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First Record of Culicoides Oxystoma, And Other Three Members of the Schultzei Group in Nigeria.

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Abstracts: *A knowledge gap exists in the study of Culicoides species in Nigeria, making it impossible to determine the possibility of evolutionary changes in Culicoides species due to climatic and ecological changes. To bridge this gap, a Taxonomic study of Culicoides species in Nigeria was conducted. Culicoides species were collected from four sites for six months using light suction traps and permanent slide mounts of collected biting midges were examined with Celestron^R LCD digital microscope model #44340 and identifications were made using various identification keys for adult Culicoides species.One thousand five hundred and sixty-five adult Culicoides species were collected and the study showed female dominance with 1,548 (93.16%) while that of the male was 107 (6.84%). Culicoides species caught around hosts was reported to be highest around cattle 931 (59.49%) and lowest around sheep 258 (16.49%). Fifteen species of Culicoides were identified in this study and these are Culicoides imicola, C. enderleini, C. oxystoma, C. nivosus, C. schultzei, C. subschultzei, C. nevilli, C. pycnostictus, C. bedfordi, C. fulvithorax, C. neavei, C.expectator, C. milnei, C. distinctipennis and C. brucei. Four of these species (C. oxystoma, C. schultzei, C. subschultzei and C. nevilli) were being reported for the first time in Nigeria. Thus, bringing the total number of Culicoides species in Nigeria to thirty-seven. With this study, we concluded that diverse species of Culicoides exist in Nigeria and further studies should be carried out to determine the source of blood for the available Culicoides species as well as detection of pathogens transmitted by these species.*

Keywords: *Ceratopogonidae, Culicoides, Nigeria, Schultzei groups*

I. Introduction

Culicoides is one of the members of the Ceratopogonidae family and are considered to have the most medical and veterinary importance due to their biting action and ability to transmit pathogens to livestock (Linley *et al*, 1983). They are small and delicate insects, measuring about 3mm in length. They have variety of habitats ranging from semi-aquatic to aquatic such as mud or moist soil around swamps, ponds, and marshes, tree holes, plant and rock cavities, rotten fruits and plants.

Adults are identified by their characteristic wing pigmentation, slightly humped thorax and multi-segmented beaded antennae which is plumose in male and pilose in female. Wing patterns and the morphology of several parts of the body, which usually requires the dissection and separation of the wings, head, abdomen and genitalia, are the main characters employed in species identification (Delecolle, 1985)

Many biting midge species are of ecological (Martinez et al., 2011), economic (Velthuis *et al*., 2010) and sanitary (Mellor *et al*., 2000) significance as blood feeders and as vectors of pathogens in humans, livestock, poultry and wildlife.

This study was conducted to bridge the knowledge gap in the study of *Culicoides* that has been in existence in Nigeria since 1976 and to give an update on the available *Culicoides* species found in Nigeria.

II. Materials And Methods

2.1 Sites of Collection

Samples were collected from four different locations (two sites each in Ibadan, Oyo State and Makurdi, Benue State) both in Nigeria (Table 1).

Ibadan is located in the south-western Nigeria (7°23'47"N 3°55'0E") in the southeastern part of Oyo State about 120 km inland northeast of Lagos and 530km southwest of Abuja, the Federal capital.The city has two zoological gardens, one at the University of Ibadan which serves as one of the two collection sites. The other site is the Teaching and Research farm, University of Ibadan.

Makurdi- the capital of Benue State is located in central Nigeria (7°43'50"N 8°32'10"E) along the Benue River. It is bordered by Nasarawa State to the north, Taraba to the east, Ebonyi and Cross River to the

south and Kogi State to the west.There are also two collection sites in Makurdi and these were the livestock market North Bank and the University livestock farm South Core.

2.2 Collection of Adult Culicoides

Adult biting midges were collected weekly for six months (February to July 2014).The protocols of Harrup (2014) were used for the selection of sites, collection of *Culicoides*, identification and storage of adult *Culicoides*.

Two distinct light traps were used: Miniature Downdraft Black-light (UV) Trap – Model 1212 and New Jersey Standards Light Trap- Model 912 (John W. Hock Ltd www.JohnWHock.com).

The batteries were charged and traps assembled according to manufacturer's instructions after which the traps were test run to ensure proper functioning. Two drops of liquid detergent (Morning Fresh Cussons^{R}) was mixed with about 100ml of water to break the surface tension of the water and allow the collected insects to sink.The collecting pots were then carefully fixed into the mesh net tube and screw in place securely over the solid fabric end of the net. The traps cables were connected to the batteries as per manufacturer's instructions and the traps were operated from dusk to dawn (6:00pm to 8:00am).

The traps were disconnected the next morning and the contents of the collecting pots were poured into the sieve. The insects were transferred into labeled sample bottles containing 70% ethanol and were kept in a secured, cool and dark area.

2.3 Sorting and Sexing

Sorting and sexing were carried out with the use of binocular stereomicroscope. Sorting involves the separation of *Culicoides* from non-*Culicoides* using characteristic features such as wing pigmentation, multi-segmented beaded antennae as well as humped thorax while the trapped *Culicoides* were sexed principally using the antennae (the male antennae are plumose and the female pilose). Counting of each species as well as the gender was done under stereomicroscope using tally counter and fine forceps and their numbers were recorded.

2.4 Species Identification

For those species whose taxonomic characters could not be identified using stereomicroscope, permanent slide mounts were prepared in Canada balsam with xylene.

Culicoides specimens were digested and cleared in10% potassium hydroxide by gently boiling for 5-10 minutes. The *Culicoides* were filtered and washed with distilled water.

Cleared *Culicoides* were passed through graded concentration of ethanol (for 24-hours in each grade) beginning from the lowest in this order: 70%, 80%, 90% and 100%.

Three drops of Canada balsam in xylene were placed on pre-cleaned well labeled microscope slide. Dehydrated *Culicoides* were placed on the drops of Canada balsam using fine stainless steel forceps. The specimens were then spread to assume the right position. Microscope cover slips were gradually lowered to prevent formation of air bubbles and the slides properly kept till dry at room temperature. The dried permanent mount slides were examined using Celestron^{R} LCD digital microscope Model #44340.

Morphological identification of *Culicoides* was carried out using several identification keys depending on the species found and their subgenus or group. This includes: Examination of the wing (presence or absence of macrotrichia and pigmentation pattern), shape, size and number of spermathecae, shape of third palpal segment andantennal segments (distribution of sensilla coeloconic). For adult male *Culicoides*, shapes of genitalia were used and are highly species-specific for identification (Meiswinkel, 1995, Meiswinkel *et al*., 2004; Borkent, 2005).

Pictures were taken at different magnifications and comparisons were made with various identification keys to arrive at species identification of the collected biting midges.

III. Results And Discussion

The total number of *Culicoides* collected for the entire sampling period was one thousand five hundred and sixty-five (1,565). One thousand four hundred and fifty-eight (93.16%) were females while one hundred and seven (6.84%) were males. Miniature Downdraft Black-light (UV) Trap - Model 1212 trapped 990(63.26%) while New Jersey Standards Light Trap - Model 912 trapped 575(36.74%) of the overall catch (Table 2). Percentage collected around Sheep, Horses and Cattle were 258(16.49%), 376(24.02%) and 913(59.49%) respectively (Table 4). Both the highest 713(45.56%) in July and the lowest 55(3.51%) in May number of collections were recorded during the rainy season (Table 2).

The number of *Culicoides* caught from various collection sites were three hundred and seventy-six (24.02%), two hundred and fifty-eight (16.49%), six hundred and eighty-four (43.71%) and two hundred and forty-seven (15.78%) at University of Ibadan Zoological garden, University of Ibadan Teaching and Research farm, University of Agriculture Livestock farm Makurdi and Livestock market North bank Makurdi respectively

(Table 3). Total catch from Ibadan, Oyo State was six hundred thirty-four (40.51%) while nine hundred and thirty-one were caught in Makurdi, Benue State (59.49%).

A total of fifteen species were identified. The most predominant was *Culicoides enderleini* 515 (32.9%)**.** Other common species were *C. imicola* 196 (12.5%), *C. nevilli* 144 (9.2%), *C. subschultzei* 135 (8.6%), *C. oxystoma* 131 (8.4%), *C. nivosus* 121 (7.7%) and *C. schultzei* 105 (6.7%). Other less common species were *C. expectator*64 (4.1%), *C. neavi* 50 (3.2%), *C. fulvithorax* 36 (2.3%), *C. distinctipennis* 28 (1.8%), *C. milnei* 13 (0.8%), *C. pycnostictus* 11 (0.7%), *C. brucei* 09 (0.6%) and *C. bedfordi* 08 (0.5%). Eleven of these were previously reported by Dipeolu (1976) in a similar work carried out at the Zoological garden University of Ibadan which happens to be one of the collection sites for this present study. The remaining four species are being reported for the first time in Nigeria and these are *C. oxystoma, C. schultzei, C. subchultzei*, and *C. nevilli*. Dipeolu (1976) reported *Culicoides imicola* as the most abundant (36.9%) of the species observed whereas in this study *C. enderleini* which was the most abundant (32.9%) was reported by Dipeolu (1976) as the second most abundant.

Other species reported by Dipeolu (1976) which were not reported in this study could be as result of deforestation which may force some of the *Culicoides* species into extinction or relocation into a new suitable habitat, especially the males which only feed on plant nectar. Furthermore, this may also be due to sampling period. Dipeolu (1976) sample period was from January to December 1974 while the period of collection in this study was six month February to July 2014) since some species may be abundant at a certain period of the year which was not covered in this study.

The new species reported in this study that were not found by Dipeolu (1976) could possibly be as a result of trans-border livestock grazing as some of the flies could accompany these animals during migratory movement. Furthermore, it may be due to sampling methods adopted in this study and could also be due to the fact that these flies have developed adaptation mechanism by which they survive in environment different to their suitable habitats.

Certain species including *Culicoides enderleini, C. oxystoma, C. nivosus, C. pycnostictus*, and *C. imicola* reported by Maryam *et al*., (2014) in Senegal were also identified in this study. However *C. kingi* reported by Maryam et al., (2014) was not identified in our study. Mame *et al*., (2013) in a similar work carried out in Senegal identified *C. oxystoma* and was the first to report it in Africa south of Sahara as we have identified it first in Nigeria. Furthermore, Alahmed (2010) also reported the presence of *C. imicola, C. oxystoma* and *C. neavei* in Saudi Arabia and Bravaman (1988) in Israel also reported the presence of *C. imicola, C. oxystoma* and *C. distinctipennis*.

The higher number of female *Culicoides* than male collected in this study is in agreement with the results obtained in Nigeria by Dipeolu (1976) who reported that 96.11% were females while 3.89% were males and also with the report of Maryam *et al*., (2014) in Senegal that 80.77% were females and 19.23% males.

Higher female to male ratio observed in this study may possibly implies that frequent bites and rapid dissemination of pathogens occurs more since only the females feed on blood and transmit diseases, also increase reproduction which subsequently leads to production of more *Culicoides* population possibly occur.

Most *Culicoides* species display some degree of host preference, which have direct implications regarding their potential role as disease vectors. Significantly more midges were collected from cattle in this study, indicating that cattle may be more attractive than sheep for midges. This was previously suggested by other authors (Garros *et al*., 2011). This has important epidemiological implications, as sheep could be a susceptible target for these vectors when cattle are absent in the vicinity.

Some species prefer to feed on birds; this group of species includes *Culicoides imicola*. Within the mammalophilic species there can be host preferences. Animals having a high body temperature attract large number of blood-sucking females. Insects tend to gather where breeding sites and hosts occur in tandem, with the highest midge concentrations in areas containing cattle, horses and pigs, and the presence of sheep encourages the midge population less than that of cattle DEFRA (2008). In certain areas midges can occur in such large numbers that they can significantly disrupt outdoor activities (for example, tourism and outdoor industry) through their biting attacks on man (Linley and Davies; 1971, Hendry and Godwin; 1988; Blackwell, 1997).

IV. Conclusion

In conclusion, this study was able to confirm the presence of a number of *Culicoides* species recorded by previous survey in Nigeria and also identified the presence of new species not previously reported (*C. oxystoma, C. schultzei, C. subschultzei* and *C. nevilli*). With these four new species reported, the total number of available *Culicoides* species in Nigeria has been raised to thirty-seven. Also, the study has showed that three (*C. enderleini, C. imicola* and *C. oxystoma*) of the 7 dominant species recorded are proven biological vectors of arboviruses.

It is therefore suggested that further studies should be carried out to determine the source of blood for the available *Culicoides* species as well as detection of pathogens transmitted by these species.

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Table 1: Collection Sites and the Species of Animals

Sites	Longitude	Latitude	Habitats
UI Zoological Garden	7.446°N	3.897° E	Horses, Donkeys, Giraffe, Hyenas, Lions, Camels, Monkeys, Duikers, Crocodiles, Giant Eland, Various birds, Humans
UI Teaching and Research Farm	7.450°N	3.880°E	Sheep, Goats
Livestock Market Makurdi	7.776°N	8.557°E	Cattle, Sheep, Goats, Dogs, poultry, Humans
UAM Livestock Farm	7.705°N	8.627°E	Cattle, Sheep, Goats, Humans

Table 2: Monthly Collection of *Culicoides* in Relation to Trap and Sex

Months	Trap 1212	Trap 912	Male	Female	TOTAL
February	77 (66.96%)	38 (30.04%)	15 (13.04%)	100 (89.96%)	115 (7.35%)
March	78 (77.23%)	23 (22.77%)	22 (21.78%)	79 (78.22%)	101 (6.45%)
April	124 (58.77%)	87 (41.23%)	30 (14.22%)	181 (85.78%)	211 (13.48%)
May	50 (90.91%)	5 (9.09%)	03 (5.45%)	52 (94.55%)	55 (3.51%)
June	152 (41.08%)	218 (58.92%)	12 (3.24%)	358 (96.76%)	370 (23.64%)
July	509 (71.39%)	204 (28.61%)	25 (3.51%)	688 (96.49%)	713 (45.56%)
TOTAL	**990**	**575**	**107**	**1,458**	**1,565**

Table 3: Number of *Culicoides* Collected from different Collection Sites

Sites of Collection	**Number Caught**
UI Zoological Garden Ibadan	376 (24.02%)
UI Teaching & Research Farm Ibadan	258 (16.49%)
Livestock Market Makurdi	247 (15.78%)
University Livestock Farm Makurdi	684 (43.71%)
TOTAL	**1,565**

Table 4: Number of *Culicoides* Collected around Species of Animals

Animal Species	No of *Culicoides* Collected
Sheep	258 (16.49%)
Horses	376 (24.02%)
Cattle	931 (59.49%)
TOTAL	**1,565**

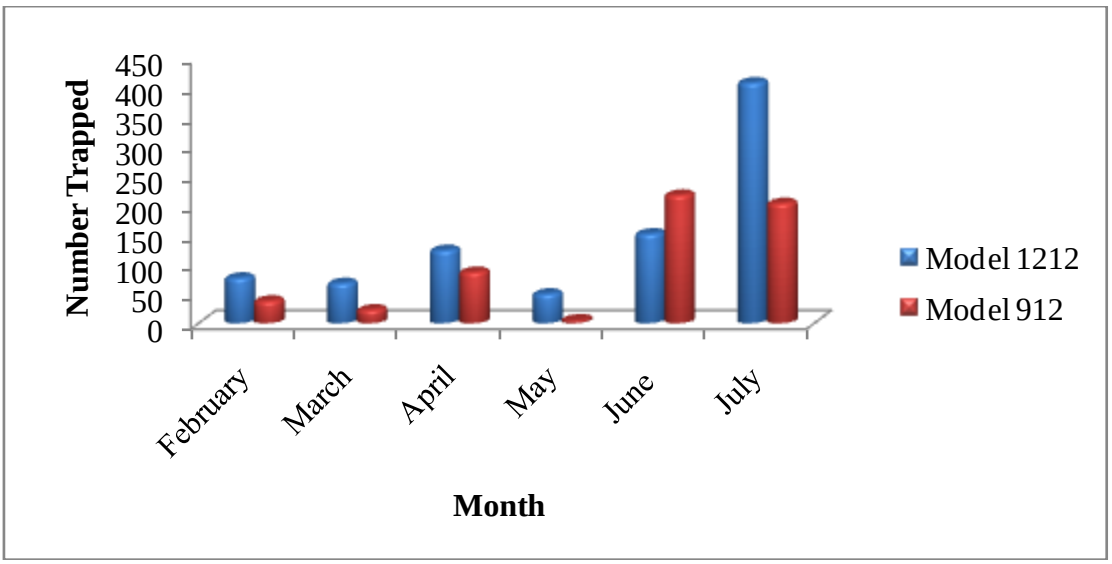

Figure 1: Monthly Collection of *Culicoides* by the Two Traps

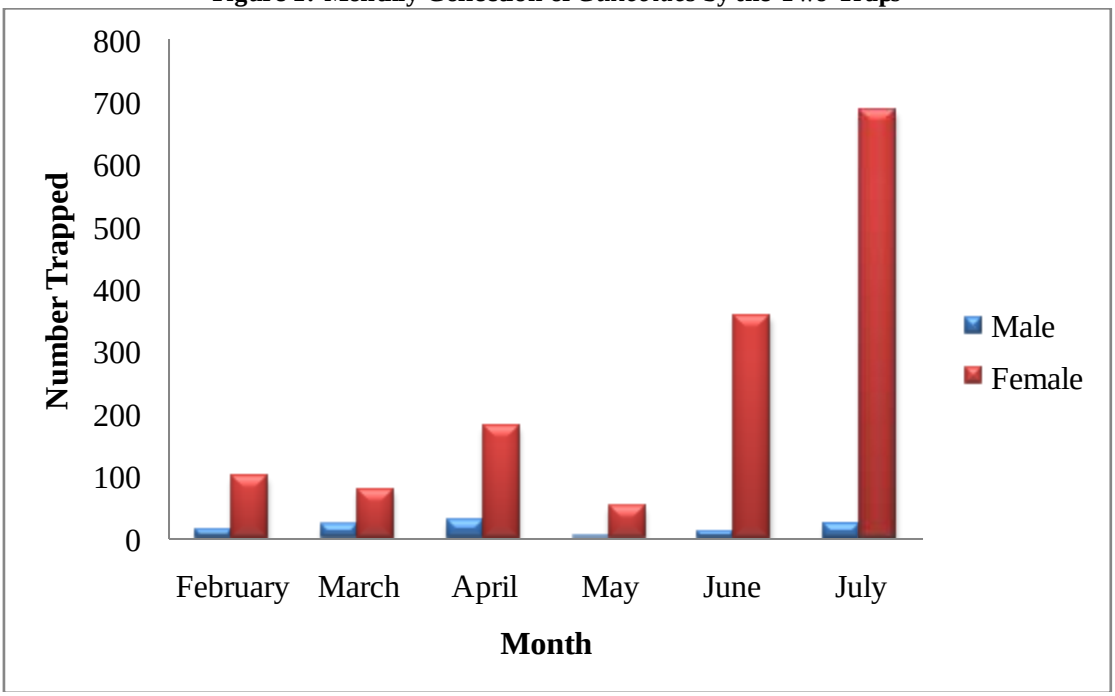

Figure 2: Monthly Collection of Male and Female *Culicoides*

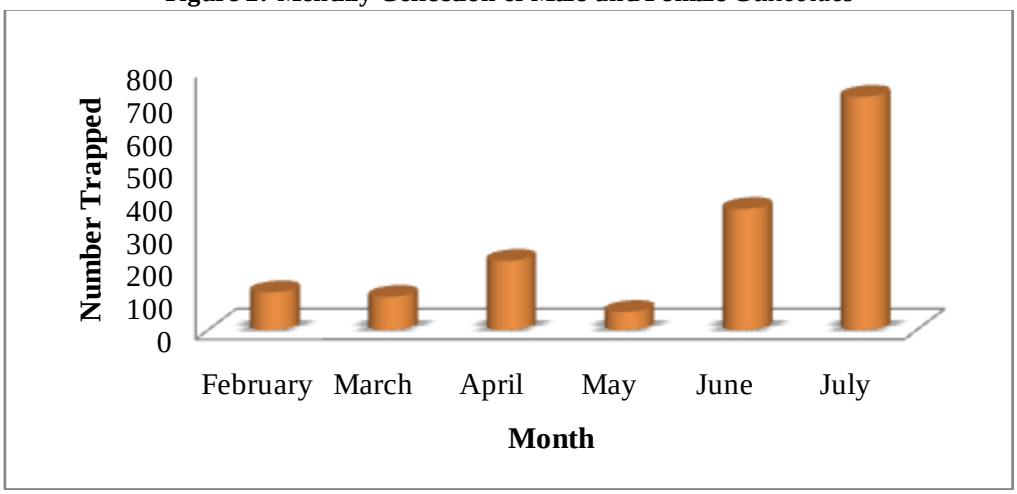

Figure 3: Total Number of *Culicoides* Collected During Period of Study

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