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Confirmation of *Culex (Culex) tritaeniorhynchus summorosus* (Diptera: Culicidae) as a separate species

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ABSTRACT

Background & objectives: *Culex tritaeniorhynchus*, a member of *Cx. vishnui* subgroup, is an important vector of Japanese encephalitis (JE) virus. *Cx. tritaeniorhynchus summorosus* considered as a variety or subspecies of *Cx. tritaeniorhynchus,* has been studied in detail to settle its taxonomic status. Surveys for the collection of *Cx. tritaeniorhynchus* from Chandigarh and adjoining areas have established the availability of *Cx. summorosus* from this area.

Methods: For the present investigation, collections have been made from Chandigarh and its adjoining areas (up to 60 km) for procuring the material. The detailed morphology including scanning electron microscopy of immatures (eggs and larvae) and adults of *Cx. tritaeniorhynchus* and *Cx. summorosus* has been studied and compared. Further, the interbreeding experiments of the two species were also conducted and efforts had been made to allow cross-breeding among the members of these two species.

Results: Comparison of egg, larval and adult morphology of *Cx. summorosus* with the parental species *Cx. tritaeniorhynchus* under the light and electron microscope, revealed significant differences. Moreover, these two species have also been found to be reproductively isolated as indicated by laboratory experiments. This settles the controversy on the status of *Cx. summorosus* and confirms its status as a distinct species.

Interpretation & conclusion: The study establishes that the two species show considerable number of differences which are sufficient to consider them as separate species rather than subspecies or variant of *Cx. tritaeniorhynchus*. Furthermore, the absence of interbreeding between these two again confirms their separate specific status according to biological species concept. But, it is yet to ascertain whether *Cx. summorosus* is a vector of Japanese encephalitis like *Cx. tritaeniorhynchus* or not.

Key words Comparative morphology; *Culex* (*Culex*) *summorosus; Culex* (*Culex*) *tritaeniorhynchus*; eggs; larva

INTRODUCTION

Occurrence of widespread variations in the species under genus *Culex* has led various workers to recognize subgenera, groups, subgroups and complexes in order to facilitate the allocation of different species, although these additional categories are not mentioned in their nomenclature. The subgenus *Culex* has thus been divided into two groups, namely *sitiens* and *pipiens*[1]. The group *sitiens* is further subdivided into five subgroups including the important *vishnui* and *sitiens* subgroups. The subgroup *vishnui* has three complexes namely *tritaeniorhynchus, vishnui* and *whitei*[2].

Cx. tritaeniorhynchus, the major vector of deadly viral disease Japanese encephalitis belongs to *tritaeniorhynchus* complex. *Cx. summorosus* which has been reportedly referred to as a variety of *Cx. tritaeniorhynchus*[3] or as the subspecies of *Cx. tritaeniorhynchus*[2, 4] is also therefore, supposed to be a vector of the same disease Japanese encephalitis, although specific comments on its vectorial status have not been made by any of the workers. Here, in these studies, the taxonomic status of *summorosus* has been confirmed but the conditions of its being a vector are yet to be verified. It may be mentioned that *Cx. tritaeniorhynchus* is widely distributed in different parts of Southeast Asia including India and its populations show a number of morphological variations. One of the notable variant was named as var. *siamensis*[3]. This variant was later recognized as a subspecies, *i.e. Cx. (Cx.) tritaeniorhynchus summorosus*[2,4] or as a variant[5] although, previously, Dyar[6] and Bram[7] had suggested specific status for the same. In the light of controversy on the true status of *summorosus*, detailed differences in the egg, larval and adult morphology of *summorosus* and *tritaeniorhynchus* have been studied and properly illustrated which confirm the specific status of *summorosus.* The laboratory experiments on the reproductive behavior of the two species have also revealed complete reproductive isolation between them, giving further support to the species status of *summorosus*. This species has also been described in detail by including the missing features in the old description.

MATERIAL & METHODS

Collection and rearing

For the present investigations, surveys were conducted from Chandigarh (30.79°N, 76.78°E), and some of the adjoining cities of Punjab and Haryana like Sirhind (District Fatehgarh Sahib, 30.38° N, 76.23° E), Khanna (District Ludhiana, 30.91° N, 75.85° E) and Ambala (Haryana, 30.38° N, 76.78° E) respectively, India. The immature stages, *i.e.* eggs, larvae and pupae were collected in plastic bowls from ponds, ditches, pools, *etc*. The larvae of different instars of *Culex* were segregated into different bowls and were fed with a mixture of yeast powder and finely crushed dog biscuits, prepared in the ratio of 2:3. The bowls containing eggs and different larval instars were kept in a biological oxygen demand (BOD; 28°C±1 and 70% RH) for further development in the laboratory. The adults were collected either with hand nets or aspirators from various resting places (cattlesheds, human dwellings, mixed dwellings, *etc*.) and breeding places (ponds, pools, puddles, submerged water plants and vegetation around water bodies).

While collecting larvae from different places, the authors have come across some specimens having relatively longer siphon which according to Colless[4] is a sure identification mark for larvae of *Cx. tritaeniorhynchus summorosus.* Such larvae were reared in laboratory and resulting adults were separated for further study, which were proved to be the adults of *Cx. tritaeniorhynchus summorosus.* Along with *summorosus* a number of larvae of *Cx. tritaeniorhynchus* were also bred in laoratory. Having a good collection of larvae and adults of *Cx. tritaeniorhynchus* and *Cx. tritaeniorhynchus summorosus,* detailed studies were made on larvae and adults of two species. The differences were rather of high order and had not been studied earlier by any worker. The noted differences prompted authors to declare *summorosus* as distinct species.

The adult morphology of *Cx. tritaeniorhynchus* has already been given by Sirivanakarn[2], Harbach[8], Reuben *et al*[9] and of *Cx. summorosus* has been given by Dyar[6] and Colless[4]. Accordingly, the noted difference in the morphology of the adults and the larvae were highlighted and listed in order to prove specific status of *Cx. summorosus*.

Interbreeding experiments

On noticing a good number of differences in the morphology of adult including male genitalia, larva and egg of *tritaeniorhynchus* and *summorosus*, their ability to interbreed was tested in the laboratory to strengthen any decision taken on the status of *summorosus* IV instar larvae of both the species were collected from the field and were individually reared in separate bowls in laboratory to procure freshly emerged adults. These freshly emerged adults were used for reproduction trials among the two species. Four sets of experiments were designed.

Experiment I: 10 pairs of males and females of *Cx. tritaeniorhynchus* were kept in a cage to allow mating within the individuals of same species.

Experiment II: 10 pairs of males and females of *Cx. summorosus* were kept in a cage again to allow mating within the individuals of same species.

Experiment III: 10 males of *Cx. tritaeniorhynchus* were kept with 10 females of *Cx. summorosus* to allow cross breeding among the members of the two species.

Experiment IV: 10 males of *Cx. summorosus* were kept with 10 females of *Cx. tritaeniorhynchus* again to allow the cross breeding among them.

All the cages containing these sets were kept in BOD (28ºC±1 and 70% RH). Readings were taken every day during morning and evening hours to note the number of fertilized females as indicated by the swollen abdomens of the females which is due to the accumulation of fertilized eggs.

RESULTS

Taxonomic observations

The differences in the morphology of two species are recorded in Table 1; and Figs. 1 and 2.

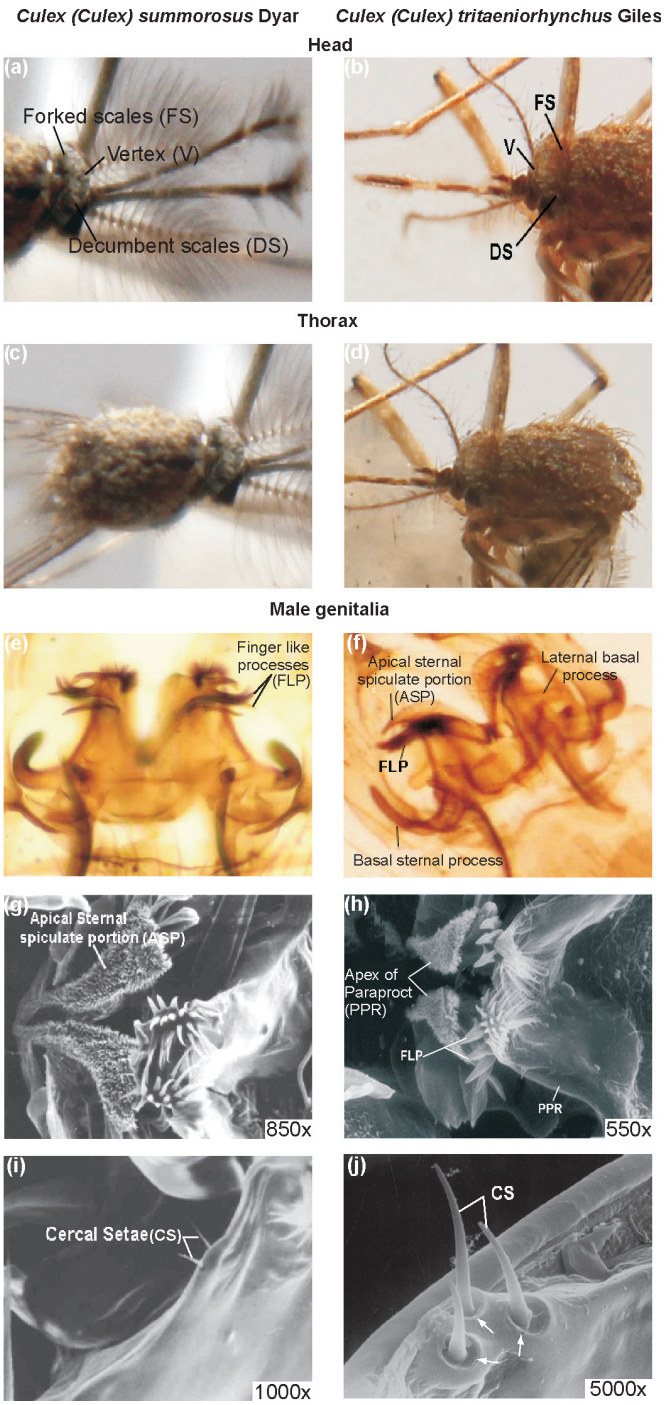
Table 1. Differences between *Cx. summorosus* and *Cx. tritaeniorhynchus*

S. No.	*Culex summorosus*	*Culex tritaeniorhynchus*
	Adult	
Head		
(1)	Narrow decumbent scales dark brown or black (Fig. 1a).	Narrow decumbent scales (DS) on head pale, white or golden (Fig 1b).
Thorax		
(1)	Mesonotal integument dark brown or black (Fig. 1c)	Mesonotal integument brown coloured (Fig. 1d).
(2)	Scutellar integument dark brown or black, exactly like mesonotal integument.	Scutellum lighter than mesonotal integument.
(3)	Scutellum with eight long setae on median lobe and seven long setae on each lateral lobe.	Scutellum with six bristles on median lobe and four on each lateral lobe.
(4)	Pleura, mainly mesoanepisternum and mesoketepimeron totally black.	Pleura lighter than mesonotum.

(Contd...)

Table 1. (Contd...)

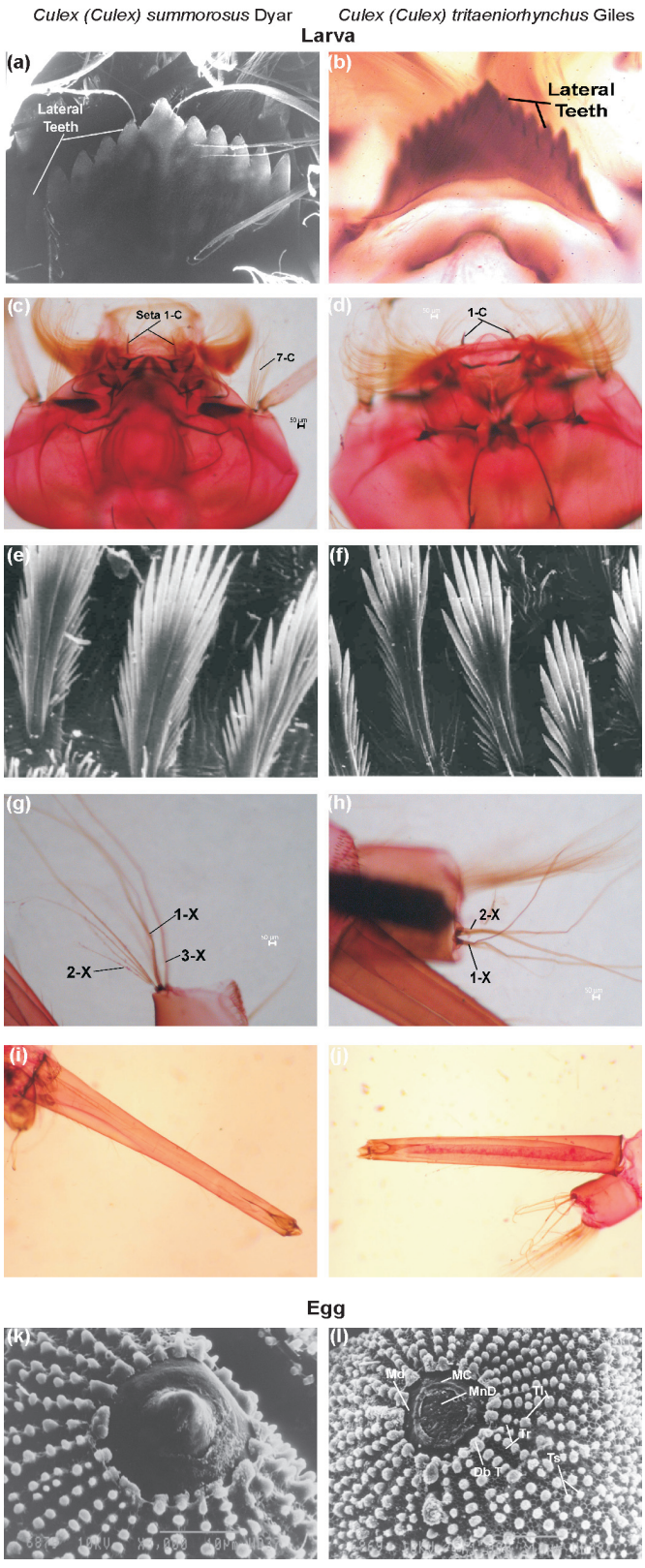
S. No.	*Culex summorosus*	*Culex tritaeniorhynchus*
(5)	Antepronotum with 7–8 long brown setae.	Antepronotum with few pale scales and 3–4 long yellowish setae.
(6)	Post-pronotum with 9–10 long and strong dark brown setae.	Post-pronotum with three weak yellowish setae.
	Female genitalia	
(1)	Upper vaginal lip with 2–4 setae.	Upper vaginal lip with 5–6 strong setae.
	Male genitalia	
(1)	Sensilla *g* on subapical lobe of gonocoxite more flattened and fan shaped.	Sensilla *g* on subapical lobe of gonocoxite leaf shaped.
(2)	Finger like processes (FLP) on the lateral plate of phallosome comparatively longer with an average length of 1.02, 0.84, 0.70 and 0.60 mm of 1st, 2nd, 3rd and 4th process respectively (Fig. 1e).	FLP on the lateral plate of phallosome comparatively shorter with an average length of 0.86, 0.69, 0.54 and 0.34 mm of 1st, 2nd, 3rd and 4th process respectively (Fig. 1f).
(3)	Apex of paraproct long (Fig. 1g).	Apex of paraproct short (Fig. 1h).
(4)	Paraproct with two cercal setae (Fig. 1i).	Paraproct with three cercal setae (Fig. 1j).
	Larva	
(1)	Mental plate with five lateral teeth on either side of median tooth (Fig. 2a).	Mental plate with six lateral teeth on either side of median tooth (Fig. 2b).
(2)	Seta 1-C long with average length of 0.19 ± 0.02 mm (Fig. 2c).	Seta 1-C short with average length of 0.10 ± 0.009 mm (Fig. 2d).
(3)	Seta 7-C with 9–10 branches	Seta 7-C with 6–7 branches
(4)	Comb scales more broad and fan shaped, and with comparatively more number of rays at the apex (Fig. 2e).	Comb scales elongated with comparatively fewer number of rays at its apex (Fig. 2f).
(5)	Seta 2-X on saddle with four branches (Fig. 2g).	Seta 2-X on saddle double (Fig. 2h).
(6)	Respiratory siphon long with average length of 1.8 mm (Fig. 2i).	Respiratory siphon short with average length of 1.3 mm (Fig. 2j).
	Egg	
(1)	Micropylar mound evaginated outwards to form a conical structure (Fig. 2k).	Micropylar mound flat, not protruding outwards (Fig. 2l).



Figs. 1(a–j): Morphological differences between the adults of *Culex summorosus* and *Cx. tritaeniorhynchus;* (a) & (b)–Head (Narrow decumbent scales); (c) & (d)– Thorax (Dorsal view); (e) & (f)–Phallosome; (g) & (h)– Apex of paraproct; and (i) & (j)–Paraproct with cercal setae.

Results of interbreeding experiments

The above mentioned taxonomic observations have been further supported by the breeding experiments conducted between two species which clearly indicate them as separate species. Critical examination of these experimental set-ups revealed that in case of *Cx.*



Figs. 2 (a–l): Morphological differences between the larva and egg of *Culex summorosus* and *Cx. tritaeniorhynchus*; (a) & (b)–Mental plates; (c) & (d)–Head with seta 1-C; (e) & (f)–Comb scales (3000x); (g) & (h)–Setae 1-X and 2-X on saddle; (i) & (j)–Respiratory siphon (20x); and (k) & (l)–Mycropylar region of egg.

tritaeniorhynchus, 70% of the females were found gravid where as in *Cx. summorosus* 50% females were gravid. But in experiments III and IV, where interbreeding among two was allowed, none of the females was found gravid. These experiments were repeated twice during two consecutive seasons and nearly same results were obtained. The results of these experiments clearly indicate the absence of interbreeding between the individuals of these two species which confirms the biological species concept according to which the members of two different species never interbreed. Hence, in the view of the results of the experiments, it becomes very clear that these two are separate species instead of the variant or the subspecies of *Cx. tritaeniorhynchus.*

DISCUSSION

It may be mentioned that the unusual larval feature noted by Colless[4] and the observations of Dyar[6] had already indicated the separate status of *summorosus* as different species. Although Colless[4], Bram[7] and Sirivanakarn[2] also agreed with this conclusion but they preferred to call it subspecies. Colless[4] gave the subspecies status to *summorosus* on the basis of sharp cleavage of species into eastern and western forms. He further explained these two forms on the basis of finger like processes on the lateral plate of phallosome which were weakly developed in western forms (India) whereas, more strong and larger in size in eastern forms (Malaysia and Japan). Similar observations have also been reported pertaining to finger like processes of lateral plate of phallosome in the male genitalia of these species collected from Japan, Los Banos, Luzon, and Philippines[10]. These were further categorized into three types, *i.e.* A, B and C based upon observations in regard to their immature stages, cytogenetic and biochemical studies as well as reproductive behaviour. Earlier, Barraud[11-12] also mentioned *Cx. tritaeniorhynchus* in fauna of British India but, in his description, the larva resembles with *Cx. summorosus* rather than *Cx. tritaeniorhynchus.* It further indicates the presence of both the species in India. While, the variants of *Cx. tritaeniorhynchus* collected from five different geographical locations of Bellary district, Mysore and Mandya district in Karnataka (India) did not show any genetic differentiation during molecular characterization[13].

However, in the present investigations the authors have collected both the species from the same region, which confirms their status from subspecies to species level, as two subspecies of the same species never coexist. Further, the loss of interbreeding among the individuals of these

two species again confirms to the biological species concept according to which, the members of two different species never interbreed. Hence, it becomes apparent that these two are separate species instead of the variant or the subspecies of *Cx. tritaeniorhynchus.*

In spite of confirmation of *Cx. summorosus* as species distinct from *Cx. tritaeniorhynchus*, information on the vectorial potential of the former will be a useful contribution for the health workers.

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