



Pollination Biology of *Phaius tankervilleae* (Banks ex L' Herit) Bl. (Orchidaceae)

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ABSTRACT

The pollination biology of *Phaius tankervilleae* (banks Ex. I' Herit) Bl. (Orchidaceae) has been studied for three consecutive year 2012 – 2014 at two different localities, Viz., , Dissoi Valley Reserve forest (26°28'47'' N and 94°16'14''E) in Jorhat District of Assam state and at Natunmati, Ward No. 4. (26°38'35'' N & 94°17'45'' E) in Mariani township, Jorhat district, Assam of the North-east India. Blooming in *Phaius tankervilleae* takes place from February to May. The flowers of *P. tankervilleae* are self-compatible but require some biotic - pollinator to complete the process of pollination. The flowers with tubular labellum are non- rewarding but emit strong sweet fragrance which attracts various insects belonging to order Hymenoptera, Diptera and Lepidoptera. However, out of all these visitors only Carpenter bees *Xylocopa violacea* of order Hymenoptera have been observed to pollinate the flowers during their visits to the flowers. Anthecological studies reveal the attachment of pollinaria on the dorsal surface of thorax of *X. violacea* which are the only pollinators of *P. tankervilleae* at the investigated sites in Assam. The mode of pollination is "Nototribic".

Keywords : *Phaius tankervilleae*, *Xylocopa violacea*, Biotic - pollinators, Nototribic pollination..

INTRODUCTION

The members of family Orchidaceae are well known for their floral structure which ensures cross-pollination (Darwin 1890, Van der Pijl & Dodson 1966, Dressler 1993). *Phaius tankervilleae*, commonly known as "Nun's Orchid" belongs to sub-family Epidendroideae. It is a critically endangered and medicinally as well as ornamentally important terrestrial orchid. The dried tubers are commonly used as tonic in traditional healing system by various ethnic groups (Pant *et al.* 2007).

The paste of the pseudo bulbs is used to cure swellings of hands and legs. The leaves and flowers are used to extract natural dyes (Mahanta & Tiwari 2005). It has been observed that the illegal and indiscriminate

collection and habitat degradation are the major threats for the decline of the population of *P. tankervilleae* in its natural habitat. However, according to Briggs and Leigh (1996), *P. tankervilleae* has been categorized as endangered species, under the Environmental Protection and Biodiversity Conservation Act.

Genus *Phaius* with about 40 species are widely distributed all over the tropical Africa, tropical and subtropical Asia and Oceania (Dressler 1993). The pollination biology of *P. tankervilleae* becomes an interesting phenomenon due to various modes of pollination reported by the earlier workers like Van der Pijl & Dodson (1966), Gandawidjaja & Arditti (1982) and Van der Cingle (2001). In cliestogamous species the

pollinaria drops on to the stigma of unopened floral buds, whereas, in chasmogamous species the process of pollination takes place during the separation of sepals and petals at the time of opening of flowers (Gandawidjaja & Arditti 1982). However, Li *et al.* (2010) have reported bumble bee pollination in an alpine species of genus *Phaius*, *Viz.*, *P. delavayi*, which is endemic to temperate Southwestern China. According to Li *et al.* (2010), the flowers of *P. delavayi* are devoid of rewards, but they get pollinated by the “learning and avoidance” process applied by insect visitors which probe the flowers for reward and this is termed as deceptive pollination. According to these authors the mechanism of insect pollination in *P. tankervilleae* is similar to that of genus *Cattleya* as described by Darwin (1890). However, Pijl & Dodson (1966) have reported bee pollination in *P. tankervilleae*.

The present studies on the mechanism of pollination of *P. tankervilleae* reveal the similar last resort of the deceptive pollination by Carpenter bees (*Xylocopa violacea*) in this non rewarding species at two different localities of Assam which is one of the North-eastern states of Indian sub-continent and considered as one of the twelve hottest hot spots of the biodiversity in the World.

MATERIAL AND METHODS

The studies on the pollination biology of *P. tankervilleae* were conducted at two sites, *Viz.*, Dissoi valley reserve forest, 26° 28' 47" N and 94° 16' 14" E in Jorhat District of Assam, and Natunmati, Ward No. 4, 26° 38' 35" N & 94° 17' 45" E, at Mariani town in Jorhat district of Assam State of North-eastern region of Indian sub-continent. A series of experiments related to the pollination biology have been performed on the flowers of *P. tankervilleae*. To see the occurrence of autogamy the inflorescences were bagged before anthesis by the nylon net bags. The experiments for self pollination to test self compatibility were conducted by inserting the pollinaria of a flower into its own stigma. Cross pollination between the flowers of the same plant was performed to see the results of geitonogamy, cross pollination with the pollinaria from the flowers of other population, of *P. tankervilleae*, growing at a distance of 10 meters was performed to see the results of

Xenogamy. After performing various experiments the flowers were bagged by the nylon net bags. However, one of the populations was left open as control to see the results of natural pollination. The experiments were performed in the month of March 2012 and 2014 and the fruit set was counted at the end of May 2012 and 2014 respectively. The insect visitors and pollinators were captured with the help of wide mouthed glass test tubes and a watch glass so as to avoid the removal of pollinaria from the body of the insects. The insect visitors of *P. tankervilleae* were sent to the ZSI, Shillong (regional office) and ZSI, Kolkata (Head Office) for authentic identification. Anthecological studies were conducted under the Leica Stereo Zoom Microscope. Field photographs were taken with the help of Sony digital camera, 16 MP. For the confirmation of occurrence of autogamy (if any), ten inflorescences were selected at random at both the study sites and covered by nylon net bags and observed till the wilting of the last flower. Repeated observations were made during the period of flowering, so as, to note the visiting behavior of the insects and to determine the pollinators as well as the mechanism of pollination in the open population of *P. tankervilleae*.

RESULTS

The inflorescences of *P. tankervilleae* attain a height of 55 to 90 cm and bear 10 to 18 flowers. Widely spread, large sepals and petals are off white in colour on the outer surface and reddish brown in colour on inner surface. Labellum is reddish pink with white stripes towards the base. The two lateral lobes of petals overlap each other above the column, thus forming a tunnel shaped structure. The whitish yellow spur is present at the base of the labellum which is narrow, cylindrical 0.8-1.2 cm in length and 1.5- 2.0 mm. in diameter. Spur shows some exudate on the inner surface but no perceptible nectar has been found inside the spur. During sunny days, in the afternoon, when temperature rises, a sweet, strong fragrance is emitted by the flower due to which a variety of insects are attracted towards the flowers. These insects belong to various orders, *Viz.*, Hymenoptera, Lepidoptera, and Diptera (Table 1) and were found visiting the flowers of *P. tankervilleae* at different times of the day. The body length of all the visitors, except for

Xylocopa violacea, is smaller than the length of the column as well as the labellum and therefore, they crawl down along the labellum to reach the spur and come out of it without even touching the column (Table 4). However, it has been found that the pollinator carpenter bees forage only one or two flowers of an inflorescence during a single visit.

Mechanism of Pollination—The carpenter bees, *Xylocopa violacea*, during their foraging visit perch over the labellum, stretch the body and enter into the labellum chamber to reach the spur in search of forage (Fig. 1B). This act of *X. violacea* exerts pressure on the labellum and in turn on the column of the flower. During this process the apical portion of column comes in contact with the thorax of the visitor bees. Since, the viscidium of pollinarium is also hangs out of the anther situated at the apical portion of the column, it gets stick over the thorax of the visitor carpenter bees. While these bees withdraw themselves out of the labellum chamber, either the whole pollinarium or the broken portion of stipe along with viscidium come along attached over the thorax (Figs. 1 C-F). When such bees, with pollinaria attached on their thorax, visit other flowers in search of the forage and repeat the same process of entering the labellum chamber, the pollinaria attached over the thorax become inserted into the sub-apically situated stigmatic hole and get glued in to it. At the time of retraction, of these bees, out of the labellum chamber, either the complete

pollinarium or a broken part of it left glued into the stigmatic hole because of the strong sticky glue present inside the stigmatic hole (Fig. 1G). Thus, the process of pollination in *P. tankervilleae* is completed. Maximum of 16 pollinaria were found attached on the thorax of a single carpenter bee (Fig. 1F). However, maximum of 4 pollinaria have been found inserted into the stigmatic hole. Further, it has been found that *X. violacea* visit flowers until the pollinaria become attached on the thorax but once the pollinaria become attached over the thorax they immediately leave the flower and fly away. Although, the senescence of flowers initiates after 36 hrs of pollination, yet, the wilting of petals and sepals takes place after 4 to 5 days of pollination. The development of ovary into the completely mature fruit takes about 10 months after pollination.

The hand pollination and bagging experiments exhibits complete absence of autogamy, whereas, the self pollinated flowers show 88.04% fruit set. The geitonogamous and xenogamous experiments show 78.48% and 74.20% fruit set respectively. However, the open pollinated flowers show only 20.10% of fruit set at site 1 (Table 2). At site -2 also, complete absence of autogamy has been observed. Self pollinated flowers show 77.44% of fruit set, whereas, geitonogamous and xenogamous experiments show 81.11% and 76.6% fruit set. The open pollination at site- 2 shows only 13.95% fruit set (Table 3).

Table 1– Insect Visitors / pollinators of *P. tankervilleae* in Assam-

Order	Family	Genus and Species
Hymenoptera	Apidae	<i>Apis cerana indica</i>
	Apidae	<i>Amegilla zonata</i>
	Apidae	<i>Xylocopa violacea</i>
	Vespidae	<i>Epsilon</i> sp.
	Vespidae	<i>Rhynchium brunneum brunneum</i>
Lepidoptera	Hesperiidae	<i>Notocrypta curvifascia</i>
	Hesperiidae	<i>Baoris farri</i>
Diptera	Syrphidae	<i>Eristalis tenax</i>
	Calliphoridae	<i>Phaenicia sericata</i>
	Muscidae	<i>Muscina prolapsa</i>

Table 2 – Pollination system and fruit set of *Phaius tankervilleae* at site- 1, Dissoi valley reserve forest, Assam

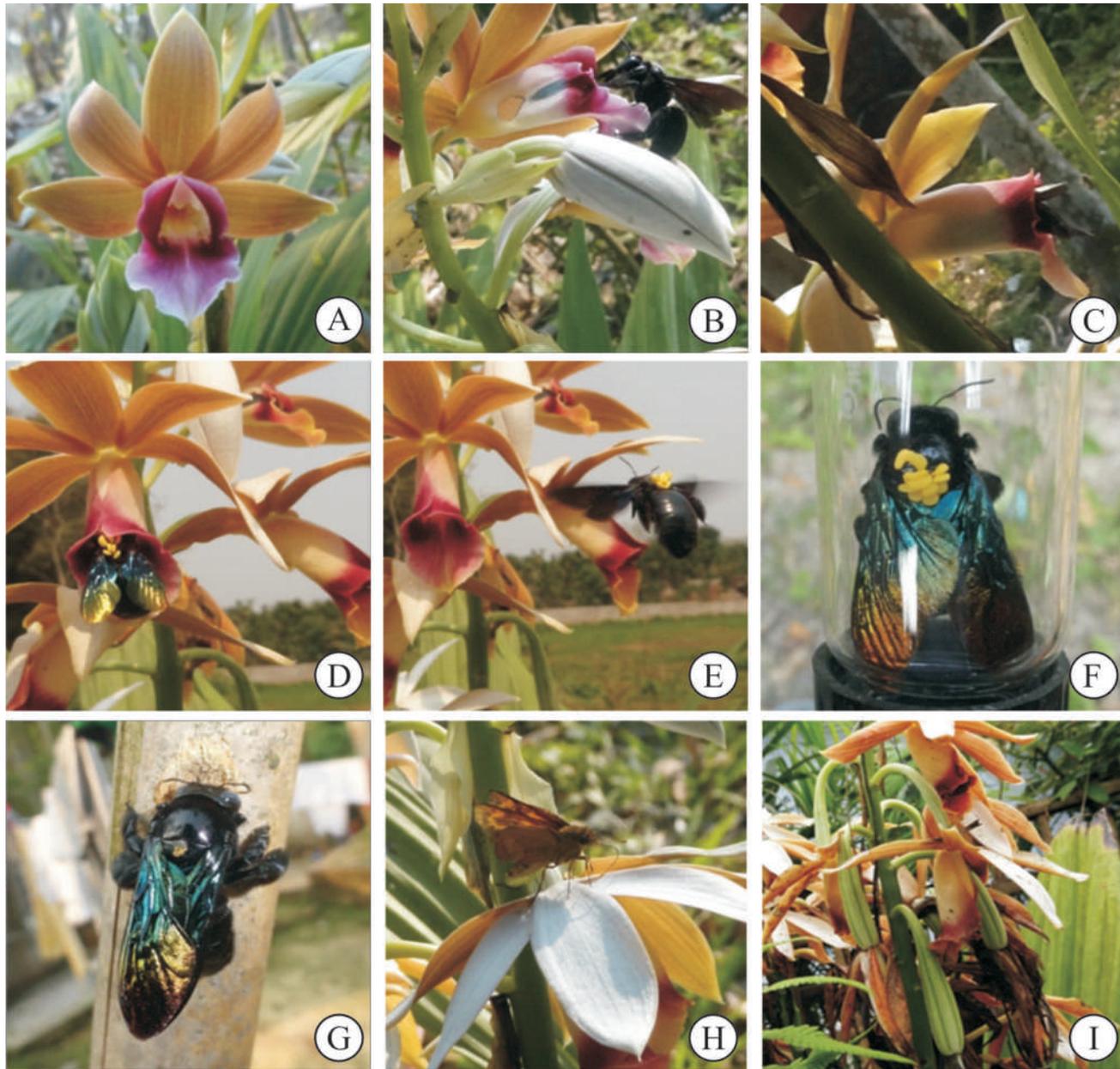
Treatment	No. of Flowers				No. of Capsule				Fruit Set %			
	2012	2013	2014	Mean	2012	2013	2014	Mean	2012	2013	2014	Mean %
Autogamy (Inflorescence were covered by plastic net bags)	72	60	96	76	Nil	Nil	Nil		Nil	Nil	Nil	
Self-pollination	25	20	22	22.33	22	18	19	19.66	88	90	86.36	88.04
Geitonogamous experiments	15	26	24	21.66	11	21	19	17	73.33	80.76	79.16	78.48
Xenogamous experiments	14	27	21	20.66	10	21	15	15.33	71.42	77.77	71.42	74.20
Open Pollination	180	144	216	180	30	35	42	35.66	16.66	24.30	19.44	20.10

Table 3 – Pollination system and fruit set of *Phaius tankervilleae*, at site- 2, Natunmati, Ward No. 4, Mariani, Assam

Treatment	No. of Flowers				No. of Capsule				Fruit Set %			
	2012	2013	2014	Mean	2012	2013	2014	Mean	2012	2013	2014	Mean %
Autogamy (Inflorescence were covered by plastic net bags)	60	60	72	64	Nil	Nil	Nil		Nil	Nil	Nil	
Self Pollination	28	14	17	19.66	22	10	14	15.33	78.57	71.42	82.35	77.44
Manipulated Geitonogamy	18	20	15	17.66	15	16	12	14.33	83.33	80	80	81.11
Manipulated Xenogamy	10	14	16	13.33	7	11	13	10.33	70	78.57	81.25	76.6
Open Pollination	216	242	264	240	26	31	45	39	12.03	12.80	17.04	13.95

Table 4 – *Phaius tankervilleae*, size of the labellum chamber as well as the body of *Xylocopa violacea*

Floral Measurement	Size (cm) Mean	Body parts	Size (cm) Mean
Length of Labellum chamber	3.8	Total body length	3.2
Width of the Labellum chamber	1.05	Width of thorax	1.05
Distance between floor of labellum and anther	0.8	Height of thorax	0.85
Distance from viscidium to the end of spur	3.0	Length from proboscis to metathorax	2.9
Length of the spur	1.15	Length of proboscis	1.3



Figs. 1 A-I – *Phaius tankervilleae* flowers, pollinators, visitors and fruit set. A. single flower; B. *Xylocopa violacea* perched on the labellum; C. *X. violacea* entering the labellum chamber; D. *X. violacea* coming out of the labellum chamber with a bunch of pollinaria attached on the thorax; E. *X. violacea* flying away with a bunch of pollinaria attached on the thorax; F. *X. violacea* magnified view of the thorax showing the attachment of yellow bunch of pollinaria; G. *X. violacea* magnified view of the thorax showing attachment of a viscidium; H. Lepidopteran perching over the flower; I. Fruits of *P. tankervilleae* at the basal portion of the inflorescence.

DISCUSSION & CONCLUSION

The present studies conclude that *Phaius tankervilleae* is self-compatible but non- autogamous at two study sites, Mariani town in Jorhat district of Assam State of North-eastern region of Indian sub-continent. Although, it exhibits self compatibility, yet, for pollination it requires a biotic agent. Various insects belonging to order Hymenoptera, Lepidoptera and Diptera visit the flowers of *P. tankervilleae* but *Xylocopa violacea* (carpenter bees) has been found as the only pollinators at Dissoi valley reserve forest as well as, at Mariani town in Jorhat district of Assam in North-eastern region of Indian sub-continent. Similar mode of bee pollination has also been reported by Van der Pijl & Dodson (1966) in *P. tankervilleae*. The autogamy, as reported by Catling (1990), Van der Cingle (2001) and Gandawidjaja & Arditti (1982) for various species of genus *Phaius* is absolutely absent in *P. tankervilleae* at the investigated localities. Since, the transfer of pollinaria from anther to stigma takes place through the dorsal surface of the thorax, the mode of pollination in *P. tankervilleae* is “Nototribic”. The open pollination rate is very low due to specificity of pollinators and the mechanism of pollination. The flowers to fruit ratio has been found as 5:1 at study site-1 and 6:1 at study site-2. As reported by Pellegrino *et al.* (2010) the fruiting in open pollinated flowers of *P. tankervilleae* takes place towards the basal parts of the inflorescence but absent at the upper part of the inflorescence, may be assigned to the “learning and avoidance” behavior of the pollinators as reported by Li *et al.* (2010) for an alpine species of *Phaius*, *Viz.*, *P. delavayi*. However, except for *X. violacea*, attachment of pollinaria has not been found on the body of any other visitors. As reported by Li *et al.* (2010) for *P. delavayi* and Jin *et al.* (2005) for *Holcoglossum rupestris* the attachment of pollinaria on more than one places of the body of insect pollinators has not been found in *P. tankervilleae*. However, as reported by Chaturvedi (2009), Chaturvedi & Chaturvedi (2010) and Buragohain *et al.* (2015) the attachment of pollinaria of orchids on the body parts of a particular pollinator is genus specific, has been found true for *P. tankervilleae* too. This specific attachment of pollinaria on a particular body part of a pollinator can be assigned as an adaptation to prevent the inter- generic hybridization in nature.

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