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Original Article

NEST STRUCTURE, DEVELOPMENT AND NATURAL ENEMIES OF CERATINA HIEROGLYPHICA SMITH, A STEM NESTING BEE COLONIZING CASHEW TREES IN HILLY TERRAINS

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Abstract

***Ceratina hieroglyphica* nesting sites were located in dried tiny twigs of cashew trees, and the life stages were observed through periodical collection of nests. Nests were located in the pithy region up to a maximum of 20 cm deep, and individual cells of 3.5 - 4 mm were separated by partitions. In 2017, one hundred and two nests were collected, of which twenty-two had been abandoned. Older cells were at the bottom of nests, while young ones towards the entrance. Among the different stages, the most in the nests were adults (51.8%), followed by pupal stages. Periodical collection of nests and the observations on developmental stages of the bees indicated that the nesting period was found to occur between October and March. Each egg was laid on a pollen provision located in separate cells and the incubation period lasted for 3.1±0.29 days. The larval period and pupal period lasted for 8.4±0.63 days and 7.3±01.41 days, respectively. Adults survived up to fourteen days in lab conditions with 10% honey solution. Parasitoids, predators and pathogens recorded on this bee species are also presented here.**

Keywords: behaviour, biology, *Ceratina hieroglyphica*, nest, parasitism, pollen

INTRODUCTION

Pollination plays a vital role in flowering plants. Insects are the prime pollinating agents, among which, bees (*Apis* and non-*Apis* species) are considered important for the pollination of a wide range of crops. Except for bees of genus *Apis*, all bees are known as non-*Apis* bees, wild bees or pollen bees (Aslam, Rafi, & Zia, 2017). Among the non-*Apis* bees, only a handful as *Bombus terrestris*, *Megachile rotundata*, *Nomia melanderi*, *Osmia* sp. and some stingless bee species (Westerkamp & Gottsberger, 2000; Hogendoorn et al., 2006; Hoehn et al., 2008) have been used extensively in agriculture, even though non-*Apis* bees have been shown to be as effective pollinators, if not better than, honey bees on certain crop plants (Greenleaf & Kremen, 2006).

Wild bees belonging to the genus *Ceratina latreille* (Apidae: Ceratinini) consist of twenty-three subgenera and are found on all continents (Terzo, 2000). These bees referred as small carpenter bees can be easily separated from Halictids by the mouthparts with a long glossa and the hindwings with a tiny jugal lobe (Grissell, 2017). They visit the flowers of cultivated plants and several weeds (Van der Vecht, 1952) and make nests in dead wood, stems or pith (Dutt, 1912; *Warrit, Michener, & Lekprayoon,* 2012; Anonymous, 2018). Many species of genus *Ceratina* are solitary, but few are subsocial or rarely eusocial (*Warrit, Michener, & Lekprayoon,* 2012; Anonymous, 2018). Although genus *Ceratina* is taxonomically diverse in the tropics, the biology of Neotropical species is still poorly known (Gonzalez, Moreno, & Richards, 2004).

The cashew tree (*Anacardium occidentale* L.) is a cross pollinated crop with sticky pollen and requires insects for pollination. It is grown in several countries in the world as an important commercial nut crop. Though multiple insect species visit cashew flowers, bees are important for pollination (Sundararaju, 2000). Recent surveys in cashew plantations showed that a wild bee, *Ceratina hieroglyphica* Smith (Apidae: Hymenoptera) serves as one of the common pollinators of cashew in the Puttur region

of Karnataka, India (Vanitha & Raviprasad, 2016) and is found in India, China, Malaysia Myanmar (Sharma, Kumar, & Gupta, 2016), Sri Lanka (Wijesekara, 2001), Hongkong and the Philippine Islands (Van der Vecht, 1952) pollinating several cultivated and wild flowers. Vanitha & Raviprasad (2018) documented in their study that *C. hieroglyphica* started foraging on cashew flowers depending on the sun shine, and peak activity was noticed between 11.00 and 13.00 hrs. The time spent per flower was 2-6 seconds, the foraging rate was 3-5 flowers/trip and the mean pollen load per bee was 74-334 pollen grains. Besides *C. hieroglyphica*, *Braunsapis* spp., *Apis* spp., *Seledonia* sp., *Lasioglossum* sp., *Pseudapis* sp. and *Tetragonula* sp. also visit cashew flowers for pollen and nectar (Vanitha & Raviprasad, 2016). Even though *C. hieroglyphica* alone contributes to 11.4% of total bee species visiting cashew trees in Puttur, India (Vanitha & Raviprasad, 2018), many aspects of this bee species are not known. Hence, attempts have been made to generate information about nesting sites, ecological environment and natural enemies of these bees for their conservation and management. The results of nest biology may help in devising conservation measures of this bee species and give insight on artificial nesting sites for these bees so as to assist in the cashew pollination.

MATERIAL AND METHODS

Nesting behavior and development of *C. hieroglyphica* were observed periodically during 2017 in 120 ha of cashew plantations of ICAR - Directorate of Cashew Research, Puttur, Karnataka, India. The region is a hilly track between the west coast and the Western Ghats of India, located at 12.77°N and 75.22°E at an average elevation of 87 m. The vegetation cover of the study site was dominated by cashew trees which were grown as a mono crop. The temperature in the area varied from 16.0 to 39.0°C. The region had an annual mean rainfall of 3970 mm but received heavy rain during the southwest monsoon between June and September. The relative humidity varied from 43 to 98% and generally above 90% from June to November.

Nests of *C. hieroglyphica* can be easily identified by the presence of a clear circular entrance hole of 2.5-3.5 mm diameter at the tip of dried cashew twigs with an exposed pith region. Such nests were cut beyond 15-20 cm from the tips and care was taken not to cut into the brood. During observations, a total of 102 nests were randomly collected (nest count varied with location, season) at fortnightly intervals between 9:00 and 10:00 except during the heavy rain period from mid June to mid July. The adults that escaped out of the nests during collection were counted and noted separately. The individual nests were split open starting at the entrance and parallel to the stick length carefully little by little using a secateur without disturbing the cell contents and the life stages present inside were observed carefully and recorded. The nests that had the same entrance diameter with such signs of occupation as cavity size, cavity wall colour and fine wood particles, but without any life stages were considered as abandoned nests. Individuals in the occupied nests were counted and observed for their developmental stages besides the presence of natural enemies. The sticks on which nests were made were measured using a standard digital veneer Caliper (Mitutoyo), while, eggs and pollen provision were measured using VIMAGE 2016 software installed in a Nikon stereo zoom microscope (SMZ 745T model) attached with 5 MP microscope camera (UC501S model).

From the nests collected from cashew plantations, eggs freshly laid on pollen provisions without any partitions near the entrance were observed for their sequential developmental duration till adulthood. The freshly laid eggs were whitish and translucent in nature. Portions of split opened nests with undisturbed fresh eggs were placed in glass 10 x 2 cm Petridishes® and covered. Fourteen such eggs were observed at ambient laboratory temperature and humidity conditions. The maximum and minimum temperatures were 31.7±1.5°C and 25.4±2.1°C respectively, and morning and evening relative humidity were 75±3 and 58±4% respectively. Adult bees in split-opened nests were kept in

glass 10 x 2 cm Petridishes® along with two or three tiny rearranged dry sticks of abandoned nests. A small cotton swab soaked in 10% honey solution was placed in the Petridishes® and the longevity was recorded.

The presence or absence and position (head or abdomen showing through the nest entrance) of *C. hieroglyphica* females at the nest entrance during the survey were observed in the field to record if there were any bee guarding behaviour. The field collected nests were dissected carefully to record any natural enemies, especially predators and pathogenic infection. Further, the nests were closely observed for any colour change in the larvae and pupae or disturbances in nest architecture which indicated parasitism. Such nests were kept separately in 10 x 2 cm Petridishes® till the emergence of parasitoids. These specimens were preserved in 70% ethanol and later identified at the ICAR-National Bureau of Agricultural Insect Resources, Bengaluru, India. Descriptive statistics was used to analyze all the data with the software SPSS 16 and the values were expressed as mean ± SD.

RESULTS

A *C. hieroglyhica* nest was only a narrow simple burrow in the pithy region of a dried cashew twig (Tab. 1 and Fig. 1a and 1b), but was rarely found on either fresh stems or a burrow already made by wood boring insect. The burrow walls were smooth but no particular lining was observed. Most of the nests had an entrance diameter of 3.2±0.22 mm. Some collections and observations showed that *C. hieroglyphica* bees also sometimes used preexisting burrows made by ants and other small insects, but the facultative cavity nesting nature of this species requires further observations.

Nest entrance narrowing made of saw dust was only rarely observed when bees occupied preexisting burrows with more than 4 mm diameter. Most *C. hieroglyphica* nests were found in dried thin twigs of cashew of 5.0 – 20.0 mm thickness. Nests were commonly seen on the twigs with exposed pith especially on the pruned cut ends. A number of nests was noticed more in the

Table 1.

Nest architecture of *Ceratina hieroglyphica*

Number of nests collected for observations (N = 102)		
Occupied nests (Nos)	80	
Abandoned nests (Nos)	22	
Particulars	(Mean±SD)	Range
Entrance diameter (mm)	3.2±0.22	3.0-3.5
Cashew stick thickness (mm)	10.6±4.68	5.0-20.0
Nest thickness (mm) (inner space)	3.2±0.43	2.8-4.0
Occupied nest space (Nest length) (cm)	6.4±2.54	2.5-10.8
Vestibular cell length (cm)	8.8±3.62	4.0-16.0
Nest cavity depth (cm)	11.5±4.96	6.0-21.0
Individual cell length (mm)	7.1±0.37	7.0-8.0
Partition thickness (mm)	1.7±0.48	1.0-2.5
No. of cells/ nest	6.2±3.64	1.0-14.0
No. of individuals/ nest	5.9±3.27	1.0-14.0
No. of adults/ nest during collection	2.5±1.42	1.0-6.0

Fig. 1. a. *C. hieroglyphica* adult at nest entrance, b. nest of *C. hieroglyphica* in a dried cashew stick – spilt opened.

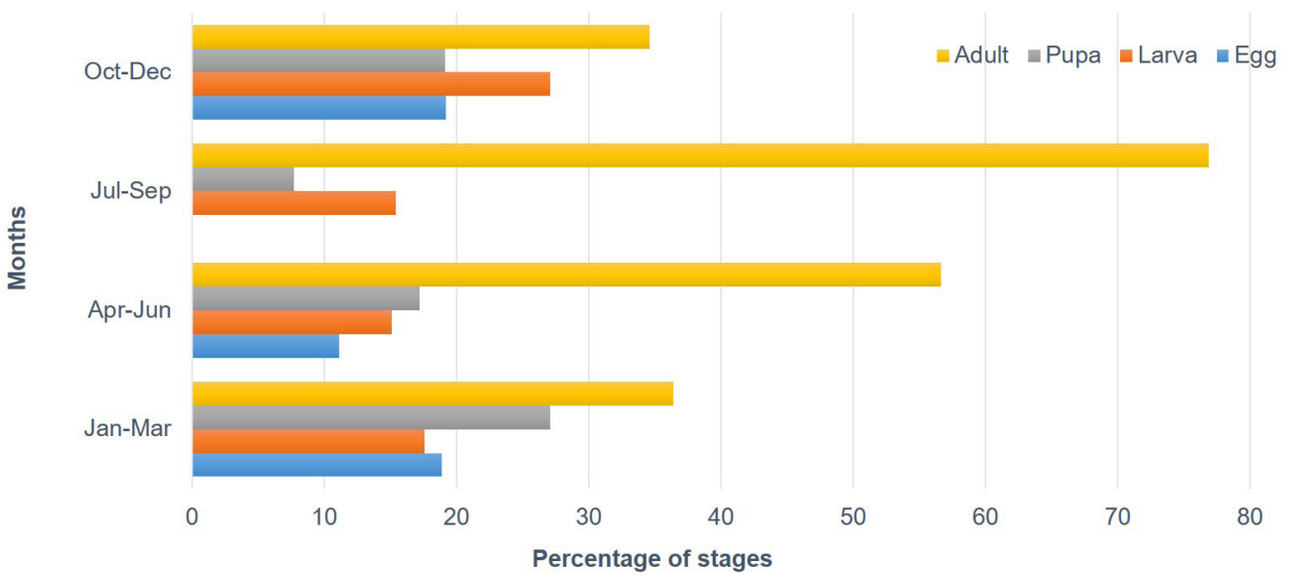

Fig. 2. Frequency of *C. hieroglyphica* stages in nests collected in one year period

pruned cashew trees compared to unpruned ones in a particular location. Out of 102 nests collected, twenty-two were abandoned.

The nests had typical compartments known as cells of 7.1±0.48 mm length, arranged linearly along the stick, and separated by cylindrical partitions made of fine wood particles of 1.0 -2.5 mm thickness (Tab. 1). Cell length was almost equal to the length of adult bees, and the cells were of equal size except for a very few. They were continuous without any empty spaces. The nests invariably had only one entrance with older ones present at the inner end and the younger ones near the entrance. They were 6.0-21.0 cm deep.

When a desired depth was reached, adult female bees placed pollen provision which is a mixture of pollen grains and nectar collected upon frequent visits to flowers. *C. hieroglyphica* is polylectic, and the pollen provisions were semisolid to solid and coloured creamy, yellow or orange depending on the bee flora available (e.g. cashew, *Lantana camara*, *Ixora* sp., *Vedalia trilobata*), and cylindrically shaped arranged with its main axis along the tunnel.

Eggs were laid on the flat side of pollen provisions commonly at the posterior upper end, each with its main axis parallel to the main axis of the pollen provision (Fig. 3b). The shape of eggs was a slightly curved cylinder with convexed ends. On average, they were 3.08 mm in length and 1.17 mm in width, whitish, trans-

Table 2.

Duration of developmental stages of *C. hieroglyphica* in laboratory condition (N= 14)

Life stage of the bee	Mean duration (±SD) days	Range (days)
Egg	3.1±0.29	3-4
Larva	8.4±0.63	8-10
Pre defecating larva	4.4±0.39	4-5
Post defecating larva	4.0±0.24	4-5
Pupa	7.3±1.41	7-9
Pupa with creamy eyes	2.0±0.0	2
Pupa with brown eyes	1.5±0.51	1-2
Pupa with black eyes	1.4±0.51	1-2
Black pupa	2.4±0.39	2-3
Total developmental period	18.8±1.34	18-22
Adult (with 10 % honey solution)	9.8±2.25	6-14
Total life cycle (in lab)	28.6±2.40	26-34

lucent with smooth and shiny chorion with a shape index of 37.98. The pollen provision in the cell was packed in a section of 5.5 to 6.5 mm (6 mm mean) along the stick axis.

The oldest progeny at the base of the nest developed first and stayed in the cell for 2-3 days and moved out. A maximum of six adults were seen in a nest. The frequency of *C. hieroglyphica* stages in the nests collected indicated that adults were more compared to its immature stages followed by pupal stages (Fig. 2). Among the different periods observed,

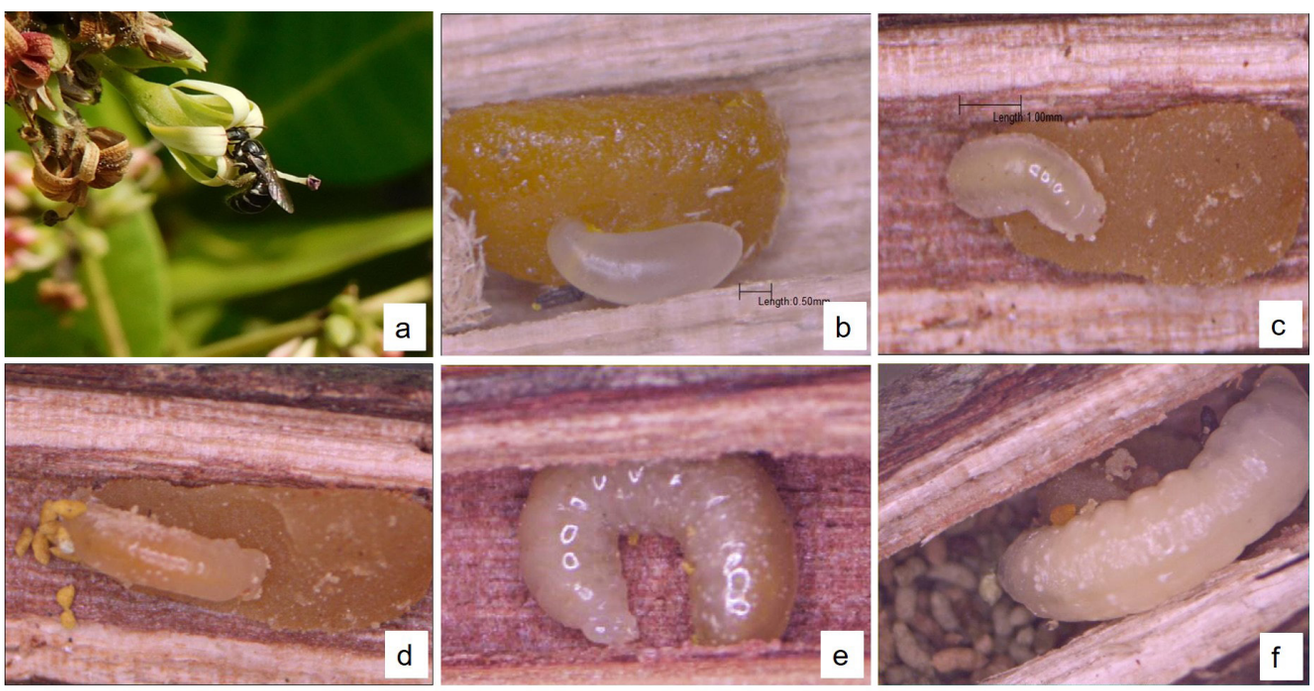

Fig. 3. *C. hieroglyphica* a. adult bee foraging a cashew flower, b. Egg, c. 1st day larva, d. 2nd day larva, e. pre-defecating larva, f. post defecating larva.

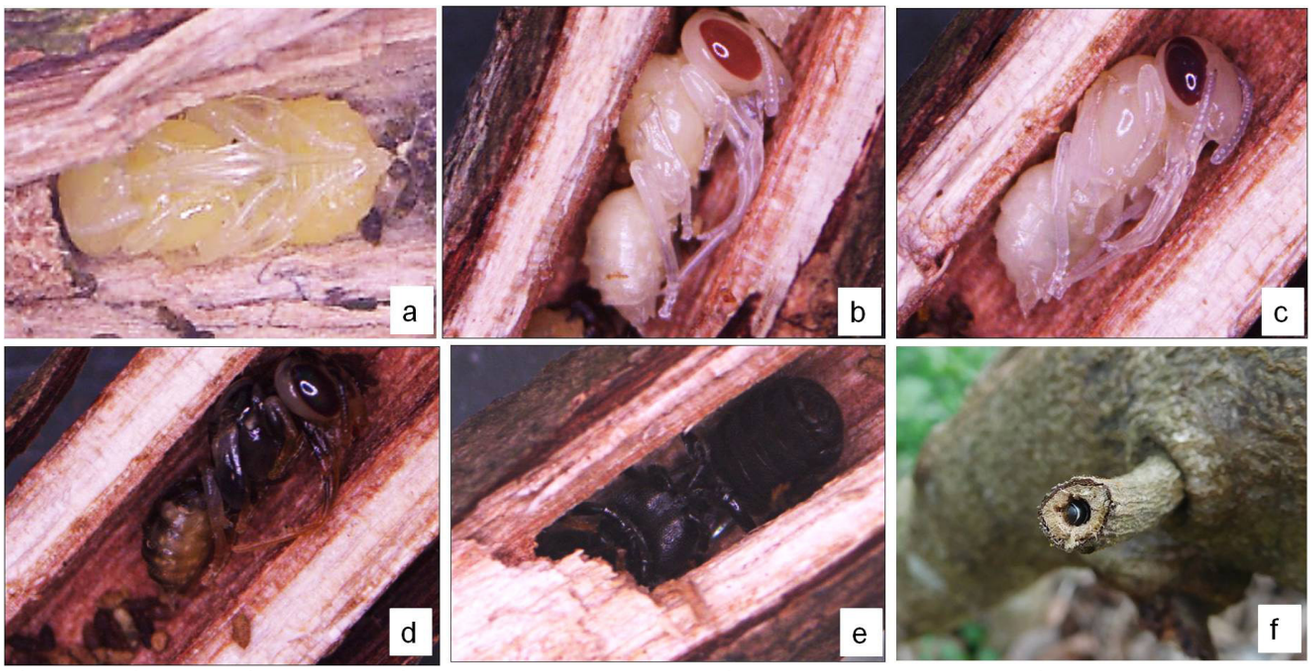

Fig. 4. *C. hieroglyphica* a. Pupa with creamy eyes, b. pupa with brown eyes, c. pupa with black eyes, d. black pupa, e. fresh adult- fully developed, f. nest guarding by adult bee at entrance.

there were more egg stages from October to December and January to March which coincided with the cashew flowering season, while during the rainy season between July and September the majority were adults. However, the number of generations of this bee species in a year is unknown.

The life cycle parameters of *C. hieroglyphica* are presented in Tab. 2. The egg stage lasted for three to four days. The first instar larvae were translucent, rested on top of nearly intact food masses and ate the pollen provision. The non-active larval stage after finishing feeding without any feces was recognized as pre-defecating larvae. Post-defecating larvae were identified by feces evident inside the cells before pupation (Fig. 3e).

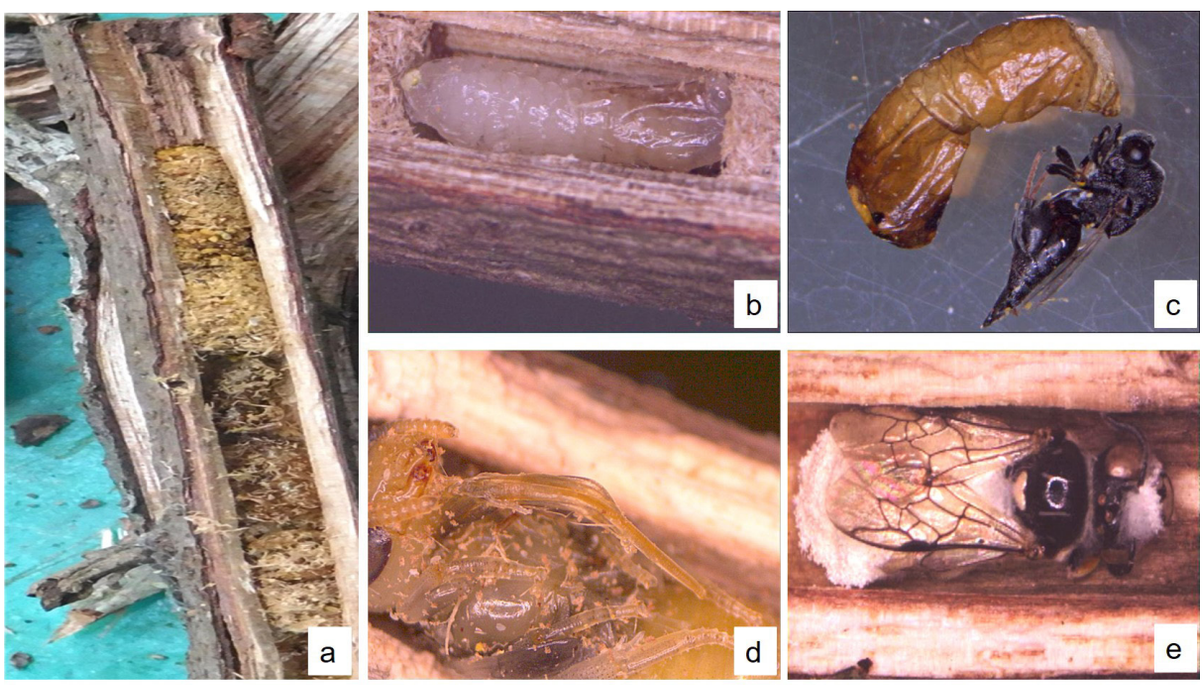

Fig. 5. *C. hieroglyphica* a. parasitized nest showing dried pupae without any partitions between cells at base, b. parasitized larva, c. *Neochalcis* sp. emerged from *C. hieroglyphica* pupa, d. mites on pupa, e. mummified adult bee.

The larval stage lasted for eight to ten days. Three consecutive types of pupae were easily identified on the basis of eye colour (Fig. 4a, 4b and 4c). Pupal stage was completed in seven to nine days. The newly emerged adults were recognized by their whitish undeveloped wings and found inside their breeding cells, and the fully developed adults moved to the entrance later. Adults survived up to fourteen days in lab conditions with 10% honey solution. The total life cycle lasted twenty six to thirty four days (Tab. 2). Out of fourteen eggs observed, thirteen (92.8 %) successfully developed into adults, and one egg dried for unknown reasons.

Excellent guarding behavior was observed in *C. hieroglyphica.* When female bees were present in the nest they could be seen at the entrance with their apical metasomal terga (Fig. 4f). When the nest was disturbed as by stem movement or touch, female bee blocked the nest entrance with the apical metasomal tergum adopting a guarding posture and pumping the abdomen. Most frequently when a female intruder approached either by flying or walking, a guarding resident female blocked the entrance will be touched and pushed by the intruder.

Natural enemies of *C. hieroglyphica* recorded in the present investigation include parasitoids, predators and pathogens. In cashew plantations, the larvae of *C. hieroglyphica* were parasitized by *Neochalcis* sp. (Hymenoptera: Chalcidoidea: Chalcididae) to an extent of 13.4 % during April. The parasitized brood was easily identified by the presence of disturbed partitions and discoloured larvae and pupae (Fig. 5a, 5b and 5c). Parasitized grubs were sometimes noticed in the nests where adult bees were also present. Parasitoids emerged out as adults after nearly twelve to twenty four days and survived for ten to nineteen days in lab conditions with 10 % honey solution. From April to January, mites were found (undetermined sp.) on larvae and pupae (Fig. 5d), but still those larvae and pupae developed into healthy adult bees, suggesting those mites might be phoretic in nature. Ants were very rarely noticed in the nests; however no bee stages were present in those nests. During the rainy season, adults were found to be infected with the entomopathogenic fungus, *Beauveria bassiana* (Bals.-Criv.) Vuill. (Fig. 5e), and a maximum of 16.0 per cent of mummified adults were noticed from August to October 2017 alone.

DISCUSSION

In the present investigation, nests of *C. hieroglyphica* were observed in dried thin cashew twigs. Earlier, nests of *C. viridissima* Friese had been recorded in hollow reeds, thatch and excavated tunnels in dried pithy tree branches and those of *C. chiangmaiensis* in broken or cut dead *Lantana* sp. stems. Observations of nesting behavior in the present study revealed that *C. hieroglyphica* exhibited solitary behavior as that observed in such bee species as *Manuelia postica* Spinola (Michener, 1969). The nests consisted of a tunnel with compartments known as cells arranged linearly along the stick, and separated by cylindrical partitions. Such a structure strongly suggests a lack of contact between the immature siblings, and between them and their mother, as reported for other bee species like *M. postica* but not all Xylocopine species (Sakagami & Michener, 1987; Maeta, Sakagami, & Michener, 1992). *C. hieroglyphica* nests invariably had only one entrance and were 6-21 cm deep with older bees present at the interior end and younger ones near the entrance; while *C. mexicana currani* nests also consisted of an unbranched tunnel up to 16 cm in length but without cell partitions (Gonzalez, Moreno, & Richards, 2004). Most of the *C. hieroglyphica* nests had an entrance of 3.0-3.5 mm in diameter, were found in dead thin cashew twigs of 5-20 mm thickness and were seen on sticks with pith exposed especially on pruned ends, while the of *Xylocopa nasalis* Westwood nests had an average length of 25.4±6.95 cm and a mean branch diameter of 17.9±6.00 mm (Hongjamrassilp & Warrit, 2014). The number of cells per *C. hieroglyphica* nest ranged between one and fourteen, and up to six adult bees was recorded. Whereas for *X. nasalis*, it was between one and eight cells per nest and up to seven adults were seen in a nest (Hongjamrassilp & Warrit, 2014). According to Dutt (1912), the mother *C. viridissima* bee always confines herself to her own nest. Furthermore,

the presence of two or three females inside a nest suggests either that a resident female allowed the entry of an intruder female, or that females of same nest remained together after hibernation (Stark et al., 1990; Hogendoorn & Velthuis, 1993).

The egg, larval and pupal periods of *C. hieroglyphica* lasted for three to four, eight to ten and seven to nine days, respectively. The *C. viridissima* bees took four to five weeks to develop, in which the egg, larval and pupal periods lasted three to four, ten to thirteen and eleven to eighteen, respectively (Dutt, 1912). No empty spaces were recorded between cells in *C. hieroglyphica*, but empty spaces were recorded in other such species as *C. australensis* (Perkins) (Michener, 1962) and *C. propinqua* Cameron (Yogi & Khan, 2014). The present study recorded that *C. hieroglyphica* bees rarely used other insects' preexisting burrows which requires further observations, while *C. dallatorreana* Friese and *Pithitis smaragdula* F. frequently did so (Daly, 1966). The cell walls of *C. hieroglyphica* had no particular lining, but according to Sakagami & Laroca (1971) those of *C. dallatorreana* were water-proof which could be due to an invisible lining of waxy secretion.

The total life period of *C. hieroglyphica* is not certain since the longevity of adults is not known in this study. The presence of food provisions continuously for six to seven months as well as the duration of immature stages, suggests more generations per year. Their nests were 6-21 cm deep with older ones present at the interior end and the younger ones near the entrance, while those of *C. calcarata* Robertson ranged from 20 to 30 cm deep (Rau, 1928) and those of *C. dallatorreana* ranged from 3-19 cm deep (Daly, 1966). According to Daly (1966), the absolute number of cells depends upon how deep the nest was excavated.

Rau (1928) had reported the emergence behaviour of *C. calcarata* and observed that the oldest chewed apart the cell cap above and packed it at the base of its own cell. In which, if the bee above was not mature it was carefully moved down to rest on the new "floor", but if mature, the eldest passed it by and worked on the cell cap above, passing the pithy material to the younger bee or bees beneath. These bees packed the material at the base of the nest, moving and adjusting any remaining pupae. Thus the mature bees at the base of the nest gained freedom by a process of displacement, gradually shifting the material behind them as they made their way to the top. The present study shows the behavior of *C. hieroglyphica* is possibly the same as the progeny at the base developed first and moved out through entrance, but empty cells of developed adults were not seen in any of the examined field collected nests and the brood was arranged continuously. Thus, adults would have begun to gnaw their way out before the others above them were ready, which may pose a special problem because the bees do not emerge laterally through the side of the stem, but vertically through all the other cells.

Two important advantages of guarding behaviour in social nesting bees are more extended foraging trips by a female while another one remains at the nest entrance and hence a higher accumulation of pollen loads and defence against conspecific or heterospecific invaders (Hogendorn & Velthuis, 1993). Parasitism by chalcid wasps had earlier been reported on *C. viridissima* by Dutt (1912). In the present investigation, 13.4 % parasitism by *Neochalcis* sp. and up to 16 % pathogenic infection by *B. bassiana* were observed in *C. hieroglyphica* from August to September, which could be due to rain from June to August that favours fungal infection. Similarly, *C. calcarata* nesting in sunny microclimates experienced less parasitism (Vickruck & Richards, 2012). The presence of parasitized grub stages even when there were adult *C. hieroglyphica* bees indicated that a guarding female could not prevent parasitism. Besides, attempts by conspecific individuals to enter the nests were observed, as reported in *M. postica* (Prado, Chiappa, & Niemeyer, 2008).

This paper describes the nesting biology and natural enemies of *C. hieroglyphica*. The main characteristics are cell construction and food provisioning strategies typical of solitary bees and no contact among immature siblings. The life cycle is short and the females guard their

nests. *Neochalcis* sp. parasitism and *B. bassiana* mycosis were recorded as natural enemies. These findings may help to understand and develop conservation measures for *C. hieroglyphica* and thereby increasing pollination of crops including the cashew trees.

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