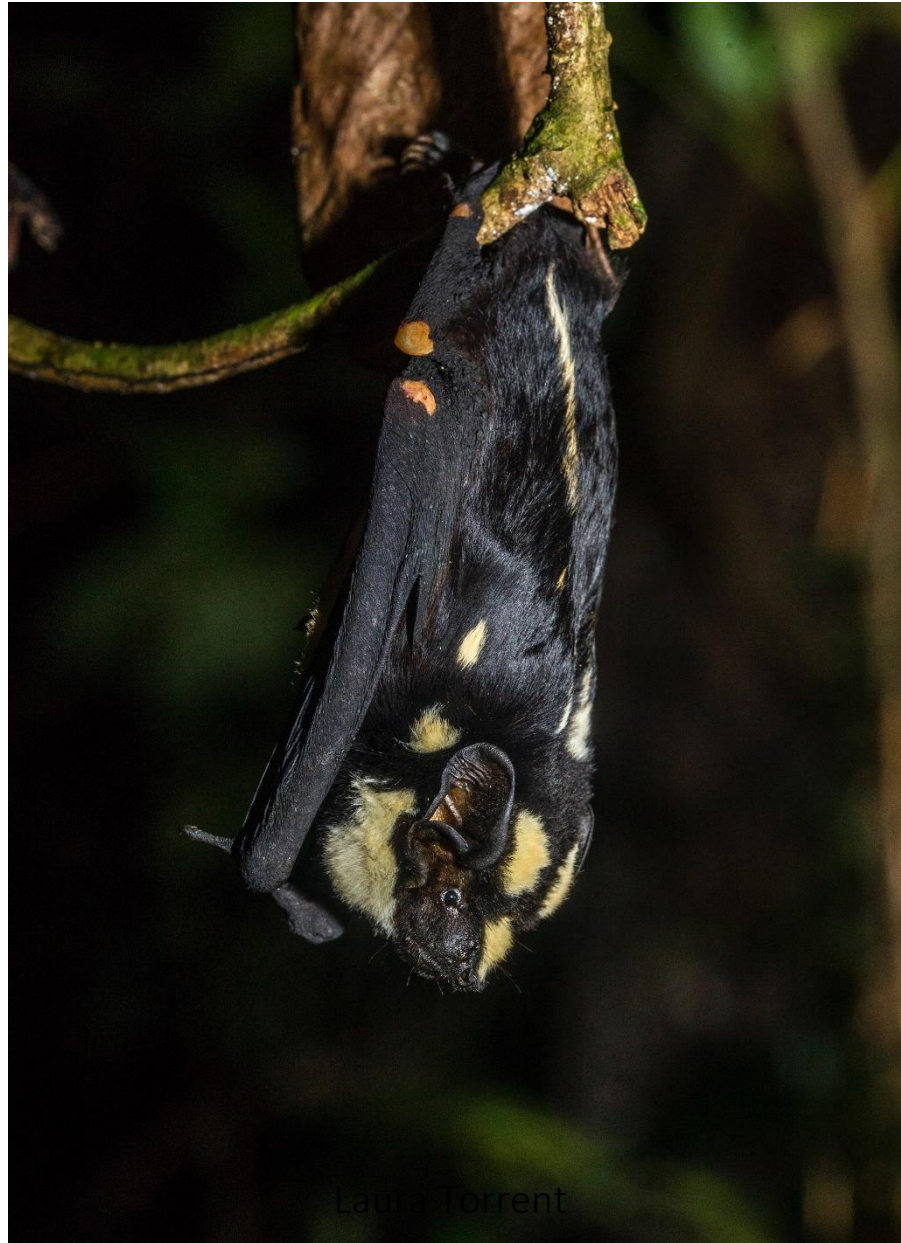


Beneath the canopy: a taxonomic review of Equatorial Guinea bat species to root an urgent Bat Conservation Policy



Natural Sciences Museum of Granollers – Grup de Recerca en Biodiversitat i Bioindicadors (BiBio)

CIBIO - BIOPOLIS - Research Centre in Biodiversity and Genetic Resources

Estación Biológica de Doñana - CSIC

Contents

Project Summary.....	2
Introduction	3
Project Results & Impact.....	4
Objective 1 – Bat Species Checklist.....	4
Objective 2 – Updated Gazetteer and maps preparation.....	5
Objective 3 – Cryptic species confirmation	5
Lessons Learned	8
Conclusion.....	9
Appendices.....	11
Appendix 1	11
Appendix 2	11
Appendix 3	19

Beneath the canopy: a taxonomic review of Equatorial Guinea bat species to root an urgent Bat Conservation Policy

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Project location and dates: Equatorial Guinea-Spain, from 08th March 2021 to 25th June 2022

Project Summary

To fulfil the first aim of my project (i.e. the elaboration of the first comprehensive checklist of the bat species from the mainland region of Equatorial Guinea), I examined 1124 bat specimens stored at the Doñana Biological Station (EBD-CSIC), Sevilla, Spain. I reviewed the morphological and craniodontal characters of all specimens and extracted 64 skulls and 28 bacula from specimens kept in alcohol. Moreover, we applied Sanger sequencing from fresh tissue samples collected during recent field expeditions (2020-2022) to genetically confirm the species identification of cryptic complexes. Combining the information from the EBD-CSIC scientific collection and from recent field data, we have confirmed the presence of 54 bat species for mainland Equatorial Guinea. Out of them, 31 are new records for the country; 33 species were confirmed from data collected in 2018-2022 expeditions and 21 species from specimens deposited in the EBD-CSIC scientific collection. With the compiled data I have created a gazetteer and mapped the 89 surveyed localities for the study area. Moreover, I expanded the distribution range for rare and endangered species like *Hipposideros curtus* and *Glauconycteris superba*. To improve future bat field work in the country I have elaborated a draft dichotomous key (both in English and in Spanish) to be tested in 2023 expedition with our local partners from the National Institute for Forest Development and Management of the Protected Areas (INDEFOR-AP).

In an attempt to confirm two rare specimens from the collection, samples were sent to an external laboratory. They applied molecular Illumina Next Generation Sequencing techniques to amplify and sequence the DNA. I expected the process would have been easier but it was not as the preservation in formalin did severely damage the DNA. Finally, we could not obtain any useful mitochondrial Cytb sequences, and the two specimens remain unidentified. The aim that has not been fully achieved is the species IUCN conservation status assessment for the country. This was due, on one hand, to still ongoing fieldwork monitoring, and on the other, because we are discussing the option to do the assessment at national level rather than only to the continental region of the country.

The impact of these data is the first checklist of bat species for the country and their distribution. From this baseline information we expect to develop specific monitoring programs for endangered

species, organise capacity building actions for local researchers and students and contribute to the management plans of the protected areas.

Introduction

The combination of an entirely unstudied scientific collection of bats from the 90s of more than 1.000 specimens from over 85 localities within Equatorial Guinea (EG) compiled by the Spanish Cooperation Program and deposited at Doñana Biological Station (EBD-CSIC) combined with additional samples from recent expeditions (2018-2022) hold the key to properly describe EG bat fauna and the groundwork for Bat Conservation. I carried out an evaluation of the bat diversity from EG mainland, Central Africa, a region located in one of the most important biodiversity hotspots of Africa, to lay the foundations for local authorities properly direct their efforts to protect its biodiversity. Due to recent offshore oil extraction the country has experienced a rapid environmental decay with large infrastructures and urban growth which are threatening the country's biodiversity. The governmental institution INDEFOR-AP oversees biodiversity conservation of protected areas, trying to keep logging activities under the regulations and preserving the biodiversity. Due to challenging fieldwork conditions, limited funding and capacity, INDEFOR's actions are limited. With elusive and nocturnal bats' habits, EG bat fauna is woefully understudied compared with other mammals and birds. Thus, there is an urgent need to know the bat fauna and prepare new conservation plans for EG. So far, the most recent research on bats was conducted in the 90s and focused on the islands' species. The only publication about the bat fauna from mainland EG is from the 70s and is out-of-date. The possibility of new technics (e.g. molecular) is being key to fulfil the project aims and to the comprehension of the African diversity. For instance, thanks to molecular identification using the mitochondrial gene Cytb African bats phylogeny has significantly improved and several new genera and species have been described in recent years.

The evaluation of the bat species from EG will contribute to the country's fauna conservation by providing a realistic knowledge of the existing species and their distribution as well as reference

material like an identification key for the field—basic information for any later conservation effort or strategy design for the country.

Project Results & Impact

Objective 1 – Bat Species Checklist

To elaborate the checklist, I reviewed 1124 bat specimens deposited at EBD-CSIC, the largest scientific collection of bats from EG. Thanks to the KBA support, I travelled three times to Sevilla to review the collection: 8-26th March; 3-28th May; and 13-19th September 2021.

The studied bat collections date from the 1960s till early 1990s and had been identified according to the knowledge in the 90s. The specimens were stored using different methods: entirely body in alcohol, body in alcohol and skull extracted- or full skeleton extracted, and body dried (Figure 8, Figure 9, Figure 10). I examined individually all the specimens and re-identified most of them using the latest bibliography based on external and craniodental traits, with the help of magnifying lenses and a calliper (to the nearest 0.1 mm for external and 0.01 mm for craniodental characters) (Figure 7).

For this project, 64 skulls were extracted from specimens kept in alcohol by the technicians of the EBD-CSIC. Furthermore, to gather as much morphological data as possible, we extracted the baculum of those specimens from those in which this bone could represent an important systematic tool (Figure 11). As a result, the bacula of 28 specimens were removed and stained with alizarin red after a 5% KOH maceration. The stained bacula were preserved in 100% glycerol after being measured and photographed under a Zeiss 2 stereo microscope and being finally incorporated into the EBD-CSIC mammals' collections (Figure 12).

The EBD-CSIC scientific collection allowed the confirmation of 26 species. Besides, additional 5 species were only confirmed thanks to the recent field expeditions. We have confirmed so far a check-list of 54 bat species belonging to eight families, from which 31 are described for the first time in this study whereas 23 were already described by other authors (Table 2).

Objective 2 – Updated Gazetteer and maps preparation

The gazetteer was elaborated from the coordinates provided in the available literature and from our own unpublished data (Table 3). In order to validate a species record we established that the minimum information should include: a) a proper description of the specimen and b) coordinates or a sufficient description of the locality. We included data from five publications: Jones, (1971) for 19 species, Hassanin et al., (2015) for *Scotonycteris bergmansi*, Andersen (1912) for *Nycteris nana*, Andersen (1906) for *Hipposideros beatus*, and Thomas (1901) for *Glauconycteris beatrix*. Coordinates from the EBD-CSIC scientific collection were reviewed and confirmed thanks to a printed map from 1986 by “Instituto Geográfico Nacional” (scale 1:500.000).

Based on the gazetteer I have prepared a map of mainland EG surveyed localities that can help identify the least explored areas for future bat expeditions (Figure 1). Moreover, I started mapping the species distribution such as *Rhinolophus alcyone* and *R. landeri* (Figure 3).

Objective 3 – Cryptic species confirmation

Molecular Characterization

The mitochondrial cytochrome b gene (Cytb) was extracted from 70 fresh wing punch samples we obtained from captured, and safely released, bat individuals in 2020 and 2022 expeditions. Thanks to this methodology we were able to confirm 22 species from which 5 were not possibly identified in the field based on external traits. A good example is the case of *Scotonycteris bergmansi* and *S. zenkeri* which can only be told apart through using Cytb sequences. We confirmed the presence of both species in the study area, expanding the known range for *S. zenkeri* (Figure 4).

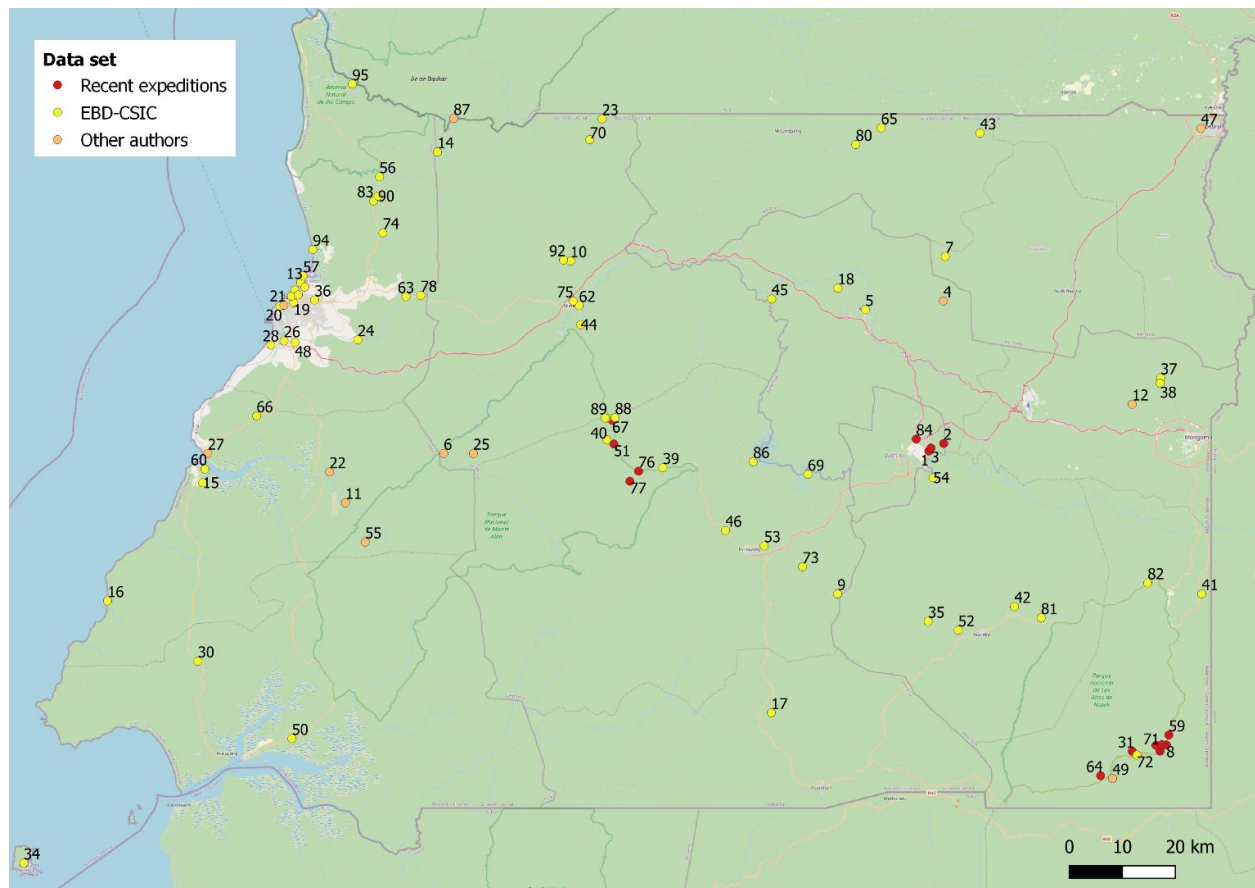


Figure 1. Surveyed localities in the continental region of Equatorial Guinea. Data from field surveys in 2018-2022 in red, data from the EBD-CSIC scientific collection in the period between the 1960-1990s in yellow, and data from previous authors in orange.

For 11 individuals we were not able to reach out a species confirmation as the sequences matched only between 86 and 96% with available sequences in GenBank. The problematic samples are from 6 Vespertilionids, 3 Molossids and 2 Nycterids bats. These three families of African bats contain taxonomic and systematic conundrums yet to be solved at a continental level.

EBD-CSIC old specimens' samples

Two Vespertilionid specimens (*Nycticeinops cf eisentrauti* [EBD19104M] and *Nycticeinops cf crassulus crassulus* [EBD17653M]) did not quite match with any of the existing described species based on craniodental and baculum details. According to external characters and bacula morphology, the specimen EBD19104M (Figure 2) could not be allocated within any of the

recognised forms. The closest species were *Nycticeinops eisenrauti* and *Nycticeinops macrocephalus*. However, *N. eisenrauti* is only known from Mount Cameroon, and *N. macrocephalus* from Eastern Democratic Republic of Congo, respectively ca. 350 km and ca. 2.200 km away from the locality our specimen was captured.

At EBD-CSIC molecular lab (LEM) and with the support of the lab technicians, we attempted to sequence and amplify DNA from tissue samples from the two conflictive specimens. However, the severe DNA degradation of the samples, caused by a long exposure to formalin solution, made impossible to recover a minimum sequence of 200 base pairs. Thus, we contracted an external service hoping they could apply molecular Illumina Next Generation Sequencing techniques to amplify and sequence the DNA. Moreover, we sent them Cytb sequences from fresh tissue samples we collected in our last expedition in 2020. The samples come from individuals we captured, and released, and we believe that they can be used as scaffold sequences to obtain the Cytb sequence of the EBD19104M *Nycticeinops* cf *eisenrauti*. The aim was to use these Cytb sequences as a reference sequence to reconstruct the highly fragmented DNA from the old specimen. Sadly, due to technical difficulties to the designing of the primers and the condition of the tissues it was not possible to reach any fruitful conclusions.

We will gather new data in the field and test other methods to sequence and amplify DNA from the specimens. Untangle these taxonomical mysteries is crucial as some of these individuals could represent new species for the science, particularly EBD19104M specimen (Figure 2).



Figure 2. Left, general view of the specimen EBD19104M *Nycticeinops* cf *eisenrauti* and right, detail of its baculum.

Lessons Learned

Considering the lack of available and reliable information on the bat fauna from EG with this project we have successfully: 1) compiled a list of more than 50 species, 2) gathered new craniodental and bacula measurements and photos, 3) extracted Cytb sequences for many taxa, 4) expanded many species distribution ranges and 5) elaborated a dichotomous key to identify species in the field.

The greatest challenge I encountered to fulfil the first aim, creating a checklist of mainland EG bat species, has been the lack of reliable comparative data. The taxonomic review of several groups required special efforts (e.g. Molossidae and Nycteridae), and among them, the Vespertilionid bats were particularly complicated. Their systematics has been traditionally entangled because the species show a very similar morphological pattern. Therefore, the advent of molecular techniques has been crucial to clarify the taxa consideration and to inspect their relationships to produce a stable overall picture for the whole family. And still for some rare taxa there is not enough information. It is the case of the two EBD-CSIC specimens we cannot properly identify because there is not enough reference material to help confirm the species identification. After unsuccessfully trying to address the problem of DNA amplification and sequencing in the LEM facilities of the EBD-CSIC, we decided to hire an external service. However, we found out it requires specialized people on the topic and more economic investment to test several methods. As I do not have more resources the specimen's identification has reached a dead end for the moment.

On the other hand, we have successfully obtained Cytb sequences in the LEM facilities from the fresh wing punch tissues collected in the recent expeditions. Still, we have encountered the problem of the lack of reference sequences in GenBank. For future projects I will better gauge my expectations to untangle complex bat taxonomical questions as I realised it requires more time, data, and funds. Moreover, I will seek advice from other African bat taxonomist, like Dr. Ara Monadjem, to guide me and perhaps establish a collaboration.

The aim that has not been achieved yet is the preparation of the species IUCN conservation status assessment due to the incompleteness of the fieldwork. I expect to accomplish this aim by end-2023, once the last field expedition, planned for January-February 2023, is finished. Therefore, I

will not be able to include it on the KBA final report. Nonetheless, the report includes the most updated IUCN Red List categories for all the described species for EG (Table 2). From a conversation I had with Dr. Dave Waldien, Red List Authority Coordinator, I learned it is best to gather more data before assessing species conservation status at global level. Thus, I will work on improving EG bat species conservation categories once all fieldwork is completed. An example species that will benefit from this is *Glauconycteris superba* (cover photo), one of the scarcest species of the genus, that was captured in EG and currently is classified as “Least Concern” rather than “Data Deficient”.

Finally, we are not ready to present a first draft of a Conservation Plan for INDEFOR-AP as I planned with this project. However, I am collaborating with the creation of a new protected area, National Park “Ciudad de la Paz”, by carrying out bat surveys in 2023. This new protected area will connect with two existing parks creating the greatest corridor for fauna in the country. Moreover, it will allow university students from the Afro-American University of Central Africa (AAUCA) to carry out field surveys and contribute to EG biodiversity conservation.

Conclusion

We provide the most comprehensive list of the bat species for the continental region of EG which includes 54 confirmed species. The list includes 31 first record species for the country: 3 Emballonurids, 4 Molossids, 1 Miniopterid, 14 Vespertilionids, 2 Nycterids, 4 Hipposiderids and 3 Pteropodids. Nonetheless, 37 EBD-CSIC specimens and 29 wing punch samples require further analysis to be confirmed.

Besides, and mainly thanks to the EBD-CSIC scientific collection, we have contributed to update the taxonomy, morphological data, and distribution of many rare African bat species. For example, we expand the distribution range for the endangered *Hipposideros curtus* thanks to 9 specimens from EBD-CSIC collection. We compiled 89 surveyed localities across the study area combining both data sets: EBD-CSIC scientific collection and recent expeditions. Finally, thanks to all

morphological data gathered we elaborated a dichotomous key to identify EG bats based on external traits. To test its efficiency and flaws we will use it in 2023 expedition.

To sustain this project in a long term, we want to tackle two main aspects: data collection and capacity building. On one hand, we are empowering local researchers to apply for grants, such as the Conservation Action Research Network (CARN) (<https://conservationactionresearch.net/aspire-grant-program.php>) and the Rufford Foundation (<https://www.rufford.org/>), and to connect with researchers and organisations from other African countries. We are also encouraging local enthusiastic people to participate on the *Eidolon helvum* citizen science project (<https://www.eidolonmonitoring.com/>). On the other hand, we are planning to organise a series of workshops for local researchers and university students from the University of Equatorial Guinea (UNGE) and the AAUCA. In January 2023 we are going to meet with the AAUCA rector and carry out a small workshop in Bioko Island. We plan to engage to these projects the TROPIBIO group (<https://cibio-tropibio.pt/en/>) from CIBIO, in Portugal, and the Harrison Institute (<https://www.harrison-institute.org/>), from UK.

The further work that would be useful for the conservation of *H. curtus* is find out its roosts and monitor its colonies in EG. We are planning to establish a monitoring protocol that is being implemented by Dr. Iroro Tanshi in Nigeria and Cameroon, the only other two known countries where the species has been found.

Appendices

Appendix 1

Table 1. Financial report showing actual expenditure against budgeted amounts.

Items	Supplier	Actual expenditure	Equivalent in GBP	Budgeted amounts
Train Barcelona to Sevilla	Renfe	81.15 €		
Accommodation Sevilla from 8 to 20 March 2021	Airbnb	244.47 €		
Accommodation Sevilla from 21 to 27 March 2021	Owner flat	112.00 €		
Train Sevilla to Barcelona	Renfe	89.35 €		
Food and public transport in Sevilla		58.87 €		
Train Barcelona to Sevilla	Renfe	89.90 €		
Accommodation Sevilla from 9 to 29 May 2021	Paco	250 €		
Food and public transport in Sevilla		56.47 €		
TOTAL Food & Accommodation & Transport Laura		982.21 €	£834.17	£1,500.00
Wing punch - 70 samples	EBD-CSIC	870.00 €	£738.88	£1,000.00
Illumina - 2 samples	AllGenetics	3,447.00 €	£2,927.48	£2,000.00
			£4,500.53	£4,500.00

Appendix 2

Other relevant information – data analysis, copies of publications, news media, theses or dates when these will be available to share, etc.

Table 2. List of all bat species confirmed in the continental region of Equatorial Guinea.

Species	Family	First record for EG	IUCN Red List
<i>Afronycteris nana</i>	Vespertilionidae	No	LC
<i>Chaerephon pumilus</i>	Molossidae	Yes	LC
<i>Coleura afra</i>	Emballonuridae	Yes	LC
<i>Doryrhina cyclops</i>	Hipposideridae	No	LC
<i>Eidolon helvum</i>	Pteropodidae	No	NT
<i>Epomophorus pusillus</i>	Pteropodidae	Yes	LC
<i>Epomops franqueti</i>	Pteropodidae	No	LC

<i>Glauconycteris alboguttata</i>	Vespertilionidae	Yes	LC
<i>Glauconycteris argentata</i>	Vespertilionidae	No	LC
<i>Glauconycteris beatrix</i>	Vespertilionidae	No	LC
<i>Glauconycteris curryae</i>	Vespertilionidae	Yes	DD
<i>Glauconycteris egeria</i>	Vespertilionidae	Yes	DD
<i>Glauconycteris humeralis</i>	Vespertilionidae	Yes	DD
<i>Glauconycteris poensis</i>	Vespertilionidae	No	LC
<i>Glauconycteris superba</i>	Vespertilionidae	Yes	LC
<i>Glauconycteris variegata</i>	Vespertilionidae	Yes	LC
<i>Hipposideros beatus</i>	Hipposideridae	No	LC
<i>Hipposideros curtus</i>	Hipposideridae	Yes	EN
<i>Hipposideros fuliginosus</i>	Hipposideridae	Yes	LC
<i>Hipposideros ruber</i>	Hipposideridae	Yes	LC
<i>Hypsignathus monstrosus</i>	Pteropodidae	No	LC
<i>Hypsugo musciculus</i>	Vespertilionidae	Yes	DD
<i>Kerivoula phalaena</i>	Vespertilionidae	Yes	LC
<i>Macronycteris gigas</i>	Hipposideridae	Yes	LC
<i>Megaloglossus woermanni</i>	Pteropodidae	No	LC
<i>Mimetillus moloneyi</i>	Vespertilionidae	Yes	LC
<i>Miniopterus inflatus</i>	Miniopteridae	Yes	LC
<i>Mops brachypterus</i>	Molossidae	No	LC
<i>Mops nanulus</i>	Molossidae	Yes	LC
<i>Mops petersonii</i>	Molossidae	Yes	NT
<i>Mops spurrelli</i>	Molossidae	No	LC
<i>Mops thersites</i>	Molossidae	No	LC
<i>Myonycteris angolensis</i>	Pteropodidae	Yes	LC
<i>Myonycteris torquata</i>	Pteropodidae	No	LC
<i>Myopterus whitleyi</i>	Molossidae	Yes	LC
<i>Myotis bocagii</i>	Vespertilionidae	No	LC
<i>Nycteris arge</i>	Nycteridae	No	LC
<i>Nycteris grandis</i>	Nycteridae	Yes	LC

<i>Nycteris hispida</i>	Nycteridae	No	LC
<i>Nycteris major</i>	Nycteridae	Yes	DD
<i>Nycteris nana</i>	Nycteridae	No	LC
<i>Nycticeinops c. crassulus</i>	Vespertilionidae	Yes	LC
<i>Nycticeinops grandidieri</i>	Vespertilionidae	Yes	DD
<i>Pseudoromicia brunnea</i>	Vespertilionidae	Yes	NT
<i>Pseudoromicia tenuipinnis</i>	Vespertilionidae	Yes	LC
<i>Rhinolophus alcyone</i>	Rhinolophidae	No	LC
<i>Rhinolophus landeri</i>	Rhinolophidae	No	LC
<i>Rousettus aegyptiacus</i>	Pteropodidae	No	LC
<i>Saccolaimus peli</i>	Emballonuridae	Yes	LC
<i>Scotonycteris bergmansi</i>	Pteropodidae	No	LC
<i>Scotonycteris zenkeri</i>	Pteropodidae	Yes	NT
<i>Scotophilus nux</i>	Vespertilionidae	Yes	LC
<i>Taphozous mauritanus</i>	Emballonuridae	Yes	LC

Table 3. Gazetteer with a unique number for each locality, its coordinates and predominant habitat.

Number	Name	Latitude	Longitude	Habitat
1	AAUCA DORMS	1.604	10.85531	Over water, Building
2	AAUCA PRIMARY	1.61681	10.8805	Primary forest
3	AAUCA SECONDARY	1.6092	10.8591	Secondary forest
4	ABAM	1.858	10.88	Forest
5	ABONISI	1.84327	10.74781	Forest
6	ABUMENSOK	1.6	10.03333	Over water
7	ADURELANG	1.93333	10.88333	Forest
8	AKOCSAKIRA	1.106711	11.258153	Culvert
9	AKOGA	1.36234	10.70041	Forest
10	ALOSA	1.92566	10.24808	Cave, Over water
11	ANSOM	1.51666	9.86666	Forest
12	ASOGOMANSUEIÑ	1.68333	11.2	Over water

13	ASONGA	1.88898	9.79074	Forest
14	AYAMIKEN	2.11004	10.0225	Forest
15	AYASONG	1.5505	9.62473	Forest
16	AYE	1.35055	9.46344	Beach, bushes, coconuts
17	AYEBE	1.1611	10.58836	Forest
18	AYENE	1.87971	10.70119	Forest
19	BATA	1.85504	9.77871	Urban area
20	BATA60	1.84931	9.75558	Urban area
21	BATA66	1.85086	9.76232	Building
22	BENITO	1.56861	9.83956	Over water
23	BESABEBA	2.16603	10.30161	Forest
24	BICOMO BATA	1.79215	9.88757	Over water
25	BILENE	1.6	10.08333	Over water
26	BOARA	1.79041	9.76254	Forest
27	BOLONDO	1.6	9.63333	Over water
28	BOME	1.7833	9.74016	Over water
29	BOMUDI BATA	1.88155	9.79766	Urban area, crops
30	BONOBONO	1.24857	9.61656	Forest
31	CAVE ACOATATAN	1.096008	11.200228	Cave, Over water
32	CAVE AKOCSAKIRA	1.106963	11.250215	Cave
33	COCOS BATA	1.87635	9.78084	Beach, bushes, coconuts
34	CORISCO	0.90678	9.3215	Beach, bushes, coconuts
35	CRTA EVINA-ACONI	1.315681	10.854271	Culvert
36	CRTA KM 3 BATA-NIEF	1.85963	9.81427	Culvert
37	CRTA MONG ASORK	1.72764	11.24842	Forest
38	CRTA MONG EBEB	1.71872	11.24739	Forest
39	CRTA NCUMIEKIE	1.575761	10.404553	Culvert
40	CUEVA ESONG	1.622972	10.309972	Cave
41	CUEVA NDUMU	1.36235	11.31796	Cave
42	CUEVA NZAS	1.34085	11.00041	Cave
43	CULVERT NCOMOYOS	2.14195	10.94203	Culvert

44	CULVERT NIEF	1.817894	10.265497	Culvert
45	DEDE	1.86132	10.58903	Forest
46	EBALOWA	1.46963	10.51059	Forest
47	EBEBIYIN	2.15	11.31666	Cave
48	EKUKU	1.78773	9.78095	Over water
49	ELON	1.05	11.16666	Over water
50	ENCURE	1.11753	9.77591	Forest
51	ENGONG	1.61599	10.3217	Over water
52	ETOM	1.30103	10.90531	Forest
53	EVINAYONG	1.44359	10.57631	Forest
54	EVOARDULU	1.55901	10.86267	Building
55	EVOARYULU	1.45	9.9	Forest
56	EXFOSA	2.06809	9.92432	Secondary forest
57	HOTEL BATA	1.90033	9.7947	Building
58	LA SALLE, BATA	1.86824	9.7865	Building
59	MBAM	1.123509	11.262197	Culvert, Building
60	MBINI	1.5737	9.62863	Forest
61	MEDJEE	1.096227	11.247339	Over water
62	MFAMAN NIEF	1.85013	10.26253	Cave
63	MICAN BATA	1.8652	9.96917	Urban area
64	MICOMO	1.05455	11.14656	Over water
65	MIKEMELE	2.15086	10.7745	Forest
66	MIKONIBE	1.6634	9.71608	Forest
67	MOCA	1.65604	10.31876	Culvert, Building
68	MONTE ALEN	1.65984	10.30925	Over water
69	MOSOC	1.56464	10.65037	Forest
70	MVUBA	2.13104	10.28072	Forest
71	NCAMA	1.101437	11.246421	Over water
72	NCOMO NSORK	1.089507	11.208015	Forest
73	NDUBU	1.4082	10.64128	Over water
74	NGONO	1.97335	9.93007	Forest

75	NIEFANG	1.85777	10.25287	Building
76	NKUMEKIEN	1.56989	10.36346	Village, crops
77	NKUMEKIEN RIO	1.55296	10.34869	Over water
78	NSING	1.86731	9.99407	Forest
79	NSORK	1.106126	11.240376	Over water
80	NSUA	2.12274	10.73157	Cave, Over water
81	NTUGU	1.32177	11.0457	Forest
82	NVOM	1.38088	11.22631	Building
83	OTOCON	2.02718	9.9144	Forest
84	OYALA	1.62434	10.83402	Village, crops
85	POLITEC BATA	1.86612	9.77515	Beach, bushes, coconuts
86	RIO BICABA	1.58595	10.55768	Over water
87	RIO CAMPO	2.16666	10.05	Over water
88	RIO LOBO	1.66042	10.32368	Over water
89	RIO MBON	1.65995	10.30647	Over water
90	SAN JOAQUIN	2.03552	9.9219	Village, crops
91	SAN PEDRO BATA	1.86824	9.7865	Urban area
92	SERVFOR NIEF	1.92737	10.23619	Secondary forest
93	SWAMP AKOCSAKIRA	1.106963	11.250215	Swamp
94	UTONDE	1.94487	9.81141	Beach, bushes, coconuts
95	YENGUE	2.22501	9.8783	Forest

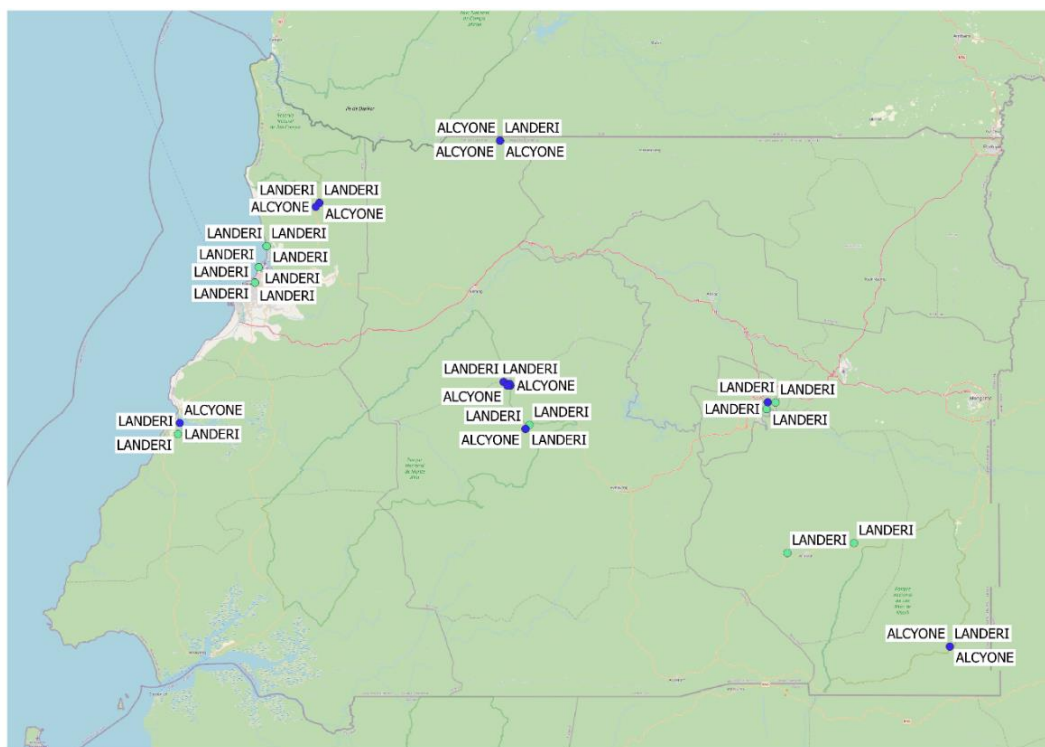


Figure 3. Distribution of *Rhinolophus alcyone* and *Rhinolophus landeri* in the continental region of Equatorial Guinea based on data from EBD-CSIC specimens and recent expeditions (2018-2022).

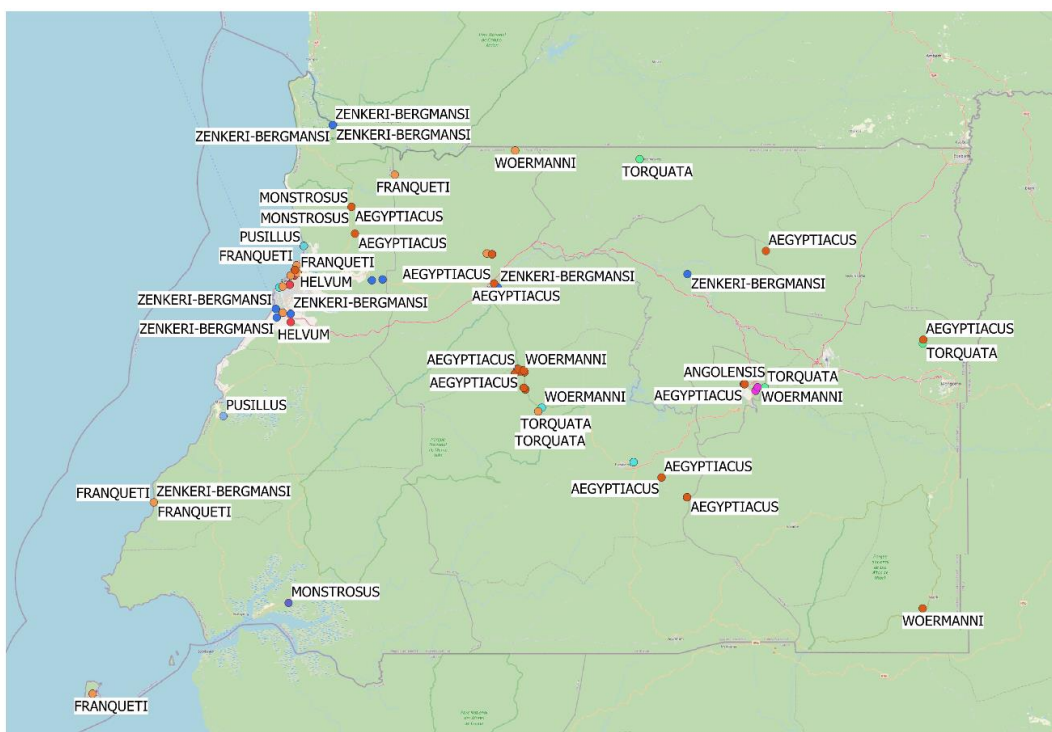


Figure 4. Distribution of the Pteropodidae species in the continental region of Equatorial Guinea based on data from EBD-CSIC specimens and recent expeditions (2018-2022).



Figure 5. Cover photo and interview about the project at the Bat News magazine, Spring 2022.



Figure 6. First slide of the presentation I did at the National Bat Conference in September 2022.

Appendix 3

Photos illustrating the research.



Figure 7. Taking external and cranial measurements of EBD-CSIC specimens.



Figure 8. Lateral view of the skull and mandibles of two specimens of *Hipposideros fuliginosus*.



Figure 9. Two specimens of *Hipposideros curtus* from EBD-CSIC scientific collection.



Figure 10. Specimen of *Saccolaimus peli* from EBD-CSIC scientific collection.



Figure 11. Left, removal of the penis and right, comparative size of two bacula with a coin.

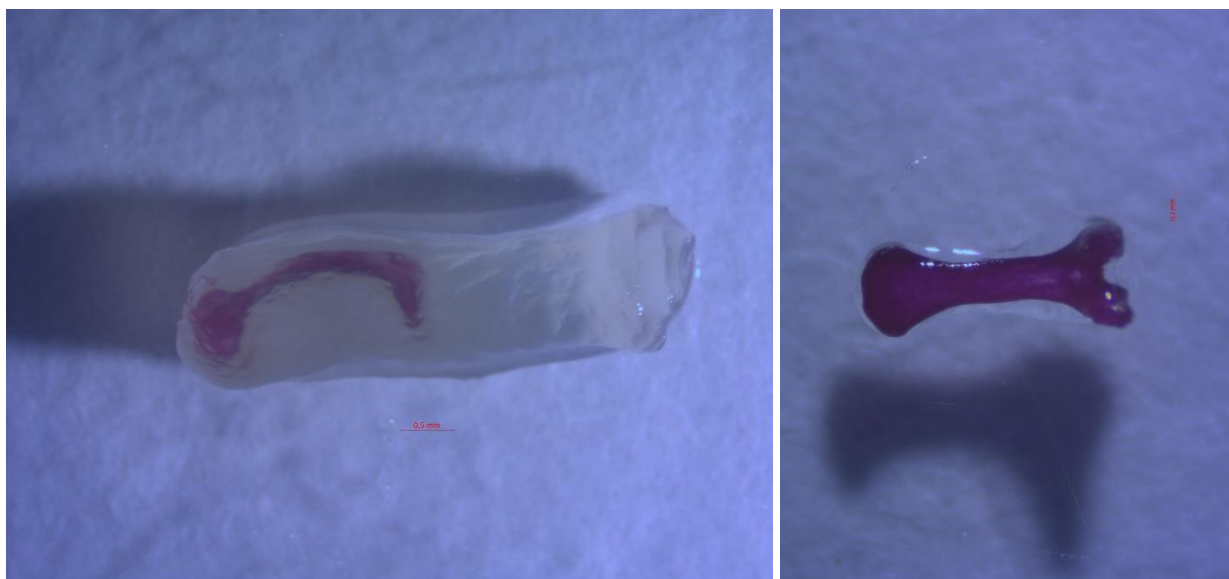


Figure 12. Left, lateral view of *Pseudoromicia brunnea* baculum inside the penis and right, dorsal view of *Afronycteris nana* baculum. Scale: 0.5 mm.