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Phoretic fauna and flora of domestic non-biting flies in Ile-Ife, South-Western Nigeria

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Abstract

Living organisms have evolved several means of migration, dispersal and locomotory supports. This usually takes subtlety in way of ectosymbiosis among several groups of insects including non-biting flies. It is informative to identify the spectrum of phoretic fauna and flora associated with non-biting flies. Non-biting flies were caught from various filthy sites using fine insect sweep nets. Externally attached organisms were isolated. Identified organisms include larvae of *Cyathostoma* sp, *Dictyocaulus* sp, *Dicrocoelium lanceatum*, hookworm and *Strongyloides stercoralis*. Phragmospore of *Conidia*, mould spore of *Drechslera* sp and *Epicocum* sp spores were isolated from the flies. *Dermatophagoides pteronyssinus*, was found attached to Calliphorid and Muscid flies. *Musca domestica* had the highest phoront load. *Dicrocoelium lanceatum* and prolotitides of *Taeniarhynchus* sp were found attached to *M. domestica*. More fungal spores were found on flies followed by nematode larvae. More complex relationships are likely to be present and far-reaching than mere phoresy.

Keywords: phoresy, mites, nematode, spore, fungi, non-biting flies

Introduction

Domestic non-biting flies are non-stylate, haustellate, dipteran flies. Adults are true flies with spongy-sucking type of mouthparts with the exception of the biting stable fly. The flies are widely distributed in the sub-phylum Cyclorhapha, having unrestricted eco-territorial boundaries [1, 2]. Well known members include house flies, flesh flies, blowflies and their allies. They are closely associated with decaying organic matter, dead and living tissues for feeding and reproductive purposes. Overtly, they are usual irritants owing to their inherent humming and swarming on diverse substrates. The annoyance associated with large populations of non-biting flies is considerable [3]. Adult non-biting flies swarm on meat, faeces, flowers, nectar, animal carcasses, garbage, human food and decaying organic matter [4]. Non-biting flies pick up different organisms including microbes and metazoans while crawling and feeding on filthy surfaces. Ubiquity, reproductive and feeding habits and anatomical structures enhance their carriage potential [5]. The nature of the flies' bodily structure as well as the intimate association with animals affects the degree of success in mechanical carriage of organisms and particles [6]. Bigger particles are transported by flies on their exoskeleton [7]. Passive transport of several bacteria, fungi and protozoa had been demonstrated on domestic filthy flies. Moreover, it has been recognized that small particles and organisms readily adhere to the exterior surfaces of flies [8]. The exoskeletons of these flies also have certain electrostatic charges, and any particle with a different charge or neutral charge will adhere to the fly body surface [9].

Phoresy is the use of larger animal (host) by smaller attaching animal (phoront) for migratory movement. Many invertebrate animals often use other animals as objects of attachment and means of dispersal. This may translate into ectosymbiosis or ectoparasitism *sensus stricto*. The alliance of phoronts and hosts always create a diverse community of species. The phoront has the advantage of dispersal by superficially attaching to its hosts [10] while the host's obvious harm or benefit is unknown. Parasitism may originate in free-living members to form facultative and cohesive community and thus side-step phoresy. Occasionally, phoresy may take the form of commensalism with assumption that the host remained unaffected. In some cases, the interaction becomes mutualistic or costly to the host [11], and thus considered parasitic. However, the tendency of attachment and carriage in living organisms enable many basic life functions such as nutrition, reproduction and movement [12].

The host usually have various attachment devices which are grouped according to the physical mechanism, biological functions and time of attachment. The attachment may be permanent, temporary or transitory depending on continuity for life. In many invertebrates including nematodes, insects, benthic animals and wave-swept shores inhabitants, the most common biological attachment devices are suckers, hooks, foot pads, claws, spines and clamps [13]. In addition, attachment mechanisms may involve wet adhesion through the production of a sticky substance functioning as biological glue [12, 14]. Many phoronts have a wide range of carriers, characterised with different surface morphology of the body. There has been report of attachment to body surfaces of various insects with smooth, hairy, setae-covered body surfaces [15]. Deutonymph mites have greater attachment affinity for other insect body part covered with setae [12]. Like most insects, non-biting domestic flies have their bodies covered with hair-like bristles and setae. Again, a small claw and fleshy little sticky pads on the tarsi permit flies easy clinging to surfaces. Higher tendency for attachment to non-biting synantropic flies is amplified by the presence of tarsi and bristles use in trapping particles and smaller organisms. The structural modifications of non-biting flies typified by their segmented bodies, appendages and integuments with fine setae or hair are succinct attachment points [2]. The adhesive device, the pulvilli, a pad-like structure between the tarsal claws of the legs, is present in the blowfly (Calliphoridae), housefly and relatives (Muscidae), and flesh fly (Sarcophagidae). This provides anatomical support for the successful carriage of metazoan organisms [16]. There has been report of phoretic abilities of dipteran flies as seen in other animal population especially, the phoresy of macrochelid mites have been found and recorded on stable flies, *Stomoxys*

calcitrans, a muscid [17].

The extent and biotic significance of phoresy in synantropic non-biting flies remains inconclusive. It may be maintained as biological or non-biological. There has been report to show the extent of permanent identifiable depression scars on adult stable fly carrying mite after separation [17]. Broadly, phoresy occurs because both parties are migrants, exploring the same unstable habitat. Permanence of relationship whether commensalism, mutualistic, parasitism, symphorism, or some other type of association is indicated by frequent occurrence on the host [18]. This study was carried out to identify and report the carriage of organisms on the exoskeletons of non-biting flies. However, what remained un-clarified is the associative imbalance that may ensue from the organisms being carried about by these flies. Nonetheless from this study, we recorded phoretic fauna and flora retrieved from the exoskeleton of non-biting flies collected from various sites in Ile-Ife, Nigeria.

Materials and Methods

Collections of non-biting flies were carried out in Ile-Ife (7°28' to 7°45' N; 4°30' to 4°34'E) south-western Nigeria. The terrain of the town is fairly plain with warm tropical areas and about 300-600m above sea level. A fine insect sweep net with mesh size of 0.5µm was used to collect flies by lowering it to the surface of the substrates where the flies swarm. Area sampled were abattoirs, refuse dump sites, open markets and latrines. Adult flies were caught before noon between Dec 2014 and May 2015. The caught flies were anaesthetized by asphyxiation in 100% ethyl acetate soaked in cotton wool. The trapped flies were then transported to Laboratory in Department of Medical Microbiology and Parasitology, Obafemi Awolowo University, Ile-Ife, Nigeria.

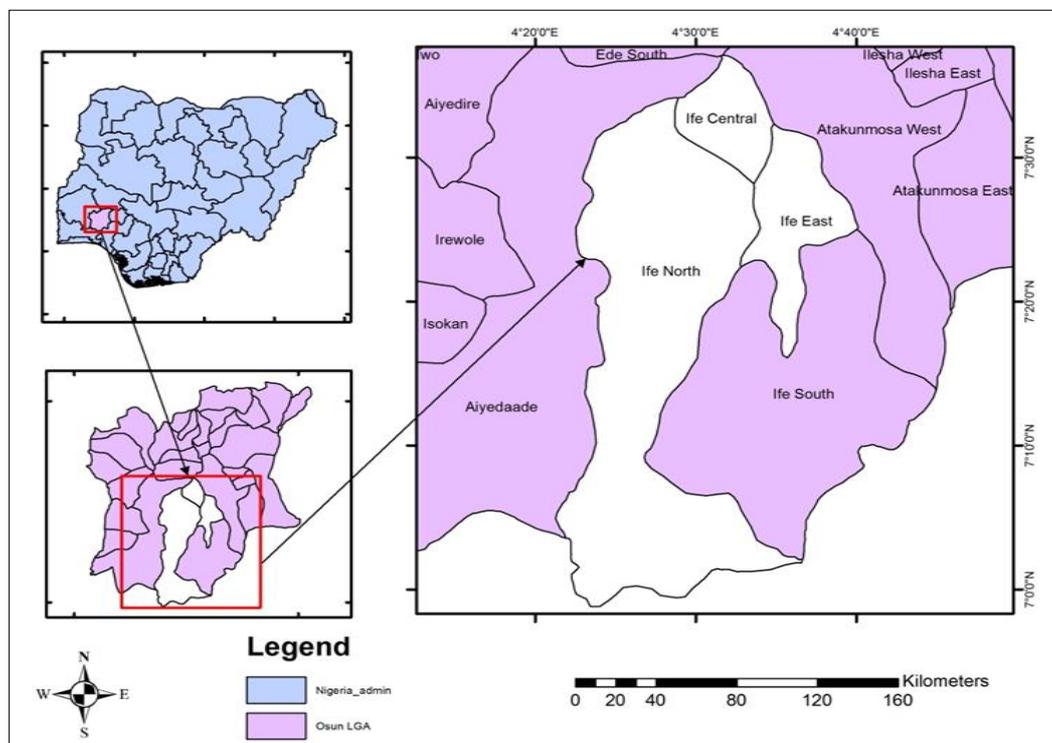


Fig 1: Map of the study area

Flies were first observed under stereo zoom microscope and identification of flies was done as described by Ogunniyi and colleagues [19]. After which the caught flies were dipped in normal saline and rocked mechanically to dislodge attached organisms. The washings generated were centrifuged at 2000

rpm for fifteen minutes. The supernatant was decanted. A drop of Lugol solution was added to sediment in the vials, thoroughly mixed and examined under light microscope using X10 and X40 objective lenses to identify the dislodged organism harboured externally. Identification of nematodes

was done based on morphological comparison from the work of Thienpont^[20]. Other organisms were identified on the basis of morphological characteristics from Natural History Museum of Obafemi Awolowo University, Nigeria.

Results

Seven hundred and eleven non-biting flies belonging to four families, seven genera and nine species of dipteran flies were caught from different filthy sites. Identified families include Calliphoridae, Muscidae, Sarcophagidae and Stratiomyidae. These comprised seven genera including *Calliphora*, *Lucilia*, *Fannia*, *Musca*, *Sarcophaga*, *Tricholoprocta* and *Hermatia*. A total of nine species caught from the collection sites were

Fannia scalaris, *Musca domestica*, *Musca vetustissima* (Family: Muscidae) *Calliphora stygia*, *Lucilia illustris* (Family: Calliphoridae) *Tricholoprocta hardyi*, *Sarcophaga haemorrhoidalis* (Family: Sarcophagidae) *Hermetia illuscens* (Family: Stratiomyidae) (Table 1). *M. domestica* had the highest species occurrence from all the sites except animal slaughter houses. Highest number of flies (200) was recorded from latrines and the lowest from open markets (143). Meanwhile, seven species of non-biting flies were identified from open markets as opposed to three species identified from latrines (Table 1). The behavioural, ecological and morphological characteristics of these flies have been previously reported^[19].

Table 1: non-biting fly species collected from different sites

Fly Species	Market places	Slaughter houses	Latrines	Refuse sites
<i>Fannia sclaris</i>	18	2	93	58
<i>Calliphora civinia</i>	29	48	-	37
<i>Musca domestica</i>	53	21	86	83
<i>Hermetia illuscens</i>	3	-	-	-
<i>S. haemorrhoidalis</i>	-	54	21	-
<i>Lucillia illustris</i>	-	34	-	-
<i>Calliphora stygia</i>	13	-	-	-
<i>T. hardyi</i>	11	25	-	-
<i>Musca Vetustissima</i>	16	-	-	-
Total	143	184	200	184

Spores of three species of fungi were isolated from some of the flies caught. These include *Epicorum* sp (spores) from *C. stygia* and *M. domestica*. The microcetes spores (*Hendersonia septem-septate*) of *Drechslera* sp were found attached to *M. domestica*, *C. vicinia*, *C. stygia*, *T. hardyi*, *S. haemorrhoidalis* and *H. illuscens*. Phragmospore, an asexual spore of *Conidia* sp was found on *C. vicina*, *C. stygia* and *M. domestica*. Several species of nematodes were found attached to *F. scalaris*. These include larvae of *Dictyocaulus* spp,

Trichonema/Cyathostoma sp (horse intestinal parasite) and *Strongyloides stercoralis* (Family; Strongyloidae) were isolated from flies caught from refuse dump sites and slaughter houses. Eggs of *Dicrocoelium lanceatum* (Platyhelminthes, Family; Dicrocoeliidae) were isolated from *M. domestica* caught from latrines. House hold dust mite, *Dermatophagoides pteronyssinus* was found among flies caught in all the categories of sites sampled (Table 2).

Table 2: Phoretic association between non-biting flies and other organisms

Flies	Phoretic organisms identified									
	A	B	C	D	E	F	G	H	I	J
<i>C. vicinia</i>	X	✓	✓	X	X	✓	X	X	X	✓
<i>C. stygia</i>	✓	✓	✓	X	X	X	X	X	X	X
<i>F. scalaris</i>	X	✓	✓	✓	✓	X	✓	X	✓	✓
<i>H. illuscens</i>	X	✓	X	X	X	X	X	X	X	X
<i>L. illustris</i>	X	✓	X	X	X	X	X	X	X	X
<i>M. domestica</i>	✓	✓	✓	✓	✓	X	X	✓	✓	✓
<i>M. vetustissima</i>	X	X	X	X	✓	X	X	X	X	X
<i>T. hardyi</i>	X	X	X	X	X	X	X	X	X	X
<i>S. haemorrhoidalis</i>	X	X	X	X	X	✓	X	X	X	X

Key: Presence (✓), Absence (X) A; *Epicocum* spore, B; *Drechslera* sp spore, C; Phragmospore of *Conidia*, D; larvae of *Strongyloides stercoralis* E; hookworm larvae, F; *Cyathostoma/Trichonema* spp, G; *Dictyocaulus* sp, H; *Taenirhyncus* sp, I; *Dicrocoelium lanceatum*, J; *Dermatophagoides pteronyssinus*

The highest percentage of identified spores of fungi (11.89%) was recorded among flies caught from markets and the least from latrines (3.80%). *Drechslera* sp was the most frequently found spore followed by *Phragmospore* of *Conidia* sp. The only species of mite identified was found most frequently among flies caught from the open markets (3.50%) followed by refuse sites (2.18%). Flies caught from the slaughter houses had more nematodes (4.30%) attached to their

exoskeleton than flies collected from sites. Eighty one (81) of the total flies caught had one or more organisms attached to its body apart from other unidentified ones and particles (Table 3). Xenobiotic relationship was identified in house dust mite, *Dermatophagoides pteronyssinus* (Family; Pyroglyphidae) among *C. vicinia*, *F. scalaris*, *M. domestic*, *M. vetustissima* and *Lucillia illustris*.

Table 3: percentage of flies in phoretic association with other organisms

sites	Number of		Nematodes		Platyhelminthes
	Flies	Spores (%)	Mites(%)	(%)	(%)
Open markets	143	17(11.89)	5(3.50)	1 (0.70)	-
Slaughter houses	184	7(3.80)	2(1.10)	8(4.30)	2(1.10)
Laterines	200	13(6.50)	2(1.00)	4(2.00)	-
Refuse sites	184	12(6.52)	4(2.18)	4(2.20)	-

Discussion

Domestic non-biting flies swarm or are attracted to various decaying organic matter as shown in this study. The variety of non-biting flies observed in the open market is as a result of variety of food substances present in the area. Highest spore attachment was recorded among flies caught from open markets. This may be due to various decaying substance of plants and animal origins. This study showed that mites were found easily attached to *M. domestica*. Similar position has been reported previously in that *Macrocheles muscaedomesticae* and *M. subbadius* mites had several attachment points on *M. domestica* [21]. However, by extension, we report the attachment of *D. pteronyssinus* on *F. scalaris* and *M. vetustissima* (Family; Muscidae) and *C. vicina*, (Family; Calliphora). From this study, *M. domestica* and *F. canicularis* were identified as carriers of mites as was earlier reported except that *F. canicularis* was not reported as carrier of mites in previous work done in mite bred manure [21]. Meanwhile, *F. scalaris* as identified in this study was readily found associated with mite. It thus signifies that presence of mite or organism is not as important as the preference of phoront in readiness for carriage. Additionally, even in the presence of several species of mite, some insects have been reportedly found with only one species of mite [18]. This shows some extent of preference and not necessarily specificity in carriage.

Other aspect of mite behaviour which is of utmost important in their attachment to dipteran flies is the production of pedicel, a sticky, mushroom-shaped attaching structure that enables temporary attachment of mites to its carrier [12]. This is a major underlining factor in mite attachment to various flies as reported in this study. More importantly, phoretic attachment is common in the class Acarina (to which *D. pteronyssinus* is classified) and occurs often on insect like fleas, domestic flies and hippoboscids [20]. Another salient supporting fact about the attachment of mites to house flies is premised on the fact that some mites bear setae on the front leg tarsi which sense water-soluble sugar compound produced by pupae and adult of both sexes of stable fly and house fly [17].

The close and species-specific associations among organisms are certainly a biologically interesting phenomenon. However, most insect-fungi relationships are diffused and loose as seen in this study and as previously reported [23]. Several reports have shown the isolation of several species of insect, especially *M. domestica*, as carriers of fungi species [24]. This study also revealed *M. domestica* as carrying the highest load of fungi spores. Insects involved in fungal association include members of the coleopteran, diptera, homoptera, hymenoptera and isopteran. Although Insects' composition varies spatially and temporally, some insects are mycophagous while most are secondarily or not at all mycophagous. Fungi-feeding insects may attach spores on their body surfaces but most times have spores contained in their guts. This condition has been well documented among Drosophilids and Muscids flies

[23]. However, in either case, the isolated spores are not morphologically different from the intact spores as seen from the spores isolated from the flies in this study.

The seeming biological complex process of spore dispersal have various behavioural and adaptability components. For example, the stinkhorn fungi (Phallales) attracts various insects with its characteristic strong odour and by its extremely sticky nature may remain glued to the body surface of dipteran flies which in turn is dispersed as flies move about. Also attraction of flies to fungi spore may be for food [25]. The sticky nature of some of the spores reported must have influenced their carriage on flies' exoskeletons. In the process of spore dispersal by flies, certain entomopathogenic fungi had been reported on some flies [26]. This makes the spectrum of insect-fungi associations a symbiosis rather than migratory. Incidentally, none of the fungi reported in this study was entomopathogenic. *D. pteronyssinus* are common in house dust consists of leftover human skin (keratin source), fungal hyphae (which consist chitin and the fibrous material of the fabric (which is a source of cellulose). The association of fungi and mite was obvious in terms bio-interconnectivity as mites and fungi were collectively found together on some of the flies [29, 30].

Many nematodes species have intimate heterospecific association with vertebrates, invertebrates and plants in symbiotic associations [26]. And so the identification of nematodes in this study from non-biting flies is not coincidental. The symbiotic association between nematodes and insect are diverse and can be classified into phoretic relationship, facultative parasitism and obligate parasitism [27]. In general, it worth highlighting that phoretic organism can have negative consequences on host fecundity and ability to travel naturally [11]. Insect parasitic nematodes in the genus *Steinernema* (*Neoaplectana*) and *Heterorhabditis* have been reported to be pathogenic to a wide range of insects including *M. domestica* [28]. Several species of nematodes identified in this study are parasites of human and livestock. For instance, hookworm, *S. stercoralis* and from this study, *Cyathostoma* sp, intestinal parasite of horse, was identified. These and other nematodes identified are not known to parasitize the flies. To the best of our knowledge, this is the first report of biological association of *Dictyocaulus* sp and *D. lancentum* among flies of Muscidae and Calliphoridae.

Conclusion

Zoohoria or phoresy is a passive mechanism of dispersal as it emphasizes transport rather than symbiosis. It is clear that association between the organisms reported in this study may span parasitism, paraphagism, symphorism, or some other type of association (physiological, reproductive or hormonal) entwining phoresy. So, more complex relationships between non-biting flies and mites, nematodes, and fungi are likely to be present. This co-existence or permanence of relationship is premised on various needs of the phoretic organisms and anatomical and behavioural capabilities of the host.

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