ORIENTAL INSECTS, 2016
http://dx.doi.org/10.1080/00305316.2016.1272500

Nesting biology of two species of the large carpenter bees *Xylocopa pubescens* and *Xylocopa fenestrata* (Hymenoptera: Apidae) in north-western Pakistan

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ABSTRACT

The large carpenter bees are very promising pollinators for many agriculture crops. The nesting biology and some foraging activities of these large carpenter bees are documented from the north-western Pakistan, most extent of its natural distribution, as these species are potentially important pollinators of cucurbit crops and some agriculture crops in the region. Nests of the large carpenter bees, *Xylocopa pubescens* Spinola, 1838 and *Xylocopa fenestrata* Fabricius, 1798, around the Ismaila village, Swabi, Khyber Pakhtunkhwa Province, Pakistan, were observed and sampled from June to August 2012 and 2013. Nest details were recorded and foraging times on various floral species were documented, with bees preferentially nesting in dead wooden stems of Poplar (*Populus deltoides*) and Sesbania (*Sesbania bispinosa*). The dissection of nests was carried out and both nest architecture and nesting biology of the species were described. The importance of such studies on wild bees in Pakistan is important for the melittologists, as it will help in conservation of wild bees. The study is also useful in using large carpenter bees for pollination purpose.

ARTICLE HISTORY
Received 9 May 2016
Accepted 12 December 2016

KEYWORDS
Large carpenter bees; pollinator; Poplar; Sesbania; foraging; North-western Pakistan

Introduction

The large carpenter bees (*Xylocopa* Latreille) are a widespread and significantly diverse lineage of Xylocopinae (Hymenoptera: Apidae), with approximately 500 species (Minckley 1998), many of which are pale yellowish in coloration, males, and dark coloured, females, or sometimes both sexes largely are dark coloured (Michener 2007). There are eight known species of the large carpenter bees recorded from Pakistan. The recorded species are *Xylocopa* (*Ctenoxylocopa*) *basalis*

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Smith, 1854; *X.* (*Maaiana*) *bentoni* Cockerell, 1919; *X.* (*Ctenoxylocopa*) *fenestrata* Fabricius, 1798; *X.* (*Koptortosoma*) *pubescens* Spinola, 1838 and *X.* (*Proxylocopa*) *rufa* Friese, 1901 (Ascher and Rasmussen 2010). In addition, another species was included to the previous list *X. valga* Gerstaecker, 1872 (Guershon and Ionescu-Hirsch 2012). Some species like *X. dissimilis* Lepel, 1841 and *X. rufescens* Smith, 2000, were recorded from the fruit orchards of the Himalayan foot hills (Hussain et al. 2012). Most of the large carpenter bee species are important for plant pollination. Species like *X. basalis* plays a major role in pollinating of alfalfa crop in Pakistan (Ahmed 1976).

There are several studies on the large carpenter bees' nesting biology that usually nest in dead wood, bamboo and other nesting wooden materials, especially solid wood (Sihag 1993a; Engel et al. 2012). In Saudi Arabia, two species', *Xylocopa aestuans* Linnaeus, 1758 and *Xylocopa sulcapites* Maa, 1970, nesting biology was studied (Engel et al. 2012). Some species build unbranched or linear nests while others build branched nests (Gerling et al. 1989). In South Asia, some bee biologists like Maeta et al. (1985), Kapil and Dhaliwal (1968) and Hongjamrassilp and Warrit (2014) studied large carpenter bees' nesting biology.

X. pubescens belongs to the subgenus *Koptortosoma* and is very similar with another species of the same genus *X. aestuans* (Mordechai et al. 1978; Engel et al. 2012), whereas *X. fenestrata* Fabricius, 1798, belongs to the subgenus *Ctenoxylocopa* and is very confused with *X. sulcatipes* Maa, 1970. Both *X. aestuans* and *X. sulcatipes* have not been recorded so far from Pakistan. All four species could be distinguished by the structure of the male genitalia (Maa 1970; Engel et al. 2012; Guershon and Ionescu-Hirsch 2012).

So far, there is lack of information on the nesting biology of the large carpenter bees in Pakistan. We provide here some details on the nesting biology and some foraging activities of two species of the large carpenter bees. The work will be used for conservation and manipulation of large carpenter bees for pollination and betterment of agriculture in the region. This work is part of ongoing efforts to understand wild bee pollinators within the region and their possible economic use (Ali et al. 2016).

Material and methods

Study site

The study was carried out around the village of Ismaila, Swabi District (34°13′50.3″N, 72°14′48.5″E), Khyber Pakhtunkhwa Province, in north-western Pakistan. Swabi is the fourth most populous district of the province and lies between the Indus and Kabul Rivers, and somewhat centrally between Kashmir and Afghanistan with a humid climate and heavy rains during summer. The area is rich in cultivated plants, particularly alfalfa, (*Medicago sativa* L; Fabaceae), Egyptian clover (*Trifolium alexandrinum* L; Fabaceae), sunflower (*Helianthus* *annuus* L.; Asteraceae), canola (*Brassica napus* L; Brassicaceae) and varied vegetable crops. During a quick sampling of bees around Ismaila, numerous nesting sites were discovered for small and large carpenter bees, particularly those of latter, which were preferentially nesting in Poplar (*Populus deltoides*) dead stem and Sesbania (*Sesbania bispinosa*) stem (Figures 1 and 2). Ongoing surveys are focused on locating the nests of additional species of large and small carpenter bees. Small carpenter bee *Ceratina smaragdula* Fibricius, 1787 (Hymenoptera: Apidae) was also abundant in the area and represents another group of potential pollinator for regional crops (Ali et al. 2016).

Bee sampling and identification

Bees were sampled by sweep netting cultivated flowers throughout the summer season (June through August) of 2012 and 2013, with sampling taking place twice per week. Plants surveyed were dead wooden stems of Poplar (*Populus deltoides* W.Bartram ex Marshall; Salicaceae) and Sesbania (*Sesbania bispinosa* Willd. Pers.; Fabaceae). The whole insect life cycle was observed for almost two months during the study period. Several nests were observed to detect the whole duration of the bees' development from egg to the immature and mature stages in both species. Periods of activity were observed from six nests over a period of five days during the study period. For foraging, the adults of three nests were marked from both species with paint markers and were observed for five days. Their activities were recorded on different flowers.

After preparations of male terminalia, when necessary, they were dissected from fresh or relaxed bees. Genital sclerites were cleared with a weak solution of potassium hydroxide (10% KOH) for at least one-half day before transferring to distilled water for further dissection. All individuals were identified to species through comparison with an authoritatively identified reference collection of King Saud University Museum of Arthropods (KSMA), Riyadh, Saudi Arabia. Different keys and illustrations were used for the identification confirmation (Maa 1970; Terzo and Rasmont 2011; Engel et al. 2012). Species samples were deposited in the King Saud University Museum of Arthropods (Riyadh, Saudi Arabia).

Nest sampling

A total of 10 nests for *X. fenestrata* and 10 nests for *X. pubescens* were sampled from 2 locations within the study area, separated by approximately 200 m. Nests were collected at dusk and after all foraging, bees had returned to the nest. Entrances were closed with tape and general features such a height from the soil surface and branch length were measured. After collection, the nests were refrigerated for 8–10 h to kill the inhabitants. External nest and branch features were measured before dissection. Nests were opened starting at the entrance and parallel to the length of the branch by slowly and gently splitting the stem with a sharp

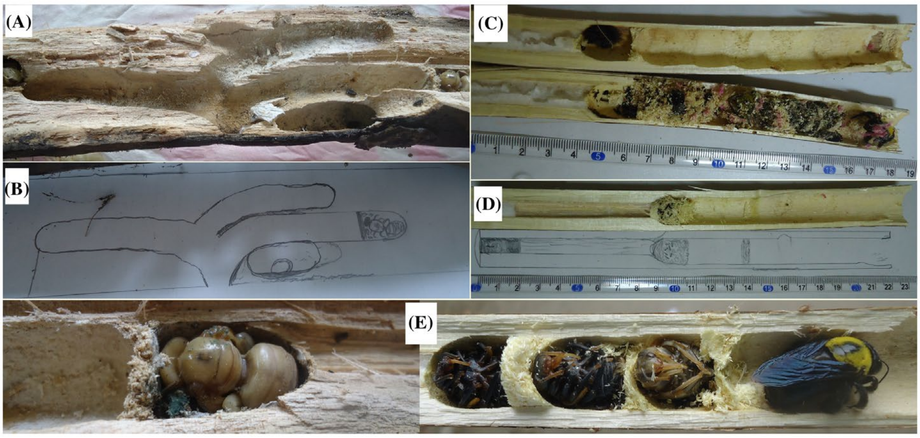

Figure 1. Photograph of nest architecture of *X. pubescens* (photos by Hussain Ali); (A) Branched nest; (B) Sketch of the branched nest; (C) Unbranched nest (scale in mm); (D) Sketch of the unbranched nest (scale in mm); and (E) Section of a nest with cell partitions with a series of different developmental stages from pupa to adult (not to same scale).

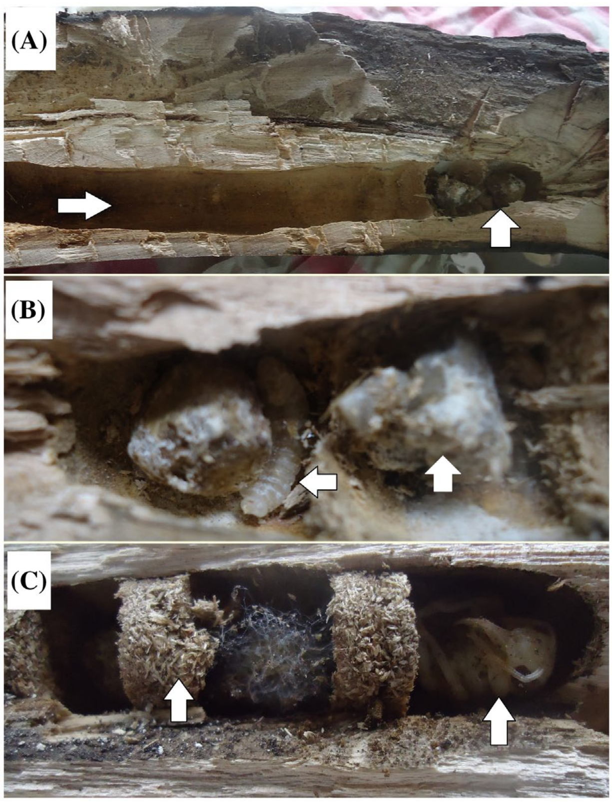

Figure 2. Photograph of nest architecture of *X. fenestrata* (photos by Hussain Ali); (A) Branched nest and entrance; (B) Section of nest containing characteristic pollen masses and larvae; and (C) Section of nest with completed cell partitions containing pupa (not to same scale).

knife. Once nests were fully exposed, all contents were recorded (e.g. number of immature stages; presence of pollen masses) and photographs were taken with a Sony DSC 160 digital camera followed by preparation of sketches. Nest data were recorded and descriptive statistics were applied on the data. Means and standard deviation of the data were calculated.

Results

Nest architecture of X. pubescens *and* X. fenestrata

The large carpenter bees' nests could be branched or unbranched with one or more gallery. The branched nests were found in Poplar (*P. deltoides*) dead stem, while the single branched nests were found in Sesbania (*S. bispinosa*) stem (Figures 1 and 2). No failed nests (i.e. abandoned over the season; filled with fungus) were observed. Some fungal and mite attacks were observed in some nests, which could possibly be the parasites for these species. Nests were mostly found close to each other. The opening directions/entrance of the nests were mostly towards east and south.

The nest was built on dead wood of Poplar and Sesbania on the top portion of the dead stems about 226.85 ± 15.46 cm above the soil surface. For the 10 nests sampled of *X. pubescens,* the branches containing the nests had an average (±SD) branch length of 24.30 ± 5.15 cm, average nest length of 19.03 ± 6.88 cm, branch diameter of 3.91 ± 1.57, average nest diameter of 1.59 ± 0.15 cm and an average nest entrance of 15.20 ± 2.92 mm (Table 1). *X. fenestrata* nests were built on the top of dead wood parts of the same plants on an average (±SD) 210.39 ± 15.61 cm above the soil surface with a total of 10 nests. The average branch length, nest length, branch diameter, nest diameter and nest entrance were 22.59 ± 2.48 cm, 22.89 ± 4.44 cm, 4.03 ± 1.15 cm, 1.69 ± 0.16 cm and 13.00 ± 1.78 mm, respectively (Table 2).

Life cycle of X. pubescens *and* X. fenestrata

Data on the nesting biology and the contents of the galleries of the immature and mature stages of *X. pubescens* and *X. fenestrata* are summarized in Tables 3 and 4. The development time ranged from 45 to 49 days during the study period. In *X. pubescens*, number of galleries (mean 2.10 ± 1.04), number of cells (mean 3.72 ± 1.10) and the cell length (mean 18.50 ± 1.62 mm) were recorded. The mean number of eggs, larvae, pupa and adults was 0.91 ± 0.53, 0.90 ± 0.53, 1.52 ± 0.92, and 2.00 ± 1.18, respectively (Table 3 and Figure 1). In *X. fenestrata,* the mean number of galleries, cells and cells length was 2.00 ± 0.77, 2.91 ± 0.83, and 18.91 ± 1.57 mm, respectively. The mean number of eggs, larvae, pupa and adults was 1.00 ± 0.63, 1.32 ± 0.45, 1.51 ± 0.92 and 1.91 ± 0.70, respectively (Table 4). The provisioned cells were separated from each other by partitions made from bamboo particles built by the female (Figure 1(D)).

Table 1. Measurements of sample nests of *X. pubescens* during 2012–2013.

	Nest measurements of *X. pubescens*						
Nest no & date of dissection	Distance from surface (cm)	Branch length (cm)	Length of nest (cm)	Branch diameter (cm)	Nest diameter (cm)	Nest entrance (mm)	No of galleries
1. June12, 2012	264.16	26.11	18.21	5.91	1.52	10	4
2. June 13, 2012	241.31	36.20	33.63	6.23	1.40	13	3
3. June 18, 2012	213.36	21.23	11.12	2.70	1.81	20	1
4. July 11, 2012	226.06	21.91	13.20	2.11	1.55	15	1
5. July 14, 2012	215.90	17.94	12.71	1.96	1.52	14	1
6. June 19, 2013	222.12	18.62	22.13	5.34	1.74	14	3
7. June 23, 2013	215.33	21.83	14.56	2.32	1.61	13	2
8. July 13, 2013	210.41	27.30	18.64	4.75	1.43	18	1
9. July 17, 2013	234.54	23.52	17.53	2.97	1.52	16	2
10. August 1, 2013	225.32	28.36	28.62	4.81	1.87	19	3
Mean ± SD	226.85 ± 15.46	24.30 ± 5.15	19.03 ± 6.88	3.91 ± 1.57	1.59 ± 0.15	15.20 ± 2.92	2.10 ± 1.04

Table 2. Measurements of sample nests of *X. fenestrata during* 2012–2013.

	Nest measurements of *X. fenestrata*						
Nest no & date of dissection	Distance from surface (cm)	Branch length (cm)	Length of nest (cm)	Branch diameter (cm)	Nest diameter (cm)	Nest entrance (mm)	No of galleries
1. June 14, 2012	240.22	24.33	21.81	4.73	1.71	11	3
2. June 15, 2012	210.21	28.31	31.24	5.21	1.95	12	3
3. June 15, 2012	213.31	23.22	20.33	3.71	1.51	15	1
4. July 26, 2012	198.73	22.10	16.51	2.60	1.43	13	2
5. August 2, 2012	191.20	21.24	29.22	2.32	1.81	11	1
6. June 10, 2013	220.14	18.61	18.22	4.14	1.60	15	2
7. June 12, 2013	196.20	21.31	22.51	5.31	1.51	14	2
8. July 27, 2013	203.95	20.83	19.81	2.92	1.72	16	3
9. July 29, 2013	196.92	24.11	25.63	5.85	1.81	12	1
10. August 4, 2013	233.11	21.91	23.73	3.51	1.90	11	2
Mean ± SD	210.39 ± 15.61	22.59 ± 2.48	22.89 ± 4.44	4.03 ± 1.15	1.69 ± 0.16	13.00 ± 1.78	2.00 ± 0.77

Table 3. Nest contents of *X. pubescens* during 2012–2013.

Nest no.	No. of galleries	No. of cells per gallery	Cells length (mm)	Contents of gallery			
				Eggs	Larva	Pupa	Adult
1	4	2	22	0	0	1	1
2	3	5	17	1	1	1	1
3	1	3	19	0	0	3	1
4	1	4	18	1	1	1	4
5	1	5	19	1	1	0	4
6	3	4	19	1	1	1	1
7	2	3	17	1	2	2	2
8	1	5	16	2	1	2	2
9	2	4	20	1	1	3	1
10	3	2	18	1	1	1	3
Mean ± SD	2.10 ± 1.04	3.72 ± 1.10	18.50 ± 1.62	0.91 ± 0.53	0.90 ± 0.53	1.52 ± 0.92	2.00 ± 1.18

Table 4. Nest contents of *X. fenestrata* during 2012–2013.

Nest no.	No. of galleries	No. of cells per gallery	Cells length (mm)	Contents of gallery			
				Eggs	Larva	Pupa	Adult
1	3	4	21	1	1	2	1
2	3	3	19	0	2	1	1
3	1	4	20	2	1	1	2
4	2	2	18	1	1	0	2
5	1	2	17	1	1	1	1
6	2	3	16	1	2	3	2
7	2	3	20	1	2	1	2
8	3	2	19	2	1	2	3
9	1	4	21	0	1	3	3
10	2	2	18	1	1	1	2
Mean ± SD	2.00 ± 0.77	2.91 ± 0.83	18.91 ± 1.57	1.00 ± 0.63	1.32 ± 0.45	1.51 ± 0.92	1.91 ± 0.70

Foraging activities

Near the nesting area, individuals were found foraging on a variety of cultivated crops. Most important for the region, we recorded the species visiting flowers of some important vegetables including cucurbits and okra (*Abelmoschus esculentus* Moench; Malvaceae). During the investigation period, both bee species started flying in the early morning around 6.59 am ± 36 min and returned from first flight after an average (±SD) 12.83 min ± 1.77 min with pollen, ended their daily foraging bouts in the late afternoon around 5.55 pm ± 25 min and spent an average 55.50 ± 15.58 s on flower (Table 5). Most species of the large carpenter bees are polylectic visiting a wide range of floral resources.

Discussion

X. pubescens and *X. fenestrata* are the most commonly known species of the large carpenter bees of the eight known species from Pakistan. In north-western Pakistan, both carpenter bees were found nesting in Poplar (*P. deltoides*) dead stem and Sesbania (*S. bispinosa*) stem. This observation differs from the variety of plants used elsewhere in its range, such as bamboo, dates and *Calotropis procera*

Table 5. Foraging time/activities of *X. pubescens* and *X. fenestrata.*

Nest no.	Foraging starting time (am)	Returning time after first forage with pollen (min)	Foraging ending time (pm)	Average time on different flowers (s)
1 (*X. pubescens)*	6.50	12.00	5.30	30.00
2 (*X. pubescens)*	7.10	10.00	5.55	45.00
3 (*X. pubescens)*	6.30	15.00	5.40	65.00
4 (*X. fenestrata)*	6.47	13.00	5.52	80.00
5 (*X. fenestrata)*	6.15	15.00	6.08	55.00
6 (*X. fenestrata)*	7.07	12.00	5.45	58.00
Mean ± SD	6.59 ± 0.36	12.83 ± 1.77	5.55 ± 0.25	55.50 ± 15.58

(Aiton) in the tropics and arid areas (Iwata 1938; Maeta et al. 1985; Silveira 2002; Engel et al. 2012). Moreover, nest architecture of both species looks similar to *Xylocopa* (*Biluna*) *nasalis,* especially *X. artifex areas* (Silveira 2002). Overall, the nest construction did not differ from that described for the species elsewhere and within other plant hosts (Gerling et al. 1989; Vicidomini 1998). It is the first report of nesting biology of both carpenter bees from Pakistan.

X. pubescens and *X. fenestrata* showed almost similar nest structure. *X. pubescens* has little larger and wider nest entrance compared to *X. fenestrata,* probably due to the differences in the size of the bees. In both species, the nests were linear and branched with only one nest entrance (Gerling et al. 1989; Hongjamrassilp and Warrit 2014). In one of the previous study, the nest length and the branch diameter were found to be 25.40 ± 6.95 cm and 17.94 ± 6.00 mm, respectively (Hongjamrassilp and Warrit 2014). In one study in India, the subtropical carpenter bee, *X. fenestrata*, prefers to nest in the dead wood of *Arundo* sp. and bamboo to build a long tunnel of 23–30 mm with internal diameter of 10–12 mm (Sihag 1993a; Rahman and Deka 2011). This previous study is in agreement with our data recorded in this study. *X. pubescens's* nest architecture, biology and floral resource were extensively studied in the Middle East (Borollosy and Ismail 1973); no report has been appeared so far in Pakistan. Usually, the female lays one or a few eggs along the tunnel and provisions them with considerable amount of pollen balls and separates each cell by partition of masticated wood (Keasar 2010). The maternal care of the female is well known which involves guarding of the immature stages (Gerling et al. 1981). Unfortunately, we did not observe such type of nesting behaviour in this study. We also observed less male compared to females in our study and this observation is similar to the observation of Sihag (1993b), who reported female-biased brood in *X. fenenstrata*.

As noted previously, *X. pubescens* and *X. fenestrata* are polylectic, typical for a species with such a broad ecological and distributional range, and are likely important pollinators in Pakistan, with the potential for use in agro-ecosystem on cucurbits as has been done elsewhere (Raju and Rao 2006). The large carpenter bees are polylectic (Hurd and Moure 1960; Gerling et al. 1989; Burgett et al. 2005) and therefore may be found visiting numerous and diverse floral resources and play beneficial role in the buzz-pollinated plants (Keasar 2010). Both carpenter bees would be some ideal candidates for managed pollination service in north-western Pakistan, with the ease of finding and trapping the bees making it likely to encourage use by local farmers. Poplar (*P. deltoides*) dead stem and Sesbania (*S. bispinosa*) stem seem to be used as artificial nesting substrate in Pakistan for future managed nesting operations. Farmers could be encouraged to place such stalks horizontally above the surface of the ground (232.15 ± 17.13 cm and 215.60 ± 13.61 cm) in accordance with the natural nests we observed, and to conserve natural habitats for wild bees surrounding crop environments.

Although there is a remarkable diversity of species of *Xylocopa* in Asia (Raju and Rao 2006), there have been comparatively few studies on their nesting biology and floral associations outside of Middle East, most having been undertaken on a subset of relatively common taxa (Iwata 1938; Maeta et al. 1985; Silveira 2002; Sadeh et al. 2007; Engel et al. 2012). Given that there are at least 263 species of pollinating and cleptoparasitic bees in Pakistan (Ascher and Rasmussen 2010), with numerous more to be documented, the potential is great for nesting and pollination studies throughout the varied habitats and elevations of the country. These realities stress the importance of preserving and protecting natural habitats in Pakistan, with such endeavours vital to the country's agricultural production and broader ecosystem health. Local educational and outreach programmes are needed to educate farmers, who often operate at a small scale within isolated villages, of the need to conserve natural areas, particularly those bordering crop fields. Although there are already robust apicultural traditions in the region, investments are needed for the training of a new generation of Pakistani melittologists to undertake biotic inventories and develop species level hypotheses, revisions and methods of modern identification for the country's melittofaunal resources (Engel 2011; Gonzalez et al. 2013).

In both, no major differences were found in their nest architecture and biology. This study will be useful as it quantified the nest structure and description and can use in conservation programmes. This can also be used in trapping these species in artificial nests and manipulating them for pollination purpose on different agronomically important crops. Some early scientists like Junqueira et al. (2012) used trap nests for brood enhancement and management of large carpenter bees. Further studies should be carried through the year to get more comprehensive information about species.

Acknowledgement

The authors extend their appreciation to the Deanship of Scientific Research at the King Saud University for funding the work through the research group project No. RGP-189. Special thanks to Dr. Md. Hannan, Guelph University, Canada for his guidance during the course of the study.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the Deanship of Scientific Research at the King Saud University through the research group project No. RGP-189.

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