Pollination Studies in the Genus *Habenaria* Willd. (Orchidaceae) from Western Ghats, India

20

B. T. Dangat and R. V. Gurav

Abstract

Orchidaceae is one of the largest families of angiosperms with diverse reproductive mechanisms and an ideal plant group for pollination studies. In the genus *Habenaria*, structure of flowers is designed in such a way that it permits access to nectar in long, narrow tubes called as spur that project away from the lip. Insects with proboscis equaling the length of spur are able to withdraw nectar from such spur. During the present study, nine species of *Habenaria* were studied for pollination biology, out of which pollination of *H. longicorniculata* by *Agrius convolvuli* and diurnal pollination in *H. foliosa* var. *foetida* by blue tiger butterfly and hawk moth belonging to the genus *Dysgonia* and *H. furcifera* by *Parotis marginata* and *Hydriris sp.* have been carried out successfully, which undoubtedly forms new pollination record for India.

Keywords

Habenaria · Orchidaceae · Pollination biology · Western Ghats

20.1 Introduction

Orchidaceae shows incredible range of diversity in habit, shape, size, colour, fragrance of flowers, etc. Orchid flowers have developed interesting characters such as shape, colour, opening of flower, nectariferous spur and species-specific scent that attract various pollinators and visitors. This shows their reliance on cross-pollination

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S. M. Khasim et al. (eds.), *Orchid Biology: Recent Trends & Challenges*,
https://doi.org/10.1007/978-981-32-9456-1_20

by specific pollinators to enhance reproductive success. Orchidaceae is one of the largest families of angiosperms with diverse reproductive mechanisms and an ideal plant group for pollination studies. Orchid pollination and evolution are common topics in evolutionary biology. Perhaps the highly specialized mechanisms by which orchids are fertilised have been the subject of many studies, with the most well known being *The Various Contrivances by which Orchids are fertilised by Insects* by Darwin (1862). Darwin in his book famously argued that the long spurs (ca. 32 cm) of *Angraecum sesquipedale*, a Madagascan orchid species, represent an example of floral specialization for pollination by a long-tongued hawk moth. Arditti (1992) and Dressler (1993) suggested that the orchid floral structure is generally specialized to avoid spontaneous self-fertilization and promote insect-mediated outcrossing. From various reports, it is confirmed that the settling moth and hawk moth pollination in the Orchidiinae is widespread (Nilsson and Jonsson 1985; Nilsson et al. 1992; Johnson and Liltved 1997; Singer and Cocucci 1997; Singer 2001; Singer et al. 2007; Peter et al. 2009; Pedron et al. 2012; Dangat and Gurav 2014).

Habenaria is one of the largest terrestrial orchid genera represented in both the New and Old World tropics and subtropics, with a few species extending into temperate areas, particularly in eastern Asia (Pridgeon et al. 2001). The genus has a temperate and pantropical distribution with the major and main centres of diversity in Brazil, southern and central Africa and East Asia (Kurzweil and Weber 1992). The number of species is assessed earlier by various researchers, but due to continuous additions and deletion of several species, it is quite difficult to estimate exact number of the species; current estimates are about 835 species in the world (WCSP 2018). Brazil, with approximately 170 species (Hoehne 1940; Pabst and Dungs 1975), is the country with the largest number of *Habenaria* species in the New World and possibly also worldwide. The genus is distributed throughout India and represented by ca. 60–90 species including 35–40 endemic ones. In Western Ghats, it is represented by ca. 50 species with 25–30 endemic species (Misra 2007; Choudhury et al. 2011; Jalal and Jayanthi 2012).

The structure of flowers is designed in such a way that it permits access to nectar in long, narrow tubes called ‘spur’ that project away from the lip. Insects with proboscis equaling the length of spur are able to withdraw nectar from such spur. The insects with mouthparts capable of reaching into such long, narrow tubular flowers belong to Lepidoptera and long-tongued hawk moths. The proboscis of the hawk moths (Sphingidae) is among the most highly developed of Lepidoptera and adapted to probing and accessing nectar from the flowers with long tubes (Miller 1997). The size and flight patterns of long-tongued hawk moths combine to make them ideal agents for carrying pollen or pollinaria rapidly and efficiently over long distances between individual plants (Grant 1985; Nilsson et al. 1992; Johnson 1995; Johnson and Liltved 1997). In most of the moth-fertilised species pollinaria of *Habenaria* species have been reported to adhere to various smooth body parts of pollinators, such as the surface of the eye, proboscis and distal parts of the forelegs.

In many floristic works, species of *Habenaria* are poorly represented as they escape attention of workers due to their short lifespan. Due to the same reason,

Table 20.1 Pollination biology studies of *Habenaria* species in the world

Sr. no.	Name of the species	Name of the pollinators	Name of the authors	Year of studies
01	*Habenaria obtusata*	Mosquito	Thien	1969
02	*H. obtusata*	Mosquito	Thien and Utech	1970
03	*H. pleiophylla*	Passion vine butterfly	Moreira et al.	1996
04	*H. gourlieana*	Hawk moth	Singer and Cocucci	1997
	H. hieronymi			
	H. paucofilia	Settling moth		
	H. rupicola	Small moths or mosquitoes		
	H. montevidensis	(postulated)		
05	*Habenaria parviflora*	Crane-fly females and Pyralid moths	Singer	2001
06	*H. epipactidea*	Hawk moth	Peter et al.	2009
07	*H. johannensis*	Butterfly (Genus *Urbanus*)	Pedron et al.	2012
	H. macronectar	*Manduca rustica*		
	H. megapotamensis	*M. sexta*		
	H. montevidensis	*Eumorpha labruscae*		
		M. cf. *lucetius*		

aspects such as floral morphology, pollination biology and adaptive strategies are very poorly studied. Unfortunately, pollination biology of *Habenaria* has remained a neglected branch of study. Till date, a detailed study on pollination biology of 13 species of *Habenaria* from abroad has been carried out (Table 20.1), while in India there is not a single report (excluding Dangat and Gurav 2014) on the pollination biology of this beautiful genus. *Habenaria* is variously assessed and claimed to be a very large polymorphic genus comprising ca. 800 species, but unfortunately not more than 2% of the total species in the world are studied for pollination and pollination biology.

During various field study tours to different localities; size of area, accessibility to the area, other vegetation around the population, number of population, number of flowers per plant, opening and closing of flower, scent or odour of the flower, etc. were studied and noted in field notebook. For a detailed study of pollination biology, the method of Peter et al. (2009) was slightly modified and used during the present study, which is described below.

20.1.1 Selection of Study Site

Study site was selected depending upon observations on number, type and area of population including probable visitors and pollinators for that orchid population. A careful observation was done especially on the flowers for pollinaria removal or adhering of pollinia to stigma. The population showing removal of pollinaria or adhering of pollinaria to the stigma was selected at first sight; e.g. in the case of *Habenaria longicorniculata,* it was observed that in many of the flowers, stigma get

Table 20.2 List of *Habenaria* species selected for pollination biology study

Sr. no.	Name	Area of study	Year of studies	No. of days observation was carried out
1	*H. crinifera*	Amba, Maharashtra	2012–2016	25 25
2	*H. foliosa var. foetida*	Botanical garden, Department of Botany, SUK, Maharashtra	2013–2016	26
3	*H. furcifera*	Kasar-Kandgaon, Ajara, Maharashtra	2013–2016	16
4	*H. suaveolens*	Chowkul, Amboli, Maharashtra, Morjai Plateau, Gaganbawda, Maharashtra	2012–2016	25 18
5	*H. grandifloriformis*	Masai Plateau, Panhala, Maharashtra Shantiniketan, Morewadi, Kolhapur, Maharashtra	2012–2016	29 45
6	*H. heyneana*	Masai Plateau, Panhala, Maharashtra	2012–2016	17 22
7	*H. longicorniculata*	Bugate Alur, Karnataka	2012–2016	25
8	*H. rariflora*	Chowkul, Amboli, Maharashtra, Morjai Plateau, Gaganbawda, Maharashtra	2012–2016	27 18
9	*H. roxburghii*	Suttgati Ghat, Karnataka	2013–2016	25

fertilised or pollinaria were deposited on the stigma naturally at Bugate Alur, Ajara-Amboli Road, Karnataka, India. Population studied for present work was monitored since 2009 for its phenological details. As most of the species were white coloured, these were nocturnally fertilised therefore upon selection of final site, depending upon climatic conditions; i.e. without rain or in slightly drizzling conditions around 6:00 pm, sites were screened for the species morphology.

The present study includes nine species (Table 20.2) selected depending upon their population details and accessibility to the location.

(i) ***Habenaria longicorniculata***: The 12–15 cm-long spur of this species was the major reason and fascination for selection of this species for pollination studies. Earlier work suggested that such long spur species was fertilised by the long-tongued hawk moth only. Other criteria such as white colour of flower, nocturnal opening of flower and nocturnal emission of sweet scent were also the reasons for selection of this species.

(ii) ***H. foliosa var. foetida***: The typical foetid odour was the major reason for selection of this species. During frequent visit to the botanical garden where this species is growing in earthen pot, it has been observed that in sunny days around 10:00 to 11:00 am, the odour of the flower was very strong and lots of butterflies get attracted towards this species.

(iii) ***H. furcifera*:** During routine field tours while collecting this species, it has been observed that one green-coloured moth got stuck in a spiderweb. After critical investigation, it has been seen that pollinaria get attached to the proboscis of this moth. Green-coloured flowers, 2–3 cm-long nectariferous spur and camouflage of moth were the main reason for selection of this species.

(iv) ***H. rariflora* and *H. suaveolens*:** White-coloured large flowers, nocturnal emission of sweet scent, 4–5 cm-long nectariferous spur and both species growing together were the main reason for selection of this species.

(v) ***H. crinifera*:** This species also commonly known as dancing doll due to its incredible shape of flower was the major fascination for selection of this species along with other criteria such as white colour and long nectariferous spur for the study of pollination biology.

(vi) ***H. grandifloriformis*:** After the June–July rain, this was the first species which emerged out and grew profusely covering the entire ground. Nocturnal opening of the flower, white colour of the flower and long nectariferous spur were the main criteria behind selection of this species.

(vii) ***H. heyneana*:** Commonly known as toothbrush orchid, whitish yellowish small flowers with about 2 cm long nectariferous spur attracts many butterflies and small insects. This was the major reason behind selection of this species.

(viii) ***H. roxburghii*:** Small bright white-coloured flowers crowded in dense raceme with 3–4 cm-long nectariferous spur were the main reason behind selection of this species.

20.1.2 Species Morphology

Morphological data related to pollination biology such as colour, opening, size, smell or odour of flowers, labellum, spur size, number of population, number of flowers per plant, number of flowers carrying pollinaria or the number of flowers from which single or both pollinaria were removed was observed, and data was recorded in the field notebook. Observations were recorded from 6:00 pm to 8:00 am. This time schedule was adopted since previous observations indicated that flowers of most of the species (e.g. *Habenaria grandifloriformis*, *H. longicorniculata*, *H. suaveolens*, *H. rariflora*) open and emit sweet, pleasant fragrance at dusk (ca. 8:00 pm) suggesting nocturnal pollination. In the case of *H. foetida*, it emits foetid smell throughout the day; photographs were taken with a Nikon D90 digital SLR camera. Digital images were edited and assembled on plates using Adobe Photoshop 7.0 (San Jose, CA, USA).

20.1.3 Pollinator Behaviour

Pollinator and visitor behaviour towards inflorescence and a particular flower was recorded with the help of low-power torch/flashlight. After every 10–15 min.

Intervals, patches of study area were flooded with flashlight to detect probable visitors or pollinators. All available inflorescences were observed for the visits of pollinators. Since most of the observations were made in the dark, it directly refers to pollinator behaviour as was perceived. It was not possible to confirm when the insects arrived or how many inflorescences they had already visited until they were noticed. Once the activities of pollinators or visitors were sighted, careful observation was made on the activity of the probable pollinators or visitors with torch.

Its foraging, landing pattern, insertion of proboscis and removal of pollinaria were observed by doing photography in the field. Insect effectively removing pollinaria or the insect with adhered pollinaria on body part such as eye, head or proboscis were considered as potential pollinators, while those lacking all these features were considered as probable visitors. After photography, an effort was made to count the total number of pollinators; depending upon their number, two or three potential pollinators were captured with the help of a butterfly net, so that it would not hamper or disturb their natural population. Pollinators and visitors were captured with the help of butterfly net and observed for number of pollinaria attached, site of attachment and proboscis length. These observations were recorded with the help of field notes and photographs. Visits to flowers made by each visitor insect were recorded, along with their number, surrounding environmental conditions (local weather and cloud cover), number of individual open flowers and number of flowers visited. Upon capturing the potential pollinator, it was brought back to the tent and observed carefully for attachment site of pollinaria. In laboratory, it was treated with formaline and mounted on thermacol sheet with wing and proboscis spread out using blunt head pins. Measurement such as length and width of entire body, wing span, proboscis and colour of the potential pollinator was recorded.

20.1.4 Scent

Scent of fresh inflorescence was determined by the human nose at an interval of 10–15 min between varying times for different species, e.g. for *Habenaria longicorniculata* J. Graham from 6:00 pm to 8:00 am. Hundred individuals per species were sampled for this purpose.

20.1.5 Male Efficiency Factor

Male efficiency factor was calculated by Nilsson et al. (1992) method. Percentage of fertilised flowers divided by the percentage of flowers acting as pollen donors was calculated for 50 inflorescences. The main criteria for selection of the inflorescence were its freshness. Stigmatic surfaces have to be fresh in order to confirm that they are fertilised or the flower withered naturally.

20.2 Pollination in Genus *Habenaria*

During the present study, nine species of *Habenaria* were selected for pollination biology studies. Detailed list of target species, number of attempts made and sites of study is provided in Table 20.2. Among the nine species, pollination of three spp., viz. *Habenaria foliosa var. foetida* Blatt. and McCann, *H. furcifera* Lindl. and *H. longicorniculata* J. Graham, has been carried out successfully, which undoubtedly forms new pollination record for India.

(a) Pollination of *H. longicorniculata* J. Graham by *Agrius convolvuli* L.
(b) Diurnal pollination in *H. foliosa* var. *foetida* (Blatt. and McCann) Bennet by blue tiger butterfly (*Tirumala limniace*) and hawk moth belonging to genus *Dysgonia*.
(c) Pollination of *H. furcifera* Lindl. by *Parotis marginata* Hampson and by a moth (*Hydriris* sp.) was reported for the first time from India.

20.2.1 Pollination Biology of *Habenaria longicorniculata* J. Graham (Figs. 20.1 and 20.2)

H. longicorniculata flower is characterized by long nectariferous spur up to 15 to 20 cm in length and therefore locally called 'long-tail orchid'.

Floral Morphology *H. longicorniculata* is the only Indian species having the longest tube-like slender nectariferous spur ca.12–20 cm (Fig. 20.1a, b). Among the studied population, a range of 10–15 cm-long spur has been observed with a mean of 13 cm, frequently containing abundant nectar. The population shows inflorescence height up to ca. 70 cm, bearing maximum five flowers per inflorescence, with mean flower number of three. The white-coloured flowers with sweet scent, which is high during dusk compared to other times, open by evening from 5:30 to 6:30 pm. Individual flower remains open for a period of 2–3 weeks until they are either fertilised or eventually wilt and drop down from the inflorescence stalk, which also dries up if there is no fruit set. The number of flowers open on a single plant is generally 3–4. The flowering pattern was found to be inconsistent at different localities, which depends on rainfall. Plants start to grow in late July and bloom in the month of August which may remain up to mid-September according to climatic conditions.

A typical flower shows entirely white petals with tri-lobed lip (Fig. 20.1c, e), lateral lobes of the lip broader than the middle with entire margin. Sepals are green coloured forming a dome-like structure, consisting of a column (Fig. 20.1c, d) on which sticky green stigma is raised; stigma is divided into two lobes situated on

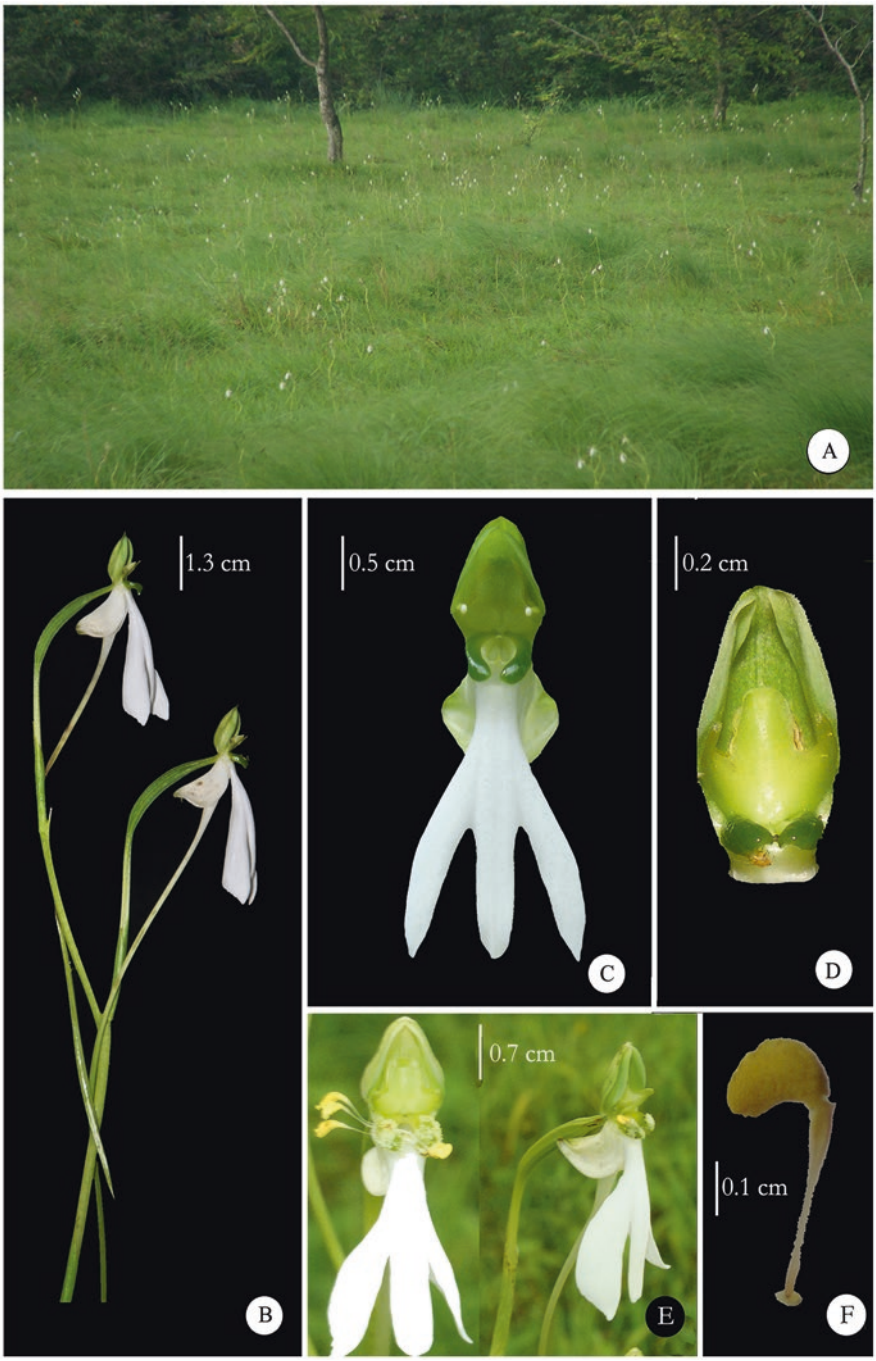

Fig. 20.1 Morphology of *Habenaria longicorniculata* J. Graham. (**a**) Habitat. (**b**) Inflorescences. (**c**) Entire flower. (**d**) Column view. (**e**) Fertilised flowers (front and lateral view). (**f**) Single pollinarium

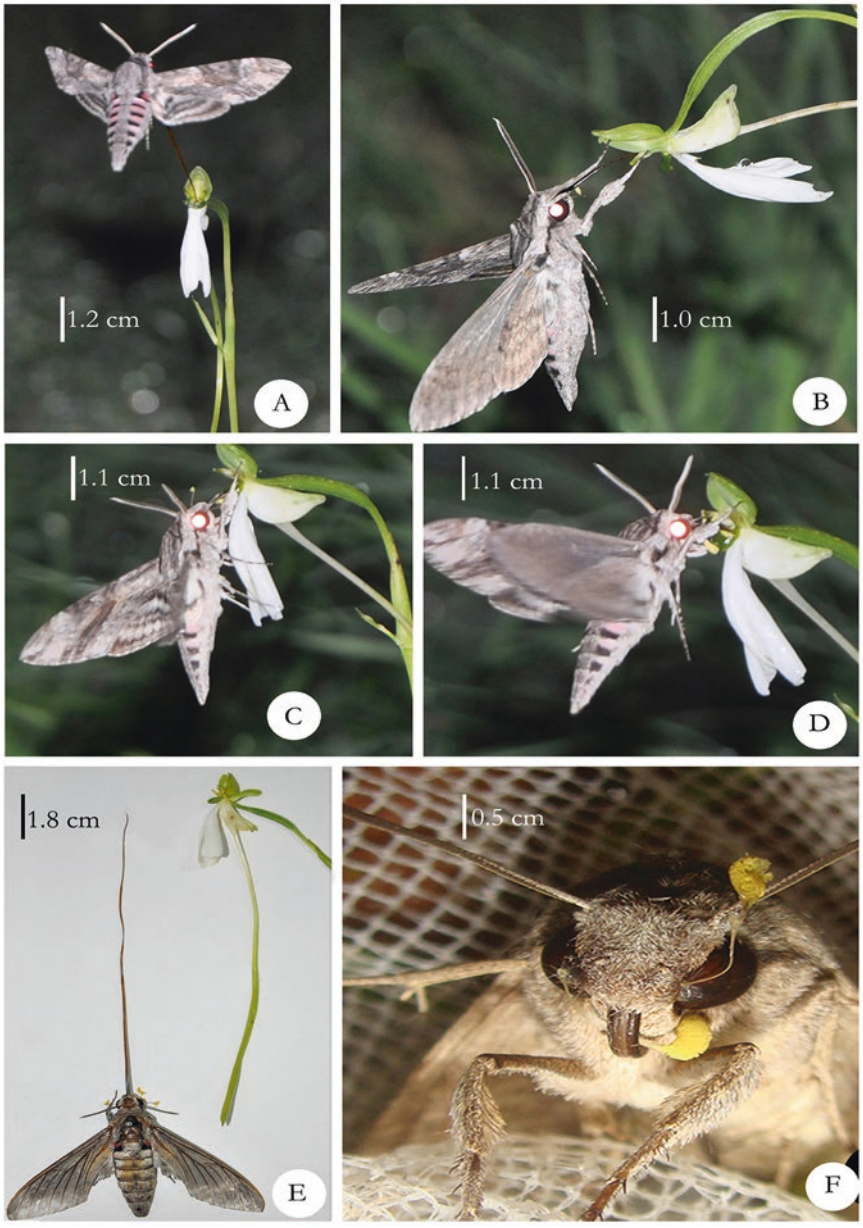

Fig. 20.2 Nocturnal activity of hawk moth during pollination. (**a**) *Agrius convolvuli* probing flower of *Habenaria longicorniculata.* (**b**) *A. convolvuli* with proboscis fully inserting into the spur. (**c**) *A. convolvuli* landing on labellum. (**d**) Transfer of pollinaria on stigma. (**e**) Comparison of proboscis and spur. (**f**) An individual of *A. convolvuli* bearing pollinaria

both sides of the opening to the spur, which is very narrow and therefore affords a passage for the proboscis of Lepidopterids only. Rostellum is present on the opening of the long slender nectariferous spur and is distinctive with the equidistantly separated viscidia from each other. Anther sacs containing the pollinaria are concealed behind the rostellum. Pollinaria (Fig. 20.1f) length varies from 0.5 to 1 cm, yellowish, consists of pollinia and composed of numerous massulae with numerous tetrahedral to rounded pollen grains. Pollinia are attached with elastic thread-like caudicle with a disc-shaped sticky viscidium at the base.

Pollinator Behaviour (Fig. 20.2) After about 120 h of critical and careful observation, 11 individual hawk moth visiting bouts to flowers were recorded. Duration of visit of the hawk moth varies greatly with rainfall and local climatic conditions. They generally are not in flight in heavy rains and strong winds. During overcast, cloudy and drizzly conditions without rain and cool breeze, 12 hawk moths came out to visit the flowers for food. Maximum activity was seen between 8:30 and 11:00 pm, while minimal activity was seen beyond midnight onward up to 3:00 am. An individual visit of a hawk moth to a particular flower ranges between 2 and 10 s. Cold breeze spreads sweet scent in the air and attracts the hawk moths.

Upon arriving on the flower, hawk moths hover in front of the flowers twice or thrice, with coiled proboscis. Then, the moth bends slightly to probe the entrance of an individual flower (Fig. 20.2a, b). It uncoils the proboscis and inserts it gently into the flower. For complete insertion of proboscis into the spur, it lands on the labellum. During this course of probing, it lands its forelegs on the labellum, then for complete insertion of proboscis, it anchors the hind legs on the connate head of sepals of flowers (Fig. 20.2c, d). This action of foraging takes about 2–5 s. During this action, the inflorescence axis bends at an angle of nearly 40°–60° towards the ground (Fig. 20.2b). After foraging and feeding on the available nectar, the hawk moth withdraws its proboscis and flies away. Upon continuous flashing torch lights, it flew up into sky to a height of about 30 m.

Pollinator Morphology and Identity Hawk moth caught on flowers with the help of butterfly net was identified as *Agrius convolvuli* L. with a common name hawk moth. Normally it keeps the proboscis coiled under the head and extends it when probing flowers for feeding. The proboscis length was 13 cm in the female, while in the male it was up to 10 cm (Fig. 20.2e). Due to limited number of moths, only three individuals were collected, so that their population will not be disturbed. During this study other visitors observed are *Leucophlebia lineata*, *Leucophlebia emittens*, *Pygospilatyres*, *Syntomoides imaon*, *Trigonodes hyppasia*, *Neoscona rumpfi*, *Oxyopes birmanicus* and *Hesperotettix speciosus*.

Pollination Mechanism Hawk moth hovers on to the flowers to insert proboscis in the spur; at the same time it presses the sticky viscidium with the base of its head. The pressure of head and sticky glue on the viscidium detaches the pollinaria by

pulling them out on the attached stalks. This involves the momentary lifting of the 'cap' that covers the pollinaria as they detach. Viscidium was found attached to the surface of the eyes also in one case (Fig. 20.2c, f). Once attached to the hawk moth, the pollinaria rest against the head or base of the proboscis. When a hawk moth carrying pollinaria probes another flower, the head comes up and flushes against the lower inner part of the column. Here the pollinaria are easily transferred and massulae comes in contact with the sticky stigma (Fig. 20.2d).

Scent At evening near about 6:00 pm, flowers start emitting a sweet and pleasant scent which is at maximum during 9:00–11:00 pm. As time passes, gradually it decreases and around 3:00 am it reduces greatly.

Male Efficiency Factor To calculate male efficiency factor, 220 fresh flowers from 50 inflorescences were screened. About 169 (77%) flowers had their stigma fertilised, while 127 (58%) flowers were found to act as pollen donors. It was also observed that 97 (44%) flowers had only one pollinarium removed, while 69 (31%) flowers had both pollinaria removed. The male efficiency factor was ca.1.33, i.e. 1.3 flowers fertilised per pollinarium removed.

20.2.2 Pollination Biology of *Habenaria foetida* (Blatt. and McCann) Bennet

This species is characterized by foul/foetid smell of flowers throughout the day. The present study involved observations of population of *Habenaria foetida* growing in the Botanical garden, Shivaji University Campus, Kolhapur, from the emergence of inflorescence up to fruit setting, both during the day and night.

Species Morphology (Fig. 20.3) *H. foetida* is a tuberous, perennial, robust herb, widespread throughout Western Ghats. Stem is erect and stout, up to 50 cm; leaves scattered along the stem; inflorescence lax, 35–50 cm (Fig. 20.3a, b); length of spur ca. 4 cm; flowers 12 per inflorescence, white with green tinge, subsessile, foetid smell during the day; sepals unequal, 3-nerved, green; petals white, 2-partite, 3-nerved, lip greenish white, 3-partite to the base, spurred, up to 4 cm (Fig. 20.3c, d); pollinia 2, obliquely ovate in outline, caudicle slender, dilated towards the base and attached to a small, yellow, sticky viscidium (Fig. 20.3e); stigma sub-cylindrical, blunt, recurved; and entrance to the spur lies between their base. Flower remains open for a period of 12–15 days until pollination or finally wilting. It was observed that in different localities, due to variation in climate, period of vegetative growth and flowering slightly varies. Generally plants start to grow in early August and are in full bloom after a month and half up to mid-September.

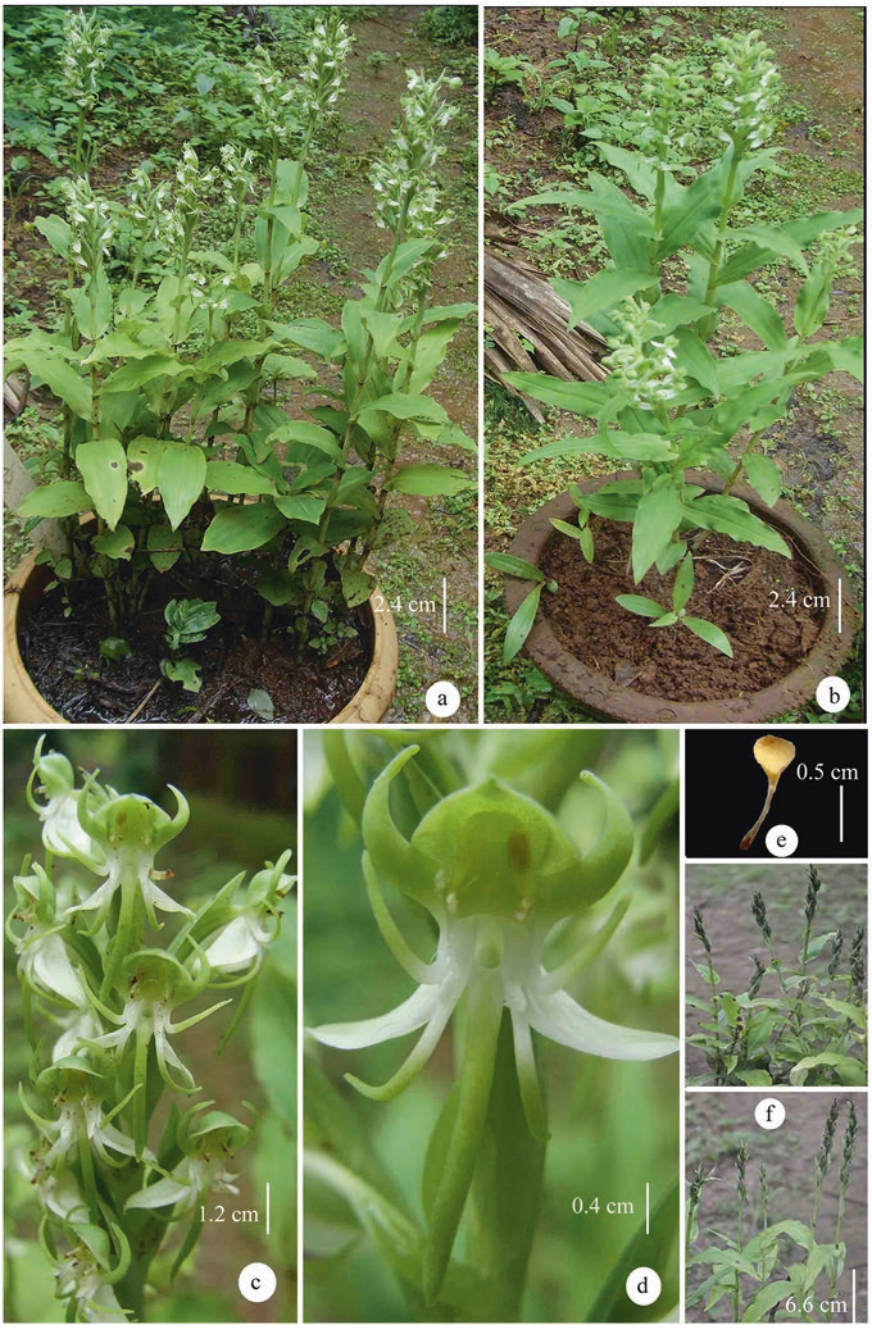

Fig. 20.3 Morphology of *Habenaria foetida* Blatt. and McCann. (**a** and **b**) Habit. (**c**) Inflorescence. (**d**) Entire flower. (**e**) Single pollinarium. (**f**) Capsule formation after pollination

Pollinators Present study confirmed that (hawk moth) *Dysgonia* sp. and (blue tiger butterfly) *Tirumala limniace* Cramer are the pollinators of *Habenaria foetida*. The length of proboscis in blue tiger is 4 cm and in hawk moth 3.6 cm.

Pollination Mechanism (Fig. 20.4) Blue tiger butterfly and moth visits were recorded. The time of visits vary greatly. They were not seen in heavy rain or strong wind. Butterfly visited in sunny condition, but hawk moth even visited during overcast and cloudy conditions. Visitors such as *Danaus chrysippus*, *Euploea core*, *Neoscona rumpfi* and *Hesperotettix speciosus* were also observed. Activity of blue tiger butterfly was from 10:30 to 11:30 am. In the case of moth, activity was observed during 8:30 to 9:30 pm. A single visit to a flower by blue tiger butterfly took between 10 and 30 s, whereas visit by the moth took 2–6 s. Both pollinators show similar activity like hovering around the inflorescence with coiled proboscis, landing on the labellum, uncoiling of proboscis and inserting into the spur.

While inserting the proboscis, insects bend their head which nearly enters into the connate hood of sepals (Fig. 20.4a–g). The foraging lasts for 10–30 s; blue tiger butterfly withdraws its proboscis and moves to the second flower by walking or flying. Hawk moth withdraws its proboscis and hovers around the inflorescence again. Moth hovers, while blue tiger butterfly sits on the flower to insert proboscis in the spur. Head of the insect presses the sticky viscidium at the base, detaches and pulls the pollinaria out on the attached stalks. It involves momentary lifting of the 'cap' that covers the pollinaria. Viscidium with pollinarium was found on the proboscis and sometimes on other parts of the head in hawk moths (Fig. 20.4n) and to the eyes or antennae in the blue tiger butterflies (Fig. 20.4f, g). When a moth carrying a single or many pollinaria probes another flower, the head comes up and flushes against the lower inner part of the column. The pollinarium/pollinaria gets transferred onto the sticky stigma (Fig. 20.4m). The butterfly carried more pollinia than the moth, i.e. it may be a more efficient pollinator than the moth.

Male Efficiency Factor Fresh flowers about 160 collected from 22 inflorescences were screened, of which 145 (90%) flowers were fertilised. 112 (70%) flowers had lost their pollinia, of which 87 (54%) flowers had only one pollinarium removed and 67 (35%) had both removed. The male efficiency factor is 1.29.

20.2.3 Pollination Biology of *Habenaria furcifera* Lindl

This species is characterized by robust habit, with several large cauline leaves, densely lax raceme up to 40–45 cm. Flowers are dark greenish to yellowish green with equally tri-lobed lips with deflexed side-lobes and thin lip; spurs are longer than the ovaries. This species is often confused with *H. ovalifolia*. But it has a distinctive species status on the basis of midlobe of the lip which is touching the sepals in *H. ovalifolia* while spreading and deflexed in *H. furcifera* (Plate – 21). Present

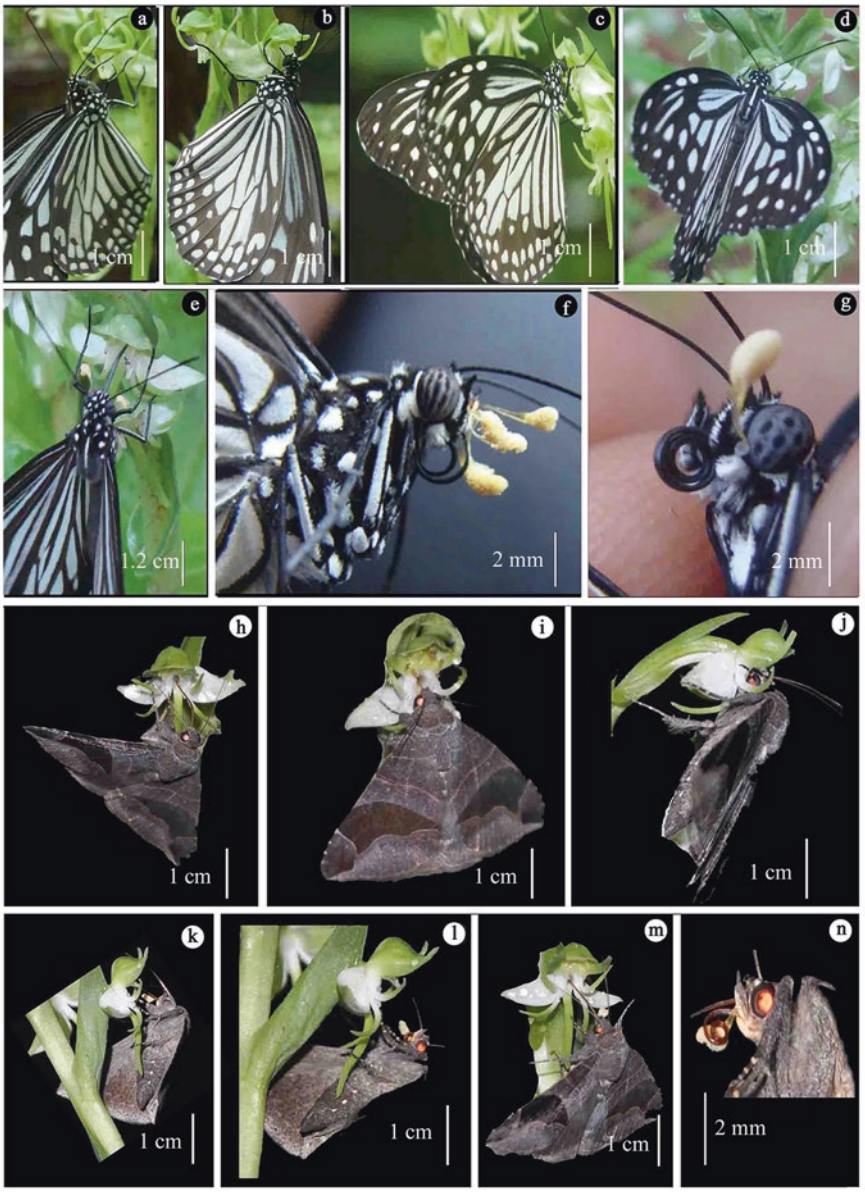

Fig. 20.4 Activity of butterfly and hawk moth during pollination. (**a**–**g**) *Triumala limniace* Cramer. (**a**–**d**) Probing flower of *Habenaria foetida.* (**e**) Removal of pollinaria on its eyes. (**f**) An individual bearing pollinaria on its eyes. (**g**) Eye attachment of single pollinarium. (**h**–**n**) *Dysgonia* sp. (**h**–**j**) Probing flower. (**k**–**l**) Pollinarium removed from the flower. (**m**) Transfer of pollinarium to another flower. (**n**) Pollinarium attached to head region

study involved observations of population of *H. furcifera* growing under dense forest or under the canopy of trees, at Kasar-Kandgaon, Tal-Ajara, Dist.-Kolhapur, Maharashtra, India, from the opening of flower up to fruit setting, throughout day and night. Flowers are dark green to yellowish green, flowering starts late in the month of September and fruiting up to December (highly dependent on local rainfall). Flower remains open for a period of 10–15 days until fertilised or up to natural withering or wilting.

Species Morphology (Fig. 20.5) Plants are 30–70 cm tall, green, erect, terete, slender, and glabrous with four to seven leaves which are long, spreading, cauline and in a cluster in the basal one-third portion of the stem (Fig. 20.5a). Inflorescence is long, stout, erect, bracteate, glabrous, densely lax, raceme with many flowers and rachis is 20–40 cm long (Fig. 20.5b). Flowers are small, 6–15 mm in size, pale to dark greenish in colour, shortly pedicellate and bracteate and in long stout, erect, densely lax inflorescence (Fig. 20.5c). Sepals are spreading or reflexed, subequal or unequal, 3-nerved, rounded to sub acute while petals are long, erect, broadly oblong-lanceolate, retuse, sub-falcate, slightly broader than the sepals as long as the dorsal sepal, forming a hood with middle and dorsal sepal over the column. Lip is longer than the sepals, linear, deeply divided at the base into three lobes and distinctly trilobed. Spur is very slender, cylindric, pendulous, 1.7–3.0 cm long, longer than the ovary, laterally compressed and curved at the apex. In present analysis 3 cm is the longest length of the spur.

Pollinator Morphology and Identity (Figs. 20.6 and 20.7) Two moths were captured pollinating different flowers of the present species. After critical examination and consulting with experts, one is identified as *Parotis marginata* Hampson (green-coloured moth) and *Hydriri ssp*. (brown colour). Both are small in size having wing span about 4–4.5 cm. Proboscis in green-coloured moth is up to 3–4 cm, while in brown-coloured moth, it is 3–3.6 cm. Seven green-coloured moths and five brown-coloured moths were observed pollinating different flowers on the same or different inflorescences. Considering orchid conservation and current threats to orchids and their pollinators due to various anthropogenic pressures, only one moth pollinator is captured for detailed study such as site of attachment of pollinia, spur length, etc. No visitors were observed.

Pollinia Transfer by Hawk Moth (Fig. 20.6) (i) ***Parotis marginata*** – It gets camouflaged and is very difficult to locate in the dark. It shows activity in the evening or dusk; at about 7:00–7:30 pm, it comes out for foraging. This activity continues up to 11:30 pm. Only one moth was observed foraging on plants at 1:40 pm. Maximum activity of this moth is observed between 7 and 9 pm. As number of plants was more, i.e. 14 plants growing in vicinity of each other, and number of moths foraging on this plant was also large, i.e. 7, it was quite difficult to identify the individual visitation

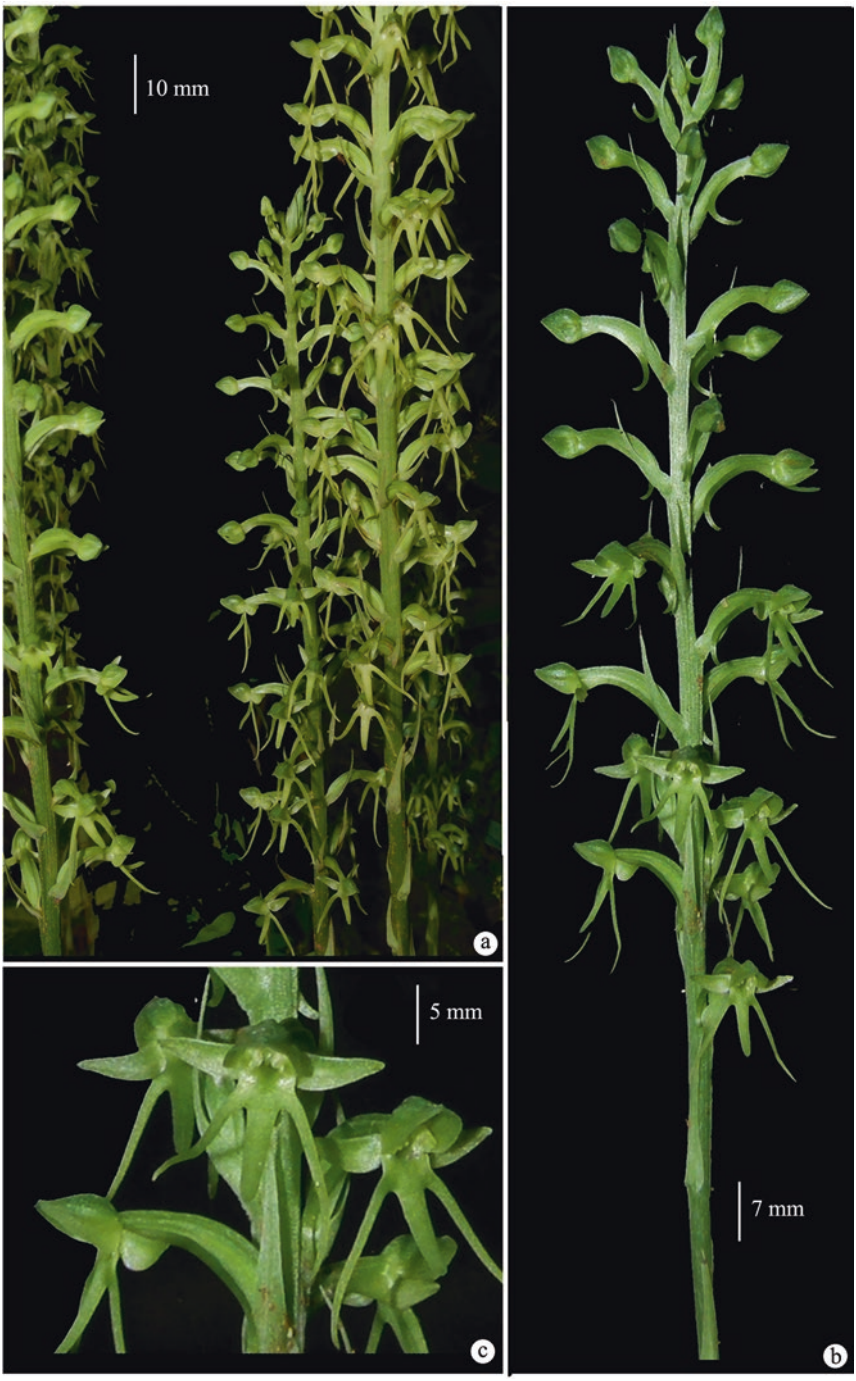

Fig. 20.5 Morphology of *Habenaria furcifera* Lindl. (**a**) Habit. (**b**) Inflorescence. (**c**) Close-up of flowers

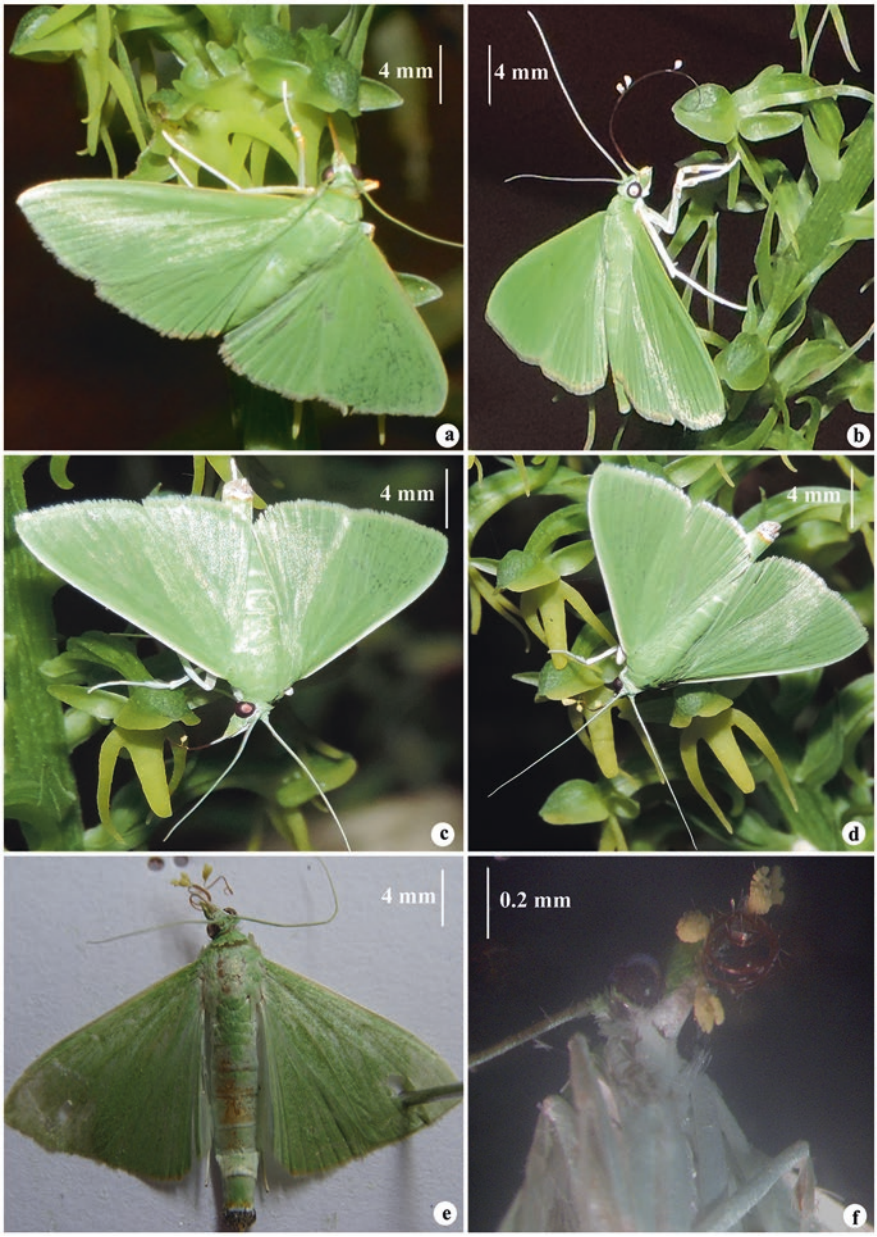

Fig. 20.6 Nocturnal activity of *Parotis marginata* Hampson. (**a**) Moth probing flowers of *Habenaria furcifera* with proboscis completely inserted into the spur, (**b**) removal of pollinaria, (**c**) transferring pollinaria to other flowers (cross-pollination), (**e**) entire moth with pollinaria attached to proboscis, and (**f**) proboscis attachment of pollinaria

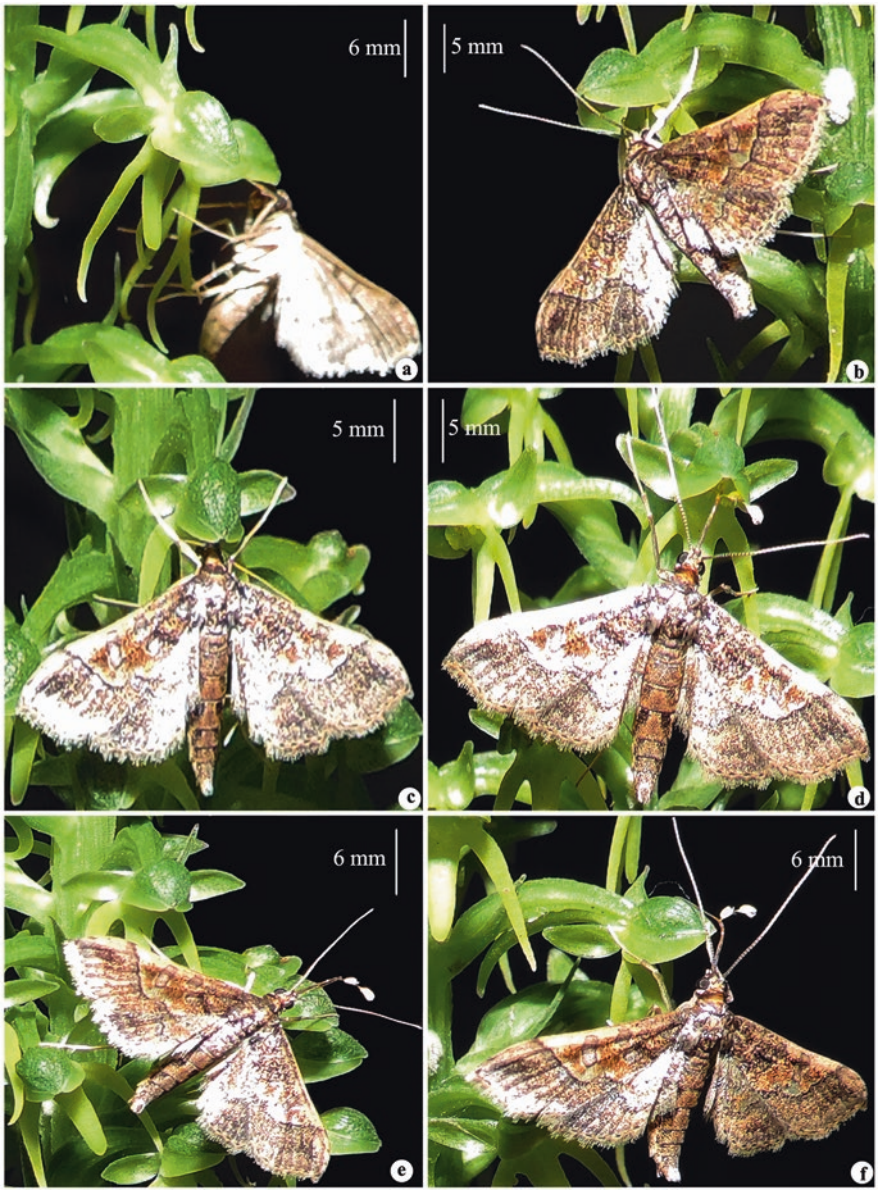

Fig. 20.7 Nocturnal activity of moth (*Hydriris* sp.). (**a**–**c**) Moth-probing flowers of *Habenaria furcifera*, (**d**) removal of pollinarium, (**e**) transferring pollinaria to other flower (cross-pollination), and (**f**) proboscis attachment of pollinaria

bouts. This moth flies over the inflorescence with coiled proboscis and lands on the flower, sometimes upside down, uncoil its proboscis and probe the flower. Probing of flower continues from a few seconds to 1 min, and then it walks on the inflorescence and probes another flower (Fig. 20.6a–f). As the inflorescence is dense, compact and many-flowered, it is quite difficult to reveal whether it probes the flower sequentially or arbitrarily. As length of its proboscis is longer than the spur, it simply inserts proboscis into the spur without inserting its head into the connate hood, which generally occurs in case of *H. longicorniculata*. Since there is no contact of head region to the column while probing, only pollinia get attached to the proboscis. At a time one to two pollinia get attached near the middle of the proboscis, and a maximum four pollinia were observed adhering to the proboscis. Once pollinia get attached, and the moth tries to forage another flower, they get transferred to the other flower, ensuring effective cross-pollination. Pollination and foraging activity is strictly restricted during night. This moth is a little phototactic; when torch light is flashed, it flies away for a few minutes and comes back immediately after 5–10 min when it is dark. As the flowers do not have any smell, this moth is attracted for the nectar only. Hence, food deception plays important role in this studied species.

(ii) ***Hydriris sp.*** (Fig. 20.7) – This moth shows activity at night, i.e. strictly nocturnal; it arrives at the inflorescence around 9–9:30 pm. It hovers over the inflorescence with coiled proboscis and lands on the flower, where labellum plays a role of landing platform. After arriving at the flower, it uncoils the proboscis to insert it into the spur. As length of the proboscis is same or little smaller than the spur, it partially inserts its head into the connate hood formed by sepals and petals. While probing, pollinia get attached to the distal end or sometimes to the middle of the proboscis. Individual visitation bouts range four to five per moth in between 9 and 11 pm. Maximum activity of this moth is observed during 9–9:30 pm; as time passes, activity gradually decreases, and after 11 pm, activity of this moth completely stops. Foraging of single flower lasts for a few seconds, i.e. 2–10 s (Fig. 20.7a–f). After this, it flies away and comes back again in 5–10 min. It is highly negatively phototactic; upon illumination of light, it immediately flies away and comes back after 10–15 min. Once pollinia get attached while probing one flower, when it comes for probing another flower, pollinia effectively get transferred to the other flower; thereby efficient and effective cross-pollination takes place.

Male Efficiency Factor 340 fresh flowers from 12 inflorescences were screened, among which 207 (61%) flowers confirmed pollination, while 122 (36%) flowers were pollen donors. It was also observed that 97 (44%) flowers had only one pollinarium removed, while 69 (31%) flowers had both pollinaria removed. The male efficiency factor was ca.1.69, i.e. 1.7 flowers fertilised per pollinarium removed.

During the present study, nine species were targeted for pollination biology studies, of which pollination biology of three species, viz. *Habenaria longicorniculata*,

H. foliosa var. foetida, and *H. furcifera*, was successfully carried out. The remaining six species, viz. *H. crinifera*, *H. suaveolens*, *H. grandifloriformis*, *H. heyneana*, *H. rariflora* and *H. roxburghii*, were also observed. Visitors, such as butterflies and unidentified moths, were observed visiting flowers of *H. heyneana*, *H. roxburghii* and *H. crinifera*. In the case of *H. grandifloriformis*, *H. rariflora* and *H. suaveolens*, no visitors and pollinators were observed maybe because of climatic conditions, as all of these species grow during heavy monsoon. During this period, either there is heavy rainfall or heavy fog which directly affects the activity of pollinators and visitors, so it is quite difficult to observe probable visitors and pollinators. It has been observed in previous studies that during heavy rainfall moths or butterflies never come out for foraging. This may be the reason leading to unsuccessful studies in pollination biology of these species.

During the present studies, it has been observed that either visual cues such as colour, shape and size or olfactory cues such as scent/odour play an important role in pollination biology of *Habenaria*. Food deception is the main reason behind pollination of all the species studied; nectar is rewarded for the pollinator as a food.

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