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SEM studies on the worker legs of arboreal bicoloured ant, *Tetraponera rufonigra* with reference to the sensilla present on it (Hymenoptera: Formicidae)

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Abstract
Scanning electron microscopic (SEM) studies on the legs of the arboreal bicoloured ant, *Tetraponera rufonigra* was investigated. The ants were collected from the colony of arboreal bicoloured ant on the *Ficus benghalensis* tree observed in RTM Nagpur University Campus, Nagpur during the period of January to February 2017. The SEM structure revealed that the entire surface of coxa consists of two types of sensilla, sensilla trichoidea (ST) and fine setae-like hairs, trichomes (TRC) while, trochanter contain sensilla trichoidea. The femur showed sensilla trichoidea (ST) arises from the hexagonal cuticular plate (CP). The mid and hind tibia showed a reduced spine-like tibial spur (TBS) with fine cuticular bristles (CCB) differentiated into CCB-I and CCB- II. The metatarsus of foreleg showed three types of sensilla, ST, TRC and comb like bristles (CMB) however; the metatarsus of mid and hind legs contain macrosetae, ST and TRC. All tarsomeres bear numerous bristles, setae and TRC on the entire surface while, surface of foreleg pretarsus showed macrosetae and sensilla trichoidea curvata (STC). The pretarsi contain sensilla and the mid dorsal surface of claws shows ST, STC and TRC.

Keywords: Arboreal ant, *Tetraponera rufonigra*, SEM, Legs

1. Introduction
Ant *Tetraponera* is commonly slender in shape in the family Formicidae and are characterised by their arboreal nature. The worker builds nest on the tree under the bark and the leg play important role in the nest building habitat of ant [1]. Each leg is divided into six segments, coxa, trochanter, femur, tibia, tarsus and pre-tarsus with well-developed paired claws. The morphology of leg in *Diacamma* species and distribution of sensilla is studied with the help of scanning electron microscope [2], reveals that an antenna cleaner is present on the tibio-tarsal portion of the leg. Tibio-tarsal antenna cleaner appeared in the form of a pore region on the surface of the basitarsus part of leg [2, 3].The sensilla used for cleaning of the antenna during communication [2]. A gland has been analysed which connected to the tibio-tarsal region of the forelegs. This cleaning apparatus is found in all Hymenoptera [4]. The receptors are the main tools of insect chemical communications and are mainly located on the legs. These receptors are tune feeding preferences and recognize host plant odors [5, 6].
There are reports on the morphology and scanning electron microscopic studies on the other ants, but reports are not available on the *Tetraponera*. Therefore, the present work was carried out to know the morphological structure of legs, and various sensilla present on the different parts of the legs of the worker of bicoloured arboreal ant *T. rufonigra* with the help of scanning electron microscope.

2. Material and Methods
2.1 Material
During the present study the adult worker ants were collected from the colony of arboreal bicoloured ant *T. rufonigra* on the *Fecus benghalensis* tree observed in RTM Nagpur University Campus, Nagpur during the month January to February 2017. After collection of animals they brought into the department of Zoology RTM Nagpur University where the present study was conducted.

2.2 Methods

2.2.1 Morphological Preparations (In Situ)

The collected ants were immobilized on ice and preserved in 70% alcohol for further study. For morphological study legs were dissected out in binocular microscope, the legs were treated with 10% KOH at 80ºC for 15 minutes. They were washed several times in the water and latter on dehydrated by passing through ascending grades of alcohol, cleared in Xylene and Mounted in DPX.

2.2.2 Scanning Electron Microscopy

The separated legs were fixed in 70% alcohol for a period of 12 hours after that dehydrated in ascending grades of alcohol and cleared in acetone. The air dried legs, fixed on the pre coated carbon strip metallic stub at different angles and proceeded for Gold coating and scanned under the Field Emission Gun Scanning Electron Microscope (SEM) Jeol (JSM 6380 A) at Indian Institute of Technology (IIT), Bombay, Powai Mumbai India.

2.2.3 Statistical Analysis

Sensilla on leg were measured, identified and counted amongst worker individuals. Measurements were taken from individuals and the means were calculated with standard deviation. The values obtained in Standard deviation were used to measure standard error.

3. Results

3.1 Morphology of leg *in T. rufonigra*

Three pairs of legs are well developed in the adult *T. rufonigra.* Each leg was divided into six segments, the coxa, trochanter, femur, tibia, tarsus, and pretarsus with well-developed paired claws. (Fig. 1A, 2A, 3A).

3.1.1 Coxa

It is the first segment articulated in the thoracic region. It is dorso-ventrally flattened highly muscular, triangular structure and broad towards the anterior joint while, narrow towards the posterior end. The dorsal and ventral surface of coxa of each leg in worker consists of two types of sensilla, trichoid sensilla and fine setae-like hairs, trichomes. The trichoid sensilla (ST) are differentiated into two types ST-I and ST- II. (Fig. 1B, 2B).

3.1.2 Trochanter

It is second segment of leg, triangular plate-like structure, extended from the posterior articulation of coxa and attached to the proximal region of femur. The entire surface of trochanter of each leg consists of fine trichoid type of sensilla. The size and distribution of sensilla are variable among fore, mid and hind legs. (Fig. 1B, 2B, 3B).

3.1.3 Femur

The femur is third segment of the leg, long and strong. The structure is highly muscular and dorso-ventrally flattened, broad towards the anterior while, narrow towards the posterior distal end. The femur is variable in size among adult polymorphic forms. The femur is larger in worker. The entire surface of fore leg femur shows sensilla trichoidea (ST) arises from the hexagonal cuticular plate (CP). The ST show similar distribution pattern on fore, mid and hind legs femur except their sizes (Fig. 1C, 2C, 3C).

3.1.4 Tibia

Tibia is the fourth segment of the leg. It is muscular longer and dorso-ventrally flattened, broad towards the anterior while, narrow towards the distal end. The well defined fibula like structure, the strigil is observed on the terminal tibio-tarsus junction of fore tibia. The fore tibial strigil (STR) consists of cuticular bristles on dorsal surface and comb teeth-like bristles (CMB) with variable length on the ventral surface (Fig. 1D).

The mid tibia contains a reduced spine-like tibial spur (TBS) while, hind tibia contains long comb like tibial spur with fine cuticular bristles (CCB) differentiated into CCB-I and CCB-II throughout the surface. The dorsal and ventral surface of tibia of all legs consists of macrosetae (MAC) and trichoid sensilla (ST) (Fig. 2C, 3C).

The macrosetae are long, broad towards the base and pointed towards the tip and sparsely located on the distal surface of tibia. The sensilla trichoidea are long, broad and pointed towards the apex. Both ST and MAC arise from a spherical base with tetragonal hexagonal cuticular plates (CP).

3.1.5 Tarsus

The tarsus is the fifth segment of the leg. It consists of four tarsal segments where the first is extensively large and termed as the metatarsus while, remaining four segments is the tarsomeres. The dorsal and ventral tarsal surface shows different types of sensillary hairs on each leg.

1. **Metatarsus:** The metatarsus is a long slender tubular segment. The dorsal and ventral surface of metatarsus shows different types of sensillary hairs on each leg. The metatarsus of foreleg shows three types of sensilla, sensilla trichoidea, trichomes and comb like bristles (CMB) however the mid and hind metatarsus shows macrosetae, sensilla trichoidea and the trichomes (Fig. 2C, 3C).
2. **Tarsomeres:** All the four tarsomeres of fore, mid and hind legs bear numerous bristles, setae and trichomes on the entire surface similar to that of metatarsus. Tarsomeres sensilla are similar in morphology with metatarsus sensilla but differ in sizes (Fig. 3D).

3.1.6 Pretarsus

The pretarsus is the sixth terminal segment as the foot. It is characterized by the presence of terminally curved, a pair of well-developed pectinated claws on each leg. A cushion-like median lobe, the arolium is well distinct and lying in between the terminal claws. The arolium is smaller in worker ants. The entire surface of pretarsus of foreleg shows macrosetae (MIC) and sensilla trichoidea curvata (STC). The mid dorsal surface of claws shows trichoid sensilla and sensilla trichoidea curvata with MIC and TRC. (Fig. 2D, 3D).

Table 1: Morphological observation of legs in *Tetraponera rufonigra* (± Standard Error)

Parts of leg	Foreleg		Mid leg		Hind leg	
	Length(µm)	Width(µm)	Length(µm)	Width(µm)	Length(µm)	Width(µm)
Coxa	0.764±0.004	0.529±0.005	0.764±0.004	0.529±0.005	766.66±0.27	466.66±0.27
Trochanter	100±0.50	250±1.78	0.235±0.004	0.294±0.004	300±2.86	266.66±0.27
Femur	1300±2.86	250±1.78	1235±2.16	0.352±0.005	1400±2.86	344.56±0.35

Tibia	1250±3.63	250±1.78	1058±2.16	0.235±0.004	1290±3.63	320.10±0.21
Spur	450±2.48	50±2.48	435±2.16	45±2.16	460.10±0.09	43±2.55
Tarsus	650±2.48	150±2.48	0.705±0.004	0.117±0.006	690±3.94	179±3.94
Metatarsus	600±2.86	150±2.48	0.941±0.004	0.117±0.006	650±2.48	176±1.78

Table 2: Length and width of various sensilla present on legs of *Tetraponera rufonigra*

Sr. No.	Foreleg			Mid leg			Hind leg		
	Sensilla	Length (µm)	Width (µm)	Sensilla	Length (µm)	Width (µm)	Sensilla	Length (µm)	Width (µm)
Coxa	ST I	50±1.07	3.33±0.68	ST I	128.57±0.74	14.28±0.74	ST I	200±1.71	33.33±0.15
	ST II	20±1.07	3.33±0.68	ST II	42.85±0.63	14.28±0.54	ST II	133.33±0.07	33.33±0.15
Trochanter	ST I	93.75±0.75	6.25±0.73	ST I	120.23±0.73	14.16±0.73	STR	485.71±0.15	71.42±0.63
	ST II	37.5±0.73	6.25±0.73	ST II	40.36±0.63	14.18±0.73	ST	98.88±0.14	6.45±0.61
Femur	ST I	76.92±1.14	7.69±0.48	ST I	110±0.65	10±0.04	ST I	180.56±0.12	29.30±0.14
	ST II	30.76±1.39	7.690.48	ST II	40±0.44	10±0.04	ST II	123.12±0.29	29.23±0.14
Tibia	ST I	81.85±1.51	5.99±0.88	ST I	171.42±0.61	14.28±0.54	ST I	396.23±0.58	65.25±0.56
	ST II	33.25±1.23	5.99±0.88	ST II	57.14±1.61	14.28±0.54	ST II	89.23±0.08	7.12±0.62
	TBS	384.61±1	30.76±1.64	TBS	885.71±0.69	71.42±1.38	TBS	456.55±0.37	51.45±0.08
Tarsus	BT	98.88 ±1.16	27.99±0.62	BT	99.11±1.17	27.85±0.16	BT	100±0.53	28.57±0.09
	CMB	19.97±1.24	6.76 ±0.74	CMB	20.10±0.48	6.98±0.64	CMB	21.42±0.12	7.14±0.62
	Cl	55.10±1.60	40.76±0.97	Cl	51.11±0.44	39.47±0.25	Cl	57.14±0.24	42.85±0.07
	MT	145.76±1.67	30.11±0.36	MT	138.11±0.18	29.12±0.19	MT	135.71±0.27	28.57±0.12
	ST I	69.20±0.89	3.76 ±0.31	ST I	70.26±0.20	3.48±0.61	ST I	76±0.17	4±0.06
	ST II	34.12 ±0.40	3.11±0.17	ST II	36.36±0.39	4.33±0.71	ST II	36±0.75	4±0.06
	Sw. C	74 ±0.61	5.98 ±0.14	Sw. C	73.36±0.21	16.98±0.14	Sw. C	76±0.17	8±0.08
Metatarsus	ST I	25.11±0.33	2.10 ±0.14	ST I	27.5±0.05	2.5±0.06	ST I	28.10±0.12	2.9±0.05
	ST II	31.98±0.32	1.98±0.08	ST II	35±0.43	2.5±0.06	ST II	32±0.37	2.1±0.04
	MAC	59.16 ±0.49	8.78 ±0.36	MAC	65±0.46	10±0.04	MAC	63±0.80	9.80±0.04
	ST	139.31±0.51	17.30±0.43	ST	128.27±0.60	14.28±0.54	ST	127.11±0.10	13.98±0.07
	STC	111.28±0.61	39.11±0.41	STC	114.28±0.57	42.85±0.17	STC	113±0.26	41.18±0.06
	TRC	151.11±0.66	25.10±0.34	TRC	157.14±0.64	28.57±0.17	TRC	155.33±0.29	26.19±0.32
	SB	3.71±0.20	1.18±0.07	SB	3.75±0.69	1.25±0.02	SB	3.70±0.11	1.21±0.05
Tarsomere	TAR I	440±1.78	200±1.71	TAR I	430±0.10	191±0.36	TAR I	435±0.09	198 ±1.26
	TAR II	500±1.07	80±1.31	TAR II	480±0.46	75±0.69	TAR II	485±0.12	77±0.80
	TAR III	500±1.07	140±1.11	TAR III	478±0.46	71±0.66	TAR III	478±0.11	71±0.72
	ST I	161.53±0.86	7.69±0.34	ST I	155.53±0.48	5.97±0.06	ST I	158.53±0.44	6.11±0.12
	ST II	61.53±1.19	7.69±0.34	ST II	55.78±0.13	6.90±0.09	ST II	66.10±0.13	6.98±0.12
	Sw. C	135±1.07	20±1.07	Sw. C	130±1.01	18±0.26	Sw. C	132 ±1.05	19±0.21
	Un. C	105±1.64	20±1.07	Un. C	110±0.65	21±0.39	Un. C	109 ±0.55	18±0.19
	BS I	4±0.85	0.5±0.007	BS I	3 ±0.06	0.3±0.02	BS I	3±0.06	0.4±0.028
	BS II	1.5±0.13	0.5±0.007	BS II	1.8±0.06	0.7±0.03	BS II	1.6±0.04	0.6±0.042
	ST	41.42±0.36	2.85±0.14	ST	44.42±0.08	2.98±0.05	ST	40.40±0.28	2.75±0.03
	TRC	71.42±0.47	5.71±0.24	TRC	71.78±0.70	5.79±0.12	TRC	71.11±0.14	5.61±0.07

Abr: ST- Sensilla trichoidea, TBS- Tibial spur, TAR- Tarsus, Sw. C- Swollen claw, Un. C- Un-pointed tip claw, BS- Basiconic sensilla, TRC- Trichomes, MAC- Macrosetae, STC- Sensilla trichoidea curvata, STR- Strigil, BT- Basitarsus, CMB- Comb like bristle, Cl- Claw, MT- Microtrichia, Ar- Arolium.

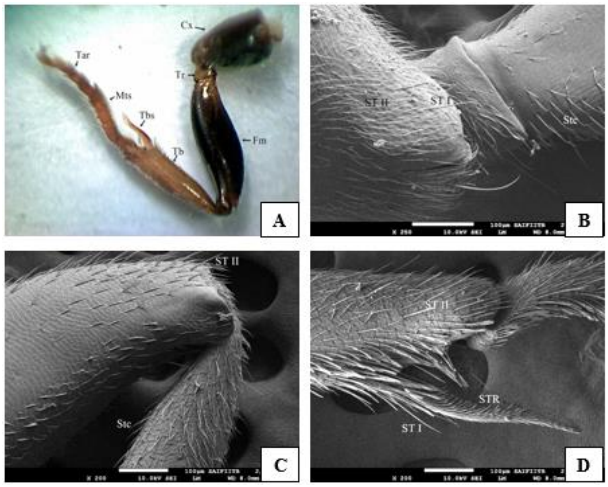

Fig 1: SEM structure of fore leg of *Tetraponera rufonigra*
A: Fore leg showing various parts, B: Coxa and Trochanter with sensillae, C: Femur and Tibia showing sensillae, D: Magnified view of tibia and tibial spur showing sensillae, Abr.: Cx- Coxa, Tr- Trochanter, Fm- Femur, Tb- Tibia, Tar- Tarsus, Mts- Metatarsus, Tbs- Tibial spur, ST- Sensilla trichoidea, Stc- Sensilla trichoidea curvata, STR- Strigill.

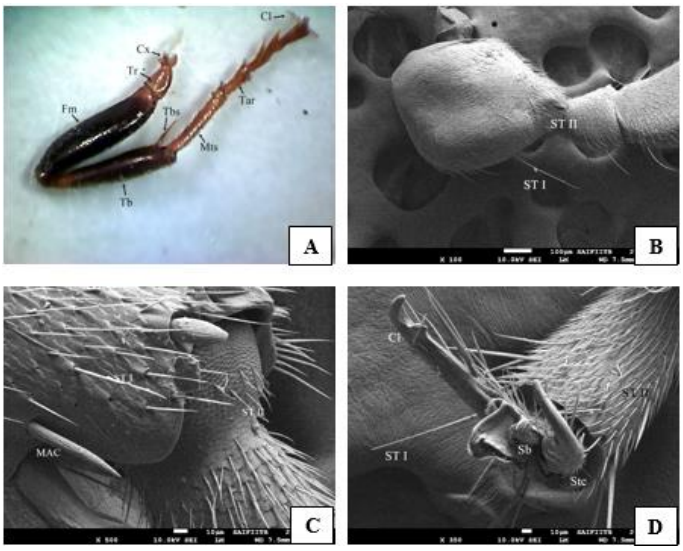

Fig 2: SEM structure of mid leg of *Tetraponera rufonigra*
A: Parts of mid leg, B:Coxa and Trochanter showing sensillae, C: Magnified view of tibia and metatarsus showing sensillae, D: Magnified view of pretarsus with claw showing sensillae.
Abr.: Cx- Coxa, Tr- Trochanter, Fm- Femur, Tb- Tibia, Tar- Tarsus, Mts- Metatarsus, Tbs- Tibial spur, ST- Sensilla trichoidea, Stc- Sensilla trichoidea curvata, Cl- Claw, SB- Sensilla basiconica, MAC- Macrosetae.

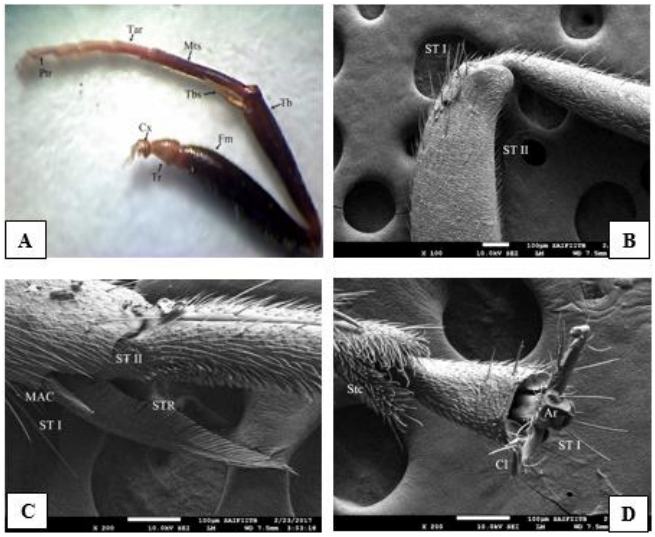

Fig 3: SEM structure of hind leg of *Tetraponera rufonigra*
A: Parts of hind leg, B: Femur and Tibia with sensillae, C: Magnified view of tibia and metatarsus showing sensillae, D: Magnified view of pretarsus showing sensillae.
Abr.: Cx- Coxa, Tr- Trochanter, Fm- Femur, Tb- Tibia, Tar- Tarsus, Mts- Metatarsus, Tbs- Tibial spur, Ptr- Pretarsus, ST- Sensilla trichoidea, STR- Strigills, Stc- Sensilla trichoidea curvata, MAC- Macrosetae, Cl- Claw, Ar- Arolium.

4. Discussion

In present study it has been observed that the legs are composed of six segments as coxa, trochanter, femur, tibia, tarsus and pretarsus All segments are well jointed to each other. The comb of notch together with the comb of fore tibial spur is present on the leg of worker ant *T. rufonigra*. A similar result was recorded by Ocada [2] in Ponerinae ant, *Diacamma*. Ocada [2] postulated that both sexes of *Diacamma* possess the comb like structures on the inner side of fore tibia known as comb of notch. A single comb is present in male and female for extensive cleaning of L-shaped antennae in *Diacamma* [10]. *T. rufonigra* contain a spur used for cleaning of antenna. The comb of notch in *T. rufonigra* consists of two rows of broad bristles while single tibial spur. A similar result was recorded by the Ocada [2] in the Ponerinae ant, *Diacamma* and Barsagade [3] in *Componotus sericeus* respectively. In the present observation it is noticed that the comb of tibial spur working as an antenna cleaner, as found similar in *Dicamma* [10] and *Sphecomyrma freyi* [7].

In the present investigation the four types of sensilla were recorded namely, trichoide asensilla, macrosetae, sensilla trichoidea curvata and trichomes on the legs of *T. rufonigra*. Similar types of sensilla were recorded on the legs of ants and honey bee, *Apis mellifera* [11, 12, 13]. In *T. rufonigra* the mid and

hind tibial spur are slender, straight laterally lined with sharp pointed teeth (CCB- I) and covered with numerous small cuticular teeth (CCB- II) in workers. The mid and hind tibial spur is slender and straight with serrated edges as reported earlier Saini and Dhillion [14] in most of the *Aculeata*.

Different size of sensilla trichoidea and sensilla trichoidea curvata are prominently observed, on the fore, mid and hind pretarsi in the workers of *T. rufonigra*. The trichomes are widely distributed along the claws. The similar types of trichomes distribution on the pretarsi of male and queen was noted by Ocada [2] in Ponerinae ant, *Diacamma* species. Hashimoto [8] and Lensky Y, Slabezki [12] suggested the chemosensory function of pretarsal sensilla in *Apis mellifera*. The presence of various types sensilla on the pretarsus of *T. rufonigra* may function as chemosensory in nature. In *Diacamma* females, long setae in parallel position with each tarsal claws has been reported and known as dorsal claws microsetae [16]. Similar types of dorsal claws microsetae were present on the pretarsi of workers of *T. rufonigra.* This finding is supported by the study of earlier workers in *C. cericeus* Barsagade [3], in *Tridomyrmex humilis* Markin and Zacharuk [17, 18].

The presence of paired pectinated claws and arolium in worker ants plays an important role in sticking to the vertical places especially to the rough surfaces in various species of insects [9, 15, 19, 20]. In the present investigation it has been observed that the pretarsus of *T. rufonigra* contain claws and well developed arolium. Such types of claws and arolium were noted in male of *Diacamma* species [2]. The sensilla trichoidea curvata, sensilla trichoidea, trichomes and microsetae were noted on the pretarsi and arolium of *T. rufonigra* in the present study and confirmed sensillary nature of the pretarsi and arolium.

Pair of horned shaped bifurcated claws was found on the pretarsi of *T. rufonigra*. These types of pretarsus morphology were also reported in arboreal species of genus *Pachycondyla* [20]. The presence of horned shaped claws in *T. rufonigra* suggests novel adaptation for fast-walking mode of locomotion and the wood dwelling habitats.

5. Conclusion

The present investigations contribute to the preliminary knowledge of the different sensillum present in the legs of arboreal bicoloured ant *T. rufonigra*. Therefore, this information provides knowledge about the understanding of behaviour nest preparation and biology of arboreal bicoloured ant *T. rufonigra*.

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