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Morphology of antennal cleaner in some selected ant species: A scanning electron microscopy study

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ABSTRACT: Antennal cleaners are cuticular structures found in insects like ants which help them in antennal grooming. A well groomed antenna is important for better olfactory sensory perception. Scanning electron microscopy studies on the morphological features of antennal cleaners in some selected ants revealed structural differences like the presence of abundant brushes on the tarsal notches, tarsal comb with abundant tines, and presence of spines among the antennal cleaners of the ants. Differences even if subtle point towards different strategies of antennal grooming adopted by the ants. Bristles, brushes and spines present on the antennal cleaners are components of the antennal cleaner used for different grooming tasks such as adhesion and scraping mechanisms. Further significant differences in the morphometrical features of the antennal cleaners, which probably have a bearing with the life styles of each ants were reported. © 2018 Association for Advancement of Entomology

KEY WORDS: Ant species, SEM, antennal cleaner, tarsal notches, tibial spur

INTRODUCTION

Insects face the constant challenge to keep their body parts clean from the various types of contaminants they are exposed to from the environment. Bacteria, spores, pollens and inorganic particles like dust, salt adhere to their body parts and interfere with their bodily functions. Therefore for insects, the removal of these micro particles is most important. Grooming help insects to get rid of such particles adhered to their body. In many insects the legs, wings and antenna are modified for the purpose of grooming (Hölldobler and Wilson, 1990). Antennal cleaners in particular, located on the forelegs of ants and other hymenopterans are examples of insect legs and its parts evolved for grooming. They are complex cuticle structures which are modified spurs of tarsus of the legs and possess many components like brushes, bristles and combs which in case of ants, is found effective to remove dust, bacteria, virus, pollen, fungal spores, salt or other particulate matters adhered to the antenna (Szebenyi, 1969; Elawami and Dent, 1995). Adherence of such debris on antennal surface interferes with olfactory sensory perception (Böröczky *et al.,*2013). Each cuticular component of the antennal cleaner is to function in removing particles of different sizes from the antennal surface.

Life style induced adaptations are often reflected in the body parts an insect possess. Antennal

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cleaner though not sensory in function are important fora debris free antennal surface which is important for an efficient sensory perception through the diverse types of antennal sensilla (Reber *et al.,* 2011). Though there have been few studies that help us understand the working mechanism of the antennal cleaner and its structural details in a phylogenetic context (Basibuyuk and Quicke, 1994). Studies relating to the structural modifications of the antennal cleaner in relation to the sensory ecology of ants are few. In the present study we explored the morphological features of the antennal cleaner of a few ants which exemplify contrasting life styles and behaviours. Sensilla profile of the antenna often reflects the life style of the insects and an efficient antennal grooming is important for a better sensory perception. *Diacamma rugosum* (Le Guillou, 1842), *Messor barbarus* (L), *Myrmicaria brunnea* (Saunders, 1842) and *Oecophylla smaragdina* (Fabricius, 1775) ant species were selected for the studies. *O. smaragdina* generally is considered to be an arboreal ant species; *D. rugosum*a generalist forager is commonly found to be foraging in open spaces, gardens and detritus; *M. brunnea* is a subterranean ant species; *M. barbarus* is the granivorous seed harvesting ants (Plowesand Holldobler, 2013; Wriedt *et al*., 2008). Structural modifications of the antennal cleaner reflect different ways of antennal grooming. Though there have been studies on the antennal cleaner of ants in pursuit of deducing phylogenetic relationships (Schonitzer *et al.,* 1996). Studies aimed to trace the antennal cleaner architecture in relation to the ants lifestyles have not been carried out yet. The present study is an attempt in this direction where we explored the structural details of the antennal cleaner in these ants to ascertain the differences.

MATERIALS AND METHODS

D. rugosum, *M. barbarus*, *M. brunnea* and *O. smaragdina* ant species were selected for the studies. Ant species for the present study were collected using polythene bags and insect collection aspirators from different localities in and around Changanassery, Kerala and brought to the laboratory and cold anaesthetized by keeping it in the refrigerator for 24 hours. Legs were dissected out from the anaesthetised specimens and immediately fixed in 4% paraformaldehyde. Leg preparations were dehydrated in graded ethanol series of 30-100% ethanol for sixty minutes in each step. The dehydrated specimens were finally cleared in methyl salicylate. After drying the specimens at a critical point, the specimens were mounted on the stub using a double side adhesive tape. The preparations were gold sputtered and dried in the desiccator. Samples were scanned under 15k Vemission current and desired images of various magnifications were captured by SEM JEOL JSM 6390.

Statistical analysis: One way ANOVA and Kruskal –Wallis tests were conducted to assess the significant differences in the morphometric features of the ants selected for the study

RESULTS

The tarsal notch and the tibial spur are the prominent features of the antenna cleaner of ants. Further the notch and spur bears common features like comb, brush, bristles (setae) and spines, which all together make up the composite structure of the antennal cleaner.

Antennal cleaners of the ants show structural similarities in general, but the subtle and significant differences are noteworthy. The tarsal notch is most prominent in *O. smaragdina;* the concavity of the tarsal notch runs deep and is richly endowed with the paddle or oar shaped brushes (setae), which are distributed along the entire concavity of the tarsal notch. The paddle shaped brushes are clubbed and densely distributed in comparison to the other ants considered for the present study. Comb of tarsal notch of *O. smaragdina* has a significantly higher number of brushes (setae) by virtue of their closely arranged packing (Table 1). Tibial spur has a length of 300 µm. There were more than 100 comb tines on the tibial spur, which accounts for the highest number in the case of the ants in the present study. Individual length of the tine is approximately 30 µm, It was observed that the setae of tarsal notch of the ants have a unique pattern of distribution

Table 1. Morphometrical features of the antennal cleaners of ant species

Ant species	Tibial spur comb length (µm)	Tarsal notch comb length (µm)	Tarsal spur and tibial notch comb tine length (µm)	Tarsal notch (brush) (Total number)	Tibial notch Spines
Oecophylla smaragdina	300 ± 12µm[b]	300 ± 6µm[a] 110 ± 8.6[a]	30 ± 4.6µm[a]	32 ± 8.4µm[a] Dense distribution along the entire concavity of the tarsal notch (Tip of the Setae of the comb tapers and ends in a pointed tip).	-
Myrmicaria brunnea	200 ± 8µm[a]	300 ± 8µm[a]	60 ± 6.2µm[b]	56 ± 6[b] Distribution partially and scarcely along the tarsal notch.	-
Messor barbarus	200 ± 9.4µm[a]	150 ± 6µm[b]	30 ± 3.4 µm[a] 25 ± 4.6 µm	52 ± 4.8[b] Sparse distribution along the concavity of the tibial notch (Oar like appearance of the setae with the terminal portion flat).	-
Diacamma rugosum	350 ± 6.8µm[c]	300 ± 9.6µm[a]	50 ± 6,6[c d] 80 ± 11.4	60 ± 4c Dense distribution along the entire concavity of the tarsal notch. longest ones with approximately 125 µm in length.	Stout and long spines on the outer margin of the tarsal notch.

Mean with the same letter are not statistically significant; All values are mean ± SD, n=10;
[*]Indicates significant differnces from each other: PÂ0.01, n=10

compared to other ants (Fig. 2a-b).The subterranean ant *M. brunnea* also has similar morphometric features, however its tarsal notch has two types of tines; the distal ones are oar shaped and comprises 10-15 in number, and the rest of the tines are of similar length but has bluntly ending tips (Fig.1a-b). *M. barbarus* has a significantly lower length of the comb tine on the tarsal spur and tibial notch respectively (Fig. 2c-d). The total length of the comb is also significantly less. The distal tarsal tines appear as oar shaped and the rest of the comb tines end bluntly and have relatively similar number of combtines all together on the tarsal and tibia combs. *D. rugosum* has the most contrasting antennal cleaner among the studied ants. The tines of the tarsal notch have 50µm length whereas the tibial spur tines are approximately 110µm. The tibial notch is conspicuously endowed with an unique array of long and prominent spines (Fig.1a-b) The finger like projections of the inner tibial notch (brush) is densely distributed and has a similar appearance to that of *O. smaragdina.*

DISCUSSION

Ants selected for the present study are considered as good foragers (Plowesand Holldobler, 2013). They forage on different substrata and are likely to encounter dust, mud, clay, bacteria, virus, pollen, fungal spores, salt or other particulate matters on the antennal surface in different levels by their for aging preferences and feeding habits.The differences revealed in the organisation of the antennal cleaner of the ants, throws some light into different antennal grooming methods adopted by the ants. *O. smaragdina* is arboreal, group forager and carnivorous. They also are found to be mutually associated with mealy bugs and other homopterous like aphids to devour honey dew those organisms produce. Previous studies indicated that brushes present on the tarsal notch help in adhesion of particles of size less than 5µm (Hackmann *et al.,* 2015), suggesting that the dense distribution of brushes on the tarsal notch of *O. smaragdina* points towards a heavy reliance on adhesion

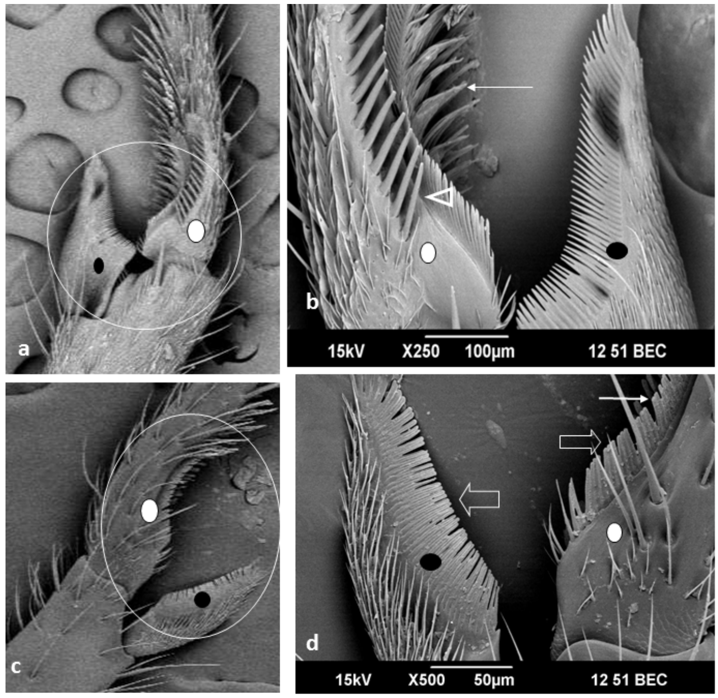

Fig. 1a. SEM microphotograph of the antennal cleaner of *Diacamma rugosum;* **1b**. Enlarged view of the antennal cleaner. the black dot corresponds to the tibial comb;white dot corresponds to tarsal comb. Tail less arrow point to the stout spines of *Diacamma,*the thin white arrow corresponds to the paddle like bristles of the tarsal comb; **1c**.SEM microphotograph of the antennal cleaner of *Messor barbarus*; **1d**. White short arrows corresponds to the tarsal and tibial comb respectively, thin arrow corresponds to paddle shaped bristles

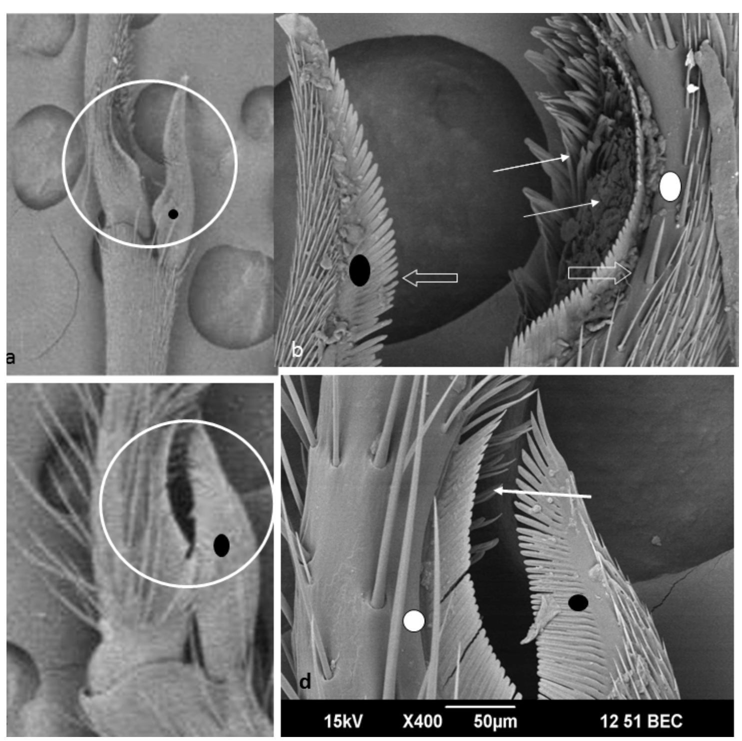

Fig. 2a.SEM microphotograph of the antennal cleaner of *Oecophylla smargdina*. Thin white arrows corresponds to the mat like structure and the paddle like bristles on the tarsal comb; **2b**.Thin white arrow corresponds to the paddle like bristles of the tarsal comb of *Myrmicaria brunnea*

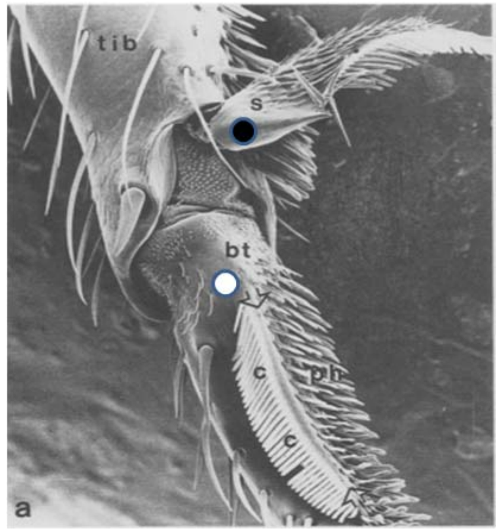

Fig. 3a. SEM microphotographof the antennal cleaner of *Messor rufitarsis*

mechanism of particles less than 5μm (Fig.1a-b). Further the highest number of the tarsal notch setae also suggests a heavy mode of scraping mechanism involved in their antennal grooming behaviour. However, it is difficult to draw definite conclusions, as conclusive evidences are required to ascertain whether the brushes of the tarsal notches are the main components involved in grooming demands associated with similar life styles. Antennal sensilla profile of *O. smaragdina* shows the presence of many unique sensilla like the thermo receptive ampullaceum and CO_2 receptive sensilla coeloconicum,which probably needs an intense cleaning because they are located below the antennal surface and opens through the antennal exterior through characteristic pores which are visible on the antennal surface. Clogging of such sensory pores through the detrimental particles can certainly interfere with the sensory perception. We speculate that the dense distribution of the brushes and the spongy mat like structures on the tarsal notches of *O. smaragdina* could certainly be helpful in such ways of antennal grooming

M. brunnea is a ground forager and lives in subterranean nests. It is considered a food opportunist and has a broad food spectrum. Though their tibial spur and tarsal notch bear morphometrical features similar to *O. smaragdina*, the tines of the combs are reduced significantly albeit with an increased length of the comb. The dichotomous pattern of the comb make up with oar shaped setae placed in the distal end is not so closely placed pattern compared to the tapering ended setae which constitutes the rest of the comb setae suggest the possibility more of scraping and the presence of very few paddle like brushes on the ventral side of the tarsal notch a comparatively less mode of adhesion mode of grooming in this ant. *M. barbarus* is the common harvester ants found in the grasslands and semi-arid areas, they are group foragers on the ground surface. In the present studies a significant difference in the total comb length of the tibial spur and the tarsal notch with a subsequent reduction in the length of the respective length of the comb setae, was noticed and noted that *M. barbarus* bears a miniature antennal cleaner among the ants in the present study. How ever compared results based on the study on the antennal cleaner of the European Harvester ant adapted to the temperate habitats of the Mediterranean areas - *Messor rufitarsis* (Schonitzer *et al*., 1996), showed a highly contrasting architecture of the antennal cleaner with the presence of densely present paddle shaped brushes on the tarsal notch meant for the purpose of adhesion; setae on the comb are distributed in a more spaced manner and they have blunt ends. *M.*

barbarus ants have brushes on the tarsal notch scarcely, which suggest of a relatively less dependence on adhesion mechanisms. It is obvious that *M. rufitarsis* (Fig. 3a) relies more on adhesion and scraping mechanisms than *M. barbarus.*

D. rugosum the largest of the ants in the study did not show a corresponding allometrical growth of the antennal cleaner, but the many features of the antennal cleaner is contrasting from that of the other ants taken in the study. The morphometric features of the combs of the tibial spur and the tarsal notches are similar to that observed in other ants in the study. How ever, the setae of the tibial spur have the highest length with pointed and slender ends and uniformly distributed throughout the tarsal notch. In addition, the presence of widely spaced thick spines which line the tarsal notch is also a significant feature of the antennal cleaner of *D. rugosum* (Fig. 3a). As spines are the reliable structures meant for the purpose of scraping, the study results point to a heavy mode of scraping in the ant, and a heavy distribution of the paddle shaped brushes densely distributed along the tarsal notch endorses the dependence on grooming mechanisms of adhesion in this antalso. Antennal cleaner of ants are the most elaborate ones in their architecture and represents complexities in their functional mechanisms (Basibuyuk and Quicke, 1994). However what appear more intriguing is the variations within the group. Though the general structure of the antennal cleaner is retained in these ants, significant deviations are evident from the statistical analysis. *M. brunnea* and *M. barbarus* though belong to the *Myrmicinae* subfamily are clearly distinct with their granivorous and carnivorous lifestyles and associated behaviours. *M. barbarus* possess a miniature antennal cleaner which is modest in its morphometric measurements in comparison to other ants. Contrastingly, the European harvester ants adapted to the temperate habitats and probably based on the resource availability possess contrasting antennal cleaner architecture Schonitzer *et al.* (1996).

REFERENCES

Basibuyuk Hasan and Quicke Donald (1994) Evolution of antennal cleaner structure in the Hymenoptera (Insecta). Norwegian journal of Agricultural Sciences 16: 199-206

Basibuyuk H.H. and Quicke D.L.J. (1995) Morphology of the antenna cleaner in the Hymenoptera with particular reference to non-aculcate families (Insccta). Zoologica Scripta 24: 157- 177.

Böröczky K., Wada Katsumata A., Batchelor D., Zhukovskaya M. and Schal C. (2013) Insects groom their antenna etoenhanceol factory acuity. Proceedings of the National Academy of Sciences, USA 110: 3615–3620.

Elawami I.O. and Dent D.R (1995) The interaction of surface and dust particle-size on the pick-up and grooming behavior of the German cockroach *Blattella germanica*. Entomologia Experimentalis et Applicata 7(1): 81–87

Hackmann, Alexander, Henry Delacave, David Labonte, and Walter Federle (2015) Functional Morphology and Efficiency of the Antenna Cleaner in *Camponotus rufifemur.* Royal Society Open Science 2(7): 1501-1529.

Hölldobler B. and Wilson E.O. (1990) The Ants. Cambridge, MA: Harvard University Press.

Plowes Nicola, Johnson Robert and Hölldobler Bert (2013) Foraging behavior in the ant genus Messor. Myrmecological News 18: 33-49.

Reber A., Purcell J., Buechel S.D., Buri P., Chapuisat M. (2011) The expression and impact of antifungal grooming in ants. Journal Evolution Biology 24(5): 954–996

Szebenyi A.L. (1969) Cleaning behaviour in *Drosophila melanogaster*. Animal Behaviour 17(4): 641–651.

Schönitzer K., Dott. H. and Melzer R. (1996) The antenna cleaner gland in Messor rufitarsis (Hymenoptera, Formicidae). Tissue and cell 28: 107- 113.

Schönitzer K. and Renner M. (1984) The function of the antennacleaner of the honeybee *(Apis mellifica)*. Apidology 15: 23–32

Wriedt J., Mezger D., Chong L. and Pfeiffer M. (2008) Observations on the foraging behaviour of *Myrmicaria brunnea sub carinata* (Smith) (Hymenoptera: Formicidae) in a tropical rainforest in Sarawak (Malaysia). Asian Myrmecology 2: 109-120

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