

# Aquatic Phoretic Mites (Acari: Hydrachnidia) Associated with Ectoparasitism of Mosquitoes (Diptera: Culicidae) in the Midwest Region of Brazil

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## Abstract

Water mites (Hydrachnidia) are common external parasites of the medically important mosquitoes (Culicidae). Between April 2014 and April 2015, 64 mites from three genus, *Arrenurus* 71.9%, *Limnochares* 3.1%, and *Hydrachna* 25.0%, were collected from female mosquitoes in two rural locations near Cuiabá, Mato Grosso, Brazil. Dipterous species parasitized by water mites belonging to seven species: *Anopheles (Nys.) darling*, *An. evansae*, *Aedes (Och.) scapularis*, *Ae. serratus*, *Mansonia (Man.) wilsoni*, *Psorophora (Jan.) ferox*, *Ps. varipes*. The most common specimens to accommodate the water mites were *Anopheles (Nys.) darlingi* and *Psorophora (Jan.) varipes*. The prevalence of parasitism of mosquitoes by water mites found in this study was less than 5. However, few studies have addressed the ecological role of mites and their biotopes, as well as host-parasite interactions in Brazil.

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## Keywords

Ectoparasitism, Hydracarina, Culicidae, Mites

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### 1. Introduction

Like most animals, insects belonging to Dipteran may be parasitized. In particular, water mites are known to parasitize the larvae and pupae of these insects by piercing the exoskeleton and ingesting the circulating hemolymph [1] [2]. These mosquitoes can transmit numerous pathogens to their hosts, such as protozoa, viruses, bacteria, nematodes, cestodes, and trematodes [3] [4].

Water mites are found in various environments that are accessible to animals, with many specimens found in soil, plant shoots, animal hair, feathers, human hair follicles, and insect hosts [5]. The habitats for water mites are very diverse. For instance, they are able to live in hot springs, wetlands, temporary pools, ponds, deep lakes, torrential waterfalls, and oceans [1]. According to Smith and Cook [1], water mites are the most abundant and diverse arthropods that exist in moist environments, and they have a life cycle that includes three larval and nymphal stages (ninfocrisalida, deutonymph, and imagocrisalida).

Water mites (Hydrachnida) are an interesting group of microarthropods, with about 6000 described species among 50 families [1] [6]. These organisms are commonly found in vegetation margins of lakes and rivers [1] and occasionally they use to live in water.

Mites are known to parasitize vertebrates as well as insects and other arthropods [7] in study conducted in Egypt indicate that larvae of several species of water mites are ectoparasites of adult mosquitoes and midges, the majority of which belong to the genera *Thyas* (Thyasidae) and *Arrenurus* (Arrenuridae). Nielsen [8] in the Denmark also found in their studies, water mites parasitizing adult mosquitoes of the genera *Aedes* and *Anopheles*; the majority of mites identified as the genus *Thyas* and *Arrenurus*.

The subfamily Arrenurinae Wolcott, 1905 (Arrenuridae Thor, 1900), particularly the genus *Arrenurus*, has the greatest abundance of species among water mites, with more than 950 known species [9].

Larvae of the *Arrenuridae* family are ectoparasites of mosquitoes of medical importance, for example, genera *Aedes* and *Anopheles* [10]. According to Uchida & Miyazaki [11], larvae *Arrenurus* spp. are the most common water mites parasites of mosquitoes, being associated as hosts of various Dipteran genera of medical and veterinary importance (*Culex*, *Anopheles*, *Mansonia* and *Coquillettidia*).

These water mites are recognized as potential natural enemies of mosquitoes such as *Anopheles* spp. (the vectors of malaria). *Anopheles* and *Mansonia* are the most frequent hosts in the tropics and subtropics; at times 80% of their populations may be parasitized [12]-[15] suggested in their studies, and these mites can be used as markers of dispersion studies of mosquitoes and as indicators of the age of this dipteran.

In this study, we aimed to identify mite isolated from female Culicidae specimens, as well as to document the preference and occurrence on Culicidae specimens captured in two areas located in the rural municipality Cuiabá, state of Mato Grosso, central region of Brazil, has a tropical climate, conducive to mosquito breeding and represented by three biomes: Amazon, Cerrado, and Pantanal. This might be the first record of genera of water mites hosted in isolated and identified mosquitoes in region Midwestern of Brazil.

### 2. Materials and Methods

The first collection site was located under a bridge where motorway MT-040 crosses the Aricazinho river at 15°56'09.93"S and 55°58'25.48"W; the second was located next to motorway MT-050 near the district of Praia Grande at 15°45'49.82"S and 56°08'53.91"W.

The mite-isolated stocks occurred related to collections of mosquitoes in the research project Medical Entomology Laboratory of the Federal University of Mato Grosso and identifying mosquitoes noted the existence of some winged specimens parasitized by mite bodies.

Mosquitoes were captured, between April 2014 and April 2015 from two forest areas in the rural area of Cuiabá, Mato Grosso, Brazil. They were conducted 12 insert the field in four areas assessed, during the seasons of the year, where culicids were captured quarterly. Mite specimens were identified in mosquitoes collected in these two areas with proximity to rivers and streams. The collections of winged specimens were conducted in

the evening at twilight, comprising the period from 17:00 to 20:00, with three hours of capture on three consecutive days.

Using the suction tube designed by Forattini [16] and Marcondes [17] and transported to the Laboratory of Entomology, Department of Health, state of Mato Grosso mosquitoes were killed by placing in a  $-20^{\circ}\text{C}$  freezer for 5 min. Then, they were each examined on a slide and using a stereo microscope at  $40\times$  magnification were identified to the species level with the taxonomic keys of Consoli & Lourenço-de-Oliveira [18] and Forattini [16].

The anatomical sites where each parasite was located were observed closely. Mites were photographed, their attachment sites recorded, and then removed from their host for identification with the aid of a stereoscopic microscope, and decryption keys and identification of species [9] [19]-[28] for reference.

### 3. Results

Mosquitoes (1362) were collected and classified into 17 species. Of the total number of mosquitoes captured and examined, 30 specimens were infested, with a prevalence of 2.2%. Four different genera: *Anopheles*, *Aedes*, *Mansonia* and *Psorophora* were found parasitized, these seven species of insects: *Anopheles (Nys.) darlingi* (9; 30%); *Anopheles (Nys.) evansae* (1; 3.3%), *Aedes (Och.) scapularis* (6; 20%), *Aedes (Och.) serratus* (2; 6.7%); *Mansonia (Man.) wilsoni* (1; 3.3%); *Psorophora (Jan.) ferox* (5; 16.7%), and *Psorophora (Jan.) varipes* (6; 20%) (Table 1).

These ectoparasites were from the families Arrenuridae, Limnocharidae, and Hydrachnidae, comprising three genera: *Arrenurus* spp. (46; 71.9 %) (Arrenuridae: Arrenurinae) were found in species of *Anopheles (Nys.) darlingi*, *Anopheles (Nys.) evansae*, *Aedes (Och.) scapularis*, *Psorophora (Jan.) varipes*, and *Psorophora (Jan.) ferox*. Two specimens (2.3%) of *Limnochares* spp. (Limnocharidae: Limnocharinae) were isolated: one derived from *Anopheles (Nys.) darlingi* (Figure 1) and the other from *Aedes (Och.) scapularis*; and specimens (16; 25%) of *Hydrachna* spp. (Hydrachnidae: Hydrachninae) were isolated from *Aedes (Och.) serratus*, *Psorophora (Jan.) varipes*, and *Mansonia (Man.) wilsoni*. The 15 male mosquitoes captured in this study were not parasitized. All females captured and identified showed higher parasitism rates: *Anopheles (Nys.) darlingi* (25; 39.1%), followed by *Psorophora (Jan.) varipes* (13; 20.3%).

When we considered the influence of temporal and spatial distribution in two sites, we obtained an index of species richness of parasitic mites of 1.11, which is considered as low richness. Mites are in greater quantity in the genera of *Anopheles* mosquitoes and *Psorophora*. When the abundance of parasitic mites on mosquitoes was

**Table 1.** Water mite parasitism of mosquitoes captured Aricazinho and Praia Grande river in Cuiabá, Mato Grosso, Brazil, 2014-2015.

Mosquito Species	Mosquitoes captured		Parasitized mosquitoões		Mites		Mites genus
	N	%	N	%	N	%	
<i>Anopheles (Nys.) darlingi</i> (Root, 1926)*	414	34.4	9	30.0	25	39.1	<i>Arrenurus</i> spp./ <i>Limnochares</i> spp.
<i>Anopheles (Nys.) evansae</i> (Brethes, 1926)*	23	1.9	1	3.3	2	3.1	<i>Arrenurus</i> spp.
<i>Aedes (Och.) scapularis</i> (Rondani, 1848)*	212	17.6	6	20.0	8	12.5	<i>Arrenurus</i> spp./ <i>Limnochares</i> spp.
<i>Aedes (Och.) serratus</i> (Theobald, 1901)*	18	1.5	2	6.7	5	7.8	<i>Hydrachna</i> spp.
<i>Mansonia (Man.) wilsoni</i> (Barreto & Coutinho, 1944)*	48	4.0	1	3.3	1	1.6	<i>Hydrachna</i> spp.
<i>Psorophora (Jan.) ferox</i> (Von Humboldt, 1819)*	78	6.5	5	16.7	10	15.6	<i>Arrenurus</i> spp.
<i>Psorophora (Jan.) varipes</i> (Coquillett, 1904)*	410	34.1	6	20.0	13	20.3	<i>Arrenurus</i> spp./ <i>Hydrachna</i> spp.
<b>Total</b>	<b>1203</b>	<b>100</b>	<b>30</b>	<b>100</b>	<b>64</b>	<b>100</b>	

\*Gaffigan *et al.* (2015) Systematic Catalog of Culicidae. WRBU-New Mosquito Classification [59].

compared; *Anopheles (Nys.) darlingi* hosted the largest number, 25 mites (39.1 %) belonging to species *Arrenurus* spp. For the insect genus *Psorophora*, we found 10 (15.6 %) individuals in *Arrenurus* spp., *Psorophora (Jan.) ferox* (Figure 2).

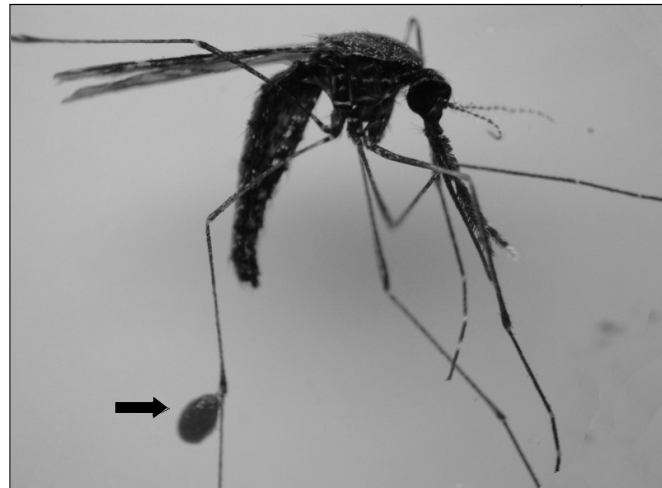
The distribution patterns of mites collected in the regions of Praia Grande and Aricazinho River were different for mosquito species: *Anopheles (Nys.) darlingi*, *Aedes (Och.) serratus* and *Psorophora (Jan.) varipes* were found to host *Arrenurus* spp. and *Hydrachna* spp. On the other hand, mosquitoes collected on the Aricazinho river, *Anopheles (Nys.) darlingi*, *Anopheles (Nys.) evansae*, *Aedes (Och.) scapularis*, *Mansonia (Man.) wilsoni*, *Psorophora (Jan.) varipes* and *Psorophora (Jan.) ferox*, were found parasitized by *Arrenurus* spp., *Limnochares* spp., and *Hydrachna* spp.

These aquatic mites were distributed on the basis of the anatomical region of the mosquitoes as follows: in the head along with the neck: three on *Anopheles (Nys.) darlingi*, five on *Aedes (Och.) serratus*, one on *Mansonia (Man.) wilsoni*, and six on *Psorophora (Jan.) varipes*; in the region of the abdomen: in regions I and II tergites, seven mites on *Anopheles (Nys.) darlingi*; II and III, two mites on *Aedes evansae*; III and IV, three mites on *Aedes scapularis*; and IV and V, one on *Aedes (Och.) scapularis*. In the abdominal tergites (I to V) of an *Anopheles (Nys.) darlingi* were isolated from 10 *Arrenurus* spp. mites parasitizing (Figure 3). In the pronotum, we found four mites on *Anopheles (Nys.) darlingi* and *Psorophora (Jan.) varipes* (Table 2).

**Table 2.** Distribution anatomical region of mosquitoes; parasitized by water mite captured Aricazinho and Praia Grande river in Cuiabá, Mato Grosso, Brazil, 2014-2015.

	Subfamily Anophelinae						Subfamily Culicinae						Geral			
	A.d.	%	A.e.	%	A.s.a.	%	A.s.	%	M.w.	%	P.v.a.	%	P.f.	%	Total	%
<b>I. Anatomical region</b>																
Head/neck	3	12	-	-	5	100	-	-	1	100	6	46	-	-	15	23
Chest	4	16	-	-	-	-	4	50	-	-	7	54	8	80	23	36
Abdome	17	68	2	100	-	-	4	50	-	-	-	-	2	20	25	39
Legs	1	4	-	-	-	-	-	-	-	-	-	-	-	-	1	2
Total	25	100	2	100	5	100	8	100	1	100	13	100	10	100	64	100
<b>II. Abdome distribution</b>																
I ao V	10	58.8	-	-	-	-	-	-	-	-	-	-	-	-	10	40
I e II	7	41.2	-	-	-	-	-	-	-	-	-	-	-	-	7	28
II e III	-	-	2	100	-	-	-	-	-	-	-	-	2	100	4	16
III e IV	-	-	-	-	-	-	3	75	-	-	-	-	-	-	3	12
IV e V	-	-	-	-	-	-	1	25	-	-	-	-	-	-	1	4
Total	17	100	2	100	-	-	4	100	-	-	-	-	2	100	25	100
<b>III. Chest distribution</b>																
Mesopleura	2	50	-	-	-	-	2	50	-	-	2	29	4	50	10	43
Sternumpleura	-	-	-	-	-	-	2	-	-	-	2	29	2	25	6	26
Pronotum	2	50	-	-	-	-	-	-	-	-	2	29	-	-	4	18
Escutellum	-	-	-	-	-	-	-	-	-	-	1	13	2	25	3	13
Total	4	100	-	-	-	-	4	50	-	-	7	100	8	100	23	100
<b>IV. Legs distribution</b>																
Posterior tibial	1	100	-	-	-	-	-	-	-	-	-	-	-	-	1	100

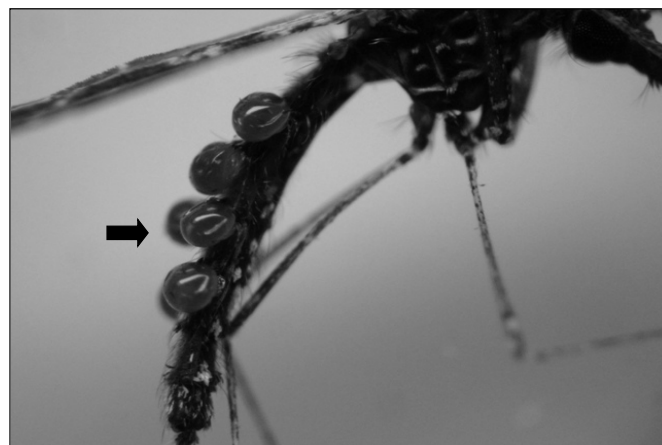
Legend: A.d. = *Anopheles (Nys.) darlingi*; A.e. = *Anopheles (Nys.) evansae*; A.s.a. = *Aedes (Och.) serratus*; A.s. = *Aedes (Och.) scapularis*; M.w. = *Mansonia (Man.) wilsoni*; P.v.a. = *Psorophora (Jan.) varipes* and P.f. = *Psorophora (Jan.) ferox*.



**Figure 1.** Aquatic mites *Limnochares* on the posterior tibial *Anopheles (Nys.) darlingi* female (40× magnification) (picture by Diniz P. Leite-Jr.). The arrow indicates the insertion site.



**Figure 2.** Aquatic mites *Arrenurus* on the abdomen of *Psorophora (Jan.) ferox* female (40× magnification) (picture by Diniz P. Leite-Jr.). The arrow indicates the insertion site.



**Figure 3.** Aquatic mites *Arrenurus* on the abdomen of *Anopheles (Nys.) darlingi* female (40× magnification) (picture by Diniz P. Leite-Jr.). The arrow indicates the insertion site.



## 4. Discussion

The larvae of aquatic mites are the most common types of ectoparasites Culicidae with medical importance such as *Aedes* spp. and *Anopheles* spp. In our study, the overall prevalence of infestation of mites on mosquitoes was 2.2%. This percentage is relatively high, as compared to other previous findings. In the United States, Milne [29] found a prevalence of 0.27%. In Australia, McCallister [30] reported 0.005%, Williams and Proctor [31] found 0.27%. In New Zealand, Snell and Heath [32] found 0.52%. Kirkhoff [33] reported 2.6% in Pennsylvania and Spurrier [34], in Wyoming, found in mosquitoes a prevalence of 0.42% and 0.76% prevalence over a period of six years. In India, Sharma & Prasad [34] found a higher prevalence (4.1%).

Most species of water mites (Acari: Hydrachnidae) are common parasites of emergent aquatic insects and have larvae that are obligate parasites on adult insects order: Odonata, Hemiptera, and Diptera, primarily in the Nematocera, especially Chironomidae, but also including the Culicidae [28] [35].

There are several records of mite parasitism of insects in different circumstances like Pymotidae [36], Odonatas [37] [38], Coleoptera [39], Sand flies [40] [41], Hemiptera [42] [43], and Muscidae [7] [44] [45].

The interaction between mites and mosquitoes has been investigated by several authors in Canada [46], India [35], United States [29] [30], Australia [31], New Zealand [32], and Pennsylvania [33]. In Brazil, there is a report of parasitized mosquitoes in São Paulo city [47]. In Mato Grosso, the country's central region, no reports on activity of mites on mosquitoes have been documented.

A behavior observed in many families of mites is phoresy phenomenon, which consists of passive transport of an organism for the purpose of dispersion [44]. The dispersion is an important process for many species of mites that allows the expansion of their populations, the colonization of different areas and the escape of natural enemies. In general, structural, physiological and behavioral adjustments associated with the scattering mechanism are common and are varied in mites [44] [48]-[50].

Most water mites that are found to parasitize mosquito larvae belong to the genus *Arrenurus* [10] [51]. This is confirmed by the findings of this study, in which we removed *Arrenurus* spp. from mosquitoes [51]. According to Mullen [23], water mites most commonly parasitizing mosquitoes are species of *Arrenurus* (Arrenuridae) and *Thyas* (=Parathyas) (Thyasidae).

Members of Arrenuridae, Hydryphantidae, and Erythracidae were described by Williams and Proctor [31], when they analyzed infected mosquitoes of the genera *Anopheles*, *Culex*, *Ochlerotatus*, and *Tripteroides* in Australia. The first three of these genera were also found in mosquitoes parasitized by larval *Arrenuridae* and *Microtrombidiidae* in New Zealand [32]. In 2013, Kirkhoff [33] identified the genera *Aedes*, *Anopheles*, *Coquillettia*, *Culex*, *Ochlerotatus*, *Orthopodomyia*, and *Psorophora* parasitized by mites of the genus *Parathyas* sp. and *Arrenurus* sp.; 33 different associations and parasitism of individual mosquitoes by more than one species of mites were observed. Our study corroborates this, as we collected and identified mosquitoes of the genus *Anopheles*, *Aedes*, *Mansonia*, and *Psorophora* parasitized by aquatic micro invertebrates.

Mullen [23] gathered 238 worldwide records of acarine parasites of mosquitoes that were always attached to membranous areas of the host body. In 2010, researchers in Egypt, recorded mite parasites attached in other groups activities: Muscidae, Ceratopogonidae and other dipterans [7]. Mites isolated in this study were located in the ventral and dorsal abdomen and at various positions on both sides of the chest, neck, and legs of mosquitoes.

Mites of the genus *Arrenurus* (71.9%) were most commonly found in membranous areas between the thorax and abdominal segments I, II, II, IV and V. We observed a strong tendency for mites to set up on the abdomen of winged hosts. This distribution is probably explained to the ease of attachment to the membranes in the ventral segments and adherence to the abdominal region, which allows easy attachment for mites and access to hemolymph (Table 2).

According Nielsen [8] describes in his study, that mites were always found attached to membranous areas of the host body. In all mosquito species, the most common attachment site was the back of coxae.

With regard to the fixing of ectoparasites to anatomical sites, we observed parasites on the abdomen, chest, head/neck, and legs. Milne [29] and Snell & Heath [32] reported a similar pattern of attachment of the abdomen mites (Table 2).

The distribution of mites on their hosts may be specific for each species [36]. Indeed, most examples have characteristic locations of connection, which depend on the species, but may also vary with the host species and genera [12] [24] [43] [46].

The parasitic mite families Arrenuridae, Hydrachnidae and Limnocharidae which belong to the genera *Arrenurus*, *Hydrachna* and *Limnochares*, are not uncommon, with a number of records involving water mites of these families, parasitizing insects [1] [29] [32] [33].

Water mites are a group of mites living in water and playing an important role as predators in aquatic biocenoses. The distribution and the dispersion degree of different species of water mites are varied depending on the parasitized insects. The water mites parasitizing insects whose imagines constantly stay outside the water are decidedly the more expansive and in general more frequent species in the current fauna [52].

According to Gledhill [53] that predation occurs depending on the migration ability of parasitized insects water and can be divided in three groups: 1) Parasites of insects whose imagines are permanently connected with water; 2) the parasites of insects whose imagines periodically leave water; 3) the parasites of insects whose imagines permanently live out of water.

According to Zawal [52] [54] the third group is composed of the remaining species of water mites, which parasitize flying insects of the orders Odonata, Ephemeroptera, Plecoptera, Heteroptera, Trichoptera and Diptera. These insects are both fairly large and easily accessible for parasites. Dipterans are less intensively infested (several parasites on one host).

Several authors have reported in their casuistic that the most frequently infested insects are family Odonata and dipterans of the families Culicidae [33] [52] [55]-[58].

Water mite larvae parasitize many aquatic insect species, including aquatic Ephemeroptera, Plecoptera, Heteroptera, Trichoptera and Diptera. Although this situation may cause damaging impacts to the hosts, the mites can disperse and colonize new localities in this way. In Brazil, little is known about the frequency of water mite ectoparasitism amongst the aquatic dipterans in Middle West in the Country. In this study, larval water mite parasitism on aquatic dipterans, families Culicidae and Anophelinae that have been collected from different localities in Cuiabá city, was evaluated. These are the first records for larval mite parasitism on Middle West region in Brazil.

The high diversity of the taxon, described by some authors, exemplifies its importance. However, there are few studies on the ecological role of mites on their biotopes and their parasite-host interactions. This paper aims to describe the association of these aquatic ectoparasites, first described in Cuiabá, Mato Grosso, Brazil.

Mites can thrive amongst various biological control mosquito populations. We suggest that further studies are needed to map the studied species of mites for Brazil and in State of Mato Grosso, as well as to identify interactions and associations with other parasitized animal species, aiming to use them as biological markers in the control of insects of medical importance. Further study is necessary to know the extent of competition/ nature and mechanism of toxic substance used by the ectoparasites.

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## Conflict of Interest

The authors declare that there are no conflicts of interest.

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