



Taxonomic Morphology of Biting Midges (*Diptera: Ceratopogonidae*)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.56557/UPJOZ/2023/v44i223739

Editor(s):

(1) Prof. Telat Yanik, Atatürk University, Turkey.

Reviewers:

(1) Panat Anuracpreeda, Mahidol University, Thailand.

(2) Abul Faiz, Barama College, India.

Original Research Article

Received: 13/08/2023

Accepted: 20/10/2023

Published: 06/11/2023

ABSTRACT

Ceratopogonidae is a family of small nematoceros flies ranging approximately 1 to 3mm in length. *Ceratopogonidae* which was previously categorised as a subfamily of Chironomidae is distinguished by the presence of its biting mouthparts. Ceratopogonids commonly known as biting midges are the vectors of numerous pathogens such as viruses, protozoans and nematodes. Hence its transmission has a great veterinary and medical importance. This family is divided into four subfamilies: Leptoconopinae, Forcipomyiinae, Dasyhilinae and Ceratopogoninae. The flies were collected with the help of aspirator, Diptera net and UV light trap. The collected specimens were preserved in the 70% ethanol and later dissected out with the help of Digital Motic Dissecting Binocular. *Ceratopogonidae* include widely diversified morphotaxonomic character describing various subgroups. This variable character which include colour pattern, sizes of the body, proportions of various morphological parts are not very reliable for taxonomic identification. The study found that the morphotaxonomic feature like external genitalia of male and female, armature and arrangement of wing venation are much more stable. There are several puzzling problems in

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the determination of disputed species, can be resolved on the basis of taxonomic characters of male and female genitalia. The present paper will resolve the problem related to plethora of taxonomic character with special reference to biting midges.

Keywords: Morphology; biting midges; Ceratopogonidae.

ABBREVIATIONS

Ac	: Acrostichals	Md	: Mandible
Ae	: Aedeagus	MP	: Maxillary palp
Al	: Alula	P	: Paramere
An	: Anal cell	Pa	: Prealars
An 1	: Anal vein first	Pc	: Pedicel
AP	: Antepnotum	Pe	: Preepisternum
ApP	: Apicolateral process	Pes	: Preepisternals
ApPs	: Apicolateral process seta	PgP	: Postgenital plate
Aps	: Antepnotals	Pm1.5	: Palpomeres
Ar	: Arculus	Pn	: Postnotum
AS	: Analpleural suture	Pp	: Pronotum
B	: Brachiolium	PS	: Parapsidal suture
C	: Costa	R	: Radius
Cer	: cercus	r1	: Cell r1
Clw	: Claws	r2	: Cell
Cly	: Clypeus	r2 r5	: Cell r5
Co	: Cornua	RM	: Cross-Vein-r-m
CP	: Cibarial pump	SAC	: Sub apical Cercus
Cu	: Cubitus cell	SCf	: Sensilla Campaniformia
Cu	: First cubitus vein	Scp	: Scape
Cu	: Second cubitus vein	Scu	: Scutum
Dc	: Dorsocentralis	SE	: Spermathecal eminence
Emp	: Empodium	Sc	: Subcosta
Fe	: Femur	Sct	: Scutellum
Flg	: Flagellomeres	SGR	: Schlerotized gland rod
FM	: False vein m1	S-IX	: Sterum IX
FM	: False vein m2	Spt	: Spermatheca
FM	: False vein M3+4	Sq	: Squama
FT	: Frontal tubercle	SVo	: Superior volsella
G	: Gland	T-IX	: Tergum IX
Go	: Gonocoxite	T	: Tergite
Gs	: Gonostylus	Ta	: Tarsomeres
H	: Humerals	Ti	: Tibiae
Hat	: Hlatere	TS	: Temporal setae
HP	: Humerals Pit	TrS	: Transverse suture
Hyp	: Hypopharynx	Vrt	: Vertex
IoS	: Interocular seta		
IVo	: Inferior Volsella		
La	: Labia		
Lab	: Labium		
Lbr-epi	: Labrum-epipharynx		
M	: Media		
m1	: First media cell		
M1	: First media Vein m2 - Second media cell		
M2	: Second media Vein		
M3+4	: Media vein		
M3+4 Max	: Maxilla		

1. INTRODUCTION

Ceratopogonidae commonly called as “biting midges”. Occurrence of *Ceratopogonidae* in great numbers in nearly every types aquatic habitat including lake, ponds, temporary pools, rivers, waterfalls and around which sheep and other cattle are going for grazing. A limited number of species are living in salty region such as salt Lake of Rajasthan. The flies are plentiful in the warmer months and are most active at dusk in the early morning; most of the flies are living near cattle, animals and human beings.

Some of *Forcipomyia* and *Dasyhelea* forming swarms above moist surface, vegetation and shrubs. *Leptoconops* forming swarms under tree in the late evening, *Culicoides* and *Forcipomyia* over cattle, under old building where surface is dried. Ceratopogonids are attracted towards artificial light sources in the night [1,2]. It is the well-established fact that the biting midges are the vectors of numerous pathogens such as viruses, protozoans and nematodes, these vectors transfer numerous pathogens to the sheep, cattle, horses, birds and even in the human [3,4]. The veterinary and medical importance of this transmission is particularly obvious and the economic impact of the veterinary diseases transmitted by the fly has created a research area in the pure virology. The number of viruses isolated from biting midges is impressive and belong mainly to the Simbu group, Orbiviruses and Rhabdoviruses.

Before 1917 *Ceratopogonidae* was considered as subfamily of family Chironomidae (non-biting midges). After discovery of biting behaviour and drastic modifications in the mouth parts, Malloch a [5] created it as a separate and independent family *Ceratopogonidae*. Till 1926 it has been in matter of discussion whether *Ceratopogonidae* having an independent status of family or not. It was Edward [6,7], who listed the differences between these two families and contrasted blood sucking ceratopogonids mouth parts with reduced mouth parts of the Chironomidae. The independent status of family *Ceratopogonidae* is accepted, although many characters used to define the *Ceratopogonidae* are plesiomorphic.

Recent studies also indicate that the biting midges are the vector of numerous protozoans, especially sporozoans. Information regarding transmission of Protozoa with special reference to India are not available, whatever information are available on this aspects from the countries of United States in Florida, Atkinson 1988 studied the epizootic of *Haemoproteus meleagridis* and he suspected *C. edeni* and *C. hinmani* to be principal natural vector, these two species represent 95% of the *Culicoides* present in that region.

Morphology of taxonomic significance of the different life stages of Biting midges, particularly their characters useful for determination of independent status of species, have been described in detail by various workers. But only those aspects relevant for an understanding of

the *Ceratopogonidae* especially imagoes are considered here.

In most of the taxonomic studies the workers have used character like sensilla coeloconica, some indexes, length of probosis, palpal ratio and patches on the wings. These taxonomic characters are not enough to resolve the problem of disputed species. Therefore, there is a urgent need to find more taxonomic characters in male and female genitalia [8]. An attempt has been made to provide more than 20 characters especially in male and female external genitalia of taxonomic importance have been listed in the present study.

2. MATERIALS AND METHODS

2.1 Collection Techniques

Swarm forming species of biting midges were collected by Diptera sweeping net. An aspirator was used for collecting the scattered individuals. An UV- light trap methods were used for collecting a variety of species in the field during night.

2.2 Preservation and Transport of Material

Adults were preserved in the 70 % ethanol in the collection tubes.

Some adult male and female were pinned in the field itself. Pinned specimens were placed in the thermocole boxes and were transported to the laboratory. This material was freezed in the freezer for one month. Such material is uncollapsible and very usefull for the study of some characters those cannot be observed in the slide mounted specimens.

2.3 Dissection and Examination

The imagoes were dissected out under Motic Digital Dissecting Bionocular and Borkent method of mounting was followed.

Terms for structures and illustrations: Saethers [9] and Wirth [10] terminology was followed for imagoes with some exceptions.

Drawings were completed with the aid of Drawing Tube on compound stereoscopic microscope. Photography was made by Motic Photographic Digital Microscope (Series B2)

3. RESULTS AND DISCUSSION

3.1 Morphology of Imagoes (Plate 1 to 8)

1. Antenna: Scape well developed, often large and bare in male, comparatively smaller and setose in female (except in both sexes of Palpomyiini where it is bare). Pedicel rounded, broad, long and setose. Flagellum with usually 13 flagellomere, plumose in male, rarely pilose, female flagellomeres pilose, flagellomeres more or less fused in some, but separated in female, flagellomeres are of variable shaped and sculptured in *Dasyhelea*, globular in *Leptoconops*, beaded in *Atrichopogon*, while in others it is moniliform, conical shaped and cylindrical, in stilobezziini collar brownish distinct, flagellomeres with reticulations proximad to the verticils (*Dasyhelea*), sensilla coeloconica present on flagellomeres in Culicoidini and Ceratopogonini. In male last 3-4(except in Palpomyiini) and in female last 5 flagellomeres elongated. 11th,12th, or 13th flagellomere in male longest but in female ultimate is longest. Apex of ultimate flagellomere in both sexes rounded and pointed (distinguished pointed apex in Forcipomyiinae).

2. Head: Spherical to subspherical, coronal suture complete to absent, frontal tubercle well developed, poorly developed to absent. Temporal setae numerous (*Forcipomyia*) to few. Eyes pubescent (*Dasyhelea*) poorly pubescent (Culicoidini and Ceratopogoninae) and bare, eyes are dichoptic in *Leptoconops*, holoptic in other; ommatidia small to large dorsomedially single interocular seta and a transverse suture usually present (Ceratopogoninae) and absent in other groups. Clypeus V-shaped long, broad margin with 4 or more than four setae, in *Dasyhelea* clypeus inverted V-shaped or U-shaped with clypeal membrane, in *Dasyhelea* clypeus & membrane jointly form fulcrum with a few setae. Maxillary palp with usually 5 palpomere, in Ceratopogoninae 2-5, *Dasyhelea* with 4 palpomere, densely covered with setae (*Forcipomyia*), bare (in Palpomyiini) usually third palpomere elongated, ovoid, spatulate, slender and cylindrical with a small, moderate, large sensory pit, bearing capitate sensilla, in some species pit absent, without sensilla or sensilla scattered on the surface, ultimate palpomere bearing 5-8 setae at the apex, ultimate palpomere club-shaped (in Stilobezziini and Palpomyiini) slender and inwardly curved in Sphaeromyiini.

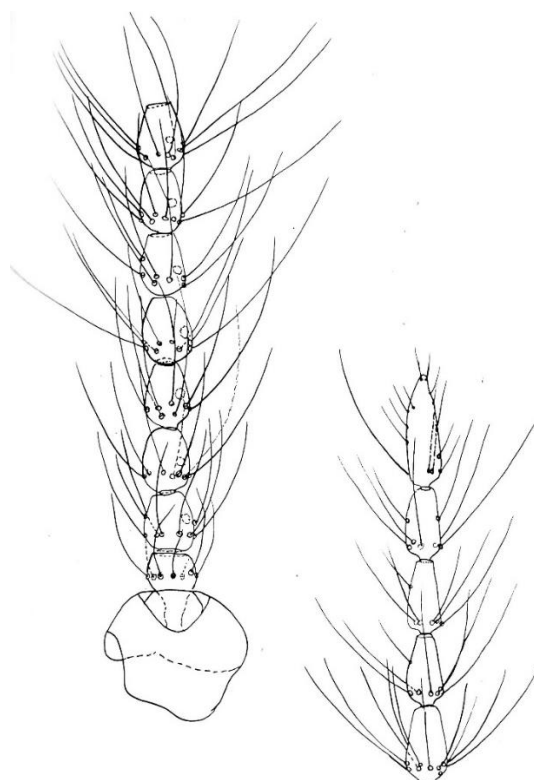


Plate 1. Distribution of sensilla of antenna in *Ceratopogonidae* female

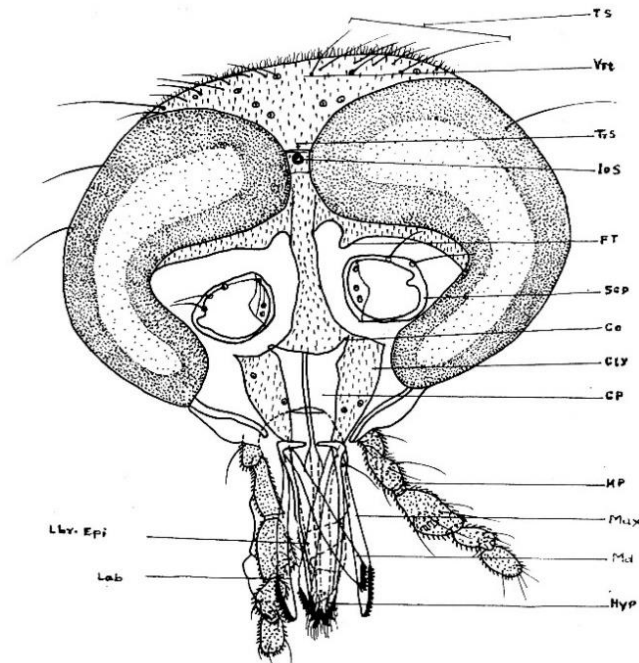


Plate 2. Generalized structures of the head of the *Ceratopogonidae*

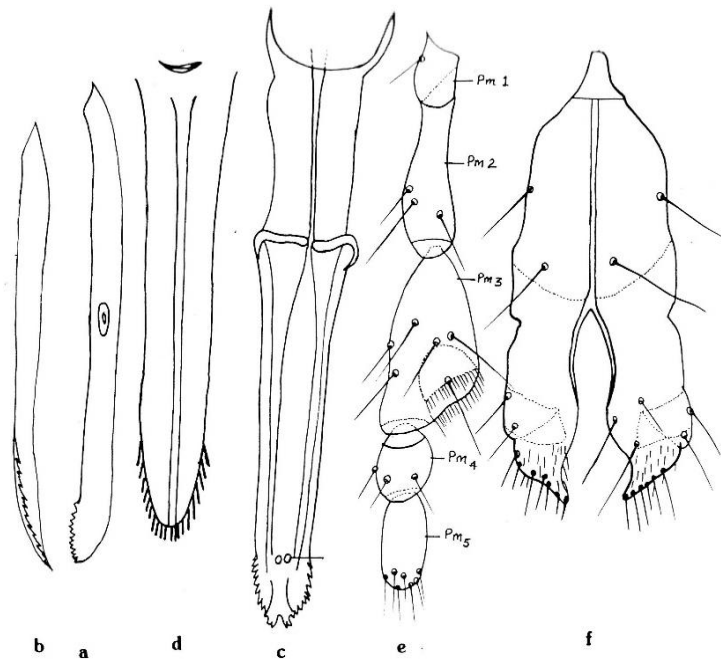


Plate 3. Mouth parts of *Ceratopogonidae*: a. mandible; b. maxilla; c. labrum epipharynx; d. hypopharynx; e. maxillary palp; f. labium

Proboscis with well-developed cibarial pump. In most of the female specimens (*Leptoconopinae*, *Forcipomyiinae* and *Ceratopogoninae*) mouth parts are piercing and blood sucking but in *Dasyheleinae* mouth parts are reduced. Maxillary palps extends beyond proboscis. Cornua long,

short, pointed, blunt, laterally to upwardly directed. In females mouth parts include; mandible strong, distal half serrated with 6 to 42 teeth, tapering apex, teeth are minute to large, sharp to blunt, straight to inwardly curved and a gland is present at the centre of the mandible (in

some Ceratopogoninae), ;maxilla slender to scalpel shaped, distal end tapering, inwardly curved, serrated with few triangular, minute teeth; in some Forcipomyiinae and all Dasyheleinae mandible and maxilla distal end with sharp tip, without teeth and setae; Labrum-epipharynx tubular, rounded to pointed and toothed distal end, 2 to 3 pairs of long teeth at the apex (Leptoconopinae, Ceratopogoninae), in Dasyheleinae and forcipomyiinae distal end usually pointed without teeth, distal end bare; hypopharynx tubular, distal end rounded and having schlerotization (Ceratopogoninae), in others distal end usually pointed without Schlerotization, In male mouth parts are reduced; maxilla and mandible slender with pointed distal end, mandible setose distally, maxilla bare; labrum-epipharynx tubular, pointed, setose to bare, labium in both sexes narrow to broad, setose and fleshy.

3. Thorax: Thorax narrows to broad anteriorly without anteromedian spine (except in some Stenoxenini). Moderately covered with macrotrichia (in *Forcipomyia* densely covered). Thorax convex anteriorly projecting over head. Humeral pit prominent (Culicoidini), small (Ceratopogonini) and usually absent in other biting midges, Parapsidal suture present. Anteprenotum well developed, with 3-6 setae (Forcipomyiinae), medially fused with or without setae. Acrostichals and dorsocentralis arranged

in well-defined rows, while scattered in Forcipomyiinae. Prelars present or absent. Anapleural suture present. Katepisternal present in Ceratopogonini, absent in others. Preepisternals present in Leptoconopinae. Postnotum usually bare.

4. Wing: Hyaline, light to dark brown with dark patches (Culicoidini, Ceratopogonini), milky (Ceratopogonini), with black spots (*Alluaudomyia*). Costa short to long. Radial sector slightly to densely covered with microtrichia. First and second radial cell present, both may be absent or only one is absent in some spp.; radial cell long and narrow in Sphaeromyiini, Palpomyiini, Stenoxenini; second radial cell much longer than first in Stilobezziini. Cross vein *r-m* absent (Leptoconopinae), present in other groups. Media always bifurcate; bifurcation proximal or at or distal to cross vein *r-m*; base of media well developed to obsolete; media completely obsolete (Rhynchohelea). Macrotrichia of wing membrane absent or minute or large and conspicuous ; macrotrichia dense, suberect and foliate (Forcipomyia) to simple in other groups. Fringe on posterior border of wing complex erect, suberect hairs, not arranged in regular pattern (Forcipomyia) simple alternating long and short simple straight hairs in other groups. Sensilla campaniformia present on brachiolum, subcosta and on radial sector. Alula and squama with or without macrotrichia.

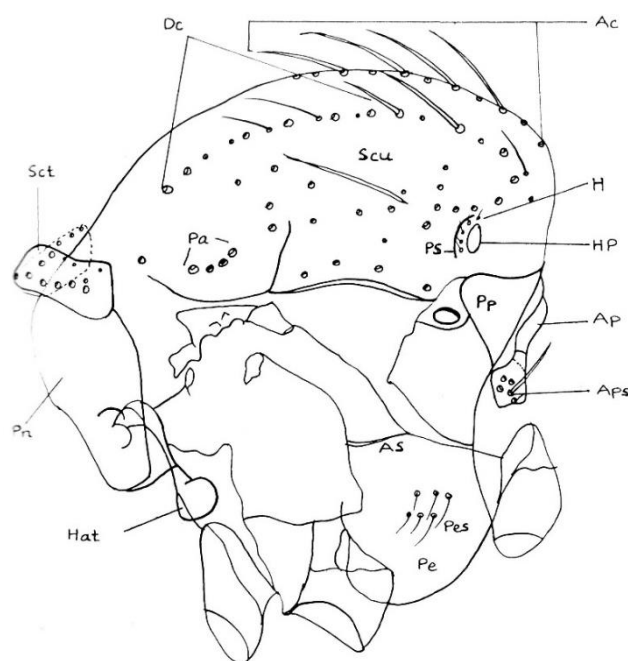


Plate 4. Detailed structure of thorax of *Ceratopogonidae*

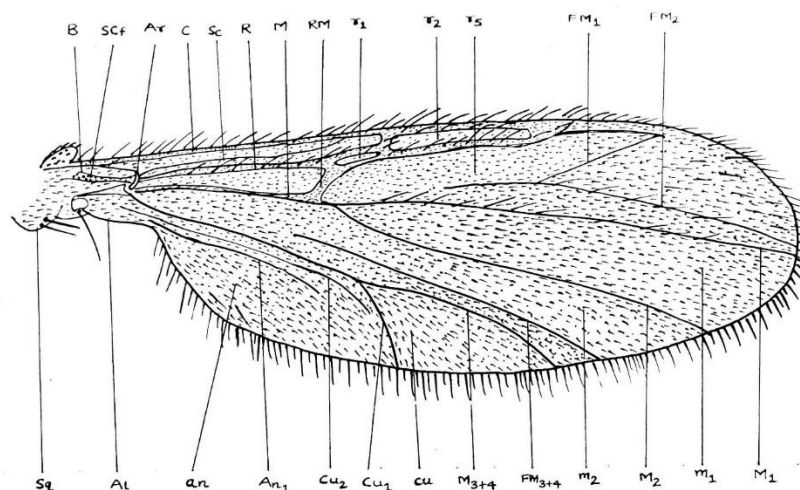


Plate 5. A generalized wind venation in *Ceratopogonidae*

5. Legs: Femora and tibia usually slender, with light brown, dark brown, light and dark patches, fore femur greatly swollen with 14 black, blunt spines (*Serrobezzia*), hind femur greatly swollen (*C. lalsinghi*), fore femur slender, with 2-3 black, blunt spines (Palpomyiini). Usually fore and hind tibial spurs present, while mid tibial spur branched and small (Culicoidini, Ceratopogonini) and absent in Sphaeromyiini. Width at the apex of tibia narrow or broad. Fore tibia with usually a comb and rarely absent; hind tibia with two combs, first comb composed with few long, thick spines, first spine usually longest, distal to spur, while in others second and third spine longest, second comb with numerous small, thin spines;

in some fore mid and hind tibial apex with 1 to 3 spine. First tarsomere's base with 1-3 spines and apex with 1 to 2 pseudo spurs. Palisade setae usually present on first four tarsomeres of hind leg, 3rd or 4th tarsomere cordiform, Ultimate tarsomere without spines (unarmed); fore mid and hind leg ultimate tarsomere with 5-7 pair of black, blunt spines (Sphaeromyiini), fore and mid leg with single pairs of sharp, ventral spines (*Stilobezzia* and *Parastilobezzia*). Claws generally small and equal, large and unequal in females of Ceratopogonini Stilobezziini, Sphaeromyiini and Heteromyiini, equal and small in male, Empodium present (Forcipomyiinae), poorly developed or absent in some.

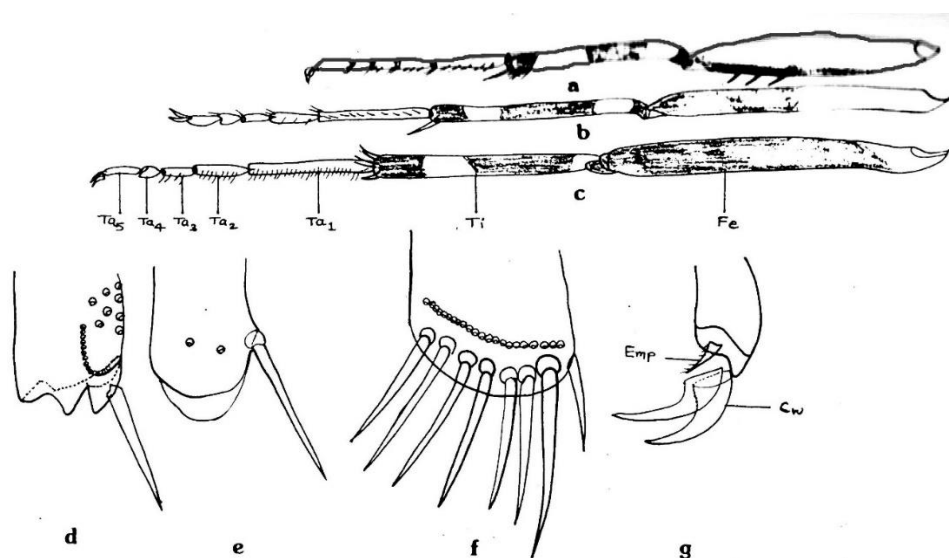


Plate 6. Legs of *Ceratopogonidae*: a. fore leg; b. mid leg; c. hind leg; d. fore tibial apex; e. mid tibial apex; f. hind tibial apex; g. apex of ultimate tarsomere

6. Genitalia: Female abdomen broad, balloon shaped, elongated, slender. IX tergum broad and narrow in some spp., with or without medioposterior notch. VIII and IX abdominal segment usually narrow to broad. Lateral margins of abdomen with globules and tuft of setae, internal sclerotized gland road usually absent, while present in *Palpomyiini* and *Stenoxenini*. Spermathecae usually 2 to 3 (*Leptoconopinae*), 1-3 (*Forcipomyiinae*, *Ceratopogoninae*), while 4 in *F. utteranchali* and *C. tetraspermathecae* *Dasyhelea* with usually one and in some two spermathecae, spatulate; oval, rounded, spherical and pears shaped, spermathecae situated in the VII, VIII and in IX abdominal segment, spermathecae with or without spermathecal neck, comma-shaped in *D. ramaensis* sp. Nov., spermathecal duct straight to convoluted, usually joining as a common duct opens in the spermathecal eminence, a sclerotized ring usually present in *Culicoidini*. Coxasternapodeme simple, poorly to well developed and often fuses with ramus, notum absent to poorly developed (*D. upiensis*). Gonapophysis VIII simple to divided in to two to three lobes namely dorso, median and ventral. Intergonocoxal membrane present in some. Gonostylus reduce in female *Leptoconops*. Transverse sternapodeme present in some groups. Spermathecal eminence is guarded by the comb like structure called Ktenoid in *Leptoconops* only. A membranous setose to bare

labia present in some spp. Post genital plate absent to reduced (*Leptoconops*), or well developed in other groups. Cerci elongated (*Leptoconops*) broad, rounded, foliate, semilunar in shape and setose in others.

In male, abdomen usually slender in some *Forcipomyia*, macrotrichia foliate. IX tergum broad at base, narrow at distal end, with or without notched, apicolateral processes present or absent, with or without distal seta. IX terga narrow to broad, with medioposteriorly shallow to deep caudomedian excavation. In *A. palisadi* caudomedian excavation almost circular. In some *Dasyhelea* IX sternum with two lateral and one median lobe. Gonocoxite elongated, tubular, lobular, cylindrical, slender and usually broad at base, narrow distally, inwardly curved superior volsella in *Dasyhelea* and *Palpomyiini*. Gonostylus usually simple, while bifurcate in *D. bifurcata*, trifurcate in *D. trifurcata*, gonostylus tip simple, pointed, rounded and hooked while megaseta in *Leptoconops*, crista dorsalis in *Stilobezziini*, *Palpomyiini*; presence of superior, median and inferior volsella variable in different spp. Subapical cerci usually present in *Culicoidini* and *Ceratopogonini*, absent in other groups. Parameres fused, separate, asymmetrical (*Dasyhelea*) symmetrical in other groups, parameres short to long, tubular, filamentous, foliate. Aedeagus triangular, rectangular, V-shaped, Y-shaped in different spp.

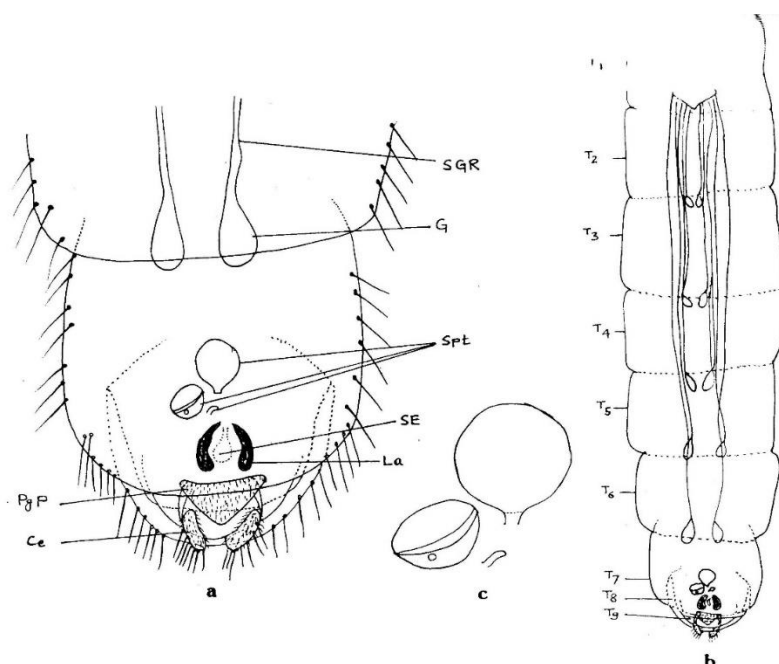


Plate 7. Female external genitalia of *Ceratopogonidae*: a. Abdomen with genital terminalia; b. female external genitalia; c. spermathecae

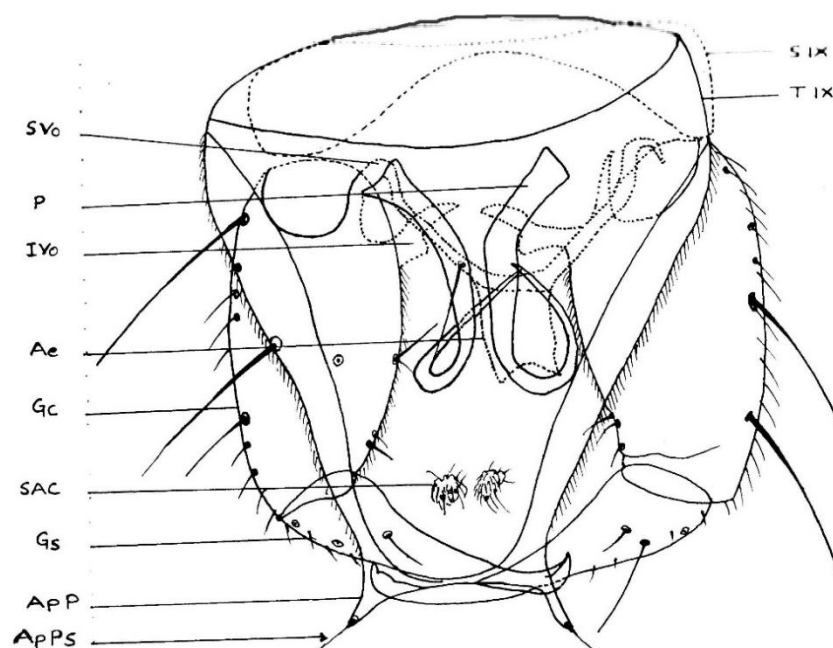


Plate 8. Male external genitalia of *Ceratopogonidae*

4. DISCUSSION

Family *Ceratopogonidae* (Diptera: Nematocera) was also subjected to a similar range of widely diversified morphotaxonomic character for the description of various subgroup including subfamilies, tribes and genera. This wide range of variable characters which included color pattern, sizes of the body, proportions of various morphological parts resulted in a great deal of confusion because color pattern and body sizes, as it now understand are not very reliable tools for taxonomic investigations. This is because these characters are subject to great deal of variations under the influence of ecological factors and hence not considered dependable for the authentic identification of the biting midges. In some cases the characters are considered for the identification of elements is not even belonging to the *Ceratopogonidae*. In course of time the emphasis from these characters shifted to more stable and reliable morphotaxonomic features like male and female external genitalia, armature and arrangement of wing venation. Thanks to the affords of Townes [11] and Moellar (1964-1966) who categorically proved that the color is unreliable in the determination of adult biting midges and further stated that the specimen developing at low temperature have greater amount of dark pigmentation than those developing at higher temperature. Prior to Townes [11] Goetghebuer and Lenz (1936-1962)

had already started employing the characteristic of male genitalia for the identification of adult *Ceratopogonidae*, but they did not realize the importance of these character, for separating different subfamilies, tribes and genera. They use the male external genital characters only for delineating the biting midges at the specific level. The recent workers such as Wirth and Hubert [10] and Borkent (1989-1999) has published a series of publications on the biting midges and used the character of the male genitalia in their identification. As far as the characters of female genitalia are concerned, they have used only the number of spermathecae in the delineation of different taxa. In the present work we have used more than 25 characters of female external genitalia and found that these are the most reliable tools in the systemic work.

5. CONCLUSION

Morphology of the *Ceratopogonidae* has not greatly advanced during last few years. It has been observed that the colour pattern of the body as well as comparative size of the various parts is not a reliable tool in the determination of the independent status of the species. Maheshwari and maheshwari [12] and [8] has given major emphasis to the male and female genitalia of the *Ceratopogonidae* in the identification of the species. They considered gonopophysis VIIIth and IXth as a significant tool in the determination

of cryptic species. It is to be hoped that microcharacter of female genitalia shall resolve some of the puzzling problems concerning the status of the subgenera and subgroups of the biting midges. In the present investigations major emphasis has been given to the most reliable tool of taxonomic importance with special reference to the external genitalia of male and female and it is hoped that it will resolve the problem related to the taxonomic character up to a considerable level.

ACKNOWLEDGEMENT

We are thankful to Dr. Girish Maheshwari, former Professor and Head, School of Entomology, St. John's College Agra for reading the MS of this paper and suggestions. We are grateful to Prof. Anurag Shukl, Principal, Agra College Agra for facilities and encouragement.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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