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DIVERSITY AND HOST SPECIFICITY OF NYCTERIBIID BAT FLIES (DIPTERA: NYCTERIBIIDAE) IN KENYA

A Thesis
Presented to
The Faculty of the Department of Biology
Western Kentucky University
Bowling Green, Kentucky

In Partial Fulfillment
Of the Requirements for the Degree
Master of Science

By
Taylor Verrett

August 2021

DIVERSITY AND HOST SPECIFICITY OF NYCTERIBIID BAT FLIES (DIPTERA: NYCTERIBIIDAE) IN KENYA

Date recommended 28 June 2021

Carl W. Dick, Director of Thesis

Jarrett Johnson

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Associate Provost for Research and Graduate Education

This thesis is dedicated to my mentor and friend of many years, Dr. Johanna Harvey. Without the countless hours she spent training and supporting me during my time as an undergraduate, and the advice, opportunities, and inspiration she provided afterward, I have every confidence I would not be where I am today. Thank you, Johanna!

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PREFACE

Understanding the full extent of parasite biodiversity is crucial for biological conservation and for characterizing the role of parasites in disease transmission. Parasites are uniquely vulnerable to artificial species boundaries due to their relative morphological conservatism, and the use of integrative approaches to species delimitation is key to accurately quantifying parasite diversity. Molecular genetic techniques have provided a valuable tool for re-assessing morphological species, and have accordingly recovered a substantial degree of cryptic speciation in parasites. Verification of putative parasite species identities with molecular genetic evidence or division of nominal species into morphologically cryptic lineages is ecologically impactful, often modifying our understanding of the distributional ranges of parasites across geography and associated hosts. Host specificity is one of the most defining characteristics of parasites, and is a measure often used to appraise a parasite's possible impact as a vector of infectious disease.

In this thesis, I will take two approaches to exploring diversity and host-parasite associations in a dipteran parasite of bats. Nycteribiid bat flies (Diptera: Nycteribiidae) are flightless, hematophagous parasites of bats, and generally exhibit high host specificity. Despite being associated with hosts (African bats) at the forefront of zoonotic disease risk, bat fly diversity remains poorly understood in many geographic regions. Although appearing together in this MS thesis, these chapters are presented as two complete yet independent manuscripts that will be submitted to scientific journals. Chapter 1 presents a comprehensive catalog of all nycteribiid bat flies recorded from Kenya, a region largely unexplored in terms of nycteribiid diversity, and their respective

hosts. This chapter synthesizes historical records and an extensive survey of 4,255 bats across many of Kenya's most biodiverse areas between 2006 and 2015. Chapter 2 narrows its focus to investigate the possibility of cryptic diversity in a single Kenyan bat fly species, *Penicillidia fulvida*. *P. fulvida* demonstrates unusually low host specificity to an extent greater than any other known nycteribiid species, a phenomenon indicating either cryptic speciation or a more meaningful potential for inter-host disease transmission by bat flies than has previously been suggested. Together, these papers contribute to our understanding of diversity and host associations in an obscure but ecologically important parasite group.

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Taylor Verrett August 2021 82 Pages

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Bat flies (Diptera: Nycteribiidae and Streblidae) are hematophagous ectoparasites of bats distributed globally. Members of Nycteribiidae are morphologically constrained relative to streblids, and are united by their lack of wings, dorso-ventrally compressed bodies, and a primary distribution across the Eastern hemisphere. Bats are principal reservoirs of infectious diseases, including viral zoonoses of important consideration to human health, but the overall high host specificity of bat flies has largely been thought to curb their potential as inter-specific vectors of bat-borne pathogens. However, nycteribiid diversity and host associations remain critically understudied in some geographic regions, and rare examples of nycteribiid bat flies demonstrating low host specificity have been documented. In this thesis, two approaches are used to investigate the diversity and ecology of nycteribiid bat flies in Kenya, a country with understudied nycteribiid diversity despite its exceptional richness of bats. The first approach consolidates all historical records of nycteribiid bat flies in Kenya with records from the recent 9-year Bats of Kenya survey to generate a comprehensive species catalog. This catalog describes seven nycteribiid genera and 18 species in total, including 5 species unknown from Kenya prior to the Bats of Kenya survey, in addition to their respective host associations and geographic distributions. The second approach uses molecular techniques to investigate the potential for cryptic diversity in a single Kenyan bat fly species with

unusually low host specificity, *Penicillidia fulvida*. Undetected cryptic diversity can conceal higher host specificity in morphologically conserved parasites, a possible explanation for the existence of anomalous host-generalist bat fly species. However, the use of mitochondrial COI and nuclear 28S did not reveal genetic structure in *P. fulvida* across 6 bat families, suggesting *P. fulvida* truly represents a single morphological and genetic species capable of parasitizing phylogenetically distant bats. Overall, this study enhances our understanding of nycteribiid diversity and host associations, and addresses important ecological factors obscuring the potential of this parasite group as vectors of infectious disease.

CHAPTER 1: NYCTERIBIID BAT FLIES (DIPTERA: NYCTERIBIIDAE) OF KENYA

Abstract:

Bat flies (Diptera: Nycteribiidae and Streblidae) are hematophagous ectoparasites of bats characterized by viviparous pupiparity and generally high host specificity. Nycteribiid bat flies are wingless, morphologically constrained, and are most diverse in the Eastern Hemisphere. Africa hosts 20% of global bat biodiversity, and over half of all African bat species occur in its most bat-rich country, Kenya. However, records of nycteribiid bat fly diversity in Kenya remain sparse and unconsolidated. This paper combines all past species records of nycteribiid bat flies with records from a survey of 4,255 Kenyan bats ("Bats of Kenya" survey led by B. D. Patterson) across 157 localities between 2006 and 2015. A total of seven nycteribiid genera and 18 species are recorded, with 5 species representing previously undocumented contributions from the Bats of Kenya survey. Host associations and geographic distributions based on all available records are also described. This comprehensive species catalog addresses and further emphasizes the need for similar investigations of nycteribiid biodiversity across Africa.

Key Words: Bat Flies, Chiroptera, Ectoparasites, Kenya, Nycteribiidae

Introduction

Bat flies (Diptera: Nycteribiidae and Streblidae) are hematophagous, obligate ectoparasites of bats worldwide. Like other members of superfamily Hippoboscoidea, they are characterized by reproduction via adenotrophic viviparity, wherein a single egg hatches and the larval instars develop within a female, nourished by specialized glands (Dick and Patterson 2006). The primary disassociation of bat flies from their hosts occurs when gravid females traverse the roost substrate to deposit prepupae, when flies are completing their pupal development, and when newly eclosed adults must locate a suitable host.

Bat fly morphology is well-suited for clinging to the pelage or membranes of bats. Most species of both families possess rows of spiny ctenidia, modified setae, and legs tipped in recurved claws to anchor themselves to their hosts. Many taxa have secondarily evolved winglessness (Theodor 1957a). Bat flies in the family Streblidae possess relatively diverse body plans across the genera, ranging from dorsoventrally flattened to laterally compressed and from wingless to fully flighted. In contrast, members of Nycteribiidae are morphologically constrained; all species are wingless, dorsoventrally flattened and superficially spider-like, and differ primarily in size (Dick and Patterson 2006).

Though both Streblidae and Nycteribiidae are globally ubiquitous, particularly in the tropics and subtropics, nycteribiid bat flies are most diverse in the Eastern hemisphere (Dick and Patterson 2006), with only about 20% of described species occurring in the Western Hemisphere. Nycteribiidae is comprised of 275 species across 3 subfamilies and 12 genera (Dick and Patterson 2006). Nycteribiid bat flies may exhibit lower host

specificity than streblids, but there have been few large and carefully collected surveys of nycteribiids. Further, some bat flies have been identified as vectors of bacterial pathogens and haemosporidian parasites (Megali et al. 2010, Wilkinson et al. 2016), and can harbor viruses related to bat-associated zoonoses (Bennett et al. 2020, Ramírez-Martínez et al. 2021). Therefore, a more complete understanding of nycteribiid diversity and host associations is important for characterizing their role in disease transmission among bats.

Bat biodiversity follows a typical latitudinal trend, with nearly 80% of species concentrated in the tropics (Willig et al. 2003). Over two hundred bat species have been described on the African continent (Happold and Happold 2013), of which 108 species have been recorded in Kenya (Patterson and Webala 2012). Despite Kenya's position as the most bat-rich East African country (Patterson and Webala 2012), the diversity of nycteribiid bat flies associated with this wealth of potential hosts remains mostly unexplored. Here I compile all known historical species records of bat flies in Kenya, in addition to identifying and cataloging nycteribiid bat flies from the Bats of Kenya survey of 4,255 bats across 157 Kenyan localities between 2006 and 2015. This species account contributes to our understanding of the diversity, distribution, and host associations of nycteribiid bat flies in an understudied region.

Materials/Collection Methods

The Bats of Kenya survey was conducted by the Field Museum of Natural History in collaboration with the National Museums of Kenya, Kenya Wildlife Service, Karatina University and Maasai Mara University between 2006 and 2015 (Monadjem et al. 2020) and contributed the bulk of species records in this study. Bats were captured across 157 localities, primarily in western, central, and eastern Kenya. Sampling of bats was

conducted using both mist nets erected along trails and roadways, or at entrance/exit flyways to roosting sites. Additional bats were collected via hand nets within roosts. The properties of roosting sites were variable, ranging from natural and anthropogenic permanent structures to transient roosts like trees. Following capture, bats were transferred to clean, individual cloth bags to minimize parasite disturbance transfers. Bats were euthanized using halothane for collection as museum specimens then fumigated with ethyl ether to ease the extraction of their ectoparasites. Museum specimen collection was performed in accordance with American Society of Mammalogist guidelines (Sikes 2016) and with the approval of the Field Museum's Institutional Animal Care and Use Committee (2012-003). Bat flies were immediately transferred to tubes containing 95% ethanol. At the lab, nycteribiid flies were identified using keys and species accounts from Theodor (1967) and reference specimens in the Field Museum of Natural History Collection of Hippoboscoid Diptera, where all specimens collected in this survey are currently stored.

Species Accounts

The following species accounts address all known species of nycteribiid bat flies from Kenya, including historical and current records. Each account lists previous records, hosts, and distributions, Kenyan records (if any), Bats of Kenya records, distribution, hosts, and comment where applicable. The previous records are based on unpublished FMNH records and data compiled by T. C. Maa. Note that host species identities reported in historical records cannot be confirmed and are reported as published, and we have attempted to update zoological nomenclature as well as names of political units.

Family Nycteribiidae Samouelle, 1819

Subfamily Cyclopodiinae Maa, 1965

Genus Cyclopodia Kolenati, 1863

***Cyclopodia greeffi greeffi* Karsch, 1884**

Previous records, hosts, distributions:

From *Eidolon helvum* (Senegal, "French West Africa", Mali, Liberia, Togolese Republic, Nigeria, Cameroon, Sao Tome, Democratic Republic of the Congo (DRC), Uganda, Sudan, Kenya), *Rousettus angolensis* (Tanzania), *Rousettus* sp. (DRC), *Epomophorus* sp. (Guinea-Bissau), *Pteropus voeltzkowi* (Zanzibar). *Hipposideros commersoni* (Ghana), *Nycteris thebiaca* (Ghana), *Arvicanthis niloticus* (!) (Nigeria). From hosts undetermined (Tanzania, DRC, Guinea-Bissau, Sao Tome, Sierra Leone, Nigeria, Dahomey, Ghana, Senegal, Ivory Coast, "French Equatorial Africa", Bioko).

Kenyan records:

Kamosi: m6, f4 from *Eidolon helvum*, D. E. McInnes, December 1948 (Theodor 1967:465).

Bats of Kenya records (6 records, 26 specimens)

KAKAMEGA: f12, m14 from *Eidolon helvum* (Mbale), January 2012.

Distribution:

Generally sub-Saharan West Africa (including Sao Tome), Central Africa, East Africa to Kenya and Tanzania (including Zanzibar)

Hosts:

Species of the subfamily Cyclopodiinae are largely limited to pteropodid bats. The historical records from *Eidolon helvum*, and potentially those from species of *Rousettus*, *Pteropus*, and *Epomophorous* are likely legitimate associations. In Kenya, *Cyclopodia greeffi greeffi* were collected exclusively from *E. helvum*. Historical records from *Hipposideros*, *Nycteris*, and *Arvicanthis* (grass rat) are likely erroneous associations.

Genus *Dipseliopoda* Theodor, 1955

***Dipseliopoda biannulata* (Oldroyd, 1953)**

Previous records, hosts, distributions:

From *Myonycteris angolensis* (Cameroon, DRC), *R. aegyptiacus* (DRC, Ghana), *Rousettus* sp. (Kenya), *Epomophorus* sp. (DRC), *Rhinolophus eloquens* or *R. landeri/logatus* (Sudan), *Tadarida faini* (DRC). From hosts undetermined (Nigeria, Uganda).

Kenyan records:

Kakamega: 20 specimens from *Rousettus* sp., Carcasson (Theodor, 1957b: 529).

Bats of Kenya records (1 record, 1 specimen)

TRANS NZOIA: f1 from *Epomophorus crypturus* (Saiwa Swamp National Park), December

2011.

Distribution:

Subsaharan West, Central, and East Africa, apparently excluding South African subregion.

Hosts:

Species of the subfamily Cyclopodiinae are largely limited to pteropodid bats. The historical records from *Rousettus aegyptiacus, Myonycteris angolensis*, and *Epomophorous* sp. are likely legitimate. In Kenya, *Dipseliopoda biannulata* has been collected from *Epomophorus crypturus* and *Rousettus* sp. Historical records from species of *Rhinolophus* and *Tadarida* are likely erroneous associations.

***Dipseliopoda setosa* Theodor, 1955**

Previous records, hosts, distributions:

From *Rousettus lanosus* (Kenya, Tanzania), *R. aegyptiacus* (Tanzania), *Eidolon helvum* (Kenya). From host undetermined (Uganda).

Kenyan records:

Mt. Menengai: 10 specimens from *Rousettus lanosus*, Hoogsraal (Theodor 1957:532).

Ruiru: f2 from *Eidolon helvum*, van Someren (Theodor 1957:532).

Ruwenzori: f1 from unidentified host, Wollaston (Theodor 1957:532).

Kakamega: m1, f1 from *Rousettus aegyptiacus*, Buzambuli Trail 3, Kakamega Forest National Reserve, P. W. Webala, 31 October 2016.

Distribution:

Subsaharan Central and East Africa (Uganda, Kenya, Tanzania).

Hosts:

Species of the subfamily Cyclopodiinae are largely limited to pteropodid bats. The historical records from *Rousettus* spp. and possibly *Eidolon helvum* are likely legitimate.

Genus *Eucampsipoda* Kolenati, 1857

***Eucampsipoda africana* Theodor, 1955**

Previous records, hosts, distributions:

From *Rousettus aegyptiacus* (Senegal, Ghana, Sudan, Kenya, Tanzania, South Africa, Sierra Leone, Congo), *Eidolon helvum* (Cameroon, South Africa). From host undetermined (Malawi). The type series comprised ca. 175 specimens from Kenya, Sudan, Congo, Malawi, and South Africa.

Kenyan records:

Kwale: Shimoni (as *Eucampsipoda hyrtli* Kolenati) m4, f3 from *Rousettus leachi*, "Grotte A de Shimoni", Alluaud & Jeannel, 9 November 1911 (Falcoz, 1923: 549).

Bahati cave: 16 specimens (type series) from *Rousettus leachi*, Garnham (Theodor, 1955: 204).

Nakuru: 14 specimens from undetermined host, Zumpt (Theodor, 1955: 204).

Bats of Kenya records (130 records, 576 specimens)

KILIFI: f3, m1 from *Epomophorous wahlbergi* (Arabuko-Sokoke Forest, Pipit Campsite),

October 2012. 3m from *Myonycteris angolensis* (Arabuko-Sokoke Forest, Mango orchard 300m north of Kenya Wildlife Service HQ), May 2006. f105, m107 from *Rousettus aegyptiacus* (Arabuko-Sokoke Forest, Kenya Wildlife Service HQ; Pipit Campsite; Gedi Ruins; Mango orchard 300m north of Kenya Wildlife Service HQ; Gede, Watamu Cave; Malindi Marine Park), May 2006 and October 2012.

KWALE: m1 from *Miniopterus clade 2* (Fikirini, Three Sisters, Mbenyenye Cave), September

2012. f22 m42 from *Rousettus aegypticus* (Fikirini, Three Sisters, Mbenyenye Cave), September 2012.

THARAKA-NITHI: f91 m78 from *Rousettus aegypticus* (Marma Cave), December 2012.

TRANS NZOIA: f66 m57 from *Rousettus aegypticus* (Mount Elgon National Park, Kitum Cave;

Makingeny Cave), December 2011.

Distribution:

Subsaharan Africa.

Hosts:

Species of the subfamily Cyclopodiinae are largely limited to pteropodid bats. The historical records from *Rousettus aegyptiacus,* and possibly *Eidolon helvum* are likely legitimate. In Kenya, associations with *Rousettus aegyptiacus* were by far most common (intensity: 4.47, prevalence: 0.64 based on 568 bat flies from 127 hosts).

Subfamily Nycteribiinae Westwood, 1835

Genus *Basilia* Miranda Ribeiro, 1903

Subgenus *Basilia*

***Basilia ansifera* Theodor, 1956**

Previous records, hosts, distributions:

From *Pipistrellus culex* (Nigeria), *P. helios* (Sudan), *P. nanus* (Ivory Coast, Benin, DRC), *P. stampflii* (= *P. minusculus*) (Sierra Leone, Liberia), *Pipistrellus* sp. (Ivory Coast, Sudan), *Eptesicus rendalli* (Gambia, Sudan), *E. tenuipennis* (Sierra Leone), *Scotophilus* sp. (Ghana), *Tadarida pulila* (mixed with *Pipistrellus nanus*) (Ivory Coast). From hosts undetermined (DRC, Malawi).

Kenyan records:

Bats of Kenya records (5 records, 9 specimens)

MERU: f1 m1 from *Nycticeinops schlieffeni* (Meru National Park, Kinna), January 2013.

SAMBURU: f2 m2 from *Nycticeinops schlieffeni* (Samburu National Game Reserve, Samburu Game Lodge), January 2013. f1 m2 from *Scotoecus albigula* (Samburu National Game Reserve, Vervet campsite), January 2013.

Distribution:

Subsaharan Africa, especially West Africa.

Hosts:

Basilia ansifera has been reported from a variety of host bats, including species of *Pipistrellus* and *Eptesicus*. In Kenya, most specimens were associated with *Nycticeinops schlieffeni* and *Scotoecus albigula*. This is the first record of *B. ansifera* from Kenya.

***Basilia robusta* Theodor, 1956**

Previous records, hosts, distributions:

From *Pipistrellus kuhli* (Zimbabwe), *P. nanus* (Ethiopia, DRC), *Eptesicus capensis* (Sierra Leone), *E. tenuipennis* (DRC), *Eptesicus* sp. (Nigeria), from hosts undetermined (Uganda, Ethiopia, Angola). There are so few records from each that it is difficult to determine a primary host based on historical records

Kenyan records:

Bats of Kenya records (35 records, 61 specimens)

KISUMU: f1 m3 from *Neoromicia tenuipinnis* (Kisumu Impala Sanctuary, State Lodge campsite), January 2012.

LAIKIPIA: f5 m7 from *Neoromicia capensis* (Loll Daiga Hills Conservancy, Farm house; Simba campsite; Munanda Dam; Ol Jogi Conservancy, Kimboko campsite), July and August 2014. f8 m6 from unidentified *Neoromicia* sp. (Loll Daiga Hills Conservancy, Munanda Dam; Valley Dam), July 2014. f1 m2 from unidentified *Pipistrellus* sp. (*aero* or *hesperidus*) (Ol Jogi Conservancy, Kiboko campsite), August 2014. f10 m6 from *Pipistrellus* cf. *hesperidus* (Loll Daiga Hills Conservancy, Kambi Dam; Main house; Munanda Dam; Shaita Dam; Valley Dam), July 2014. m1 from *Scotophilus clade 2* (Loll Daiga Hills Conservancy, Shaita Dam), July 2014.

MARSABIT: f4 m2 from unidentified *Pipistrellus* sp. (*aero* or *hesperidus*) (Marsabit National Park and Reserve, 12.09 km SW of campground; 6.07 km SW of campground), July 2015. f1 m2 from *Pipistrellus* cf. *hesperidus* (6.07 km SW of campground), July 2015.

NAROK: m1 from *Pipistrellus* cf. *hesperidus* (Masai Mara National Reserve, Mara Simba Lodge), January 2014.

SAMBURU: m1 from *Neoromicia capensis* (Samburu National Game Reserve, Vervet campsite), January 2013.

Distribution:

Subsaharan Africa, apparently excluding South Africa.

Hosts:

Basilia robusta has previously been reported from a variety of host bats, including species of *Pipistrellus* and *Eptesicus*. In Kenya, 61 specimens were collected, largely associated with bat species in the genera *Neoremecia* and *Pipistrellus*. The single specimen from *Scotophilus* may be an erroneous association. This is the first record of *B. robusta* from Kenya.

Subgenus *Tripselia* Scott, 1917

***Basilia blainvillii blainvillii* (Leach, 1817)**

Previous records, hosts, distributions:

From *Taphozous mauritianus* (Sierre Leone, Ivory Coast, Cameroon, DRC, Angola, Sudan, Tanzania, Mozambique, Assumption Islands), *T. peli* (DRC), *T. perforatus* (Egypt), *Nycteris thebaica* (Tanzania), *Pteropus* sp. (Comoros), *Rousettus* sp. (Dahomey, Kenya), *Epomophorus anurus* (Tanzania), from hosts undetermined (Gold Coast).

Kenyan records:

Kyambu: f3 from unidentified host, Garnham (Theodor 1956: 359).

Bats of Kenya records: None

Distribution:

Subsaharan Africa and Egypt.

Hosts:

Basilia blainvillii blainvillii has previously been reported from a variety of host bats, including species of *Taphozous, Nycteris,* and several genera of pteropid bats. Theodor (1956) stated that this species was largely associated with *Taphozous mauritianus.*

Subgenus *Paracyclopodia* Scott, 1917

***Basilia bouvieri* (Falcoz, 1924)**

Previous records, hosts, distributions:

From *Scotophilus leucogaster* (Senegal, Uganda), *S. nigrita* (Senegal, Sierra Leone, Ghana, Sudan), *Scotophilus* sp. (Sudan), *Eptesicus phasma* (=*Neoromicia rendalli*) (Sudan). From hosts undetermined (Tanzania).

Kenyan records:

Bats of Kenya records (7 records, 25 specimens):

NAROK: m1 from *Scotophilus clade 4*, January 2014.

MARSABIT: f19 m5 from *Scotophilus andrewreborii* (Marsabit National Park and Reserve, 12.09 km SW of campground; campground near headquarters), July 2015.

Distribution:

Subsaharan Africa, apparently excluding South Africa.

Hosts:

Basilia bouvieri has previously been reported from a variety of host bats, primarily species of *Scotophilus*. In Kenya, 25 specimens were collected and all but one were associated with *S. andrewreborii*. This is the first record of *B. bouvieri* from Kenya.

Genus *Nycteribia* Latreille, 1796

Subgenus *Nycteribia*

***Nycteribia latitergum* Theodor, 1957**

Previous records, hosts, distributions:

Mt. Menangai near Nakuru: ca. 30 specimens (type series) from mixed samples of *Minipterus arenarius* (=*Miniopterus natalensis* A. Smith) and *Myotis tricolor*, Hoogstrall, 8 June 1948 (Theodor 1957b: 471).

Kenyan records:

Previously known only from the type series.

Bats of Kenya records (5 records, 7 specimens)

LAIKIPIA: f2 from *Neoromicia capensis* (Loll Daiga Hills Conservancy, Munanda Dam; Gilgil, Diatomite Cave), July 2014.

NAKURU: f2 m3 from *Myotis tricolor* (Menengai Crater, Mau Mau Cave), June 2014.

Distribution:

Kenya (Theodor 1967 listed “East Africa”).

Hosts:

Nycteribia latitergum has been previously reported from *Miniopterus natalensis.* Specimens collected during the Bats of Kenya project were found in association with *Myotis tricolor* and *Neoromicia capensis.*

***Nycteribia schmidlii scotti* Falcoz, 1923**

Previous records, hosts, distributions:

From *Miniopterus inflatus* (Cameroon, French Guinea, DRC), *M. minor* (Kenya, DRC), *M. schriebersii* (Sudan, Kenya, Mozambique, South Africa), *Miniopterus* sp. (Sudan), *Pipistrellus culex* (Nigeria), *P. nanus* (Cameroon, South Africa), *Eptesicus* sp. (Sudan), *Rhinolophus clivosus augur* (South Africa), *R. hildebranti* (DRC), from mixture of *R. capensis* and *Eptesicus capensis* (South Africa), *Hipposideros caffer* (DRC), *Triaenops afer* (Mozambique), *Tadarida condylura niveiventer* (DRC), *Nycteris capensis* (Zimbabwe), from undetermined hosts (Zambia, Sao Tome Island).

Kenyan records:

Kwale: Shimoni (as *Nycteribia scotti* Falcoz) m3, f2 from *Miniopterus minor*, m1, f1 from *Hipposideros caffer*, "Grotte A de Shimoni", Alluaud & Jeannel, 9 November 1911 (Falcoz, 1923: 548).

Ngong near Mt. Elgon: 14 specimens from *Miniopterus* sp., Cade (Theodor 1957: 465).

Mt. Elgon: 60 specimens from *M. schreibersii*, Edwards (Theodor 1957: 465).

Mt. Menangai: 6 specimens from unknown host, Hoogstraal (Theodor 1957: 465).

Kapretwa, Kitale: 6 specimens from *M. schreibersii*, Hopkins (Theodor 1957: 466).

Bats of Kenya records (177 records, 408 specimens)

KAJIADO: f36 m35 (Mount Suswa, Cave 14C; Cave 18A) from *Miniopterus clade 8*, August 2011.

KAKAMEGA: f55 m40 from *Miniopterus* clade 10 (Kakamega Forest, Lirhanda Hill Cave; Mahiakalo Cave), January 2012.

KILIFI: f9 m9 from *Coleura afra* (Watamu, Makuruhu Cave), October 2012. m2 from *Miniopterus* clade 2 or 5 (Watamu, Makuruhu Cave), February 1966.

KWALE: f1 m1 from *Coleura afra* (Mwaluganje Community Elephant Sanctuary, Ngomeni Cave), September 2012. f63 m74 from *Miniopterus* clade 2 (Fikirini, Pare Cave; Three Sisters, Kisimani Cave; Three Sisters, Mbenyenye Cave; Three Sisters, Pangani Cave; Mwaluganke Community Elephant Sanctuary, Ngomeni Cave), September 2012. m2 from *Miniopterus* clade 3 (Mwaluganke Community Elephant Sanctuary, Ngomeni Cave), September 2012.

LAIKIPIA: f12 m9 from *Miniopterus* clade 7 (Loll Daiga Hills Conservancy, Simba Campsite Dam), July 2014.

NAKURU: f3 m6 from *Miniopterus* clade 1 (Gilgil, Kariandusi Mines; Menengai Crater, Mau Mau Cave), January, June, and August 2014. f7 m11 from *Miniopterus* clade 1 or 4 (Gilgil, Kariandusi Mines; Menengai Crater, Mau Mau Cave), June and August 2014. f1 from *Miniopterus* clade 4 (Menengai Crater, Mau Mau Cave), August 2014. f1 from *Rhinolophus* cf. *landeri* (Gilgil, Kariandusi Mines), June 2014.

NYERI: f2 from *Miniopterus* clade 1 (Mount Kenya National Park, Narumoru Gate), January 2013.

TAITA-TAVETA: f7 m4 from *Miniopterus* clade 5 (Mount Kilimanjaro, Lake Jipe), February 1966. f3 m3 from *Miniopterus* clade 7 (Marungu Cave), April 2006.

TRANS NZOIA: f8 m5 from *Miniopterus* clade 1 (Mount Elgon National Park, Kitum Cave; Makingeny Cave), December 2011.

Distribution:

Subsaharan Africa, including South Africa.

Hosts:

Nycteribia schmidlii scotti has been previously reported from a variety of microchiropteran bats, including species of *Miniopterus, Pipistrellus, Eptesicus, Rhinolophus, Hipposideros, Triaenops, Tadarida* and *Nycteris capensis*. However, the recent collection efforts in Kenya recovered 408 specimens, the vast majority of which were associated with various species/clades of *Miniopterus* and to a far lesser extent

Coleura afra. The single specimen collected from *Rhinolophus* cf. *laderi* may well be erroneous.

Genus *Penicillidia* Kolenati, 1863

Subgenus *Penicillidia*

***Penicillidia fulvida* (Bigot, 1885)**

Previous records, hosts, distributions:

From *Miniopterus schriebersii* (South Africa, Mozambique, Kenya), *M. inflatus* (DRC, Cameroon), *Miniopterus* sp. (Sudan), *Myotis tricolor* (South Africa, Kenya), *Rhinolophus blasii* (Yemen), *R. clivosus* (South Africa), *R. eloquens* (Sudan), *R. foxi* (Cameroon), *R. hildebranti* (Tanzania), from *R. keniensis* (Kenya), *Rhinolophus* sp. (South Africa, DRC, Sudan, Dahomey), *Hipposideros caffer* (Mozambique, Kenya, DRC), *Nycteris thebaica* (South Africa, Mozambique, DRC), *Choleura gallarum* (Sudan), *Eidolon helvum* (South Africa).

Kenyan records:

Kericho: f1 from *Hipposideros caffer*, Dobbs (Theodor 1957b: 513).

Mt. Elgon: 21 specimens from *M. schreibersi*, f1 from *Rhinolophus clivosus*, Edwards (Theodor 1957b: 513).Mt. Menengai, Rift Valley: 10 specimens from *M. schreibersii* and *Myotis tricolor*, Hoogstraal (Theodor 1957b: 513).

Bats of Kenya records (58 records, 65 specimens)

KAKAMEGA: f4 m2 from *Miniopterus* clade 10 (Kakamega Forest, Lirhanda Hill Cave; Mahiakalo Cave), January 2012 and September 2014.

KWALE: f5 m1 from *Miniopterus* clade 2 (Fikirini, Pare Cave; Three Sisters, Kisimani Cave; Three Sisters, Mbenyenye Cave), September 2012. f1 from *Miniopterus* clade 3 (Mwaluganje Community Elephant Sanctuary, Ngomeni Cave), September 2012. m1 from *Nycteris thebaica* clade 4 (Shimba Hills National Reserve, Sable Bandas), October 2012. f1 from *Rhinolophus fumigatus* clade 8 (Fikirini, Pare Cave), September 2012. f1 from *Taphozous hildegardeae* (Mwaluganje Community Elephant Sanctuary, Ngomeni Cave), September 2012. f1 from *Triaenops afer* (Fikirini, Three Sisters, Mbenyenye Cave), September 2012.

MARSABIT: f1 from *Rhinolophus* cf. *landeri* (Marsabit National Park and Reserve, campground near headquarters), July 2015. m1 from *Rhinolophus fumigatus* clade 2 or 3 (Marsabit National Park and Reserve, 6.07 km SW campground near headquarters), July 2015. f5 from *Rhinolophus fumigatus* clade 3 (Marsabit National Park and Reserve, campground near headquarters; 6.07 km SW of campground; 1.3 km SE of campground), July 2015.

NAKURU: f3 m4 from *Miniopterus* clade 1 (Gilgil, Kariandusi Mines), January and August 2014. f9 m2 from *Miniopterus* clade 1 or 4 (Gilgil, Kariandusi Mines; Menengai Crater, Mau Mau Cave), June and August 2014. f1 m1 from *Miniopterus* clade 4 or 7 (Gilgil, Pipeline Cave), August 2014. f1 from *Miniopterus* clade 8 (Gilgil, Kariandusi Mines), September 2014. f7 m3 from *Myotis tricolor*, June and August 2014 (Menengai Crater, Mau Mau Cave;

Soysambu Conservancy, Monkey Bridge campsite). m1 from *Rhinolophus* cf. *landeri* (Gilgil, Kariandusi Mines), August 2014. m2 from *Rhinolophus clivosus* clade 2 (Gilgil, Kariandusi Mines), January and September 2014. f1 from *Rhinolophus fumigatus* clade 4 (Gilgil, Pipeline Cave), September 2014. TAITA-TAVETA: f4 m1 from *Coleura afra* (Marungu Cave; Tsavo West National Park, Shetani Caves), April and May 2006. m1 from *Pipistrellus* sp. (Marungu Cave), April 2006.

Distribution:

Subsaharan Africa, including South Africa; Arabian Peninsula (Yemen).

Hosts:

This species has been reported in association with a remarkable variety of bats in the families Vespertilionidae, Emballonuridae, Rhinolophidae, Hipposideridae, Nycteridae, and Pteropodidae. Theodor (1967) remarked that this species is apparently quite unspecific [to host species of bats] and had been reported from 14 species, 7 genera, and 5 families of bat. The 65 specimens of *P. fulvida* collected recently in Kenya were also recovered from a wide variety of host bats, with little evidence of population structure among the specimens (Verrett et al. In Prep). *Penicillidia fulvida* is a rarity among bat flies in its lack of host specificity, even at the bat family level.

***Penicillidia pachymela* Speiser, 1901**

Previous records, hosts, distributions:

From *Hipposideros caffer* (Mozambique, DRC, Tanzania, Zambia), *Hipposideros* sp. (DRC, Cameroon), *Rhinolophus hildebranti* (Mozambique), *R. landeri* (DRC, Cameroon), from mixture of *R. eloquens* and *R. lobatus* (Sudan), *Nycteris thebaica* (Mozambique), *Nycteris* sp. (Tanzania), from undetermined hosts (Somalia, "French Equatorial Africa").

Kenyan records:

Nairobi: m1 from undetermined host, February 1912 (Theodor 1967: 374).

Ngong hills (near Nairobi): m1 from undetermined host, 19 September 1934, van Someren (Theodor 1967: 374).

Tana Bridge; m2 from undetermined hosts, 1 February 1948, van Someren (Theodor 1967: 374).

Bats of Kenya records (1 record, 1 specimen):

> NAKURU: m1 from *Hipposideros caffer* (Lake Nakuru National Park, Lion Hill Cave), August 2011.

Distribution:

Subsaharan Africa, apparently excluding South Africa.

Hosts:

This rarely encountered species has been reported in association with a variety of microchiropteran species. The single specimen collected during the bats of Kenya survey

was associated with *Hipposideros caffer*. Too few specimens exist to determine whether *P. pachymela* exhibits the low specificity of *P. fulvida*.

Genus *Phthiridium* Hermann, 1804

***Phthiridium hoogstrali* (Theodor, 1957)**

Previous records, hosts, distributions:

From *Rhinolophus eloquens* (Sudan), *R. hildebrandti* (DRC), Rhinolophus sp. (Sudan).

Kenyan records:

Bats of Kenya records (*45 records, 130 specimens*):

KISUMU: f2, m1 from *Rhinolophus fumigatus* clade 1 (Kisumu Impala Sanctuary, State Lodge campsite), January 2012.

LAIKIPIA: f3, m1 from *Rhinolophus fumigatus* clade 1 (Loll Daiga Hills Conservancy, Simba Campsite Dam), July 2014.

NAKURU: f7, m8 from *Rhinolophus fumigatus* clade 1 (Lake Nakuru National Park, Lion Hill Cave), August 2011 and January 2012. f59, m24 from *Rhinolophus fumigatus* clade 1 or 4 (Lake Nakuru National Park, Lion Hill Cave), August 2011 and January 2012. f13 m12 from *Rhinolophus fumigatus* clade 4 (Gilgil, Pipeline Cave; Lake Nakuru National Park, Lion Hill Cave), August 2011, January 2012, and August 2014.

Distribution:

Subsaharan Africa, excluding South Africa.

Hosts:

Phthiridium hoogstrali has previously been reported from at least two species of *Rhinolophus*. In Kenya, 130 specimens were collected and all were collected from *Rhinolophus fumigatus*. This is the first record of *Phthiridium hoogstrali* from Kenya.

***Phthiridium inopinata* (Theodor, 1957)**

Previous records, hosts, distributions:

From *Rhinolophus alcyone* (Cameroon)

Kenyan records:

Bats of Kenya records (1 record, 2 specimens)

KAKAMEGA: f1 m1 from *Hipposideros beatus* clade 1 (Kakamega Forest, Ikhondo campground), January 2012.

Distribution:

Subsaharan Africa (Cameroon, Kenya).

Hosts:

Phthiridium inopinata is apparently scarce in nature and has previously been reported from *Rhinolophus alcyone*, which is distributed in west central Africa. In Kenya, two

specimens were collected from *Hipposideros beatus* in Kakamega (western Kenya). This is the first record of *Phthiridium inopinata* from Kenya.

***Phthiridium rhodesiense* (Theodor, 1957)**

Previous records, hosts, distributions:

From *Rhinolophus hildebrandi* (Rhodesia), *R. darling* (Rhodesia), *Nycteris thebaica capensis* (Rhodesia), from undetermined host (Malawi).

Kenyan records:

Bats of Kenya records *(3 records, 4 specimens)*

MAKUENI: m2 from *Rhinolophus hildebrantii* clade 1 (Chyulu Hills National Park, Kisula Cave), May 2006.

TAITA-TAVETA: f2 from *Rhinolophus hildebrantii* clade 1 (Tsavo West National Park, Shetani Caves), May 2006.

Distribution:

Subsaharan east Africa (Kenya, Malawi, Rhodesia).

Hosts:

The historical records of Phthiridium rhodesiense have largely been associated with species of Rhinolophus. In Kenya, four specimens were collected from two individuals

of *Rhinolophus hildebrantii.* This is the first record of *Phthiridium inopinata* from Kenya.

***Phthiridium scissum* (Speiser, 1901)**

Previous records, hosts, distributions:

From *Rhinolophus capensis* (South Africa), R. darling (South Africa), R. hildebrandtii (Mozambique), *R. clivosus* (Namibia, South Africa), from mixture of *R. eloquens*, *Hipposideros caffer*, and *Nycteris capensis* (Namibia).

Kenyan records:

Bats of Kenya records (56 records, 134 specimens)

MARSABIT: f4 m3 from *Rhinolophus fumigatus* clade 2 (Marsabit National Park and Reserve, 12.09 km SW of campground near headquarters; 6.07 SW of campground near headquarters), July 2015. f44 m36 from *Rhinolophus fumigatus* clade 2 or 3 (Marsabit National Park and Reserve, 1.3 km SE of campground and headquarters; 12.09 km SW of campground near headquarters; 6.07 km SW of campground near headquarters), July 2015. f20 m19 from *Rhinolophus fumigatus* clade 3 (Marsabit National Park and Reserve, 1.3 km SE of campground near headquarters; 12.09 km SW of campground near headquarters; 6.07 km SW of campground near headquarters; campground near headquarters), July 2015.

NAKURU: f4 m2 from *Rhinolophus clivosus* clade 2 (Gilgil, Kariandusi Mines), January 2014.

TAITA-TAVETA: f2 from *Rhinolophus fumigatus* clade 2 (Tsavo West National Park, Shetani Caves), May 2006.

Distribution:

Subsaharan Africa (Kenya, South Africa, Namibia, Mozambique).

Hosts:

The historical records of *Phthiridium scissum* have largely been associated with species of *Rhinolophus*, but records exist for species of *Hipposideros* and *Nycteris*. In Kenya, 134 specimens were collected, all from *Rhinolophus fumigatus.* These are the first records of *Phthiridium scissum* from Kenya.

***Phthiridium tectum* (Theodor, 1957)**

Previous records, hosts, distributions:

From *Miniopterus schrieberesii arenarius* (= *Miniopterus natalensis*) (recorded as *Rhinolophus schreibersii arenarius* (Kenya), *Miniopterus* sp. (Kenya), *Eptesicus* sp. (Sudan), *Rhinolophus darlingi* (Aouth Africa), *Rhinolophus deckeni* (=*Rhinolophus silvestris*) (Uganda), *Rhinolophus* sp. (Tanzania), *Hipposideros caffer* (Zimbabwe).

Kenyan records:

Ngong near Mt. Elgon: f1 (holotype) from *Miniopterus* sp., Cade (Theodor 1957b: 485).

Kapretwa, Kitale: f1 from *Miniopterus schrieberesii arenarius*, Theodor, 15 January 1957 (Theodor 1967: 178).

Bats of Kenya records: None

Distribution:

Subsaharan Africa (Kenya, Sudan, Uganda, Tanzania, Zimbabwe, South Africa).

Hosts:

The historical records of *Phthiridium tectum* have been associated with a variety of bats in the genera *Miniopterus, Rhinolophus, Eptesicus, and Hipposideros*. This species is apparently rare in Kenya, and no recent collections were made during the Bats of Kenya survey.

***Phthiridium* sp. nov.? from *Rhinolophus clivosus* (only male flies)**

Three male specimens collected from two "Rhinolophus clivosus 2" represent a putative new species. At this time we are not inclined to describe this new species using only males.

Kenyan records:

Bats of Kenya records (2 records, 3 specimens)

NAKURU: m3 from *Rhinolophus clivosus* clade 2 (Gilgil, Kariandusi Mines), June 2014.

Distribution:

Known only from Kenya.

Hosts:

The three known specimens were all collected from one *Rhinolophus clivosus* clade 2, at the Kariandusi mines near Gilgil.

Discussion

This species record represents the most extensive catalog of nycteribiid biodiversity in Kenya to date, and one of the most thorough efforts to summarize nycteribiid diversity in an Afrotropical region. The Bats of Kenya survey contributes 5 nycteribiid species previously unknown from Kenya (*Basilia robusta*, *Phthiridium hoogstrali*, *P. inopinata*, *P. rhodesiense*, and *P. scissum*), and a possible new species in genus *Phthiridium*, bringing the diversity of nycteribiid bat flies cataloged from Kenya to a total of 18 species across 7 genera.

The geographic sampling distribution of bats across Kenya was reasonably thorough with respect to biodiversity centers. Localities sampled in the Bats of Kenya survey were concentrated in tropical forests containing much of Kenya's bat biodiversity, whereas gaps in coverage comprised less bat-rich desert and savanna. Notable exceptions are some stretches of coastal forest at or near the Somalian border, including the Boni and

Dodori National Reserves, which could not be sampled due to the presence of armed groups during survey years. Kenya's coastal forests are recognized as global biodiversity hotspots with high degrees of endemism (Myers et al. 2000), and further sampling efforts should target this area when it is safe to do so. Further, although the unsampled northwestern and northeastern regions of Kenya are composed mainly of savanna or desert habitat with relatively few bat species (Herkt et al. 2016), they may contain unique bat and bat fly communities (Monadjem and Reside 2008) and warrant future survey attention.

The most biodiverse habitats in Kenya are also those most prone to habitat modification and fragmentation, as areas with higher water availability are attractive for anthropogenic use in an overall arid country (Bennun and Njoroge 2000). It is crucial for Kenyan biodiversity to be more thoroughly explored as it is depleted by habitat loss. Moreover, habitat fragmentation can affect the size and isolation of populations, influencing transmission dynamics of vector-borne diseases in patterns mediated by host and parasite ecology (Suzán et al. 2012). Land conversion and habitat fragmentation, particularly in highly biodiverse areas, also increase the probability of human-wildlife interaction and can facilitate the spread of zoonotic disease (Johnson et al. 2020). Nycteribiid bat flies are vectors of bacterial pathogens in genus *Bartonella* (Wilkinson et al. 2016) and of haemosporidian parasites of bats (Megali et al. 2010). Bat flies are also becoming increasingly linked to viral pathogens related to bat-associated zoonoses, though their role as potential vectors or principal carriers of such diseases remains unclear (Bennett et al. 2020, Ramírez-Martínez et al. 2021). As the role of bat flies in disease transmission is further elucidated, bat fly diversity must be understood at a

fundamental level in areas where it remains largely unexplored. The need to investigate bat fly vector potential and diversity is especially salient in continental Africa, which harbors 20% of all bat biodiversity (Happold and Happold 2013) and accounted for over half of all emerging infectious disease outbreaks between 1996 and 2009 (Chan et al. 2010).

County	Locality	Locality description	Latitude	Longitude
Embu	1	Nyamindi River cave	-0.556	37.388
Homa Bay	2	Ruma National Park, Fig tree campsite	-0.646	34.335
Homa Bay	2	Ruma National Park, houses outside HQ gate	-0.652	34.343
Kajiado	3	Amboseli National Park, Elephant Research Camp	-2.679	37.267
Kajiado	3	Amboseli National Park, Amboseli Serena Lodge	-2.705	37.266
Kajiado	4	Mount Suswa Conservancy, Cave 18A	-1.132	36.405
Kajiado	4	Mount Suswa Conservancy, Cave 14C	-1.133	36.402
Kakamega	5	Kakamega Forest, Colobus Circuit 1	0.356	34.861
Kakamega	5	Kakamega Forest, Colobus Circuit 2	0.356	34.861
Kakamega	5	Kakamega Forest, Buyangu Kenya Wildlife Service HQ	0.355	34.866
Kakamega	5	Kakamega Forest, Buzambuli Trail 1	0.350	34.861
Kakamega	5	Kakamega Forest, Buzambuli Trail 3	0.344	34.857
Kakamega	5	Kakamega Forest, Ikhondo Campsite	0.352	34.865
Kakamega	5	Kakamega Forest, Ikhondo Junction	0.353	34.862
Kakamega	5	Kakamega Forest, Isiukhu River Trail	0.332	34.879
Kakamega	5	Kakamega Forest, Mukangu Village	0.368	34.870
Kakamega	5	Kakamega Forest, Kakamega Dam	0.359	34.866
Kakamega	5	Kakamega Forest, Litali Trail	0.360	34.861
Kakamega	5	Kakamega Forest, Buyangu Village	0.352	34.865
Kakamega	5	Kakamega Forest, Buyangu, Glade 1	0.349	34.870
Kakamega	6	Kakamega Forest, Malava, Edge	0.458	34.860
Kakamega	6	Kakamega Forest, Malava, Glade 1	0.458	34.852
Kakamega	6	Kakamega Forest, Malava, Glade 2	0.458	34.853
Kakamega	6	Kakamega Forest, Kambi ya Mwanza	0.458	34.853
Kakamega	7	Kakamega Forest, Buyangu Reserve, Edge	0.383	34.891
Kakamega	7	Kakamega Forest, Buyangu Reserve, Glade 2	0.383	34.891
Kakamega	7	Kakamega Forest, Kisere Reserve, Edge	0.387	34.891
Kakamega	7	Kakamega Forest, Kisere Reserve, Glade	0.387	34.891
Kakamega	7	Kakamega Forest, Kisere Reserve, Kisere Bridge	0.396	34.883
Kakamega	7	Kakamega Forest, Kisere Reserve, Kisere Farm	0.400	34.879
Kakamega	7	Kakamega Forest, Mungokho Village	0.375	34.898
Kakamega	8	Kakamega Forest, Isiukhu River	0.328	34.880
Kakamega	8	Kakamega Forest, Isiukhu River Trail 1	0.332	34.879
Kakamega	8	Kakamega Forest, Salazar Trail	0.335	34.874
Kakamega	9	Mbale	0.096	34.721
Mbale Vihiga	9	Ilwanda Roost Site	0.097	34.739
Kakamega	10	Kakamega Forest, Lirhanda Hill Cave	0.218	34.897
Kakamega	10	Kakamega Forest, Mahiakalo Cave	0.248	34.906
Kakamega	11	Shamberere	0.378	34.847
Kakamega	12	Kakamega Forest, Lirhanda Cave	0.212	34.899
Kilifi	13	Arabuko-Sokoke Forest, Anametra Forest	-3.305	39.937
Kilifi	13	Arabuko-Sokoke Forest, Brachystegia Forest	-3.282	39.971
Kilifi	14	Arabuko-Sokoke Forest, Jilore Staff Quarters	-3.216	39.925
Kilifi	15	Arabuko-Sokoke Forest, Kenya Wildlife Service HQ	-3.300	39.995
Kilifi	15	Arabuko-Sokoke Forest, 2.1 km NW camp	-3.287	39.982
Kilifi	15	Arabuko-Sokoke Forest, Pipit Campsite	-3.300	39.995
Kilifi	15	Arabuko-Sokoke Forest, Gedi Ruins	-3.309	40.018
Kilifi	15	Arabuko-Sokoke Forest, mango orchard 300m north of KWS HQ	-3.303	39.999

County	Locality	Locality description	Latitude	Longitude
Kilifi	16	Gede, Kaboga Cave	-3.335	40.031
Kilifi	16	Gede, Watamu Cave	-3.351	40.014
Kilifi	17	Hodihodi Cave	-3.850	39.810
Kilifi	18	Kakuyuni Dispensary	-3.27	39.983
Kilifi	19	Malindi Marine Park, Kenya Wildlife Service HQ	-3.255	40.132
Kilifi	20	Watamu, Makuruhu Cave	-3.323	40.042
Kirinyaga	21	Sagana Tunnel	-0.117	34.541
Kisumu	21	Kit Makayi	-0.117	34.541
Kisumu	22	Kisumu Impala Sanctuary, Impala public campsite	-0.109	34.746
Kisumu	22	Kisumu Impala Sanctuary, Ecolodge	-0.115	34.744
Kisumu	22	Kisumu Impala Sanctuary, State Lodge campsite	-0.110	34.746
Kitui	23	Mutha, Ngelani Baobab	-1.698	38.467
Kitui	24	Mwingi, Baobab tree	-0.992	38.330
Kitui	24	Mwingi, Mutyangome Dam	-0.959	38.337
Kitui	24	Mwingi, Mwalo Dam	-1.019	38.326
Kitui	24	Mwingi, Khaluku Rock Dam	-0.992	38.330
Kwale	25	Fikirini, Pare Cave	-4.590	39.331
Kwale	26	Fikirini, Three Sisters, Kisimani Cave	-4.615	39.353
Kwale	26	Fikirini, Three Sisters, Mbenyenye Cave	-4.614	39.354
Kwale	26	Fikirini, Three Sisters, Pangani Cave	-4.614	39.354
Kwale	26	Kisite-Mpunguti Marine Park and Reserve	-4.647	39.378
Kwale	26	Shimoni, "Slave Cave"	-4.647	39.380
Kwale	27	Kenya Forest Service, Kwale Office	-4.174	39.452
Kwale	28	Mwaluganje Community Elephant Sanctuary, Ngomeni Cave	-4.082	39.483
Kwale	29	Shimba Hills National Reserve, Sable Bandas	-4.215	39.451
Kwale	30	Wasini Island, Wasini Village, Wasini Lodge & Restaurant	-4.658	39.368
Laikipia	31	Loll Daiga Hills Conservancy, Samaki Dam	0.144	37.115
Laikipia	32	Loll Daiga Hills Conservancy, Kambi Dam	0.201	37.130
Laikipia	33	Loll Daiga Hills Conservancy, Konambaya	0.183	37.146
Laikipia	33	Loll Daiga Hills Conservancy, Main gate	0.170	37.163
Laikipia	33	Loll Daiga Hills Conservancy, Main house	0.212	37.122
Laikipia	33	Loll Daiga Hills Conservancy, Farm house	0.201	37.130
Laikipia	33	Loll Daiga Hills Conservancy, Mugumo campsite	0.170	37.163
Laikipia	33	Loll Daiga Hills Conservancy, Valley Dam	0.172	37.148
Laikipia	34	Loll Daiga Hills Conservancy, Munanda Dam	0.227	37.117
Laikipia	34	Loll Daiga Hills Conservancy, Shaita Dam	0.230	37.110
Laikipia	34	Loll Daiga Hills Conservancy, Shaita Valley	0.228	37.113
Laikipia	34	Loll Daiga Hills Conservancy, Simba Campsite Dam	0.204	37.105
Laikipia	34	Loll Daiga Hills Conservancy, West Kiburuti Borehole Dam	0.188	37.082
Laikipia	35	Ol Jogi Conservancy, Kiboko Campsite	0.317	36.911
Laikipia	35	Ol Jogi Conservancy, Ol Jogi Dam	0.325	36.935
Laikipia	35	Ol Jogi Conesrvancy, Pyramid Camp	0.309	36.076
Laikipia	35	Ol Jogi Conservancy, Water Treatment Site	0.304	36.925
Laikipia	36	Nanyuki, Kenya Wildlife Service Station	-0.015	37.091
Laikipia	37	Monkey Hills, Mpala Research Centre	-0.308	36.887
Laikipia	38	Loll Daiga Hills Conservancy, Kiburuti Bridge	0.309	37.150

County	Locality	Locality description	Latitude	Longitude
Laikipia	38	Loll Daiga Hills Conservancy, Kiburuti Camp	0.308	37.152
Laikipia	39	Loll Daiga Hills Conservancy, Ngainito Hill	0.338	37.111
Makueni	40	Chyulu Hills National Park, campground	-2.499	37.940
Makueni	41	Chyulu Hills National Park, Guano cave 1	-2.321	37.693
Makueni	41	Chyulu Hills National Park, Guano cave 3	-2.340	37.709
Makueni	42	Chyulu Hills National Park, Kisula Cave	-2.529	37.853
Makueni	43	Kiboko, Kenya Wildlife Service Station	-2.203	37.714
Makueni	43	Kiboko, Hunter's Lodge	-2.211	37.714
Marsabit	44	Marsabit National Park and Reserve, 1.3km SE campground and headquarters	2.309	38.000
Marsabit	44	Marsabit National Park and Reserve, campground near headquarters	2.320	37.994
Marsabit	44	Marsabit National Park and Reserve, Marsabit Lodge	2.309	37.966
Marsabit	45	Marsabit National Park and Reserve, 12.09km SW of campground	2.235	37.927
Marsabit	45	Marsabit National Park and Reserve, 6.07km SW of campground	2.283	37.954
Meru	46	Meru National Park, Bwatherongi Campground	0.165	38.208
Meru	46	Meru National Park, Kinna	0.170	38.194
Meru	47	Meru National Park, Kanjoo Gate Compound	0.220	38.065
Meru	48	Meru National Park, Leopard Rock Lodge	0.223	38.201
Meru	49	Meru National Park, Murera Gate bandas	0.268	38.129
Meru	49	Meru National Park, Murera Gate guardhouse	0.268	38.121
Meru	50	Meru National Park, Ura Gate	0.024	38.066
Nairobi	51	Karura Forest caves	-1.250	36.837
Nakuru	52	Gilgil, Diatomite cave	-0.430	36.174
Nakuru	53	Gilgil, Jaika Cave	-0.564	36.254
Nakuru	53	Gilgil, Kwapotea Cave	-0.564	36.254
Nakuru	54	Gilgil, Kariandusi Mines	-0.451	36.282
Nakuru	55	Gilgil, Pipeline Cave	-0.539	36.294
Nakuru	56	Kenya Wildlife Service, Hippo Camp	-0.742	36.432
Nakuru	57	Lake Nakuru National Park, Rift Regional Headquarters	-0.306	36.082
Nakuru	57	Lake Nakuru National Park, backpackers' campsite	-0.317	36.084
Nakuru	57	Lake Nakuru National Park, headquarters	-0.306	36.082
Nakuru	58	Lake Nakuru National Park, Lion Hill Cave	-0.346	36.119
Nakuru	59	Menengai Crater, Mau Mau Cave	-0.217	37.137
Nakuru	60	Soysambu Conservancy, Field Study Office	-0.392	36.242
Nakuru	60	Soysambu Conservancy, Serena Elementaita Lodge	-0.403	36.239
Nakuru	61	Soysambu Conservancy, Monkey Bridge Campsite	-0.392	36.211
Narok	62	Masai Mara National Reserve, Research Station	-1.547	35.306
Narok	62	Masai Mara National Reserve, Mara Simba Lodge	-1.547	35.306
Narok	62	Masa Mara National Reserve, Sarova Mara Lodge	-1.531	35.320
Narok	63	Mara Conservancy, Eluai Public Campsite	-1.397	35.004
Narok	63	Mara Conservancy, Iseiya Public Campsite	-1.401	35.018
Narok	64	Mara Conservancy, Mara River	-1.331	34.994
Narok	65	Mara Conservancy, Ngiro-Are Anti-Poaching Unit Station	-1.414	34.794
Narok	66	Masai Mara National Reserve, Keekorok Lodge	-1.590	35.234
Nyeri	67	Aberdares National Park, Ruhuruini Campground	-0.388	36.818

County	Locality	Locality description	Latitude	Longitude
Nyeri	68	Mount Kenya National Park, Kisio Munyao Campground	-0.176	37.147
Nyeri	68	Mount Kenya National Park, Narumoru Gate	-0.175	37.143
Samburu	69	Samburu National Game Reserve, Larsen's Camp	0.572	37.572
Samburu	70	Samburu National Game Reserve, Samburu Game Lodge	0.572	37.537
Samburu	70	Samburu National Game Reserve, Vervet Campsite	0.567	37.536
Taita-Taveta	71	Lake Jipe, Mt Kilimanjaro	-3.56	37.75
Taita-Taveta	72	Marungu Cave	-3.61	38.74
Taita-Taveta	73	Taita Discovery Centre	-3.706	38.776
Taita-Taveta	74	Tsavo East National Park, Sala Gate, visitors' toilets	-3.076	39.217
Taita-Taveta	75	Tsavo West National Park, Chyulu Gate Ranger Post	-2.902	38.133
Taita-Taveta	76	Tsavo West National Park, Komboyo Campground	-2.754	38.115
Taita-Taveta	76	Tsavo West National Park, park headquarters	-2.747	38.131
Taita-Taveta	77	Tsavo West National Park, Shetani Caves	-2.855	38.001
Tharaka-Nithi	78	Marma Cave	-0.264	37.687
Trans Nzoia	79	Cherangani Forest Station	1.036	35.326
Trans Nzoia	80	Mount Elgon National Park, Kitum Cave	1.029	34.756
Trans Nzoia	80	Mount Elgon National Park, Makingeny Cave	1.036	34.753
Trans Nzoia	81	Mount Elgon National Park, Rongai Campground	1.031	34.778
Trans Nzoia	82	Saiwa Swamp National Park, Bushbuck Nature Trail	1.095	35.118

Table 1.1. Gazetteer of localities sampled in Bats of Kenya survey. Latitude and longitude are presented in decimal degrees. Localities with considerable geographic overlap are assigned the same identifying number.

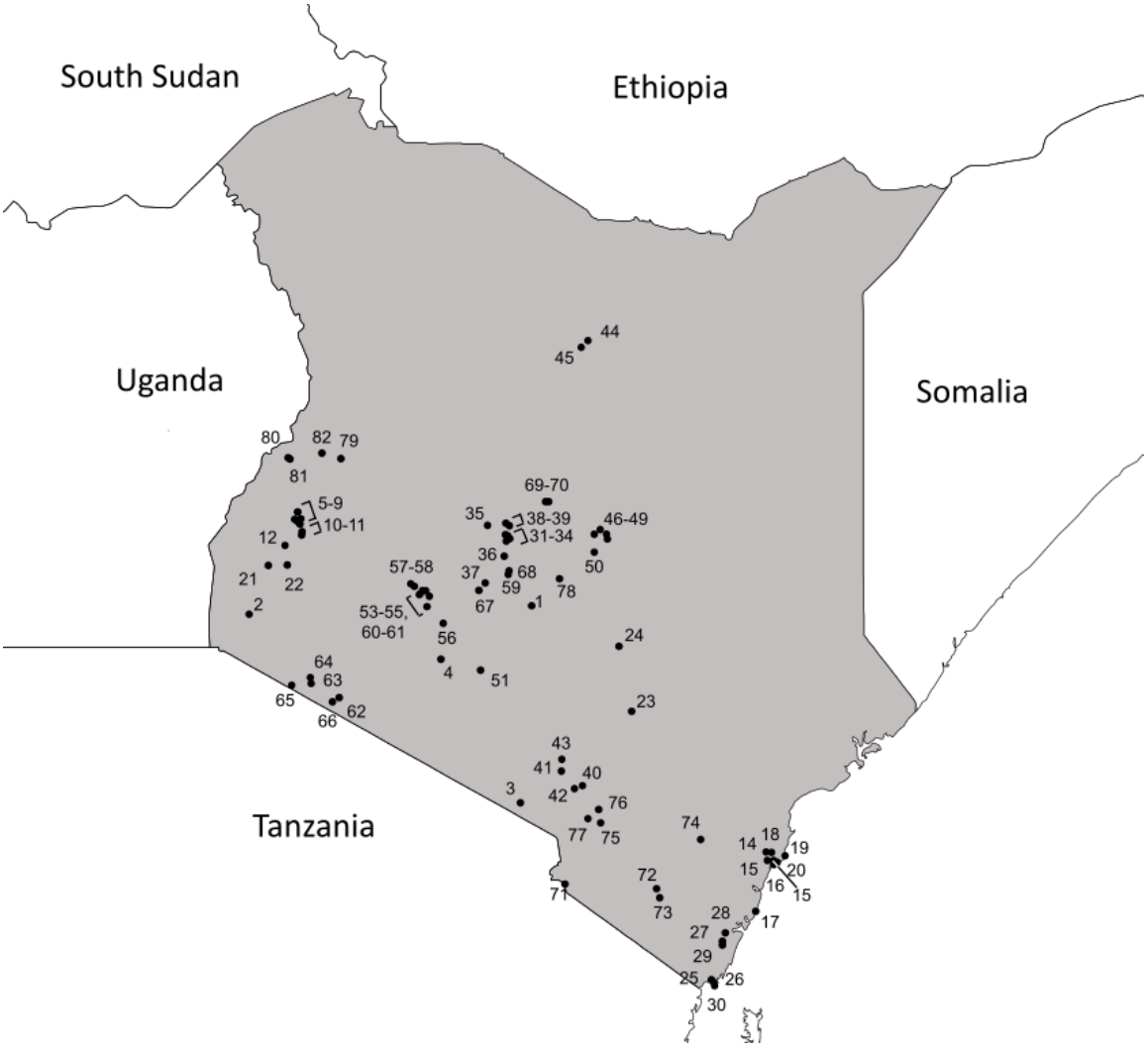

Figure 1.1. Map of localities sampled in Bats of Kenya survey. Gazetteer located in Table 1. Localities with considerable geographic overlap are assigned the same identifying number.

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APPENDIX 1

List of 18 nycteribiid bat fly species known from Kenya. New country records from Bats of Kenya survey are denoted by an asterisk.

Cyclopodia greeffi greeffi (Karsch 1884)

Dipseliopoda biannulata (Oldroyd 1953)

Dipseliopoda setosa (Theodor 1955)

Eucampsipoda africana (Theodor 1955)

Basilia ansifera (Theodor 1956)

**Basilia robusta* (Theodor 1956)

Basilia blainvillii blainvilli (Leach 1817)

Basilia bouveri (Falcoz 1924)

Nycteribia latitergum (Theodor 1957)

Nycteribia schmidlii scottii (Falcoz 1923)

Penicillidia fulvida (Bigot 1885)

Pencillidia pachymela (Speiser 1901)

* *Phthiridium hoogstrali* (Theodor 1957)

* *Phthiridium inopinata* (Theodor 1957)

* *Phthiridium rhodesiense* (Theodor, 1957)

* *Phthiridium scissum* (Speiser, 1901)

Phthiridium tectum (Theodor 1957)

* *Phthiridium sp. nov.* ex. *Rhinolophus clivosus*

CHAPTER 2: REMARKABLY LOW HOST SPECIFICITY IN THE BAT FLY *PENICILLIDIA FULVIDA* (DIPTERA: NYCTERIBIIDAE) AS ASSESSED BY MITOCHONDRIAL COI AND NUCLEAR 28S SEQUENCE DATA

Abstract:

The recognition and delineation of morphologically indistinguishable cryptic species has broad implications for wildlife conservation, disease ecology, and accurate estimates of biodiversity. Discoveries and estimates of undetected cryptic diversity are climbing with advances in molecular systematics. Parasites are intriguing in the study of cryptic speciation because unique evolutionary pressures and diversifying factors are generated by ecological characteristics of host-parasite relationships, including host specificity. Bat flies (Diptera: Nycteribiidae and Streblidae) are obligate, hematophagous ectoparasites of bats that generally exhibit high degrees of host specificity. One rare exception is *Penicillidia fulvida* (Diptera: Nycteribiidae), an African bat fly found in association with numerous and phylogenetically distant hosts. One explanation for *P. fulvida*'s unique polyxeny is that it may represent a complex of host-specific yet cryptic species, an increasingly common finding among molecular genetic studies of supposed generalist parasites. However, the use of two genetic markers (nuclear 28S and mitochondrial COI) did not indicate that cryptic speciation or host-specific genetic structure is present in *P. fulvida*, instead supporting its putative status as a rare example of a single bat fly species with primary host associations spanning multiple bat families. Gene flow among *P. fulvida* utilizing multiple host species may be promoted by

polyspecific roosting behavior in bats, and host preference may also be malleable based on the bat assemblages occupying common roosts. The proclivity of generalist parasites to switch hosts makes them more likely to vector or opportunistically transmit pathogens across species boundaries. The presence of polyxenous bat flies is therefore of important consideration to disease ecology as bat flies become increasingly known to be associated with pathogens of bats.

Key Words: Bat Flies, Chiroptera, Ectoparasites, Kenya, Nycteribiidae, Host specificity, Cryptic species, Molecular ecology

Introduction

Although the species is a basic unit of organization in biology and is of fundamental importance in the study of ecology and evolution, the definition of a species is famously contentious. At least 32 species concepts have been described (Zachos 2016), but are often broadly united by few criteria (Perkins 2000): the ability to successfully reproduce (e.g. Biological Species Concept; Mayr 1942), distinct morphology (e.g. Morphological Species Concept; Cronquist 1978), and shared evolutionary descent (e.g. Phylogenetic Species Concept; Cracraft 1983). An integrative approach to species delimitation addressing several species concepts is often desirable, but some criteria are difficult to satisfy in practice or categorically inapplicable to certain study systems (Perkins 2000).

The non-universality of species concepts is demonstrated by cryptic species that are morphologically indistinguishable but genetically and often ecologically distinct

(Bickford 2006, de Leon and Nadler 2010). The evolutionary processes associated with cryptic speciation are variable: cryptic speciation is known to occur in allopatry (Norman et al. 2014), sympatry (Whiteman et al. 2006), and parapatry (Dennis and Hellberg 2010). Recent advances in molecular systematics have significantly increased the rate of intentional and unintentional discovery of cryptic species (Bickford 2007, de Leon and Nadler 2010). The recognition and delimitation of cryptic species is not only critical to accurately quantifying biodiversity, but misidentification of cryptic species complexes as single species have profound consequences for wildlife conservation and disease ecology, potentially complicating efforts to control and prevent invasive pests and pathogens (Bickford 2007, de Leon and Nadler 2010).

Parasites are compelling candidates for the study of cryptic speciation because they experience evolutionary pressure from their hosts, which may be strong enough to result in speciation (Hafner and Nadler 1988, Light and Hafner 2007, Tortosa et al. 2013). The shift from morphological to molecular techniques in delimiting parasite species has uncovered significant cryptic diversity, including the revision of 175 morphologically identified species of avian malaria parasites to an estimated 10,000 using mitochondrial DNA markers (Bensch et al. 2004). However, the division of nominal parasite species into cryptic species complexes has significance beyond contribution to our knowledge of parasite biodiversity. Cryptic species of parasites may inform our understanding of host-parasite coevolution and cospeciation (Engelbrecht et al. 2014), have different host invasion pathways (Miura 2006) and exhibit different degrees of host specificity (Smith et al. 2005, Whiteman et al. 2006).

Host specificity is a measure of the frequency with which a parasite species associates with a single species of host (Poulin 2007). Parasites limited to only one species of host are said to be host specialists, and less discriminate parasites found in association with multiple host species are host generalists. Terms coined by Wenzel et al. (1966) describe specificity in varying degrees based on host relatedness: strict specialists associated with a single host species are "monoxenous" (Wenzel et al. 1966), parasites confined to related species are stenoxenous (congeneric hosts) or oligoxenous (confamilial hosts), and true generalists associated with multiple hosts irrespective of phylogenetic distance are polyxenous.

Morphological conservatism is an alternative explanation for some generalist parasites identified solely by morphological attributes (Whiteman et al. 2006). The value of using molecular genetic techniques to more accurately delimit levels of host specificity is increasingly recognized (Poulin and Keeney 2008), and several nominal species of supposed generalist parasites have been revealed as complexes of host-specific cryptic species using genetic markers, including mites and ticks (McCoy et al. 2001, Whiteman et al. 2006). Accurately determining host specificity of parasite species is of wildlife conservation and human health concern because generalist parasites may be more capable of vectoring pathogens to novel hosts (Tompkins and Poulin 2006). Generalist parasites are also more competent invaders of new environments than specialists (Tompkins and Poulin 2006), a trait that warrants attention as anthropogenic change brings invasive parasites into contact with novel habitats and hosts more frequently (Daszak et al. 2000).

Host-parasite cospeciation, or the tendency for a parasite's evolutionary history to parallel that of its host, is one potential driver of parasite diversification (Hafner and

Nadler 1988). However, perfect congruence of host-parasite phylogenies (i.e., strict cospeciation) is rare, and evolutionary histories of parasites are more frequently colored by a combination of cospeciation and host-switching events (Huyse et al. 2005). Therefore, ecological characteristics affecting a parasite's ability to switch hosts may also affect host-parasite coevolutionary dynamics and parasite diversification (Hafner and Nadler 1988), including cryptic speciation (Falk and Perkins 2013). Population genetic structure in parasites is thought to increase with host specificity due to restricted gene flow across host species (Huyse et al. 2005).

Bat flies (Diptera: Streblidae and Nycteribiidae) are obligate, blood-feeding ectoparasites of bats. Members of the Nycteribiidae are uniformly spider-like in appearance and wingless, restricting their ability to disperse independently of their hosts (Dick and Patterson 2006). Bat flies reproduce via viviparous pupiparity, and a gravid female will leave her host only to adhere a single pupa to the roost substrate (Ching and Marshall 1968). Bats are known reservoirs for an exceptionally diverse array of pathogens, including many zoonotic viruses relevant to human health (Calisher et al. 2006). As obligate parasites feeding exclusively on bat blood, bat flies warrant increased attention as potential vectors of these pathogens (Dick and Dittmar 2014). Bat flies have been identified as vectors of protozoan parasites (Megali et al. 2010) and bacterial pathogens in genus *Bartonella* (Wilkinson et al. 2016), and have recently been found to harbor bat-associated viruses related to medically impactful zoonoses (Bennett et al. 2020, Ramírez-Martínez et al. 2021).

Bat flies were historically understood to possess relatively low host specificity due to frequent host-switching opportunities, ostensibly facilitated by polyspecific

roosting behavior in bats (Theodor 1957), paired with the significant portion of their life cycle spent off-host due to a reproductive reliance on roost substrate (Dick and Patterson 2007). In actuality, most bat flies are strictly monoxenous, or found in reliable association with only one species of bat (Dick and Dittmar 2014). In many parasites, patterns of host specificity are governed by host and parasite ecological characteristics facilitating reliable host-parasite encounters and eventual evolutionary associations. However, host specificity in bat flies seems unaligned with host-independent dispersal ability (e.g., vagility) or other ecological associations that could provide host-switching opportunities (ter Hofstede et al. 2004, Dick and Patterson 2007). High host specificity in bat flies may therefore be maintained by host immunocompatibility or the decreased probability of encountering suitable mates on non-primary hosts ("reproductive filter"; Dick and Patterson 2007). Bat flies associated with multiple host species are often stenoxenous or oligoxenous, infesting only closely related or congeneric bats (Dick and Patterson 2007). Previous studies examining gene flow in oligoxenous bat flies using mitochondrial genetic markers have uncovered little geographic or host-structured population genetic structure (Wilson et al. 2007, Olival et al. 2013).

Penicillidia fulvida (Diptera: Nycteribiidae) is an African bat fly species that exhibits unusually low host specificity (Theodor 1967) and is thus apparently polyxenous. Collections summarized by Theodor (1967) associated *P. fulvida* with 14 host species, and noted a wide range of host associations relative to other bat fly species. *Penicillidia fulvida* specimens collected from Kenya and currently stored in the Field Museum of Natural History Collection have been recovered from 16 putative host species representing 7 genera and 6 families of bat. In contrast to the monoxenous or oligoxenous

majority of bat flies (Dick and Patterson 2007), *P. fulvida* is known to parasitize bats belonging to families that diverged as many as 58.9 million years ago (Agnarsson et al. 2011).

The rarity of polyxenous host associations among bat flies, in concert with the increasingly recognized prevalence and ecological importance of cryptic parasite species, suggests that *P. fulvida* may represent a complex of cryptic, and possibly more host-specific, bat fly species. Alternatively, *P. fulvida* may truly represent a single generalist species capable of colonizing distantly related hosts, and such information may inform the potential of some bat flies as interspecific vectors of disease. The questions addressed in this study are 1) do patterns of genetic differentiation exist within *P. fulvida*, potentially indicative of cryptic speciation?, and 2) are any patterns of genetic differentiation in *P. fulvida* observable among sympatric hosts, indicating cryptic host specificity?

Materials and methods

Sampling

All *P. fulvida* specimens used in this study were collected during field expeditions of the Bats of Kenya project between 2006 and 2015. *P. fulvida* were recovered from 14 localities, comprising mostly tropical broadleaf forests in the southern and western regions of Kenya (Figures 1 and 2). A total of 65 specimens were collected (Table 1); all are referenced to describe host associations (Table 2), and 59 were sequenced. During collection, bats were captured in mist nets or in hand nets at roosting sites and stored individually in clean cloth bags to minimize the risk of parasite disturbance transfers.

Each bat was then euthanized with halothane following guidelines by the American Society of Mammalogists (Sikes 2016), under the approval of the Field Museum's Institutional Animal Care and Use Committee (2012-003). Bats were then fumigated with ethyl ether and checked thoroughly for parasites. Once extracted from the host, bat flies were immediately stored in 95% ethanol and later identified under a light microscope using species keys and descriptions from Theodor (1967) as well as reference specimens from the Field Museum of Natural History Collection of Hippoboscoid Diptera. All specimens are housed in the Field Museum collection (currently on long term loan to C. W. Dick at Western Kentucky University).

Two congeneric African bat flies, *Penicillidia pachymela* and *P. leptothrinax*, were also sequenced to better elucidate "species-level" divergence in target genes. *P. pachymela* is posited to be closely related to *P. fulvida* based on morphological characteristics, and is also assigned to the *P. fulvida* group of species (Theodor 1967). *P. pachymela* was collected in sympatry with *P. fulvida* but is exceedingly rare across their shared range in Kenya, with only a single specimen collected during this cumulative 9-year sampling effort. *P. leptothrinax* is a more distantly related and smaller-bodied species endemic to Madagascar.

DNA extraction, amplification and sequencing

One or two legs were removed from each *P. fulvida* specimen for genetic analysis to allow retention of morphological vouchers. Prior to DNA extraction, each leg was lacerated with sterile forceps to expose the muscle tissue beneath the exoskeleton and transferred to a 1.5 ml microcentrifuge tube. Whole genomic DNA extractions were

performed according to manufacturer protocol using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, California), with the final elution divided into two steps at 35 and 65 μl volumes to optimize DNA concentration. All extractions were assessed for quality using a NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts).

A 658-bp fragment of the cytochrome c oxidase subunit I gene (COI) was amplified using the invertebrate-specific primer pair LCO1490 (5'-GGTCAACAA ATCATAA-AGATATTGG-3') and HCO2198 (5'-TAACTTCAGGGT GACCAAAAAATCA-3') (Folmer et al. 1994). Polymerase chain reactions (PCR) were conducted in 25 μl total volumes composed of 12.5 μl GoTaq MasterMix (Promega, Madison, Wisconsin), 9 μl of nuclease-free water, 1 μl each of 10 μM forward and reverse primers, and 1.5 μl of DNA template (averaging 14 ng/ul). A negative control replacing template DNA with nuclease-free water was included with every set of reactions. Thermal cycler conditions for COI were as follows: initial denaturing at 95 degrees for 1 minute, followed by 35 cycles of 95 degrees for 2 minutes, 50 degrees for 20 seconds, and 72 degrees for 1 minute, then final extension at 72 degrees for 5 minutes.

Mitochondrial DNA has rapid substitution rates, making mitochondrial sequence data favorable for parsing relatively shallow, species-level phylogenetic relationships (Moore 1995). Though segments of COI have been proposed as "universal barcodes" suitable for species delimitation almost ubiquitously across taxa (Hebert et al. 2003), sole reliance on mitochondrial markers disregards other modes of inheritance (Rubinoff 2006) and can obscure potential effects of introgression or infection with *Wolbachia*, an arthropod-associated bacterial endosymbiont capable of disrupting patterns of

mitochondrial inheritance (Jiggins 2003). For a multilocus approach, I also amplified 28S, a nuclear gene commonly used alongside COI for species-level analyses in arthropods (Smith et al. 2005, Kuhlmann et al. 2009).

The D2 region of the nuclear 28S gene was amplified using the primers F2 (5-AGAGAGAGTTCAAGAGTACGTG-3') and 3DR (5'-TAGTTCACCATCTTTCGGGTC-3') (Belshaw et al. 2001). Reaction components and volumes were identical to those used to amplify COI. Thermal cycler conditions for 28S were as follows: initial denaturing at 98 degrees for 2 minutes, followed by 35 cycles of denaturing at 94 degrees for 3 minutes, annealing at 51 degrees for 30 seconds, and extension at 72 degrees for 2 minutes, then final extension at 72 degrees for 8 minutes.

PCR products were verified for size and quality via electrophoresis on a 1.5% agarose gel stained with SybrSafe (Invitrogen, Carlsbad, California) and visualized under a blue LED light. Sanger sequencing was performed at North Carolina State Genomic Sciences Laboratory (Raleigh, North Carolina) using forward and reverse primers for both genes.

Phylogenetic analyses

Sequences were trimmed and assessed for ambiguous bases by eye, then aligned using the MUSCLE algorithm (Edgar 2004) as implemented in Geneious 6.0 (Kearse et al. 2012). A hippoboscid fly, *Pseudolynchia canarensis*, was included as an outgroup (sequence data retrieved from GenBank). Haplotype groups were identified using DnaSP 6 (Rozas et al. 2017), and a minimum-spanning haplotype network was constructed with PopART (Leigh and Bryant 2015).

A maximum parsimony tree was created in PAUP* 4.0 (Swofford 1998) using a heuristic search algorithm and non-parametric bootstrap method. The initial tree was obtained using stepwise addition, with 10 trees held at each step. Branch-swapping was performed with the tree-bisection-reconnection (TBR) algorithm, with the steepest descent option enabled and 100 random addition replications. Nodal support was calculated using 1,000 bootstrap replicates, which were used to craft a 50% majority-rules consensus tree.

Substitution models for Bayesian analysis were estimated with jModeltest (Posada 2008). Optimized substitution models based on Akaike's Information Criterion were GTR + G for COI and HKY + I for 28S. Bayesian analysis was performed in MrBayes 3.2 (Ronquist and Huelsenbeck 2003) using the default burn-in and 10 million Markov Chain Monte Carlo (MCMC) generations. Average standard deviation in split frequencies fell below 0.01 at 380,000, then went above 0.01 and again below at 650,000 generations and remained under this threshold until the final generation, indicating stationarity was reached. Posterior probabilities for clade support were calculated using the trees remaining after the default burn-in of 25%.

Analysis of molecular variance

A hierarchical analysis of molecular variance (AMOVA) was used to determine to what extent genetic variation in *P. fulvida* is allocated among host taxa. Because AMOVA requires groups to be defined *a priori*, haplotypes were pooled by host family for a conservative approach. Host families with insufficient sample sizes for statistical analysis (Emballonuridae, Nycteridae and Hipposideridae, from which only 1-3 *P.*

fulvida were recovered or sequenced) were excluded; therefore, only specimens from Miniopteridae (n=33), Rhinolophidae (n=11) and Vespertilionidae (n=9) were compared. AMOVA was performed in Arlequin 3.5.2.2 (Excoffier and Lischer 2010) using a locus-by-locus method to accommodate missing data. The significance of pairwise fixation indices (F_{st}, an F-statistic measuring variance in allele frequency among populations) was calculated using 1,023 permutations, each assigning parasite haplotypes to host families at random to generate a null distribution.

Results

Phylogenetic analyses

12 COI haplotypes were present within *P. fulvida*, with 11 variable sites (Figure 3). One specimen (BDP4273-74) could not be assigned a COI haplotype due to an ambiguous base at a variable site, and was excluded from the haplotype network. Only a single 28S haplotype was recovered from *P. fulvida*, but 28S haplotypes were distinct among the three described species within *Penicillidia* (1.8% pairwise uncorrected p-distance between *P. leptothrinax* and most common *fulvida* haplotype, 1.6% between *P. pachymela* and *fulvida*, and 3.2% between *P. leptothrinax* and *pachymela*). The inclusion of 28S in phylogenetic analyses yielded poorly resolved relationships among the three putative species and was too conservative to inform population genetic structure within *P. fulvida*. Only COI-based trees are displayed, as *P. pachymela* and *P. leptothrinax* were only included for comparative purposes and resolving relationships among putative species within *Penicillidia* is outside the scope of this study.

Bayesian and maximum parsimony analyses yielded incongruent topologies (Figure 4). Maximum parsimony analysis placed two specimens collected from *Miniopterus* (clade 1 or 4; see Demos et al. 2019a) and *Taphozous hildegardeae* peripheral to the primary *P. fulvida* clade and supported an additional interior clade, which was not structured by host identity or geography (Figure 4). One clade was supported by both Bayesian and maximum parsimony analyses, comprised of 6 specimens from the host family Rhinolophidae but from a single, distant locality (Marsabit; Figures 1 and 4). Neither analysis placed any outgroups within the in-group, and relationships among described *Penicillidia* species aligned with past delineations based on morphology (Theodor 1967).

Analysis of molecular variance

Hierarchical AMOVA rejected the null hypothesis of haplotype homogeneity among host families ($p < 0.001$; Table 3). The significance of pairwise F_{st} values indicated allele frequencies were unique in *P. fulvida* haplotype groups from rhinolophid bat hosts when compared to haplotype groups from both miniopterid and vespertilionid bats (Table 4). However, this significant AMOVA appears artifactual when regarded alongside phylogenetic structure and sampling composition: genetic structure associated with Rhinolophidae aligns more reliably with sampling locality (Table 1, Figure 4); 7 of 11 *P. fulvida* recovered from rhinolophid bats were also recovered from Marsabit, and 6/6 of "rhinolophid" clade were from Marsabit bats). Regarded cumulatively, these results do not suggest host-based genetic differentiation is present within *P. fulvida*.

Discussion

Parasites are susceptible to placement in artificial species groups because of their morphological conservatism relative to their hosts (Whiteman et al. 2006). The advent of molecular genetic techniques has provided a valuable strategy for scrutinizing morphologically indistinguishable but ecologically unique parasites, and has routinely resulted in the division of host-generalist nominal species into more host-specific cryptic species complexes (Bensch et al. 2004, Smith et al. 2005, Whiteman et al. 2006). Using two genetic markers (mitochondrial COI and nuclear 28S), we have uncovered no such evidence of host-mediated genetic structure in the polyxenous bat fly *P. fulvida*. This finding is concordant with other studies evaluating patterns of population genetic structure in non-monoxenous (oligoxenous) nycteribiid bat flies (Olival et al. 2013, Wilson et al. 2007), but to our knowledge represents the first such investigation of a truly polyxenous bat fly species. These results indicate inter-host gene flow is occurring in *P. fulvida*, and support its putative status as a single morphological and phylogenetic species capable of parasitizing phylogenetically distant bat species.

Although no genetic structure could be reliably attributed to host identity, there is some evidence *P. fulvida* is not uniformly panmictic across Kenya. Both maximum parsimony and Bayesian phylogenetic analyses supported a clade of 6/7 *P. fulvida* from a single disparate sampling site in Marsabit Forest (Figures 3 and 4). Isolation by distance alone is insufficient for explaining this geographic differentiation, as *P. fulvida* is essentially panmictic (with respect to the localities sampled, and based on 53 specimens) across the comparable 700-kilometer distance between Kakamega and Fikirini (Figure 1). Kenya is composed of diverse biomes, and Marsabit Forest is well-representative of this

mosaic: a tropical broadleaf forest sustained by volcanic soil and fully surrounded by swaths of rocky desert (Figure 2). Further, all *P. fulvida* from Marsabit Forest were collected from horseshoe bats (5/6 from *Rhinolophus fumigatus* (clade 2-3; see Demos et al. 2019b), and 1/6 from *Rhinolophus* cf. *clivosus*), which are reliant on the availability of suitable permanent roosts and are relatively weak fliers (B. D. Patterson, personal observation). Marsabit Forest's geographic isolation may stymie gene flow in *P. fulvida* by restricting the dispersal of its bat hosts. However, due to a small sample size and a paucity of intermediate sampling sites closer to Marsabit Forest that may more firmly implicate the surrounding desert as a barrier to gene flow, the origin of this site-related genetic structure remains speculative.

One *P. fulvida* haplotype from Marsabit fell outside the Marsabit-associated clade despite being collected from the same individual specimen of *R. fumigatus* as a within-clade parasite, instead mirroring a haplotype from a *Miniopterus* host in Kakamega Forest over 400 kilometers away. This is tentatively indicative of some gene flow occurring between Marsabit and other populations, but absent additional data this finding cannot be explored in more detail.

An alternative explanation for the lack of genetic structure in *P. fulvida* is insufficient variety and variability in the genes used. Nuclear 28S was conservative among the three *Penicillidia* species sequenced and invariable in *P. fulvida*. Consequently, only mitochondrial COI was used to assess patterns of intraspecific, inter-host variation in *P. fulvida*. Drawing conclusions from one mitochondrial gene can be precarious, as mito-nuclear discordance and the small proportion of the overall genome represented in single-gene phylogenies can cause incongruence (Rubinoff and Holland

2005). Further, reliance on mtDNA is unfavorable in some insect taxa, including bat flies, due to possible infection with bacterial *Wolbachia* (Szentiványi et al. 2019). *Wolbachia* is maternally transmitted and can cause sweeping disruptions in mitochondrial inheritance, resulting in the overrepresentation of certain haplotypes in a population or the absence of polymorphism altogether (Jiggins 2003). Although speculative, the possibility of *Wolbachia* infection obscuring genetic structure in *P. fulvida* cannot be discarded based on our data at this time.

Understanding host specificity and the processes responsible for its evolution and maintenance across parasite communities is crucial to understanding the evolutionary context of host-parasite associations and the role of parasites in disease transmission. High host specificity in parasites has historically been regarded as the default trend in parasite evolution and specialization, and also as an evolutionary "dead end", in which the degree of morphological specialization required for a highly specific parasite to efficiently exploit its host makes a specialist lineage's "return" to a generalist strategy improbable (Poulin et al. 2006). However, the evolution of ecological resource specialization is not actually characterized by fixed directionality (Thompson 1994, Poulin et al. 2006). Different degrees of host specificity can arise multiple times in the evolutionary history of a parasite lineage, and each strategy may be associated with unique trade-offs (Jaenike 1990). Extensive biodiversity surveys have found that bat fliespossess considerably high host specificity as a group, and some incidences of low specificity in bat flies may be attributable to poorly understood species boundaries (Dick and Dittmar 2014). Using molecular techniques, this study demonstrates that, although rare, the existence of polyxenous bat fly species is tenable.

Host ecology serves as an important evolutionary driver of patterns of host specificity in parasite communities. Multiple bat species often aggregate closely in a single roost, a behavior which may confer anti-predator benefits or is simply a result of species gathering to exploit a limited resource (Stensland et al. 2003). Mixed-species groups provide host-switching opportunities to parasites dependent on their hosts for dispersal; as a result, polyspecific bat roosts were a historical precedent for proposing universally low host specificity in bat flies (Jobling 1949, Theodor 1957). *Penicillidia fulvida* was collected from multiple host species in 8 of 14 roosts and multiple host families in 7 of 14 roosts, indicating polyspecific roosting behavior may be a proponent of *P. fulvida*'s broad host range and high gene flow across hosts. Further, although it is now recognized that specialist bat flies can maintain their high specificity irrespective of host-switching opportunities (Dick and Patterson 2007), bat assemblages in shared roosts may influence host preference in generalist bat flies (Seneviratne et al. 2009). Accordingly, there is some indication parasitism by *P. fulvida* is based on host availability. *P. fulvida* occasionally parasitizes the African sheath-tailed bat *Coleura afra* (4/63 total host associations in Bats of Kenya survey; Table 2), but associations with *C. afra* were only recorded in the absence of potential miniopteran and rhinolophid hosts (Table 1). *C. afra* was present in 3 shared roosts containing *Miniopterus* hosts of *P. fulvida*, but *P. fulvida* was never recovered from *C. afra* when these alternative hosts were available. This pattern suggests that although host selection in *P. fulvida* is unconstrained by phylogenetic distance, *P. fulvida* may still demonstrate tiers of host preference, which could function to increase fitness by mitigating local competition or selecting optimally nutritious host blood (Dick and Dittmar 2014).

Vector ecology is a valuable determinant of pathogen spread and potential zoonotic spillover (Plowright et al. 2017). Low host specificity in parasites may promote exposure to a wider range of infectious agents and facilitate disease transmission to new hosts and geographic areas (Daszak et al. 2000). Bats are reservoirs for a nearly unmatched diversity of pathogens, a product of immunological or ecological predisposal (Brook and Dobson 2015) or plainly of their high diversity relative to most mammalian taxa (Mollentze and Streiker 2020). Nycteribiid bat flies, including *P. fulvida,* can serve as vectors or reservoirs facilitating the transmission of several bat pathogens: haemosporidian parasites in genus *Polychromophilus* (Megali et al. 2010) and bacterial bartonellae (Wilkinson et al. 2016). Moreover, research linking nycteribiid bat flies to impactful bat-associated viral zoonoses is expanding (Bennett et al. 2020, Jansen van Vuren 2016). Within the scope of this study, Kenyan hosts of *P. fulvida* are known reservoirs of paramyxoviruses (*Miniopterus* spp.; Conrardy et al. 2014), SARS coronaviruses (*Rhinolophus fumigatus* and *R. landeri*; Warahiu et al. 2017), and lyssaviruses (*Miniopterus* spp.; Kuzmin et al. 2008).

Because this study failed to detect significant genetic differentiation among *P. fulvida* parasitizing bats across three phylogenetically distant families that could not be explained geographically, its findings reinforce that *P. fulvida* is a rare polyxenous species of bat fly. However, it is important to acknowledge this study is not comprehensive with respect to *P. fulvida*'s geographic and host range. *P. fulvida* has a broad range outside Kenya, comprising sub-Saharan Africa (Theodor 1967) and possibly Madagascar (C.W. Dick, unpublished data). Additionally, *P. fulvida* was collected from three host families in Kenya with relatively uninformative sample sizes (Emballonuridae,

Hipposideridae and Nycteridae) and has previously been recorded from Pteropodidae, though this association is tenuously based on a single record (*Eidolon helvum* in South Africa; Theodor 1967). Based on associations recorded in this study and past surveys, it seems probable that bats from Miniopteridae, Rhinolophidae, and Vespertilionidae represent *P. fulvida*'s primary host range, whereas other, infrequently observed hosts are non-primary associations indicative of host-switching events. Increased, targeted sampling efforts may be useful for further parsing host associations and for testing hypotheses associated with geographic differentiation in *P. fulvida*.

Host specificity is one of the most basal ecological characteristics of parasites and likely subject to strong selection. Accurate measurement of host specificity is vital to understanding parasite biodiversity, vulnerability to population decline, and the role of parasites in disease transmission. Moreover, accurately estimating host specificity relies on acknowledging the presence of cryptic diversity and using integrative approaches to delimiting parasite species. This study provides molecular genetic evidence the nycteribiid bat fly *P. fulvida* does not exhibit cryptic host specificity, and instead represents a single species with a wide range of phylogenetically distant bat hosts.

Bat fly ID	Host family	Host species	Locality description	Locality	Latitude	Longitude	COI	COI haplotype	28S
BDP5586	Miniopteridae	*Miniopterus* clade 10	Kakamega Forest Station, Lirhanda Hill Cave	1	0.218	34.897	Yes	7	Yes
BDP5580	Miniopteridae	*Miniopterus* clade 10	Kakamega Forest Station, Lirhanda Hill Cave	1	0.218	34.897	Yes	8	Yes
BDP5631	Miniopteridae	*Miniopterus* clade 10	Kakamega Forest, Mahiakalo Cave	2	0.244	34.907	Yes	1	Yes
BDP5630	Miniopteridae	*Miniopterus* clade 10	Kakamega Forest, Mahiakalo Cave	2	0.244	34.907	Yes	2	Yes
BDP5665	Miniopteridae	*Miniopterus* clade 10	Kakamega Forest, Mahiakalo Cave	2	0.244	34.907	Yes	6	Yes
PWW2955	Miniopteridae	*Miniopterus* clade 10	Kakamega Forest, Mahiakalo Cave	2	0.248	34.907	No	-	Yes
BDP6082	Rhinolophidae	*Rhinolophus fumigatus* 8	Fikirini, Pare Cave	3	-4.59	39.331	Yes	1	Yes
BDP6058	Miniopteridae	*Miniopterus* clade 2	Fikirini, Pare Cave	3	-4.59	39.331	Yes	6	Yes
BDP6059	Miniopteridae	*Miniopterus* clade 2	Fikirini, Pare Cave	3	-4.59	39.331	Yes	6	Yes
BDP6033	Miniopteridae	*Miniopterus* clade 2	Fikirini, Three Sisters, Kisimani Cave	4	-4.615	39.353	Yes	6	Yes
BDP6038	Miniopteridae	*Miniopterus* clade 2	Fikirini, Three Sisters, Kisimani Cave	4	-4.615	39.353	Yes	6	Yes
BDP6009	Hipposideridae	*Triaenops afer*	Fikirini, Three Sisters, Mbenyenye Cave	4	-4.61	39.354	Yes	6	Yes
BDP5971a	Miniopteridae	*Miniopterus* clade 2	Fikirini, Three Sisters, Mbenyenye Cave	4	-4.61	39.354	Yes	6	Yes
BDP5971b	Miniopteridae	*Miniopterus* clade 2	Fikirini, Three Sisters, Mbenyenye Cave	4	-4.61	39.354	Yes	6	Yes

Table 2.1. Summary of all *P. fulvida* (n=65) collected, including host identity, collection locality and coordinates, specimens used for sequencing, and COI haplotype groups

Bat fly ID	Host family	Host species	Locality description	Locality	Latitude	Longitude	COI	COI haplotype	28S
BDP6165	Emballonuridae	*Taphozous hildeaardeae*	Mwaluganje Community Elephant Sanctuarv.	5	-4.082	39.483	Yes	3	Yes
BDP6144	Miniopteridae	*Miniopterus* clade 3	Mwaluganje Community Elephant Sanctuarv.	5	-4.082	39.483	Yes	6	Yes
BDP6188	Nycteridae	*Nycteris thebaica* 4	Shimba Hills National Reserve. Sable Bandas	6	-4.215	39.451	Yes	9	No
BDP7337	Rhinolophidae	*Rhinolophus cf. landeri*	Marsabit National Park and Reserve. southeast	7	2.32	37.994	Yes	10	Yes
BDP7509	Rhinolophidae	*Rhinolophus fumigatus* 3	Marsabit National Park and Reserve, southeast	7	2.309	38	Yes	10	Yes
BDP7314	Rhinolophidae	*Rhinolophus fumigatus* 3	Marsabit National Park and Reserve, southeast	7	2.32	37.994	Yes	10	Yes
BDP7363	Rhinolophidae	*Rhinolophus fumiaatus* 3	Marsabit National Park and Reserve. southwest	8	2.283	37.954	Yes	10	Yes
BDP7368	Rhinolophidae	*Rhinolophus fumiaatus* 2-3	Marsabit National Park and Reserve. southwest	8	2.283	37.954	Yes	10	Yes
BDP7359a	Rhinolophidae	*Rhinolophus fumiaatus* 3	Marsabit National Park and Reserve. southwest	8	2.283	37.954	Yes	10	Yes
BDP7359b	Rhinolophidae	*Rhinolophus fumiaatus* 3	Marsabit National Park and Reserve. southwest	8	2.283	37.954	Yes	2	Yes
BDP6910	Miniopteridae	*Miniopterus* clade 1	Gilgil, Kariandusi Mines	9	-0.451	36.282	Yes	6	Yes
BDP6909a	Miniopteridae	*Miniopterus* clade 1	Gilgil, Kariandusi Mines	9	-0.451	36.282	Yes	12	Yes
BDP6909b	Miniopteridae	*Miniopterus* clade 1	Gilgil, Kariandusi Mines	9	-0.451	36.282	Yes	1	Yes
PWW2758	Miniopteridae	*Miniopterus* clade 1 or 4	Gilgil, Kariandusi Mines	9	-0.451	36.282	Yes	4	Yes

Table 2.1 (cont.). Summary of all *P. fulvida* (n=65) collected, including host identity, collection locality and coordinates, specimens used for sequencing, and COI haplotype groups.

Bat fly ID	Host family	Host species	Locality description	Locality	Latitude	Longitude	COI	COI haplotype	28S
PWW2746	Miniopteridae	*Miniopterus* clade 1	Gilgil, Kariandusi Mines	9	-0.451	36.282	Yes	6	Yes
PWW2727	Rhinolophidae	*Rhinolophus cf. landeri*	Gilgil, Kariandusi Mines	9	-0.451	36.282	Yes	6	Yes
PWW2751	Miniopteridae	*Miniopterus* clade 1 or 4	Gilgil, Kariandusi Mines	9	-0.451	36.282	Yes	6	Yes
PWW2755	Miniopteridae	*Miniopterus* clade 1 or 4	Gilgil, Kariandusi Mines	9	-0.451	36.282	Yes	6	Yes
PWW2756	Miniopteridae	*Miniopterus* clade 1 or 4	Gilgil, Kariandusi Mines	9	-0.451	36.282	Yes	6	Yes
PWW2850	Rhinolophidae	*Rhinolophus clivosus* 2	Gilgil, Kariandusi Mines	9	-0.451	36.282	Yes	6	Yes
PWW2747a	Miniopteridae	*Miniopterus* clade 1	Gilgil, Kariandusi Mines	9	-0.451	36.282	Yes	1	Yes
PWW2747b	Miniopteridae	*Miniopterus clade* 1	Gilgil, Kariandusi Mines	9	-0.451	36.282	Yes	1	Yes
BDP6905	Rhinolophidae	*Rhinolophus clivosus* 2	Gilgil, Kariandusi Mines	9	-0.451	36.282	Yes	6	Yes
BDP6932	Miniopteridae	*Miniopterus* clade 1	Gilgil, Kariandusi Mines	9	-0.451	36.282	Yes	6	Yes
PWW2815	Miniopteridae	*Miniopterus clade* 4 or 7	Gilgil, Pipeline Cave	10	-0.539	36.294	Yes	5	Yes
PWW2812	Miniopteridae	*Miniopterus* clade 4 or 7	Gilgil, Pipeline Cave	10	-0.539	36.294	Yes	6	Yes
PWW2893	Rhinolophidae	*Rhinolophus fumigatus* 4	Gilgil, Pipeline Cave	10	-0.539	36.294	No	-	No
PWW2918	Miniopteridae	*Miniopterus* clade 8	Soysambu Conservancy, Diaotmite Cave	11	-0.430	36.174	Yes	11	Yes

Table 2.1 (cont.). Summary of all *P. fulvida* (n=65) collected, including host identity, collection locality and coordinates, specimens used for sequencing, and COI haplotype groups.

Bat fly ID	Host family	Host species	Locality description	Locality	Latitude	Longitude	COI	COI haplotype	28S
PWW2437	Vespertilionidae	*Myotis tricolor*	Soysambu Conservancy, Monkey Bridge Campsite	11	-0.392	36.211	Yes	6	Yes
PWW2737	Vespertilionidae	*Myotis tricolor*	Menengai crater, Mau Mau cave	12	-0.217	37.137	Yes	6	Yes
PWW2767	Miniopteridae	*Miniopterus* clade 1 or 4	Menengai crater, Mau Mau cave	12	-0.217	37.137	Yes	6	Yes
PWW2775a	Miniopteridae	*Miniopterus* clade 1 or 4	Menengai crater, Mau Mau cave	12	-0.217	37.137	Yes	6	Yes
PWW2775b	Miniopteridae	*Miniopterus* clade 1 or 4	Menengai crater, Mau Mau cave	12	-0.217	37.137	Yes	6	Yes
PWW2778	Miniopteridae	*Miniopterus* clade 1 or 4	Menengai crater, Mau Mau cave	12	-0.217	36.055	Yes	6	Yes
PWW2365a	Vespertilionidae	*Myotis tricolor*	Menengai crater, Mau Mau cave	12	-0.252	36.055	Yes	1	Yes
PWW2365b	Vespertilionidae	*Myotis tricolor*	Menengai crater, Mau Mau cave	12	-0.252	36.055	Yes	6	Yes
PWW2367a	Vespertilionidae	*Myotis tricolor*	Menengai crater, Mau Mau cave	12	-0.252	36.055	No	-	No
PWW2367b	Vespertilionidae	*Myotis tricolor*	Menengai crater, Mau Mau cave	12	-0.252	36.055	Yes	1	Yes
PWW2368	Vespertilionidae	*Myotis tricolor*	Menengai crater, Mau Mau cave	12	-0.252	36.055	Yes	6	Yes
PWW2410	Miniopteridae	*Miniopterus* clade 1 or 4	Menengai crater, Mau Mau cave	12	-0.252	36.055	Yes	6	Yes
PWW2369	Vespertilionidae	*Myotis tricolor*	Menengai crater. Mau Mau	12	-0.252	36.055	Yes	1	Yes
PWW2364	Vespertilionidae	*Myotis tricolor*	Menengai crater, Mau Mau cave	12	-0.252	36.055	Yes	6	Yes

Table 2.1 (cont.). Summary of all *P. fulvida* (n=65) collected, including host identity, collection locality and coordinates, specimens used for sequencing, and COI haplotype groups.

Bat fly ID	Host family	Host species	Locality description	Locality	Latitude	Longitude	COI	COI haplotype	28S
PWW2363	Vespertilionidae	*Myotis tricolor*	Menengai crater, Mau Mau cave	12	-0.252	36.055	Yes	6	Yes
PWW2763	Miniopteridae	*Miniopterus* clade 1 or 4	Menengai crater, Mau Mau cave	12	-0.217	37.137	Yes	6	Yes
PWW2770	Miniopteridae	*Miniopterus* clade 1 or 4	Menengai crater, Mau Mau cave	12	-0.217	37.137	Yes	6	Yes
BDP 4273-4a	-	*Pipistrellus* sp. or *Coleura afra*	Marungu Cave	13	-3.61	38.74	Yes	1 or 2	Yes
BDP 4273-4b	-	*Pipistrellus* sp. or *Coleura afra*	Marungu Cave	13	-3.61	38.74	No	-	No
BDP 4254	Emballonuridae	*Coleura afra*	Marungu Cave	13	-3.61	38.74	No	-	No
BDP 4658	Emballonuridae	*Coleura afra*	Tsavo West National Park, Shetani Caves	14	-2.855	38.001	No	-	No
BDP 4650	Emballonuridae	*Coleura afra*	Tsavo West National Park, Shetani Caves	14	-2.855	38.001	No	-	No
BDP 4660	Emballonuridae	*Coleura afra*	Tsavo West National Park, Shetani Caves	14	-2.855	38.001	No	-	No

Table 2.1 (cont.). Summary of all *P. fulvida* (n=65) collected, including host identity, collection locality and coordinates, specimens used for sequencing, and COI haplotype groups.

Host family	**# *P. fulvida***	**Host species**	**# *P. fulvida***	**% total**
Miniopteridae	34	*Miniopterus* clade 1	7	11.1
		Miniopterus clade 1 or 4	11	17.5
		Miniopterus clade 10	6	9.5
		Miniopterus clade 2	6	9.5
		Miniopterus clade 3	1	1.6
		Miniopterus clade 4 or 7	2	3.2
		Miniopterus clade 8	1	1.6
Rhinolophidae	12	*Rhinolophus clivosus* 2	2	3.2
		Rhinolophus fumigatus 2-3	1	1.6
		Rhinolophus fumigatus 3	5	7.9
		Rhinolophus fumigatus 4	1	1.6
		Rhinolophus fumigatus 8	1	1.6
		Rhinolophus cf. *landeri*	2	3.2
Vespertilionidae	10	*Myotis tricolor*	10	15.9
Emballonuridae	5	*Coleura afra*	4	6.3
		Taphozous hildegardeae	1	1.6
Hipposideridae	1	*Triaenops afer*	1	1.6
Nycteridae	1	*Nycteris thebaica*	1	1.6

Table 2.2. Concise summary of 63 *Penicillidia fulvida* host associations in Kenya. Two specimens with uncertain host identities (BDP4273-4a and b) are excluded. Proportionally, *Miniopterus* "clade 1 or 4" and *Myotis tricolor* hosted the most *P. fulvida* (17.5% and 15.9% respectively).

Source of variation	Sum of squares	Variance	Percent variation	F-statistic	P-value
Among host families	5.804	0.08851	19.42387	0.19424	<0.001
Within host families	37.818	0.36717	80.57613		

Table 2.3. AMOVA analysis of genetic structure in *Penicillidia fulvida* as it corresponds to host family.

	Rhinolophidae	Miniopteridae	Vespertilionidae
Rhinolophidae		0.26579*	0.27479*
Miniopteridae	0.26579*		0.01145
Vespertilionidae	0.27479*	0.01145	

Table 2.4. Pairwise F_{st} values among host families of *Penicillidia fulvida*. *Significant at $P < 0.05$ in permutation test.

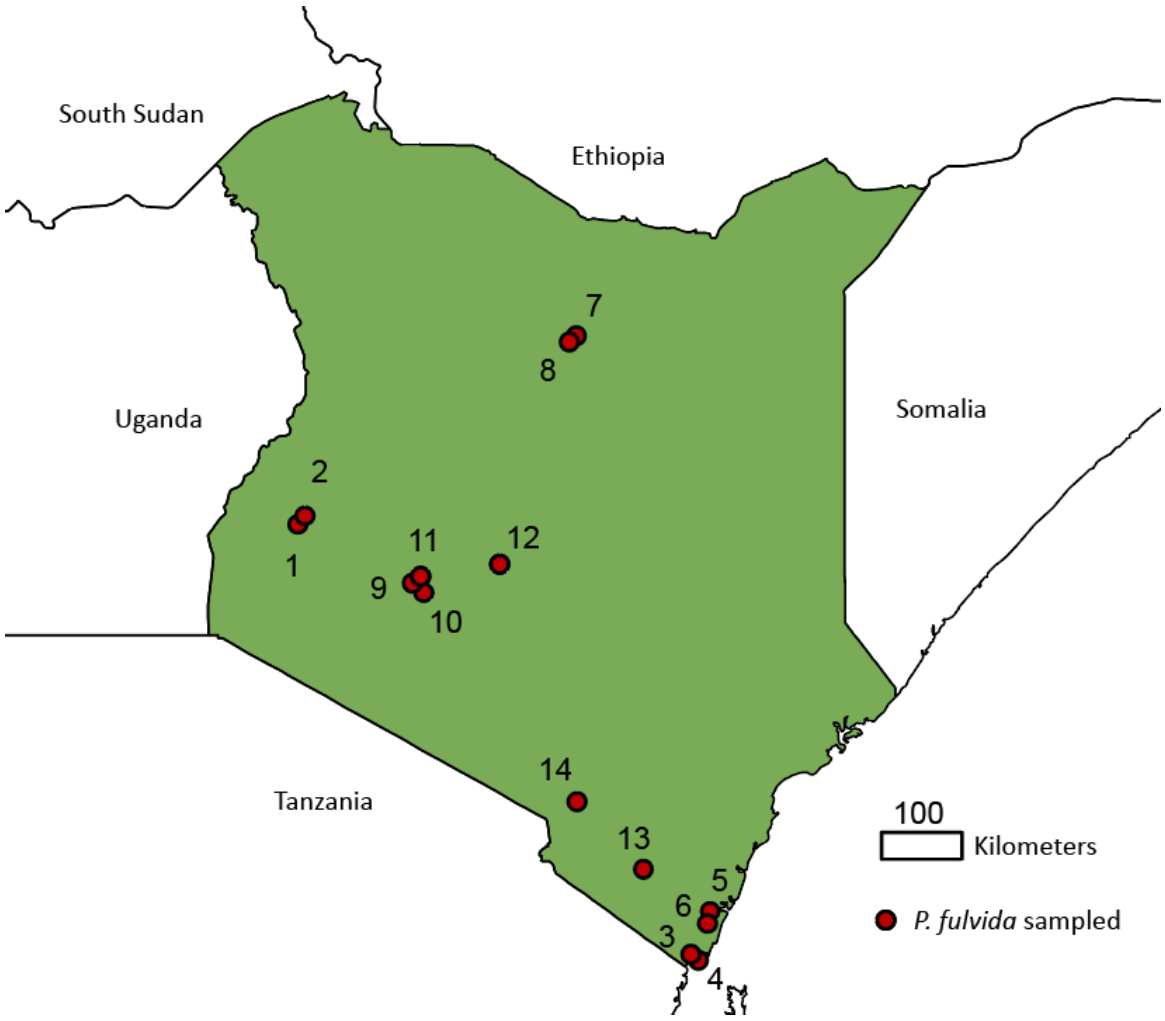

Figure 2.1. Map of Kenya featuring 14 sampling localities yielding *P. fulvida*. Gazetteer of sampling localities is located in Table 1.1.

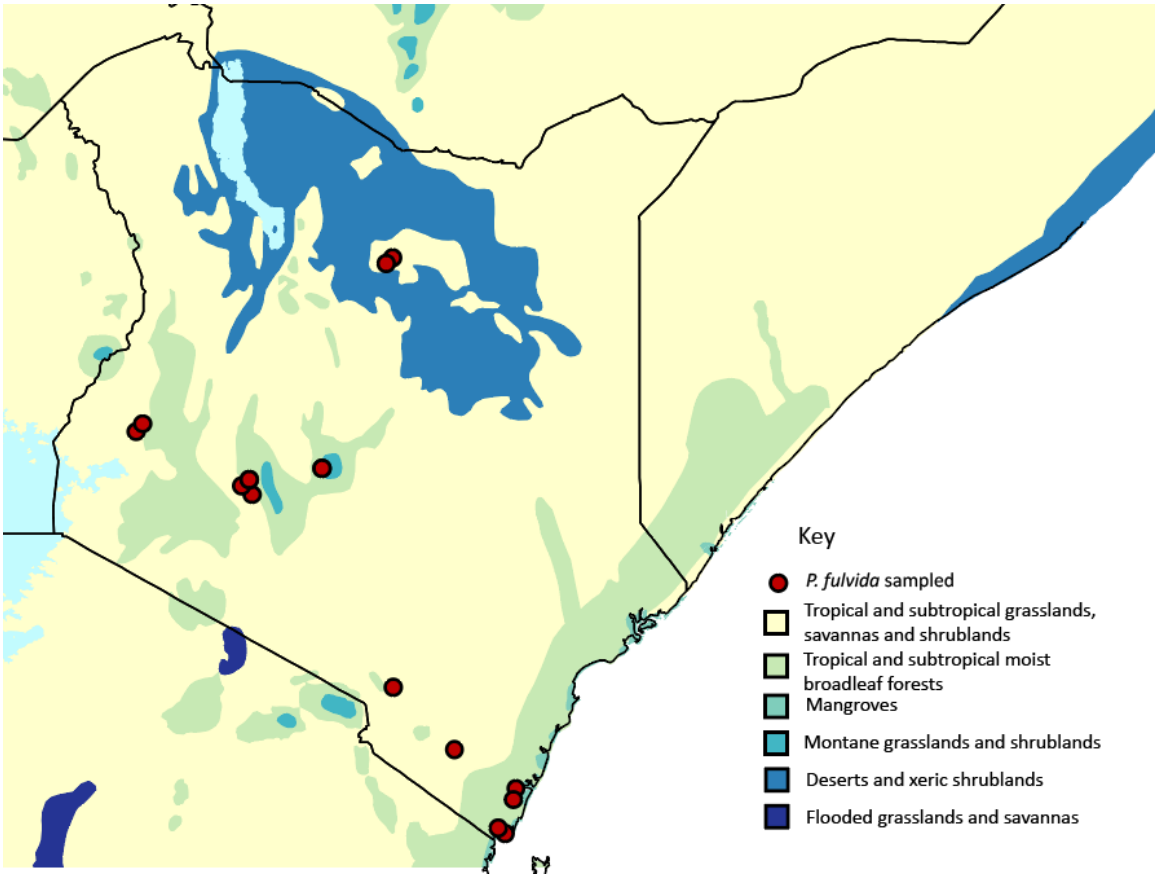

Figure 2.2. Map of Kenya featuring 14 sampling localities yielding *P. fulvida* and biomes as delineated by Olson et al. 2001.

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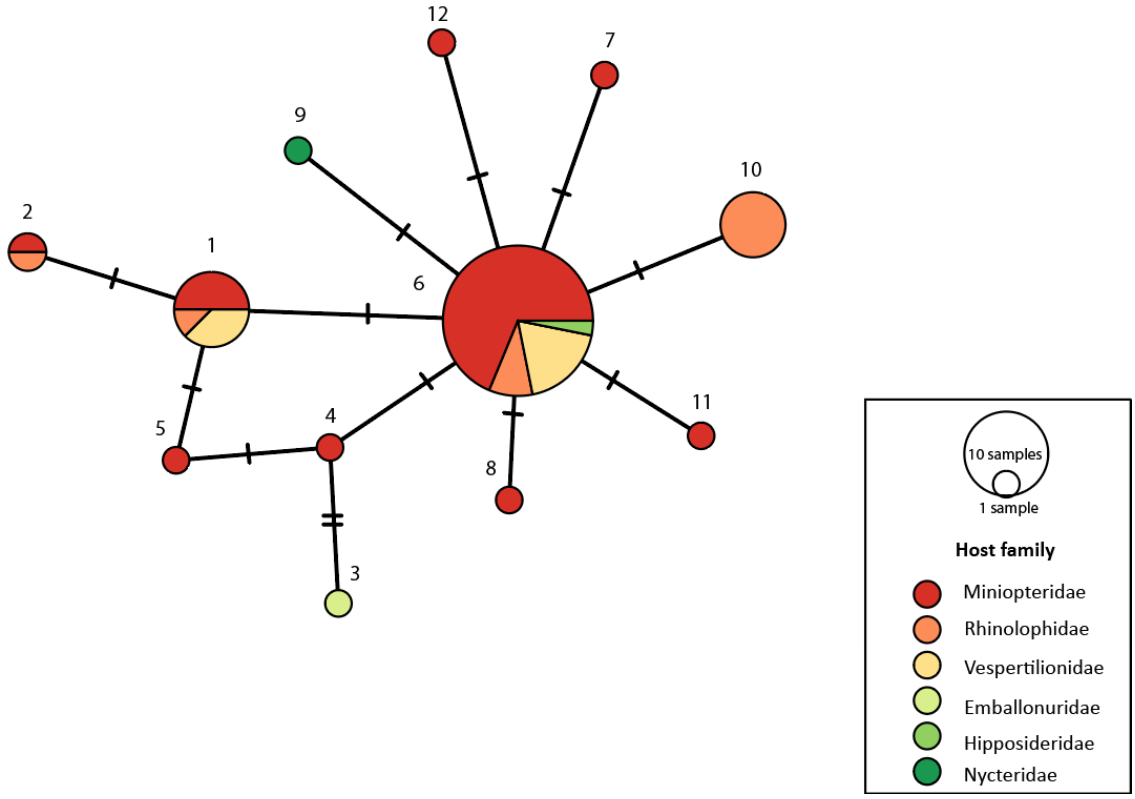

Figure 2.3. Minimum-spanning haplotype network of 12 unique *P. fulvida* COI haplotypes, allocated by host family.

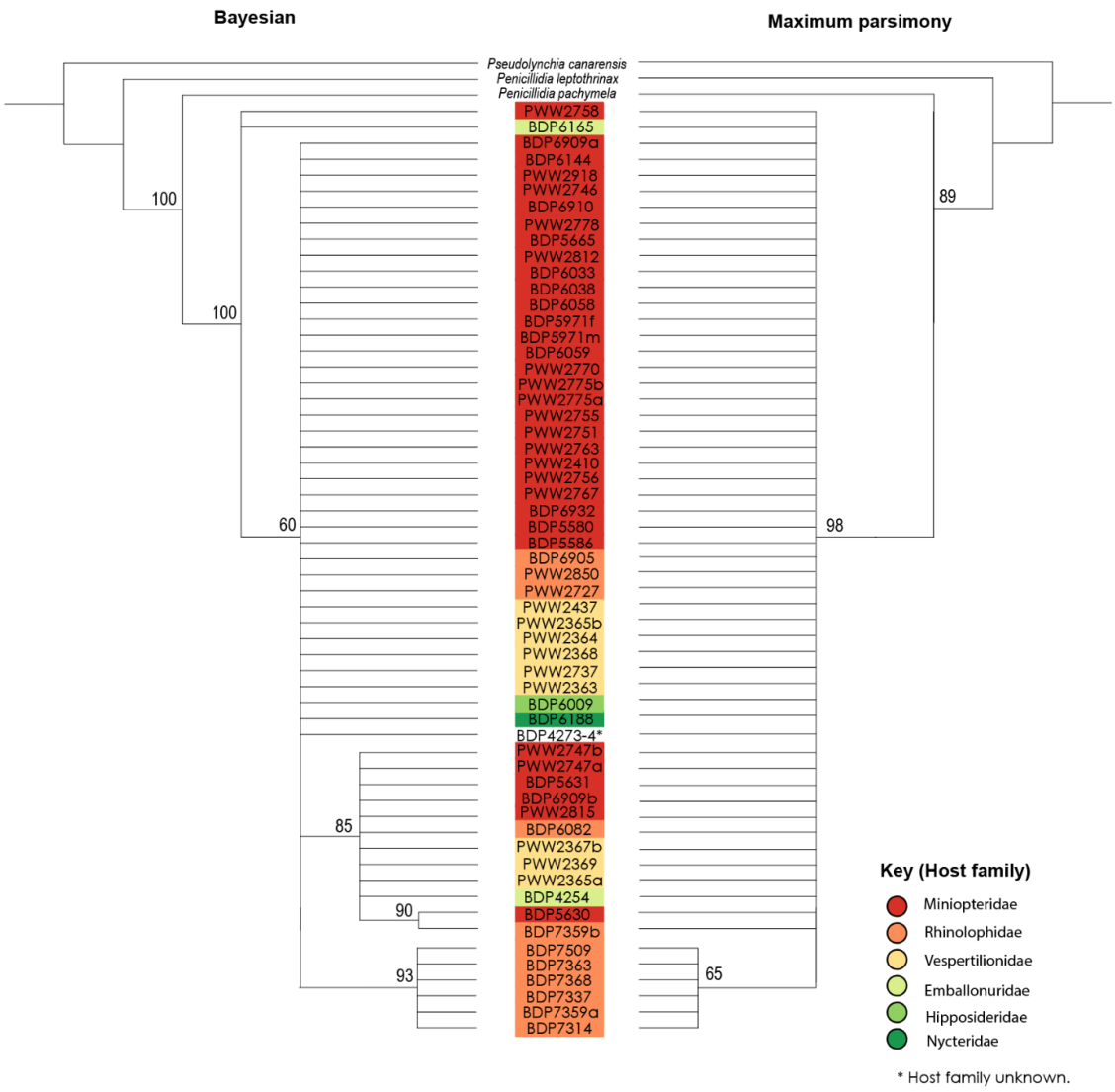

Figure 2.4. Phylogenetic trees (*P. fulvida*, congeners *P. pachymela* and *P. leptothrinax*, and outgroup *P. canarensis*) constructed using COI. Bayesian phylogeny used GTR + G substitution model and 10 million generations, and posterior probabilities for clade support are presented on a 0-100 scale for visual comparison to bootstrap values. Maximum parsimony phylogeny used 1,000 bootstrap permutations to calculate nodal support and is displayed as a majority-rules consensus tree.

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