Evidence of *Culiseta* mosquitoes as vectors for *Plasmodium* parasites in Alaska

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ABSTRACT: Mosquito vectors play a crucial role in the distribution of avian *Plasmodium* parasites worldwide. At northern latitudes, where climate warming is most pronounced, there are questions about possible changes in the abundance and distribution of *Plasmodium* parasites, their vectors, and their impacts to avian hosts. To better understand the transmission of *Plasmodium* among local birds and to gather baseline data on potential vectors, we sampled a total of 3,909 mosquitoes from three locations in south-central Alaska during the summer of 2016. We screened mosquitoes for the presence of *Plasmodium* parasites using molecular techniques and estimated *Plasmodium* infection rates per 1,000 mosquitoes using maximum likelihood methods. We found low estimated infection rates across all mosquitoes (1.28 per 1,000), with significantly higher rates in *Culiseta* mosquitoes (7.91 per 1,000) than in *Aedes* mosquitoes (0.57 per 1,000). We detected *Plasmodium* in a single head/thorax sample of *Culiseta*, indicating potential for transmission of these parasites by mosquitoes of this genus. *Plasmodium* parasite DNA isolated from mosquitoes showed a 100% identity match to the BT7 *Plasmodium* lineage that has been detected in numerous avian species worldwide. Additionally, microscopic analysis of blood smears collected from black-capped chickadees (*Poecile atricapillus*) at the same locations revealed infection by parasites preliminarily identified as *Plasmodium circumflexum*. Results from our study provide the first information on *Plasmodium* infection rates in Alaskan mosquitoes and evidence that *Culiseta* species may play a role in the transmission and maintenance of *Plasmodium* parasites in this region. ***Journal of Vector Ecology* 44 (1): 68-75. 2019.**

Keyword Index: Mosquito, *Plasmodium*, *Culiseta*, *Aedes*, infection rate, vectors, Alaska.

INTRODUCTION

Avian haemosporidians are well-studied protozoan parasites with a nearly worldwide distribution and include species from the genera *Haemoproteus, Leucocytozoon,* and *Plasmodium*. Avian malaria, caused by infection with *Plasmodium* parasites, has been associated with avian mortality events around the world, particularly in isolated, naïve populations (Van Riper et al. 1986, Ots and Hõrak 1998). All *Plasmodium* species, including those that infect birds, mammals, and reptiles, require competent invertebrate (mosquito) vectors to transmit parasites between vertebrate hosts (Valkiūnas 2005). Avian *Plasmodium* parasites are transmitted by a rather broad suite of ornithophilic mosquito species from multiple subfamilies (Valkiūnas 2005, Njabo et al. 2009), and occur in a broad array of avian hosts. Worldwide, 11 mosquito genera comprising over 80 species have been identified as viable vectors for avian *Plasmodium* parasites (Valkiūnas 2005, Santiago-Alacron et al. 2012). Species of *Aedes* and *Culex* mosquitoes comprise the majority of these vectors, having greater than 50 species identified as viable vectors between them (Santiago-Alacron et al. 2012). Similar trends exist in North America, where *Aedes* and *Culex* mosquitoes make up the majority of the 29 identified species from six genera that are known *Plasmodium* vectors (Valkiūnas 2005, Santiago-Alacron et al. 2012). Previous research has helped elucidate the role mosquitoes play in the transmission and movement of *Plasmodium* parasites among avian populations (Ejiri et al. 2008, 2011, Ishtiaq et al. 2008, Kimura et al. 2010, Carlson et al. 2011). However, given the diversity of these vectors and complex host/parasite dynamics, combined with the large geographic distribution of *Plasmodium*, knowledge about vector ecology is still limited or absent in many regions.

Alaska is home to large populations of mosquitoes comprising over 30 species from four genera: *Aedes, Anopheles, Culex,* and *Culiseta* (Gjullin et al. 1961, Darsie and Ward 2005). With rapid warming trends occurring in northern regions, concerns about possible climate-driven changes to the distribution and abundance of these vectors have become more pronounced (Garamszegi 2011, Van Hemert et al. 2014). *Plasmodium* parasites are responsive to environmental factors, requiring ambient temperatures above a certain threshold to complete their life cycle (Valkiūnas 2005). Longer growing seasons and increased temperatures could allow expansion and persistence of mosquito species into new regions and for longer periods throughout the year, exposing previously naïve avian populations to potentially pathogenic infections. However, the vector species responsible for transmitting *Plasmodium* in these regions have not yet been identified, making projections about such changes difficult. Thus, collection of baseline information on mosquito vectors in Alaska is a necessary step for understanding possible future impacts of avian malaria on resident (non-migratory) bird populations.

Information pertaining to avian malaria in Alaskan birds has increased considerably in the past decade, with multiple studies examining the prevalence of *Plasmodium* infections in

various avian host taxa (Loiseau et al. 2012, Ramey et al. 2012, Oakgrove et al. 2014, Reeves et al. 2015, Smith et al. 2016, Wilkinson et al. 2016) and several providing evidence of local transmission in some parts of the state (Loiseau et al. 2012, Oakgrove et al. 2014, Ramey et al. 2015, Smith et al. 2016, Wilkinson et al. 2016). Notably, *Plasmodium* prevalence in certain resident hosts in south-central Alaska is relatively high (Smith et al. 2016, Wilkinson et al. 2016), suggesting common occurrence of the parasite in this boreal region. There has been little to no work published, however, on mosquitoes relative to *Plasmodium* transmission in Alaska, and there is a dearth of information about vectors in the region in general.

To gather baseline information on *Plasmodium*/mosquito relationships in Alaska, and to determine which genera of mosquitoes could facilitate transmission of *Plasmodium* parasites to local birds, we collected mosquitoes throughout the summer of 2016 in the south-central region of the state and screened them for the presence of *Plasmodium* parasites using molecular methods. The results of our study provide preliminary identification of *Plasmodium* vectors in south-central Alaska, as well as some of the first genetic information on *Plasmodium* lineages in vector species from the region. Additionally, we present results from microscopic examination of peripheral blood smears collected from resident avian hosts at our vector-sampling locations.

MATERIALS AND METHODS

Sample collection

We collected mosquitoes from three wooded parklands (Campbell Creek: 61.164° N, 149.777° W; Eagle River: 61.234° N 149.271° W; Mirror Lake: 61.428° N 149.425° W) in or near the city of Anchorage in south-central Alaska, U.S.A., during the summer of 2016. Study areas ranged in elevation from 56 to 155 m above sea level and were mixed deciduous–coniferous forest dominated by paper birch (*Betula papyrifera*) and white spruce (*Picea glauca*). We selected sites at which we had previously studied *Plasmodium* prevalence in black-capped chickadees (Wilkinson et al. 2016). We assembled one modified CDC model 512 miniature light trap (John Hock Company, Gainesville, FL, U.S.A.) in early May, 2016, at each location. We affixed traps to stands approximately 1.5 m tall and fitted each with kill-jar assemblies containing 100% ethanol and supplemental CO_2 systems to aid in mosquito attraction. Traps were opened for two 24-h periods per week from 8 May through 11 August in 2016. Compressed CO_2 was vented from tanks at approximately 100–150 ml/min during open periods and all kill-jars were collected at the end of each 24-h period. After collection, we transferred mosquitoes to separate containers and stored them in 100% ethanol until identification and subsequent DNA extraction.

Mosquito identification and dissection

We identified all mosquitoes collected in our CDC light traps to genus using morphological keys (Aquatic Biology Associates Inc., Corvallis, OR, U.S.A.). Further identification to species level was not possible due to the use of ethanol to preserve specimens for genetic analysis, causing discoloration and preventing species identification. Mosquitoes were sorted by genus, sampling location, and sampling period prior to dissection.

To maximize the probability of detecting *Plasmodium* infections and to decrease screening time, we combined mosquitoes from each sampling occasion in pools of 1 to 25 individuals of the same genus and sampling period. Mosquitoes were placed on a glass slide under a magnifier and the wings and legs removed using forceps and a scalpel. We then separated each individual mosquito abdomen from the thorax; these were placed in separate 1.5 ml microcentrifuge tubes and dried at 56° C for a minimum of 60 min. To prevent possible contamination between pools, we sterilized all scalpels, forceps, and glass slides with 10% bleach solution between each dissection. After drying, we removed tubes from the incubator and crushed the contents with sterile probes until all mosquito parts were homogenized.

DNA extraction

We extracted DNA from all samples using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA, U.S.A.) following a modified protocol derived from the Qiagen supplementary insect DNA extraction handbook and a procedure specially optimized for extracting DNA from mosquito tissues (Plichart et al. 2006). After all mosquito parts were homogenized, we added 200 µl of buffer ATL to each sample, followed by 30 µl of proteinase K, then vortexed, spun briefly, and incubated in a two-step process. We incubated samples at 56° C overnight, re-vortexed, added 200 µl of lysis buffer (buffer AL) and 10 µl of proteinase K, vortexed again, and incubated for 10 min at 56° C. After the second incubation, we added 200 µl of 100% ethanol to each sample, then vortexed and centrifuged at 13,000 RPM. The supernatant was then transferred to the spin silica column for DNA binding. The washing steps followed those published by Plichart et al. (2006) and included washing each column twice with 500 µl buffer AW1 and once with 500 µl buffer AW2, followed by spinning twice at 13,000 RPM to ensure each filter was sufficiently dry. Finally, we eluted DNA from each column twice using 100 µl AE buffer for each elution step.

In addition to the above protocol, we ran all head/thorax samples from each pool through an additional wash series to help remove any PCR-inhibiting substances (Arez et al. 2000). We pipetted the eluate from each head/thorax sample into a new silica spin column and washed these in the same series as before (two washes with AW1 buffer and one with AW2 buffer plus a final drying spin) and then re-eluted to 200 µl. Tests on mosquitoes used to optimize this extraction method showed that this additional wash series greatly increased DNA yield from head/thorax samples.

DNA amplification

To ensure the viability and quality of our DNA extractions, we put each sample through a positive-control PCR reaction using universal insect primers LCO1490 and HCO2198, which amplify a fragment of the cytochrome oxidase subunit I (COI) gene of insects' mitochondrial DNA (Folmer et al. 1994). We considered an extraction to be positive via this

COI control if bands were present when visualized on 1% agarose gels stained with Gel Red Nucleic Acid Gel Stain (Biotium, Hayward, CA, U.S.A.). We subsequently screened all mosquito pools verified via our COI positive control for the presence of *Plasmodium* parasites using a variation of the nested-PCR protocol described by Hellgren et al. (2004). This protocol uses one primer set (HaemNF1/HaemNR3) that amplifies a larger, outer section of the cytochrome *b* (cyt *b*) gene and two additional primer pairs (HaemF/HaemR2 and HaemFL/HaemR2L) that amplify smaller regions within the first amplified section. This allows for simultaneous detection of three genera of blood parasites: *Haemoproteus, Plasmodium,* and *Leucocytozoon*. Since our goal was to identify *Plasmodium* infections in our samples, we screened our samples using only a single nested primer pair, HaemF/HaemR2. All samples were screened two to three times to account for imperfect detection of *Plasmodium* infection (Ramey et al. 2012, Meixell et al. 2016) and visualized on 1% agarose gels as described previously. For all positive samples, we bidirectionally sequenced a 479 bp fragment of *Plasmodium* cyt *b* gene using identical primers from PCR. PCR products were purified with Exo-SapIT (USB Inc., Cleveland, OH, U.S.A.) at a 3:7 dilution and sequencing was conducted using Big Dye Terminator v3.1 mix (Applied Biosystems, Foster City, CA, U.S.A.) and analyzed on an ABI 3730xl automated DNA sequencer (Applied Biosystems, Foster City, CA, U.S.A.). Sequence data were edited using Sequencher 5.0.1 software (Gene Codes Corp., Ann Arbor, MI, U.S.A.).

Microscopic examination

To identify *Plasmodium* morphospecies in resident birds at our study sites, we screened a subset of blood smears from black-capped chickadees collected as part of a separate, ongoing study. Detailed capture methods are described in Handel et al. (2010) but, briefly, birds were caught using funnel traps or mist nets between September, 2014, and April, 2015. Peripheral blood smears were made for each sampled bird, fixed in absolute methanol, and stained with Giemsa. Slides from birds confirmed to be positive for *Plasmodium* by PCR (M.M. Smith, unpublished data) were visualized using light microscopy under 1000× magnification, and *Plasmodium* morphospecies were identified using taxonomic keys following Valkiūnas (2005).

Statistical analysis

We used a maximum likelihood estimation method (MLE), PooledInfRate (www.cdc.gov), to calculate the *Plasmodium* infection rate per 1,000 mosquitoes. This method accounts for the possibility of more than one positive mosquito in each pool and can accommodate data consisting of pools of varying size. For the sake of this estimate, we combined results from both the head/thorax and abdomen pools and considered any detections of *Plasmodium* a single infection for that pool.

RESULTS

We collected a total of 3,929 mosquitoes from three sites near Anchorage, Alaska, during the summer of 2016 (Table 1). A single pool containing 20 *Aedes* mosquitoes failed to amplify via our COI positive control and was excluded from further analysis, leaving a total of 3,909 mosquitoes for *Plasmodium* screening. We identified the majority of these as *Aedes* mosquitoes (n = 3,536), with a smaller number of individuals belonging to the genus *Culiseta* (n = 373). After grouping samples by genus, capture site, and date we screened a total of 241 pools of each abdomen and head/thorax segments, for a total of 482 screening reactions. We successfully amplified *Plasmodium* mtDNA in 2.07% of our combined head/thorax and abdomen pools (5/241; Table 1), three consisting of *Culiseta* mosquitoes and two of *Aedes* mosquitoes. Our MLE infection rate for all mosquitoes was 1.28 per 1,000 (95% CI: 0.48–2.82; Table 1). Estimated infection rates were 0.57 per 1,000 (95% CI: 0.10–1.85) for *Aedes* mosquitoes and 7.91 per 1,000 (95% CI: 2.16–20.90; Table 1) for *Culiseta*. Both PCR detections of *Plasmodium* in *Aedes* mosquitoes were in the abdomens only. *Plasmodium* DNA was amplified from both the abdomen and head/thorax samples of one of the *Culiseta* pools, with the remainder of the detections (n = 2) originating from abdomens pools only.

Analysis of *Plasmodium* mtDNA sequences revealed a single *Plasmodium* lineage in all of our positive samples (GenBank accession number MH458252). Comparison to previously published lineages in the MalAvi (Bensch et al. 2009) and GenBank databases showed a 100% identity match between our samples and the BT7 (P43) lineage previously reported in Alaskan birds, as well as in many other avian species worldwide.

Microscopic examination of blood smears collected from black-capped chickadees (M.M. Smith, unpublished data) revealed infection by *Plasmodium* parasites and an unknown species of *Hepatozoon*. Positive identification of *Plasmodium* species was difficult due to low parasitemia levels and poor quality of blood smears, but morphological characteristics strongly resembled those of *Plasmodium circumflexum* as described by Valkiūnas (2005). Most notably, we observed erythrocytic meronts and gametocytes growing around the nucleus of infected erythrocytes, sometimes completely encircling the nucleus without displacing it laterally (Figure 1). Additionally, pigment granules in erythrocytic meronts tended to be found in a single, loose clump near one end of observed meronts (Figure 1).

DISCUSSION

Our study represents the first published report of *Plasmodium* parasites in vectors in Alaska and provides genetic information about *Plasmodium* lineages in mosquitoes from this region. The significantly higher estimated infection rates in *Culiseta* mosquitoes and detection of *Plasmodium* in a *Culiseta* head/thorax pool provides evidence of active transmission of *Plasmodium* by mosquitoes of this genus. The 100% identity match of all *Plasmodium* lineages identified

Table 1. Number of mosquitoes (*Aedes* and *Culiseta* spp.) collected at three sites by month in south-central Alaska, U.S.A., during May–August, 2016. Number of pools screened for *Plasmodium*, number (%) of *Plasmodium*-positive pools, and estimated infection rates per 1,000 mosquitoes calculated by maximum likelihood estimation, with 95% lower (LCL) and upper (UCL) confidence limits. Asterisk indicates sample that was positive for *Plasmodium* in both abdomen and head/thorax pools.

Genus	Site[a]	Month	Mosquitoes	Pools		Infection rate		
				Total	Positive (%)	Estimate	LCL	UCL
Aedes	ML	May	331	19	0			
		June	619	33	0			
		July	1,537	69	1 (1.45)	0.64	0.04	3.12
		August	78	5	0			
	ER	May	128	7	0			
		June	347	20	0			
		July	213	13	0			
		August	8	3	0			
	CC	May	76	5	0			
		June	96	9	1 (11.11)	10.38	0.62	51.04
		July	102	9	0			
		August	1	1	0			
	Total		3,536	192	2 (1.04)	0.57	0.10	1.85
Culiseta	ML	May	122	9	0			
		June	14	5	0			
		July	5	3	1 (33.33)	175.15	12.56	592.89
		August	0	0	0			
	ER	May	162	10	0			
		June	21	7	0			
		July	0	0	0			
		August	0	0	0			
	CC	May	31	7	1 (14.29)	29.31	1.95	134.09
		June	13	5	2 (20.00)*	65.23	4.71	273.69
		July	5	3	0			
		August	0	0	0			
	Total		373	49	3 (6.12)	7.91	2.16	20.90
Grand total			3,909	241	5 (2.07)	1.28	0.48	2.82

[a]ML: Mirror Lake, ER: Eagle River, CC: Campbell Creek.

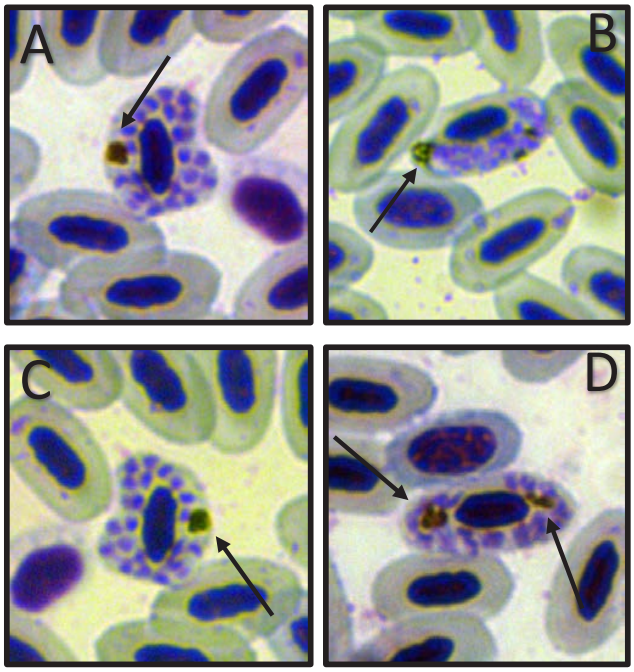

Figure 1. Erythrocytic meronts of *Plasmodium* in blood smears from black-capped chickadees (*Poecile atricapillus*) sampled at locations in south-central Alaska, U.S.A. Fully grown meronts completely surrounding the host cell nucleus (**A, C**) without displacing it laterally. Growing erythrocytic meronts (**B, D**) beginning to encompass the host cell nucleus. Arrows indicate pigment granules loosely clumped near one or both ends.

in our study to the BT7 lineage, which has been identified previously in black-capped chickadees at our study sites, provides further evidence for active transmission of this parasite lineage by mosquitoes in the area. Additionally, the preliminary identification of *P. circumflexum* in blood smears collected from birds at these locations provides some of the first information on *Plasmodium* morphospecies infecting Alaskan bird hosts.

The life cycle of *Plasmodium* parasites involves a sexual phase (sporogony) that occurs in the midgut of mosquitoes, located in the abdomen (Valkiūnas 2005). The length of time it takes to complete sporogony can vary by *Plasmodium* species, vector viability, and ambient temperature. *P. circumflexum*, for example, takes 8–16 days to complete sporogony in certain *Culiseta* species (Valkiūnas 2005). At that stage, sporozoites penetrate the mosquitoes' salivary glands and can be actively transmitted to vertebrate hosts (Valkiūnas 2005). Thus, lineages isolated from head/thorax segments potentially represent infective stages of the *Plasmodium* parasite. We successfully identified *Plasmodium* mtDNA in one *Culiseta* head/thorax pool, lending support for *Culiseta* mosquitoes as viable *Plasmodium* vectors in south-central Alaska. Detection of *Plasmodium* lineages in abdominal samples of both *Culiseta* and *Aedes* mosquitoes, while informative, does not demonstrate vector viability since abdominal detections may represent noninfective stages of the parasite. It is possible these detections represent *Plasmodium* parasites that had not yet completed sporogony at the time of sampling, or, alternatively, were picked up in a recent blood-meal from an infected bird prior to capture but were unable to undergo sexual reproduction in that mosquito species. Varying ranges of vector viability exist for avian *Plasmodium* parasites, and some *Plasmodium* species are only able to fully complete their life cycles in certain species of mosquitoes (Valkiūnas 2005). Further work, including the collection and identification of mosquitoes to the species level or experimental infections with local vectors, would help to better understand these vector–parasite relationships.

The difference in the abundance of mosquitoes from each genus we captured was notable, with *Aedes* mosquitoes caught at a rate nearly ten times that of *Culiseta* species. However, the estimated infection rate of *Plasmodium* was unexpectedly more than ten times greater in *Culiseta* mosquitoes than in *Aedes* mosquitoes, possibly indicating that *Culiseta* species play a larger role as vectors of *Plasmodium* in this region. The fact that *Plasmodium* was detected in the head/thorax of a *Culiseta* pool also supports *Culiseta* mosquitoes as probable vectors. Additionally, *P. circumflexum* is only known to be transmitted by mosquitoes from the *Culiseta* and *Mansonia* genera and are not known to complete sporogony in *Aedes* species (Valkiūnas 2005).

Most studies examining prevalence of haemosporidian parasite infections in Alaskan birds over the past decade have found relatively low levels of *Plasmodium* infection, ranging from 0–24%, depending on host taxa and sampling location (Ramey et al. 2012, Oakgrove et al. 2014, Reeves et al. 2015, Smith et al. 2016, Wilkinson et al. 2016). The highest prevalence of *Plasmodium* reported in recent years (24%), and the most relevant to our study, was detected in black-capped chickadees sampled at the same locations in south-central Alaska by Wilkinson et al. (2016). The low *Plasmodium* infection rate in mosquitoes from these sites during the summer (1.28 per 1,000) was somewhat surprising, particularly when compared to prevalence in black-capped chickadees. As this species is resident year-round and birds disperse only short distances from their natal home range (Foote et al. 2010), *Plasmodium* infections detected in both adult and juvenile birds can be considered locally transmitted. The rate at which mosquitoes can spread *Plasmodium* infections throughout a resident population of bird hosts is relatively unknown. Infective sporozoites can persist in the salivary glands of mosquitoes for several weeks (Valkiūnas 2005) and, assuming female *Culiseta* or *Aedes* mosquitoes can take multiple blood meals throughout their lifetime, it is plausible that low infection rates of *Plasmodium* in viable mosquitoes could lead to much higher prevalence of infections in avian hosts. It is also important to note that *Plasmodium* infections in birds can become chronic, possibly persisting for years in certain individuals (Valkiūnas 2005, Kim et al. 2009). This makes estimation of the time of initial transmission of *Plasmodium* from vector to host very difficult, and it is unclear whether the prevalence of *Plasmodium* we detected in local vector populations would be adequate to maintain moderate rates of transmission in resident avian hosts, or if detections in local black-capped chickadees mostly represent chronic or

recrudescent *Plasmodium* infections.

Previous studies have shown that transmission and prevalence of *Plasmodium* infections in avian hosts vary by season and that these parasites are responsive to certain climatic factors, such as temperature and precipitation (Cosgrove et al. 2008, Zamora-Vilchis et al. 2012). *Plasmodium* parasites are the most temperature-sensitive genus of avian haemosporidians and are only able to complete their life cycle in mosquitoes if the ambient temperature is above a certain threshold (Valkiūnas 2005). Due to low prevalence of *Plasmodium* infection, we were unable to analyze possible effects of environmental factors on *Plasmodium* infection in mosquitoes. Wilkinson et al. (2016) reported a positive correlation between summer temperatures and *Plasmodium* presence in black-capped chickadees at these sampling locations. Although it is possible that temperatures during our sampling period were not consistently high enough to promote *Plasmodium* sexual reproduction in mosquito midguts, typical temperature ranges across our study area are above the threshold for *Plasmodium* development (Wilkinson et al. 2016), so thermal limitations are unlikely to fully explain the low parasite prevalence we observed in mosquitoes.

Our trapping methods employed CDC miniature light traps baited with CO_2, and previous studies have found evidence that mosquito sampling methods can influence the abundance, species diversity, and physiological state and age of mosquitoes caught (Chen et al. 2011, Carlson et al. 2015). CO_2-baited light traps have been shown to perform well in attracting a broad range of mosquito species in greater abundance when compared to other trap types but may collect a higher number of unfed, nulliparous females (Reisen and Pfuntner 1987, Carlson et al. 2015). These unfed mosquitoes would thus have never come into contact with *Plasmodium*-infected birds through a blood meal. To alleviate potential bias from oversampling unfed mosquitoes, broader sampling efforts should be used in future studies, including the use of gravid traps, which are known to collect older mosquitoes that have digested at least one blood meal. Additionally, dissecting individual mosquitoes would help determine mosquito parity rates, while also allowing for positive identification of *Plasmodium* life stages in mosquito mid-gut and salivary glands.

Our preliminary identification of *P. circumflexum* in the peripheral blood of black-capped chickadees, combined with the 100% nucleotide identity match between all *Plasmodium* lineages isolated from vectors and those detected by Wilkinson et al. (2016), supports the presence and local transmission of this *Plasmodium* species in Alaska. Erythrocytic meronts and gametocytes present in blood smears corresponded with characteristics identical to those described by Valkiūnas (2005) as *P. circumflexum*. It is important to note, however, that morphospecies identification of *Plasmodium* species can be difficult in blood smears collected from wild birds, where parasitemia is rarely found in the acute stage. Also, *P. circumflexum* has a wide range of vertebrate hosts and has been detected on every continent besides Antarctica (Valkiūnas 2005). It is possible that multiple *Plasmodium* species with nearly identical blood stages could be identified as *P. circumflexum*, given its range and host diversity. Future work involving molecular methods paired with the collection of additional blood smears from avian hosts during times when parasitemia is likely at its peak (e.g., breeding season) or experimental infections in controlled environments would allow for more definitive identification of *Plasmodium* species in Alaskan birds.

All sequences from our study shared a 100% nucleotide identity with the BT7/P43 lineages published on Malavi and GenBank, respectively. BT7 is one of the most common *Plasmodium* lineages detected across the globe and has been isolated in 41 bird species to date (Bensch et al. 2009). In Alaska, this lineage has been reported in waterfowl (Reeves et al. 2015), grouse (Smith et al. 2016), and passerines (Oakgrove et al. 2014, Wilkinson et al. 2016). The detection of the BT7/P43 lineage in mosquitoes at our sampling locations was expected, based on the findings of Wilkinson et al. (2016), who reported the same lineage in all 417 of their positive samples. The 100% match with our *Plasmodium*-positive *Culiseta* head/thorax sample suggests that this genus of mosquito is responsible for at least a portion of the *Plasmodium* infections being transmitted. This, combined with the higher detection rate of *Plasmodium* in *Culiseta* rather than *Aedes* species suggests that *Culiseta* mosquitoes would be good candidates for any future work on *Plasmodium* vectors in Alaska. Targeted sampling of *Culiseta* mosquitoes and the identification of samples to the species level would allow for better resolution of host-vector relationships in this region.

Our findings of low rates of *Plasmodium* infection among mosquitoes in south-central Alaska provide important baseline information for future studies of *Plasmodium* vectors in northern regions. The higher infection rate found in *Culiseta* mosquitoes and evidence for active transmission suggest that mosquitoes of this genus may play an important role in the transmission and maintenance of *Plasmodium* parasites in the region. Additional sampling and expansion of the geographic scope of mosquito collections are needed to examine the dynamics of *Plasmodium* vectors in Alaska on a greater scale. Future studies incorporating focused sampling of *Culiseta* mosquitoes, identification of specimens to species level, and dissection of individual mosquitoes are needed to determine which *Culiseta* species are viable *Plasmodium* vectors. Additional work focusing on the combination of molecular and microscopic methods in this region will help confirm the relationship between *Plasmodium* mtDNA lineages and morphospecies identification. Future research incorporating these methods would help clarify the distribution of *P. circumflexum* and other *Plasmodium* parasites in Alaska, which is important for future studies focusing on possible changes in *Plasmodium* infection rates or expansion into new regions.

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