Molecular detection of Trypanosoma spp. and Hepatocystis parasite infections of bats in Northern Nigeria

J. Kamani1, Y. J. Atuman1, D. A. Oche2, A. Shekaro1, O. Werb3, I. Ejotre3,4 and J. Schaer3

1Parasitology Division, National Veterinary Research Institute (NVRI), PMB 01, Vom, Plateau State, Nigeria; 2Animal Health & Production, Akgeran Orshi Polytechnic Yandev, Gboko, Benue State, Nigeria; 3Department of Molecular Parasitology, Institute of Biology, Humboldt University, Berlin, Germany and 4Department of Biology, Muni University, Arua, Uganda

Abstract

Bats are mammalian hosts to a large diversity of eukaryotic protozoan blood parasites, including different genera of haemosporidians and diverse species of trypanosomes. Phylogenetic studies suggest that bats, particularly in Africa, have played an important role in the evolutionary history of these parasite groups. However, our understanding of the diversity and distribution of chiropteran haemosporidians and trypanosomes in Africa remains tenuous. We investigated the prevalence and phylogenetic relationships of the blood parasites in different bat species in Northern Nigeria using molecular methods. A low prevalence of Hepatocystis parasites was detected in a potentially rare host species, the African straw-coloured fruit bat (Eidolon helvum) confirming yet another fruit bat species in the diverse range of African bat hosts. Trypanosome infections were identified in 3 different bat species. The trypanosomes of Mops cf. pumilus were recovered as a distinct lineage that is related to Trypanosoma erneyi, a species which is closely related to Trypanosoma dionisi and Trypanosoma cruzi. Nycteris cf. macrotis bats were infected with trypanosomes that are related to the distinct lineage of Trypanosoma cf. livingstonei parasites. Further, 2 different lineages of trypanosomes in E. helvum bats share highest nucleotide identities with Trypanosoma livingstonei and a group of Trypanosoma sp. parasites that are closely related to T. cf. livingstonei and T. livingstonei, respectively. The findings of this study confirm the notion that trypanosomes of African bats are phylogenetically diverse and that African bats might harbour a variety of yet undescribed trypanosome species.

Introduction

Bats, a species-rich group with over 1454 species (Simmons, 2005; Miller-Butterworth et al., 2007; Lack et al., 2010) are mammalian hosts to a large diversity of eukaryotic protozoan blood parasites. These comprise different genera of haemosporidian parasites and diverse species of trypanosomes (e.g. Lima et al., 2013; Schaer et al., 2013; Clement et al., 2020).

The apicomplexan parasites of the order Haemosporida infect diverse vertebrate hosts, comprising mammals, squamates and birds and use different dipteran hosts as vectors (Garnham, 1966; Levine, 1988). The over 500 described haemosporidian parasites are classified in the genus Plasmodium that includes the human-infecting species that cause the malaria disease, plus 9 additional genera (Martinsen and Perkins, 2013). Among mammals, bats harbour the most diverse set of haemosporidians parasites and results of previous phylogenetic studies suggest that bats have played an important role in the evolutionary history of haemosporidian parasites (Duval et al., 2007; Schaeer et al., 2013; Perkins and Schaeer, 2016; Galen et al., 2018).

The kinetoplastid flagellated Trypanosoma parasites feature complex lifecycles and are mainly transmitted to the vertebrate host by haematophagous arthropods or leeches (Simpson et al., 2006). In Africa, the Trypanosoma species Trypanosoma brucei sensu lato, Trypanosoma vivax and Trypanosoma congolense present a threat to both humans and livestock (Morrison et al., 2016; Büscher et al., 2017). Previous studies have identified a high diversity of Trypanosoma species in bats and revealed that bats also played an important role in the evolutionary history of these parasites (e.g. Hamilton et al., 2012; Lima et al., 2012, 2013; Clement et al., 2020; Austen and Barbosa, 2021). The investigation of the diversity, distribution and phylogenetic relationships of trypanosome species of wildlife, including bats, is essential for a better understanding of the evolution of the whole parasite group.

With about 85 species of bats, Nigeria represents a diversity hotspot in Africa (Simmons and Cirranello, 2022). However, the knowledge about haemosporidian parasites and trypanosomes in Nigerian bats is scarce (Atama et al., 2019). Here, we investigated the prevalence and phylogenetic relationships of haemosporidian and trypanosome parasites in different bat species in Northern Nigeria using molecular methods.
Materials and methods

Bats were sampled in Northern Nigeria between December 2018 and March 2019 in the states of Bauchi, Benue, Katsina, Nasarawa and Plateau. The sampling sites comprised 10 peri-domestic roosting sites (trees, caves, ceiling/eaves of residential buildings) (Fig. 1).

Field sampling and microscopy

Bats were captured using improvised traps produced from fishing nets and wooden poles. The nets were set every capture night in some distance to their roosting sites. The bats were gently removed from the nets and transferred into individual bags and genus and/or species were identified following the keys available in Patterson and Webala (Patterson and Webala, 2012). Bats were captured and sacrificed within the scope of a larger project focusing on viral and bacterial infections in bats (e.g. Kamani et al., 2021). The bats were anaesthetized using a combination of 5 mg kg⁻¹ ketamine plus 2 mg kg⁻¹ of xylazine injected intramuscularly. The voucher specimens were preserved in 70% (vol vol⁻¹) ethanol and stored in the Parasitology Division NVRI Vom, in Nigeria. Blood samples were collected by venous puncture of the brachial vein and collected in capillary tubes. For the investigation of blood parasites, blood samples were used to spot blood dots onto DNA FTA cards (Whatman) and to prepare thin blood smears on slides. The blood smears were dried and fixed in 99–100% (vol vol⁻¹) methanol solution for 3 s and later stained with 10% Giemsa solution for 50 min. Giemsa-stained blood smears were examined for the presence of haemosporidian and trypanosome parasites using light microscopy at a magnification of ×1000 with immersion oil.

Molecular methods

Whole genomic DNA was extracted from the dried blood dots on DNA FTA cards using the DNeasy extraction kit (Qiagen, Hilden, Germany) (e.g. Schaer et al., 2017). The protocol for animal tissues was performed and samples were eluted in 60 μL AE buffer. Polymerase chain reactions (PCRs) were performed using the AllTaq Master Mix Kit (QIAGEN) with 4–5 μL of genomic DNA as the template, and 1 μL of each primer (10 mM).

To confirm morphological bat identifications, most of the samples were genotyped using 1 or more of the following genetic markers for the bat hosts: the mitochondrial cytochrome b (cytb) and the nuclear introns Acyl-CoA oxidase 2, intron 3 (acox2), and Beta-fibrinogen gene, intron 7 (fgb) (Table S1). A part of the cytb gene was sequenced for 47 of the 60 E. helvum individuals, which featured 100% nucleotide identity with E. helvum reference sequences in NCBI GenBank. For 11 E. helvum individuals, 650 bp of the fgb were additionally or instead sequenced, which again resulted in a nucleotide identity of 100% with published E. helvum reference sequences. To identify 3 individuals that were morphologically identified as Tadarida sp. to species level, cytb sequences were generated which resulted in highest nucleotide identities of 99.2% with reference sequences of Mops condylurus (e.g. GenBank accession number EF474030) and thus might represent M. condylurus or a close related species and are therefore termed M. cf. condylurus. Five out of 8 individuals that were morphologically identified as Chaerephon sp. were genotyped with cytb (featuring identical cytb sequences) and highest nucleotide identities of 98.4% were recovered with reference sequences for Mops pumilus (e.g. GQ489157). Again, these bats might represent the species M. pumilus or a close related species and are termed M. cf. pumilus. Twelve out of 16 Nycteris sp.
individuals were genotyped with cyt b and highest nucleotide identities of 94.7% (sample number KJ-M), 96.98% (sample numbers KJ-B, KJ-C, KJ-D, KJ-J) and 97.3% (remaining samples) were recovered with the reference sequence for Nyctericis macrotis (accession number MTS86790). The sequences of the nuclear acox2 for 2 of the Nycteris sp. samples (samples numbers KJ-E, KJ-F) featured a 98.7% nucleotide identity with the reference sequence for Nyctericis macrotis complex sp.1 (MK837356). We, therefore, tentatively refer to the individuals of the study as Nyctericis macrotis.

The mitochondrial cytochrome b (cyt b) gene was targeted for detection and subsequent phylogenetic analysis of haemosporidian parasites (e.g. Schaar et al., 2013). Four genes were used for molecular detection of haemosporidian parasites: the mitochondrial genes cytochrome b (cyt b) and cytochrome oxidase 1 (cox1); the apicoplast caseinolytic protease gene (clpC); and the nuclear gene elongation factor 2 (ef2) (Table S1) (Schaer et al., 2013).

For the detection of trypanosomes, a nested-PCR approach was carried out for the amplification of the partial sequence of about 640 bp of the small subunit 18S ribosomal RNA gene (18S rRNA) following Noyes et al. (1999). A second gene, the nuclear glycosomal glyceraldehyde phosphate dehydrogenase (gGAPDH), was subsequently targeted for all positive samples in a nested-PCR approach following Clement et al. (2020). All primers are listed in Table S1. All positive PCR products were sequenced with the amplification primers and run on an ABI-373 sequencer. The software Geneious Prime 2022.1.1 (https://www.geneious.com) was used to quality-check and manually edit all nucleotide sequences. Ambiguous base calls or missing data were coded with N’s or the corresponding ambiguity code. Sequences were aligned using the MAFFT algorithm (Katoh et al., 2002; Katoh and Standley, 2013). Sequences for the analysis of the phylogenic relationships of Hepatocystis comprised 531 nucleotides (nt) of the partial cyt b gene. Sequence alignments for the analysis of Trypanosoma spp. included 1022 nt for 18S rRNA and 894 nt for gGAPDH, respectively. Reference sequences were retrieved from GenBank and added to the alignments (all accession numbers for analysis of Hepatocystis parasites are listed in Table S2, all accession numbers for 18S rRNA and gGAPDH analyses are listed in the corresponding phylogenetic tree figures and Table S3). The software ModelTest-NG was used to test different DNA substitution models (Darriba et al., 2020). Phylogenetic relationships were evaluated with maximum likelihood (ML) analysis and carried out in the software raxmlGUI version 2.0.6 (Edler et al., 2021). The Hepatocystis analysis of the partial cyt b was carried out using the substitution model GTR + I (proportion of invariant sites) + Gamma (rate heterogeneity) and the taxon Leucocytozoon as outgroup. Nodal support was evaluated using 1000 replicates (through bootstrap). The trypanosome ML analysis of the 18S rRNA and gGAPDH gene were carried out using the models TIM3 + I + G and GTR + I + G, respectively, and 1000 replicates and Trypanosoma lewisi as outgroup taxon. Phylogenetic trees were displayed in FigTree (http://tree.bio.ed.ac.uk/software/figtree/).

**Results**

A total of 95 bats belonging to 3 bat families were investigated. The greater part of the captured bats (n = 68) belonged to the African fruit bat species Eidolon helvum. Further, 16 Nycteris cf. macrotis of the family Nycteridae, 8 Mops cf. pumilus and 3 Mops cf. condylurus (Molossidae) were screened for haemosporidian parasite and trypanosome parasite infections (Table 1).

**Table 1. Investigated bat species and prevalences of haemosporidian and trypanosome parasite infections**

| Bat species (Bat family) | Total number of individuals | Haemosporidian parasite prevalence (%) | Trypanosoma sp. prevalence (%) |
|-------------------------|----------------------------|---------------------------------------|---------------------------------|
| Mops cf. condylurus (Molossidae) | 3 | 0/3 (0) | 0/3 (0) |
| Mops cf. pumilus (Molossidae) | 8 | 0/8 (0) | 3/8 (37.5) |
| Nycteris cf. macrotis (Nycteridae) | 16 | 0/16 (0) | 11/16 (68.8) |
| Eidolon helvum (Pteropodidae) | 68 | 4/68 (5.9) | 2/68 (2.9) |
| Total | 95 | 4/95 (4.2) | 16/95 (16.8) |

**Prevalence and phylogenetic characterization of haemosporidian parasites**

Haemosporidian parasite infections were limited to only 4 individuals of E. helvum, which correspond to a prevalence of 5.9% (4/68) in E. helvum and an overall prevalence of 4.2% (4/95). No infections were detected in the species Nycteris cf. macrotis, Mops cf. condylurus and Mops cf. pumilus (Tables 1 and S4). The molecular analysis identified the parasite infections in E. helvum as Hepatocystis parasites. Microscopic screening of the corresponding Giemsa-stained thin blood smears of the 4 PCR-positive samples did not result in detection of parasite stages which points to subpatent infections with a very low parasitaemia. The partial cyt b sequences of Hepatocystis sp. of 2 samples (sample numbers KJ72, KJ82) were identical, the third sample (sample number J53) featured a few ambiguous base calls which point to a mixed haplotype infection but was otherwise identical to the other 2 samples. The Hepatocystis sequences are identical to published parasite sequences from fruit bat hosts in Nigeria and South Sudan (Schaer et al., 2017; Atama et al., 2019). The 4th sequence (sample number KJ63) featured about 96% identity with the other 3 sequences that were recovered from Hepatocystis parasite infections in E. helvum in this study. Interestingly, all 4 sequences showed higher similarities to parasite sequences from other bat host species than to the sole published sequence from Hepatocystis sp. from E. helvum in Gabon. The phylogenetic analysis that was performed to assess the phylogenetic relationships and possible geographic and/or host specific patterns of Hepatocystis parasites from Nigeria confirmed Hepatocystis as a monophyletic group and as sister clade to the rodent- and bat-infecting Plasmodium (Vinckeia) parasite clade as shown before (Fig. 2) (e.g. Galen et al., 2018). Within the African bat Hepatocystis clade, the Hepatocystis sequence from sample KJ63 groups basal, whereas the other 3 sequences fall within the main clade (Fig. 2). Overall, the Hepatocystis sp. sequences of different African bat species from several distant related African countries neither show geographic nor host specificity patterns confirming previous analysis (e.g. Schaar et al., 2017).
Trypanosome infections were detected in 16 individuals from 3 out of the 4 bat species of the study (16/95, overall prevalence of 16.8%) (Tables 1 and S4). Two infections were confirmed in E. helvum (2/68, prevalence of 2.9%), 3 infections in Mops cf. pumilus (3/8, 37.5%) and a high prevalence of 68.8% was found in Nycteris cf. macrotis (11/16). No infections were recorded for 3 individuals of Mops cf. condylurus (Table 1). The trypanosomes of Mops cf. pumilus (Molossidae) were identified as species Trypanosoma cf. erneyi based on the blastn (https://blast.ncbi.nlm.nih.gov/) search of the 18S and gGAPDH sequences and the phylogenetic analysis. All 3 infected individuals (sample numbers VC6, VC9, VC13) featured identical 18S rRNA sequences which shared highest sequence identities (96.5%) with a reference sequence of a trypanosome from Mops condylurus in Mozambique (JN040989). One gGAPDH sequence was successfully generated (for sample number VC13), which showed highest identity (95.2%) with a trypanosome reference sequence from Tadarida sp. (Molossidae) again from Mozambique (JN04095). The species Trypanosoma erneyi has been described from bat species of the family Molossidae in Africa and the sequences of this study group closely to the reference sequences of T. erneyi, but in their own separate clade, in the 18S rRNA phylogenetic analysis as well as in the gGAPDH analysis (Figs 3 and 4). This finding indicates that the sequences of this study might represent a distinct species or subspecies, however with...
The lack of morphological information of these parasites (no parasite stages were detected in the blood smears), they are tentatively referred to as *T. cf. erneyi*.

The trypanosome infections of the host species *Nycteris cf. macrotis* belong to 1 parasite haplotype of *Trypanosoma cf. livingstonei*. One sequence from the parasites from *E. helvum* hosts basal to *T. livingstonei* parasites, whereas the other one was recovered as separate lineage to *Trypanosoma* sp. parasites. The parasite sequences of *N. cf. macrotis* and *E. helvum* of the study are highlighted in bold blue, the parasite sequences of *Mops cf. pumilus* in red. The trypanosome sequences from *Mops cf. pumilus* hosts of the study group as distinct lineage to *T. ernyei* parasites with high support (bootstrap value 93).

**Discussion**

This study adds information to the scarce knowledge about haemosporidian parasite infections in Nigerian bats. *Hepatocystis* parasites were detected in the straw-coloured fruit bat species *E. helvum*, which is only the second time that this bat species was recovered as host to *Hepatocystis*, the only other single evidence has been reported from Gabon (*Boundenga et al*, 2018). The *Hepatocystis* prevalence in *E. helvum* in this study (4 infected individuals, prevalence of 6%, 4/68) was quite low, compared to reports of *Hepatocystis* infections in African epauletted fruit bat.
species where prevalences can range from 25% up to about 90% (e.g. Schär et al., 2013; Lutz et al., 2016; Schär et al., 2017; Boundenga et al., 2018; Atama et al., 2019). The phylogenetic analysis recovered the Hepatocystis sequences from *E. helvum* within the African bat Hepatocystis clade without any apparent geographic or host species specific pattern following previous findings (e.g. Schär et al., 2017). Yet, as the morphological verification of parasite stages in the blood of the vertebrate host (proof that a parasite has undergone asexual reproduction) is lacking for *Hepatocystis* parasites in *E. helvum* hosts, the findings must be considered with caution. In some cases, the sensitivity of PCR methods might verify haemosporidian parasite DNA (transmittable sporozoite stages) in wrong hosts, where the parasite life cycle has been aborted (Valkiunas et al., 2009). Interestingly, another African fruit bat species, *Rousettus aegyptiacus* that represents a true but perhaps rare host species of *Hepatocystis* (1 published record of a single infected individual) has been also documented from Nigeria (Atama et al., 2019). Systematic serial surveys of the 2 specific African fruit bat species *R. aegyptiacus* and *E. helvum* (Pteropodidae) along with the commonly infected epauletted bat host species are needed to understand the different prevalences and transmission dynamics of *Hepatocystis* infections in African bats. Both *R. aegyptiacus* and *E. helvum* are common fruit bat species that form large colonies in caves and trees, respectively, and feature an extensive distribution across sub-Saharan Africa (e.g. Cooper-Bohannon, 2016; Korine, 2016). With the reported bat host records, Nigeria might be a particular suitable country/area to investigate *Hepatocystis* infections in African fruit bats.

The trypanosome infections that were detected in the 3 bat species *E. helvum*, *Mops cf. pumilus* and *Nycteris cf. macrotis* belong to 2 or 3 different *Trypanosoma* species. The trypanosomes of *Mops cf. pumilus* (Molossidae) are related to *T. erneyi*, a species which is closely related to *T. dionisii* and *T. cruzi* and that has been described from different bat species exclusively of the family Molossidae in Africa (Lima et al., 2012). The parasites of the study might represent a subspecies of *T. erneyi* as the phylogenetic analysis recovered their sequences in their own separate clade (Figs 3 and 4). The trypanosome parasites of the host species *Nycteris cf. macrotis* are closely related to the lineage of *T. cf. livingstonei* parasites that has been reported from e.g. *Nycteris thebaica* bats from South Africa (Clement et al., 2020). *T. cf. livingstonei* infections appear to be common in different African insectivorous bat species and future investigations will determine whether the divergent lineage *T. cf. livingstonei* might represent a distinct species or a subspecies of *T. livingstonei* (Lima et al., 2013; Clement et al., 2020). Lacking the gGAPDH sequence for the trypanosome infections in the 2 individuals of *E. helvum* in this study did not allow an unambiguous assignment to 1 trypanosome species. One sample showed highest 18S rRNA nucleotide identity with *T. livingstonei*, whereas the other sample was recovered as basal to a clade that comprises *Trypanosoma* sp. sequences.
from different African bats, that are closely related to *T. cf. livingstonei* and *T. livingstonei* parasites (Fig. 3). The findings of this study confirm the notion that trypanosomes of African bats are phylogenetically diverse and might harbour a variety of yet undescribed species (Clement et al., 2020). Understanding the diversity and the phylogenetic relationships of bat trypanosomes are necessary to improve our understanding of the whole group of parasites that comprise species that are a threat to humans (Hamilton et al., 2009; Lima et al., 2012). Many trypanosome lineages of the *T. cruzi* clade might have evolved in African bat species (e.g. Lima et al., 2013; Clement et al., 2020) and therefore, a more targeted systematic sampling and a subsequent molecular characterization of trypanosome species from African bats is of particular importance.

**Supplementary material.** The supplementary material for this article can be found at [https://doi.org/10.1017/S0031182022000890](https://doi.org/10.1017/S0031182022000890).

**Data availability.** All Hepatocystis and trypanosome sequences of the study are available at GenBank (NCBI) with the accession numbers ON326584 – ON326587, ON332819 – ON332820, ON494560 – ON494563, ON571545 – ON571548.

**Acknowledgements.** We thank Kamani EV, Bishir Jibrin, Nyor Delahan and Adamu O. Ngbede for assistance during field sampling. We thank Lara Palm for helping with the molecular work in the laboratory.

**Author contributions.** J. K. conceived and designed the study. J. K., A. Y. J., S. A. and O. D. A. carried out field work and bat sampling. J. S., J. K., O. W., I. E. performed molecular work and phylogenetic analysis. All authors conducted data gathering and wrote the article.

**Financial support.** J. S. is funded by an individual research grant from the German Research Foundation (DFG; project number 437846632). I. E. is supported by a PhD scholarship of the German Academic Exchange Service (DAAD).

**Conflict of interest.** The authors declare there are no conflicts of interest.

**Ethical standards.** This study was approved by the Institutional Animal Use and Care Committee (IAUCC), National Veterinary Research Institute (NVRI), Vom, permission numbers AEC/02/59/18 and AEC/03/65/19. All work was performed in accordance with the relevant guidelines and regulations regarding care and use of animals.

**References**

Atama N, Manu S, Ivande S, Roskopf SP, Matuschekvi Ch and Scharer J (2019) Survey of *Hepatocystis* parasites of fruit bats in the Amurum forest reserve, Nigeria, identifies first host record for *Roussetus aegyptiacus*. *Parasitology* 146, 1550–1554.

Austen JM and Barbosa AD (2021) Diversity and epidemiology of bat trypanosomes: a one health perspective. *Pathogens* (Basel, Switzerland) 10, 1148.

Boudengda L, Ngoubangoye B, Mboomo TM, Tsoubmou TA, Renaud F, Rougeron V and Prugnolle F (2018) Extensive diversity of malaria parasites circulating in Central African bats and monkeys. *Ecology and Evolution* 8, 10578–10586.

Bücher P, Cecchi G, Jamonneau V and Priotto G (2017) Human African trypanosomiasis. *The Lancet* 390, 2397–2409.

Clement L, Dietrich M, Markotter W, Fasel NJ, Monjadem A, Lopez-Bauccells A, Scaravelli D, Theeuw P, Pigault R, Ruedi M and Christe P (2020) Out of Africa: the origins of the protozoan blood parasites of the *Trypanosoma cruzi* clade found in bats from Africa. *Molecular Phylogenetics and Evolution* 145, 106705.

Cooper-Bohannon R (2016) *IUCN Red List of Threatened Species: Eidiolomon helvum*. IUCN Red List of Threatened Species. Available at [https://www.iucnredlist.org](https://www.iucnredlist.org).

Darriba D, Posada D, Kozlov AM, Stamatakis A, Morel B and Flouri T (2020) ModelTest-NG: a new and scalable tool for the selection of DNA and protein evolutionary models. *Molecular Biology and Evolution* 37, 291–294.

Duval L, Robert V, Csoora G, Hassanin A, Randrianarivelojosia M, Walston J, Nhim T, Goodman SM and Ariey F (2007) Multiple host-switching of Haemosporidia parasites in bats. *Malaria Journal* 6, 157.

Edler D, Klein J, Antonelli A and Silvestro D (2021) raxmIGL 2.0: a graphical interface and toolkit for phylogenetic analyses using RAxML. *Methods in Ecology and Evolution* 12, 373–377.

Galen SC, Borner J, Martinsen ES, Schaer J, Austin CC, West CJ and Perkins SL (2018) The polyphyly of *Plasmodium*: comprehensive phylogenetic analyses of the malaria parasites (order Haemosporida) reveal widespread taxonomic conflict. *Royal Society Open Science* 5, 171780.

Garnham PCC (1966) *Malaria Parasites and Other Haemosporida*. Blackwell Scientific Publications, Oxford.

Hamilton PB, Adams ER, Njiokou F, Gibson WC, Cuny G and Herder S (2009) Phylogenetic analysis reveals the presence of the *Trypanosoma cruzi* clade in African terrestrial mammals. *Infection, Genetics and Evolution* 9, 81–86.

Hamilton PB, Cruickshank C, Stevens JR, Teixeira MM and Mathews F (2012) Parasites reveal movement of bats between the New and Old Worlds. *Molecular Phylogenetics and Evolution* 63, 521–526.

Kamani J, Harrus S, Ocholi RA, Yague II, Nyango PG, Gonzalez-Miguel J and Koizumi N (2021) Molecular detection and characterization of pathogenic *Leptospira* species in bats (Chiroptera) roosting in human habitats in Nigeria, West Africa. *Zoonoses and Public Health* 68, 908–916.

Katoh K and Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* 30, 772–780.

Katoh K, Misawa K, Kuma K and Miyata T (2002) MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research* 30, 3092–3096.

Korine C (2016) *IUCN Red List of Threatened Species: Roussetus aegyptiacus*. IUCN Red List of Threatened Species. Available at [https://www.iucnredlist.org](https://www.iucnredlist.org).

Lack JB, Rochs ZP, Stanley CE, Ruedi M and Van Den Bussche RA (2010) Molecular phylogenetics of *Myostis* indicate familial-level divergence for the genus *Cistugo* (Chiroptera). *Journal of Mammalogy* 91, 976–992.

Levine ND (1988) The *Protozoan Plasmodium Apicomplexa*: Volume 2. CRC Press, Boca Raton, Florida.

Lima L, Silva FM, Neves L, Attia Ch, Takata CS, Campaner M, de Souza W, Hamilton PB and Teixeira MM (2012) Evolutionary insights from bat trypanosomes: morphological, developmental, and phylogenetic evidence of a new species, *Trypanosoma (Schizotrypanum) ernyi* sp. Nov., in African bats closely related to *Trypanosoma (Schizotrypanum) cruzi* allied species. *Protist* 163, 856–872.

Lima L, Espinosa-Alvarez O, Hamilton PB, Neves L, Takata CS, Campaner M, Attia M, de Souza W, Camargo EP and Teixeira MM (2013) *Trypanosoma livingstonei*: a new species from African bats supports the bat seeding hypothesis for the *Trypanosoma cruzi* clade. *Parasites and Vectors* 6, 221.

Lutz HL, Patterson BD, Kerbis Peterhans JC, Stanley WT, Weblea PW, Gnoske TP, Hackett SJ and Stanhope MJ (2016) Diverse sampling of East African haemosporidians reveals chiropteran origin of malaria parasites in primates and rodents. *Molecular Phylogenetics and Evolution* 99, 7–15.

Martinsen ES and Perkins SL (2013) The diversity of *Plasmodium* and other haemosporidians: the intersection of taxonomy, phylogenetics and genomics. In Carlton JM, Perkins SL and Deitsch KWK (eds), *Comparative Genomics, Evolution, and Molecular Biology*. Boca Raton, Florida.

Miller-Butterworth CM, Murphy WJ, O’Brien SJ, Jacobs DS, Springer MS and Teeling EC (2007) A family matter: conclusive resolution of the taxonomic position of the long-fingered bats, *Miniopterus* (Chiroptera). *Molecular Phylogenetics and Evolution* 24, 1553–1561.

Morrison LJ, Veza L, Rowan T and Hope JC (2016) Animal African trypanosomiasis: time to increase focus on clinically relevant parasite and host species. *Trends in Parasitology* 32, 599–607.

Noves H, Stevens JR, Teixeira M, Phekan J and Holz P (1999) A nested PCR for the *ssrRNA* gene detects *Trypanosoma bennetti* in the platypus and *Trypanosoma sp.* in wombats and kangaroos in Australia. *International Journal for Parasitology* 29, 331–339.

Patterson BD and Weblea PW (2012) Keys to the bats (Mammalia: Chiroptera) of East Africa. *Fieldiana Life and Earth Sciences* 6, 1–60.

Perkins SL and Scharer J (2016) A modern menagerie of mammalian malaria. *Trends in Parasitology* 32, 772–782.
Schaer J, Perkins SL, Decher J, Leendertz FH, Fahr J, Weber N and Matuschewski K (2013) High diversity of West African bat malaria parasites and a tight link with rodent Plasmodium taxa. Proceedings of the National Academy of Sciences 110, 17415–17419.

Schaer J, Perkins SL, Ejotre I, Vodzak ME, Matuschewski K and Reeder DM (2017) Epauletted fruit bats display exceptionally high infections with a Hepatocystis species complex in South Sudan. Scientific Reports 7, 6928.

Simmons NB (2005) Order Chiroptera. Mammal Species of the World: A Taxonomic and Geographic Reference, DE Wilson and DM Reeder Eds. Washington, DC: Smithsonian Institution Press.

Simmons NB and Cirranello AL (2022) Bat Species of the World: A Taxonomic and Geographic Database. Available at https://batnames.org (Accessed 1 May 2022).

Simpson AGB, Stevens JR and Lukeš J (2006) The evolution and diversity of kinetoplastid flagellates. Trends in Parasitology 22, 168–174.

Valkiunas G, Iezhova TA, Loiseau C and Sehgal RN (2009) Nested cytochrome B polymerase chain reaction diagnostics detect sporozoites of hemopiridian parasites in peripheral blood of naturally infected birds. Journal of Parasitology 95, 1512–1515.