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Short communication

Nycteria and *Polychromophilus* parasite infections of bats in Central Gabon[☆]

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ABSTRACT

Haemosporida are arthropod-borne blood parasites that infect a wide range of vertebrate hosts, including numerous species of bats. Here, we present data of haemosporidian infections in different bat species that were surveyed in Ngounié province, Gabon. We detected *Nycteria* parasites in *Rhinolophus* bats and *Polychromophilus* in *Miniopterus minor*, a rare and poorly known bat species. Strikingly, no *Hepatocystis* parasites, which are abundant in epauletted fruit bats elsewhere in Africa, were detected. Our findings suggest that *Hepatocystis* infections in bats display diverse regional patterns of distribution and transmission dynamics, that cannot be predicted from host abundance. *Nycteria* parasites are widely distributed in several African rhinolophid species and *Polychromophilus* parasites of diverse *Miniopterus* species worldwide belong to the same parasite species.

1. Introduction

Haemosporidia (Apicomplexa) are arthropod-borne parasites that infect blood and different tissues of diverse vertebrate hosts and include *Plasmodium*, the causative agent of malaria (Garnham, 1966). Besides *Plasmodium,* eighteen haemosporidian genera have been described to date; revealing their evolution and parasite/host adaptations is key to understanding this important human disease (Galen et al., 2018). Members of the genera *Hepatocystis*, *Nycteria* and *Polychromophilus* lack erythrocytic schizogony, which is the distinct replication phase inside red blood cells and the exclusive cause of clinical symptoms and complications of malaria. Besides bats, *Hepatocystis* parasites infect a range of mammals, including primates, whereas *Nycteria* and *Polychromophilus* have been found exclusively in bats (Garnham, 1966).

Studies focusing on identification and molecular analyses of haemosporidian parasites in Afrotropical bats are still scarce and refer to few locations only (Duval et al., 2012; Schaer et al., 2013, 2015, 2017; Lutz et al., 2016; Obame-Nkoghe et al., 2016). Furthermore, knowledge of the ecology and distribution of many host species is also rudimentary but essential to comprehend parasite biology. Therefore, additional sampling and molecular data with regard to host taxa and localities are needed. Here, we present data from investigations of haemosporidian infections in bats in the province Ngounié, Gabon and add important information to the distribution, prevalence and phylogeny of these neglected parasites.

2. Methods

A rapid survey of haemosporidian infections in bats was conducted at the beginning of the dry season in June 2015, at five different study sites around Fougamou and in the Waka National Park in Gabon (Held et al., 2017). Bats were captured with standard mist netting methods, using a combination of different canopy and ground level mist nets that were placed in different habitats (Kunz and Parsons, 2009). Captured

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[☆] Nucleotide sequence data reported in this paper are available in the GenBank database under accession nos. MK098839-MK098860.

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Table 1
Investigated bat species and prevalence of haemosporidian infections.

Bat family	Bat Species	[a]Prevalence	Parasite genus
Pteropodidae	*Micropterопus pusillus*	0/28 (0%)	–
	Epomops franqueti	0/21 (0%)	–
	Rousettus aegyptiacus	0/17 (0%)	–
	Megaloglossus woermanni	0/11 (0%)	–
Rhinolophidae	*Rhinolophus landeri*	2/2 (100%)	*Nycteria*
	Rhinolophus sp. 1 (*ferrumequinum*-group)	0/2 (0%)	–
	Rhinolophus sp. 2 (*capensis*-group)	3/3 (100%)	*Nycteria*
	Rhinolophus sp. 3 (*capensis*-group)	0/1 (0%)	–
Hipposideridae	*Hipposideros* aff. *ruber*	0/3 (0%)	–
Miniopteridae	[b]*Miniopterus minor occidentalis*	2/2 (100%)	*Polychromophilus*
Vespertilionidae	*Pipistrellus nanulus*	0/1 (0%)	–
Molossidae	*Chaerephon pumilus*	0/1 (0%)	–

[a] Number of infected individuals/total number of investigated individuals (prevalence in %).

[b] The individuals of *Miniopterus minor* of this study belong to *Miniopterus minor occidentalis*, a subspecies only recorded from few localities in the Democratic Republic of the Congo and the Congo Brazzaville.

bats were individually held in cloth bags and released after identification and sampling. The rare bat species *Miniopterus minor*, listed as “Data Deficient” by the IUCN Red List, was documented for the first time for this country (IUCN, 2018). Out of four *Rhinolophus* species, only *Rhinolophus landeri* was identified to species level, whereas the other three species are preliminarily assigned to species groups (based on molecular and morphological data), and further identification is subject to ongoing research (Table 1).

In total, 92 bats in six bat families were screened for haemosporidian infections (Table 1). Blood samples (approximately 50 μl) were collected as thin blood smears for morphological identification of blood parasites and preserved on DNA FTA cards for molecular identification. Blood was obtained from the venous puncture of the antebrachial or interfemoral vein. Approval to capture and study bats was granted by the “Centre national de la recherché scientifique” (CENAREST), the responsible wildlife authority in Gabon (N:AR0016/15/MESRS/CENAREST/CG/CST/CSAR).

Microscopy and molecular methods were used to detect and identify the haemosporidian parasites and to investigate their phylogenetic relationships among the large group of malaria parasites (Haemosporida) following Schaer et al. (2013, 2015). This included light microscopy of Giemsa-stained thin blood smears at a magnification of 1,000× and extraction of genomic DNA from DNA FTA cards using the DNeasy extraction kit (Qiagen). Four genes from the three parasite genomes were amplified (mitochondrial *cytb*, *cox1;* apicoplast *clpC*; nuclear *EF2*). To identify potential sub-microscopic infections, fruit bat (Pteropodidae) samples were additionally screened using *Hepatocystis*-specific *cytb*-primers (HepF3/HepR3; Schaer et al., 2013). PCR products were sequenced in both directions using BigDye v3.0 (Applied Biosystems) and run on an ABI-373 sequencer (primers and accession numbers in Tables S1 and S2). Phylogenetic analyses of the concatenated alignments were carried out with PartitionFinder v.2 (Lanfear et al., 2017) and MrBayes v3.2.6 (Huelsenbeck and Ronquist, 2001). Bayesian inference methods were conducted with two runs of four chains (three heated, one cold, temperature = 0.02) each for 10 million generations. The first 25% of trees were discarded as burn-in. Mixing and convergence of runs, and effective sample size (ESS > 500) were valued with Tracer v1.6 (Rambaut et al., 2014).

3. Results and discussion

The majority of samples belonged to fruit bats (Pteropodidae; 77 out of 92), including 49 epauletted fruit bats (*Micropteropus pusillus,* $n = 28$ and *Epomops franqueti,* $n = 21$) (Table 1). Interestingly, no infections were detected in the latter, which is in marked contrast to previous reports, where epauletted fruit bat species showed constant (high) prevalences of 30%–100% of *Hepatocystis* infections in different areas of Africa throughout different seasons (Schaer et al., 2013, 2017; Lutz et al., 2016). This is the first description of a sampling area where *Hepatocystis* transmission is either very low or completely absent. However, the survey was conducted in the dry season and our findings underscore the need for more cross-sectional and longitudinal surveys towards a better understanding of the ecological factors that drive parasite transmission in different habitats across Africa. The other two pteropodid species of this study, *Rousettus aegyptiacus* ($n = 17$) and *Megaloglossus woermanni* ($n = 11$), have not been described as infected by haemosporidian parasites (*e.g.* Schaer et al., 2013; Lutz et al., 2016) and our results support this finding (Table 1).

We detected *Nycteria* infections in five out of eight individuals of *Rhinolophus,* (Table 1). This might indicate that the local infectivity can occasionally be higher than the reported previously prevalences of 28% and 9% (Schaer et al., 2015; Lutz et al., 2016). Our data (together with findings in previous studies) suggest that *Nycteria* parasites are common in certain species of the species-rich genus *Rhinolophus*. For instance, *Rhinolophus landeri* exhibits high prevalences of up to 100% across its distribution range (*e.g.* Guinea, Sierra Leone, South Sudan), whereas other species of *Rhinolophus* are uninfected according to our current knowledge (*e.g.* Schaer et al., 2015; Lutz et al., 2016). In our study, both individuals of *R. landeri* as well as all three individuals of *Rhinolophus* sp.2 (*capensis*-group) were infected with *Nycteria* (Fig. 1). The blood stages resemble those of *Nycteria congolensis* and *Nycteria gabonensis* (Krampitz and de Faveaux, 1960; Rosin et al., 1978), but could not unequivocally be assigned to the morphospecies (Fig. 1A). Based on phylogenetic analysis the sequences group within a clade of *Nycteria* parasites from other African *Rhinolophus* bats, including *N. gabonensis* and *N.* cf. *congolensis* (Schaer et al., 2015; Karadjian et al., 2016) (Fig. 1C). Together with previous findings (Schaer et al., 2015; Lutz et al., 2016), our data indicate that *Nycteria* parasites are common and widespread in African rhinolophids. Further investigations are needed to understand potential host species specificity and correlations with the life histories of host bat species.

Polychromophilus parasites were identified in the two individuals of *Miniopterus minor* (Table 1), based on gametocyte morphology and phylogenetic analysis (Fig. 2). The sequences group closely with *Polychromophilus melanipherus,* a species that is common in *Miniopterus* bats worldwide and possibly represents a species complex (Duval et al., 2012). This finding underlines the clear genetic distinction, most likely at the species level, between *Polychromophilus* parasites of Vespertilionidae and Miniopteridae (Witsenburg et al., 2012). Two morpho-species of *Polychromophilus* have been described from African *Miniopterus* species, *Polychromophilus corradetti* from *Miniopterus inflatus* in Gabon and *Polychromophilus adami* from *Miniopterus minor* in the Democratic Republic of the Congo (Landau et al., 1980). However, the close genetic relationship of the parasites of *M. minor* in this study with *P. melanipherus* parasites from Europe and Africa do not point to an assignment to a distinct species or to any of the two aforementioned morphospecies respectively. As suggested before, *P. corradetti* and *P. adami* could belong to the same monophyletic *P. melanipherus* species complex (Duval et al., 2012). Unexpectedly, two *Polychromophilus* parasites from West Africa from vespertilionid bats, from *Pipistrellus* aff. *grandidieri* and *Neoromicia capensis* from Guinea (Schaer et al., 2013), form a distinct group, which is basal to the *melanipherus/murinus* clades. We hypothesize that these samples might represent a different species of *Polychromophilus.* Future studies in this region are warranted to support or disprove this assumption.

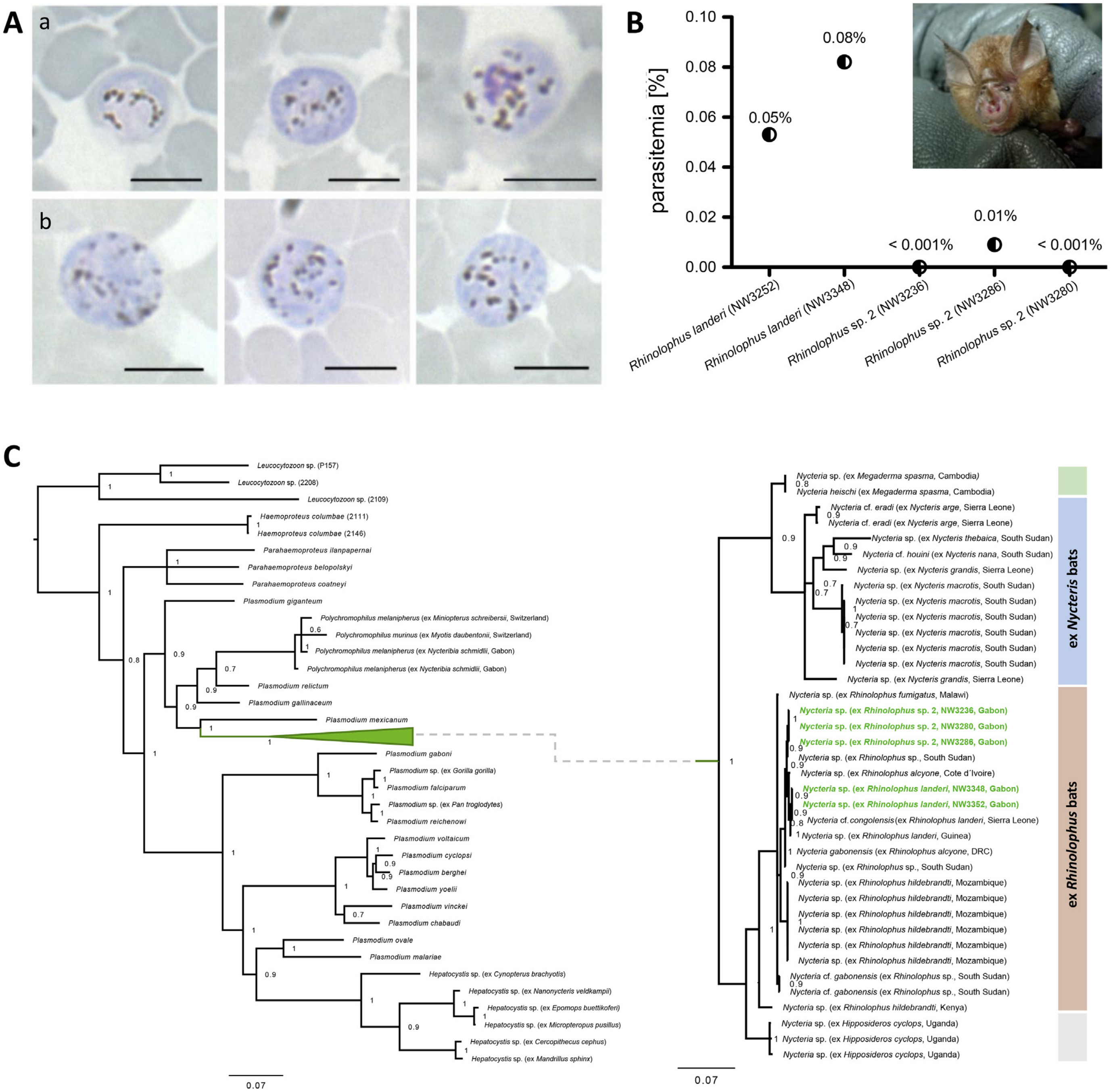

Fig. 1. (A) Representative micrographs of gametocytes of *Nycteria* parasites from *Rhinolophus* host species in Gabon. Bar = 5 μm. (a) ex *R. landeri* (NW3252), (b) ex *R.* sp.2 (*capensis*-group) (NW3286). (B) *Nycteria* parasitemia in %. Parasitemia values in the two infected *R. landeri* (0.05%, 0.08%) and in the three infected *Rhinolophus* sp.2 (*capensis*-group) (0.1% to < 0.001%), were lower than the reported values (mean of 0.2%) from Schaer et al. (2015). Inserted photograph of *R. landeri* (NW3252) (courtesy of N. Weber) (C) Three-genome phylogeny of *Nycteria* parasites in the context of the major haemosporidian parasite clades recovered by Bayesian analysis. Posterior probability values are given. Phylogenetic analyses were conducted on the concatenated alignment (total of 3138 bp) of the mitochondrial cytochrome *b* (1119 bp) and cytochrome oxidase I (978 bp), the nuclear elongation factor 2 (513 bp), and the apicoplast caseinolytic protease (528 bp). Placement of *Nycteria* parasites (collapsed clade on the left) as sister to a clade that contains the lizard and bird *Plasmodium* species, as shown before (Schaer et al., 2015). The uncollapsed clade (on the right) includes all published *Nycteria* sequences to date. *Nycteria* parasites of *Rhinolophus* (brown clade), *Nycteris* (blue clade), *Hipposideros* (grey clade) and *Megaderma* (green clade) group in their own host bat family specific clades. The sequences of the current study (highlighted in bold green) group well between *Nycteria* parasites sequences of other African rhinolophid hosts that were assigned to *Nycteria gabonensis* and *Nycteria congolensis.* (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

In conclusion, our results show that *Hepatocystis* infections in African epauletted fruit bat species cannot be predicted from previous findings of very high prevalence. Apparently, *Hepatocystis* distribution covers a complete range from locally abundant to common and absent. This unexpected finding poses interesting questions on *Hepatocystis* transmission dynamics and highlights the need to identify the vector that transmits these parasites to bats. This study also adds important molecular information on the two neglected haemosporidian genera *Nycteria* and *Polychromophilus* and their host specificity, and encourages larger efforts for systematic taxon sampling across the continent.

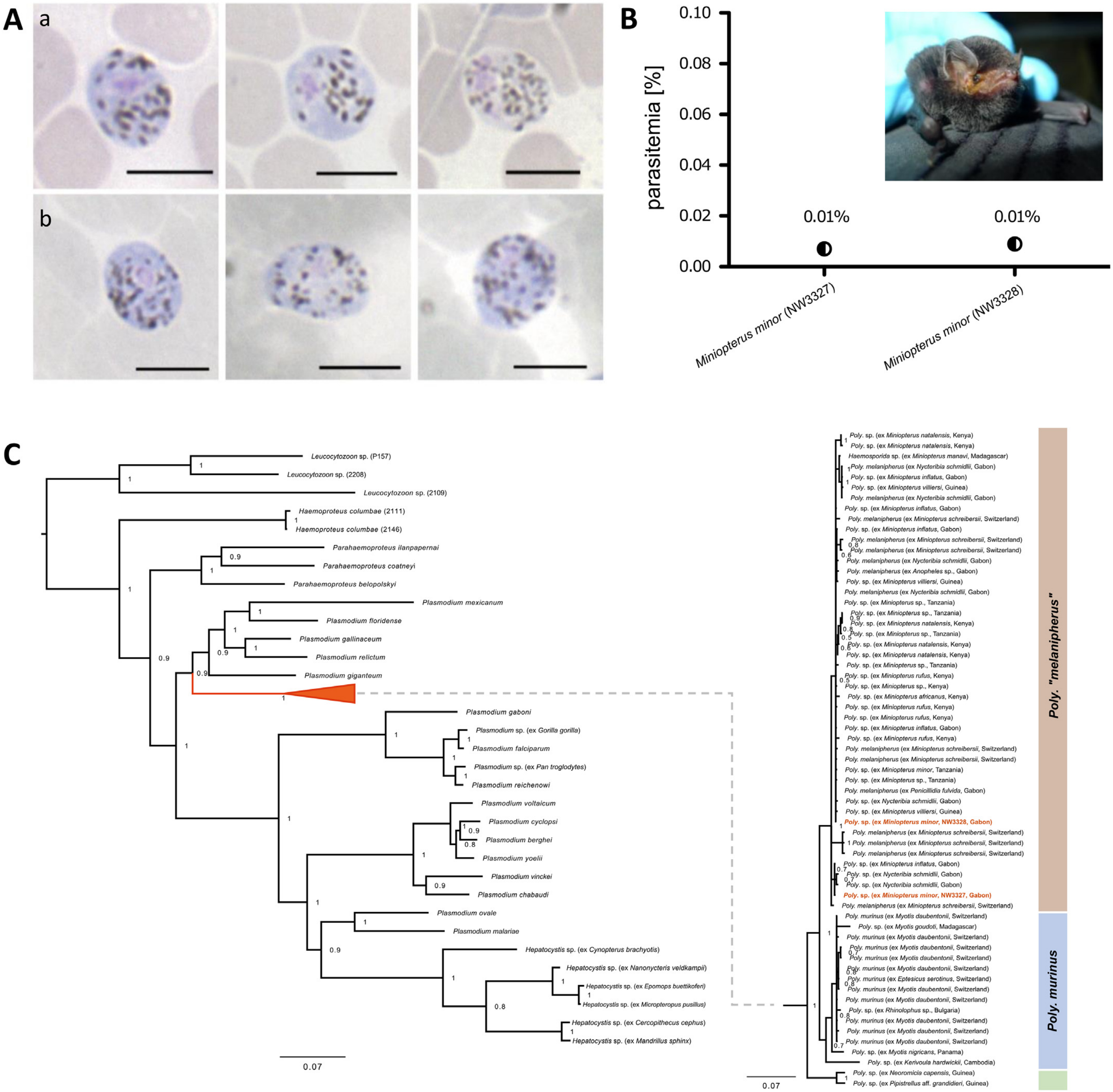

Fig. 2. (A) Representative micrographs of gametocytes of *Polychromophilus* from *Miniopterus minor* in Gabon. Bar = 5 μm. (a) ex NW3328, (b) ex NW3327. (B) *Polychromophilus* parasitemia in %. Both individuals exhibited low parasitemia values of 0.01%. Inserted photograph of *M. minor occidentalis* (NW3328) (courtesy of N. Weber) (C) Three-genome phylogeny of *Polychromophilus* parasites in the context of the major haemosporidian parasite clades recovered by Bayesian analysis. Posterior probability values are given. Phylogenetic analyses were conducted on the concatenated alignment (total of 3138 bp) of the mitochondrial cytochrome *b* (1119 bp) and cytochrome oxidase I (978 bp), the nuclear elongation factor 2 (513 bp), and the apicoplast caseinolytic protease (528 bp). Placement of *Polychromophilus* parasites (collapsed clade on the left) as sister to a clade that contains the lizard and bird *Plasmodium* species, as shown before (Witsenburg et al., 2012). The uncollapsed clade (on the right) includes representatives of published *Polychromophilus murinus* sequences and sequences from all undetermined *Polychromophilus* species as well as *Polychromophilus melanipherus* samples from all reported *Miniopterus species* and associated Nycteribiidae fly hosts (*e.g.* Obame-Nkoghe et al., 2016). Sequences fall in two main clades, representing *P. melanipherus* and *P. murinus,* respectively. Only the two parasite samples from *Pipistrellus* aff. *grandidieri* and *Neoromicia capensis* from Guinea (Schaer et al., 2013) group basal to the *melanipherus/murinus* clades and might represent a different species of *Polychromophilus* (green clade). The two samples of the current study, isolated from *Miniopterus minor,* group well within the *melanipherus*-clade (highlighted in bold and red, brown clade) and represent two haplotypes (cyt*b* sequences differ by 1%). *Polychromophilus melanipherus* possibly represents a complex of species (*e.g.* Duval et al., 2012). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.meegid.2018.11.022.

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