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Comparative studies on *Culex bitaeniorhynchus* Giles (1901) and its *tenax* variant (Diptera: Culicidae) in Chandigarh, India

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ABSTRACT: During present investigations the detailed morphotaxonomic studies of *Culex bitaeniorhychus* revealed that it exists in two forms *i.e typical* and *tenax* in and around Chandigarh. Various intraspecific variations in the morphology and male genitalia were observed in both these forms. The main aim of this study is to distinguish these two forms of *Cx. bitaeniorhynchus* and their separation from other closely related species. The intraspecific variants of these forms have been studied further with respect to phallosome regions of the male genitalia. For assessing the significant differences among their phenotypic characteristics one–way ANOVA was done along with pair-wise comparisons of samples means. Some of the earlier workers have considered *tenax* form, a synonym of *Cx. infula* which is another closely related species of *Cx. bitaeniorhynchus.* But, on the basis of remarkable differences observed between *tenax* and *infula*, it is suggested that these two are separate taxons and should not be synonymised.

KEY WORDS: *Culex bitaeniorhynchus*, *Cx. infula, typical*, intraspecific variation

INTRODUCTION

Culex (Oculeomyia) bitaeniorhynchus Giles (1901) complex has six closely related species viz; *Culex bitaeniorhynchus*, *Cx. infula*, *Cx. longicornis*, *Cx. luzonensis*, *Cx. pseudosinensis* and *Cx. selangorensis*. Several species of this subgroup are widely distributed from Southeast to Southwest Asian countries like Africa, Egypt, Japan, Korea and Eastern Palearctic regions (Tanaka, 1979; Sirivanakarn, 1976; Harbach 1988; Reuben 1994; Tanaka, 2004), and in Indian subcontinents, particularly India, Bangladesh, Nepal, and Pakistan. *Culex bitaeniorhynchus* is well known as a rural species which breed in marshes, puddles, rice fields and the habitats rich in water containing Spirogyra, a filamentous green-algae (Harbach, 1988; Sirivanakarn, 1976). It has a major role in spreading various arboviral diseases like Japanese encephalitis, filariasis in tropical and subtropical areas of Asia (Iyengar 1938; Carter 1948; Benerjee et al. 1975, 1978; Reuben et al. 1994). *Cx. bitaeniorhynchus* is a highly plastic species which shows intraspecific variations in both of its morphology and male genitalia. These variations have been observed with respect to erect scales on center of vertex of head, thorax, legs, wings, apical bands of abdominal terga III–IV and inner

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and outer division of phallosome region of male genitalia. Previous researchers have reported considerable variations in *Cx. bitaeniorhynchus* and proposed these variations as subspecies, varieties or forms of *Cx. bitaeniorhynchus* (Edwards, 1922; Barraud, 1934; Bram, 1967; Sirivanakarn, 1973). This species group also includes many binomial names and hence due to the complexity of various synonyms (Sirivanakarn, 1976), it is difficult to distinguish or classify the species accurately.

During present communications, morphotaxonomic studies were made on *Cx. bitaeniorhynchus* from Chandigarh and its surrounding areas. In addition to morphological variants the allied form '*tenax'* was also found in the area. *Cx. bitaeniorhynchus* (including form '*tenax'*) was found widely distributed in most areas of Chandigarh. In India, no one has made any attempt to study '*typical'* and its allied '*tenax'* form of *Cx. bitaeniorhynchus* along with intraspecific variations. It was Barraud (1934) who for the first time replaced three forms *i.e* '*ambiguus'*, '*tenax'* and one unnamed *var*. into varieties from Indian region. After almost decades, the taxonomic studies have not been made in India. Thus, in current analysis, various phenotypic attributes have been re-examined in detail in 154 samples from study areas, under three years of surveillance to explore its variability. The morphometric analysis of distinguishing characters on the phallosome of male genitalia of *Cx. bitaeniorhychus* and its '*tenax'* form were also done to know the exact taxonomic status.

MATERIALS AND METHODS

Study area: The Chandigarh, which is located near foothills of the Shivalik range of Himalayas in Northwest India (30.74º N, 76.79º E) has variety of favourable habitats for mosquito breeding like thick vegetation which cover around 8.77 per cent of total geographical areas, 3245.30 hectares forest area, green belts of gardens, lakes full of flora and fauna (also attracts various species of migratory birds from parts of Siberia and Japan in the early winter season), paddy fields and slum areas on its outskirts. There are different seasons like summer (March–May), pre-monsoon (June–July), monsoon (August–September), post-monsoon (October–November) and winter (December–Mid March). The surveys were conducted during the transitional period of the season from pre-monsoon to post-monsoon seasons.

Mosquito collections, Identification and analysis*:* Daily visits were made from different categorized habitats *viz*; Developed areas, Garden belts, Villages and Slums of Chandigarh (Fig. 1). Adult specimens were collected from different resting sites near temporary or permanent urban pools, grasses, gardens, slum areas, villages having cattle sheds, and in and around pig enclosures, with the help of mouth aspirators and hand net traps under three years of entomological surveillance from June 2017– November 2019. The adults were collected during dawn and dusk from indoor and outdoor resting places. Field–caught samples were held in the laboratory to record the variability and distribution pattern. Adult specimens were preserved in insect collection boxes after pinning and identified with the help of identification keys (Barraud, 1934; Sirivanakarn, 1973, 1976). Siverly & Shroyer (1974) methodology was adopted to prepare slides for male genitalia. The terminology used for naming different parts of male genitalia was revealed by Sirivanakarn and Reuben. After careful identification, the adults were photographed under Stereo Zoom Trinocular Microscope attached with digital camera. Slides of male genitalia were studied and photographed under research microscope. The morphometric measurements of phenotypic attributes of phallosome were studied using compound microscope calibrated with stage micrometer and digital camera. Further, for statistical analysis, the mean (M) and Standard deviations (SD) of the various parts of phallosome was computed by parametric tests using one–way ANOVA.

RESULTS

During present work, 154 adults of *Cx. bitaeniorhynchus* were collected from four majorly categorized habitats *viz*; developed urban areas, villages, garden belts and slums of Chandigarh and its surrounding areas and identified. It has been

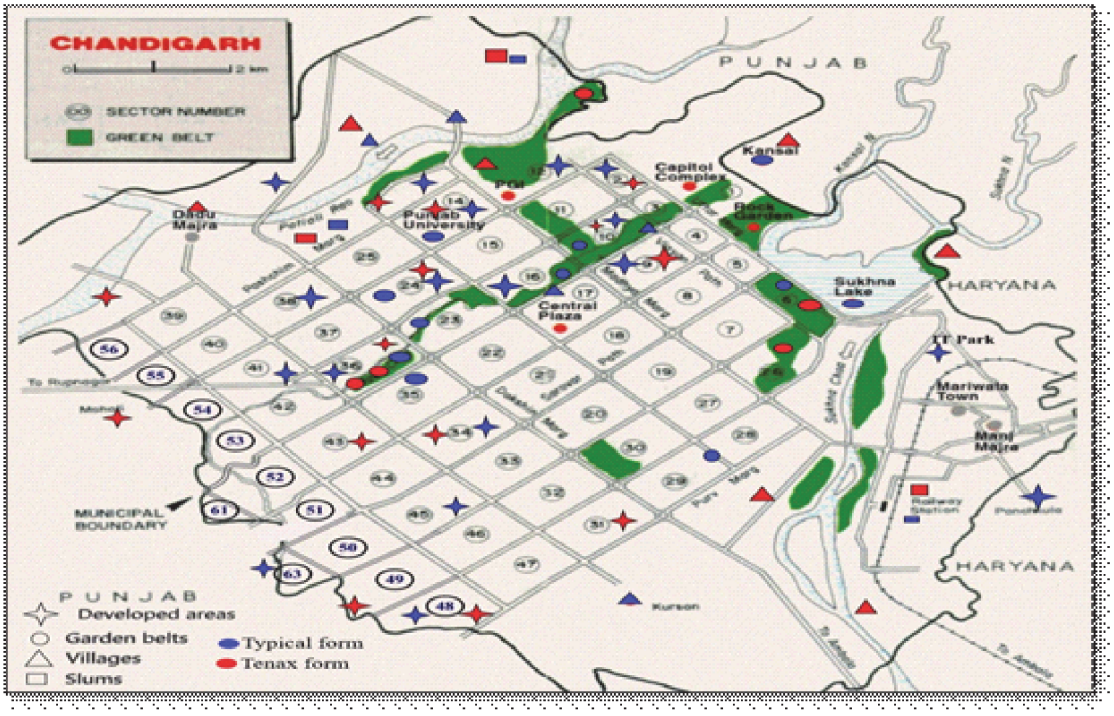


Fig. 1 Map of Chandigarh showing various ecological habitats surveyed during Jun 2017–Nov 2019.

Developed area		Garden belts	Villages		Slums
1.Sector4	16.Baltana	1.Bougainvellia Garden	1.Sector4	16.Baltana	1.Khuda Lahora
2.Sector 8	17.Sector 31	2.Leisure Valley	2.Sector 8	17.Sector 31	2.Mauli Jagran
3.Sector14	18.Sector 40	3.Rose Garden	3.Sector14	18.Sector 40	3.Bapu Dham Colony I
4.Sector 15	19.Bal Bhawan	4.Fragnance Garden	4.Sector 15	19.Bal Bhawan	4.Palsora
5.Sector 38	20.Mohali	5.Terrace Garden	5.Sector 38	20.Mohali	5.Burail
6.Sector 39	21.Nada sahib	6.Topiary Garden	6.Sector 39	21.Nada sahib	6.Dadu Majra
7.Sector 46	22.Panchkula	7.Pointsettian&Ixora	7.Sector 46	22.Panchkula	7.Sector 25
8.Sector 48	23.Zirakpur	8.Botanical Garden (Sec-14)	8.Sector 48	23.Zirakpur	8.Madanpur
9.Sector 2	24.Manimajra	9. Hibiscus Garden	9.Sector 2	24.Manimajra	9.Raipur khurd
10.Sector 9	25Sukhna Lake	10.Dahlia Garden	10.Sector 9	25.Sukhna Lake	10.Makkanmajra
11.Sector 16	26.New Lake	11.Garden of Silence	11.Sector 16	26.New Lake	11.Railway Colony
12.Sector 24	(&other areas)	12. Butterfly Garden	12.Sector 24	(&other areas)	12. BapuDham Colony II
13.Sector 34		13.Valley of Animal	13.Sector 34		
14.Sector 36		14. Garden of Springs	14.Sector 36		
15.Sector 45		15. Garden of Palms	15.Sector 45		
		16. Japanese Garden			
		(& more)			

Abbreviations used:

SAL – Sub Apical Lobe
ASP – Apical Sternal Speculate Process
BSP – Basal Sternal Processes
ASA – Apical Sternal Angle
LBP – Lateral Basal Process
ASSP – Apical Sternal Spiculate Portion/ Inner Division
GC-–Gonocoxites
GS–Gonostylus
PPR –Paraproct
FP –Folliform Processes
ATA – Apical Tergal Angle
OD – Outer Division
AM – Apical Margin
TE – Terga
P – Proboscis
MF – Mid Femur
FF – Fore Femur
W – Wing
ES– Erect Scale

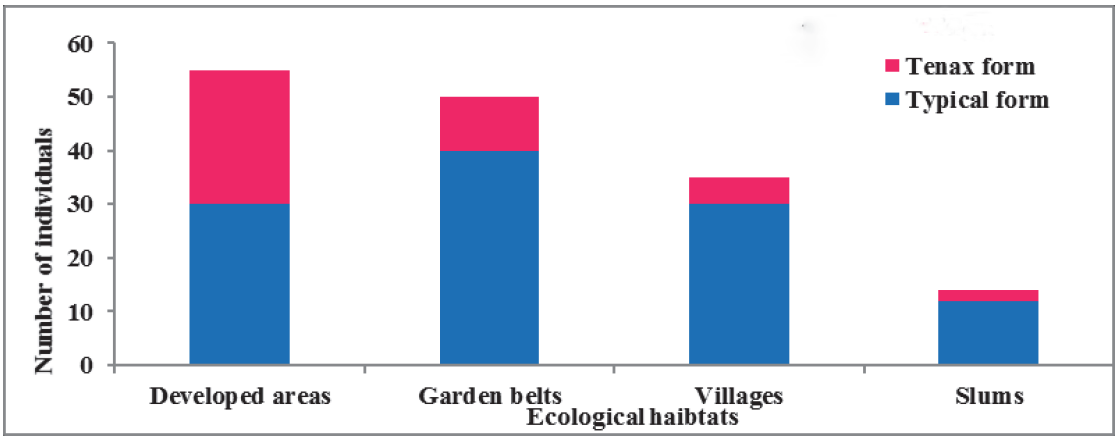


Fig. 2 Ecological distribution of *Culex bitaeniorhynchus* typical and *tenax* from Chandigarh (June 2017-Nov 2019)

observed that *Cx. bitaeniorhychus* exists as both '*typical'* as well as '*tenax'* form, which comprised 72.7 and 27 per cent respectively. Out of these four ecologically divided habitats, both *typical* and *tenax* has shown maximum abundance in developed areas (47.4%), followed by garden belts (31.8%), villages (16.2%) and slums (4.54%) during three years i.e June 2017–November 2019 (Fig. 2). These adult populations started appearing in June, showed an upward trend in July and then reached to peak in August. Thereafter, population density declined during September and October and greatly reduced in November.

The taxonomic investigations of both *typical* and *tenax* form of *Culex bitaeniorhynchus* along with their intraspecific variations have been done in detail in the present communication. It has been observed that these two forms differ with respect to the erect scales on center of vertex of head, colour of scales on scutum of thorax, speckling of scales on the legs as well as on the wings, apical and basal bands on abdominal terga and the phallosome attributes of male genitalia. The phenotypic differences in the morphology of respective forms '*typical'* and '*tenax'* along with their intraspecific variants has been elucidated (Table 1, 1a).

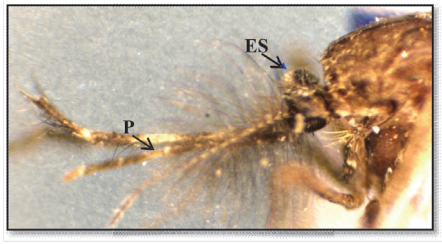
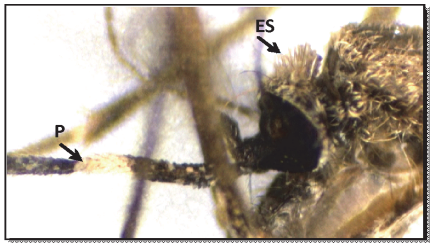
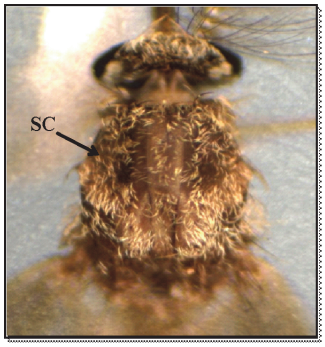
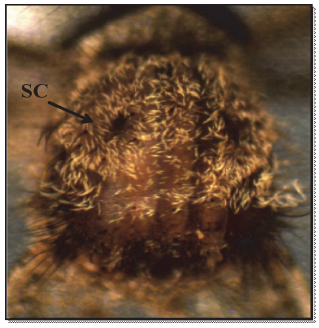
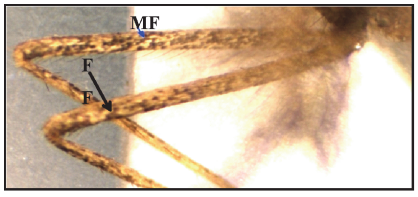
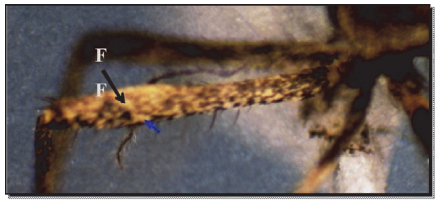
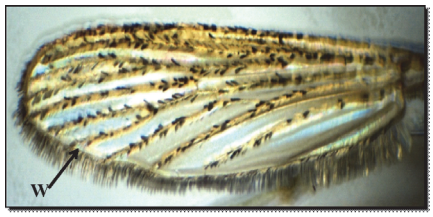
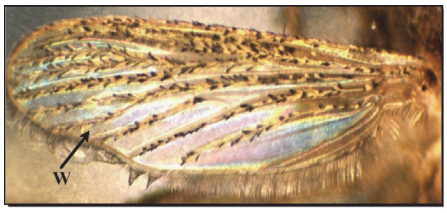
Male Genitalia (♂)

In *Cx. bitaeniorhynchus,* the gonocoxites (**GC**) are slender or conical in shape and gonostylus (**GS**) are elongate with apex sometimes swollen in shape and has long spiniform setae and hair majorly on lateral sides. Sub Apical Lobe (**SAL**) of gonostylus is small with a basal rod like structure. Apical region of Paraproct (**PPR**) is dark and covered with setae. In phallosome, apical speculate portion (**ASP**) of inner division to apex is typically beaklike sharp, vary in length, along with smoothly curved apical margin. Apical tergal angle blunt; basal sternal process of proctiger slender and vary in length, while the apical sternal angle strongly produced sterned into sharp beak; inner tergal surface with distinct lobe bearing 2–3 budlike processes called folliform Processes (**FP**). Outer division (OD) is in shape of broad acuminate leaf with variable structure; lateral basal processes (LBP) is mostly knoblike (Fig. 4A, Fig. 4B).

Lateral Plate of Phallosome: The lateral plate of phallosome of '*typical'* and '*tenax'* along with their intraspecific variations is described below.

'*Typical'* (Fig. 5A): Apical Sternal Angle **(ASA)** of apical sternal speculate portion of inner division strongly produced into typically sharp sterned beak, 0.96mm in length, covering with smoothly curved apical margin. Apical Tergal Angle **(ATA)** is satiny curved; Basal Sternal Lobe (**BSL**) of proctiger slender, vary in length, while the inner tergal surface with distinct lobe bearing 2–3 budlike fused Folliform processes (**FP**). Outer division (**OD**) is in shape of broad acuminate leaf with average 0.591mm

Table 1. Comparative phenotypic details of morphotaxonomy of *'typical'* and *'tenax'* form of *Culex bitaeniorhynchus*

	'Typical' form	*'Tenax' form*
Head		
Thorax		
Legs		
Wings		

horizontal length; Lateral basal process **(LBP)** is mostly knoblike, measuring 0.253mm (Table 2).

'*Tenax*' (Fig. 5B): Apical Sternal Angle (**ASA**) of inner division of an apical sternal speculate part strongly produced sterned into a sharp beak with interiorly curved apex, 0.396mm in length with little distinct emargination on apical margin. Apical Tergal Angle **(ATA)** protrude outwardly but not beaked; inner tergal surface with distinct lobe bearing 2–3 budlike fused Folliform processes (**FP**), these buds varying in length. Outer division (**OD**) is in shape of a broad acuminate leaf pointing downward with 0.603mm horizontal length; Lateral Basal Portion **(LBP)** is mostly cuboidal, measuring 0.381mm while Basal Sternal lobe (**BSL**) is slender in shape and vary in length (Table 2).

Typical - ***Variant*–I (Fig. 6A):** Apical Sternal Angle (**ASA**) of sternal speculate portion of inner division is beaked, average measuring 0.35mm along with slight curved apical margin of 0.81mm.

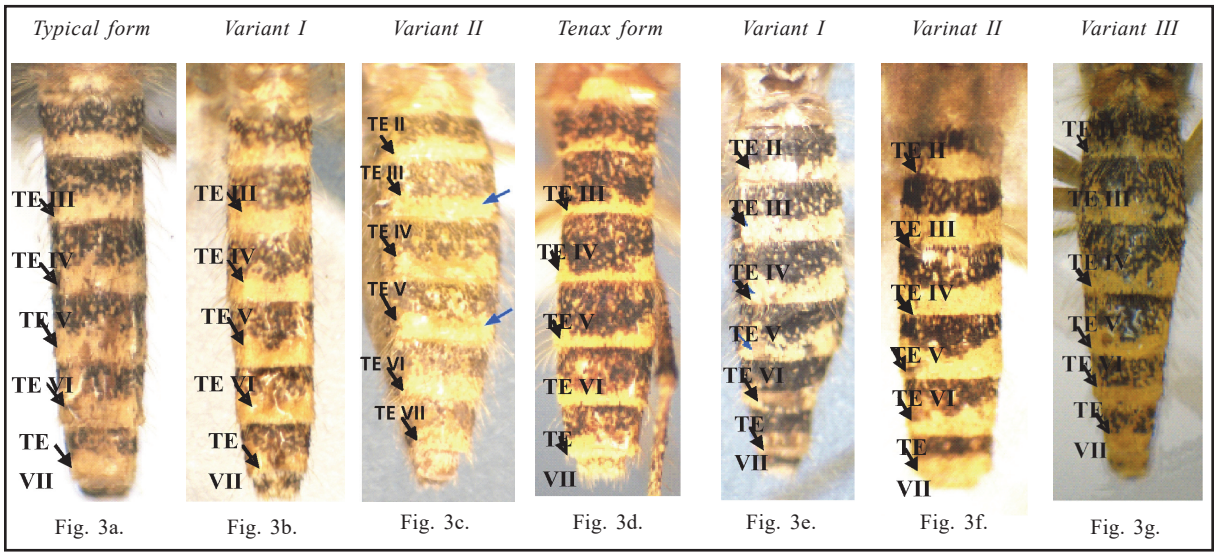
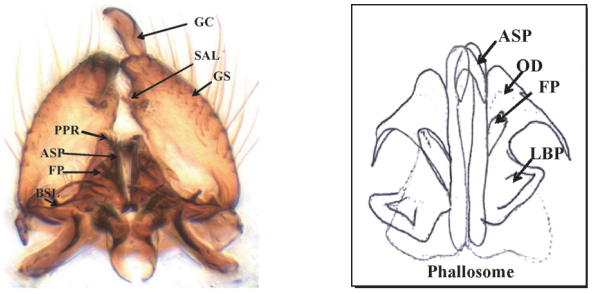
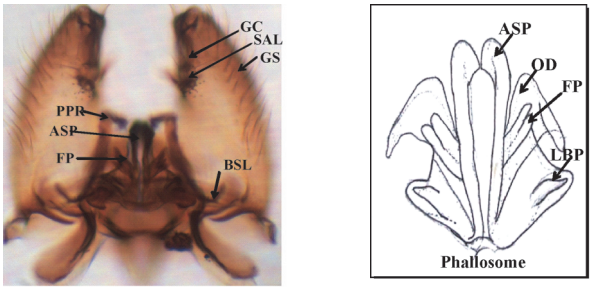


Fig. 3 Phenotype of Abdominal Terga (TE) of both '*typical* and '*tenax'* forms with its intraspecific variants of *Cx. bitaeniorhynchus*



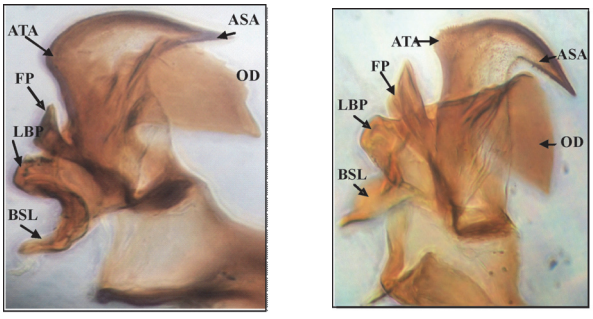
General view

Fig. 4A. Male Genitalia of *Culex bitaeniorhychus* ('*typical'*)



General view

Fig. 4B. Male Genitalia of *Culex bitaeniorhychus ('tenax')*



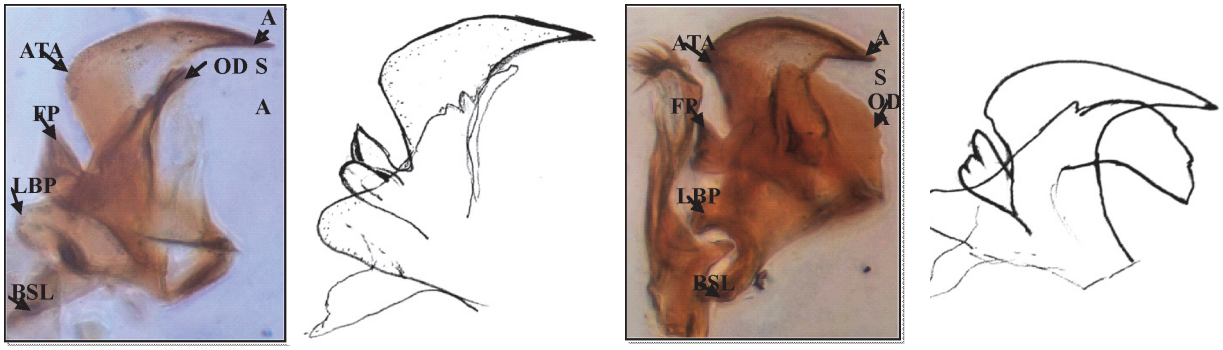
'Typical' form (Fig. 5A)

'*Tenax'* form (Fig. 5B)

Fig. 5 Lateral view of phallosome of male genitalia

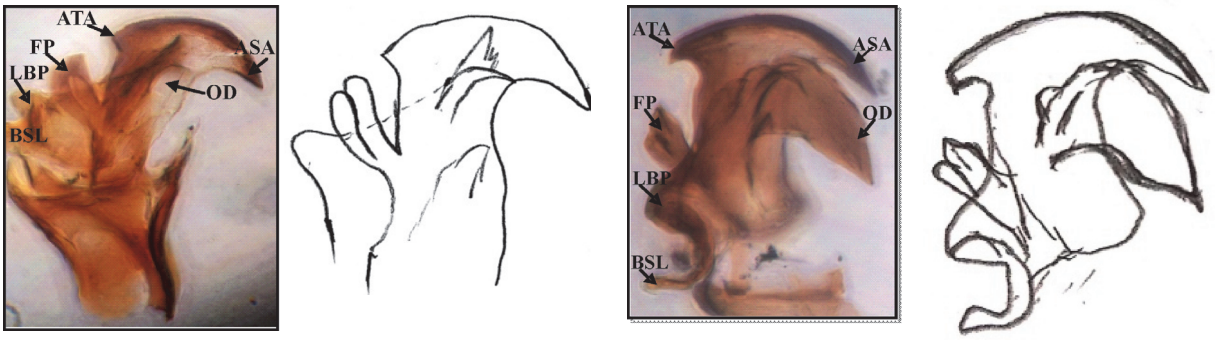
Apical Tergal angle (**ATA**) mainly blunt or obtuse in shape. The inner tergal surface with distinct lobe bearing distinct 2–3 fingerlike folliform processes (**FP**), in which basal folliform process is short (0.30mm). Outer division (**OD**) is in shape of short acuminate leaf with average 0.51mm horizontal length; Lateral basal process **(LBP)** is mostly slender, measuring 0.23mm, whereas, Basal Sternal lobe (**BSL**) is slender in shape (Table 2).

Intraspecific variations in the lateral plate of phallosome of male genitalia



Variation I (Fig. 6A) *Variation II* (Fig. 6B)

Fig. 6 *'typical'* form



Variation I (Fig. 7A) *Variation II* (Fig. 7B)

Variation III (Fig. 7C)

Fig. 7 *'tenax'* form

Table 1a. Morphotaxonomic details of different variants in *Typical* and *Tenax* forms of *Cx. bitaeniorhynchus* Giles

Phenotype	*Typical* Form		*Tenax* Form	
	Typical	Intraspecific variations	*Tenax*	Intraspecific variations
Head (colour of erect scales on center of vertex)	Whitish or pale scales	***Variation* I** Whitish/golden pale ***Variation* II** Bronzy brown	Dark brownish to partially pale with some pale scales in center	***Variation* I** Whitish pale ***Variation* II** Dark brown ***Variation* III** Bronzy brown
Thorax (colour of scales on Scutum)	Anterior 2/3 pale, whitish, yellowish, Posterior ¼ some pale scales	***Variation* I** Golden or pale/ some whitish pale scales ***Variation* II** whole SC with Bronzy pale with white mottling	Anterior 2/3 Pale to bronzy brown Posterior ¼ few white scales	***Variation* I** Dense Whitish/Few whitish pale scales ***Variation* II** Dense brownish/ dark scales ***Variation* III** Dense pale brownish /without pale scales
Legs	Extensively speckled with black and pale scales	***Variation* I** Extensively speckled with more pale scales ***Variation* II** Lightly speckled with black and pale scales	Light to moderately speckled	***Variation* I** Light speckled with more dark scales ***Variation* II** Moderately speckled ***Variation* III** Light to moderately speckled
Wings	Extensively speckled with black and pale scales on all veins	***Variation* I** Extensively speckled with black and pale scales on all veins ***Variation* II** Lightly speckled with black and pale scales on all veins	Lightly to moderately speckled	***Variation* I** Heavily speckled with golden pale scales ***Variation* II** Heavily speckled with whitish pale scales ***Variation* III** Heavily speckled with more pale scales on all veins
Abdomen	Broad apical and basal bands on terga II-VII, tergaVIII with both basal and apical scales(Fig. 3a)	***Variation* I** Broad golden pale apical bands present on terga II-VII and apical and basal bands on VIII terga. (Fig. 3b)Black tergal area speckled with pale scales ***Variation* II** Moderatly broad whitish pale apical bands present on terga II-VII and apical and basal bands on VIII terga (Fig. 3c) Black tergal area lightly speckled with pale scales.	Terga II-IV with Pale yellowish apicolateral spots forming very narrow apical bands; terga V-VIII with narrow apical yellowish bands connecting with large yellowish apicolateral spots at sides. (Fig. 3d)	***Variation* I** Terga III-IV with whitish Pale apicolateral spots forming narrow apical bands; terga V-VIII with narrow apical palish bands connecting with large yellowish apicolateral spots at sides (Fig. 3e) ***Variation* II** Terga II-IV with Pale yellowish apicolateral spots forming broad apical bands; terga V-VIII with narrow apical yellowish bands connecting with large yellowish apicolateral spots at sides (Fig. 3f) ***Variation* III** Terga II-V with Pale yellowish apicolateral spots forming very narrow apical bands; terga V-VIII without narrow apical yellowish bands at sides (Fig. 3g)

Typical - ***Variant*–II (Fig. 6B):** Apical Sternal Angle (**ASA**) of inner division of sternal portion is beaked, measuring 0.43mm average length along with curved apical margin. Apical Tergal Angle (**ATA**) mainly smoothly curved. The inner tergal surface with distinct lobe bearing distinct 2–3 fused fingerlike folliform processes (**FP**) measuring 0.588mm average size of basal folliform process. Outer division (**OD**) is in shape of short acuminate leaf with 0.808mm horizontal length; Lateral Basal processes **(LBP)** are mostly slender with down projection, measuring 0.269mm and Basal Sternal lobe (**BSL**) is slender with down projection (Table 2).

***Tenax* - *Variant*–I (Fig. 7A):** Apical Sternal Angle (**ASA**) of sternal speculate portion of inner division to apex is beaked, measuring 0.395mm along with curved apical margin. Apical Tergal Angle (**ATA**) mainly sharply beaked. The inner tergal surface with distinct lobe bearing two long fused fingerlike folliform processes (**FP**), in which the basal folliform process is measuring 0.493mm. Outer division (**OD**) is in shape of short acuminate leaf with 0.598mm horizontal length; Basal Sternal lobe (**BSL**) is cuboidal in shape while, Lateral Basal processes **(LBP)** are mostly slender with down projection, measuring 0.227mm (Table 2).

***Tenax* - *Variant*–II (Fig. 7B):** an Apical Sternal Angle (**ASA**) of sternal speculate part of inner division to apex is sharp beaked, measuring 0.352mm along with curved apical margin. Apical Tergal Angle (**ATA**) mainly bluntly beaked. The inner tergal surface with distinct lobe bearing distinct 2–3 fused fingerlike folliform processes (**FP**), in which basal process is 0.499mm in length. Outer division (**OD**) is in shape of short acuminate leaf shaped with 0.836mm in horizontal length; Lateral Basal processes **(LBP)** is slightly oval in shape while the Basal Sternal lobe (**BSL**) are mostly slender with down projection, measuring 0.316mm (Table 2).

***Tenax* - *Variant*–III (Fig. 7C):** an Apical Sternal Angle (**ASA**) of sternal speculate portion of inner division to apex is sharp beaked**,** measuring 0.889mm along with curved apical margin. Apical Tergal Angle (**ATA**) mainly shortly beaked. The inner tergal surface with distinct lobe bearing distinct 2–3 fused finger shaped folliform processes (**FP**) measuring 0.481mm. Outer division (**OD**) is in shape of short acuminate leaf with 0.558mm horizontal length; Lateral Basal Processes **(LBP)** are mostly oval with down projection, measuring 0.384mm while Basal Sternal lobe (**BSL**) is slender shaped and projected downwardly (Table 2).

Table 2. Morphometric analysis of various parts of phallosome of *Cx. bitaeniorhynchus*

S.no.	Phenotypics variations	*n*	AM	ASSP	OD	FP	LBP
1.	***Typical* form**	90	0.964	0.414	0.591	0.458	0.253
2.	*variant* I	16	0.815	0.358	0.518	0.304	0.179
3.	*variant* II	6	0.918	0.432	0.888	0.588	0.269
			0.89±0.08	0.40±0.05	0.66±0.17	0.45±0.15	0.23±0.04
4.	***tenax* form**	23	0.891	0.396	0.603	0.538	0.381
5.	*variant* I	10	0.766	0.395	0.598	0.493	0.227
6.	*variant* II	5	0.969	0.352	0.836	0.499	0.316
7.	*variant* III	4	0.889	0.275	0.558	0.481	0.384
			0.87±0.11	0.35±0.08	0.64±0.11	0.50±0.06	0.32±0.08

* Length measured in mm (Mean±SD); Number of mosquito sample collected-n

One factor ANOVA along with parametric tests was performed to confirm statistical significance in morphological differences in '*typical'* as well as '*tenax'* form and their respective intraspecific variants of *Cx*. *bitaeniorhynchus*. The significant p value (Confidence interval of 95%) for both of these forms of *Cx*. *bitaeniorhynchus* has been found to be <0.05, Hence, these two forms are statistically significant and rejecting the null hypothesis and accepting the alternate hypothesis. Furthermore, pair wise comparison of each phenotypic variant along its naturally occurring forms was also done statistically in a single analysis (Table 3).

DISCUSSION

Culex bitaeniorhynchus subgroup comprises of species which are extremely similar and difficult to identify because of overlapping suites of shared phenotypic features. Previous taxonomic workers (Edward, 1922; Colless, 1959; Sirivanakarn, 1973, 1976) have studied *Cx. bitaeniorhynchus* along with different forms on the basis of striking and discontinuous variations in coloration of scales on different parts of the body. They also mentioned the presence of different variants in *Cx. bitaeniorhynchus*. Edward (1922) distinguished *Cx*. *bitaeniorhynchus* (type form) into three varieties like '*tenax'*, '*ambiguus'*, '*domesticus'* and two unnamed forms from Oriental region. Barraud (1934) extensively surveyed all parts of India and studied *Cx. bitaeniorhynchus* along with male genitalia. He indicated the presence of two or more variabilities in genitalic characters of *Cx. bitaeniorhynchus* and also mentioned three varieties i.e *var*. '*ambiguus'*, *var*. '*tenax'* and one unnamed variety in the Fauna of British India. Bram (1967) reported 3 forms from Thailand i.e common or typical form, '*tenax'* form, '*ambiguus'* form and one intermediate form. After many years, Sirivanakarn (1973) recognized number of forms within *Cx*. *bitaeniorhynchus i.e*. '*tenax'* Theobald, '*domesticus'* Leicester, '*ambiguus'* Theobald, '*taeniarostris'* Theobald, '*sarawaki'* Theobald from Southeast Asia. In 1976, he mentioned five species under *Cx*. *bitaeniorhynchus* complex *viz*; *bitaeniorhynchus* (typical form), *infula*, *luzonensis* (*luzon* form), *selangorensis* (*selagor* form), *pseudosinensis* and *longicornis*. He also mentioned that all these component species show many overlapping characteristics with one another in their phenotype, so usually pose great difficulty in their correct identification. He lumped all available multiple variants like '*ambiguus'*, '*tenax' var. 'ocellata',* '*domesticus'*, '*taeniarostris', 'infula'* and *'sarawaki'* into single species i.e. *infula.* Darsie & Pradhan (1990) found only female of '*ambiguus'* from Nepal and agreed provisionally

Table 3. Results of one-way ANOVA for comparing means of phenotypic attributes in both forms of *Cx. bitaeniorhynchus*

	***Typical* form**	**Var. I**	**Var. II**			***Tenax* form**	**Var. I**	**Var. II**	**Var. III**		
Phenotypic Attributes	**Mean± SD**	**Mean± SD**	**Mean± SD**	**F**	**Sign. (p)**	**Mean± SD**	**Mean± SD**	**Mean± SD**	**Mean± SD**	**F**	**Sign. (p)**
AM	0.96± 0.02	0.81± 0.005	0.91± 0.10	4.21	0.07	0.89± 0.16	0.7± 0.01	0.9± 0.05	0.8± 0.09	1.76	0.22
ASSP	0.41± 0.03	0.35± 0.02	0.43± 0.06	1.77	0.18	0.39± 0.08	0.3± 0.004	0.3± 0.004	0.2± 0.13	1.07	0.04
OD	0.59± 0.06	0.51± 0.03	0.88± 0.007	0.01	0.01*	0.6± 0.05	0.8± 0.03	0.83± 0.03	0.5± 0.03	41.3	0.001*
FP	0.45± 0.005	0.30± 0.002	0.58± 0.18	21.0	0.04	0.5± 0.008	0.4± 0.006	0.4± 0.006	0.4± 0.14	0.23	0.726
LBP	0.25± 0.01	0.17± 0.009	0.26± 0.02	16.7	0.02*	0.38± 0.89	0.3± 0.005	0.3± 0.005	0.3± 0.08	2.23	0.016*

with Sirivanakarn about '*ambiguus'* a synonym of *infula* and left it pending for further studies.

However, during present investigations, both male and female of 'typical' and '*tenax'* has been found and studied. After detailed morphotaxonimic studies and careful observations on important characteristics of male genitalia it has been concluded that '*tenax'* totally resemble with the '*tenax'* mentioned by Sirivanakarn (1973), not with '*infula'* as mentioned by him in 1976. According to him, apical spiculate portion of inner division of phallosome of '*infula'* is strongly pigmented and is with deep distinct emargination proximad of apical tergal angle. The folliform processes on inner tergal lobes are 4–5 in number. However, in '*tenax'* it has been observed that the interiorly emargination on apical sternal speculate part is not much deeply distinct and apical sternal angle of inner division strongly produced sterned into sharp beak with a little pigmentation. The folliform processes on inner tergal lobes are 2–3 in number. Moreover, outer divisions of phallosome is lumped inferiorly in '*infula'* while, it is in the shape of a broad acuminate leaf which points downward with distinct horizontal length in '*tenax'*. The basal sternal process of proctiger in '*infula'* is relatively stronger (Sirivanakarn, 1973, 1976) but, in '*tenax'* it is usually slender in shape. Hence, '*tenax'* which is strikingly different from '*infula'* should not be synonymised with '*infula'*.

The present study revealed the occurrence of *Cx. bitaeniorhynchus* in Chandigarh as '*typical'* and '*tenax'* forms. The '*tenax'* form was synonymised provisionally with species '*infula'* by earlier workers. But, in the present exploration, it has been perceived that '*tenax'* is incredibly different from '*infula'* in morphology and male genitalia and hence, should not be synonymised with species name '*infula'*. It is also evident from the statistical analysis which showed significant difference between these two forms.

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