

Prevalence of *Haemoproteus* spp. infections in blood samples from Allen's hummingbirds (*Selasphorus sasin*)

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Background

- Haemoparasite infections have been revealed to be pathogenic in wild birds and to reduce the survival of wild bird populations.^{1,2}
- In the hummingbird family Trochilidae, microscopy and nested polymerase chain reaction (PCR) have identified three *Haemoproteus* spp. haemoparasites (*H. archilochus*, *H. trochili* and *H. witti*) transmitted by Dipteran vectors of the family Ceratopogonidae and Hippoboscidae, also known as biting midges (Figure 1).^{3,4,5}
- Common to coastal California, Allen's hummingbirds (*Selasphorus sasin*, ALHU, Figure 2) have adapted to suburban and urban areas and are important pollinators for endangered and threatened coastal plants.
- *Haemoproteus* spp. have been identified in Rufous hummingbirds (*Selasphorus rufus*), however a population study of the *Selasphorus* genus has not been performed.³
- ALHU have a different habitat range compared to other species that show *Haemoproteus* spp. infections, the Anna's (*Calypte anna*, ANHU) and Black-chinned hummingbirds (*Archilochus alexandri*, BCHU) as shown in Figure 3. ANHU and BCHU gravitate to riparian forest areas, which are potentially better suited for vectors to fly, reproduce, and land on hosts due to the presence of freshwater and lack of wind compared to the coast.

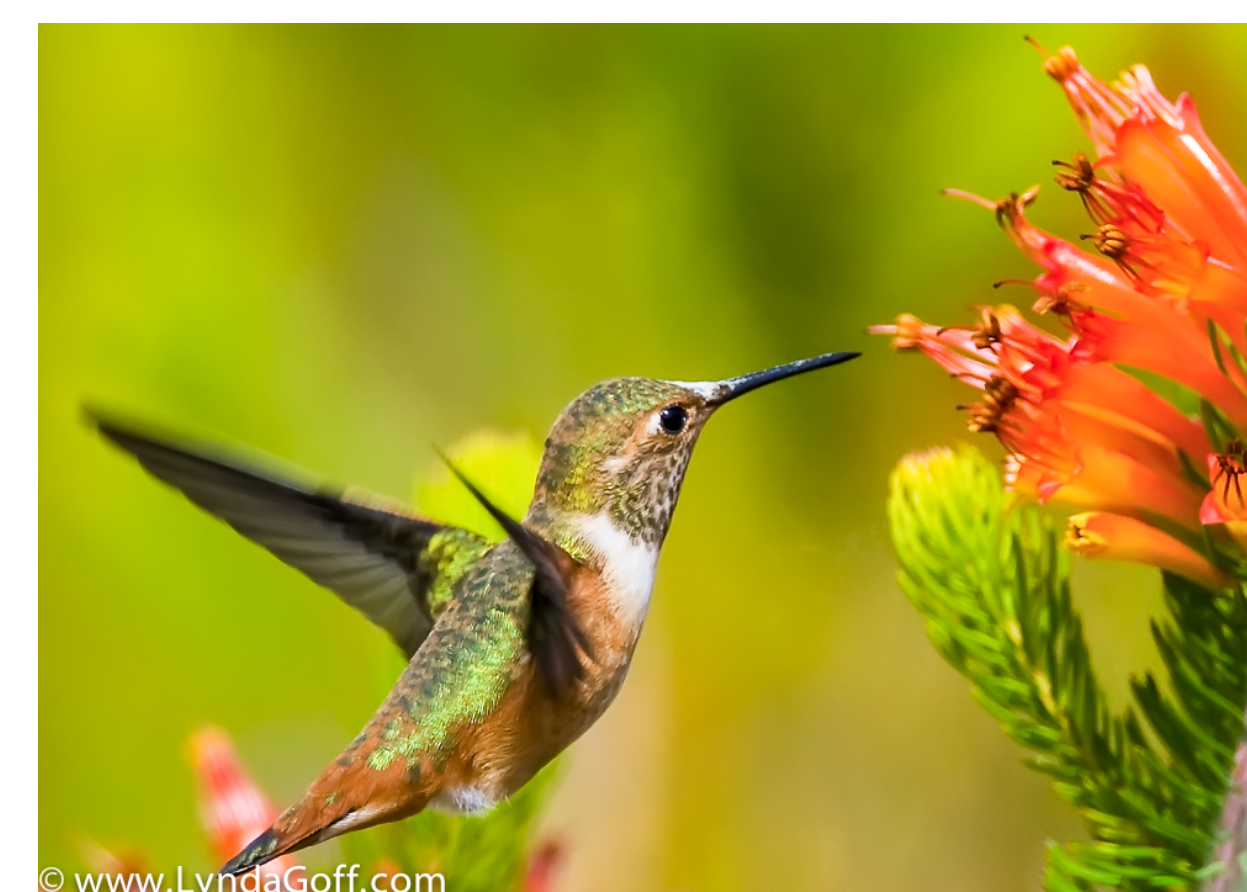


Figure 2. Allen's hummingbird.



Figure 3. Maps of habitat ranges of California native hummingbirds. From left to right, the distribution of ALHU, ANHU, and BCHU in California.



Figure 1. Dipteran vector of *Haemoproteus* spp., the biting midge.



Figure 4. Hall drop net and feeder.

Methods

- ALHU (n = 100) were captured using Hall drop nets (Figure 4) at feeders from March 2016 to March 2017 at locations throughout California (Avalon, Malibu, Inverness, and Beverly Hills).
- Birds were banded with unique ID bands following UC Davis IACUC approved protocol #18605.
- Blood was collected by clipping a distal toenail and dabbing onto Nobuto filter paper strips (Figure 5).
- DNA was extracted from dried blood samples by Promega Wizard® SV Genomic DNA Purification System. DNA presence was confirmed by NanoDrop.
- DNA samples were tested for parasites using nested PCR. Primers for the cytochrome *b* gene lineage in mitochondrial DNA were used to screen for *Haemoproteus* spp. Primers HaemNF and HaemNR2 were used to amplify a 478 bp fragment (excluding the primers) from *Haemoproteus* and *Plasmodium* spp and HaemF and HaemR for the second nested PCR.
- PCR products and a positive control from an ANHU were viewed on 1.8% agarose gels and visualized using GelStar (Figure 6). Positive PCR products were purified and sequenced.
- Data was compared to published data on *Haemoproteus* spp. infections in ANHU and BCHU.⁵



Figure 5. Blood sample from an ALHU on a Nobuto filter paper strip.

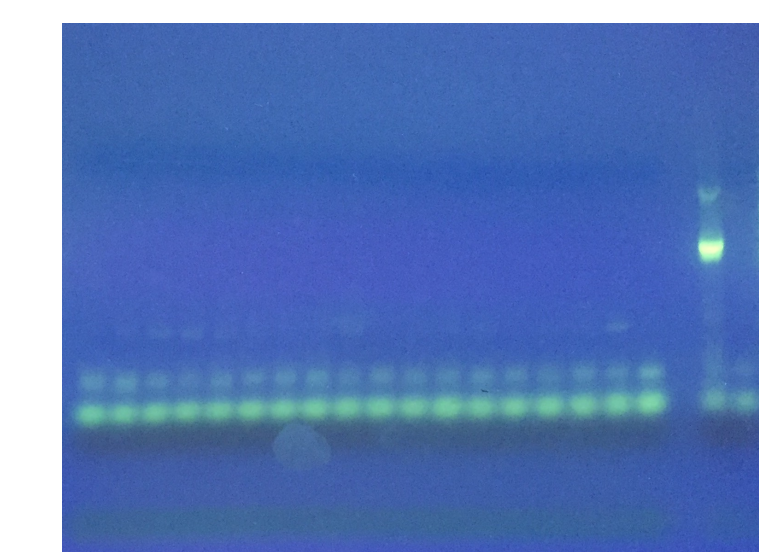


Figure 6. Gel electrophoresis of PCR products of ALHU blood samples. From left to right, 18 negative ALHU samples, a positive control from an ANHU, negative control, and 100 bp ladder.

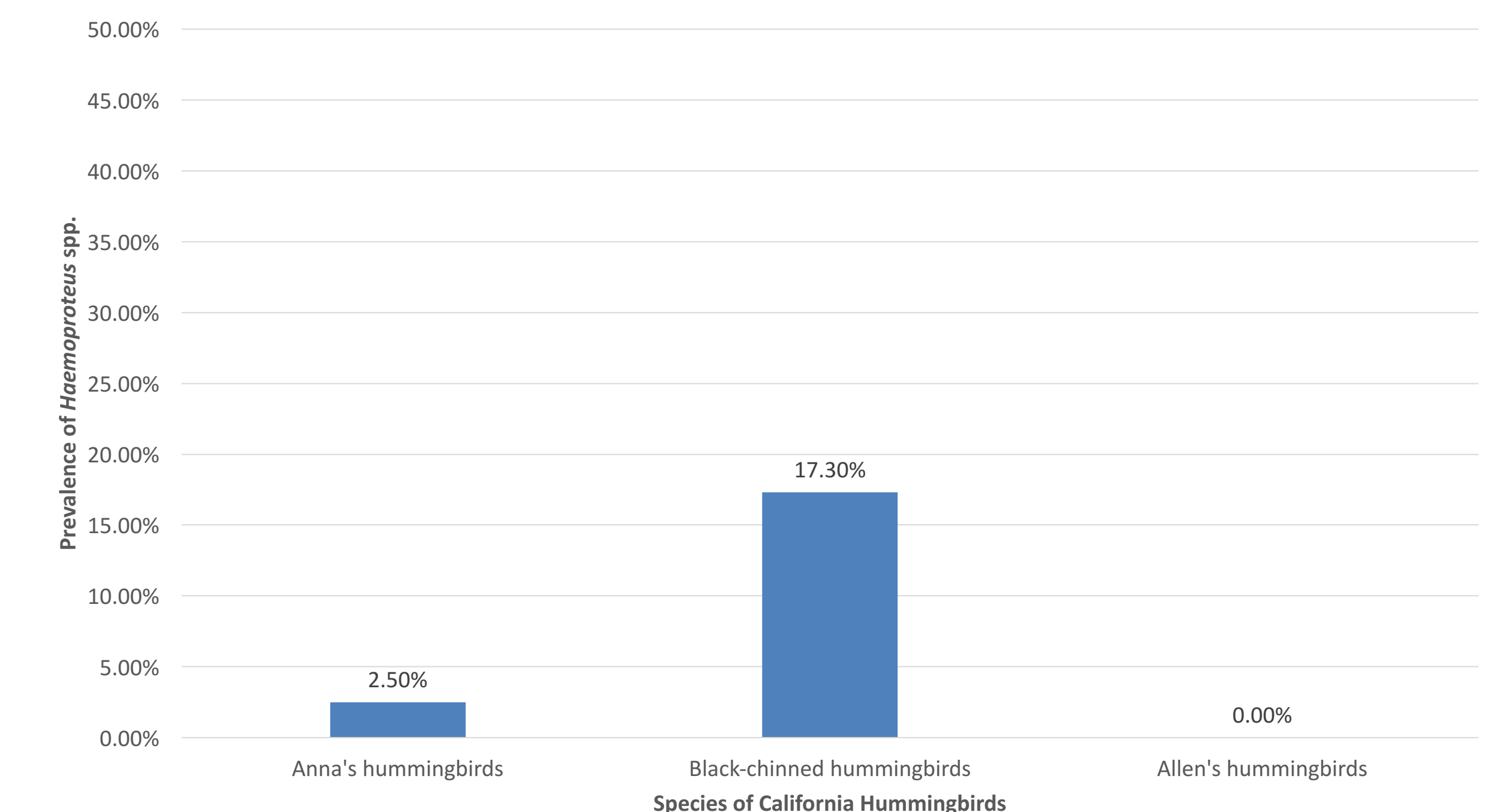


Figure 7. Prevalence of *Haemoproteus* spp. in three species of California hummingbirds. Data for ANHU and BCHU were taken from previously published findings.⁵

Conclusions

- Compared to previously published data on *Haemoproteus* spp. prevalence in ANHU and BCHU, the prevalence in ALHU is lower in a limited sample set (n=100).⁵
- For the PCR product of one sample (#41) that showed up as a bright single band, a sample positive for *Plasmodium* spp. could cross-react with *Haemoproteus* spp. primers to reveal a band not homologous to *Haemoproteus* spp. positive controls.
- This study supports that the prevalence of *Haemoproteus* spp. infections in hummingbird species can vary due to habitat differences in avian hosts and dipteran vectors.

Future Work

- Investigate vector populations and feeding behavior on hosts.
- Uncover the host-pathogen relationship between hummingbirds and haemoparasites.
- Evaluate a greater number of samples from all hummingbirds from different geographical regions and obtain higher concentrations of DNA from blood samples.

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Study Objectives and Hypothesis

- To determine the prevalence of *Haemoproteus* spp. in ALHU by using nested PCR to test blood samples.
- To compare the prevalence of *Haemoproteus* spp. infections in ALHU to previously published data on ANHU and BCHU and contribute to the detection and monitoring of future disease threats to these avian pollinators.⁵
- **Hypothesis:** There is a higher prevalence of *Haemoproteus* spp. infections in ANHU and BCHU than in ALHU due to potentially greater host-vector contact rates in riparian compared to coastal habitats.

Results

- Out of 100 ALHU samples, all birds were negative for *Haemoproteus* spp. infections (Figure 6).
- On gel electrophoresis, the PCR product of one blood sample #41 showed up as a bright single band not homologous to where the band was for the positive control of *Haemoproteus* spp. from an ANHU. The evaluation of this sample still remains to be determined.
- The prevalence of *Haemoproteus* spp. in ALHU was 0%. Previously published data for ANHU (n=157) and BCHU (n=104) was 2.5% and 17.3% respectively, with four distinct *H. archilochus* cyt *b* lineages (Figure 7).⁵