Nesting biology of allodapine bee *Braunsapis picitarsis* (Cameron) from South India

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The allodapine bee, *Braunsapis picitarsis* (Cameron) (Allodapini: Xylocopinae: Apidae) is polylectic and found to be an important pollinator for many agricultural and horticultural crops. As *B. picitarsis* was found to be cryptic to other species, DNA barcoding was done to confirm the species identity. The nesting sites were located in dried twigs of peacock flower tree, *Caesalpinia pulcherrima* (L.) and a total of 83 nests were collected from 2019 to 2021. The bees preferred nesting sites with an entrance diameter of 2.83 ± 0.06 mm and a nest length of 5.38 ± 0.30 cm. The total development period was 56.85 ± 0.84 days in the laboratory. Pupa exhibited a difference in eye colour and body pigmentation during development.

Keywords: Agricultural and horticultural crops, *Braunsapis picitarsis*, DNA barcoding, life cycle, nesting architecture.

POLLINATION is a key process to bioresource mobilization in order to fulfil the needs of a rising human population[1–4], which aids in increasing the productivity of agro-ecosystems and other natural ecosystems[5–7]. The importance of pollinators to global agricultural stability is well documented[8,9]. Up to 75% of the crops used as human food require insect pollination[8]. An estimated 35% of crop production, including many of our most nutritious foods, benefits from insect pollination worldwide[10]. Among insects, bees pollinate more than 66% of the world's 1500 crop species[11]. Bees are the most specialized insects with efficient morphological adaptations to collect, manipulate, transport and store pollen efficiently[12,13]. Besides honey bees, many native bees play an important role as pollinators in the cropping ecosystem; however, they are undervalued[14]. Native bees have diverse requirements for their habitat and the type of substrate they use for constructing their nesting sites varies according to species[15]. Most bees build their nests in wood, soil, hollow pithy stems or tunnels left behind by other wood-boring insects[16].

Among native bees, *Braunsapis* spp. are found to be efficient and abundant pollinators of many horticultural crops in Kerala, India. *Braunsapis picitarsis* (Cameron) is a major pollinator of many cucurbit crops, viz. bitter gourd, oriental pickling melon, ash gourd, pumpkin and cucumber. *B. picitarsis* is a primitively eusocial stem-nesting bee[17–19], which rears its brood in an undivided communal tunnel without any cell partitions[20,21]. Most *Braunsapis* spp. have life cycles involving more than one egg with pollen provisions provided by the mother bees[22]. *Braunsapis* spp. construct their nests in dried sticks and pruned stems of cashew, mussanda, bamboo and drilled wooden blocks[21,23,24].

Destruction of natural nesting habitats of these native bees will have a direct negative impact on the valuable pollination provided by them. The decreasing trend in abundance and species richness of native bees in agricultural landscapes due to habitat loss and lack of foraging resources has been well reported[2,25,26]. Understanding the nesting behaviour, nest architecture and life cycle of the native bees will enable us to provide better pollination services to various ecosystems. Studies on nesting behaviour of Indian species of *B. picitarsis* are meagre. Hence the present study was undertaken to examine the nesting architecture and life cycle of the allodapine bee *B. picitarsis*, which may help design conservation strategies.

Materials and methods

Studies on nesting architecture and life cycle of *B. picitarsis* were carried out at the Kerala Agricultural University (KAU), Thrissur campus (10.54°N and 76.27°E; elevation 23 m amsl) between October 2019 and January 2021. The area receives a mean annual rainfall of 3198 mm. During the study period the temperature ranged from 20°C to 39°C and the average relative humidity (RH) varied from 47% to 89%. The maximum RH was recorded in July–October (70–90%) and the minimum during December–March (50–60%). Regular surveys were conducted to locate the nests of *Braunsapis* bees in areas where they were found to visit the flowers of many crops and weeds in and around the university campus, viz. bitter gourd, oriental pickling melon, pumpkin, ash gourd, snake gourd, touch-me-not, little tree plant, white buttercup, copper-pod tree, peacock flower tree, etc.

DNA barcoding of *Braunsapis picitarsis*

The adults of *B. picitarsis* are highly cryptic and closely resemble *Braunsapis mixta* Smith, which was previously reported from South India. Hence adult specimens of *B.*

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picitarsis were collected to confirm their identity at the molecular level. DNA extraction was carried out in the Molecular Laboratory of All India Network Project (AINP) on Agricultural Ornithology, KAU, Thrissur, using the whole body of an adult bee specimen ($N = 3$) with the DNeasy blood and tissue kit (Qiagen). The purity of the extracted DNA sample was tested at the Molecular Laboratory of AINP on Agricultural Ornithology using a spectrophotometer (model NanoDrop-1000, Thermo Scientific™) and the purity of DNA was recorded at 260 and 280 nm wavelengths. The DNA extracts were subjected to a polymerase chain reaction (PCR) following the standard protocol[27]. The universal barcode primers[28] specific to mitochondrial cytochrome oxidase I (mtCOI) were used[28], viz. forward primer (LCO 1490: 5′-GGTCAACAAATCATAAAGATATT0GG-3′) and reverse primer (HCO 2198: 5′-TAAACTTCAGGGTGACCAAAAAATCA-3′). The PCR reaction was carried out in 96-well plates with 20 µl reaction volume containing 2 µl template DNA (50 ng/µl), 10 µl PCR mastermix, 0.6 µl forward primer, 0.6 µl reverse primer and 6.8 µl sterile distilled water. Thermocycling consisted of an initial denaturation at 94°C for 2 min followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 52°C for 1 min and extension at 72°C at 45 sec. The final extension was carried out at 72°C for 10 min. The PCR reaction was performed using a thermal cycler (PCR-Invitrogen Veriti Thermal cycler, Applied Biosystems). The amplified products were subjected to agarose gel (1.2%) electrophoresis using a 100 bp DNA ladder (Thermo Scientific™) and viewed for the presence of DNA using the gel documentation unit (Invitrogen Life Technologies E-gel Imager). The amplified product was sequenced using Sangers sequencing (Agrigenome Labs, Cochin). The forward and reverse sequences were aligned using CAP3 assembly program to generate the DNA contigs[29]. The contigs developed were analysed for the presence of stop codons using the MEGA 7 software. The sequences generated from the study were analysed for sequence homology using nucleotide BLASTn at National Center for Biotechnology Information and submitted to BankIt, GenBank to generate an accession number. Details of the species were uploaded to the Barcode of Life Data Systems (BOLD Systems v4) to generate the barcodes.

Nesting architecture and life cycle of *B. picitarsis*

The nesting substrates having soft pithy or hollow stems, viz. peacock flower tree *Caesalpinia pulcherrima* (L.), Tecoma sp., *Rosa* spp., *Peltophorum pterocarpum* (DC) and *Lantana* sp. were regularly monitored for a period of 30 days. Nests of *B. picitarsis* were located only on hollow twigs of *C. pulcherrima* during the study. A total of 83 nests were collected randomly from *C. pulcherrima*. All the nests were collected during evening hours so as to ensure the presence of adult bees inside. Nests were cut beyond 10–25 cm from the tip of the twigs so that the broods were unharmed and the nest entrance was covered with a small cotton plug to prevent the escape of adult bees.

Individual nests were dissected carefully with a sharp blade to give a gentle split lengthwise and classified into four categories[30,31], viz. hibernacula nests, founding nests, active brood nests and full brood nests according to the life stages of bees and conditions of nests constructed by them. Hibernacula nests included remnants of previously built nest cells, darkened walls and foecal remains, pollen residues or molted skins from the previous breeding season as well as the presence or absence of adult bees in them. Founding nests were those with adult bees actively working to construct new nests devoid of immature stages and pollen provisions. Active brood nests contained pollen provisions with freshly laid eggs or immature stages. Full brood nests carried various immature stages of bees with pollen provisions that showed active feeding of pollen by the larva[32].

The nest architecture of *B. picitarsis*, including entrance diameter, thickness of nesting stem, occupied nest length, number of immature stages per nest, number of adults in the nest during collection and height of nest from the ground, was recorded. The immature stages of bees collected from the nests were reared in the laboratory (28°± 2°C and 75% ± 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 the developmental duration of different life stages. A cotton swab soaked in 10% honey was kept in the rearing boxes and the adult longevity was recorded. Descriptive statistics was performed to analyse the data with the SPSS 21 software.

Results and discussion

B. picitarsis is a major native bee pollinator of cucurbit crops and intensive searches for locating its habitat revealed the presence of nesting sites in dried and pruned stems of *C. pulcherrima* (Figure 1). A limited number of nests were located in plants like *Rosa* spp. and *Lantana camera*. As the number of nests in these plants was limited, studies on

Figure 1. Adult bee *Braunsapis picitarsis* (Cameron).

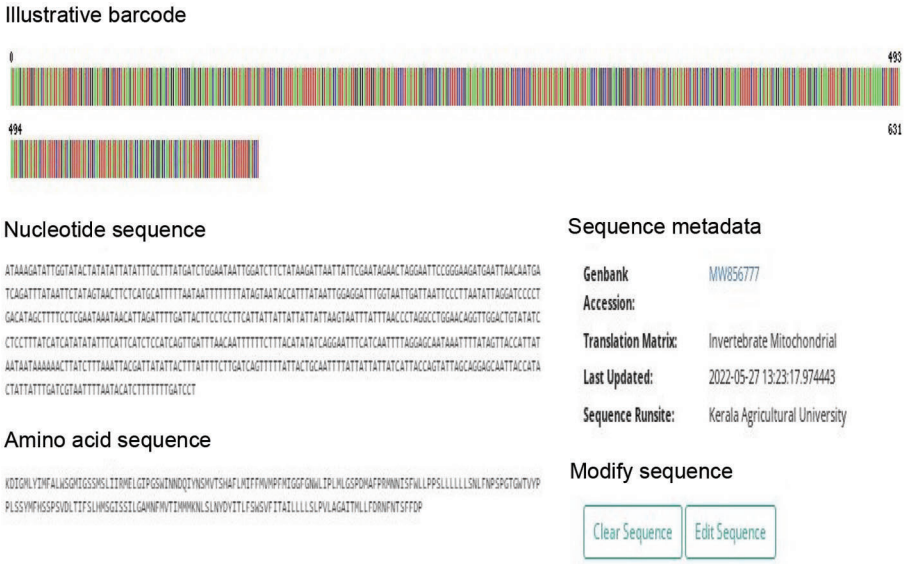

Figure 2. Barcode of *Braunsapis picitarsis*.

Table 1. Nest architecture of *Braunsapis picitarsis*

Particulars	Mean (± SEM)	Range ($N = 30$)
Entrance diameter (mm)	2.83 ± 0.06	2.10–3.50
Twig thickness (mm)	6.96 ± 0.12	5.50–8.00
Inner diameter of nest (mm)	2.90 ± 0.03	2.50–3.00
Occupied nest length (cm)	5.38 ± 0.30	3.00–9.00
Number of immature stage/nest	7.24 ± 0.31	3.00–13.00
Number of adults/nest	0.96 ± 0.09	0.00–2.00
Height of nest from ground level (cm)	100.58 ± 10.25	17.90–172.40

the nest architecture and life cycle of *B. picitarsis* were done using the nesting sites in *C. pulcherrima*. *B. picitarsis* was found to forage on several plant species other than cucurbits on the campus, viz. *Mimosa* spp., *Biophytum sensitivum* (L.), *Cassia fistula* L., *Sphagneticola trilobata* (L.), *Cyanthillium cinereum* (L.), *Cleome rutidosperma* DC, *Antigonon leptopus* Hook. & Arn., and *Lantana camera* L. during the study period.

DNA barcoding

Molecular identification of the bee species revealed that it was *B. picitarsis*. The sequences showed 100% similarity with *B. picitarsis* in BLASTn sequence analysis at NCBI. The sequence generated was submitted to GenBank, NCBI and the accession number was generated (MW856777). The DNA sequences were submitted to BOLD Systems v4 and a barcode for *B. picitarsis* was generated (Figure 2).

Nest architecture of B. picitarsis

B. picitarsis constructed linear nests in soft pithy stems of *C. pulcherrima*, which were unbranched with an average length of 5.38 ± 0.38 cm ($N = 30$) and twig thickness of 6.96 ± 0.12 (Table 1). The length of allodapine bee nests varied greatly according to species and their nesting substrate in *Braunsapis mixta*[16], the length of the nests was reported to vary from 12 to 174 mm (ref. 16), whereas in *Braunsapis sauteriella* (Cockerell) it varied from 6.0 to 106.0 mm (ref. 33). Out of the 83 nests collected, 7.22% was hibernacula nests with most of them abandoned and some occupied by adult bees. Active brood nests were 9.63%, followed by founding nests (13.25%) and full brood nests (69.87%). Female bees were found in a defensive position, either showing their head or abdomen facing upwards to guard the immature bees inside the nest. A study in Karnataka also reported the same defensive behaviour of allodapine bees in their nesting sites[16]. Most of the collected nests were occupied by one or two adult female bees, whereas several studies have reported the presence of 2–5 females in a single nesting site[34]. However, similar studies on *Braunsapis vitrea* (Vachal) recorded 23% of the nests occupied by single female bees[35].

The entrance diameter of the nests in *B. picitarsis* was found to be 2.83 ± 0.06 mm. In other studies, it ranged from 1.1 to 2.5 mm in *B. mixta*[16] and 1.8 to 3.5 mm in *B. sauteriella*[33]. The nests were averaged with an inner diameter of 2.90 ± 0.03 cm, in which pollen grains were spread throughout the extreme end of the nest wall. Eggs were

Table 2. Life cycle descriptions of *B. picitarsis*

Life-stage description	Mean duration (± SEM; days)	Range (N = 30)
Egg	4.44 ± 0.14	3.18–7.00
Larva		
First instar larva	3.17 ± 0.11	2.12–4.12
Second instar larva	2.66 ± 0.08	2.00–3.60
Third instar larva	2.91 ± 0.07	2.00–4.00
Pre-defecating larva	3.39 ± 0.09	3.00–4.60
Post-defecating larva	2.48 ± 0.09	2.00–3.60
Total larval period	14.62 ± 0.25	12.18–17.12
Pupa		
White-eyed pupa	3.57 ± 0.09	3.00–4.60
Pink-eyed pupa	1.51 ± 0.07	1.00–2.00
Brown-eyed pupa	2.60 ± 0.10	1.18–3.60
Black-eyed pupa	3.20 ± 0.13	2.12–4.60
Pupa with 1/2 body pigmentation	2.86 ± 0.09	2.00–3.60
Pupa with 3/4 body pigmentation	2.73 ± 0.09	2.00–3.60
Pupa with full body pigmentation	4.62 ± 0.12	3.18–5.60
Total pupal period	21.11 ± 0.32	17.60–24.22
Adult longevity	16.66 ± 0.64	11.12–24.60
Total life cycle	56.85 ± 0.84	49.32–66.46

Figure 3. Linearly arranged immature stages of *B. picitarsis* bees inside *Caesalpinia pulcherrima* twigs.

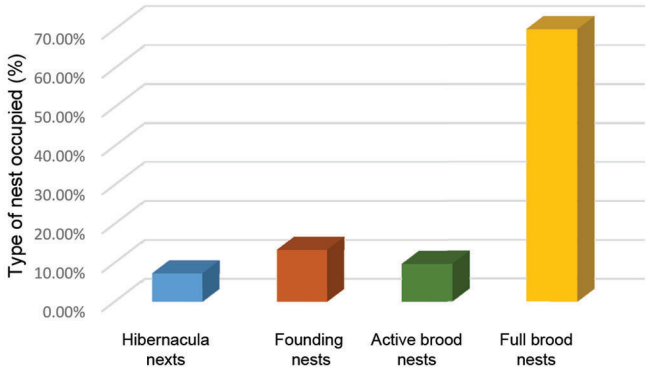

Figure 4. Classification of *B. picitarsis* nests found in *C. pulcherrima*.

laid in groups towards the bottom of the nest, where all of them were coated with pollen grains, ensuring immediate availability of food for the hatching larvae. There were no separate pollen provisions for the immature stages of *B. picitarsis* compared to the common stem nesting small carpenter bees (*Ceratina* spp.) in which each egg in the nest was deposited in separate pollen provisions procured by the mother bees[15,36]. Unlike the *Ceratina* bee nests, there were no cell septum partitions in the nests of *B. picitarsis*. Similar observations have been recorded with *B. mixta*[16]. All the immature bees were arranged linearly in the nest, with the mature ones placed towards the outermost end, whereas the younger ones were placed towards the innermost end of the nest (Figure 3). Mother bees continuously interacted with their offspring, as there was no cell partitioning. This might help the mother bees to properly provision and monitor their broods. The immature bees averaged 3 to 11 in number in the nests, with one or two adult bees guarding them. The nests were located about a metre from the ground level (100.58 ± 10.25 cm).

Life cycle of B. picitarsis

Observations on the life cycle of *B. picitarsis* were made on the nests in *C. pulcherrima* twigs. Of the 83 nests located, 13.25% were founding nests with only adult bees in them (Figure 4). The walls of the founding nests were clean, with

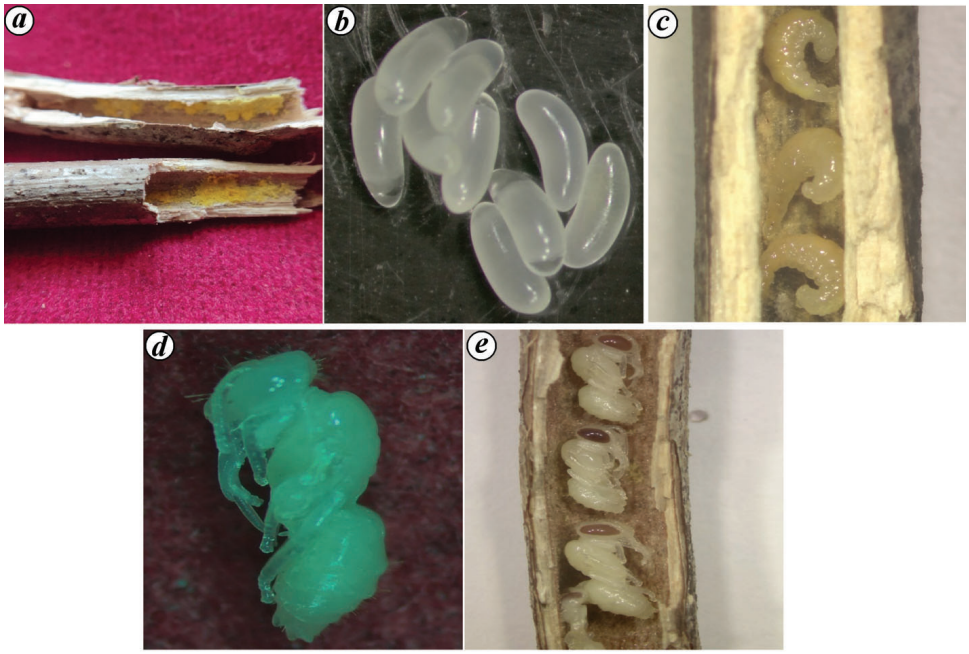

Figure 5. ***a***, Pollen provision in *Braunsapis* nest. ***b***, Eggs laid in groups. ***c***, Larva in bee nest. ***d***, White-eyed pupa of *B. picitarsis*. ***e***, Pink-, brown- and black-eyed pupa in bee nest.

no signs of pollen grains inside. Eight nests (9.63%) were active brood nests, with freshly laid eggs in groups blanketed with pollen grains. Pollen grains were spread throughout the innermost sides of the nest wall (Figure 5 *a*), ensuring food for the immature bees for their whole development period. Fifty-eight nests (69.87%) were full brood nests inhabited with mature larva and pupa.

The total developmental period of *B. picitarsis* from egg to adult stage was 56.85 ± 0.84 days (Table 2). The adult bees laid eggs in groups at the base of the nests. The eggs hatched at an average of 4.44 ± 0.14 days. *B. mixta* took an average of six days to hatch with a mean period of 5.38 ± 0.67 days (ref. 16). The eggs were white, translucent, banana-shaped and mostly laid in groups (Figure 5 *b*). They were covered with pollen grains. The apodous larva fed actively on the pollen grains and took an average of 14.62 ± 0.25 days to complete development and pupate (Figure 5 *c*). The post-defecated larvae metamorphosed into white-eyed pupae (Figure 5 *d*), which showed various types of eye pigmentation like pink, brown and black (Figure 5 *e*). The black-eyed pupa gradually attained pigmentation in the body and the total pupal period lasted for an average of 21.11 ± 0.32 days. The adult longevity was 16.66 ± 0.64 days under laboratory conditions. However, adults of *B. mixta* could survive up to 75 days, with male bees having less longevity compared to female bees[16].

Conclusion

The present study shows that dried hollow twigs of *C. pulcherrima* provide nesting sites for the allodapine bee, *B. picitarsis*. The linear nests constructed by the bees measured 5.38 ± 0.38 cm in length with an entrance diameter of 2.83 ± 0.06 mm. The number of immature stages average 7.24 ± 0.31 in number per nest, with one or two adult bees. The total development period was 56.85 ± 0.84 days in the laboratory, where the pupa exhibited a difference in eye colour and body pigmentation during development. In this study, the peacock flower tree was found to be a good host for *B. picitarsis* bees. Thus it can be used as a hedge in crop fields for maximum utilization of pollination services and better farm scaping.

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