Role of Scanning Electron Microscopy in Understanding Insect Corneal Nipple and Other Structures

Sudip Dey[*]

Electron Microscope Division, Sophisticated Analytical Instrumentation Facility, North Eastern Hill University, Bijni Complex, Laitumkhrah, Shillong – 793003, Meghalaya, India

Scanning electron microscopic studies have been found to be important in revealing corneal nipple varieties in some insects other than nocturnal moths. The study could present some modifications in certain classical concepts regarding the relations between corneal nipple and phylogenic position of insects. The study further revealed the special role played by corneal nipples in nocturnal, crepuscular and social insect species. In addition, scanning electron microscopy was found to be important in studying the detailed morphology of facet hairs suitable for functioning as infra-red receptors.

Keywords: corneal nipple; facet; nocturnal moths; microscopy; insect eye; SEM.

1. General Introduction

The characteristic feature of an eye, either camera type (or compound type is the presence of a refracting surface, which is essential for the formation of image. It is a well known fact that sometimes small reflections are produced in the refracting surface in the region where there is a sharp transition in the refractive index. In vertebrate eye, these reflections are easily detected as in the case of Purkinje image, caused by reflection from the surface of cornea and lens.

When the refractive surface reflects light, the result is some adverse effects on the image produced. The loss of image intensity and degradation of image quality due to glares and ghosts become evident. The intensity of reflection from ocular refractive surface, as calculated from Fresnel formula is found to be 4% of the incident beam. A similar type of reflection is also produced from optical lens and the lenses of microscope and camera. To overcome this effect, good quality lenses are coated with thin films designed to reduce this reflection [1].

In a search for possible anti-reflection device in ocular refractive surface in animal kingdom, it was found by some scientists that corneal cuticle of certain species of insects is relatively non- reflective. While comparing the front surface reflections of the eye of a honey bee and a nocturnal moth, Bernhard et al., (1965) [2] observed a bright reflection from the eye of the former, while the later exhibited relatively non- reflective ocular surface.

Electron microscopical observation revealed the presence of an array of conical cuticular protuberances, termed as corneal nipples, which were found to be responsible for reduced reflection observed in the moth cornea [2-4]. Bernhard et al. (1970) [3] reported that corneal nipple is the characteristic feature of nocturnal moth, but it is also present in some diurnal Lepidoptera which contain tapeta in their eyes. Corneal nipple acting as anti-reflection coating in compound eye of insects other than Lepidoptera is rare [3]. The compound eye of most of the insects studied so far (other that of Lepidoptera) was found to have smooth corneal surface and it was reported that the reflections from corneal surface of the grasshopper (*Schistocera*) and fly (*Musca*) are white and correspond in intensity to that from a plane glass surface [1].

Bernhard et al. (1965) [2] classified corneal nipples on the basis of their height as Group I (50 nm or less), Group II (between 50 nm and 200 nm) and Group III (More than 200 nm) protuberances. The

[*] E-mail: sudipdeyrsic@yahoo.com

formation of the lower height Group I protuberances, although detected on the facet surface of many insect species belonging to different orders, is totally unknown.

Group II and Group III nipples on the other hand, play a definite anti-reflection role due to their conical shape, regular periodicity and height.

The explanation on the role of Greater height corneal nipples as anti-reflection coating was put forward by Millar, (1979) [1]as follows:

It can be considered that the cornea with a refractive index $n_3 = 1.5$ is covered with the corneal nipple, which can be treated as a thin film n_2 of thickness d. However the corneal nipple array is different from thin film of uniform refractive index (n), since the average refractive index (n) in the nipple increases smoothly from refractive index (n) of air at the tip to that of cornea at the base. It is known that if n_2 is a gradual transition between n1 and n_3 with a thickness $d = \lambda_0/2$, a broad band anti reflection effect is achieved.

2. Corneal nipple in insects other than nocturnal moth

2.1. Epacromia dorsalis

2.1.1. Introduction

As already stated, greater height corneal nipples (Group II and Group III protuberances), which act as effective anti-reflection device is reported mostly in nocturnal moths. In an attempt to find out whether or not anti-reflection device exists in the ocular refractive surface of other nocturnal insect species, we made some studies on a nocturnal grasshopper, *Epacromia dorsalis.* Although, transmission electron microscopy is relevant in studying corneal nipples as far as the precise understanding of the nipple height is concerned, it is not able to reveal the detailed arrangement, distribution pattern and density of the nipple on the corneal surface. The larger depth of field of scanning electron microscope appears to be important in this regard.

2.1.2. Material & Methods

The compound eye samples of the nocturnal grasshopper, *Epacromia dorsails* were fixed in 2.5 % glutaraldehyde prepared in 0.1 M Na-cacodylate buffer for 2 hr. After washing in buffer, they were fixed in the secondary fixative, osmium tetroxide, dehydrated through increasing concentration of acetone, cleared in toluene and air dried, as suggested by Wooley and Vossbrinck (1977) [5] for SEM studies of chitinous cuticle of arthropods. The specimen was secured horizontally to the brass stub (30mm x 20mm high) by electro-conductive paint and was coated with gold in a fine coat ion sputter, JFC 1100 (Jeol). Observations were made on the Scanning Electron Microscope JSM 35 CF (Jeol) operated at 15 KV. Tilt control was fixed at Oº for setting the specimen stage in a horizontal position.

2.1.3. Observation

The corneal cuticle was found to be thickly coated with conical protuberances with a density of 24 nipples per µm area (Fig. 1). When the nipples of *Epacromia dorsalis* were compared with those of the moth *Antheraea* sp. similarities were observed between the two [6], as far as their height and periodicity was concerned. The height of the nipple (distance between base and tip) as revealed in scanning electron micrograph was 100 nm and the periodicity (centre to centre distance of the nipple) was found to be about 130 nm. However, although most of the nipples were Group II or III greater height nipples (G), a few lower height nipples (L) were also present.

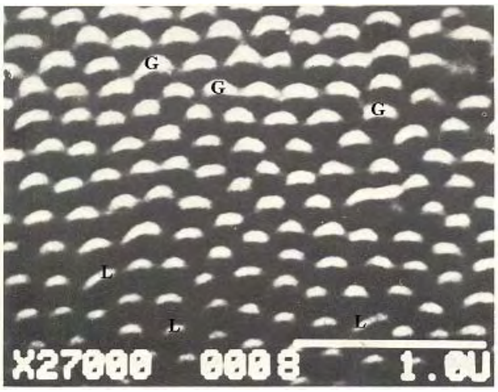

Fig 1. A representative scanning electron micrograph of corneal nipple pattern in a single facet of the grasshopper *Epacromia dorsalis*. Bar = 1.0 μ

G, greater height nipple; L, lower height nipple

2.1.4. Discussion

It is important to note that the grasshopper, *Epacromia dorsalis* is attracted by light. Hence, the corneal nipple array detected on the corneal surface is likely to play definite role in increasing the intensity of light transmitted in the ocular media by reducing the reflection of incident beam from refractive surface. Further, the anti-reflection helps the insect in its scotopic vision by ensuring the retention of all the available photons, some of which would have otherwise escaped through reflection. The study thus suggests that corneal nipple, relevant to anti-reflection device need not necessarily be the characteristic features of moths, and it may be present in other groups as well, depending upon their physiological requirement for the same.

2.2. *Cloeon* sp.

2.2.1. Introduction

The striking event in the evolutionary history of arthropods is their transition from an aquatic way of life to a terrestrial one. This transition led to several rearrangements of different organs including that of the photoreceptors. The great majority of aquatic arthropods possess compound eyes, and, the lower amphibiotic insects are able to use compound eyes in both aquatic and subaerean environment. In view of the physical differences between the aquatic and terrestrial environment, significant differences in the corneal lens of aquatic nymph and the terrestrial adult are exp3ected. However, earlier workers [7], while studying the dioptric apparatus of the aquatic nymphs and terrestrial adults of the dragonfly *Coenagrion puella* (L) could not find any remarkable difference between the two except their size. On the basis of their observations, Minelli and Pavan (1974) [7] came to the conclusion that ommatidial modifications during metamorphosis in both amphibiotic and terrestrial insects follow a generalized pattern which needs no different alternatives in relation to the different physical properties of water and air. Since the approach in the aforementioned study was based on optical microscopy with limited resolution and magnification, it is quite logical to seek the expected differences between the dioptric apparatus of aquatic nymphs and terrestrial adults of amphibiotic insect species at ultrastructural level.

On that consideration we made a comparative study on the dioptric apparatus of aquatic nymphs and terrestrial adult of an amphibiotic insect species, *Cloeon* sp. (*Ephemeroptera* : *Baetidae*) [8].

2.2.2. Materials & Methods

Different nymhal stages of the mayfly, Cloeon sp were collected from their natural habitat and were reared in aerated aquaria to obtain adults. The compound eyes of both the nymph and adults were prepared for scanning electron microscopic studies through routine procedures [5, 6] and were viewed in JSM 35 CF (Jeol) scanning electron microscope at an accelerating voltage of 15 Kv.

2.2.3. Observation

The facet surface was found to be smooth throughout the nymphal stages indicating the absence of corneal nipples. However, in the subimago, the facets were found to be coated with corneal nipples, which persisted in the imaginal stage also. One of the interesting observations was that the nipples showed variations in length, height and periodicity in different regions of the eye. In the central region, the length of the nipples ranged between 210 to 263 nm, the height between 78 to 108 nm and the periodicity between 263 and 315 nm. In the peripheral region on the other hand, the length of the nipple was found to be around 161 nm, height was about 64-96 nm and the periodicity ranged between 161 and 193 nm.

2.2.4. Discussion

The significance of the observations is that the occurrence of corneal nipple in the terrestrial subimago and imago of *Cloeon* sp. and their absence in the nymph seems to be one of the remarkable differences in the structural features of the eye of an aquatic nymph and that of a terrestrial adult in an amphibiotic insect in relation to the difference between the physical properties of water and air. It is important to note that the observed differences could be obtained only with the help of Scanning electron microscope. Apart from corneal nipple, other ultrastructural differences may exist between aquatic nymph and terrestrial adults of amphibiotic insects as an aquatic and terrestrial adaptation. Thus it appears that many biological phenomenon interpreted through optical microscopical approach may have to be reviewed and confirmed through electron microscopy because of the higher resolution of the later. As far as the differences observed in the height and periodicity of the nipple in different portions of the facet is concerned, the optical significance, if any, is at present unresolved.

2.3. *Forficula* sp.

2.3.1. Introduction

Forficula, which belongs to the group earwig (Dermaptera) is a screpuscular insect and has scotopic vision. Since anti-reflection coating in the form of greater height corneal nipple was reported to be important in scotopic vision of nocturnal moths [1-3] and other nocturnal insects [6], it was felt that investigations on the facet surface of crepuscular insects with reference to corneal nipple and anti-reflection may be important.

2.3.2. Materials & Methods

The samples were prepared for scanning electron microscopy through routine procedures [5-6] and were viewed in the secondary electron emission mode of a JSM 35 CF (Jeol) scanning electron microscope at an accelerating Voltage of 15 KV.

2.3.3. Observations

The Scanning electron micrograph of the corneal surface of *Forficula* sp. revealed certain interesting features [9]. When the surface pattern of a single facet of *Forficula* sp. was compared with those of a nocturnal moth (*Attacus atlas*) and a diurnal fly (*Musca domestica*), corneal protuberances of *Furficula* sp. exhibited a number of similarities as well as differences with the corneal nipple of *Attacus* and

Musca. The height of the corneal protuberances of *Forficula* sp. (70-140 nm) was found to be more or less the same as that of the moth, *Attacus atlas*, on most of the facet parts. However, the periodicity (centre to centre distance) of the protuberances was not constant unlike the regular periodicity of the corneal nipple of the moth. Further, in some parts of the facet, the corneal protuberances were found to be flat, similar to the lower height corneal nipples of the fly *Musca domestica*.

2.3.4. Discussion

The pioneering work of Bernhard et al. (1970) [3] suggested that greater height corneal nipple (> 50 nm to 200 nm or more) tend to occur in insects of advanced orders and lower height nipple (< 50nm) are the characteristic features of insects representing less advanced order. Since the lower height corneal nipple (< 50nm) cannot fulfill the physical conditions necessary for acting as anti-reflection device, it was suggested that insects representing lower order, have no anti-reflection device associated with their dioptric apparatus [3].

In our study on the dioptric apparatus of an earwig, *Forficula* sp. representing a less advanced order (Dermaptera), the occurrence of greater height nipples (70-14 nm) on the major portions of the facets, indicates a definite anti- reflection mechanism [1]. The study thus appears to be an exception to the aforementioned hypothesis of Bernhard et al. (1970) [3]. However, the greater height corneal nipples detected on the facet surface of *Forficula* sp. may not be as efficient as those of the nocturnal moth, as far as the anti-reflection property is concerned, because of the irregularities in their periodicity. Nevertheless, they are likely to help the scotopic vision of the insect by reducing the reflection from the ocular refractive surface to a certain extent, thereby increasing the intensity of transmitted light.

2.4. Provespa berthelemyi (Worker)

2.4.1. Introduction

Provespa berthelemyi is a nocturnal hymenopteran species of the genus *Provespa* which is reported from the Northeast India by Matsuura and Yamane (1990) [10]. The body of the wasp is yellow-brown in colour and it bears a pair of large well developed ocelli, in addition to compound eye. Ecological understanding of the three species of the genus *Provespa* was poor, until Van der Vecht (1957) [11] reported that they are nocturnal and are attracted to light. Later, it was observed that *Provespa* are the most specialized group of the subfamily Vespinae [10]. Detail studies have been carried out on ecological characters of *Provespa* [10], but morphological and ultrastructural specialization of the group, particularly in relation to its nocturnal behaviour is not well documented in literature.

Since in this nocturnal social insect, the two main jobs of the workers, i.e. building work and food collection are to be carried out at night, it is expected that their visual system is extremely sensitive and well developed.

To analyze the mechanism whereby the eye of the worker performs at night, its delicate role in catching the suitable prey for feeding the larvae, building the nest with specific plant parts [10], and keeping the nest tidy, we investigated the ultrastructural features of the compound eye, which, we felt might provide some explanation for efficient night vision. The objective of our study was to identify the morphological and ultrastructural specialization, if any, in the visual system of the insect, relevant to its night vision and social achievements.

2.4.2. Materials & Methids

Specimens used in the study were collected from a number of nests in the hole of trees from different localities of Sikkim, a province situated in the North-Eastern part of India. Incidentally, the only species of the genus, *Provespa*, i.e. *P. berthelemyi* reported from the Northeast India was described from the state of Sikkim [10]. The nocturnal behaviour of the wasp was confirmed by close watch on several nests for a few days and also from the reports of the local people. The workers of *P. berthelemyi* were collected by light trapping and were sacrificed by exposing them to benzene vapour. A total of 20 individual workers of the wasp collected from different nests were studied in order to confirm whether or not the ultrastructural features were characteristics of the species. The compound eye samples excised from the head were processed through routine preparatory procedures for scanning electron microscopic studies [5-6]. Observations were made in the secondary electron emission mode of a JSM 35 CF (jeol) scanning electron microscope operated at 15 kv.

2.4.3. Observation

In the frontal part of the eye, the facets were found to be hexagonal in shape with an average diameter of about 18 µm and average number of about 14000 facets/ mm^2 (Table 1) .

Table 1. The number shape and size of facets in different zones of the eye of *P. berthelemyi*. .

Zone	Shape	Size (diameter)	Number
Frontal	Hexagonal	18 µ	14000/mm^2
Lateral	Tetragonal with spherical mini-facets on the four corners	Largest facet = 20 µ Mini-facets = 2 µ	Largest tetragonal = 4000 / mm^2 Mini-facet = 12000 / mm^2
Central	Hexagonal	23 µ	3500 / mm^2

The lateral part of the eye consists of tetragonal facets with small spherical structures on the four corners of the tetragon (Fig. 2). The tetragonal facets were found to be about 20 µm in diameter, while the small spherical structures located on the four corners of the tetragonal facets were found to be around 2 µm in diameter. The number of the tetragonal facets was about 4000/ mm^2, while that of the small spherical structure, which can be called as mini-facets were about 12000/ mm^2 (Table 1).

In the central zone of the eye, the facets were found to be hexagonal in shape with a diameter of about 23µm. The number of the facets was found to be about 3500/mm^2. (Table 1).

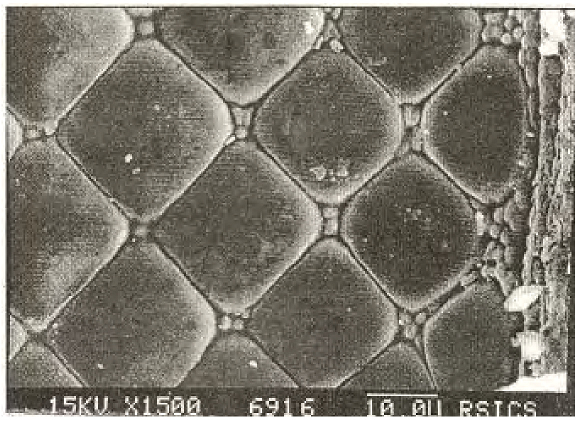

Fig 2. Rhomboidal arrangement of facets at the lateral part of eye. Small circular or oval structures filling up the gaps between adjacent facets are seen (bar = 10 µ);

Corneal nipple: The surface of all the facets were found to be adorned with conical protuberances, the corneal nipple with uniform height (about 280 nm) and periodicity (about 80 nm), comparable to the corneal nipple of moth eye. The scanning electron micrograph of the small spherical or oval structures covering the gaps between adjacent facets, also exhibited the corneal nipple having the same height and periodicity as those of the facets.

Facet hair: Margin between some adjacent facets revealed the presence of some hair, about 85 µ long, 25 µ broad at the base and 8 µ broad at the tip. A well developed socket, about 4.1µ in diameter was also found to be present (Fig. 3). The socket was found to be surrounded by a collar-like structure. The floor of the socket appeared to be a thin membranous cuticle with folds, which possibly is involved with the movement of the peg. The dimension and structural features of the hair was found to be similar to the sensory peg reported as the infra-red receptor [26] (Table 2).

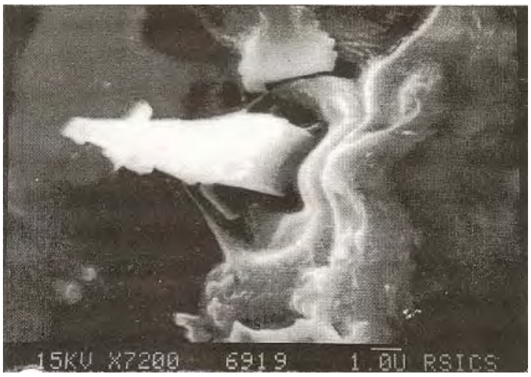

Fig 3. Facet hair similar to IR-sensitive sensory peg (bar = 1 µ) in compound eye of P. berthelemyi.

Table 2. Comparative data on structural features, dimension and location on the facet hair of *P. berthelemyi.* and possible infra-red sensitive sensory pegs.

Sl/No	Parameters	Facet hair	Possible IR-sensitive sensory peg (Amrine & Lewis, 1978) [26]
1.	Location	Margin between adjacent facet	Fronto-clypeal and dorsal region of the head
2.	Length	8.5 ± 3 µ	8.0 µ
3.	Breadth of the base	2.0 ± 2 µ	0.5 µ
4.	Breadth of the tip	0.8 ± 1.0 µ	0.5 µ
5.	Socket diameter	4.1 µ	3 µ
6.	Structural features of the floor	Thin membranous cuticle with folds	Thin membranous cuticle

Our study [12] on the dioptric apparatus of a wasp, *Provespa berthelemyi* thus presented a number of special features, which may be related to the social behaviour of the insect. Social insects are among the great achievement of evolution, since they exemplify the full sweep of ascending levels of organization from molecule to society. Insect colony, which is also called super-organism, displays many social phenomena that are analogous to the physiological properties of organs and tissues.

The cuticular sensory system of some social insects was reported to be more specialized as compared to non-social insects [13-17]. However, similar types of studies on ocular structures in relation to social behaviour are not well documented in existing literature.

Our study [12] is one of the very few of its kind to understand the structural specialization of ocular tissues in relation to nocturnal behaviour of a social wasp. Since for night vision, the compound eye of the wasp has to work under minimum available light, and since the worker of the wasp species has to carry out much more delicate and specific jobs, as compared to solitary nocturnal species, its eye is expected to adopt a greater degree of specialization to perform its duty. As evident from our scanning electron microscopic study, the number of facet or ommatidia is very high in the worker of *Provespa*, which is certainly one of its prime requirement because large number of facets are known to be directly related to better visual performances [18] . Similarly the large diameter of the facets is also considered to be an advantage for the insect's visual acuity [18] and it has been shown that the square of the diameter of the facet is proportional to the radius of curvature of the whole compound eye [19]. The hexagonal shape of the facet over major parts of the compound eye in *Provespa berthelemyi* indicates minimum spatial arrangement of spherical outer surface of the lens [19]. The tetragonal facets observed in the dorso- and ventrolateral edges of the eye of *P. berthelemyi* have also been reported in some crustacean

[19] and also in some dipteran species [20]. It is suggested that when facets are flat rather than convex on the outside, minimum spatial arrangement is overruled by the cellular composition [19]. In *Provespa berthelemyi* on the other hand, the tetragonal facets on the dorso- and ventrolateral edges are not true tetragon because they are not angular at the four corners, which are curved to remarkable extent. This brings convexity to the tetragon-like facets unlike other insects, where they are more or less flat.

The change in lattice of ommatidia from frontally to laterally, from hexagonal configuration (called La by Stavenga, 1975) [21] via a square lattice (Lb) to a new hexagonal arrangement (Lc) have been reported in a number of dipteran species [20]. This change in orientation implies the presence of a gradient in the interommatidial angles over the eye [22-24], which possibly has a relation with the orientation behaviour of the insect [25]. A similar relation between change in ommatidial lattice configuration and orientation behaviour is likely to exist in *Provespa berthelemyi*. The regularity in the ommatidial lattice observed in *Provespa* through scanning electron microscopy is not general among insects, and is considered to be an important criterion for visual efficiency. Any kind of irregularity in the matrix causes fading of the image of the surroundings [20].

The corneal nipples present on the facet surface of *P. berthelemyi* appear to be the most significant adaptation of the wasp worker in relation to its nocturnal behaviour, and social achievement. Our study involving scanning electron microscopy seems to be the first report on the occurrence of greater height nipple (Group III) in a hymenopteran species (*P. berthelemyi*) indicating the existence of anti-reflection device in their eyes. The corneal nipple in the eye of *Provespa berthelemyi* obviously ensures an increase in intensity of light transmitted in the ocular media, and helps in scotopic vision of the insect. However, a remarkable specialization about the distribution of corneal nipple is that each and every corner and gap between adjacent tetragonal facets are covered with corneal nipples a situation that had never been reported in any insect.

Besides the corneal nipple, the other remarkable specialization of the *P. berthelemyi* facet is the occurrence of facet hair at places as revealed from scanning electron microscopy. These hairs are likely to be involved in the reception of infra-red waves, since their structural features, dimension etc. have striking similarity with those of sensory peg described in other arthropods as infra-red receptors [26]. Apart from similarity in structural features and dimension, the facet hairs like infra-red sensitive sensory pegs have protected location between adjacent ommatidia. Several authors have sought a correlation between the dimensions of some sensilla and infra-red sensitivity, and it was suggested that structural features, shape and size of sensory pits were suitable for detection of infra-red wavelength ranging from 2μ and 8μ [37]. Measurement of di-electric values and physical dimensions of some sensory spines were found to meet the requirements of di-electric Infra-red aerials 27-30]. It was also suggested that compound eyes of some insects could be an optic electromagnetic thermal radiometer [31. The importance of Infra-red emitter and detector system to nocturnal insect species is amply highlighted in literature. Heat radiation of female *Platysamia cecropia* was measured by Duane and Tyler (1950) [32] and its importance as an attractant for male was discussed. Callahan (1965a) [33] measured distinctive heat radiation from female *Heliothis zea* and found that the species is attracted to an Infra-red source of 8 μ to 13 μ wavelength emitted from 4 W mercury-arc-argon discharge tube covered with black tape.

The facet hair detected in *P. berthelemyi* in our study is similar to the infra-red receptive sensory peg described by Amrine and Lewis (1978) [26]. This seems to be an important adaptation for the wasp, *P. berthelemyi* and is likely to be associated with the function of its compound eye as an optic electromagnetic thermal radiometer [31]. This specialization in the compound eye appears to be important for nocturnal behaviour and social achievements of the insect.

Conclusion

Our study suggests that Scanning electron microscopy can play a very important role in understanding some of the unique features of the ocular refractive structures of insect compound eye. The device also appears to be extremely important in revision of some of the classical concepts on insect ocular refractive structures to gather additional information.

In this context it is to be noted that the role of scanning electron microscopy in understanding corneal nipple array of butterflies [34], natural nanostructures on insects [35], optical properties and wettability of nanostructured biomaterials [36] etc. has been highlighted in some recent literature.

References

[1] Miller, WH. *Handbook of Sensory Physiology.* (H. Autrum ed). Springer, Berlin. Vol. VII / 6A, pp- 70-143 (1979).
[2] Bernhard, CG., Miller, WH. & Mollar, AR. *Acta. Physiol. Scand*. 63 (Suppl-234): 1-79 (1965).
[3] Bernher, CG., Gemne, G. & Sallstrom, J. *Z. Vergl. Physiol*. 67: 1-25 (1970).
[4] Miller, WH., Moller, AR. & Bernhard, CG. *Functional Organization of the Compound Eye* (Bernhard, CG.ed). Pergamon Press. Oxford. pp-21-23 (1966).
[5] Wolly, TA. & Vossbrinck, CR. *SEM / II / IIT Research Institute*. Chicago, IL 60616. 645-652 (1977)
[6] Dey, Sudip. *Vision Res.* 28. 9: 975-977 (1988)
[7] Minelli, A. & Pavan, F. *Zoologischer Anzieger.* 192 (1/2). 5: 10-14 (1974).
[8] Gupta, S., Dey, Sudip., Varman, AR., Michael, RG. *Vision Research*. 28. 8: 1037-1039 (1989)
[9] Dey, Sudip & Dkhar, B. *Micron and Microscopica Acta*. 23. 3: 337-339 (1992).
[10] Matsuura, M. & Yamane, S. *Biology of the Vespine Wasp*. Springer-Verlag. Barlin. Heidelberg (1990).
[11] van der Vecht. *Zool. Verh. Leiden.* 341-383 (1957).
[12] Dey, Sudip & Raghu Varman, A. *Journal of Animal Morphology and Animal Physiology*. 49. Nos. 1 & 2: 31-40 (2002)
[13] Mark, H. Z. *Vergl. Physiol.* 45: 475-569 (1962).
[14] Wilson, EO. *Animal Behaviour.* 10(102): 134-364 (1962).
[15] Getz, WM. & Smith, KB. *Journal of Comperative Physiology.* 160A: 239-246 (1987).
[16] Getz, WM. & Page, RE. *Ethology*. 87: 298-315
[17] Dey, Sudip. *Proceedings of National Academy of Science,* India. 68(B). 1: 37-44 (1998).
[18] Horridge, GA. *The compound Eye and Vision of Insects*. Claredon Press. Oxford.
[19] Kuiper, JW. *Proceedings of the International Symposium on the Functional Organization of the Compound Eye*. Pergamon Press. Oxford & New York.
[20] Beersma, DGM., Stavenga, DG. & Kuiper, JW. *Journal of Comparative Physiology*. 119: 207-220 (1977).
[21] Stavenga, DG. *Journal of Comperative Physiology*. 102: 297-304 (1975).
[22] Braitenberg, V. *Kyber-netik*. 7: 235-242 (1970).
[23] Braitenberg, V. *Proceeding in the Visual System of Arthropods* (R. Wehner ed). Berlin-Heidenberg-New York. Springer. pp- 3-16 (1972)
[24] Braitenberg, V. & Strausfeld, NJ. *Handbook of Sensory Physiology*. (R. Jung ed) Springer. Berlin-Heigenberg-New York. Vol. VII / 3A. pp- 361-660 (1973).
[25] Beersma, DGM., Staveng, DG. & Kuiper, JW. *Journal of Comparative Physiology*. 102: 305-320 (1975)
[26] Amrine, JW. & Lewis, RE. *Journal of Parasitology.* 64(2): 343-358 (1978).
[27] Callahan, PS. *Annals of Entomological Society of America*. 58: 727-745 (1965 b).
[28] Callahan, PS. *Miscellaneous Publications of Entomological Society of America*. 5: 315-347 (1967).
[29] Callahan, PS. *University of Gaeorgia Research Bulletin*. 54: 1-105 (1969)
[30] Callahan, PS., Tachenberg, EF. & Carlysle, T. *Annals of Entomological Society of America*. 4: 934-937 (1968).
[31] Neville, AC. *Biology of Arthropod Cuticle.* Springer-Verlag. Berlin, Heidelberg. New York.
[32] Duane, JP. & Tyler, JE. *Interchemical Review*. 9: 25-28 (1950).
[33] Callahan, PS. *Nature* (Lond). 207: 1172-1173 (1965 a).
[34] Stavenga, DG., Foletti, S., Palasantzas, G. & Arikawa, K. *Proceedings of Royal Society*. B. 273: 661-667 (2006).
[35] Watson, GS. & Watson, JA. *Applied Surface Science*. 235: 139-144 (2004)
[36] Palasantzas, G., De Hosson, J., Michielsen, KFL. & Stavenga, DG. *Optical Properties and Wettability of Nanostructural Biomaterials: Moth eyes, Lotus Leaves and Insect Wings. In: Handbook of Nanostructural Biomaterials and their Application in Biotechnology*. Vol. I: Biomaterials (HS Nalwa ed). Americal Scientific Publishers. Stevenson Rench. CA. (2005).
[37] Grant, GRM. Proceedings of Royal Society of Queensland. 60: 80-98.