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

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**Supplemental Material**

* **Supplementary Figure S1**. Micrographs of trypanosomes of the study
* **Supplemental Table S1:** Nucleotide primers used for parasite screening and sequencing
* **Supplemental Table S2:** GenBank accession numbers for phylogenetic analysis of *Hepatocystis* parasites
* **Supplementary Table S3**: GenBank accession numbers for *Hepatocystis* and trypanosome parasite sequences of the study
* **Supplementary Table S4:** Overview of investigated bat individuals of the study, parasite infections

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**Supplemental Figure S1. Micrographs of trypanosomes.** *Trypanosoma* cf. *livingstonei* parasites detected in Giemsa-stained blood smears of *Nycteris* cf. *macrotis* bat hosts (a = sample I; b = sample no KJ2). Magnification of 1000x. Bars indicate 5µm. Due to the low quality of the blood smears, no assessment of the morphological characteristics was possible.

**Supplemental Table S1:** Nucleotide primers used for parasite screening and sequencing

|  |  |  |  |
| --- | --- | --- | --- |
| **Gene** | **Primer name** | **Sequence (5´- 3´)** | **Reference** |
| ***cytb***  (haemosporidian parasites) | Hep-F3 | CTTACCTTGGGGACAAATGAGTTATT | Schaer *et al*., 2013 |
| Hep-R3 | CTCTAGCACCAAATGTCATTTTAAATTG | Schaer *et al*., 2013 |
| DW2 | TAATGCCTAGACGTATTCCTGATTATCCAG | Perkins and Schall, 2002 |
| DW4 | TGTTTGCTTGGGAGCTGTAATCATAATGTG | Perkins and Schall, 2002 |
| 3932-F | GGGTTATGTATTACCTTGGGGTC | Perkins and Schall, 2002 |
| 3932-R | GACCCCAAGGTAATACATAACCC | Perkins and Schall, 2002 |
| ***cox1***  (haemosporidian parasites) | Cox1-F | CTATTTATGGTTTTCATTTTTATTTGGTA | Martinsen *et al*., 2008 |
| Cox1-R | AGGAATACGTCTAGGCATTACATTAAATCC | Martinsen *et al*., 2008 |
| Cox-in-F | ATGATATTTACARTTCAYGGWATTATTATG | Martinsen *et al*., 2008 |
| Cox-in-R | GTATTTTCTCGTAATGTTTTACCAAAGAA | Martinsen *et al*., 2008 |
| Cox-mid-F | TTATTCTGGTTTTTTGGTCATCCAG | Martinsen *et al*., 2008 |
| Cox-mid-R | CTGGATGACCAAAAAACCAGAATAA | Martinsen *et al*., 2008 |
| ***clpc***  (haemosporidian parasites) | Clpc-out-F | AAACTGAATTAGCAAAAATATTA | Martinsen *et al*., 2008 |
| Clpc-out-R | CGWGCWCCATATAAAGGAT | Martinsen *et al*., 2008 |
| Clpc-in-F | GATTTGATATGAGTGAATATATGG | Martinsen *et al*., 2008 |
| Clpc-in-R | CCATATAAAGGATTATAWG | Martinsen *et al*., 2008 |
| ***ef2***  (haemosporidian parasites) | EF2-F | GTTCGTGAGATCATGAACAAAAC | Schaer *et al*., 2013 |
| EF2-R | CCTTGTAAACCAGAACCAAA | Schaer *et al*., 2013 |
| **18S rRNA**  (trypanosomes) | TRY927F | GAAACAAGAAACACGGGAG | Noyes *et al*., 1999 |
| TRY927R | CTACTGGGCAGCTTGGA | Noyes *et al*., 1999 |
| SSU561F | TGGGATAACAAAGGAGCA | Noyes *et al*., 1999 |
| SSU561R | CTGAGACTGTAACCTCAAAGC | Noyes *et al*., 1999 |
| **gGAPDH**  (trypanosomes) | G5 | ACMAGRTCCACCACRCGGTG | Hamilton *et al*., 2004 |
| G3 | TTYGCCGYATYGGYCGCATGG | Hamilton *et al*., 2004 |
| G1 | CGCGGATCCASGGYCTYMTCGGBAMKGAGAT | Hamilton *et al*., 2004 |
| G4A | GTTYTGCAGSGTCGCCTTGG | Hamilton *et al*., 2004 |
| ***cytb***  (bat hosts) | L14724 | CGAAGCTTGATATGAAAAACCATCGTTG | Päabo 1989 |
| H15915 | GGAATTCATCTCTCCGGTTTACAAGAC | Irwin *et al*. 1991 |
| ***fgb***  (bat hosts) | FGB-F | CCACAACRGCATGTTCTTCAGCAC | Hassanin & Ropiquet, 2007 |
| FGB-R | GTATCTGCCATTTGGATTGGCTGC | Hassanin & Ropiquet, 2007 |
| ***acox2***  (bat hosts) | ACOX2-F1 | CCTSGGCTCDGAGGAGCAGAT | Salicini *et al*., 2011 |
| ACOX2-R1 | GGGCTGTGHAYCACAAACTCCT | Salicini *et al*., 2011 |

*cytb*, cytochrome b; *cox1*, cytochrome oxidase I; *clpc*, apicoplast caseinolytic protease; *ef2*, nuclear elongation factor 2; 18S rRNA*,* SSU rRNA, a component of the eukaryotic ribosomal small subunit; gGAPDH, Glyceraldehyde-3-phosphate dehydrogenase; Nuclear introns for bat genotyping: *acox*2 (Acyl-CoA oxidase 2, intron 3); *rogdi* (Rogdi-like protein gene, intron 7); *fgb* (Beta-fibrinogen gene, intron 7)

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**Supplemental Table S2:** GenBank accession numbers for phylogenetic analysis of *Hepatocystis* parasites (samples from this study highlighted in bold)

|  |  |  |
| --- | --- | --- |
| **Parasite (host group)** | **Sample (host species)** | ***cytb*** |
| *Leucocytozoon* (Aves) | *Leucocytozoon* sp. (2109) | EU254518 |
| *Leucocytozoon* sp. (2208) | EU254520 |
| *Leucocytozoon* sp. (P157) | EU254519 |
| *Haemoproteus* (Aves) | *HHaemoproteus columbae* (2111) | EU254548 |
| *Haemoproteus columbae* (2146) | EU254553 |
| *Haemoproteus columbae* | FJ168562 |
| *Plasmodium* (Primates) | *Plasmodium falciparum* | DQ642845 |
| *Plasmodium gaboni* | FJ895307 |
| *Plasmodium reichenowi* | AJ251941 |
| *Plasmodium* sp. (ex *Pan troglodytes*) | HM235391 |
| *Plasmodium* sp. (ex *Gorilla gorilla*) | HM235288 |
| *Plasmodium* (Rodentia) | *Plasmodium berghei* | DQ414645 |
| *Plasmodium chabaudi* | DQ414649 |
| *Plasmodium vinckei* | DQ414651 |
| *Plasmodium yoelii* | AY099051 |
| *Plasmodium* (Chiroptera) | *Plasmodium cyclopsi* | KF159710 |
| *Plasmodium voltaicum* | KF159671 |
| *Polychromophilus* (Chiroptera) | *Polychromophilus* sp. (ex *Miniopterus villiersii*) | KF159681 |
| *Polychromophilus melanipherus* | JN990709 |
| *Polychromophilus murinus* | HM055583 |
| *Polychromophilus* sp. (ex *Pipistrellus* aff. *grandidieri*) | KF159714 |
| *Hepatocystis* (Chiroptera) | *Hepatocystis* sp. (ex *E. pusillus,* Guinea) | KF159683 |
| *Hepatocystis* sp. (ex *E. pusillus,* Guinea) | KF159680 |
| *Hepatocystis* sp. (ex *E. pusillus,* Guinea) | KF159693 |
| *Hepatocystis* sp. (ex *E. pusillus,* Guinea) | KF159704 |
| *Hepatocystis* sp. (ex *E. pusillus,* Guinea) | KF159683 |
| *Hepatocystis* sp. (ex *E. pusillus,* Cameroon) | MZ460922 |
| *Hepatocystis* sp. (ex *E. pusillus,* Cameroon) | MZ460918 |
| *Hepatocystis* sp. (ex *E. pusillus,* Cameroon) | MZ460915 |
| *Hepatocystis* sp. (ex *E. pusillus,* Nigeria) | MK634490 |
| *Hepatocystis* sp. (ex *E. pusillus,* Nigeria) | MK634507 |
| *Hepatocystis* sp. (ex *E. pusillus,* Nigeria) | MK634505 |
| *Hepatocystis* sp. (ex *E. pusillus,* Nigeria) | MK634501 |
| *Hepatocystis* sp. (ex *E. pusillus,* Nigeria) | MK634496 |
| *Hepatocystis* sp. (ex *E. pusillus,* Nigeria) | MK634503 |
| *Hepatocystis* sp. (ex *E. pusillus,* Nigeria) | MK634487 |
| *Hepatocystis* sp. (ex *E. pusillus,* Nigeria) | MK634497 |
| *Hepatocystis* sp. (ex *E. pusillus,* Nigeria) | MK634489 |
| *Hepatocystis* sp. (ex *E. pusillus,* Nigeria) | MK634506 |
| *Hepatocystis* sp. (ex *E. pusillus,* Nigeria) | MK634488 |
| *Hepatocystis* sp. (ex *E. pusillus,* South Sudan) | KY753527 |
| *Hepatocystis* sp. (ex *E. pusillus,* South Sudan) | KY753525 |
| *Hepatocystis* sp. (ex *Epom.* sp*.,* Kenya) | KY753518 |
| *Hepatocystis* sp. (ex *Epom.* sp*.,* Kenya) | KY753519 |
| *Hepatocystis* sp. (ex *Epom.* sp.*,* South Sudan) | KY753506 |
| *Hepatocystis* sp. (ex *Epom.* sp.*,* South Sudan) | KY753507 |
| *Hepatocystis* sp. (ex *Epom.* sp.*,* South Sudan) | KY753513 |
| *Hepatocystis* sp. (ex *Epom.* sp.*,* South Sudan) | KY753510 |
| *Hepatocystis* sp. (ex *Epom.* sp.*,* South Sudan) | KY753516 |
| *Hepatocystis* sp. (ex *E. buettikoferi*, Guinea) | KF159706 |
| *Hepatocystis* sp. (ex *E. buettikoferi*, Guinea) | KF159703 |
| *Hepatocystis* sp. (ex *E. franqueti*, Uganda) | KT750344 |
| *Hepatocystis* sp. (ex *E. franqueti*, Uganda) | KT750356 |
| *Hepatocystis* sp. (ex *E. franqueti*, Uganda) | KT750351 |
| *Hepatocystis* sp. (ex *E. franqueti*, Uganda) | KT750353 |
| *Hepatocystis* sp. (ex *E. franqueti*, South Sudan) | KY753503 |
| *Hepatocystis* sp. (ex *E. franqueti*, South Sudan) | KY753504 |
| *Hepatocystis* sp. (ex *Hipposideros* sp.*,* South Sudan) | KY753520 |
| *Hepatocystis* sp. (ex *H. monstrosus,* Liberia) | KF159689 |
| *Hepatocystis* sp. (ex *H. monstrosus,* Liberia) | KF159712 |
| *Hepatocystis* sp. (ex *H. monstrosus,* South Sudan) | KY753521 |
| *Hepatocystis* sp. (ex *M. leptodon,* Ivory Coast) | KF188066 |
| *Hepatocystis* sp. (ex *M. leptodon,* Liberia) | KF159705 |
| *Hepatocystis* sp. (ex *M. torquata,* Uganda) | KT750356 |
| *Hepatocystis* sp. (ex *M. torquata,* Uganda) | KT750357 |
| *Hepatocystis* sp. (ex *M. torquata,* Uganda) | KT750342 |
| *Hepatocystis* sp. (ex *N. veldkampii,* Guinea) | KF159698 |
| *Hepatocystis* sp. (ex *N. veldkampii,* Guinea) | EU254528 |
| *Hepatocystis* sp. (ex *N. veldkampii,* Liberia) | KF159698 |
| *Hepatocystis* sp. (ex *R. aegyptiacus,* Nigeria) | MK634508 |
| *Hepatocystis* sp. (*ex Eidolon helvum, Gabon)* | MG602649 |
| ***Hepatocystis* sp. (ex *Eidolon helvum*, J53, Nigeria)** | **ON494563** |
| ***Hepatocystis* sp. (ex *Eidolon helvum*, KJ72, Nigeria)** | **ON494561** |
| ***Hepatocystis* sp. (ex *Eidolon helvum*, KJ82, Nigeria)** | **ON494560** |
| ***Hepatocystis* sp. (ex *Eidolon helvum*, KJ63, Nigeria)** | **ON494562** |

Mozam. = Mozambique, Switz. = Switzerland; SL = Sierra Leone, SoSu = South Sudan

**Supplemental Table S3**: GenBank accession numbers for *Hepatocystis* and trypanosome parasite sequences of the study

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Sample number** | **Bat species\*** | ***cytb* (*Hepatocystis*)** | **18S rRNA (trypanosome)** | **gGAPDH (trypanosome)** |
| J53 | *Eidolon helvum* | ON494563 | NA | NA |
| KJ2 | *Eidolon helvum* | NA | ON326584 | - |
| KJ14 | *Eidolon helvum* | NA | ON326585 | - |
| KJ63 | *Eidolon helvum* | ON494562 | NA | NA |
| KJ72 | *Eidolon helvum* | ON494561 | NA | NA |
| KJ82 | *Eidolon helvum* | ON494560 | NA | NA |
| VC9 | *Mops* cf*. pumilus* | NA | ON332819\*\* | - |
| VC13 | *Mops* cf*. pumilus* | NA | ON332820\*\* | ON571548 |
| A | *Nycteris* cf*. macrotis* | NA | - | ON571545\*\*\* |
| E | *Nycteris* cf*. macrotis* | NA | ON326586\*\*\* | ON571546\*\*\* |
| G | *Nycteris* cf*. macrotis* | NA | ON326587\*\*\* | - |
| KJ107 | *Nycteris* cf*. macrotis* | NA | - | ON571547\*\*\* |

\*Please note that almost all 95 bat individuals have been genotyped; \*\*18sRNA sequences identical for all infected *M*. cf. *pumilus*; \*\*\*all eleven infected *N*. cf. *macrotis* featured identical trypanosome 18sRNA and gGAPDH sequences; NA = not applicable (no infection with the respective parasite taxon); - = sequence could not be successfully amplified

**Supplemental Table S4:** Overview of investigated bat individuals of the study, parasite infections

|  |  |  |  |
| --- | --- | --- | --- |
| **Sample**  **Number** | **Bat species** | **Infection with**  **haemosporidian parasite** | **Infection with trypanosome** |
| J201 | *Eidolon helvum* | - | - |
| J204 | *Eidolon helvum* | - | - |
| J206 | *Eidolon helvum* | - | - |
| J208 | *Eidolon helvum* | - | - |
| J210 | *Eidolon helvum* | - | - |
| J216 | *Eidolon helvum* | - | - |
| J224 | *Eidolon helvum* | - | - |
| J231 | *Eidolon helvum* | - | - |
| J233 | *Eidolon helvum* | - | - |
| J239 | *Eidolon helvum* | - | - |
| J240 | *Eidolon helvum* | - | - |
| J242 | *Eidolon helvum* | - | - |
| J25 | *Eidolon helvum* | - | - |
| J40 | *Eidolon helvum* | - | - |
| J50 | *Eidolon helvum* | - | - |
| J51 | *Eidolon helvum* | - | - |
| J53 | *Eidolon helvum* | *Hepatocystis* sp. | - |
| J55 | *Eidolon helvum* | - | - |
| J56 | *Eidolon helvum* | - | - |
| J57 | *Eidolon helvum* | - | - |
| J58 | *Eidolon helvum* | - | - |
| J59 | *Eidolon helvum* | - | - |
| J61 | *Eidolon helvum* | - | - |
| J62 | *Eidolon helvum* | - | - |
| J63 | *Eidolon helvum* | - | - |
| J64 | *Eidolon helvum* | - | - |
| J65 | *Eidolon helvum* | - | - |
| J66 | *Eidolon helvum* | - | - |
| J67 | *Eidolon helvum* | - | - |
| J68 | *Eidolon helvum* | - | - |
| J82 | *Eidolon helvum* | - | - |
| KJ1 | *Eidolon helvum* | - | - |
| KJ10 | *Eidolon helvum* | - | - |
| KJ11 | *Eidolon helvum* | - | - |
| KJ111 | *Eidolon helvum* | - | - |
| KJ12 | *Eidolon helvum* | - | - |
| KJ121 | *Eidolon helvum* | - | - |
| KJ125 | *Eidolon helvum* | - | - |
| KJ126 | *Eidolon helvum* | - | - |
| KJ127 | *Eidolon helvum* | - | - |
| KJ129 | *Eidolon helvum* | - | - |
| KJ13 | *Eidolon helvum* | - | - |
| KJ130 | *Eidolon helvum* | - | - |
| KJ131 | *Eidolon helvum* | - | - |
| KJ132 | *Eidolon helvum* | - | - |
| KJ14 | *Eidolon helvum* | - | *Trypanosoma* cf. *livingstonei* |
| KJ15 | *Eidolon helvum* | - | - |
| KJ16 | *Eidolon helvum* | - | - |
| KJ17 | *Eidolon helvum* | - | - |
| KJ18 | *Eidolon helvum* | - | - |
| KJ19 | *Eidolon helvum* | - | - |
| KJ2 | *Eidolon helvum* | - | *Trypanosoma* cf. *livingstonei* |
| KJ21 | *Eidolon helvum* | - | - |
| KJ3 | *Eidolon helvum* | - | - |
| KJ4 | *Eidolon helvum* | - | - |
| KJ5 | *Eidolon helvum* | - | - |
| KJ6 | *Eidolon helvum* | - | - |
| KJ63 | *Eidolon helvum* | *Hepatocystis* sp. | - |
| KJ69 | *Eidolon helvum* | - | - |
| KJ7 | *Eidolon helvum* | - | - |
| KJ70 | *Eidolon helvum* | - | - |
| KJ72 | *Eidolon helvum* | *Hepatocystis* sp. | - |
| KJ8 | *Eidolon helvum* | - | - |
| KJ82 | *Eidolon helvum* | *Hepatocystis* sp. | - |
| KJ87 | *Eidolon helvum* | - | - |
| KJ9 | *Eidolon helvum* | - | - |
| KJ90 | *Eidolon helvum* | - | - |
| KJ92 | *Eidolon helvum* | - | - |
| GB118 | *Mops* cf. *condylurus* | - | - |
| GB119 | *Mops* cf. *condylurus* | - | - |
| GB120 | *Mops* cf. *condylurus* | - | - |
| VC11 | *Mops* cf. *pumilus* | - | - |
| VC12 | *Mops* cf. *pumilus* | - | - |
| VC17 | *Mops* cf. *pumilus* | - | - |
| VC3 | *Mops* cf. *pumilus* | - | - |
| VC5 | *Mops* cf. *pumilus* | - | - |
| VC6 | *Mops* cf. *pumilus* | - | *Trypanosoma* cf*. erneyi* |
| VC9 | *Mops* cf. *pumilus* | - | *Trypanosoma* cf*. erneyi* |
| VC13 | *Mops* cf. *pumilus* | - | *Trypanosoma* cf*. erneyi* |
| KJ-A | *Nycteris* cf. *macrotis* | - | *Trypanosoma* cf. *livingstonei* |
| KJ-B | *Nycteris* cf. *macrotis* | - | *Trypanosoma* cf. *livingstonei* |
| KJ-C | *Nycteris* cf. *macrotis* | - | *Trypanosoma* cf. *livingstonei* |
| KJ-D | *Nycteris* cf. *macrotis* | - | - |
| KJ-E | *Nycteris* cf. *macrotis* | - | *Trypanosoma* cf. *livingstonei* |
| KJ-F | *Nycteris* cf. *macrotis* | - | *Trypanosoma* cf. *livingstonei* |
| KJ-G | *Nycteris* cf. *macrotis* | - | *Trypanosoma* cf. *livingstonei* |
| KJ-H | *Nycteris* cf. *macrotis* | - | - |
| KJ-I | *Nycteris* cf. *macrotis* | - | *Trypanosoma* cf. *livingstonei* |
| KJ-J | *Nycteris* cf. *macrotis* | - | - |
| KJ-K | *Nycteris* cf. *macrotis* | - | *Trypanosoma* cf. *livingstonei* |
| KJ104 | *Nycteris* cf. *macrotis* | - | *Trypanosoma* cf. *livingstonei* |
| KJ107 | *Nycteris* cf. *macrotis* | - | *Trypanosoma* cf. *livingstonei* |
| KJ128 | *Nycteris* cf. *macrotis* | - | *Trypanosoma* cf. *livingstonei* |
| KJ-L | *Nycteris* cf. *macrotis* | - | - |
| KJ-M | *Nycteris* cf. *macrotis* | - | - |