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Bio-ecology of blue banded bees, *Amegilla zonata* L. (Apidae: Hymenoptera)

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Abstract
The most common blue banded bee occurring in Tamil Nadu, India is *Amegilla zonata* (Linnaeus). Nests are often built together in one place forming nest congregation. Females of blue banded bees have a well-defined brood cell cycle involving cell construction, waxing, provisioning, egg laying and cell capping. Red soil and potting mixture are found to be the best substrates for nesting. Each female bee digs out her own nest burrow of depth 4 to 30 cm using her strong mandibles and push out the excavated soil outside with her legs forming a tumulus. Sometimes they build a turret of varying height over the nest opening using discarded soil. The nest shaft extends from nest entrance to a cluster of earthen brood cells. Brood cells are cylindrical, urn shaped and internally lined with thick, water proof linings. Each brood cell was mass provisioned with of bee bread on which creamy white egg is laid individually by a female bee. The first instar larva is straight. The second instar larva has slightly pigmented mandibles. The third instar larva is ‘C’ shaped and found along with a small amount of unutilized brood food. The fourth instar larva almost occupies the brood cell without any unconsumed larval food. It consumes also the wax linings and shrinks slightly before turning into a prepupa. Pupal eye colouration changes from yellow to orange and then to brown and finally black. As it matures pupa transforms into adult within a fortnight. Heavy rains and brood mites (*Rhizoglyphus* sp. and *Histiostoma* sp.) attack affects the development of bees.

Keywords: Blue banded bees, *Amegilla*, nest architecture, biology, India

1. Introduction
Blue banded bees are soil nesting solitary creatures. They are fossorial bees, they build their nests gregariously. Species of *Amegilla* excavate nests in the soil either in banks or on flat ground. Most species of *Amegilla* make non-aggregated nests on flat ground, but some are known to aggregate nests in vertical clay banks [10]. Since blue banded bees are solitary bees, they build nests often close to one another there by forming nest congregations. Dug out soil on soil surface is often loose soil or arranged in a delta shape with irregular pellets [4]. They prefer soft sand stone to burrow in and areas of this type of rock can become riddled with many bee tunnels. They also like mud-brick houses and often burrow into the mortar in old buildings. Nesting is also seen in poorly maintained brick walls and crevices of rocks. Each female builds her own nest burrow but many bees often seen building nests together in one place. They use nectar to soften the soil during excavation [14]. Females recharge their crop with liquid at the time of digging [8]. Cells at the end of tunnels contain an egg with pollen or pollen nectar mixture which is provided as food for emerging larva. Research studies on bioecology of blue banded bees are inadequate. Keeping in mind their inadequate research in India, present research work was done to expose their nest architecture and developmental biology.

2. Materials and methods
2.1 Nesting biology
2.1.1 Studies on nesting site preference
Pot culture experiments were conducted to find out the soil preferred by bees for nesting. Two soil types *viz.*, black soil and red soil were taken for comparison. Since the bees commonly nest in pots filled with potting mixture used for growing plants, potting mixture was also included as one of the treatments. The chosen substrates were filled in medium sized pots. The pots were kept in the shade at a location 10 m away from nest congregation having several nests of blue banded bees. The mean number of nests constructed in each substrate was worked out for making comparisons.

Nest dissection studies

Plastic pots filled with red soil were kept in shade closer to the nest congregation site. Nest dissection was taken up after two months.

- Wet the soil slightly with water
- Wait for 10 min for the water to percolate
- Keep a wooden plank on floor.
- Turn the pot upside down and keep it on the plank.
- Tap the bottom and the sides of the pot gently to loosen the soil.
- Use a hot knife to cut the pot alone into two equal halves.
- Separate the two cut halves carefully.
- Remove the soil slowly starting from periphery using injection needles and fine forceps to expose the nest components.

2.1.2 Nest architecture

The following metric data were taken pertaining to various nest components of the nest like nest entrance, turret, tumulus, shaft, brood cells and nearest neighbor distance

2.2 Developmental biology

Observations were taken on colour, consistency and the quantum of brood food provided in each brood cell. Similarly colour, shape and mode of deposition of egg were also recorded. Details were also collected on larval shape and quantity of unconsumed food available in the brood cell. Differentiations were made among young larva, grown up larva, prepupa and pupa based on certain structural details. The number of larva present per brood cell was recorded to find out cleptoparasitism if any. The activities of adult cleptoparasites were also monitored at the nest entrance. The immature stages (larvae and pupae) were examined for the presence of ectoparasitic mites. The mites were collected and preserved in 70 per cent ethanol. Permanent slides were prepared using Hoyer's medium and their identity was fixed. Cursory observations were also taken on the mode of pupation, site of pupation and changes of pupal eye colouration.

2.2.1 *In vitro* rearing of bees

An attempt was made to rear bees under *in vitro* conditions. Eppendorf tubes and small glass specimen tubes were the artificial containers used for rearing. The brood cell contents (egg plus bee bread or larva plus bee bread or pupa) were transferred to artificial containers. One set of containers was kept inside BOD incubator at 28^{o}c. Another set of containers was kept at room temperature. To keep the brood food moist a few drops of honey were added whenever necessary. A razor blade was used to cut open the brood cells to observe the internal nest linings and faecal deposits, various life stages (egg, larvae, prepupa, pupa and adults), brood food (consistency, colour and smell) and natural enemies if any.

3. Results

3.1 Nesting Biology

Blue banded bees built their nests in the soil. The bees showed special preference for soil kept in flower pots. More nests were found closer to the edge of the pot (Fig 1) rather than in the central zone of the pot. Thus they formed nest congregation in flower pots. They also built several nests along the sides of well shaded irrigation channels and also in the imported soil found in the mini bamboo wood lot created in a Japanese garden in the college campus. Occasionally the nests were also found in the brick walls of old buildings where clay was used as mortar and a mixture of sand and red soil was used for plastering.

3.1.1 Preference of nesting substrate by *Amegilla zonata*

Amegilla zonata showed almost equal preference (Table 1) for both red soil and potting mixture. However, nest dissection was easy when nests were built in red soil. These bees did not prefer black soil for nesting.

Table 1: Nesting substrates preferred by *Amegilla zonata*

Pot no.	Number of nests constructed in 30 days		
	Black soil	Red soil	Potting mixture
1	0	34	21
2	0	13	19
3	0	26	22
4	0	15	11
5	0	21	31
6	0	26	26
7	0	24	16
Mean	0	22.71	20.85

3.1.2 Nest architecture (Table 2)

Each female bee dug her own nest burrow but many bees were often seen building nests together in one place. Nest construction activity was observed throughout the day. Bees constructed their nest by digging the soil using their mandibles and pushing the mud outside using their legs (Fig 2).

The diameter of the entrance hole ranges from 0.4 to 0.8 cm (n=25). The nest burrows were as close as 0.5 cm to 8.0 cm. Nest burrows were plugged with small clods of soil occasionally. The nest entrance had a well defined rim in some nests. A small heap of dug out soil (acentric tumulus) (Fig 3) was also present near the entrance hole.

In some nests a small cylindrical earthen tube *viz.*, turret was attached to the entrance hole. Nest opening was present either on the apex of the turret (Fig 4) or on the side wall of the turret. The height of the turret was ranging from 1.2-4.8 cm (n=12). The interior surface of the turrets was smooth and exterior surface was rough.

Each nest had an entrance hole leading into a shaft of varying depth (4.0 to 30 cm, n=7). Initially the tunnel was dug out vertically but later it was laterally extended for a certain distance and then descended vertically. At the end of the shaft a cluster of earthen brood cells was found. They constructed brood cells in pots from a depth of 4 cm. The numbers of brood cells in isolated nesting sites are ranging from two to six. In aggregated nests (Fig 5) it was ranging from two to twenty one. Brood cells were cylindrical (Fig 6), urn-shaped, not easily separable from the matrix. The brood cell cap was thick with water proof lining (Fig 7). The height of brood cell ranged from 1.3 to 2.0 cm and diameter of brood cell ranged from 0.4 to 0.7 cm (n=38). Waxy coatings were found along the inner cell wall. The inner brood cell linings were pliable, smooth, thin, waxy and translucent which prevented water entry. Larval faeces were deposited at bottom of the cell, they do not spin cocoon. The cast skin was found inside the brood cell.

3.2 Developmental biology

The various life stages (Fig 8) of blue banded bees are described below

Egg

Eggs were present only in brood cells which are adequately provisioned with larval food or bee bread. The bee bread had a semisolid consistency. Sometimes larval provisions were semi liquid only at surface. The pollen bread was kept at the bottom and a small quantity of nectar was placed over it. The colour of the bee bread varied according to the pollen source. Provisions were with strong odour of fermenting cheese, and. The average weight of bee bread was 0.07 gm (n=13). Egg was creamy white in colour, slightly arched and singly laid on the brood food (Fig 9). It was cylindrical in shape with bent ends. The bent caudal ends of the egg were just touching the brood food. Middle part of the egg did not touch the brood food. Mean length of egg was 2.8 mm (n=4).

Larva

Newly hatched larva was straight and found on the top of the brood food. As the larva grew the body became 'C' shaped and robust. Second instar larva had slightly pigmented mandibles. The grownup larva was distended and only a small amount of unutilized brood food was left out in the brood cell. The fourth instar larva consumed the remaining provisions and also the cell linings (Fig 10). Head capsule was well developed with two well-developed mandibles. It was elongate without any legs in thorax and abdomen. The dorsal blood vessel was faintly visible in the mature larva. Similarly Malpighian tubules were also visible through the transparent larval cuticle. The grown up fourth instar larva voided the black colour faeces at the bottom of the brood cell.

Prepupa

The body of the fully grown larva shrank to form the prepupa. The cell housing the prepupa neither had any larval food nor wax linings. Prepupa moulted into an exarate pupa without any covering within the brood cell.

Pupa

Newly formed pupa was yellow in colour with yellow coloured compound eyes, As the pupa matured the eye colour also changed (Fig 11) from yellow to orange and then to brown and finally to black. Sexing was possible even during the pupal stage based on the number of bands present on the metasoma. Male pupa had five bands (Fig 12) where as female pupa had four bands (Fig 13) only. The pupa transformed into an adult bee within a fortnight under *in vitro* conditions.

Adult

The newly emerged adult, the callow remained within the earthen cell for two to three days and then came out of the nest and walked for a while near the nest. It cleaned its compound eyes with its fore legs and rubbed its gaster with its hind legs and fluttered its wings. Activity of blue banded bee was reduced markedly especially during rainy seasons. Eggs and larvae did not transform into adults under *in vitro* conditions.

3.2.1. Natural enemies of blue banded bees

A Sphecid wasp (*Liris* sp) was found in the nest congregation of blue banded bees found in a pot. In one of the nests, a paralyzed cricket was found protruding from nest entrance. Dissection of that nest yielded six earthen brood cells of Sphecid wasp which were reared *in vitro* conditions. Six adult wasps emerged after a week from the brood cells of the wasp. Two mite species were found to be ectoparasitic on larvae and pupae (Fig 14) of blue banded bees. They were not found on pollen provisions. The mite population was more in pupae rather than in larvae. The mites were also found even on dead pupae. Approximately 70 mites were found per dead pupa. Both the feeding (adult) and non-feeding stage (hypopal) were found on the immature stages of bees. These two mite species were identified as *Rhizoglyphus* sp. and *Histiostoma* sp.

Table 2: Parameters of nesting architecture

Parameters	Range		N	Standard error
	Minimum	Maximum		
Entrance hole (dia)	0.4 to	0.8 cm	25	0.62 ± 0.02
Closeness of nest burrows	0.5 to	8 cm	44	5.05 ± 0.33
Height of the turret	1.2 to	4.8 cm	12	3.1 ± 0.30
Shaft depth	4 to	30 cm	7	16.33 ± 1.81
Height of brood cell	1.3 to	2 cm	38	1.67 ± 0.04
Brood cell (dia)	0.4 to	0.7 cm	38	0.56 ± 0.01
Weight of bee bread	0.04 to	0.07gm	13	0.05 ± 0.003

Fig 1: Nests constructed near pot edge

Fig 2: Female of *Amegilla zonata* digging soil

Fig 3: Tumulus around nest entrance

Fig 4: Turret with apical entrance hole

Fig 5: Aggregation of brood cells of adjacent nests

Fig 6: Cylindrical brood cells

Fig 7: Internal wax lining of brood cells

Fig 8: Life stages of Amegilla zonata

Fig 9: Egg on brood food

Fig 10: Fourth instar larva feeding on cell linings

Fig 11: Change in eye colouration of pupa of *Amegilla zonata*

Fig 12: Male pupa of *Amegilla zonata*

Fig 13: Female pupa of *Amegilla zonata*

Fig 14: Ectoparasitic mites on pupa of *Amegilla zonata*

4. Discussion

The research study about these solitary bees has yielded many interesting results. Bees nest in diverse earthen habits, ranging from weathered sandstone and the mortar of adobe walls to embankments of dense clay, deep alluvial silts, and sand dunes of deserts and beaches. Fossorial nesting is characteristic of Andrenidae, Fideliidae, Halictidae, Melittidae, Oxaeidae and Stenotritidae. The majority of Colletidae and Anthophorini of Apidae also nest underground [3]. The Anthophorine blue banded bees build nest in soil. They are variously called burrowing bees or digger bees as they build subterranean nests in soil burrows or dig the soil for nest construction using their strong and sturdy mandibles. The female bees select the nesting sites with particular edaphic attributes. The soil preference shown by different groups of soil nesting bees vary widely. Dawson burrowing bee (*A. dawsoni*) prefer clay pan [1]. Sand stone and soft clay offered ideal nesting sites for *Amegilla cingulata*.

Alkali bees (*Nomia melanderi*) showed special preference for salt affected soils. In our studies *A. zonata* preferred both red soil and potting mixture and also made up soil used for raising bamboo lot in the Japanese garden. Shade, good drainage and absence of excess roots in pots were found to be the major factors in deciding the choice of nesting sites. Shaded places were always useful to keep their nest cool. Many soil nesting bees also show similar preferences in nest site selection.

The finding of that blueberry bee (*Habropoda laboriosa*), another Anthophorine bee preferred deep and well drained sandy soil lends support to our finding [3]. Similarly another finding of the above author that the mortar of prehistoric adobe walls was one of the nesting habitats of soil nesting bee is in agreement with wall nesting behaviour of *A.zonata*. The depth of nesting in our studies varied according to the pot size and was ranging from 4 to 30 cm. The impact of adverse soil temperature will be low whenever nests are deeply placed. The observation of [16] that nesting female of the alkali bee, *N. melanderi* responded to ambient temperatures by excavating nest burrows more deeply in the soil supports the present finding.

Soils nesting solitary bees often construct their nest close together forming dense aggregations, though each bee digs its own nest. The gregarious nesting behavior was reported in several species of *Amegilla*. [11] Attributed that female philopatry was the primary reason for the aggregation of nests, as neighbouring seemingly equivalent sites in the same embankments remained unused. The phenomenon of philopatry where female bee is particularly attracted to sites having holes or depressions where other females are already constructing nests which results in the formation of nest congregation. In such a situation there is over crowding of nests and as a result the nearest neighbor distance becomes very low. Usually both horizontal and vertical nests were built only in well drained soils.

Probably pheromone odour emanating from the bees or the soil of existing or previous nests serve as the attractant and release hole-searching, digging and nest constructing behaviours [2]. In our nest dissection studies we also found out that the soil surrounding the nest when removed while performing nest dissection also attracted *A.zonata* bees. The finding of [9] that *Amegilla* females were even attracted from outdoors to soil taken from a distant nesting site of their species and kept in a box inside a building is in consonance with our observations.

Nest congregations were observed both in pots and in the bamboo lot of the Japanese garden. Several nests were found closer to the edge of the pot rather than in the centre. Nest when built near the periphery of the pot the side wall of the pot offered good support to the nest. Nest congregations in solitary bees not only ensure reproductive success but also resulted in natural enemies dilution effect because of overcrowding of nests and reduction in nearest neighbor distance. The female bee used either water or nectar for making the soil suitable for digging [14]. However, in our studies we did not observe this behaviour as the soil in the nesting sites had sufficient moisture.

The excavated soil was heaped near the entrance as tumulus usually and sometimes a turret was also built over the entrance. The main role of the turret is thermoregulation, prevention of the entry of debri and excavated soil into the nest, denial of easy access to the natural enemies and serving as a passage way [8]. In many nests either tumuli or turrets were not seen near the nest which could be attributed to periodical watering of pots and removal of tumuli due to winds.

At the end of the shaft brood cells were built either singly or in a cluster or in a linear series in different soil nesting bees. In our study the brood cells were found in small clusters. The water entry into the brood cell was blocked by the internal wax linings present in every brood cell. The study by [5] indicated that these linings are produced from the secretions of Dufour's gland and mainly consisting of triglycerides.

Fossorial solitary bees build several earthen brood cells in their nests for rearing the immature stages. These brood cells are provided with water repellent wax linings to prevent the entry of water from surrounding soil. As a rule the solitary bees rear their young ones through mass provisioning in contrary to the progressive provisioning found in eusocial *Apis* spp. Since the bee development is taking place under captivity, these bees could not be reared successfully in our studies under *in vitro* conditions when egg and larvae were transferred to artificial containers. However, pupae could be reared successfully as adults. The finding of [12] that only grown up larva, prepupa and pupa alone could be reared outside the nest is in partial agreement with our findings. The developmental duration of all life stages could not be worked out in the present study as planned. Employing non-invasive methods like Computerized Tomography (CT) scanning could be a possible alternative to study the developmental biology as it was successfully used for monitoring nest structures, nesting behavior, adult female activity and natural enemies of *A. holmesi* [7].

However, the four larval instars and prepupa were successfully differentiated based on the shape, colouration of the mandible, amount of left over brood food present inside the brood cell and absence of wax linings in brood cells. The first instar larva was straight and found on the top of the brood food. The second instar larva was slightly curved with pigmented mandibles. The third instar larva was typically 'C' shaped along with a small quantity of left over brood food. The fourth instar larva was plumpy with distended body without any larval food in its cell. The fully grown larva also ate the wax linings which are a source of lipid produced from Dufour's gland as a special nutritious secretion in these bees. The body of the matured larva shrank and turned into prepupa. The prepupa did not have distinct cephalic, thoracic or abdominal regions and remained motionless as earlier reported by [15]. At this stage defaecation occurred. The delayed defaecation at the end of the larval stage helped in avoiding the pollution of brood cells. As in the larvae of *Apis* spp. the midgut of *Amegilla* larva is also occluded and it gets connected to the hind gut only in the grown up larval stage. The eye colour of the pupa showed several changes over a period of one fortnight. Similar colour changes were also reported in European bees, *Apis mellifera.* The age of the pupa could be determined based on the eye colour. In newly moulted pupa it was yellow and in well matured pupa it was black.

In our studies brood mortality was more during rainy season. Such brood mortality was also reported in soil nesting sweat bee (*Halictus* spp.) during hottest and driest part of the summer [13]. Brood mite infestation was found to be another factor contributing to brood mortality in our studies. Two mite species *viz., Rhizoglyphus* sp. and *Histiostoma* sp. were found to infest especially the pupal stage. Earlier reports by [6] about brood death caused by the mite, *Pymotes ventricosus* in *Amegilla* sp provide support to our present finding.

Cuckoo bees (*Thyreus* sp.) are common cleptoparasites associated with *Amegilla* sp [6], but no cleptoparasitism was found in the nest of *Amegilla* in our present studies Similarly adult cuckoo bees were not found at the nesting sites but they were found at the foraging sites. Since invariably in all brood cells only a single larva was found and all the larvae found in the brood cells were similar except for size and shape. However, a digger wasp (*Liris* sp) was found to nest in the vacant nest of blue banded bees found in a nest congregation. Our observations clearly indicated that the Sphecid wasp used the nest of blue banded bees only for rearing its progenies.

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