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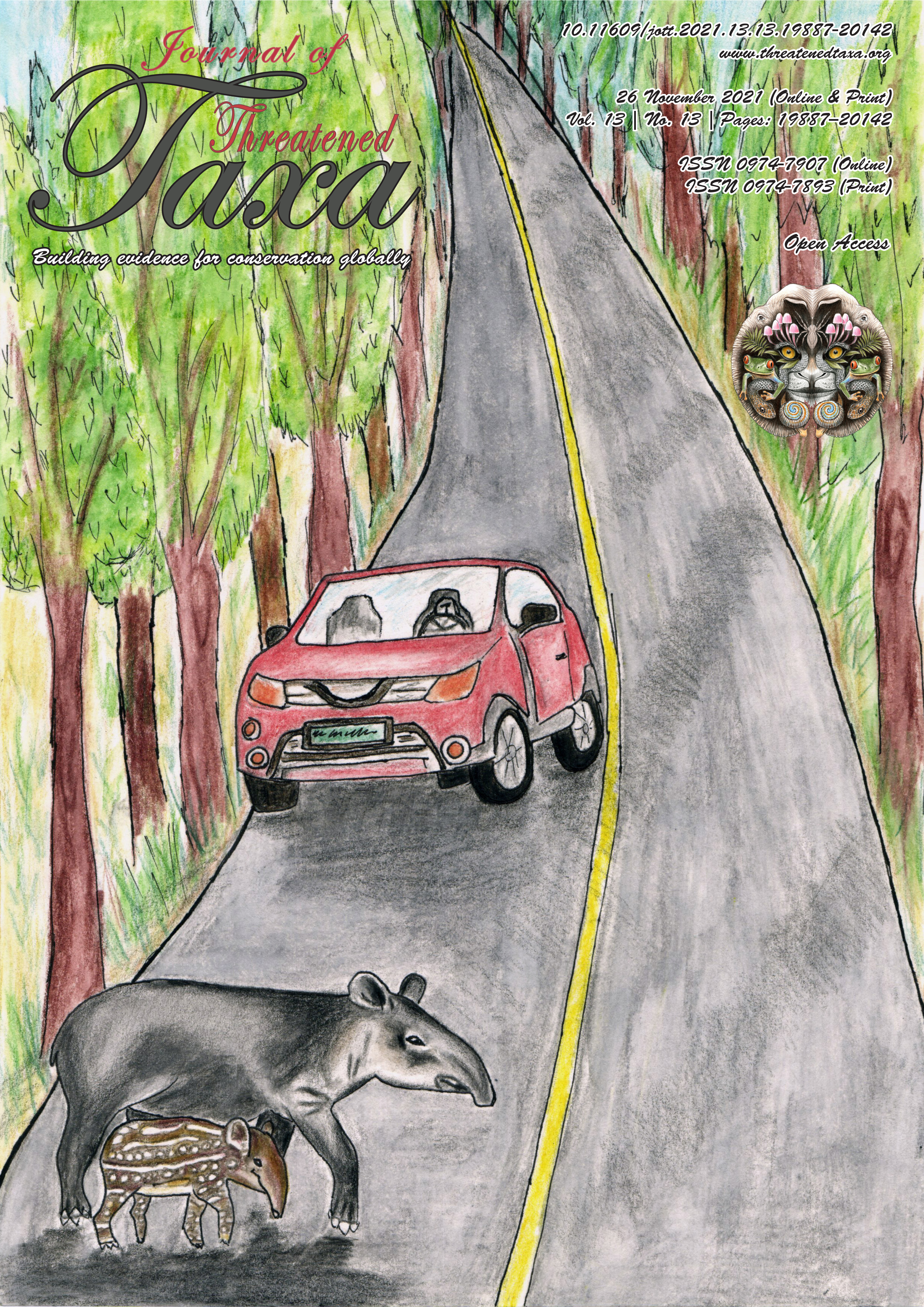

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Caption: Lowland Tapir *Tapirus terrestris* (Medium—watercolours on watercolour paper) © Aakanksha Komanduri.

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COMMUNICATION

Reproductive biology of *Ophiorrhiza caudata* C.E.C.Fisch. (Rubiaceae), an endemic and endangered creeping perennial herb of the Western Ghats, India

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Abstract: *Ophiorrhiza caudata* is a creeping, perennial herb distributed along wet and shady areas. The species is distylous with two distinct floral morphs: pin and thrum. Flowering usually occurs during the monsoon season. No particular difference was noticed in the flowering phenology of the two morphs. Presently the species is self-incompatible, however, it shows a tendency towards intramorph compatibility. Fruit set is above 60% in open pollination and intermorph pollination. Bees and butterflies are the major pollinators. The pollen flow between the two floral morphs varies depending upon floral morphology and pollinators. Fruit is a bi-valved capsule which dehisces by a splashing drop mechanism. The seeds are very minute. The rate of seed germination and seedling establishment in the wild condition is very poor due to adverse climatic factors. *Ophiorrhiza caudata* is struggling for survival in its natural habitat, where habitat fragmentation, climatic factors and poor seedling establishment could account for its narrow distribution.

Keywords: Floral morphs, flowering phenology, perennial herb, pollination, seed germination.

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Author details: Dr. Maria Theresa has been awarded PhD by the University of Kerala. She joined as assistant professor (on contract) in the department of botany, Newman College, Thodupuzha, Idukki. Dr. A.K. Sreekala is working as Principal scientist in the division of conservation biology, JNTBGRI, Palode. She has published more than 85 research papers in national and international journals. Also presented 60 research papers in national and international conferances. Dr. Jayalakshmi Mohanlal has been awarded PhD by the University of Kerala and presently she is working as guest lecturer in the department of botany, Vimala College (Autonomous), Thrissur.

Author contributions: All the three authors contributed equally for the study as well as MS preparation.

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INTRODUCTION

The genus *Ophiorrhiza* L. (Rubiaceae) is believed to be Indo-Malaysian in origin (Mabberly 2008), and different species are scattered throughout tropical-subtropical Asia, New Guinea, Australia, and the Pacific islands. The roots of these herbaceous plants were used against snake venom from ancient times, and various plant parts are characterised by the presence of the alkaloid camptothecin and its derivatives (Yamazaki et al. 2003). Camptothecin is an inhibitor of the enzyme topoisomerase-1 (Uday & Kondapi 2010) and has anti-cancerous properties. Some *Ophiorrhiza* species such as *O. barnesii*, *O. brunonis* and *O. incarnata* are also under threat of extinction due to climatic change, habitat disturbance, natural calamities and obstruction to pollination mechanisms and reproduction.

The genus has been considered as distylous (Deb & Mondal 1977) with two floral morphs: pin (long-styled) and thrum (short-styled). Distyly is usually associated with self-incompatibility, that is, the flower of one morph cannot be fertilized by the pollen from the same flower or from another flower of same morph. This kind of heteromorphic incompatibility is reported from 25 plant families including Rubiaceae (Ganders 1979; Lloyd & Webb 1992). Darwin (1877) considered distyly as an adaptation to promote cross breeding. *Ophiorrhiza caudata* C.E.C.Fisch. is a creeping herb distributed along the wet and shady regions of southern Western Ghats. The species was considered extinct (Deb & Mondal 1997), and IUCN (1997) included it under the extinct category. It was rediscovered in 2009 (Joseph & Joseph 2009), 70 years after its last report. Considering the medicinal value and present status of this endemic distylous species, a study on its reproductive mechanism is needed for conservation. The present investigation was carried out during 2013–2016 to examine the flowering phenology, floral biology, breeding system, pollination and seed biology of *O. caudata*.

MATERIALS AND METHODS

Study area

A clumped population of *O. caudata* was located in the Mankulam and Kallar forest areas of Idukki district (Image 1a) in Kerala (10^{0}11.9230”N 76^{0}92.884”E, 340–2,102 m). The average rainfall in the area ranges from 2,500–3,000 mm, with 70% occurring during the south-west monsoon; mist and frost prevail during the winter months and high humidity during the monsoon season. *O. caudata* is distributed along the wet and shady areas of Mankulam and Kallar (Figure 1). *Ophiorrhiza mungos, O. barberi*, and *O. barnesii are* the other *ophiorrhiza* species distributed along this area. *Dictyospermum montanum, Neanotis decipiens, Plectanthus malabaricus, Cleome speciosa, Impatiens elegans, Impatiens maculata, Cyanotis pilosa*, and *Pilea melastomoides* are other associated plants in the area.

Phenology and floral morphology

Twenty-five healthy individuals of approximately the same age from both floral morphs (i.e., pin and thrum) were marked, and periodic observations were made on different developmental events from the emergence of vegetative buds up to seed germination and successful establishment of seedlings. The observations were made as per the method suggested by Dafni (2007). The time of anthesis and anther dehiscence, flower colour and odour, nectar production, stigma type, and flower longevity were noticed. The flower morphology of each morph was studied with the help of hand lens and dissection microscope. Floral measurements were taken in millimetres by using a digital vernier calliper. For this purpose, 20 flowers of each morph were collected from the field, preserved in 70% ethanol and detailed study was conducted in the laboratory. This was helpful to analyse heterostyly in the species.

The mean number of pollen grains per flower was calculated by dissecting a single anther in a drop of acetocarmine: glycerine (3: 1) on a microscopic slide and counting all the grains; the number obtained was multiplied by five (the number of anthers per flower). In this way the pollen count was taken from anthers of 10 flowers (from 10 different individuals of each morph) and the mean number of pollen grains per flower was calculated. The average number of ovules per ovary was counted by dissecting young pistils under a microscope. Pollen-ovule ratio was calculated as per the method suggested by Cruden (1977).

Pollination biology

Field observations of flower visitors were carried out from 0600 to 1630 h. Insect foraging activity was noted by visual observation. Number of floral visits per hour by each pollinator, their foraging behaviour, time spent by the insect on each flower and stigma touch were recorded. Temporal activities of the insects on pin and thrum morphs were distinctly noted.

Pollinators were trapped using insect nets, pan traps and sticky traps (Toler et al. 2005). Trapped insects were preserved individually in small screw cap vials (10 ml)

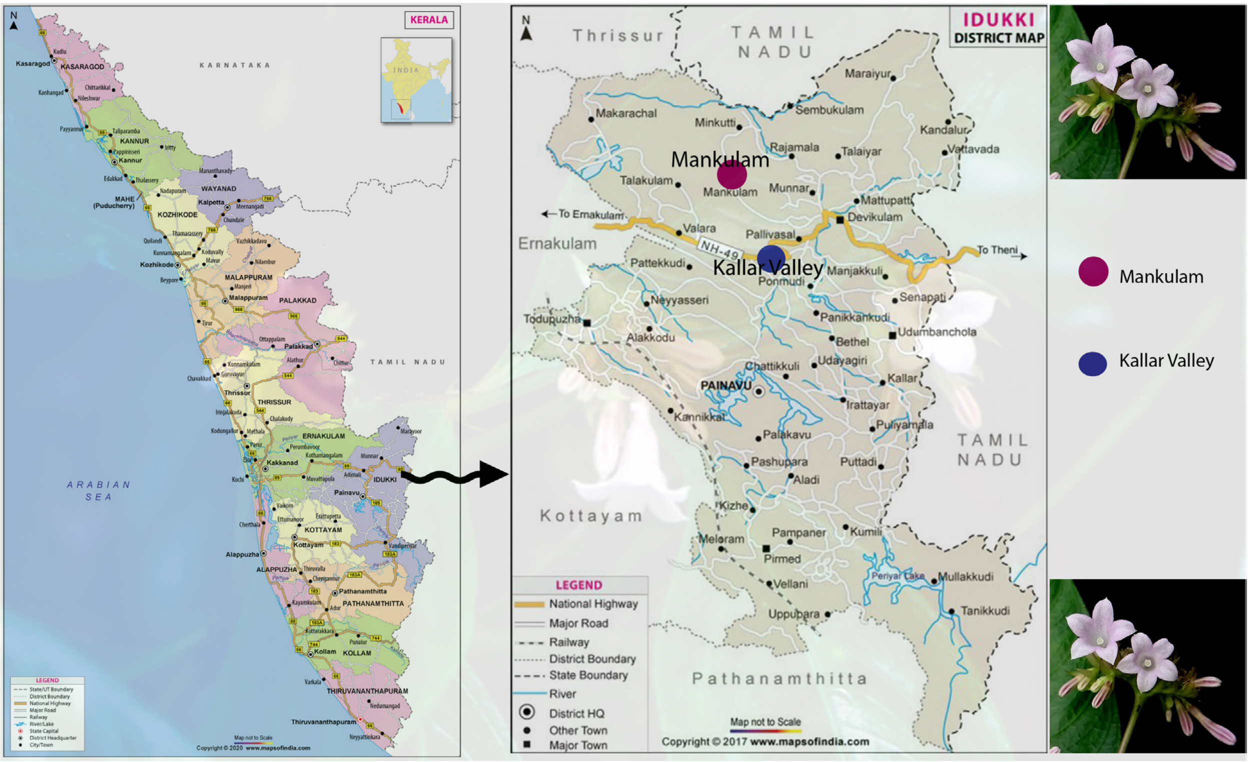

Figure 1. Study area in Idukki district—Mankulam and Kallar Valley

containing 4 ml of ethyl alcohol. The vials were vigorously shaken for two minutes to remove pollen grains from the insect's body. The insects were taken out from the vial and the suspension was allowed to evaporate. After evaporation, pollen grains were mounted in a few drops of acetocarmine-glycerin stain and observed under a microscope. The number of stained and unstained pollen grains of the selected plant species was counted. The pollinators were identified with the help of entomologists from the school of biology, IISER, Thiruvananthapuram, and an insect manual.

Breeding system

The mating system of the distylous species was analysed by fruit set comparisons in the field after various breeding experiments such as self-pollination, intramorph pollination, intermorph pollination, emasculation & bagging, and bagging without emasculation. Fruit set after these experiments were compared with the fruit set after open pollination. For each of the breeding experiment, 150 flowers were chosen from different individuals. The results were compared with Student's t-test. (SPSS ver.16.0 at the significance level of $\alpha = 0.05$)

Self-pollination was conducted to check whether the species is self-compatible. For this, flower buds one day before anthesis were bagged using butter paper bags and pollinated the next day using pollen from flowers of the same individual. The pollinated flowers were bagged again, and periodically observed for fruit set. For intramorph pollination, flower buds were emasculated and bagged one day before anthesis using butter paper bags. On the next day, pollen grains were transferred from flowers of other individuals of the same morph into the opened flower. For intermorph pollination, the flower buds were emasculated, bagged and were pollinated with pollen grains from flowers of the other morph. Another set of flowers were emasculated and bagged to check the occurrence of apomictic fruit test. Several flower buds were bagged without emasculation to test autogamous self-pollination within each morph.

Fruit and Seed biology

Fruit development was observed from the day of pollination until its maturation and dehiscence. Mature fruits from each morph were harvested and seeds collected. The average number of seeds developed per fruit/capsules was calculated. For analysing the reproductive success of flowers after pollination, the number of flowers per day in a 10 x 10 m quadrate of the population and the number of mature fruits developed from these flowers were scored and the flower-fruit ratio was calculated.

For the evaluation of seed dispersal, laboratory experiments were preferred because the seeds of *Ophiorrhiza* were minute to count from the intact soil. Peduncles with an open capsule containing mature seeds were placed in individual bottles and set on a floor covered with three square meters of white paper for easy detection of the scattered seeds. One 2 ml syringe fixed at a height of 2 m and water drops were allowed to fall onto the open capsule. Each water drop was approximately 0.1 ml. After the seeds scattered by water droplets, the longest distance from the peduncle to the seed was measured. The experiments were repeated at least five times for each morph (Nakanishi 2002).

For analysing seed germination, mature fruits were covered with paper bags before dehiscence and seeds were collected from each morph separately. Seeds were stored under laboratory conditions and allowed to germinate in petri dishes under different conditions: 1) Whatman filter paper (Grade 1); 2) soil from natural habitat; 3) soil from JNTBGRI campus. Samples of seeds were also allowed to germinate in the natural habitat to assess the influence of environmental factors on seed germination. Five replicates of 30 seeds of each morph were allowed to germinate every month to determine the optimal month for seed germination and seedling establishment. Quantitative features such as the number of days taken for seed germination and percentage of seed germination and seedling establishment in the field as well as in the laboratory conditions were analyzed periodically.

RESULTS

Phenology and floral morphology

O. caudata is a perennial creeping herb that sprouts at the end of May. Vegetative buds arise from the nodes of creeping stems during the monsoon, and the young leaves are yellowish-green. When mature, it becomes dark green in its upper surface and brownish green in the lower surface. The plants started to bloom in June and continued to January (Figure 2). New seedlings take 75–86 days to flower. Peak blooming was noticed from mid-August to mid-September (Figure 2). When the fruit matures, each fruit contains 45–110 minute brownish seeds which are dispersed by rain water

The inflorescence of *O. caudata* is a terminal capitate cyme with 1–6 flowers, with the flowers arranged in a centrifugal manner. Flower development completes within 7–10 days (Image 1d). Anthesis was noticed in the morning hours between 0600–0845 h and anther dehiscence occurs soon after flower opening. Flowers of thrum morphs open earlier than pin ones. Flowers are white with a mild fragrance and the nectar is located on the semicircular disc present above the ovary. Average life span of each flower is 20–24 hours. Blooming lasts for an average of 215 days in a year. Fruit development was completed within 25-35 days after pollination. During heavy rain, dehiscence of fruits occurs by splashing drop mechanism.

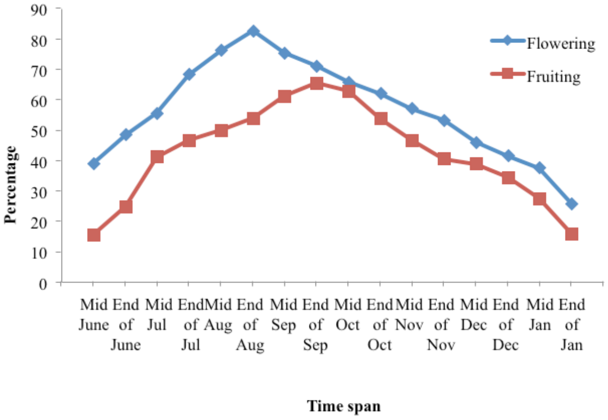

Figure 2. Flowering and fruiting phenology of *Ophiorrhiza caudata*.

The inflorescence and peduncles are glabrous, and 2.5–3.5 cm long. The flowers are white, lanceolate and 9.3–13.5 mm long. Pedicels are 1–1.5 mm long. Hypanthium is cup shaped. Calyx lobes are 5, ovate-lanceolate, acute and shortly keeled at back. Corolla is white and infundibuliform with 5 lobes. They are 8–10 mm long and glabrous outside with a ring of hairs on the throat of corolla tube. Stamens are 5 and are epipetalous; anthers are oblong and longitudinally dehiscent. Average length of the stamen is different in the two different morphs, 2.45 mm in pin flowers and 7.19 mm in thrum flowers. Ovary is obovoid, 0.59–0.85mm x 0.31–0.41 mm in pin flowers and 0.65–0.81mm x 0.33–0.41 mm in thrum flowers. Style is slender in both morphs but its length varies among the two different morphs, 5.95 mm in pin flowers and 1.79 mm in thrum flowers. Stigma-bifid and capitate in pin flowers and lanceolate in thrum flowers (Image 1b,c).

Pollination Biology

Butterflies, flies, bees and ants were the major floral visitors of *O. caudata,* and they were attracted by the mild fragrance of flowers. Flowering was in rainy season, and rainwater promotes pollination (Hydrophily) in *O. caudata*. One unidentified insect visits the flowers frequently; it spend around 45 ± 15 sec per flower in

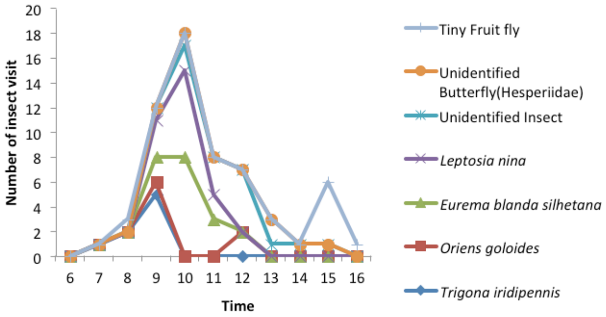

Figure 3. Foraging activity of pollinators: *Ophiorrhiza caudata* – pin morph.

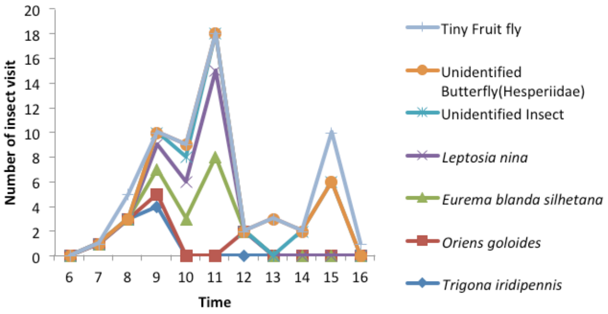

Figure 4. Foraging activity of pollinators: *Ophiorrhiza caudata* – thrum morph.

pin morphs and 46 ± 21 sec per flower in thrum morphs (Figure 3, 4). This insect either collects pollen from the exposed anthers of thrum flowers or enters the corolla tube to collect nectar. In pin morph, they enter into the corolla tube for collecting both pollen and nectar. Pollen flow into the thrum morphs by this insect was comparatively poor because the stigma is positioned under the throat of the corolla tube and therefore there was a reduced stigma touch. The prime floral visitor was stingless bee (*Trigona iridipennis*). It can enter up to the throat of the corolla tube and collects both pollen and nectar. The tiny fruit flies also make irregular visits and collects nectar. *Oriens goloides* is a butterfly visitor with more foraging time comparing to the other butterflies. *Eurema blanda silhetana* and *Leptosia nina* are the other butterflies visiting on the same time (Figure 3, 4). However, they spend only less time per visit but have more frequent visits per flower. These tiny butterflies make frequent movements in the flower which facilitates cross-pollination. Butterflies normally rest on the corolla, exert their proboscis and collect the nectar present on the disc above the ovary. Therefore, the pollen transfer from the inserted anthers of pin morph flowers into the stigma of thrum flowers which is also located inside the corolla tube will be comparatively less.

Table 1. Percentage of fruit set in six pollination methods in pin and thrum morphs of *Ophiorrhiza caudata*.

	Treatment	Number of flowers examined	Fruit set (%)
1	Self-pollination		
	Pin	150	00
	Thrum	150	00
2	Intramorph pollination		
	Pin x Pin	150	6.67
	Thrum x Thrum	150	3.33
3	Intermorph pollination		
	Pin (female) x Thrum(male)	150	72. 51
	Thrum(female) x Pin(male)	150	69. 07
4	Emasculation and netting		
	Pin	150	00
	Thrum	150	00
5	Netting without emasculation		
	Pin	150	00
	Thrum	150	00
6	Open pollination		
	Pin	150	66. 76
	Thrum	150	64. 13

Breeding System

Fruit set was not observed after self-pollination, emasculation and netting, netting without emasculation experiments in both the morphs. This indicated that the species was self-incompatible and there is no apomixis or parthenocarpic fruit development in this species. The percentage of fruit set after intramorph pollination in pin and thrum morphs are 6.67% and 3.33%, respectively. These results indicated that *O. caudata* has a tendency towards intramorph compatibility. Pin (female) x Thrum (male) and Thrum (female) x Pin (male) crosses resulted in 72% and 69% fruit set respectively and no notable difference between the morphs (t= 3.21, P >0.05). Therefore, the male and female organs of both the morphs were functional. Open pollination resulted in a fruit set of 66% in pin morphs and 64% in thrum ones. Comparison of these results with the manual intermorph pollination treatments showed no notable difference (Pin (female) x Thrum (male), inter-morph pollination vs. open pollination, 72% vs. 66%, t= 4.33, P >0.05 and thrum (female) x pin (male), inter-morph pollination vs. open pollination, 69% vs. 64%, t= 4.90, P >0.05) (Table 1).

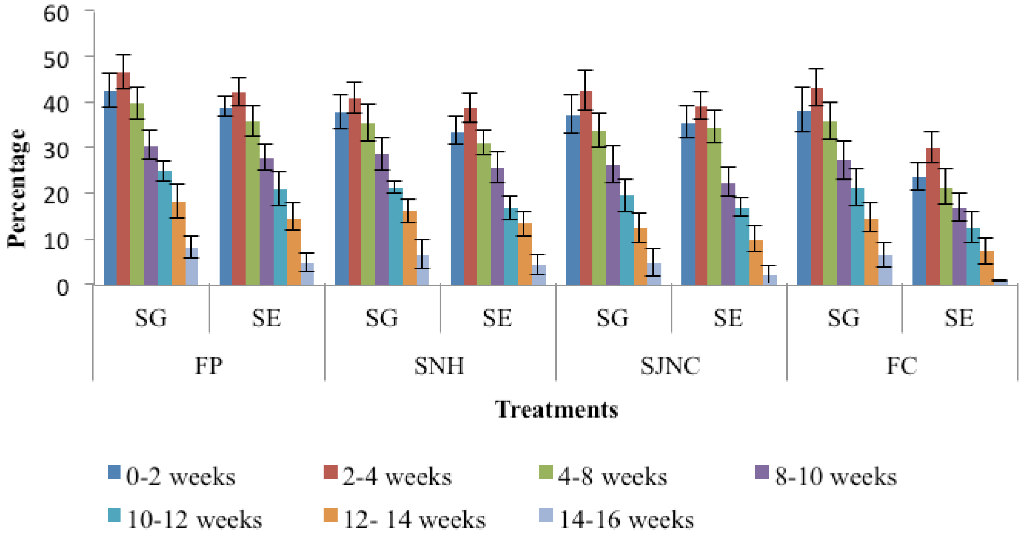

Figure 5. Seed germination of O. caudata (pin morph) at different conditions: SG—seed germination | SE—seedling establishment | FP—filter paper | SJNC—soil from JNTBGRI campus | SNH—Soil from natural habitat | FC—field condition.

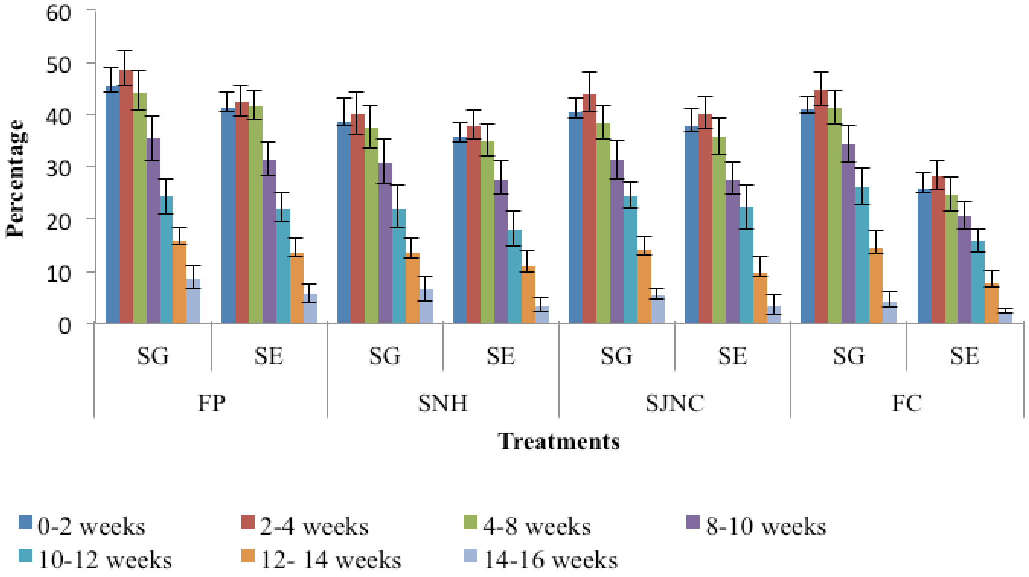

Figure 6. Seed germination of O. caudata (thrum morph) at different conditions: SG—seed germination | SE—seedling establishment | FP—filter paper | SJNC—soil from JNTBGRI campus | SNH—soil from natural habitat | FC—field condition.

Fruit and seed biology

The fruit is a boat-shaped, bi-valved capsule which dehisces along the dorsal surface (Image 1e). The capsule attains its maximum size (6.7 ± 0.51 mm x 5.4 ± 0.72 mm in pin morphs and 6.7 ± 0.32 mm x 5.5 ± 0.63 mm in thrum morphs) within 25–35 days after pollination. Each fruit contains 86 ± 12 seeds in both morphs and the number of seeds in the two locules may vary. The seeds were minute (0.68 ± 0.06 mm x 0.54 ± 0.05mm in pin plants and 0.64 ± 0.07 mm x 0.58 ± 0.08 mm in thrum plants), angular, glabrous and were brown coloured. Flower-fruit ratio of pin morphs in natural condition was calculated as 1.5:1 and that of thrum flowers was 1.6:1.

Seed dispersal

The seed dispersal mechanism in *O. caudata* was splash seed dispersal by raindrops. During rain, the water drops were collected in the boat-shaped capsule containing the seeds, which are splashed out and flushed away over certain distance. Thus the raindrops provide

Image 1. Floral biology of *Ophiorrhiza caudata* C.E.C. Fisch.: a—population | b—flower of pin morph | c—flower of thrum morph | d—flower development | e—fruit | f—seed germination.

energy for the seeds to come out of the capsule. The maximum dispersal distance of seeds under laboratory conditions in pin morphs was 93 ± 5.9 cm and 93 ± 5.6 cm in thrum morph seeds.

Seed germination and seedling establishment

The freshly harvested seeds of *O. caudata* shows dynamic germination in all the conditions, which indicates the seeds, are recalcitrant. Seed germination was hypogeal. In both morphs, maximum seed germination 40–45 % was noticed within 2–4 weeks after harvest (Figure 5, 6; Image 1f). The rate of germination was noticed to be declining in the next weeks. But the seedling establishment was at a reduced level while comparing to the seed germination rate in the natural habitat. This poor rate of seedling establishment may due to the heavy rain in the fruiting season. In natural condition, during heavy rain, the minute seeds are either buried in the mud or carried away by the rain water and only an average of 25% seedlings were established in the wild. However, seeds germinated in the plastic pots when transferred to the natural habitat also failed to establish due to heavy rainfall. In the natural condition, about 3% seeds inside the capsule exhibit viviparous germination which can be considered as an adaptation for establishment of young seedlings during heavy precipitation.

DISCUSSION

The genus *Ophiorrhiza* L. is characterised by white or pinkish white flowers and most of the species exhibit heterostyly. Majority of the *Ophiorrhiza* species reported from Western Ghats exhibit the similar pattern of flowering except the varieties of *O. brunonisis* which flowers during the summer months (Deb & Mondal 1997). Anthesis occurs in both the morphs in the early morning around 0600 h, and all the flowers completely open within around 0845 h. Distylous species with white tubular flowers in Rubiaceae such as *Psychotria carthagenesis* (Consolaro et al. 2011) follow the same pattern of anthesis. Simultaneous opening of flowers of both the morphs have a positive influence on the pollen transfer between them.

The family Rubiaceae is characterised by different pollen transfer mechanisms and functional gender of pin and thrum morphs with various pollination systems (Wolff & Liede-Schumann 2007). Several investigations were conducted on the pollination biology of heterostylous plants in Rubiaceae, and there are species which have evolved functional dioecy in the family (Li et al. 2010). Flowering of *O. caudata* is during monsoon. The earlier studies (Wolda 1988; Fonseca et al. 2006; Silva et al. 2011) reported highest foraging activities of Hymenoptera and Lepidoptera during the rainy season.

This observation agrees with the pollinators of candidate species, and can be considered an adaptation to suitable conditions for pollination, i.e., species tend to flower when vector availability is higher, as reported in other plant groups (Almeida & Alves 2000; Koptur et al. 1988; Martin-Gajardo & Morellato 2003).

The stingless bee *Trigona iridipennis* is a major pollinator of the selected species. Pollination by *Trigona* was observed in other members of Rubiaceae, including *Psychotria barbiflora* (Texeira & Machado 2004) and *Manettia cordifolia* (Consolaro et al. 2005). They visit the flowers in morning immediately after anthesis. High level of nectar concentration in morning hours stimulated the visit of *Trigona spinipes* in both the morphs of *Psychotria poeppigiana* (Valois-Cuesta et al. 2009). In *Ophiorrhiza*, the nectar is present in trace amounts and was too viscous in the selected species to be measured by conventional hand refractometers. *Trigona*, the tiny bees can enter into the corolla tube, feed pollen and nectar from both morphs. *Trigona* and fruit flies can enter the narrow corolla and comes in contact with the short stigma of thrum flowers but other hymenopterans and lepidopterans are less able to enter the narrow corolla tube of thrum morphs and moreover there is a ring of hairs along the corolla tube above the stigma. If these hairs are absent, even short stigmas of thrum flowers can effectively receive pollen grains from pin flowers (Stone & Thomson 1995) *Eurema blanda silhetana* and *Leptosia nina* are the common butterflies visiting *Ophiorrhiza caudata*. They usually visit the flowers after two hours of anthesis and there is a competition between them for nectar. These restless butterflies spend less time per flower but have frequent visits. *Oriens goloides* and an unidentified butterfly from Hesperiidae visits *O. caudata* for nectar. Butterflies usually alight near or on flowers when foraging for nectar (Naiki & Kato 1999), and in *Ophiorrhiza caudata* they took the exudates secreted from the disc above the ovary by extending their proboscides into the corolla tube. Therefore, butterflies most often come in contact with the exposed floral parts from the corolla tube; anthers of thrum flowers and stigmas of pin flowers. Thus the pollen transfer from pin morphs to thrum was comparatively poor. No significant difference was observed in the foraging time, foraging period, and number of visits per flower and stigma touch among the morphs of the selected species.

According to some authors, thrum (short-styled) flowers are efficiently pollinated by insects with longer mouthparts (Beach & Bawa 1980; Lloyd & Webb 1992), while short-tongued insects would be more efficient pollinators of pin (long-styled) flowers (Beach & Bawa 1980). In another view, reproductive interference might interrupt the proper functioning of disassortative pollination between short-level organs, which promote asymmetric pollen flow due to extremely narrow corollas of the species pollinated by Lepidopterans (Marten-Rodriguez et al. 2013). *Ophiorrhiza caudata* do not totally depend on lepidopterans for their pollination; bees, fruit flies and even rain water act as pollinating agents. Here, lepidopterans are efficient pollinators of pin morphs and asymmetry in pollen flow by insect vectors is noticed because of the narrow corolla tube. Fruit flies and *Trigona* can enter the narrow corolla and comes in contact with the short stigma of thrum flowers but other Hymenopterans and Lepidopterans are less able to enter the narrow corolla tube of thrum morphs. There is a ring of hairs along the corolla tube above the stigma. If these hairs are not present, even short stigmas of thrum flowers can effectively receive pollen grains from pin flowers (Stone & Thomson 1995). Even though the pollen grains from pin flowers were attached to the proboscis of an insect, most of them would be easily swept off by the hairs in a thrum flower, which results in asymmetric pollen flow between the pin and thrum flowers (Naiki & Kato 1999). When considering pollination by lepidopterans, some butterflies collect the nectar without the stigma touch (Naiki & Kato 1999). However, these butterflies efficiently transfer pollen from the exposed anthers of thrum flowers to the exposed stigmas of pin flowers with their proboscis and other mouthparts or with legs and wings.

Manual pollination treatments confirmed that the species is self-incompatible and no fruits are developed by apomixis. After interpreting the results of illegitimate pollination, it is found that a small percentage of fruit set is obtained after intra-morph crossing. This indicates that the species shows a tendency towards intra-morph compatibility. Heterostylous species are usually self- and intra-morph incompatible and produce fruits only after legitimate (intermorph) pollination. However, self- and intra-morph compatibility was reported from both distylous (Ornduff 1976) and tristylous (Barrett 1985; Eckert & Barrett 1994) species. Intra-morph compatibility accompanied by self-incompatibility is reported from tristylous *Narcissus triandrus* (Barrett et al. 1995) in Amaryllidaceae, from the distylous *Anchusa hybrid* (Dulberger, 1970), and from *Anchusa officinalis* (Philipp & Schou, 1981) in Boraginaceae. In Rubiaceae, partial intramorph compatibility was reported from several self- incompatible distylous species like *Psychotria nuda* (Castro & Araujo 2004), *P. homalosperma* (Watanabe et al. 2014) and *Gaertnera vaginata* (Pailler & Thompson 1997) where fruit set was obtained after illegitimate pollination in one morph and no fruit set in the other one. Intramorph incompatibility may gradually decrease in the species and may become intramorph and self-compatible in the future. The heterostylous species which are self-compatible were considered to be derived from self-incompatible ancestors (Baker 1966; Ganders 1979).

Fruit of *O. caudata* is a bi-valved capsule which dehisces along the dorsal surface. According to Deb & Mondal (1997), many seeded dehiscent fruit of *Ophiorrhiza* which releases and disperses seeds is a primitive character while comparing to the few seeded indehiscent fruit. Flower-fruit ratio in the two morphs specifies that above 60% fruit set was obtained in natural conditions. Seed dispersal mechanism is splash or ballistic seed dispersal by rain drops in which the seeds are dispersed into the surroundings due to the pressure exerted by the rain drops falling in the loculicidal capsule. This kind of seed dispersal was reported in *O. japonica* in which the seeds are dispersed to a maximum distance of 95.0 ± 6.2 cm (Nakanishi 2002). Nakanishi (2002) reported the same mechanism of seed dispersal in *Sagina* spp. (Caryophyllaceae), *Sedum* spp. (Crassulaceae), *Gentiana* spp. (Gentianaceae) and several members of Saxifragaceae and Scrophulariaceae. All these plants are herbaceous and splash rain dispersal might be an advantage for small plants because dispersal is not affected by plant height. Occasionally, in the absence of rain, the seeds of *Ophiorrhiza* sp. are dispersed by wind.

The minute seeds of *Ophiorrhiza* showed maximum seed germination within 2–4 weeks after the harvest. The seeds of *Ophiorrhiza caudata* showed more than 40–45 % germination in all the conditions, but only a few seedlings are establishing in the wild habitat. In natural conditions, most of the seeds are leached along with the rain water or buried in the muddy soil. The germinating seeds were also destroyed by heavy rain. There is increasing evidence that the events which occur during seedling establishment influences the distribution and abundance of adults in a plant community (Marks 1974; Platt 1975; Werner 1977; Rabinowitz 1978; Gross & Werner 1982). Seeds leached out by the rain water which may germinate in a long distance apart which leads to habitat fragmentation.

CONCLUSION

Ophiorrhiza caudata is an endangered species endemic to the southern Western Ghats. It exhibits heterostyly, but shows some deviations from the typical characters of a heterostylous species. Its breeding system is self-incompatible but shows some degree of intra-morph compatibility, which has an evolutionary significance. Poor seedling establishment in wild conditions, habitat fragmentation, and anthropogenic activities are the major threats for the survival of the species. Only 25% of seedlings were established in the wild condition due to climatic problems. The seedlings were successfully established in Jawaharlal Nehru Tropical Botanic Garden and Research institute campus. Conservation of this rediscovered medicinal plant is of great significance in the present scenario. The self-incompatible heteromorphic species which is phasing environmental and other threats in its natural habitat is conserved in our campus. Both the morphs are protected, thereby promote cross pollination and further establishment of the species.

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