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Using MALDI-TOF MS to identify mosquitoes collected in Mali and their blood meals

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Abstract

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has been recently described as an innovative and effective tool for identifying arthropods and mosquito blood meal sources. To test this approach in the context of an entomological survey in the field, mosquitoes were collected from five ecologically distinct areas of Mali. We successfully analysed the blood meals from 651 mosquito abdomens crushed on Whatman filter paper (WFPs) in the field using MALDI-TOF MS. The legs of 826 mosquitoes were then submitted for MALDI-TOF MS analysis in order to identify the different mosquito species. Eight mosquito species were identified, including *Anopheles gambiae* Giles, *Anopheles coluzzii*, *Anopheles arabiensis*, *Culex quinquefasciatus*, *Culex neavei*, *Culex perexiguus*, *Aedes aegypti* and *Aedes fowleri* in Mali. The field mosquitoes for which MALDI-TOF MS did not provide successful identification were not previously available in our database. These specimens were subsequently molecularly identified. The WFP blood meal sources found in this study were matched against human blood ($n = 619$), chicken blood ($n = 9$), cow blood ($n = 9$), donkey blood ($n = 6$), dog blood ($n = 5$) and sheep blood ($n = 3$). This study reinforces the fact that MALDI-TOF MS is a promising tool for entomological surveys.

Introduction

Mosquito-borne infectious diseases are a public health concern in tropical countries, and an emerging problem in temperate areas (Becker *et al.* 2010). The main mosquito vectors, which may transmit pathogens during their blood meals, belong to three main genera, namely *Aedes*, *Culex* and *Anopheles* (Becker *et al.* 2010). *Aedes* spp. mosquitoes are vectors for several arboviruses including the Yellow Fever, Dengue, Chikungunya and Zika viruses, which have come to the world's attention in recent years (Gardner and Ryman, 2010; Vasilakis *et al.* 2011; Caglioti *et al.* 2013). *Culex* mosquitoes are responsible for West Nile Virus (WNV) and Japanese encephalitis virus transmission around the world (Komar, 2003; Anosike *et al.* 2005; de Wispelaere *et al.* 2017). *Anopheles* mosquitoes are the primary vectors of malaria. Female *Anopheles* mosquitoes are able to transmit six species of *Plasmodium* to humans: *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale wallikeri*, *P. ovale curtisi*, *P. knowlesi* and *P. simium* (WHO, 2016; Brasil *et al.* 2017). Malarial transmission remains high in Africa, with 117 886 deaths in 2015 (WHO, 2016). In Mali, West Africa, 1544 deaths were recorded as being attributable to malaria in 2015 (WHO, 2016). It is reasonable to assume that the number of malaria-associated deaths remains underestimated.

The precise identification of mosquito fauna is essential in entomological surveys, and in order to plan control measures and monitor their impact (Bass *et al.* 2007). Furthermore, the identification of mosquito blood meal sources is essential to understanding the biting behaviour of mosquito vectors (anthropophilic or zoophilic) (Muturi *et al.* 2013).

Mosquitoes are most frequently identified at the genus and species levels by morphological characteristics and using molecular tools. Morphological identification requires well-trained entomologists using dichotomous identification keys (Gillies MT 1987). Morphological identification continues to be the standard approach for arthropod studies. However, it presents some limits in terms of discriminating cryptic or sibling species. In recent years, molecular tools have emerged and can identify mosquitoes by amplifying different target genes. The target gene, such as the cytochrome *c* oxidase (COI), internal transcribed spacer 2, IGS regions of rDNA, has been used to satisfactorily identify mosquitoes up to sibling species with great specificity and sensitivity (Folmer *et al.* 1994).

Several approaches have been developed to identify the host vertebrate blood source of mosquito meals. The main tools include a serological approach which involves precipitin tests and enzyme-linked immunosorbent assays (ELISA) (Fyodorova *et al.* 2006; Gomes *et al.* 2013). However, these techniques present several limitations, including the availability of specific antisera against a broad diversity of host species and the cross-reactivity of antibodies for close species. To this end, molecular methods have also been developed to identify

mosquito blood meal sources, such as mammalian blood and avian blood from *Culex pipiens* complex (Gomes *et al.* 2013). However, molecular methods also present several constraints, such as their cost, the time they take and the need for bulky equipment.

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has recently been used as an alternative tool for rapid arthropod identification. The mass spectrum from a new sample, generated using MALDI-TOF MS, is compared with a library of spectra from a reference database. In our laboratory, the MALDI-TOF MS approach has been routinely used to identify arthropods such as ticks (using their legs) (Yssouf *et al.* 2013, 2015; Kumsa *et al.* 2016), fleas (bodies of fleas without the abdomen) (Yssouf *et al.* 2014b), sand flies (using their thoraces, wings and legs) (Lafri *et al.* 2016), adult mosquitoes (using their legs) (Yssouf *et al.* 2013, 2014a) and mosquito larvae (using whole mosquitoes) (Dieme *et al.* 2014). Preliminary studies have also reported that MALDI-TOF MS may be used for mosquito blood meal identification. When the MS spectra obtained from the abdomen of mosquitoes which had been experimentally engorged on different blood meals source were tested, the MS protein profiles were clearly distinct according to the origin of the mosquito blood meals, up to 24 h post-feeding (Niare *et al.* 2016). During entomological surveys, it may be difficult to preserve samples, and entomologists frequently use Whatman filter papers (WFPs) to preserve mosquito blood meals in the field by crushing the engorged abdomens onto WFPs.

In this study, the goal was to use the proteomic MALDI-TOF MS approach to identify mosquitoes collected in Mali and determine the sources of their blood meals. For this purpose, mosquitoes were collected in different ecological areas of Mali and tested by MALDI-TOF MS in Marseille, France. The abdomens of engorged female mosquitoes were crushed onto WFP to determine the blood meal sources using MALDI-TOF MS.

Materials and methods

Ethics statement

Consent was obtained from the heads of families where the mosquitoes were collected. Ethical approval for the collection of mosquito was granted by authorities from the National Malaria Control Program (NMCP) and approved by the Faculty of Medicine Ethical Committee, Bamako, Mali (N°2016/113/CE/FMPOS). The mosquito samples were processed and stored in line with the World Health Organization (WHO) Good Laboratory Practices guidance and documents on mosquito sampling handling procedures.

Collection sites

This study was conducted in three different localities in Mali, namely Donéguébougou, Bougoula-hameau and Bamako. In Bamako, the collection was performed in the three semi-urban areas of Sotuba, Yirimadio and Missabougou. The geo-positions of each collection site are as follows: Bougoula-hameau ($-5^{\circ} 66'13.1''$, $11^{\circ}30'95.2''$ E); Donéguébougou ($-7^{\circ}98'39.8''$ N, $12^{\circ} 80'44.9''$ E) and the semi-urban areas of Bamako, Sotuba ($-9^{\circ} 18'65.7''$ N, $8^{\circ}23'07.4''$ E), Yirimadio ($-9^{\circ}18'56.5''$ N, $6^{\circ}23'01.8''$ E) and Missabougou ($-9^{\circ}18'77.5''$ N, $8^{\circ}23'03.9''$ E).

Mosquito collection

Mosquitoes were collected from the various sites during the middle of the rainy season between July and August 2016 (WHO,

1992). The peak densities and consequentially of anopheline mosquitoes in Mali occur in August (Sogoba *et al.* 2007). Mosquitoes were collected over three consecutive days per week. On each day, mosquitoes were aspirated from 10 houses using a mouth aspirator (Model 612, John W Hock, Gainesville, Florida, USA). All mosquitoes were collected indoors in the morning between 8 am and noon. The mosquito specimens were identified using morphological criteria (Gillies MT 1987). After being collected, mosquito specimens were kept at room temperature (RT) between 2 and 4 h during the female abdomens crushed process and then were stored at -20° C. Each mosquito specimen was then individually transferred to a 1.5 mL Eppendorf tube labelled with a reference number, the gender of the specimen, the date and site of collection.

Mosquito abdomens with visible blood meals were crushed on WFPs (Whatman International Ltd., Maidstone, England, approved by BSI). Following the entomological stage, all samples were transported to Aix-Marseille University for mosquito and blood meal identification using MALDI-TOF MS in September and October 2016.

Preparation of samples for MALDI-TOF MS analysis

Mosquito identification

The legs of the specimens were cleaned in 70% (v/v) ethanol for between one to two minutes, then rinsed in high performance liquid chromatography (HPLC) grade water. The legs from each mosquito were individually placed in 1.5 mL Eppendorf tubes with glass powder (Sigma, Lyon, France), 15 μ L of 70% (v/v) formic acid (Sigma, Lyon, France), and 15 μ L of 50% (v/v) acetonitrile (Fluka, Buchs, Switzerland). The samples were crushed using a TissueLyser device (Qiagen, Hilden, Germany) over three cycles of 30 $m s^{-1}$ for 60 s (Nebbak *et al.* 2016). The samples were centrifuged at 200 g for one minute, and 1.5 μ L of supernatant of each homogenate was deposited on the MALDI-TOF target plate in quadruplicate (Bruker Daltonics, Wissembourg, France) and covered with 1.5 μ L of CHCA matrix solution composed of saturated α -cyano-4-hydroxycinnamic acid (Sigma, Lyon, France), 50% acetonitrile (v/v), 2.5% trifluoroacetic acid (v/v) (Aldrich, Dorset, UK), and HPLC grade water (Yssouf *et al.* 2013; Nebbak *et al.* 2016). The target plate was dried for several minutes at RT and placed in the Microflex LT MALDI-TOF Mass Spectrometer (Bruker Daltonics, Wissembourg, France) for analysis (Yssouf *et al.* 2013, 2016; Nebbak *et al.* 2016).

Bloody Whatman filter papers (BWFPs)

A piece of the WFPs (i.e. about 1 mm^2) containing crushed abdomens from engorged mosquitoes was individually cut using a sterile scalpel and transferred to a new 1.5 mL Eppendorf tube (Niare *et al.* 2017). For each piece of WFPs, 20 μ L of formic acid (70%, v/v) plus 20 μ L of acetonitrile (50% v/v) (Fluka, Buchs, Switzerland) was added and incubated for 10 min at RT. After a fast spin (i.e. 10 000 rpm for 20 s), 1 μ L of the supernatant of each sample was loaded onto the MALDI-TOF target plate in quadruplicate and covered with 1 μ L of CHCA matrix (Niare *et al.* 2016). After drying for several minutes at RT, the MALDI-TOF target plate was placed in the Microflex LT MALDI-TOF Mass Spectrometer (Bruker Daltonics, Bremen, Germany) for analysis. To control loading on mass spectra steel, matrix quality and MALDI-TOF apparatus performance, the matrix solution was loaded in duplicate onto each MALDI-TOF plate with or without a bacterial test standard (Bruker protein Calibration Standard I) (Niare *et al.* 2016).

Spectral analysis

Protein mass profiles were acquired using a Microflex LT MALDI-TOF Mass Spectrometer, with detection in the linear positive-ion mode at a laser frequency of 50 Hz within a mass range of 2–20 kDa. The acceleration voltage was 20 kV, and the extraction delay time was 200 ns. Each spectrum corresponded to ions obtained from 240 laser shots performed in six regions of the same spot and automatically acquired using the AutoXecute of the Flex Control v.2.4 software (Bruker Daltonics, Bremen, Germany). The spectrum profiles obtained from mosquito legs and bloody WFPs were visualized with Flex analysis v.3.3 software and were exported to ClinProTools version v.2.2 (Bruker Daltonics, Bremen, Germany) and MALDI-Biotyper v.3.0. (Bruker Daltonics, Bremen, Germany) for data processing (smoothing, baseline subtraction, peak picking) and evaluated using cluster analysis. Spectra of low quality were excluded from the study.

MALDI-TOF identification of mosquitoes

We used our in-lab arthropod MALDI-TOF database, which includes spectra obtained from various arthropods listed in Table 1. The database was upgraded with the spectra of three *Culex quinquefasciatus* mosquitoes and one *Culex neavei* mosquito collected and molecularly identified during this study. A comparison of the spectrum of each specimen of mosquito legs from Mali was evaluated against the home-made MS reference spectra database using the MALDI-Biotyper software v3.0. tool (Bruker Daltonics, Bremen, Germany). The level of significance was determined using the log score values (LSVs) provided by the MALDI-Biotyper software v.3.3. corresponding to a matched degree of signal intensities of mass spectra of the query and the reference spectra. LSVs ranged from zero to three. To determine the origin of blood meals, MALDI-TOF MS spectra from the

abdominal proteins of engorged mosquitoes crushed on WFPs were also blindly queried against the database. A sample was considered to be correctly and significantly identified at the species level when the queried spectrum had a log score value (LSV) ≥ 1.8 (Niare *et al.* 2016).

Cluster analysis

Cluster analysis on MSP (MSP, Main Spectrum Profile) spectra was performed and the comparison of the main spectra given by the MALDI-Biotyper software was clustered according to protein mass profile (i.e. their mass signals and intensities). We performed hierarchical clustering of the mass spectra of two specimens per mosquito species using the MSP dendrogram function. The clustering analyses were performed to visualize the homogeneity level of MS spectra from specimens belonging to the same species level. The resulting MSP dendrogram shows how samples are related to one another.

Molecular identification

A molecular tool was used to confirm MALDI-TOF MS identification in randomly selected mosquitoes. Molecular identification was also conducted for specimens whose spectra did not match with any mosquito spectrum in our database. When it was demonstrated that a high quality spectrum had been obtained from a mosquito species missing from our database, this new spectrum was added to the database. DNA extractions from individual mosquito heads and thorax samples were performed using the EZ1 DNA Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's recommendations. A set of primers specifically amplifying a fragment of 710 bp of the mosquito's cytochrome *c* oxidase I gene (mCOI) was used (LCO1490 (forward): 5'-GGTCAAC

Table 1. List of the arthropod species present in our home-made MALDI-TOF MS^a database.

Mosquitoes	<i>Imago: Aedes albopictus, Ae. excrucians, Ae. vexans, Ae. rusticus, Ae. dufouri, Ae. cinereus, Ae. fowleri, Ae. aegypti, Ae. caspius, Anopheles gambiae</i> Giles, <i>An. coluzzii, An. funestus, An. ziemanni, An. arabiensis, An. wellcomei, An. rufipes, An. pharoensis, An. coustani, An. claviger, An. hyrcanus, An. maculipennis, Culex quinquefasciatus, Cx. pipiens, Cx. modestus, Cx. insignis, Cx. neavei, Mansonia uniformis, Culiseta longiareolata, Orthopodomyia reunionensis, Coquillettidia richiardii and Lutzia tigripes.</i> <i>Larvae: Aedes aegypti, Ae. albopictus, Anopheles gambiae</i> Giles, <i>An. coluzzii, Cx. pipiens, Cx. molestus, Culiseta sp.</i>
Sand flies	<i>Phlebotomus papatasi, P. (Larrousius) longicuspis, P. (Larrousius) perfilliewi, P. (Larrousius) perniciosus, P. (Paraphlebotomus) sergenti and Sergentomyia minuta</i>
Triatomines	<i>Triatoma infestans, Rhodnius prolixus, Rh. pictipes, Rh. robustus, Eratyrus mucronatus and Panstrongylus geniculatus</i>
Ticks	<i>Legs: Amblyomma cohaerens, Am. gemma, Am. variegatum, Dermacentor marginatus, D. reticulatus, Haemaphysalis leachi, Hae. concinna, Hae. spinulosa, Hyalomma marginatum rufipes, H. truncatum, H. detritum, Rhipicephalus decoloratus, Ixodes hexagonus, I. ricinus, Rh. bergeoni, Rh. e. evertsi, Rh. praetextatus, Rh. pulchellus, Rh. sanguineus, Rh. sulcatus, Rh. microplus, Rh. annulatus, Rh. turanicus and Rh. bursa.</i> <i>Hemolymph: Am. variegatum, D. marginatus, H. marginatum rufipes, Rh. bursa and Rh. sanguineus.</i>
Mites	<i>Leptotrombidium chiangraiensis, L. imphalum and L. deliense</i>
Bedbugs	<i>Cimex lectularius</i>
Lice	<i>Pediculus humanus, Damalinia bovis, D. caprae, D. ovis, Haematopinus eurysternus, Linognathus vituli and L. africanus</i>
Fleas	<i>Ctenocephalides felis, Ct. canis, Archaeopsylla erinacei, Xenopsylla cheopis and Stenoponia tripectinata</i>
Abdomen of mosquitoes engorged	<i>Anopheles gambiae</i> Giles fed on: <i>Homo sapiens, Equus caballus, Ovis aries, rabbit, Balb/C mouse, Rattus norvegicus, Canis familiaris, Bos taurus, Capra hircus, Gallus gallus, Equus asinus, Tapirus indicus, Tapirus terrestris, Carollia perspicillata, Thraupis episcopus, Erythrocebus patas and Callithrix pygmaea</i> blood <i>Aedes albopictus</i> fed on: <i>Homo sapiens</i> blood
<i>Anopheles gambiae</i> Giles blood meals from Whatman Filter paper	<i>Ovis aries</i> and <i>Homo sapiens</i> blood

^aMALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.

AAATCATAAGATATTGG-3'; HC02198 (reverse): 5'-TAAAC TTCAGGGTGACCAAAAAATCA-3' (Folmer *et al.* 1994). We used gene Acetylcholinesterase-2 to amplify a fragment of 610 bp of *Culex pipiens* and a fragment of 274 bp of *Cx. quinquefasciatus*. The primers set were ACEquin (forward): 5'-CCTT CTTGAATGG CTGTGGCA-3', ACEpip (forward): 5'-GGAAA CAACGACGTATGTACT-3', B1246s (reverse): 5'-TGGAGCC TCCTCTCACGGC-3' (Smith and Fonseca, 2004).

A set of primers specifically amplifying a fragment of 310 bp of the *Anopheles gambiae* mosquito complex Acomplex_28S_MBF 5'-AGCKCGTCTTGGTCTGGGG-3' and Acomplex_28S_MBR 5'-GCCGACAAGCTCAYTAGTGT-3' was designed in our laboratory based on the work by Fanello *et al.* and PCR reactions were processed as described (Fanello *et al.* 2002). Molecular identification of the blood was carried out on the bloody WFPs from 41 specimens randomly selected from the Malian samples, as previously described (Niare *et al.* 2016). Positive PCR products were then purified and sequenced using the same primers with the BigDye version 1-1 Cycle Sequencing Ready Reaction Mix (Applied Biosystems, Foster City, CA) and an ABI 3100 automated sequencer (Applied Biosystems, Foster City, CA). The sequences were assembled and analyzed using the ChromasPro software (version 1.34) (Technelysium Pty. Ltd., Tewantin, Australia) and the NCBI BLAST website (<http://blast.ncbi.nlm.nih.gov>).

Results

Identification of the mosquitoes by MALDI-TOF MS

A total of 865 mosquitoes were captured by aspiration in Mali from various collection sites, including 257 in Bougoula-hameau,

168 in Donéguébougou, 230 in Sotuba, 125 in Missabougou and 85 in the Yirimadio semi-urban zones of Bamako (Fig. 1). All specimens collected were morphologically identified to genus level as *Anopheles* spp. (287/865; 33.18%), *Culex* spp. (573/865; 66.24%) and *Aedes* spp. (5/865; 0.58%).

For MALDI-TOF analysis, MS spectra of good quality were obtained from 272 legs of *Anopheles* spp. Of these 272 *Anopheles* spp. tested against the arthropod MS database, 97% ($n = 264/272$) were identified with a log score value (LSV) ranging between 1.70 and 2.575. These 264 *Anopheles* specimens were identified as *Anopheles gambiae* Giles (95.80%, $n = 253/264$), *Anopheles coluzzii* (3.40%, $n = 9/264$) and *Anopheles arabiensis* (0.80%, $n = 2/264$) (Fig. 2) by MALDI TOF MS. The remaining eight *Anopheles* spp. were subjected to molecular identification.

We tested the MS spectra from the legs of 549 *Culex* spp. against our arthropod database.

Of these 549 *Culex* spp. high-quality spectra, 98% ($n = 537/549$) were identified as species contained in our database. The 537 *Culex* specimens were identified by MALDI-TOF MS as *Cx. quinquefasciatus* (98%, $n = 527/537$) and *Cx. neavei* (2%, $n = 10/537$) from Mali (Fig. 3). These 537 *Culex* obtained LSVs ranging from 1.713 to 2.611. The remaining twelve *Culex* spp. were subjected to molecular identification.

The five *Aedes* specimens were identified by MALDI-TOF MS as *Aedes fowleri* ($n = 4$) and *Aedes aegypti* ($n = 1$), with log score values ranging between 2.128 and 2.418.

The MS spectra comparison from different mosquito species with Flex analysis software revealed an intra-species reproducibility and an inter-species specificity (Fig. 4). Visually, the signals and intensity of mosquito species' protein profiles (Fig. 4) were consistent for MALDI-TOF identification and revealed eight



Fig. 1. Ecological patterns and geographic distribution of mosquito collection in Mali. Sikasso: Bougoula-hameau (rural area), Bamako: Sotuba (peri-urban area), Missabougou, Yirimadio (urban areas) and Kati: Doneguebougou (rural area).

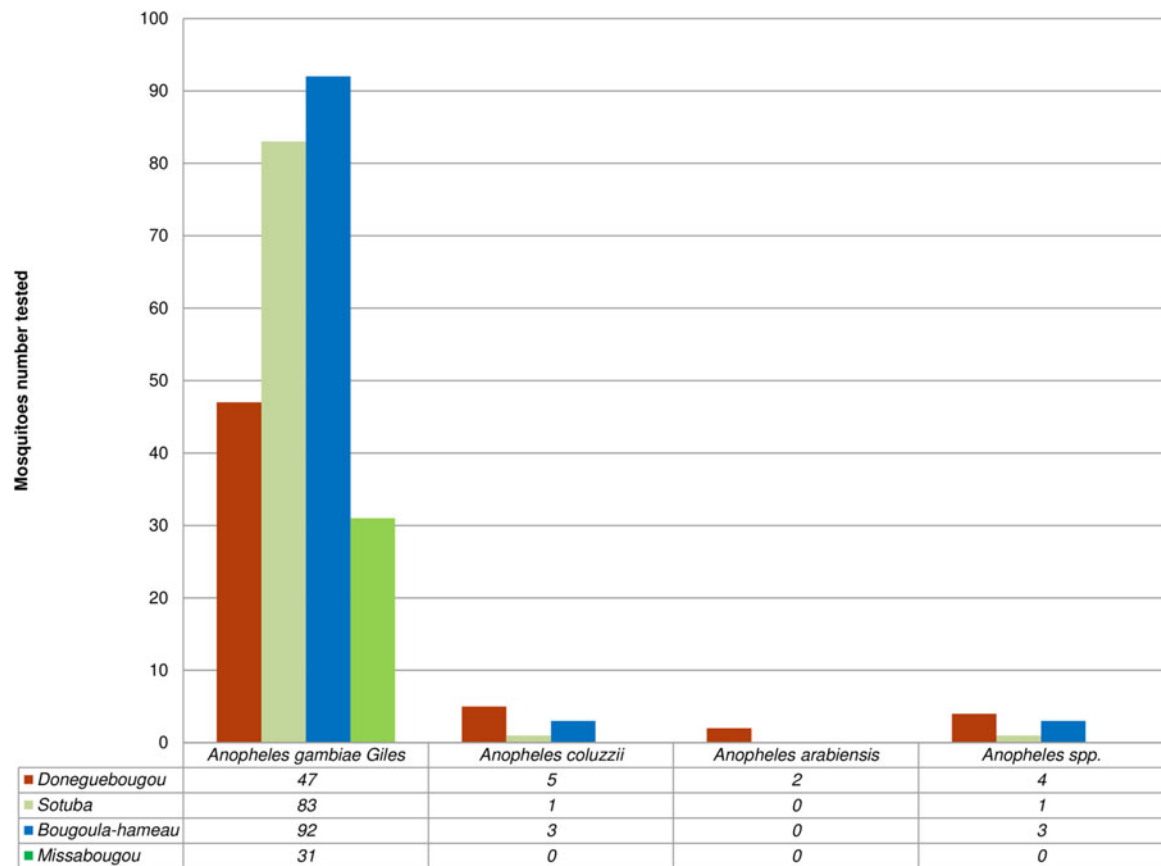


Fig. 2. MALDI-TOF MS Identification of 272 *Anopheles* spp. collected in Mali.

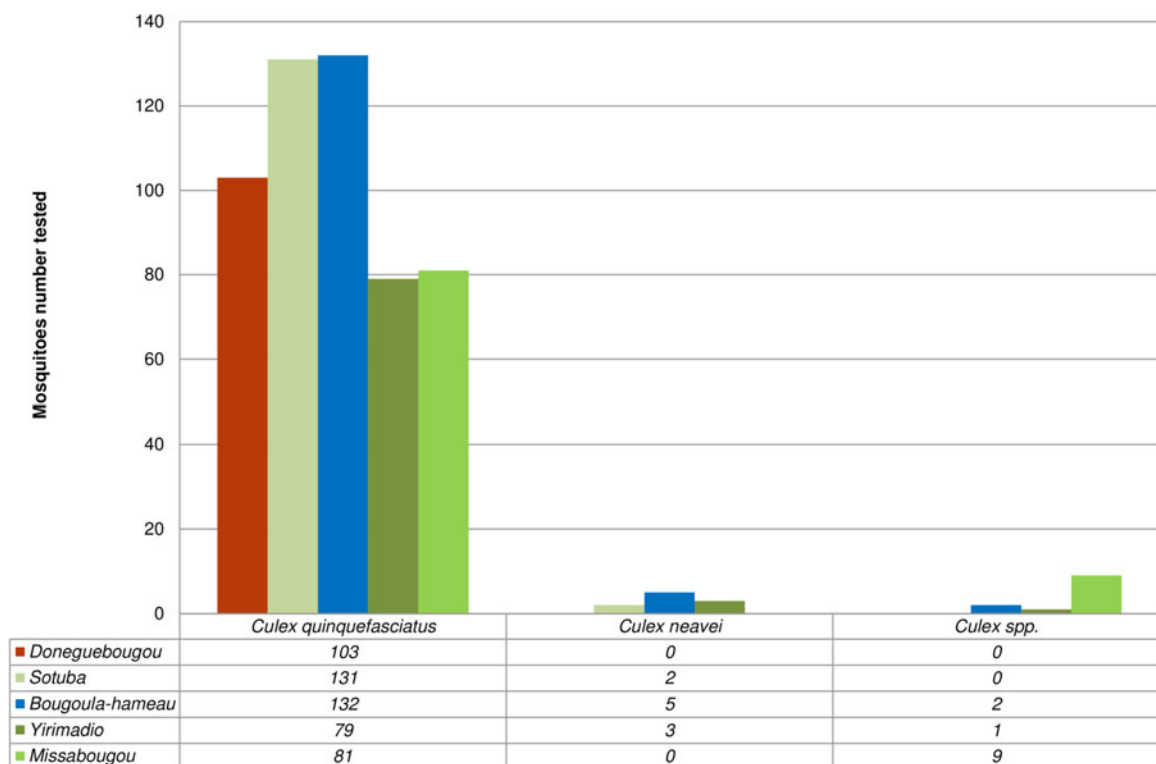


Fig. 3. MALDI-TOF MS Identification of 549 *Culex* spp. captured in Mali.

different species, namely *Anopheles gambiae* Giles, *An. coluzzii*, *An. arabiensis*, *Cx. quinquefasciatus*, *Cx. neavei*, *Culex perexiguus*, *Ae. fowleri* and *Ae. aegypti*. Clustering analysis of MSP spectra

from two specimens per mosquito species was used to generate a dendrogram. Clustering analysis revealed a gathering on distinct branches, following the eight species which were loaded

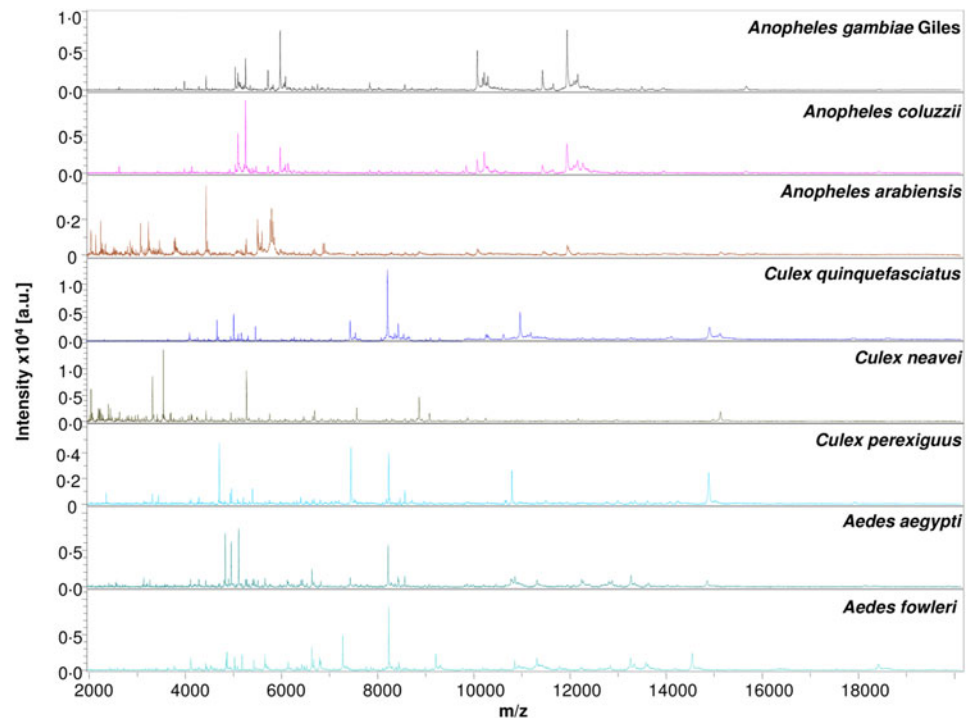


Fig. 4. Comparison of MALDI-TOF MS profiles of eight mosquito species collected in Mali. Spectra analysis was performed using Flex analysis 3.3 software. Abbreviations: a.u., arbitrary units; m/z , mass-to-charge ratio.

(*Anopheles gambiae* Giles, *An. coluzzii*, *An. arabiensis*, *Cx. quinquefasciatus*, *Cx. neavei*, *Cx. perexiguus*, *Ae. fowleri* and *Ae. aegypti*) (Fig. 5). The clusters formed were consistent with the intra-species reproducibility and inter-species specificity visually observed on protein profiles.

Molecular identification of mosquitoes collected in Mali

Molecular biology was performed to confirm the mosquito identification resulting from the MALDI-TOF MS analyses. For this purpose, we randomly selected 20/253 *An. gambiae* Giles, 2/9 *An. coluzzii*, 15/527 *Cx. quinquefasciatus*, 1/10 *Cx. neavei* for sequencing. The 28S gene sequencing of *Anopheles* corroborated the MALDI-TOF MS identification in all cases, with between 97.51 and 99.27% identity with Genbank sequences (Table 2).

The acetylcholinesterase-2 and *COI* genes were used to identify the *Culex* species. Sixteen specimens of *Cx. quinquefasciatus* ($n = 15$) and *Cx. neavei* ($n = 1$) were randomly selected for sequencing.

The molecular results were found to be highly consistent with the MALDI-TOF MS identification. Sequences obtained from *Cx. quinquefasciatus* and *Cx. neavei* were shown to share between 98.90 and 100% identity with Genbank (Table 2).

Molecular biology was also carried out on the mosquitoes that were not identified by MALDI-TOF MS (low scores), including the eight *Anopheles* spp. and 12 *Culex* spp. Sequencing of the 28S gene was performed to identify the eight *Anopheles* spp. (3%, $n = 8/264$). The matching sequences corresponded to *Anopheles gambiae* Giles ($n = 4$) and *An. coluzzii* ($n = 4$), which were shown to share between 98.52 and 100% identity with Genbank (Table 2).

The acetylcholinesterase-2 and *COI* genes were amplified to identify the 12 *Culex* spp. which were misidentified (2%, $n = 12/549$) by MALDI-TOF MS. The sequences obtained correspond to the *Cx. quinquefasciatus* ($n = 11$) which were shown to share between 98.90 and 100% identity with Genbank and 100% identity with *Cx. perexiguus* ($n = 1$) (Table 2).

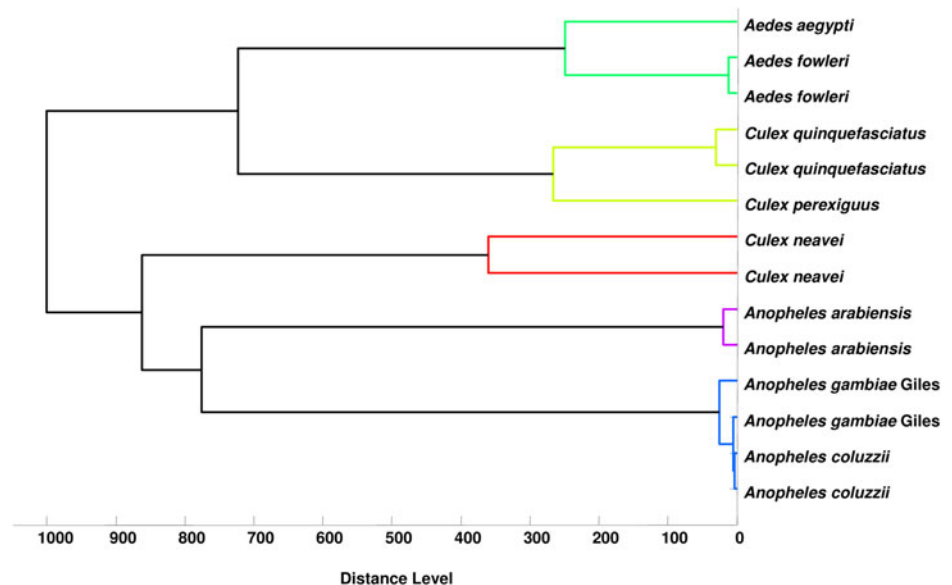


Fig. 5. MSP (Main Spectrum Profile) dendrograms of MALDI-TOF MS spectra of eight mosquito species collected in Mali. Clustering analysis was performed using MALDI Biotyper software. Distance unit corresponds to the relative similarity calculated from the distance matrix.

Table 2. Molecular identification of mosquitoes collected in Mali

Collection sites	Morphological identification	MALDI-TOF MS identification	Log score value	Genes	Molecular identification	% Identities Genbank	Accession number
Sotuba	<i>Anopheles</i> spp.	Human	[1.754]	28S	<i>Anopheles coluzzii</i>	100	AF470112.1
Donéguébougou	<i>Anopheles</i> spp.	<i>Anopheles gambiae</i> Giles	[2.470]	28S	<i>Anopheles gambiae</i> Giles	99.25	AF470116.1
Donéguébougou	<i>Anopheles</i> spp.	Human	[1.854]	28S	<i>Anopheles coluzzii</i>	98.51	AF470113.1
Donéguébougou	<i>Anopheles</i> spp.	Less relevant	[1.553]	28S	<i>Anopheles coluzzii</i>	98.51	AF470113.1
Donéguébougou	<i>Anopheles</i> spp.	Less relevant	[1.610]	28S	<i>Anopheles coluzzii</i>	99.25	KT284724.1
Donéguébougou	<i>Anopheles</i> spp.	Human	[2.008]	28S	<i>Anopheles gambiae</i> Giles	98.14	AF470116.1
Donéguébougou	<i>Anopheles</i> spp.	<i>Anopheles coluzzii</i>	[1.864]	28S	<i>Anopheles coluzzii</i>	98.51	AF470113.1
Bougoula -hameau	<i>Anopheles</i> spp.	<i>Anopheles gambiae</i> Giles	[2.383]	28S	<i>Anopheles gambiae</i> Giles	98.88	AF470116.1
Bougoula-hameau	<i>Anopheles</i> spp.	<i>Anopheles gambiae</i> Giles	[2.389]	28S	<i>Anopheles gambiae</i> Giles	99.25	AF470115.1
Bougoula-hameau	<i>Anopheles</i> spp.	<i>Anopheles gambiae</i> Giles	[2.404]	28S	<i>Anopheles gambiae</i> Giles	99.25	AF470116.1
Bougoula-hameau	<i>Anopheles</i> spp.	Less relevant	[1.581]	28S	<i>Anopheles gambiae</i> Giles	98.51	AF470115.1
Bougoula-hameau	<i>Anopheles</i> spp.	Human	[2.286]	28S	<i>Anopheles gambiae</i> Giles	98.88	AF470115.1
Bougoula-hameau	<i>Anopheles</i> spp.	Human	[1.961]	28S	<i>Anopheles gambiae</i> Giles	98.88	AF470116.1
Bougoula-hameau	<i>Anopheles</i> spp.	<i>Anopheles coluzzii</i>	[1.964]	28S	<i>Anopheles coluzzii</i>	98.51	AF470113.1
Bougoula -hameau	<i>Anopheles</i> spp.	<i>Anopheles gambiae</i> Giles	[2.237]	28S	<i>Anopheles gambiae</i> Giles	99.27	AF470116.1
Bougoula -hameau	<i>Anopheles</i> spp.	<i>Anopheles gambiae</i> Giles	[2.406]	28S	<i>Anopheles gambiae</i> Giles	99.27	AF470116.1
Bougoula -hameau	<i>Anopheles</i> spp.	<i>Anopheles gambiae</i> Giles	[2.390]	28S	<i>Anopheles gambiae</i> Giles	98.59	AF470116.1
Missabougou	<i>Anopheles</i> spp.	<i>Anopheles gambiae</i> Giles	[2.099]	28S	<i>Anopheles gambiae</i> Giles	99.27	AF470116.1
Missabougou	<i>Anopheles</i> spp.	<i>Anopheles gambiae</i> Giles	[2.401]	28S	<i>Anopheles gambiae</i> Giles	98.90	AF470116.1
Missabougou	<i>Anopheles</i> spp.	<i>Anopheles gambiae</i> Giles	[2.283]	28S	<i>Anopheles gambiae</i> Giles	98.54	AF470116.1
Missabougou	<i>Anopheles</i> spp.	<i>Anopheles gambiae</i> Giles	[2.258]	28S	<i>Anopheles gambiae</i> Giles	98.90	AF470116.1
Missabougou	<i>Anopheles</i> spp.	<i>Anopheles gambiae</i> Giles	[2.368]	28S	<i>Anopheles gambiae</i> Giles	98.91	AF470116.1
Bougoula-hameau	<i>Anopheles</i> spp.	<i>Anopheles gambiae</i> Giles	[2.293]	28S	<i>Anopheles gambiae</i> Giles	98.16	AF470116.1
Bougoula-hameau	<i>Anopheles</i> spp.	<i>Anopheles gambiae</i> Giles	[2.293]	28S	<i>Anopheles gambiae</i> Giles	98.13	AF470116.1
Bougoula-hameau	<i>Anopheles</i> spp.	<i>Anopheles gambiae</i> Giles	[2.049]	28S	<i>Anopheles gambiae</i> Giles	99.26	AF470116.1
Bougoula-hameau	<i>Anopheles</i> spp.	<i>Anopheles gambiae</i> Giles	[2.311]	28S	<i>Anopheles gambiae</i> Giles	97.51	AF470116.1
Bougoula-hameau	<i>Anopheles</i> spp.	<i>Anopheles gambiae</i> Giles	[1.704]	28S	<i>Anopheles gambiae</i> Giles	97.84	AF470116.1
Bougoula-hameau	<i>Anopheles</i> spp.	<i>Anopheles gambiae</i> Giles	[2.110]	28S	<i>Anopheles gambiae</i> Giles	98.50	AF470116.1
Bougoula-hameau	<i>Anopheles</i> spp.	<i>Anopheles gambiae</i> Giles	[2.233]	28S	<i>Anopheles gambiae</i> Giles	97.70	AF470116.1
Donéguébougou	<i>Anopheles</i> spp.	<i>Anopheles gambiae</i> Giles	[2.204]	28S	<i>Anopheles gambiae</i> Giles	98.13	AF470116.1
Bougoula-hameau	<i>Culex</i> spp.	<i>Culex pipiens</i>	[2.022]	Ace2	<i>Culex quinquefasciatus</i>	99.26	FJ416029.1

(Continued)

Table 2. (Continued.)

Collection sites	Morphological identification	MALDI-TOF MS identification	Log score value	Genes	Molecular identification	% Identities Genbank	Accession number
Bougoula-hameau	<i>Culex</i> spp.	<i>Culex pipiens</i>	[2.229]	Ace2	<i>Culex quinquefasciatus</i>	98.90	FJ416029.1
Donéguébougou	<i>Culex</i> spp.	<i>Culex quinquefasciatus</i>	[1.791]	Ace2	<i>Culex quinquefasciatus</i>	99.26	FJ416029.1
Missabougou	<i>Culex</i> spp.	Human	[2.182]	COI	<i>Culex quinquefasciatus</i>	99.38	KU920694.1
Missabougou	<i>Culex</i> spp.	Less relevant	[1.610]	COI	<i>Culex perexiguus</i>	100	KU380476.1
Missabougou	<i>Culex</i> spp.	<i>Culex pipiens</i>	[1.761]	Ace2	<i>Culex quinquefasciatus</i>	99.63	FJ416025.1
Missabougou	<i>Culex</i> spp.	Human	[2.042]	COI	<i>Culex quinquefasciatus</i>	99.08	KU920694.1
Missabougou	<i>Culex</i> spp.	<i>Culex pipiens</i>	[2.139]	Ace2	<i>Culex quinquefasciatus</i>	98.90	FJ416025.1
Missabougou	<i>Culex</i> spp.	<i>Culex pipiens</i>	[2.231]	Ace2	<i>Culex quinquefasciatus</i>	98.90	FJ416025.1
Missabougou	<i>Culex</i> spp.	Human	[2.514]	COI	<i>Culex quinquefasciatus</i>	99.84	KU920694.1
Missabougou	<i>Culex</i> spp.	<i>Culex pipiens</i>	[2.088]	Ace2	<i>Culex quinquefasciatus</i>	98.90	FJ416029.1
Missabougou	<i>Culex</i> spp.	Human	[2.294]	COI	<i>Culex quinquefasciatus</i>	99.69	KU920694.1
Yirimadio	<i>Culex</i> spp.	<i>Culex pipiens</i>	[2.032]	Ace2	<i>Culex quinquefasciatus</i>	98.90	FJ416029.1
Donéguébougou	<i>Culex</i> spp.	<i>Culex quinquefasciatus</i>	[2.000]	Ace2	<i>Culex quinquefasciatus</i>	99.26	FJ416029.1
Donéguébougou	<i>Culex</i> spp.	<i>Culex quinquefasciatus</i>	[2.009]	Ace2	<i>Culex quinquefasciatus</i>	99.27	FJ416025.1
Donéguébougou	<i>Culex</i> spp.	<i>Culex quinquefasciatus</i>	[2.207]	Ace2	<i>Culex quinquefasciatus</i>	98.90	FJ416025.1
Bougoula-hameau	<i>Culex</i> spp.	<i>Culex quinquefasciatus</i>	[2.406]	Ace2	<i>Culex quinquefasciatus</i>	98.90	FJ416019.1
Bougoula-hameau	<i>Culex</i> spp.	<i>Culex neavei</i>	[2.839]	COI	<i>Culex neavei</i>	99.16	KU380473.1
Bougoula-hameau	<i>Culex</i> spp.	<i>Culex quinquefasciatus</i>	[2.037]	Ace2	<i>Culex quinquefasciatus</i>	99.11	FJ416029.1
Bougoula-hameau	<i>Culex</i> spp.	<i>Culex quinquefasciatus</i>	[2.197]	Ace2	<i>Culex quinquefasciatus</i>	99.55	FJ416029.1
Bougoula-hameau	<i>Culex</i> spp.	<i>Culex quinquefasciatus</i>	[2.146]	Ace2	<i>Culex quinquefasciatus</i>	99.56	FJ416029.1
Bougoula-hameau	<i>Culex</i> spp.	<i>Culex quinquefasciatus</i>	[2.383]	Ace2	<i>Culex quinquefasciatus</i>	99.56	FJ416029.1
Bougoula-hameau	<i>Culex</i> spp.	<i>Culex quinquefasciatus</i>	[2.322]	Ace2	<i>Culex quinquefasciatus</i>	99.13	FJ416029.1
Donéguébougou	<i>Culex</i> spp.	<i>Culex quinquefasciatus</i>	[2.445]	Ace2	<i>Culex quinquefasciatus</i>	99.57	FJ416029.1
Donéguébougou	<i>Culex</i> spp.	<i>Culex quinquefasciatus</i>	[2.067]	Ace2	<i>Culex quinquefasciatus</i>	98.71	FJ416029.1
Donéguébougou	<i>Culex</i> spp.	<i>Culex quinquefasciatus</i>	[2.151]	Ace2	<i>Culex quinquefasciatus</i>	100	FJ416029.1
Missabougou	<i>Culex</i> spp.	<i>Culex quinquefasciatus</i>	[2.244]	Ace2	<i>Culex quinquefasciatus</i>	98.68	FJ416029.1
Missabougou	<i>Culex</i> spp.	<i>Culex quinquefasciatus</i>	[2.159]	Ace2	<i>Culex quinquefasciatus</i>	99.56	FJ416029.1

MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; Ace 2: acetylcholinesterase-2; COI: the cytochrome c oxidase; %, per cent.

Table 3. Identification of the blood meals of mosquitoes collected in distinct ecological areas in Mali

Sites	Morphological ID	Mosquito identified by MALDI-TOF MS	Blood meals identified by MALDI-TOF MS							Total	
			Human	Chicken	Cow	Donkey	Dog	Sheep	Not identified		
Bougoula-hameau	<i>Anopheles</i>	<i>Anopheles gambiae</i> Giles	87		3	5			3	98	
	<i>Anopheles</i>	<i>Anopheles coluzzii</i>	3							3	
	<i>Culex</i>	<i>Culex quinquefasciatus</i>	86	5			1			92	
	<i>Culex</i>	<i>Culex neavei</i>	3							3	
Donéguébougou	<i>Anopheles</i>	<i>Anopheles gambiae</i> Giles	46		6	1			2	4	59
	<i>Anopheles</i>	<i>Anopheles coluzzii</i>	5								5
	<i>Anopheles</i>	<i>Anopheles arabiensis</i>	2								2
	<i>Culex</i>	<i>Culex quinquefasciatus</i>	93	1			1			5	100
Missabougou	<i>Anopheles</i>	<i>Anopheles gambiae</i> Giles	13							2	15
	<i>Culex</i>	<i>Culex quinquefasciatus</i>	69	1						12	82
Sotuba	<i>Anopheles</i>	<i>Anopheles gambiae</i> Giles	51					3	1	6	61
	<i>Culex</i>	<i>Culex quinquefasciatus</i>	97							2	99
	<i>Culex</i>	<i>Culex neavei</i>	1								1
Yirimadio	<i>Aedes</i>	<i>Aedes aegypti</i>	1								1
	<i>Culex</i>	<i>Culex quinquefasciatus</i>	61	2						16	79
	<i>Culex</i>	<i>Culex neavei</i>	1								1
Total			619	9	9	6	5	3	50	701	

ID, Identification; MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.

Identification of the bloody WFPs sources by MALDI-TOF MS

A total 701 abdomens of engorged mosquitoes were crushed in WFPs in the field in Mali. The 701 bloody BWFPs were submitted for MALDI-TOF MS analysis in Marseille one month after sampling. Of the 701 BWFPs, 651 (93%) high-quality spectra were obtained. The 651 BWFPs MS high-quality spectra were queried against our blood source MALDI-TOF MS database for identification. They matched with spectra from our database, including those of mosquito abdomens engorged with human blood ($n=619$), chicken blood ($n=9$), cow blood ($n=9$), donkey blood ($n=6$), dog blood ($n=5$) and sheep blood ($n=3$) (Table 3). These blood meals were identified using MALDI-TOF MS with log score values (LSVs) ranging from 1.707 to 2.731. The MS spectra comparison of different host blood revealed an intra-species reproducibility and an inter-species specificity by Flex analysis (Fig. 6).

Molecular identification of the bloody mosquito WFPs

A total of 41 bloody WFPs identified by MALDI-TOF MS as mosquito abdomens engorged with human blood ($n=21$), donkey blood ($n=5$), chicken blood ($n=4$), cow blood ($n=5$), dog blood ($n=4$) and sheep blood ($n=2$) were randomly selected for sequencing by *COI* gene amplification. Thirty-three bloody WFPs sequences were obtained which confirmed the accuracy of the MS identification. However, for eight bloody WFPs, no quality sequences could be obtained. The results of the PCR based on bloody WFP sequencing highly correlated with the

results of MALDI TOF MS identification (Table 4). The sequences obtained from seventeen bloody WFPs had identities between 98.52 and 100% against Genbank NCBI (Table 4).

Discussion

The goal of this work was not to provide precise data on the presence and abundance of various mosquito species in specific areas in Mali. Indeed, these data vary according to the type of climate and the seasons. However, we did want to test the usefulness of MALDI-TOF MS using mosquitoes collected in the field, as most preliminary studies have used laboratory specimens.

The use of MALDI-TOF MS has recently emerged in medical entomology, including for the identification of arthropods, their blood meals and the detection of potential microorganisms (Schaffner *et al.* 2014; Yssouf *et al.* 2016). The choice of the arthropod body part is critical for specimen identification by MALDI-TOF MS (Yssouf *et al.* 2016). For example, the legs from adult mosquitoes have been shown to be sufficient for identification, whereas whole specimens have been used for aquatic stages (larvae) (Nebbak *et al.* 2017).

Here, the MS spectra from mosquito legs collected in Mali, including 264 *Anopheles*, 549 *Culex* and five *Aedes*, permitted MALDI-TOF MS identification. The MS spectrum analyses from the mosquito legs revealed an intra-species reproducibility and inter-species specificity consistent with molecular validation (Fig. 5). Accurate identification of mosquitoes queried against the home-made MS database corresponded to 100% concordance with molecular identification results (Table 2). The consistent

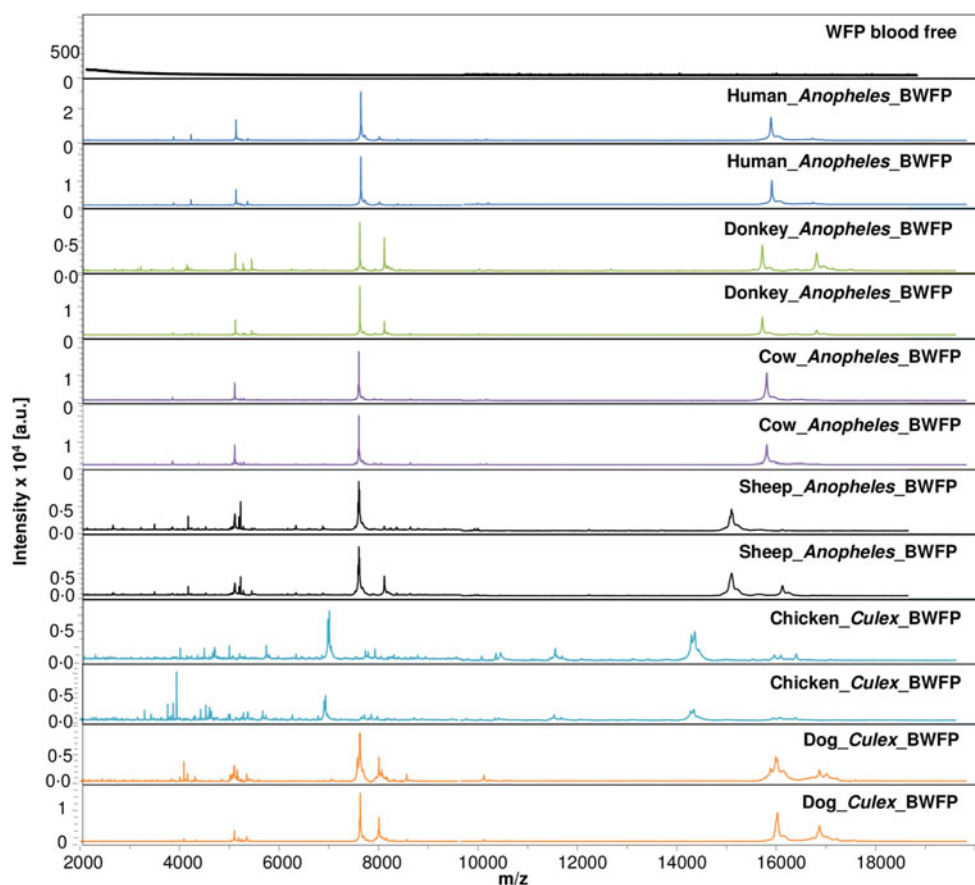


Fig. 6. The MS spectrum alignment from mosquito abdomen engorged on vertebrate host bloods and then crushed on Whatman filters. All bloody WFPs (BWFPs) were obtained from the field mosquitoes collected in Mali and crushed on WFPs. The MS spectrum alignment was performed by Flex analysis 3.3 software. The WFP blood free corresponds to the MS profiles of WFPs where no mosquito blood meal was released. The representative MS spectra from abdominal protein corresponds to *Anopheles gambiae* Giles abdomens BWFPs feed on human, donkey, cow and sheep blood, and *Culex quinquefasciatus* abdomens feed on chicken and dog blood. a.u. arbitrary units; m/z mass-to-charge ratio.

Table 4. Molecular identification of the blood from mosquito's abdomens crushed on Whatman filter papers

Mosquito blood meals sources identification by MALDI-TOF MS	Mosquito blood meals sources identification by COI gene amplification	% Identities Genbank	Accession number Genbank
Human	<i>Homo sapiens</i>	100	KM102136.1
Human	<i>Homo sapiens</i>	99.84	KY595668.1
Human	<i>Homo sapiens</i>	99.64	HM185231.1
Human	<i>Homo sapiens</i>	98.52	KM102136.1
Human	<i>Homo sapiens</i>	98.86	KY595669.1
Human	<i>Homo sapiens</i>	98.72	MF058292.1
Human	<i>Homo sapiens</i>	99.68	KF161694.1
Human	<i>Homo sapiens</i>	99.68	MF058210.1
Human	<i>Homo sapiens</i>	99.34	MF058210.1
Human	<i>Homo sapiens</i>	100	MF058210.1
Human	<i>Homo sapiens</i>	99.37	MF058210.1
Human	<i>Homo sapiens</i>	98.68	MF058210.1
Human	<i>Homo sapiens</i>	99.67	MF058210.1
Human	<i>Homo sapiens</i>	99.18	AY275535.2
Human	<i>Homo sapiens</i>	99.52	MF588867.1
Human	<i>Homo sapiens</i>	99.36	MF057217.1
Human	<i>Homo sapiens</i>	99.18	MF588867.1
Human	<i>Homo sapiens</i>	99.05	AY922271.1
Human	<i>Homo sapiens</i>	99.52	MF588867.1
Human	<i>Homo sapiens</i>	99.35	KM101695.1
Human	<i>Homo sapiens</i>	99.36	KF163046.1
Donkey	<i>Equus asinus</i>	99.37	KX683425.1
Chicken	<i>Gallus gallus</i>	99.68	KX781318.1
Chicken	<i>Gallus gallus</i>	99.22	KX781318.1
Chicken	<i>Gallus gallus</i>	99.21	KX781318.1
Cow	<i>Bos taurus</i>	99	KY650678.1
Donkey	<i>Equus asinus</i>	98.91	KX683425.1
Cow	Failed	-	-
Cow	<i>Bos taurus</i>	99	KY650678.1
Cow	Failed	-	-
Cow	Failed	-	-
Donkey	Failed	-	-
Chicken	<i>Gallus gallus</i>	98.52	KX781318.1
Dog	Failed	-	-
Dog	Failed	-	-
Dog	Failed	-	-
Donkey	<i>Equus asinus</i>	99.52	KX683425.1
Donkey	<i>Equus asinus</i>	100	KX683425.1
Sheep	<i>Ovis aries</i>	99.84	KP998473.1
Sheep	<i>Ovis aries</i>	99.21	KR868678.1
Dog	Failed	-	-

MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; COI, the cytochrome c oxidase; %, per cent.

identification between molecular biology and MALDI-TOF MS was validated by the choice of the 28S gene for *Anopheles* species identification, and the acetylcholinesterase-2 and COI genes for *Culex* species identification. As shown in previous studies, the choice of these genes was highly relevant to discriminate and assess the phylogenetic relation between different mosquito species (Folmer *et al.* 1994; Fanello *et al.* 2002; Smith and Fonseca, 2004). Here, the quality of spectra was a very important element for identification, as more than 98% of the good quality spectra were identified with LSVs >1.8. The MALDI-TOF MS reference database has been updated with other mosquito species. It is necessary to create a reference database, which could subsequently be shared, and open access could be provided for routine arthropod identification. In this study, *Aedes* mosquitoes collected in Mali were correctly identified as *Ae. fowleri* using a database containing reference spectra of this species collected from La Reunion Island only, which is located in the Pacific Ocean. Therefore MALDI-TOF MS appears as an efficient tool for the identification of arthropods collected from distant geographical areas.

For Raharimalala *et al.* (2017), the usefulness and accuracy of MALDI-TOF MS as a tool to identify vector mosquito species requires the creation of an international database (Raharimalala *et al.* 2017). In this study, 2.40% of inconsistent MS leg results were attributed to low-quality MS spectra for identification. The MS spectra of some legs ($n = 9$) (Table 2) that matched with reference spectra of mosquito abdomens engorged with human blood were attributed to traces of blood on the legs during the abdomen crushing process onto WFPs. This phenomenon of low-quality spectra, leading to lower identification rates have been reported in arthropod identification such as at the aquatic mosquito stage (Dieme *et al.* 2014). According to the reproducibility of MS spectra, the hierarchical clustering showed that all specimens from the same species were grouped in the same branch. These results are similar to previous studies supporting inter-species reproducibility for mosquito identification (Yssouf *et al.* 2013). Additionally, we stress that MS cannot yet be considered a reliable tool for the phylogenetic study of mosquito species (Yssouf *et al.* 2013).

Our results showed that 95% of the collected mosquitoes had fed on human blood. This result is not surprising because all mosquitoes were collected inside homes. The advantage of our MALDI-TOF approach is its rapidity, effectiveness and reliability in determining bloody WFPs, since more than 100 bloody WFPs specimens were processed per day. Previously, the authors had demonstrated that the profiles of abdominal spectra of mosquito females engorged on human blood are the same, regardless of whether they were crushed or not crushed on WFPs (Niare *et al.* 2017). Indeed, the home-made database contains filter papers with *Anopheles gambiae* engorged blood such as human blood and sheep blood. These authors tested WFP either with the crushed abdomen of a non-engorged mosquito or simply as a control (Niare *et al.* 2017). These results suggest that MALDI-TOF MS is not time-consuming in comparison with molecular tools and serological techniques. The eight bloody WFPs which failed molecular biology identification may be attributed to blood meal digestion. As previously reported, the time of the host blood digestion in the mosquito has an impact upon blood meal identification by MALDI-TOF MS and molecular biology (Niare *et al.* 2016). Moreover, the molecular biology results of the seventeen BWFPs sequences obtained by COI gene amplification corroborated the MALDI-TOF MS identification (Table 4).

Interestingly, as we have recently found that MALDI-TOF may also recognize mixed blood meals (unpublished), we did not find any mixed blood meals either by molecular tools nor by MALDI-TOF. The authors experimentally engorged *An. gambiae* Giles mosquitoes with a mixture of blood from distinct vertebrate

hosts, such as human, sheep and dogs. Their results demonstrate that mixed mosquito blood meals can be successfully identified, depending on the concentration ratio (unpublished). Recently, some authors have also used the proteomic approach to identify the sources of tick mixed blood meals (Onder *et al.* 2013).

Of the mosquitoes identified by MALDI-TOF MS, *A. gambiae* Giles and *Cx. quinquefasciatus* were widely distributed across all collection sites. Our work enabled *Cx. neavei* and *Cx. perexiguus* to be detected for the first time in Mali. Currently, few studies have been carried out on the *Culex* species in Mali, particularly on their abundance, ecology and the infectious pathogens transmitted by these vectors. *Culex* species are widely distributed in West Africa and are found in any type of breeding sites (clear and polluted water), whereas the *Anopheles* species colonizes sunny, fresh water (Becker *et al.* 2010). There is an abundant literature on these mosquitoes, the well-known distribution of *Cx. neavei* and *Cx. perexiguus* in sub-Saharan Africa and their implication in the transmission of many arboviruses (Jupp *et al.* 1986; Fyodorova *et al.* 2006; Nikolay *et al.* 2012; Fall *et al.* 2014; Gould *et al.* 2017). The presence of these potential vectors in Mali might be of epidemiological importance.

Our study is the first to use MALDI-TOF MS as a tool for monitoring field mosquitoes in Africa, particularly in Mali, an endemic malarial area. Moreover, when the MALDI-TOF MS device is bought for clinical microbiology purposes, it can also be used for medical entomology at no additional cost. For example, at the Dakar hospital in Senegal, the MALDI-TOF MS equipment that was initially bought for clinical microbiology has been used for field entomology surveys and has successfully identified *Culicoides* (Sambou *et al.* 2015). In Senegal, the acquisition of MALDI-TOF MS equipment has revolutionized bacteriology laboratories and clinical microbiology domains, suggesting that this technique can be used as a front-line tool in tropical countries (Lo *et al.* 2015).

Although the time period for blood meal source determination by MALDI-TOF MS was shorter than that of molecular biology or ELISA, the rapidity and low cost of the reagents made this proteomic method a financial and reliable competitive strategy. However, the relatively high cost of the machine could be an impediment to implementation of this innovative tool in laboratories. The cost of purchasing the MALDI-TOF MS equipment in under-developed countries such as Mali (sub-Saharan Africa) could be a limitation to estimating the local vector-borne risk. However, when the device is bought by a microbiology lab it can be used in medical entomology at no additional cost.

Concluding remarks

The present study successfully identified field mosquitoes and the sources of their blood meals using MALDI-TOF MS. The mosquitoes collected in Mali were correctly identified based on reproducibility and specificity from the protein profiles of leg extracts. The innovative MALDI-TOF MS tool enabled the rapid identification of eight mosquito species in Mali during entomological surveys. The challenge is to maintain and develop collaboration between north and south to facilitate the acquisition of the MALDI-TOF MS equipment.

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Author contributions. PP, TF and NS designed and developed the protocol. TF and NS performed the protocol. PP, TF, NS and ML analysed the data. KKA, DZA, OA, BMJ, OD and RD contributed reagents/materials/analysis tools. PP, TF and NS wrote the paper. OD and RD contributed to editing the paper. All authors agreed to publication.

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