

Supplementary Materials for

Hidden in plain sight: Cryptic and endemic malaria parasites in North American white-tailed deer (*Odocoileus virginianus*)

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Table S1. Mosquito species screened by nested PCR for *Plasmodium* infection for the study. Sampling sites included the National Zoological Park in Washington, D.C. (DC), and sites within San Diego County, California (CA). Mammalian malaria parasites were only isolated from *Anopheles punctipennis* mosquitoes in this study.

Mosquito species and sampling site	Number of individuals screened by PCR
<i>Aedes albopictus</i> (DC)	21
<i>Aedes aegypti</i> (DC)	4
<i>Aedes japonicas japonicus</i> (DC)	145
<i>Aedes triseriatus</i> (DC)	16
<i>Aedes vexans</i> (DC)	35
<i>Anopheles barberi</i> (DC)	1
<i>Anopheles crucians</i> (DC)	1
<i>Anopheles punctipennis</i> (DC)	35
<i>Anopheles quadrimaculatus</i> (DC)	3
<i>Coquillettidia perturbans</i> (DC)	1
<i>Culex erythrothorax</i> (CA)	299
<i>Culex erraticus</i> (DC)	6
<i>Culex pipiens</i> or <i>Culex restuans</i> (DC)*	1138
<i>Culex quinquefasciatus</i> (CA)	59
<i>Culex salinarus</i> (DC)	14
<i>Culex stigmatosoma</i> (CA)	11
<i>Culex tarsalis</i> (CA)	128
<i>Culex territans</i> (DC)	5
<i>Culiseta incidens</i> (CA)	37
<i>Culiseta melanura</i> (DC)	1
<i>Culiseta morsitans</i> (DC)	3
<i>Culiseta particeps</i> (CA)	1
<i>Orthopodomyia signifera</i> (DC)	1
<i>Psorophora columbia</i> (DC)	2
<i>Psorophora ferox</i> (DC)	1
<i>Uranotaenia sapphirina</i> (DC)	10

**Culex pipiens* and *Culex restuans* were distinguished by PCR analysis for infected individuals.

Table S2. County and state locations for the wild ungulate species sampled in our study and proportion found to be infected with malaria parasites at each site by nested PCR. State abbreviations are as follows: AL=Alabama, AR=Arkansas, CO=Colorado, CT=Connecticut, DC=District of Columbia, GA=Georgia, ID=Idaho, KS=Kansas, KY=Kentucky, LA=Louisiana, MS=Mississippi, NC=North Carolina, NY=New York, SC=South Carolina, TX=Texas, VA=Virginia, WV=West Virginia, CA=California, MT=Montana, SD=South Dakota, AZ=Arizona, and OR=Oregon.

Host species	County	State	#infected/ #sampled
White-tailed deer	Marshall	AL	0/5
	Shelby	AL	2/5
	Hemstead	AR	0/5
	Lawrence	AR	0/6
	Larimer	CO	0/1
	Fairfield	CT	0/4
	Washington	DC	3/20
	McIntosh	GA	1/12
	Latah	ID	0/1
	Crawford	KS	0/1
	Elk	KS	0/1
	Johnson	KS	0/1
	Labette	KS	0/1
	McPherson	KS	0/1
	Osage	KS	0/1
	Saline	KS	0/1
	Ballard	KY	3/11
	Boone	KY	0/4
	Hardin	KY	1/5
	Knott	KY	0/2
	Madison	KY	1/5
	Owen	KY	0/4
	Avoyelles Parish	LA	1/5

	Concordia Parish	LA	0/5
	Adams	MS	0/5
	Dare	NC	2/11
	Westchester County	NY	4/76
	Beaufort	SC	1/9
	Bosque	TX	0/3
	Coke	TX	0/4
	Duval	TX	0/1
	Henderson	TX	0/1
	Hopkins	TX	0/1
	Tarrant	TX	0/1
	Tom Greene	TX	0/1
	Warren	VA	12/46
	Hardy	WV	2/5
	Knott	WV	0/2
	Lewis	WV	3/5
	Mason	WV	1/5
	Mineral	WV	0/5
	Pocohontas	WV	1/5
	Taylor	WV	1/5
	Upshur	WV	2/5
	Witt	WV	0/5
Elk	Contra Costa	CA	0/3
	Kern	CA	0/3
	Solano	CA	0/3

	Gallatin	MT	0/2
	Custer	SD	0/30
Pronghorn	Coconino	AZ	0/12
	Maricopa	AZ	0/12
	Navajo	AZ	0/12
	Yavapai	AZ	0/12
Mule/Black-tailed deer	Cochese	AZ	0/29
	Pima	AZ	0/29
	Benton	OR	0/2
	Lincoln	OR	0/1

Table S3. Collection animals screened at the NZP's campuses in Washington, DC, and Front Royal, VA, for malaria parasite infection by nested PCR.

Collection species	Number of Individuals Screened
Alpaca (<i>Vicugna pacos</i>)	2
Dama Gazelle (<i>Nanger dama</i>)	4
Domestic Cow (<i>Bos taurus</i>)	2
Domestic Donkey (<i>Equus africanus asinus</i>)	4
Domestic Goat (<i>Capra aegagrus hircus</i>)	4
Eld's Deer (<i>Panolia eldii</i>)	6
Przewalski's Horse (<i>Equus ferus przewalskii</i>)	5
Scimitar-horned Oryx (<i>Oryx dammah</i>)	8
Tufted Deer (<i>Elaphodus cephalophus</i>)	4

Table S4. GenBank accession numbers for *Plasmodium* sequences generated from WTD and *A. punctipennis* and used in the phylogenetic analyses in this study.

Parasite lineage	Host species	Sampling location	GenBank accession nos.			
			<i>cytb</i>	<i>col</i>	<i>clpc</i>	<i>asl</i>
<i>Plasmodium</i> sp. (ANPUN01)	<i>Anopheles punctipennis</i>	Washington, D.C., USA	KU133755	KU133758	KU133766	KU133762
<i>Plasmodium</i> sp. (ANPUN02)	<i>Anopheles punctipennis</i>	Washington, D.C., USA	KU133748	KU133757	KU133765	KU133761
<i>Plasmodium</i> sp. (ODVIR01)	<i>Odocoileus virginianus</i>	Virginia, USA	KU133754	KU133756	KU133764	KU133760
<i>Plasmodium</i> sp. (ODVIR02)	<i>Odocoileus virginianus</i>	Virginia, USA	KU133751	KU133759	KU133767	KU133763
<i>Plasmodium</i> sp. (ODVIR03)	<i>Odocoileus virginianus</i>	Virginia, USA	KU133749	N/A	N/A	N/A
<i>Plasmodium</i> sp. (ODVIR04)	<i>Odocoileus virginianus</i>	Virginia, USA	KU133750	N/A	N/A	N/A
<i>Plasmodium</i> sp. (ODVIR05)	<i>Odocoileus virginianus</i>	Virginia, USA	KU133752	N/A	N/A	N/A
<i>Plasmodium</i> sp. (ODVIR06)	<i>Odocoileus virginianus</i>	Virginia, USA	KU133753	N/A	N/A	N/A