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# Haemosporidian infection in passerine birds from high elevation in the Tian Shan, Kyrgyzstan

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**Abstract.** The study of blood parasites in alpine environments allows us to look into host-parasite relationships under very specific conditions. The objective of our study was to detect the presence of blood parasites in Alpine accentors (*Prunella collaris*) living in the Central Tian Shan region, Kyrgyzstan. However, an examination of the specimens of the genus *Prunella* confirmed the absence of blood parasites in all tested samples (species: *P. atrogularis, P. collaris rubida, P. fulvescens*). In addition to the target species, other songbirds were also examined to determine the presence of haemosporidian parasites in the region. A total of 52 birds of 21 species of 6 families were examined. In the samples, the presence of parasites of the genus *Haemoproteus* (8 positives individuals) and *Leucocytozoon* (4 positives individuals) was detected in the host species: *Emberiza buchanani, Luscinia megarhynchos, Motacilla cinerea, Phoenicurus caeruleocephala, Serinus pusillus*.

*Key words:* blood parasites, *Haemoproteus, Leucocytozoon*, *Prunella* sp., high altitude

## Introduction

Mountain regions are characterized by specific and demanding conditions and they vary geographically based on altitude, water availability, and seasonality (Grabherr *et al.* 2010; Körner *et al.* 2011), all of which determine the biotic composition of alpine communities (Winkler *et al.* 2019). The patterns of climate and landscape are major determinants of the distribution of biodiversity (Pearson and Dawson 2003; Foley *et al.* 2005), the principles of which are also true for parasites (Pérez-Rodríguez *et al.* 2013). Understanding the patterns and factors that determine and shape parasite community composition leads to our knowledge of host-parasite relationship and dynamics. The environmental conditions generally influenced by climate change, like temperature, humidity, availability of vectors, constitute a good model to examine variation in host-parasite interactions. Of the abiotic variables, temperature is one of the most important environmental factors that affects the structure, distribution and diversity of species along elevational gradients (Oommen and Shanker 2005). In this sense, generally, parasite abundance declines with elevation (Badyaev 1997; Álvarez-Ruiz *et al.* 2018).

Parasites from order Haemosporida, whose species (mainly *Haemoproteus*, *Plasmodium*, and *Leucocytozoon*) infect birds, have a broad range of vertebrate host and vectors (blood-sucking insects - Diptera) worldwide (Valkiunas 2005). Increased parasite infection can have negative effects on the host such as a diminished immune response (Merino *et al.* 2000; Tomás *et al.* 2007), reduced reproductive success and growth of birds (Hamilton and Zuk 1982; Marzal *et al.* 2005; Valkiunas *et al.* 2006), as well as a reduction in fitness (Schmid-Hempel 2011; Asghar *et al.* 2015). The epidemiology of avian malaria is regulated by many dynamic spatio-temporal factors, but particularly by ecological (season, habitat quality, elevation), demographical (host and vector density, age host), and environmental factors (temperature, precipitation) (see e.g. Ishtiaq and Barve 2018; Wood *et al.* 2007; Cosgrove *et al.* 2008; Paaijmans *et al.* 2009; Lachish *et al.* 2011; van Rooyen *et al.* 2013; Liao *et al.* 2017). Many factors affect the prevalence of malaria parasites, but the keys to this prevalence seem to include the birds' habitat, precipitation and ambient temperature (which decreases at higher elevations). The prevalence of parasitemia fall as elevation increases, because vectors decline in numbers and become more seasonal at higher altitudes (Atkinson 2005). Monitoring haemosporidian parasites on the altitudinal gradient indicates the correlation of parasites species with altitude. At lower altitude a higher occurrence of *Plasmodium* and *Haemoproteus* can be observed (van Riper *et al.* 1986; Harrigan *et al.* 2014; Zamora-Vilchis *et al.* 2012), while several studies have confirmed the common occurrence of *Leucocytozoon* parasites at higher altitudes (Haas *et al.* 2012; Imura *et al.* 2012; van Rooyen *et al.* 2013; Lotta *et al.* 2015).

To understand host-vector-parasite interactions, and the infection dynamics of haematozoa, it is important to investigate the prevalence of haematozoa among bird communities in specific environmental conditions. Despite a significant number of studies in the last two decades (Marzal 2012), there are few studies about haemoparasites of avifauna located over 2000 m a.s.l. worldwide, (Gonzalez *et al.* 2015) as well as a lack of publications from the Central Asia region.

 

The objective of the study was to estimate the prevalence of haemosporidian infection in passerine birds at high elevations. Our previous results from the West Carpathians (High Tatras Mountains), in the species *Prunella collaris* confirm zero prevalence (Haas and Kisková 2010). We sought to discover if the prevalence of blood parasites in the genus *Prunella* is similar in another alpine region. We also wanted to collect data on infection with Haemoproteids in other passerine birds, living in the same biotope. The birds were trapped during a research expedition in Kyrgyzstan in August-September 2008.

The Tian Shan is the largest mountain system located in Central Asia and is also the largest isolated east-west stretching mountain range. This region is a distinctly continental climate with cold, snowy winters contrasting with hot, dry summers. The climatic conditions are further modified by the mountainous terrain which creates microclimates and pronounced vertical zonality in the climate and ecology (IUCN 2016). Several species of genus *Prunella* are naturally found in this area, especially: *P. collaris rubida*, *P. fluvescens, P. atrogularis* (Hatchwell 2005).

## Material and Methods

A total of 52 birds of 21 species and 6 families were captured and examined for the presence of haematozoan parasites using the PCR method of blood analysis. Microscopic examination of blood smears was only perfomed in genus *Prunella*.

*Study area*

Birds were captured at five sites using ornithological mist nets or ornithological clap traps.

*Site 1:* Karaburra pass N: 42° 12'11.62" E: 71° 36'41.13"

This site is located in West Tian-Shan in the Chatkal range at an altitude of 3000 m a.s.l. The terrain was rocky, with grazed alpine meadows and extensive screes sparsely covered with juniper. There was a small stream in the vicinity. Birds were captured in mist nets between August 26-28th 2008.

*Site 2:* Too-Ashu pass N: 42° 20'39.65" E: 73° 49'46.42"

This site is located in the Northern Tian-Shan range close to a major North-South traffic route. The area is covered with alpine meadows and heavily grazed. It is located in the vicinity of a small stream. Altitude is 3050 m a.s.l. Birds were captured with mist nets between August 23-24th 2008.

*Site 3:* Too-Ashu valley N: 42° 20'34.36" E: 73° 50'12.66"

This site is located approximately 300 m above Site 2 (3350 m a.s.l.) in rocky terrain with sparse vegetation in the vicinity of a small stream. Birds were captured between August 21-22nd 2008.

*Site 4:* Ak-Sai valley N: 42° 32' 4.76" E: 74° 31' 45.03"

This site is located in the Northern Tian-Shan range approximately 30 km north of the Kyrgyz capital Bishkek in Ala Archa National Park. Birds were captured with mistnets and fall-traps on two occasions between September 10-12 2008 in the vicinity of a climbing camping site at an altitude of 3370 m a.s.l. The site is located in the alpine zone with rocky terrain and sparse grass vegetation.

*Site 5*: Altyn-Arashan valley N: 42° 23' 20.95" E: 78° 35' 37.15"

This site is located at an altitude of 2500 m a.s.l. in the Central Tian-Shan range of the Altyn-Arashan valley in the Karakol province. The location was a grassland used for cattle grazing, covered with scattered shrubs and low trees. Birds were captured with mist nets on September 6, 2008.

*Field procedure and laboratory analysis*

After trapping was complete, standard morphometric measurements were taken for each individual. Adult birds were sexed. The blood was taken by puncture from the vena brachialis. Bleeding was stopped using pressure with a paper swab and thus a blood sample for DNA analysis was obtained. The blood smears were only made from birds of genus *Prunella*. These were further processed by staining according to Pappenheim (Doubek *et al.* 2003). The smears were examined microscopically under 1000× magnification for the presence of blood parasites.

Genomic DNA was extracted from dry blood spots on a paper swab (after blood collection) using the QIAamp DNA Mini Kit (Qiagen, Germany). PCR amplification was carried out according to previous studies (Bensch *et al.* 2000; Hellgren *et al.* 2004; Waldenström *et al.* 2004). Based on the sequence homology between aligned sequences of the blood parasites, initial primers were HaemNFI (5'-CATATATTAAGAGAAITATGGAG-3') and HaemNR3 (5'-ATAGAAAGATAAGAAATACCATTC-3') used to amplify parasite mitochondrial DNA (gene of the cytochrome b, 617 bp large fragment) from both genera of *Haemoproteus*, and *Leucocytozoon*. For the second PCR the following primers were used: for *Haemoproteus* spp. HaemF (5'-ATGGTGCTTTCGATATATGCATG-3') and HaemR2 (5'-GCATTATCTGGATGTGATAATGGT-3') to amplify a 480 bp large fragment, for Leucocytozoon spp. HaemFL (5'-ATGGTGTTTTAGATACTTACATT-3') and HaemR2L (5'-CATTATCTGGATGAGATAATG-3') to amplify a 478 bp large fragment. The first PCR (using HaemNFI–HaemNR3 primers) was performed in using 20 µl reaction volumes, which included 50 ng of total genomic DNA, 1x reaction buffer (15mM Tris-HCl, (pH 8.2 at 25° C) 30mM KCl, 5mM $(NH_4)_2SO_4$, 2.5 mM $MgCl_2$, 0.02 % BSA), 200 µM of each dNTP, 0.5 µM of each primer and 0.5 U DynaZyme DNA polymerase (Finnzymes OY). The PCR amplification protocols were as follows: initial denaturation 94° C for 10 min, then 20 cycles of 94° C for 30 s, 50° C for 30 s and 72° C for 1 min, finally 72° C 10 min.

The product of the first PCR was taken (2 µl) as the template for the second PCR, 2 µl for *Leucocytozoon* spp. (HaemFL-HaemR3L) and 2 µl for *Haemoproteus* spp. (HaemF-HaemR2). These PCR's were performed separately in 20 µl volumes with the same proportions of reagents as in the initial PCR reactions. The thermal profile of the PCR was identical to the initial PCR but performed for 35 cycles. The PCR products were shown on a 2 % agarose gel stained with ethidium bromide.

## Results

In the investigated birds the predominant parasite was *Haemoproteus* with 8 infected birds (15.7 %). The *Haemoproteus* was found in three birds species: *Emberiza buchanani*, *Luscinia megarhynchos* and *Serinus pusillus*. *Leucocytozoon* was detected in four birds (7.8 %) in the following species: *Motacilla cinerea*, *Phoenicurus caeruleocephala* and *Serinus pusillus*. *Serinus pusillus* was infected by both parasites. The investigetion of blood samples (including blood smears) confirmed our previous assumption of the absence of haemosporidian parasites in Prunellidae living in the alpine zone (Table 1).

## Discussion

Parasitism is a strong selective force in nature and significantly affects host fitness, thereby affecting its ability to survive and reproduce. The host-parasite relationship thus depends on ecology, behaviour and life history of both host and parasite (Vicente *et al.* 2007; Woodhams *et al.* 2008). More than 200 malarian parasites of the genera *Plasmodium*, *Haemoproteus* and *Leucocytozoon* have been morphologically described among the 4000 bird species investigated worldwide (Valkiunas 2005). The prevalence of blood parasites is highly dependent on diagnostic method, season, and region (John 1997). Presently, the polymerase chain reaction (PCR) is widely used to identify haematozoa parasites in bird blood (e.g. Križanauskiené *et al.* 2010; Bell *et al.* 2015; Ishtiaq *et al.* 2017; Chaisi *et al.* 2019). PCR methods are more sensitive than microscopy, but in the case of chronic infections because numbers of parasites are low, microscopy is still considered the „gold standard" for malaria diagnosis (Atkinson 2005). We chose the PCR method for easier handling and transport of samples. Our resultes based on PCR, confirm the appearance of parasites from genera *Haemoproteus* and *Leucocytozoon* in the study sites, all at an elevation of more than 2500 m a.s.l. We assume this is a pilot study on blood parasites in this region.

The family Fringillidae was classified as highly susceptible to infection by blood parasites (Atkinson and Van Riper 1991). Yakunin and Zhazyltaev (1977) report positive findings in the species

| **Birds species** | **n** | **Sex** | ***Haemoproteus*** | ***Leucocytozoon*** |
|---|---|---|---|---|
| | | **Male/Female/ Juvenile** | **Male/Female/ Juvenile** | **Male/Female/ Juvenile** |
| *Anthus trivialis* (*richardi*) | 1 | 0/1/0 | | |
| *Calliope pectoralis* | 1 | 0/0/1 | | |
| *Cardualis caniceps* | 1 | 0/1/0 | | |
| *Carpodacus* sp. | 1 | 0/1/0 | | |
| *Cinclus cinclus* | 1 | 0/1/0 | | |
| ***Emberiza buchanani*** | 2 | 2/0/0 | 1/0/0 | |
| ***Luscinia megarhynchos*** | 1 | 1/0/0 | 1/0/0 | |
| *Luscinia* sp. | 1 | 1/0/0 | | |
| *Luscinia svecica* | 1 | 0/1/0 | | |
| ***Motacilla cinerea*** | 1 | 1/0/0 | | 1/0/0 |
| *Motacilla personata* | 1 | 0/0/1 | | |
| ***Phoenicurus caeruleocephala*** | 4 | 1/3/0 | | 1/0/0 |
| *Phoenicurus erythrogaster* | 1 | 1/0/0 | | |
| *Phoenicurus ochruros* | 1 | 1/0/0 | | |
| *Phylloscopus inornatus* | 1 | NI | | |
| *Phylloscopus trochiloides* | 1 | 1/0/0 | | |
| *Prunella atrogularis* | 1 | 1/0/0 | | |
| *Prunella collaris rubida* | 10 | 6/3/1 | | |
| *Prunella fulvescens* | 5 | 2/3/1 | | |
| *Rhodospiza obsoleta* | 1 | 0/1/0 | | |
| *Saxicola torquata* | 2 | 1/0/1 | | |
| ***Serinus pusillus*** | 12 | 6/4/2 | 3/2/1 | 1/0/1 |
| **Total** | **51** | **25/19/7** | **5/2/1** | **3/0/1** |

**Table 1.** Occurrence of haematozoa in investigated passeriform birds from Tian-Shan (NI - unidentified sex).

*Emberiza buchanani* and *Motacilla cinerea* with same genera of parasites as the wild birds of Kazakhstan. However they did not record a positive finding in *Phoenicurus caeruleocephala* (Yakunin and Zhazyltaev 1977). The absense of Haemosporida in *Serinus pusillus* is documented by Nourani *et al.* (2018) from Iran.

There is a higher prevalence of infection by genus *Haemoproteus*, which is in contrast to studies that show a higher prevalence of genus *Leucocytozoon* at higher altitudes (Haas *et al.* 2012; Imura *et al.* 2012; van Rooyen *et al.* 2013; Lotta *et al.* 2015). This conclusion may be due to the fact that *Haemoproteus* had a high prevalence in *Serinus pusillus*, who was also infected with *Leucocytozoon*. A possible explanation for the presence of blood parasites is the ecology of the host species, which is an altitudinal migrant. Elevational migrants may hmave more parasites as they are exposed to high prevalence areas at low elevations with optimal climatic conditions for parasite transmission (Waldenström *et al.* 2002). They have also adapted physiologically to fluctuations in environmental hypoxia during elevation migraqtion and through changes in physiological parameters associated with blood oxygen-carrying capacity such as haemoglobin concentration and haematocrit (Ishtiaq and Barve 2018). Bird distribution in the high mountains is very variable. Some species strictly inhabit narrow altitudes during a specific period of the year e.g. during breeding, while others live at cold high elevations year-round (Price *et al.* 2011; Dixit *et al.* 2016). Elevational migrants, in the wintering grounds, encounter a diverse fauna of parasites as compared to sedentary species and may act as reservoirs for blood parasites.

The main aim of testing birds for the presence of blood parasites in this region was to detect haemosporidian infection in *Prunella collaris*. Our previous findings confirmed the absence of blood parasites in the species *P. collaris* from the High Tatras, Slovakia (Haas and Kisková 2010) as well as in individuals *P. collaris* from Rila Mountain, Bulgaria (2010; unpublished data). In Tian Shan, (Kyrgyzstan) we did not detect infection through the PCR method or microscopic examination in ten caught individuals. The infection was also not detected in other species of *Prunella* - *P. atrogularis* and *P. fulvescens*. The only species in this family where haemosporidian has been documented to date is *P. modularis*, which occurs at lower altitudes (e.g. Merino *et al.* 1997; Palinauskas *et al.* 2005; Hauptmanová *et al.* 2006; Haas *et al.* 2012). For the resident strongly high elevation adapted bird species, the key factor in the development of infection is the ambient temperature on which the haematophagous arthropod vectors depend (Dunn *et al.* 2013; Ishtiaq and Barve 2018). Higher elevation, low temperatures, fewer water reservoirs and windy conditions can help to reduce both the development of avian haematozoa and the abundance of parasite vectors, and hence parasite prevalence (Zamora-Vilchis *et al.* 2012). An alternative explanation for the absence of blood parasites could be either a low host density or insufficient time for the co-evolution of the host, vectors and parasites (Bennett *et al.* 1992; Rytkönen *et al.* 1996; Valera *et al.* 2003).

Variation in ambient temperature can increase or decrease host condition and parasite virulence can lead to a critical influence on the host-parasite interactions, even over relatively small temperature ranges (Thomas and Blanford 2003). In the context of climatic changes, it is presumably that with increasing temperatures of environments, parasites adapt to the biotic and abiotic conditions of the highlands (Gonzalez *et al.* 2015) which can effect vector distribution, leading to the emergence of infectious disease in the new hosts (Imura *et al.* 2012). Knowledge of how different environmental factors affect host-parasites as well as how different environmental factors affect host-parasite interactions will allow us to predict future parasite impacts and their potential effects on biodiversity.

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