Polyctenidae (Hemiptera: Cimicoidea) species in the Afrotropical region: Distribution, host specificity, and first insights to their molecular phylogeny

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Funding information
Tématerületi Kiválósági Program 2020, Grant/Award Number: 2020-4.1.1-TKP2020; National Research, Development and Innovation Fund of Hungary, Grant/Award Number: TKP2020-NKA-01; National Research Foundation of South Africa, Grant/ Award Number: 91496, 85756, 92524 and 98339; South African Research Chair Initiative of the Department of Science and Technology

Abstract
Polyctenidae bugs are rarely studied, hematophagous, and highly specialized ectoparasites of bats. There are only 32 described species worldwide, including six species in the Afrotropical region. Knowledge on these parasites is limited, and most studies are restricted to the New World polyctenid species. Here we report additional records of Adroctenes horvathi from Kenya and South Africa, as well as Hypoctenes faini from Rwanda. We present an updated list of published polyctenid records in the Afrotropical region indicating their host specificity and their geographical distribution. We report global infection patterns and sex ratio of polyctenids based on previously published data, including Old and New World species. Lastly, we demonstrate the first molecular phylogeny of Polyctenidae, showing their phylogenetic relationship with the closely related family Cimicidae.

KEYWORDS
bat bug, Chiroptera, Cimicidae, distribution, ectoparasite, Polyctenidae, specificity

TAXONOMY CLASSIFICATION
Entomology
INTRODUCTION

1.1 | Polycetenid diversity worldwide

Bats host a wide variety of parasites, including ectoparasitic bugs. Bat bugs (Hemiptera: Cimicidae and Polycetidae) are blood-sucking parasites, belonging to the superfamily Cimicoidea. Cimicids (especially the bed bugs, *Cimex lectularius* and *C. hemipterus*) are a well-studied parasitic group as they are a public health concern due to their vectorial potential of several diseases, including *Trypanosoma cruzi* toward humans (Delaunay et al., 2011; Salazar et al., 2015). Additionally, the ecology, distribution, and phylogeny of some cimicids species parasitizing bats, particularly *C. adjunctus*, *C. pipistrelli*, *C. lectularius*, and closely related species, are relatively well studied (Balvín et al., 2014, 2013; Bartoníčka, 2008, 2010; Hornok et al., 2018, 2021, 2017; Queglas et al., 2012; Reinhardt et al., 2007, 2008). By contrast, Polycetidae is an extremely understudied ectoparasitic family. They are represented by 32 species worldwide belonging to two subfamilies and five genera (*Adroctenes* Jordan, 1912, *Eoctenes* Kirkaldy, 1906, *Hypoctenes* Jordan, 1922, *Polycetes* Giglioli, 1864 within the Polycetinae and *Hesperoctenes* Kirkaldy, 1906 within the Hesperoctetinae).

Polycetenid subfamilies occur in different biogeographical regions. Subfamily Polycetinae only found in the Eastern Hemisphere (Africa, Asia and Australia), whereas Hesperoctetinae is restricted to the Western Hemisphere (South and North America) (Dick & Bindokas, 2007; Maa, 1964). In the Eastern Hemisphere, there are 16 species in total, out of which six species occur in the African continent. In the subfamily Polycetinae, *Eoctenes* is the most species rich genus with seven species (*E. coleurae* Maa, 1964, *E. ferrisi* Maa, 1964, *E. intermedius* Speiser, 1904, *E. maaei* Bhat, Sreenivasan and Ilkal, 1973, *E. nycteridis* (Maa, 1964) and references therein), *E. sinae* Maa (1961) and *E. spasmae* (Waterhouse, 1879)). *Eoctenes intermedius* is the most widespread species with several records from Australia, Africa, and Asia (e.g. Malaya, Philippines, Sudan, Sumatra and Thailand) (Dick & Bindokas, 2007). By contrast, *Eoctenes coleurae* and *E. nycteridis* are endemic to the African continent. Additionally, three endemic species are found in the African region which are *Adroctenes horvathi*, *Hypoctenes clarus*, and *H. faini*. The most recent records of polycetids from the African region indicate the occurrence of *Hypoctenes clarus* from Kenya, which was also a new observation to the country (Patterson et al., 2018). Nevertheless, the last polycetenid records were published nearly two decades ago from the continent (Kock et al., 1998), which suggests either biased sampling efforts, the difficulty of collecting polycetids, or possibly the rarity of these parasites.

1.2 | Phylogenetic relation with Cimicidae

The phylogenetic relationship of polycetid species with other groups has previously received little attention. It has been shown that, based on morphological characters, the phylogenetic relationship between cimicids and polycetids represents two different monophyletic groups, but molecular data were missing from polycetids (Schuh et al., 2009). Polycetids are generally excluded from molecular phylogenetic reconstruction of the superfamily Cimicoidea, due to the lack of available specimens and molecular data on these species (Jung & Lee, 2012; Roth et al., 2019; Schuh et al., 2009). Only a cytochrome c oxidase subunit 1 mitochondrial gene (COI) fragment of a North American species, *Hesperoctenes fumarius*, has been previously published (Smit & Miller, 2019). Additionally, fossil records of polycetids are not available.

1.3 | Reproduction biology of polycetids

Our knowledge about the basic biology and ecology of these bat bugs is currently based on some long-standing observational work, based on a few common species. The whole life cycle of polycetids takes place on their hosts (Jordan, 1911; Marshall, 1982a), in contrast with cimicids, which only feed on the host but lay eggs on a substrate, such as the host’s roost wall. Polycetids show strong morphological and physiological adaptation to their parasitic lifestyle; they are viviparous, dorsoventrally flattened, eyeless, and wingless, and these features might strongly affect their host specificity and abundance through limited dispersal ability.

1.4 | Host specificity and infection patterns

Previously published data have suggested that polycetids show a high specificity to their host species. Most species are described as oixenous (i.e., specific to one certain host species) and/or stenoxenous (i.e., occurring on two or more congenic host species) (Maa, 1964; Marshall, 1982a). An experimental study has shown that *Hesperoctenes fumarius*, a New World species, is able to survive and actively feed on different host species, when dispersal barriers are removed (Dick et al., 2009), although congeneric host species were used during this experiment. Overall, specificity and host preferences of polycetenid species are mostly unknown.

Limited data are available about the infection patterns, such as prevalence and abundance of polycetid species on their hosts. *Hesperoctenes fumarius* showed prevalence of 21% on *Molossus rufus* as well as intensity of infestation (mean number of bat bugs on infected hosts) of $2.22 \pm 2.86$ (Esbérard et al., 2005). Presley (2011) also reported the infection patterns of *H. fumarius* on two hosts. The prevalence of *H. fumarius* was 26.8% and 13% on *Molossus molossus* and *M. rufus*, respectively. Additionally, he observed mean abundance (mean number of bat bugs per host) of $0.5 \pm 1.14$ and $0.4 \pm 1.49$ as well as mean intensity of $2.0 \pm 1.43$ and $3.2 \pm 3.00$ on *M. molossus* and *M. rufus*, respectively (Presley, 2011). *Hesperoctenes* species tend to show sex-biased parasitism toward female bat hosts and in some cases, their abundance is affected by host morphological characters, such as body mass and/or forearm length, which may indicate the body condition of their hosts.
(Presley & Willig, 2008). Data on the sex ratio of polyctenids are scarce. Some studies reported mostly female biased sex ratio in adults, although sex ratio at emergence was unknown (Maa, 1964; Marshall, 1981, 1982a).

Our aim was to describe the specificity, sex ratio, and distributional patterns of polyctenids using published and field collected data along with specimens retrieved from museum collection, extending the current knowledge on the Polyctenidae family. Furthermore, we aimed to gain insights to the phylogenetic relationship of this family in relation to the closely related family Cimicidae, for the first time.

2 | MATERIAL AND METHODS

2.1 | Sampling and species identification

Opportunistic ectoparasite sampling was carried out by the Centre for Viral Zoonoses at University of Pretoria at several sites in South Africa, Rwanda, and Botswana. This was part of bio surveillance in both frugivorous and insectivorous bat species between 2008 and 2017. Bat species were identified based on morphological characters (Meester, 1986; Van Cakenberghe et al., 2017). Currently valid bat names are used throughout this work, whenever possible, based on batnames.org (Simmons & Cirranello, 2022). Parasites were individually placed into 70% ethanol. Voucher specimens are deposited at Museum of Zoology, Lausanne, Switzerland. Additionally, further polyctenid specimens were examined at the collection of California Academy of Sciences in San Francisco, CA (USA), and previously unpublished data were also added to this work. Morphological identifications were performed using Maa (1964) and Greenwood (1991).

2.2 | DNA extraction and molecular analyses

Polyctenid samples were extracted non-invasively (whole body), keeping whole specimens from external damage. Specimens were placed in separate tubes at 56°C for overnight digestion, using 20 μl Proteinase-K and 180 μl ATL buffer (per sample) (Qiagen). DNA was extracted using DNeasy Blood and Tissue Kits (Qiagen) based on the protocol provided by the manufacturer. We targeted the COI gene (658 bp long fragment) for the molecular analysis, and we used the following primers: Lep1F (5’-ATT CAA CCA ATC ATA AAG ATA TGG G-3’), Lep1Fdeg (5’-ATT CAA CCA ATC ATA AAG ATA TNG G-3’), and Lep3R (5’-TAT ACT TCA GGG TGT CCG AAA AAT CA-3’) (Balvin et al., 2015). Polymerase chain reaction (PCR) master mix was prepared based on previously published protocol (Hornok et al., 2017). During amplification, the following steps were used: 1 cycle of 95°C for 5 min, 40 cycles of 94°C for 40 s, 53°C for 1 min, and 72°C for 1 min. Final extension of 1 cycle of 72°C for 10 min (Veriti 96-Well Thermal Cycler, Applied Biosystems). Additionally, we targeted the 16S gene fragment (381–384 bp), with the primers 16S LR-J (5’-TTA CGC TGT TAT CCC TAA-3’) and 16S LR-N (5’-CGC CTG TTT ATC AAA AAC AT-3’) (Kambhampati & Smith, 1995; Simon et al., 1994). Fragments were amplified using PCR premix (AccuPower PCR Premix, BIONEER) under the following conditions: 1 cycle of 95°C for 5 min, 35 cycle of 95°C for 30 s, 48°C for 30 s, and 72°C for 30 s. Final extension of 1 cycle of 72°C during 5 min (Veriti 96-Well Thermal Cycler, Applied Biosystems).

Furthermore, we targeted the 18S gene fragment (1200 and 800 bp long fragments), using primer pairs 18S-1 (5’-CGT GTT GAT CCT GCC AGT AGT-3’) and 18S-3 (5’-GGT TAG AAC TAG GGC GGT ATC T-3’), and 18S-2 (5’-AGA TAC CGC CCT AGT TCT AAC C-3’) and 18S-4 (5’-GAT CCT TCT GCA GGT TCA CC-3’) (Tian et al., 2008); however, only the shorter region (800 bp) was successfully retrieved. Lastly, the 28S rRNA gene fragment was also targeted using the primers 1274 (5’-GAC CGG TCT TAA AAC ACG GA-3’) and 1275 (5’-TGG ACC GGA ACC ACC AGC TAC TA-3’) (Markmann & Tautz, 2005). Another PCR was also used targeting an approx. 700-bp-long part of the 28S rRNA gene, with the primers 28S-FF (5’-TTA CAC ACT CCT TCG CGG AT-3’) and 28S-DD (5’-GGG ACC CGT CTT GAA ACA C-3’) (Hillis & Dixon, 1991). However, the amplification and sequencing of the 28S rRNA gene of Hypoctenes faini were not successful with two different primer sets. PCR reactions of 18S and 28S amplifications were performed as reported (Hornok et al., 2021).

PCR products were visualized on 1.5% agarose gel. Biomi Ltd. and Microsynth AG performed purification and high-throughput Sanger sequencing of the PCR products.

Sequences (in the following order: 16S rRNA, COI, and 18S rRNA) were concatenated in the Geneious Prime 2019.2.3 (Kearse et al., 2012) software. The alignment of the concatenated sequences was done with MAFFT algorithm (Katoh et al., 2002). The best fitting evolutionary model was selected as general time reversible (GTR)+G+I model by MEGA 11.0.10 (Kumar et al., 2018; Tamura et al., 2021), as it takes into account most parameters. A Bayesian consensus tree was created using the MrBayes (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003) Geneious plugin, with GTR model with gamma distribution and invariant sites (GTR+G+I). The stationarity of posterior distribution was also examined using the Geneious plugin. The chain length was set to 5,000,000, sampling frequency to 500 and burn-in length to 100,000. The gene partitions were treated as unlinked. The random seed was set to 21,231. The analysis of the Bayesian tree was done with the MEGA11 11.0.10 (Kumar et al., 2018; Tamura et al., 2021) software. Distribution maps of parasites were produced by using QGIS version 2.16.2.

References sequences of A. horvathi and H. faini can be obtained in GenBank under accession numbers: ON157489–ON182061.

3 | RESULTS

3.1 | Polyctenidae collected during this study

Three polyctenids (2 female adults and 1 nymph) were found belonging to two species: Adroctenes horvathi (n = 1, female) and Hypoctenes faini (n = 2, female and nymph), from one female...
of *Rhinolophus simulator* (in South Africa, 26. 09. 2017) and one female of *Otomops martiensseni* (Rwanda, 13. 12. 2008), respectively.

Five specimens of previously unidentified and unpublished polyctenids were recorded, representing *Adroctenes horvathi* in the collection of the California Academy of Sciences in San Francisco, CA (USA). The specimens were collected by James D. Hawkins (1 female, 25. 02. 1971, Busia District, N Mambale, Kenya; 2 females, 1 male, 1 nymph, 11. 03. 1971) from *Rhinolophus* spp. We included these records in Table 1.

### 3.2 | Geographical distribution of African polyctenids

We collected distributional data of all six African polyctenid species, which have been reported from 14 countries to date (Figure 1a–f, Table 1). Our records of *A. horvathi* and *H. fainii* are the second published occurrence of these species to both Rwanda and South Africa. Finally, *H. fainii* and *A. horvathi* are reported for the first time from *Otomops martiensseni* and *Rhinolophus simulator*, respectively. We excluded records with unspecified data, when exact country was not given (e.g. “Central Africa”).

### 3.3 | Infection patterns and sex ratio in Polyctenidae

Published and new records of Polyctenidae prevalence are shown in Table 2, including Old and New World species. Altogether, records of at least 2175 screened host individuals and 1716 parasites were obtained covering broad geographic scale. Most frequently, recorded prevalence rates are known from the New World genus *Hesperoctenes*. Sex ratio is often female biased in both New and Old World species; however, there is no clear evidence for strong female biased occurrence due to low sampling effort and lack of data. In total, 645 females and 381 males were reported from previous works, indicating female biased sex ratio (Table 2).

### 3.4 | Molecular analysis of COI, 16S, 18S, and 28S rRNA gene

Based on BLAST search, for the COI gene fragment the closest match for *H. fainii* and *A. horvathi* was 83.09% *Psacasta exanthematica* (MF162983) (Scutelleridae) and 83.18% *Ceratocapsidea* (MW984087), respectively. The 16S sequences of *H. fainii* and *A. horvathi* showed the highest similarity of 84.29% *Tetraphleps aterrimus* (NC_042679) (Anthocoridae) and 83.65% *Primicipimex cavernis* (MG596876) (Cimicidae), respectively. For the 18S fragment, *H. fainii* and *A. horvathi* showed the highest similarity to 97.37% and 95.50% *Latrocimex spectans* (MZ378786) (Cimicidae), respectively. Lastly, the BLAST search of the 28S gene fragment of *A. horvathi* (28S) showed a 90.12% similarity with *Cimex lectularius* (KJ461188) (Cimicidae). Amplification and sequencing of the 28S rRNA gene of *H. fainii* were unsuccessful with two different primer sets.

Overall, within Cimicoidea, as reflected by the topology of the Bayesian tree based on three genetic markers (COI, 16S, and 18S rRNA genes) (Figure 2, Table 3), the monophyly of Cimicidae can only be maintained if it includes Polyctenidae.

### 4 | DISCUSSION

#### 4.1 | Distribution of polyctenidae in Africa

Currently, six species of polyctenids are known from the African region. *Adroctenes horvathi* has been recorded in the African continent only and has the widest distribution, being present in Eastern and Southern Africa and is the most common species among all the known African polyctenids. The primary host species of *A. horvathi* belong to the family Rhinolophidae, which are widely distributed in continental Africa and *A. horvathi* may be present in additional countries where its presence has not yet been observed.

*Eoctenes* is the most species-rich genus in Africa, with three different species. Nevertheless, *E. coleurae* seems to be the most rarely collected polyctenid species among all the African Polyctenidae as it has been recorded only once in Sudan and has not been reported since its description (Maa, 1964), making additional conclusions on its distribution problematic. Nevertheless, its host *Coleura afra* is a widely distributed species, known from several Central, Eastern, and Western African countries. Consequently, *E. coleurae* might occur within its host distribution (if *C. afra* is the main host of this species). Future studies focusing on family Emballonuridae and its parasitic fauna should give more insights to the distribution of *E. coleurae*.

*Eoctenes nycteridis* is also endemic to the African continent and has been mostly reported from the central countries with some additional records, such as Eritrea and Liberia; therefore, it is expected to occur in other regions within the distribution range of its hosts, family Nycteridae. Species belonging to family Nycteridae occur in Africa but some parts of Asia as well.

*Eoctenes intermedius* is a widely distributed species with several records from Asia (Maa, 1961, 1964, Theodor & Moscona, 1954), Australia (Maa, 1964), and Africa (Jordan, 1912; Maa, 1964; Speiser, 1904). In Africa, the species has a Northern and Central African distribution but has also been recorded once in Guinea, Western Africa (Aellen, 1956). Its hosts, *C. afra* and *Taphozous* spp., are widely distributed in Africa, *T. perforatus* occurring in several parts of Asia as well. Within its global distribution, *E. intermedius* shows a strong preference toward *Taphozous* species; therefore, its distribution is expected where these hosts occur (Maa, 1964).
| Polycetenid species | Host species (current/valid name) | Host family | Host habitat type | Country | Location | References |
|---------------------|----------------------------------|-------------|------------------|---------|----------|------------|
| *Adroctenes horvathi* Jordan (1912) | *Rhinolophus blasii* | Rhinolophidae | Caves | Malawi | Viphya Plateau | Kock et al. (1998) |
| *Adroctenes horvathi* Jordan (1912) | *Rhinolophus eloquens* | Rhinolophidae | Caves | Kenya | Mt. Elgon, Kapsakwany | Ferris and Usinger (1939), Kock et al. (1998) |
| *Rhinolophus eloquens* | *Rhinolophus eloquens* | Rhinolophidae | Caves | South Africa | Gauteng (Formerly Transvaal) | Zumpt (1966) |
| *Rhinolophus eloquens* | *Rhinolophus eloquens* | Rhinolophidae | Caves | South Sudan | Equatoria | Maa (1964) |
| *Rhinolophus fumigatus* | *Rhinolophus fumigatus* | Rhinolophidae | Caves | Malawi | Zomba | Kock et al. (1998) |
| *Rhinolophus landeri* | *Rhinolophus landeri* | Rhinolophidae | Caves | Democratic Republic of the Congo | Kasongo | Cooreman (1955) |
| *Rhinolophus simulator* | *Rhinolophus simulator* | Rhinolophidae | Caves | South Africa | Matlapits cave, GaMafefe, Limpopo Province | This study |
| *Rhinolophus sp.* | *Rhinolophus sp.* | Rhinolophidae | Caves | South Sudan | Torit | Maa (1964) |
| *Rhinolophus sp.* | *Rhinolophus sp.* | Rhinolophidae | Caves | Kenya | N Mambale | James D. Hawkins, Unpublished record (California Academy of Sciences, CA, USA) |
| unknown | unknown | – | – | Somalia | Upper Sheika | Jordan (1912) |
| *Eoctenes coleurae* Maa (1964) | *Coleura afra* | Emballonuridae | Underground sites including caves | Sudan | | Maa (1964) |
| *Eoctenes intermedius* Speiser (1904) | *Coleura afra* | Emballonuridae | Underground sites including caves | Guinea | | Aellen (1956) |
| *Eoctenes intermedius* Speiser (1904) | *Taphozous mauritianus* | Emballonuridae | Mixed (no caves mentioned) | Democratic Republic of the Congo | | Cooreman (1951) |
| *Eoctenes intermedius* Speiser (1904) | *Taphozous perforatus* | Emballonuridae | Underground sites including caves | Democratic Republic of the Congo | grotte Dethioux (Kataga) | Anciaux de Faveaux (1965), Benoit (1958); Leleup (1956) |
| *Eoctenes intermedius* Speiser (1904) | *Taphozous perforatus* | Emballonuridae | Underground sites including caves | Egypt | Luxor, Abu Rawash, Cairo | Maa (1961, 1964), Speiser (1904) |
| unknown host | unknown host | – | – | Sudan | | Jordan (1912), Kellogg & Paine (1911) |
| Polycetenid species | Host species (current/valid name) | Host family | Host habitat type | Country       | Location                              | References                                       |
|---------------------|-----------------------------------|-------------|------------------|---------------|---------------------------------------|-------------------------------------------------|
| Eoctenes ncteridis  | *Nycteris arge*                   | Nycteridae  | Mixed (no caves mentioned) | Liberia       |                                       | Ferris and Usinger (1939)                        |
|                     | *Nycteris grandis*                | Nycteridae  | Mixed (no caves mentioned) | Congo         |                                       | Cooreman (1951)                                 |
|                     | *Nycteris hispida*                | Nycteridae  | Underground sites including caves | Tanzania     | Victoria Nyanza, Shirati              | Maa (1964) and references therein               |
|                     |                                   |             |                  |               |                                       | Benoit (1958)                                   |
|                     | *Nycteris hispida*                | Nycteridae  | Underground sites including caves | Rwanda       |                                       |                                                 |
|                     | *Nycteris macrotis*               | Nycteridae  | Underground sites including caves | Democratic Republic of the Congo | Katanga              | Anciaux de Faveaux (1965), Benoit (1958), Maa (1964) |
|                     | *Nycteris thebaica*               | Nycteridae  | Mixed but also caves | Rwanda       |                                       | Benoit (1958)                                   |
|                     | Unknown host                       |             |                  | Democratic Republic of the Congo | Katanga              | Anciaux de Faveaux (1965)                      |
|                     | Unknown host                       |             |                  | Rwanda       |                                       |                                                 |
|                     |                                       |             |                  | Eritrea      | Sembel                                | Maa (1961, 1964)                                |
|                     |                                       |             |                  | Uganda       |                                       | Jordan (1912), Maa (1964)                       |
| Hypoctenes clarus   | *Chaerephon pumilus* (currently *Mops pumilus*) | Molossidae  | Mixed (no caves mentioned) | Congo |                                       | Benoit (1958)                                   |
|                     | *Mops thersites*                  | Molossidae  | Mixed (no caves mentioned) | Cameroon      |                                       | Jordan (1922)                                   |
|                     | *Mops thersites*                  | Molossidae  | Mixed (no caves mentioned) | Ghana         | Eastern Region                        | Maa (1970)                                      |
|                     | *Otomops harrisoni*               | Molossidae  | Caves            | Kenya         |                                       | Patterson et al. (2018)                         |
| Hypoctenes faini    | *Chaerephon pumilus* (currently *Mops pumilus*) | Molossidae  | Mixed (no caves mentioned) | Kenya         | Lake Naivasha                         | Greenwood (1991)                                |
|                     | *Otomops martiensseni*            | Molossidae  | Underground sites including caves | Rwanda     | Ruhengeri                            | This study                                      |
|                     | *Tadarida fulminans*              | Molossidae  | Underground sites including caves | Rwanda     |                                       | Benoit (1958)                                   |
The genus *Hypoctenes* includes two species, *H. clarus* and *H. faini* exclusively found in the African continent. The African representatives of this genus are rarely collected, and records seem to be limited in a relatively narrow distribution, when compared to other species in the family. *Hypoctenes clarus* has been reported from Cameroon, Democratic Republic of Congo, Ghana, and Kenya (Benoit, 1958; Jordan, 1922; Maa, 1970; Patterson et al., 2018). It might have additional populations in other regions where host species are distributed. Family Molossidae is one of the most species rich bat families occurring in all continents (except Antarctica) (Ammerman et al., 2012). *Hypoctenes clarus* and *H. faini* are known to occur on the members of this family but reports are scarce.

*Hypoctenes faini* is also a rarely observed species, with only two published records, representing two specimens (Benoit, 1958; Greenwood, 1991). During our work, two specimens of *H. faini* have been found in Rwanda for the second time (Figure 1). It might be expected from additional countries where its potential hosts from the Molossidae family are present. *Otomops martiensseni*, which we recorded in Rwanda as host species, occurs mainly in Central Africa but has populations in the southern and western part of the continent; therefore, the occurrence of *H. faini* is possible in these areas.

### 4.2 | Host specificity

Based on literature and field collected data, all polyctenid species appeared to be oligoxenous, meaning that they occur on two or more congeneric host species. However, the number of sampled individuals is low and conclusions cannot be drawn on the preferred host species, if any. Nevertheless, all polyctenid species exclusively occur on the members of a single bat family. The level of dispersal ability of polyctenids is unknown, although Marshall (1981) stated that biased sex ratio occurs in polyctenids due to males being the more mobile sex (Marshall, 1981), which could affect their dispersal ability and their specificity. Phylogenetic specificity (rather than ecological specificity) is supported by the fact that some host species often form mixed colonies with bats belonging to different families, which are not known as polyctenid hosts (McDonald et al., 1990; van der Merwe 1987). In conclusion, dispersal barriers do not likely influence polyctenid host specificity.

**FIGURE 1** Distribution of Polyctenidae species in the African countries. Collection sites (whenever known) are indicated with black stars. *Adroctenes horvathi* (a), *Eoctenes coleurae* (b), *E. intermedius* (c), *E. nycteridis* (d), *Hypoctenes clarus* (e), and *H. faini* (f).
| Parasite species       | Host species                               | Hosts screened | Infected hosts (n) | Parasites (n) | Prevalence (%) | Female (n) | Male (n) | Nymph (n) | Biased sex ratio | Location                        | References                     |
|-----------------------|--------------------------------------------|----------------|-------------------|---------------|----------------|-------------|-----------|-----------|------------------|-------------------------------|--------------------------------|
| Adroctenes horvathi   | Rhinolophus spp. (+unknown host species)   |                | 19                |               | 13             | 2           | 4         |           | Female           | Africa (various countries)    | Maa (1964) and references therein |
|                       | Rhinolophus simulator                      | 41             | 1                 | 1             | 2.4            | 1           | 0         | 0         |                  | South Africa                  | This study                     |
| Eoctenes coleurae     | Coleura afra                               |                | 4                 |               | 2              | 1           | 1         |           |                  | Sudan                         | Maa (1964)                     |
| Eoctenes intermedius  | Taphozous spp.                             |                | 44                |               | 25             | 13          | 6         |           | Female           | Australia, Asia, Africa        | Maa (1964) and references therein |
| Eoctenes nycerisidis  | Nycteris spp.                              |                | 26                |               | 14             | 1           | 11        |           |                  | Africa (various countries)    | Maa (1964) and references therein |
| Eoctenes spasmae      | Megaderma spasma                           | 27             | 23                | 370           | 85.2           | 241         | 129       | –          | Female           | Malaysia                      | Marshall (1982a)               |
|                       | Megaderma spasma (+unknown)                |                | 102               |               | 51             | 27          | 24        |           | Female           | Asia (various countries)       | Maa (1964) and references therein |
|                       | Megaderma spasma                           |                | –                 |               | –              | –           | 0         |           |                  | Philippines                   | Amarga & Yap (2017)            |
| Hesperoctenes angustatus | Molossus molossus                         | 20             | 1                 | 1             | 5              | –           | –         | –          |                  | Peru                          | Bonifaz et al. (2020)          |
| Hesperoctenes cartus  | Cynomops planirostris and C. abrasis       |                | 13                |               | 13             | 13         |           |           |                  | Argentina                     | Autino et al. (2020)           |
| Hesperoctenes fumarius| Molossidae/emballonuridae/mormoopidae      |                | 148               |               | 45             | 53          | 50        |           |                  | South-America (various countries) | Ueshima (1972)               |
| Hesperoctenes fumarius| Molossus rufus                             | 762            | 161               | 387           | 21             | –           | –         | –          | –                | Brazil                        | Esbérard et al. (2005)         |
|                       | Molossus molossus                          | 228            | 70                |               | 26.8          | –           | –         | –          | –                | Paraguay                      | Presley (2011)                 |
|                       | Molossus rufus                             | 100            | 27                |               | 13             | –           | –         | –          | –                | Paraguay                      | Presley (2011)                 |
|                       | Molossus molossus                          | 228            | 62                | 106           | 27.1           | 31          | 28        | 47         | No               | Paraguay                      | Presley (2012)                 |
|                       | Molossus bondae                            | –              | 71                |               | 3              | –           | –         | –          | –                | Columbia                      | Marinkele & Grose (1979)       |
|                       | Molossus molossus                          | 3              | –                 | 6             | –              | 6           | 0         |           |                  | Lesser Antilles               | Smit & Miller (2019)           |
| Hesperoctenes longiceps | Eumops patagonicus                         | 526            | 89                | 135           | 16.9           | 52          | 33        | 50         | Female           | Paraguay                      | Presley (2012)                 |
| Hesperoctenes parvulus | Molossops temminckii                       | 160            | 30                | 41            | 18.7           | 11          | 10        | 20         |                  | Paraguay                      | Presley (2012)                 |
| Parasite species       | Host species                  | Hosts screened | Infected hosts (n) | Parasites (n) | Prevalence (%) | Female (n) | Male (n) | Nymph (n) | Biased sex ratio | Location | References                          |
|-----------------------|-------------------------------|----------------|--------------------|---------------|----------------|-------------|----------|-----------|-------------------|----------|-------------------------------------|
| Hesperoctenes vicinus | Molossops temminckii         | –              | 1                  | 1             | –              | –           | 1        | 0         | –                 | Argentina| Autino et al. (2020)               |
| Hesperoctenes sp.     | Eumops glaucinus              | 56             | 24                 | 136           | 42.8           | 54          | 35       | 47        | Female           | Paraguay| Presley (2012)                     |
|                       | Molossus molossus             | 3              | 1                  | 3             | 33             | –           | –        | –         | –                 | Colombia| Calonge-Camargo & Pérez-Torres (2018) |
| Hesperoctenes spp.    | Molossidae/emballonuridae/mormoopidae | –          | –                  | 84            | –              | 46          | 14       | 24        | Female           | South-America (various countries) | Ueshima (1972) |
| Hypoctenes clarus     | Otomops harrisoni             | 20             | 5                  | 5             | 25             | 2           | 3        | 0         | –                 | Kenya   | Patterson et al. (2018)            |
|                       | Tadarida spp.                 | –              | –                  | 3             | –              | 2           | 1        | 0         | –                 | Africa (various countries) | Maa (1964) and references therein |
|                       | Tadarida thersites (currently Mops thersites) | –          | –                  | 12            | –              | 8           | 1        | 3         | Female           | Ghana   | Maa (1970)                         |
| Hypoctenes faini      | Tadarida fulminans            | –              | –                  | 1             | –              | 1           | 0        | 0         | –                 | Rwanda  | Maa (1964) and references therein |
|                       | Otomops martiensensi         | 1              | 1                  | 1             | (100)          | 1           | 0        | 0         | –                 | Rwanda  | This study                          |
| Hypoctenes hutsoni    | Tadarida pusillus (currently Mops pusillus) (+unknown) | –              | –                  | 23            | –              | 10          | 10       | 3         | No                | Seychelles| Maa (1970)                        |
| Polycnenis molossus   | Megaderma lyra (currently Lyroderma lyra) (+unknown) | –              | –                  | 16            | –              | 8           | 2        | 6         | Female           | Asia (various countries) | Maa (1964) and references therein |

Note: African species are highlighted in bold.
Common characteristics of polyctenid hosts include insectivore behavior; however, emballonurids occasionally consume fruits. Infected bat species mostly roost in underground places, such as caves. The microclimate of these roosts might be preferred or required by polyctenids.

4.3 | Sex ratio and infection patterns

Biased sex ratio in ectoparasitic insects is common and has been explored in the case of bat-associated parasites (Dick & Patterson, 2008; Dittmar et al., 2011; Szentiványi et al., 2017). Several factors may cause biased sex ratio, such as difference between body size, mobility, dispersal ability between sexes, or the presence of reproduction manipulating bacteria or inbreeding (Dick & Patterson, 2008; Duron et al., 2008; Patterson et al., 2008; Szentiványi et al., 2017). We found some evidence of female biased sex ratio in polyctenid bat bugs, similarly to previous suggestion (Marshall, 1982a). Overall, it is currently unknown if polyctenid bat bugs show biased sex ratio at birth, such as in the case of bat flies (Dittmar et al., 2011), or if female biased sex ratio occurs a later life stage. If natural polyctenid populations are truly female biased, some scenarios (or combinations of them) might explain this phenomenon. Local mate competition (LMC) could be one explanation. LMC results a female biased sex ratio in parasite populations, due to dispersal limited, isolated, and inbred populations, which could all be true in the case of polyctenids. LMC implies a female biased sex ratio, since males compete for mating opportunities, and mothers try to decrease sexual competition by maximizing female success through reducing the number of male offspring (Hamilton, 1967). Marshall (1982a, 1982b) suggested that biased sex ratio occurs because males are more active than females and therefore more exposed to predation by their hosts (Marshall, 1981, 1982a). Additionally, if there is different mobility and dispersal ability between sexes, it might also affect the capture success and thus implies a apparent bias in sex ratio. Furthermore, different longevity between females and males might also strongly influence sex ratio. Dispersal ability and mobility differences between female and male polyctenids are currently unknown on their hosts; however, off-host both sexes are incapable of moving (Marshall, 1982a). Additionally, Wolbachia, which is a genus of Gram-negative bacteria known to be able to alter sex ratios, has been found at least in one polyctenid species, Hesperoctenes fumarius (Sakamoto et al., 2006), and is common in other bat ectoparasites (Morse et al., 2012; Wilkinson et al., 2016). Nevertheless, there is a lack of evidence if they occur in a wide range of polyctenid species, and if they affect their reproduction. Future studies should address polyctenid sex ratios and their driving factors. Prevalence of polyctenids shows a wide variation on their hosts, ranging from 2.4% to 85.2%. We currently have little understanding on what affects prevalence of these ectoparasites, although it is likely shaped by several factors, such as host availability, dispersal ability, seasonality, and population dynamics of each species. Furthermore, data on potential host sex bias are not available or scarce; however, one study found equal infection between female and male hosts (Marshall, 1982b). Prevalence and infection pattern between host sexes need to be explored in future studies.

4.4 | Phylogenetic relationship of Polyctenidae

Previous phylogenetic trees involving Polyctenidae were based on morphological data (Schuh et al., 2009). Our genetic analysis placed Primicimicinae (Primicimex and Bucimex) to the base of the tree. Polyctenid species cluster close to Primicimicinae, forming a separate clade at the base of Cimicidae. Based on these results, Polycteninae is a sister clade to Primicimicinae. Subfamily Cacodminae also shows monophyly, with two separated clusters for the genus Cimex encompassing the genus Paracimex, which supports previous findings (Balvin et al., 2015; Roth et al., 2019). Additionally, the 18S sequence of H. faini was only 93.5% identical to Curalium cronini (EU683128) suggesting that Curaliidae is not a sister group of Polyctenidae (unlike in Schuh et al. (2009); figure 10). Until today, there is a single 18S sequence available for Curalium cronini, representing family Curaliidae, and further conclusion cannot be drawn regarding its relationship to the Polyctenidae family.

Based on previous works, we expected Polyctenidae and Cimicidae to be two separate monophyletic group on their own; our results strongly suggest that the monophyly of Cimicidae can only be maintained if it includes Polyctenidae. However future studies including more polyctenid species are needed to draw final conclusions. Overall, family Polyctenidae (or subfamily Polycteninae) may be considered as a subfamily of Cimicidae.

4.5 | Potential as vectors

Polyctenidae have not been identified as vectors of any pathogens. However, they may have a potential role in disease transmission. Closely related bat bug species belonging to family Cimicidae are competent or suspected vectors of several pathogens, such as Trypanosoma, Bartonella, and Kaeng Khoi virus (Gardner & Molyneux, 1988; Reeves et al., 2005; Salazar et al., 2015; Van Den Berghe et al., 1963; Williams et al., 1976). The vector of Nycteris (Haemoporsid) parasites, which have been shown to infect, e.g., Rhinolophidae and Nycteridae species (Schaer et al., 2015), is not known and as some polyctenids parasitize these families, it is possible that they play a vectorial role in Nycteris transmission.
| Species                  | Host species | Host family | Country | COI          | 16S          | 18S          |
|--------------------------|--------------|-------------|---------|--------------|--------------|--------------|
| Acanthocrios furnarii    | Furnarius rufus | Furnariidae | Brazil  | MG596830     | MG596866     | MG978385     |
| Adroctenes horvathi      | Rhinolophus simulator | Vespertilionidae | South Africa | This study | This study | This study |
| Afrocimex constrictus    | Rousettus aegyptiacus | Pteropodidae | Kenya    | MG596840     | MG596842     | MG978385     |
| Aphrania barys           | Neoromicia capensis | Vespertilionidae | Namibia  | MG596820     | MG596847     | MG978385     |
| Aphrania elongata        | Neoromicia capensis | Vespertilionidae | South Africa | MG596825     | MG596861     | MG978385     |
| Aphrania recta           | Pteropodidae   | Pteropodidae | Kenya    | MG596816     | MG596863     | MG978385     |
| Bucimex chilensis        | Unknown        | Unknown     | Chile    | MG596840     | MG596842     | MG978385     |
| Cacodmus sparsilis       | Pipistrellus dhofarensis | Vespertilionidae | Oman    | MG596813     | MG596849     | MG978385     |
| Cacodmus vicinus         | Pipistrellus sp. | Vespertilionidae | Spain    | MG596814     | MG596853     | MG978385     |
| Cimex adjunctus          | Myotis cf. alcathoe | Vespertilionidae | Bulgaria | MG596837     | MG596874     | MG978385     |
| Cimex emarginatus        | Myotis cf. alcathoe | Vespertilionidae | Canada  | MF609526     | MF609517     | MG978385     |
| Cimex hirundinis         | Delichon urbica | Hirundinidae | Switzerland | MG596864     | MG596846     | MG978385     |
| Cimex kripkei            | Myotis rufus | Vespertilionidae | Chile    | MG596818     | MG596848     | MG978385     |
| Cimex latipes            | Myotis lucifugus | Vespertilionidae | Canada  | MG596834     | MG596873     | MG978385     |
| Species                  | Host group | Host species       | Host family   | Country | COI       | 16S       | 18S       |
|-------------------------|------------|--------------------|---------------|---------|-----------|-----------|-----------|
| Cimex latipennis        | Bat        | Myotis volans      | Vespertilionidae | Canada  | KF018757  | KF018733  | KF018719  |
| Cimex lectularius       | Human      | Homo sapiens       | Hominidae     | Czechia | GU985524  | GU985546  | GU985563  |
| Cimex lectularius       | Human      | Homo sapiens       | Hominidae     | UK      | MG596836  | MG596873  | MG978394  |
| Cimex pipistrelli       | Bat        | Pipistrellus sp.   | Vespertilionidae | UK      | GU985534  | GU985556  | MG978393  |
| Cimex pipistrelli       | Bat        | Chiroptera         | -             | Spain   | MG596835  | MG596872  | MG978392  |
| Cimex vicarius          | Bird       | Petrochelidon pyrhorota | Hirundinidae | USA     | GU985541  | GU985563  | MG978407  |
| Cyanolicimex patagonicus| Bird       | Cyanoliseus patagonus | Psittidae     | Argentina | MG596833  | MG596869  | MG978388  |
| Haematosiphon inodoros  | Bird       | Falco mexicanus (nest) | Falconidae | USA     | MG596829  | MG596865  | MG978384  |
| Hypoctenes faini        | Bat        | Otomops martienensi | Molossidae     | Rwanda  | This study | This study | This study |
| Latrocimex spectans     | Bat        | Noctilio leporinus | Noctidae      | Belize  | MW269881  | MW270938  | MZ87876  |
| Leptocimex duplicatus   | -          | Unknown            | -             | Israel  | MG596810  | MG596847  | MG978365  |
| Ornithocoris pallidus   | Bird       | Delichon urbicum (nest) | Hirundinidae | USA     | MG596827  | MG596863  | MG978382  |
| Ornithocoris pallidus   | Bird       | Delichon urbicum (nest) | Hirundinidae | USA     | MG596828  | MG596864  | MG978383  |
| Paracimex avium         | Bird       | Aerodramus salanganus | Apodidae     | Indonesia | MG596807  | MG596844  | MG978360  |
| Paracimex cf chaeturus  | Bird       | Aerodramus brevirostris | Apodidae     | China   | MF680531  | MF680520  | MG978362  |
| Paracimex setosus       | Bird       | Aerodromus sp.     | Apodidae     | Malaysia | KFO18761  | KFO18735  | KFO18721  |
| Primicimex cavernis     | Bat        | Tadarida brasiliensis | Molossidae | Mexico  | MG596839  | MG596876  | MG978398  |
| Psitticimex urutui      | Bird       | Myiopsitta monachus | Psittacidae   | Argentina | MG596831  | MG596867  | MG978386  |
| Stricticimex namru      | Bat        | mixed species bat colony | -         | Iran    | MG596811  | MG596848  | MG978366  |
| Stricticimex sp.        | Bat        | Nyctinomus thomasi (currently Tadarida aegyptiaca) | Molossidae | Oman    | MG596817  | MG596853  | MG978372  |
| Synxenoderus comosus    | Bird       | Aeronautes saxatalis (nest) | Apodidae     | USA     | MG596832  | MG596868  | MG978387  |
| Amphareus obscurecens   | Outgroup   | Anthocoridae       | Anthocoridae | USA     | GQ92178   | GQ258358  | GQ258393  |
| Anthocoris confusus     | Outgroup   | Anthocoridae       | Anthocoridae | USA     | KM022525  | GQ258359  | GQ258401  |
| Blaptostethus aurivilus | Outgroup   | Anthocoridae       | Anthocoridae | USA     | KF36463   | GQ258388  | GQ258400  |
| Buchananiella crassicornis | Outgroup | Anthocoridae     | Anthocoridae | USA     | GQ92145   | GQ258364  | GQ258407  |
| Capsus ater             | Outgroup   | Miridae            | Miridae       | USA     | AY252977  | AY252712  | EU683117  |
| Dysepicritus rufescens  | Outgroup   | Anthocoridae       | Anthocoridae | USA     | GQ92210   | GQ258386  | GQ258399  |
| Eteoneus angulatus      | Outgroup   | Tingidae           | Tingidae      | USA     | EF523481  | EF487290  | EF487311  |
| Himacerus apterus       | Outgroup   | Nabidae            | Nabidae       | USA     | KR034788  | GQ258381  | GQ258425  |
| Lasiocclus japonicus    | Outgroup   | Anthocoridae       | Anthocoridae | USA     | GQ92187   | GQ258367  | GQ258410  |
| Loricula elegantula    | Outgroup   | Microphysidae      | Microphysidae | USA     | KM022867  | EU683098  | EU683151  |
AUTHOR CONTRIBUTIONS

Tamara Szentiványi: Conceptualization (equal); data curation (equal); formal analysis (equal); investigation (equal); methodology (equal); project administration (equal); resources (equal); supervision (equal); visualization (equal); writing – original draft (equal); writing – review and editing (equal). Sándor Hornok: Conceptualization (equal); data curation (equal); formal analysis (equal); investigation (equal); methodology (equal); supervision (equal); writing – original draft (equal); writing – review and editing (equal). Áron Botond Kovács: Formal analysis (equal); methodology (equal); writing – review and editing (equal). Nóra Takács: Methodology (equal); writing – review and editing (equal). Miklós Gyuranecz: Formal analysis (equal); software (equal); writing – review and editing (equal). Wanda Markotter: Data curation (equal); funding acquisition (equal); investigation (equal); supervision (equal); writing – review and editing (equal). Philippe Christe: Conceptualization (equal); project administration (equal); supervision (equal); validation (equal); writing – original draft (equal); writing – review and editing (equal). Olivier Glaizot: Conceptualization (equal); project administration (equal); supervision (equal); validation (equal); writing – original draft (equal); writing – review and editing (equal).

ACKNOWLEDGMENTS

First and foremost, we are grateful to Malcolm Greenwood and David Happold, who kindly offered their suggestions and literature copies during the preparation of this work. We are grateful to Mine Altinli who gave suggestions to improve the phylogenetic analysis. In addition, we are thankful to Christopher Grinter who helped to access the undetermined polycentrid species in the collection of the California Academy of Sciences. We are grateful to the reviewers for their insights and for their constructive comments. The authors acknowledge the contributions of staff and students from the Biosurveillance and Ecology of Emerging Zoonoses Research Group, Centre for Viral Zoonoses, University of Pretoria, and the Centre for Emerging Zoonotic and Parasitic Diseases, National Institute of Communicable Diseases, AfricanBats, Ditsong National Museum of Natural History and Rwanda Tourism and National Park’s authority for field work associated with this study. Funding was obtained from the South African Research Chair Initiative of the Department of Science and Technology and National Research Foundation of South Africa, UID 98339, 92524, 85756, and 91496 (WM). Molecular laboratory work at the Department of Parasitology and Zoology, University of Veterinary Medicine, Budapest was funded by Project no. TKP2020-NKA-01 implemented with the support provided from the National Research, Development and Innovation Fund of Hungary, financed under the "Tématerületi Kiválósági Program 2020" (2020-4.1.1-TKP2020) funding scheme (SH, NT).

CONFLICT OF INTEREST

The authors declare no competing interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in GenBank at ncbi.nlm.nih.gov/genbank/, reference number ON157489 - ON182061.
ETHICS APPROVAL

Ethical approval was obtained from the University of Pretoria (Pretoria, South Africa; EC054-14) and Research was performed under Section 20 approval of the Department of Agriculture, Land Reform and Rural Development, South Africa.

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How to cite this article: Szentiványi, T., Hornok, S., Kovács, Á., Takács, N., Gyuranecz, M., Markotter, W., Christe, P., & Glaizot, O. (2022). Polyctenidae (Hemiptera: Cimicoidea) species in the Afrotropical region: Distribution, host specificity, and first insights to their molecular phylogeny. Ecology and Evolution, 12, e9357. https://doi.org/10.1002/ece3.9357