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Stinging apparatus of apoid wasps and bees as never seen before

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ABSTRACT: The stinging apparatus is expected to vary depending on the type of prey taken and the way it is carried in apoid wasps and the purpose of defense it serves in bees. To understand the differences in sting morphology, members of two apoid wasp families (Ampulicidae and Crabronidae) and a bee family (Halictidae) were studied. Scanning Electron Microscope images of lancets revealed tooth like projections on dorso-lateral aspect in *Ampulex compressa* (Fabricius, 1781) and blunt barbs on the lancets of *Liris aurulentus* (Fabricius, 1787) and *Tachysphex bengalensis* Cameron, 1889 whereas, in *Halictus fimbriatellus* Vachal, 1894 barbs are arranged in two rows on lancet, which includes four barbs on one side and three barbs on the other side of lancet which are not acutely pointed. The SEM images also indicated the presence of campaniform sensilla on the lancets of *A. compressa*. These findings help us to know the possible relationships of hunting behavior and modification of the sting in accordance.

KEYWORDS: Barbs, Hymenoptera, lancet, prey, sting.

INTRODUCTION

The order Hymenoptera is the third largest order of insects in the world, next to Coleoptera and Lepidoptera (Gaston, 1993; Sharkey, 2007). Hymenoptera is the only endopterygotan order with well-developed ovipositor which is plesiomorphic retention, and is considered as one of the key factors in their diversification (Gauld and Bolton, 1988). Stinging apparatus of Aculeate Hymenoptera evolved under selection in relation with hunting behavior and modification of the sting is expected to vary depending on the prey carrying type (Steiner, 1981).

The Scanning Electron Microscope (SEM) study of certain sclerites, particularly the gonostyli and the lancets shafts has revealed the presence of sensory structures in the former and of different barbs shapes in the latter, which are of considerable interest. The sensory structures vary in shape, density and distribution among the species studied (Gadallah and Assery, 2004). The cast specific ultrastructural specialization of the sting of the worker and queen of *Apis dorsata* was explored by Paliwal and Tembhare (1998) and as the external fine structure of sting apparatus of worker and queen honeybee was illustrated.

In the present study we have made an effort to take a closer look of lancets of *Ampulex compressa, Tachysphex bengalensis*, *Liris aurulentus* and *Halictus fimbriatellus*. All the four studied species nested in the ground. The prey of *A. compressa*

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was cockroaches, while *Tachysphex bengalensis* hunted for grasshoppers and *Liris aurulentus* took crickets. *Halictus fimbriatellus* collected pollen. Thus, there were differences between the species in their behaviour with references to the food stored in the nest cells for their offspring.

MATERIALS AND METHODS

Specimens of apoid wasps and bees were collected from nesting sites in different ecosystems in and around Bangalore and in Gandhi Krishi Vignan Kendra (GKVK) Campus, and representative specimens were preserved in 70% alcohol. In the laboratory, the specimens were relaxed and the sting apparatus was separated using a fine forceps and a pair of hooked minuten pins mounted on a holder, and was transferred to 10% KOH and left for 12-24 hours based on the sclerotization for clearing of soft tissues. After clearing, the sting apparatus and the lancets were separated under a stereo-binocular microscope and washed with distilled water for 5–10 minutes. This was followed by dehydration through a graded series of ethanol (20 min each in 70, 80, 90, and 100%) and then the lancets were dried in a critical point drying apparatus mounted on aluminum stubs.

SEM studies were conducted at Insect Systematic Laboratory, Department of Entomology, Indian Agricultural Research Institute, New Delhi with Zeiss EVOMA 10 Scanning Electron Microscope at 20 KV/EHT and 10 pa at different magnification, after 24 nm palladium gold coating.

A brief outline of the terminologies used for the major components of the sting apparatus is as follows. Each half of the divided terga of the 7th and 8th gastral segment is referred to as the 7th and 8th hemitergite, these have often been termed as spiracular and quadrate plates, respectively (Sollman, 1863; Beyer, 1891; Snodgrass, 1956). General description of ovipositor of Hymenoptera is given in Fig. 1. The first valvifer, which originates from the appendage of the 7th gastral segment, has commonly been referred to as the triangular plate (Cameron, 1882; Snodgrass, 1956) or gonangulum (Scudder, 1961; Kristensen, 1991). Basally gives rise to a long thin process called first valvula. The basal part of the first vavula is the first ramus and the more apical part of the lancet which itself gives rise to the valvilli (lancet). The appendage derived structures if the eight gastral segment called second valvifers. These are termed as oblong plates by same authors (Sollman, 1863; Snodgrass, 1956). Basally the second valvifers give rise to second valvulae. Initially these are narrow, separated and form second rami, but apically they are fused to form the sting shaft. The upper valve (sting shaft–fused second valvulae) is interlocked with each lower valve (lancets or first valvula) by a longitudinal tongue and groove joint referred to as the olistheter. The tongue of rhachis situated ventro-laterally on each side of the upper valve is ‘T’ shaped in transverse section and these rhachis runs within the ‘T’ shaped groove or aulax (Fig. 2), which is on the dorso-lateral face of lower valve (Quicke *et al*., 1995).

RESULTS AND DISCUSSION

The stinging apparatus includes sclerites like 7th and 8th hemitergite (Spiracular plate and quadrate plate), oblong plate, Triangular plate, and gonostylus, sting shaft lancets all these sclerites are linked with each other and operates effectively when needed. There is direct heavy muscles attachment to quadrate plate and oblong plate. These sclerites further gives rise to ramus which are extended and modified into sting shaft and lancets. Hence lancets are the one which penetrate first in to the victim’s skin. In order to penetrate, the lancets which are placed along the sides of semicircular sting shaft are moved forward in alternate strokes, each sliding on its track against the sting shaft. They are either entirely smooth along their lengths or they may be furnished with barbs of week teeth near their tips. This may be correlated with their function during stinging in relation to the kind of body sclerotization of their prey (Radovic, 1985) and it may also be a useful phylogenetic and taxonomic character (Wahl and Sharkey, 1993; Wahl and Gauld, 1998; Gadallah, 2001).

The lancets of *A. compressa* are very slender and long with minute, blunt barbs, with campaniform sensilla and dorsoapical teeth like structure on the aulax of the ridges. The lancets are also hollow

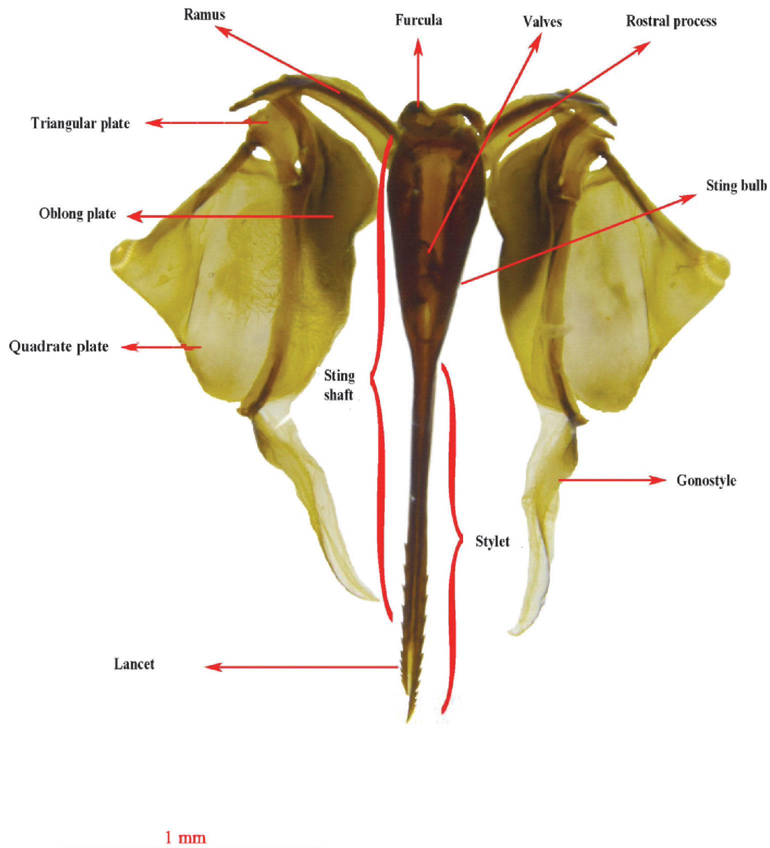

Fig. 1 The detailed description of sting apparatus

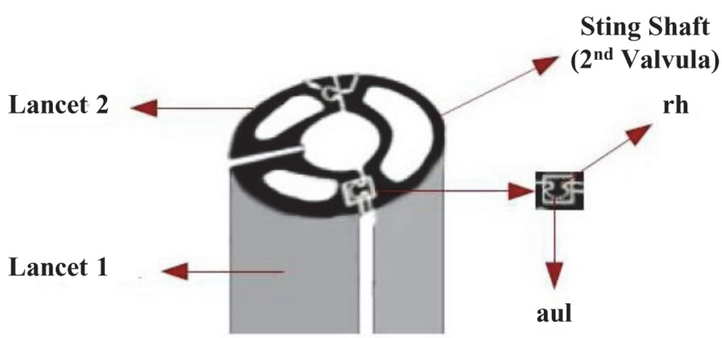

Fig. 2 Diagrammatic representation of transverse section from mid-region of braconid ovipositor (adopted from Quicke et al., 1995) (rh- rhachis, aul-aulax)

(Fig. 3), which probably helps them to be flexible during oviposition. Lancets of *T. bengalensis* and *L. aurulentus* are also slender and thin and equipped with six and five blunt dentate barbs (Figs. 4a, b, c and 5a), respectively. The Barbs are more prominent in *T. bengalensis* (Fig. 4c) compared to *L. aurantalus* (Fig. 5c). Dorso-apical tooth like projections are present (Figs. 4d and 5b) on the ridges of the aulax in both species. Interestingly, punctations on the surface of the lancets are prominent in *L. aurulentus* where the barbs are blunter, while in *T. bengalensis* the punctations are not as prominent but the barbs are more distinctly dentate. This is the first report on the presence of blunt barbs on the lancets of *L. aurulentus* and *T. bengalensis* along with confirmation of teeth like

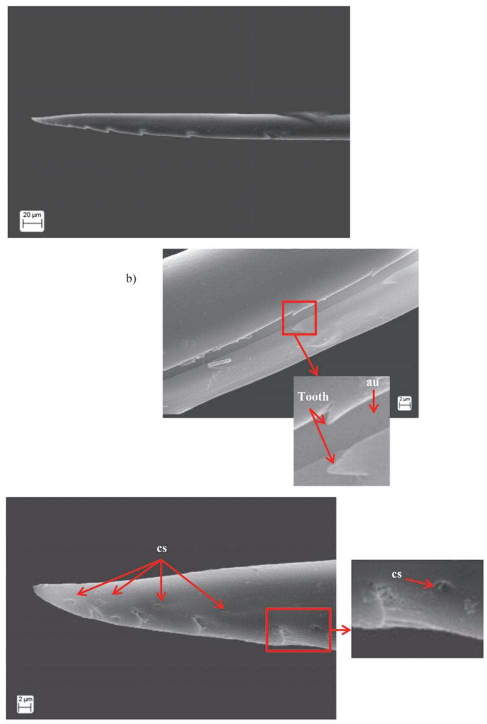

Fig. 3 SEM images of *Ampulex compressa* lancets a) lancet with blunt barbs b) presence of aulax (au) and tooth-like structure dorso-apically c) presence of camponiform sensilla

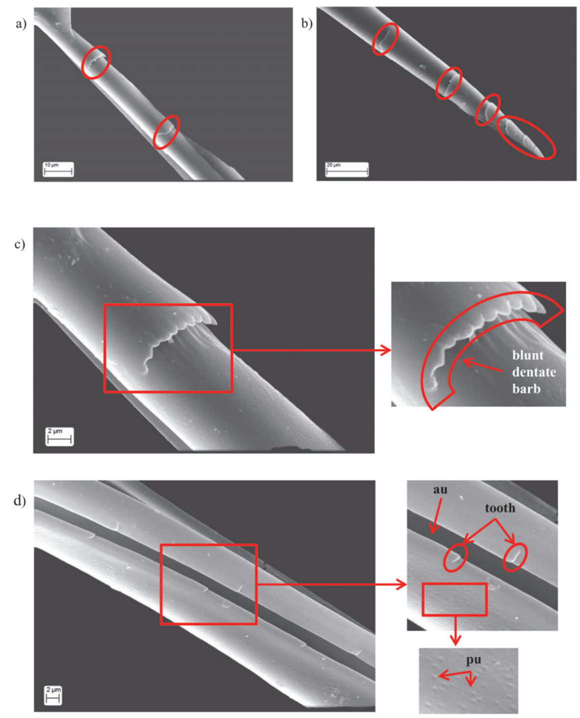

Fig. 4 SEM images of *Tachysphex bengalensis* lancets a) and b) lancet with blunt dentate barbs c) dentate barb d) presence of aulax (au), presence of tooth-like structure and minute punctation (pu).

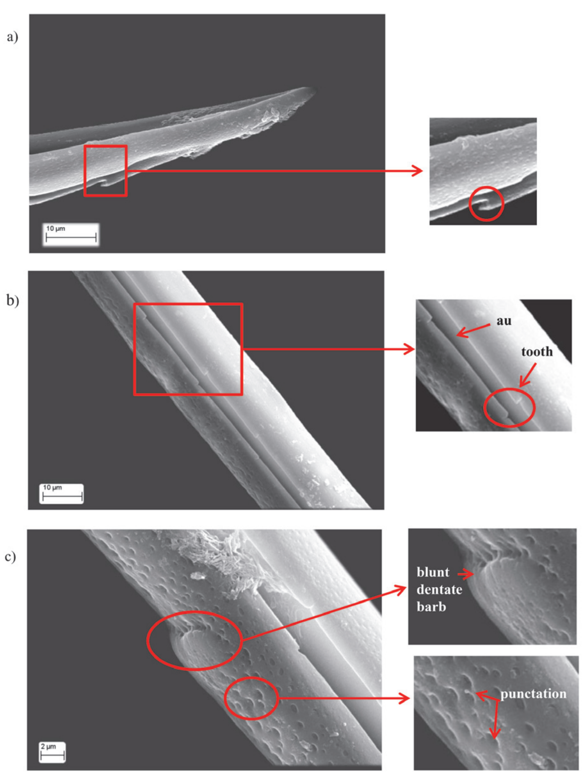

Fig. 5 SEM images of *Liris aurulentus* lancets a) the hooked blunt barb b) presence of aulax (au) and tooth-like structures c) presence of less dentate barb and prominent punctations.

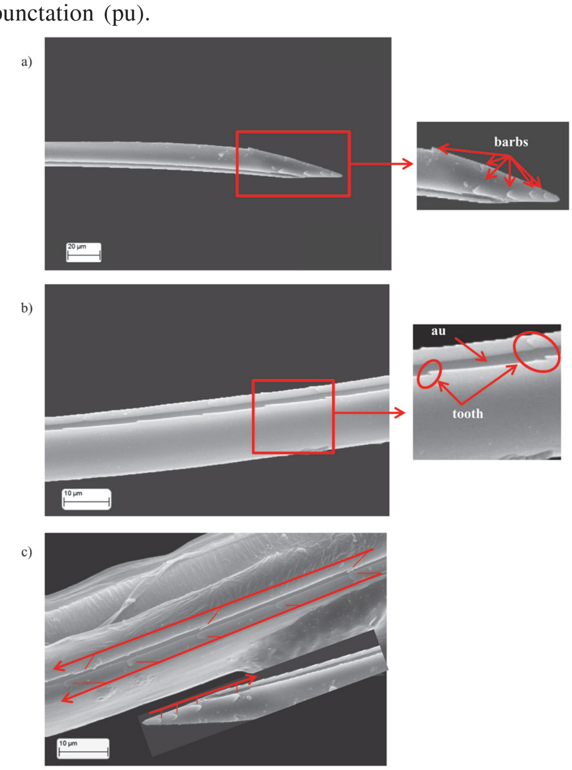

Fig. 6 SEM images of *Halictus fimbriatellus* lancets a) two rows blunt barbs on the lancet b) presence of aulax (au) c) presence of tooth-like projection on dorso-lateral of lancet which are in opposite direction of barbs.

projections on dorso-lateral aspect in *A. compressa* which has significance in understanding the phylogenetic relationships of the group.

The lancets of *H. fimbriatellus* show diverse type of arrangement of barbs. The barbs are arranged in two rows. On a single lancet, four barbs on one side and three barbs on the other side are present and the barbs are blunt (Fig. 6a). The teeth like projections on the ridges of aulax of lancets run in opposite direction of the barbs present on lancets (Figs. 6b and c).

SEM images of *A. compressa*, *L. aurantalus*, *T. bengalensis* and *H. fimbriatellus* indicated the presence of Aulax for the first time, which was previously described in Braconidae (Quicke *et al*., 1995); *Bembix rostrata* (Fabricius) (Matushkina, 2011) and in *Ampulex compressa* (Gal *et al*., 2014). Along with this, teeth-like projections were also noticed dorso-laterally in the three species. Such projections were earlier recorded in Braconidae by Quicke *et al*. (1995) who suspected the structure to enhance the ovipositor steering mechanism.

Apart from bembicine wasps (Crabronidae: Bembicinae), barbed sting has been found in *Sericophorus relucens* F. Smith (Crabronidae: Crabroninae) which possesses spines on the first and second valvulae that may fasten the prey during its transportation on the sting (Radovic and Susic, 1997). Species in which the lancet shaft is smooth are those in which the prey is heavily sclerotized, as in the case of *Gastrosericus waltlii* and *Larra anathema* (Larrinae) which provision their nests exclusively with gryllids and gryllotalpids (Honore, 1942; Bohart and Menke, 1976; Radovic, 1985). On the other hand, many species of apoid wasps which possess barbed stings prey on less sclerotized insects like caterpillars, aphids, cockroach nymphs and mantids (Radovic, 1985), which needs further investigation.

The SEM observation of lancets of the *A. compressa* revealed the presence of barbs; the barbs were progressively present at certain intervals, clustered at the tip (Fig. 3a) and blunt and not pointed as in *Apis* spp. The long and slender lancets may be useful for preying on Cockroaches as reported by Bohart and Menke (1976). SEM images also revealed the presence of campaniform sensilla in *A. compressa* (Fig. 3c) as previously described by the Ogawa *et al*. (2011) in *Apis mellifera*; Matushkina (2011) in *Bembix rostrata* (Fabricius) and Gal *et al*. (2014) in *Ampulex compressa*. According to Gal *et al*. (2014), *A. compressa* uses sensory input from its stinger to differentiate between the brain and other tissues inside the head capsule of its prey cockroach. Scanning Electron Microscope study of species uncovered the presence of various kinds, shapes and numbers of sensory bristles and sensory pore clustered at the tip of the gonostylus which are mechanoreceptors. These appears to be absent in bees (Packer, 2003) except in Megachilidae and some of the sphecids (Blum and Hermann, 1978; Le Relac *et al*., 1996). Comparative morphology of sting barbs of genus *Apis* is presented by Weiss (1978) and various authors have been described some sensory receptors associated with stings of certain Hymenoptera, including *Apis mellifera* (Hermann and Dongles, 1976).

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