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Case Study for Undetermined Mosquito Species by Polymerase Chain Reaction in Western Burkina Faso

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Abstract

Introduction: Malaria eradication campaigns all over the world are largely based on parasite and vector control. Vector identification, whether morphological or molecular, is an essential component of vector control. This study analyzed the possible causes of indeterminate polymerase chain reaction (PCR) results for mosquito species in Western part of Burkina Faso. **Methodology:** From July 2021 to November 2021, mosquitoes were collected during the period of high malaria transmission in the village of Séguéré, Houet province, Burkina Faso, and morphologically identified. After DNA extraction, samples were amplified by sine 200× PCR to identify species of the *Anopheles gambiae* complex. Indeterminate samples were then selected for further analysis. The parameters studied were: DNA dilution, the effect of protocol adjusting, and the type of protocol used. **Results:** A total of 130 "indeterminate" DNAs diluted 1:10 were analyzed. After dilution, the mean amount was 14.73 ± 3.59 ng/µL and absorbance 1.71 ± 0.1. PCR chain reaction yielded 94.62% (123/130) anopheline species in SINE PCR, 5.38% (7/130) "negative". A significant difference between SINE PCR before dilution and after dilution was observed ($P < 0.001$). Identification tests carried out using other protocols gave no positive results. From these results, we note that the adaptation of the protocol significantly reduced the polymerase amplification results of the species. **Conclusion:** It is therefore necessary to respect the amplification protocols. However, the persistence of "indeterminate" results suggests that further studies should be carried out to shed more light on the subject.

Keywords

Malaria, Vector, DNA, PCR

1. Introduction

Malaria, a vector-borne disease, is caused by the protozoan parasites of *Plasmodium* spp transmitted to humans through the bite of an infected *Anopheles* female mosquito [1] [2]. Globally, a total of 494 species of the subfamily Anophelinae are currently recognized, and Approximately 70 species of formally recognized Anopheles are human malaria vectors [3] [4]. Burkina Faso, four vectors transmit almost all malaria parasites: *Anopheles gambiae*, *Anopheles coluzzii*, *Anopheles arabiensis* and *Anopheles funestus [5]*. Nowadays, major programs to control and eliminate malaria are based on the treatment of clinical cases using artemisinin-based therapeutic combinations (ACT), chemo-prevention of seasonal (SMC) malaria and vector control [6] [7].

Vector control appears to be one of the main mass prevention methods applicable against malaria. This method includes physical destruction of breeding sites, biological and chemical control, and genetic control [8] [9]. Malaria control strategy choice must therefore be based on all available knowledge of vector populations [8] [10]. The characterization and identification of malaria vectors have always been a major concern in vector control. Vector identification is not just an academic exercise for taxonomists, but it is a fundamental phase in any control operation, from planning to evaluation. Species recognition is mandatory for measuring the role played by each species in transmission, particularly in sympatric areas, and for identifying and therefore "targeting" vectors in a control program.

Species were initially described using essentially morphological criteria, based on morphological characters and the use of "determination keys" established for each major zoogeographical region because vectors differ according to these regions [8]. However, other methods have been used in taxonomy, including the cytogenetic method based on the observation and study of polytene chromosomes [11]; the study of para-centric inversions of polytene chromosomes, and more recently molecular analyses. We also have the iso-enzymatic method, a technique based on the electrophoretic mobility of certain enzymes due to the polymorphism of amino acid sequences. Nowadays, DNA amplification, mainly by polymerase chain reaction or PCR, coupled with DNA polymorphism analysis, is the method of choice for mosquito species molecular identification and characterization. This tool can be applied to any stage of development, using specimens simply preserved in a dry state or in ethanol, and a small part of the insect is sufficient (mosquito legs, for example). Thus, the same specimen can be identified by PCR from leg DNA and undergo several other tests, for hybridization experiments or for ELISA tests (on the head-thorax for the detection of cir-

cumsporozoite proteins, on the abdomen for the identification of the blood meal) or be preserved as a collection specimen. It allows the discrimination between species having a very similar, or even indistinguishable, morphological appearance, which were previously designated under the same term [8].

The identification of anopheline species using the molecular method can lead to the risk of "indeterminate" results, *i.e.* individuals who, on the basis of morphology, should normally be identified as a species. In the case of "indeterminate" results, the causes may include problems with mosquito samples, incorrect morphological identification, the existence of unknown species, or changes to protocols by different laboratories. Indeterminate results are rarely taken into account in laboratories, especially when the number of mosquito samples to be analyzed has been reached but these samples can provide important information. In the present study, we analyze the possible causes of indeterminate species PCR results for anopheline species in western Burkina Faso using PCR developed by different authors.

2. Methodology

2.1. Study Site and Mosquito Collection

We conducted an experimental study from July 2021 to November 2021, the high transmission period, in the village of Séguéré. The village is located (11˚29'39.12"N 4˚14'0.01"W) in Dandé health district, in the Hauts Bassins region of western Burkina Faso. In this region, *An. gambiae* s.l. and the remaining *An. arabienses*, *An. funestus* represent the majar vecor. In the *An. gambiae* s.l. complex, *An. gambiae* s.s. and *An. coluzzi* are predominant [12] [13].

Mosquitoes were captured using the residual fauna collection (RFC) technique through indoor residual spraying (IRS) with pyrethrin and derivatives. Mosquitoes were collected from white sheets spread over the entire floor of the room. This method made it possible to collect endophilic species, as well as semi-gravid, gravid, and fed or no fed specimens [14].

After collection, specimens were brought back from the field to the Institut de Recherche en Sciences de la Santé de Bobo-Dioulasso (IRSS/DRO) molecular biology laboratory and asleep in the cold. *Anopheles* mosquitoes were morphologically identified according to Gillies and De Meillon keys [15]. After this identification, each species was placed in individual tubes and stored at −20˚C in a freezer. These specimens were later used for DNA extraction.

2.2. Sampling of Indeterminate Mosquitoes

DNA from the thorax and heads of each mosquito was extracted using the CTAB (cetyltrimethylamonium bromide) protocol. Briefly, the thorax heads were ground in 200 µl of 2% CTAB solvent, then placed for 5 minutes in a Water Bath at 65˚C for inactivation of cellular nucleases. After removal of the CTAB, 200 µl of chloroform was added to each grind and mixed by inversion, then centrifuged for 5 minutes at 12,000 rpm to separate the nucleic acid in the superna-

tant from the debris. The supernatant was removed and placed in another 1.5 ml eppendorf tube, into which 200 µl of isopropanol was added. The whole mixture was then mixed by inversion and centrifuged for 15 minutes at 12,000 rpm. After centrifugation, the supernatant-isopropanol mixture was emptied and the tube was drained on paper towels. Next, 200 µl of 70% ethanol was added to the tube for centrifugation for 5 minutes at 12,000 rpm, to allow further purification or elution of residual salts. The 70% ethanol was then added to the tube and the pellet dried for a maximum of 5 minutes on speed-vac (eppendorf concentrator Plus)Finally, the DNA was suspended at room temperature overnight in 20 µl of ultrapure water and stored at −20˚C [16]. DNAs "indeterminate" in SINE 200× PCR were selected for further experimentation.

2.3. DNA Dilution

The previous extraction was not performed using whole mosquitoes but head or thorax, which are enough to obtain DNA, so the amplification protocol was adapted using a 2 µl volume of pure, undiluted DNA. According to the standard protocol, a 1/10 dilution should be performed. To verify the hypothesis of DNA dilution in false-negative mosquitoes, we therefore carried out dilutions prior to PCR testing. In a sterile 1.5 ml tube, pure DNA was diluted by taking 2 µl of DNA for 18 µl of sterile distilled water. The mixture was then vortexed and centrifuged to homogenize the pipetted DNA. Only this dilution is used for all assays.

2.4. DNA Quantification

Selected DNAs were assayed by nanodrop (Thermo Scientific NanoDrop Lite) in order to eliminate the hypothesis of poor extraction in the previous tests. Spectrophotometry was used for the nucleic acid assay to verify the purity of the extracted DNA. Nucleic acid concentration was determined at 260 nm against a "blank" sample. DNA purity is represented by the A260/A280 ratio, with a value for "pure" DNA commonly in the range of 1.8 to 2, and concentration in the range 0.2 to 27500 ng/µL [17]. This step was necessary to ensure the quantity and quality of the DNA because a poor extraction technique can negatively influence the amplification and consequently the identification of samples.

2.5. Amplifications

PCR SINE 200X

A series of amplifications were carried out with various protocols to exclude the sensitivity and specificity of the diagnostic techniques. Samples remaining "indeterminate" after the first reaction underwent a second amplification using the SINE 200× PCR of Santolamazza *et al.* [18] to exclude human operator error. The forward and reverse primer sequences used were: SINE 200X.6.1. F: 5'-TCGCCTTAGACCTTGCGTTA-3' and SINE 200X. 6.1. R:5'-CGCTTCAAG AATTCGAGATAC-3'Amplification was carried out according to the following

program: denaturation at 94˚C for 10 min, allowing total denaturation of the DNA and activation of the Taq polymerase, followed by 35 cycles consisting of a denaturation step at 94˚C for 30 s, hybridization at 54˚C for 30 s and extension at 72˚C for 1 min. The last extension step is extended by holding at 72˚C for 10 min. The expected sizes of the species are 479 base pairs (bp) for *An. coluzzii*, 249 bp for *An. gambiae* and 223 bp for *An. arabiensis*.

Following this second SINE 200X amplification, the still negative samples were amplified by other protocols initially used in the IRSS/DRO molecular biology laboratory.

PCR Favia

The "indeterminate" samples were amplified using the protocol of Favia *et al.* (2001) [19] to distinguish species *An. coluzzii* and*An. gambiae,* respectively old molecular form M *(Mopti) S (Bamako/Savana).* This protocol uses 4 primers: R3: 5'CGAATTCTAGGGAGCTCCAG3'; R5: 5'GCCAATCCGAGCTGATAG CGC3'; Mopint: 5'GCCCCTTCCTCGATGGCAT3'; B/S: 5'ACCAAGATGGTT CGTTGC3'. The amplification program was as follows: 3 minutes at 94˚C, 28 cycles of 30 seconds at 94˚C, 30 seconds at 63˚C and 45 seconds at 72˚c and finally 5 minutes at 72˚c for extension. Typical bands for molecular forms M and S were obtained at 727 bp and 475 bp respectively.

PCR Scott-Fanello

It is a technique for the simultaneous identification of the anopheline species of the *An. gambiae* complex. This method combines protocols established by Scot *et al.* (2013) and Favia *et al.* (1997) to identify all *An. gambiae* s.l and form M (*An. coluzzii*), and form S (*An. gambiae*) within *An. gambiae* s.s. It targets the ribosomal DNA (rDNA) region in the intergenic space (IGS). It begins with amplification for differential identification using three pairs of primers. The first primer UN (5'GTGTGCCGCTTCCTCGATGT3') is universal and amplifies the IGS part of the complex, the second AA (5'CTGGTTTGGTCGGCACGTTT3') specific to *An. arabiensis* and the last primer AG (5'AAGTGTCCTTCTCCATC CTA3') identity *An. gambiae*. Amplification was performed according to the following program: 94˚C for 3 min; 40 cycles of 94˚C for 30 s, 50˚C for 30 s and 72˚C for 30 s and finally 72˚C for 5 min. The expected sizes after amplification were 315 bp for *An. arabiensis* and 390 bp for *An. coluzzii* and *An. gambiae* species. In a second step, an enzymatic digestion with the Hhal (*Haemophilus haemolyticus*) enzyme is performed to differentiate *An. gambiae* and *An. coluzzii* species. The amplification products were distributed (10 µl) in the 96-well PCR plate, containing each 15 µl of the "Master MIX" previously prepared on ice (**Table 1**). Expected sizes are 257 bp for *An gambiae*, and 367 bp for *An. coluzzii* after digestion.

Cohuet *et al.* (2003)

Based on the hypothesis of morphological misidentification, we performed amplification targeting the *An. funestus* complex. This PCR is based on interspecies variations in the internal transcribed space 2 (ITS2) of ribosomal DNA (rDNA) using the primer pair FUN F 5'-TGT GAA CTG CAG GAC ACA T 3' et FUN

Table 1. Composition of the reaction mix for the different protocols.

Protocols	Master mix Reagents	Vol. for 1 rxs* at 25 ul
SINE 200X	Buffer 5×	4
	25 Mm $MgCl_2$	2
	5 Mm d'NTPS	0.8
	Primer F (20 Um)	0.3
	Primer R (20 Um)	0.3
	Taq DNA polymerase (5 U/ul)	0.07
	D dH_2O	10.53
	DNA template (1/10 CTAB)	2
Favia	Master mix Reagents	Vol. for 1 rxs at 25 ul
	Buffer 5×	5
	25 Mm $MgCl_2$	1
	5 Mm d'NTPS	1
	R3 (10 Um)	0.75
	R5 (10 Um)	0.75
	B/S (10 Um)	1.75
	Mop (10 Um)	1.75
	Taq DNA polym (5 U/ul)	0.05
	D dH_2O	11.95
	DNA template (1/10 CTAB)	1.5
Scott	Master mix Reagents	Vol. for 1 rxs at 25 ul
	5× Buffer	5
	25 Mm $MgCl_2$	1.5
	5 Mm d'NTPS	1
	UN (10 Um)	0.5
	AG (10 Um)	0.5
	AR (10 Um)	0.5
	Taq DNA polym (5 U/ul)	0.05
	D dH2O	13.95
	DNA template (1/10 CTAB)	2
Cohuet	**Master mix Reagents**	**Vol. for 1 rxs at 25 ul**
	5× £Buffer	5
	25 Mm $MgCl_2$	1
	5 Mm d'NTPS	1
	FUN F (10 µM)	0.5

Continued

FUN R (10 µM)	0.5
Taq DNA polym (5 U/ul)	0.1
D dH_2O	13.9
DNA template (1/10 CTAB)	3

*Volume for one reaction.

5'-GCA TCG ATG GGT TAA TCA TG 3'. Three microliters of 1:10 diluted DNA were added after dispensing 22 µl of the master mix (Table 1) into 96-well PCR plates. Amplification conditions were: 94˚C for 2 minutes, followed by 36 cycles of 94˚C for 30 seconds, 45˚C for 30 seconds, 72˚C for 40 seconds, and finally 72˚C for 5 minutes. *An. funestus* group was indicated by the presence of fluorescent bands at 505 bp [10].

All amplifications took place in the Applied Biosystems 2720 thermocycler, and migration of PCR products on 2% agarose gel was performed in migration cuvettes. Bands were visualized using a transluminator after staining with ethidium bromide. Table 1 shows the composition of the reaction mix for the different protocols.

2.6. Data Processing and Analysis

The data collected were entered using Microsoft Excel 2016. Statistical processing and analysis were carried out using R software version 4.0.3. Graphs and tables were produced using Excel 2016. Word version 2016 was also used to format tables and graphs. The significance threshold for comparing two proportions was set at $P < 0.05$.

3. Results

A total of 130 "indeterminate" DNAs were analyzed during our study. These DNAs were selected during the five months of the peak malaria transmission period.

3.1. *A*nopheles Mosquito DNA Quantification through Nanodrop Assay

The average amount of DNA before dilution (172.83 ± 37.59 ng/µl) was approximately 12 times greater than the amount of DNA assayed after dilution (14.73 ± 3.59 ng/µl). The same pattern was found with optical density (OD), with mean ODs of 1.82 and 1.71 before and after dilution respectively. These mean amounts were within the range required to initiate amplification.

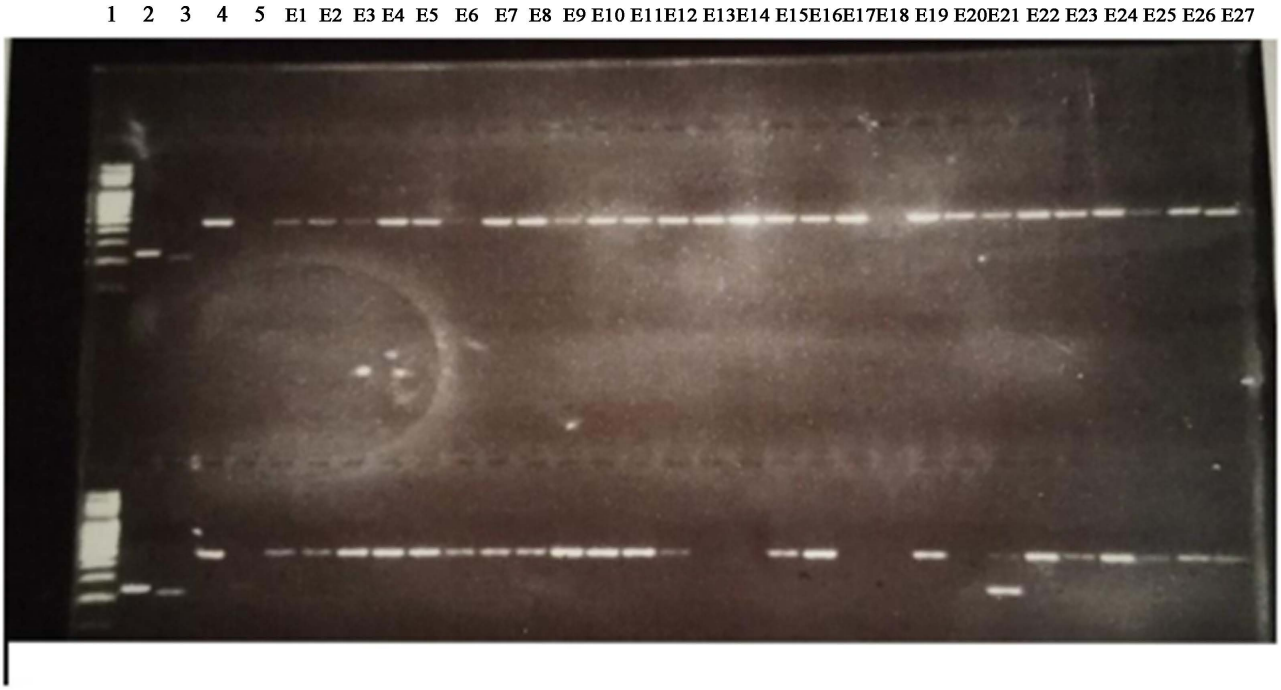
3.2. PCR SINE 200X

SINE PCR performed before dilution and after dilution after the second amplification differs in the number of species identified in each test. Table 2 shows the results obtained.

Of the 130 diluted DNA samples, the SINE 200X protocol identified 94.61%

Table 2. SINE 200X PCR result before and after DNA dilution.

	PCR positive (sample number)	PCR négative (Sample number)	P-value
Before dilution	0	130	$P < 0.001$
After dilution	123	7	



lane1 = 1 kb ladder, lane 2 = *An. gambiae* control, lane 3 = *An. arabiensis* control, lane 4 = *An. coluzzii* control, lane 5 = negative control, lane E1 to E27 = samples

Figure 1. DNA band after electrophoresis with sine 200× protocol.

anopheline species, of which 3.07% were *An. gambiae*, 0.76% *An. arabiensis* and 90.76% *An. coluzzii*. Statistical analysis showed a significant effect between SINE PCR results before dilution and after 1/10 dilution ($LRTX_2$ 1 = 90.9, $P < 0.001$).

Figure 1 shows species positivity. Dilution appears to have had an effect on amplification success.

The two amplification protocols, Favia *et al.* (2001) and Fanello *et al.* (2002), previously used by the laboratory for the identification of anopheline species and still used in the case of identification problems, failed to produce a band after migration.

The hypothesis of morphological misidentification led us to carry out a PCR of the *An. funestus* group. The protocol used to identify *An. funestus* gave no positive result on the 07 "indeterminate" samples.

4. Discussion

In this study, our aim was to investigate the causes of the "indeterminate" PCR results obtained on mosquitoes of the *An gambiae* complex, by suggesting a number of hypotheses. The DNA quantification was used to determine the im-

pact of DNA concentration on PCR results. An insufficient quantity of DNA does not allow for adequate amplification, while too much DNA can inhibit the reaction due to the accumulation of contaminants [20]. Our results showed that the extracted DNA was quantitatively sufficient to be amplified both before and after dilution. Indeed our maximum and minimum quantities were within the concentration range to initiate amplification [17] [21] [22]. This result confirms that the extraction technique used was appropriate.

Because the DNA used did not come from whole mosquitoes, a protocol adaptation was made. This involved dispensing with the dilution stage. We applied the protocol described, diluting the samples to 1/10, and identified the majority of species. The adaptation of the protocol therefore had an effect on the SINE 200× PCR results, as shown by the significant effect of the statistical analyses. In view of these results, we can see that the dilution probably had a positive effect on PCR test results. Thus, even if we are dealing with mosquito head and thorax DNAs, dilution is necessary for the complete identification of the samples.

The most common species was *An. coluzzii*, followed *An. gambiae* and *An. arabiensis*. These results are similar to previous results [23], which used the same SINE 200× PCR identification technique to identify these three species with mosquitoes from the village of Bama, in western Burkina Faso.

Analyses using the other protocols (PCR Favia and Fanello) gave no successful results for seven samples. The PCR identification of *An. funestus* was therefore carried out assuming poor morphological identification of these samples. Indeed, mosquitoes in poor conditions can be difficult to identify morphologically. After amplification and migration, the PCR identification of *An. funestus* also gave no positive result. There are three hypotheses which can explain the failure of identifying *Anopheles funestus*. Firstly, another group of *Anopheles* other than *funestus*, given that we did not use protocols from other groups of species. These include species belonging to the *An. nili complex*, the *An. rufipes* group and the *An. coustani* group. Secondly, the limited sensitivity of the SINE 200× protocol, which would not detect all mosquitoes, and thirdly, speciation over time. Indeed, speciation is an evolutionary mechanism [24] during which mutations accumulate over time, and differences between paired species can arise. In fact, mosquito species live in a partially or totally anthropized environment (modified by human societies, placed under their influence, transformed by them) which is constantly changing through development, degradation, exploitation of resources, etc. These modifications to the environment are carried out by a number of factors, including the evolution of the mosquito. These environmental modifications have been taking place at an ever-increasing pace over the last few centuries, and are a powerful driving force behind the speciation phenomena that are still underway [8] [25].

5. Conclusion

This study showed that the quantity of DNA was sufficient for PCR testing. The

SINE 200× PCR performed after dilution identified most of the samples analyzed. It would therefore be worthwhile continuing this study to identify these seven samples, if possible, using more recent and more sensitive protocols than those we used, or using primers from other species groups, as it is possible that these species are not part of the *An. gambiae* complex or the *An. funestus* group.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Sumruayphol, S., *et al.* (2020) Seasonal Dynamics and Molecular Differentiation of Three Natural Anopheles Species (Diptera: Culicidae) of the Maculatus Group (Neocellia Series) in Malaria Hotspot Villages of Thailand. *Parasites and Vectors*, **13**, Article No. 574. https://doi.org/10.1186/s13071-020-04452-0

[2] Riehle, M.M., Guelbeogo, W.M., Gneme, A., Eiglmeier, K., Holm, I., Bischoff, E., *et al.* (2011) A Cryptic Subgroup of *Anopheles gambiae* Is Highly Susceptible to Human Malaria Parasites. *Proceedings of the National Academy of Sciences of the United States of America*, **108**, 15037-15042.

[3] Service, M. (2012) Medical Entomology for Students. Cambridge University Press, Cambridge. https://doi.org/10.1017/CBO9781139002967

[4] Hay, S.I., *et al.* (2010) Developing Global Maps of the Dominant Anopheles Vectors of Human Malaria. *PLOS Medicine*, **7**, e1000209.

[5] Coetzee, M., Hunt, R.H., Wilkerson, R., Della Torre, A., Coulibaly, M.B. and Besansky, N.J. (2013) *Anopheles coluzzii* and *Anopheles amharicus*, New Members of the *Anopheles gambiae* Complex. *Zootaxa*, **3619**, 246-274. https://doi.org/10.11646/zootaxa.3619.3.2

[6] Plewes, K., Leopold, S.J., Kingston, H.W.F. and Dondorp, A.M. (2019) Malaria: What's New in the Management of Malaria? *Infectious Disease Clinics of North America*, **33**, 39-60. https://doi.org/10.1016/j.idc.2018.10.002

[7] OMS (2022) Rapport Paludisme 2022. Geneva.

[8] Carnevale, P. and Robert, V. (2009) Les Anophèles, IRD Edition, IRD, Marseille. https://doi.org/10.4000/books.irdeditions.10374

[9] Nkya, T.E., Fillinger, U., Sangoro, O.P., Marubu, R., Chanda, E. and Mutero, C.M. (2022) Six Decades of Malaria Vector Control in Southern Africa: A Review of the Entomological Evidence-Base. *Malaria Journal*, **21**, Article No. 279. https://doi.org/10.1186/s12936-022-04292-6

[10] Cohuet, A., Simard, F., Toto, J.C., Kengne, P., Coetzee, M. and Fontenille, D. (2003) Species Identification within the *Anopheles funestus* Group of Malaria Vectors in Cameroon and Evidence for a New Species. *American Journal of Tropical Medicine and Hygiene*, **69**, 200-205. https://doi.org/10.4269/ajtmh.2003.69.200

[11] Coluzzi, M., Sabatini, A., Della Torre, A., Di Deco, M.A. and Petrarca, V. (2002) A

Polytene Chromosome Analysis of the *Anopheles gambiae* Species Complex. *Science* (80-.), **298**, 1415-1418. https://doi.org/10.1126/science.1077769

[12] Dabiré, K.R., *et al.* (2009) Distribution of Insensitive Acetylcholinesterase (ace-1R) in *Anopheles gambiae* s. l. Populations from Burkina Faso (West Africa). *Tropical Medicine & International Health*, **14**, 396-403. https://doi.org/10.1111/j.1365-3156.2009.02243.x

[13] Kaboré, D.P.A., *et al.* (2023) Mosquito (Diptera: Culicidae) Populations in Contrasting Areas of the Western Regions of Burkina Faso: Species Diversity, Abundance and Their Implications for Pathogen Transmission. *Parasites and Vectors*, **16**, Article No. 438. https://doi.org/10.1186/s13071-023-06050-2

[14] Carnevale, P. (1974) Comparaison de trois méthodes de capture pour l'échantillonnage d'une population d'Anophdes nili (Theobald), 1904. *Cahier. O.R.S.T.O.M., série Entomologie médicale et Parasitologie*, **22**, 135-144.

[15] Gillies, M.T. and Coetzee, M. (1987) A Supplement to the Anophelinae of Africa South of the Sahara. South African Institute for Medical Research, Johannesburg, No. 55. http://www.metoffice.gov.uk/public/weather/climate/u1214qgj0

[16] Alout, H., *et al.* (2014) Interplay between Plasmodium Infection and Resistance to Insecticides in Vector Mosquitoes. *The Journal of Infectious Diseases*, **210**, 1464-1470. https://doi.org/10.1093/infdis/jiu276

[17] Scientific, T.F. (1975) T042-Technical Bulletin NanoDrop Spectrophotometers 260/280.

[18] Santolamazza, F., Mancini, E., Simard, F., Qi, Y., Tu, Z. and della Torre, A. (2008) Insertion Polymorphisms of SINE200 Retrotransposons within Speciation Islands of *Anopheles gambiae* Molecular Forms. *Malaria Journal*, **7**, Article No. 163. https://doi.org/10.1186/1475-2875-7-163

[19] Favia, G., Lanfrancotti, A., Spanos, L., Sidén-Kiamos, I. and Louis, C. (2001) Molecular Characterization of Ribosomal DNA Polymorphisms Discriminating among Chromosomal Forms of *Anopheles gambiae* s.s. *Insect Molecular Biology*, **10**, 19-23. https://doi.org/10.1046/j.1365-2583.2001.00236.x

[20] Bi, I.V., du Jardin, P., Mergeai, G. and Baudoin, J.P. (1997) Optimisation et application de la RAPD (Random Amplified Polymorphic DNA) dans un programme de sélection récurrente chez le cotonnier (Gossypium spp.). *Biotechnology, Agronomy and Society and Environment*, **1**, 142-150.

[21] Ahn, S.J., Costa, J. and Emanuel, J.R. (1996) PicoGreen Quantitation of DNA: Effective Evaluation of Samples Pre- or Post-PCR. *Nucleic Acids Research*, **24**, 2623-2625. https://doi.org/10.1093/nar/24.13.2623

[22] Haque, K.A., Pfeiffer, R.M., Beerman, M.B., Struewing, J.P., Chanock, S.J. and Bergen, A.W. (2003) Performance of High-Throughput DNA Quantification Methods. *BMC Biotechnology*, **3**, Article No. 20. https://doi.org/10.1186/1472-6750-3-20

[23] Wadaka, M. (2011) Stratégies de survie en saison sèche chez les vecteurs majeurs du paludisme au Burkina Faso: physiologie, morphologie, comportement et métabolisme. Université polytechnique de bobo-dioulasso, Dindéresso.

[24] Coyne, J.A. (1998) The Evolutionary Genetics of Speciation. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, **353**, 287-305. https://doi.org/10.1098/rstb.1998.0210

[25] Coluzzi, M. (1999) The Clay Feet of the Malaria Giant and Its African Roots: Hypotheses and Inferences about Origin, Spread and Control of *Plasmodium falciparum. Parasitologia*, **41**, 277-283.