ECOLOGY, BEHAVIOR AND BIONOMICS

Filamentous Fungi Associated with Mosquito Larvae (Diptera: Culicidae) in Municipalities of the Brazilian Amazon

Eleny da S Pereira[1], Maria I de M Sarquis[2], Ruth Leila Ferreira-Keppler[1], Neusa Hamada[1], Yamile B Alencar[1]

[1]Coordenação de Pesquisas em Entomologia, Instituto Nacional de Pesquisas da Amazônia, C. postal 478, 69011-970, Manaus, AM; leny_spbr@yahoo.com.br; ruth@inpa.gov.br; nhamada@inpa.gov.br; yamile@inpa.gov.br
[2]Depto de Micologia, Instituto Oswaldo Cruz-Fiocruz, C. postal 926, 21045-900, Av. Brasil, 4365, Manguinhos, RJ; sarquis@ioc.gov.br

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Fungos Filamentosos Associados a Larvas de Mosquitos (Diptera: Culicidae) em Municípios da Amazônia Brasileira

RESUMO - Algumas espécies da família Culicidae são importantes vetores de doenças em humanos e em outros animais. Estágios imaturos são filtradores não seletivos de partículas orgânicas e microrganismos. Estudos da diversidade microbiológica podem contribuir para a descoberta de novas substâncias que podem ser usadas em indústrias farmacêuticas, para alimentação ou para controle biológico. O objetivo deste estudo foi isolar e identificar os fungos associados a larvas de Culicidae encontradas em diferentes tipos de criadouros (natural e artificial), como casca de frutos, buracos em pedras, lagoas, plantas aquáticas, bráctea de palmeira e potes de cerâmica, em vários municípios da Amazônia Brasileira, principalmente no Amazonas e em Rondônia. O total de 38 isolados foram obtidos a partir de larvas de *Aedes aegypti* (L.), *Aedes fluviatilis* (Lutz), *Trichoprosopon digitatun* (Rondani), *Anopheles argyritarsis argyritarsis* Robineau-Desvoidy, *Anopheles darlingi* Root, *Aedeomyia squamipennis* (Lynch Arribálzaga), *Mansonia titillans* (Walker) e *Uranotaenia* sp. Os fungos que ocorreram nas larvas de Culicidae foram: *Acremonium kiliense*, *Aspergillus sydowii*, *Fusarium sacchari* var. *sacchari*, *Fusarium merismoides* var. *merismoides*, *Gliocladium viride*, *Paecilomyces* sp., *Penicillium citrinum*, *Penicillium sclerotiorum*, *Penicillium melinii*, *Penicillium oxalicum*. Características macro-microscópicas dos isolados foram apresentadas, assim como informações sobre a distribuição geográfica.

PALAVRAS-CHAVE: Fungo anamorfo, inseto aquático, microrganismo, Amazonas, Rondônia

ABSTRACT - Several species of the family Culicidae are important vectors of diseases in humans and other animals. Immature stages are filter-feeders of organic particulate matter and microorganisms. Studies on microbial diversity can contribute to the discovery of new substances that can be used in the pharmaceutical industry for food or for biological control. The aim of this study was to isolate and identify the fungi associated with Culicidae larvae found in different habitats (natural and artificial), such as fruit shells, rock holes, lakes, aquatic plants, palm bracts and ceramic pots, in several municipalities of Brazilian Amazonia, especially in the states of Amazonas and Rondônia. A total of 38 fungal lineages were isolated from larvae of *Aedes aegypti* (L.), *Aedes fluviatilis* (Lutz), *Trichoprosopon digitatum* (Rondani), *Anopheles argyritarsis argyritarsis* Robineau-Desvoidy, *Anopheles darlingi* Root, *Aedeomyia squamipennis* (Lynch Arribálzaga), *Mansonia titillans* (Walker) and *Uranotaenia* sp. The following fungi occurred associated with the larvae of Culicidae: *Acremonium kiliense*, *Aspergillus sydowii*, *Fusarium sacchari* var. *sacchari*, *Fusarium merismoides* var. *merismoides*, *Gliocladium viride*, *Paecilomyces* sp., *Penicillium citrinum*, *Penicillium sclerotiorum*, *Penicillium melinii* and *Penicillium oxalicum*. Macro- and microscopic characteristics of the lineages are presented, as well as information on their geographical distribution.

KEY WORDS: Anamorphic fungi, aquatic insect, microorganism, Amazonas, Rondônia

Female Culicidae mosquitoes are haematophagous and some are vector of etiological agents of diseases such as yellow fever, malaria and filaria to humans and other animals. The immature stages are non-selective filter-feeders of organic particles suspended in the water suspension and of microorganisms such as bacteria, viruses, protozoans and fungi (Forattini 2002).

The fungi are heterotrophic, filamentous and pluricellular organisms. They occur in all environments of the planet and are important parasites, decomposers or saprophytes. Some can be pathogenic due to toxin production (Mallozzi & Corrêa 1998), while others can be beneficial and play an important ecological role in degrading organic matter (Putzke & Putzke 1998). In recent years, the interest in searching for persistent microorganisms that multiply easily and limit host resistance acquirement, as natural alternative ways to control pest populations without harming the environment has increased (Alves 1998).

Some fungi can occasionally attack insects or develop symbiotic relationships (Lichtwardt 1986). Studies on entomopathogenic fungi have shown their promise as biological control agents of mosquito vectors of tropical diseases (Messias 1989).There are many examples worldwide related to the interactions of fungi and Culicidae larvae (e.g. Agarwala *et al* 1999, Lucarotti & Shoulkamy 2000, Scholte *et al* 2004, Pereira *et al* 2005). However, the present paper is the first study of Hyphomycetes fungi associated with Culicidae larvae in the Brazilian Amazon region.

In this investigation we isolated and identified Hyphomycetes associated with Culicidae larvae in different habitats in Amazonia, thereby contributing information on the distribution and taxonomy of these fungi. The taxonomic section includes descriptions of species with published names and others that are not named (*Paecilomyces* sp.) due to an insufficient number of collected specimens. These studies can be useful both in efforts to discover biological control agents of insect vector and in the indication of substances with larvicidal action for pest insects in agriculture.

Material and Methods

This study was conducted from April to December 2004 in different localities in the states of Amazonas and Rondônia. In Amazonas, the collection sites were located in Manaus municipality, in the vicinity of the AM 010 road at the Reserva Florestal Adolpho Ducke (02º57'S; 59º57'W) and Bairro Educandos (03º08'S; 60º00'W); in Iranduba municipality, at the lago Camaleão, ilha da Marchantaria (03º15'S; 59º58'W) and, in Rio Preto da Eva municipality at the Vale Piratininga - km78/AM-010 (02º42'S; 59º43'W). In Rondônia state, the collection was done in Porto Velho municipality (Rio Abunã, Cachoeira Fortaleza do Abunã) (09º46'S; 65º30'W).

Culicidae larvae were collected using forceps, pipettes, sieves and nets in different types of standing water (temporary, semi-permanent and permanent) on holes in stones, palm tree bracts, fruit shells, fish-culture tanks and floating aquatic macrophytes, placed in containers and stored under refrigeration until being dissected in the laboratory.

The species were identified according to Forattini (2002). Voucher specimens were deposited in the Invertebrate Collection of the Instituto Nacional de Pesquisas da Amazônia (INPA), Manaus, Amazonas, Brazil.

The collected insects were surface-sterilized by consecutive washing in sterile distilled water and 70% alcohol for 1 min, and then dissected, separating the head and the breathing siphon of the body under aseptic conditions on a vertical laminar flow hood following Alencar *et al* (2003). For each Culicidae species collected, the bodies of 10 last-instars were macerated in 0.2 ml saline solution (0.9%). The macerated samples were processed according to Alves (1998) and seeded onto Petri dishes containing Potato Dextrose Agar (PDA), Malt Extract Agar (MEA) or Czapeck Yeast Agar (CYA) (DIFCO), to which 0.05 g per l of chloranphenicol was added. Plates were incubated at 28ºC and examined every three days for 20 days. Selected colonies were then transferred to tubes with PDA. Cultures were identified by microscopic characteristics (sexual and asexual) using slide culture techniques and specific literature (Raper & Fennell 1965, Samson 1974, Gerlach & Nirenberg 1982, Pitt 1985, Klich & Pitt 1998, Putzke & Putzke 1998, Humber 1998, Klich 2002). The material was mounted on semi-permanent slides using Amann lactophenol plus cotton blue and observed under oil immersion using an optical microscope.

The representative cultures studied were preserved in PDA under mineral oil (Putzke & Putzke 1998) and incorporated into the Fungus Culture Collection of The Coordenação de Pesquisas em Entomologia do Instituto Nacional de Pesquisas da Amazônia (INPA), Manaus (AM) and in the Coleção de culturas de Fungos do Departamento de Micologia do Instituto Oswaldo Cruz-Fiocruz (IOC), Rio de Janeiro (RJ).

Results and Discussion

A total of eight species of Culicidae larvae were collected: *Aedes aegypti* (L.), *Ae. fluviatilis* (Lutz), *Trichoprosopon digitatum* (Rondani), *Anopheles argyritarsis* Robineau-Desvoidy, *An. darlingi* Root, *Aedeomyia squamipennis* (Lynchi Arribálzaga), *Mansonia titillans* (Walker) and *Uranotaenia* sp.

A total of 38 fungal lineages were isolated from the Culicid larvae. The semi-permanent slides with genera and species of fungi had a predominance of anamorphic fungi: *Acremonium kiliense*, *Aspergillus sydowii*, *Fusarium sacchari* var. *sacchari*, *Fusarium merismoides* var. *merismoides*, *Gliocladium viride*, *Paecilomyces* sp., *Penicillium citrinum*, *Penicillium sclerotiorum*, *Penicillium melinii* and *Penicillium oxalicum*.

After being transferred to several sporulation media, ten isolates did not have reproductive structures and were placed into the "form genus" (sterile mycelium) *Mycellia sterilia*. They were grouped according to their macroscopic characteristics. However, a study of the DNA sequences is necessary to confirm the generic and specific status of these isolates (Borazjani *et al*1998, Tymon & Pell 2005).

Acremonium kiliense (Fig 1a)

Acremonium kiliense was isolated in MEA medium, and shows the following microscopic characteristics: septate hyphae; phialides solitary, erect; these are differentiated from hyphae by a septum, fine walls slightly tapering at the apex or intercalary, 13-30 μm in length. At the apices of the phialides are the conidia, ellipsoidal to short-cylindrical, 4-7 × 2-3 μm in size. This fungus is characterized microscopically by an agglomeration of conidia at the superior extremity of the phialides.

Species of this genus are filamentous and cosmopolitan, common in decomposing organic matter, fallen plants and soil. Some species are contaminants and parasitize live fungi and algae (Bott & Rogenmuser 1980). *Acremonium kiliense* has been recognized as a human pathogen mainly in imunocompromised patients and in other animals such as dogs (Mendoza *et al*1985, Simon *et al* 1991, Fridkin 1996, Pastorino *et al* 2005). It also has enzymatic properties on account of the production of *Cephalosporium acremonium* with proteolytic activity (Heyningen *et al* 1971). Rodrigues *et al* (2005) reported the association of *A. kiliense* with *Atta sexdens rubropilosa* Forel nests where this fungus can coexist in a dormant phase. The present study is the first report of the association of this fungus with Culicidae larvae.

Mosquito host species. *Mansonia titillans*

Sampling site. Iranduba municipality, by Ferreira-Keppler RL and Pereira ES in 30/xi/2004.

Habitat. Mosquito larvae were collected from groups of *Eichhornia crassipes* in floodplain lakes. In this environment, the immature mosquitoes of this gender remain fixed by

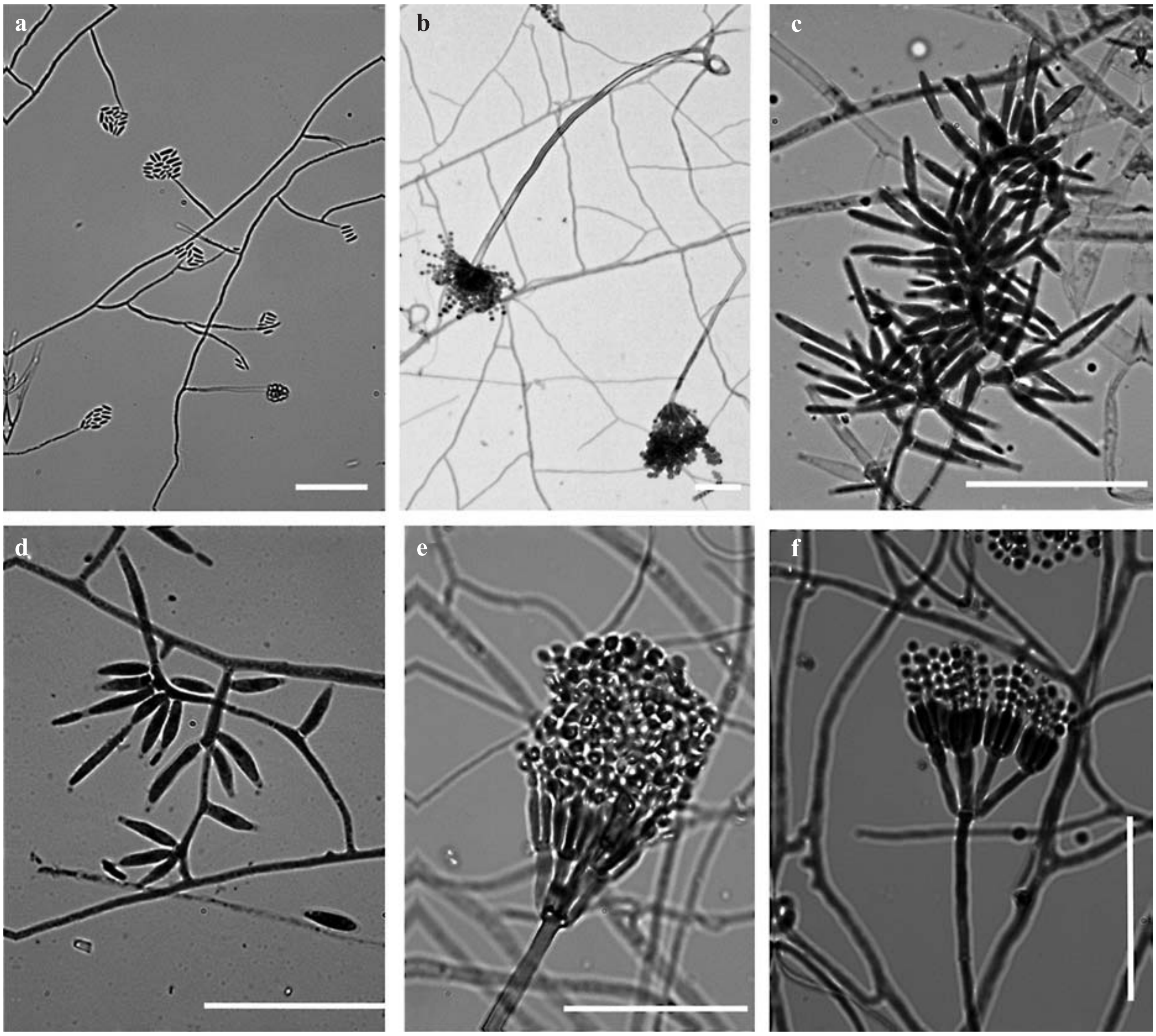

Fig 1 Microscopic characteristics of: a) *Acremonium kiliense* (general features); b) *Aspergillus sydowii* (general features); c) *Fusarium sacchari* var. *sacchari* (detail of conidiophore); d) *Fusarium merismoides* var. *merismoides* (detail of conidiophore); e) *Gliocladium viride* (detail of conidiophore: stipes, phialides and metulae with disposition detail to conidia; f) *Penicillium citrinum* (detail of conidiophore: phialides and metulae); scale = 30 μm.

their respiratory siphon and attached to roots of aquatic macrophytes to obtain oxygen, food, and shelter (Ferreira 1999).

Aspergillus sydowii (Fig 1b)

Aspergillus sydowii was isolated in MEA and CYA media and had radiate conidial heads; stipes 127-387 µm, thick-walled, smooth, colorless to pale brown, expanding to clavate vesicles 9-12 µm in width; aspergilla biseriate; metulae and phialides present; conidia spherical, rough to spinose, 2-4 µm in diameter; diminutive conidial structures similar to simple penicilliate heads.

The genus *Aspergillius* has more than 180 species. Some are rare and some are common. An important characteristic that distinguishes this fungus from others is that the phialides appear simultaneously from the vesicles (Klich 2002). Many species of *Aspergillus* have been used by industry for the production of enzymes and acids, amilase and citric acid, among others (Klich 2002). However, this group has problems in their use as some can degrade agricultural products (Mallozzi & Corrêa 1998). In addition, some species are pathogenic or allergenic to humans and other animals. Bioassays performed by Moraes *et al* (2001) showed high pathogenicity in the lineages *Aspergillus ochraceus*, *A. kanagawaensis* and *A. sulphureus* for larvae of *Ae. fluviatilis* and *Culex quinquefasciatus* Say.

Aspergillus sydowii is widely distributed and has been reported from many substrates, but it is mainly found associated with soil (Moraes *et al* 1998, Klich 2002).

Isolated mosquito species. *Aedes fluviatilis*

Sampling site. State of Rondônia, by Ferreira-Keppler RL and Silva JO, in 02/vi/2004.

Habitat. In this work, larvae were collected in stone holes, with water retention, at locations generally of low depth (3 to 20 cm), containing sand and little organic matter. These locations are placed in open areas along rivers or small streams. This species is known to colonize these environments, presenting adaptations for its development and being common in different conditions of temperature according to the incident solar energy.

***Fusarium* spp.**

Species of the genus *Fusarium* can be distinguished from other anomorphic genera by their two types of conidia: macro, moon shaped and micro, spherical or oval, both produced by phyalides (Putzke & Putzke 1998). These are filamentous, cosmopolitan, saprophytic or opportunistic plant parasites, which can be found in fruit, seeds and soil (Putzke & Putzke 1998, Almeida *et al* 2005). Some species are considered human opportunistic pathogens causing superficial and systemic infections, mainly when the patient is immunodeficient (Anaissie *et al* 1988).

Fusarium sacchari* (Butler) var. *sacchari (Fig 1c)**.** The fungous was isolated in PDA medium displayed septate hyphae, conidiophores, phialides and macroconidia. Conidiophores arising laterally on hyphae, loosely ramose; monophialidic, being sometimes polyphialidic, with a strong tendency to proliferate, slender, almost cylindrical; macroconidia uniform, slightly, falcate, canoe form, with two or more cells measuring 10-15 × 2-3 µm. We found two lineages of *Fusarium sacchari*.

Isolated mosquito species. *Mansonia titillans*

Sampling site. Iranduba municipality, by Ferreira-Keppler R L and Pereira E S in 30/xi/2004.

Habitat. See item 1.

Fusarium merismoides* var. *merismoides (Fig 1d)**.** The fungous was isolated in PDA media, had septate hyphae, conidiophores, phialides and macroconidia. Conidiophores arising as single lateral phialides on hyphae, more or less irregularly branched; monophialidic almost cylindrical or obclavate (5-20 µm long); macroconidia were observed with great variation in size (6-12 × 2-3µm), straight to slightly curved in the extremities, canoe shaped in two or more cells. We found one lineage.

Isolated mosquito species. *Mansonia titillans*

Sampling site. Iranduba municipality, by Ferreira-Keppler

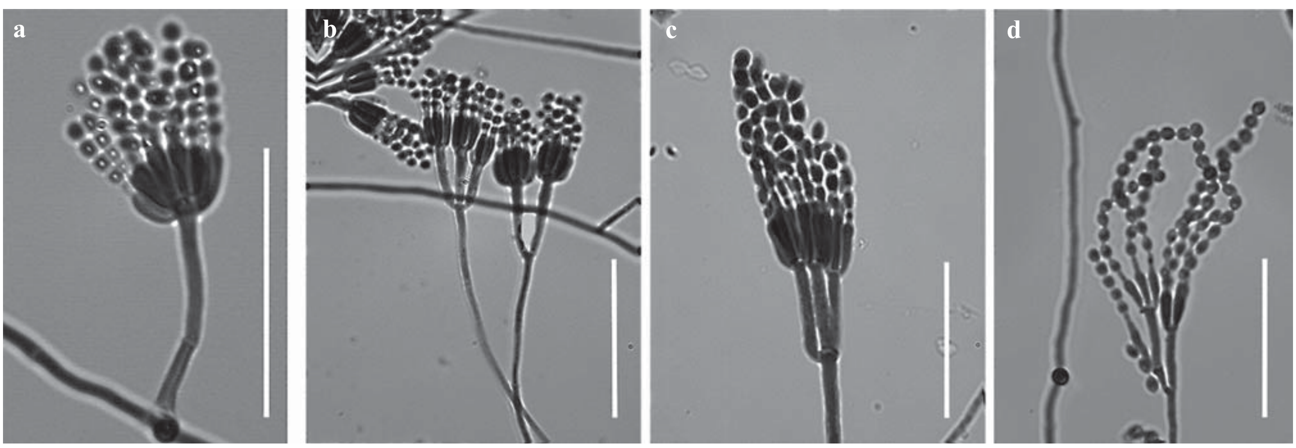

Fig 2 Microscopic characteristics of: a) *Penicillium sclerotiorum* (detail of conidiophore: stipes, metulae and disposition to conidia); b) *Penicillium melinii* (conidiophore with phialides); c) *Penicillium oxalicum* (detail of conidiophore: stipes, phialides and metulae); d) *Paecilomyces* sp (detail of conidiophore: phialides, metulae with disposition detail to conidia); scale = 30 µm.

RL and Pereira E S in 30/xi/2004.
Habitat. See item 1.

Gliocladium viride (Fig 1e)**.** The fungous was isolated in MEA and CYA media and had septate hyphae, conidiophores, phialides and conidia. Conidiophores are erect, terminated by a dense brush-like branching system bearing tapered phialides; phialides (measuring 11-13 μm long) of the terminal branches give rise to flask shaped; conidia measure 2-3 μm in diameter, they are one-celled, ovoid to cylindrical accumulating in the shape of a ball or in a loose column.

This is a widely distributed filamentous fungus that can be isolated from decomposing plants and soil (Itoh *et al* 1980, Pandey *et al* 1990). It was not reported as causing disease in humans and animals; however, it is considered to be a "contaminant" fungus.
Isolated mosquito species. *Anopheles darlingi*
Sampling site. Rio Preto da Eva municipality, by Ferreira-Keppler R L, Pereira E S, Oliveira A F and Martins M S.
Habitat. Larvae were collected along a lake in locations with sufficient suspended organic matter (e.g. small fruits, leaves, kindlings) between grasses. Females of *An. darlingi*, main malaria vector in the Amazon region, generally search for places with permanent clean water and relatively covered by vegetation for oviposition (Zeilhofer *et al* 2007).

Penicillium spp.

***Penicillium citrinum* Thom** (Fig 1f)**.** The fungous was isolated in MEA and CYA media. Conidiophores borne from surface of hyphae; stipes 87-287 μm long, smooth walls terminating in well-defined verticils of 3 divergent metulae and vesiculate; metulae usually of uniform length 15-23 μm, less usually with metulae interleaving, spathulate or terminally in vesiculate; phialides ampulliform 10-14 μm long; conidia spherical, 2 μm diameter with smooth walls originating in long well-defined columns, one per metula.

Penicillium citrinum is found in decaying vegetation and in the air. It is also a biodeteriogen and causes losses principally in foods, textiles, paintings and plastics (Pitt 1985). Russel *et al* (2001) reported *P. citrinum* parasitizing eggs of *Ae. aegypti* in Australia by the production of mycotoxins that inhibit the complete development of the eggs during the dry season, thereby diminishing the incidence of mosquitoes during the rainy season. In Brazil, da Costa and Oliveira (1998) isolated *P. citrinum* from adults and larvae of Culicidae.
Isolated mosquito species. *Aedeomyia squamipennis*, *An. darlingi* and *Ae. fluviatilis*.
Sampling sites. The Culicidae species were collected in Amazonas. In the municipality of Rio Preto da Eva from larvae of *Ad. squamipennis* and one *An. darlingi* were collected by Ferreira-Keppler R L, Pereira E S, Oliveira A F and Martins MS, in 30/ ix/ 2004. On the Abunã River, in Rondônia state, from larvae of *Ae. fluviatilis*, *Ad. squamipennis* by Ferreira-Keppler, R L and Silva J O, in 02/ vi/ 2004.
Habitat. *Aedeomyia squamipennis* and *An. darlingi* were collected in a natural lake (item 4) and *Ae. fluviatilis* in stone holes (item 2). In *Ad. squamipennis*, monotype species, immature forms were collected from a laminar water flow at locations covered by aquatic macrophytes, frequently in artificial lakes, as fish tanks. They cohabit with species of *Anopheles* and *Mansonia* which does not have the same medical importance.

Penicillium sclerotiorum (Fig 2a)**.** The fungous was isolated in MEA and CYA media presented conidiophores arising from surface or subsurface hyphae; stipes 20-42 μm long, slender with thin and smooth walls, finishing in well defined verticillate, strictly monoverticillate; phialides numerous, ampulliform, 6-9 μm long; conidia ellipsoidal 1-3 μm in diameter, growing in well-defined columns and becoming irregular.

This fungus is commonly found in the soil but can occur in tissue biodeteriorative situations of tissue (Pitt 1985) and foods along with other species (Amoa-Awua *et al* 1997). *Penicillium sclerotirum* catalyzed the bioconversion of herbertenediol into dimers: mastigophorenes A and B, neurotrophically active compounds (Harinantenaina *et al* 2005). In this study we found one lineage.
Isolated mosquito species. *Anopheles argyritarsis* and *An. darlingi*.
Sampling sites. Both Culicidae species were collected in Rio Preto da Eva municipality by Ferreira-Keppler R L, Pereira, E S, Oliveira A F and Martins M S, in 30/ix/2004.
Habitat. The species *op cit* were collected in a lake, according to item 4. They can grow in a variety of locations, which can be related to reservoirs built for hydroelectric constructions (Tadei *et al* 1998, Forattini 2002). In the transmission of malaria, *An. argyritarsis* females, contrary to *An. darlingi*, may not be anthropophilous (Forattini 2002).

Penicillium melinii (Fig 2b)**.** The fungous was isolated in MEA and CYA media had conidiophores arising from the surfaces of hyphae; stipes measuring 12-120 μm with walls roughened, bearing terminal verticils of 2-4 metulae, integrated with short monoverticillate conidiophores; metulae rough walled, 9-16 μm long; phialides ampulliform, 7-10 μm long; conidia spherical, spinose, 1-2 μm in diameter, arising in short to long chains, in disordered to well-defined columns.

Penicillium melinii appears to be exclusively a soil fungus. In general, the new colonies produce characteristic pigments (Pitt 1985).
Isolated mosquito species. *Uranotaenia* sp.
Smapling site. Rio Preto da Eva municipality, by Ferreira-Keppler R L and Pereira E S, in 30/xi/ 2004.
Habitat. *Uranotaenia* sp. was collected in a lake according to the description for the species *Anopheles* and *Aedeomyia* (items 4 and 5). Portions of this environment are mainly liquid collections as in waterlogged conditions where they were found in locations with abundant vegetation, at the superior water level. The medical and sanitary aspects of these species, at present, are unknown.

***Penicillium oxalicum* Corrie and Thom** (Fig 2c)**.** The fungous was isolated in MEA and CYA media and had the following microscopic characteristics: conidiophores arising from surface mycelium; stipes 12-292 μm in length with thin, smooth walls, characteristically terminating in verticils of 2-3 metulae; metulae 16-23 µm long; phialides acerose, 12-17 µm long, with short collula; conidia ellipsoidal, with walls smooth, 3-5 µm × 1-3 µm.

This fungus is widely distributed and although common in the soil its main habitat is rotting vegetation (Pitt 1985). In Brazil, da Costa & de Oliveira (1998) isolated *P. oxalicum* from *Mansonia* spp. (Culicidae) larvae and adults.

Santamarina *et al* (2002) extracted metabolites of *P. oxalicum* lineages demonstrating their antagonistic effect against bacteria, fungi and insects. Bioassays were undertaken to test the pathogenicity of *P. oxalicum* against *Fusarium oxysporum* lycopersici and *Fusarium oxysporum gladioli*. *Penicillium oxalicum* has shown good capability as a biocontrol agent against species of *Fusarium* (Santamarina *et al* 2003).

Species of *Penicillium* can be found in soil, rotting plants and fruits or in dry places such as seeds and wood; dry spores of this fungus are disseminated by wind and insects (Trabulsi *et al* 1999). Some species can play important roles in the natural processes of biological recycling (Pitt 1985) and in the control of infectious diseases. Others are contaminants or opportunistic pathogens due to their production of mycotoxins (Mallozzi & Corrêa 1998). According to Pitt (1985), *Penicillium* is a large genus with at least 150 or maybe 300 species. *Penicillium* was found in larvae and adults of some species of *Anophele*s, *Aedes*, *Culex* and *Mansonia* in the southeast and northern regions of Brazil: *P. canescens*, *P. chrysogenum*, *P. citrinum*, *P. corylophilum*, *P. decumbens*, *P. expansum*, *P. fellutanum*, *P. implicatum*, *P. janthinellum*, *P. oxalicum*, *P. purpurogenum*, *P. viridicatum* and *P. waksmanii*. Bioassys were undertaken with *P. corylophilum* and *P. janthinellum* indicating that they were pathogenic to larvae of *Ae. fluviatilis*, *Ae. aegypti*, *An. aquasalis* and *Cx. quinquefasciatus*, demonstrating that these lineages have a potential for use in biological control of Culicidae vectors (da Costa & de Oliveira 1998, da Costa *et al* 1998).

Isolated mosquito species. *Mansonia titillans*
Sampling site. Iranduba municipality by Ferreira- Keppler RL and Pereira ES, in 30/xi/ 2004.
Habitat. See items 1 and 3.

***Paecilomyces* sp.** (Fig 2d)

Paecilomyces sp. was recorded and illustrated but not named. It was isolated in MEA and CYA media had the following microscopic characteristics: septate hyaline hyphae with flat walls; conidiophores, phialides and conidia are observed; conidiophores 55-112 µm wide, branched with phialides at the extremity; phialides measuring 8-12 µm, swollen at their bases and tapering towards their apices; conidia are unicellular, hyaline, flat, oval measuring 2-3 µm in diameter. *Paecilomyces* sp. is distinguished from *Penicillum* by the irregular or verticillate forms of the phialides and the divergent conidial chains, and from *Acremonium* by verticillate conidiophores and flask-shaped phialides (Samson 1974). It is a cosmopolitan genus that can be found in decomposing plants and soil. Some species are considered to be insect parasites. Kalkar *et al* (2006) isolated *Paecilomyces reniformis* from Orthoptera (Insecta) in Indonesia. Scholte *et al* (2004) reported the presence of *Paecilomyces farinosus* and *Paecilomyces lilacinus* in larvae of *Cx. pipiens* and *Ae. aegypti*.

Isolated mosquito species. *Anopheles darlingi*
Sampling site. Rio Preto da Eva municipality, by Ferreira-Keppler R L, Pereira E S, Oliveira A F and Martins M S in 30/ ix/ 2004.
Habitat. See items 4 and 5.

This paper is a contribution to a collection of insect-related microorganisms in the Brazilian Amazon. Five genera were identified: *Acremonium*, *Aspergillus*, *Fusarium*, *Gliocladium* and *Penicilium*. Species of these genera are known for their importance in the production of secondary metabolites, especially antibiotics and mycotoxins. Some of the lineages isolated, as cited above, have been reported in the literature as having biotechnological potential. Further studies in this area can be undertaken with the objectives of selecting industrially important biocontrol agents.

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