

This is the peer reviewed version of the following article:

Hripsime A. Atoyán, Mariam Sargsyan, Hasmik Gevorgyan, Marko Raković, Igor Fadeev, Vahagn Muradyan, Ahmad Daryani, Mehdi Sharif, Sargis A. Aghayan. Determinants of avian malaria prevalence in mountainous Transcaucasia. in *Biologia* (2018): 73, 1123–1130.

doi: <https://doi.org/10.2478/s11756-018-0128-0>

This work is licensed under Creative Commons - Attribution-Noncommercial-No Derivative Works 4.0 International

## Determinants of avian malaria prevalence in mountainous Transcaucasia

Hripsime A. Atoyán, Mariam Sargsyan, Hasmik Gevorgyan, Marko Raković, Igor Fadeev, Vahagn Muradyan, Ahmad Daryani, Mehdi Sharif, Sargis A. Aghayan

### Abstract

Deforestation, urban development, and global climate change can lead to dramatic changes of ecological communities and increase prevalence of infectious diseases at higher latitudes and altitudes. Identification of factors responsible for the prevalence of parasites is of crucial importance to understand the dynamics of parasite distribution in a changing environment. Mountain areas are especially suitable for studies of factors governing parasite distribution and prevalence due to heterogeneity of landscapes, climatic regimes, and other biotic and abiotic conditions. We examined 903 avian blood smears collected in mountains of Transcaucasia for prevalence of *Haemoproteus* and *Plasmodium*. We found that the haemoparasites prevalence differed among bird species and localities, highlighting the environmental components affecting disease distribution. The prevalence of both *Haemoproteus* and *Plasmodium* was significantly higher in males, adults, and migratory species than in females, juveniles, and resident species. Geographic Information System (GIS) and linear regression analyses revealed that elevation and monthly average precipitation were strongly correlated with proportion of infected birds with *Plasmodium*, indicating that the prevalence increased with increase of monthly average temperature and elevation. Birds from forested and high grassed areas were also more infected with avian haemosporidia. Our study provides

baseline data for modelling of parasites distribution under global climate change scenarios, which is of great importance for monitoring and management of communities and environment for conservation and human health.

Keywords Avian malaria . Haemoproteus . Plasmodium . Prevalence determinants . Biotic and abiotic factors

## Introduction

Malaria and related Haemosporidia are highly virulent pathogens of a wide range of vertebrates and are especially diverse in birds (Valkiunas 1997). Avian Haemosporidia are key models for studying the dynamics of naturally occurring infections under different biotic and abiotic conditions, including environmental, behavioural etc. (Benning et al. 2002; Garamszegi 2011; Aghayan 2012). In spite of their importance, the processes responsible for acquisition, control and suppression of malaria infections remain poorly understood (Videvall et al. 2015). Our knowledge about the environmental factors affecting the distribution of avian Haemosporidia is especially limited (Illera et al. 2017).

Avian Haemosporidia are protozoans of the genera Plasmodium, Haemoproteus and Leucocytozoon which need intermediate avian hosts and arthropods to complete their life cycle. Many studies have examined this obligate relationships between blood parasites and their hosts such as condition or sexually selected traits and many aspects of the biology of Haemosporidia and their interactions with hosts (Bennett et al. 1980; Ricklefs et al. 2004; Fallon et al. 2005; Morand et al. 2006). This in turn provides a rich dataset to understand host-parasite interactions also touching evolution, biogeography and ecology of the parasites. Recently, it has been shown that interactions between host and parasites also vary among genera of parasites (Bensch et al. 2007; Drovetski et al. 2014), suggesting that genera can differently react to the same biotic and abiotic factors (Dawson and Bortolotti 1999; Illera et al. 2017).

Climatic variables were also reported to have an influence on distribution of haemosporidian parasites and their invertebrate vectors (Zhou et al. 2007; Paaijmans et al. 2010; Garrett et al. 2013; Ehrmann et al. 2017). Majority of studies on how climatic and other variables affect avian malaria distribution are related to single host species or communities inhabiting limited landscapes like forests, deserts etc.. However, studies on community level using high number of samples and more climatic variables are lacking (Laurance et al. 2013; Calero Riestra and García 2016; Marzal et al. 2016). Due to the heterogeneity of climatic and habitat conditions, mountains are well-suited systems to disentangle environmental factors driving distribution of parasites (Zamora-Vilchis et al. 2012; Meléndez et al. 2014).

Thus, the goal of the current study is to determine factors affecting prevalence of avian Haemoproteus and Plasmodium parasites using bird communities and mountainous landscapes of Transcaucasia. Our study area contains various landscapes including semi-desert to forests, subalpine belt and elevation varying from below 600 m up to 2600 m above sea level

(a.s.l.). This study area represents an ideal system to test the effect of different environmental variables on parasites' distribution (Illera et al. 2017; Padilla et al. 2017). We used microscopy method to investigate intensity and prevalence of avian Haemoproteus and Plasmodium in 903 individual birds sampled during the 2013–2014 breeding season. Microscopy was reported as an adequate technique to study distribution and prevalence of parasites (Valkiūnas et al. 2008). According to published literature climatic variables such as temperature, are better predictors of parasites distribution than type of the landscape. The latter is explained by climate playing a major role in vector abundance (Watts et al. 1987; Chaves et al. 2011; Pérez-Rodríguez et al. 2013).

Thus we want to improve knowledge on environmental biotic and abiotic predictors of parasites distribution using mountainous landscapes of Transcaucasia and avian Haemoproteus and Plasmodium as a model system.

## Materials and methods

### Sampling

We collected 903 blood samples of 90 avian species at 15 localities in the Lesser Caucasus (Armenian Highlands) in April–July 2013 and 2014 (Online resource 1). In our study, birds hatched in the current year were considered as juveniles and comparison was conducted between those and other individuals. Birds were captured with mist nets. Each mist net was opened for 2–3 days in the same place. Blood samples were obtained by brachial venipuncture with a sterile needle and collected using a heparin-free glass capillary. Then the blood was transferred onto a glass slide with smearing and air-dried in the field. Afterwards, we fixed the smears in 100% methanol by immersing the preparation in the alcohol for about 5 min (Valkiunas 1997).

We stained the smears with 10% Giemsa's stain in a special container. The staining was performed for 30 min at a temperature of approximately 25 °C. After the staining, the slides were washed with water, air-dried, and examined with a light microscope.

### Microscopy

We examined the smears first at low magnification ( $\times 400$ ). Positives were examined under higher magnification ( $\times 1000$ ) with an oil immersion objective (Valkiunas 1997). The intensity of parasitisation was calculated as the number of blood cells containing gametocytes of *Haemoproteus* spp. and *Plasmodium* spp. parasites per 10,000 cells. At least 50,000 cells for each bird were examined (Valkiūnas et al. 2008).

### Data analyses and GIS application

To determine how environmental biotic and abiotic factors affect avian malaria prevalence, we selected the following components which are the most suitable for mountainous landscapes (Muradyan et al. 2016; Illera et al. 2017): 1) relief and its morphological characteristics including elevation, slope and aspect, 2) climatic conditions, including average monthly precipitation and temperature, and 3) vegetation condition based on Normalized Difference Vegetation Index (NDVI).

Based on the digital elevation model (DEM) ([https:// earthexplorer.usgs.gov/](https://earthexplorer.usgs.gov/)) (EarthExplorer 2018), we obtained derivative thematic elevation, slope layers and aspects of the territory of Armenia using the ArcGIS 10.1 with 3D Analyst expansion module. Vegetation condition maps were obtained from Landsat OLI multispectral satellite image using NDVI (Muradyan et al. 2016). The NDVI is used for monitoring and assessing the vegetation condition (Kaufman and Tanre 1992). In general, NDVI values range from  $-1.0$  to  $1.0$ , with negative values indicating clouds and water, positive values near zero indicating bare soil, and higher positive values of NDVI ranges from sparse vegetation ( $0.1$ – $0.5$ ) to dense green vegetation ( $0.6$  and above). After this fixed range of densities ( $0.7$ ) increase of the volume of green vegetation slightly increases the value of NDVI (Milich and Weiss 2000). We used the temperature and precipitation data from Hydrometeorology and Monitoring State Service of Armenia SNPO, Ministry of Emergency Situations of the Republic of Armenia. With the input of GPS coordinates into GIS software the sampling points were set on the map and for each point the biotic and abiotic data were obtained (Table 1) and a GIS database was created. Collation between avian malaria prevalence and environmental components was done by a linear regression statistical method, which helped to establish correlation between these data. We used the GIS package ArcGIS 10.1, with expansion moduli 3D Analyst, Spatial Analyst (ESRI Inc.) for our spatial analysis.

Table 1. Biotic and abiotic factors values and prevalence of infections by avian haemosporidians for each locality.

| Locality    | Lat   | Lon   | Environmental biotic and abiotic factors |       |      |      |      |      | N   | Ninf | Prev | <i>Haemoproteus</i> |      | <i>Plasmodium</i> |      |
|-------------|-------|-------|--|-------|------|------|------|------|-----|------|------|---------------------|------|-------------------|------|
|             |       |       | Aspect                                   | Slope | Elev | NDVI | Prec | Temp |     |      |      | Ninf                | Prev | Ninf              | Prev |
| Agarak      | 38.86 | 46.21 | SE                                       | 3.2   | 580  | 0.05 | 61   | 14.5 | 4   | 1    | 25%  | 1                   | 25%  | 0                 | 0%   |
| Aknalich    | 40.14 | 44.17 | S  | 1.89  | 850  | 0.3  | 58   | 12.5 | 97  | 25   | 26%  | 23                  | 24%  | 5                 | 5%   |
| Armavir     | 40.16 | 44.05 | SE                                       | 0.95  | 880  | 0.1  | 62   | 11.3 | 49  | 9    | 18%  | 8                   | 16%  | 3                 | 6%   |
| Zuar        | 40.05 | 46.24 | E  | 14.46 | 1445 | 0.34 | –    | –    | 33  | 11   | 33%  | 10                  | 30%  | 0                 | 0%   |
| Kuchak      | 40.49 | 44.45 | W  | 3.48  | 1902 | 0.49 | 67   | 4.6  | 118 | 39   | 33%  | 35                  | 30%  | 6                 | 5%   |
| Lichk       | 40.17 | 45.24 | NE                                       | 0.66  | 1916 | 0.27 | 68   | 5.4  | 19  | 16   | 84%  | 16                  | 84%  | 3                 | 16%  |
| Meghri      | 38.9  | 46.22 | E  | 17.72 | 943  | 0.03 | 61   | 14.3 | 98  | 42   | 43%  | 41                  | 42%  | 4                 | 4%   |
| Mt.Aragats  | 40.41 | 44.25 | S  | 8.81  | 2398 | 0.29 | 74   | –2.6 | 3   | 2    | 67%  | 2                   | 67%  | 0                 | 0%   |
| Nerkin Hand | 39.06 | 46.52 | SE                                       | 10.44 | 707  | 0.26 | 72   | 12   | 250 | 102  | 41%  | 93                  | 37%  | 12                | 5%   |
| Tatev       | 39.4  | 46.24 | SE                                       | 19.81 | 1753 | 0.38 | 70   | 7.1  | 50  | 35   | 70%  | 40                  | 80%  | 9                 | 18%  |
| Tkhut       | 39.01 | 46.21 | SE                                       | 25.49 | 1450 | 0.48 | 68   | 6.8  | 27  | 18   | 67%  | 19                  | 70%  | 3                 | 11%  |
| Tsakhkadzor | 40.52 | 44.71 | NS                                       | 5.42  | 2003 | 0.42 | 72   | 5.4  | 15  | 4    | 27%  | 2                   | 13%  | 2                 | 13%  |
| Upper Kaler | 39.58 | 46.29 | N  | 18    | 2686 | 0.45 | 71   | 8.2  | 4   | 2    | 50%  | 2                   | 50%  | 0                 | 0%   |
| Vedi Hills  | 39.95 | 44.71 | SE                                       | 26.42 | 1126 | 0.07 | 60   | 12.4 | 29  | 14   | 48%  | 13                  | 45%  | 2                 | 7%   |
| Zikatar     | 41.13 | 44.91 | NS                                       | 16.2  | 1226 | 0.69 | 76   | 11.8 | 107 | 70   | 65%  | 73                  | 68%  | 18                | 17%  |
| Total       |       |       |  |       |      |      |      |      | 903 | 390  | 43%  | 378                 | 42%  | 67                | 7%   |

Lat, latitude; Lon, longitude; Elev, elevation; Prec, precipitation; Temp, temperature; N, number of birds; Ninf, number of infected birds; Prev, prevalence

## Results

In total, over 43% of the 903 tested birds were infected by avian *Haemoproteus* spp. (n = 378) and *Plasmodium* spp. (n = 87). Out of them, 75 samples appeared to carry parasites belonging to both genera. The overall prevalence varied greatly among sampled avian species ranging from no positives in *Troglodytes troglodytes* to 72.7% in *Sturnus vulgaris* and *Sylvia communis* (Table 2). The prevalence of *Haemoproteus* spp. separately was higher in *Sturnus vulgaris* (72.7%) and lower in *Troglodytes troglodytes* with no positives. Twelve species had no *Plasmodium* in blood smears (*Acrocephalus palustris*, *Chloris chloris*, *Erithacus rubecula*, *Hippolais languida*, *Hirundo rustica*, *Luscinia megarhynchos*, *Periparus ater*, *Remiz pendulinus*, *Sitta tephronota*, *Sturnus vulgaris*, *Sylvia borin*, and *Troglodytes troglodytes*), however, the most infected species was *Aegithalos caudatus* with prevalence of 16.7%. In the calculation only species with sample size higher than 9 individuals were considered to escape coincidence (Table 2).

Table 2. Prevalence of avian *Haemoproteus* and *Plasmodium* in individual bird species (sample size >9)

| Bird species   | N  | Ninf | Prev  | <i>Haemoproteus</i> |       | <i>Plasmodium</i> |       |
|--|----|------|-------|---------------------|-------|-------------------|-------|
|  |    |      |       | Ninf                | Prev  | Ninf              | Prev  |
| <i>Acrocephalus arundinaceus</i> (Linnaeus, 1758)      | 15 | 7    | 46.7% | 7                   | 46.7% | 2                 | 13.3% |
| <i>Acrocephalus palustris</i> (Bechstein, 1798)        | 21 | 11   | 52.4% | 11                  | 52.4% | 0                 | 0.0%  |
| <i>Aegithalos caudatus</i> (Linnaeus, 1758)            | 12 | 8    | 66.7% | 8                   | 66.7% | 2                 | 16.7% |
| <i>Anthus trivialis</i> (Linnaeus, 1758)               | 14 | 2    | 14.3% | 2                   | 14.3% | 1                 | 7.1%  |
| <i>Carduelis carduelis</i> (Linnaeus, 1758)            | 43 | 16   | 37.2% | 15                  | 34.9% | 3                 | 7.0%  |
| <i>Chloris chloris</i> (Linnaeus, 1758)                | 10 | 6    | 60.0% | 6                   | 60.0% | 0                 | 0.0%  |
| <i>Carpodacus erythrinus</i> (Pallas, 1770)            | 22 | 12   | 54.5% | 11                  | 50.0% | 3                 | 13.6% |
| <i>Cettia cetti</i> (Temminck, 1820)                   | 20 | 7    | 35.0% | 5                   | 25.0% | 2                 | 10.0% |
| <i>Cyanistes caeruleus</i> (Linnaeus, 1758)            | 23 | 5    | 21.7% | 5                   | 21.7% | 2                 | 8.7%  |
| <i>Dendrocopos major</i> (Linnaeus, 1758)              | 19 | 8    | 42.1% | 8                   | 42.1% | 1                 | 5.3%  |
| <i>Erithacus rubecula</i> (Linnaeus, 1758)             | 22 | 4    | 18.2% | 4                   | 18.2% | 0                 | 0.0%  |
| <i>Fringilla coelebs</i> (Linnaeus, 1758)              | 44 | 32   | 72.7% | 31                  | 70.5% | 3                 | 6.8%  |
| <i>Hippolais languida</i> (Hemprich & Ehrenberg, 1833) | 14 | 9    | 64.3% | 9                   | 64.3% | 0                 | 0.0%  |
| <i>Iduna pallida</i> (Hemprich & Ehrenberg, 1833)      | 14 | 8    | 57.1% | 7                   | 50.0% | 1                 | 7.1%  |
| <i>Hirundo rustica</i> (Linnaeus, 1758)                | 10 | 2    | 20.0% | 2                   | 20.0% | 0                 | 0.0%  |
| <i>Lanius collurio</i> (Linnaeus, 1758)                | 17 | 9    | 52.9% | 9                   | 52.9% | 1                 | 5.9%  |
| <i>Luscinia megarhynchos</i> (Brehm, CL, 1831)         | 10 | 7    | 70.0% | 7                   | 70.0% | 0                 | 0.0%  |
| <i>Periparus ater</i> (Linnaeus, 1758)                 | 12 | 3    | 25.0% | 3                   | 25.0% | 0                 | 0.0%  |
| <i>Parus major</i> (Linnaeus, 1758)                    | 93 | 28   | 30.1% | 26                  | 28.0% | 8                 | 8.6%  |
| <i>Passer domesticus</i> (Linnaeus, 1758)              | 24 | 11   | 45.8% | 10                  | 41.7% | 2                 | 8.3%  |
| <i>Passer montanus</i> (Linnaeus, 1758)                | 45 | 10   | 22.2% | 10                  | 22.2% | 2                 | 4.4%  |
| <i>Phylloscopus collybita</i> (Vieillot, 1817)         | 12 | 7    | 58.3% | 7                   | 58.3% | 1                 | 8.3%  |
| <i>Phylloscopus sindianus</i> (Brooks, WE, 1880)       | 26 | 10   | 38.5% | 9                   | 34.6% | 3                 | 11.5% |
| <i>Remiz pendulinus</i> (Linnaeus, 1758)               | 13 | 2    | 15.4% | 2                   | 15.4% | 0                 | 0.0%  |
| <i>Sitta europaea</i> (Linnaeus, 1758)                 | 10 | 3    | 30.0% | 2                   | 20.0% | 1                 | 10.0% |
| <i>Sitta tephronota</i> (Sharpe, 1872)                 | 11 | 6    | 54.5% | 6                   | 54.5% | 0                 | 0.0%  |
| <i>Sturnus vulgaris</i> (Linnaeus, 1758)               | 11 | 8    | 72.7% | 8                   | 72.7% | 0                 | 0.0%  |
| <i>Sylvia atricapilla</i> (Linnaeus, 1758)             | 26 | 17   | 65.4% | 16                  | 61.5% | 3                 | 11.5% |
| <i>Sylvia borin</i> (Boddaert, 1783)                   | 13 | 4    | 30.8% | 4                   | 30.8% | 0                 | 0.0%  |
| <i>Sylvia communis</i> (Latham, 1787)                  | 22 | 16   | 72.7% | 15                  | 68.2% | 2                 | 9.1%  |
| <i>Sylvia crassirostris</i> (Cretzschmar, 1830)        | 19 | 11   | 57.9% | 11                  | 57.9% | 3                 | 15.8% |
| <i>Sylvia curruca</i> (Linnaeus, 1758)                 | 16 | 8    | 50.0% | 8                   | 50.0% | 1                 | 6.3%  |
| <i>Troglodytes troglodytes</i> (Linnaeus, 1758)        | 13 | 0    | 0.0%  | 0                   | 0.0%  | 0                 | 0.0%  |
| <i>Turdus merula</i> (Linnaeus, 1758)                  | 33 | 17   | 51.5% | 16                  | 48.5% | 2                 | 6.1%  |

N, number of birds; Ninf, number of infected birds; Prev, prevalence

Our results show that males were more susceptible to avian haemosporidia than females ( $p = 0.01$ ). The prevalence was significantly higher in adults than in juveniles ( $p = 0.0001$ ). Additionally, Fisher Exact Probability Test also showed that migrants were more infected than resident birds ( $p = 0.01$ ) (Fig. 1).

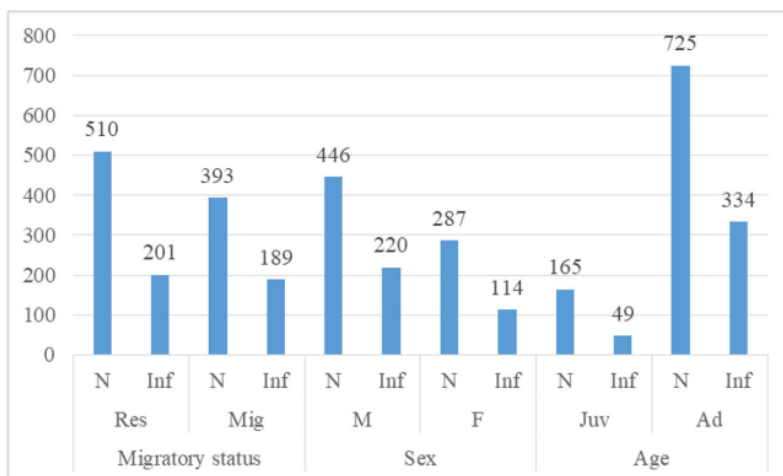


Fig. 1 Number of infected birds by host age, sex and migratory behaviour. N – number of birds, Inf – number of infected birds, Res – resident birds, Mig – migratory birds, M – male, F – female, Juv – juveniles, Ad – adults

The prevalence of avian haemosporidian parasites differed among localities (Table 1). Among localities with  $\geq 10$  samples, the lowest prevalence was observed in Armavir (18%;  $n = 9/49$ ) and the highest one in Tatev (80%;  $n = 40/50$ ). The highest prevalence of both *Haemoproteus* and *Plasmosium* was in Tatev (80% and 18%, respectively). The lowest prevalence of *Haemoproteus* was recorded in Armavir (16%) and no *Plasmodium* was reported from Zuar ( $n = 0/33$ ).

To better understand differences in prevalence of haemosporidia among localities we selected seven bird species (*Carduelis carduelis*, *Cettia cetti*, *Dendrocopos major*, *Fringilla coelebs*, *Parus major*, *Passer montanus* and *Turdus merula*) sampled in at least two localities with sample size greater than 10 individuals and from each locality having at least 6 blood samples. Utilizing Fisher exact test for  $2 \times 2$  and To highlight the abiotic and biotic factors contributing to  $2 \times 3$  tables, we found that in 3 of 7 species the prevalence was significantly varying among localities (Table 3). To highlight the abiotic and biotic factors contributing to avian malaria prevalence, we conducted GIS analyses and found that the prevalence of *Plasmodium* was significantly positively correlated with elevation and precipitation (Table 4). There was a marginally significant ( $0.05 < p < 0.1$ ) positive correlation between NDVI and aspect and *Plasmodium* prevalence. We have also observed high correlation ( $0.4 < r > 0.4$ ) between overall haemosporidian prevalence and precipitation and slope, *Haemoproteus* prevalence and slope, *Plasmodium* prevalence and temperature (negative correlation), but none of them was significant or marginally significant ( $p > 0.1$ ).

Table 3. Prevalence of avian haemosporidians in selected bird species (sample size  $>9$  and presented in  $>1$  localities and sample size in each locality  $>5$ ) from different localities

| Bird species               | Locality    | N  | Ninf | Prev | <i>p</i> values |
|----------------------------|-------------|----|------|------|-----------------|
| <i>Carduelis carduelis</i> | Armavir     | 7  | 0    | 0%   | 0.00078         |
|                            | Nekin Hand  | 11 | 9    | 82%  |                 |
|                            | Kuchak      | 21 | 6    | 29%  |                 |
| <i>Cettia cetti</i>        | Aknalich    | 13 | 1    | 8%   | 0.0012          |
|                            | Lichk       | 7  | 6    | 86%  |                 |
| <i>Dendrocopos major</i>   | Zikatar     | 7  | 2    | 29%  | 0.314           |
|                            | Nerkin Hand | 8  | 5    | 63%  |                 |
| <i>Fringilla coelebs</i>   | Zikatar     | 15 | 14   | 93%  | 0.0097          |
|                            | Nerkin Hand | 12 | 9    | 75%  |                 |
|                            | Kuchak      | 14 | 6    | 43%  |                 |
| <i>Parus major</i>         | Nerkin Hand | 52 | 15   | 29%  | 0.244           |
|                            | Zikatar     | 15 | 8    | 53%  |                 |
|                            | Kuchak      | 8  | 2    | 25%  |                 |
| <i>Passer montanus</i>     | Aknalich    | 22 | 7    | 32%  | 0.253           |
|                            | Armavir     | 16 | 2    | 13%  |                 |
| <i>Turdus merula</i>       | Kuchak      | 6  | 4    | 67%  | 0.872           |
|                            | Nerkin Hand | 12 | 7    | 58%  |                 |
|                            | Zikatar     | 7  | 5    | 71%  |                 |

*N*, number of birds; *Ninf*, number of infected birds; *Prev*, prevalence

Table 4. Correlations of environmental components and haemosporidian prevalence (only localities with sample size  $>5$  are included)

| Factors       | Overall prevalence |          | H prevalence |          | P prevalence |          |
|---------------|--------------------|----------|--------------|----------|--------------|----------|
|               | <i>r</i>           | <i>p</i> | <i>r</i>     | <i>p</i> | <i>r</i>     | <i>p</i> |
| Aspect        | 0.19               | 0.5701   | 0.12         | 0.7278   | 0.53         | 0.0964   |
| Slope         | 0.46               | 0.1572   | 0.45         | 0.1631   | 0.15         | 0.6548   |
| Elevation     | 0.37               | 0.2643   | 0.3          | 0.3649   | <b>0.61</b>  | 0.0461   |
| NDVI          | 0.33               | 0.3263   | 0.3          | 0.377    | 0.57         | 0.07     |
| Precipitation | 0.43               | 0.1841   | 0.37         | 0.2662   | <b>0.65</b>  | 0.0297   |
| Temperature   | -0.28              | 0.4106   | -0.23        | 0.5045   | -0.48        | 0.1338   |

Correlations marked in bold are significant at  $p < 0.05$

*H prevalence*, prevalence of *Haemoproteus*; *P prevalence*, prevalence of *Plasmodium*

## Discussion

Infections with avian haemoparasites cause disease in birds (Jarvi et al. 2003; DeGroot and Rodewald 2010; Podmokla et al. 2017), and sometimes they are lethal (Atkinson et al. 2000; Freed and Cann 2013; Asghar et al. 2015). During the past decade, the global climate change caused profound and complex changes in the prevalence or severity of infectious diseases (Altizer et al. 2013; Garrett et al. 2013), and it is highly important to determine factors contributing to prevalence of diseases (Padilla et al. 2017). The latter is more suitable to be investigated in the mountainous landscapes as they provide variety of habitats, climatic, and other environmental conditions (Illera et al. 2017).

Our results show significant role of sex, age and migratory behaviour of birds in the prevalence of infection with avian Plasmodium and Haemoproteus, particularly suggesting that prevalence is higher in males, adults and migrants than in females, juveniles and resident birds. Interestingly, all three mentioned factors were also shown to be related to prevalence of parasites (Bennett et al. 1980; Dunn et al. 2011; Jenkins et al. 2012; Isaksson et al. 2013; Karadjian et al. 2013; Rivera et al. 2013; Kulma et al. 2014; Calero-Riestra and García 2016; Anneti et al. 2017; Freeman-Gallant and Taff 2017). Bennett et al. (1980) stated that migratory birds carry more avian haemosporidian infections than native resident birds in Jamaica, but in contrast to our results they found identical prevalence in adult and young birds. In a microscopy-based study of Haemosporidia in the American kestrel (*Falco sparverius* Linnaeus, 1758), Dawson and Bortolotti (1999) showed for females a trend to have more mixed infections or double infection than males, however, they did not find significant differences in overall parasite prevalence between the sexes. The authors also found no difference in overall prevalence between young and adult birds, when young birds included individuals in their second year. In our study, birds of the first year were considered as juveniles and comparison was conducted between those and other individuals. In the same way Karadjian et al. (2013) by studying *Haemoproteus syrnii* in tawny owl (*Strix aluco* Linnaeus, 1758) from France showed 60% prevalence of haemosporidian infection in adults and 3% in juveniles, supporting our finding. Our results on juveniles being less infected could be explained by increasing exposure of aging hosts to vectors that transmit infections (Kataoka et al. 2017). Similar to our results, prevalence of infection was highly variable between species and sampling localities in other studies (Baker 1975; Drovetski et al. 2014; Soares et al. 2016; Ellis et al. 2017). To better describe the role of sex and age in prevalence of infection, more experimental studies with involvement of the same bird species inhabiting the same locality are required (Dimitrov et al. 2015; Matthews et al. 2016; Granthon and Williams 2017). The factors in a locality include components of the environment such as vector abundance (Svobodová et al. 2014), bioclimatic conditions (da Amaral et al. 2017) and their change (Fuller et al. 2012; Spurgin et al. 2012; Cornuault et al. 2013). Unfortunately, during the current study we have not obtained data on vector abundance in the study sites, but by utilizing GIS techniques we were able to test some environmental biotic and abiotic factors that affect the prevalence of the avian parasites. We found that prevalence of *Plasmodium* spp. was significantly varying with precipitation and elevation changes and showed marginally significant relationship to NDVI and aspect. The other tested correlations were not significant. Similarly to our investigation, Illera et al. (2017) studied factors determining prevalence and richness in avian haemosporidians (*Plasmodium*, *Haemoproteus* and *Leucocytozoon*) at the community level along elevation gradients in two mountain ranges located around the northern and southern limits of the Iberian Peninsula, Spain. Examining 1460 breeding birds of 68 species they demonstrated an important role of climatic and landscape variables in explaining prevalence and species spectrum of the avian parasites in the Iberian mountains. Particularly, they showed *Haemoproteus* affinities to woodland areas. This result was supported by Laurance et al. (2013) who stated that the prevalence of *Haemoproteus* was higher in continuous forests rather than in fragmented areas, which could be explained first of all by higher vector abundances (Zhou et al. 2007; Mangudo et al. 2017). The latter was shown also to be significantly dependent on elevation, vegetation and distance from water reservoirs (Zhou et al. 2007; Chaves et al. 2011; Liao et al. 2017). However, in contrast to vector abundance which is shown to decrease with higher altitude, our results indicate that the prevalence of avian haemoparasites was positively correlated with increasing elevation. This could be explained by the fact that animals were more

susceptible to infection by haemoparasites at lower temperature, oxidative stress and lower food availability (Álvarez-Ruiz et al. 2018), showing vulnerability of higher elevation inhabitants to parasites.

Our results highlight the predictors of avian Haemoproteus and Plasmodium prevalence in mountainous landscapes providing baseline for modelling distribution of the parasites under global warming scenarios (Gonzalez-Quevedo et al. 2014; Liao et al. 2017), which is of crucial importance from environmental, conservation and human health perspectives (Reiter and LaPointe 2007; Aghayan et al. 2013; Atkinson et al. 2014; Abella-Medrano et al. 2018).

**Acknowledgments** This work was made possible by a research grant from the Armenian National Science and Education Fund (ANSEF) based in New York, USA (grant number: zoo- 2983). We would like to thank Sergei V. Drovetski from Laboratories for Analytical Biology, National Museum of Natural History, Smithsonian Institution, Washington DC, USA for valuable contribution during field work and manuscript preparation. Fieldwork in Armenia for Marko Raković was supported by Natural History Museum of Belgrade grant “Ptice zapadnog Palearktika”.

## Compliance with ethical standards

**Ethical statement** All biomaterials (birds blood smears collected in 2013 and 2014) used in the study were collected under the permission from the Ministry of Nature Protection of Republic of Armenia given to the Scientific Center of Zoology and Hydroecology, Yerevan, Armenia.

**Conflict of interest** The authors declare that they have no conflict of interest.

## References

- Abella-Medrano CA, Ibáñez-Bernal S, Carbó-Ramírez P, SantiagoAlarcon D (2018) Blood-meal preferences and avian malaria detection in mosquitoes (Diptera : Culicidae) captured at different land use types within a neotropical montane cloud forest matrix. *Parasitol Int* 67:313–320. <https://doi.org/10.1016/j.parint.2018.01.006>
- Aghayan SA (2012) Migratory birds as a tool to colonize new territories for avian haemosporidians. *J Nat Sci* 1:20–24
- Aghayan SA, Hovhannisyan TS, Ghasabyan MG, Drovetski SV (2013) Preliminary report on avian malaria infection in breeding colony of lesser kestrel in Armenia. *Raptors Conserv* 26:96–99
- Altizer S, Ostfeld RS, Johnson PTJ, Kutz S, Harvell CD (2013) Climate change and infectious diseases: from evidence to a predictive framework. *Science* 341:514–519. <https://doi.org/10.1126/science.1239401>
- Álvarez-Ruiz L, Megía-Palma R, Reguera S, Ruiz S, Zamora-Camacho FJ, Figuerola J, Moreno-Rueda G (2018) Opposed elevational variation in prevalence and intensity of endoparasites and their vectors in a lizard. *Curr Zool* 64:197–204. <https://doi.org/10.1093/cz/zoy002>
- Anneti KL, Rivera NA, Andrews JE, Mateus-Pinilla N (2017) Survey of Haemosporidian parasites in resident and migrant game birds of Illinois. *J Fish Wildl Manag* 8:661–668. <https://doi.org/10.3996/082016-JFWM-059>
- Asghar M, Hasselquist D, Hansson B, Zehndjiev P, Westerdahl H, Bensch S (2015) Hidden costs of infection: chronic malaria accelerates telomere degradation and senescence in wild birds. *Science* 347:436–438. <https://doi.org/10.1126/science.1261121>
- Atkinson CT, Dusek RJ, Woods KL, Iko WM (2000) Pathogenicity of avian malaria in experimentally-infected Hawaii amakihi. *J Wildl Dis* 36:197–201. <https://doi.org/10.7589/0090-3558-36.2.197>
- Atkinson CT, Uzzurum RB, Lapointe DA, Camp RJ, Crampton LH, Foster JT, Giambelluca TW (2014) Changing climate and the altitudinal range of avian malaria in the Hawaiian islands - an ongoing conservation crisis on the island of Kaua’i. *Glob Chang Biol* 20: 2426–2436. <https://doi.org/10.1111/gcb.12535>
- Baker JR (1975) Epizootiology of some haematozoic protozoa of English birds. *J Nat Hist* 9:601–609. <https://doi.org/10.1080/00222937500770491>
- Bennett GF, Witt H, White EM (1980) Blood parasites in some Jamaican birds. *J Wildl Dis* 16:29–38. <https://doi.org/10.7589/0090-3558-16.1.29>
- Benning TL, LaPointe D, Atkinson CT, Vitousek PM (2002) Interactions of climate change with biological invasions and land use in the Hawaiian islands: modeling the fate of endemic birds using a geographic information system. *Proc Natl Acad Sci* 99:14246–14249. <https://doi.org/10.1073/pnas.162372399>
- Bensch S, Waldenström J, Jonzen N, Westerdahl H, Hansson B, Sejberg D, Hasselquist D (2007) Temporal dynamics and diversity of avian malaria parasites in a single host species. *J Anim Ecol* 76:112–122. <https://doi.org/10.1111/j.1365-2656.2006.01176.x>
- Calero-Riestra M, García JT (2016) Sex-dependent differences in avian malaria prevalence and consequences of infections on nestling growth and adult condition in the tawny pipit, *Anthus campestris*. *Malar J* 15:178. <https://doi.org/10.1186/s12936-016-1220-y>



- Chaves LF, Hamer GL, Walker ED, Brown WM, Ruiz MO, Kitron UD (2011) Climatic variability and landscape heterogeneity impact urban mosquito diversity and vector abundance and infection. *Ecosphere* 2:art70. <https://doi.org/10.1890/ES11-00088.1>
- Cornuault J, Khimoun A, Harrigan RJ, Bourgeois YXC, Milá B, Thébaud C, Heeb P (2013) The role of ecology in the geographical separation of blood parasites infecting an insular bird. *J Biogeogr* 40:1313–1323. <https://doi.org/10.1111/jbi.12098>
- da Amaral HLC, Bergmann FB, dos Santos PRS, Silveira T, Krüger RF (2017) How do seasonality and host traits influence the distribution patterns of parasites on juveniles and adults of *Columba livia*? *Acta Trop* 176:305–310. <https://doi.org/10.1016/j.actatropica.2017.08.023>
- Dawson RD, Bortolotti GR (1999) Prevalence and intensity of hematozoan infections in a population of American kestrels. *Can J Zool* 77: 162–170. <https://doi.org/10.1139/z98-206>
- DeGroot LW, Rodewald PG (2010) Blood parasites in migrating woodwarblers (Parulidae): effects on refueling, energetic condition, and migration timing. *J Avian Biol* 41:147–153. <https://doi.org/10.1111/j.1600-048X.2009.04782.x>
- Dimitrov D, Palinauskas V, Iezhova TA, Bernotienė R, Ilgūnas M, Bukauskaitė D, Zehntindjiev P, Ilieva M, Shapoval AP, Bolshakov CV, Markovets MY, Bensch S, Valkiūnas G (2015) *Plasmodium* spp: an experimental study on vertebrate host susceptibility to avian malaria. *Exp Parasitol* 148:1–16. <https://doi.org/10.1016/J.EXPPARA.2014.11.005>
- Drovetski SV, Aghayan SA, Mata VA, Lopes RJ, Mode NA, Harvey JA, Voelker G (2014) Does the niche breadth or trade-off hypothesis explain the abundance-occupancy relationship in avian Haemosporidia? *Mol Ecol* 23:3322–3329. <https://doi.org/10.1111/mec.12744>
- Dunn JC, Cole EF, Quinn JL (2011) Personality and parasites: sex-dependent associations between avian malaria infection and multiple behavioural traits. *Behav Ecol Sociobiol* 65:1459–1471. <https://doi.org/10.1007/s00265-011-1156-8>
- Ehrmann S, Liira J, Gärtner S, Hansen K, Brunet J, Cousins SAO, Deconchat M, Decocq G, De Frenne P, De Smedt P, Diekmann M, Gallet-Moron E, Kolb A, Lenoir J, Lindgren J, Naaf T, Paal T, Valdés A, Verheyen K, Wulf M, Scherer-Lorenzen M (2017) Environmental drivers of *Ixodes ricinus* abundance in forest fragments of rural European landscapes. *BMC Ecol* 17:31. <https://doi.org/10.1186/s12898-017-0141-0>
- Ellis VA, Medeiros MCI, Collins MD, Sari EHR, Coffey ED, Dickerson RC, Lugarini C, Stratford JA, Henry DR, Merrill L, Matthews AE, Hanson AA, Roberts JR, Joyce M, Kunkel MR, Ricklefs RE (2017) Prevalence of avian haemosporidian parasites is positively related to the abundance of host species at multiple sites within a region. *Parasitol Res* 116:73–80. <https://doi.org/10.1007/s00436-0165263-3>
- Fallon SM, Bermingham E, Ricklefs RE (2005) Host specialization and geographic localization of avian malaria parasites: a regional analysis in the Lesser Antilles. *Am Nat* 165:466–480. <https://doi.org/10.1086/428430>
- Freed L, Cann R (2013) Vector movement underlies avian malaria at upper elevation in Hawaii: implications for transmission of human malaria. *Parasitol Res* 112:3887–3895. <https://doi.org/10.1007/s00436-013-3578-x>
- Freeman-Gallant CR, Taff CC (2017) Age-specific patterns of infection with haemosporidians and trypanosomes in a warbler: implications for sexual selection. *Oecologia* 184:813–823. <https://doi.org/10.1007/s00442-017-3919-z>
- Fuller T, Bensch S, Müller I, Novembre J, Pérez-Tris J, Ricklefs RE, Smith TB, Waldenström J (2012) The ecology of emerging infectious diseases in migratory birds: an assessment of the role of climate change and priorities for future research. *Ecohealth* 9:80–88. <https://doi.org/10.1007/s10393-012-0750-1>
- Garamszegi LZ (2011) Climate change increases the risk of malaria in birds. *Glob Chang Biol* 17:1751–1759. <https://doi.org/10.1111/j.1365-2486.2010.02346.x>
- Garrett KA, Dobson ADM, Kroschel J, Natarajan B, Orlandini S, Tonnang HEZ, Valdivia C (2013) The effects of climate variability and the color of weather time series on agricultural diseases and pests, and on decisions for their management. *Agric For Meteorol* 170:216–227. <https://doi.org/10.1016/j.agrformet.2012.04.018>
- Gonzalez-Quevedo C, Davies RG, Richardson DS (2014) Predictors of malaria infection in a wild bird population: landscape-level analyses reveal climatic and anthropogenic factors. *J Anim Ecol* 83:1091–1102. <https://doi.org/10.1111/1365-2656.12214>
- Granthon C, Williams DA (2017) Avian malaria, body condition, and blood parameters in four species of songbirds. *Wilson J Ornithol* 129:492–508. <https://doi.org/10.1676/16-060.1>
- Illera JC, López G, García-Padilla L, Moreno Á (2017) Factors governing the prevalence and richness of avian haemosporidian communities within and between temperate mountains. *PLoS One* 12:e0184587. <https://doi.org/10.1371/journal.pone.0184587>
- Isaksson C, Sepil I, Baramidze V, Sheldon BC (2013) Explaining variance of avian malaria infection in the wild: the importance of host density, habitat, individual life-history and oxidative stress. *BMC Ecol* 13:15. <https://doi.org/10.1186/1472-6785-13-15>
- Jarvi SI, Farias MEM, Baker H, Freifeld HB, Baker PE, Van Gelder E, Massey JG & Atkinson CT (2003) Detection of avian malaria (*Plasmodium* spp) in native land birds of American Samoa. *Conserv Genet* 4:629–637. <https://doi.org/10.1023/a:1025626529806>
- Jenkins T, Thomas GH, Hellgren O, Owens IPF (2012) Migratory behavior of birds affects their coevolutionary relationship with blood parasites. *Evolution* 66:740–751. <https://doi.org/10.1111/j.1558-5646.2011.01470.x>
- Karadjian G, Puech M-P, Duval L, Chavatte J-M, Snounou G, Landau I (2013) *Haemoproteus symii* in *Strix aluco* from France: morphology, stages of sporogony in a hippoboscoid fly, molecular characterization and discussion on the identification of *Haemoproteus* species. *Parasite* 20:32. <https://doi.org/10.1051/parasite/2013031>
- Kataoka H, Nakano J, Kondo Y, Honda Y, Sakamoto J, Origuchi T, Okita M (2017) The influence of aging on the effectiveness of heat stress in preventing disuse muscle atrophy. *Physiol Int* 104:316–328. <https://doi.org/10.1556/2060.104.2017.4.1>

- Kaufman Y, Tanre D (1992) Atmospherically resistant vegetation index (ARVI) for EOS-MODIS. *IEEE Trans Geosci Remote Sens* 30:261–270. <https://doi.org/10.1109/36.134076>
- Kulma K, Low M, Bensch S, Qvarnström A (2014) Malaria-infected female collared flycatchers (*Ficedula albicollis*) do not pay the cost of late breeding. *PLoS One* 9:e85822. <https://doi.org/10.1371/journal.pone.0085822>
- Laurance SGW, Jones D, Westcott D, Mckeown A, Harrington G, Hilbert DW (2013) Habitat fragmentation and ecological traits influence the prevalence of avian blood parasites in a tropical rainforest landscape. *PLoS One* 8:e76227. <https://doi.org/10.1371/journal.pone.0076227>
- Liao W, Atkinson CT, LaPointe DA, Samuel MD (2017) Mitigating future avian malaria threats to Hawaiian forest birds from climate change. *PLoS One* 12:e0168880. <https://doi.org/10.1371/journal.pone.0168880>
- Mangudo C, Aparicio JP, Rossi GC, Gleiser RM (2017) Tree hole mosquito species composition and relative abundances differ between urban and adjacent forest habitats in northwestern Argentina. *Bull Entomol Res* 108:203–212. <https://doi.org/10.1017/S0007485317000700>
- Marzal A, Balbontín J, Reviriego M, García-Longoria L, Relinque C, Hermosell IG, Magallanes S, López-Calderón C, de Lope F, Møller AP (2016) A longitudinal study of age-related changes in Haemoproteus infection in a passerine bird. *Oikos* 125:1092–1099. <https://doi.org/10.1111/oik.02778>
- Matthews AE, Ellis VA, Hanson AA, Roberts JR, Ricklefs RE, Collins MD (2016) Avian haemosporidian prevalence and its relationship to host life histories in eastern Tennessee. *J Ornithol* 157:533–548. <https://doi.org/10.1007/s10336-015-1298-y>
- Meléndez L, Laiolo P, Mironov S, García M, Magaña O, Jovani R (2014) Climate-driven variation in the intensity of a host-symbiont animal interaction along a broad elevation gradient. *PLoS One* 9:e101942. <https://doi.org/10.1371/journal.pone.0101942>
- Milich L, Weiss E (2000) GAC NDVI interannual coefficient of variation (CoV) images: ground truth sampling of the Sahel along north-south transects. *Int J Remote Sens* 21:235–260. <https://doi.org/10.1080/014311600210812>
- Morand S, Krasnov B, Poulin R (2006) *Micromammals and macroparasites*. Springer-Verlag, Tokyo. <https://doi.org/10.1007/978-4-431-36025-4>
- Muradyan VS, Asmaryan SG, Saghatelian AK (2016) Assessment of space and time changes of NDVI (biomass) in Armenia's mountain ecosystems using remote sensing data. *Curr Probl Remote Sens Earth from Sp* 13:49–60. <https://doi.org/10.21046/2070-74012016-13-1-49-60>
- Paaijmans KP, Blanford S, Bell AS, Blanford JI, Read AF, Thomas MB (2010) Influence of climate on malaria transmission depends on daily temperature variation. *Proc Natl Acad Sci U S A* 107:15135–15139. <https://doi.org/10.1073/pnas.1006422107>
- Padilla DP, Illera JC, Gonzalez-Quevedo C, Villalba M, Richardson DS (2017) Factors affecting the distribution of haemosporidian parasites within an oceanic island. *Int J Parasitol* 47:225–235. <https://doi.org/10.1016/j.ijpara.2016.11.008>
- Pérez-Rodríguez A, Fernández-González S, de la Hera I & Pérez-Tris J (2013) Finding the appropriate variables to model the distribution of vector-borne parasites with different environmental preferences: climate is not enough. *Glob Chang Biol* 19:3245–3253. <https://doi.org/10.1111/gcb.12226>
- Podmokła E, Dubiec A, Drobniak SM, Sudyka J, Krupski A, Arct A, Gustafsson L, Cichoń M (2017) Effect of haemosporidian infections on host survival and recapture rate in the blue tit. *J Avian Biol* 48:796–803. <https://doi.org/10.1111/jav.01108>
- Reiter ME, LaPointe DA (2007) Landscape factors influencing the spatial distribution and abundance of mosquito vector *Culex quinquefasciatus* (Diptera: Culicidae) in a mixed residential/agricultural community in Hawai'i. *J Med Entomol* 44:861–868. <https://doi.org/10.1093/jmedent/44.5.861>
- Ricklefs RE, Fallon SM, Bermingham E (2004) Evolutionary relationships, cospeciation, and host switching in avian malaria parasites. *Syst Biol* 53:111–119. <https://doi.org/10.2307/4135399>
- Rivera J, Barba E, Mestre A, Rueda J, Sasa M, Vera P, Monrós JS (2013) Effects of migratory status and habitat on the prevalence and intensity of infection by haemoparasites in passerines in eastern Spain. *Anim Biodivers Conserv* 36:113–122
- Soares L, Escudero G, Penha VAS, Ricklefs RE (2016) Low prevalence of haemosporidian parasites in shorebirds. *Ardea* 104:129–141. <https://doi.org/10.5253/arde.v104i2.a8>
- Spurgin LG, Illera JC, Padilla DP, Richardson DS (2012) Biogeographical patterns and co-occurrence of pathogenic infection across island populations of Berthelot's pipit (*Anthus berthelotii*). *Oecologia* 168:691–701. <https://doi.org/10.1007/s00442-0112149-z>
- Svobodová M, Weidinger K, Peške L, Volf P, Votýpka J, Voříšek P (2014) Trypanosomes and haemosporidia in the buzzard (*Buteo buteo*) and sparrowhawk (*Accipiter nisus*): factors affecting the prevalence of parasites. *Parasitol Res* 114:551–560. <https://doi.org/10.1007/s00436-014-4217-x>
- Valkiunas G (1997) *Bird Haemosporida*. Acta Zool Lith 3–5 (Monography)
- Valkiunas G, Iezhova TA, Križanauskienė A, Palinauskas V, Sehgal RNM, Bensch S (2008) A comparative analysis of microscopy and PCR-based detection methods for blood parasites. *J Parasitol* 94:1395–1401. <https://doi.org/10.1645/GE-1570.1>
- Videvall E, Cornwallis CK, Palinauskas V, Valkiunas G, Hellgren O (2015) The avian transcriptome response to malaria infection. *Mol Biol Evol* 32:1255–1267. <https://doi.org/10.1093/molbev/msv016>

- Watts DM, Burke DS, Harrison BA, Whitmire RE, Nisalak A (1987) Effect of temperature on the vector efficiency of *Aedes aegypti* for dengue2 virus. *Am J Trop Med Hyg* 36:143–152.<https://doi.org/10.1139/b89-069>
- Zamora-Vilchis I, Williams SE, Johnson CN (2012) Environmental temperature affects prevalence of blood parasites of birds on an elevation gradient: implications for disease in a warming climate. *PLoS One* 7:e39208.  
<https://doi.org/10.1371/journal.pone.0039208>
- Zhou G, Munga S, Minakawa N, Githeko AK, Yan G (2007) Spatial relationship between adult malaria vector abundance and environmental factors in western Kenya highlands. *Am J Trop Med Hyg* 77: 29–35 Available at: <https://earthexplorer.usgs.gov/>