Trigona iridipennis (stingless bees) are presumably endowed with remarkable visual sensory perceptional capabilities. Compound eyes and the three ocelli, which constitute their visual system, occupy a large area of their head surface, which indicates the likelihood of an elaborate visual system. It is therefore reasonable to assume that visual sensory modalities have a crucial role in the social life of these bees. Scanning Electron (SEM) and light microscopic investigations were carried out to understand structural sophistications of compound eyes and ocelli to get insights into their visual adaptations of these bees. The general organization of the brain areas concerned with visual sensory inputs processing were also analysed. Results of our studies suggest that Trigona iridipennis employ a complex way of perceiving light differentially at different areas of the eyes. Ommatidial facets of different diameters of the compound eyes suggest a differential perceptional mechanism in these bees. Further, the complex arrangement of rhabdoms within the eye and the organization of the visual neuropils corroborate the argument that these bees have complex visual sensory adaptations, which probably might have evolved in relation to a lifestyle that heavily relies on visual sensory inputs.

**Keywords:** Compound eye, Ocelli, Ommatidia, neuropils

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**Introduction**

Compound eyes of insects are remarkable for their architectural design and functional adaptations. Evolutionary background of the insects and their life style are important factors that seem to influence the developmental design of compound eyes (Greiner, 2005). For example, nocturnal and diurnal insects show striking features in their structural designs that enable them to work in and feeble and ample light conditions respectively (Warrant et al. 2006, Warrant et al. 2011). Evolutionary mechanisms have enabled external and internal innovations within their eyes, which help the insects to adapt in diverse environmental niches. (Greiner 2005, Borges and Walle 2009) In many social insects, the physically and physiologically distinct castes show polymorphism in the structure and function of the compound eyes, underlining the principle of insects eyes development in sync with the visual demands associated with their life style. An insect, which relies primarily on visual cues for many of its life style requirements like foraging, enemies, mate recognition etc. possess significantly large compound eyes that occupies a significant portion...
of its head.(Mishra 2009, Raderschall et al. 2013). In contrast, insects those rely on chemo sensory information have compound eyes, which are relatively less sophisticated; however, they have evolved olfactory/gustatory cuticular sensors to detect important chemo sensory signals. Visual predators like Dragonflies and Praying mantis are typical examples of insects with acute visual sensory perception orchestrated by large sized compound eyes(Berry et al. 2007, Singh and Mohan 2013).

Compound eyes of insects are marvelous in their architectural intricacies. On the surface, the light capturing lenses of insect eye - called Ommatidia are arranged intact. Ommatidia number in insects varies from 50000 to a hundreds or in some insect even less than that. It can be assumed that by virtue of the higher number of lenses in their compound eyes Dragonflies have a more efficient way of capturing the photons, which may help them in better vision. In insects with an acute sense of vision, symmetrical and compact arrangement of ommatidial facets are observed.

Stingless bees, commonly known as Dammar bees are the smallest of the honey-producing bees, residing in permanent colonies. They make a special wax, which is secreted and subsequently mould to construct their colonies in old walls, logs, crevices and other concealed places. (Pavithra and Jayaprakash, 2013). The bees derive their names from the absence of stings, which is present in all other bees. Stingless bees differ from other honeybees in their morphology and contrasting behaviors(Rasmussen 2013).

Though commonly found in our localities, we still lack in a good amount of knowledge about many behavioral aspects of stingless bees and a clear understanding about the functional organization of their sensory repertoire and adaptations at the many levels of the eye structure. On the other hand, with the knowledge about the completely sequenced genome in silico and well-delineated behaviors, Honeybee spp has emerged as a favorite model system in biological research. However, comparative studies on Stingless bees and other lesser-known insects are required to derive insights into diverse adaptations of insect sensory systems. As evolutionary innovations leads to sensory adaptations and survival in contrasting niches, it could be worthy to study the Stingless bees because their unique life style and behaviours are apt for addressing questions about adaptive sensory mechanisms. In the present studies, we explored the compound eyes and ocelli of stingless bees to understand the morphological and anatomical intricacies associated with a life style that requires sensing light in feeble and ample conditions for survival. Stingless bees forage on different plants during the daytime and reside in nests which are constructed in locations where light is feeble.

**Methods**

*Trigona irridipennis* were collected from nearby localities and cold anaesthetized by keeping them...
in the refrigerator for a few hours. The heads of the experimental specimens were split in half and fixed overnight at 4°C in a mixture of 2% paraformaldehyde and 2.5% glutaraldehyde buffered to a pH of 7.4. Specimens were rinsed again in the same buffer and washed in distilled water several times. Subsequently specimens were dehydrated in graded series of ethanol (50%-100%) and immersed in acetone for 1 day. Finally, the specimens were embedded in wax, and sections for light microscopy were taken on a rotary microtome at 5µm thickness.

**Haematoxylin and Eosin staining (H & E Staining)**

Tissue sections were immersed in the filtered Hematoxylin for 1 minute and rinsed with tap water. Sections were immersed in Eosin stain for 1-2 minute and rinsed with tap water. Sections were dehydrated in ascending grades of alcohol solutions(50%-100%). Specimens were cleared in xylene (2X) and mounted in DPX.

**Scanning electron microscopy (SEM)**

Scanning electron microscopic studies were carried out to understand the detailed morphology of the compound eyes of *Trigona irridipennis*. Isolated heads of *Trigona irridiennis* were immersed in 70% acetone. The specimens were then dehydrated in ascending series of alcohol grades. The antenna were then mounted on a brass stub and gold sputtered for about 2 minutes (SPI-Module Gold Sputter Coater). Observations were made using a JEOL JSM -5800VL Scanning electron microscope.

**Results and Discussion**

The visual sensory system of stingless bees comprises of two prominent compound eyes and three simple eyes called ocelli. The compound eyes are located on the dorso lateral sides of the head and are made up of several thousands of small lenses or facets called ommatidia(fig.1a). Each eye has about 1700-1800 ommatidia (facets). Ommatidia are typically hexagonal in shape, however many ommatidia which appear nearly rectangular or spherical were noticed from different regions of the eye (fig.1 b-e). The typical horizontal patterning of ommatidia was observed in *Trigona* with ommatidia arranged compact and loosely in different loci of the eye(fig. 1b-e). Hair like structures, were seen projecting from some corners of the corneal facets between the facets termed as ommatrichia, which are believed to be mechano sensory in function (fig. 1a). In each compound eye, we could count about 1800 ommatidia (fig1.b-e). Each ommatidium has a transparent corneal layer, a crystalline cone layer, primary pigment layer, retinula cells. Together, the lens and the crystalline cone form a dioptric apparatus that refracts incoming light down into a receptor region containing visual pigment. The retinula cells forms the photoreceptor organ, which is known as the rhabdom. Two types of crystalline cones were observed from the haematoxylin and eosin (H and E) stained sections. Further, H and E stained sections showed a clear difference in the secondary pigment distribution and the rhabdom organization of the compound eye (fig. 2.c-d).
The three Ocelli of *Trigona irridipennis* are laid out on top of the head in a linear pattern instead of triangular pattern seen in other insects. This pattern of ocelli arrangement has not been reported from other insects. The three simple eyes are placed in the middle of the head and they have different diameters. The right one is 110ìm, the middle one is 110ìm and the left one is 100ìm in diameter (fig. 3). The distance between each eye is 95 ìm in length. Anatomy of the ocelli reveals a transparent lens below which columnar cells and pigmented cells are present.

The visual neuropils (brain areas of vision) of *Trigona irridipennis* are prominent and occupy the bulk of the brain of this bee (fig.2.a). Visual neuropils like lamina (La), medulla (Me) and lobula (Lo) appear well organized and probably occupy a large volume compared to other sensory centres of the brain of *Trigona irridipennis*.

![Fig. 1. (a) SEM image of the compound eye of *Trigona irridipennis*; (b to e) light microscopic images of ommatidial facets from different areas of the eye](image1)

![Fig. 2. (a) Light microphograph of the visual neuropils of *trigona irridipennis*. [R-retina L-lamina, M-medulla,Lo-Lobula] (b). (C-cornea,CC-crystalline cornea, PC-primary pigment cell,SPC-secondary pigment cell.White arrow refers to Rhabdom)(c-d). differntial organization of the compound eye anatomy from different area of the eye. Note the difference in the pattern of Crystalline cone and rhabdoms](image2)

*Trigona irridipennis* shows broad similarities to the general features of compound eye of insects. However, subtle differences in the organization of visual system are significant which provides us valuable information about the visual sensory adaptations of these insects. The pattern of ommatidial facets observed in this bee (size and their arrangement over the eye) probably indicates functional complexities. Previous studies have reported similar visual adaptations in other insects (Yilmaz et al. 2014). A comparatively less
compact arrangement of the ommatidial facets over the compound eye surface points towards a weak level of light capturing mechanisms of the compound eye. Differences in the size and shape of the ommatidial facets points towards the differential visual acuity of compound eyes of Trigona iridipennis suggesting the possibility that the compound eyes of Trigona iridipennis perceives light differentially through their different domains of the compound eye. However, further studies need to be conducted for a comprehensive understanding of these mechanisms. The two types of crystalline cones and structural differences noticed in the rhabdoms from the periphery of the compound eyes garner more evidence to support the argument that the compound eyes of Trigona iridipennis are organized in a differential manner that is likely to enable appropriate visual adaptations at feeble and ample light conditions.

Fig. 3: SEM image of the three ocelli of Trigona iridipennis
Note the linear arrangement of the individual ommatidium

The ocelli of Trigona iridipennis shows a linear arrangement (fig. 3), which is in stark contrast to the ocelli arrangement found in other insects where ocelli arranged are arranged at three points of a triangle. The linear arrangement presumably corresponds to a different wiring of the neurons associated with it and there is the likelihood of ocelli assuming functions unknown hitherto in these bees(Berry et al. 2011). The brain areas concerned with vision occupy bulk of the brain mass in Trigona iridipennis Greater investment in the visual neural tissue points to the greater dependence on the vision related sensory processing and memory(Stieb et al. 2010).

References


