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Diversity of Dipterans Associated with Mushroom, Volvariella volvacea Bull ex Fr. Singer Cultivation

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Abstract

Determining the associated Dipteran flies in *Volvariella volvacea* mushroom cultivation is important since these flies are purportedly potential pests of mushrooms especially in their immature stages. To address this, mushroom bed establishment and adult fly sampling at different growth stages of *V. volvacea* mushrooms by net-sweeping and use of sticky boards were done at weekly intervals. Net-sweeping revealed catches of adult flies which were identified using appropriate taxonomic keys; however, insects collected by sticky boards were mutilated and identification was not possible.

Eight species of flies found exhibited the breadth of dipteran diversity; three families represented the suborder Nematocera (Scatopsidae, Sciaridae, and Empididae) and five of the suborder Brachycera namely, Mycophila (Cecidomyiidae), *Pipunculus* (Pipunculidae), *Porcenus* (Heleomyzidae), *Leptocera* (Sphaeroceridae), and *Desmometopa* (Milichiidae). The most abundant fly was *Desmometopa* in the eight weekly samplings signifying that the mushroom substrate was supporting this fly population. *Desmometopa,* as well as *Porcenus* and Scatopsidae flies are known detritivores, while Sciaridae and Cecidomyiidae represented by *Mycophila* are known fungivores. *Pipunculus* and the Empididae fly are endoparasite and predator, respectively.

Key Words: detritivore flies, dipteran diversity, endoparasitic flies, fungivorous flies, rice straw mushroom

Introduction

Both adult and immature flies (order Diptera) are touted as nuisance in mushroom growing. Their presence alone in or near cultivation beds connote possible damage on the developing hyphae, either by feeding of immature flies on fruiting bodies (Hosaka & Uno, 2012), vectoring mites (Hanski, 2012), and damaging the development of hyphae (Gratwick, 1992) and pinheads (Momgkynrih et al., 2017). Adult flies can likewise oviposit and sustain pest populations throughout the growing period. Infested mushrooms often discolor, turn to brown and become leathery, and rendered inedible. Aside from reducing both quantity and quality of mushrooms, postproduction damages also lessen

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marketability due to presence of maggots and pupae in the produce. Kielbasa and Snetsinger (1980) reported that the injury threshold of commercial mushroom production is 108 females of *Lycoriella mali*, (Sciaridae fly) per square meter. This threshold sets up the conditions for economic loss. Other researchers were able to determine that four larvae per square meter during a spawn-run can result to substantial yield loss (White, 1970; Wyatt, 1970). Owing to the reproductive potential inherent in flies, as much as 3.5 million larvae per square meter has been observed.

Very few studies deal with flies associated with mushroom cultivation, even more so for *Volvariella volvacea.* It is one of the common choices of mushroom growers in Asian countries since rice straw is considered as the best substrate, if not the main substrate, for its cultivation. In addition, the high and constant demand for rice produces rice straw as a by-product, making cultivation of the mushroom an economical option.

Resource use by flies can be a basis to explain the concept of ecological niche and succession of biota on substrates, as well as its applications on possible pest infestations (mushroom fruiting body grazers) in mushroom cultivation. Alternatively, it can even be viewed as a positive ecological interaction such as probable dispersal of spores by associated mites being carried by flies.

This research aimed to determine the species of flies associated with *V. volvacea* cultivation and address the paucity of information with regards to fly occurrence. The result of this study is considered an initial report on the diversity of adult flies in rice straw mushroom cultivation in the Philippines.

Materials and Methods

Four mushroom beds measuring 2 x 0.75 x 0.75 ft. (L x W x H) were prepared following that of the bed-type approach detailed by Reyes et al. (2004) using dried rice straw. Minor modification from the previous approach was employed, that is, beds were raised using a steel platform to preclude proliferation of soil dwelling insects in the sampled collections.

Collection of insect samples commenced eight days from spawning by which time hyphal run was established. A total of eight samplings were collected by net sweeping (50 net-sweeps) on tops and sides of mushroom beds at weekly intervals. Fly collection was done in the mornings since contrasting reports state the best times of the day for collections (Beyer, 2002; Mazin et al., 2019; Singh & Sharma, 2016). However, since the set-up was under a shed and beds were covered with black plastic, mornings were considered as the best collection time since most flies reported were nocturnal or active in the dark; hence, mornings would have the least light penetration. The last sample collection was undertaken one week after the last full harvest. Samples were sorted based on distinguishing morphological characteristics, and identified from the family level to the lowest taxon possible using appropriate keys for taxonomic identification. Photographs of distinguishing and diagnostic parts and digitized measurements were taken using Optika Microscope and Software. Parts measured for all specimens wherein applicable are shown in Table 1. These were modified from that of McAlpine's (1981) morphology and terminologies in Diptera.

Results and Discussion

Fly Populations in *V. volvacea* Cultivation

The weekly populations of the different fly species are shown in Figure 1. The Milichiidae fly, represented by *Desmometopa* had the highest population throughout the sampling period. Within the

first four to five weeks, the numbers ranged from nine to 16, but plunged in the seventh week and surged again in the eighth week. The Heleomyzidae fly was next in rank in terms of occurrence with fluctuating numbers ranging from two to eight within the 2-month period. Both fly families, Milichiidae and Heleomyzidae are known to be associated with decomposition. Scatopsidae flies are known to occur in decaying plant matter. Hence, the presence of these three was due to the decomposition of the *Volvariella* substrate.

Table 1

Detailed Description of Fly Parts Measured (mm) and Indices Computed (Ruiz-Fiegalan, 2003)

Part	Meaning	Description
BL	Body Length	straight distance from distal edge of pedicel to tip of abdomen
ThL	Thorax Length	distance from anterior total margin to apex of scutellum
WL	Wing Length	distance from humeral cross vein to wing apex
WW	Wing Width	maximum wing width
FW/HW	Frontal Width/Head Width	measurement across frons/ that of widest head width
ch/o	cheek proportion with eye	maximum width of gena/maximum diameter of eye
Prorb	proclinate orbital bristle	proclinate orbital/posterior reclinate orbital
Rcorb	reclinate orbital bristle	anterior reclinate orbital/ posterior reclinate orbital
Vb	Vibrissae	subvibrissal seta/ vibrissa
Dcl	dorsocentral bristle	anterior dorsocentral seta/ posterior dorsocentral seta
Sctl	scutellar bristle	basal scutellar/ apical scutellar
Orbito	pertains to relative position of setae as described	distance between proclinate and posterior reclinate orbitals/distance between inner vertical and posterior reclinate orbital
Dcp	pertains to relative position of setae as described	length of distance between inner vertical and posterior reclinate orbital
Sctlp	pertains to relative position of setae as described	distance between basal and apical scutellars/ cross distance between apical scutellars

The known fungi-feeders were the flies belonging to the family Cecidomyiidae, Sciaridae, and Sphaeroceridae. These were also the families of flies reported by other researchers found associated with mushroom cultivation (Erler & Polat, 2015; Greenslade & Clift, 2004; Mazin et al., 2019; Singh & Sharma, 2016; Smith et al., 2006). The numbers of Sphaerocerid fly, *Leptocera*, was higher than the other fungivorous flies during the first week. Only one sciarid fly was caught throughout the sampling

period, and this was in the second week, when the mushrooms were in the pre-button stage. Also, starting the middle part to the end of the two-month sampling duration, the cecid fly, *Mycophila*, had higher numbers than *Leptocera* (Sphaeroceridae).

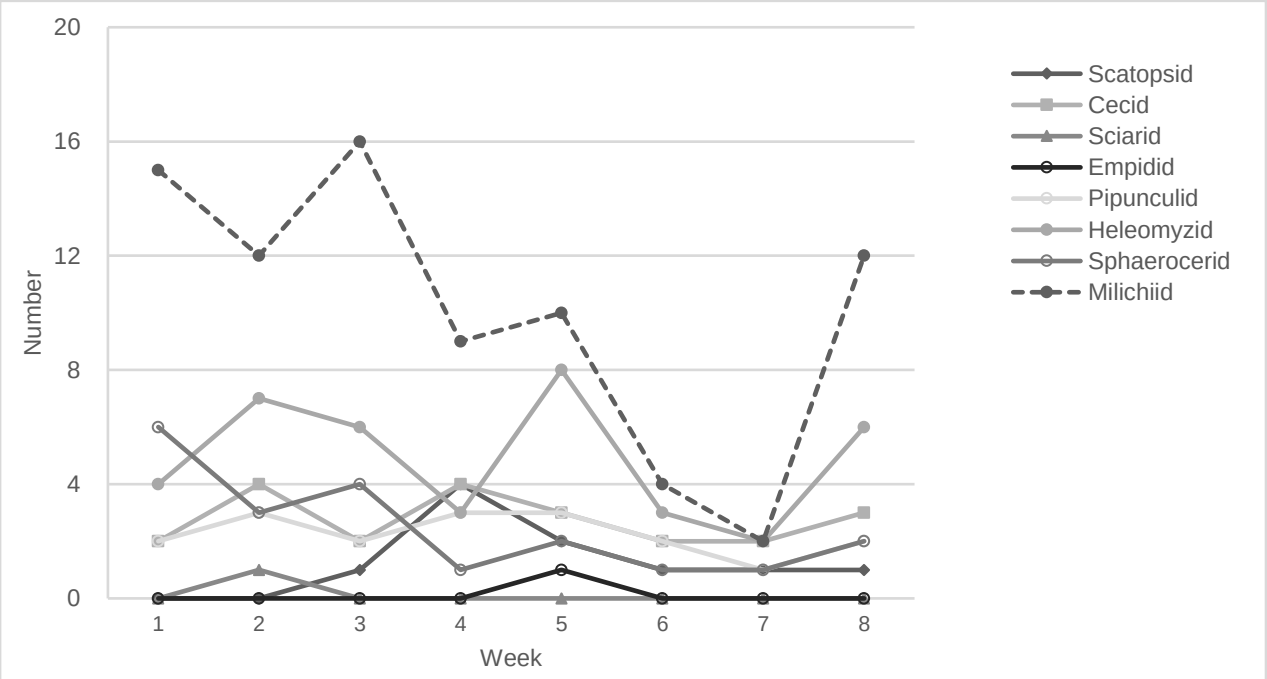
The other two families of flies, Pipunculidae and Empididae are known natural enemies; the former is an endoparasite, while the latter is predaceous on smaller arthropods. There was only one Empidid fly caught (5th week), while *Pipunculus* of the Pipunculid family was more frequent in the samples.

A trend between *Mycophila* and *Pipunculus* was noted wherein a surge in the former brought a subsequent surge of the latter. *Mycophila* is a genus under the family Cecidomyiidae reported to be a fungivore, while *Pipunculus* is a known endoparasite.

Studies in India (Singh & Sharma, 2016) reported three Dipterans (Sciaridae, Cecidomyiidae and Phoridae) found infesting mushrooms (*Agaricus, Pleurotus,* and *Volvariella*). Research in the United States have already dealt with yield impact of flies on mushrooms, mode of damage, and rearing of individual pests for biological studies. Research dealing on the subject matter of this experiment is sparse and deals only with one group of flies such as Shin et al. (2012) in South Korea. Other research are focused on coevolution of flies and mushroom and are far too specific to be of practical application (Zhang et al., 2016).

Figure 1

Weekly Populations of Dipterans Associated in Volvariella C *Growing paddy straw mushroom*



Species of Flies

A total of 191 flies (Order Diptera Linnaeus 1758) were identified. The flies found associated in *Volvariella* mushroom cultivation represented both suborders, Nematocera and Brachycera, signifying the breadth of dipteran diversity. Of the suborder Nematocera, three families were represented, i.e. Scatopsidae, Cecidomyiidae, and Sciaridae. The suborder Brachycera which is further subdivided into Cyclorrhapha and Orthorrhapha also had representative families, the latter with one representative family (Empididae). In addition, the division Cyclorrhapha which is segregated into two series, namely series Aschiza and series Schizophora. Pipunculidae was the family represented in the series Aschiza while Schizophoran families included Heleomyzidae, Sphaeroceridae, and Milichiidae. Table 2 shows the listing of families represented based on the classification by Colless and McAlpine (1991).

Other families under the Order Diptera that were not found in the samplings but are reported by other workers include Phoridae (Erler & Polat, 2015; Mazin et al., 2019; Smith et al. 2006), and Drosophilidae (Greenslade & Clift, 2004; Nongkynrih et al., 2017).

Table 2

List of adult flies from Volvariella volvacea *mushroom beds, showing the classification and taxon name*

Suborder	Division	Series	Family	Species
Nematocera	_	_	Scatopsidae	
Nematocera	_	_	Cecidomyiidae	*Mycophila barnesi*
Nematocera	_	_	Sciaridae	
Brachycera	Orthorrhapha	_	Empididae	
Brachycera	Cyclorrhapha	Aschiza	Pipunculidae	*Pipunculus* sp.
Brachycera	Cyclorrhapha	Schizophora	Heleomyzidae	*Porcenus johnsoni*
Brachycera	Cyclorrhapha	Schizophora	Sphaeroceridae	*Leptocera* sp.
Brachycera	Cyclorrhapha	Schizophora	Sphaeroceridae	*Desmometopa* sp.

Of the eight species found, two were identified to species, three to genus level, and three up to family level, namely: Scatopsidae, Sciaridae, and Empididae. Distinguishing characteristics to identify the genus of the Scatopsidae or sp. 1 was not clear, while for Sciaridae and Empididae, there was only one specimen each and parts pertinent to identification beyond the family level were either lost or damaged. The five species identified to the level of genera were *Mycophila*, *Pipunculus*, *Porcenus, Leptocera,* and *Desmometopa*, of which two were pinned down to species level, *Mycophila barnesi* and *Porcenus johnsoni*. The following details the taxonomic treatments for the eight species found.

Family Scatopsidae

1. Scatopsidae

Diagnosis. This family was distinguished based on the predominant dark brown color (Figure 2); thickened and dark radial veins (Figure 3); mid and hind tibiae is without apical spurs; wings without markings; discal cell absent, but ocelli present; mesonotum without V-shaped suture.

Measurements. BL = 0.72 mm; ThL = 0.20 mm; WL = 0.5 mm; WW = 0.2 mm.

Indices. FW/HW = 0.4(0.3 – 0.5); ch/o = 0.02 (0.1 – 0.2); prorb = 0.2 (0.1 – 0.2); rcorb = 0.3 (0.1 = 0.3); vb = 0.2 (0.1 -0.2); dcl = 0.2 (0.1 – 0.2); sctl = 0.3 (0.2 – 0.4); orbito = 0.2 (0.1 – 0.2); dcp = 0.2 (0.1 – 0.4); sctlp = 0.4 (0.3 – 0.5).

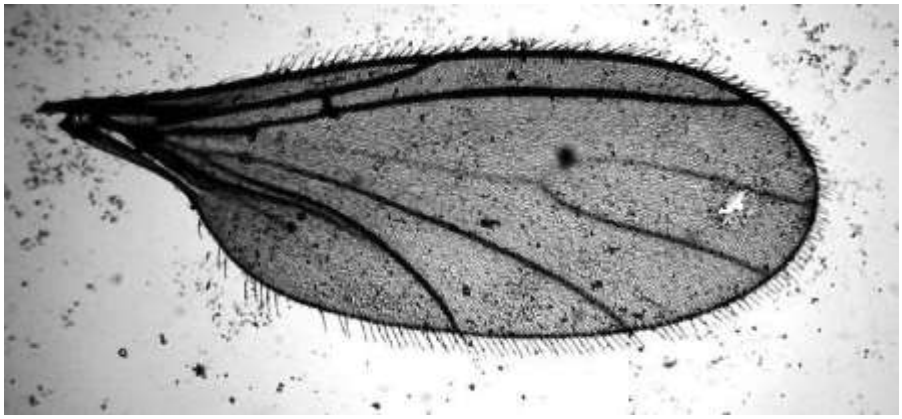
Remarks. Only five individuals out of the total 10 collected were measured. This appears very similar to the following species and was distinguished only based on the presence of post-vertical peak.

Figure 2

Scatopsidae (Head, Thorax and Part of Abdomen in Focus)

Figure 3

Wing of Scatopsidae



Family Cecidomyiidae

2. *Mycophila barnesi* (Figure 4)

Diagnosis. This genus belongs to a large family described as being small or minute flies, most with delicate hairy wings with reduced venations. The wings of these flies are without markings (Figure 5) and with a single complete anal vein reaching wing margin; discal cell and/or ocelli absent, mesonotum without V-shaped suture, moniliform antennae, eyes bridged above the antennal sockets, tibiae without strong purs and coxae usually not elongated.

The Genus *Mycophila* was distinguished from other genera in having 8 to 10-segmented flagellum; absence of genital rod; and wing vein R_1 not more than twice as long as R_5.

Measurements. BL = 0.80 mm (0.60 – 0.90); ThL = 0.22 mm (0.19 – 0.24); WL = 0.50 mm (0.47 – 0.52); WW = 0.23 mm (0.22 – 0.25).

Indices. FW/ HW = 0.6 (0.3 – 0.9); ch/o = 0.3 (0.2 – 0.5); prorb = 0.2 (0.1 – 0.3); rcorb = 0.19 (0.11 – 0.20); vb = 0.14 (0.11 -0.14); dcl = 0.05 (0.01- 0.05); presctl = 0.42 (0.38 – 0.42); sctl = 0.21 (0.19 – 0.25); orbito = 0.17 (0.17 – 0.19); dcp 0.8 (0.5- 0.8); sctlp = 0.39 (0.30 – 0.39).

Remarks. This could be a new record. Based on the key to species, this was identified as *Mycophila barnesi* because the flagellum has 9 segments and the ninth segment of which is a compound segment (Figure 6). This belongs to the Subfamily Lestrimiinae and Tribe Micromyini. The dipteran inventory by Baltazar (1990) listed this species. Based on the assessment done by Baltazar on dipteran taxonomy, there were three works listed for this family which are all by E. P. Felt, two publications in 1915 and one in 1919. Thus, she suggested that this group of flies was a good subject for study.

Ten of the 22 individuals collected were measured.

Figure 4

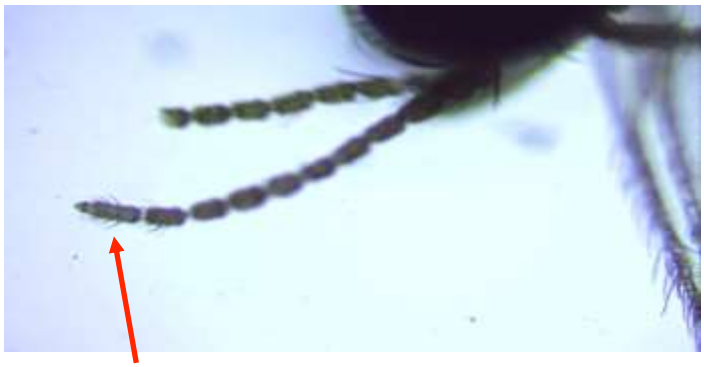
Mycophila barnesi (Head and Thorax, Lateral Aspect)

Figure 5

Wing of Mycophila barnesi *(Cecidomyiidae)*

Figure 6

Antennal Flagella of Mycophila barnesi *(Arrow, Compound 9th Segment*)



Family Sciaridae

3. **Sciaridae** (Figure 7)

This family is widespread with members adapted to a wide variety of climates. Sometimes, it is treated as subfamily of Mycetophilidae because they resemble in adult habits. Eyes of these flies are usually connected dorsally above the antennae (Figure 8); distinct midpleural pit usually present below the wing base; wings with characteristics venation without such markings; discal cell absent, ocelli present. Only one individual was found.

Figure 7

Sciaridae with Left Wing Spread Out

Figure 8

Head of Sciaridae sp. -2 Showing the 9-segmented Flagellomere of the Antennae

Family Empididae

1. Empididae

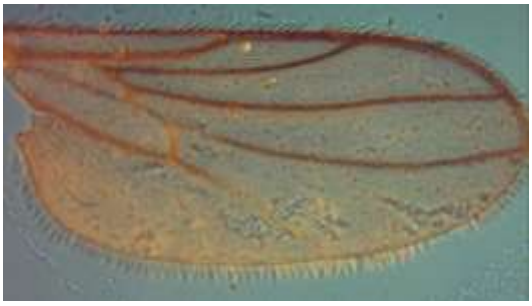
A small- to medium-sized flies that range in size from 1-15 mm. They are darkish to light in color and are rarely metallic green. The head is variously shaped and usually narrower than the thorax. The compound eyes are usually large and holoptic (Figure 9). The antenna has one segmented, variously shaped first flagellomere, a pedicel usually without a thumb-like conus inserted into first flagellomere; stylus that are short and elongated and usually two segmented, situated apically or when situated more dorsally appearing arista-like; proboscis is short to elongated; ocellar bristles present; thorax usually rectangular in dorsal outline; wing varied in size sometimes broadened and the wing color hyaline (Figure 10); Rs vein originating well distal to the level of crossvein h and have crossvein r-m distal to the basal fourth of the wing.

Figure 9

Head of Empididae

Figure 10

Wing of Empididae



Remarks. Only one individual of this species was found throughout the duration of the study. A phoretic mite was found attached near the cervix as shown in Figure 11.

Family Pipunculidae

5. ***Pipunculus* sp.** This family was distinguished based on the absence of ocellar bristles (Figure 12); eyes with straight hind margin, males with holoptic eyes; possession of pterostigma; scutum with evenly distributed hairs.

Head mainly occupied by the two compound eyes, holoptic; ocelli present; ocelli bristles absent; antenna with first flagellomere appearing as single segment, obtuse to filiform in shape with dorsal arista; occiput shining in upper part; maxillary palps with relatively long marginal setae (Figure 13); thorax with darkish ground color; proepisternum sometimes bearing fanlike tuft of bristles; scutum with evenly distributed hairs at least in anterior half (Figure 14); wings long and narrow usually hyaline, iridescent in direct light, pterostigma present (Figure 15), cell r_{4+5}, cubital vein and anal vein always meet before reaching wing

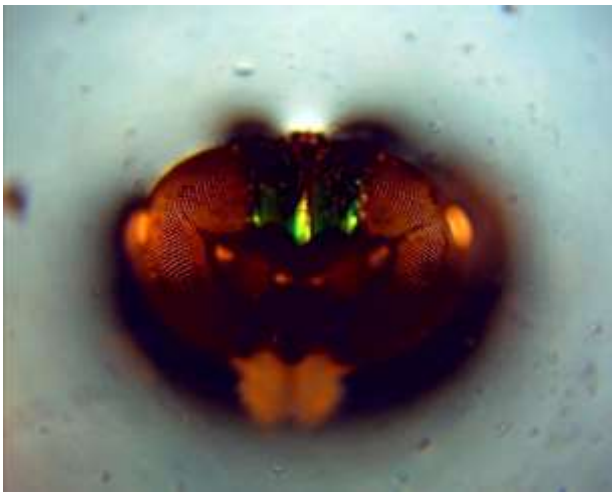
margin; abdomen subcylindrical, coloration and tomentum similar to thorax, occasionally with yellow markings, tergite 1 usually with row or cluster of bristles laterally.

Figure 11

Phoretic Mite (Arrow) Attached Near the Cervix

Figure 12

Pipunculus sp. – *Head (Frontal)*



Measurements. BL = 1.77 mm (1.68 – 1.90); ThL = 0.63 mm (0.59 – 0.64); WL = 1.01 mm (0.97 – 1.04); WW = 0.41 mm (0.38 – 0.41).

Indices. FW/HW= 0.05 (0.05 -0.06); ch/o = 0.04 (0.03 - 0.06); vb = 0.06 (0.02 – 0.06).

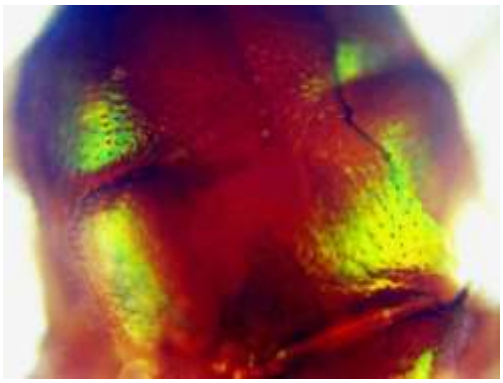
Remarks. Ecologically, these are known endoparasites.

Figure 13

Maxillary palp (arrow) of Pipunculus *sp*.

Figure 14

Notum of Pipunculus *sp.*



Family Heleomyzidae

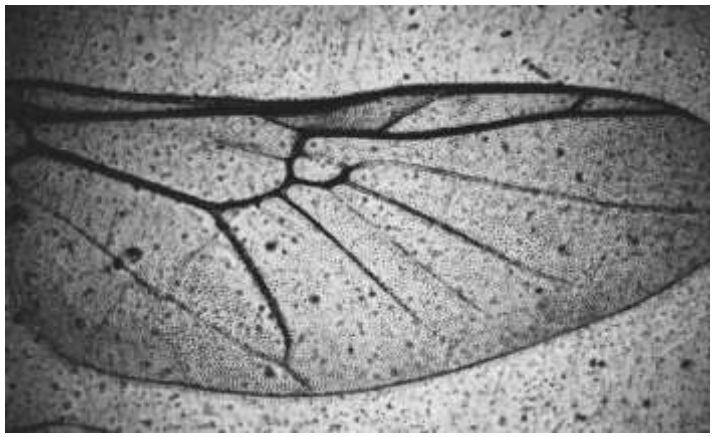
1. ***Porcenus johnsoni*** (Figure 16)

Diagnosis. Postvertical bristles convergent; fronto-orbital bristles usually reclinate; sternopleural and usually presutural bristles present; tibiae usually with preapical dorsal bristles; costa weakened or broken at end of Sc (Figure 17); cell CuP and M closed; male postabdomen with S6 and S7 usually laterally displaced or absent.

Subfamily Suillinae having oblique frontal plates, directed inward anteriorly and distinctly separated from eye margins; propleural bristles absent, sixth vein not reaching wing margin; fronto orbital bristles on each side.

Figure 15

Wing of Pipunculus *sp.*



Porcenus johnsoni Darlington having supra alar bristles 1+1, scutellum with a pair of strong apical bristles, much weaker lateral bristles on one side only; anterior crossvein of wing darkened, posterior crossvein very slightly if at all darkened; humeral bristles absent; having one pair of dorsocentral bristles.

Measurements. BL = 0.62 mm (0.62 – 0.71); ThL = 0.26 mm (0.20 – 0.29); WL = 0.47 mm (0.37 – 0.51); WW = 0.23 mm (0.21 – 0.25).

Indices. FW/HW = 0.03 (0.03 – 0.05); ch/o = 0.04 (0.03 – 0.08); prorb = 0.30 (0.27 – 0.31); vb = 0.22 (0.21 – 0.25); dcl = 0.03 (0.02 – 0.05); sctl = 0.06 (0.05 – 0.11); orbito = 0.02 (0.01 – 0.03); dcp = 0.03 (0.02 – 0.05); sctlp = 0.04 (0.03 – 0.06).

Remarks. Identification had been pinned down to species level, *Porcenus johnsoni.*

Family Sphaeroceridae

1. *Leptocera* sp.

Diagnosis. Small to very minute flies with distinct vibrissae; coxae of each pair little or not separated; proboscis developed; subcranial cavity not much reduced; wings usually present; Sc either indistinct distally, or not ending in costa independently of R_1 or joined to R_1 by sclerotization of the intervening region; R_1 not setulose; incurved lower fronto-orbitals absent; mesoscutum large; scutellum present; hind basitarsus much swollen, or shortened and compress; vibrissae distinct; fore leg not raptorial; tarsal claws normal.

Figure 16

Habitus of Porcenus johnsoni–*Heleomyzidae*

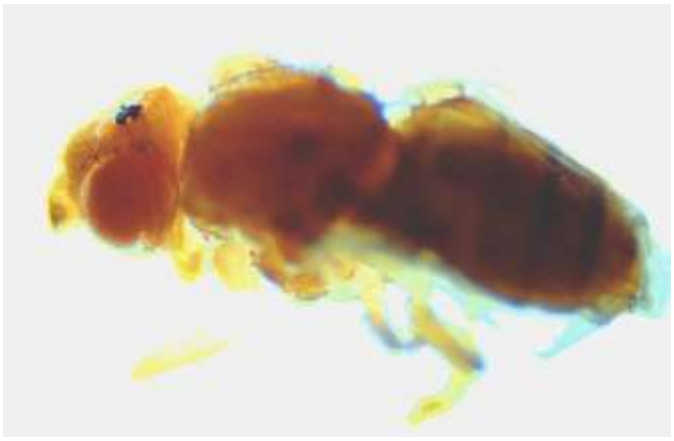
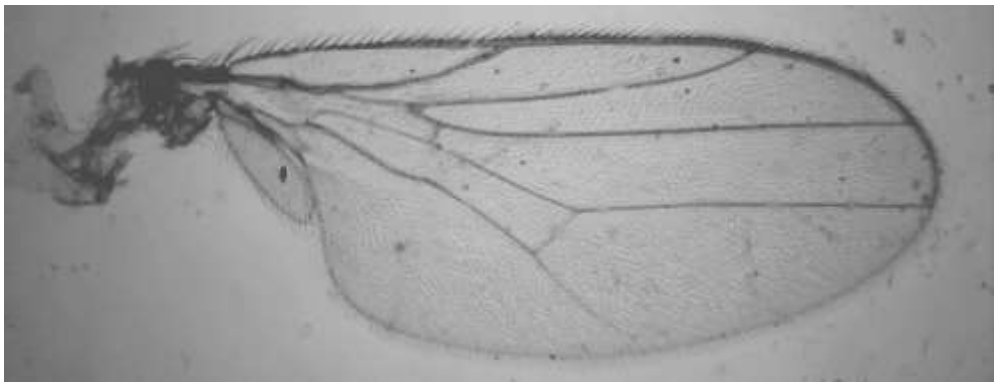


Figure 17

Wing of Porcenus johnsoni



Head. Head predominantly dark brown with distinctive hairs; eyes brownish; ocellar triangle brown, setulae convergent; ocelli creamy white; frontal vittae brownish to black, with three rows of hairs on each side, middle row, and row near eye margin directed outward and innermost row, directed inward; frontoorbital plate brownish; face brown; carina brown; clypeus brownish black; gena blackish; occiput black; pedicel kidney-bean shaped, pollinose black; first flagellomere yellowish to brown; arista pubescent with small hairs; palpus creamy to brownish.

Thorax. Scutum, scutellum, mesopleuron, and postpronotal lobe brownish to black; postpronotal setae, 1 strong and two shorter setulae; acrostichal rows covering whole scutum, 4 rows

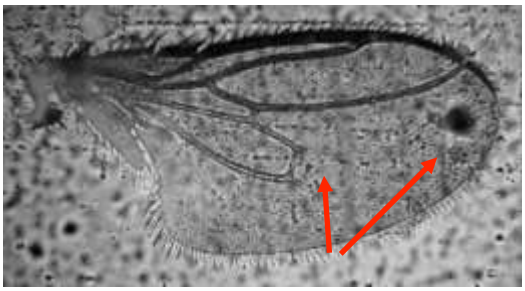
between 1 pair of dorsocentrals (Figure 18); scutellar setae, 2 pairs with smaller pair located midline between basal and apical setae; midline scutellars parallel each other, apical scutellars convergent; wings (Figure 19) hyaline with no dark spots, veins light brown; R_{2+3} vein not curved towards margin; R_{4+5} and M_1 veins not distinct; C_1 setae distinct with one strong setulae near base or before humeral break; haltere creamy white; legs light brown, with distinct setulae; apical and preapical bristles not distinct.

Figure 18

Notum of Leptocera *showing the four rows of acrostichals between the pair of dorsocentral*

Figure 19

Wing of Leptocera *Showing Indistinct R_{4+5} and M_1 Veins (Arrows)*



Abdomen. No distinctive markings, dark brown to black; male surstylus armed with long setae; genitalia as described by Tenorio (1968).

Remarks. Sphaerocerid adults have been constantly associated with decaying matter of a diverse nature as stated by Tenorio (1968), thus they are usually referred to as dung flies. Larvae have been basically scavengers in horse manure, cow manure, human excrement, urine, dog dung, fungi, dead animals, ant and wasp nests, and a variety of decaying plants. Life cycle of these flies is about 12 days. However, other sources deemed this family as flies of mycological composts.

Measurements. BL = 0.51 mm (0.52 - 0.66); ThL = 0.22 mm (0.18 – 0.23); WL = 0.32 mm (0.29 – 0.34); WW = 0.19 mm (0.17 – 0.22).

Indices. FW/HW = 0.03 (0.02 – 0.04); ch/o = 0.04 (0.03 – 0.05); prorb = 0.03 (0.01 - 0.06); rcorb = 0.02 (0.01 – 0.02); vb = 0.01 (0.01 – 0.01); dcl = 0.06 (0.04 -0.06); sctl = 0.19 (0.04 – 0.02); orbito = 0.02 (0.01 – 0.02); dcp = 0.02 (0.01 – 0.02); sctlp = 0.03 (0.01 – 0.03).

Family Milichiidae

8. ***Desmometopa* sp.** This genus belongs to subfamily Madizinae, distinguished for lacking a deep indentation in the costa. The family is characterized as having convergent (or parallel) postvertical bristles (Figure 20); lower frontoorbital bristles incurved, upper ones otherwise directed; vibrissae present; prosternal bridges present, or the prosternum reduced; costa twice broken (Figure 21); cells M and CuP closed. Tergum with characteristic patterns (Figure 22).

Measurements. BL = 0. 83 mm (0.70 - 1.05); ThL = 0.30 mm (0.25 - 0.35); WL = 0.47 mm (0.43 - 0.55); WW = 0.23 mm (0.19 - 0.27);

Indices. FW/HW **=** 0.07 (0.05 - 0.09); ch/o = 0.11 (0.10 - 0.15); prorb = 0.01 (0.01 - 0.01); vb = 0.01 (0.01 - 0.01); dcl = 0.03 (0.01 - 0.05); orbito = 0.04 (0.01 - 0.05); dcp = 0.04 (0.02 - 0.05); sctl = 0.05 (0.02 - 0.05); sctlp = 0.03 (0.01 - 0.05).

Remarks. It was noted that phoretic mites (Figure 23) were found attached around the cervix, postscutellum and parts with notches.

Figure 20

Head of Desmometopa *sp.-Milichiidae*

Figure 21

Wing of Desmometopa *sp.-Milichiidae*

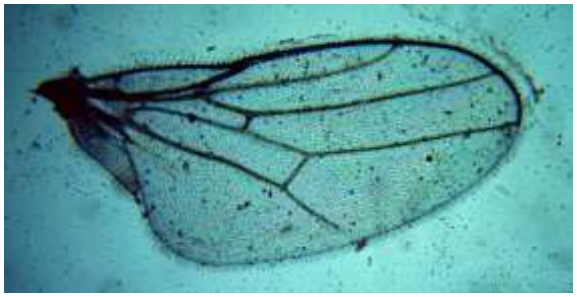


Figure 22

Abdomen of Desmometopa *sp.-Milichiidae, Showing Characteristic Blackish-brown P*atterns

Of the eight species of flies found associated with *Volvariella* mushroom cultivation, three of which are fungivorous, namely: *Mycophila* (family Cecidomyiidae) and an unidentified species belonging to family Sciaridae, both of the Nematoceran suborder of the Order Diptera, and *Leptocera* (Sphaeroceridae) which is classified under Series Schizophora, Division Cyclorrhapha of the suborder Brachycera. The other three are known detritivores, subsisting on decaying matter, as follows: *Desmometopa* (family Milichiidae) and *Porcenus* (family Heleomyzidae), both under series Schizophora, Division Cyclorrhapha, suborder Brachycera. The third unidentified species belongs to the family Scatopsidae classified under suborder Nematocera.

The remaining two are categorized as natural enemies: one an endoparasite, *Pipunculus* (family Pipunculidae, of the series Aschiza, division Cyclorrhapha of the suborder Brachycera) and the other unidentified species, which is a known predator, belonging to the family Empididae, (classified under Division Orthorrhapha of the suborder Brachycera).

Figure 23

Phoretic mites (arrow) near the cervix of Desmometopa *fly*

Conclusion

In conclusion, results imply the breadth of dipteran diversity which was apparent; ranging from the most primitive fly (Suborder Nematocera) to the most advanced (Division Cyclorrhapha, series Schizophora), representing flies with varied trophic levels such as fungivores, detritivores as well as predator and parasite. Further research on a bigger scale of *Volvariella* cultivation could reveal more insights on the dynamics of fly population and yield loss assessment. Studies on the impact of fly especially the immature stage on mushroom yield is likewise recommended. Also, there were other families of flies (Drosophilidae and Phoridae) reported in other researches associated with mushroom cultivation. However, these were not specifically found in association with *Volvariella volvacea*.

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