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Batkoa apiculata (Thaxter) Humber affecting *Anopheles* (Diptera: Culicidae) in the municipality of Una, Southern Bahia, Brazil.

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Abstract

Méndez Sánchez SE, Humber RA, Lage Freitas A, Pinheiro AMCM. 2010. *Batkoa apiculata* (Thaxter) Humber affecting *Anopheles* (Diptera: Culicidae) in the municipality of Una, Southern Bahia, Brazil. Entomotropica 25(2): 63-68.

Surveys for fungal pathogens affecting adult mosquitoes from the genus *Anopheles* were conducted in flooded and swamp-like natural breeding sites near residences in the center and suburbs of the city of Una as well as the nearby village of Outeiro in southern Bahia. Surveys of 54 mosquito breeding sites over a period of 24 months yielded 150 sample collections with a total of 194 *Anopheles* cadavers infected by the pathogenic fungus *Batkoa apiculata* (Entomophthorales: Entomophthoraceae). Apart from their basic scientific value, these results also offer some hope about the possibilities to use fungus-based biocontrol against *Anopheles* species that vector malaria in Brazil.

Additional key words: Fungi, Entomophthoromycotina, mosquito vector, malaria, Surveys.

Resumen

Méndez Sánchez SE, Humber RA, Lage Freitas A, Pinheiro AMCM. 2010. *Batkoa apiculata* (Thaxter) Humber infectando *Anopheles* (Diptera: Culicidae) en la municipalidad de Una, sur de Bahia, Brasil. Entomotropica 25(2): 63-68.

Muestreos de mosquitos adultos del género *Anopheles* fueron realizados en criaderos y pantanos naturales próximos a residencias urbanas y suburbios de la ciudad de Una asi como en la villa Outeiro, situada en los alrededores, al sur de Bahia. De 54 muestreos en locales de reproducción de mosquitos, por un período de 24 meses, 150 muestras fueron colectadas, de las cuales un total de 194 presentaron cadáveres de *Anopheles* infectados por el hongo patogénico *Batkoa apiculata* (Entomophthorales: Entomophthoraceae). Estos resultados, a pesar de originarse de una investigación básica, son estudios relevantes por su relación con las posibilidades de control microbial alternativo para *Anopheles* spp., principal vector de la malaria en Brasil.

Palabras clave adicionales: Hongo, Entomophthoromycotina, mosquito vector, malaria, muestreos.

Introduction

Mosquitoes of the genus *Anopheles* (Diptera: Culicidae) are the principal vectors of the genus *Plasmodium*, the causative agent of malaria, in Brazil and other tropical countries. The control

of these pests is based mainly on the application of chemical insecticides such as organochlorines, organophosphates, carbamates, pyrethroids and even DDT against adult mosquitoes, but their behavioral tendency to spend much time outdoors (for both feeding and resting on plants) strongly decreases the likelihood of contacts with these chemicals since they are customarily used only for indoor applications (Berti et al. 1993; Zimmerman and Berti 1994). In addition to these behavioral complications for Brazilian anopheline vectors of malaria, the prospects for chemically based strategies to reduce malarial transmission continue to be problematic due to the rise of insecticidal resistance in many vector species throughout the world, and because the complex genetics of *Plasmodium* species have effectively prevented the development of antimalarial vaccines. These difficulties emphasize the need to develop improved control strategies against the mosquito vectors (including the use of fungal or other microbial diseases of these vectors) for the indirect reduction of malarial transmission, supplementing the ongoing quest to find direct means to control the malarial parasites transmitted by these mosquitoes.

Whether Brazilian anophelines are becoming physiologically resistant to the insecticides used against them or (at least in the area studied here) exhibit behaviors making them less likely to contact those insecticides, there is no doubt that malarial parasites are well established, and continue to be transmitted and dispersed within the country. Presently, studies on the habitats for larvae of these vectors are being made in various countries using geographical information systems to produce ecological maps that aid the monitoring and evaluation of mosquito control programs (Gabinaud 1987; Barrera et al. 1998, 1999; Bergo et al. 2007). In Brazil, there are pilot programs for integrated management of malarial vectors that are successful for larvae, but these programs lack much basic information on vector ecology to understand the spatial and temporal distribution of the vectors and how various biotic and abiotic factors of their habitats might determine the presence in and preference for particular environments. In Bahia, in spite of their decreasing incidences, malarial outbreaks continue to be observed in recent years. This state is, therefore, susceptible, although classified as low risk for the transmission of the disease. Nonetheless, a high density of anophelines makes at least 110 municipalities vulnerable to malaria, as is the city of Ilheus because of its proximity to the municipality of Una where malaria is known to occur. The risk of contracting malaria is not geographically uniform within a country (especially one so large as Brazil), and varies notably within a region; and also is not temporally linked with the seasons of the year or the fluctuating populations of vectors.

In this study, our prime objective was to survey, map and monitor natural breeding grounds of urban environments with ample presence of anopheline mosquitoes in the municipality of Una and to detect possible occurrences of mosquito cadavers naturally infected by the fungus *Batkoa apiculata* (Thaxter) Humber (Entomophthorales: Entomophthoraceae). This study extends our understanding of the presence and significance of this fungus as a mortality factor for populations of *Anopheles* vectors of malaria in this part of Brazil, and also provides a better basis for understanding how the fungus might serve to aid in the control of these important insect pests.

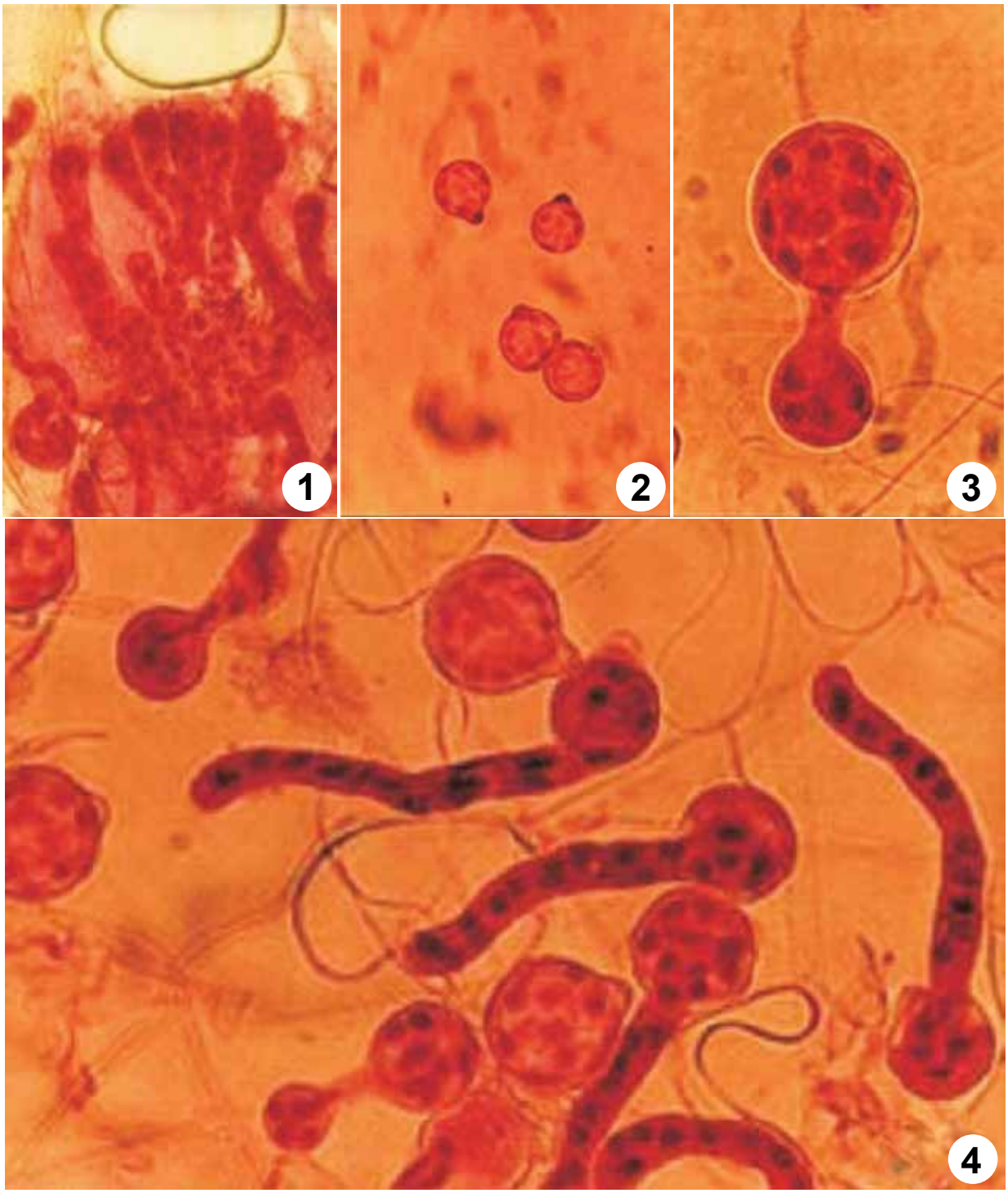
Material and Methods

All the surveys were made in the municipality of Una, the urban center and the periphery of the city, and in the village of Outeiro at 13 Km of the city of Una. Adult *Anopheles* mosquitoes were sampled from April 2008 to April 2010, during which 54 natural breeding sites in flooded areas and swamps near residences were visited, and yielded a total of 150 sample

collections. The breeding sites we sampled were previously identified and marked according to their varying geographical habitats and visited once or twice a month at random. Samples were collected with the help of a small entomological kit consisting of a 7-liter polystyrene box, self-adhesive labels, magnifying glass, flexible-tipped tweezers, brush, flasks with 65 % alcohol + 5 % glycerin, and sterilized 100 x 15 mm glass Petri dishes into which infected mosquito cadavers were placed after their removal from the substrates. Specimens were promptly returned to the entomological laboratory at UESC for identification of the insects (Gorham et al. 1967), as well as for recording the characteristics and symptoms associated with the pathologies caused by the entomophthoralean fungi. All samples were analyzed within 72 hours of their collection from the field. In the laboratory, the following sequence of analytic approaches was used to obtain critical data: 1. Macroscopic examinations determined the physical characteristics of the external mycelium growing on the cuticle of mycotized cadavers. 2. Microscopic examination of slide preparations of conidiogenous material from the surfaces of infected hosts or hyphae and of hyphal bodies from the hemocoel were mounted in a drop of LP/AO (lactophenol/aceto-orcein, 1:1) for examination of the size and nature of stained granulations in the nuclei of this entomophthoraceous fungus. 3. Incubation in humid chambers for 80 min to observe the production and discharge of primary and secondary conidia after 80 and 180 min, respectively.

Results and Discussion

The Una municipality, with a population of ca. 31 000, is located about 50 Km from the city of Ilhéus on the coast of southern Bahia (15° 17´ 23´´ - altitude 20 m). This municipality supports cattle ranches and farms. This region includes some of the few remaining areas of Atlantic rain forest and has a warm, humid climate with average annual temperature of 25 °C, no well defined dry season while rainfall is regularly distributed throughout the year. The area's diverse plant communities help make it an attractive tourist destination, and the city of Una and the village of Outeiro are located near Comandatuba Island and the Una Biological Research that is a natural habitat of the highly endangered golden-headed lion tamarin. Of the 54 surveyed breeding sites and 150 collected samples, 194 *Anopheles* cadavers infected by the pathogenic fungus *Batkoa apiculata* were evaluated positive within the results still considered preliminary, which demonstrated the occurrence, infection and colonization of cadavers of adult anophelines by the fungus *Batkoa apiculata*. This pathogen has primary conidia with multiple nuclei showing granular contents staining in LP/AO. The conidia are 32.5 – 35 x 30 – 33 µm (averages from four sets of 20 spores from different insects), with a rounded (but often slightly pointed or apiculate) basal papilla (Figure 2); secondary conidia similar to the primaries although smaller (27-28 x 25 µm) (Figure 3); primary conidia germinating with their young germs tubes, 3.0-3.5 µm diam, containing a total of 16 – 20 nuclei (this is also probably the number of nuclei in ungerminated conidia) (Figure 4). The developing conidiophores are unbranched (simple) that are slightly broader at the apex where an obviously narrower extension (not illustrated) grows out from the apex before producing the primary conidium (Figure 1); neither rhizoids nor resting spores were observed. Mycotized cadavers were characterized by the proliferation of grayish white (to yellowish in older specimens) bands of conidiophores emerging at first through the intersegmental membranes or, when more fully developed, forming a generalized covering of the back and sides of the host. Most infected anophelines were collected in humid habitats, especially in the interiors of residences adjacent to flooded areas or swamps.



Figures 1-4. *Batkoa apiculata* (Thaxter) Humber mounted in lactophenol/aceto-orcein (This stain differentiates the nuclei and the granular nature of the highly condensed DNA that is a permament feature of their contents). **1.** Young conidiophores forming on the exterior of an infected mosquito; the broadly rounded apices of these cell will produce a narrow extension before the development of the single apical conidium. **2.** Multinucleate, globose primary conidia with rounded basal papilla. **3.** Early development of a (smaller) secondary conidium by a primary conidium; the nuclei and their granular contents are distinctly visible. **4.** Primary conidia germinating by formation of germ tubes in which the large nuclei are usually visible in a single-file arrangement.

The potential use of many diverse fungi whose potential for use in mosquito control have been reviewed by Scholte et al. (2004). Among these mosquito fungi are the hypocrealean (clavicipitoid) conidial fungi *Metarhizium anisopliae, Beauveria bassiana, Culicinomyces clavisporus* and *Tolypocladium cylindrosporum*; the fungus-like straminipilan oomycetes *Leptolegnia chapmanii* and *Lagenidium giganteum*; the many species of *Coelomomyces* (now classified in the class Blastocladiomycetes)*;* as well as zygomycetous (or trichomycetous) fungi (species of *Smittium* and other genera now classified in the subkingdom Kickxellomycotina) and a few entomophthoralean fungi (now in the subkingdom Entomophthoromycotina that is not yet assigned to any kingdom). The correct identifications of entomophthoraleans from mosquitoes (e.g. as in Scholte et al. 2004) according to the current classification of this order (Humber 1989) have remained somewhat confused, largely because their comparatively low incidences have not provided sufficiently detailed characterizations to assure their correct placements. The new, phylogenetically based classifications of these fungi (and former fungi) mentioned here follows that adopted by Hibbett et al. (2007) and Sung et al. (2007). However, nearly all of these fungi–with the possible exceptions of *Metarhizium* and *Beauveria* species as well as of *Leptolegnia chapmanii*–either have comparatively low virulence for their mosquito hosts and/or present significant challenges for their production *in vitro*, formulation, or application in the field.

The persistence of malaria in some parts of Brazil, combined with the costs and undesirable environmental effects of using chemical pesticides to attempt to manage the mosquito vectors of this disease, underline the need to find suitable biological alternatives for mosquito control (Costa et al. 1991a, b). Relatively little effort has been expended to find and to identify microbial pathogens affecting mosquitoes or other dipteran vectors of human and livestock diseases in the Brazilian state of Bahia despite the common occurrence of both suitable environmental conditions and malarial vector species. Some preliminary surveys for entomophthoralean pathogens affecting insects in Bahia (Sanchez et al. 2002) noted the presence of *Batkoa apiculata* (although tentatively and incorrectly identified then as either *Entomophaga tipulae* or *Entomophaga domestica*) affecting *Anopheles*. species in the municipality of Una. During that 2002 survey there was no sampling of the types of vegetation present around anopheline breeding sites; this is now known to affect whether larvae of these vectors are likely to be present (Savaje et al. 1990).

There is no doubt from the findings reported here that there are high populations of anopheline mosquitoes near brooks and swamps in this region, and that the ambient temperatures and humidities are highly favorable for the development of substantial populations of both the vector mosquitoes and for the initiation and maintenance of outbreaks of entomophthoralean mycoses in these mosquito populations. We acknowledge that the poor (to nonexistent) level of general sanitation characteristic of residences in or near our study sites contributes to the proliferation of vector mosquito populations and to the ongoing transmission of malaria to both the local inhabitants and to casual visitors to these areas.

Further studies of *Batkoa apiculata* will seek to determine whether any significant difference might be found based on the sexes, the life stages, or which species of *Anopheles* is attacked by this fungus. Future studies will sample a wider range of biotic and abiotic factors that might affect the incidence of mosquito mycoses and will monitor a greater number of breeding sites.

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