



Dominance and Diversity of *Anopheles gambiae* s.l (Female Mosquitoes) Collected from Six Local Government Areas in Adamawa State, Northeastern Nigeria

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ABSTRACT: Malaria continues to pose a formidable health challenge, particularly in regions where *Anopheles* mosquitoes are highly effective vectors for disease transmission. Hence the objective of the paper was to assess the dominance and diversity of *Anopheles gambiae* s.l (adult female mosquitoes) collected from six Local Government Areas in Adamawa State, North-eastern Nigeria using Coetzee's identification key. The morphological identification revealed that *Anopheles gambiae* s.l was significantly the dominant species across all the areas studied, ranging from 97.3% in Hong to 99.7% in Gombi of all identified species, with 1.67% *Anopheles pretoriensis* identified in Hong LGA only. The presence of *Anopheles pretoriensis* in Hong LGA and its absence in other LGAs indicates geographical variations in species distribution. Molecularly, *Anopheles coluzzii* was the dominant sibling species observed across all Local Government Areas (LGAs), with its presence ranging from 66.7% in Mubi South to 83.3% in Mubi North, Gombi, Jada, and Mayo-Belwa of all identified *Anopheles gambiae* sensu lato (s.l). While a few samples of *Anopheles gambiae* and *Anopheles arabiensis* were identified, these were not found in all LGAs. Therefore, *Anopheles gambiae* s.l emerged as the dominant species identified morphologically, while molecularly, *Anopheles coluzzii* was the most prevalent sibling species for *Anopheles gambiae* s.l found in all six (6) LGAs. To mitigate the transmission risk of malaria, it is imperative to implement a multifaceted vector control strategy that effectively targets both exophilic and endophilic mosquito species.

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Anopheles gambiae sensu lato (s.l) significantly impacts malaria transmission and human health, especially in Africa. Malaria continues to pose a formidable health challenge, particularly in regions where *Anopheles* mosquitoes are highly effective vectors for disease transmission. The predominant malaria vectors are the Anophelines which comprise

approximately 480 species, of which only about 80 are considered malaria vectors (Dahan *et al.*, 2020). The composition of *Anopheles* species varies, depending on the geographic region, consequently, the vectors responsible for malaria transmission differ across regions (WHO, 2012). *Anopheles gambiae* sensu lato is the most significant malaria

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vector in Africa. This group consists of seven morphologically indistinguishable sibling species: *Anopheles gambiae* sensu stricto, *Anopheles arabiensis*, *Anopheles coluzzii*, *Anopheles melas*, *Anopheles merus*, *Anopheles amharicus*, and *Anopheles bwambae*. Collectively, these species are referred to as the "gambiae complex" or "gambiae group." (Ebenezer *et al.*, 2012). *Anopheles gambiae* sensu stricto exists in two molecular forms, M (now *Anopheles coluzzii*) and S (now *Anopheles gambiae*), distinguishable by differences in a 4-Mb region on the X chromosome (Ranford *et al.*, 2016). This increases the number of species to 8. Research shows that *Anopheles gambiae* sensu stricto, *Anopheles coluzzii*, and *Anopheles arabiensis* exhibit exceptional capacity for malaria transmission, making them the focus of vector control efforts (Coetzee, 2013). Additionally, the adaptability of *Anopheles melas*, *Anopheles merus*, and *Anopheles bwambae* to mineral-rich or saline environments demonstrates the diverse ecological niches these species can occupy (Kengne, 2019). Their public health significance arises from their strong attraction to humans and their ability to adapt to various environmental conditions influenced by human activities, either directly or indirectly (Kabula, 2011). This suggests that they can develop different mechanisms for survival depending on the diverse environmental factors defined by various physicochemical parameters. These differences may result in different medical importance of the sibling species of a complex and can also have important implications in vector control (WHO, 2012).

It is well known that studying the dominance and diversity of *Anopheles gambiae* s.l, especially in regions of high malaria transmission would provide valuable insights that inform effective and targeted interventions based on its diversity, distribution, and ecology. Therefore, the objective of the paper is to assess the dominance and diversity of *Anopheles gambiae* s.l (adult female mosquitoes) collected from six Local Government Areas in Adamawa State, Northeastern Nigeria

MATERIALS AND METHODS

Study area: This study was carried out in six Local Government Areas of Adamawa State. Adamawa State is one of Nigeria's largest states in the northeastern part, covering approximately 38,700 km² with an estimated population of 4,502,132 (NIPC, 2020). It lies between latitude 9°19'60.00"N and longitude 12°09'59'99" E. Yola is the administrative and commercial capital. It borders Borno State to the northwest, Gombe State to the west, and Taraba State to the southwest. The eastern border of Adamawa State forms the national boundary with Cameroon. It

has major vegetation formations comprising Guinea and Sudan Savannas (NIPC, 2020). The mean annual rainfall ranges from 700mm to 1600mm (Akosim *et al.*, 1999). The rainiest months are August and September, drought begins from November to April. January through March recorded the lowest relative humidity ranging from 20% to 30% with a steady increase from April to a peak of about 80% around August and September (Adebayo *et al.*, 2020). The six LGAs selected for the study were Mubi-North, Mubi-South, Hong, Gombi, Jada and Mayo-Belwa. Mubi-North is located at Latitude 10°06'-10°07'-13°30'E, Longitude 13° 07'-13°30'E; Mubi-South is located at Latitude 10°06'-10°29'N, Longitude 13°07'13°30'E; Hong is located at Latitude 9°58'-10° 35'N, Longitude 12° 35'-13°E; Gombi is located at Latitude 9°59'-10° 27'N, Longitude 12°14'-12°50'E; Jada is located at Latitude 8°27'-8°54'N, Longitude 11°55'-12°48'E and Mayo-Belwa is located at Latitude 8°33'-9°12'N, Longitude 11°41'-12°11'E (Adebayo *et al.*, 2020).

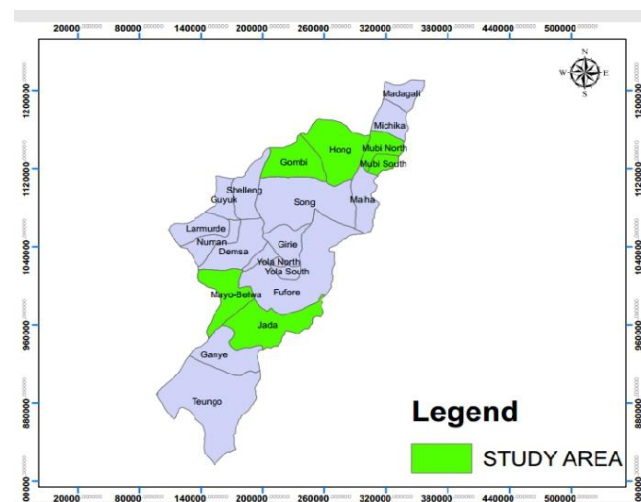


Fig. 1: Map of Adamawa State highlighting the study area.

Ethical consideration: Ethical cooperation and support of the Malaria Elimination Program offices at both the State and Local Government levels were sought. Community leaders at the various sentinel sites where mosquito larvae were collected, were approached and informed about the study, their cooperation was requested to ensure the successful conduct of the research.

Larval collection: *Anopheles* mosquito larvae were collected according to the Standard Operational Procedure described by WHO (2012). Briefly, a ladle was used to collect *Anopheles* mosquito larvae, transferred to a container, and transported to the Institute of Infectious Diseases of Poverty (IIDP) laboratory in Modibbo Adama University (MAU)

Yola. The samples were reared to adulthood. The emerged adult mosquitoes were fed with a 10% sugar solution.

Morphological identification: Three thousand six hundred (3600) female *Anopheles* mosquitoes were identified using Coetzee's identification key (Coetzee, 2020) and stored in appropriately labeled Eppendorf tubes containing silica gel.

Polymerase Chain Reaction for Sibling Species Identification: A total of one hundred and eighty (180) female *Anopheles gambiae* s.l were selected, with thirty samples taken from each Local Government Area (LGA), to represent the entire population. These samples were sent to the Nigerian Institute of Medical Research (NIMR) for sibling species identification. The identification was performed using the method outlined by Wilkins *et al.* (2006).

Primer sequences

IMP-UN 5' GCTGCGAGTTGTAGAGATGCG 3'
AR-3T 5' GTGTTAAGTGTCTTCTCCGTC 3'
GA-3T 5' GCTTACTGGTTTGGTCGGCATGT 3'
ME-3T 5' CAACCCACTCCCTTGACGATG 3'
QD-3T 5' GCATGTCCACCAACGTAAATCC 3'
IMP-S1 5' CCAGACCAAGATGGTTCGCTG 3'
IMP-M1 5' TAGCCAGCTCTTGTCCACTAGTTTT 3'

Sibling species identification: Sibling species identification was carried out using the method described by Wilkins *et al.* (2006). This method of *Anopheles gambiae* complex discrimination is based on species-specific single nucleotide polymorphisms (SNPs) in the intergenic spacer region (IGS). Primers were added to this method to simultaneously elucidate the Ribosomal DNA type. These additional primers also incorporate intentional mismatches into the primers (Intentional Mismatch Primers (IMPs)) to increase the specificity (Wilkins *et al.* 2006).

Procedure: Enzyme mix was prepared for one 12.5µl PCR reactions, reagents were added in the order; ddH₂O 5.5µl, Pre mix (×5) 2.5µl, IMP-UN 0.5µl, AR-3T 0.5µl, GA-3T 0.5µl, ME-3T 0.5µl, QD-3T 0.5µl, IMP-S1 0.5µl, IMP-M1 0.5µl and DNA template 1.0µl

PCR cycle conditions

95°C/5min x 1 cycle; (95°C/30sec, 59.2°C/30sec, 72°C/30sec) x 30 cycles; 72°C/5min x 1 cycle 4°C hold. Samples were run on a 1.5% agarose gel stained with EtBr; 10 µl samples were loaded. Primers create fragments of 636 *An. quadriannulatus*, 528 *An.*

merus, 463 *An. gambiae* s.s, 387 *An. arabiensis*, 333 *An. coluzzi* M and 221 *An. gambiae* S.

Data analysis: Data were analyzed using SPSS version 29.0 for Windows. Descriptive statistics were employed to present the results in tables and percentages.

RESULTS AND DISCUSSION

The composition of species indicates that *Anopheles gambiae* s.l and *Anopheles pretoriensis* were the two *Anopheles* mosquito species identified morphologically. *Anopheles gambiae* s.l was the significant ($P = <0.0001$) dominant sibling species recorded in all the six LGAs with 99.7%, 99.5%, 99.3%, 98.6%, and 97.3% in Gombi, Mayo-Belwa, Mubi-South, Jada, and Mubi-North LGAs, respectively. Meanwhile, 1.67% of the *Anopheles* mosquitoes identified in Hong LGA were *Anopheles pretoriensis*. However, 1.33%, 0.66%, 1.00%, 0.33%, 0.66%, and 0.50% of samples from Mubi North, Mubi South, Hong, Gombi, Jada, and Mayo Belwa, respectively, were unidentified (Table 1).

The distribution of sibling species within *Anopheles gambiae* s.l indicates that *Anopheles coluzzii* was the significantly ($P = <0.0001$) dominant sibling species recorded in all six LGAs with 83.3% in Mubi North, Gombi, Jada, and Mayo-Belwa LGAs, 73.3% in Hong LGA, and 66.7% in Mubi South LGA. Meanwhile, 3.3% of the *Anopheles gambiae* s.l identified in Gombi LGA, and 6.7% of those identified in Mubi North LGA were *Anopheles gambiae*, while 3.3% of the *Anopheles gambiae* s.l identified in Hong LGA, and 6.7% of those identified in Mayo Belwa LGA were *Anopheles arabiensis*. However, 33.3%, 23.3%, 13.3%, 16.7%, and 10.0% of samples from Mubi-South, Hong, Gombi, Jada, Mubi-North, and Mayo-Belwa, respectively, did not amplify (Table 2).

Anopheles gambiae s.l was the significant dominant species identified across all six Local Government Areas (LGAs), this indicates that *Anopheles gambiae* s.l is the most prevalent species of *Anopheles* mosquito in these regions. This species is recognized as a major vector for malaria, and its dominance implies that it significantly contributes to malaria transmission in these areas. (Santolamazza *et al.*, 2008). The prevalence of *Anopheles gambiae* s.l in this study aligns with numerous investigations throughout Africa identifying this species complex as the main vector of malaria. (Coetzee *et al.*, 2013; Yoriyo *et al.*, 2014; Garba *et al.*, 2017; Oduola *et al.*, 2019; Wahedi *et al.*, 2020). However, this is contrary to studies conducted in neighboring Gombe State that

reported *Anopheles pretoriensis* as the dominant *Anopheles* species (Abba *et al.* 2024)

Anopheles pretoriensis was identified only in Hong LGA and was not found in any of the other LGAs. The presence of *Anopheles pretoriensis* in Hong LGA and its absence in other LGAs indicates geographical variations in species distribution. The region's climate and geography might provide a favorable environment for the proliferation of *An. pretoriensis* (Adeogun *et al.*, 2024). The presence of *An. pretoriensis* alongside other malaria vectors in the same area can complicate control efforts as extant malaria vector control strategies in Nigeria primarily centered on endophilic and endophagic mosquitoes, which might not effectively control exophilic species like *An. pretoriensis* (Abba *et al.*, 2024). This underscores the importance of localized vector surveillance for effective malaria control. (Simka *et al.*, 2012). *Anopheles pretoriensis* has been identified as a secondary malaria vector responsible for *Plasmodium falciparum* transmission in certain areas of Sub-Saharan Africa. (Goupeyou-Youmsi *et al.*, 2020; Bedasso *et al.*, 2022; Assa *et al.*, 2023; Saili *et al.*, 2023).

A few samples from each LGA were unidentified. This could be due to various factors, such as damage to the specimens, making them difficult to identify, or a species that is not easily identifiable using the morphological methods employed.

Anopheles coluzzii was identified as the predominant sibling species across all six Local Government Areas (LGAs) using PCR-RFLP analysis. The dominance of *Anopheles coluzzii* has also been reported in certain regions of Adamawa State (Wahedi *et al.*, 2020) and Taraba State (Lamidi *et al.*, 2017). Additionally, the prevalence of *Anopheles coluzzii* over *Anopheles gambiae* and *Anopheles arabiensis* has been documented in Benin (Djouaka *et al.*, 2008; Yadouleton *et al.*, 2010) and Lagos (Oduola *et al.*, 2012). The presence of *An. coluzzii* is likely due to habitat segregation and strong assortative mating in natural environments. The predominance of *Anopheles gambiae* was reported, which differed from our findings (Awolola *et al.*, 2018; Ebenezer *et al.*, 2012; Ebenezer *et al.*, 2014; Obembe *et al.*, 2018; Oduola *et al.*, 2019).

Anopheles gambiae and *Anopheles arabiensis* were identified in small numbers, but not in all LGAs. Wahedi *et al.* (2020) reported the presence of several *Anopheles gambiae* and *Anopheles arabiensis* collected from various locations in Adamawa State.

In a similar study, Obembe *et al.* (2018) observed a low occurrence of *Anopheles arabiensis* in two communities within the Guinea Savannah region of Kwara State.

Both *Anopheles coluzzii* and *Anopheles gambiae* are naturally anthropophilic (preferring to feed on humans) and endophilic (preferring to live indoors), making them efficient vectors for malaria in Africa (Killeen, 2014). In contrast, *Anopheles arabiensis* is known to be zoophilic (preferring to feed on animals) and exophilic (preferring to live outdoors) (Mayagaya *et al.*, 2015). *Anopheles gambiae* is better suited to small, temporary breeding sites, while *Anopheles coluzzii* favors more permanent, flooded breeding habitats (Gimonneau *et al.*, 2014). Meanwhile, *Anopheles arabiensis* tends to breed in ephemeral, sunlit water pools.

Adamawa State is characterized by floodplains that contain numerous rivers flowing through the Fadama lands across the region (Adewumi, 2013). These floodplains may have offered relatively permanent breeding sites that were preferred by *Anopheles coluzzii* larvae, which could explain their higher prevalence compared to *Anopheles gambiae* and *Anopheles arabiensis* in all the areas examined in this study.

The unidentified samples may represent other potential vector species or genetic variations that require further investigation. This emphasizes the importance of ongoing research to identify and understand the full range of malaria vectors. A significant proportion of samples from each Local Government Area (LGA) failed to amplify, with rates ranging from 10.0% to 33.3%. This could be due to issues with the DNA extraction procedure or PCR processes, suggesting that some *Anopheles gambiae* s.l could not be accurately identified at the species level using the molecular methods employed. Therefore, it is crucial to use robust molecular techniques for accurate species identification.

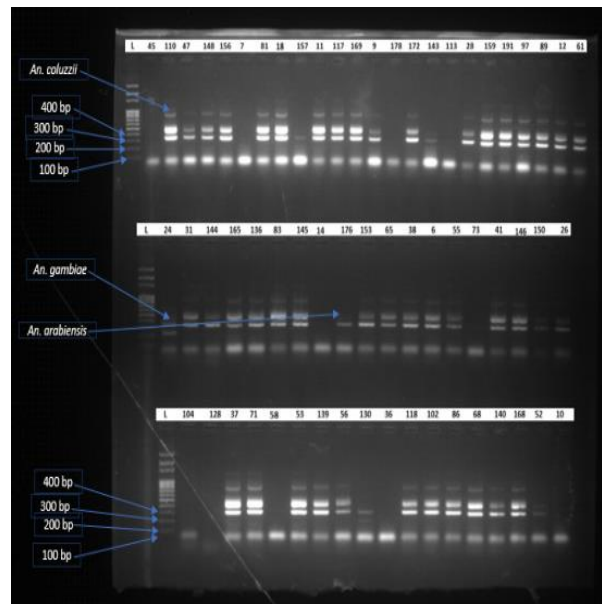
These findings offer valuable insights into the composition of *Anopheles* species in the studied areas, which are essential for developing effective vector control strategies. The dominance of *Anopheles gambiae* s.l, particularly *Anopheles coluzzii*, underscores the importance of directing control measures towards these specific species. Additionally, the presence of other species and unidentified samples in this study indicates the need for continuous surveillance and identification efforts.

Table 1: Morphological identification of *Anopheles* mosquitoes in the study area

LGAs	Number Identified (N)	Species Composition (%)		
		<i>An. gambiae</i> s.l	<i>An. pretoriensis</i>	Unidentified
Mubi-North	600	592(98.6)	0(0)	8(1.33)
Mubi-South	600	596(99.3)	0(0)	4(0.66)
Hong	600	584(97.3)	10(1.67)	6(1.00)
Gombi	600	598(99.7)	0(0)	2(0.33)
Jada	600	596(99.3)	0(0)	4(0.66)
Mayo-Belwa	600	597(99.5)	0(0)	3(0.50)
Total	3600	3,563(99.0)	10(0.28)	27(0.75)

Table 2: Diversity of sibling species of *Anopheles gambiae* s.l in the study area

LGAs	No. Examined	Sibling Species (%)			No amplification (%)
		<i>Anopheles coluzzii</i>	<i>Anopheles gambiae</i>	<i>Anopheles arabiensis</i>	
Mubi-North	30	25 (83.3)	2 (6.7)	0 (0)	3 (10.0)
Mubi-South	30	20 (66.7)	0 (0)	0 (0)	10 (33.3)
Hong	30	22 (73.3)	0 (0)	1 (3.3)	7 (23.3)
Gombi	30	25 (83.3)	1 (3.3)	0 (0)	4 (13.3)
Jada	30	25 (83.3)	0 (0)	0 (0)	5 (16.7)
Mayo-Belwa	30	25 (83.3)	0 (0)	2(6.7)	3(10.0)
Total	180	142 (78.9)	3(1.67)	3(1.67)	32(17.8)

**Fig. 2:** Gel electrophoresis of PCR-RFLP product for the identification of *Anopheles gambiae* s.l sibling species. Fragments of 387= *An. arabiensis*; 333=*An. coluzzii* (M-form); and 221=*An. gambiae* (S-form)

Conclusion: These findings revealed the complex and diverse nature of *Anopheles* mosquitoes in Adamawa State. The dominance of *Anopheles gambiae* s.l, along with the prevalence of specific sibling species and some unidentified samples, underscores the importance of continuous surveillance, suggesting potential shifts in species composition or local environmental factors influencing these populations. Given these insights, it is imperative to implement a multifaceted vector control strategy that effectively targets both exophilic and endophilic mosquito

species. Such an approach is crucial for enhancing vector control efficacy and mitigating the transmission risk associated with malaria and other diseases carried by these vectors.

Declaration of conflict of interest: The authors declare no conflict of interest

Data availability statement: Data are available upon request from the first author.

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