Exp Appl Acarol (2016) 69:323–333
DOI 10.1007/s10493-016-0045-2

Mites (Acari: Trombidiformes) parasitizing mosquitoes (Diptera: Culicidae) in an Atlantic Forest area in southern Brazil with a new mite genus country record

Emili Bortolon dos Santos[1] · Mario Arthur Favretto[2] · Samuel Geremias dos Santos Costa[3,4] · Mario Antonio Navarro-Silva[1]

Received: 14 December 2015 / Accepted: 8 April 2016 / Published online: 16 April 2016

Abstract In this study, a total of 4146 culicids collected in an Atlantic Forest area in Paraná state, southern Brazil were examined for the presence of mites. Forty larval Parasitengone mites (*Arrenurus* spp., Arrenuridae; *Durenia* spp., Trombellidae; *Microtrombidium* spp., Microtrombidiidae) parasitized 25 specimens of mosquitoes, with the intensity varying from one to nine mites attached. Most mites were found on *Aedes serratus/nubilus*, *Culex vomerifer*, *Cx. pedroi* and *Cx. sacchettae*. The overall percentage of parasitized mosquitoes was 0.6 %. The highest intensity of mites encountered was in an individual of *Cx. pedroi* with nine attached mites. Regarding the attachment site, most mite specimens were attached to the abdomen (n = 25), whereas 15 were located on the thorax. Specimens of *Arrenurus* spp. were only found on the abdomen of mosquitoes, and the same was observed for *Microtrombidium* spp., while *Durenia* spp. attached to both the thorax (n = 15) and abdomen (n = 4). This is the first record for the genus *Durenia* in Brazil. Additionally, some species of mosquitoes were, for the first time, reported as being parasitized by mites.

Keywords *Arrenurus* · Brazil · Culicidae · *Durenia* · *Microtrombidium*

✉ Emili Bortolon dos Santos
emilibortolon@hotmail.com; emili.bsantos@gmail.com

[1] Post-graduation Program in Entomology, Federal University of Paraná, Curitiba, Paraná State 81531-980, Brazil

[2] Post-graduation Program in Ecology and Conservation, Federal University of Paraná, Curitiba, Paraná State 81531-980, Brazil

[3] Federal University of Minas Gerais, Belo Horizonte, Minas Gerais State 31270-901, Brazil

[4] Museum of Biological Diversity, Ohio State University, Columbus, OH, USA

Introduction

The parasitism of Parasitengona mites on insects is common, usually observed in aquatic mites for insects that have at least one life stage in the water, but also occur on other taxa. Among the insect hosts, Diptera (Culicomorpha) is one of the main groups that is parasitized by water mites (Smith and Oliver 1986). Regarding the culicids, *Arrenurus* spp. and *Parathyas barbigera* are the taxa best known for parasitizing a large number of mosquito species (Di Sabatino et al. 2000).

DeGeer made the first record of a culicid parasitized by mites, at the time describing the mite species *Trombidium culicis* (Mullen 1975). Back then, the culicids were not well studied, as people did not know their role in the transmission of agents causing diseases in humans and other animals. In 1876, Patrick Manson discovered that mosquitoes could spread nematode worms responsible for causing filariasis. This was the first time that the relationship between insects and disease transmission was established (Eldrige 1992).

Nowadays, it is known that culicids can spread several etiological agents. Besides filariasis, they can also transmit dengue, chikungunya, zika virus, yellow fever, encephalitis and malaria, among others diseases. The last disease is responsible for about 198 million cases each year and more than 580 thousand deaths per year (Fang 2010; WHO 2015). Given the importance of this group of insects for public health, it is logical to search for potential biological agents for these vectors. Many studies indicate that mites impose negative impacts on mosquitoes, because they reduce the life expectancy and reproductive capacity (Lanciani and Boyt 1977; Smith and McIver 1984a; Snell and Heath 2006).

Mites belonging to the sub-order Parasitengona have a life cycle including three active instars, a larva that is parasitic of various arthropod species including Diptera, heteromorphic deutonymphs and adults that are free-living predators, interspersed with inactive, non-motile instars (Welbourn 1983; Smith 1988; Wohltmann 2000).

The mites that parasitize mosquitoes are mainly aquatic and need to feed in order to reach the deutonymphal stage. Later, as adults, they commonly act as predators of insects eggs and larvae (Esteva et al. 2007). After the appearance of the host adult stage, the mite becomes a parasite and remains with the insect until its return to the water for reproduction or oviposition. The mites can also detach from the hosts on land (Di Sabatino et al. 2000). Terrestrial Parasitengona mites, e.g. *Microtrombidium* spp. (Microtrombidiidae), can attach to mosquitoes that have the immature stages in the accumulated water in the soil, such as *Aedes serratus*, which may develop in temporary puddles and less frequently in permanent breeding sites (Forattini 2002).

The studies with mites parasitizing mosquitoes are concentrated in some countries, such as the USA (Tsai et al. 1969; Spurrier 1998; Kirkhoff et al. 2013), Pakistan (Reisen and Mullen 1978), Germany (Werblow et al. 2015), Australia (Williams and Proctor 2002) and New Zealand (Snell and Heath 2006). Meanwhile, studies with mites in Brazil, in their majority, are concentrated in species descriptions, associating the specimens to the parasitized organism (e.g. Treat and Flechtmann 1979; Haitlinger 1987, 2004). In this country, the studies about mites' parasitism in mosquitoes are scarce, not having systematic surveys, restricted to the study of Flechtmann (1974), where there was a record of a mosquito species being parasitized by several mites. In addition, Mullen (1975) made some records of mosquitoes being parasitized by unidentified mites in Brazil.

In this context, taking into account the importance of increasing knowledge regarding the interaction mite-Culicidae in the Neotropical region, the aim of this study was to

conduct a research on the species of mosquitoes that are parasitized by mites in an Atlantic Forest fragment in the state of Paraná, in southern Brazil.

Methods

Mosquitoes and mites were collected in an Atlantic Forest area, in the *Floresta Estadual do Palmito*, located in the municipality of Paranaguá, in Paraná state, in the South of Brazil (25°35′S, 48°32′W). The area has 530 ha of native vegetation and is a conservation park of sustainable use (Fig. 1). It is bordered by PR-407 Road in the south, by Cotinga's Canal (Paranaguá Bay) in the north, by the Almeidas' River in the east and by *Ribeirão das Correias* in the west.

The studied area is characterized as having Cfa subtropical climate. The average temperature in the coldest months is about 18 °C and in the warmest months is about 22 °C. Frosts are infrequent and the summer is usually warm and rainy, there is no well-defined dry season, according to the Köeppen classification (Iapar 2010). The annual average precipitation is about 1950 mm and the rainiest months are January and February, however, there is rain distribution throughout the year, which makes the place very humid; the average relative air humidity in a year is 85 % (Boeger and Wisniewski 2003).

The vegetation of the area is classified as Dense Ombrophilous Forest of Low Lands, however, the area has vegetation with influence from the sea (sandbank), it has mangrove areas and areas modified by humans. In *Floresta Estadual do Palmito* the forest formation is composed by individuals that can reach more than 20 m high.

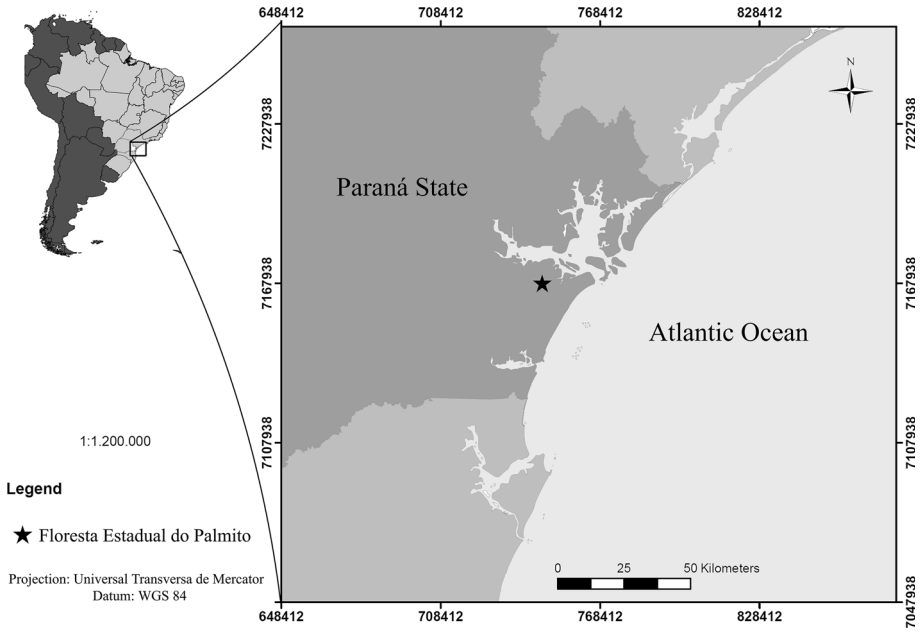

Fig. 1 Location of *Floresta Estadual do Palmito*, Paranaguá, Paraná state, Brazil, where the collections were performed

Regarding the collections of culicids, they were performed between June 2014 and May 2015, with the exception of July and August 2014. Mosquitoes were collected with the Nasci aspirator, searching for the insects in the middle of low vegetation. After collections, culicids were stored in a freezer until sorting. In the sorting, parasitized mosquitoes were separated from non-parasitized ones. The attachment site in the mosquito's body was divided in two categories: abdomen and thorax. They were identified with help of dichotomous keys from Rozeboom and Komp (1950), Galindo et al. (1954), Consoli and Oliveira (1994), Sallum and Forattini (1996) and Forattini (2002). We followed the abbreviation system of Culicidae genera and subgenera accordingly to Reinert (2009). The specimens are deposited in the *Coleção Entomológica Padre Jesus Santiago Moure* at the Federal University of Paraná, Curitiba, in the state of Paraná.

The mites were mounted using Hoyer's medium, which was prepared according to the protocol described by Krantz and Walter (2009), then kept in the stove at 50 °C for four days to dry, in the third day the borders were cleaned and after the fourth day the slides were sealed with glaze. Mites were identified with keys to family from Krantz and Walter (2009) and Mullen (1974). The genera were identified using the keys from Southcott (1994) and Saboori et al. (2003, 2005). Three mite genera are illustrated by photos (Fig. 2) taken with the Nikon Eclipse 90i compound microscope equipped with a Nikon DS-fi1 camera and Adobe Illustrator imaging software. The specimens were deposited for future consultation in the Taxonomic Collection of the Laboratory of Systematics and Evolution of Acariform Mites, with the codes: UFMG-AC (1301053–1301060 and 1301062–1301095), at the Federal University of Minas Gerais, Belo Horizonte, in the state of Minas Gerais.

Results

Of the 4146 specimens of Culicidae collected, 1639 mosquitoes were identified and divided into nine genera and 42 species. From these, 25 individuals were parasitized by mites, 19 females and six males (Table 1). The taxon with the highest parasitism index was *Ae. serratus/nubilus*, which corresponded to 28 % (n = 7) of the total of parasitized mosquitoes, followed by *Culex vomerifer* (16 %; n = 4), *Culex pedroi* and *Culex sacchettae*, both representing 12 % (n = 3). Forty mites in total were found attached to the mosquitoes, ranging from one to nine mites attached to each, represented by the genera *Arrenurus* (47.5 %; n = 19), *Durenia* (47.5 %; n = 19) and *Microtrombidium* (5 %; n = 2).

Overall, the percentage of infestation for mites was 0.6 % throughout the collection period. *Aedes serratus/nubilus* and *Ae. hortator* were only parasitized by the genus *Arrenurus*. *Culex vomerifer* was parasitized by one *Arrenurus* larva and three *Durenia* larvae. *Culex pedroi* was also parasitized by *Arrenurus* and *Durenia*, as well as *Cx. sacchettae*. We collected two specimens of *Uranotaenia mathesoni*, both were parasitized.

Culex pedroi was the species with the largest number of mites attached (n = 11), being that one specimen was parasitized by nine mites, six *Durenia* and three *Arrenurus*. *Culex vomerifer* was parasitized by eight mites, two specimens were parasitized with three *Durenia* mites each. *Aedes serratus/nubilus* specimens had eight attached mites, however, only one had two *Arrenurus* mites attached, the others had only one mite each.

In this study we observed mites being attached more to the mosquitoes' abdomen (n = 25), and less frequently to the thorax (n = 15). The genus *Arrenurus* only parasitized

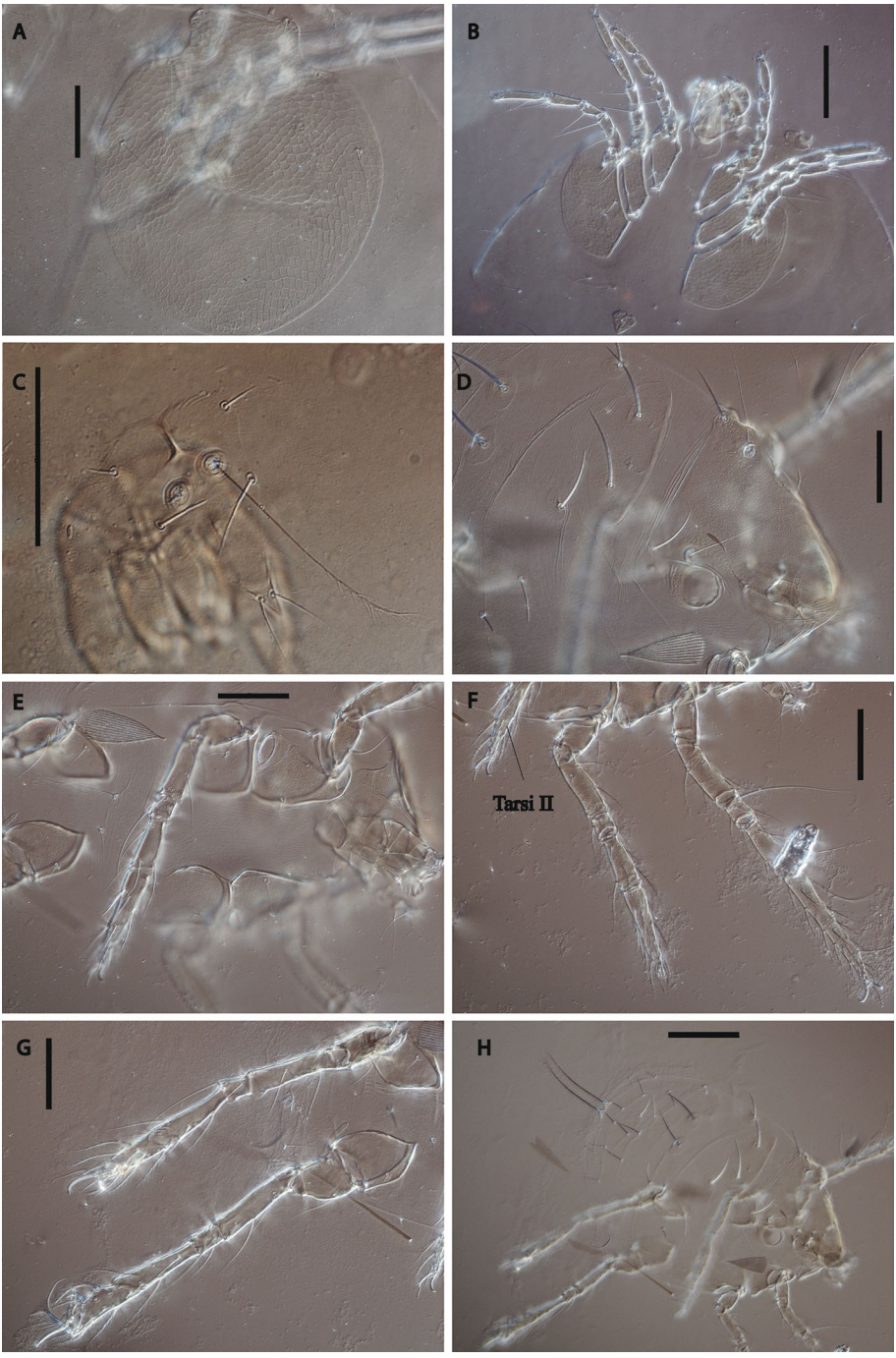

Fig. 2 *Arrenurus* sp. (**a**) Scutum; (**b**) Ventral view. *Durenia* sp. (**c**) Scutum. *Microtrombidium* sp. (**d**) Scutum; (**e**) Ventral view; (**f**) Leg I and II; (**g**) Leg III; (**h**) General view. *Scale* **a**, **b**, **h**: 100 μm; **c**–**g**: 50 μm

Table 1 Parasitized mosquitoes collected in an Atlantic Forest area in southern Brazil

Culicidae species	Total no. mosquitoes	No. parasitized mosquitoes	Mite abundance		
			Microtrombidium	*Arrenurus*	*Durenia*
Anopheles cruzii	138	0	0	0	0
Aedes fulvus	1	0	0	0	0
Aedes oligopistus	1	0	0	0	0
Aedes scapularis	32	0	0	0	0
Aedes serratus	17	0	0	0	0
Aedes serratus/nubilus	443	7♀	0	7	0
Aedes hortator	89	1♀	0	1	0
Psorophora ferox	197	0	0	0	0
Culex bidens	1	0	0	0	0
Culex declarator	4	0	0	0	0
Culex lygrus	1	0	0	0	0
Culex mollis	71	0	0	0	0
Culex neglectus	1	0	0	0	0
Culex nigripalpus	75	0	0	0	0
Culex dunni	1	0	0	0	0
Culex pedroi	4	3♀	0	1	2
Culex pedroi/ribeirensis	2	0	0	0	0
Culex pilosus	3	0	0	0	0
Culex sacchettae	81	2♀, 1♂	0	1	2
Culex spissipes	8	0	0	0	0
Culex vomerifer	4	4♂	0	1	3
Culex zeteki	2	1♀	0	1	0
Culex aphylactus	3	0	0	0	0
Culex imitator	8	1♀	1	0	0
Culex (*Microculex*) spp.	31	1♀	1	0	0
Limatus durhamii	41	0	0	0	0
Runchomyia reversa	95	0	0	0	0
Trichoprosopon pallidiventer	1	0	0	0	0
Wyeomyia leucostigma	19	0	0	0	0
Wyeomyia sabethea	1	0	0	0	0
Wyeomyia davisi	12	0	0	0	0
Wyeomyia edwardsi	1	0	0	0	0
Wyeomyia fuscipes	1	0	0	0	0
Wyeomyia pallidoventer	106	0	0	0	0
Wyeomyia quasilongirostris	1	0	0	0	0
Wyeomyia theobaldi	3	0	0	0	0
Wyeomyia cf. *lassali*	120	0	0	0	0
Wyeomyia confusa	1	0	0	0	0
Wyeomyia coenonus/tarsata	44	0	0	0	0
Wyeomyia shannoni	1	0	0	0	0
Uranotaenia mathesoni	2	2♀	0	2	0

Table 1 continued

Culicidae species	Total no. mosquitoes	No. parasitized mosquitoes	Mite abundance		
			Microtrombidium	*Arrenurus*	*Durenia*
Uranotaenia pallidoventer	2	0	0	0	0
Uranotaenia spp.	62	1♀, 1♂	0	1	1

mosquitoes' abdomen (n = 19), none was found on the thorax. The same was observed for *Microtrombidium* spp. On the other hand, most *Durenia* spp. were attached to the thorax (n = 15), although some were found on the abdomen (n = 4). Specimens of *Ae. serratus/nubilus* only were parasitized by mites on the abdomen (n = 8), the same was observed for *Ae. hortator* (n = 2). *Culex pedroi* had three *Arrenurus* mites attached to the abdomen and seven *Durenia* attached to the thorax and one to the abdomen. A similar pattern was observed for *Cx. vomerifer*, which had one *Arrenurus* mite attached to the abdomen and six *Durenia* to the thorax and one to the abdomen (Table 2).

Discussion

There are just a few studies on mites parasitizing mosquitoes in Brazil. We can cite Flechtmann (1974), a study where one specimen of *Culex fatigans* (synonym: *Cx. quinquefasciatus*) was found parasitized by 15 *Arrenurus* mites. We did not find other related studies, thus, our study is probably the first to do a systematic investigation on mosquitoes being parasitized by mites in Brazil. The overall percentage of parasitized mosquitoes in the period from June 2014 to May 2015 was 0.6 %. Kirkhoff et al. (2013) collected 929,873 mosquitoes in Pennsylvania, USA, among them, 0.2 % were parasitized by mosquitoes (n = 1836). A similar pattern was observed in Australia, where from 19,280 mosquitoes collected, 0.3 % were parasitized by mites (Williams and Proctor 2002). On the other hand, there are studies where the percentage is higher, such as in Ontario, Canada (6.7 %) (Smith and McIver 1984b) and in another study, also in Ontario, Smith and McIver (1984a) found a much higher percentage: 87.5 %.

This variation of percentage of parasitism in different countries may be related to the species composition of the studied areas and fresh water availability for the presence of mosquitoes (Milne et al. 2008). Some abiotic factors may strongly influence the distribution and abundance of mosquitoes. Rainfall, for instance, can alter the abundance and type of breeding sites for mosquitoes (Shaman and Day 2007), thus, according to the climate of a certain region, some species of mosquitoes can vary their development. That is, each region of the planet has its own Culicidae fauna, which can also vary during the year due the climate.

We collected 25 parasitized mosquitoes, of these, 19 were composed by females, while only six were males. Mites apparently attach more in the females because after they become adults they return to the water to lay their eggs. In many dipterans species only the female return to the water for oviposition, so if the mites attach males, their life cycles could not be completed (Mullen 1974; Smith and McIver 1984a; Lanciani 1988; Rolff 2001).

Mites were most often found on the abdomen of the mosquitoes in this study. Snell and Heath (2006) also found mites attached mainly to the abdomen, and in lower numbers on the thorax and on the legs as well. Milne et al. (2008) generally observed aquatic mites attached

Table 2 Attachment sites of each mite genus on the parasitized mosquitoes collected in an Atlantic Forest area in southern Brazil

Culicidae species	*Microtrombidium*		*Arrenurus*		*Durenia*		Total
	Abdomen	Thorax	Abdomen	Thorax	Abdomen	Thorax	
Ae. serratus/nubilus	0	0	8	0	0	0	8
Ae. hortator	0	0	2	0	0	0	2
Cx. imitator	1	0	0	0	0	0	1
Cx. pedroi	0	0	3	0	1	7	11
Cx. sacchettae	0	0	1	0	1	2	4
Cx. vomerifer	0	0	1	0	1	6	8
Cx. zeteki	0	0	1	0	0	0	1
Cx. (*Mcx.*) sp.	1	0	0	0	0	0	1
Ur. mathesoni	0	0	2	0	0	0	2
Ur. (*Ura.*) sp.	0	0	1	0	1	0	2
Total	2	0	19	0	4	15	40

to the abdomen of mosquitoes, the same was found in this study, where *Arrenurus,* an aquatic mite, only parasitized the abdomen. Mitchell (1959) in a study on *Arrenurus* demonstrated that the site each species of mite chose to attach to was directly related to the way that the Odonata species laid their eggs. We emphasize that the total amount of parasitized mosquitoes was low, so inferences about host and attachment site preferences are premature.

We found that *Arrenurus* was the genus which parasitized the greatest diversity of mosquitoes. This probably occurred due this genus's biology, as it lives in different types of freshwater environments, allowing it to encounter different host species (Mlynarek et al. 2015). *Arrenurus* is the genus most reported parasitizing mosquitoes, whose activity had already been documented in several countries, reinforcing its flexibility regarding the hosts. There are several species of *Arrenurus* mites which may parasitize these insects (Tsai et al. 1969; Reisen and Mullen 1978; Williams and Proctor 2002; Morales and Miranda 2008; Werblow et al. 2015).

All species of mosquitoes collected in this study were found being parasitized by *Arrenurus*, with the exception of species of the subgenera *Microculex.* This can be an indication that this genus perhaps is not developing in bromeliads, which is the common container in which *Microculex* is found (Lourenço-de-Oliveira et al. 1986). This is confirmed by the other species collected in this study which were not parasitized and may develop in bromeliads, such as *Anopheles cruzii* and *Wyeomyia* (*Phoniomyia*) (Consoli and Oliveira 1994; Forattini 2002).

Regarding the other species parasitized by *Arrenurus* collected in this study, *Ae. serratus* specimens have their immature stages developing mainly in temporary puddles in the soil, formed by rain. The same is true for the other collected species of *Culex*, belonging to the subgenera *Melanoconion. Aedes hortator* can develop in stone holes and also in temporary puddles in the soil (Forattini 1965, 2002; Hutchings et al. 2002). Immature stages of *Uranotaenia* species develop in swamps among aquatic vegetation (Galindo et al. 1954). Therefore, in this study *Arrenurus* was only found parasitizing mosquitoes which have a life phase in the soil, demonstrating a distribution pattern, as was observed by Williams and Proctor (2002) in South Australia.

Microtrombidium was collected in only two mosquitoes, *Cx. imitator* and an unidentified species of *Culex* (*Microculex*). The immature stages of these mosquitoes can develop in natural containers such as tree holes and bromeliads, especially the ones located on stones and in the soil (Kumm 1933; Lourenço-de-Oliveira et al. 1986). In contrast to the majority of mites which parasitize mosquitoes, *Microtrombidium* mites live in the soil. They have been reported parasitizing Culicidae (e.g. Michener 1946) and it is understandable that mosquitoes who can develop in bromeliads located in the soil, for instance, can be attached by larval mites on land.

Regarding the genus *Durenia*, little information is known, there is one study about its relation as parasites of mosquitoes (Mullen 1975). Despite the reduced number, almost half of all mites parasitizing mosquitoes in the present study belonged to *Durenia*, which could indicate that this genus could be easily found parasitizing Culicidae, at least in Brazil. However, a major quantity of information about the relation *Durenia*-Culicidae is necessary, mainly because here we present the first record of this genus in Brazil.

Mites probably carry out a natural control of mosquitoes and a lot of other insect taxa as well. Besides reducing life expectancy and egg reproduction, mites are known for delaying sexual maturity and reducing the flight capability. In all cases, the impact of parasitism depends on the quantity of parasites attached to the insects and can also affect the whole population of a certain species (Smith et al. 2001). García et al. (1994) observed the parasitism by mites in natural populations of Culicidae and conclude that the prevalence and number of mites in each host were low.

We emphasize here that we made the first records of some species of mosquitoes being parasitized by mites: *Ae. hortator*, *Cx. imitator*, *Cx. pedroi*, *Cx. sacchettae*, *Cx. zeteki* and *Ur. mathesoni*. In addition, based on a literature search, this is the first record of the genus *Durenia* for Brazil. We also found some specimens new to science; the description process is underway. It is evident that the studied area needs to be better explored in order to improve knowledge regarding mites parasitizing mosquitoes in this country.

Acknowledgments The authors want to thank CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) and CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) for the grants to EBS, MAF and SGSC, this last with the government program "Ciência sem Fronteiras". Our special thanks to Aristides Fernandes, from School of Public Health, University of São Paulo, for the identification of some Culicidae species. We are grateful to Hans Komplen from Ohio State University and Warren C. Welbourn, from University of Florida, for their valuable contribution for the improvement of this study. We also want to thank to the reviewers who contributed to the improvement of this manuscript as well.

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