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Research Article
Antennal Sensilla-types and Distribution in Two Species of Genus *Creatonotos* Hübner (Arctiidae: Lepidoptera)

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Abstract

Background and Objective: The different morphological types of sensilla and their distribution on antennae in two species i.e., *Creatonotos gangis* (Linnaeus) and *Creatonotos transiens* (Walker) (Arctiidae: Lepidoptera) have been scanned and studied in detail. **Materials and Methods:** The adult moths of two species were collected from Himachal Pradesh and identified as *Creatonotos gangis* (Linnaeus) and *Creatonotos transiens* (Walker) referable to family Arctiidae. After following proper procedure, the SEM Studies were carried out in the Instrumentation Centre, Punjabi University, Patiala. **Results:** In *Creatonotos gangis* (Linnaeus), 8 morphologically different types of sensilla had been found on antennae namely sensilla basiconica, böhm's bristles, sensilla campaniformia, sensilla chaetica, sensilla coeloconica, sensilla quamiformia, sensilla trichodea and uni-porous peg sensilla whereas In *Creatonotos transiens* (Walker), 5 types of sensilla were present on antennae such as sensilla auricillica, sensilla basiconica, böhm's bristles, sensilla chaetica and sensilla trichodea. In *Creatonotos gangis* (Linnaeus) 3 subtypes of sensilla trichodea and 2 subtypes of sensilla chaetica were present whereas, 4 subtypes of sensilla trichodea were found in *Creatonotos transiens* (Walker). **Conclusion:** The presence of distinct types and subtypes of sensilla on different parts of antennae in both the species has been examined and it is concluded that their presence is found to be species specific.

Key words: *Creatonotos*, *gangis*, *transiens*, sensilla, antennae, SEM, Arctiidae

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Insects, particularly moths, possess antennae which are usually filiform, pectinate or bi-pectinate. These are packed with minute sensory structures called sensilla which play an important role in their behaviors during their entire lifespan and also play an important role in mating, host orientation, oviposition etc[1]. Byers and Wigglesworth observed that the antennae are the primary olfactory organs in insects[2,3]. In moths, the volatile cues are detected by olfactory sensory neurons (OSNs) enclosed in sensilla distributed across the antennal surface[4,5]. Traditionally, sensilla have been classified on the basis of morphology and location. In Lepidoptera, there are ten different types of sensilla namely sensilla auricillica, sensilla basiconica, böhm's bristles, sensilla campaniformia, sensilla chaetica, sensilla coeloconica, sensilla squamiformia, sensilla styloconica, sensilla trichodea and uni-porous peg sensilla recognized on antennae. All these sensilla have peculiar structures.

The SEM studies have been extensively carried out on insects referable to orders Diptera and Coleoptera but only few scattered works are there on order Lepidoptera. The present work has been initiated with the aim to explore the ultrastructure of sensilla present on antennae. Scanning electron microscopic studies had been carried out to examine these ultra-structures on antennae of two species of genus *Creatonotos* Hübner referable to family Arctiidae. According to Elkinton and Cardé elaborate antennae may provide an increased sensitivity to the pheromone by increasing the "active space" of the signal[6]. Antennal sensilla also play an important role in sexual dimorphism. In the present studies, the location and structure of sensilla on antennae of these species has been described and illustrated in detail.

MATERIAL AND METHODS

Collection and preservation: The adult representatives of family Arctiidae were collected in the month of September, 2017 from Mandi District of Himachal Pradesh with the help of light trap. The collected material was killed with ethyl acetate. The freshly killed specimens were pinned, properly stretched and preserved for further studies. Each specimen was tagged with field data such as locality, date of collection and name of collector.

Identification and consultation of literature: The identification was done with the help of relevant literature i.e., Hampson[7] and Singh[8]. The identification was also confirmed by making comparison with the identified collections lying in the Department of Zoology and Environmental Sciences, Punjabi University, Patiala. An exhaustive search of Biological abstracts and net surfing led to the collection of large number of references and procurement of research papers.

Preparation of samples and procedure for SEM studies: To prepare samples for Scanning electron microscopic studies, the desired parts i.e., antennae of preserved specimens were carefully detached with the help of forceps. The sample material was washed with water and detergent to remove the debris.

Fixation: The material was fixed in 2.5% glutaraldehyde and then transferred into phosphate buffer solution (PBS) with pH 7.4 overnight at 4°C. After that the material was kept in 10% KOH for 30 min.

Dehydration, mounting and sputtering: After washing, the material was dehydrated in graded alcohol. After proper dehydration, the material was mounted in aluminium stubs with double sided sticky carbon adhesive tape and sputter coated with gold.

Scanning: The sputtered material was observed under Scanning Electron Microscope (JEOL) JSM-6100 in the Instrumentation Centre, Punjabi University, Patiala. Subsequently, the material was photographed according to the distribution of sensilla and other ultra-structures.

Terminology: The sensilla were classified according to the nomenclature systems of Schneider[1] and Zacharuk[9]. The terminology used by Zheng *et al.*[10] has been followed in the present study.

RESULTS

Type species: *Creatonotos interruptus* Linnaeus.

Distribution: Throughout India, China, Java, Myanmar and Sri Lanka.

Remarks: This genus is represented by three species i.e., *gangis* (Linnaeus), *transiens* (Walker) and *ananthakrishanani* Kirti and Kaleka from India. In the present studies, scanning electron microscopy has been performed on antennae of two species of this genus.

Fig. 1(a-f): Antenna of *Creatonotos gangis* (Linnaeus), (a) Scape, (b) Pedicel and (c-f) Flagellum

On antenna of *Creatonotos gangis* (Linnaeus): Eight types of sensilla are found on antenna. The different morphological types of sensilla on the antennae of both the species are summarized in Table 1 (Fig. 1a-f).

Scape

Sensilla Basiconica: Two sensilla basiconica are found on the surface of scape. Each sensillum is with rod-like morphology having blunt tip and without basal socket.

Böhm's bristles: These bristles are present on the surface of scape. These are small spine-like structures with blunt tips, rising out of sockets. These are quite less in number.

Sensilla chaetica: Large numbers of sensilla chaetica are found on the surface of scape. These are almost similar to sensilla trichodea but with broader base, set in sockets and can be identified by their thick walls. Each sensillum is long in size with blunt tip and without longitudinal lines on its surface.

Table 1: Summarizing different morphological types of sensilla on antennae of *Creatonotos gangis* (Linnaeus) and *Creatonotos transiens* (Walker)

	Scape		Pedicel		Flagellum	
	Creatonotos gangis	*Creatonotos transiens*	*Creatonotos gangis*	*Creatonotos transiens*	*Creatonotos gangis*	*Creatonotos transiens*
Sensilla auricillica		✓				
Sensilla basiconica	✓	✓		✓	✓	✓
Böhm's bristles	✓	✓	✓	✓		
Sensilla campaniformia			✓			
Sensilla chaetica	✓	✓	✓	✓	✓	✓
Sensilla coeloconica					✓	
Sensilla squamiformia	✓		✓			
Sensilla styloconica						
Sensilla trichodea	✓	✓		✓	✓	✓
Uni-porous peg sensilla					✓	

Sensilla squamiformia: Single sensillum squamiformia is located on the scape. The surface of sensillum looks like scale but narrower with longitudinal lines. It has a basal socket. No pore is found on surface of this sensillum.

Sensilla trichodea: These sensilla are present on the surface of scape and less in numbers. Each sensillum is long in size with pointed tip. There is no basal socket present. Not all, but some of them are with slightly curved distal end.

Pedicel

Böhm's bristles: These are found on the surface of pedicel in large numbers. Each bristle is small spine-like structure with basal socket.

Sensilla campaniformia: Single sensillum is found on the pedicel. It has cap-shaped structure and is situated inside tight socket.

Sensilla chaetica: Sensilla chaetica are also present on the pedicel. Each sensillum is with pointed tip and having a basal cavity. Some of these are slightly curved from distal end. These are very less in number.

Sensilla squamiformia: These sensilla are found on the pedicel and only two in number. Each sensillum has scale-like appearance with pointed tip. No pore is found on its surface.

Flagellum

Sensilla basiconica: These are randomly distributed on the flagellomeres. Each sensillum has blunt tip and rod-like morphology and is without basal socket. The number of sensilla basiconica is less in comparison to sensilla trichodea. Single sensillum is also found near the joint between flagellum and pedicel.

Sensilla chaetica: These sensilla are found on lateral margins of the flagellum. Each sensillum has arc-like morphology having blunt tip and basal socket. Fine longitudinal lines are found on the surface of sensilla. Three-four sensilla are found on flagellomeres. Two sensilla are also found near the joint between flagellum and pedicel. These are small in size having pointed tips and basal sockets.

Sensilla coeloconica: Single sensillum is found on the ventral surface of antennal flagellum. It is grooved peg settled in a pit which is surrounded by cuticular spines and form a circle around the peg. No sensillum is found near the joint.

Sensilla trichodea: These sensilla are found in large numbers on each antennal flagellum. Three types of sensilla found on the antennal flagellum. One (Tr1) is long in size with pointed tip and slightly curved distal end. These are large in numbers. Second (Tr2) one is short in size with blunt tip. These are less in number. Third (Tr3) one is small in size with pointed tip and slightly curved from distal end. These are large in numbers. These are also found near the joint between flagellum and pedicel. These are small in size with pointed tips.

Uni-porous peg sensilla: These are usually present on each antennal segment. Each sensillum is small in size, surrounded with socket and pore is present at its distal end. No uni-porous peg sensilla are found near the joints. Distal crown is found on the antennal flagellum.

On antenna of *Creatonotos transiens* (Walker): Five types of sensilla are found on different parts of the antenna (Fig. 2a-f).

Scape

Sensilla auricillica: Single sensillum is found on the surface of scape. It has tongue-like appearance and situated inside a wide socket.

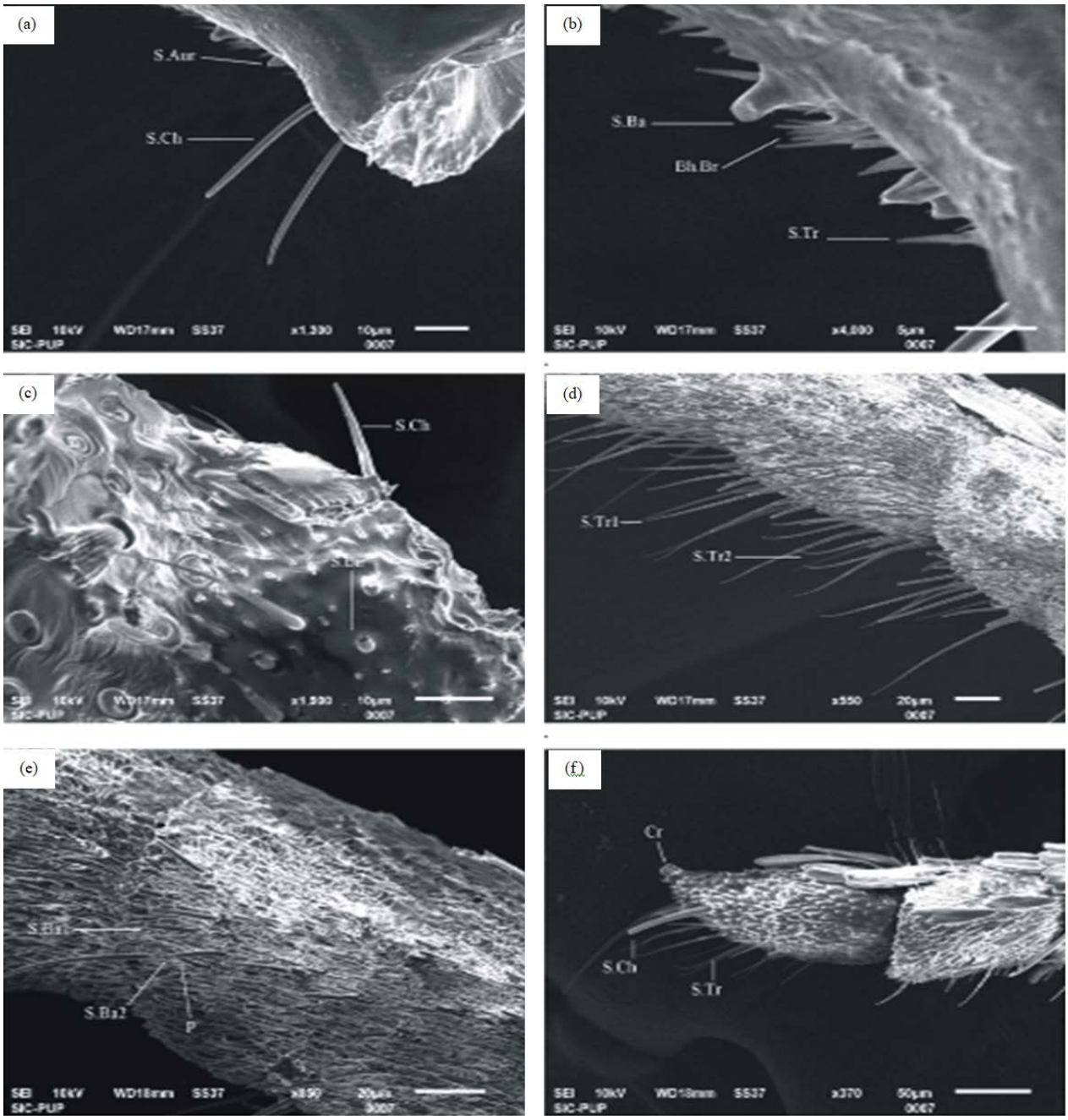

Fig. 2(a-f): Antenna of *Creatonotos transiens* (Walker), (a) Scape, (b) Pedicel and (c-f) Flagellum

Sensilla basiconica: These are also found on the surface of scape and are very less in number. Each sensillum has rod-like appearance having blunt tip and without basal socket. The pore is present at the terminal end.

Böhm's bristles: These bristles are found on the surface of scape and are few in number. Each sensillum is of small spine-like appearance with basal socket.

Sensilla chaetica: These are present near sensilla trichodea and less (2-3) in number than sensilla trichodea. Each sensillum is large in size having blunt tip and basal socket.

Sensilla trichodea: These sensilla are found on the surface of scape and are large in numbers. These are present in a single row. Each sensillum is small in size having pointed tip and without basal socket.

Pedicel

Sensilla basiconica: Single sensilla basiconica is found on the surface of pedicel. It has rod-like appearance with blunt tip. No basal socket and terminal pore are found.

Böhm's bristles: These bristles are found on the pedicel and are less in number. Each sensillum has spine-like appearance having blunt tip and basal socket.

Sensilla chaetica: These sensilla are found on the pedicel and are also very less (2) in number. Each sensillum is large in size having blunt tip and basal socket. No longitudinal lines are found on its surface.

Sensilla trichodea: These sensilla are found on the surface of pedicel and are of short type. Each sensillum is having blunt tip and without basal socket. These are less in number.

Flagellum

Sensilla basiconica: Two types of sensilla basiconica are found on flagellum. These are randomly distributed on surface of the antennal flagellum. Each sensillum is with rod-like appearance having blunt tip and without basal socket. First type (Ba1) of sensillum is with pore at its terminal end and second type (Ba 2) without any pore at its terminal end. These are not present near joints.

Sensilla chaetica: These sensilla are found on lateral margin of flagellomeres and two sensilla are present on distal segment of flagellum. Each sensillum is straight having blunt tip and with basal socket. Longitudinal lines are not found on the surface of sensilla. Arc-like morphology is seen in sensilla present on distal segment. These are not present near joint between pedicel and flagellum.

Sensilla trichodea: Two types of sensilla trichodea are found on the surface of flagellum. These are randomly distributed on the antennal segment. One type (Tr1) is long having pointed tip and slightly curved at distal end. Second (Tr2) is short, relatively straight having blunt tip. Both types are without basal sockets.

Distal crown is found at tip of flagellum.

DISCUSSION

The present study revealed that sensilla trichodea and sensilla chaetica are the most abundant type of sensilla on antennae. In *Creatonotos gangis* (Linnaeus) 8 different types of sensilla such as sensilla basiconica, böhm's bristles, sensilla campaniformia, sensilla chaetica, sensilla coeloconica, sensilla squamiformia, sensilla trichodea and uni-porous peg sensilla are found on antennae. In *Creatonotos transiens* (Walker) 5 types of sensilla are found on the antennal surface namely sensilla auricillica, sensilla basiconica, böhm's bristles, sensilla chaetica and sensilla trichodea. Generally, Sensilla auricillica have tongue-shaped appearance and its surface is deeply concaved. Sensilla auricillica have already been described in both sexes in different moth species such as *Adoxophyes orana* (Fischer von Roslerstamm), *Ostrinia nubililas* (Hubner) and *Manduca sexta* (Linnaeus)[11,4,12]. The same are found on scape of *Creatonotos transiens* (Walker). Sensilla basiconica have short hairs with blunt tip, curved rod-like morphology and without basal socket. This type is found in both the species on scape as well as flagellum. Böhm's bristles are special type of sensilla chaetica. These are small spine-like structures with blunt tips. These bristles are also observed on scape and pedicel of both the species. Böhm's bristles have been observed in other families of order Lepidoptera, e.g., Pyralidae, Tortricidae, Sesiidae[13-15]. Sensilla chaetica are long sensilla and their size decreases from base to apex. These sensilla are found on scape, pedicel and flagellum in both the species. These sensilla have also been observed in many other moth species, viz. *Cydia nigricana* (Fabricius), *Bactra furfurana* (Haworth) and *Zamagiria dixolophella* (Dyar)[16,17,13]. Sensilla coeloconica appear as grooved peg, sunk in a deep pit surrounded by cuticular spines which are pointing inward and form a circle around the peg. These are observed on flagellum in *Creatonotos gangis* (Linnaeus) only. Pophof reported these in *Bombyx mori* Linnaeus[18]. Sensilla squamiformia look like scales and are observed on scape and pedicel in *Creatonotos gangis* (Linnaeus). Sensilla styloconica have peg-like appearance, set on cylindrical projection or style and attached to a membranous socket. These are not found on any part of antenna in both the species. Sensilla trichodea has hair-like appearance with pointed or tapering ends. These are observed on scape and flagellum of both the species and found on pedicel in *Creatonotos transiens* (Walker). Subtypes of this sensilla have been found in *Synanthedon scitula* (Harris) and *Ostrinia nubilalis* (Hübner)[15,4]. Uni-porous peg sensilla are small in size, sunk in a deep pit, surrounded with mortar-like basal socket membrane. These are observed only on flagellum in *Creatonotos gangis* (Linnaeus). Moths specially males have the ability to detect and respond to female sex pheromones over impressively long distances, even though female produce very small quantities of sex pheromone in the order of nano-grams or even Pico-grams[19-22]. Body size is closely related with the length of antennal structures of many insects[23-25]. In the present studies, different types of sensilla on antennae

of two species of tiger moths have been examined in detail. These studies will provide significant information for taxonomy and phylogeny of Lepidoptera and prove significant for differentiation of closely related taxa.

CONCLUSION

On the basis of present investigations, it is concluded that in *Creatonotos gangis* (Linnaeus) 8 different types of sensilla such as sensilla basiconica, böhm's bristles, sensilla campaniformia, sensilla chaetica, sensilla coeloconica, sensilla squamiformia, sensilla trichodea and uni-porous peg sensilla are found on antennae whereas, in *Creatonotos transiens* (Walker) 5 types of sensilla are found on the antennal surface namely sensilla auricillica, sensilla basiconica, böhm's bristles, sensilla chaetica and sensilla trichodea. In *Creatonotos gangis* (Linnaeus) three subtypes of sensilla trichodea were present i.e., short with blunt tip (Tr1), short with pointed tip (Tr2) and long with pointed tip (Tr3). Two subtypes of sensilla chaetica were also found such as chaetica with longitudinal lines (Ch1) and chaetica without longitudinal lines (Ch2). In *Creatonotos transiens* (Walker) 4 subtypes of sensilla trichodea were found and these included small with blunt tip (Tr1), small with pointed tip (Tr2), long with blunt tip (Tr3) and long with pointed tip (Tr4). The presence of distinct sensilla on different parts of antennae is species specific. These features can be used for differentiation of different taxa and will also help in sorting out species complexes.

SIGNIFICANCE STATEMENT

These studies will strengthen the morpho-taxonomy and these ultrastructures will help in characterization and differentiation of various taxa. The present study will act as a foundation to explore the ultrastructures in insects and definitely help in sorting out the species complexes of tiger moths.

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