Article

A preliminary comparative study on structure and main characteristics of compound eyes in four Mexican cone borers *Conophthorus spp* (Coleoptera: Scolytinae)

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Received 19 April 2014; Accepted 25 June 2014; Published online 1 September 2014

Abstract

The compound eyes of four Mexican cone borers: *Conophthorus ponderosae* Hopkins,*C. conicolens* Wood, *C.michoacanae* Wood and *C.teocotum* Wood were studied and compared by main internal and external structures as are: number of facets, eye length, ventral eye width, dorsal eye width, facet diameter, primary and secondary pigments diameter, ommatidium length and cone length, where this last seem be larger in females than males; It is described the main characteristics of *dioptric apparatus* and *photoreceptor layer*. For 2 cone borer species studied. In general all the *Conophthorus* species showed identically kidney-shaped eyes where the number of *ommatidia* or facets quantified no differed significantly between species (P= 0.0149) and sex, except for the case of *C.teocotum* W; the other parameters or characteristics compared are too seem for all species group studied. It was described that the general structures of the dioptric apparatus and photoreceptor layer for *C.ponderosae* H and *C.conicolens* W, where were identified the presence of 8 *rhabdomeres*, confirms the fact that this number of receptors is common in scolitids, which give them the possibility of a UV-sensitive navigation system added to a green sensitive motion detecting their hosts.

Keywords cone borers; *Dioptric apparatus*; *Photoreceptor layer*; *ommatidium*; *rhabdom*; *cornea*; *acone* type; *Conophthorus ponderosa*e H; *Conophthorus conicolens* W; *Conophthorus michoacanae* W; *Conophthorus teocotum* W; SEM; TEM micrographs.

Arthropods
ISSN 2224-4255
URL: http://www.iaees.org/publications/journals/arthropods/online-version.asp
RSS: http://www.iaees.org/publications/journals/arthropods/rss.xml
E-mail: arthropods@iaees.org
Editor-in-Chief: WenJun Zhang
Publisher: International Academy of Ecology and Environmental Sciences

1 Introduction

Pine cone beetles of the genus *Conophthorus* (Coleoptera: Curculionidae) are destructive pests of pine trees in North America (Hedlin et al., 1980), and periodic outbreaks may destroy 15-60% of a cone crop over large acreages. Adult beetle bores into an immature second year cone in pines. Usually each cone is attacked by one pair of beetles, boring into the stalk of the cone and tunnelling into the axis. The female lays its eggs in the tunnel, which now becomes an egg gallery, then vacates the cone, plugging the entrance hole with frass.

Larvae are present late June-August, pupate and transform into adults which remain in the cone until the following spring. The attack kills the cone and destroys all potential seeds. Damaged cones drop to the ground in early summer (Del Rio and Mayo, 1988).

The study of the Scolytinae compound eyes and visual role in locating their hosts has received little attention from entomologists and to date only abundant research concerning sexual behavior between individuals and the release of pheromones in aggregative and antiagregatives mostly focused in the colonization of their host trees and chemical defense mechanisms, so that the development of *semiochemicals* for monitoring and control methods has made significant progress.

Taxonomically, the compound eyes of Scolytinae are not of value and used for species identification or morphological comparisons (Thompson, 1992; Morimoto and Kojima, 2003; Wood 1982, 1977; Wood and Bright, 1992; de Groot 1981) and more even are few referenced debt to apparently similitude of external structure of eyes in many species studied.

The objective of this paper is contributing to preliminary knowledge of the main compound eye structures for *Conophthorus* species.

Regarding the importance of visual orientation of Scolytinae species for localization of their hosts, only have the reference of Henson (1967) that reported the highest percent of pines cones attacked by *Conophthorus coniperda* on trees with dense canopies in, suggesting that the cone borers are possibly guide visually by shadows at the location of their hosts, although the dispersal flight of scolitids has been shown to be initiated by an appropriate balance of internal feedback information and environmental conditions (Borden, 1974) where initially flight is dominated by an attraction towards higher intensity light (Shepherd, 1966) and when this insects group are relatively unresponsive to olfactory stimuli. Indeed, it has been shown that visual orientation of the bark beetles and woodboring beetles are attracted primarily to dark surfaces and both have a negative phototropism away white objects, when there is not chemical stimuli or olfactory(Bell, 1990; Bernays and Chapman, 1993; Leather, 2005); Moreover, once localized and choiced the more accurate host, the last area is limited in pine crown and reflects individual features of tree or trees group, level of physiological state and as its or their function(s) odor concentration and odor quality which is determined by correlation of some terpenoids depending the tree species and locality (Smelyanets, 1977), afterwards, the feedback(+) is closed when the *Conophthorus* species free their proper semiochemicals during the attack to pine cones (Rappaport et al., 2000).

Aylesworth (2002) studied the integration of visual and olfactory cues by host -seeking bark beetles (Scolytinae) and wood borers (Cerambycidae), which can integrate visual and olfactory information to avoid non- host angiosperms while flying.

2 Materials and Methods

2.1 Collect and samples preparation

Conophthorus specimens alive into pine cones were collected during summer time from different forest stands sampling sites in Mexico in 2010 year: *Conophthorus conicolens* W specimens were collected from *Pinus pseudostrobus* cones in San Lorenzo(19 31´37" N, 102 04´50 "W), *C.teocotum* W in Paracho (19° 39´N, 102° 05´W) and *C. michoacanae* W from the hill "El Panadero" in Ziracuaretiro (18° 01 ´N, 102° 22 ´W), all them localities belonging to Michoacán state, whereas *Conophtorus ponderosae* H was collected from *P. montezumae* cones in "Los Humeros", Puebla near of Perote, Veracruz.

Pine cones attacked by cone borers were stored under laboratory conditions and labeled in plastic bags, taken to and examined at Jacobs University laboratory (Bremen, Germany) where after they were opened to extract the beetles.

The cones were opened using common razor blades by tearing apart the cone completely and carefully. The insects were found along the central part of each cone, collected and stored in glass test tubes whose openings were covered with duck tape and labeled with the species name. The cones were divided into sets, splitting around 5 cones and collecting the whole content during different times of the day; the first group was collected and stored during natural daylight hours and fixed down immediately after during the same period. There was a second group collected and fixed during night time hours.

Males and females were separated by sexual dimorphism according to Mora and Tuchina, 2011 (except *C.michoacanae* Wood specimens), research down light microscope and immediately decapitated, heads split in halves during daytime hours under room light conditions before being fixated for 1 day in a modified Karnovsky's (1965) active solution, containing 2% paraformaldehyde and 2.5% glutaraldehyde, buffered with 0.1M cacodylate (pH 7.4) and fixed. For night time treatment, the procedure was followed during night time hours and by covering the light microscope source of light to prevent changes in ommatidia structure.

After a 2-h period of postfixation in 2% OsO4 solution, also buffered with 0.1M cacodylate (pH 7.4), the samples were rinsed 3 times in the same buffer. The samples were dehydrated in a graded series of ethanol before being passed through different acetone/ Epon mixtures (3:1, 1:1, 1:3, pure Epon). Pure Epon-812 resin was used for final embedding and the samples were then polymerized at a temperature of 60°C for 3 days.

2.2 Transmission and scanning electron microscopy

For light microscopy (LM), semi-thin sections were cut on an ultramicrotome (RMC, Boeckeler instruments Inc., Tucson, AZ) with a glass knife and stained with 0.5% aqueous solution of toluene blue on a hot plate for 30s. Ultrathin sections were cut with a diamond knife and picked up on formvar-coated copper slot grids. The ultrathin sections were stained with lead citrate and 2% aqueous uranyl acetate for 20min each (modified after Reynolds, 1963). The sections were mainly examined under Zeiss EM 900 and JEOL JEM-1011 transmission electron microscope (TEM), operated at 80KV. The TEM was calibrated with a calibration grid (Plano S104) before any measurements commenced. For scanning electron microscopy (SEM), osmium-fixed heads were dehydrated in a graded series of ethanol and then subjected to critical-point-drying (EMITECH 850, Emitted Ltd., Ashford, UK). The dried samples were coated with a layer of approximately 15nm gold in a sputter coater (Quorum Q150T S, Quorum Technologies Ltd. East Grinstead, UK) and observed under JEOL JSM-5900 SEM, operated at 20KV.

2.3 Morphometric analyses and statistical analyses

Measurements on the *omattidium* were taken using ImageJ software (http://rsbweb.nih.gov/ij/). SEM pictures were used to determine dorsoventral and anterior-posterior measurement of the eye (i.e., height and width, respectively), total number of *ommatidia* per eye, facet diameters, and diameters of the *ocelli*. Longitudinal sections for LM were used for measurements of *ommatidial lengths*, radii of curvature of eye, and corneal facets as well as *interommatidial angles*. The radius curvature of the eye was measured on the broadest anterior-posterior extension (eye width) of the eye, in sections done in dorsoventral direction, by laying a circular measurement tool over the eye surface in the image analysis software. The *interommatidial angles* were measured at the point of intersection of the normal to the eye surface through the *ommatidial axes* of central *ommatidia* TEM micrographs of cross-sections were used to measure *rhabdom* diameters, shapes, and diameters of pigment granules of primary pigment cells (PPC) and secondary pigment cells (SPC) as well as *retinula* cells. Measurements of *rhabdom* diameters were taken at the distal tip of the *rhabdom* and registered for internal process of analysis as aprotocol of laboratory and only from few images obtained of light micrographs are enclosed here as general description of Dioptric apparatus and Photoreceptor layer; Longitudinal sections are used to determine *cone length*, *corneal thickness*, and shapes and positions of the pigment granules within *retinula* cells.

Compound eyes of each sex were used for measurements under the SEM. For examinations involving LM and TEM, eyes of either sex were used. All measurements were gathered by image analysis software (Image J, Rasband, W.S., U.S. National Institutes of Health, Berthesda, MD) and based on an identical number of objects per specimen to avoid a weighting in the statistics later.

Tukey-Kramer multiple comparisons test was performed to compare the number of facets between *Conophthorus* species studied, using GraphPadInStatsoftware versión 3.06.32 (2003) and Prisma 6 (2014) GraphPad Software.

3 Results and Discussion

3.1 Description of the general external organization of the eye

The SEM micrographs (Figs. 1-2) reveal that all *Conophthorus* species showed kidney-shaped compound eyes, with anterior margin emarginated (notched or indented) and the long axis perpendicular to the substrate when the insect is walking and where the *ommatidium* quantified no differed significantly between most species .The individual facets are lightly hexagonal towards the center of the compound eye but the margins are curved near the periphery. The facet lens surfaces are smooth and corneal nipples or ridges absents.

It is noted there are not over the whole compound eye any interface hairs distributed randomly which are presents in some small beetles as *Sartallus signatus* (Coleptera: Staphylinidae) (Meyer Rockow, 1972), where likely are mechanoreceptor related to cleaning reflexes.

From the *Conophthorus* species studied, *C.conicolens* W and *C. ponderosae* H are both the more closely related phylogenetically and morphologically than others of the same genus (Wood, 1982; Cognato et al., 2005), so these cone bores share same hosts and the prevalence populations mixed in the neovolcanic edge of Mexico, this is the reason why these species were selected as representatives to describe the fine structures and the photoreceptor layer regarding light micrographs forward seen.

3.1.1 Facets

Total eye length was measured, dorsally and ventrally, aiming at finding a difference between the sexes on the same species, due to other scolytids groups as the coffee berry borer, *Hypothenemus hampei*, where the male individuals have much fewer facets than females (Vega et al., 2014).

Chapman (1972) indicated that ommatidial number ranges from approximately 150 per eye in *T. Zineatwn* to around 300 in D. *pseudotsugae*. The relatively small number of facets is consistent with an adult life that is spent within the bark or wood of a host tree.

A total of 37 specimens in good conditions from 111 adults collected of diferent sex for the species of pine cones borers mentioned were considered in our study to quantify and measure the number of *facets* or *ommatidia* and rest of other features measured, dissected and prepared according to the methodology described for only obtain information regarding the number of facets and the results are:

The number of facets per eye compound rate between species (Table 1) represented by a mean minimum for *Conophthorus teocotum* female specimens of 93.333 (n= 6) to an average maximum of facets per eye for the case of *C.michoacanae* unsexed specimens of 194.33 (n= 3), although as can see on the table 2, according to statistical test one way analysis of variance(ANOVA) and Tukey-Kramer multiple comparisons test performed, there is not significance difference between number of facets by species and sex, except for *C. michoacanae* unsexed specimens and *C.teocotum* females and *C.ponderosae* males versus *C.teocotum* female specimens studied. However, seem it is clear that due to the low number of measurements for these two pairs of comparisons we have the only real difference is in the case of *C.teocotum* in difference to the other three species of borers cones. All this let suppose that the number of facets compound eye *Conophthorus* species is a function of body size of this group of insects, as representing the species *C. michoacanae* and *C.tecototum*, largest and the smallest in that order (Wood, 1982; Del Rio and Mayo, 1988).

Table 1 Number of facets per compound eye in fourth cone borers species studied.

Group	Number of observations	Minimum value	Maximun value	Mean	Standard of mean (E)
C.conicolens(fe)	10	100	208	151.7	14.33
C.conicolens(ma)	7	100	192	135.57	14.661
C.michoacanae	3	181	208	194.33	7.796
C.ponderosae(fe)	4	110	207	165.00	22.498
C.ponderosae(ma)	7	109	207	164.71	15.24
C.teocotum(fe)	6	47	145	93.333	17.091

Simbology: *(fe)*, female specimens; *(ma)*, male specimens.

Table 2. Results of Tukey-Kramer multiple comparisons test, referent to number of facets between *Conophthorus* (Coleoptera: Scolytinae) species studied.

Comparison	Mean difference	Q	P value	
C.con(fe) vs *C.con*(ma)	16.129	1.125	ns	P>0.05
C.con(fe) vs *C.michoa*	-42.633	2.226	ns	
C.con(fe) vs *C.pon*(fe)	-13.300	0.7728	ns	
C.con(fe) vs *C.pon*(ma)	-13.014	0.9078	ns	
C.con(fe) vs *C.teo*(fe)	58.367	3.885	ns	
C.con(ma) vs *C.michoa*	-58.762	2.927	ns	
C.con(ma) vs *C.pon*(fe)	-29.429	1.614	ns	
C.con(ma) vs *C.pon*(ma)	-29.143	1.874	ns	
C.con(ma) vs *C.teo*(fe)	42.238	2.610	ns	
C.michoa vs *C.pon*(fe)	29.333	1.320	ns	
C.michoa vs *C.pon*(ma)	29.619	1.475	ns	
C.michoa vs *C.teo*(fe)	101.00	4.910	*	P<0.05
C.pon(fe) vs *C.pon*(ma)	0.2857	0.01567	ns	P>0.05
C.pon(fe) vs *C.teo*(fe)	71.667	3.817	ns	P>0.05
C.pon(ma) vs *C.teo*(fe)	71.381	4.410	*	P<0.05

Simbology: *(fe)*, female specimens; *(ma)*, male specimens.

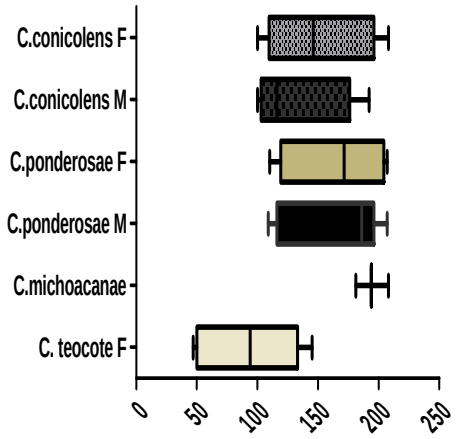

Fig. 1 Graphic comparison showing number of facets per eye, minimal, median and maximal value (Simbology: F, female specimens; M male specimens).

Moreover, if we compare the length of the compound eyes of *Conophthorus ponderosae* males and females, with the evidence of the few measurements made about, we see that females have statistically the largest eyes, but if we compare the number of facets of the compound eyes of the species studied can see that it is possible conclude in general that the size of the compound eyes is seem between species and between individuals of both genders, as the number of facets and angle of these is of little variation and leads us to infer the respect.

In the Table 3 are add other characteristics or features of the *Conophthorus* compound eyes.

3.1.2 Other external or internal features and structures

Below we present the results of some of the internal and external measurements of the compound eyes of the 4 species of borers cones characteristics mentioned in this study.

In general, *C. conicolens* males showed larger eye coverage than females (see Table 1). Also, males showed an important difference where ventral section was measured, the size of this area is significantly larger than female eyes' display, interestingly when the dorsal measurements were taken, the difference is less obvious, but yet, for full confirmation of this fact would be make major morphological studies and above all, increase the number of measurements.

Table 3 Some measurements of eigth diferent components featuring the cone borers compound eyes(Values expressed in nm.).

Species	Eye length	Ventral eye width	Dorsal eye width	Facet diameter	Primary pigments diameter	Secondary pigments diameter	Faces Length	Ccone length	Rhabdom length	Cornea thickness
Male *C. Conícolens*	414.9	232.19	225.69	16.47	813.75	671.85	--------	------	DI	DI
	39.3	51.6	62	2	45.2	71.5				
Female *C. Conícolens*	392.27	180.65	201.93	16.53	723.12	652.40	13.04	-------	DI	DI
					59.5					
Male *C. Ponderosae*	408.91	156.7	193.14	18.11	813.75	*	18.77 *	16.24	26.1nm	8.85nm
	29.5	10.6	*	1.9	45.2			2.1	1.9	*
Female *C. Ponderosae*	465.43	174.85	212.09	18.71	645.48	571.2	27.35 *	21.48	34.19nm	9.93nm
	4.6	18.2	4.6	2.4	*	*		9.1	15.7	*
C.michoacanae Sex undetermined	408.91	156.7	193.14	18.11	813.75	-------	18.77 *	16.24	26.1nm	8.85nm
	29.5	10.6	*	1.9	45.2			2.1	1.9	*
C. teocotum Female	465.43	174.85	212.09	18.71	645.48	571.2	27.35 *	21.48	34.19nm	9.93nm
	4.6	18.2	4.6	2.4	*	*		9.1	15.7	*

Simbology: Mean ;------ no measured; SD; * single measurement

Measurements on *C. ponderosae* specimens showed a different current. Female measurements showed larger than males eyes, which can be doubt to a evolutionary adaptation to help in the visual localization of

green cones, since the females of cone bores are beginning the attack on the second year cones .The most obvious difference between sexes in this species was found on dorsal eye's measurement, whereas the ventral size difference shown was less significant, although as it was said before, our study is preliminary and overall, our size samples are not sufficient statistically to conclude these possible differences. On the other hand, There were no significant differences between *omattidium* diameter measures on both sexes belonging to the same species.

The most significant difference shown was between species *omatidium* diameters, for both sexes, *C. ponderosae* showed bigger *omattidium* diameter measurements than *C. conicolens* (Table 1).

3.1.3 Dioptric apparatus

Technically all the compounds of *dioptric apparatus* functions as an efficient window for the *ommatidium*, with light transmission greater than 70% throughout the visible spectrum; Then, that light is focused at the level of the photoreceptor cells, sharply when light-adapted and partially when dark-adapted , optimizing resolution over a wide range of light levels (Meyer-Rochow, 1974). Here mention the basic components of this apparatus related *Conophthorus* species studied.

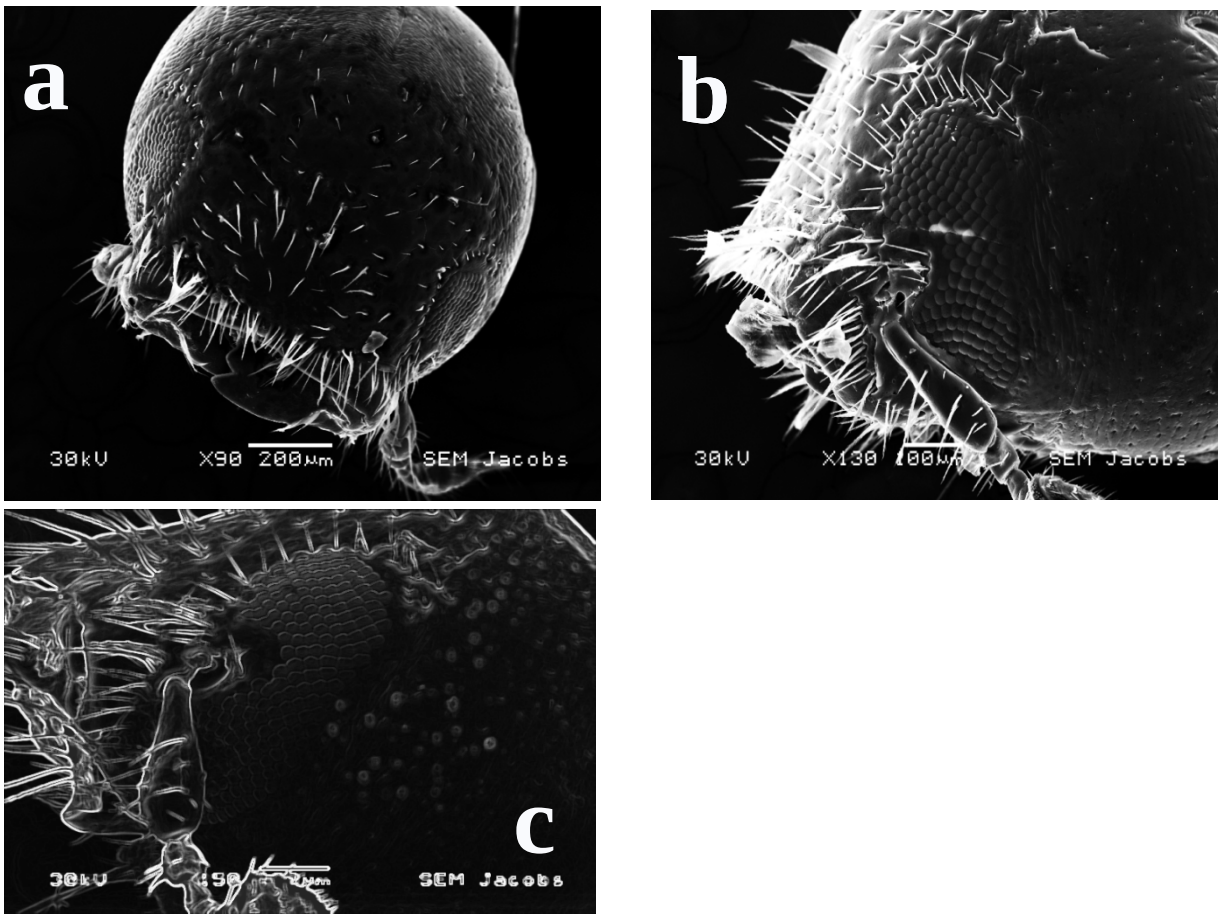

Fig. 2 Scanning electron micrographs of the compound eye of *Conophthorus conicolens* Wood: a) male specimen: head-capsule (frontal view); b) compound eyes (lateral view); c) female, left side of head with typical compound eyes kidney-shaped compound eyes.

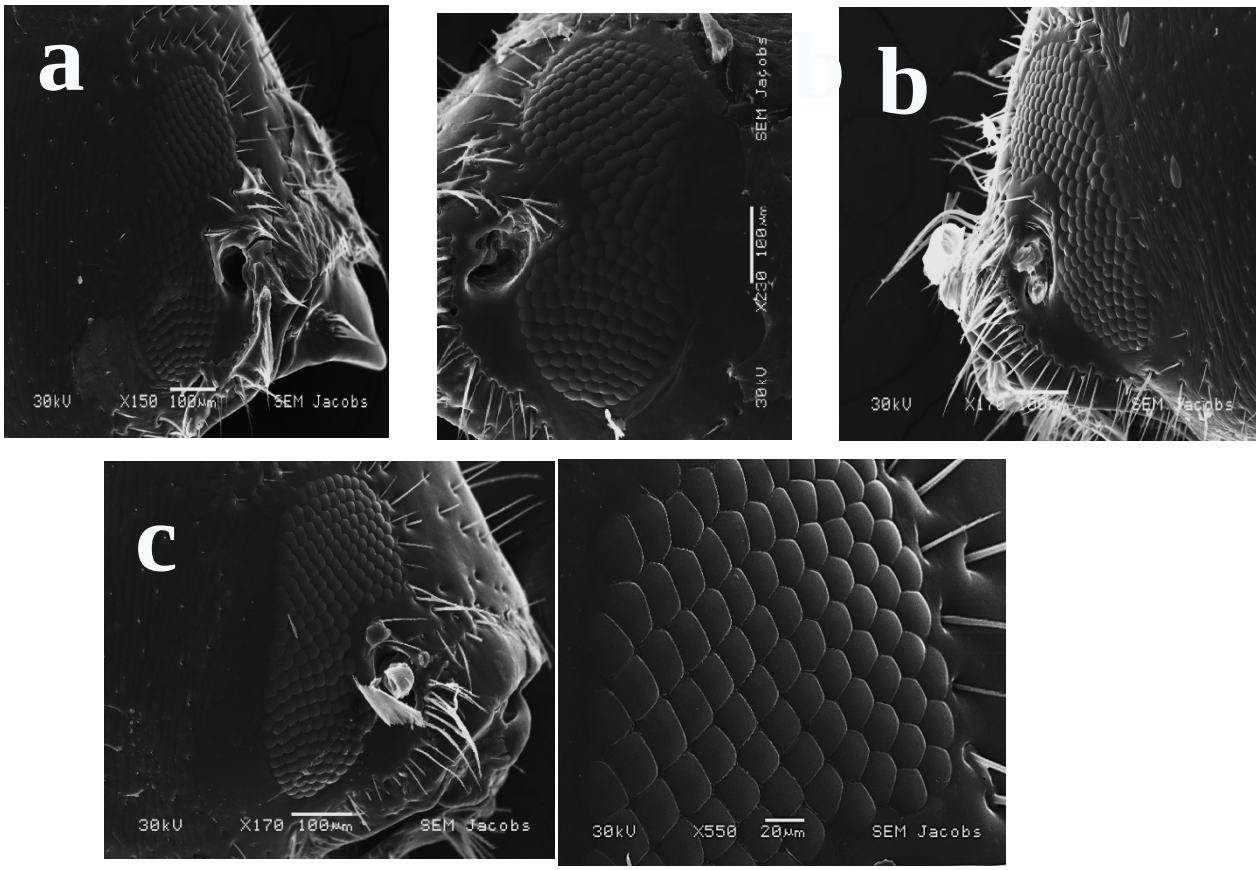

Fig. 3 Pictures micrographs of the compound eyes of: a) *Conophthorus ponderosae* H, left and right lateral views, female specimens dissected ; b) *Conophthorus ponderosae* H, head: left side, male specimens; c) *Conophthorus michoacanae* W: right side of head (left picture) and several ommatidia.

The cornea and cone are more significant of the *dioptric system* in beetle compound eyes, where the morphology of these structures provides the basis for the classification of compound eyes.

As is observed, cones and receptor layer are not separated by a clear zone and typical for the apposition eyes.

In our case, the cone is really represented by an *acone* type of crystalline cones and its measures can read on Table 3; For the case of female specimens in *C.ponderosae* H and *C.teocotum* W is clear *acone length* is notoriously up in females than these observed in the male specimens (Female *C. Ponderosae* and *C.teocotum*, 21.48 nm versus male *C.ponderosae*, 16.24 and *C.michoacanae*, idem, although in this specie were not determined the sex for specimens estimated), condition too can be related to their elemental mail activity as pioneers in looking for pine green cones of second year growing before attack them and it implicate, logically, a major captation of sunlight during this process.

Secondary pigment cells pictures were taken from *Conophthorus conicolens* Wood and *C.ponderosae H* and genders after a previous exposition at day-light and dark-time period separately.

The *ommatidia* or *facets* are confined in a discoid space beneath the cuticle by an underlying and concentric *apodeme* projecting downward from the margins of the compound eye, so the cuticular *apodeme* forms the supporting structure for the *ommatidia*, except for a central opening where the basement membrane completes the inner limits of the compound eye; According to our results, the internal structure of the cone

bores compound eyes are seem these described for *Dendroctonus pseudotsugae* Hopkins by Bennet (1978).

Below in the next pictures we can identify the major components of the fine structures representing dioptric apparatus and part of the components of the *photoreceptor layer* for *Conophthorus ponderosae* H.

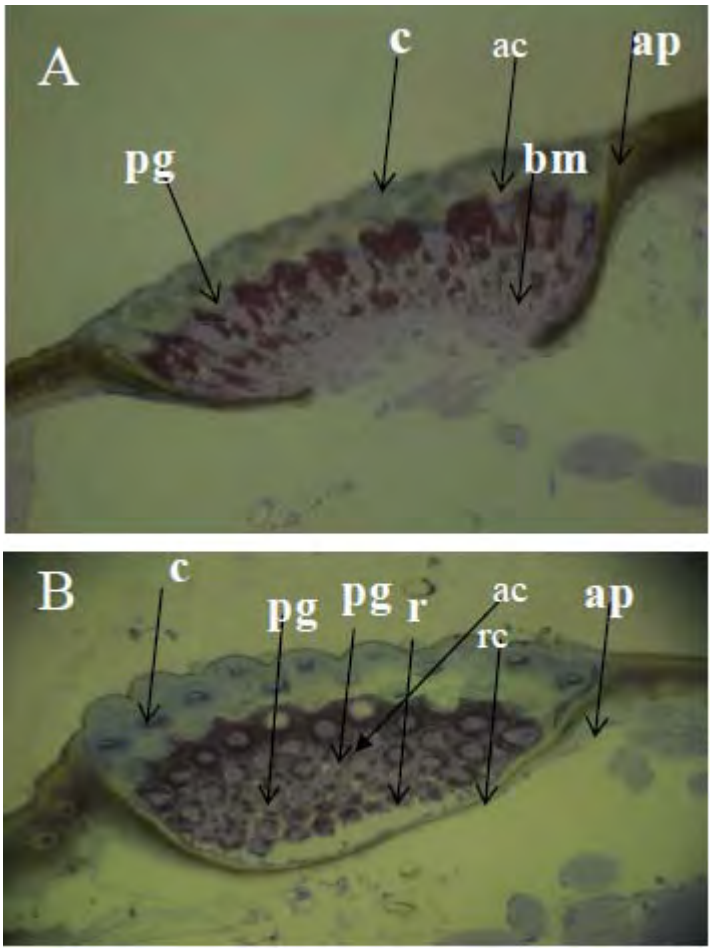

Fig. 4 Light micrographs of the internal structure of the compound eye of *Conophthorus ponderosae* Hopkins; A) Longitudinal section through entire compound eye showing arrangement of ornrnatidia and supporting apodeme-structure. (Scale = 100 µm). B) Transverse section through part of compound eye with ommatidia cut in cross section, revealing rhabdomeric ring structure (Scale = 100 µm). Simbology: Cornea (c), acone (ac), retinula cells (rc), rhabdomeres (r), pigment granules (pg), apódeme (ap), basement membrane (bm).

As shown previously, surrounding each *facet* (*ommatidium*) and shielding it from its neighbours are pigment cells and several of them are attached to the base of each lens and seem to dark-staining granules around the cone and along the length of each ones.

At next picture is possible see the four cells containing rather clear protoplasmic material with a few cell inclusions and exhibit an arrangement typical for fluids surrounded by thin membranes:

3.2 Photoreceptor layer

The fields of view of the photoreceptor cells are determined by the dimensions and anatomical arrangement of the optical part of the *ommatidium.* The dimensions, and therefore the fields of view of the ommatidia are also related across the eye (Horridge, 1980), hence the importance of measurement and properties of the dioptric components mentioned in advance.

As has been reported for other species of scolytids (Chu et al, 1976; Bennet, 1978) the *photoreceptor layer* for in *Conophthorus* species contains too eight *retinula* cells per *ommatidium*, with the *rhabdomeres* arranged as a peripheral ring around central *rhabdom* (Figs 6-8), which suggest the spectral preferences near UV-violet (below 425 nm) and the blue-green (500-525 nm) as in other scolytid species (Bennet, 1978; Meyer, 1976; Shönherr, 1971). For this principle of the visual orientation of scolitids, the monitoring of bark beetles populations is commonly by using lindgren funnel traps and for the case of *Conophthorus* species the Japanese beetle traps are effectives (Rappaport et al., 2000; de Groot and DeBarr, 1998) and these are pick up on the third upper of the trees crown made and made of color yellow or dark green.

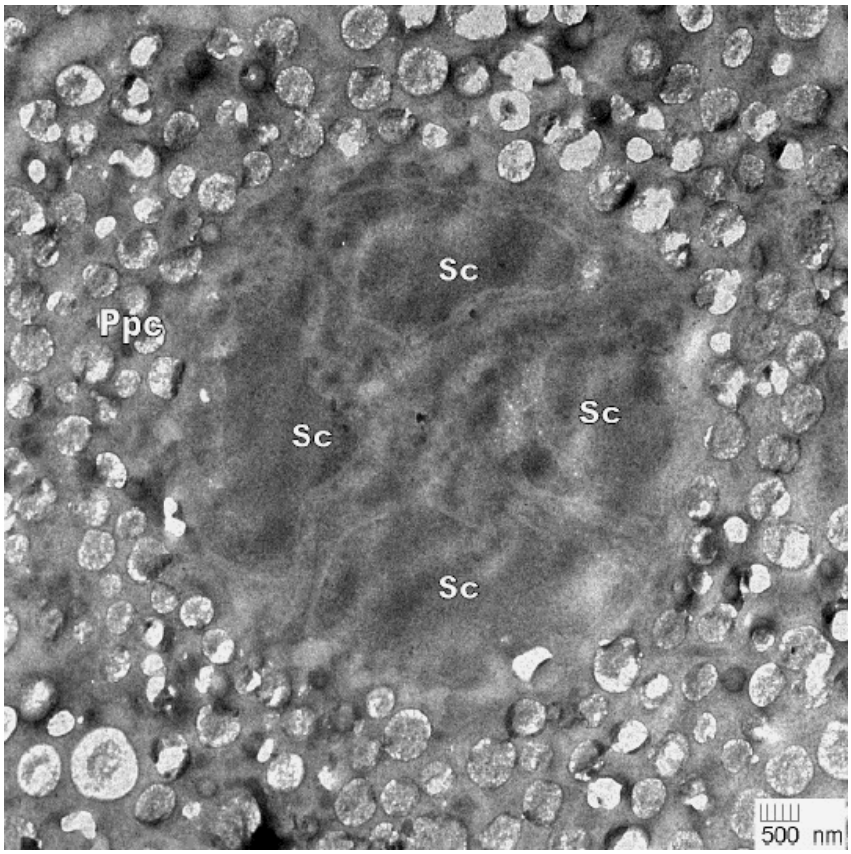

Fig. 5 *Conophthorus conicolens* W: Cross section of the acone structure. The four semper cells conforming the cone. Simbology: PPc. Primary pigment cells. Sc. Semper cells or acone.

As mentioned above, Bennet, 1978 suggested that the *peripheral rhabdomeres* (1-6) could be the green receptors and central ones (7-8) the blue receptors.

This fact seems has been an process of evolution where once separated has become to *rhabdom* more efficient even and that for each *ommatidium* now has equal number of *axons* and lead increasing the number of images formed with the same number of *ommatidia* and this explain too as was before mentioned, the relative small number of them in the bark beetles in general.

On the other hand, the *cytoplasm* of the *retinula* cells forms the light are between the peripheral and central *rhabdomeres* as well as between them and the surrounding pigment cells.

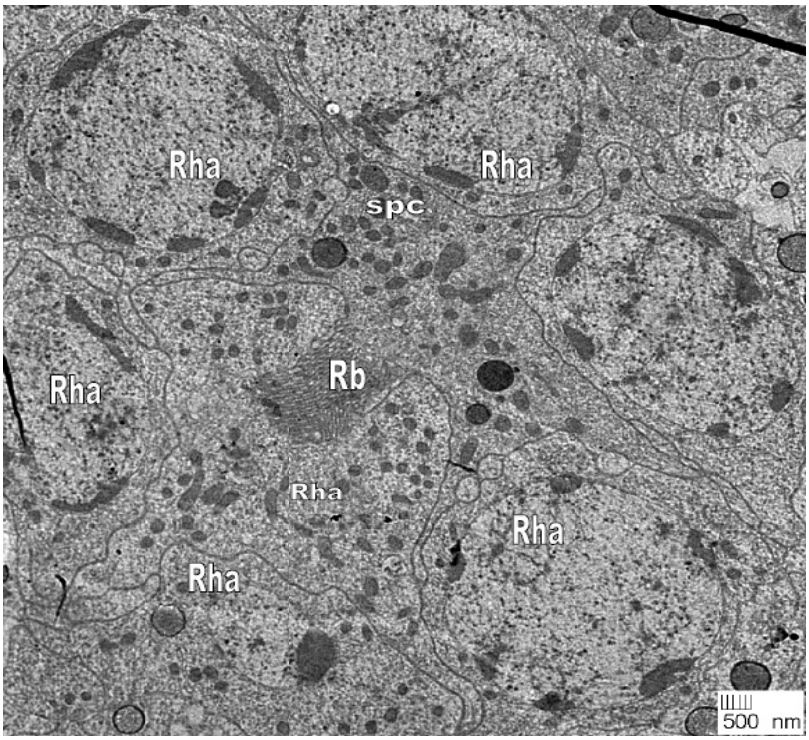

Fig. 6 *Conophthorus ponderosae* H: Light micrographs of thephotoreceptor layer, showing the arrangement of cell within the internal ommatidium structure, surrounded by secondary pigment cells as supports. Simbology: Rha. Rhabdom cells surrounding the rhabdom Rb Spc. Secondary pigment cells.

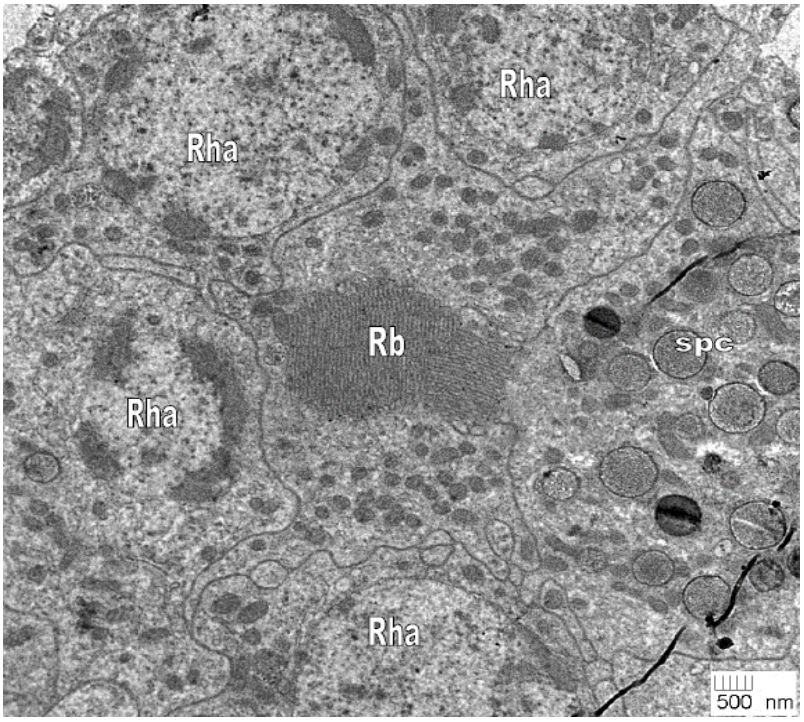

Fig. 7 The arrangement of the rhabdomeres inside the main rhabdom. The pattern on rhabdomeres cells structure. Simbology: Rha. Rhabdom cells. Rb. Rhabdom structure. Spc. Secondary pigment cells.

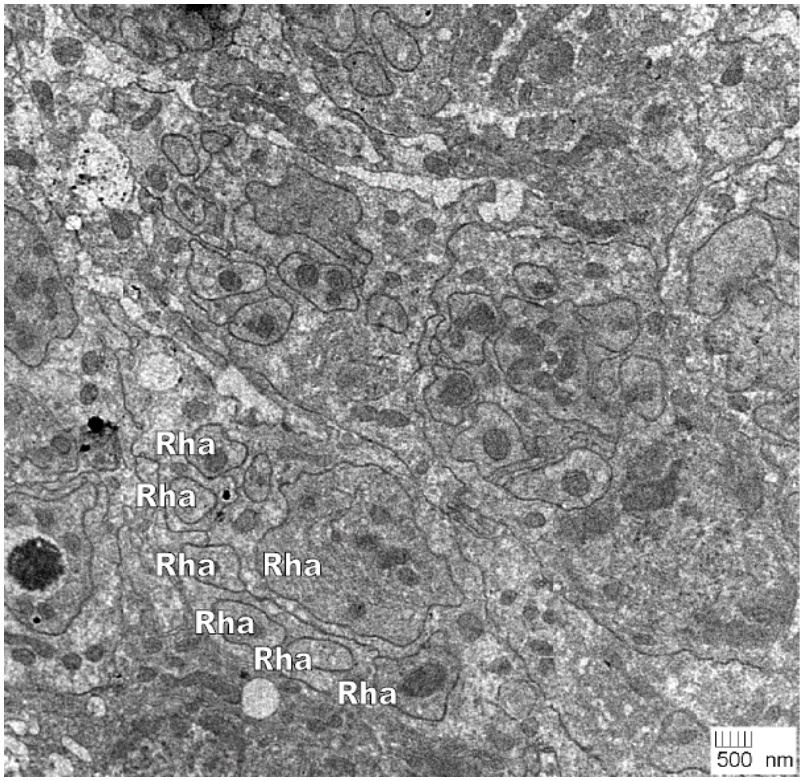

Fig. 8 Near the basal grid, the rhabdom cells display its axons conections: Rha. Rhabdomere cells, basal location.

Acknowledgements

We wish to express very specially our deep appreciation to Dr. VB Rochow for valuable advice and facilities for the accomplishment of this work, and the Jacobs University, School of Engineering and Science, Bremen, Germany. Thanks you so much for your important participation in all work performed in the preparation of specimens, measurements, SEMand light micrographs performed in Jacobs University laboratories, Bremen, Germany., To: Stefan Fischer ,PhD Student (Biology) in Jacobs University Bremen, Germany, PhD Oksana Tuchina in Max Planck Institute for Chemical Ecology, Jena, Germany and to M.C. Bisbrian Alheli Nava Gonzalez in Instituto de Investigaciones sobre Recursos Naturales (INIRENA), México, Universidad Michoacana de San Nicolas de Hidalgo

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