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Trichomycete Fungi (Zygomycota) Associated with Mosquito Larvae (Diptera: Culicidae) in Natural and Artificial Habitats in Manaus, AM Brazil

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Fungos Trichomycetes (Zygomicota) Associados com Larvas de Mosquitos (Diptera: Culicidae) em Criadouros Naturais e Artificiais em Manaus, AM

RESUMO - Espécies de fungos da classe Trichomycete (Zygomycota) estão associados com o trato digestivo dos Arthropoda. A relação entre esses fungos e seus hospedeiros ainda é pouco conhecida, podendo ser comensal, benéfica ou deletéria. Conhecimentos da estrutura de comunidades parasitas/ patógenos e os habitats de larvas de Culicidae podem ser importantes em estudos que utilizam medidas combinadas para o controle populacional. Larvas de Culicidae e os fungos Trichomycetes associados foram coletados no município de Manaus, AM; amostras de criadouros incluindo plantas (habitat natural) e reservatórios antrópicos (habitat artificial). O total de 1518 larvas foi coletado, 913 em criadouros naturais e 605 em criadouros artificiais, distribuídas em 12 espécies de sete gêneros. O total de 661 indivíduos (4º estádio) foi dissecado para verificar a presença de fungos Trichomycetes no intestino médio e posterior. Infecção de fungos Trichomycetes no intestino posterior foram observados em 15% de *Culex urichii* Coquillett, 13% de *Culex* (*Culex*) sp1, 9% of *Limatus* spp., 49% de *Aedes aegypti* Linnaeus e 1% de *Ochlerotatus argyrothorax* Bonne-Wepster & Bonne. Somente em *Ae. aegypti* foram observados fungos Trichomycetes na matriz peritrófica, no intestino médio; porém este fato é provavelmente, um resultado de um desenvolvimento anormal deste fungo.

PALAVRAS-CHAVE: Inseto aquático, *Smittium culisetae*, criadouro, infecção de larvas, Amazônia Central

ABSTRACT - Fungal species of the class Trichomycete fungi (Zygomycota) are associated with the digestive tracts of Arthropoda. The relationships between these fungi and their hosts are still little understood: they may be commensal, beneficial or deleterious. Knowledge of the community structure of parasites/ pathogens and of the habitats of each species of Culicidae larvae can be important in studies that intend to use combined approaches to population control. Larvae of Culicidae and their associated trichomycete fungi were collected in Manaus County, AM, Brazil; sampling habitats included plants (natural habitat) and anthropic containers (artificial habitats). The total of 1,518 larvae were collected, 913 of which were in natural habitats and 605 were in artificial habitats, distributed in 12 species of seven genera. The total of 661 individuals (4th instar) were dissected to verify the presence of trichomycete fungi in the mid and hindgut. Infection of trichomycete fungi in the hindgut was observed in 15% of *Culex urichii* Coquillett, 13% of *Culex* (*Culex*) sp1, 9% of *Limatus* spp., 49% of *Aedes aegypti* Linnaeus and 1% of *Ochlerotatus argyrothorax* Bonne-Wepster & Bonne. Only in *Ae. aegypti* were trichomycete fungi observed in the peritrophic matrix, in the midgut; however, this fact is probably, a result of abnormal development of the fungi.

KEY WORDS: Aquatic insect, *Smittium culisetae*, breeding habitat, larvae infection, Amazon Basin

Some Arthropods are hosts for fungi of the class trichomycete fungi (Misra 1998). Species in the genus *Smittium* are known to be associated with the hindgut wall of Culicidae, Chironomidae, Simuliidae, Ceratopogonidae, Tipulidae and Thaumaleidae larvae. However, the relationships of the fungi with their hosts are not yet well

understood and can be commensal, beneficial or deleterious. *Smittium morbosum* Sweeney is known to be lethal to some Culicidae species.

Some species of Culicidae have both medical and economic importance, being vectors of pathogenic agents to man and other animals. The female lays eggs in a large variety of aquatic environments where immature stages develop. There is a preference for some kinds of environments as habitats, such as holes in the ground or in stones, swamps and lakes, besides artificial containers such as cans and tires. Frank & Lounibos (1983) reported that approximately 400 species (15 genera) occur in many plant environments (e.g. Bromeliaceae, Palmae, Heliconiaceae, Araceae). However, information on habitats of Culicidae in Manaus is scarce (Lopes *et al*. 1983, 1985, Tadei *et al.* 1988, Hutchings 1994, Ferreira 1999, Ferreira *et al*. 2001).

Knowledge of trichomycete fungi associated with Culicidae larvae in the Amazon region may provide, in the future, new tools to help control the populations of species of medical or economic importance. The present study has as its objective the identification of trichomycete fungi associated with Culicidae larvae in natural and artificial habitats in urban and natural environments in Manaus County.

Material and Methods

Culicidae larvae were collected at sites located in Manaus city (urban environment), AM, Brazil, including the Instituto Nacional de Pesquisas da Amazônia (campus /INPA) (03°06'S; 59º54'W), Universidade Federal do Amazonas (campus/ UFAM) (03º05'S, 59º58'W), Reserva Particular da Associação Brasil Soka Gakkai Internacional (03º06'S; 59º54'W) and four cemeteries (São João Batista (03º06'S, 60º01'W), São Francisco de Assis (03º08'S, 59º59'W), Parque de Manaus (03º03'S, 06º04'W) and Santa Helena (03º07'S, 60º02'W). Collection in natural environments were made in the Reserva Florestal Adolpho Ducke (02º57'S; 59º57'W) located 26 km from Manaus city. Fieldwork was conducted from September 1999 to July 2000. Larval habitats were classified as permanent or semi-permanent in artificial and natural conditions. In natural habitats, the habitats considered permanent were the Bromeliaceae and Rapataceae plant families, and the habitats considered semi-permanent were holes in stones or on the ground, bamboo knots, fruit shells and palm bracts. Artificial habitats considered permanent were water wheels; semi-permanent habitats were pots, disposable containers, canoes, cans and tires.

Larvae were collected with forceps, sieves and spoons (according to the environment) and then transported to the laboratory in plastic containers with water from the habitat. Only 4th-instar larvae were dissected to observe fungal associations, because, in general, morphological characters used to identify the specimens to species are present only at this stage.

Larvae were dissected alive in distilled water; the digestive-tube content was cleaned off and then the hindgut and peritrophic matrix were transferred to a slide in a drop of distilled water and covered with a coverslip; observations were made under a compound microscope. If the preparation was positive for the presence of trichomycete fungi, the thallus, holdfast and trichospore, when present, were measured, and finally, the trichomycete fungi were stained with lactophenol-cotton blue; the coverslip was sealed with uncolored fingernail polish, using the technique of Lichtwardt (1986). Taxonomic identification was made to the lowest level possible. Three species of *Limatus* were obtained from larvae reared in the laboratory, however they cannot be distinguished at the larval stage; therefore species of *Limatus* were grouped to determine the trichomycete infection rate. Two unidentified species of *Culex* were denominated as sp1 and sp2. One species in the genus *Haemagogus* was not identified because all of the larvae were dissected to check for trichomycete fungi infections. The classification we used followed Harbach & Kitching (1998), Reinert (2000), and the abbreviation of genera and subgenera followed Reinert (2001). Identification of adults and immatures was done using the keys of Consoli & Oliveira (1994), Valencia (1973), Lane (1953a, b) and Forattini (2002). Identification of trichomycete fungi was based on Misra & Lichtwardt (2000).

Results and Discussion

The total of 1,518 culicid larvae were collected; 913 of them in natural and 605 in artificial habitats, distributed among 12 species and seven genera in the subfamilies Anophelinae and Culicinae (Aedini, Sabethini, Culicini and Toxorhynchitini). The Culicinae identified to the species level were: *Aedes aegypti* Linnaeus, *Ochlerotatus argyrothorax* Bonne-Wepster & Bonne, *Haemogogus* sp, *Culex urichii* Coquillett, *Culex* (Culex) sp1, *Culex* (*Culex*) sp2, *Limatus durhami* Theobald, *Limatus pseudomethysticus* (Bonne-Wepster & Bonne), *Limatus flavisetosus* Oliveira Castro, *Anopheles eiseni* Coquillett, *Toxorhynchites haemorrhoidalis* (Fabricius) and *Thrichoprosopon digitatum* (Rondani). All of the species were found in natural habitats; however *Ae. aegypti*, *Culex* (*Culex*) sp1, *Limatus* spp., *Haemagogus* sp. and *Tx. haemorrhoidalis* were also found in artificial environments (Table 1).

The total of 661 4th-instar larvae were dissected to verify the presence of trichomycete fungi. Only the species *Smittium culisetae* Lichtwardt was observed in the posterior intestine of 120 Culicidae larvae, representing 18% of the total (Table 1). This fungal species had already been reported in Culicidae in other studies, associated to *Ae. aegypti*, *Culiseta incidens* (Thomson), *Aedes albopictus* (Skuse), *Aedes vexans* (Meigen) with wide distribution in United States (Colorado, Nebraska, Kansas, California and Hawaii), Japan, Austria, New Zeeland and France (Lichtwardt 1986). Infection in the midgut (peritrophic matrix), probably, by *S. culisetae* was detected in 20 larvae of *Ae. aegypti*, representing 3% of the total dissected specimens (Fig. 1a,b). This occurrence, according to Lichtwardt *et al*. (1997), resulted from an abnormal development where the trichospores extrude prematurely and the sporangiospores stick to the peritrophic matrix on their way to the hindgut

Table 1. Number (n) of Culicidae (Diptera: Nematocera) larvae collected, number of larvae infected (+) by *S. culisetae* (Trichomycetes) and Infection Rate (IR) of Culicidae larvae of each species per habitat.

Species / Habitats	1			2			3			4			5			6		7		8		9		10		Total examined	Total infected	IR (%)
	n	+	IR (%)	n	+	IR (%)	n	+	IR (%)	n	+	IR (%)		+	IR (%)	n	+	n	+	n	+	n	+	n	+			
Stereaceae	-	-	-	-	-	-	15	5	33	1	-	0	36	6	17	2	0	8	0	-	-	-	-	-	-	62	11	18
Plastic containers	80	56	70	-	-	-	-	-	-	1	-	0	88	12	14	-	-	-	-	-	-	2	0	-	-	171	68	40
Bambu	-	-	-	-	-	-	-	-	-	-	-	-	23	0	0	-	-	-	-	-	-	-	-	-	-	23	0	-
Palm bracts	14	0	0	-	-	-	26	1	4	-	-	-	26	0	0	-	-	-	-	-	-	1	0	3	0	70	1	1
Bromeliaceae	-	-	-	-	-	-	-	-	-	-	-	-	16	0	0	-	-	-	-	-	-	-	-	-	-	16	0	-
Holes in stone	-	-	-	104	1	1	-	-	-	15	2	13	-	-	-	-	-	1	0	-	-	-	-	-	-	120	3	3
Water tank	-	-	-	-	-	-	-	-	-	21	1	5	-	-	-	-	-	-	-	-	-	-	-	-	-	21	1	5
Rapataceae	-	-	-	-	-	-	-	-	-	-	-	-	4	0	0	-	-	-	-	-	-	-	-	-	-	4	0	-
Canoe	-	-	-	-	-	-	-	-	-	8	3	38	-	-	-	-	-	4	0	-	-	-	-	-	-	12	3	25
Ceramic container	46	26	57	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10	0	56	26	46
Can	25	2	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	0	-	-	-	-	-	-	26	2	8
Holes in the ground	-	-	-	-	-	-	-	-	-	32	2	6	-	-	-	-	-	-	-	3	0	-	-	-	-	35	2	6
Tire	6	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6	0	-
Fruit shells	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	20	0	-	-	20	0	-
Well	-	-	-	-	-	-	-	-	-	10	3	30	-	-	-	-	-	-	-	-	-	-	-	-	-	10	3	30
Ditch	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9	0	9	0	-
Total	171	84	49	104	1	1	41	6	15	88	11	13	193	18	9	2	0	14	0	3	0	23	0	22	0	661	120	18
RA (%)	25.9			15.7			6.2			13.3			29.2			0.3		2.1		0.5		3.5		3.3		100		

n = examined; + = positive cases; IR (%) = Infection rate; RA (%) = Relative abundance of specie
1- *Ae. aegypti*; 2 - *Oc. argyrothorax*; 3 - *Cx. urichii*; 4 - *Cx.* (*Culex*) sp 1; 5 - *Limatus* spp.; 6 - *An. eiseni*; 7 - *Tx. haemorrhoidalis*; 8 - *Cx.* (*Culex*) sp 2; 9 - *Tr. digitatum*; 10 - *Haemagogus* sp.

(Fig. 1c,d). In the peritrophic matrix these sporangiospores do not find the minimum conditions needed to develop. *S. culisetae* has already been reported associated with larvae of *Ae. aegypti* under experimental conditions in the laboratory (Williams & Lichtwardt 1972).

Sweeney (1981) stated that fungi of this species cause no damage to their hosts under natural conditions, being released with the cuticle during ecdysis. In the Central Amazon, Alencar *et al*. (2003) isolated, for the first time, *S. culisetae* from the hindgut of *Culex* sp., but did not observe zygospores. We also found no zygospores in the dissected larvae, in the present study.

Larvae with *S. culisetae* were found either in natural or artificial habitats (Table 1). *Ae. aegypti* was the most abundant species in the artificial habitats (RA = 25.9) and in plastic containers, has been found the one with highest infection rate by *S. culisetae* (IR = 70%); however, this fungal species did not occur in all of the places where this mosquito species was collected. In general, *Ae. aegypti* is found in clean water with a large quantity of organic matter (Consoli & Oliveira 1994, Pinheiro & Tadei 2002). However, we observed this species in habitats such as cans and pots with dirty water and acidic pH, indicating a flexibility of *Ae. aegypti* when compared with other species. This result corroborates the work of Silva *et al*. (1999), who mentioned adaptations in this species to certain polluted areas.

We collected *Oc. argyrothorax* in stone holes at the confluence of the Rio Negro and the Rio Solimões (Upper Amazon River). This habitat is considered temporary because it is periodically flooded; only one individual of this species had *S. culisetae* in its hindgut.

Limatus was the second genus to have high frequency of *S. culisetae* in the hindgut in larvae collected in water retained in Stereaceae fungi; this habitat was located in a shaded area of Campinarana (Ferreira *et al*. 2001). *Limatus* larvae also were collected with *S. culisetae* in plastic containers but did not occur in bamboo, palm bracts, Bromeliaceae and Rapataceae (Table 1).

S. culisetae infesting *Cx.* (*Culex*) sp1 larvae did not occur in plastic containers and Stereaceae fungi (Table 1). However this result might be masked by the low number of collected larvae (Table 1), because other species collected in the same habitat were infected by *S. culisetae*. Species of *Cx.* (*Culex*) are adapted to a wide variety of habitats, that in general are maintained by rain water and have a great amount of decayed matter such as leaves, stems and fruits (Consoli & Oliveira 1994).

Infected *Cx. urichii* by *S. culisetae* was collected in Stereaceae (33%) and in palm bracts (4%). These habitats were frequently located in shaded areas and had large amounts of organic matter. This species is known to

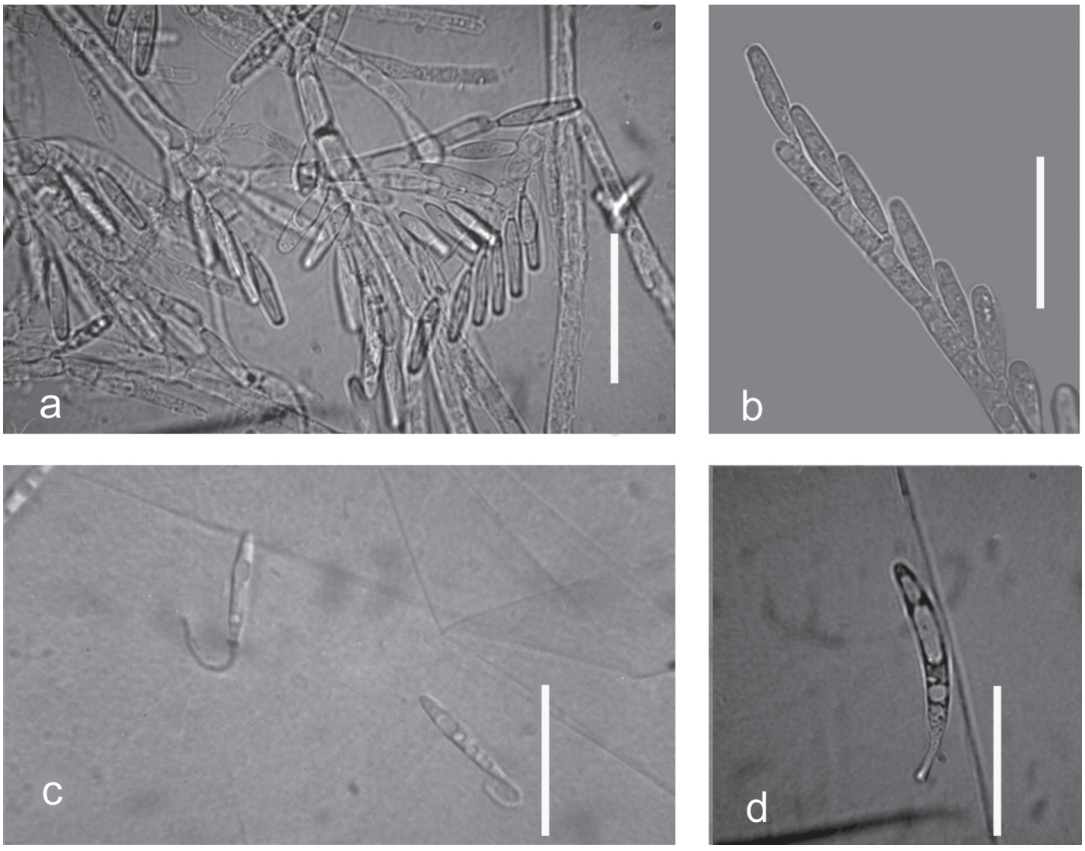

Figure 1. *S. culisetae* (Trichomycetes: Harpellales). a) General aspect of the thallus and trichospores in the larval hindgut (scale bar = 10 μm), b) Thallus with trichospore (scale bar = 25μm), c) and d) Trichospore developing in the peritrophic matrix, larval midgut (scale bar = 10 μm and 25 μm, respectively).

colonize natural habitats, such as *Heliconia* flower bracts, bromeliads, holes in trees and palm leaves (Valencia 1973).

An. eiseni, *Tx. haemorrhoidalis*, *Cx.* (*Culex*) sp2, *Tr. digitatum* and *Haemogogus* sp. did not host *S. culisetae*. Of these, *Toxorhynchites*, *Trichoprosopon* and some *Cx.* (*Culex*) are predators (Forattini 2002). Misra (1998) states that predatory insects are not hosts of trichomycete fungi. In general, Culicidae larvae feed on plankton, algae, bacteria, fungal spores and other organic particles (Consoli & Oliveira 1994). The non-selective ingestion of particles by the larvae makes the ingestion of contaminant spores easier.

S. culisetae was already found infecting Culicidae larvae inhabiting environments such as pineapple bracts, Bomeliaceae and swamps, in Costa Rica (Lichtwardt 1994). This fungus has been found throughout the world (Lichtwardt *et al*. 2001) indicating its wide geographical distribution. Another equally widespread species of *Smittium*, *S. culicis* Manier, normally found in Culicidae but with a wide host range, was not found in this study.

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