CARN ASPIRE Grant 2023 Application

COVER PAGE (1-page maximum)

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Date of birth*: Jan 1, 1989

Gender*: Female

Nationality*: Cameroon

Affiliation* University of Dschang

Current Position or Job Title* Student

Project title* Haemosporidian parasites of insectivorous pest-eating birds in Cameroonian cocoa farms

Project summary* Previous Research in Cameroon has shown evidence that 12 species of insectivorous birds eat brown capsids (Africa's #1 cocoa pest), and can be used for pest biological control in coca farms. However, these species are subject to haemosporidian parasites infections *(Plasmodium, Haemoproteus and Leucocytozoon spp.)* identified as a potential cause of extinction and population decline in many bird species. Better understanding the pathogenicity of avian haemosporidiosis might ultimately contribute to improvement of health and wildlife conservation policies.

Requested funding* $5000

Example application is adapted from 2022 awardee Mélanie Adèle Tchoumbou's project:
https://www.conservationactionresearch.net/projects/which-native-shade-trees-will-attract-pest-eating-birds-to-cameroonian-cocoa-farms

PROJECT PROPOSAL

Please provide a detailed description of the proposed project as described below.

Background and Rationale*

Chocolate is among resources highly required worldwide and the chocolate market heavily depends on the production of cocoa, about 70% of which is cultivated in Africa. In Cameroon, previous studies integrating community field data and DNA metabarcoding provides proof that 12 species of birds (*Alethe castanea, Bleda notatus, Elminia longicauda, Camaroptera brachyura, Ispidina lecontei, Hylia prasina, Phyllastrephus sp., Criniger sp., Diaphorophyia castanea, Ispidina picta, Muscicapa comitata, Terpsiphone sp., Rhinolophus alcyone, Hipposideros ruber, Hipposideros cyclops, Nycteris sp., Nycteris grandis* and *Hypposideros sp*.) consume different cocoa pests, including considerable numbers of Brown Capsids (*Sahlbergella singularis*), the most destructive cocoa pest on Earth (Babin *et a*l., 2011).

It is obvious that birds provide substantial benefit for crop production through their ecosystem services and can be used for pest's biological control in cocoa farms. However, over 68% of bird species are susceptible to haemosporidian parasites (*Haemoproteus, Plasmodium* and *Leucocytozoon spp*.) known to reduce the fitness of the hosts and in some cases lead to death (Cannell e*t al*., 2013; Paxton et al., 2016). Recent histopathology findings showed that haemoproteids can cause severe and even lethal avian diseases due to damage of various internal organs by exo-erythrocytic stages (Duc et al., 2021; Himmel et al., 2021). Their effect on bird eating pests in Cameroonian cocoa farms remains unknown. Better understanding the pathogenesis of avian haemosporidiosis in insectivorous birds might ultimately contribute to improvement of health and wildlife conservation policies.

The main objective of this project is to assess the pathogenicity of haemosporidian parasites in insectivorous pest-eating birds in Cameroonian cocoa farms. Specifically, we will determine 1) prevalence and intensity of haemosporidian parasites in the targeted species, 2) seasonal variation in parasite prevalence and intensity, and 3) risk factors associated to parasite prevalence.

Goal and objectives*

The main goal of this project is to improve the conservation success of birds in Cameroonian cocoa farms. Given that one of the biggest challenges for the current conservation effort is lack of knowledge about how habitat choice and disease prevalence impacts existing bird populations, this project seeks to assess the differences in infected bird numbers across cocoa farms. We will focus on three main objectives: (1) prevalence and intensity of haemosporidian parasites in the targeted species (2) seasonal variation in parasite prevalence and intensity, and (3) risk factors associated to parasite prevalence.

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Methods*

Study area

The field work will take place in 10 cocoa farms in the west region of Cameroon. Cocoa cultivation is among the main economic activities in this part of the country. The targeted area is located between 5°160N and 9°580E. The annual average temperature is 22.5 °C., and relative humidity is 92%. Its climate is equatorial of the Guinean type and has four unequal seasons, namely: the long rainy season which runs from mid-August – October ; the small rainy season (from March to June); the great dry season (mid-October to March) and the short dry season (June – mid-August). Laboratory analyses of bird blood will be carried out in a laboratory of the 6 Applied Biology and Ecology Research Unit, Department of Animal Biology, University of Dschang.

Materials and methods

Two weeks field research will be conducted during each season from December 2023 to January 2024. Targeted birds will be captured in each cocoa plantation using 10 mist nets (12 m long, 4 shelves, 2.6 m high, 30 mm mesh) set in parallel and perpendicularly with sampling effort of 6 h per day (from 6 a.m. to 12 p.m.). Opened nets will be checked every 15 min, and all captured birds will be identified using standard reference (Borrow and Demey, 2014). Birds will then be weighed using a Pesola scale of 100 and 1000 g; measured using a manual caliper of with precision of 0.05; banded with numbered rings; sampled for blood and then released, after bleeding had stopped. The date, plantation sites, GPS coordinates, common and scientific names of birds, families, band numbers and other related information will be noted at for each targeted birds.

Same day recaptures identified through leg bands will not be included in the study. Blood samples from all captured birds will be collected by venipuncture from the brachial vein. Immediately following blood collection, two thin blood films will be quickly prepared, fixed in absolute methanol for at least 1 min, air-dried and packed into slide boxes for subsequent staining in the laboratory. Once in the laboratory, all the blood films will be stained for 1 h with Giemsa diluted in 1/10 with phosphate buffer (obtained by dissolving 1g of Na2HPO4 and 0.7g of KH2PO4 in 1 liter of distilled water), and rinsed in tap water. After staining, blood films will be air dried and examined at high magnification (X100) under a light microscope using immersion oil. Morphometric features and parasites identification will be made according to Valkiunas (2004).

Statistical analyses

The prevalence of parasite will be determined as the number of infected birds over the total number of sampled birds. Intensity of infections will be estimated as a percentage by actual counting of the number of parasites per 1000 red blood cells for heavy infections (>1 parasites per microscopic field) or per 10 000 red blood cells for light infections (<1 parasites per

microscopic field) (Godfrey et al., 1987). Seasonal effect on the prevalence and intensity of parasite will be assessed using chi-square test, while the variation of parasite prevalence among risk factors will be performed using Kruskal-wallis test.

Indicators and Outputs*

To communicate the results of this study, we will prepare a paper to be submitted to peer review publication journals such as Nature and Science. We will also prepare a one-pager for and work directly with the Cameroonian Parks Service to share our findings so that they are able to incorporate new information about the prevalence of the parasite into upcoming conservation policies. Finally, should the prevalence of infected birds appear extensive (>80% infection rate), we will prepare a flyer that can be used to communicate the impact of the pest to cocoa farmers in the region, as well as provide suggestions for how to manage the pest prevalence in a way that does not interfere significantly with crop production or other conservation goals.

Who will benefit?*

Beneficiaries will include cocoa farmers in the region. From our previous research, we have existing relationships with 10 cocoa farms in the west region of Cameroon (these are the same locations at which we will conduct the research for this project). These farmers see the value of preserving bird species as a means of biological pest control for their cocoa crops and have thus indicated their interest in participating in the conservation effort. Additionally, they have existing positive relationships with other farmers not directly included in our study, and have indicated willingness to share their learnings and/or connect us in the future.

Preliminary results*

From our previous research using fecal metabarcoding, we identified many species of insectivorous bird that eat brown capsids (Africa's#1 cocoa pest), and other pests in Cameroonian cocoa farms. These species are important both biologically and economically—by identifying the birds' role as biological pest control, our work emphasizes that they are of conservation priority. However, the status of these species as connected to common global avian pathogens remains unknown, thus limiting possible conservation initiatives. The CARN funding would help address this important gap.

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Timeline*

Activity	Time Period
Finalizing field sites, preparing travel, ordering necessary materials	December 2022
Monitoring of bird and data collection	January 2023-October 2023
Data analysis and results	November 2023-December 2023

Figure and Table
Not applicable

References*
Babin R, Anikwe JC, Dibog L, Lumaret J-P (2011) Effects of cocoa tree phenology and canopy microclimate on the performance of the mirid bug Sahlbergella singularis: Two factors affecting cocoa mirid performance. Entomol Exp Appl 141:25–34. https://doi.org/10.1111/j.1570-7458.2011.01164.x

Borrow, N., & Demey, R. (2014). *Birds of Western Africa* (0th ed.). Princeton University Press.

Duc M, Ilgūnas M, Kubiliūnaitė M, Valkiūnas G. First Report of *Haemoproteus* (Haemosporida, Haemoproteidae) Megalomeronts in the Brain of an Avian Host, with Description of Megalomerogony of *Haemoproteus Pastoris*, the Blood Parasite of the Common Starling. Animals (Basel). 2021 Sep 27;11(10):2824. doi: 10.3390/ani11102824. PMID: 34679845; PMCID: PMC8532782.

Godfrey, R. D., Jr., A. M. Fedynich, AND D. B. Pence. (1987). Quantification of hematozoa in blood smears. Journal of Wildlife Diseases 23: 558-565.

Himmel T, Harl J, Matt J, Weissenböck H. A citizen science-based survey of avian mortality focusing on haemosporidian infections in wild passerine birds. Malar J. 2021 Oct 23;20(1):417. doi: 10.1186/s12936-021-03949-y. PMID: 34688278; PMCID: PMC8542282.

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BUDGET

Budget*

Category	Items	Unit Cost (USD)	Quantity	Total Price (USD)
Field Equipment: bird capture & blood sampling	Mist net	50	10	500
	GPS	140	1	140
	Compass	15	1	15
	Bird rings	1.99	15	29.85
	Slides	3.25	15	48.75
	2.5 mL syringes	9.99	15	149.85
	Slides storage box	12.99	15	194.85
Lab Equipment: pathogen screening	Buffer solution	21.77	2	43.54
	Immersion oil	24.99	1	24.99
	Giemsa	19.75	1	19.75
	Methanol	19.99	2	39.98
Personnel & Administrative costs	Accommodation (per night)	22.99	56	1287.44
	Transport	12	56	672
	Food (Per day)	17	56	952
	Administrative budget	32	1	32
	Health	100	1	100
	Field assistant & local facilitator payment	150	5	750
Total Cost				**5000**

Budget Justification*

Category	Items	Justification
Field Equipment: bird capture & blood sampling	Mist net & bird rings	Capture birds for sampling and avoid double-counting
	GPS & compass	ensure save navigation between field sites and record field location data
	Slides, syringes, slides storage box	draw and store blood samples
Lab Equipment: pathogen screening	Methanol	fix red blood cells before staining
	Giemsa & buffer solution	Immersion oil to stain blood samples, buffer solution to dilute the stain
	Immersion oil	Needed to observe the slides under a microscope
Personnel & Administrative costs	Accommodation (per night)	covers the lodging for 4 team members to stay near the field site during field trips (4 trips, 14 days each = 56 total days)
	Transport	includes petrol and taxi costs to move between field sites and field sites and home
	Food (Per day)	cover the meals of the entire team during the 4 2 week field trip periods
	Administrative budget	books, journals, publications, photocopies, printing, internet and other office consumables.
	Health	Covers purchase of first aid medical box for prophylaxis and therapy to take to the field
	Field assistant & local facilitator payment	Two field assistants will be needed to help collecting data in the field, and one local facilitator from each collection region will be recruited

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CURRICULUM VITAE (CV) or Resume * (2 pages maximum)

CURRICULUM VITAE

Name: Mélanie
Position:
Date of Birth:
Email:
Telephone:

EDUCATION

SEMINAR AND WORKSHOP

WORK EXPERIENCES

GRANTS

3

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PUBLICATIONS

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LANGUAGE PROFICIENCY

REFERENCES

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LETTERS OF RECOMMENDATION

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