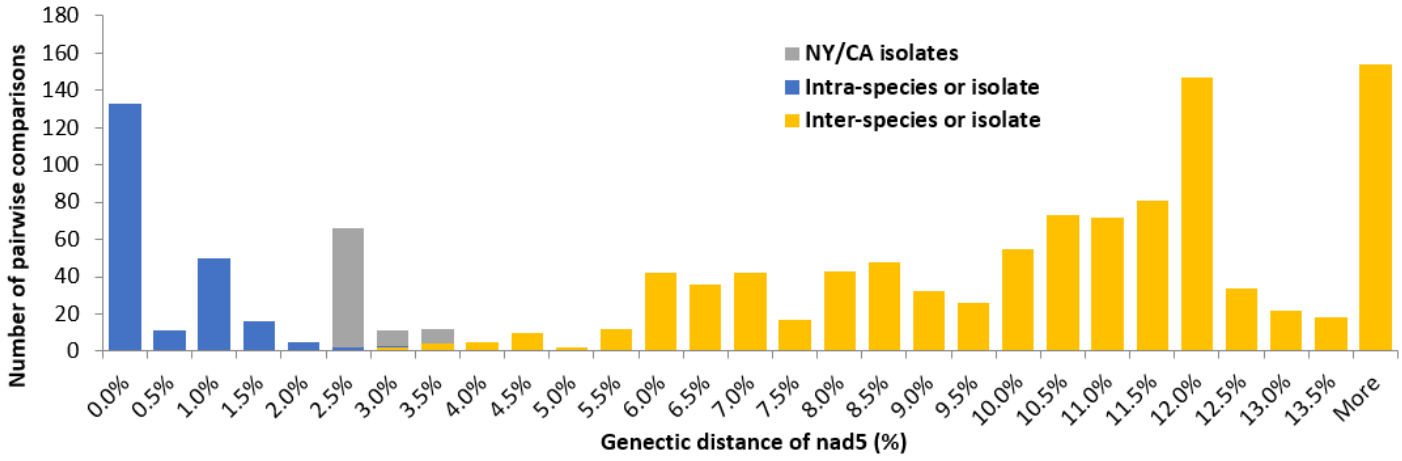

Figure 3

Intra-isolate versus inter-isolate pairwise comparisons at the nad5 genetic marker The number of base substitutions per site are calculated and the evolutionary divergence is estimated between sequences. Each bar represents the total amount of pairwise comparisons of the nad5 gene, or nucleotide sequence divergence, from 51 different Onchocerca species or isolates. Evolutionary analysis was done using MEGA X and a Tamura-Nei model with gamma distribution. Blue bars indicate supposed intra-isolate comparisons and orange bars indicate supposed inter-isolate comparisons of all Onchocerca species or discovered isolates. Lake County, CA and Los Angeles, CA isolate comparisons have been treated as intra-specific species. Gray bars indicate NY-CA isolate comparisons.

Supplementary Files

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- GraphicalAbstract.png