International Journal for Parasitology: Parasites and Wildlife 25 (2024) 100986

Contents lists available at ScienceDirect

International Journal for Parasitology: Parasites and Wildlife

journal homepage: www.elsevier.com/locate/ijppaw

# *Polychromophilus* (Haemosporida: Plasmodiidae): A review of association with bats (Mammalia, Chiroptera) and the first record in the Neotropical bat, *Myotis albescens* (Chiroptera, Vespertilionidae) from Colombia

Diego Fernando Ceballos-Pérez [a,b], Johnathan Alvarez-Londoño [b,c], Héctor E. Ramírez-Chaves [b,d], Fredy A. Rivera-Páez [b,*]

[a] *Programa de Biología, Facultad de Ciencias Exactas y Naturales, Universidad de Caldas, Calle 65 No. 26-10 A.A 275, 170004, Manizales, Caldas, Colombia*
[b] *Grupo de Investigación en Genética, Biodiversidad y Manejo de Ecosistemas (GEBIOME), Departamento de Ciencias Biológicas, Facultad de Ciencias Exactas y Naturales, Universidad de Caldas, Calle 65 No. 26-10 A.A 275, 170004, Manizales, Caldas, Colombia*
[c] *Maestría en Ciencias Biológicas, Facultad de Ciencias Exactas y Naturales, Universidad de Caldas, Calle 65 No. 26-10 A.A 275, 170004, Manizales, Caldas, Colombia*
[d] *Centro de Museos, Museo de Historia Natural, Universidad de Caldas, Calle 58 No. 21-50, 170004, Manizales, Caldas, Colombia*

ARTICLE INFO

*Keywords:*
Chiroptera
Hemoparasites
Malaria
Neotropics
Vespertilionidae

ABSTRACT

Some species within the family Plasmodiidae (Haemosporida) have been extensively studied due to their implications for human health. However, for other haemosporidians that infect wild animals the knowledge is limited. Species within the genus *Polychromophilus* have thus far been documented exclusively as hemoparasites of bats. Records of *Polychromophilus* are primarily from Africa, Europe, and Southeast Asia, with limited information available for the Americas. Here, we assessed the state of knowledge on *Polychromophilus* species infecting bats worldwide and searched for the presence of *Polychromophilus* in blood samples of neotropical bats from Colombia. We found a total of 65 records of *Polychromophilus* in 46 bat species belonging to the families Emballonuridae, Hipposideridae, Miniopteridae, Rhinolophidae, Rhinonycteridae, and Vespertilionidae worldwide, except for Antarctica. In the Americas, records of the genus *Polychromophilus* are exclusively from Vespertilionidae bats in Brazil, Colombia, the United States, and Panama. The morphological and molecular analyses of blood from 125 bats, belonging to 39 species and captured in seven localities within the departments of Arauca and Caldas (Colombia), confirmed the presence of *Polychromophilus deanei* in a silver-tipped myotis, *Myotis albescens* (Vespertilionidae). This finding represents the first morphological and molecular confirmation of *P. deanei* in the Americas. Additionally, it expands the knowledge on the diversity and distribution of *Polychromophilus* in Neotropical bats.

## 1. Introduction

Haemosporidians of the family Plasmodiidae (Haemosporida: Apicomplexa) represent a diverse group of blood parasites transmitted by vectors that infect a wide range of vertebrate hosts worldwide (Liu et al., 2010; Galen et al., 2018; Harris et al., 2019). These haemosporidians exhibit complex and similar life cycles, involving sexual reproduction stages in the arthropod vectors and asexual stages in vertebrate hosts (Garnham, 1966; Valkiunas, 2004; Perkins, 2014). Over the past decade, the implementation of morphological and molecular tools has facilitated the identification of a wide diversity of morphospecies and lineages of haemosporidians (Perkins and Schaer, 2016; Lutz et al., 2016; Arnuphapprasert et al., 2020). However, the true diversity and phylogenetic relationships are not well-established due to descriptions based either only on morphology or isolated molecular data (Valkiunas, 2004; Perkins, 2014; Sándor et al., 2021).

In mammals, haemosporidian infections within Plasmodiidae are primarily known in primates and rodents due to their significance in human health research (e.g., *Plasmodium falciparum*, *Plasmodium malariae*, *Plasmodium vivax*) (Schaer et al., 2013; Lalremruata et al., 2015; Borner et al., 2016; Minozzo et al., 2021). Currently, Plasmodiidae includes six genera that infect mammals, i.e., *Dionisia, Hepatocystis, Nycteria, Plasmodium, Rayella,* and *Polychromophilus* (including *Biguetiella*), all of which have been detected in bats (Chiroptera), except

* Corresponding author.
*E-mail address:* fredy.rivera@ucaldas.edu.co (F.A. Rivera-Páez).

https://doi.org/10.1016/j.ijppaw.2024.100986
Received 1 August 2024; Received in revised form 4 September 2024; Accepted 5 September 2024
Available online 7 September 2024

*Rayella*, and three exclusively infecting these hosts (i.e., *Dionisia, Nycteria* and *Polychromophilus*) (Perkins and Schaer, 2016). In this context, bats are a crucial group for the taxonomic, systematic, and evolutionary study of haemosporidians in mammals (Lutz et al., 2016). *Polychromophilus* is the genus with the widest global distribution, primarily associated with insectivorous bats of the families Miniopteridae and Vespertilionidae in temperate and tropical regions (Garnham, 1973; Perkins and Schaer, 2016). These haemosporidians are transmitted to bats by highly specialized dipterans of the family Nycteribiidae (Megali et al., 2011; Ramasindrazana et al., 2018). There are five known species of *Polychromophilus*: *Polychromophilus adami* and *Polychromophilus corradetti*, described from Africa; *Polychromophilus melanipherus* and *Polychromophilus murinus*, described from Europe; and *Polychromophilus deanei*, described from South America (Garnham et al., 1971; Perkins and Schaer, 2016).

In the Americas, the initial reports of *Polychromophilus* were based solely on morphological traits of records coming from the USA, Brazil, and Colombia (Wood, 1952; Garnham, 1966; 1970; 1971; Foster, 1979; Marinkelle, 1995). The first molecular record of *Polychromophilus* sp. corresponds to a DNA sequence in *Myotis nigricans* from Panama (Borner et al., 2016). Subsequently, eight additional sequences of *Polychromophilus* sp. were reported in *Neoeptesicus diminutus*, *Myotis ruber*, *Myotis riparius*, and *Myotis* sp. in Brazil (Minozzo et al., 2021; Mathias et al., 2023). However, there are no studies that combine both morphological and molecular methods for identifying haemosporidians of the genus *Polychromophilus* infecting bats across the continent. Given the existing information gaps on *Polychromophilus* species, and the fact that up to 222 bat species have been recorded in Neotropical countries like Colombia (Ramírez-Chaves et al., 2024), this research aimed to gather available information of *Polychromophilus* infecting bats worldwide and provide morphological and molecular information to enhance the understanding of the biology of *Polychromophilus*.

## 2. Materials and methods

### 2.1. Literature review

To compile the available information on *Polychromophilus* associated with bats on a global scale, we conducted a comprehensive literature search in the Web of Science and Scopus databases (from January 1960 to May 2024) using the search term "*Polychromophilus*". To ensure the quality and replicability of the studies, we limited the search to peer-reviewed articles (Bohada-Murillo et al., 2021). To gather the most comprehensive set of *Polychromophilus* reports, we included only studies presenting novel and non-duplicated information. Additionally, we included a report not part of the initial search, concerning the species *Bioccala deanei*, now known as *P. deanei* (Marinkelle, 1995). The review followed the methodology proposed in the PRISMA statement (Page et al., 2021).

The initial search yielded 75 articles, which were reduced to 48 after removing duplicates. Subsequently, 27 articles were retained after excluding those that did not contain relevant information for the study. The selected articles were thoroughly reviewed to determine if they met the following inclusion criteria: (1) morphological and/or molecular detection method, (2) detection in bats, (3) georeferenced location or a detailed description of the sampling area, and (4) reports of infections in wild environments. The inclusion of articles was performed by the same person (DFCP) to avoid potential reviewer bias. As a result, we excluded four articles that did not meet the inclusion criteria, resulting in a total of 23 articles that provided 65 reports of *Polychromophilus* (Fig. 1). With this information, we generated a table with the extracted information from the selected studies, indicating the bat family, country record, detected *Polychromophilus* species, and bibliographic reference (Table 1).

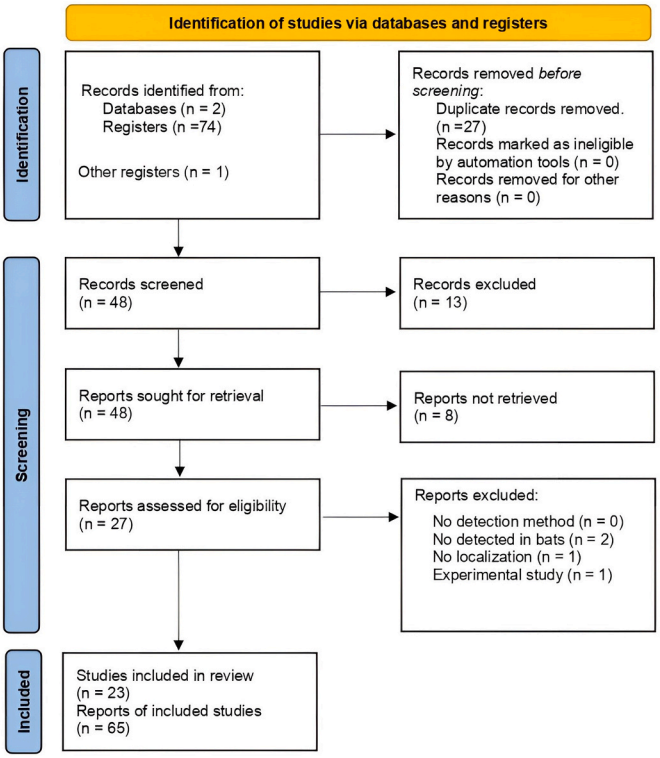


**Fig. 1.** Flow diagram of elements of reporting for systematic reviews and Meta-Analysis (PRISMA; 2020 version) summarizing the selection of articles included in this review.

### 2.2. Searching for Polychromophilus in Neotropical bats

To explore the presence of *Polychromophilus* in Neotropical bats, we conducted field work between October and November 2021, and November 2022, in six localities across four municipalities (La Dorada, Manizales, Palestina, and Villamaría) in the Department of Caldas and one locality in the municipality of Arauca in the Department of Arauca, Colombia (Table 2, Fig. 2). The Department of Caldas is situated in the central-western region of Colombia (5°17′N and 75°21′W; central point), covering an approximate area of 7888 km$^2$. Caldas encompasses a wide diversity of ecosystems along the Central and Western Andes and the inter-Andean valleys of the Cauca and Magdalena rivers, with elevations ranging from 140 to 5350 m a.s.l. (Cardona-Salazar et al., 2020). The average monthly temperature varies from 13 °C to 27 °C, and monthly precipitation ranges from 150 to 400 mm, with peak rainfall occurring during April–May and August–November (Escobar-Lasso et al., 2013; Ruiz and Melo, 2022). The Department of Arauca is in the northern part of the Colombian Orinoquia (6°37′ N, 70°59′ W; central point) and covers a total area of 23,818 km$^2$. It is situated within the warm thermal floor, with an average annual temperature of 27 °C and an average annual precipitation of 1500 mm, following a monomodal precipitation regime (lower rainfall from December to February) known as the dry season (McNish, 2007). The landscape of the Orinoquia alluvial plain, dominated by savanna, is characterized by herbaceous vegetation associations with scattered trees and seasonal water availability patterns dictated by a markedly dry climatic season (Rippstein, 2001).

To capture bats, we installed between two and six mist nets at each locality and operated from 17:30 to 20:00 h. To confirm the taxonomic identity, captured bats were euthanized and collected following the animal care recommendations proposed by Sikes and Animal Care and Use Committee of the American Society of Mammalogists (2016). Tissue samples from the sacrificed specimens were also collected to search for other pathogens (e.g., *Anaplasma, Babesia, Borrelia, Coxiella, Rickettsia*). Some of these data have already been published and are available in the

**Table 1**
Records of species of *Polychromophilus* (Plasmodiidae) in bats (Chiroptera) around the world. The information of the family and bat species, and country of the record is included.

| Host family | Bat species | Locality | *Polychromophilus* species | References |
|---|---|---|---|---|
| Rhinonycteridae | *Paratriaenops furculus* | Madagascar | *Polychromophilus melanipherus* | Ramasindrazana et al. (2018) |
| Hipposideridae | *Hipposideros semoni* | Australia | *Polychromophilus melanipherus* | Perkins and Schaer (2016) |
| | *Hipposideros caffer* | Congo | *Polychromophilus murinus* | Perkins and Schaer (2016) |
| | *Hipposideros gentilis* | Thailand | *Polychromophilus* sp. | Poofery et al. (2023) |
| Rhinolophidae | *Rhinolophus ferrumequinum* | Romania | *Polychromophilus murinus* | Sándor et al. (2021) |
| | *Rhinolophus hipposideros* | Romania | *Polychromophilus murinus* | Sándor et al. (2021) |
| | *Rhinolophus mehelyi* | Romania | *Polychromophilus murinus* | Sándor et al. (2021) |
| Miniopteridae | *Miniopterus schreibersii* | Romania, Hungary, Switzerland, Italy, Turkey, Russia, Israel, Congo, Portugal, Spain, France, Croatia, Slovakia | *Polychromophilus melanipherus* | Perkins and Schaer (2016); Sándor et al. (2021); Szentiványi et al. (2020) |
| | *Miniopterus inflatus* | Gabon | *Polychromophilus melanipherus* and *Polychromophilus corradetti* | Duval et al. (2012); Perkins and Schaer (2016) |
| | *Miniopterus orianae* | Australia | *Polychromophilus melanipherus* | Holz et al. (2019); Holz et al. (2020) |
| | *Miniopterus africanus* | Kenya | *Polychromophilus melanipherus* | Lutz et al. (2016) |
| | *Miniopterus* cf. *fraterculus* | Kenya | *Polychromophilus melanipherus* | Lutz et al. (2016) |
| | *Miniopterus natalensis* | Kenya | *Polychromophilus melanipherus* | Lutz et al. (2016) |
| | | South Africa | *Polychromophilus* sp. | Szentiványi et al. (2020) |
| | *Miniopterus rufus* | Kenya | *Polychromophilus melanipherus* | Lutz et al. (2016) |
| | *Miniopterus minor* | Tanzania | *Polychromophilus melanipherus* | Lutz et al. (2016) |
| | | Gabon and Congo | *Polychromophilus adami* | Perkins and Schaer (2016); Rosskopf et al. (2019) |
| | *Miniopterus fuliginosus* | Japan | *Polychromophilus melanipherus* | Rosyadi et al. (2022) |
| | *Miniopterus villiersi* | Guinea | *Polychromophilus* sp. | Schaer et al. (2013) |
| | *Miniopterus gleni* | Madagascar | *Polychromophilus* sp. | Rasoanoro et al. (2021); Perkins and Schaer (2016) |
| | *Miniopterus ambohitrensis* | Madagascar | *Polychromophilus* sp. | Rasoanoro et al. (2021) |
| | *Miniopterus egeri* | Madagascar | *Polychromophilus* sp. | Rasoanoro et al. (2021) |
| | *Miniopterus griveaudi* | Madagascar | *Polychromophilus* sp. | Rasoanoro et al. (2021) |
| | *Miniopterus manavi* | Madagascar | *Polychromophilus* sp. | Perkins and Schaer (2016) |
| | *Miniopterus* sp. | Madagascar, Vanuatu, Australia | *Polychromophilus melanipherus* | Ramasindrazana et al. (2018); Perkins and Schaer (2016) |
| Vespertillionidae | *Myotis silogorensis* | Thailand | *Polychromophilus murinus* | Arnuphapprasert et al. (2020) |
| | *Myotis daubentonii* | Romania, Switzerland | *Polychromophilus murinus* | Witsenburg et al. (2012), (2014); Sándor et al. (2021) |
| | *Myotis myotis* | Bulgaria | *Polychromophilus murinus* | Sándor et al. (2021) |
| | *Myotis macrodactylus* | Japan | *Polychromophilus murinus* | Rosyadi et al. (2022) |
| | *Myotis blythii* | Romania | *Polychromophilus murinus* | Sándor et al. (2021) |
| | *Myotis albescens*[a] | Colombia | *Polychromophilus deanei* | This study |
| | *Myotis nigricans* | Brazil | *Polychromophilus deanei* | Garnham et al., (1971) |
| | | Panama | *Polychromophilus* sp. | Borner et al. (2016) |
| | *Myotis tricolor* | Kenia | *Polychromophilus* sp. | Perkins and Schaer (2016) |
| | *Myotis austroriparius* | United States | *Polychromophilus* sp. | Foster (1979) |
| | *Myotis goudoti* | Madagascar | *Polychromophilus murinus* and *Polychromophilus* sp. | Perkins and Schaer (2016); Ramasindrazana et al. (2018); Rasoanoro et al. (2021) |
| | *Myotis riparius* | Brazil | *Polychromophilus* sp. | Minozzo et al. (2021); Mathias et al. (2023) |
| | *Myotis ruber* | Brazil | *Polychromophilus* sp. | Minozzo et al. (2021); Mathias et al. (2023) |
| | *Myotis* sp. | Israel, England Russia, Switzerland | *Polychromophilus murinus* | Perkins and Schaer (2016) |
| | | Brazil | *Polychromophilus* sp. | Mathias et al. (2023) |
| | *Vespertilio murinus* | Italy | *Polychromophilus murinus* | Perkins and Schaer (2016) |
| | *Pipistrellus* aff. *grandidieri* | Guinea | *Polychromophilus* sp. | Schaer et al. (2013) |
| | *Neoromicia capensis* | Guinea | *Polychromophilus* sp. | Schaer et al. (2013) |
| | *Eptesicus fuscus* | Colombia | *Polychromophilus* (*Bioccala*) *deanei** | Marinkelle (1995) |
| | *Neoeptesicus diminutus* | Brazil | *Polychromophilus* sp. | Minozzo et al. (2021) |
| | *Nyctophilus bifax* | Australia | *Polychromophilus* sp. | Perkins and Schaer (2016) |
| | *Scotophilus kuhlii* | Thailand | *Polychromophilus* sp. | Chumnandee et al. (2021) |
| | *Scotophilus robustus* | Madagascar | *Polychromophilus* sp. | Rasoanoro et al. (2021) |

(*continued on next page*)

**Table 1** (*continued*)

| Host family | Bat species | Locality | *Polychromophilus* species | References |
|---|---|---|---|---|
| | *Kerivoula hardwickii* | Cambodia | *Polychromophilus* sp. | Perkins and Schaer (2016) |
| | *Vespadelus pumilus* | Australia | *Polychromophilus melanipherus* | Perkins and Schaer (2016) |
| Emballonuridae | *Taphozous melanopogon* | Thailand | *Polychromophilus melanipherus* | Arnuphapprasert et al., (2020) |

*Bioccala* is a subgenus of *Polychromophilus* (discussed by Witsenburg et al., 2012).
[a] New association reported in this study.

literature (Mancilla-Agrono et al., 2022; Velásquez-Guarín et al., 2024; Ossa-López et al., 2024a, 2024b). The morphological identification of the collected bats was based on taxonomic keys (e.g., Gardner, 2008). We took blood samples of each bat via cardiac puncture, and we prepared at least two blood smears per individual and fixed in absolute methanol for 3 min. The smears were transported to the laboratory and stained with 4% Giemsa solution, pH 7.2, for 45 min at 17 °C (Alvarez-Londoño et al., 2022). The remaining blood was stored in absolute ethanol for subsequent molecular analyses.

Sample collection was conducted under the framework of the permit granted by the National Environmental Licenses Authority (ANLA) to the Universidad de Caldas, as stipulated in resolution No. 02497 of December 31, 2018, updated by resolution No. 000026 of January 9, 2024. Additionally, no species registered in the red list of threatened species of Colombia, as stated in resolution No. 1912 of 2017, updated by resolution No. 0126 of February 6, 2024, were collected. The capture and collection of wild mammals were approved by the Comité de Bioética of the Facultad de Ciencias Exactas y Naturales of the Universidad de Caldas (June 2, 2017, and September 20, 2019). All collected samples and specimens were deposited in the mammals and ectoparasites collections of the Museo de Historia Natural of the Universidad de Caldas (MHN-UCa). Additionally, we determined the percent prevalence of *Polychromophilus* in the captured bats using the equation [(number of infected individuals/number of examined individuals) * 100] (Bush et al., 1997).

### 2.3. Identification

We performed the morphological identification of haemosporidians of the genus *Polychromophilus* by observing blood smears using an Olympus BX43 light microscope at 40X and 100× magnification, examining approximately 100–150 fields per objective (Valkiunas, 2004; Campbell, 2015). We morphologically identified the parasites using the original descriptions of the genus *Polychromophilus* (Garnham, 1966, Garnham et al., 1971; Landau et al., 1984; Marinkelle, 1995). We captured images of the parasites with an Olympus DP28 digital camera and edited using Olympus CellSens Standard software v3.22.11. We also identified the ectoparasites of bats infected by *Polychromophilus* following Brown (2016).

To molecularly determine *Polychromophilus* infection in bat blood samples, we extracted DNA using the Promega Wizard Genomic DNA Purification Kit (Promega Corporation, Madison, USA), following the manufacturer's suggested instructions. To detect *Polychromophilus* DNA, nested Polymerase Chain Reaction (PCR) assays were conducted on a fragment of the cytochrome *b* (*cyt b*) gene. For the initial PCR, primers AE064/AE066, which amplify a 1109 bp fragment for the major haemosporidians genera, were used (Pacheco et al., 2018). The second PCR used the initial PCR amplicon with primers HaemF/HaemR2, which amplify a 525 bp fragment specific to Plasmodiidae (Hellgren et al., 2004). All PCR reactions included negative controls ($H_2O$dd) and positive controls (*Plasmodium homopolare*).

Additionally, to confirm the taxonomic identity of the bat and its ectoparasites, we conducted PCR assays targeting the mammalian *cyt b* gene using primers L14816/H15173 (Parson et al., 2000) and the invertebrate cytochrome oxidase 1 (COI) gene using primers LCO1490/HCO2198 (Folmer et al., 1994). Following the confirmation of the hemoparasite, to search for haemosporidian DNA in the liver tissue of the individual and its ectoparasites, we conducted a PCR assay All PCR products were visualized by horizontal electrophoresis on 1% agarose gels stained with SYBR SAFE (Thermo Fisher Scientific, Waltham, USA) and viewed using a UVP GelDoc-It® 2310 photodocumenter (Thermo Fisher Scientific, Waltham, MA, USA). We sent the positive samples to Macrogen (Seoul, South Korea) for purification and Sanger sequencing, and we edited the sequence in Geneious Prime (2023.0.4. https://www.geneious.com/) and compared with sequences deposited in the GenBank database. All sequences obtained were deposited in

**Table 2**
Information on the department, municipality, and locality of the bats captured and analyzed in this study. Locality numbers are shown in Fig. 2.

| Department | Municipality | Locality | Locality number | Latitude | Longitude | Elevation (meters above sea level) | Habitat type |
|---|---|---|---|---|---|---|---|
| Caldas | Palestina | Vereda El Retiro | 1 | 5.136722 | −75.678944 | 823 | Agroforestry matrix in dry forest |
| | | Kilómetro 35 | 2 | 5.099806 | −75.698583 | 847 | National road edge in dry forest |
| | Manizales | Jardín Botánico Universidad de Caldas | 3 | 5.055833 | −75.495278 | 2131 | Botanical garden in urban matrix |
| | | Vereda Rosario | 4 | 5.027472 | −75.581917 | 1241 | Agroforestry matrix in tropical submontane forest |
| | Villamaría | Reserva Forestal Protectora Bosques de la CHEC | 5 | 5.024611 | −75.395778 | 2635 | Private peri-urban forest reserve |
| | La Dorada | Vereda La Atarraya | 6 | 5.710056 | −74.727444 | 178 | Agroforestry and livestock matrix in dry forest |
| Arauca | Arauca | Los Trompillos | 7 | 6.779556 | −70.716667 | 120 | Cattle grazing area in floodable savanna |

GenBank.

### 2.4. Phylogenetic analysis

We inferred phylogenetic relationships among haemosporidians using Bayesian Inference (BI) and Maximum Likelihood (ML) methods, incorporating our sequence along with partial *cyt b* gene sequences obtained from GenBank (Table S1), and a sequence from *Leucocytozoon buteonis* (**HF543630**) as an outgroup. Sequences were aligned using MEGA11 software (Tamura et al., 2021), resulting in a total alignment length of 490 bp. The General Time Reversible (GTR) + I + G model was selected based on the corrected Akaike Information Criterion using

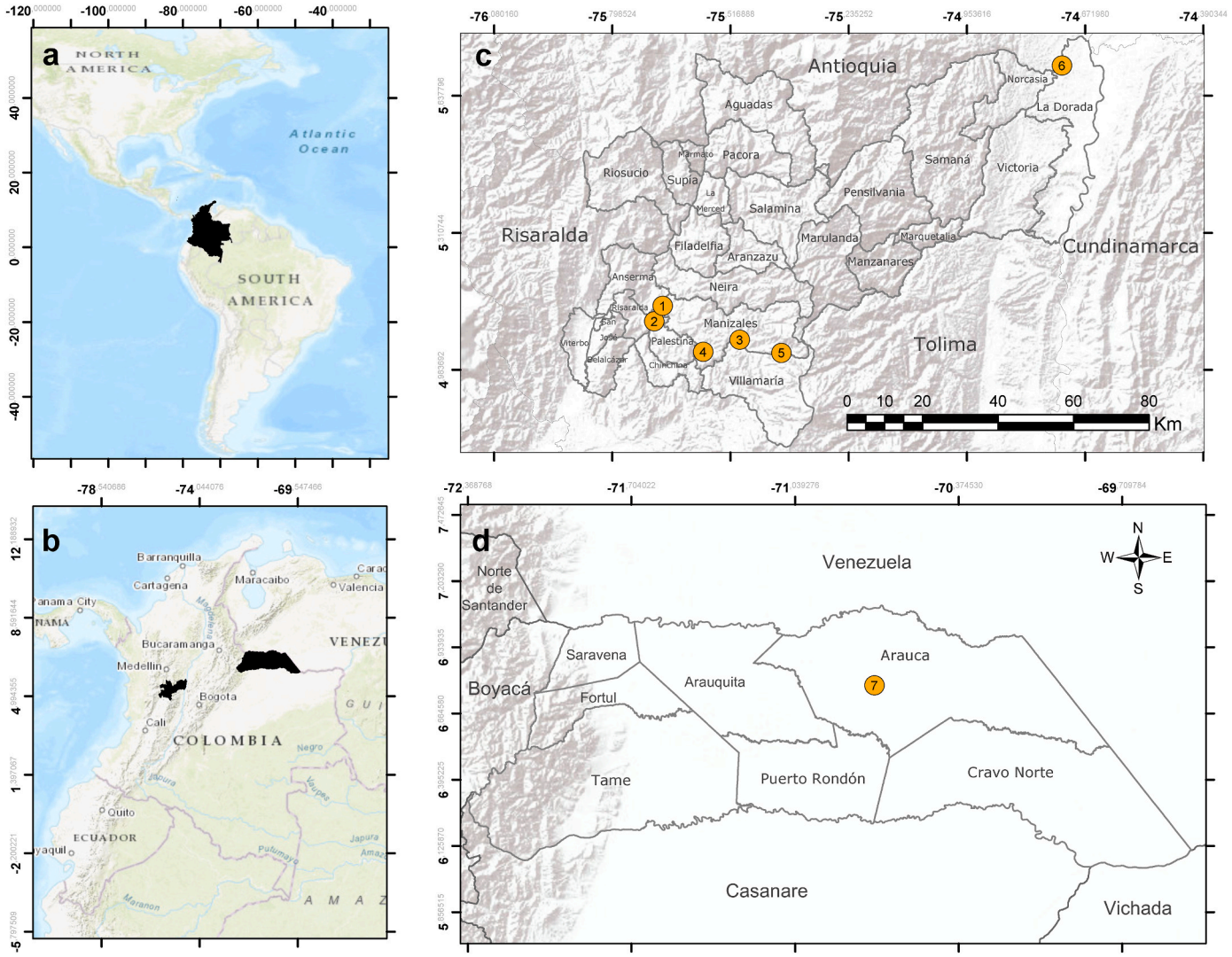


**Fig. 2.** Study area. (a) America with Colombia (black), (b) map of Colombia with the departments of Arauca and Caldas (black), (c) Department of Caldas, and (d) Department of Arauca. Sampled localities in the municipalities of Palestina (1, vereda El Retiro; 2, Kilómetro 35), Manizales (3, Jardín Botánico de la Universidad de Caldas; 4, vereda Rosario) Villamaría (5, Reserva Forestal Protectora Bosques de la CHEC) La Dorada (6, vereda La Atarraya) and Arauca (7, Los Trompillos).

**Table 3**
Bat species examined for *Polychromophilus* detection in the departments of Arauca and Caldas, Colombia. Localities are shown in Fig. 2.

| Host family | Species | Number of bats examined | Number of bats per locality | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Emballonuridae | *Saccopteryx leptura* | 1 | | | | | | | 1 |
| Molossidae | *Cynomops greenhalli* | 1 | | | | | | 1 | |
| | Cynomops milleri | 1 | | | | | | | 1 |
| | *Cynomops planirostris* | 9 | | | | | | | 9 |
| | *Eumops auripendulus* | 1 | | 1 | | | | | |
| | *Eumops glaucinus* | 2 | | | | | | | 2 |
| | *Molossops griseiventer* | 1 | | | | | | 1 | |
| | *Molossops temminckii* | 3 | | | | | | | 3 |
| | *Molossus coibensis* | 1 | | | | | | | 1 |
| | *Molossus molossus* | 14 | 10 | | | 3 | | | 1 |
| | *Molossus pretiosus* | 6 | | | | | | | 6 |
| | *Molossus rufus* | 4 | | | | | | | 4 |
| Noctilionidae | *Noctilio albiventris* | 6 | | | | | | | 6 |
| Phyllostomidae | *Artibeus lituratus* | 4 | | | 3 | | | 1 | |
| | *Artibeus obscurus* | 1 | | | | | | 1 | |
| | *Carollia brevicauda* | 5 | | 1 | 1 | | | 2 | 1 |
| | *Carollia castanea* | 3 | | 1 | | | | 2 | |
| | *Carollia perspicillata* | 4 | | | | | | 4 | |
| | *Carollia sp.* | 4 | | | 4 | | | | |
| | *Dermanura anderseni* | 5 | | | | | | 5 | |
| | *Desmodus rotundus* | 1 | | | | | | | 1 |
| | *Glossophaga soricina* | 4 | | | 2 | | | 2 | |
| | *Lophostoma nicaraguae* | 3 | | | | | | 3 | |
| | *Phyllostomus discolor* | 2 | | | | | | 1 | 1 |
| | *Phyllostomus hastatus* | 1 | | | | | | 1 | |
| | *Platyrrhinus helleri* | 2 | | | | | | 2 | |
| | *Sturnira* cf. *parvidens* | 1 | | | | | | 1 | |
| | *Sturnira erythromos* | 3 | | | | | 3 | | |
| | *Sturnira giannae* | 3 | | | | | | 3 | |
| | *Sturnira luisi* | 2 | | | | | | 2 | |
| | *Sturnira parvidens* | 1 | | | | | 1 | | |
| | *Trinycteris nicefori* | 1 | | | | | | 1 | |
| | *Uroderma convexum* | 1 | | | | | | 1 | |
| | *Uroderma magnirostrum* | 1 | | | | | | | 1 |
| Vespertilionidae | *Myotis albescens*[a] | 5 | 5 | | | | | | |
| | *Myotis handleyi* | 5 | | | | | | | 5 |
| | *Myotis keaysi* | 1 | | | | | 1 | | |
| | *Myotis* sp. | 3 | | | | | | | 3 |
| | *Neoeptesicus orinocensis* | 9 | | | | | | | 9 |
| **Total** | | **125** | **15** | **3** | **10** | **3** | **5** | **34** | **55** |

[a] *Polychromophilus deanei* positive species.

jModeltest v.2.1.6 (Darriba et al., 2012).

We performed the BI analysis using MrBayes 3.2.7a (Ronquist and Huelsenbeck, 2003) via the CIPRES Science Gateway v3.3 (Miller et al., 2010). We estimated posterior probability distributions (PP) using two independent Markov Chain Monte Carlo (MCMC) chains, run for 15 million generations with four chains sampled every 1000 generations. Convergence was assessed using Tracer (Rambaut and Drummond, 2007), with 25% of the trees discarded as burn-in and the remaining trees used to construct a consensus tree based on a 50% majority rule. We conducted the ML analysis using PhyML version 3.0 (Guindon and Gascuel, 2003), with nodal support values calculated via Bootstrap (BS) analysis using 1000 replicates. The phylogeny was visualized using FigTree v1.3.1 (Rambaut, 2009).

## 3. Results

From the 23 studies analyzed, we found 65 records of *Polychromophilus* in 46 bat species distributed across all continents except Antarctica (Table 1). Most *Polychromophilus* reports were detected in bats from the families Vespertilionidae (22 species), Miniopteridae (16 species), Hipposideridae (3 species), Rhinolophidae (3 species), with Rhinonycteridae and Emballonuridae having one species each (Table 1). The records were obtained in 30 countries and the most detected species was *P. melanipherus* (n = 14) and *P. murinus* (n = 12), *P. adami* and *P. corradetti* had a single record each, as did the species *P. deanei*, to which the record from this study is added and *Polychromophilus* sp. had 22 records.

Specifically, in America, *Polychromophilus* infections have been recorded exclusively in bats of the family Vespertilionidae, including *Eptesicus fuscus, Myotis austroriparius*, *M. nigricans*, *M. riparius*, *M. ruber*, *Myotis* sp., and *N. diminutus* in Brazil, Colombia, the United States, and Panama (Table 1). The searched for new records of *Polychromophilus* in bats from Colombia included 125 individuals belonging to 39 species and 5 families captured in seven localities (Table 3). The families with the highest number of bat individuals were Phyllostomidae (n = 52), Molossidae (n = 43), Vespertilionidae (n = 23), and Noctilionidae (n = 6), while the family Emballonuridae was represented by only one individual (Table 3). The species with the highest number of individuals captured were *Molossus molossus* (n = 14), *Cynomops planirostris* (n = 9), *Neoeptesicus orinocensis* (n = 9), *Molossus pretiosus* (n = 6), and *Noctilio albiventris* (n = 6) (Table 3). We detected only one individual *of Myotis albescens* (Vespertilionidae) infected with *Polychromophilus* through morphological analysis of blood smears and through molecular analysis of the blood, since it was not possible to detect it molecularly in the liver samples. The *M. albescens* captured in the municipality of Palestina was infected with *P. deanei* (prevalence = 0.8%). In this individual, we detected morphologically intraerythrocytic sexual forms (gametocytes) in blood smears, matching the original description of *P. deanei* (Fig. 3). The mature macrogametocytes measured between 5.5 and 7.5 μm in length and 4.0–5.0 μm in width (Fig. 3h–l, n, o). Microgametocytes were

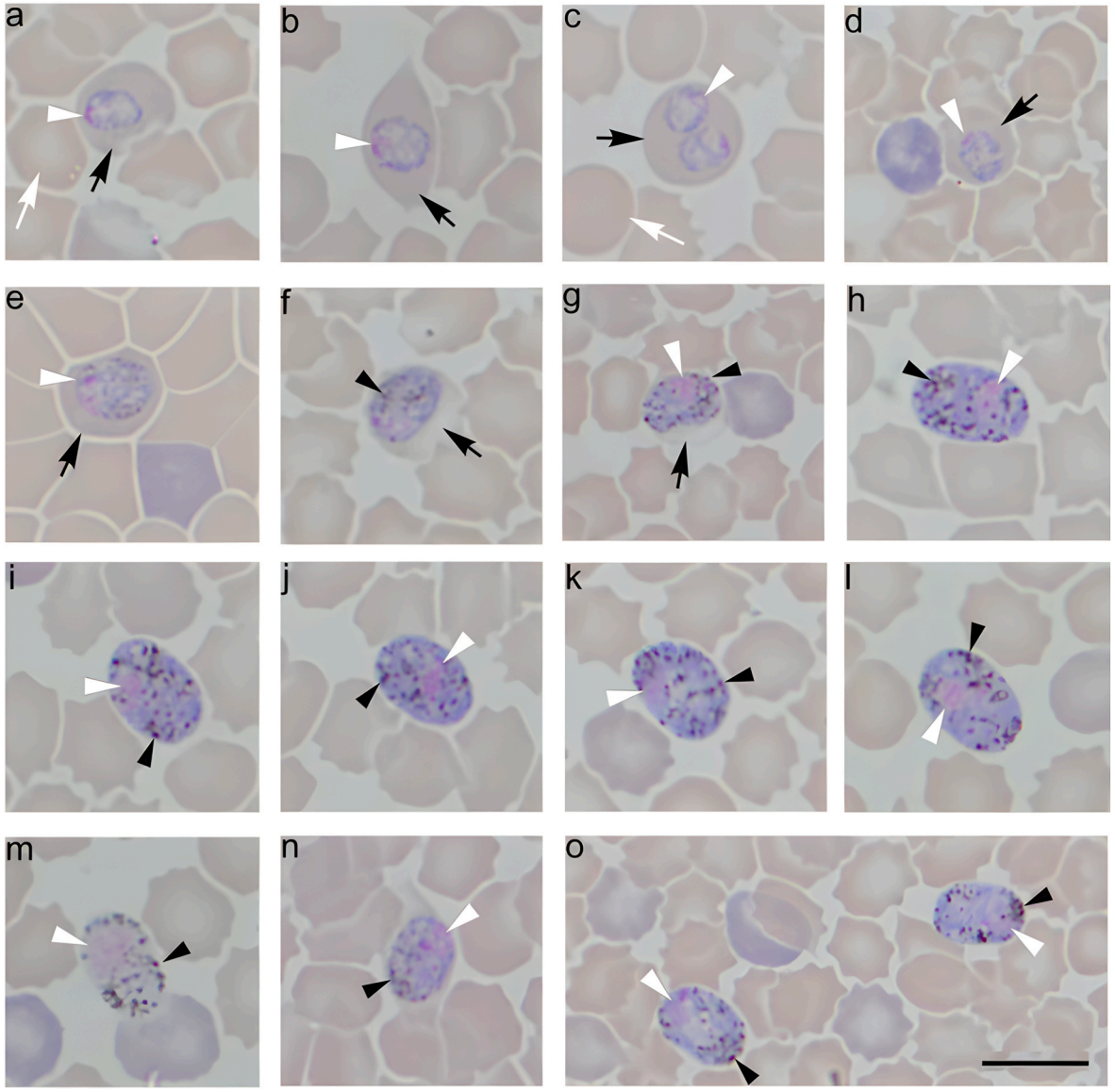


**Fig. 3.** Erythrocytic forms of *Polychromophilus deanei* found in *Myotis albescens*. (a–d) trophozoites, (e–g) immature gametocytes, (h-l, n, o) mature macrogametocytes, (m) mature microgametocyte. Short white arrow indicates healthy erythrocytes. White arrowhead indicates parasite nucleus. Black arrowhead indicates hemozoin granules. Short black arrow indicates infected erythrocyte cytoplasm. Scale bar = 10 μm.

less abundant, measuring approximately 7 μm in length and 4.5 μm in width (Fig. 3 m). The number of hemozoin granules of gametocytes varied between 24 and 35 (Fig. 3h–o). Molecular analyses of the hemoparasite *cyt b* gene showed 100% identity with *Polychromophilus* sp. sequences (**MW984522** and **MW984518**) from Brazil in *M. ruber* and *M. riparius*. The GenBank accession code for the *P. deanei* sequence obtained in this study is (**PP971136**). Additionally, we molecularly confirmed the taxonomic identity of the infected bat (*M. albescens*) (GenBank accession: **PP975259**). During capture, this bat was parasitized by mites, identified morphologically and molecularly as *Spinturnix americanus* (Fig. 4). The GenBank accession codes for the (COI) gene of *S. americanus* are (**PP967722** and **PP967723**).

Our phylogenetic reconstruction of the haemosporidians grouped all *Polychromophilus* sequences into a well-supported clade (PP = 1 and BS = 100%; Fig. 5). Our sequence formed a clade with sequences identified as *Polychromophilus* sp. found in *Myotis* bats from Brazil and Panama (genetic distances ≤0.8%), with a segregated sequence of *Polychromophilus* sp. detected in *N. diminutus* in Brazil (Table S2; Fig. 5). Furthermore, these sequences formed a clade with sequences of *P. murinus* reported in bats and flies of the family Nycteribiidae from the Old World (Madagascar and Switzerland) (genetic distance = 2%; Table S2; Fig. 5).

## 4. Discussion

The results of our review indicate that *Polychromophilus* has been recorded infecting bats worldwide, except in Antarctica, with a higher concentration of cases in Africa, Europe, and Asia. Most of these reports have been in bats of the family Vespertilionidae, with *Myotis* being the most frequently host globally (Megali et al., 2011; Witsenburg et al., 2014; Ramasindrazana et al., 2018; Arnuphapprasert et al., 2020; Rosyadi et al., 2022). Similarly, *P. melanipherus* and *P. murinus* are the most documented species, primarily in bats of the families Miniopteridae and Vespertilionidae, respectively (Witsenburg et al., 2012; Perkins and Schaer, 2016; Lutz et al., 2016; Sándor et al., 2021). In contrast, *P. adami* and *P. corradetti* have been recorded only in miniopterids from Africa (Congo and Gabon) (Perkins and Schaer, 2016). Similarly, *P. deanei* has only been reported in vespertilionids from South

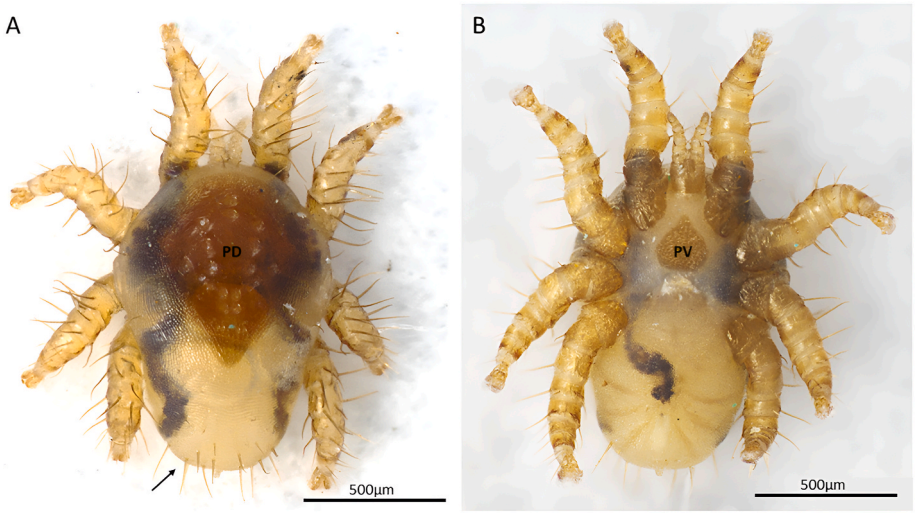


**Fig. 4.** Female *Spinturnix americanus* collected on a *Myotis albescens* in Colombia. (A) dorsal view (B) ventral view. PD = dorsal plate, PV = ventral plate. Scale bar = 500 µm.

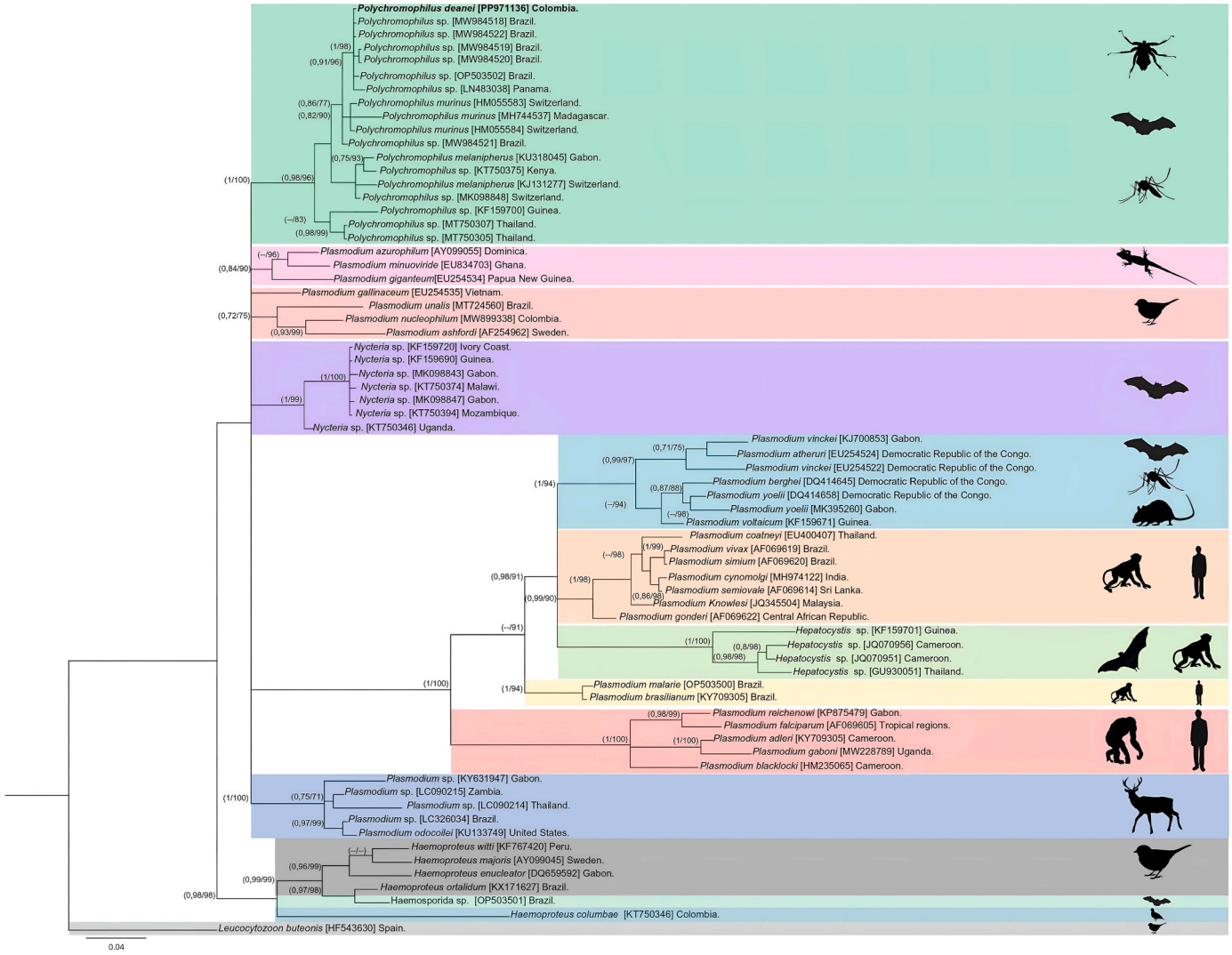


**Fig. 5.** Bayesian phylogeny based on 71 partial sequences of the mitochondrial cytochrome *b* (*cyt b*) gene of parasites of the order Haemosporida (Table S1). *Leucocytozoon buteonis* (**HF543630**) was used as an outgroup. Posterior probability (PP)/bootstrap (BS) values are shown above the nodes. Scale bar = 0.04 substitutions per site.

America (Brazil, Colombia) (Garnham et al., 1971; Marinkelle, 1995). Additionally, many of the yet undescribed species of *Polychromophilus* are found in the tropical regions, which are known for the high diversity of bats (Schaer et al., 2013; Borner et al., 2016; Arnuphapprasert et al., 2020; Chumnandee et al., 2021; Mathias et al., 2023). this underscores the need to expand research on *Polychromophilus* in these regions.

Our study represents the first report of *P. deanei* in *M. albescens* for the Americas. Morphologically, the immature intraerythrocytic forms (trophozoites) and sexual forms (gametocytes) of *Polychromophilus* match the original description of *P. deanei* in *M. nigricans* proposed by Garnham et al. (1971). Furthermore, our observations were similar to reports of *Polychromophilus* sp. in *Myotis austroriparius* from the United States (Foster, 1979) and *P. deanei* (as *Bioccala deanei*) in *Eptesicus fuscus miradorensis* from Colombia (Marinkelle, 1995). Additionally, our sequence of *cyt b* of *Polychromophilus* (**PP971136**) grouped with sequences identified as *Polychromophilus* sp. reported in Vespertilionidae bats in America in three previous studies (Borner et al., 2016; Minozzo et al., 2021; Mathias et al., 2023). However, these studies only used molecular techniques for detecting *Polychromophilus*, so there is no morphospecies associated with these sequences (Borner et al., 2016; Minozzo et al., 2021; Mathias et al., 2023). We recommend integrating morphological and molecular tools for understanding the interactions between *Polychromophilus* and bats, considering that both sources of information are key to resolve deeper phylogenetic discrepancies in Haemosporida (Outlaw and Ricklefs, 2011). Phylogenetic relationships evidenced a separation between *Polychromophilus* species in bats within Vespertilionidae from the Americas and the old World *P. murinus*. These results align with the differentiation of *Polychromophilus* in vespertilionid bats from different zoogeographic regions (Garnham et al., 1971; Perkins and Schaer, 2016). In this context, bats appear to be a crucial link in the circulation of haemosporidian of the Plasmodiidae in vertebrates, particularly parasites of the genus *Polychromophilus* (Perkins and Schaer, 2016). Therefore, understanding the interactions between bats, *Polychromophilus*, and their vectors is crucial to understand their zoonotic potential (Witsenburg et al., 2012).

In this study, we reported a prevalence of 0.8%, similar to values obtained in Brazil (Minozzo et al., 2021; Mathias et al., 2023). However, the prevalence reported in the study by Mathias et al. (2023), considering only individuals of the genus *Myotis*, was approximately 21.7%, compared to the ~7.1% found in this study. This discrepancy could be explained by the greater representation of *Myotis* in the Brazilian samples, as well as by ecological variables still unexplored in the Neotropics (e.g., seasonality and age of the bats) (Dew and McMillan, 1970; Witsenburg et al., 2014). These results contrast with high absolute prevalences (>40%) found in studies conducted in bats in countries in Africa and Europe (Schaer et al., 2013; Witsenburg et al., 2014; Lutz et al., 2016). It has been suggested that in temperate regions, the infection cycles of *Polychromophilus* might respond to habitat characteristics (e.g., seasonality) or to ecological traits of their hosts (e.g., age or reproductive periods) (Dew and McMillan, 1970; Witsenburg et al., 2014). Therefore, future research should gather information to contrast the unimodal seasonal profile of the tropics with the ecological and reproductive aspects of *Polychromophilus* reservoirs. This is particularly relevant in the Neotropical context, where seasonal dynamics may differ from those in temperate regions. Additionally, a high diversity of non-competent hosts reduces the probability of infection by vector-borne parasites (Garrido et al., 2021), and this could particularly occur in the Neotropics, where a higher diversity of bats has been recorded compared to temperate zones in Europe or tropical regions in Africa (Mickleburgh et al., 2002; Mena et al., 2011; Ramírez-Chaves et al., 2024).

Additionally, we identified wing mites of the species *S. americanus* on an individual of *M. albescens* infected with *P. deanei*. This mite species is exclusive to bats and is distributed from southern Canada to southern Brazil (Rudnick 1960; Dick et al., 2003). However, reports of its presence on *M. albescens* in Colombia are scarce and have been limited to morphological confirmations (Tarquino-Carbonell et al., 2015). In this study, we report for the first time COI gene sequences for the mite *S. americanus*. Since mites can act as vectors of pathogens, it is crucial to further investigate the possible role of *S. americanus* in disease transmission in bats, as this relationship could have important implications for the ecology and health of bats in areas where these mites are present.

Finally, this study represents the first morphological and molecular report of *P. deanei* in *M. albescens* in America. Additionally, we confirmed the presence of *Polychromophilus* in bats in Colombia, almost 30 years after the first and only report by Marinkelle (1995). Given these findings, it is necessary to expand the diversity of examined bat hosts, as well as use additional molecular markers to perform more detailed phylogenetic analyses. New studies should include morphological observations and molecular identification in other tissues of bat hosts, as well as perform these same analyses for their infective forms in the vector. This will provide a comprehensive picture of the biology of *Polychromophilus* species.

## Funding

This work was funded by the Program: “Relación, distribución, taxonomía de especies de garrapatas asociadas a mamíferos silvestres en zonas endémicas de rickettsiosis en Colombia. Un acercamiento a la comprensión de la relación vectores patógenos-reservorios”, granted by the Ministerio de Ciencia, Tecnología e Innovación - Minciencias (Code: 120385270267 and CTO 80740-200-2021) - Project: Garrapatas asociadas a mamíferos silvestres en el departamento de Caldas: Diversidad, detección de patógenos y distribución. Funded by Minciencias (Code: 71717). and also by the Project: Garrapatas (Acari: Ixodidae) de pequeños mamíferos en Arauca (Arauca, Colombia): una aproximación al ciclo de transmisión de borreliosis o enfermedad de Lyme. Funded by Vicerrectoría de investigaciones y Posgrados - Universidad de Caldas; Code: 0277620. Additionally, we received support from the Call for Economic Resources Support to Research Groups of the University of Caldas. - 2023 - Vicerrectoría de Investigaciones y posgrados - Universidad de Caldas.

## CRediT authorship contribution statement

**Diego Fernando Ceballos-Pérez:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Johnathan Alvarez-Londoño:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Héctor E. Ramírez-Chaves:** Writing – review & editing, Writing – original draft, Visualization, Validation, Resources, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Fredy A. Rivera-Páez:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgments

We thank all members of the GEBIOME group, particularly Dr. Paula A. Ossa-López and the researchers Erika M. Ospina-Pérez, María José Narváez-Moreno, Estefani T. Martínez-Sánchez, the members of the Natural History Laboratory at the Museo de Historia Natural de la

Universidad de Caldas (IZBD), Alison Guerrero-Diaz, and José J. Henao-Osorio, Wendy Juliana Muñoz-Márquez as well as all members of the Semillero de Investigación en Genética Molecular (SeGeM) for their valuable collaboration and support during the development of this research.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijppaw.2024.100986.

## References


Alvarez-Londoño, J., Cardona-Romero, M., Martínez-Sánchez, E.T., Ossa-López, P.A., Pérez-Cárdenas, J.E., Gonzalez, A.D., Rivera-Páez, F.A., Castaño-Villa, G.J., 2022. Avian haemosporidian (Haemosporida: *Plasmodium* and *Haemoproteus*) in the department of Arauca, Colombian Orinoquia region. Parasitol. Res. 121 (6), 1775–1787. https://doi.org/10.1007/s00436-022-07511-w.

Arnuphapprasert, A., Riana, E., Ngamprasertwong, T., Wangthongchaicharoen, M., Soisook, P., Thanee, S., Bhodhibundit, P., Kaewthamasorn, M., 2020. First molecular investigation of haemosporidian parasites in Thai bat species. Int. J. Parasitol.: Parasites Wildl 13, 51–61. https://doi.org/10.1016/j.ijppaw.2020.07.010.

Bohada-Murillo, M., Castaño-Villa, G.J., Fontúrbel, F.E., 2021. Effects of dams on vertebrate diversity: a global analysis. Diversity 13, 528. https://doi.org/10.3390/d13110528.

Borner, J., Pick, C., Thiede, J., Kolawole, O.M., Kingsley, M.T., Schulze, J., Cottontail, V. M., Wellinghausen, N., Schmidt-Chanasit, J., Bruchhaus, I., Burmester, T., 2016. Phylogeny of haemosporidian blood parasites revealed by a multi-gene approach. Mol. Phylogenet. Evol. 94 (Pt A), 221–231. https://doi.org/10.1016/j.ympev.2015.09.003.

Brown, A.D., 2016. Inferring Haplotype Diversity and Population Structure of the Mite *Spinturnix Americanus* between Two Host Species, *M. lucifugus* and *M. Septentrionalis*, via Cytochrome B Sequencing.

Bush, A.O., Lafferty, K.D., Lotz, J.M., Shostak, A.W., 1997. Parasitology meets ecology on its own terms: Margolis et al. revisited. J. Parasitol. 575–583 https://doi.org/10.2307/3284227.

Campbell, T.W., 2015. Evaluation of the blood film. Clin. Lab. Med. 35 (3), 703–721. https://doi.org/10.1016/j.cll.2015.05.016.

Cardona-Salazar, L.J., Benavides-Ossa, Y.A., Vargas-Daza, Á.M., Betancurt-Grisales, J.F., Bohada-Murillo, M., Martínez-Sánchez, E.T., Cardona-Romero, M., Busi, A., Tobón-Escobar, W.D., Ortíz-Giraldo, M., Gómez Castillo, D., Alvarez-Londoño, J., Molina-Marin, D.A., Rodas-Rua, J.C., Grisales-Muñoz, L.D., Velásquez-Trujillo, V., Salazar-Ramírez, L.F., Pineda-Gómez, D.M., Hoyos-Jaramillo, A., Santisteban-Arenas, R.R., Salazar-Ramírez, J.G., Orozco-Montoya, G.P., Ramos-Valencia, S.A., Gómez-Londoño, G., Ospina-Bautista, F., Estévez-Varón, J.V., Ossa-López, P.A., Rivera-Páez, F.A., Pérez Cárdenas, J.E., Fontúrbel, F.E., Castaño-Villa, G.J., 2020. A morphological, reproductive, and molt phenology database for 379 bird species from the Colombian Tropical Andes. Ecol. 101 (6), e03016 https://doi.org/10.1002/ecy.3016.

Chumnandee, C., Pha-obnga, N., Werb, O., Matuschewski, K., Schaer, J., 2021. Molecular characterization of *Polychromophilus* parasites of *Scotophilus kuhlii* bats in Thailand. Parasitology 148 (4), 495–499. https://doi:10.1017/S003118202000222X.

Darriba, D., Taboada, G.L., Doallo, R., Posada, D., 2012. jModelTest 2: more models, new heuristics and parallel computing. Nat. Methods 9 (8), 772. https://doi.org/10.1038/nmeth.2109.

Dew, B.B., McMillan, B., 1970. Seasonal variation of *Polychromophilus melanipherus* (Sporozoa: Haemoproteidae) in the bent-winged bat *Miniopterus schreibersii* (Chiroptera) in new South Wales. Parasitology 61 (1), 161–166. https://doi.org/10.1017/S0031182000040956.

Dick, C.W., Gannon, M.R., Little, W.E., Patrick, M.J., 2003. Ectoparasite associations of bats from central Pennsylvania. J. Med. Entomol. 40 (6), 813–819. https://doi.org/10.1603/0022-2585-40.6.813.

Duval, L., Mejean, C., Maganga, G.D., Makanga, B.K., Koumba, L.B.M., Peirce, M.A., Ariey, F., Bourgarel, M., 2012. The chiropteran haemosporidian *Polychromophilus melanipherus*: a worldwide species complex restricted to the family Miniopteridae. Infect. Genet. Evol. 12 (7), 1558–1566. https://doi.org/10.1016/j.meegid.2012.06.006.

Escobar-Lasso, S., Cerón-Cardona, J., Castaño-Salazar, J., 2013. Los mamíferos de la cuenca del río Chinchiná, en la región andina de Colombia. Therya 4 (1), 139–155. https://doi.org/10.12933/therya-13-111.

Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R., 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotechnol. 3 (5), 294–299.

Foster, G.W., 1979. *Polychromophilus* from southeastern brown bats (*Myotis austroriparius*) in North-central Florida. J. Parasitol. 65, 465–466. https://doi.org/10.2307/3280300.

Galen, S.C., Borner, J., Martinsen, E.S., Schaer, J., Austin, C.C., West, C.J., Perkins, S.L., 2018. The polyphyly of *Plasmodium*: comprehensive phylogenetic analyses of the malaria parasites (order Haemosporida) reveal widespread taxonomic conflict. R. Soc. Open Sci. 5 (5), 171780 https://doi.org/10.1098/rsos.171780.

Gardner, A.L. (Ed.), 2008. Mammals of South America, Volume 1: Marsupials, Xenarthrans, Shrews, and Bats. University of Chicago Press. https://doi.org/10.1644/08-MAMM-R-296.1.

Garnham, P.C.C., 1966. Malaria parasites and other Haemosporidia. Am. J. Trop. Med. Hyg. 561–563. https://doi.org/10.4269/ajtmh.1967.16.561.

Garnham, P.C.C., 1973. The zoogeography of Polychromophilus and description of a new species of a gregarine (*Lankesteria galliardi*). Ann. Parasitol. Hum. Comp. 48 (2), 231–242. https://doi.org/10.1051/parasite/1973482231.

Garnham, P.C., Lainson, R., Shaw, J.J., 1970. A malaria-like parasite of a bat from Brazil. Trans. R. Soc. Trop. Med. Hyg. 64 (1), 13, 13.

Garnham, P.C.C., Lainson, R., Shaw, J.J., 1971. A contribution to the study of the Haematozoon parasites of bats: a new mammalian haemoproteid, *Polychromophilus deanei* n. sp. Mem. Inst. Oswaldo Cruz 69, 119–125. https://doi.org/10.1590/s0074-02761971000100009.

Garrido, M., Halle, S., Flatau, R., Cohen, C., Navarro-Castilla, Á., Barja, I., Hawlena, H., 2021. The dilution effect behind the scenes: testing the underlying assumptions of its mechanisms through quantifying the long-term dynamics and effects of a pathogen in multiple host species. Proc. R. Soc. A B 288, 20210773. https://doi.org/10.1098/rspb.2021.0773, 1952.

Guindon, S., Gascuel, O., 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst. Biol. 52 (5), 696–704. https://doi.org/10.1080/10635150390235520.

Harris, D.J., Santos, J.L., Borges-Nojosa, D.M., de Castro, D.P., 2019. Molecular screening of *Plasmodium* (Haemosporidia: Plasmodiidae) parasites from reptiles in Brazil. J. Parasitol. 105 (6), 913–917. https://doi.org/10.1645/18-149.

Hellgren, O., Waldenström, J., Bensch, S., 2004. A new PCR assay for simultaneous studies of *Leucocytozoon, Plasmodium*, and *Haemoproteus* from avian blood. J. Parasitol. 90 (4), 797–802. https://doi.org/10.1645/GE-184R1.

Holz, P.H., Lumsden, L.F., Legione, A.R., Hufschmid, J., 2019. *Polychromophilus melanipherus* and haemoplasma infections not associated with clinical signs in southern bent-winged bats (*Miniopterus orianae bassanii*) and eastern bent-winged bats (*Miniopterus orianae oceanensis*). Int J Parasitol Parasites Wildl 8, 10–18. https://doi.org/10.1016/j.ijppaw.2018.11.008.

Holz, P.H., Clark, P., McLelland, D.J., Lumsden, L.F., Hufschmid, J., 2020. Haematology of southern bent-winged bats (*Miniopterus orianae bassanii*) from the naracoorte caves national park, South Australia. Comp. Clin. Pathol. 29 (1), 231–237. https://doi.org/10.1007/s00580-019-03049-z.

Lalremruata, A., Magris, M., Vivas-Martínez, S., Koehler, M., Esen, M., Kempaiah, P., Jeyaraj, S., Perkins, D.J., Mordmüller, B., Metzger, W.G., 2015. Natural infection of *Plasmodium brasilianum* in humans: man and monkey share quartan malaria parasites in the Venezuelan Amazon. EBioMedicine 2 (9), 1186–1192. https://doi.org/10.1016/j.ebiom.2015.07.033.

Landau, I., Baccam, D., Ratanaworabhan, N., Yenbutra, S., Boulard, Y., Chabaud, A.G., 1984. Nouveaux Haemoproteidae parasites de chiroptères en Thailande. Ann. Parasitol. Hum. Comp. 59 (5), 437–447. https://doi.org/10.1051/parasite/1984595437.

Liu, W., Li, Y., Learn, G., et al., 2010. Origin of the human malaria parasite *Plasmodium falciparum* in gorillas. Nature 467, 420–425. https://doi.org/10.1038/nature09442.

Lutz, H.L., Patterson, B.D., Peterhans, J.C.K., Stanley, W.T., Webala, P.W., Gnoske, T.P., Hackett, S.J., Stanhope, M.J., 2016. Diverse sampling of East African haemosporidians reveals chiropteran origin of malaria parasites in primates and rodents. Mol. Phylogenet. Evol. 99, 7–15. https://doi.org/10.1016/j.ympev.2016.03.004.

Mancilla-Agrono, L.Y., Banguero-Micolta, L.F., Ossa-López, P.A., Ramírez-Chaves, H.E., Castaño-Villa, G.J., Rivera-Páez, F.A., 2022. Is *Borrelia burgdorferi* sensu stricto in South America? First molecular evidence of its presence in Colombia. Trav. Med. Infect. Dis. 7 (12), 428. https://doi.org/10.3390/tropicalmed7120428.

Marinkelle, C.J., 1995. The haemoproteid parasite, *Bioccala deanei*, from a Colombian bat, *Eptesicus fuscus* (Vespertilionidae). Ann. Trop. Med. Parasitol. 89 (1), 89–91. https://doi.org/10.1080/00034983.1995.11812935.

Mathias, B.D.S., Minozzo, G.A., Biondo, A.W., Costa, J.D.O.J., Soares, H.S., Marcili, A., Guimarães, L.D.O., Anjos, C.C.D., Santos, A.P.D., Riediger, I.N., Fecchio, A., Bueno, M.G., Pinho, J.B., Kirchgatter, K., 2023. Molecular investigation confirms *Myotis* genus bats as common hosts of *Polychromophilus* in Brazil. Microorganisms 11 (6), 1531. https://doi.org/10.3390/microorganisms11061531.

McNish, T., 2007. Bogotá. Las aves de Los Llanos de, p. 302p.

Megali, A., Yannic, G., Christe, P., 2011. Disease in the dark: molecular characterization of *Polychromophilus murinus* in temperate zone bats revealed a worldwide distribution of this malaria-like disease. Mol. Ecol. 20 (5), 1039–1048. https://doi.org/10.1111/j.1365-294X.2010.04905.x.

Mena, J.L., Solari, S., Carrera, J.P., Aguirre, L.F., Gómez, H., Herzog, S.K., Martinez, R., Jorgensen, P.M., Tiessen, H., 2011. Small mammal diversity in the tropical Andes: an overview. Clim. Change Biodiver. tropical Andes. Sao José dos Campos: Inter-American Instit. Global Change Res. Scientific Committee on Problems Environ. 260–275.

Mickleburgh, S.P., Hutson, A.M., Racey, P.A., 2002. A review of the global conservation status of bats. Oryx 36 (1), 18–34. https://doi:10.1017/S0030605302000054.

Miller, M.A., Pfeifer, W., Schwartz, T., 2010. Creating the CIPRES science gateway for inference of large phylogenetic trees. In: Proceedings of the gateway computing environments workshop (GCE),New Orleans. LApp 1–8. https://doi.org/10.1109/GCE.2010.5676129.

Minozzo, G.A., Mathias, B.D.S., Riediger, I.N., Guimarães, L.O., Dos Anjos, C.C., Monteiro, E.F., Dos Santos, A.P., Biondo, A.W., Kirchgatter, K., 2021. First molecular detection of *Polychromophilus* parasites in Brazilian bat species. Microorganisms 9 (6), 1240. https://doi.org/10.3390/microorganisms9061240.

Ossa-López, P.A., Ramírez-Chaves, H.E., Rivera-Páez, F.A., 2024a. Pathogens associated with ticks (Acari: Ixodidae) and mammals in the Orinoquia region of Colombia: an approach to understanding vector-pathogen-host interactions. Acta Trop. 256, 107282 https://doi.org/10.1016/j.actatropica.2024.107282.

Ossa-López, P.A., Ramírez-Chaves, H.E., López, M.E.Á., Villa, G.J.C., Rivera-Páez, F.A., 2024b. Bacterial community of ticks (Acari: Ixodidae) and mammals from Arauca, Colombian Orinoquia. Int J Parasitol Parasites Wildl 24, 100943. https://doi.org/10.1016/j.ijppaw.2024.100943.

Outlaw, D.C., Ricklefs, R.E., 2011. Rerooting the evolutionary tree of malaria parasites. Proc. Natl. Acad. Sci. U. S. A. 108 (32), 13183–13187. https://doi.org/10.1073/pnas.1109153108.

Pacheco, M.A., Matta, N.E., Valkiunas, G., Parker, P.G., Mello, B., Stanley Jr., C.E., Lentino, M., Garcia-Amado, M.A., Cranfield, M., Kosakovsky Pond, S.L., Escalante, A. A., 2018. Mode and rate of evolution of haemosporidian mitochondrial genomes: timing the radiation of avian parasites. Mol. Biol. Evol. 35 (2), 383–403. https://doi.org/10.1093/molbev/msx285.

Page, M.J., McKenzie, J.E., Bossuyt, P.M., Boutron, I., Hoffmann, T.C., Mulrow, C.D., Shamseer, L., Tetzlaff, J.M., Akl, E.A., Brennan, S.E., Chou, R., Glanville, J., Grimshaw, J.M., Hróbjartsson, A., Lalu, M.M., Li, T., Loder, E.W., Mayo-Wilson, E., McDonald, S., McGuinness, L.A., Stewart, L.A., Thomas, J., Tricco, A.C., Welch, V.A., Whiting, P., Moher, D., 2021. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. J. Clin. Epidemiol. 134, 178–189. https://doi.org/10.1016/j.jclinepi.2021.03.001.

Parson, W., Pegoraro, K., Niederstätter, H., Föger, M., Steinlechner, M., 2000. Species identification by means of the cytochrome b gene. Int. J. Leg. Med. 114 (1), 23–28. https://doi.org/10.1007/s004140000134.

Perkins, S.L., 2014. Malaria's many mates: past, present, and future of the systematics of the order Haemosporida. J. Parasitol. 100 (1), 11–25. https://doi.org/10.1645/13-362.1.

Perkins, S.L., Schaer, J., 2016. A modern menagerie of mammalian malaria. Trends Parasitol. 32 (10), 772–782. https://doi.org/10.1016/j.pt.2016.06.001.

Poofery, J., Ngamprasertwong, T., Narapakdeesakul, D., Arnuphapprasert, A., Nugraheni, Y.R., Thanee, S., Asada, M., Kaneko, O., Kaewthamasorn, M., 2023. Complete mitochondrial genome analyses confirm that bat *Polychromophilus* and ungulate *Plasmodium* constitute a distinct clade independent of other *Plasmodium* species. Sci. Rep. 13 (1), 20258 https://doi.org/10.1038/s41598-023-45551-z.

Ramasindrazana, B., Goodman, S.M., Dsouli, N., Gomard, Y., Lagadec, E., Randrianarivelojosia, M., Dellagi, K., Tortosa, P., 2018. *Polychromophilus* spp. (Haemosporida) in Malagasy bats: host specificity and insights on invertebrate vectors. Malar. J. 17, 1–11. https://doi.org/10.1186/s12936-018-2461-8.

Rambaut, A., 2009. FigTree. Tree figure drawing tool. http://tree.bio.ed.ac.uk/software/figtree/.

Rambaut, A., Drummond, A.J., 2007. Tracer v1. 5. Available from: http://beast.bio.ed.ac.uk.Tracer.

Ramírez-Chaves, H.E., Robles, L.N.G., Castaño, R.A., Morales-Martínez, D.M., Suárez, C. A.F., Rodríguez-Posada, M.E., Zurc, D., Concha, O.D.C., Trujillo, A., Noguera, U.E.A., Pantoja, P.G.E., González, M.J.F., Pérez, T.J., Mantilla, M.H., López, C.C., Velásquez, V.A., Zárrate, C.D., 2024. Mamíferos de Colombia. v1.14. Sociedad Colombiana de Mastozoología. https://doi.org/10.15472/kl1whs.

Rasoanoro, M., Goodman, S.M., Randrianarivelojosia, M., Rakotondratsimba, M., Dellagi, K., Tortosa, P., Ramasindrazana, B., 2021. Diversity, distribution, and drivers of *Polychromophilus* infection in Malagasy bats. Malar. J. 20, 1–11. https://doi.org/10.1186/s12936-021-03696-0.

Rippstein, G., 2001. Agroecología y biodiversidad de las sábanas en los Llanos Orientales de Colombia, No. 322. Ciat.

Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19 (12), 1572–1574. https://doi.org/10.1093/bioinformatics/btg180.

Rosskopf, S.P., Held, J., Gmeiner, M., Mordmüller, B., Matsiégui, P.B., Eckerle, I., Weber, N., Matuschewski, K., Schaer, J., 2019. *Nycteria* and *Polychromophilus* parasite infections of bats in Central Gabon.". Infect. Genet. Evol. 68, 30–34. https://doi.org/10.1016/j.meegid.2018.11.022.

Rosyadi, I., Shimoda, H., Takano, A., Yanagida, T., Sato, H., 2022. Isolation and molecular characterization of *Polychromophilus* spp. (Haemosporida: Plasmodiidae) from the Asian long-fingered bat (*Miniopterus fuliginosus*) and Japanese large-footed bat (*Myotis macrodactylus*) in Japan. Parasitol. Res. 121 (9), 2547–2559. https://doi.org/10.1007/s00436-022-07592-7.

Rudnick, A., 1960. A Revision of the Mites of the Family of Spinturnicidae (Acarina), second ed., Vol. 17. University of California Press, Berkeley, California.

Ruiz, J.F., Melo, J.Y., 2022. Informe de predicción climática a corto, mediano y largo plazo en Colombia.

Sándor, A.D., Péter, Á., Corduneanu, A., Barti, L., Csősz, I., Kalmár, Z., Hornok, S., Kontschán, J., Mihalca, A.D., 2021. Wide distribution and diversity of malaria-related haemosporidian parasites (*Polychromophilus* spp.) in bats and their ectoparasites in Eastern Europe. Microorganisms 9 (2), 230. https://doi.org/10.3390/microorganisms9020230.

Schaer, J., Perkins, S.L., Decher, J., Leendertz, F.H., Fahr, J., Weber, N., Matuschewski, K., 2013. High diversity of West African bat malaria parasites and a tight link with rodent *Plasmodium* taxa. Proc. Natl. Acad. Sci. U. S. A. 110 (43), 17415–17419. https://doi.org/10.1073/pnas.1311016110.

Sikes, R.S., Animal Care and Use Committee of the American Society of Mammalogists., 2016. 2016 Guidelines of the American Society of Mammalogists for the use of wild mammals in research and education. J. Mammal. 97 (3), 663–688. https://doi.org/10.1093/jmammal/gyw078.

Szentiványi, T., Markotter, W., Dietrich, M., Clément, L., Ançay, L., Brun, L., Genzoni, E., Kearney, T., Seamark, E., Estók, P., Christe, P., Glaizot, O., 2020. Host conservation through their parasites: molecular surveillance of vector-borne microorganisms in bats using ectoparasitic bat flies. Parasite 27. https://doi.org/10.1051/parasite/2020069.

Tamura, K., Stecher, G., Kumar, S., 2021. MEGA11: molecular evolutionary genetics analysis version 11. Mol. Biol. Evol. 38 (7), 3022–3027. https://doi.org/10.1093/molbev/msab120.

Tarquino-Carbonell, A.D.P., Gutiérrez-Díaz, K.A., Galindo-Espinosa, E.Y., Reinoso-Flórez, G., Solari, S., Guerrero, R., 2015. Ectoparasites associated with bats in northeastern Tolima, Colombia. Mastozool. Neotrop. 22 (2), 349–358.

Valkiunas, G., 2004. Avian Malaria Parasites and Other Haemosporidia. CRC press.

Velásquez-Guarín, D., Pérez Cárdenas, J.E., Serpa, M.C.A., Labruna, M.B., Faccini-Martínez, A.A., Rivera-Páez, F.A., Ramírez-Chaves, H.E., 2024. *Rickettsia* spp. seroprevalence in wild mammals from Arauca, Orinoquia region of Colombia. Mastozool. Neotrop. 31 (1), 10–31687. https://doi.org/10.31687/saremMN.24.31.01.10.e0963.

Witsenburg, F., Salamin, N., Christe, P., 2012. The evolutionary host switches of *Polychromophilus*: a multi-gene phylogeny of the bat malaria genus suggests a second invasion of mammals by a haemosporidian parasite. Malar. J. 11 https://doi.org/10.1186/1475-2875-11-53. Article 53.

Witsenburg, F., Schneider, F., Christe, P., 2014. Epidemiological traits of the malaria-like parasite *Polychromophilus murinus* in the Daubenton's bat *Myotis daubentonii*. Parasites Vectors 7 (1). https://doi.org/10.1186/s13071-014-0566-7. Article 566.

Wood, S.F., 1952. Mammal blood parasite records from southwestern United States and Mexico. J. Parasitol. 38, 85. https://doi.org/10.2307/3274183.