Diversity, Distribution, and Drivers of Polychromophilus Infection in Malagasy Bats

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Research

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Abstract

**Background:** Numerous studies have been undertaken to improve knowledge on apicomplexan parasites infecting vertebrates, including humans. Of these parasites, the genus *Plasmodium* has been most extensively studied because of its socio-economic and public health impacts. In non-human vertebrates, studies on malaria or malaria-like groups have been conducted but information is far from complete. On Madagascar, recent studies on bat blood parasites indicate that three families, namely Miniopteridae, Rhinonycteridae, and Vespertilionidae, are infected by the genus *Polychromophilus* with pronounced host specificity: *Miniopterus* spp. harbor *P. melanipherus*, while *Myotis goudoti* is infected by *P. murinus*. However, most of the individuals analyzed in these previous studies were sampled on the western and central portions of the island. The aims of this study are 1) to add new information on bat blood parasites in eastern Madagascar and 2) highlight biotic and abiotic variables driving prevalence across the island.

**Results:** In total, 222 individuals belonging to 17 bat species were analyzed. *Polychromophilus* infections were identified in two families: Miniopteridae and Vespertilionidae. Molecular data showed that *Polychromophilus* spp. parasitizing Malagasy bats form a monophyletic group composed of three distinct clades displaying marked host specificity. In addition to *P. melanipherus* and *P. murinus*, hosted by *Miniopterus* spp. and *Myotis goudoti*, respectively, we report a novel *Polychromophilus* lineage detected from a single individual of *S. robustus*. Based on the present study and the literature, different biotic and abiotic factors are shown to influence *Polychromophilus* infection in bats, which is correlated based on MaxEnt modeling.

**Conclusion:** The present study improves our knowledge on *Polychromophilus* infecting Malagasy bats and highlights the existence of a novel *Polychromophilus* lineage. Additional studies are needed to get additional representatives of this novel lineage to resolve its taxonomic relationship with known members of the genus. Further, the transmission mode of *Polychromophilus* in bats as well as its potential effect on bat populations should be investigated to complement the results provided by our modeling and eventually provide a comprehensive picture of the biology of these host-parasite interactions.

**Background**

In recent years, different studies have elucidated the biology, ecology, diversity, and evolutionary history of apicomplexan parasites infecting vertebrates worldwide [1–4]. Among these blood parasites, the genus *Plasmodium* has been the most intensively studied because of the millions of deaths that it causes each year in humans [5]. This genus also infects different groups of vertebrates such as reptiles, birds, and bats [6–8]. In fact, the genus *Plasmodium* is now recognized to be polyphyletic and species diversification has led to the evolution of different malaria or malaria-related parasite lineages. For example, a recent study has highlighted that malarial parasites in general can be the subject of different host-switching events and shifts in life-history traits, which in turn insures their maintenance within hosts further facilitated by a simplification of their life cycle [8].
Based on previous observations, bats are also reservoirs of different malaria-related parasites, including nine genera of Haemoproteidae [8–15], but only four of these, including the genus Polychromophilus, are diagnosed based on molecular tools, which calls for an in-depth investigation of haemosporidian parasites, including their epidemiology, host range, distribution, transmission, and patterns of speciation.

With respect to the genus Polychromophilus, five species have been formerly identified using morphological characters (P. adami, P. deanei, P. coradetti, P. melanipherus, and P. murinus) of which two have been molecularly characterized (P. melanipherus and P. murinus) infecting Miniopteraidae [16, 17] and Vespertilionidae [17, 18], respectively. Further molecular diagnostic has revealed two additional taxa in Vespertilionidae hosts, classified as Polychromophilus sp. 1 in Kerivoula hardwickii from Cambodia [16] and Polychromophilus sp. 2 in Pipistrellus grandidieri (currently, Pipistrellus simandouensis [19]) and Laephotis capensis from Guinea [10]. The remaining three species, Polychromophilus adami, P. deanei, and P. coradetti have only been diagnosed using morphological characters, and their taxonomic identity needs further molecular characterization.

On Madagascar, 46 species of bats are currently recognized with a level of endemism approaching 80% [20]. Bat species diversity in the eastern mesic portions of the island is lower than in the western dry zone [21, 22]. In terms of microorganisms hosted by Malagasy bats, different published studies have demonstrated the circulation of viruses, bacteria, and metazoan parasites in these nocturnal mammals [23–26]. Besides parasites of potential medical importance, bats from Madagascar also host malaria-related parasites, namely the genus Polychromophilus (Haemosporida: Plasmodiidae). Two species of Polychromophilus are currently documented in Malagasy bats: P. melanipherus infecting Miniopterus spp. and rarely Paratriaenops furculus (Rhinonycteridae) and P. murinus parasitizing Myotis goudoti (Vespertilionidae) [17, 27]. While 32 species of bats occurring in the western and the central portion of Madagascar were already screened for the presence of Polychromophilus [16, 17, 27], knowledge on Polychromophilus in bats occurring in the eastern area is not well-known and aspects on biotic and abiotic drivers of infection is far from complete. In the present study, we explored Polychromophilus diversity and distribution in bats occurring in the oriental portion of the island and predict the potential drivers of Polychromophilus infection across the island based on new and previous records.

**Methods**

**Study sites**

Fieldwork was undertaken from 2014 to 2016 at four main sites in eastern Madagascar to sample bats: Kianjavato Forest Station within the Paysage Harmonieux Protégé du Corridor Forestier Ambositra-Vondrozo and surrounding areas; Réserve Naturelle Intégrale de Betampona; Parc National de Masoala; and Parc National de Marojejy (Fig. 1, Additional file 1: Table S1). A range of habitat types including day roosts in buildings, degraded areas associated with croplands, and lowland moist evergreen-forested areas were investigated.
Bat sampling

Bats were captured using mist nets and harp traps set up across flight pathways. Additionally, a hand net was used to capture bats inside day roost sites. Upon capture, each individual bat was placed separately in a clean cloth bag. Bat voucher specimens were morphologically identified and deposited at the Université d'Antananarivo, Mention Zoologie et Biodiversité Animale (formerly Département de Biologie Animale, UADBA), Antananarivo, and in the Field Museum of Natural History (FMNH), Chicago. We follow the recent revision of Monadjem et al. [19] for the taxonomy of Afro-Malagasy members of the family Vespertilionidae.

Blood smear screening and infection rate

In the field, one thin blood smear per individual bat was prepared using a non-calibrated drop of blood. In the laboratory, blood smears were fixed with Methanol, stained with Giemsa for 10 min at room temperature, rinsed with Phosphate-Buffered Saline solution, and air-dried. Smears were subsequently examined using a Leica microscope (Leitz, Portugal) under 1000x magnification. For each blood smear, all monolayer fields were screened for the presence of haemosporidian parasites. Infection rate per species was obtained by dividing the number of infected individuals by the total number of individuals examined for each species [28, 29].

DNA extraction, PCR amplification, and sequencing

In the field, blood spots from each individual bat were conserved on Whatman filter paper, air-dried, and stored at room temperature until DNA extraction. In the laboratory, two blood spots from microscopically positive samples were lysed in 0.5% saponin solution during four hours at room temperature. Subsequently, they were washed with Phosphate-Buffered Saline solution (PBS 1X). Total DNA extraction was performed using Instagene™ Matrix (BioRad, USA) following manufacturer's protocol. PCR amplification targeting the portion of mitochondrial Cytochrome b locus (Cyt b) of hemosporidian parasites was undertaken using a previously described nested PCR protocol [16, 18, 27] using Plas 1 and Plas 2 for the first PCR and Plas 3 and Plas 4 for the second PCR. PCR products were visualized in an electrophoresis gel and subsequently sent for Sanger sequencing to Genoscreen (Lille, France) using Plas 3 and Plas 4 primers.

Phylogenetic analysis

Nucleotide sequences obtained from positive individual bats were manually edited using Geneious software [30]. Individual nucleotide sequences were subsequently aligned with other data on blood parasites in bats downloaded from Genbank using MAFFT alignment implemented in Geneious software. Prior to determining the phylogenetic relationship of the parasites identified, the best substitution model was identified using jModelTest version 2.1.3 [31, 32]. Subsequently, Bayesian inference consisting of two independent runs of four incremental Metropolis Coupled Markov Chain Monte Carlo (MC3) iterations starting from a random tree was conducted using MrBayes 3.1.2 [33]. This analysis consisted of two runs of 5,000,000 generations with trees and associated model parameters sampled every 500 generations.
The first 25% of the trees were discarded as a burn-in. New nucleotide sequences produced in the present study were deposited to Genbank under Accession numbers MW039207 to MW039233 (Additional file 2: Table S2).

Statistical analysis and Maxent modeling of *Polychromophilus*

A Generalized Linear Model (GLM) was constructed to investigate variation in *Polychromophilus* infection between *Miniopterus* species and sex classes using R version 3.0.0 [34]. Results of the GLM are expressed as adjusted odd-ratio with confidence interval at 95%. The identification of variables controlling the distribution of *P. melanipherus* was conducted using the MaxEnt software version 3.3.3 k [35]. Modeling was carried out using occurrence records obtained in the present study, as well as another recent study [27]. Environmental variables for each of the capture sites [35, 36] included the following nine bioclimatic variables: annual total evapotranspiration, maximum precipitation of the wettest month, minimum precipitation of the driest month, maximum temperature of the warmest month, minimum temperature of the coldest month, mean annual precipitation, mean annual temperature, numbers of months with a positive water balance, and annual water balance). These data were recovered at 30 arc seconds resolution for each site at www.worldclim.org [37]. Elevational data was downloaded from http://srtm.csi.cgiar.org; Geology from the simplified geological map of Madagascar [38] and vegetation cover taken from Rakotondratsimba [39].

Model accuracy was assessed by splitting occurrence data into 70% for training and 30% for testing [35]. This approach was repeated five times. A set of 10,000 random points was used as background data to generate the Area under the Curve (AUC) [40, 41]. Models with an AUC above 0.8 were considered as informative [40, 42]. The output of MaxEnt modeling is presented in map form showing the probability of suitable and unsuitable area [35].

**Results**

**Blood smear screening**

In total, 222 bats belonging to 17 species were microscopically screened for the presence of haemopsporidian parasites. Thirty-six individual bats representing six species were found positive. Four of the six screened species of *Miniopterus* (Miniopteridae), as well as *Myotis goudoti* and *Scotophilus robustus* (Vespertilionidae), were positive for haemosporidian infection (Table 1). This is the first report of haemosporidian parasites in *S. robustus*, a species endemic to Madagascar. Infection rates ranged from 20 to 54.5% in *Miniopterus* spp. (*n* = 88), 8% in *Myotis goudoti* (*n* = 25), and 33% in *Scotophilus robustus* (*n* = 3) (Table 1). Based on Generalized Linear Model analyses, infection rates presented some variations between species (*p* = 0.036) but not between sexes (*p* = 0.44). For the different species of *Miniopterus* in the present study, two, *M. gleni* (Adjusted Odd-Ratio: 2.17, CI: 0.21–22.5) and *M. griveaudi* (Adjusted Odd-Ratio: 3.86, CI: 0.35–43.05) - had a greater chance of being infected than the other analyzed species.
Table 1
Polychromophilus infection in bats from eastern Madagascar based on blood smear screening.

| Family       | Species               | N  | Infected (in %)  |
|--------------|-----------------------|----|------------------|
| Pteropodidae | Rousettus madagascariensis | 25 | 0                |
| Hipposideridae | Macronycteris commersoni | 1  | 0                |
| Emballonuridae | Coleura kibomalandy  | 9  | 0                |
|              | Paremballonura atrata  | 27 | 0                |
| Myzopodidae  | Myzopoda aurita       | 17 | 0                |
| Molossidae   | Chaerephon atsinanana  | 12 | 0                |
|              | Mops leucostigma      | 11 | 0                |
| Vespertilionidae | Myotis goudoti     | 25 | 2 (8)            |
|              | Laephotis matroka     | 2  | 0                |
|              | Pipistrellus raceyi   | 2  | 0                |
|              | Scotophilus robustus  | 3  | 1 (33)           |
| Miniopteridae | Miniopterus ambohitrensis | 9  | 3 (33)           |
|              | Miniopterus brachytragos | 6  | 0                |
|              | Miniopterus cf. aelleni | 3  | 0                |
|              | Miniopterus egeri     | 5  | 1 (20)           |
|              | Miniopterus gleni     | 43 | 17 (39.5)        |
|              | Miniopterus griveaudi | 22 | 12 (54.5)        |
| Total        |                       | 222| 36 (16.2)        |

N: number of individuals analyzed

Phylogenetic analysis

In total, 27 samples were amplified and sequenced. The Bayesian analysis based on Cyt b sequences from this study and also including parasites sequences from different areas around the world showed that the genus Polychromophilus forms a well-supported monophyletic clade (Fig. 2). Within this cluster, five branches are observed: P. melanipherus infecting Miniopteridae, P. murinus infecting members of the family Vespertilionidae, Polychromophilus sp. 1 identified in Kerivoula hardwickii from Cambodia, Polychromophilus sp. 2 infecting two species of Vespertilionidae (Parahypsugo grandidieri and Laephotis capensis) from Guinea and a novel branch of Polychromophilus, annotated Polychromophilus sp. from Scotophilus robustus (Vespertilionidae) from Madagascar. For Malagasy bats, nucleotide sequences
obtained from those captured in the eastern portion of the island are composed of three different taxa: *P. melanipherus*, *P. murinus*, and the novel lineage of *Polychromophilus* (Fig. 2).

**Distribution of Polychromophilus spp. on Madagascar**

Of the seven bat families investigated from Malagasy samples in the current and previous studies, *Polychromophilus* spp. infect mainly two bat families, Miniopteridae and Vespertilionidae, with *P. melanipherus* infecting members of the family Miniopteridae (10 species infected), *P. murinus* parasitizing a member of the family Vespertilionidae (*Myotis goudoti*) and *Polychromophilus* sp. found in another vespertilionid (*Scotophilus robustus*) sampled at Kianjavato (Figs. 3A and B).

*Polychromophilus* sp.

*Polychromophilus* sp., the novel lineage, was identified in *Scotophilus robustus* based on morphological and molecular screening, but little can be said about its distributional range due to the single known occurrence.

**Polychromophilus murinus**

*Polychromophilus murinus* was strictly associated with *Myotis goudoti* sampled in different sites across the island. *Polychromophilus murinus* was present in 11 out of the 17 sampled sites and based on current data is specific to *Myotis goudoti* (Additional file 3: Table S3).

**Polychromophilus melanipherus**

*Polychromophilus melanipherus*, associated with the genus *Miniopterus*, is a widespread and cosmopolitan parasite of this bat genus. As *P. melanipherus* was identified in 23 out of the 28 sites sampled throughout Madagascar based on previous and the present study (Additional file 3: Table S3), it was used as a model to predict its presence on the island using MaxEnt analysis. The model had high accuracy with an AUC of 0.855 and the main four variables predicting suitable habitat on the presence of *Polychromophilus* were vegetation (61.1%), geology (29.4%), annual water balance (4.9%), and annual total evapotranspiration (4.6%). The remaining eight variables were less explicative (percent contribution between 0 and 1.3%) and were omitted from the final model.

MaxEnt prediction indicated an area of highest environmental suitability in the northern and southwestern areas of Madagascar, corresponding to orange to red colors (Fig. 3B), with a probability of infection ranging from 0.7 to 1. Further, *P. melanipherus* may occur in diverse areas with different environmental conditions, corresponding to the green color and a probability of infection ranging from 0.54 to 0.69 (Fig. 3B). Based on vegetation classification [39]Gautier et al., 2018), regions of highest suitability for *Polychromophilus* included northern moist evergreen forest, southwestern dry spiny thicket, and western dry deciduous forest. It appears that on Madagascar *P. melanipherus* is tightly associated with the presence of *Miniopterus* spp.
Discussion

Polychromophilus infection in Malagasy bats and phylogenetic analyses

On the basis of the results presented herein including the analysis of seven different families of bats, Polychromophilus infection in Malagasy bats from the eastern portion of the island appears to be limited to two families (Miniopteridae and Vespertilionidae), while the other five tested families (Pteropodidae, Hipposideridae, Emballonuridae, Myzopodidae, and Molossidae) were negative. Such host-parasite associations are in keeping with previous investigations carried out worldwide [10, 17, 18, 27, 43]. To date, 10 out of the 12 species of Miniopterus tested and occurring on Madagascar are infected by P. melanipherus based on morphological or molecular screenings. Miniopterus brachytragos was represented by six individuals in the current study which were negative. Hence, the absence of P. melanipherus infection in M. brachytragos may associated with the limited sample size, as this species was previously found infected by Haemosporidae [44]. Miniopterus petersoni, with a limited geographical distribution [45], has not yet been analyzed to date due to the absence of blood samples. Based on generalized linear model analysis, M. gleni and M. griveaudi present higher risk of infection than the other Malagasy species within this genus. This may be due to their roosting behavior, as these two species occur in large day roosting colonies within caves and both species have broad geographical distributions and little genetic variability, which we assume is related to their high dispersal capacity [45–47].

Phylogenetic analysis based on a portion of cytochrome b gene of Polychromophilus spp. in bats from different localities showed that they form a monophyletic group composed of five distinct clades, of which three are documented on Madagascar. Polychromophilus melanipherus was identified in Miniopterus spp., providing further support that this blood parasite species is specific to the Miniopteridae and with a broad Old World distribution [8, 10, 48]. This parasite infects different species of Miniopterus without geographic or host species barriers [10, 17, 43, 49]. Apart from Miniopterus spp., Paratriaenops furculus (Rhinonycteridae) has already been reported to be infected with Polychromophilus melanipherus [27], whereas a closely related member of this family, Triaenops menamena, which occurs in sympatry with Paratriaenops furculus in the same day roost sites [45, 50] tested negative for this parasite [27].

The second clade represented by Polychromophilus murinus, occurred in different genera and species of the family Vespertilionidae [27, 51, 52]. Compared to infection of P. murinus in European bats, it appears that on Madagascar, within Vespertilionidae, only Myotis goudoti is infected by P. murinus. A third lineage of Polychromophilus, identified herein as Polychromophilus sp. 3 based on molecular data, was found in a single specimen of Scotophilus robustus (Vespertilionidae). Our phylogenetic tree shows that this lineage is different from what was previously published and represents an undescribed Polychromophilus taxa. However, with only one positive specimen among three captured individuals, we refrain from proving a diagnosis this taxon and describe it as new to science; further samples are needed to resolve the taxonomy of Polychromophilus sp. 3 based on morphological and molecular diagnosis.
The other two *Polychromophilus* clusters identified in Fig. 2 as *Polychromophilus* sp. 2 in *Laephotis capensis* and *Pipistrellus simandouensis* from west Africa, and *Polychromophilus* sp. 1 in *Kerivoula hardwickii* from southeast Asia [10, 16] also need further investigation to have additional information on their taxonomy. To date, 38 out of the 46 species of bats occurring on Madagascar have been screened for the presence of apicomplexan parasites. The balance of eight species needs to be examined as material becomes available.

**Distribution of Polychromophilus spp. on Madagascar**

We used *P. melanipherus* data to model the distribution of bat-borne blood parasites in members of the genus *Miniopterus*, as these bats are widespread on Madagascar from the sea level to up to 1800 m [45]. We could not use data from Raharimanga et al. [44] in the modeling analysis presented herein as the lack of molecular data associated with the identified hematoparasites does not allow positive identification. In total, 10 out of the 11 *Miniopterus* species screened for the presence of *P. melanipherus* were positive. Members of the family Miniopteridae use different roost sites such as caves, crevices or rock overhangs [45, 50] and generally live in a mix colonies with other species, often *Myotis goudoti* [45]. The dispersal capacity of members of the genus is notably high, as exemplified by *Miniopterus gleni* and *M. griveaudi* [45, 50]. *Polychromophilus melanipherus* appears to be a cosmopolitan blood parasite of *Miniopterus* spp.

Based on the Maxent analysis, vegetation and geology are the two most significant parameters and contributed more than 90% of the variables explaining the occurrence of *P. melanipherus* on Madagascar. On the island, the vegetation variable showed a notable difference between the west and east. In the western portion, three main vegetation types occur, including dry forest in the north, dry deciduous forest in the central area, and dry spiny thicket in the southwest. These vegetational gradients are important in the ecology of *Miniopterus* species associated with their feeding behavior at a local scale and their dispersal at a broader scale. In addition, throughout portions of the west are found limestone formations and often containing cave systems where different species of *Miniopterus* roost in sympatry or in syntopy within the cave. At such day roost sites, *Miniopterus*, as well as other species most notably *Myotis goudoti* occur in large to moderately large mono- or multi-specific groups, which may favor parasite transmission. The analysis indicates that the eastern and central portions of Madagascar, characterized by natural moist evergreen forests and associated climatic conditions, are less favorable for *Miniopterus*. Also, the local geology of this area lacks exposed sedimentary rock and deep cave formations and day roost sites are limited to crevices or tree holes where roosting groups tend to be relatively small. While *P. melanipherus* is associated with the genus *Miniopterus*, several abiotic help explain the lower prevalence of this parasite in the eastern portion of the island. It is important to mention that *P. melanipherus* and *P. murinus* generally co-occur within the same locality as their respective hosts sometimes live in sympatry or in syntopy within their day roost sites but no case of co-infection are known to date in a single individual.

**Drivers of Polychromophilus infection in bats**
Dick and Dittmar [53] hypothesized that the type of day roost sites, colony size, and bioclimatic aspects can affect the exposure of bats to insect vectors, and hence parasite transmission. Based on the results obtained herein and in the literature, *Polychromophilus* infection is limited to several species from three different families of Malagasy bats, and some ideas can be presented based on the analyses presented herein about potential drivers of *Polychromophilus* infection on the island. To this end, we suggest that *Polychromophilus* infection in bats is driven by two main factors: the ecological niche they occupy and the behavioral aspects of bat hosts and the presence of competent vectors.

**Ecological niche and bat behavior**

The three *Polychromophilus* taxa show a certain level of host specificity that may be related to the ecological niche and behavior of their bat hosts. As *P. melanipherus* infects almost all *Miniopterus* species on Madagascar, hypotheses regarding parasites maintenance and transmission can be suggested. The genus *Miniopterus* occurs in a wide range of habitats on the island with some of the species within the genus forming large monospecific or multispecific colonies. *Miniopterus gleni* and *M. griveaudi* often live in caves and rock shelters located near freshwater streams or in areas protected from solar radiation and with humid local conditions. Further, these two bat species often share or occur in relatively close proximity to other cave roosting bat species. *Miniopterus gleni* is the only species with a large distribution across much of the island, from sea level to 1200 m, and can also co-occur with other members of the genus [50]. Further, phylogeographic studies show little genetic variation across the different populations and it can be presumed that this species disperses widely [46]. The role of this species as a bridge for *Polychromophilus* transmission is therefore probable. Nonetheless, this supposition needs further consideration with regards to the ecology, ectoparasites, and behavior of this species.

*Polychromophilus murinus* on Madagascar is only known from *Myotis goudoti*. This endemic bat species shows little phylogeographical structure, best explained by broad dispersal [54]. *Myotis goudoti* occurs in various habitat types on the island and roosts in caves, crevices, and tree holes [45], and in small or large day roost colonies. Although this species can live in syntopy with some small or middle-sized *Miniopterus* such as *M. griveaudi*, *M. ambohitrensis*, or *M. majori* within the same day roost site, no co-infection of *P. murinus* has been reported.

**Presence of competent vectors and fly/bat specificity**

*Polychromophilus* spp. have been proposed to be transmitted by nycteribiid flies (Diptera: Hippoboscoidea: Nycteribiidae) [13, 52], which are highly specialized ectoparasites of bats [55–58]. Nycteribiid flies and their associated bat hosts’ show a strong association providing the means for parasite transmission through their respective life cycles. As a vector-borne infection, the transmission of *Polychromophilus* is presumably insured by the presence of competent vectors that are tightly associated with bat hosts. The molecular screening of 38 individual nycteribiids belonging to three species (*Penicillidia leptothrinax*, *Penicillidia* sp. cf. *fulvida*, and *Nycteribia stylidiopsis*) revealed the presence of *Polychromophilus* spp. [27]. While no detailed work on the role of Nycteribiidae as vector of
Polychromophilus has been achieved on Madagascar, three species of Nycteribiidae, namely Penicillidia leptothrinax, Nycteribia stylidiopsis and Penicillidia sp. are local candidate vectors of Polychromophilus [27]. Penicillidia leptothrinax and Nycteribia stylidiopsis have been previously reported positive for Polychromophilus melanipherus. These positive bat flies included four Penicillidia leptothrinax specimens (two sampled on Miniopterus aelleni and two on M. manavi s.l.), in addition to a single Nycteribia stylidiopsis obtained from M. gleni. These two bat fly species are common on bats of the genus Miniopterus and can be putatively considered as vectors of Polychromophilus melanipherus [27, 57]. As far as P. murinus is concerned, one specimen of Penicillidia sp. was found positive but this nycteribiid was collected on a Miniopterus individual that was negative to P. melanipherus, and this result probably indicates considerable ectoparasite exchange between bats occurring in day roost sites. Based on available information, we assume that these three species of Nycteribiidae are candidate vectors of Polychromophilus spp. in Malagasy bats.

Conclusions

Across the Old World, Polychromophilus melanipherus infection is widespread in Miniopterus spp. Members of the family Vespertilionidae are infected by two different species of Polychromophilus on Madagascar. Based on our findings, three taxa of Polychromophilus are currently known in bat species from the island. While P. melanipherus and P. murinus appeared to be broadly distributed within their identified hosts namely Miniopterus spp. and Myotis goudoti, respectively, the third taxa identified represents a new lineage. According to the data presented herein, Polychromophilus is closely related with specific hosts (species or family) and their occurrence is linked with the abiotic factors as exemplified by the MaxEnt analysis of P. melanipherus, as well as the presence of nycteribiid vectors favoring parasite transmission. Although the majority of Malagasy bat species have now been examined for the presence of Polychromophilus spp. using morphological and/or molecular screening, additional work is needed on the few non-analyzed species and to increase sample sizes for some others that have not tested positive. Future studies should focus on the life cycle of Polychromophilus spp. based on an experimental design and their potential effect on the bat hosts. Further, the importance of nycteribiid flies in the transmission of the different species of Polychromophilus in bats from Madagascar should be investigated.

Declarations

Ethical approval

The study was conducted in strict accordance with the terms of research permits issued by Malagasy authorities (Direction du Système des Aires Protégées, Direction Générale de l’Environnement et des Forêts) and following national laws (Permit number no. 313/13/MEF/ SG/DGF/DCB.SAP/SCB of 30 December 2013; no. 103/16/MEEMF/ SG/DGF/DAPT/SCBT of 29 April 2016). Bats were captured, manipulated, and euthanized in accordance with guidelines accepted by these different national authorities and the scientific community for the handling of wild animals [59].
Consent for publication

Not Applicable

Availability of data and materials

All data generated or analyzed during this study are included in this article.

Competing interests

The authors declare no competing interests.

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Authors' contributions

MR, BR and SMG designed the study, MR and BR did the experiment, MR, BR and MbR and RB performed data analyses, MR, BR, MbR, SMG, PT, KD and MiR wrote the manuscript. All authors read and approved the final manuscript.

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**Figures**
Figure 1

Localization of study sites and sites surveyed in previous study. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.
Figure 2

Phylogenetic relationship of haemosporidian parasites infecting bats based on Bayesian analysis.
Figure 3

A: Distribution of haemosporidian parasites on Madagascar: A) known distribution of Polychromophilus spp. on Madagascar. B. Predicted occurrence of P. melanipherus based on MaxEnt analysis. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

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