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The Shape Matters: Morphology of Male Genital Variations in the Large Carpenter Bees (Hymenoptera:Apidae) of Karnataka

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Abstract

Large carpenter bees (genus *Xylocopa*) are prominent members of the Indian bee fauna. The genus is represents 45 species in the Indian region. The study was undertaken to know the male genital variations among nine species of *Xylocopa* recognized from Karnataka including *X. (Biluna) nasalis, X.(Ctenoxylocopa) fenestrata, X.(Koptortosoma) hafizii, X.(Koptortosoma) pubescens, X.(Koptortosoma) ruficornis, X. (Mesotrichia) latipes, X.(Nodula) amethystina, X. (Nyctomelitta) tranquebarica* and a probable new species *X.(Zonohirsuta)* sp.1.

Bees are a large group of insects that are specialized for feeding at flowers and gathering nectar and pollen. They play an important role in the life of flowering plants by participating in their sexual reproduction. For some crops, wild bees are more effective pollinators on a per visit basis than honey bees and functionally complement the dominant visitor. There are 16,325 species of bees in the world, grouped under 425 genera, in the division Apiformes under the super family Apoidea (Michener, 2007). Species composition amongst different families of bees, Apidae constitutes 57 per cent. The Xylocopini is one of four tribes in the subfamily Xylocopinae, the other three - Allodapini, Ceratinini, and Manueliini are mostly smaller than the Xylocopini. Hurd and Moure (1963) in their revision of the tribe Xylocopini recognized three genera: *Lestis* Lepeletier & Serville, *Proxylocopa* Hedicke, and *Xylocopa* Latreille. In a cladistic analysis of the genera and subgenera based on parsimony analyses of morphological characters, Minckley (1998) concluded that the genera *Lestis* and *Proxylocopa* should be considered as subgenera of *Xylocopa* to avoid paraphyly of this genus. The large carpenter bees belong to genus *Xylocopa* and are cosmopolitan in distribution. It consists of 469 species in 51 subgenera in the world (Michener, 2007). These are prominent members of the Indian bee fauna. Gupta and Yanega (2003) recorded 45 species and subspecies under 11 subgenera in the Indian region. These are robust, fast-flying, some of which are among the largest of all the bees. As their name implies, carpenter bees excavate nest galleries in woody plant material, including dead branches, stalks, stumps, and structural timbers of buildings, hollow culms of bamboo or pithy stems. Members of subgenus *Proxylocopa* construct their nests underground. (Hurd and Moure, 1963; Gerling *et al.,* 1989). The nesting behavior ranges widely from solitary to social, which makes them of interest to behavioural ecologists because of their utility for studies of social evolution (Gerling *et al.,* 1989). Carpenter bees are also important for investigating the evolution of mating systems, because a variety of mating strategies have been found in the group. Some particular mating strategies seem to be correlated with morphological adaptations and are found in a limited number of subgenera (Leys, 2000).

Male genitalia are widely recognized as being the most variable and divergent of all morphological structures. Male genital structures are considered as important diagnostic traits in insect systematics, and there are entire groups the classification of which is based solely on the structure of male genitalia. This study represents the first description of male genital structures of the Indian large carpenter bees, to understand the genital variations among different species of *Xylocopa*. Cladistic analysis of the phylogenetic relationships by using genital characters coded for nine species of the ingroup, and to identify traits that could be used in the systematics of the genus *Xylocopa* were also attempted.

Specimen collections were made from different parts of Karnataka. Male genitalia were dissected by treating with 10 per cent KOH for 24 hours and were

transferred to test tubes containing 70 per cent ethyl alcohol. The different skeletal parts were separated under a stereo-binocular microscope and placed on a cavity block with glycerol. Genitalia of each species were stored in small eppendorf tube containing a few drops of glycerol for subsequent studies. Illustrations of genitalia were photographed using Leica 205A microscope mounted with camera Leica DFC450, plates were prepared in Adobe Photoshop CS5.The terminology used in this work is that proposed by Maa (1938), Hurd and Moure (1963), Minckley (1998) and Michener (2007). The various parts and morphological terms of male genitalia were illustrated in fig. 1. Preliminary attempts were made to construct a phylogenetic tree to reveal the patterns of genitalia evolution across the species based on the morphology of genitalia using binary character across nine species of the genus *Xylocopa* and by fixing the small carpenter bee, *Ceratina (Pithitis) binghami* as out group taxon. In a large group, such as the Xylocopini, it seemed reasonable to expect that some characters homoplasious within some clades will serve as synapomorphies of other clades. Parsimony was used as the optimality criterion. All searches were completed in NONA (Goloboff, 1999) spawned from Winclada (Nixon, 2002). All the cladograms were generated using Winclada. For this analysis the matrix developed using character states (Table. I and II) of 9 species and 12 characters of *Xylocopa* were processed using Winclada. A heuristic search was carried out holding a maximum of 1000 trees in memory, with 1000 replications and 10 trees to hold per replication, in random addition sequence. The strict consensus tree of large carpenter bees had 36 steps, a consistency index of 0.54 and a retention index of 0.60. The analysis of taxa of the carpenter bees produced seven equally parsimonious trees. Only those branches with Bootstrap and Jack-knife values above 50 were considered and are indicated above the branches or nodes.

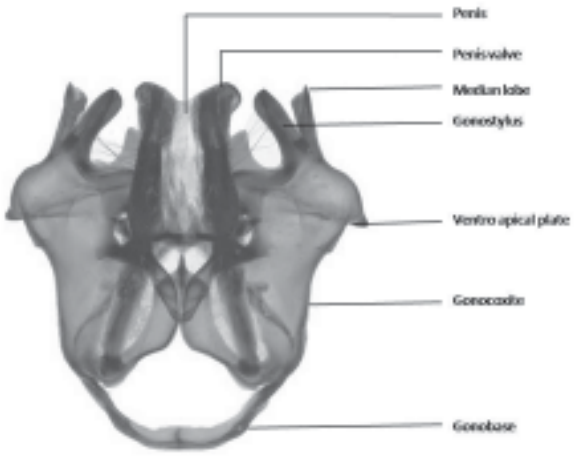

Fig. 1 : Structure male genitalia of large carpenter bee (*Xylocopa (Koptortosoma*) r*uficornis* - Dorsal vies)

Phylogenetic reconstruction of the genital variations in large carpenter bees (Fig. 2) revealed that using *Ceratina binghami* as the outgroup, *Xylocopa* proved to be monophyletic. Subgenera *Ctenoxylocopa* and *Mesotrichia* are monophyletic and the synapomorphies defining the nodes are inner margin of the gonocoxite nearly parallel and hairs on base of the penis valve absent. *Ctenoxylocopa* (Fig. 5-6) is separated from all other subgenera by genital capsule wider than long. Subgenus *Koptortosoma* is monophyletic with three species *X. hafizii, X. pubescens, X. ruficornis*. The synapomorphies that unite these three species are genital capsule narrower at base and gonostylus with slender lobe like projection. *X. ruficornis* (Fig. 15-16) is separated from *X. hafizii* and *X. pubescens* by median lobe on gonostylus arising from base. *X. pubescens* (Fig. 13-14) is separate from *X. hafizii* (Fig. 7-8) by presence of setae on apex of gonostylus and ventro apical plate of gonocoxite being weakly carinate. Subgenus *Nyctomelitta* has only one taxon *X. tranquibarica* (Fig. 17-18) with genital capsule shape almost subequal and gonostylus with sharp spine like projection. It gets separated from other groups by the ventroapical plate of gonocoxite strongly carinate and presence of hairs on base of the penis valve. The subgenus *Zonohirsuta* (Fig.19-20) is separated from the *Biluna* and *Nodula* by the penis valve being slender and parallel. *Biluna* (Fig. 11-12) is separated from *Nodula* (Fig. 3-4) by absence of short spine like projection on gonostylus and genital capsule shape narrower at base. This study strongly demonstrates that the male genital characters of *Xylocopa* species can be considered species specific since the combination of the shape and size variations and the differences in individual genital components is unique for each species.

TABLE I

Characters and character states

Characters	Character states		
	0	1	2
Genital capsule size	Longer than wide	Wider than long	Nearly subequal
Genital capsule shape	Narrower at base	Nearly subequal	-
Gonostylus at apex of gonocoxite	Slender	Rounded or conical	-
Gonostylus with projection	Absent	Present(short spine like)	Present (slender lobe like)
Setae on apex of gonostylus	Absent or very few	Present (less dense)	Present (more dense)
Median lobe on gonostylus	Absent	Present arising from apex	Present arising from base
Inner margin of gonocoxite	Diverging from the base	Weakly parallel	-
Penis	Entirely membranous	Basal half sclerotized	-
Ventoapical plate of gonocoxite	Absent	PresentWeakly carinate	Present strongly carinate
Penis valve	Apically expanded	Slender and parallel	-
Lateral edge on genital capsule	Absent		
Hairs on base of the penis valve	Absent		

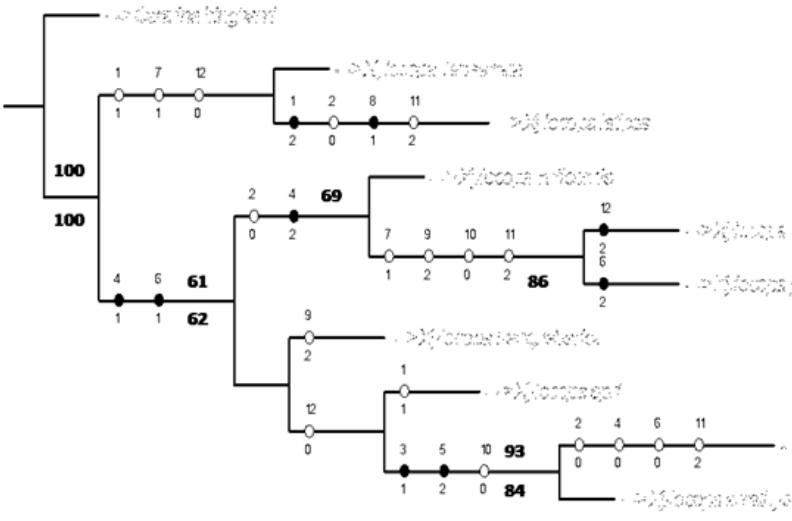

Figure 2. Strict consensus cladogram of most parsimonious t genital variations in large carpenter bees. Filled circles re characters; open circles, homoplastic characters. Number above and Jack-Knife support values respectively. (values below 50 % n

Fig. 2 : Strict consensus caldogram of most parsimonious tree showing patterns of genital variations in large carpenter beed. Filled circles represent non-homoplastic characters ; open circles, homoplastic characters. Numbers above and below) are bootstrap and Jack-Knife support values respectively. (values below 50% not shown)
CI - 54, RI-60

TABLE II

Data matrix used in the phylogenetic analyses

Species	1	2	3	4	5	6	7	8	9	10	11	12
Ceratina (Pithitis) binghami	0	1	0	0	0	0	0	0	0	1	0	1
X. (Biluna) nasalis	0	0	1	0	2	0	0	0	1	0	2	0
X.(Ctenoxylocopa) fenestrata	1	1	0	0	0	0	1	0	1	1	0	0
X.(Koptortosoma) hafizii	0	0	0	2	0	1	1	0	2	0	2	1
X.(Koptortosoma) pubescens	0	0	0	1	1	1	0	0	1	1	0	1
X.(Koptortosoma) ruficornis	0	0	0	2	0	2	1	0	2	0	2	1
X. (Mesotrichia) latipes	2	0	0	0	0	0	1	1	0	1	2	0
X.(Nodula) amethystina	0	1	1	1	2	1	0	0	1	0	1	0
X. (Nyctomelitta) tranquebarica	0	1	0	1	1	1	0	0	2	1	1	1
X.(Zonohirsuta) sp.1	2	1	0	1	1	1	0	0	1	1	1	0

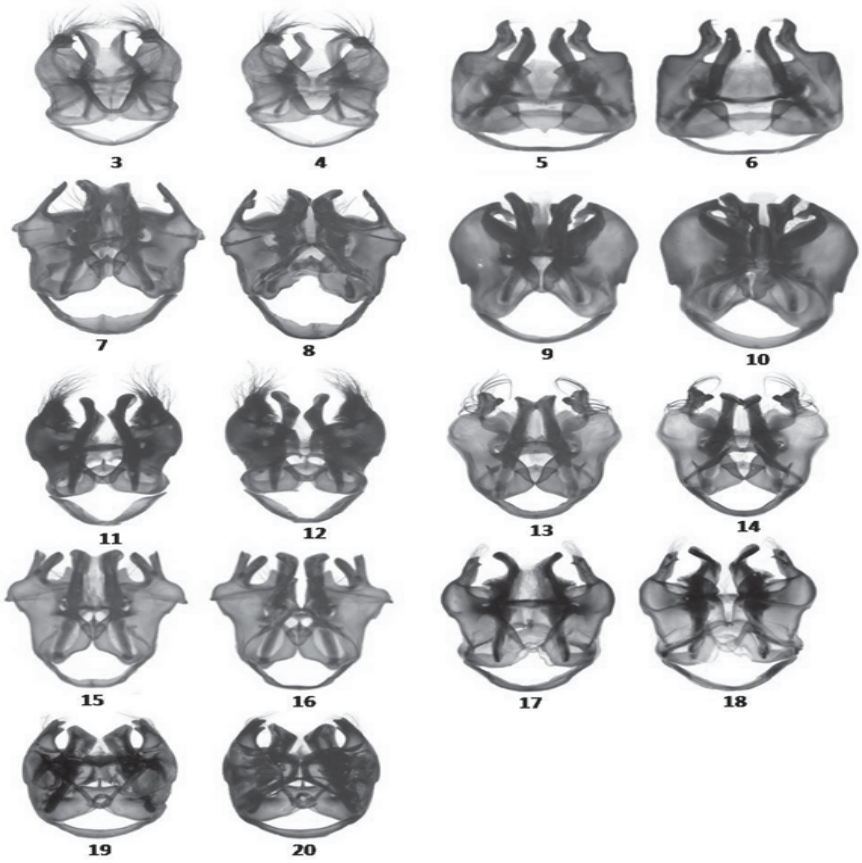

Fig. 3-20 : Genital capsule of *Xylocopa species* (dorsal and ventral view respectively)
3-4: X. (*Nodula*) *amethystina,* 5-6 (*Ctenoxylocapa*) *fenestrata,* 7-8 X (*Kotortosoma*) hafrzii, 9-10. X. (*Mesotrichia*) *latipes,* 11-12. X. (*Biluna) nasalis,* 13-14. X. (*Koptortosoma*) pubescens, 15-16. X. (*Koptorlosoma*) *ruficornis,* 17-18 X. (*Nyctomelitta*) *tranquebarica,* 19-20. X (*Zonohirsata*) sp.1

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