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First Report of a Biting Midge Culicoides Anophelis Parasitzing Mosquito *Anopheles stephensi* from North-Western India

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ABSTRACT: *Culicoides*, commonly called biting midges, are haematophagus ectoparasites of insects, humans and other animals of importance to man. The present study deals with the first report and description of one such ectoparasitic ceratopogonid *Culicoides* (*Trithicoides*) *anophelis* Edwards which was found parasitizing on a malaria vector *Anopheles* (*Cellia*) *stephensi* Liston from North-western India. The parasite having an alar expanse of 2.6 mm only was identified by the standard morphotaxonomic characters such as wing-venation, tibial comb, spermatheca, mandibles and the ratio of the head, antenna and palps. This ectoparasite was found clinging to the abdomen of the host (when collected from cattle sheds) with its proboscis completely penetrating into the abdominal cavity of the host, firmly remaining in that position even during the flight of the mosquito. What was interesting in this case was the effect of its parasitization on the biology of the host. The mosquito females infested with this ectoparasite failed to lay eggs and did not survive for long even under standard insectory conditions. Based on the infestation rate of as high as 72.72% combined with a significant reduction in fecundity and mortality of the mosquito there is ample scope for this parasite as a potential source of host diminution.

Key words: *Culicoides anophelis*, Ectoparasite, *Anopheles stephensi*, Biological control, Biting midge, Mosquito

Introduction

The ceratopogonids, commonly known as biting midges, belong to suborder Nematocera of the superfamily *Chironomidae* of order Diptera. Some of the species are important vectors of viral diseases of humans and livestock [1, 2]. However, members of this group of flies act as important pollinators of plants like cocoa and rubber while the larvae of many are predators of other aquatic organisms [2]. The adults of some biting midges also suck haemolymph from other insect hosts and act as sources of viral infections which kill the larvae of the host. Most of the studies carried out so far on these insects involved the description of medically important ceratopogonid species in which the culicoids were considered to be more important due to their parasitic behaviour. Presented here is the first report of a ceratopogonid dipteran *Culicoides* (*Trithicoides*) *anophelis* Edwards parasitizing on another dipteran *Anopheles* (*Cellia*) *stephensi* Liston from North-western region of India. *An. stephensi* is an important vector of malaria in the Oriental region; therefore the effects of this parasite on host fecundity and survival value though of preliminary nature have significance in the future biological control of mosquitoes.

Materials and Methods

The adult females of *Anopheles* (*Cellia*) *stephensi* Liston infested with the parasite, were procured during early morning field collections along with other mosquito species from the cattle sheds in the village Beladhyani on the outskirts of the township of Nangal, Punjab, India located 110 Km North-west of Chandigarh (30^{o} - 32”N, 76^{o} - 40”E). The identification of the species was carried out by following the standard keys of Wattal and Kalra [3] and from the species specific features of the larval salivary polytene X-chromosome and the autosomes [4]. The adults of *Culicoides* (*Trithicoides*) *anophelis* Edwards were seen clinging to the underside of the females of *An. stephensi* for feeding on the abdominal fluids of the host (Fig. 3). After they got detached by themselves they were processed for the study of their detailed morphological characters.

The live adults of *Culicoides* (*Trithicoides*) *anophelis* were identified by examining them under suitable magnification of the microscope after which they were slide mounted by following the standard procedure of Wirth and Marston [5]. The detailed morphotaxonomic characters were studied by following the keys of Wirth and Hubert [6] meant for South-east Asian fauna of ceratopogonids. The relationship of parasitoid species specificity with host mortality was studied by nonparametric correlation with Kendall's and Spearman's procedure performed in SPSS 15.0.

Results

C. anophelis is a tiny dipteran with an alar expanse of only 2.6 mm having one or two radial veins whose branches reach the wing margin and two branched median veins. The postnotum lacks a median longitudinal groove while the antenna usually has 13 flagellomeres. The females of the species have biting mouthparts with serrated mandibles. In addition to these characters it can also be identified by the presence of large and curved mandibular teeth numbering 15-16, in which the proximal ones are the largest, similar to those of the other mosquito parasites such as *baisasi* and *culiciphagus*. The spermathecae are similar to the members belonging to Flavescens group while the bifid tarsal claws of the present species are present on all the pairs of legs in both the sexes. (Fig.1-11):

Morphotaxonomic description

Wing: Alar expanse 2.6 mm, wing length 1.01 (0.93-1.09, n = 8) mm. Wing pattern with dark streaks along veins and moderately pale area in cells. There are two large and pale yellow spots, one centering on r-m crossvein while other on the apex of second radial cell. Apex of wing narrowly pale with costal ratio of 0.69 (0.67-0.71, n = 8). Halteres are infuscate (Fig.4).

Head: Antenna with length of flagellar segments in proportion of 21-15-15-16-17-17-18-19-24-22-26-30-43 (Fig.6), antennal ratio 1.00 (0.91-1.10, n = 8); sensilla coeloconica present on antennal segments 3, 11-15. Palpal segments with length in proportion of 10-17-22-10-11, second and third segments short and stout. Third segment with sensilla scattered on surface of distal half of segment, palpal ratio 2.1 (1.6-2.3, n = 8) (Fig.11). Proboscis short, P/H Ratio 0.38, clypeus greatly enlarged; mandibles with 15 (12-19, n = 8) curved teeth, proximal teeth largest (Fig.11).

Thorax: Mesonotum yellowish brown, dark brown on anterior fourth; scutellum, postscutellum and lower pleuron dark brown (Fig.2). Legs pale brown, fore and mid legs with pale joints, broad apical pale bands on femora and basal pale bands on tibiae, hind leg with dark joints, broad subapical pale band, sometimes indistinct in femur, tibia with base and apex broadly pale, hind tibial comb with 4 spines (Fig.7), second from the spur being longest, tarsal claws divided at the tip on all the legs (Fig.6).

Abdomen: Dark brown, terga poorly sclerotized. Spermathecae 3, slightly unequal, measuring 0.039 x 0.033 mm, 0.040 x 0.033 mm, and 0.038 x 0.031 mm; sub-spherical to slightly ovoid, necks slender and sclerotized for a short distance (Fig.6).

Parasitic effects of *C. anophelis* on An. stephensi:

Species specificity: Out of the four species of An. stephensi, An. culicifacies, An. annularis and An. maculatus collected only An. stephensi was found to be infested with the parasite.

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Infestation rate with parasite: Out of a thirty three mosquito females collected eleven were identified to be Anopheles stephensi of which only eight were found to be infested with a percentage frequency of 72.72%.

Effect on fecundity: None of the females infested with the parasite (eight) were able to lay eggs thus the reduction in fecundity of mosquito females on infestation was 100%. When the mortality rate due to infestation was calculated it was found to be 75%, Six out of eight females dying due to manifestations of infestation.

Statistical analysis:

There was significant negative correlation between infestation rate and loss of fecundity as r = -1.000 at p value of 0.000, where n=33. The relationship between parasitoid infestation and host mortality also had significant negative correlation as r = - 606 at p value of 0.001, where n=33 based on Kendall's and Spearman's methods, the values obtained were same for both the tests.

Fig. 1: ***Anopheles stephensi*** **Liston (Host) ; Fig. 2:** ***Culicoides anophelis*** **adult female (Parasite) ; Fig. 3:** ***Anopheles spp.*** **carrying** ***Culicoides anophelis*** **attached to its abdomen; Fig. 4: Wing venation of** ***C. anophelis*** **(R-M); Fig. 5: Hind tibial comb (TC) of spines and tibial spur(S); Fig. 6: Bifid tarsal claws (TCL); Fig. 7: Three spermathecae (Sp); Fig. 8: Head with compound eyes (CE) and other mouth parts; Fig. 9: Antennae (A); Fig. 10: Palpi with sensory pit (SP); Fig. 11: Mandibles with mandibular teeth (MT)**

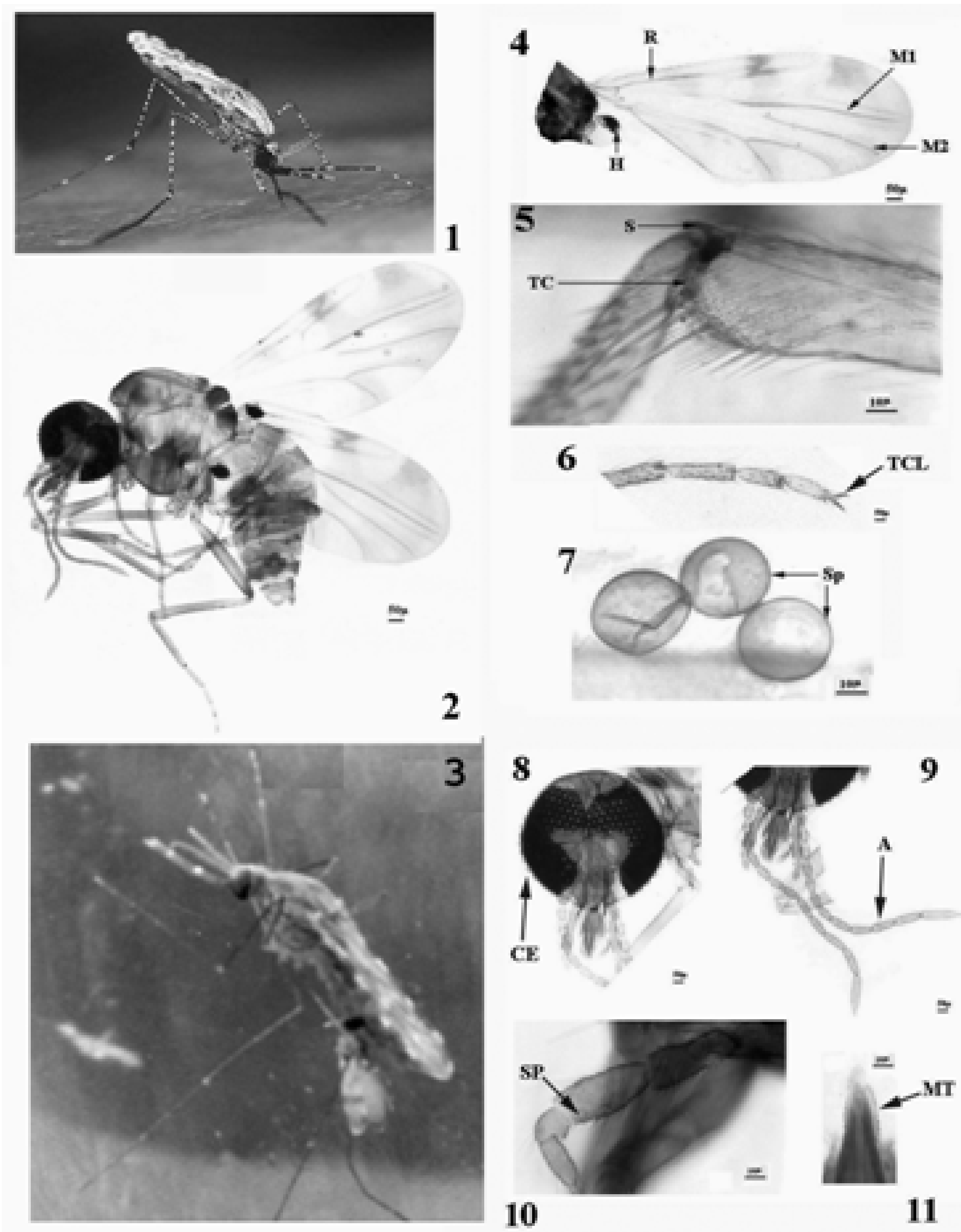

Table 1: Biological effects of parasitization of *Culicoides anophelis* on *Anopheles stephensi*

S. No	Effects of parasitization			
1	Species collected	*An. stephensi*, *An. culicifacies*, *An. annularis* and *An. maculatus*.		
2	Infestation rate	Eleven out of 33 *Anopheles* females were *Anopheles stephensi*	Eight out of eleven *Anopheles stephensi* females infested	72.72%
3	Effect on fecundity	Nil out of infested eight females laid eggs	All three non-infested females laid eggs	100% reduction in fecundity
4	Mortality rate due to parasitization	6 died	2 survived	75% mortality

Discussion

C. anophelis is the type form of the Anophelis group in the subgenus *Trithicoides* which was collected from a locality in north-western part of India. Some earlier references had reported its prevalence from the eastern part of India approx. 1700 km away from the present site [6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17].

Wirth and Hubert [6] collected it from the cattle shelters where it was found parasitizing on blood-fed mosquitoes. Sen and Das Gupta [16, 18] were of the opinion that the prolonged periods of its attachment to the abdomen of the host species ranged from 36-48 hours to complete the process of feeding. Das Gupta [14] carefully examined the well fed specimens of the species and reported that the guts of *C. anophelis*, which had fed on engorged mosquitoes, contained a pale yellow fluid that responded negatively to human and cattle antisera. This was indicative of the fact that this ectoparasitic activity was meant for feeding on the haemolymph of the host instead of stomach contents. However, the blood in the gut of the mosquito gave a positive test for cattle blood but negative for human antisera. Chastel et al. [19] also got similar results in their observations on *Aedes* mosquito.

C. anophelis is relatively species specific in its parasitic habit as out of the four species viz. *An. stephensi*, *An. culicifacies*, *An. annularis* and *An. maculatus* it was found attached to the underside of the abdomen of only *Anopheles stephensi*. Though it has been reported to parasitize other mosquitoes as well but presence of sibling/cryptic species so common in other related insect genera can not be disregarded. Its total feeding time ranged from 48-56 hours with an infestation rate as high as 72.72%. This resulted in a significant reduction in fecundity and increased mortality of the host species most important criterion for any biological control agent.

In addition to the earlier morphotaxonomic studies carried out by Patton [20], Mukerji [21, 22], Iyenger [23], Jamnback [24] and Wirth and Navai [25] the work on them has been extended further to include the valuable information about their geographical distribution, seasonal activity, PCR based molecular phylogenetics and rDNA analysis [14, 26, 27, 28, 29, 30]. However, none of these studies were strictly aimed at evaluating their effect on the rate of survival and fecundity of the host species. As for the biological control of mosquitoes a number of larval and adult stages of animals have been discovered and used for the control of vector species of mosquitoes. Consequently some of the cost effective natural enemies of mosquitoes such as fishes, nematodes, bacteria and fungi etc. have been successfully used, however, except for some success in the use of fish species, other agents could not be adopted for mass production as potential sources for field applications. Therefore, ample opportunities exist for exploiting these ectoparasites for the effective control of mosquito vectors of diseases. In the light of these efforts the present Culicoides species holds sufficient promise as an agent for limiting the reproductive potential of host species.

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