



Research Article

## BEHAVIORAL STUDY ON *HYPOTRIGONA GRIBODOI* (HYMENOPTERA, APIDAE, MELIPONINI) WITH REFERENCE TO THE PROCESS OF PROVISIONING AND OVIPOSITION (POP)

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### ABSTRACT

The behavior of *Hypotrigena gribodoi* was examined in 2008 and 2014 in Cameroon. The study focused on food provisioning and oviposition process (POP) by the workers and the queen. Several observations and 30 video recordings were made. These bees are among the smallest bees found in the world. A study on POP showed the following behavioral features: The queen stays most of her life in the brood nets except during early stages of cell construction. The brood cells are constructed in clusters and in a disorderly manner, without any particular plane at the surface to distinguish the newly constructed cells from the old ones. They exhibit semi-synchronous brood cell construction with exclusively batched queen oviposition. The workers clean their nest during the early hours of the day and dump the dirt just below the nest entrance. The coactions or interactions between the queen and the workers are mostly cordial. The queen feeds the worker in some instances. The workers were never seen laying trophic eggs and only a single queen in the colony was observed throughout the research period. *H. gribodoi* are generally calm with a single queen (monogynous) at any given time in a colony. Brood cells are constructed semi synchronously and the oviposition pattern is exclusively batched. Cell provisioning is done by extremely large number of workers and the duration of oviposition is longer than that of most stingless bees already studied. A close comparison with nearest relatives of *H. gribodoi*, shows that the queen feeds the workers in some cases instead of the workers feeding the queen as is the case with the relatives. Workers oviposition does not seem to occur in this species. A few cases of aggressive behavior was observed with workers when *Meliponula becarii* and *Apis M. adansonii* tried invading and also by the queen to workers who prolonged their stay on already provisioned cells

**Keywords:** Stingless bees, *Hypotrigena gribodoi*, POP, Apidea.

### INTRODUCTION

The stingless bees together with the honeybees represent the most advanced stage of social differentiation in the Apidea. Some etho-sociological characters suggest that their equivalent social status was attained independently

(Sakagami, 1982). In social groups which are less advanced, behavioral control of workers by queens seem frequent (Shoichi *et al.*, 1973). In Meliponinae, cells are mass-provisioned by workers before queen oviposition and subsequent cell closure, in a characteristic process

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(Sommeijer *et al.*, 1984). Workers constructs cup-like structure (cells) in which larva food is poured into from the mouth of the workers. The process of spitting larva food into the cells is known as food provisioning. After the cells must have received enough larva food, the queen then lays her egg into the provisioned cell in the process known as oviposition. The oviposition process of stingless bees proceeds with a complicated and highly group specific temporal sequence of queen-worker interactions. This behavior is unique to social insects (Kalt *et al.*, 1987). But this kind of complex organization is shared by only two eusocial bee groups; the honeybees (Apinae) and the stingless bees (Meliponinae). Taking the queen oviposition as a focus, the daily life of stingless bee colonies proceeds through an alternation of several phases, roughly divided into quiescent ( $\bar{Q}$ ), transient ( $\bar{T}$ ), patrolling ( $\bar{P}$ ), arousal ( $\bar{A}$ ), and oviposition phases ( $\bar{O}$ ). Each oviposition is further divided into several phases, basically formulated as Predischage (r) + Discharge (d) + Oviposition (O) + Operculum (s). In other words, cell construction (C), food provisioning (D), Oviposition ( $\bar{O}$ ) and cell operculum (S) form a tight link process, C.D.O.S., which passes during a short time under high excitement, often reinforced by peculiar behavior (Shôichi *et al.*, 1974). This basic pattern receives modifications in each group of stingless bee according to their group specificity (Kalt *et al.*, 1987). In this study therefore, the ethological diagnosis of *H. gribodoi* is given and the ethological features are compared with the oviposition process of some well studied taxa. Attempts were also made to analyze the peculiar

behavior of this species and compared with that of related species.

## MATERIALS AND METHODS

The colony of *H. gribodoi* used in this study was collected from Lip (Bui Division) and transferred to an observation hive in Bamenda (Mezam Division). Lip is about 100 km from Bamenda. The colony was stabilised for two days before being transferred into the observation. The observation hive was made of a styrofoam box with dimensions, 35 cm (length) x 24 cm (width) X 12 cm (height) and a transparent glass lid. The glass lid was often covered with a dark piece of cloth when observation was not taking place. The dark cloth prevented direct light rays into the hive and kept the colony in its similar dark state as in the tree cavity. An artificial nest entrance was introduced to the observation hive using a silicone tube as shown in figure 1. The colony consisted of about 1500 adult bees. Observations and filming were done using low light intensities. The observations focused on the behavioral patterns of the bees during the process of provisioning and oviposition. Several observations and 30 video recordings were made. Observations were made during the day and at nights when necessary. The experiment lasted six months (May to November). Adapted nest entrance with exit tube smeared with resin from the original nest. Bees are acquainting themselves with the introduced entrance tube trying to find their way into the observation hive.



**Figure 1.** Observation hive with nest entrance.

## RESULTS AND DISCUSSION

During the observation period, no case of guard bees attacking parts of the body was registered. However, an expression of defense behavior was observed with worker bees of *H. gribodoi* clinching on the wings of other bees intruding into the hive to collect spilt honey from ruptured honey pots. *H. gribodoi* guard bees continuously bite the wings of the intruder until the intruder is rendered helpless. The queens of other stingless bee species spend most time

shuttling between the combs and the floor of the hives, sometimes on the glass lid except during the oviposition period ( $\bar{O}$ ) in most behavioural studies. But in *H. gribodoi*, the queen turn to stay at particular areas on old brood cells and comes out regularly to visit new cells under construction. Generally, the queen does not beat the wings when she is resting but she does beat the wings with intermittent strokes when she is walking or cruising. During queen resting, workers formed a royal court around

the queen. Workers were seen coming towards the queen making forward and backward movements, sometimes touching the queen with their antennae. Interaction between the queen and the workers of *H. gribodoi* was very cordial and simple when they come in contact. An exception to this queen - worker coactions was observed in some four instances during the oviposition phase with the queen becoming aggressive to workers sitting on already provisioned cells and refusing to let the queen perform her egg laying task into that cell. In some instances when the queen suddenly comes in contact with a worker, the worker escapes to the opposite direction or enters into the nearby openings of old cells. Contacts between the proboscis of queen and workers were frequent lasting up to 90 seconds. Among 30 carefully observed buccal contacts, 4 cases actually resulted in food delivery from the queen to the worker and none from the worker to the queen. Darting appears regularly on the newly oviposited cells when the queen comes in contact with a worker. After mutual contacts with the antennae, the worker rapidly raises her mandibles and antennae and dashes towards the queen, with forelegs extended and touching the queen's face rapidly and the she retreats. Workers also used their antennae to touch the abdomen of the queen in royal court.

The type of cell construction manifested by *H. gribodoi*, is semisynchronous ( $S_m$ ) cell construction (i.e. construction of new cells starts successively but the difference between growth stages in the cells becomes gradually smaller). Cells of *H. gribodoi* are constructed by successive activities of many workers. The task abandoned half way by a worker is taken over by another worker. The

construction of new cells can occur at any place on the surface of the already oviposited cells in the cluster. The start of construction of new cells is inhibited once the cells under construction attained an advance stage ready for provisioning (collard).

The process of larva food discharge and egg laying into individual cells of *H. gribodoi* is exclusively batched ( $B_e$ ). Food discharge and egg laying into many cells constitute a batch. Normally, exclusively batched means, all cells in the batch are food provisioned and eventually receive an egg each from the queen. In *H. gribodoi* though, this was the case with some of the batches, a few (8) collard cells were not provisioned and did not receive any egg from the queen during the oviposition process. The average number of cells found unattended to by the queen after the oviposition phase (egg laying phase) in a batch were significantly low per batch ( $1.6 \pm 1.02$ ;  $n=22$ ) with a range of 1-3 unprovisioned cells (Table 1). However, in 8 of the 22 batches observed, only 1 cell each did not receive an egg and in 2 batches all the cells in the batch were provisioned with larva food and oviposited. The batch size of *H. gribodoi* ranged from 13 - 28 cells with a minimum of 2 batches per day and the mean number of cells per batch was  $19.95 \pm 5.02$  cells,  $n=22$ . A mean oviposition rate of 5.47 cells per minute ( $X=5.47 \pm 2.20$  oviposition,  $n=22$ ). The duration of the whole process of POP starting from the beginning of cell construction to end of oviposition ranged from 3:55 – 8:20 hours and the mean duration was 6:20 hours per POP. This can be summarized as follows: POP = Cell construction + Patrol phase + Oviposition Phase = 6 hours 20 minutes (mean).

**Table 1.** Numerical data on the batch size and oviposition rate in *Hypotrigona gribodoi*.

| Sessions | Total Nr. of cells in batch | Nr. Of cells oviposited | Nr. Of cells Not oviposited | Duration of O (Min:secs) | Duration of POP (H:m) | Approx nr of O per min |
|----------|-----------------------------|-------------------------|-----------------------------|--------------------------|-----------------------|------------------------|
| 1        | 16                          | 15                      | 1                           | 6:12                     |                       | 2.4                    |
| 2        | 14                          | 13                      | 1                           | 3:55                     | 5:15                  | 3.3                    |
| 3        | 17                          | 14                      | 3                           | 4:12                     |                       | 3.3                    |
| 4        | 13                          | 12                      | 1                           | 3:08                     | 6:35                  | 3.8                    |
| 5        | 17                          | 14                      | 3                           | 6:20                     |                       | 2.2                    |
| 6        | 19                          | 16                      | 3                           | 2:15                     |                       | 7.1                    |
| 7        | 17                          | 16                      | 1                           | 3:27                     | 8:20                  | 3.6                    |
| 8        | 21                          | 19                      | 2                           | 4:00                     | 5:44                  | 3.6                    |
| 9        | 20                          | 17                      | 3                           | 3:35                     | 4:07                  | 4.7                    |
| 10       | 23                          | 22                      | 1                           | 3:06                     |                       | 7.1                    |
| 11       | 20                          | 17                      | 3                           | 1:56                     |                       | 8.8                    |
| 12       | 23                          | 23                      | 0                           | 4:18                     | 3:55                  | 4.1                    |
| 13       | 27                          | 26                      | 1                           | 3:30                     | 6:45                  | 7.4                    |
| 14       | 29                          | 27                      | 2                           | 3:15                     |                       | 8.3                    |
| 15       | 25                          | 22                      | 3                           | 2:24                     |                       | 9.1                    |
| 16       | 23                          | 21                      | 2                           | 3:37                     |                       | 5.8                    |
| 17       | 19                          | 18                      | 1                           | 4:10                     | 8:18                  | 3.3                    |
| 18       | 21                          | 21                      | 0                           | 2:50                     | 5:57                  | 7.4                    |
| 19       | 31                          | 29                      | 2                           | 3:15                     |                       | 8.9                    |

|      |    |       |      |      |      |      |
|------|----|-------|------|------|------|------|
| 20   | 26 | 25    | 1    | 3:44 | 5:26 | 6.9  |
| 21   | 26 | 24    | 2    | 5:10 | 8:14 | 4.6  |
| 22   | 28 | 28    | 0    | 4:32 |      | 4.7  |
| Mean |    | 19.95 | 1.63 |      | 6:20 | 5.47 |
| STD  |    | 5.02  | 1.02 |      |      | 2.20 |

After each oviposition (end of operculation), the queen leaves the top surface clusters to her resting place which is usually underneath the cell cluster. She comes out from time to time to visits the cell cluster, cruises around checking the new cells under construction and then returns to her resting place. One or two workers could be seen attending to the new cells, inserting their body into the cell. In some cases, worker body insertions into cells can last for more than 30 seconds. When the queen arrives at a cell (queen patrol), the workers gently avoid her by just inclining their body upwards. She stays by the cell for a while, either hanging on the cell or sitting on a neighboring cell, and then leaves the cluster after walking around the cell. The number of workers attending the cells increases and the duration of each body insertion shortens. The alternation between the workers attending to the cell and workers body insertion becomes more and more frequent, indicating that the beginning of the next phase (larva food discharge or cell provisioning phase) is soon starting.

Usually, it is complex to draw a clear line between the two phases. Queen patrolling becomes more frequently as

the cells under construction progresses in height. The more closely the cell become ready to be food provisioned, the more frequent the queen patrols on the cluster surface of the cells. The behavior in the patrolling phase in *H. gribodoi* was characterized by the following key features; 1) Appearance of collard cell or completed but unprovisioned cells. 2) Congestion of workers in the brood cell area and 3) Increased and prolonged queen visits to the brood area. These features are quite distinct with *H. gribodoi* (Table 2). During the early part of the patrolling phase, the queen visits to the brood cell cluster is less frequent with the duration of each visit on the cell surface ranging from as low as 1 second to up to 58 seconds. The mean duration of queen stay of the cell surface however was:  $32.5 \pm 10.8$  seconds,  $n=11$ . In 11 observed cases, the number of visits registered ranged from 8 to 20 visits per case, with a mean number of visits:  $13.4 \pm 3.4$  visits;  $n=11$ . The duration of the whole patrolling phase was measured in 11 cases and the mean duration of the phase was:  $80.6 \pm 18.1$  minutes;  $n=11$ .

**Table 2.** Some measurements of queen behaviour related to Patrolling phase (P).

| ( $\bar{P}$ ) case nr | Nr of queen visits during $\bar{P}$ phase | Average duration of queen visits (secs) in $\bar{P}$ | Duration of $\bar{P}$ (mins) |
|-----------------------|-------------------------------------------|------------------------------------------------------|------------------------------|
| 1                     | 15                                        | 24.13                                                | 74                           |
| 2                     | 11                                        | 33.3                                                 | 57                           |
| 3                     | 12                                        | 25.25                                                | 66                           |
| 4                     | 16                                        | 31.18                                                | 89                           |
| 5                     | 9                                         | 32.22                                                | 123                          |
| 6                     | 12                                        | 45.08                                                | 67                           |
| 7                     | 14                                        | 21.14                                                | 73                           |
| 8                     | 20                                        | 58.1                                                 | 64                           |
| 9                     | 8                                         | 40                                                   | 98                           |
| 10                    | 17                                        | 26                                                   | 90                           |
| 11                    | 13                                        | 21.38                                                | 86                           |
| Mean                  | 13.36                                     | 32.52                                                | 80.63                        |
| SD                    | 3.36                                      | 10.82                                                | 18.14                        |

During this patrolling phase, the behavior of both the queen and workers were quite simple and cordial. When the queen is visiting cells, she stays on already oviposited cells, touching the brim of the cells with her antennae and she inspects the cells occasionally by inserting her head into the cells for a while. One or two workers attend to each of the cells where the queen stops during her patrol. The general behavior of the queen does not change during this phase.

Workers gradually accelerate the repeated head and metasomal insertions into the cells sometimes with or without change of individuals. The duration of workers body duration insertions in to the cells could last for up to 74seconds. When the queen arrives at a cell, the worker attendants to the cell immediately abandon the cell after attempting to make body insertion into the cells. During the patrolling, the queen spends much more of her time

cruising (walking) on the cell clusters rather than staying at a particular cell. The workers who accidentally come in contact with the queen when making body insertions, quickly withdraws from the cell and escape into nearby openings or nearby cells. Some workers will turn around and move away to the opposite direction.

The start of the arousal phase ( $\bar{A}$ ) is marked by a distinct increase in the agitation displayed by both queen and worker attendants. Congestion of workers at the brood cell area becomes even more compared to that of the patrolling phase. The behavior during the arousal phase does not differ much from those seen in patrol phase. Generally, the coaction between the queen and the worker is equally simple without any form of rituals. During the later parts of the patrolling phase and the arousal phase, queen-worker interactions are much simplified and are predominantly represented by queen inspection and worker body insertions with fewer pushing and darting. The queen walking rhythm gains considerable speed and agitation during arousal, and her wing movements tend to vibrate more continuously than in the patrol phase. Her cruising on the cluster is gradually replaced by brief visits to some collard cells. The excitement that the queen arouses at each cell sometimes culminated in a high excitement over the cell cluster but sometimes the excitement is limited to particular areas on the cell cluster.

This phase is marked by the queen arrival at the first cell to the first food discharge by the worker into a cell. As in many taxa, when most cells are structurally completed

(collared cells), they engineer an excitement gathering of workers on the surface of the cluster. The workers alternately insert their fore bodies into the cell indicating to the queen their intentions to deliver larva food discharge act into the cells. In *H. gribodoi*, the appearance of the excitement was very conspicuous. The behavior of both workers and queen was very simple in this phase. In both the arousal and the predischage phase, queen inspection and workers body insertion into cells was not ritualized. The cells that were first provisioned and oviposited were not necessarily the first cells by which the queen waited in predischage phase and were not necessarily followed by food discharge and queen oviposition in the same cell. In 25 carefully observed cases, none of cells where the queen waited was followed by food provision and eventual oviposition in same cell. The duration of the Predischage phase ( $\bar{r}$ ) range from 85-105 seconds (mean  $77.75 \pm 17.78$  seconds;  $n = 12$ ). Among 15 observations of the predischage waiting phase, queen inspection was noted in 13 cases (Table 3). Generally, the time spent by the queen on cruising is much longer than the time she spends making prolonged waiting during the arousal phase (table 3). Prolong waiting here means the queen stopping for more than two seconds at a particular cell during this phase. The queen performs relatively fewer cell inspections in the Predischage phase than in the arousal phase (table 3). The number of worker insertions in to the collard cells during the Predischage phase is almost trice as much as that in the arousal phase as seen in table 11 above.

**Table 3.** Numerical data on the arousal ( $\bar{A}$ ) and predischage behavior ( $\bar{r}$ ).

| Nr of prolonged queen waiting (W) in $\bar{A}$ | Nr of queen inspection during $\bar{A}$ (E) | Nr of queen inspection during $\bar{r}$ 1 (e) | Nr. of workers insertion during $\bar{A}$ | Nr. of workers insertion during $\bar{A}$ |
|------------------------------------------------|---------------------------------------------|-----------------------------------------------|-------------------------------------------|-------------------------------------------|
| 0                                              | 3                                           | 1                                             | 3                                         | 5                                         |
| 0                                              | 4                                           | 2                                             | 3                                         | 11                                        |
| 1                                              | 3                                           | 1                                             | 2                                         | 7                                         |
| 0                                              | 2                                           | 1                                             | 3                                         | 5                                         |
| 0                                              | 4                                           | 1                                             | 1                                         | 2                                         |
| 1                                              | 1                                           | 0                                             | 1                                         | 6                                         |
| 1                                              | 3                                           | 0                                             | 0                                         | 7                                         |
| 0                                              | 2                                           | 1                                             | 0                                         | 8                                         |
| 0                                              | 4                                           | 1                                             | 2                                         | 2                                         |
| 0                                              | 2                                           | 1                                             | 2                                         | 1                                         |
| 0                                              | 1                                           | 3                                             | 1                                         | 3                                         |
| 0                                              | 4                                           | 1                                             | 0                                         | 1                                         |
| 1                                              | 3                                           | 1                                             | 0                                         | 1                                         |
| 0                                              | 1                                           | 2                                             | 1                                         | 4                                         |
| 2                                              | 2                                           | 1                                             | 2                                         | 8                                         |
| 0.4                                            | 2.6                                         | 1.1                                           | 1.4                                       | 4.7                                       |

Finally, after several rapid body insertions, a worker discharges the first droplet of larval food from the mouth into a cell. The cell where the first food discharge takes place never coincided with the cell where the queen predischage waiting ( $\bar{r}$ ) took place. All the collard cells are food provisioned with larva food successively by many

workers. This happens after the alternation of agitated insertions by many workers and some workers contract their metasoma and discharge the larva food into the cell. After a worker makes her discharge, she quickly retreats and leaves the cell making way for the next worker to make the next discharge. This behavior is repeated for all the collard cells in the batch ready to be provisioned. After a cell receives sufficient food, a worker stays on the margins of the cell, checking the cell rim with the antennae but do performs neither body insertion nor removal. The congestion of workers around a cell undergoing provisioning gradually clears out as soon as the cell is filled with larval food, making the approach of the queen possible. Usually, just one worker take control of the cells with sufficient food and protects filled cells from any further discharges. The discharges are done successively i.e. one worker before the next. Intentional body insertions by workers are seen before the arousal and predischage phases are replaced by actual ones when the queen arrives and stays at a particular cell. After each food discharge, the worker slightly withdraws herself towards the lower part of the cell. The first discharge is immediately followed by the second and subsequent discharges. The mean number of discharges per cell was very high,  $34.2 \pm 5.9$ ;  $n=37$ . The duration of each discharge is short, mostly one second or slightly higher ( $1.10 \pm 0.38$  seconds;  $n=37$ ). In 37 cases observed: 1 second (29cases), 2seconds (7cases) and 3 seconds (1case). Postdischarge subphase (the time between the final discharge and the beginning of cell inspection by the queen) is very short and simple and virtually absent in most cases recorded. No specific measurements were taken during this phase because of it inconspicuousness.

The queen lays her egg immediately (Oviposition) following a brief cell inspection period which usually lasted under a minute. The duration of queen oviposition time (egg laying into a cell) ranged between 2-7 seconds with a

mean duration of ( $\bar{X} = 3.75 \pm 1.11$  seconds;  $n=111$ ). The interval between 2 oviposition was between 5-14 seconds with a mean interval of ( $\bar{X}=8.4 \pm 2.8$  seconds,  $n=16$ ). The queen beats her wings more or less continuously while laying egg. The duration of a complete queen oviposition into all collard provisioned cells ranged from 6.25 to 12.1 minutes, with a mean duration of  $8.75 \pm 2.23$  minutes;  $n = 12$  (Table 4). An interesting queen behavior was observed in 4 instances during the oviposition phase was observed during the study period with some 4 workers refused to allow the queen assess to lay her eggs into already provisioned cells. This resulted into a fight between the queen and the worker at the brim of the cell. At the end, the queen gave up the fight and concentrated on the other provisioned cells. This behavior lasted 10 seconds (1 case), 12 (1 case), 15 (2 cases). The workers did not lay any egg in any of the cases. After the final oviposition, the queen often stays on the cell clusters and visits the different cells under operculation (sealing of cell) by the various workers. During these visits, workers do not show any signs of aggressiveness or reaction to the queen advances. Coaction between workers and the queen is simple in this phase. Cell operculation ( $\bar{S}$ ) starts immediately after queen oviposition. Soon after the queen oviposition ends, a worker sits on the brim of ovposited cell, inserts her metasomal tip into the cell and work by rotating herself on the cell. The mean duration of the operculation for a single cell ( $\bar{S}$ ) range from 313 - 780 seconds ( $\bar{X} = 484.5 \pm 112.3$ ,  $n=48$ ). Meanwhile the mean duration of the operculation phase ranged between 455 and 815 seconds ( $\bar{X} = 646.8 \pm 64.4$  seconds,  $n = 12$ ). The rotation sub phase ( $\bar{S}_r$ ), took more than 75% of the total duration. Meanwhile the  $\bar{S}_i$  subphase was not actually noticed in *Hypotrigena*. The sidework ( $\bar{S}_s$ ) subphase lasted just a few seconds and not very clear for measurements. Change of operculator was never observed during our observation period.

**Table 4.** Duration of oviposition in some accurately measured cases (seconds).

| Cases | # of cells | Total duration of POP |             |           |             |              |               |
|-------|------------|-----------------------|-------------|-----------|-------------|--------------|---------------|
|       |            | Arousal               | Predischage | Discharge | Oviposition | Operculation | Total O phase |
| 1     | 11         | 75                    | 85          | 160       | 420         | 650          | 1390          |
| 2     | 13         | 87                    | 74          | 95        | 450         | 630          | 1336          |
| 3     | 13         | 85                    | 60          | 90        | 375         | 675          | 1285          |
| 4     | 14         | 97                    | 57          | 180       | 435         | 780          | 1549          |
| 5     | 14         | 76                    | 94          | -         | 459         | 640          | 1269          |
| 6     | 15         | 198                   | 77          | 105       | 730         | 815          | 1925          |
| 7     | 17         | 132                   | 105         | 145       | 366         | 455          | 1203          |
| 8     | 17         | 165                   | 66          | 75        | 720         | 620          | 1646          |
| 9     | 20         | 207                   | 89          | 65        | 570         | 590          | 1521          |
| 10    | 20         | -                     | 45          | 133       | 725         | 710          | 1613          |
| 11    | 21         | 208                   | 78          | 133       | 525         | -            | 944           |
| 12    | 23         | 235                   | 103         | 120       | -           | 550          | 1008          |
| Mean  | -          | 142.27                | 77.75       | 118.27    | 525         | 646.81       | -             |
| SD    | -          | 47.69                 | 17.78       | 28.53     | 136.72      | 68.46        | -             |

The total duration of oviposition process is define here as the  $\bar{A}$  (arousal phase) +  $\bar{r}$  (Predischage subphase) +  $\bar{dp}$ (discharge subphase) +  $\bar{O}$ (oviposition phase) +  $\bar{S}$ (operculation phase) was measured in 12 cases.

**Table 5.** Behavioural differences between *L. mulleri*, *H. duckei* and *H. gribodoi*.

| S.No. | Characteristic                                      | <i>Leurotrigona mulleri</i>                                                     | <i>Hypotrigona (Trigonisca) duckei</i>                                                                          | <i>Hypotrigona gribodoi</i>                                                     |
|-------|-----------------------------------------------------|---------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------|
| 1     | Rhythmic wing movement of queen                     | Relatively rare but present at walk                                             | Extremely rare                                                                                                  | Present at walk and during rest sometimes                                       |
| 2     | Cell construction                                   | Synchronous ( $S_y$ )                                                           | *Semisynchronous ( $S_m$ )                                                                                      | Semisynchronous ( $S_m$ )                                                       |
| 3     | Excitement at cell construction                     | *Relatively high                                                                | **Not conspicuous                                                                                               | inconspicuous                                                                   |
| 4     | Oviposition pattern                                 | Exclusively batched ( $B_e$ )                                                   | *Singular ( $B_e$ ) in the colony observed, probably facultatively batched ( $B_f$ ) under favorable conditions | Exclusively batched ( $B_e$ )                                                   |
| 5     | Pattern of PredischARGE queen behavior              | More cruising than waiting (CW)                                                 | More waiting than cruising (cW)                                                                                 | More cruising than waiting (CW)                                                 |
| 6     | Shortening of secondary PredischARGE waiting        | Inconspicuous                                                                   | *Occasionally conspicuous                                                                                       | *Inconspicuous                                                                  |
| 7     | PredischARGE worker behavior                        | Simple, body insertions into cell replaced by intentional ones at queen waiting | Intentional insertions rare. Peculiar behavior, presentation of metasoma to queen prevailing                    | Simple, body insertions into cell replaced by intentional ones at queen waiting |
| 8     | PredischARGE queen behavior                         | Simple, waiting short                                                           | Waiting long with violent beating of workers with antennae and fore legs                                        | Long waiting and simple                                                         |
| 9     | Number of discharges per cell                       | Low, mostly 2                                                                   | Normal, 4~6                                                                                                     | Extremely high, 30~47                                                           |
| 10    | Postdischarge escape                                | Inconspicuous                                                                   | Distinct                                                                                                        | Inconspicuous                                                                   |
| 11    | Duration of operculation                            | Normal                                                                          | Extremely long especially at the rotation subphase                                                              | Extremely long                                                                  |
| 12    | Differentiation of rotation and side work subphases | Distinct                                                                        | Indistinct due to persistence of rotation                                                                       | Distinct                                                                        |

\*= Characters that might require further confirmation.

The oviposition process of *H. gribodoi* proceeds with distinct phases as in other stingless bees like *Melipona beecheii*, *M. compressipes*, *Tetragona clavipes*, *Leurotrigona mulleri* and *M. bicolor* (Kalt *et al.*, 1987; Pioker *et al.*, 2003; van Veen, 2000). Furthermore, there are several ethological characteristics common to the other species that are also shared by *H. gribodoi*. These characteristics are: Rapid succession of food discharges by workers into cells. Rapid escapes of workers from the cell following food discharge. Increased in workers body insertions during the patrolling and arousal phase. Worker-queen relation showed repetition of pushing followed by a retreat by attendants in front of the queen, workers avoiding approaching queen and formation of royal courts around resting queen. Queen lays egg vertically on larva food. Cells are constructed by several workers successively as in honeybees, not through the work of a single worker as in bumble bees. Oviposition process can be divided into successive stages and phases which is not the case with

other social insects. Absence of workers licking the queen. Rarity of food delivery by workers to the queen. A detail comparison of the ethological features of *H. gribodoi* with those of other closely studied taxa is given below with special reference to the queen-worker coactions and oviposition process. All species listed here are South American species. The groups compared with are abbreviated as follows: The arrangement of cells in clusters as found in *H. gribodoi* is similar to that of *L. mulleri*, *Trigonisca duckei*, *T. clavipes* and neither complete combs as in *Lepidotrigona ventralis*, *Scaptotrigona postica*, *Nannotrigona testaceicornis*, nor semi combs as in *Duckeola ghillianii* and *Friesella spp.* However, *H. gribodoi* showed some peculiar characteristics in its simple and no exaggerated or ritualized behavioral patterns and extreme excitement level during oviposition. From the above ethological diagnoses, the number of character states shared ( $n_1$ ) or not ( $n_2$ ) with *H. gribodoi* are given below for each taxa as  $n_1 / n_2$  following the

descending orders of  $n_i$ : *Leurotrigona* 17/2 > *Duckeola* 13/9 > *Trigonisca* 12/5 > *Friesella* 11/7 > *Tetragona* 10/10 > *Scaptotrigona* 7/13 > *Melipona* 6/8 > *Cephalotrigona* 6/9 > *Lepidotrigona* 0/4 > *Nanotrigona* 3/9 > *Mourella* 2/7. The above character states represents a preliminary attempt towards phylogenetic and other comparative analyses in view of increasing the number of studied taxa and establishing a the phylogenetic polarities of each character. The above result shows that *H. gribodoi*, shares most ethological features with *Leurotrigona*, *Duckeola*, *Trigonisca* and *Tetragona*. Aside *Duckeola* which is a semi comb maker in the list of most shared characters with *H. gribodoi*, all the others (*Tetragona*, *Leurotrigona* and *Trigonisca*) are cluster makers like *H. gribodoi*. The least character states shared was with *Mourella* and *Lepidotrigona*. Nonetheless, *H. gribodoi* is peculiar in the following characters when compared to the others in the most shared characters: The number of food discharge per cell is exaggerated. Queen oviposition is made with leg twitching and the oviposition duration is longer than that of most studied taxa. Colony condition cannot be over looked at as a possible reason for the differences in some of the shared characters mentioned above. If not all could be characteristics of *H. gribodoi*. A closer comparison between *H. gribodoi* and *L. mulleri* and *H. duckei* is shown in Table 5. Behavioral characters common to the three groups were as follows: Cells are arranged in clusters. Involucrum is absent. Differentiation of honey and pollen pots is absent. Waste materials are thrown down from the nest entrance. Queen - worker exaggerated interactions out of oviposition process is absent. Food discharges in cells is successive. Worker oviposition is completely absent. Queen oviposition relatively short. Cell operculum started without much delay. Some characteristics common exclusively to *H. gribodoi* are as follows: The extremely high number of discharges per cell Wing beats during rest and at walks at times though not continuous Oviposition pattern is exclusively batched but 1-3 collared cells were left unprovisioned in some batches.

## CONCLUSION

*H. gribodoi* are generally calm with a single queen (monogynous) at any given time in a colony. Brood cells are constructed semisynchronously and the oviposition pattern is exclusively batched. Cell provisioning was done by extremely large number of workers and the duration of oviposition is longer than that of most stingless bees already studied. A close comparison with nearest relatives of *H. gribodoi*, shows that the queen feeds the workers in some cases instead of the workers feeding the queen as is the case with the relatives. Workers oviposition does not seem to occur in this species. Though, *H. gribodoi* are mostly calm; the workers are capable of protecting their nests from invading bees when their honey pots are damaged. This was observed with the presence of *Meliponula beccarii* and *Apis M. adansonii* invading the

hive to steal honey from ruptured honey pots. Also, during the oviposition process, the queen can suddenly become aggressive towards any worker prolong stay on already provisioned cell, by pushing and chasing the worker away from the surface of cell in order to stop the workers from laying egg. The queen in most cases succeeded in laying her egg into the provisioned cell after vigorously pushing the worker aside.

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