



E-ISSN: 2320-7078

P-ISSN: 2349-6800

JEZS 2016; 4(3): 307-313

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Received: 20-03-2016

Accepted: 21-04-2016

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A preliminary report on the reproductive system of a polistine wasp *Ropalidia montana* Carl (Hymenoptera: Vespidae)

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Abstract

Ropalidia montana is an old world social wasp with distinct caste polymorphism. The three castes i.e. queen (reproductive female), drone (reproductive male) and worker (sterile female), besides having behavioral peculiarities show distinct differences in the shape, size and color of abdomen. They also have differences in their internal anatomy. The present study deals with the reproductive system of all three castes. The reproductive system of the queen consists of paired ovaries with polytrophic ovarioles, common and lateral oviducts, a spermatheca and external genitalia. The reproductive system of drone consists of a pair of testes and seminal vesicle encapsulated in a scrotal sac like structure, a pair of mesodermal accessory glands, a pair of vas deferens, a pair of post vesicular deferent ducts, an ejaculatory duct and endophallus. The reproductive system of the worker with five rudimentary ovarioles arising from common oviduct is peculiar.

Keywords: Abdomen, polymorphism, reproductive system, *Ropalidia montana*, swarm founders, wasp

1. Introduction

The social wasps in the vespid subfamily Polistinae fall into two behaviourally well defined groups, the independent founders and the swarm founders. Among independent founders the nest size is quite small with small number of adults (<100) which are usually short lived^[1, 2]. Their colony is founded by an inseminated queen and its reproductive dominance is based on direct physical attack^[3]. This type of colony is reported to have a less distinct caste system^[1]. On the other hand swarm founding species have a colony comprising of an adult population ranging from a few dozens to several thousands. This kind of colony has a large number of workers accompanied by a smaller number of queens and drones and the reproductive dominance of queen is suggested to involve pheromonal induction^[3]. The Old World genus *Ropalidia* curiously includes both type of species unlike other Polistine genera which include either one or the other type of species. Of the independent founding species only a few have been studied in the field^[4-10], but swarm founding species which are a minority in the genus are even less known.

R. montana is a swarm founding species endemic to Peninsular India with collection records from the states of Karnataka, Kerala and Tamil Nadu^[11]. *R. montana* first entered the scientific literature when Carl^[12] described the species and its unique nest architecture. Subsequent studies on this species were related to Queen/Worker size dimorphism^[13] and their social behavior from Peninsular India^[14]. The present study aims at description of different castes of *R. montana* from their colony as well as thorough examination of their reproductive systems.

2. Materials and Methods

R. montana were collected in January 2015 from Mudumalai Wildlife Sanctuary, which lies between 11°32'N - 11°42'N and 76°20'E - 76°40'E in the state of Tamil Nadu, India. The specimens were preserved in 5% Formalin for further investigation. 2000 adult *R. montana* from each of the three collected colonies was randomly selected for study. The population was unambiguously sorted into two size categories: the specimen with larger and longer abdomen with sting (Queen), and the specimen with shorter abdomen (males and workers). The workers and the males were further distinguished on the basis of presence and absence of the stings respectively. Number of members in each caste was counted. Fifteen specimens from each

caste were randomly selected and were dissected to study the reproductive system of each Caste. Scanning Electron Microscopic study was carried out to obtain the detailed information on the structure of the abdomen and the external genitalia of each caste. The measurements of different internal structures were done by ocular and stage micrometer in a stereo binocular.

3. Results

3.1. Different Castes in the colony

The percentage of queen was consistently low in each colony and varied between 3.40-4.60%. The composition of drone ranged from 13.65-16.15% and that of worker varied from 79.95-81.9% (Table 1, Figure 1). Out of the three nests collected (Figure 2 A-C), 2000 specimens from each colony was randomly selected and sexed in order to ascertain the ratio of different castes in the colony.

Table 1: Difference in the composition of colony members across 3 nests as observed in the study

Sl. No.	No. of specimen	No. of Queen (%)	No. of Drone (%)	No. of Workers (%)
Nest 1	2000	92 (4.6)	273 (13.65)	1635 (81.75)
Nest 2	2000	71 (3.55)	291 (14.55)	1638 (81.90)
Nest 3	2000	78 (3.9)	323 (14.55)	1599 (79.95)

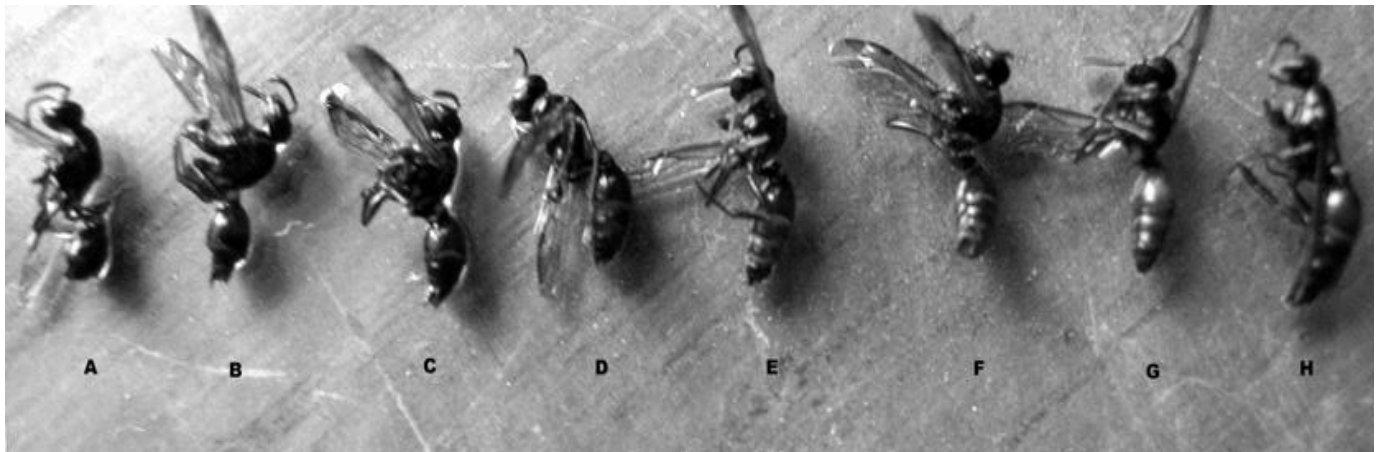


Fig 1: Different castes of *R. montana* present in a colony; **A-F:** Workers, **G:** Drone, **H:** Queen

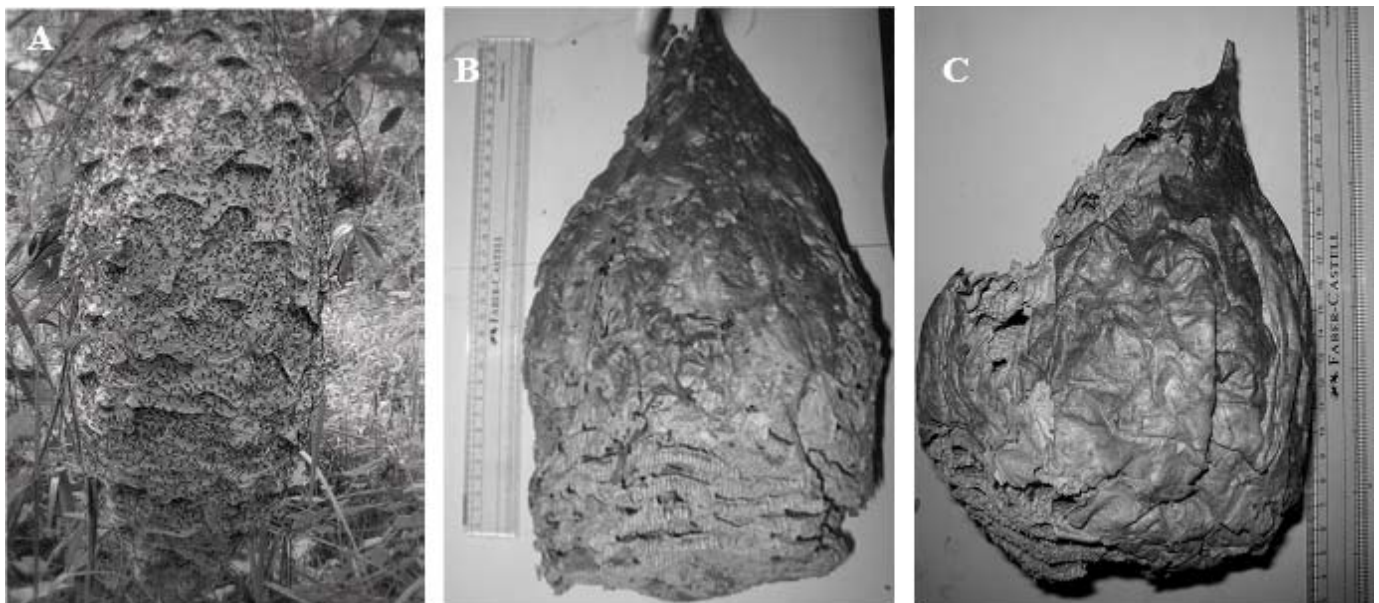


Fig 2: Nests of *R. montana* sampled. **A.** Nest 1 in Mudumalai Wildlife Sanctuary, **B** (Nest 2) and **C** (Nest 3) in laboratory

3.2. Description of the Abdomen and External Genitalia of *R. montana*

As *R. montana* is a swarm-founding social wasp, a distinct caste system is clearly observed. Variations in abdomen and abdominal tip (external genitalia) between different castes can be well recognized on close observation.

Queen

Queen has a much longer and slender abdomen ($4.0 \pm 0.707 - 5.0 \pm 0.567$ mm) as compared to the other two castes. The sting

is usually found in retracted condition and hence only its tip is visible from outside. The gaster is brown in color and the consecutive segments gradually darken downwards. The overall color of the abdomen of queens is comparatively lighter than that of workers. Their tip of the abdomen bears a barbed sting (the modified ovipositor). At the base of the sting, vagina is present positioned ventrally flanked by a pair of vulvulae. Due to the presence of vagina, the ventral sub-genital plate bears a conspicuous incision. Short sensory hairs cover the abdominal tip (Figure 3 A-E).

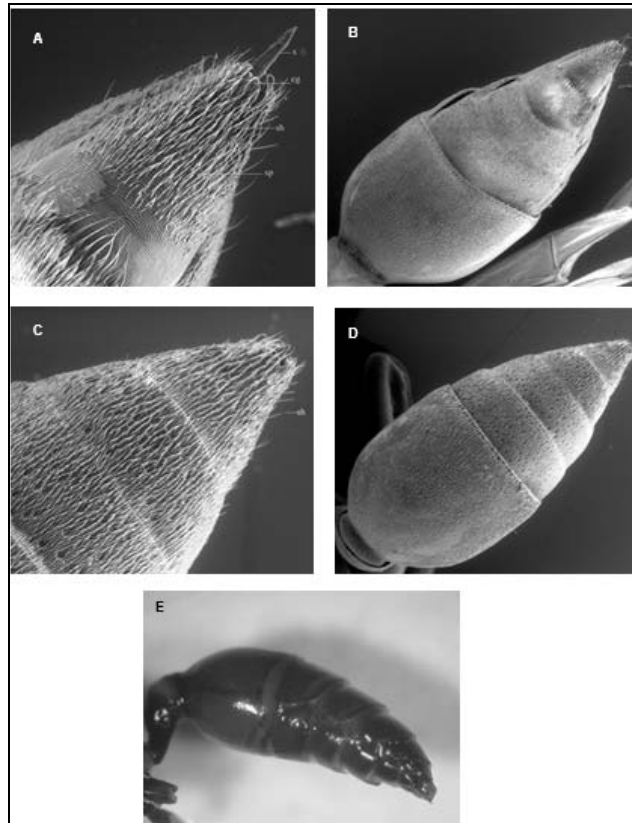


Fig 3: Abdomen of queen of *R. montana* under scanning electron microscope and dissecting binocular (eg- external genitalia; s-sting; sh-sensory hairs; sp-subgenital plate) **A.** SEM of Ventral side of the abdominal tip (150 X). **B.** SEM of Ventral side of the abdominal tip (36 X). **C.** SEM of Dorsal side of the abdominal tip (150 X). **D.** SEM of Ventral side of the abdominal tip (36 X). **E.** Lateral view of the abdomen of queen under dissecting binocular

Drone

The abdomen of drone is usually shorter ($2.7\pm 0.114 - 3.2\pm 0.0707\text{mm}$) but broader anteriorly. The gaster is light yellowish brown in color and the rest of the segments are darker. Unlike the queen, the color of the segments does not change gradually. The end of the abdomen is curved and sting

is absent. Presence of a pair of anal cerci at the tip of the abdomen is observed which flanks the tip of an endophallus. The ventral sub-genital plate has a smooth unbroken margin. The abdominal tip also bears sparse but longer sensory hairs (Figure 4A-F)

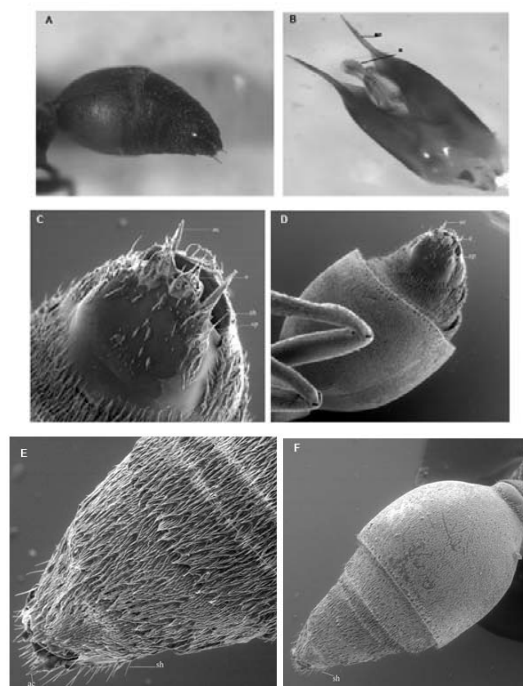


Fig 4: Abdomen of Drone of *R. montana* under dissecting binocular and scanning electron microscope (ac-anal cerci; e- endophallus, sh-sensory hairs; sp- subgenital plate) **A.** Lateral view of the abdomen of Drone under dissecting binocular **B.** External Genitalia of male along with its associate parts **C.** SEM of Ventral side of the abdominal tip (150 X) **D.** SEM of Ventral side of the abdominal tip (36 X) **E.** SEM of Dorsal side of the abdominal tip (150 X) **F.** SEM of Ventral side of the abdominal tip (36X)

Worker

The abdomen of the worker is completely black in color. The abdominal segments are retracted antero-posteriorly causing a characteristic shrunken appearance. Thus the abdomen is very short (2.0 ± 0.707 - 2.5 ± 0.141 mm), but looks quite broad due to

retraction of segments. The abdominal tip bears a long sturdy barbed sting enclosed in a sheath. External genitalia cannot be recognized externally but unlike drone the ventral sub-genital plate of worker bears a wavy margin. Abundant sensory hairs are present in the abdominal tip (Figure 5 A-E).

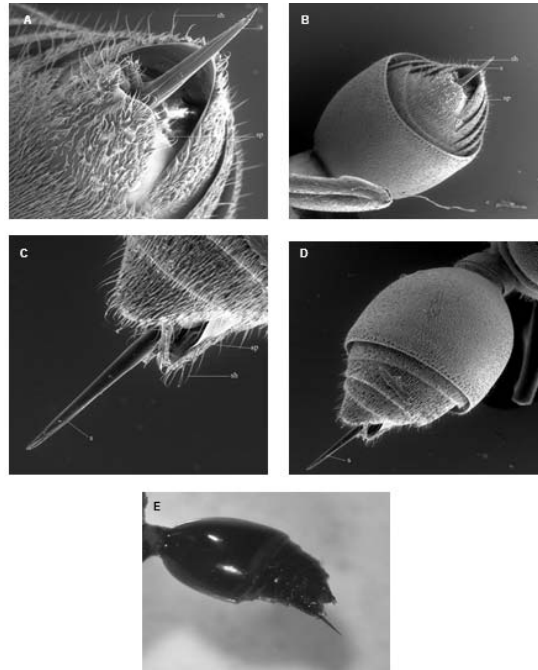


Fig 5: Abdomen of worker of *R. montana* under scanning electron microscope and dissecting binocular (s-sting; sh-sensory hairs; sp-subgenital plate) **A.** SEM of Ventral side of the abdominal tip (150 X). **B.** SEM of Ventral side of the abdominal tip (36 X). **C.** SEM of Dorsal side of the abdominal tip (150 X). **D.** SEM of Ventral side of the abdominal tip (36 X). **E.** Lateral view of the abdomen of worker under dissecting binocular

3.3. Description of Reproductive System of *R. montana* Queen

The reproductive system of the queen occupies the entire abdominal cavity, extending from gaster to the terminal end of abdomen. It consists of paired ovaries on either side of the

digestive tract. Each ovary consists of three ovarioles which arise from the lateral oviduct (0.35 ± 0.014 x 0.15 ± 0.007 mm). Two lateral oviducts in turn originate from a short (0.20 ± 0.01 x 0.18 ± 0.007 mm) common oviduct which ultimately opens in vagina (about 0.20 ± 0.018 mm long) (Figure 6).

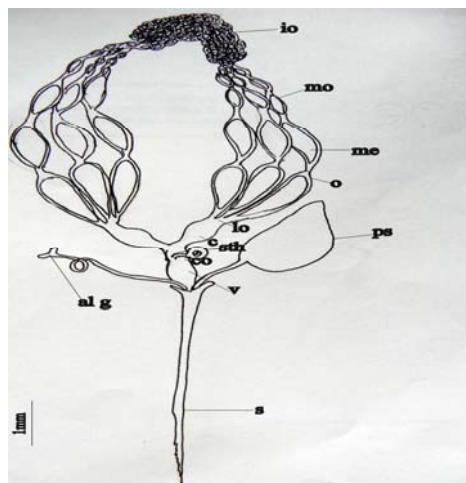


Fig 6: Schematic diagram of female reproductive system of *R. montana* (io-immature oocyte; mo-maturing oocyte; me-matured eggs; o-ovariole; lo-lateral oviduct; c-calyx; sth-spermatheca; co-common oviduct; v-vagina; alg-alkaline gland; ps-poison sac)

A spermatheca remains attached to the dorsal surface of the common oviduct. The spermatheca has a rounded head descending to a small tube by which it is attached dorsally to the common oviduct. The spermatheca measures 0.35 ± 0.007 mm in length and 0.20 ± 0.009 mm at its widest end. The rounded head of the spermatheca has an outer translucent peripheral region and an opaque centre (Figure 7A). The

ovarioles are of polytrophic type. Eggs in ovarioles are arranged in layers in order of descending size from calyx to apex (Figure 7B). The mature eggs lying close to the calyx are large (1.00 ± 0.285 x 0.35 ± 0.007 mm) while the growing oocytes lying towards the apex are smaller (0.50 ± 0.016 x 0.20 ± 0.014 mm). If uncoiled, the length of each ovariole was about 4 ± 0.707 mm. Importantly the region containing the

mature eggs was followed by a constriction. Following this constriction the apical filament of each ovariole entered the gaster where the six filaments (three from each side) formed an entangled mass measuring 2.40 ± 0.007 mm. Two other minor constrictions were also observed. The apical filament contained immature bead like eggs of various size ($0.30 \pm 0.014 \times 0.15 \pm 0.007$ mm - $0.10 \pm 0.006 \times 0.10 \pm 0.020$ mm) in various stages of development. Significantly, in multiple specimens number of mature eggs in right ovariole was less than number of mature eggs in left ovariole (Table 2). This may be attributed to the presence of poison sac in the lower right half of the abdominal cavity.

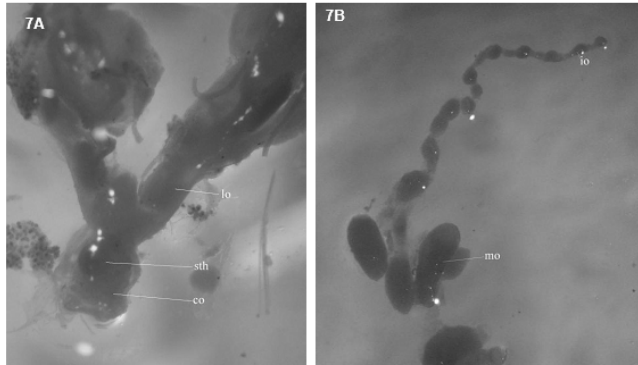


Fig 7A: Female Reproductive System of *R. montana* showing spermatheca, common oviduct lateral oviduct and ovarioles in each ovary (co- common oviduct; sth- spermatheca, lo- lateral oviduct).
Fig 7B: Part of female reproductive system showing apical portion of an ovariole containing a maturing oocyte and many immature oocytes in various stages of development (mo- maturing oocyte; io- immature oocytes.).

Table 2: Comparison of Number of eggs in right and left ovariole of queen of *R. montana*

Specimen Sl. No.	No.(Mean ±SE) of eggs in right ovariole	No. (Mean ±SE) of eggs in left ovariole
1	8±0.707	14±0.866
2	11±0.577	18±0.745
3	7±0.707	12±0.913
4	6±0.699	12±1.414
5	9±0.816	16±1.062
6	7±1.018	11±0.816
7	9±0.866	14±0.866
8	8±0.866	12±0.816
9	9±1.140	14±1.00
10	9±0.738	13±0.632
11	9±0.866	11±0.707
12	10±0.745	15±1.140
13	12±0.699	18±1.414
14	8±0.866	14±1.140
15	8±0.699	15±0.931

Drone

The male reproductive system of *Ropalidia montana* (Figure 8A, 8B) runs approximately from the junction of 2nd and 3rd abdominal segment to the terminal end of the body. It consists of a scrotal sac like structure that encapsulated the testis and seminal vesicle. It is a bilobed structure with upper lobe measuring 0.15 ± 0.016 mm and 0.20 ± 0.01 mm in length and diameter respectively and the lower larger lobe is 0.25 ± 0.011 mm in length and has a diameter of 0.30 ± 0.01 mm. A pair of accessory gland is situated on either side of the median scrotal sac like structure, with the upper gland measuring about 0.24 ± 0.009 mm in length and 0.18 ± 0.007 mm in diameter. The lower gland is slightly smaller and measures about 0.15 ± 0.018

mm in length and 0.12 ± 0.014 mm across the diameter. An elongated tube, the vas deferens arises from each gland and runs for 1.10 ± 0.018 mm before joining together to form the ejaculatory duct. Each vas deferens is about 0.05 ± 0.01 mm wide. The ejaculatory duct is shorter and wider than vas deferens (0.55 ± 0.011 mm in length and 0.08 ± 0.016 mm wide). The ejaculatory duct ultimately runs into the endophallus. The post vesicular deferent ducts from the scrotal sac meet the vas deferens midway through narrow tubules measuring about 0.15 ± 0.013 mm.

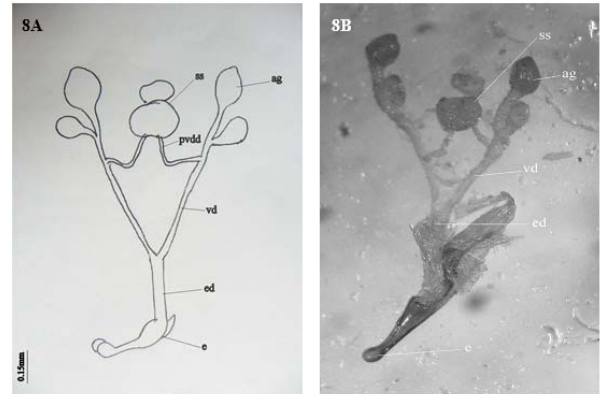


Fig 8A: Schematic diagram of male reproductive system of *R. montana* (ss-scrotal sac; ag-accessory gland; pvdd-post vesicular deferent ducts; vd-vas deferens; ed-ejaculatory duct; e-endophallus)
Fig 8B: Male Reproductive system of *R. montana* (ss-scrotal sac; ag-accessory gland; vd-vas deferens; ed- ejaculatory duct; e-endophallus)

Worker

The reproductive system of the workers (Figure 9A, 9B) is confined approximately within the last two abdominal segments. The worker females have a rudimentary reproductive system consisting of oviduct, ovarioles and external genitalia. There are five degenerated ovarioles arranged in a fan like structure wrapped around the hindgut. Each ovariole consists of a single egg inside a thin sac. The size of egg bearing ovarioles ranges from 0.40 ± 0.014 - 0.30 ± 0.032 mm x 0.08 ± 0.007 - 0.06 ± 0.014 mm with the smaller being on the right side. This may again be attributed to the presence of poison sac in the right half of the abdomen. The ovarioles diverge from a common oviduct (0.20 ± 0.017 x 0.80 ± 0.01 mm). The absence of lateral oviduct and spermatheca is noted. The common oviduct leads to the external genitalia (0.40 ± 0.007 x 0.15 ± 0.018 mm).

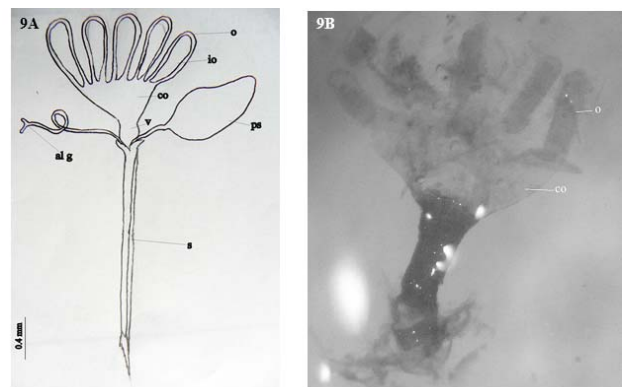


Fig 9A: Schematic diagram of reproductive system of workers of *R. montana* (o-ovariole; io-immature oocyte; co-common oviduct; v-vagina; ps-poison sac; alg-alkaline gland)
Fig 9B: Reproductive system of worker of *R. montana* (o- ovarioles with immature oocytes; co- common oviduct)

4. Discussion

The nests of *R. montana* obtained from Mudumalai were not in progressive growth stage as speculated earlier by Carl in 1934 and later by Jeanne and Hunt in 1992. Almost complete absence of brood was observed by the present workers and the colony size is much smaller, as compared to those obtained by Jeanne during the warmer months of the year. In the present study the percentage of male obtained was high (13-16%) and several fertile females with mature eggs in their ovarioles were also obtained. *R. montana* showed distinct polymorphism externally, according to the shape and size of the abdomen and thus three castes can be easily identified. The abdomen of the queen is more slender and lighter in color as compared to the other two and had an incision in sub genital plate due to the presence of external genitalia at the ventral base of the sting. The reproductive system of the queen is typically hymenopteran^[15], containing two ovaries each of which is comprised of three polytrophic ovarioles repeatedly folded on them, as consistently observed by workers^[16, 17]. Each ovarioles contained 2-4 mature eggs and a number of beads like immature eggs in various stages of development, the size of the follicles progressively increasing in size towards the calyx. Presence of a spermatheca, an alkaline gland, a poison sac and a sting the modified ovipositor was recorded. The external genitalia opened at the base of the sting. Unlike *Vespa* sp. as reported previously^[18, 19], the number of ovarioles differed in queen and worker.

The workers possessed 3+2 ovarioles as compared to a constant (3+3) ovarioles in case of queen. In workers the ovarioles arising directly from the common oviduct and complete absence of lateral oviduct was noteworthy. Also none of the ovarioles were filamentous in nature, each ovariole containing a single egg. Although the spermatheca is absent, presence of alkaline gland, poison sac and the sting is present and like the queen the external genitalia opens at the base of the sting. But externally the presence of external genitalia is not so conspicuous and the sub-genital plate instead of the incision as in case of the queen shows a wavy margin having a depression at middle of plate on the ventral side at the base of sting. The male reproductive system consist of testes, encapsulated within the scrotal sac like structure, paired accessory glands, post vesicular deferent ducts, vas deferens, ejaculatory duct and an endophallus. The accessory glands are paired and bilaterally situated. The gland is presumed to be mesodermal in origin as the ducts from the gland joins the vas deferens^[20]. The testis was found to be encapsulated within the scrotal sac like structure along with the seminal vesicle as also previously reported in primitive groups of bees^[21].

5. Conclusion

The above study provides a preliminary report on the reproductive system of *R. montana*. The presence of three ovarioles per ovary in queen appears to be a unique feature of these polistine wasps. However the occurrence of five rudimentary ovarioles in worker is a distinct peculiarity. The reproductive system in drone consists of an encapsulated testes, paired accessory glands, post vesicular deferent duct, vas deferens, ejaculatory duct and endophallus. Thus the present study attempts to reveal a marked distinctiveness in the reproductive system of different castes of *R. montana* which probably confirms their primitive status.

6. Acknowledgement

The authors would wish to thank the officials of the Forest Department of Mudumalai Wildlife Sanctuary for their

cooperation during this study. The guidance and help of Dr. Tushar Kanti Mukhopadhyay (Retired Associate Professor, Department of Zoology, Presidency College, Kolkata, India) is acknowledged. The assistance offered by Bose Institute, Kolkata during scanning electron microscopic analysis of specimens is highly appreciated. Finally the assistance and encouragement received from the faculty members of the Department of Zoology, West Bengal State University is also acknowledged.

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