A cross-sectional study of malaria transmission in suggests the existence of a potential bridge vector susceptible of ensuring the transfer of simian malaria parasites to humans

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Introduction: Despite all the efforts made to control or even eliminate malaria, the disease continues to claim the highest number of victims of vector-borne pathogens in the world and Sub-Saharan countries bear the heaviest burden. The lack of knowledge of the role of various protagonists involved in the transmission of this parasitic disease, such as mosquito vectors and the plasmodial species they transmit as well as the host species they infect in a locality, constitutes one of the main causes of the persistence of malaria. In Gabon, in several areas, entomological data on malaria transmission remain poorly known. Thus, this study aimed to determine the diversity of *Anopheles* involved in malaria transmission in different environments of the province of Nyanga in southwest Gabon.

Methods: For this, an entomological study was carried out in the four main localities of the province of Nyanga to provide answers to these shortcomings. Mosquitoes were collected over several nights using the human landing catch method. The identification of *Anopheles* and malaria parasites circulating in the different sites was achieved by combining morphological and molecular analysis tools.

Results: A total of five hundred and ninety-one (591) mosquitoes belonging to the *Culicidae* family were collected. From this collection of adult mosquitoes, nine species of *Anopheles* mosquitoes notably species of the *Anopheles nili* complex (53.46%) followed by those of *Anopheles gambiae* complex (22.01%), *Anopheles funestus* group (18.24%), *Anopheles moucheti* complex (5.66%) and *Anopheles hancocki* (0.63%). Approximately 18 percent of these *Anopheles* species were infected with *Plasmodium* spp. *Anopheles funestus*, known to be involved in malaria transmission to humans, and *An. moucheti*-like, recently discovered in Gabon, and whose status in *Plasmodium* transmission is not yet elucidated, were found to be infected with great ape *Plasmodium*.

Discussion: Our results raise the question of the potential switch of simian malaria parasites to humans. If these observations are confirmed in the future, and the infective capacity of the bridge vectors is demonstrated, this new situation could ultimately constitute an obstacle to progress in the fight against malaria.

Introduction

Malaria is the most important parasitic disease based on its impact on human populations. This disease is caused by parasites belonging to the *Plasmodium* genus and remains most pronounced in many parts of the world, in particular in Sub-Saharan Africa. Indeed, this region of the world accounts for more than 95% of all malaria deaths, and nearly 80% of deaths are among children under 5 years old (WHO, 2020, 2022). Currently, six species are known to infect humans (*Plasmodium falciparum, Plasmodium malariae, Plasmodium ovale curtisi, Plasmodium ovale wallikiri, Plasmodium vivax,* and *Plasmodium knowlesi*) (Lalremruata et al., 2017; Rougeron et al., 2022). In fact, malaria is endemic in the majority of countries in Central Africa. The situation concerning the infection varies from country to country (Gemperli et al., 2006). For example, in Cameroon, the prevalence of malaria in humans is 30% (Eleonore et al., 2020) whereas in Central African Republic, it is between 50–60% (Nzoumbou-Boko et al., 2020) and in Congo, the infection level is 52.8% (Mbouloungou et al., 2019; Mayengue et al., 2020).

Malaria remains a major public health problem in Gabon. It is the main cause of medical consultations and hospitalizations (Assele et al., 2015; Manego et al., 2017). Gabon is located in a hyper-endemic area and malaria risk is present throughout the country (Maghendji-Nzondo et al., 2016). Malaria transmission is perennial with high transmission occurring throughout the year. Available malaria data show that the prevalence ranging from 20–34% in urban areas (Maghendji-Nzondo et al., 2016; Larson-Boundenga et al., 2023; Lendongo-Wombo et al., 2023) and between 47–55% in rural areas (Assele et al., 2015; Pegha-Moukandja et al., 2016). More than 90% of malaria cases are due to *P. falciparum* (Maghendji-Nzondo et al., 2016). Moreover, multidrug-resistant *P. falciparum* malaria has long been present and documented in several areas in Gabon (Pradines et al., 1998; Ndong et al., 2003; Boukoumba et al., 2021). Recently, a study carried out in departmental capitals of the province of Nyanga revealed a high prevalence of malaria (13.9%) in asymptomatic people, however, data on the vectors involved in the *Plasmodium* transmission in this province are not available or fragmentary (Imboumy-Limoukou et al., 2020) and dating back several decades (Galliard, 1932).

In the light of these epidemiological data, malaria situations present particular modalities that could be explained by differences related to the biotope, the diversity of vertebrate hosts, the mosquitoes of the genus *Anopheles* and the parasites they transmit and also the dynamics of their interrelationships (Carnevale et al., 1984; Marsh and Snow, 1997; Mouchet, 2004; Acharya et al., 2017). Of the 131 *Anopheles* species found in tropical Africa, less than 10% are known to transmit *Plasmodium* (Sinka et al., 2012; Coetzee, 2020). In Gabon, around 41 *Anopheles* species have been recorded, however, among all species identified in different areas of the country, only some are known for their role in *Plasmodium* transmission, with *Anopheles funestus* and *Anopheles gambiae* known as the main vectors (Longo-Pendy et al., 2022). In addition, recent studies have revealed that some

opportunistic anopheline mosquitoes could play the role of bridge vectors (e.g., *Anopheles moucheti, Anopheles vinckei*) and promote the exchange of *Plasmodium* between humans and primates (Paupy et al., 2013; Makanga et al., 2016). Moreover, the fact that the vectors responsible for the transmission of malaria are found in different ecosystems (forest, rural, and urban) (Longo-Pendy et al., 2022) could be a limitation for all control strategies but it could also enhance the risk of parasites exchange between humans and animals in the rural and forest environment.

We think that a potential risk scenario for the future would be the ability of ape's malaria parasite species to infect humans like was observed with *P. knowlesi* in Southeast Asia (Setiadi et al., 2016). Thus, a better understanding of the evolution of these different factors involved in malaria transmission is needed through entomological and parasitological monitoring which could provide more information on the ecology of malaria vectors species and their ability to switch hosts and to be infected with and potentially transmit the ape's *Plasmodium* species in a specific area. In this article, we present the results of a cross-sectional entomological survey carried out for the first time in the four main localities of the Nyanga Province in southwestern Gabon in Central Africa to characterize the *Anopheles* species and to identify which of these species are involved in *Plasmodium* transmission to humans.

Materials and methods

Study sites

Bordered in the North-East by the province of Ngounié, and in the North-West by the province of Ogooué-Maritime, the province of Nyanga is a region that covers an area of 21,285 km² (Figure 1; Matoumba, 2011). This province is home to the Moukalaba-Doudou protected area, one of the largest national parks in Gabon known for its ape and chimpanzees populations. The sampling sites were selected in both urban and rural environments (Table 1). The factors "population size," "type of human activity," "proximity to forest" and "built density" were used to discriminate between urban and rural areas in this study (Table 1). Indeed, a high density of buildings and inhabitants characterizes the urban environment, while the rural environment low density of buildings and very close to the forest environment. Tchibanga (Urban, Latitude: −2.927877; Longitude: 10.997658), the provincial capital, is the first locality with a population of 30,042 (DGS, 2015). Mayumba (Urban, Latitude: −3.442609, Longitude: 10.656261; Population: 5220 inhabitants), a coastal locality, is one of the main tourist destinations in Gabon and Nyanga Province in particular. Moabi (Latitude: −2.444807; Longitude: 10.922204; Population: 3418 inhabitants) and Moulengui-Binza (Latitude: −3.483083; Longitude: 11.743834, Population: 518 inhabitants) (Rural) are villages in this province whose main activities are cassava and banana productions, respectively. These villages have the particularity of being close to

FIGURE 1
Geolocation of collection sites in Nyanga Province.
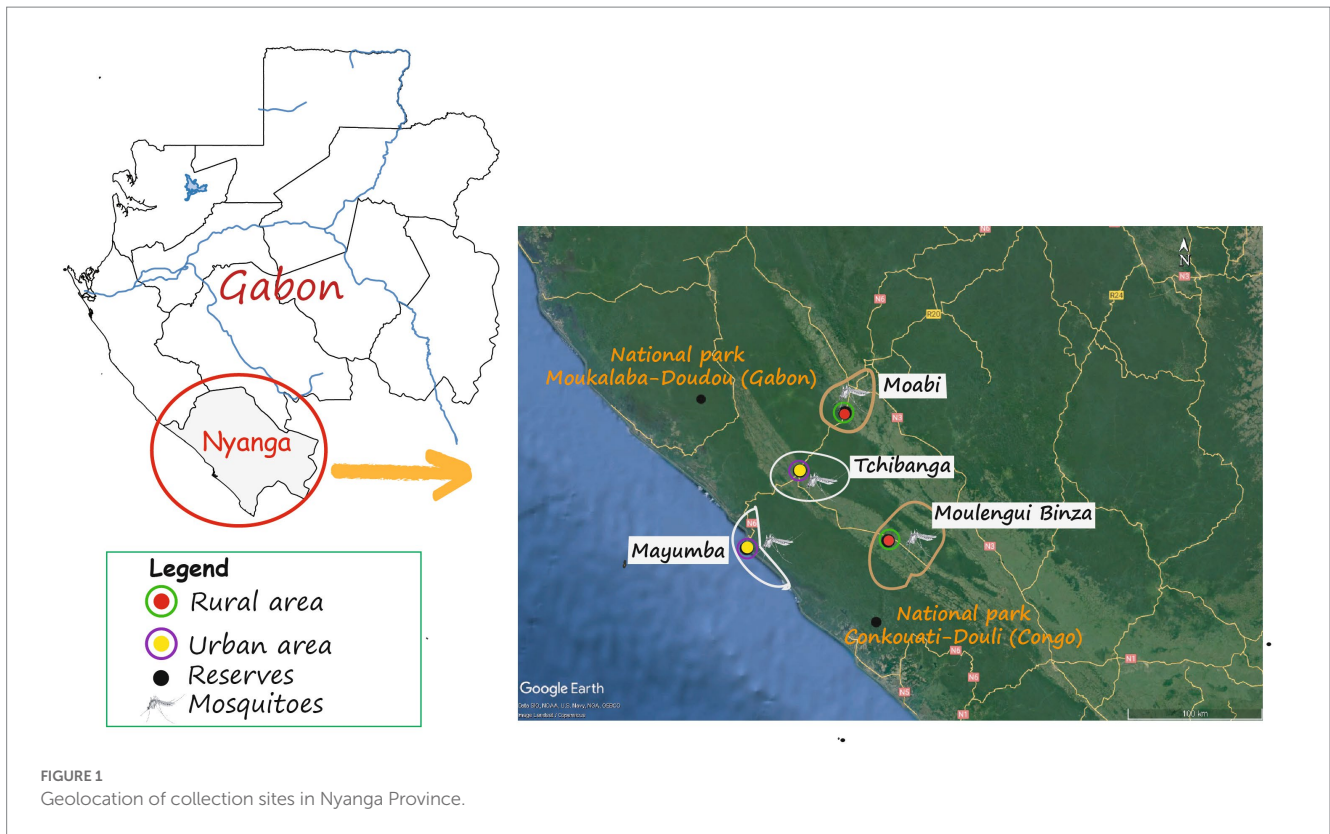
TABLE 1 Geolocation of each collection site and abundance of *Culicidae* collected.

Locality	Longitude	Latitude	Urbanization	Built density	Human population	Proximity to forest (km)	Genre mosquito	Number
Mayumba	10.6554	−3.43198	Urban	Medium	5,220	85.11	*Aedes*	0
							Anopheles	1
							Culex	252
							Mansonia	0
Moabi	11.0001	−2.5041	Rural	Low	3,418	79.59	*Aedes*	0
							Anopheles	56
							Culex	45
							Mansonia	0
Moulengui-Binza	11.5788	−3.37231	Rural	Low	518	58.45	*Aedes*	3
							Anopheles	104
							Culex	3
							Mansonia	8
Tchibanga	10.997658	−2.927877	Urban	High	30,042	60.91	*Aedes*	3
							Anopheles	26
							Culex	88
							Mansonia	2

National Parks containing a great diversity of fauna, notably great apes [(*Gorilla gorilla gorilla* and *Pan troglodytes troglodytes*) (Kami et al., 2019; Freddy and Bakouetila, 2020; Nguema, 2020; Oyaba Yinda et al., 2022)]. Indeed, Moabi is located less than 80 km from Moukalaba-Doudou National Park in Gabon and Moulengui-Binza

is less than 60 km from Conkouati-Douli National Park in the Republic of Congo (Figure 1).[1]

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1 https://fr-fr.topographic-map.com/maps/i9s0/Nyanga/

Mosquito collection

To assess the diversity of the anopheline mosquitoes feeding on humans, we performed sessions of collection on humans in both urban and rural environments. These captures were done in April 2018 during two consecutive nights (from 6: 00 PM to 6: 00 AM) in four subsites (Table 1). Captures were made by eight volunteers (volunteers were over 18 years old and all were male) of our team by site. The daily exposure time of the volunteers was 12 h. All mosquitoes were collected by using hemolysis tubes on the exposed legs of the volunteers (all protected with malaria prophylaxis). This study and the methods employed for mosquito collection, including human landing catch (HLC) were approved by Gabon's National Ethics Committee (authorization number PROT 0031/2014/SG/CNE). After morphological identification of the species using the identification key for *Culicidae* present in Central Africa and Gabon (Baldacchino and Paupy, 2010), inspired by Gillies and De Meillon (1968) and Gillies and Coetzee (1987), *Anopheles* specimens were preserved individually in tubes with desiccant.

DNA extraction and molecular analysis

For each *Anopheles* collected, DNA was extracted and purified from the head-thorax assembly without the abdomen by using the Cetyltrimethylammonium Bromide (CTAB) extraction protocol (Morlais et al., 2004). Diagnostic PCRs were selected and applied according to the outcome of the morphological identification of different complex and group of *Anopheles* (Fanello et al., 2002; Cohuet et al., 2003; Kengne et al., 2003, 2007). However, total DNA was also used to amplify a portion of the mitochondrial Cytochrome oxidase II (COII) (Rahola et al., 2014) to confirm the mosquito species, especially for specimens for which the morphological diagnose was doubtful or impossible because of poor preservation of individuals' morphological characters. For the identification of malaria parasites, we first used the mitochondrial Cytochrome b gene PCR protocol described previously in Boundenga et al. (2017). Then, all positive samples were subjected to PCR following the protocol of Snounou and Singh (2002) in order to discriminate all *Plasmodium* spp. capable of infecting humans. Small subunit ribosomal RNA (ssrRNA) were used for the phylogenetic analysis of the *Plasmodium* species. All samples that remained unidentified by this method were reanalyzed using PCR of the Cytochrome b (Cyt-b). The amplification of the portion of Cyt-b gene is based on a nested PCR with 2 sets of primers, one developed by Perkins and Schall (2002) (DW2– DW4) and one from Schwöbel et al. (2003). This combination of primers (despite the fact that some of these primers (Cytb1 and Cytb2) were originally developed to amplify *P. falciparum* Cytochrome-b gene) can amplify the Cytochrome-b from other haemosporidian parasites infecting a diverse range of hosts (Prugnolle et al., 2010, 2011; Boundenga et al., 2016; Makanga et al., 2016).

Contamination verification

All molecular analyses were repeated and verified in three different separate laboratories at the CIRMF (Entomology and Parasitology department), of which two have never manipulated

Plasmodium DNA to avoid the problem of contamination. In each laboratory, a single designated experimenter performed the molecular analyses and the three experimenters obtained identical results. In order to ensure that the results obtained were not the result of any contamination, verification manipulations were carried out. Firstly, the Cyt-b PCR protocol was carried out on thirty-seven (37) non-*Anopheles* mosquitoes, namely eight (8) *Aedes aegypti*, fifteen (15) *Aedes albopictus*, nine (9) *Aedes africanus*, three (3) *Culex* spp. and two (2) *Toxorhynchites* spp. The same protocol was carried out on a plate of 96 water samples. Then, the genetic distance between the different *Plasmodium* obtained in this study was calculated using the MEGA11 software in order to look at the similarity between the *Plasmodium* sequences. After, investigation of potential *Plasmodium* contamination within the laboratory shows that no specific bands of the expected size were amplified from the DNA of the 37 non-*Anopheles* mosquitoes and the 96 water samples. In addition, the genetic distance analysis between the great ape *Plasmodium* sequences shows that the sequences are not similar (Supplementary Table S1).

Phylogenetic analyses

For the molecular identification of *Anopheles* species and their respective malaria, we performed phylogenetic analyses using (i) COII and (ii) Cyt-b sequences for mosquitoes and parasites, respectively. Each analysis was performed using a set of previously known species sequences from different mosquito species and *Plasmodium* species infecting humans and primates. Multiple alignments of all sequences were performed using ClustalW [(v 1.8.1 in BioEdit v.7.0.9.0.) (Hall, 1999)]. Maximum Likelihood (ML) tree construction was performed based on the Cytochrome-b and mosquito COII sequences of 773 and 603 nucleotides, respectively. The best-fitting ML model according to Akaike's information criterion was the general time-reversible + γ distribution for nucleotides, as identified by ModelTest (Posada and Crandall, 1998). The most probable DNA trees and corresponding bootstrap support values were obtained by the online software PhyML[2] using nearest neighbor exchange and subtree pruning, regrafting, branch exchange, and 100 bootstrap repeats (Guindon et al., 2010). Finally, we differentiated *Plasmodium* infections of simian origin from those of human origin by analyzing the polymorphism of the sequences obtained. Thus, we identified a polymorphism at positions 3,575 and 3,617 spread over the whole mitochondrial genome and verified on human *P. falciparum* (AY282930-*P. falciparum* 3D7 and AY282924-*P. falciparum*-102) as described in Prugnolle et al. (2011).

Statistical analyses

All statistical analyses were performed using R software version 4.0.2. The comparison of *Culicidae* proportions between urban and rural areas was carried out using the "chisq. Test" function in R. The Shannon index of diversity (H′) was determined using the "diversity" function of the vegan package (Oksanen et al., 2013).

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2   http://www.atgc-montpellier.fr/

Results

Taxa of sampled mosquitoes

Five hundred and ninety-one (591) mosquitoes were sampled throughout the survey in the four study sites (Table 1). The mosquitoes mainly belonged to four genera (*Culex* spp., *Anopheles* spp., *Mansonia* spp. and *Aedes* spp.). The majority of the specimens were assigned to the *Culex* genus, which represented by 65.7%; followed by *Anopheles* spp. (31.6%), *Mansonia* spp. (1.7%) and *Aedes* spp. (1%) (See Table 1; Figure 2 for more information). Comparative analysis of the populations revealed that *Culex* species were significantly predominant in urban localities (Tchibanga and Mayumba) compared to rural localities where *Anopheles* species dominated ($\chi^2 = 289.6$, value of *p* <0.0001).

Anopheles diversity and distribution

Regarding the *Anopheles* diversity, after morphological and molecular analysis of 185 specimens belonging to this genus, our results revealed that the specimen identified belonged to nine known species, of which the most frequent were *Anopheles nili s.s* (45.41%) followed by *An. gambiae s.s* (32.43%), *An. funestus* (15.14%), *An. moucheti*-like (3.24%), *An. moucheti* (1.62%), *An. hancocki* (0.54%), *An. carnevalei* (0.54%), *An. coustani* (0.54%) and *An. funestus*-like (0.54%) (Figures 2, 3; Table 2). Rural areas (H′ = 1.27) were more diverse than urban areas (H′ = 0.16) and contained more than 85% of the anopheline fauna. In addition, we observed that this fauna was composed of species of the *Anopheles nili* complex [(53.46%) (*An. nili s.s* and *An. carnevalei*)], followed by those of the *Anopheles gambiae* complex [(22.01%) (*An. gambiae s. s*)], the *Anopheles funestus* group [(18.24%) (*An. funestus* and *An. funestus*-like)], the *Anopheles moucheti* complex [(5.66%) (*An. moucheti* and *An. moucheti*-like)], and *An. hancocki* (0.63%) (Figure 2). However, urban environments were mainly composed of *An. gambiae s.s* (96%) and one *An. coustani* (4%) specimen.

Plasmodium infection in malaria vectors

One hundred and eighty-five (185) upper thorax parts of anopheline mosquito samples were tested for the presence of malaria parasites using a Cytochrome b (Cyt-b)-based nested PCR method. Overall, thirty-three (33) samples were positive with malaria parasites, giving a prevalence of 17.84% (Table 2). Our results reveal the variation of average prevalence of *Plasmodium*-infected *Anopheles* per site (Table 2). Indeed, we observed significant differences in the rates of *Plasmodium*-infected *Anopheles* between the sites: Moabi, Tchibanga, Moulengui-Binza, and Mayumba had 30.36, 16, 11.65 and 0%, respectively (Fisher's test, value of *p* = 0.024). However, malaria transmission does not differ between urban and rural environments (Fisher's test, value of *p* = 1). Among the nine (*n* = 9) *Anopheles* species identified, only six were found to be infected, namely *An. gambiae s.s* (12/60), *An. funestus* (9/28), *An. nili s.s* (7/84), *An. moucheti like* (3/6), *An. moucheti* (1/3) and *An. funestus like* (1/1) (Table 2). Molecular and phylogenetic analyses revealed that the *Plasmodium* spp. detected belonged to three known species; one which infects human
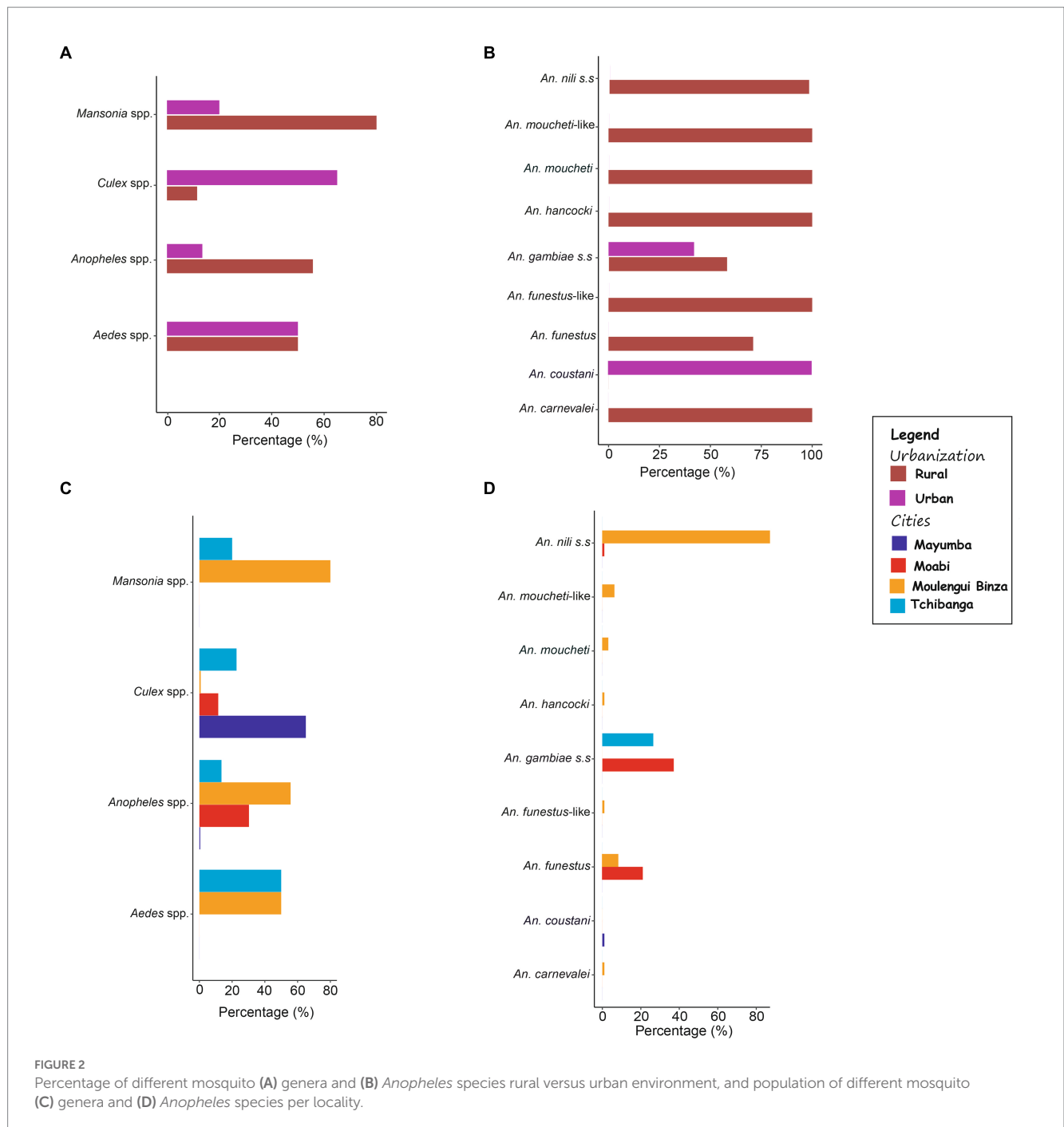
(*Plasmodium falciparum*) and two which infect apes (*Plasmodium reichenowi* and *Plasmodium praefalciparum*). Of all the *Plasmodium* detected *P. falciparum* was the most prevalent (15.68%), followed by *P. praefalciparum* (1.62%) and *P. reichenowi* (0.54%) (Table 2; Figure 4). Of the twenty-nine *P. falciparum* positives, the most infected species were *An. gambiae* s.s (41.4%) followed by *An. funestus* (24.14%) and *An. nili* s.s (24.14%). *P. falciparum* was found in the upper parts of six *Anopheles* species (*An. gambiae s.s, An. funestus, An. funestus*-like, *An. moucheti, An. moucheti* like and *An. nili s.s*) from urban and rural environments. However, anopheline mosquito infested with apes *Plasmodium* were observed only in rural areas. Indeed, *P. praefalciparum* was detected in two species (*An. funestus* and *An. moucheti*-like) from Moabi and Moulengui-Binza and *P. reichenowi* was observed in *An. funestus* form Moabi (Table 2; Figure 4).

Discussion

In this study, we sought to identify *Anopheles* species involved in human malaria transmission in urban and rural areas. To do so, we conducted a study of anopheline communities in the Nyanga Province in southern Gabon (Central Africa). Indeed, the lack of knowledge of the different vector species potentially involved in the transmission of malaria remains an obstacle to the implementation of an effective malaria control system (WHO, 2020, 2022) especially for countries located in hyper-endemic areas such as Gabon, where the most vulnerable groups are pregnant women and children under 5 years old (Jäckle et al., 2013; Mawili-Mboumba et al., 2013; Imboumy-Limoukou et al., 2020). Thus, this cross-sectional entomological study was carried out following a parasitological study which revealed a significant average malaria prevalence of 13.9% in Nyanga Province (Imboumy-Limoukou et al., 2020).

Urbanization influences on *Culicidae* composition in Nyanga

In the present study, the results showed that four groups of mosquitoes feed on human subjects in the four sites surveyed. However, the spatial distribution of the different identified mosquitoes was not homogeneous across the different environments. Indeed, mosquitoes of the genus *Culex* were significantly more abundant in urban areas, whereas in rural areas, mosquitoes of the genus *Anopheles* were predominant and very diverse. This contrasting abundance and diversity of *Anopheles* species between urban and rural areas could be explained by environmental disturbances resulting from anthropogenic land use, which in most cases could eliminate a greater diversity of larval breeding sites in urban areas than in rural areas (Johnson and Munshi-South, 2017; Epelboin et al., 2021; Nieto-Sanchez et al., 2022; Verrelli et al., 2022). Thus, the higher species diversity identified in rural areas could be explained by the stability of rural environments that favors the abundance of anopheline species (Imbahale et al., 2011; Mzilahowa et al., 2016). Indeed, studies on the impact of urbanization shows that both faunal and floral species richness along an urban–rural gradient decreases from the periphery to the middle of the city (Johnson and Munshi-South, 2017). These observations could be explained by environmental conditions such as

FIGURE 2
Percentage of different mosquito **(A)** genera and **(B)** *Anopheles* species rural versus urban environment, and population of different mosquito **(C)** genera and **(D)** *Anopheles* species per locality.

deforestation, industrial and domestic pollution of surface waters, which destroy the ecological niches of certain species and thus create new environmental conditions unfavorable to the normal life cycle of indigenous species (Clergeau et al., 1998; Chace and Walsh, 2006), particularly mosquito vectors of *Plasmodium* (Longo-Pendy et al., 2021). Seven of the nine *Anopheles* species recorded were exclusive to rural areas, namely *An. nili s.s*, *An. funestus*, *An. funestus* like, *An. moucheti*, *An. moucheti* like, *An. carnevalei* and *An. hancocki*. Therefore, only mosquito species that are able to adapt to environmental changes (such as those generated by pollution of any kind) in urban areas such as the larvae of the *Culex* genus (Doby and Mouchet, 1957; Subra, 1981) and very recently the sister species of the

Anopheles gambiae complex, *An. gambiae s.s* and *An. coluzzii* (Antonio-Nkondjio et al., 2011; Kamdem et al., 2012; Tene Fossog et al., 2012; Longo-Pendy et al., 2021; Nieto-Sanchez et al., 2022), can proliferate in mostly polluted urban breeding sites (Brown et al., 2008). This is coherent with our results, which showed that the *Culicidae* fauna in urban localities in Nyanga Province was mainly composed of adult mosquitoes of the *Culex* genus and of the species *An. gambiae s.s*. Thus, the high abundance of the genus *Culex* that we observed in the urban areas of Mayumba and Tchibanga confirms that rapid unplanned urbanization could contribute to the proliferation of *Culex* species by creating suitable environmental conditions for their development (Brown et al., 2008; Mohammed
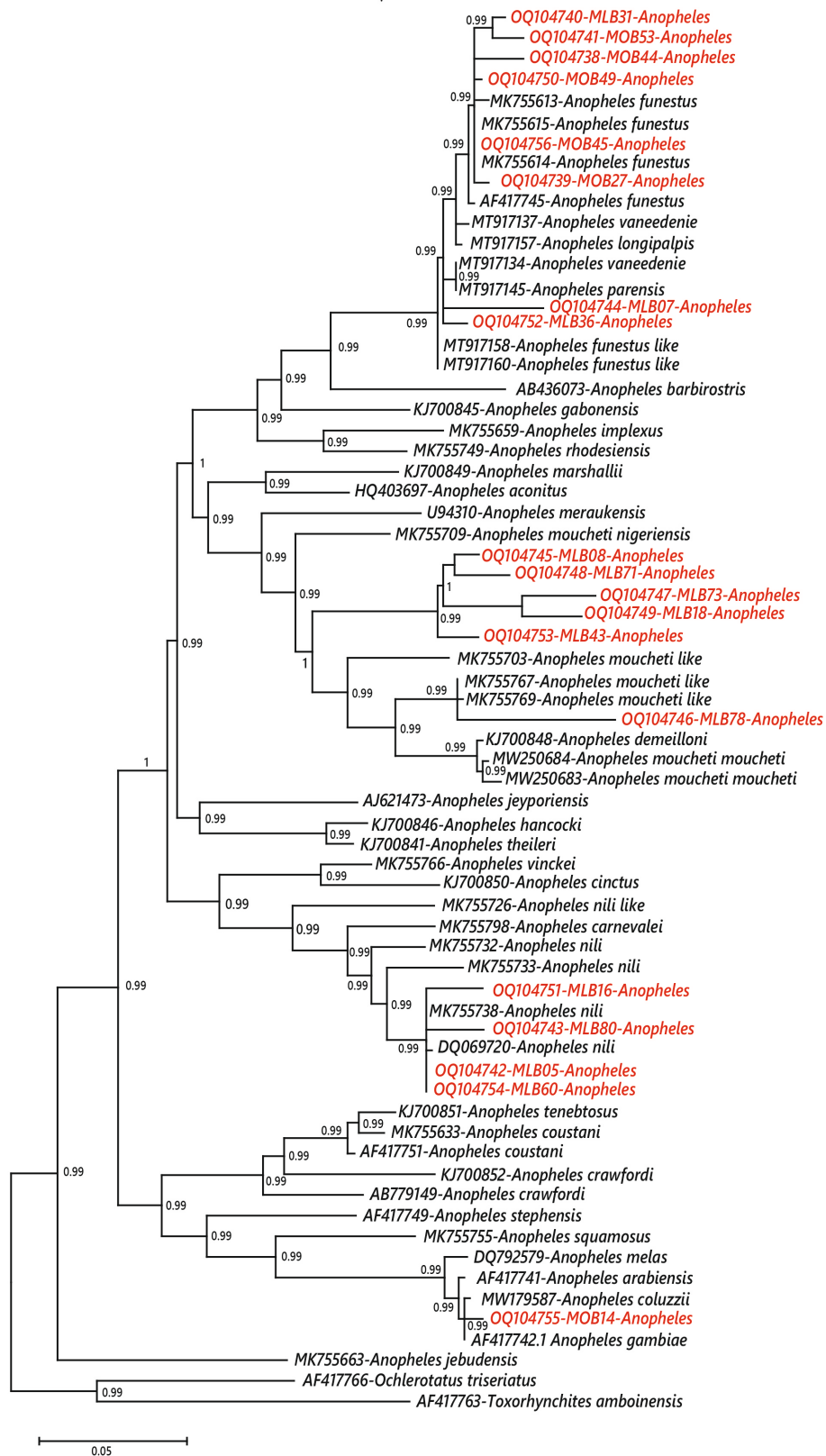
FIGURE 3
Phylogenetic position of mosquitoes of the *Anopheles* genus indeterminate by conventional species diagnostic PCR in this study. The tree was constructed from COII sequences using maximum likelihood (ML) methods. Bootstrap values are indicated at each node. The sequences obtained in this study are in red.

TABLE 2 Summary of the *Anopheles* species screened for plasmodial infection in this study.

Anopheles species	Human malaria role	Locality	Mosquito tested	*P. falciparum* n (%)	*P. praefalciparum* n (%)	*P. reichenowi* n (%)	General infection (%)
An. carnevalei	Minor	Mayumba	0	/	/	/	0
		Moabi	0	/	/	/	
		Moulengui-Binza	1	0	0	0	
		Tchibanga	0	/	/	/	
An. coustani	Minor	Mayumba	1	/	/	/	0
		Moabi	0	/	/	/	
		Moulengui-Binza	0	/	/	/	
		Tchibanga	0	/	/	/	
An. funestus	Major	Mayumba	0	/	/	/	32.14
		Moabi	**20**	**7 (35)**	**1 (5)**	**1 (5)**	
		Moulengui-Binza	**8**	/	/	/	
		Tchibanga	0	/	/	/	
An. funestus-like	Minor	Mayumba	0	/	/	/	100
		Moabi	0	/	/	/	
		Moulengui-Binza	**1**	**1 (100)**	/	/	
		Tchibanga	0	/	/	/	
An. gambiae s.s	Major	Mayumba	0	/	/	/	20
		Moabi	35	**8 (22.9)**	0	0	
		Moulengui-Binza	0	/	/	/	
		Tchibanga	25	**4 (16)**	0	0	
An. hancocki	Minor	Mayumba	0	/	/	/	0
		Moabi	0	/	/	/	
		Moulengui-Binza	1	0	0	0	
		Tchibanga	0	/	/	/	
An. moucheti	Major	Mayumba	0	/	/	/	0
		Moabi	0	/	/	/	
		Moulengui-Binza	3	**1 (33.3)**	0	0	
		Tchibanga	0	/	/	/	
An. moucheti like (Ayala et al., 2019)	Undetermined	Mayumba	0	/	/	/	50
		Moabi	0	/	/	/	
		Moulengui-Binza	6	**1 (16.7)**	**2 (33.3)**	0	
		Tchibanga	0	/	/	/	
An. nili s.s	Major	Mayumba	0	/	/	/	8.3
		Moabi	0	/	/	/	
		Moulengui-Binza	84	**7 (8.3)**	**0**	0	
		Tchibanga	0	/	/	/	
	Total		185	29 (15.68)	3 (1.62)	1 (0.54)	**17.84**

Bold was used to highlight mosquitoes positive for Plasmodium spp.

FIGURE 4
(A) Phylogenetic relationships between *Plasmodium* isolates obtained after sequencing of the Cytochrome b gene. The tree was constructed using Maximum likelihood (ML) methods. Bootstrap values are shown at each node except those below 0.5. *Leucocytozoon majoris* was used to root the tree. (B) Shown the result of comparison at the level of two diagnostic mitochondrial SNPs of our twelve suspect samples against those present in *Plasmodium falciparum* and non-human isolates.

et al., 2021). This could constitute a risk for human health in urban centers in Gabon, as *Culex* species are known to be vectors of several zoonotic pathogens such as Rift Valley fever virus (Ferraguti et al., 2021). Moreover, the very low occurrence of *Anopheles* mosquitoes

observed in Mayumba, with only one specimen detected, *An. coustani*, which is known to be a minor vector of malaria in sub-Saharan Africa and in Gabon in particular (Longo-Pendy et al., 2022), allows us to justify the observations made by Imboumy-Limoukou et al. (2020)

(Imboumy-Limoukou et al., 2020) who showed that there were no cases of malaria in this coastal town of Gabon (Imboumy-Limoukou et al., 2020). The impact of environmental changes on the spatial dynamics of mosquitoes is not only specific to *Anopheles* species but has also been observed in mosquitoes of the *Aedes* genus (Albrieu Llinás et al., 2018; Zettle et al., 2022). This modification of spatial distribution could modulate their trophic behavior and influence the occurrence of the plasmodial species they host as recently described for arboviruses (Li et al., 2013; Kolimenakis et al., 2021) and helminths in the giant toad *Rhinella horribilis* (Jacinto-Maldonado et al., 2022).

In rural areas, *Anopheles* harbor apes malaria parasites

Vectors analysis allowed us to observe a prevalence of infection of 17.84% in the *Anopheles* species surveyed. The prevalence we obtained in this study is higher than those reported in previous studies in Gabon. It has been reported that *Anopheles* infestation rates range from 0.15 to 0.8% in Libreville (Mourou et al., 2010, 2012), 0.64% in Port-Gentil (Mourou et al., 2010) and 2.2% in Lambaréné (Boussougou-Sambe et al., 2022). This difference in the level of *Anopheles* infection could be justified, as mentioned above, by the influence of environmental factors specific to each study site. However, the prevalence of malaria observed in *Anopheles* in our study seems consistent with the 14% level of malaria infection in humans observed in Nyanga Province in 2018 (Imboumy-Limoukou et al., 2020). This observation reflects a high risk of exposure to malaria infection by populations with each *Anopheles* bite in this region of Gabon. Thus, given the heterogeneity of malaria prevalence from one locality to another, the establishment of an epidemiological surveillance system in this region would allow a better characterization of the transmission facies, and consequently the adoption of more effective malaria control strategies.

Concerning malaria parasites species, our study revealed that some of these *Anopheles* species were carriers of sporozoites of three plasmodial species belonging to the subgenus *Laverania* in particular *P. falciparum*, *P. reichenowi*, and *P. praefalciparum*. Among these parasite species, the most represented was *P. falciparum* (87.9%) followed by *P. praefalciparum* (9.1%) and *P. reichenowi* (3%). The vector species of *P. falciparum* were mainly *An. gambiae s.s*, *An. nili s.s* and *An. funestus*, species known in sub-Saharan Africa and particularly in Gabon, for their strong involvement in malaria transmission (Sinka et al., 2012; Longo-Pendy et al., 2022). Surprisingly, *An. funestus* was found to be infected for the first time by *P. reichenowi* and *P. praefalciparum*, both species of simian plasmodia. This result shows that this *Anopheles* species, described in the literature as very anthropophilic, can adopt an opportunistic behavior and adapt its feeding habits depending on the environment it lives. This species would be able to take its blood meal on primates other than humans in rural areas where human populations live near forests. In addition, our study has also enabled us to highlight for the first time the presence of malaria parasite in *An. moucheti*-like, a species described very recently as being very close to the species of the *Anopheles moucheti* complex (Ayala et al., 2019). In this study, two specimens of this species were found to be infected with the gorillas *Plasmodium* (*P. praefalciparum*). All these new findings combined, allow us to add to the list of potential bridge-vectors of malaria

parasite between apes and human (Makanga et al., 2016), *An. funestus* and *An. moucheti*-like. Thus, the presence of a wide diversity of *Anopheles* species with opportunistic behavior could constitute a risk for human health and raises the question of the potential existence of zoonotic infections in this part of Gabon as already described in the Asian region with *P. knowlesi* (Setiadi et al., 2016), in Latin America with *P. simium* (Brasil et al., 2017; Mourier et al., 2021; Lempang et al., 2022), and in Central Africa with *P. vivax*-like (Prugnolle et al., 2013). Thus, it could be that the identical morphology of *Plasmodium* belonging to the subgenus *Laverania*, as seen between *P. falciparum* and *P. reichenowi* (Coatney, 1971; Martin et al., 2005), could lead to a proportion of human malaria cases being wrongly attributed to *P. falciparum* in this region of Gabon where diagnosis of malaria is done only via microscopy and malaria rapid detection test (RDT) methods. Only a molecular characterization would allow to distinguish these *Plasmodium* species. However, it would be necessary to carry out a longitudinal entomological and parasitological follow-up with molecular tools that would allow us to confirm the involvement of *An. funestus* and *An. moucheti*-like in malaria transmissions from great apes. In addition, it would be necessary to highlight the role of bridge vector in the simians malaria transmission to humans and finally to determine the occurrence of *Plasmodium* species of non-human primates in humans and the associated clinical signs.

Conclusion

Our study highlighted that among the large diversity of *Anopheles* species found in the province of Nyanga in south-eastern Gabon, one *Anopheles* species known to be highly involved in human malaria transmission, *An. funestus*, and a very recently described, *An. moucheti*-like, were infected with *P. reichenowi* and *P. praefalciparum* which are great ape *Plasmodium* species genetically very close to *P. falciparum*. One of the scenarios that could be imagined in this region of the country, would be the existence of a zoonotic transmission of malaria that is undetectable in humans with the conventional tools of plasmodial morpho-taxonomy, notably the blood smear, and which could be untreatable with artemisinin derivatives. Thus, a better characterization of this zoonotic transmission would require the implementation of molecular diagnostic tools that would allow to reveal it and to strategize ways to manage it.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found at: https://www.ncbi.nlm.nih.gov/genbank/, OP970503-OP970515 and OQ104738-OQ104756.

Ethics statement

The studies involving human participants were reviewed and approved by National Ethical Research Committee in Gabon (No. PROT 0031/2014/SG/CNE and N °001/PR/SG/CNE/2018). The

patients/participants provided their written informed consent to participate in this study.

Author contributions

N-ML-P, J-BL-D, and PK: conceptualization. BM: fieldwork and morpho-taxonomy of mosquitoes. N-ML-P, OA-E, CM-B, LaB, and SL-D: molecular analysis and sequencing. N-ML-P and LaB: phylogenetic and bio-statistical analyses. N-ML-P and LaB: writing–original draft. BM, CM-B, LeB, OA-E, LN-N, J-BL-D, PK, LaB, and N-ML-P: writing–review and editing. PK and J-BL-D: supervision. J-BL-D: project management and funding acquisition. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2023.1176687/full#supplementary-material

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