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Morphology of Female Genitalia of *Xylotrechus basifuliginosus* Heller, 1926 (Coleoptera: Cerambycidae: Clytini)

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Female genitalia have been largely neglected in studies of genital evolution, perhaps due to the long-standing belief that they are relatively invariable and therefore taxonomically and evolutionarily uninformative in comparison with male genitalia. In the present study, the female genital structure of lesser known beetle, *Xylotrechus basifuliginosus* was examined for the first time for morphological descriptions using advanced microscopy techniques and dissection methods and compared with its female congener, *Xylotrechus smei* (Castelnau & Gory). In X. basifuliginosus, the tergite and sternite are generally equal in length. Fully extended genitalia is 3.78mm long from the proximal edge of 9th segment and 0.37mm at its widest.8th abdominal segment is 0.65mm long, 0.36 mm

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wide whereas eighth abdominal segment is 1.2 times longer than wide, apico-lateral margin, covered with intermix of few short and long setae in X. smei. The genital pocket is 1.36 mm long-scissor like, cylindrical in shape, strongly sclerotized. Spermatheca duct is reduced, “J” shaped just like the genera of *Chlorophorus* and *Plagionotus* spp. Spiculum gastrale is 1.34 mm long, thin, slightly thickened at tip and almost three times the length of sternite 8 and ovipositor is 2.19 mm long, 0.85 mm wide, elongated and gradually narrows down to posterior end in *X. basifuliginosus* whereas in *X. smei,* spiculum gastrale is 3.43 mm long; ovipositor is with distinct paraproct, gonocoxite and gonostylus and surface of gonostylus is with few setae.

Keywords: Cerambycidae; beetle; Xylotrechus; morphology; genitalia.

1. INTRODUCTION

“The genus *Xylotrechus* Chevrolat, 1860 is Holarctic in origin and richest genus in Southeast Asia with at least 180 species of the genus *Xylotrechus* known across the globe [1]). *Xylotrechus basifuliginosus*is black coloured beetle (15 mm), which inhabits moist temperate forests of Chakrata hills (Dehradun district) in Garhwal region of Uttarakhand state and Chopal (Shimla) and Dharamsala (Kangra) both in Himachal Pradesh state, in the Indian Western Himalaya between ~1500-3000m” [2]. “The larval habits and host plants preferences of *Xylotrechus* spp*.* are various and the major hosts include the genera *Quercus* (*Quercus semecarpifolia* Sm.), *Picea smithiana* (Wall). ,*Pinus* sp. and *Salix* spp.” [3,4]. “*Xylotrechus basifuliginosus* is an important secondary borer which not only causes oak tree mortality in *Q. semecarpifolia* but also considerably deteriorates the quality of timber and wood” [5,6].

“Most studies on the taxonomy of longhorn beetles (Cerambycidae) focus on external morphological characters. However, morphological identification is more correctly based on the differences in male genitalia but research on external female genitalia in the *Xylotrechus spp.* is rare”. [7] “The male genitaia of *Xylotrechus basifuliginosus* and compared it with its congeners (*X. smei, X. chinensis, X. clarinus*, *X. cuneipennis* and *X. pyrrhoderus*)” [8]. “There were only a few descriptions of male or female genitalia for this family when new species were described or revisions were made” [9,10,11,12]. “In the majority of members of the family *Cerambycidae,* the tergite and sternite of the 8th abdominal segment are combined to form a flattened segment” [13,14-16]. “The tergite and sternite are generally equal in length and the apical margin generally contains setae along each side, or alternatively can be found on the lateral angle near the apical margin of the setae plexus. Morphological variation of the 8th abdominal segment is extremely variable in different species of cerambycids. Therefore, the present study for the first time describes the details of external structure of female genitalia of *X. basifuliginosus* and compared with *X. Smei* which was already described” [17].

2. MATERIALS AND METHODS

21 individuals of female *X. basifuliginosus* were handpicked from *the Q. semecarpifolia* forest in Deoban Reserve Forest (N-30.74806; E-77.86639; 2600-2815m; Fig.1), Chakrata Forest Division during June 2020. Among 21 individuals 5 specimens were studied by immersing in 75% alcohol until the apical part was dissected. Apical part of the abdomen was cut off from the body and was put in 10% KOH for overnight to soften the chitin and dissolve away the muscles and other unwanted parts. After macerated, the connections between the tergite and sternite were cut off and finally the genitalia were separated completely from the tissues and sclerites. The material was then properly washed in distilled water followed by ethyl alcohol treatment in a dehydration series of 30%, 50% and 70%, and absolute alcohol for 5 minutes at each step. Than material was cleared in xylol and mounted it Canada balsam on slides and photos of genitalia were taken under microscope. Morphological terminology for the female genitalia of the *Xylotrechus* follows [18,19,20] Photographs of genitalia were taken under OlympusSZX16 stereoscopic microscope and measurements taken with Digimizer 4 image analysis software.

Specimens and slides of female genitalia of *Xylotrechus* spp. preserved at Entomology Branch, Forest Research Institute, Dehradun.

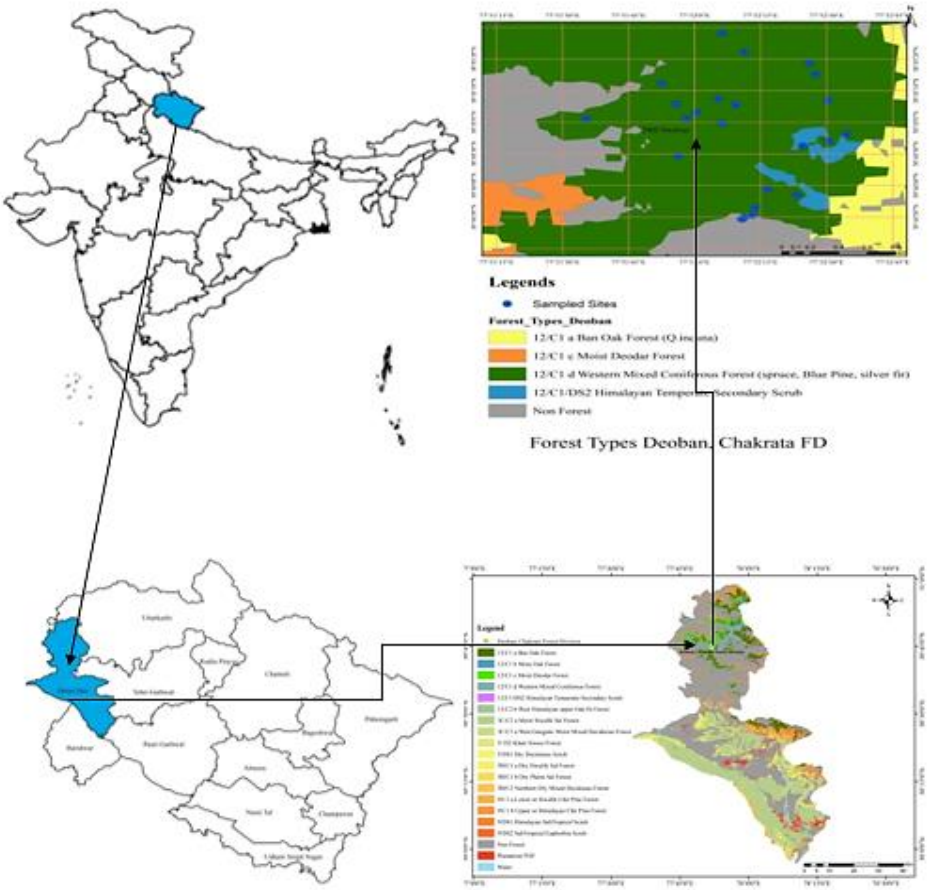

Fig. 1. Map of India depicting Uttarakhand state, Dehradun district along with the study area, Deoban Reserve Forest (2815m) in Chakrata Forest Division

3. RESULTS AND DISCUSSION

3.1 Morphology of Female Beetle

Female beetles are 12-19mm in length and 4-6 in width; black to dark brown with grey pubescence on the head, thorax and abdomen region (Fig. 2). Elytra are dark brown and covered with grey pubescence, with three yellow coloured transverse bands in female. These bands are present on the sub-basal, median and sub-apical area of elytra, while the elytra is tapered at the end. Eyes are faceted and emarginated in the head. Face with a more or less distinct median carina. Between each carina is an elongated, very finely rugulose, opaque black area with slightly raised edges. Mandibles are triangular, black, short, pointed and posses short brown setae. Head and pronotum is seperated by pale band. Antennae is 11 segmented in both the sexes, filiform type, black coloured, inserted in the middle of the frons and measures less than the half of the body length. The antenna are attached to the head capsule by a large basal membrane which is often regarded as the 'basal segment'. Prothorax is rounded, widest behind the middle and narrows gradually in front. Pronotum is dark brown colour and densely covered with grey pubescence. Abdominal sternites are densely covered with white/yellow & black alternate bands covered with long erect hairs. Legs are hairy, black to brown in colour, strong and moderate size.

Femur of foreleg and midleg is stout and longer than tibia. Shape of hind leg is slender and slightly shorter than tibia. Fore, mid and hind tibia have tibial spurs. Spur of hind tibia is long. Tarsus is four segmented. First meta-tarsomere is longer than the total length of remaining tarsomere and twice as long as 2nd and 3rd combined of remaining tarsomeres. The last tarsal segment has pair of claws. Hind femora nearly reaches but does not exceed the elytral apics.

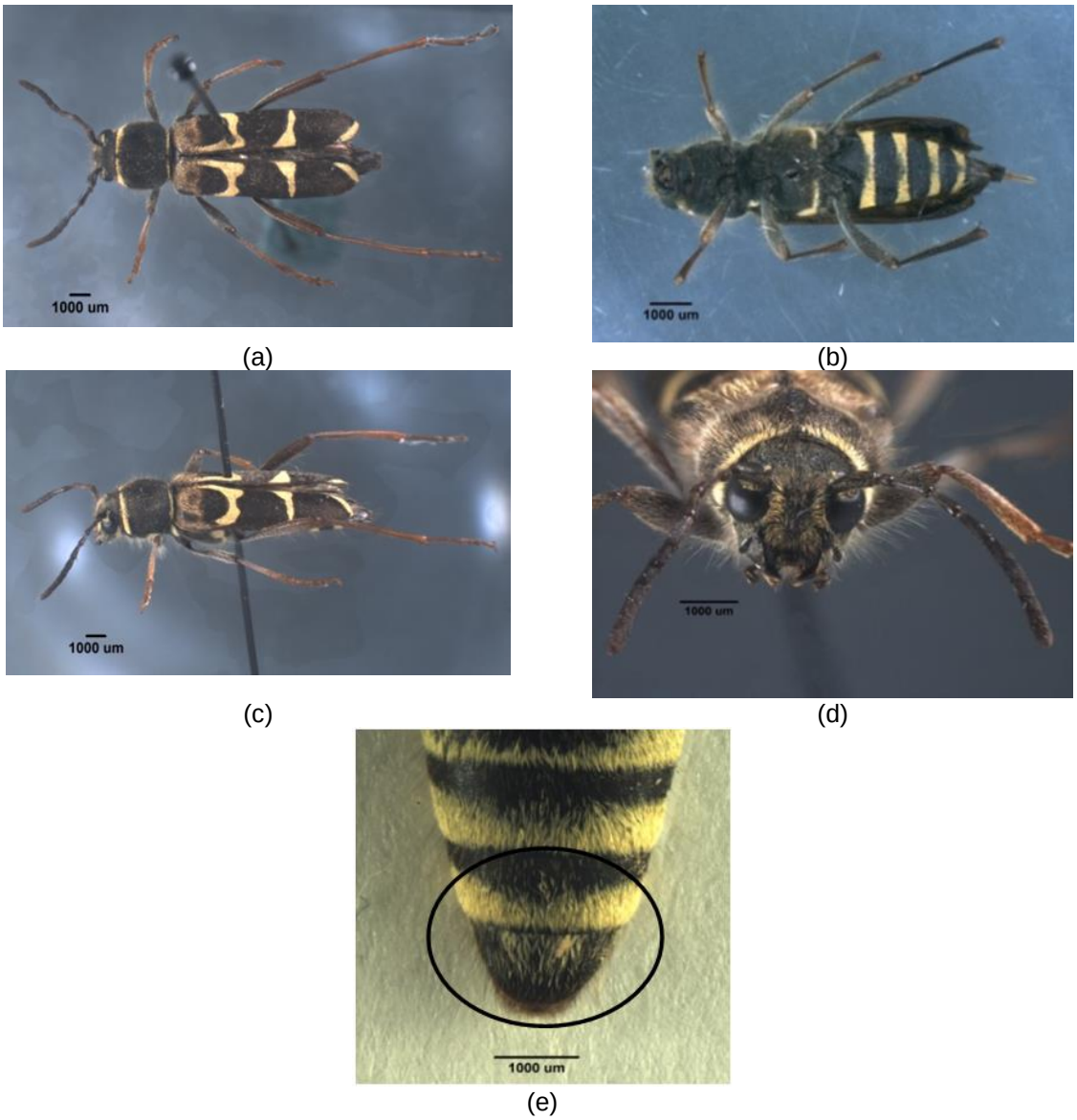

Fig. 2. Morphology of female Xylotrechus basifuliginosus:(a) Dorsal view; (b) Ventral view; (c) Lateral view; (d) Frontal view; (e) Abdominal sternites (circle indicating the part taken for genital study) (Photos taken by Gaurav Chand Ramola)

3.2 Female Genitalia of *Xylotrechus. Basifuliginosus*

Female genitalia of *X. basifuiginosus* is a long, tubular structure formed by tergite and sternite of the 8th abdominal segment combined to form a flattened segment (Fig. 3a). The tergite and sternite are generally equal in length and the apical margin generally contains setae along each side. Inside the apical margin of the 8th abdominal segment there is an inward fold, which extends to the body cavity to form a genital pocket. The fully extended genitalia is3.78mm long from the proximal edge of 9th segment and 0.37mm at its widest. 8th abdominal segment is 0.65mm long, 0.36 mm wide and apical margin generally contains sparsely distributed setae equal in length (Fig. 3b).

The apical opening of 8th sternite is the posterior opening of the genital pocket which varies from species to species. The genital pocket is 1.36 mm long-scissor like, cylindrical in shape, strongly sclerotized in which basal half portion that is broad and flattened membranous and stretches from the apex of 8th abdominal segment to the basal ovipositor and connects with the ovipositor by membrane. There are

twlique palpi at the posterior portion which are separated (Fig. 3a). Spermatheca duct is reduced, “J” shaped just like the genera of *Chlorophorus* and *Plagionotus* spp.and connected laterally with spermatheca capsule responsible for receiving, maintaining, and releasing sperm to fertilize eggs(Fig.3b).The spiculum gastrale is 1.34 mm long, thin, slightly thickened at tip and almost three times the length of sternite 8 and connected to sternite 8 by a loose, slightly sclerotized membrane connecting it with the base of the spiculum gastrale and moves freely in species like *Purpuricenus temminckii* (Guérin-Méneville).

The ovipositor is 2.19 mm long, 0.85 mm wide, elongated and gradually narrows down to posterior end which helps female beetle to pass down the egg for laying (Fig. 3d). Two palpi are located near the apex. The proctiger is rather short or absent and the pair of baculi are located on each side. Ovipositor contains pair of baculi on each side which extends from posterior of the coxite to the base of the ovipositor. The paraproct and coxite are fused to form a flattened sclerite at the base. The coxite is 0.44mm in length, cylindrical with sensory setae on it. The apex of the coxite is cylindrical and its outer part is generally swollen. The stylus is 0.36 mm in length with long sensory setae located on the tip of coxite (Fig. 3e).

3.3 Female Genitalia of *Xylotrechus smei*

“In case of female genitalai of *X. smei*. eighth abdominal segment is 1.2 times longer than wide, apico-lateral margin, covered with intermix of few short and long setae. Spiculum gastrale is 3.43 mm long; ovipositor is with distinct paraproct, gonocoxite and gonostylus and surface of gonostylus is with few setae” [17].

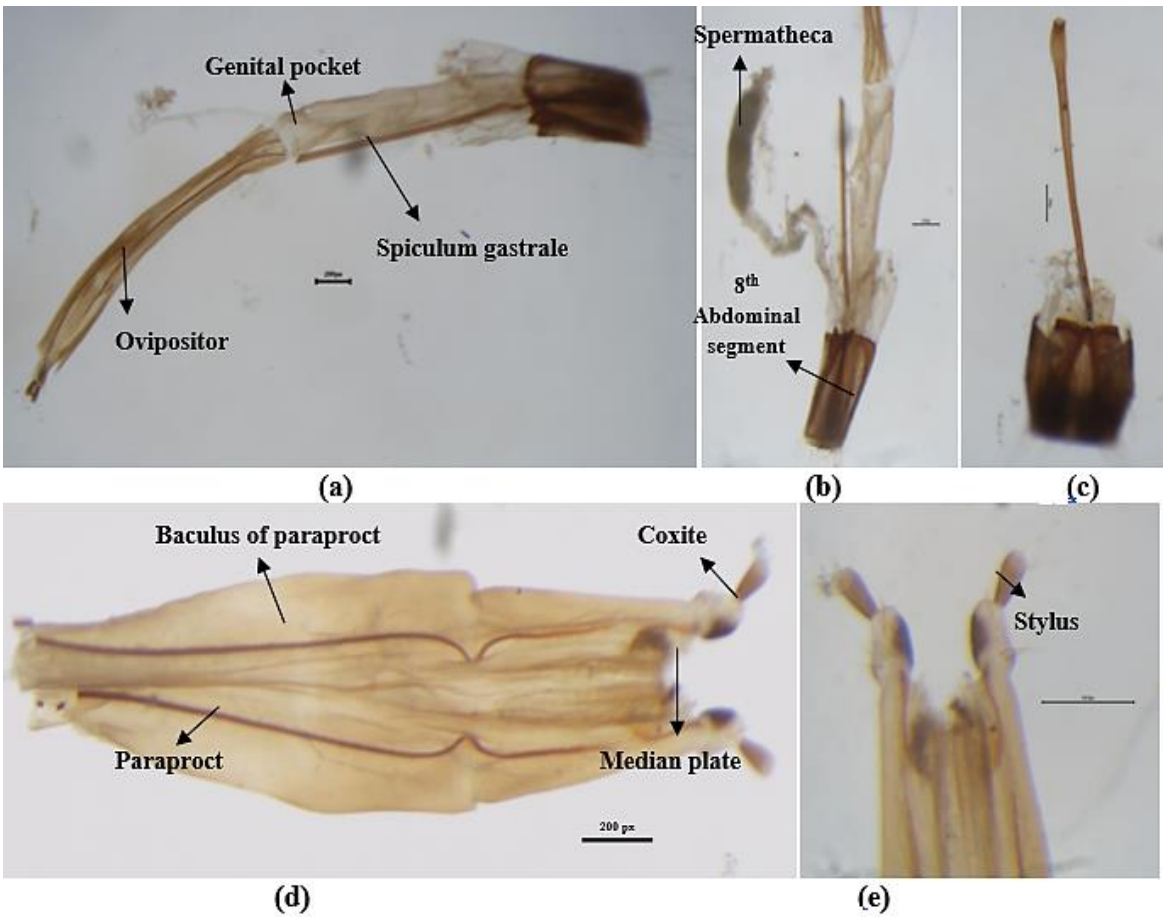

Fig. 3. Female genitalia parts of *X. basifuliginosus*: (a & b) Female genitalia; (c) Spiculum gastrale; (d) Ovipositor; (e) Apical region of ovipositor (Photos taken by Gaurav Chand Ramola)

4. CONCLUSION

This study on the female genitalia of *X. basifuliginosus* provides critical insights into the morphological variations and their potential implications for taxonomy, phylogeny, and species identification within this diverse beetle family. Unlike male genitalia, which can exhibit significant variation even within a species due to sexual selection pressures, female genitalia tend to be more conserved and less variable. This stability makes them excellent characters for systematic and taxonomic studies. By meticulously examining and documenting the genital structures of multiple species, we have highlighted the significance of these characteristics in distinguishing between closely related species and understanding evolutionary relationships.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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