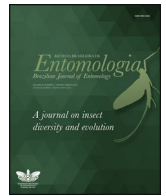




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Review

Host–parasite interaction and impact of mite infection on mosquito population



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ABSTRACT

During the present study, the host–parasite relationship between mosquitoes and parasitic mites was investigated. The 8954 individuals of male and female mosquitoes belonging to 26 genera: seven each of *Aedes* and *Culex*, six of *Anopheles* and one each of *Toxorhynchites*, *Coquillettidia* and *Uranotaenia* were collected from 200 sites. The male and female mosquitoes were collected from the State of Uttar Pradesh, located at 26.8500° N, 80.9100° E in North India by deploying Carbon dioxide-baited and gravid traps. The intensity of mite's infection, type and number of mites attached to mosquitoes, mite's preference for body parts and host sexes were the parameters used to determine host–parasite relationship. Eight species of mites: *Arrenurus acuminatus*, *Ar. gibberifrons*, *Ar. danbyensis*, *Ar. madaraszi*, *Ar. kenki*, *Parathyas barbiger*, *Leptus* sp., and *Anystis* sp., parasitized mosquitoes. Parasitic mites preferred host's thorax for attachment as compared to the head, pre-abdomen or appendages. The present study suggests phoretic relationship between parasitic mites and mosquitoes. Wide occurrence, intensity of infection, parasitic load, and attachment preferences of the mites suggested their positive role in biological control of adult mosquitoes. The present study will set the path of future studies on host–parasite relationships of mites and mosquitoes and define the role of parasitic mites in the biological control of mosquitoes.

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Introduction

Aedes, *Anopheles* and *Culex* species of mosquitoes transmit diseases to humans and animals. They are most prevalent in developing and under developed countries, and spread diseases like malaria, dengue, chikungunya, yellow fever, filaria (Esteva et al., 2007). Despite decreasing incidence of human mortality, mosquito borne diseases are still the cause of serious health issues to over 214 million people (WHO, 2015) in developing and under developed countries.

Parasitic mites are ubiquitous and prevalent in the fresh-water habitats, their population density reaches up to 500 individuals with more than 50 species within 1 m² (Di Sabatinol et al., 2010). They parasitize insects, including mosquitoes and predate upon them. Larval mites of Arrenuridae, Thyasidae, Anystidae, Hydryphantidae (Mullen, 1975; Smith, 1983) are obligate parasites, which ingest hemolymph by piercing exoskeleton of the host (Smith et al., 2009; Gerson et al., 2003). Attached to mosquito pupae as parasite, the larval stages of mites transform to adults upon

ecdysis (Smith and McLever, 1984). In contrast, *Parathyas* larvae attach to their hosts, when host returns to oviposit at the surface of the water (Mullen, 1997). Studies made by Lanciani and Boyt (1977), Lanciani and McLaughlin (1989), Rajendran and Prasad (1992), Nelson (1998), Sarkar et al. (1990), Mathieu et al. (2006), Esteva et al. (2007), Kirkhoff et al. (2013), and Worthen and Turner (2015) have generated significant interest in parasitic mites and their possible role in biological control of insects.

The biphasic (parasitic and predation) life cycle of parasitic mites consists of egg, pre-larva, larva, three nymphal stages and adult stage (Smith, 1988; Esteva et al., 2006). Parasitic mites hatch in the water, and attach to the host during emergence as a phoretic partner (Worthen and Turner, 2015). After completing parasitic phase, larval mites transform into deutonymph and adults, becoming predatory in nature and feeding upon insects and mosquitoes alike. Mites grasp and puncture prey using chelicerae, secrete stylostome to feed on digested tissues (Smith, 1988) much like plant-parasitic nematodes, which make feeding-plugs to suck host contents (Bilgrami and Gaugler, 2004). Mites can also attach to previously uninfected adults through transfers during mating (Hussell et al., 2010). The larval development completes upon dropping of mites by insects, which return to water bodies, leaving scars as indicators of parasitism (Rolf et al., 2000). Mites grow in size

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(80–90 times) during feeding and up to 47 mite's infected one mosquito individual at a time (Mitchell, 1967; Kirkhoff et al., 2013), significantly enough to affect and reduce host diversity and mosquito population in the area.

The contacts between the mosquito and mite are co-incident, except in some cases where chemical or other cues play a role (Mullen, 1997). The larval stages of terrestrial mites (e.g. Erthraeiidae and Trombellidae) affect mosquito populations (Welbourn and Young, 1988; Southcolt, 1992), whereas others e.g. *Charletonia* and *Leptus* parasitize adult mosquitoes during inactive and resting phases (Wohltmann and Wendt, 1966).

The use of chemical pesticides impacts mosquito populations but alongside, it leaves toxic and adverse effects on human and animal populations. Parasitism (Mullen, 1975; Williams and Proctor, 1991; Gerson et al., 2003) and predation (Bilgrami and Tahseen, 1992; Bilgrami, 1994; Bilgrami, 1997a,b) are ecological interactions that may act alone or concomitantly during biological control process of the pests and vectors. Such is the relationship between mosquitoes and aquatic mites (Acari: Hydrachnidia) (Esteve et al., 2006).

A few options such as *Bacillus thuringiensis*, *B. sphericus*, and *Gambusia affinis* are available to biologically control mosquito larvae but none is available to use against adult mosquitoes. The parasitic mites possessing biological control potentials, few studies made on their biology and behavior, and the need of an effective biological control agent to control adult mosquitoes have led us to carry out this study.

The present study was made on the collected individuals of 23 species of mosquitoes in order to determine prevalence, parasitic load, host preference, attachment site preference, host–parasite relationships, and biological control potential of mites against adult mosquitoes.

Materials and methods

Collection of mosquitoes

The Carbon dioxide-baited and Gravid Traps were used to collect male and female mosquitoes from more than 200 sites in the State of Uttar Pradesh, located at 26.8500° N, 80.9100° E in North India. Each trap was set from dusk to dawn, once a week between May 1st and October 30th 2014. The following morning, mosquitoes were collected and mite infested mosquitoes were sorted out based on mosquito species and parasitic mites. Mosquito individuals infected by the mites were stored at –80 °C for further analysis. No animal specimens were exported out of the country for any purpose. During present study, *Toxorhynchites splendens*, *Uranotaenia compestris* and *Coquillettidia* sp., are referred to as “others”, since they were not available in sufficient numbers. They are included in this study for comparison purposes.

Collection of parasitic mites

The parasitic mites carefully separated from mosquitoes, and preserved in the Alcohol–Glycerin–Acetic Acid solution (AGA) (Gibb and Oseto, 2006) for identification. Five to seven mites were mounted in AGA solution on a glass slide, under 12 mm circular cover slip, in such a way that the legs of the mite stayed separated (Smith et al., 2009). Mites were identified by using taxonomic keys provided by Prasad and Cook (1972), Mullen (1974, 1975) and Pesic et al. (2010).

Analysis of host–parasite relationship

The infested mosquito individuals were examined for the intensity of mite infection, type and species of mites, number of mites

attached, and preference for host species, sex and body parts. Mosquito–mite relationship was determined in terms of infection intensity (defined as the number of aquatic mites on a host individual) and the mean infection intensity (defined as the total number of parasitic mites divided by total number of parasitized hosts) (Margolis et al., 1982). Preference of mites for male or female mosquitoes was determined on the basis of the number of individuals parasitized. The attachment sites were grouped into five categories: head, thorax, pre-abdomen (between metathoracic and first abdominal segment), abdomen and appendages (legs and wings) (Kirkhoff et al., 2013).

Statistical analysis

Statistical analysis of the data was performed by using Ky-Plot version 2 (Yoshioga, 2002). Student's 't-test' and Tukey's multiple range test were applied to determine significant differences at $p \leq 0.05$.

Results

A total number of 8954 individuals belonging to six mosquito genera and 23 species i.e., seven species each of *Aedes* and *Culex*, six of *Anopheles* and one each of *Toxorhynchites*, *Coquillettidia* and *Uranotaenia* were collected (Tables 1–4). From the collection, 43.73% mosquito individuals were parasitized by eight species of parasitic mites i.e., *Arrenurus acuminatus*, *Ar. gibberifrons*, *Ar. kenki*, *Ar. danbyensis*, *Ar. madaraszi*, *Parathyas barbiger*, *Leptus* sp., and *Anystis* sp. Fig. 1 shows *Aedes* sp., infected with *Ar. danbyensis*, *Cx. pipiens* infected with *Ar. danbyensis*, and *Coquillettidia* sp. with *Leptus* sp.

Aedes parasitized by parasitic mites

Parathyas barbiger parasitized all species of *Aedes*. *Arrenurus acuminatus* and *Ar. kenki* parasitized *Ae. pallidostriatus* and *Ae. pipersalatus* whereas, *Ar. gibberifrons* infected *Ae. novalbopictus* (Table 1). *Arrenurus danbyensis*, *Ar. madaraszi*, *Leptus* sp., and *Anystis* sp., did not parasitize any individual of *Aedes*.

Parathyas barbiger parasitized maximum number of *Ae. aegypti* (63.13%) with mean infection intensity of 5.59 ($p \leq 0.05$) and parasitic load of 1–21 (Table 1). Mites parasitized fewer individuals of *Ae. albopictus* (11.49%) but the mean infection intensity (4.29) and parasitic load (1–9) was significantly higher than other *Aedes* species. The other *Aedes* species were parasitized between 2.35 and 8.21% of the collected population, with mean infection rate and parasitic load ranging between 1.48–5.7 and 1–10 respectively (Table 1).

Anopheles parasitized by parasitic mites

Arrenurus acuminatus and *Pr. barbiger* parasitized all species of *Anopheles* mosquitoes except *An. thomsoni* (Table 2). The 67.30% of *An. stephensi* were parasitized with mean infection intensity of 7.30 and parasitic load of 1–12 (Table 2). In terms of parasitized individuals, *An. thomsoni* was the second most preferred mosquito species (20.00%) ($p \leq 0.05$), which carried less parasitic load (1–6) and mean infection intensity (3.0) as compared to other species of *Anopheles*. *Arrenurus kenki* was parasitic on *An. thomsoni* and *An. quinquefasciatus*. *Anopheles barbarostris* was least preferred with only 1.25% of its population parasitized at the mean infection intensity of 3.35 and parasitic load of 1–4 (Table 2).

Culex parasitized by parasitic mites

Mites preferred *Culex* species more than others. Seven species of mites parasitized 64.24% of collected individuals of mosquitoes. *Arrenurus kenki* and *Pr. barbiger*, each was parasitic on four species

Table 1
Aedes mosquitoes parasitized by mites.

Mosquito species ^a	<i>Aedes albopictus</i>	<i>Aedes aegypti</i>	<i>Aedes pallidostriatus</i>	<i>Aedes pipersalatus</i>	<i>Aedes novalbopictus</i>	<i>Aedes vittatus</i>	<i>Aedes ramachandara</i>
Number of mosquitoes collected	436	987	543	654	203	565	156
Number of parasitized host	124	523	145	163	41	218	53
Number of mites attached	533	2928	580	340	234	324	109
Mean infection intensity	4.29	5.59	4.0	2.08	5.7	1.48	2.05
Parasitic load	1–9	1–21	1–6	1–3	1–10	1–2	1–3
<i>Arrenurus acuminatus</i>	0	0	113	85	0	0	0
<i>Arrenurus gibberifrons</i>	0	0	0	0	63	0	0
<i>Arrenurus kenki</i>	0	0	86	63	0	0	0
<i>Parathyas barbiger</i>	533	2928	381	192	171	324	109

^a *Arrenurus danbayensis*, *A. madaraszi*, *Leptus* sp. and *Anystis* sp. did not parasitize any mosquito species, hence not included in the table.

Table 2
Anopheles mosquitoes parasitized by mites.

Mosquito species ^a	<i>Anopheles barbarostris</i>	<i>Anopheles thomsoni</i>	<i>Anopheles minimus</i>	<i>Anopheles stephensi</i>	<i>Anopheles quinquefasciatus</i>	<i>Anopheles culicifacies</i>
Number of mosquitoes collected	98	768	298	1267	432	165
Number of parasitized host	14	226	161	543	121	51
Number of mites attached	47	678	916	3974	454	213
Mean infection intensity	3.35	3.0	5.68	7.31	3.75	4.17
Parasitic load	1–4	1–6	1–9	1–12	1–16	1–6
<i>Arrenurus acuminatus</i>	17	0	46	1697	244	164
<i>Arrenurus kenki</i>	0	678	0	0	23	0
<i>Parathyas barbiger</i>	30	0	870	2277	187	49

^a *Arrenurus danbayensis*, *A. gibberifrons*, *A. madaraszi*, *Leptus* sp. and *Anystis* sp. did not parasitize any mosquito species, hence not included in the table.

Table 3
Culex mosquitoes parasitized by mites.

Mosquito species ^a	<i>Culex vishnui</i>	<i>Culex infula</i>	<i>Culex nigropunctatus</i>	<i>Culex pipiens fatigans</i>	<i>Culex malayi</i>	<i>Culex tritaeniorhynchus</i>	<i>Culex bitritaeniorhynchus</i>
Number of mosquitoes collected	301	431	308	786	109	213	64
Number of parasitized host	213	321	175	543	34	106	29
Number of mites attached	468	2562	987	1574	98	371	45
Mean infection intensity	2.19	7.98	5.64	2.89	2.88	3.50	1.55
Parasitic load	1–3	1–27	1–6	1–10	1–6	1–6	1–2
<i>Arrenurus acuminatus</i>	0	0	987	347	8	0	18
<i>Arrenurus danbyensis</i>	0	1824	0	0	0	0	0
<i>Arrenurus madaraszi</i>	0	326	0	0	0	0	0
<i>Arrenurus kenki</i>	468	0	0	517	64	371	0
<i>Parathyas barbiger</i>	0	412	0	391	24	0	27
<i>Leptus</i> sp.	0	0	0	67	2	0	0
<i>Anystis</i> sp.	0	0	0	252	0	0	0

^a *Arrenurus gibberifrons* did not parasitize any mosquito species, hence not included in the table.

Table 4
Other species of mosquitoes parasitized by mites.

Mosquito species ^a	<i>Toxorhynchitis splendens</i>	<i>Uranotaenia compestris</i>	<i>Coquillettidia</i> Spp.
Number of mosquitoes collected	23	65	82
Number of parasitized host	19	29	64
Number of mites attached	45	19	368
Mean infection intensity	1.0	1.6	5.6
Parasitic load	1–2	1–2	1–43
<i>Parathyas barbiger</i>	45	19	368

^a *Arrenurus acuminatus*, *A. danbayensis*, *A. gibberifrons*, *A. madaraszi*, *A. kenki*, *Leptus* sp. and *Anystis* sp., did not parasitize any mosquito species, hence not included in the table.

of *Culex*. *Arrenurus acuminatus*, *Ar. danbyensis*, *Ar. madaraszi* and *Anystis* sp., each parasitized one species of *Culex* mosquito. *Leptus* sp., infected two species of *Culex*, whereas, *Cx. pipiens* was parasitized by four species of mites i.e., *Ar. acuminatus*, *Pr. barbiger*, *Leptus* sp. and *Anystis* sp., (Table 3). *Culex infula* was parasitized maximally (74.0%). The mean infection intensity on *Cx. pipiens* was second to *Cx. nigropunctatus*, where it was highest (7.98) ($p \leq 0.05$) with parasitic load ranging between 1 and 27. Mites preferred individuals of *Cx. tritaeniorhynchus* the least in terms of the number of

host individuals parasitized (31.19%), mean infection rate (1.55) and parasitic load (1–2) (Table 3).

Other mosquitoes parasitized by parasitic mites

Parathyas barbiger parasitized 82.0% of *Tx. splendens*, 44.61% of *Ur. compestris* and 78.04% of *Coquillettidia* sp. (Table 1). The mean infection intensity (5.6) and parasitic load (1–43) was the highest for *Coquillettidia* sp., in this group (Table 4).

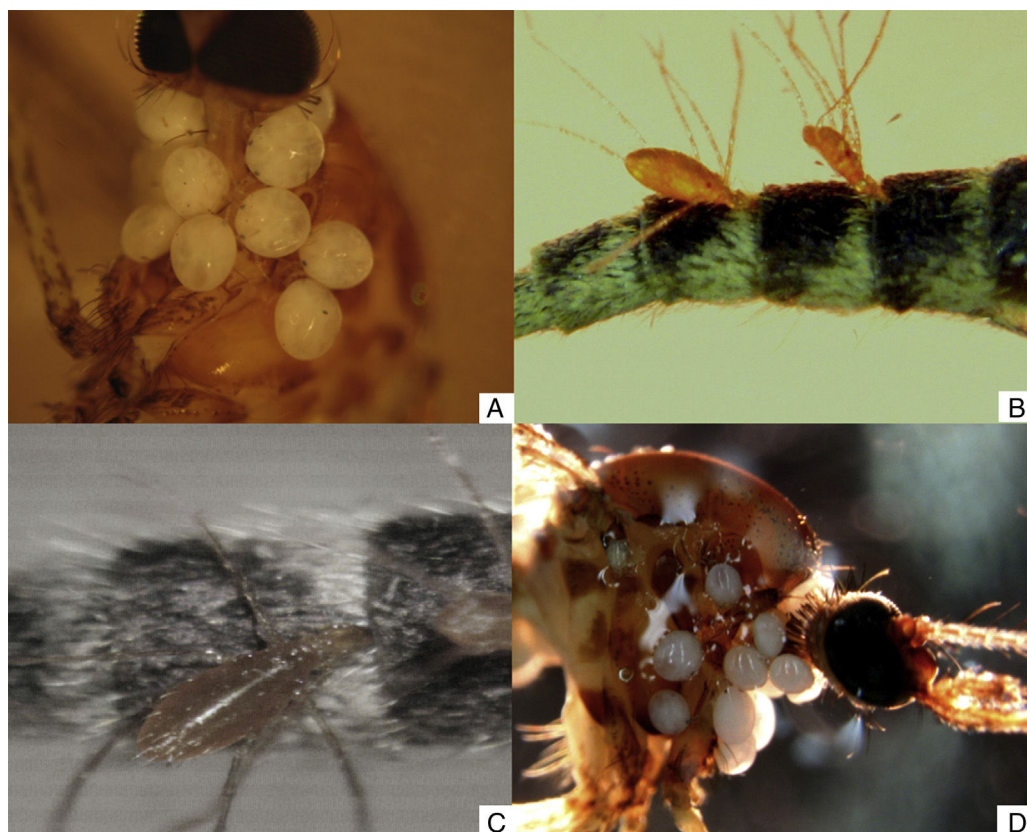


Fig. 1. (A) *Culex pipiens fatigans* infected by *Arrenurus danbyensis*; (B and C) *Coquillettidia* sp. infected with *Leptus* sp.; (D) *Aedes* sp., infected with *Arrenurus danbyensis*.

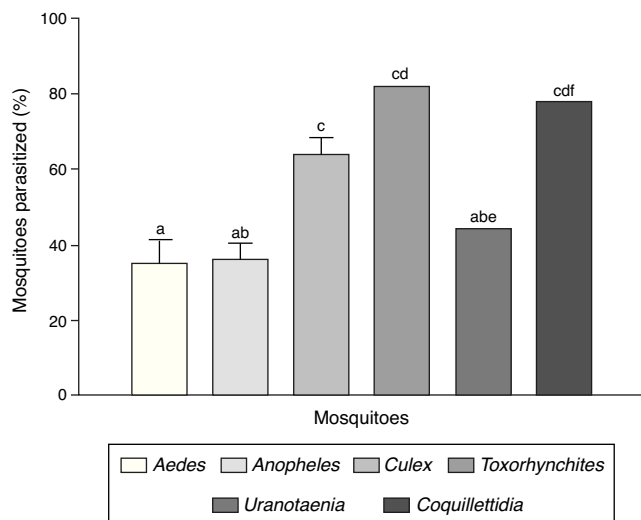


Fig. 2. Mosquitoes parasitized by mites. Tukey's multiple comparison tests were applied at 95% confidence to compare differences. Bars (\pm SE) with different letters show significant differences at $p \leq 0.05$. Bars without SE = no variations.

Preference for mosquito species

Mites parasitized *Culex* (64.02%) in greater numbers than *Aedes* (35.75%), *Anopheles* (36.45%) or *Uranotaenia* (44.0%) ($p \leq 0.05$) (Fig. 2). Mites also infected *Toxorhynchites* (82.0%) at higher numbers ($p \leq 0.05$) (Fig. 2), which may not be true representative of host preference behavior, since observations were based on fewer specimens. They are included in the study for comparison purposes.

Preference of mites for attachment sites

Parasitic mites showed preferential rates of attachment to the host body parts. All species of mites attached maximally to the thorax (41.3–79.8%), as compared to the head (3.9–18.0%), pre-abdomen (4.3–28.6%) or appendages (0.9–4.8) (Fig. 3). The rate of attachment also varied with the species of mites. *Anystis* sp., attached to the thorax (79.8%) maximally as compared to other species of mites ($p \leq 0.05$) (Fig. 3).

Sexual preference for attachment

All species of mites preferred female mosquitoes as the host (Fig. 4). Fewer than 6.5% of males were parasitized by various species of parasitic mites. No males, but all female individuals of *Tx. splendens*, *Ur. compestris* and *Coquillettidia* sp. were parasitized.

Discussion

To the best of our knowledge, this study presents the largest mosquito collection in order to study host–parasite relationships between mosquitoes and parasitic mites after Kirkhoff et al. (2013). It is also the first detailed study made on the host–parasite relationships of parasitic mites with mosquitoes in Asia.

Effective biological control agents possess several attributes of successful biological control agents (Spurrier, 1998; Bilgrami et al., 2005). Parasitic mites also possess beneficial traits such as wide spread occurrence, effective dispersal capability, moderate host preference, host body part preference for attachment, and significant parasitic load to make differences in mosquito populations as biological control agents.

The parasitic mites prefer to stay in the still or slow moving fresh water streams, as indicated by their association with 23 species of

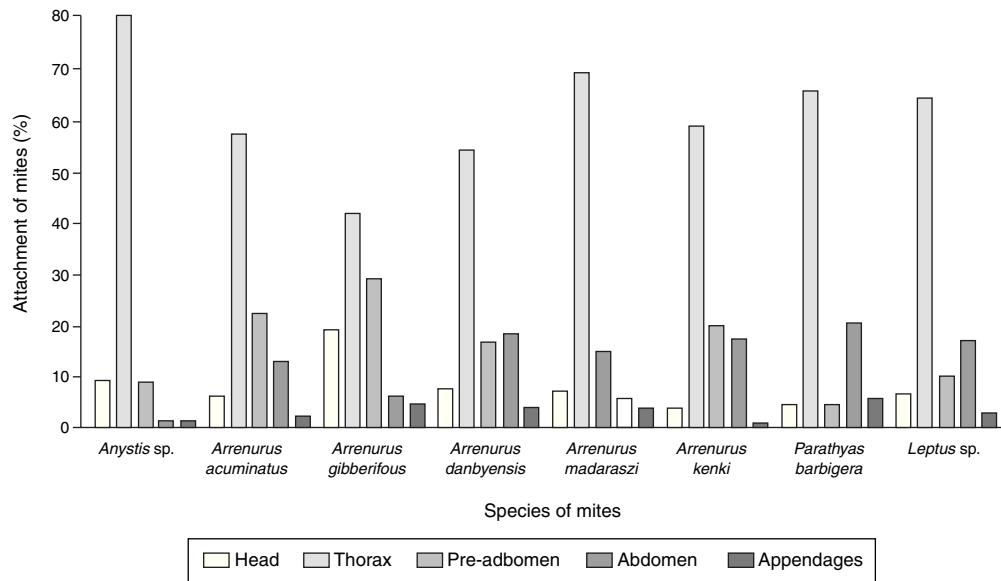


Fig. 3. Attachment preferences of mites for mosquito body parts. Bars show \pm SE, comparisons are made at 95% confidence using ANOVA. Bars without SE = no variations.

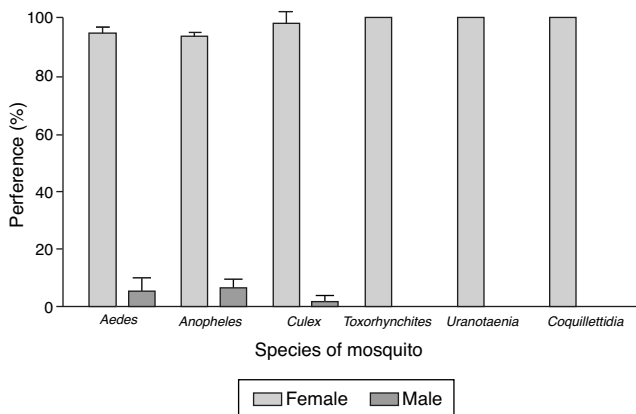


Fig. 4. Attachment preference of mites for mosquito sexes. Bars show \pm SE, comparisons are made at 95% confidence using ANOVA. Bars without SE = no variations.

mosquito. Seven species each of *Aedes* and *Anopheles*, six of *Culex* and one each of *Toxorhynchites*, *Uranotaenia* and *Coquillettida* were parasitized by different species of mites. The present study showed that five species of *Arrenurus* and one each of *Parathyas*, *Leptus* and *Anystis* were parasitic on *Aedes*, *Anopheles*, *Culex*, *Toxorhynchites* and *Uranotaenia*.

The density of mites depended upon the rain, available food, mosquito species and abundance of mosquito individuals. With one peak in the early mosquito season, density of mites remained high and plateau for some time before declining toward the end of the mosquito season (end of November, when low temperatures bring down populations of mites and mosquitoes), a phenomenon that was also observed by Spurrier (1998).

The mosquito life cycle i.e. univoltine or multivoltine may have played a role in host selection by the mites (Milne et al., 2008). In all likelihood, the rate of attachment of mites to emerging multivoltine mosquitoes with higher rates of fecundity should also be high. Mosquito females would return to oviposition sites multiple times in a single season, resulting in the increase of mosquito density many folds as compared to univoltine or other multivoltine species, which have low rates of fecundity. Therefore, mosquito density made differences in the host selection, parasitism and rate of attachment of mites. Host preference by parasitic mites

depended upon host species, number of parasitized individuals, mean infection intensity and parasitic load of mites as evident during the present study. Such preferences explain adaptive mechanisms that allow larval mites to co-evolve successfully and parallel to their mosquito hosts (Martins, 2004). *Anystis* sp., terrestrial in nature, was found attached to the individuals of *Cx. pipiens* and *Cx. malayi*, and possibly transferred by males to females during mating (Hussell et al., 2010).

Parasitic mites hatch in water, float at the surface, climb and attach to the pupae (Worthen and Turner, 2015), they wait until pupae hatch and young adult mosquito emerges. The mites use their pedipalps to attach to mosquito individuals at the time of later's emergence. During or shortly after emergence, mosquito individuals have short spans of inactivity, sufficient for parasitic mites to attach to the host. Parasitic mites use chelicerae to cut host cuticle, whereas, some species of *Arrenurus* secrete adhesive secretions to construct a feeding tube (Martins, 2004).

Thorax, that emerges immediately after the head provides mites the maximum opportunity to attach (Smith, 1988; Kirkhoff et al., 2013). Should the sequence of emerging mosquito body parts play any role in preference of host body parts then the head should have the highest number of mites attached, nonetheless mites preferred mosquito thorax over other body parts. This has resulted due to prolonged exposure of thorax at the time of mosquito emergence at the water surface that has provided extended time to mites to attach. Similar to other organisms, physio-chemical factors such as texture of attachment site or chemicals eliciting mite's responses toward specific body parts also play an important role in differential rates of attachments. As is the case with other insects (Mitchell, 1967), timing of larval mite attachment to the adult host also play a significant role in determining where mite should attach to the host. Our findings are comparable to those of Sharma and Prasad (1998), who have observed 85% of parasitic mites preferring thorax, Milne et al. (2008), however, reported mite's preference for the host abdomen.

The parasitic load of mites varied from 1.0 to 47/individual and the infection intensity from 1 to 7.98. In the present study, some mosquito species with moderate to heavy parasitic loads suggest that attached mites could hinder flight and dispersal of mosquitoes due to their weight, allowing mites to feed on the host and cause mortality. During the present study, no specific factor other than increased chances of contact between mite and host appeared to

have played any role in the attachment process. Female mosquito individuals returning to habitat multiple times to oviposit have increased chances of mites to attach to female individuals, resulting in the attachment ratio of females to male as 95:5.

Arrenurus danbyensis could be a potential candidate of biological control, which have shown detrimental effects on *Cq. perturbans* and *Aedes* spp. (Smith and McLever, 1984). Our study also showed similar phenomenon, where *Aedes*, *Culex* and *Anopheles* species were parasitized by different species of mites. *Arrenurus* species have extreme detrimental effects in terms of its diversity, density and wide geographical presence as compared to other species of mites, *Parathyas barbiger* also showed similar promise, when it comes to the rates of infection intensity and parasitic loads at which they were attached.

In conclusion, parasitic mites i.e., *Arrenurus*, *Parathyas*, *Anys-tis*, and *Leptus* showing beneficial traits of biological control agent e.g. wide host range, population abundance, high rates of attachment, infection and infection intensities, suggest that they may play a significant role in mosquito biological control (Mitchell, 1967; Smith and McLever, 1984). How effective parasitic mites be in the adult mosquito depends on future studies investigating detrimental effects of mites, and studies on population dynamics, reproductive rates, dispersal capabilities, host specificity and distribution of mites with particular reference to adult mosquito population.

Conflicts of interest

The authors declare no conflict of interest.

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