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Molecular identification of Stomoxys and musca (Diptera: Muscidae) of veterinary importance in the pasture area of Ngaoundere

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Abstract
Pasture flies are abundant pests in rangelands of Ngaoundere and information on their species composition is weak. For this reason a cross sectional entomological prospection using a Vavoua trap was conducted in the rainy season (September 2017) in Ngaoundere. Of a total of 568 Stomoxys (biting muscid) trapped, two species were identified 196 *S. niger niger* (34.51%, 9. 33 Snn/t/d) and 101 *S. omega* (17.78%, 4.80 so/t/d) as well as 271 non-biting *Musca* spp (47.71%, 12.90 m/vavoua and day). Molecular genotyping confirmed the morphological identification of *Stomoxys* species (*S. n. niger* and *S. omega*) and *Musca* species nucleotide sequences generated were 88% identical to *Musca autumnalis*.

Keywords: Stomoxyini, muscini, vavoua, PCR

Introduction
Muscids constitutes an important group of dipterous flies belonging to the family Muscidae. Individuals of the genus Stomoxys possesses biting mouthparts that permits them to suck blood from their hosts [1, 2]. An important life trait of Stomoxys is that both male and female are blood sucking [1, 2, 3]. Individuals of the genus *Musca* possesses the spongy mouth part that permits them to lick blood and body fluids from hosts and other surfaces [4, 5]. Morphologically, the size of stable fly (Stomoxys) is about the same as the housefly, measuring from 4 to 7 mm. Both individuals of the genera *Stomoxys* and *Musca* breed in dung, rotten vegetation materials plus faeces and urine [2, 6]. Stomoxys are well known mechanical vectors of dangerous diseases (protozoans, helminthes, bacteria, and viruses) [3]. Mechanical transmission by this group is possible based on the fact that they are interrupted during blood feeding by their host and can rapidly switch to another calm counterpart to continue the processing hence leading to disease transmission [3, 7]. Also, the fact that non-biting muscids feed on open sores and secretions, indicates that they could be involved in contaminative transmission of highly contagious diseases [8]. Apart from *Musca domestica* that is common around human dwellings, *Musca autumnalis* (face fly) is frequently encountered around cattle and feed on body secretions. *Musca autumnalis* is known for mechanically transmitting several diseases such as bovine keratoconjunctivitis, infectious bovine rhinotracheitis, etc. [9, 10]. Apart for the fact that individuals of both *Stomoxys* and *Musca* taxa are involved in disease transmission, they also cause direct effects to their hosts such as painful bites that stresses hosts, annoyance, decrease animal feeding time resulting in poor body condition and reduced traction power.
Based on geographical distribution of muscids, the face fly is native to Europe, Russia, China, North Africa, Korea, Japan, and Iran [11]. But Stomoxys are found in all several parts of the world [1].
Morphologically, it is difficult for an untrained eye to separate individuals of the Stomoxys group from those of the Musca group. The identification of muscids of Cameroon have been made using morphological keys, with many unidentified species signaled. To effectively control pasture flies, a sensitive identification tool such as the Polymerase Chain Reaction (PCR) is required to confirm the morphologically typed individuals to be sure of what to target. For this reason we designed a study to identify muscids from Ngaoundere using PCR.

Materials and Methods

Description of study area

The study area is located in Mbidjoro in Ngaoundere II, Vina Division of the Adamawa Region. It is about 15Km away from the town of Ngaoundere along the Ngaoundere/Tignere motorable highway. Geographically, the study herd is situated between Latitude 07° 21'452" N and Longitude 13° 32'366" E with an average altitude of 1523m *a.s.l*. The study site is a pasture area with Goudali as the most dorminant cattle breed and others like red Fulani, white Fulani, Charlorais and their crosses (metis) are also common. The climate of this area is a typical Soudano-sahelian type with vegetation consisting of savanna grasses grazed upon by cattle. The mean annual temperature is 23.1°C, mean annual humidity is 63.2%, mean annual rainfall is 1176.9 mm (rainy season) and wind usually blowing in the South East (SE) direction. Ecologically, the area consists of gallery forest, primary forest, secondary forest and open grass savanna.

Fly collection

Stomoxys spp. were trapped beside a herd around the Ngaoundere. Capture was made using a Vavoua trap [12]. Total trap exposition period was 5hours from 6:00 am to 6:00 pm. The Vavoua trap cages were emptied every evening (6:00pm). This study was conducted in September 2017 for eight consecutive trapping days.

Morphological fly identification

The captured flies from both methods were first identified at species level using the taxonomic keys of Zumpt *et al*., [1]. *Musca* species were morphologically typed using the key of Gregory [13] and Grzywacz *et al*., [14].

DNA extraction, amplification and sequencing using fly samples

An aliquot (100µl) of the genomic DNA obtained using the Wizard® Genomic DNA purification kit (Promega™) following manufactures instructions was used for the molecular confirmation of our morphologically typed species (*S. n. niger*, *S. omega*) and *Musca* sp. Morphologically identified samples were used for the PCR amplification where primer sequence for the mtDNA used was defined according to Sharpe *et al*., [15]: ITS2A (forward) (TGTGAACTGCAGGACACAT) and ITSB (reverse) (TATGCTTAAATTCAGGGGGT). It was performed in 25 µl reaction volume consisting of 0.6 µl of each primer, 5 µl 5xbuffer green, 2.5 µl $MgCl_2$, 1 µl dinucleotide Tri Phosphates (dNTPs), 0.3 µl Taq polymerase (Promega™, Madison, WI USA), 13 µl RNase-free water and 2 µl genomic DNA out of 100 µl. The reaction mixture was pipetted each into 0.2ml eppendorf tubes and placed in a Master cycler (Eppendorf®) with the following thermal cycling conditions: initial denaturation at 94°C for 4mins, final denaturation step-94 °C for 40s, annealing (55°C for 30s), elongation (72°C, 1 min for 35 cycles and final extension at 72°C for 4mins. Products were visualized in Midori green-stained 1.5% agarose gel with an expected product size of approx. 480bp [15]. Amplicons were prepared for sequencing following the EZ-Seq User guide briefly, 5 µl template and 5 µl primer in each tube (1.5ml eppendorf tubes)/well. Sanger sequencing was carried out at Macrogen. Sequences were aligned using Geneious version 10.2.3 and Evolutionary analyses were conducted in MEGA7.

Trap Apparent Density (ADT) defined as muscids caught per trap per day (s/t/d) and mathematically expressed as

$$\text{Trap Apparent Density(ADT)} = \frac{\text{Number of muscids captured}}{\text{number of traps} \times \text{number of trapping days}}$$

Results

A total of 568 Stomoxys were caught and *S. n. niger* and *S. omega* were the only species morphologically identified. The non-biting *Musca* sp. was also identified morphologically. *S. n. niger* was the most abundant biting muscid in the collection (Table 1).

Table 1: The abundance of Muscids identified in the study area

Muscidae	Number	%	ADT
***Stomoxys* species (biting)**			
Stomoxys niger niger	196	34.51	9.33
Stomoxys omega	101	17.78	4.80
***Musca* species** (non-biting)			
Musca autumnalis	271	47.71	12.90

ADT: Apparent density (number of flies caught per Vavoua trap per day)

The PCR products of the nine samples gave a positive signal with amplicons band size of 400bp which was the expected product size (Figure 1).

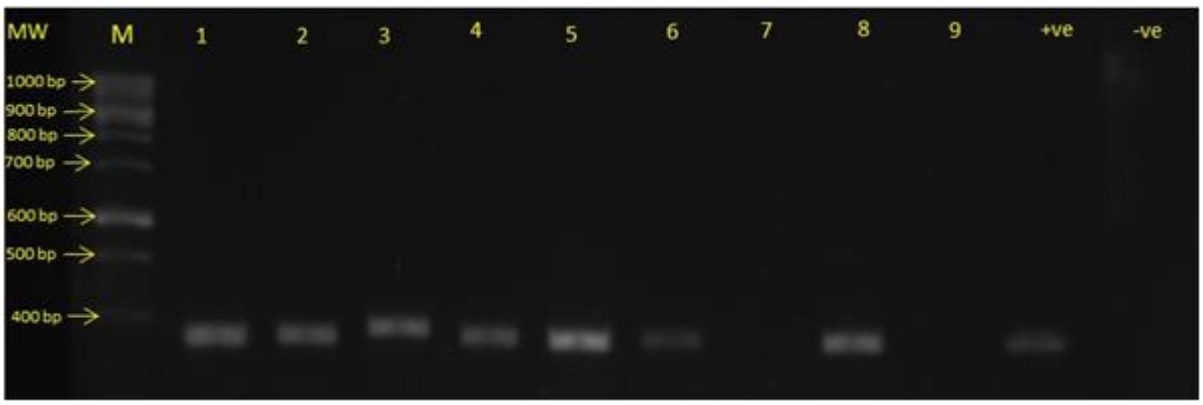

Fig 1: Gel electrophoresis of the amplicons of some muscids collected during the survey. 1 to 9 represent test samples, M: 100bp gene ladder, MW: molecular weight, +ve: positive control (*S. n. niger*), -ve: negative control.

The sequencing results resulted in nucleotide sequences from two groups namely *Musca* and *Stomoxys* groups. From the blast into the already existing nucleotide sequences in the Gene Bank, our sequence code-S51_SN (Morphological identified as *Musca sp.*) was identical to *Musca autumnalis*, S49_SN and S53_SN (morphologically identified as *S. omega*) were identical to *Stomoxys omega Sog7* and S50_SN (morphologically confirmed as *Stomoxys niger niger*) was identical to *Stomoxys niger niger Snng2*. A phylogenetic tree was constructed using sequences from the present study and reference sequences from the gene bank (Figure 2).

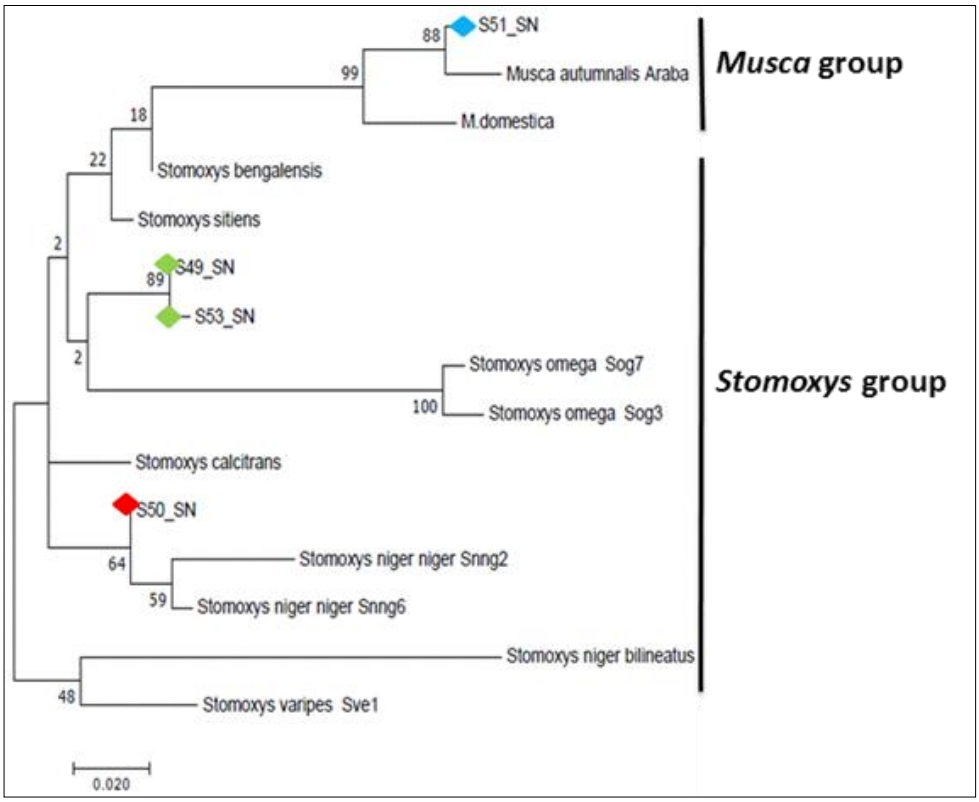

Fig 2: Molecular Phylogenetic analysis by Maximum Likelihood method showing the classification of Muscidae from the study.

The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model [16]. The tree with the highest log likelihood is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. All positions containing gaps and missing data were eliminated. Evolutionary analyses were conducted in MEGA7 [17].

Discussion

The two species of biting muscids (*S. n. niger* and *S. omega*) identified in the pasture area of Ngaoundere was not suprising as these species have already been presented in the report of Mamoudou *et al*.,[18] in east, Sevidzem *et al*., [19,20] in Ngaoundere, Sevidzem *et al*., [21] in North and Hiol *et al*., [22] in the Littoral region of Cameroon. Based on the abudance of the different species of muscids collected, *S. n. niger* recorded the highest frequency. *Stomoxys niger niger* has been reported to develop and survive well in tropical African climates [1]. *Stomoxys omega* has been reported to be abundant in the rainforest of central Africa [22, 23]. The present study revealed for the first time the presence of *Musca autumnalis* in Ngaoundere. Literature on the distribution of *M. autumnalis* shows that it is present in Northern parts of Africa [10]. The occurrence of this species in Cameroon (central African) could be due to the recent complex movement of trade and transhumance cattle in Africa hence moving along with this fly. Additionally, *M. autumnalis* was the most abundant species in our collection and this could be due to its ability to survive and breed in the study site as well as presence of cattle host for this species. The study of Sevidzem *et al*., [21] in North Cameroon indicated that *Stomoxys* and *Musca* genera shared same biotope and that the Musca group was more abundant. Apart of the fact that individuals of Stomoxys and Musca genera are involed in the mechanical transmission of dangerous diseases [3, 9], their importance in forensic entomology has been documented [14].

Conclusion

The presence of two biting muscids *S. n. niger* and *S. omega* and one non-biting muscid *Musca autumnalis* in the rangeland of Ngaoundere were confirmed through molecular techniques. *M. autumnalis* is seen for the first time and was most abundant in the present collection.

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Conflict of interest

Authors declare no conflict of interest.

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