



## A COMPARATIVE STUDY ON NEST ARCHITECTURE AND LIFECYCLE OF TWO SMALL CARPENTER BEES *CERATINA SMARAGDULA* (F.) AND *CERATINA HIEROGLYPHICA* SMITH

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### ABSTRACT

Small carpenter bees *Ceratina smaragdula* (F.) and *C. hieroglyphica* Smith (Xylocopinae: Apidae) are the major pollinators of many agricultural and horticultural crops. Nesting sites of these native bee pollinators were located at dried twigs of peacock flower tree *Caesalpinia pulcherrima*, and a total of 199 nests were collected from 2019-2021. Both species constructed linear nests at soft pithy region of stems with a maximum of 12 cm depth and individual cells ranged 6 to 10 mm in length which were separated with partitions of 2 to 4 mm. There were no significant differences in height of the nests constructed from ground level. The younger cells were near to the entrance, whereas the mature cells were arranged towards the innermost side. The nests of bees consisted of egg, larva, pupa and adult stages; and *C. smaragdula* took  $15.51 \pm 0.19$  days while *C. hieroglyphica* took  $15.93 \pm 0.27$  days for completion of larval period. Total pupal period of *C. smaragdula* ranged from  $20.71 \pm 0.26$  days whereas *C. hieroglyphica* ranged from  $18.56 \pm 0.16$  days. Total lifecycle for *C. smaragdula* and *C. hieroglyphica* took  $49.15 \pm 0.40$  and  $43.19 \pm 0.58$  days under laboratory conditions.

**Key words:** *Ceratina smaragdula*, *C. hieroglyphica*, *Caesalpinia pulcherrima*, bee pollinator, nest architecture, lifecycle, adult longevity, artificial nesting sites, active and full brood nest, polylectic bees, bee pollen

Bees (Hymenoptera: Apidae) are considered as the quintessential pollinators of terrestrial ecosystems (Ollerton et al., 2011). These provide key ecosystem services through pollinating wild flowers as well as numerous agricultural crops (Yogi and Khan, 2014). Except bees of genus *Apis*, all bees are known as non-*Apis* bees, wild bees or pollen bees (Aslam et al., 2017). Many species of bumble bees (*Bombus* spp.) and solitary bees (*Amegilla*, *Andrena*, *Ceratina*, *Halictus*, *Lasioglossum*, *Megachile*, *Nomia*, *Osmia* and *Xylocopa*) can be reared on large scale and managed for crop pollination (Abrol, 2012). Among these, the small carpenter bees (*Ceratina* Latreille) are widely distributed throughout the tropical and subtropical regions of the world (Michener, 1962).

The *Ceratina* bees are polylectic which are reported to be an excellent pollinator of wide range of crops viz., niger, safflower, linseed, mustard (Navatha and Sreedevi, 2015), alfalfa, winged bean, tomato, red gram, sunflower, raspberry, cranberry, apple (Mattu and Kumar, 2016), ridge gourd, brinjal, rape seed, carrot, marigold, safflower and yellow cosmos (Batra, 1967). *Ceratina binghami* construct their nests in dried tiny twigs and pruned pithy stems by making linear burrows

in peacock flower tree *Caesalpinia pulcherrima* (L.) (Fabaceae) plants (Udayakumar and Shivalingaswamy, 2019); and *C. hieroglyphica* was found constructing their nests in pithy region of dried twigs of cashew tree *Anacardium occidentale* (L.) (Anacardiaceae) (Kaliaperumal, 2019). The females of *Ceratina* chew the central pith of selected twig and flies out to forage pollen and nectar. They mould the collected pollen into pollen masses to oviposit on them and close the cell by septum (McIntosh, 1996). Mothers inspect the brood cells constructed by them and mostly found in the gallery between the entrance and the first brood cell often in a defensive position blocking the nest entrance to protect the broods from natural enemies (Rehan and Richards, 2010).

Bees are threatened due to destruction and fragmentation of their nesting habitats. One of the primary threats is the spread of urban settings as well as increased mechanization, all of which diminish nesting habitats such as the walls of mud houses, dried plant twigs and debris which are used by stem and cavity nesting bees (Shebl et al., 2018). Nesting biology and lifecycle of bees provides information about nesting sites and ecological requirements of bees that will help

to design the artificial nests for managed pollination of crops and appropriate tools to protect and maintain plant diversity and thereby improving agricultural productivity. A comparative study was undertaken to study the nesting biology and lifecycle of two small carpenter bees, *C. smaragdula* and *C. hieroglyphica* on *Caesalpinia pulcherrima* of which the results are explained herein.

#### MATERIALS AND METHODS

The study on nesting behavior and lifecycle of *C. smaragdula* and *C. hieroglyphica* was carried out in the University Campus of College of Agriculture, Vellanikkara as well as in the areas under Kerala Agricultural University (KAU), Thrissur, Kerala (10.54556N, 76.27323E) during October 2019 to January 2021. Regular surveys were conducted to locate the nests of *Ceratina* bees in the University Campus area. The nesting substrates having soft pithy or hollow stems viz., *Caesalpinia pulcherrima*, *Tecoma* sp., *Rosa* spp., *Peltophorum pterocarpum* and *Lantana* sp. were thoroughly monitored. A total of 199 nests were collected randomly from *C. pulcherrima*, with remnants of previously constructed cells within them. All the nests were collected during the evening hours so as to ensure the presence of adult bees inside. Nests were cut beyond 10-30 cm away from the tip of the twigs, so that no broods are harmed and nest entrance were covered with small cotton plugs to prevent the escape of adult bees from nests.

Individual nests were dissected carefully with a sharp blade to give gentle split lengthwise and classified into five categories according to Daly's (1966) classification (Rehan and Richards, 2010) viz., hibernacula nests, founding nests, active brood nests, full brood nests and mature brood nests according to the life stages of bees and conditions of nests constructed by the bees. Hibernacula nests are those with remnants of previously built nest cells with adult bees in them. Founding nests are with adult bees which are actively working for construction of new cells. Active brood nests always contain pollen masses in each constructed cells with freshly laid eggs or immature stages whereas full brood nests are those which contain various immature stages of bees with different proportion of pollen masses. Mature brood nests include the nests inhabited by adult bee interacting with their callow offsprings (Rehan and Richards, 2013).

The nest architecture of both the species of *Ceratina* including entrance diameter, thickness of nesting stem,

occupied nest length, individual brood cell length, cell septum thickness, number of cells/ nest, number of immature stages/ nest, weight of pollen provision/ brood cell and number of adults in nest during collection were recorded. The immature stages of bees collected from the nests were reared at laboratory ( $28 \pm 2^\circ\text{C}$ ,  $75 \pm 1\%$  RH), where the split stems were tied properly with rubber bands and kept in rearing boxes with proper aeration. The stems were opened on a daily basis to observe developmental duration of different life stages (Udayakumar and Shivalingaswamy, 2019). A cotton swab soaked in 10% honey solution was kept in rearing boxes and the adult longevity was also recorded (Kaliaperumal, 2019). Descriptive statistics and two sample t-test was used to analyze the data with the software SPSS 21.

#### RESULTS AND DISCUSSION

**Nest architecture:** The small carpenter bees viz., *C. smaragdula* (Fig. 1a) and *C. hieroglyphica* (Fig. 1b) were found to nest in soft pithy and dry stems of *C. pulcherrima* trees linearly. A total of 199 nests were collected from *C. pulcherrima* trees which were planted at a distance of 2 m. Out of 199 nests collected, 128 were inhabited by *C. smaragdula* and 71 nests by *C. hieroglyphica*. According to the classification of nests given by Daly (1966), nests were classified and counted separately, where *C. smaragdula* nests comprised of 19 hibernacula, 28 founding nests, 21 active brood nests, 15 full brood nests and 45 mature brood nests; *C. hieroglyphica* comprised of 8 hibernacula, 4 founding nests, 17 active brood nests, 11 full brood nests and 31 mature brood nests. The active and full brood nests of both the bee species were used to study the nest architecture (n=25).

The small carpenter bees *C. hieroglyphica* and *C. smaragdula* were found to construct linear nests in pruned dry pithy stems of *C. pulcherrima*, but was rarely found on freshly cut ends of plants. They were also observed constructing nests in various host plants viz., *Tecoma* sp., *Croton* sp. and *Rosa* spp. According to Udayakumar and Shivalingaswamy (2019) small carpenter bee *C. binghami*, also nests on *C. pulcherrima*, *Adhatoda zeylanica* and *Adenanthera pavonina*. Ali et al (2016) reported the nesting activity of *C. smaragdula* in wooden stalks of Ravenna grass (*Saccharaum ravennae*). The nests of both species had only one entrance and the entrance diameter did not differ among *C. smaragdula* and *C. hieroglyphica* (two sample t-test,  $t=0.848$ ,  $p>0.05$ ) (Table 1). These observations are in line with the study of Yogi and

Khan (2014), where they reported that the nest entrance diameters of *Ceratina propinqua* and *C. simillima* had little difference in their nest architecture. Most of the nests were found with adult bees guarding their nests either showing their head or abdomen to ward off natural enemies and thereby protecting their young ones and these observations corroborate with those of Kaliaperumal (2019).

Preferences of bees towards twig thickness varied significantly ( $t=3.365$ ,  $p<0.05$ ) whereas, inner nest diameter showed only slight significant difference ( $t=-1.357$ ,  $p>0.05$ ). Cells constructed inside were separated with pith of stem with a septum thickness of  $3.1\pm 0.10$  and  $2.70\pm 0.08$  in *C. smaragdula* and *C. hieroglyphica*, respectively. Kaliaperumal (2019) reported that the cell septum thickness of *C. hieroglyphica* ranged from  $1.7\pm 0.48$  mm. Cells constructed in individual nests were equal to the length of adult bees and were arranged continuously with one after another without any empty space between them. Individual cell length of both the species ranged 6 to 10 mm with slight significant difference in length ( $t=-5.139$ ,  $p<0.05$ ), and these observations corroborate with those of Kaliaperumal (2019) who reported that *C. hieroglyphica* constructed their cells in cashew tree twigs with a length ranged from 7 to 8 mm. Both the species showed little significant difference in their nesting attributes viz., occupied cell length ( $t=2.651$ ,  $p>0.05$ ), cell septum thickness ( $t=3.024$ ,  $p>0.05$ ), number of cells/ nest ( $t=1.568$ ,  $p>0.05$ ) and number of immature stages/ nest ( $t=-1.672$ ,  $p>0.05$ ). Most of the nests collected were found with one or two adult bees guarding their nests. Similarly, Batra (1976) reported the presence of old mother bee guarding their nests by buzzing loudly and blocking their nest entrance with the dorsum of their abdomen. Both the species constructed their nests at varied heights (*C. smaragdula*;  $61.55\pm 5.34$  and *C. hieroglyphica*;  $63.42\pm 6.74$  with no significant difference in their preference towards selection of nesting site from ground ( $t=-0.218$ ,  $p>0.05$ ) (Table 1). These observations agree with those of Yogi and Khan (2014), who reported that there was no significant difference in height of nests from ground level for *C. propinqua* and *C. simillima*.

**Lifecycle:** The females of *C. smaragdula* as well as *C. hieroglyphica* bees placed their pollen provisions which is a mixture of pollen grains and nectar in individual cells constructed in their nest. The pollen provisions are yellow to orange (Fig. 1c) which weighs  $14.80\pm 0.35$  and  $14.45\pm 0.33$  (Mean $\pm$  SE in mg;  $n=15$ )

in *C. smaragdula* and *C. hieroglyphica*, respectively. Similar observations were reported by Ali et al. (2016) that the pollen provisions were brownish, viscous, rounded and soft with a length ranging from 0.5 to 0.6 cm. In the present study, length of pollen provisions measured  $5136.56\pm 30.61$  and  $5068.74\pm 25.81$  (Mean $\pm$  SE in  $\mu\text{m}$ ;  $n=15$ ) with a width of  $3069.45\pm 13.26$  and  $3089.93\pm 18.61$  (Mean $\pm$  SE in  $\mu\text{m}$ ;  $n=15$ ) in *C. smaragdula* and *C. hieroglyphica*, respectively.

The eggs are laid dorsally on pollen provision to ensure immediate availability of food for the larvae. Eggs are translucent white (Fig. 1d) with cylindrical shape and convex ends. Eggs hatched in 3 to 5 days in both the species of bees with no significant difference (Two sample t-test;  $t=2.861$ ;  $p>0.05$ ) (Table 1). These results corroborate with those of Latha et al. (2020) who reported that *C. binghami* laid spindle shaped eggs on pollen balls which took four days for hatching into first instar larva. The first instar apodous larvae (Fig. 1e) are translucent white which actively fed on pollen provisions. Size of pollen mass varied in each cell of an active brood nest, where pollen masses were larger with early instars of larvae and vice-versa in cells with mature larvae. The first instar larvae are named as one by third size of pollen mass, which showed slight significant difference in their developmental days in *C. smaragdula* and *C. hieroglyphica* ( $t=0.690$ ,  $p<0.05$ ). The larva with two by third size of pollen mass (Fig. 1f) in both the species of bees showed significant difference in their development period ( $t=0.695$ ,  $p<0.05$ ), whereas larva with twice the size of pollen mass (Fig. 1g) did not show significant difference ( $t=0.402$ ,  $p>0.05$ ).

Udayakumar and Shivalingaswamy (2019) reported that *C. binghami* took a total larval period of  $13.67\pm 1.63$  days, as observed now, with that of *C. smaragdula* that took  $15.51\pm 0.19$  days and *C. hieroglyphica* took  $15.93\pm 0.27$  days. Pre-defecating larva (Fig. 1h) showed no significant difference in development time ( $t=-0.338$ ,  $p>0.05$ ). Post defecating larva found in their cells with feces and were metamorphosed into white pupa. Pupa appeared with difference in eye colour viz., white, pale pink, pink, pale brown, brown and black (Fig. 1i-l) in accordance with the development period. Pupa with black eye showed difference in body pigmentation and these observations corroborate with those of Kaliaperumal (2019), who reported three consecutive type of pupae based on eye colour in *C. hieroglyphica* viz., creamy, brown and black. Both the species of bees did not show any significant difference in their pupal development period up to pink eyed stage. Pale brown

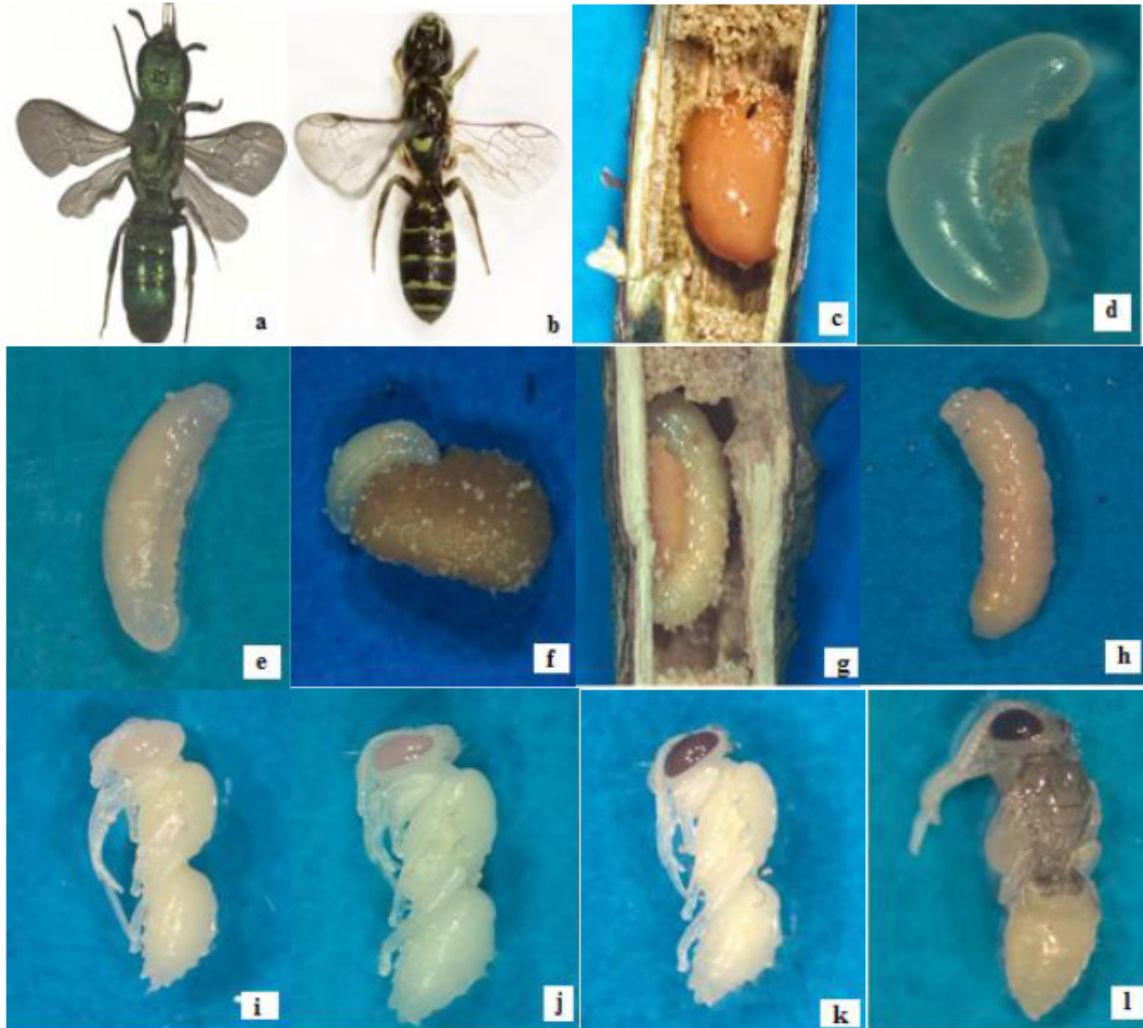


Fig. 1a-l. Carpenter bees; nest architecture and life stages; a. *C. smaragdula* adult; b. *C. hieroglyphica* adult; c. *C. smaragdula* nest with pollen provision; d. freshly laid egg by *C. hieroglyphica*; e. first instar larva of *C. smaragdula*; f. *C. hieroglyphica* larva with two third size of pollen ball; g. *C. hieroglyphica* larva with twice the size of pollen ball; h. pre-defecating larva of *C. smaragdula*; i. white eyed pupa of *C. smaragdula*; j. pink eyed pupa of *C. smaragdula*; k. brown eyed pupa of *C. smaragdula*; l. black eyed pupa of *C. hieroglyphica* with half body pigmentation.

eyed pupa of both species showed significant difference in their developmental days ( $t=4.081$ ,  $p<0.05$ ). Pupa with black eye showed significant difference in developmental period ( $t=1.779$ ,  $p<0.05$ ) which later attained varied body pigmentation. Pupa with three by fourth body ( $t=5.791$ ,  $p<0.05$ ) and with full body pigmentation ( $t=2.857$ ,  $p<0.05$ ) showed significant difference in developmental period (Table 1). Total pupal period of *C. smaragdula* ranged from  $20.71 \pm 0.26$  days whereas *C. hieroglyphica* ranged from  $18.56 \pm 0.16$  days. *C. smaragdula* showed an adult longevity of  $8.55 \pm 0.36$  days, whereas *C. hieroglyphica* showed  $4.82 \pm 0.31$  days which were not significantly different ( $t=7.706$ ,  $p>0.05$ ).

Total developmental period of both the bee species is not certain as observed now, and the adult longevity period may vary based on climate, host plants and various other factors. Ali et al. (2016) reported that *C. smaragdula* completed development within 28 to 32 days in Ravenna grass under laboratory conditions. In present study, *C. smaragdula* completed it in 45-54 days, and *C. hieroglyphica* within 43-53 days. Newly emerged adult bees were observed passing from their respective cell to uppermost cells so as to find their way out without disturbing other immature stages. Such a behavior is common for both the bee species, and these corroborate with the reports of Rau (1928) on *C. calcarata*, in which he stated that the oldest progeny

Table 1. Nest architecture and lifecycle of *Ceratina* spp.

Nest architecture (Mean± SE, n=25)		
Particulars	<i>C. smaragdula</i>	<i>C. hieroglyphica</i>
Entrance diameter (mm)	2.92± 0.07	3.00± 0.05
Twig thickness (mm)	8.08± 0.22	9.44± 0.33
Nest thickness (mm)	3.14± 0.06	3.33± 0.09
Occupied nest length (cm)	7.03± 0.47	8.82± 0.48
Cell septum thickness (mm)	3.1± 0.10	2.70± 0.08
Individual cell length (mm)	6.08± 0.53	4.32± 0.76
No. of cells/nest	4.92± 0.25	5.56± 0.31
No. of immatures/nest	4.68± 0.26	5.36± 0.31
No. of adult/nest	1.00± 0.05	0.80± 0.08
Height of nest from ground level (cm)	61.55± 5.34	63.42± 6.74
Lifecycle (Mean± SE, n=30)		
Life stage description	<i>C. smaragdula</i>	<i>C. hieroglyphica</i>
Egg	4.36± 0.12	3.87± 0.11
Larva		
One third of PB*	2.82± 0.09	2.91± 0.07
Two third of PB	2.74± 0.08	2.62± 0.14
Twice the size of PB	2.88± 0.08	2.83± 0.08
Pre-defecating larva	3.34± 0.06	3.38± 0.09
Post- defecating larva	3.71± 0.10	4.17± 0.15
Total larval period	15.51± 0.19	15.93± 0.27
Pupa		
White eyed pupa	2.93± 0.09	2.82± 0.10
Pale pink eyed pupa	1.32± 0.10	1.37± 0.07
Pink eyed pupa	1.09± 0.03	1.12± 0.04
Pale brown eyed pupa	1.61± 0.10	1.16± 0.04
Brown eyed pupa	1.36± 0.09	1.71± 0.08
Black eyed pupa	2.81± 0.09	3.02± 0.07
½ body pigmented pupa	3.01± 0.06	2.17± 0.04
¾ body pigmented	2.53± 0.14	1.53± 0.09
Full body pigmented	4.01± 0.08	3.62± 0.10
Total pupal period	20.71± 0.26	18.56± 0.16
Adult		
Adult longevity	8.55± 0.36	4.82± 0.31
Total lifecycle	49.15± 0.40	43.18± 0.58

\*Pollen ball

at the base of the nests mature and begin to gnaw their way out before the others above them are ready. As these bees do not emerge laterally through side of the stem but vertically through all the other cells of the nests, they move through chewing apart the above cell septum. If the bee next to their cell is immature (Kapil, 1969) those were carefully moved down to the cell and new cap was made. If the bee next to the cell is mature, then elder bee passed it by and gnaw the cell septum of younger bees and the displacement process carried on till reaching up to the outermost cell.

The peacock flower tree *Caesalpinia pulcherrima* is found to be the most preferred nesting site of *C. smaragdula* and *C. hieroglyphica*. The dried pithy stems

of *C. pulcherrima* can be used to trap these polylectic bees which not only help in conservation of these solitary pollen bees but also aid in better pollination services. These trees can also be planted as hedges in fields so that maximum utilization of pollination services can be obtained and better farm scaping is achieved. Thus, we need more landscape management practices to boost native pollinator densities by increasing habitat-carrying capacity.

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