

RESEARCH PAPER

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Abundance, diversity, resting and blood-feeding behaviours of malaria vectors in Ouagadougou, Burkina Faso

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ABSTRACT

Malaria remains a public health concern in Burkina Faso, as in most sub-Saharan African countries. A better control of *Anopheles* mosquito, vectors of this disease, requires an understanding of vector species composition and their behaviour. The study aims to identify malaria vector species, assess their resting behaviour, and evaluate their trophic preferences in Ouagadougou. Mosquitoes were collected indoors and outdoors using Prokopack aspirators in September 2022 from 200 households in the Zongo and 1200 Logements districts. Mosquitoes were morphologically sorted, species identification within *Anopheles* complex species, as well as the determination of the origin of the blood meal, were performed by PCR. Out of a total of 4,466 mosquitoes collected, 238 belonged to the *Anopheles gambiae* complex, including 99 males and 139 females. *An. arabiensis* was the predominant *An. gambiae* complex species with more than 86% (120/139), which species was significantly more collected outdoors (97/120) compared to indoors ( $\chi^2= 17.051$ ;  $p<0.001$ ). *An. coluzzii* was the second *Anopheles gambiae* complex species and represented 13.67% (19/139). The analysis of blood meal sources showed that both species fed on four hosts. Human exclusive blood meal represented 26.32% (5/19) of the total blood meal. The animal exclusive blood including dogs (15.79%), cows (15.79%), and pigs (5.26%) represented 36.84% (7/19). However, 36.84% (7/19) of the blood meals were mixed (human-animal). Although the study only covered two districts of Ouagadougou, the findings provide valuable information that can strengthen and guide the fight against anopheles in urban areas, by targeting the predominant vector, *An. arabiensis*.

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## INTRODUCTION

Malaria remains a serious public health challenge affecting the development of some countries worldwide, particularly in sub-Saharan Africa. An estimated 282 million cases and 610,000 deaths occurred in 2024. The WHO African Region continues to endure the greatest burden, with 11 countries contributing for about two-thirds of global cases and deaths (WHO, 2025). In Burkina Faso, about 8,324,000 cases were reported in 2024 (WHO, 2025). Although long considered a rural disease affecting primarily children and pregnant women, malaria has been presenting in Africa for over a decade with severe forms in urban adults (Diallo *et al.*, 2003)). According to the WHO, the rapid expansion of the urban population will be one of the major global health challenges of the 21st century (WHO, 2016). Indeed, in developing African countries, rapid population growth is generally unplanned, resulting in populations being exposed to health risks. Then, there is a growing concern about urban malaria, mainly due to a better adaptation to an urban or peri-urban site (De Silva and Marshall, 2012; Doumbe-Belisse *et al.*, 2021). This requires continuous monitoring and control of urban malaria to minimize the impact of urbanisation on malaria.

Malaria is transmitted to humans through the bite of an infected mosquito of *Anopheles* genus. Overall, in Africa, members of the *Anopheles gambiae* complex and *An. funestus* ensure the essential of malaria transmission (Collins and Besansky, 1994; Kelly-Hope and McKenzie, 2009). *An. gambiae* complex comprises 9 species (Coetzee *et al.*, 2013; Barrón *et al.*, 2019).

Malaria vectors showed a heterogeneous distribution and resting behaviour according to the species and level of urbanisation. Overall, while *An. arabiensis* is collected more outdoors *An. gambiae* s.s was indoor (Mahande *et al.*, 2007; Charlwood *et al.*, 2018). The blood-feeding patterns are key parameters for malaria transmission. Indeed, mosquito species that feed mostly on humans may be more dangerous to spray with pathogens. Overall, *An. gambiae* s.l is reported

to be a high anthropophilic species. However, this frequency can change in the absence of humans (Bouafou *et al.*, 2024). Some reported a zoophilic behaviour of *An. arabiensis* using odour-baited entry traps (Mahande *et al.*, 2007).

The primary malaria vectors in Burkina Faso are members of *An. gambiae* (s.l) species complex (*An. gambiae* s.s, *An. coluzzii* and *An. arabiensis*) and *An. funestus*. Furthermore, in recent years, studies conducted in Burkina Faso have focused more on vector resistance than on their diversity (Badolo *et al.*, 2012; Namountougou *et al.*, 2019; Niang *et al.*, 2021).

However, with the migration and adaptation of vector species to the urban environment, it is essential to have up-to-date data on vector abundance and diversity to better guide vector control methods. The objective of this study was to determine the abundance, diversity, resting and blood-feeding behaviours of the malaria vector in both urban and peri-urban sites of Ouagadougou, the capital city of Burkina Faso.

## MATERIALS AND METHODS

### Study sites

This study took place in September 2022 in the city of Ouagadougou (12° 21' 56.4" North, 1° 32' 2" West). Ouagadougou is the capital and largest city of Burkina Faso, with an estimated population of 2,780,000 (ONSP, 2023). The town is located roughly in the centre of the country, in the province of Kadiogo, in the inter-tropical zone. It is the administrative centre of the country. The climate is Sudano-Sahelian with an average annual rainfall of 400-800 mm (Kambire *et al.*, 2015). The study was carried out in a peri-urban site, Zongo and an urban district of 1200 Logements. In each district, 100 households were selected. A total of 200 households were visited during the study.

### Adult mosquito collection and identification

Adult mosquito collection was conducted in the morning from 6:00 a.m. to 11:00 a.m. and from 4:00 p.m. to 7:00 p.m. These periods correspond to lower

ambient temperatures, during which mosquitoes are generally more active, thereby increasing the likelihood of encountering them. All areas likely to be used as resting places for mosquitoes were visited for 15 mn. Collections were made indoors for endophilic mosquitoes and outdoors shelters for exophilic mosquitoes using a battery-operated Prokopack aspirator (Vazquez-Prokopec *et al.*, 2009). About 10 households were targeted to be sampled daily. Collected mosquitoes were sorted morphologically under a stereomicroscope using classical identification keys (Edwards, 1941; Rueda, 2004; Robert *et al.*, 2022) and stored in 1.5 ml tubes (Eppendorf®) over silica gel separately according to genus, species, repletion stages (unfed, gravid, blood fed), and collection location (inside or outside housing).

### **Molecular identification *Anopheles gambiae* complex and origin of the blood meal**

#### *Mosquito dissection and DNA extraction*

*An. gambiae* s.l. mosquitoes were dissected to separate the head-thorax from the abdomen and put separately in a 1.5 ml Eppendorf tube. Head-thorax parts were used for the molecular identification of *Anopheles* species and determination of the infection to *Plasmodium* parasite, while the abdomen for used for the detection of the origin of the blood meal.

DNA extraction was performed on the abdomen of blood-fed and partially fed females for the determination of the origin of the blood meal, while extraction was made on the head-thorax for the molecular identification.

Each sample was ground in 100 µl of Buffer A (containing 0.1 M Tris pH 9.0; 0.1 M EDTA; 1% SDS; and 0.5% DEPC) using a sterile plastic pestle and placed on a hot plate for 30 min at 70°C. At the end of incubation time, 22.4 µl of 5 M potassium acetate (KOAc) was added, and the mixed by vortexing before placing on ice for 30 min. The samples were then centrifuged at 15,000 rpm, and the supernatant was transferred to a new Eppendorf tube to which 45 µl of isopropanol was added and mixed by

vortexing. After centrifugation at 15,000 rpm for 20 min at 4 °C, the supernatant was then discarded, and the pellet containing the DNA was rinsed with 100 µl of 70% ethanol by centrifugation at 15,000 rpm for 5 min. The supernatant is discarded again and the pellet, consisting of DNA, is dried at room temperature for about 15 min. 50 µl of TE (Tris EDTA) is added to the tube to dilute the DNA. The extracted DNA is stored at -20 °C for subsequent analysis.

### **Molecular identification of *Anopheles gambiae* complex members**

Two different PCR protocols were used to identify the species members of the *An. gambiae* complex members present in the study sites. The Restriction Fragment Length Polymorphism (RFLP) PCR protocol by Scott *et al.* (1993) was used to discriminate *An. arabiensis* from *An. gambiae* s.s/*An. coluzzii*, while the protocol of Santolamazza *et al.* (2008) was used to discriminate *An. gambiae* s.s from *An. coluzzii*. For each PCR a final volume of 12.5 µl was prepared using PCR-specific primers at 10 µM and 1 µl of DNA. For the Scott *et al.* protocol amplification carried out under the following program: initial denaturation at 95 °C for 10 mn (1 cycle), followed by 30 cycles of denaturation at 95 °C for 30 s, annealing at 50 °C for 30 s, and extension at 72 °C for 30 s, with a final extension at 72 °C for 5 min, and storage at 4 °C. For the Santolamazza *et al.* (2008) protocol, the following amplification programme was used: initial denaturation at 95 °C for 10 min (1 cycle), followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 54 °C for 30 s, and extension at 72 °C for 30 s, with a final extension at 72 °C for 10 mn, and stored at 4 °C.

PCR products (10 µL) were mixed with 2.5 µL of loading dye and electrophoresed on a 1.5 % agarose gel prepared in 1× Tris-Borate-EDTA (TBE) buffer at 120 V for 30 mn. A 100 bp molecular weight marker was used to estimate fragment size. DNA bands were visualized under ultraviolet light (UV), and the species was determined by comparing the DNA migration bands with the molecular weight marker. For the Scott *et al.* protocol, a DNA band at 315 bp means *An. arabiensis* while a band at 390 bp designs *An. gambiae* s.s or *An.*

*coluzzii*. When using the Santolamazza *et al.* (2008) protocol, the DNA band of *An. gambiae* s.s was expected at 279 bp and 479 bp for *An. coluzzii*.

### Molecular determination of origin of blood-meal

The origin of the blood meal was determined by multiplex PCR, following the method of Kent and Norris (2005), which targets mitochondrial cytochrome b gene sequences. The PCR reaction mixture (final volume 12.5  $\mu$ L) contained 6.25  $\mu$ L of Master Mix, 2.25  $\mu$ L of sterile water, 0.5  $\mu$ L of each primer pair (universal, human, goat, pig, dog, and cow), and 1  $\mu$ L of genomic DNA. Amplifications were performed under the following program: initial denaturation at 95 °C for 5 min (1 cycle), followed by 40 cycles of denaturation at 95 °C for 60 s, annealing at 56 °C for 60 s, and extension at 72 °C for 60 s, with a final extension at 72 °C for 7 min, and storage at 4 °C. PCR products (10  $\mu$ L) were mixed with 2.5  $\mu$ L of loading dye and electrophoresed on a 2 % agarose gel prepared in 1 $\times$  Tris-Borate-EDTA (TBE) buffer at 120 V for 30 min. A 100 bp molecular weight marker was used to estimate fragment size. DNA bands were visualized under ultraviolet light (UV), and the host blood-meal origin was determined by comparing of the DNA migration bands with the

molecular weight marker corresponding to human (334 bp), cow (561 bp), dog (680 bp), goat (132 bp) and pig (452 bp).

### Data analysis

The mosquito abundance was compared between study and place of collection. Differences between indoor and outdoor collected mosquitoes were assessed using a Chi-square ( $\chi^2$ ) test of independence to determine whether resting site distribution differed significantly from a random expectation. The analysis was performed using R software (version 4.0.3).

## RESULTS

### Relative density of mosquito species collected

A total of 4,466 mosquitoes were collected during the study period, belonging to three major genera (Table 1). *Culex quinquefasciatus* was by far the most abundant species, accounting for 57.19% (3358/4466) of all collected. *Aedes aegypti* represented 19.37% (865/4466), while *Anopheles gambiae* s.l. contributed to 5.33% (238/4466) of the total. The remaining species *Ae. vexans* was only sporadically reported, with less than 0.1% (4/4466) of the total collected (Table 1). This composition indicates a strong dominance of *Culex* species in the sampled areas, followed by a moderate presence of *Aedes* and *Anopheles* mosquitoes.

**Table 1.** Proportion of each mosquito species collected per study sites in September 2022

Species	1200 Logements (Males)	1200 Logements (Females)	Zongo (Males)	Zongo (Females)	Total
<i>Aedes aegypti</i>	350	247	149	119	865
<i>Aedes vexans</i>	1	1	0	3	5
<i>Anopheles gambiae</i> s.l.	49	68	50	71	238
<i>Culex quinquefasciatus</i>	1054	741	884	679	3358
Total	1454	1057	1083	872	4466

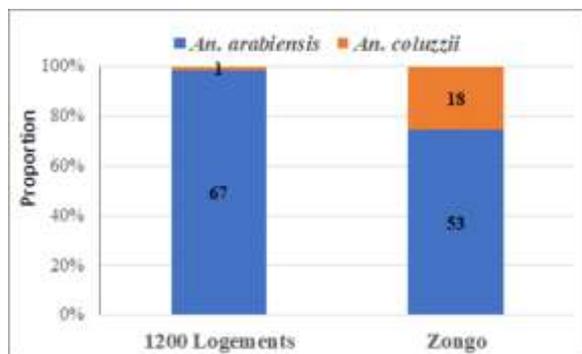
### Distribution of *Anopheles gambiae* complex members

A total of 238 *Anopheles* mosquitoes, including 139 females and 99 males were collected. However, only females were preceded to the molecular identification by PCR. All the 139 females were successfully identified by PCR. *An. arabiensis* was the predominant *An. gambiae* complex species representing 86.3% (120/139), followed by *An. coluzzii* at 13.7% (19/139)

(Fig. 1). *Anopheles gambiae* s.s was not found among *Anopheles* mosquitoes collected.

In the peri-urban site of Zongo, *An. arabiensis* represented 74.65% (53/71) and *An. coluzzii* 25.35% (18/71); in the urban district of 1200 Logements, *An. arabiensis* represented more than 98% (67/68) of the *Anopheles* mosquitoes collected. *Anopheles arabiensis* predominated in both sites, with a significant

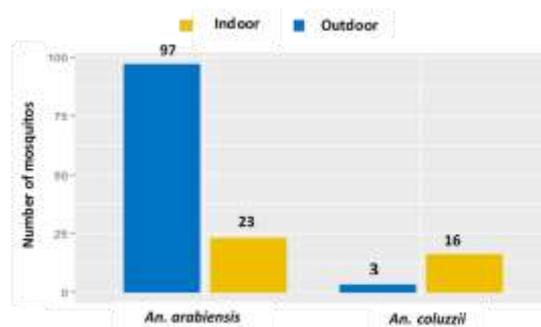
difference in its proportion according to the sites ( $\chi^2 = 18.72$ ,  $p < 0.001$ ). *Anopheles coluzzii* was less represented in both sites, although the abundance was slightly higher in Zongo; the difference was non-significant ( $\chi^2 = 1.8095$ ;  $p = 0.179$ ).



**Fig. 1.** Proportion of *An. arabiensis* and *An. coluzzii* in 1200 Logements and Zongo in September 2022

**Indoor and outdoor resting behaviour**

Overall, more *Anopheles* mosquitoes were collected outdoors than indoors (100/139). *Anopheles arabiensis* was predominantly collected outdoors (97/120) regardless the districts, while *An. coluzzii*, was more often found indoors than outdoors (16/19) (Fig. 2).



**Fig. 2.** Number of *An. arabiensis* and *An. coluzzii* collected outdoors and indoor

In both sites, similar pattern of resting behaviour was observed; indeed, *An. arabiensis* was predominantly collected outdoors in 1200 Logements (94.03% vs. 5.97%) and in Zongo (64.15% vs. 35.85%), whereas *An. coluzzii* showed an endophilic behaviour, with 100% of individuals collected indoors in 1200 Logements and 83.33% vs. 16.67% in Zongo, with significant difference in resting preference (indoors and outdoors) between the two species ( $\chi^2 = 34.38$ ,  $p < 0.001$ ). *Anopheles arabiensis* showed significantly exophilic resting behaviour ( $\chi^2 = 17.051$ ;  $p < 0.001$ ), while the outdoors vs. indoors resting behaviour of *An. coluzzii* was unclear ( $\chi^2 = 0.1979$ ;  $p = 0.656$ ).

**Table 2.** Blood meal origin and their distribution in *An. arabiensis* and *An. coluzzii* in Zongo and 1200 Logements

Blood-meal source	Zongo		1200 Logements		Total	Percentage (%)
	<i>An. arabiensis</i>	<i>An. coluzzii</i>	<i>An. arabiensis</i>	<i>An. coluzzii</i>		
Cows	2	0	1	0	3	15.79
Human	1	0	4	0	5	26.32
Pigs	1	0	0	0	1	5.26
Dogs	0	1	2	0	3	15.79
Human-cows	2	0	0	0	2	10.53
Human-dogs	1	0	4	0	5	26.32
Total blood meals	7	1	11	0	19	100.00
Anthropophilic rate (%)	57.14	0	72.72	0	—	63.16

**Blood-feeding patterns behaviour**

Out of a total of 86 mosquitoes analysed, only 19 (22.1%) were successfully amplified by PCR for the detection of the blood-meal origin of humans, cows, dogs, pigs and goats.

Human blood meal represented 63.16% (12/19) (considering a mixed blood meal) of the collected mosquitoes tested. The total animal blood meal exclusively represented 36.84% (7/19) (Table 2). A

mixed human-dog and human-cow blood meal represented 26.32% (5/19) and 10.52% (2/19) of the blood meals. Among the animal blood meals, the dog was the most predominant with a rate of 42.11% (8/19). Overall, *An. arabiensis* showed high anthropophilic behaviour in both study sites (Table 2).

**DISCUSSION**

The fight against malaria is a major public health challenge in Burkina Faso. The acceleration of

urbanization in Africa is often not accompanied by adequate sanitation plans. This can create suitable permanent breeding sites, leading to the development of disease-carrying mosquitoes (De Silva and Marshall, 2012).

Mosquito collection in the two districts, differing in levels of urbanicity, revealed a relatively higher abundance of *Culex* mosquitoes, specifically *Culex quinquefasciatus* (75.19%). The abundance of this species was common in Ouagadougou (Fournet *et al.*, 2010; Gnémé *et al.*, 2019) and may be due to its high capacity to reproduce in various types of breeding sites, particularly in the polluted environments of large cities. In both study sites, sources of polluted water, such as drainage canals and wastewater from were identified. In Burkina Faso, studies have shown a predominance of *Culex quinquefasciatus* over other mosquito species in the city of Ouagadougou (Fournet *et al.*, 2010; Gnémé *et al.*, 2019). In this study, *Anopheles* mosquitoes represented only 5.33% of the total mosquitoes collected. A similar proportion of 5.7% was reported in 2018 (Gnémé *et al.*, 2019), confirming the low abundance of *Anopheles* mosquitoes in urban environments such as Ouagadougou.

Molecular identification of species belonging to the *An. gambiae* complex showed a predominance of *An. arabiensis*, with over 86% in the city of Ouagadougou with a significant higher abundance in the urban district. This suggests a better adaptation of *An. arabiensis* to the urban environment compared to the other members of the complex, such as *An. gambiae* s.s and *An. coluzzii*. Similarly, in the second-largest city of the Burkina Faso, *An. arabiensis* has been found replacing *An. gambiae* s.s to become the predominant *Anopheles* species (Dabiré *et al.*, 2012), suggesting its better adaptation to the polluted urban environment (Jones *et al.*, 2012).

Overall, more *Anopheles* mosquitoes were collected outdoors than indoors, suggesting an exophilic behaviour. Indeed, *An. arabiensis*, the predominant

*Anopheles* mosquitoes in Ouagadougou, displays a preference for outdoor resting as collected more outdoors. *An. arabiensis*, is recognized as the exophilic species within the *Anopheles gambiae* complex (Fournet *et al.*, 2010; Doumbe-Belisse *et al.*, 2018; Fournet *et al.*, 2022). The exophilic behavior observed in *An. arabiensis* can be a response to the increased use of insecticide-treated bed nets and indoor spraying of insecticides (Perugini *et al.*, 2020).

The determination of the origin of blood meals showed an average anthropophilic rate of 63.16%, suggesting a preference for human blood for *An. arabiensis*, the predominant species as already reported in Ouagadougou (Gnémé *et al.*, 2019). *An. arabiensis* seems to display a heterogeneous blood feeding preference as some reported a zoophilic rate of nearly 40% (Perugini *et al.*, 2020), and 90.3 % attraction for cattle odour than human using odour-baited entry traps (Mahande *et al.*, 2007). Indeed, *An. Arabiensis* opportunistic blood-feeding behaviour may depend on hosts availability. However, during this, a link between host availability and the blood-feeding behaviour has been tested. Also, the high proportion of individuals for which the origin of blood-feed could not be identified represents a limitation of the study. Further analyses should extend blood-meal identification to additional hosts.

## CONCLUSION

This study reports that *An. coluzzii* and *An. arabiensis* occur in sympatry in Ouagadougou, with *An. arabiensis* is the predominant malaria vector. Well-adapted to the urban environment, *An. arabiensis* exhibits exophilic behaviour and a strong preference for human hosts, specifically in urban districts. This predominance, combined with its exophilic and anthropophilic behavior, makes *An. arabiensis* a major contributor to malaria transmission in Ouagadougou. These findings should be considered when designing and implementing sustainable vector control strategy to prevent the expansion of the urban malaria.

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