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Cover: *Saproamanita praeclara*: Sporocarp in habitat © Kantharaja. R.

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COMMUNICATION

Reproductive biology of two threatened and highly traded medicinal plants, *Salacia gambleana* and *Salacia oblonga*, from the Western Ghats of India

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Abstract: *Salacia* is a genus of flowering plants in the family Celastraceae, consisting of woody climbers distributed in tropical America, Africa, and Asia. In India it is represented by 21 species, of which 15 occur in peninsular India. Most species of the genus have been used in traditional medicine, mainly the Ayurvedic system. Apart from overexploitation for medicinal purposes, the low fruit set and infestation of seeds have affected natural regeneration, and led to the rarity of *Salacia* species in their natural habitats. The reproductive biology of *Salacia oblonga* and *S. gambleana* was studied for the first time to understand the reproductive constraints of these threatened and medicinally important species. The flowering phenology, pollen viability, germination, stigma receptivity, and insect-pest interaction were analyzed. The obligatory entomophily coupled with insufficient pollinators and seed pest infestation were found to be the main reproductive constraints responsible for the low fruit set and poor natural regeneration of these species.

Keywords: Conservation, health care, management, medicinal genetic resource, reproductive constraints, rarity.

Malayalam: സപുഷ്പികളായ സലേഷ്യവർഗ്ഗം സെലാസ്ട്രേസ്യേ സസ്യകുടുംബാംഗമാണ്. പ്രധാനമായും മരവള്ളികളായ ഇനങ്ങൾ മദ്ധ്യഅമേരിക്ക, ആഫ്രിക്ക, ഏഷ്യ എന്നീ ഭൂഖണ്ഡങ്ങളിലാണ് വിന്യാസം ചെയ്യപ്പെട്ടിട്ടുള്ളത്. ഇന്ത്യ ഉപഭൂഖണ്ഡത്തിൽ പ്രതിനിധാനം ചെയ്യപ്പെട്ടിട്ടുള്ള 21 സസ്യഇനങ്ങളിൽ 15 ഇനങ്ങൾ പശ്ചിമഘട്ടത്തിൽ മാത്രമായി കണ്ടുവരുന്നു. സലേഷ്യ വർഗ്ഗത്തിൽ പെടുന്ന സസ്യങ്ങൾ പരമ്പരാഗത വൈദ്യസമ്പ്രദായത്തിൽ പ്രത്യേകിച്ച് ആയുർവേദചികിത്സയിൽ ഉപയോഗിച്ചുവരുന്നവയാണ്. ഔഷധ ആവശ്യങ്ങൾക്കുവേണ്ടിയുള്ള അമിത ചൂഷണത്തിനു പുറമെ ഫല ഉൽപാദനത്തിലെ കുറവും ഗുണമേന്മയുള്ള വിത്തുകളുടെ അഭാവത്തിലും സസ്യഇനങ്ങൾ ആവാസമേഖലകളിൽ വംശനാശം നേരിട്ടുകൊണ്ടിരിക്കുകയാണ്. ഈ പശ്ചാത്തലത്തിൽ സലേഷ്യ ഒബ്ളോങ്ങ, സലേഷ്യ ഗാബ്ളിയാന (പൊൻകൊരണ്ടി) എന്നീ ഇനങ്ങളുടെ പ്രത്യുൽപാദന-ജീവശാസ്ത്ര ഘടകങ്ങൾ പഠനവിധേയമാക്കുകയും പൂവിടൽ മുതൽ പരാഗണങ്ങളുടേയും സ്റ്റിഗ്മയുടേയും ജീവനക്ഷമത, പ്രാണി - കീട പ്രവർത്തനങ്ങൾ തുടങ്ങിയവ വിശകലനം നടത്തുകയും ചെയ്തു. പ്രാണി ആശ്രിതമായ ഈ സസ്യങ്ങളുടെ പരാഗണത്തിന് പരാഗണകാരികളുടെ അപര്യാപ്തതയും വിത്തുകളിലെ കീടബാധയും സസ്യങ്ങളിലെ ഫലദൗർലഭ്യത്തിനും വിരളമായ സ്വാഭാവിക പുനരുൽപാദനത്തിനും പ്രധാന പരിമിതികളാണെന്ന് കണ്ടെത്തി .

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Author contributions: PSK—carried out the study by her as a part of MSc Dissertation programme. PAJ—Served research supervisor for M.Sc. Dissertation of PSK and manuscript correction and editing. KS—helped in reproductive biological studies in the laboratory and preparation of map, figures and Plate for the article. TVS—helped in field data collection in the medicinal garden.

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INTRODUCTION

The genus *Salacia* contains important medicinal plants with a wide range of therapeutic properties. In India 21 species are reported; 15 occur in peninsular India including the Western Ghats. The majority of *Salacia* species are over-harvested for their roots, which are used in the Ayurveda and Unani systems of medicine for treating diabetes, asthma, leukemia and ear infections. *Salacia* species mainly contain salacinol, kotalanol, neosalacinol, several phenolic compounds, sesquiterpenes, and triterpenes (Kirtikar & Basu 1975; Prakash et al. 2008; Wang et al. 2012). These components have medicinal properties and significant applications in modern medicine. Besides their overexploitation, these species show a low fruit set and poor natural regeneration leading to low population size and rarity. Therefore, the existing populations of *Salacia* species and their natural habitats warrant urgent conservation and management measures.

The rarity of a plant often stems from its biological functions, particularly its reproductive biology, which has a significant contribution to the sexual reproduction of flowering plants. Reproductive biology studies help in the conservation of genetic resources. The reproductive patterns are some of the key factors leading to the abundance, distribution and genetic diversity of the species. There is a widespread consensus that reproductive biology studies of threatened species can help determine strategies for in situ and ex situ conservation. Several levels of problems can be seen in the reproduction of threatened plants, such as infrequent flowering, flower bud fall, flower infestations, lack of pollinators and low fruit set. Knowledge on anthesis, pollen viability and germination, stigma receptivity, pollen-ovule ratio, breeding behavior, and pollinators are prerequisites to unravel the biological constraints leading to endangerment of the species.

MATERIALS AND METHODS

Study area

The study was conducted at the herbal garden of Kerala Forest Research Institute (KFRI) Peechi, Kerala, South India. KFRI is situated about 20 km east of Thrissur district, spread over a 28 ha reserve forest area adjacent to Peechi-Vazhani Wildlife Sanctuary at 10.530 N latitude and 76.347 E longitude with an altitude of 186 m (Figure 1). The study was carried out from January to June 2019.

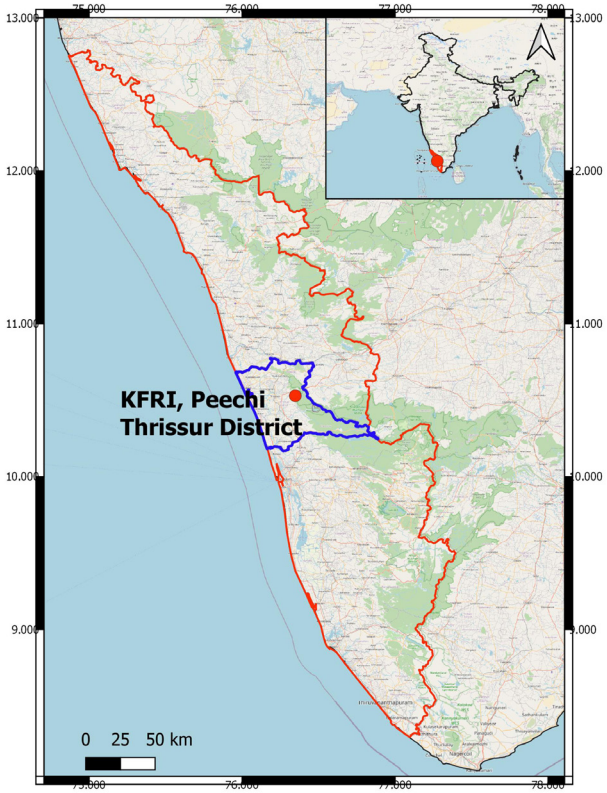

Figure 1. Western Ghats of Kerala showing the study area.

Salacia gambleana Whiting & Kaul (Syn. *Salacia talbotii* Gamble)

Scandent shrubs, endemic to the southern Western Ghats, occur in evergreen and semi-evergreen forests. This species has been assessed as threatened by Sasidharan (2017). Flowering and fruiting were recorded from January to July.

Salacia oblonga Wall. ex Wight & Arn. (Syn. *Comocladia serrata* Blanco)

Stout climbers, found in the evergreen and semi-evergreen forests up to an altitude of 1,500 m. The species has a global distribution in the Western Ghats and Sri Lanka. It has been assessed as Vulnerable based on the IUCN Criteria A2cd (Ved et al. 2015). Flowering and fruiting were recorded from March–May. The root-bark is used to treat rheumatism, gonorrhea, and skin diseases (Kirtikar & Basu 1975). This species has shown antiperoxidative properties, and is also used to treat renal complications (Krishnakumar et al. 2000).

Methods

The study covered day-to-day monitoring and recording of flowering phenology, such as bud initiation, development, anthesis, stigma receptivity, pollen viability, pollen-ovule ratio, pollination, pollinators,

blooming period, pest incidence, and fruit set. The data presented as the average values of each trial (Sreekala et al. 2008; Jose & Pandurangan 2012, 2013; Swarupanandan et al. 2013; Gopalakrishnan & Thomas 2014).

Reproductive phenology

Data on reproductive phenology, including the number of inflorescence per branch, number of flowers per inflorescence, flower/ inflorescence development, blooming period, fruit initiation and development,were recorded daily. Five inflorescences per plant for both species were subjected for data collection. Each inflorescence was tagged and monitored for the flower development from bud to full bloom. The average days taken for each bud to bloom were calculated and recorded. The number of flower buds with pest incidence was also recorded. Each flower was tagged to observe fruit formation.

Pollen viability

Pollen grains from fully matured flower buds were dusted into a cavity slide containing a solution of acetocarmine, kept for one hour and then observed under a compound microscope. The pollen grains stained red were treated as viable and others as non-viable. A viability test was carried out in two-hour intervals.

Pollen germination

Pollen grains from fully matured flower buds were transferred to a cavity slide containing germination medium (Sucrose 10%). Pollen germination was counted after one hour using a compound microscope. The pollen tubes with a longer length than pollen diameter were treated as germinated. The experiment was repeated in two-hour intervals from the anthesis.

Stigma receptivity

Visual observations using a hand lens and chemical methods using hydrogen peroxide (H_2O_2) were conducted. In the visual method, the stigma with wetness, turgidity and oily nature was considered receptive and the rest as non-receptive. In the chemical test, a drop of hydrogen peroxide was added to the stigma of a freshly opened flower. The effervescence resulting from the peroxidase enzyme activity was observed in the receptive stigma, and the duration of stigma receptivity was calculated (Dafni et al. 2005).

Pollen-Ovule ratio

The number of pollen grains in anthers per flower was counted using a haemocytometer (Shivanna & Rangaswamy 1992). The number of ovules per ovary was counted by the cross-section of the ovary (Cruden 1977). The pollen-ovule ratio was calculated using the following formula:

$$\text{Pollen-ovule ratio} = \frac{\text{Pollen count per anther x No. of anthers per flower}}{\text{No. of ovules per flower}}$$

Pollination and insect interaction

Bagging experiments were carried out to understand the mode of pollination. The physical observation was made throughout the flowering period, and the insect interactions were recorded day and night. Adhesive tapes were kept on flowering branches to collect insects for identification. The taxonomic identification of insects was made with the relevant literature.

Fruit phenology

Fruit phenology such as fruiting primordia, period of development including premature abscission and pest incidence was monitored and recorded.

RESULTS

Salacia gambleana

Reproductive phenology

Bulbous and light green flower buds were observed during the second week of January. It took about 25 days for the bud to develop into full bloom (Figure 2). The flower started opening from 0430 h to 0500 h and was fully opened by 0930 h. Anther dehisced through the horizontal slit from 0500 h and 0530 h. Stigma found receptive prior to the anther dehiscence (protogynous condition).

Pollen viability and stigma receptivity

Fresh pollen grains (on anthesis) showed 100% viability, and gradual reduction was noticed to 98, 86, 80% after 6, 10, and 12 hours, respectively. A drastic decline to 52% was noted after 28 hours (Table 1). The hydrogen peroxide application followed by effervescence formation confirmed the stigma receptivity up to 58 hours. The stigma then turned brown, lost turgidity, and became non-receptive.

Pollen germination

At the time of anthesis, 96% of pollen grains were germinated. A gradual decrease in pollen germination was observed in succeeding hours, and 24% pollen

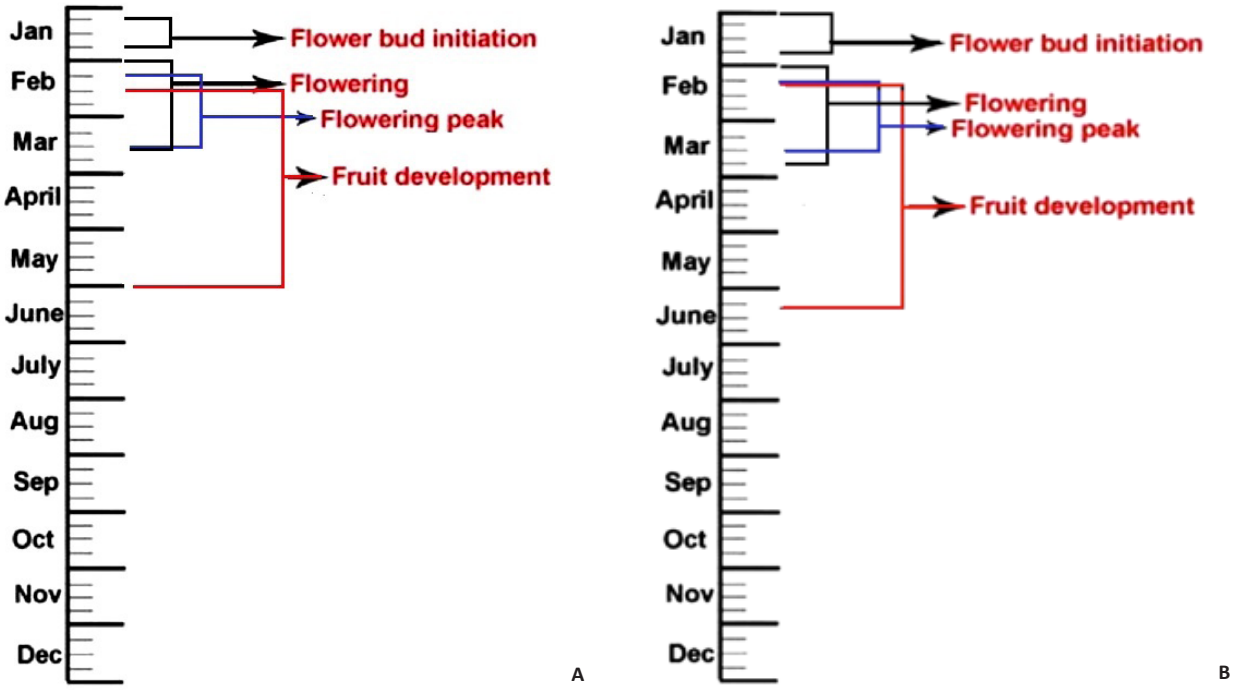

Figure 2. Phenology calendar of *Salacia gambleana* (A) and *S. oblonga* (B).

germination was recorded at 11 hours after anthesis.

Pollen-Ovule ratio

A flower contains three to four anthers, and approximately 277.1±165.7 pollen grains are present per anther. Hence, the pollen count per flower was calculated as 834.56±510.5. A flower has 4.45±1.5 ovules, and pollen-ovule ratio thus estimated to 187.54:1.

Mode of pollination and insect interaction

Flowers are not fragrant. Ants such as *Tetraponera* sp., *Ocecophylla smaragdina*, and *Anoploilepis gracilipes* were usually found foraging during the flowering period. *O. smaragdina* is an arboreal ant that forms colonies on the host plant using leaves stitched together. The maximum incidence of ants was observed in the peak of anthesis hours 0830–0930 h. Bagging experiments resulted in no fruit set; however, floral arrangements per se facilitated self-pollination (Anthers placed over stigma). Further, the incidence of ants during flowering was also found promoting cross-pollination. A larval infestation was found in developing fruits, which later caused the abscission of young fruits. The adult ants were collected for identification.

Salacia oblonga

Reproductive phenology

Flower bud initiation was noted from the first week of January. The development of bud to bloom was observed for 30 days (Figure 2). Flower opening initiated from 0130 h to 0330 h and fully opened by 0830 h. Anthers dehisced through the longitudinal slit from 0430 h and 0530 h. The stigma was receptive prior to anther maturity (protogynous condition).

Pollen viability and stigma receptivity

Fresh pollen grains showed 100% viability for up to six hours from anthesis. A gradual reduction in pollen viability was recorded, viz., 97, 85, 64% after 8, 12, 37 hours, respectively (Table 1). Stigma was found receptive up to 39 hours and later turned brown, lost turgidity, and became non-receptive.

Pollen germination

At the time of anthesis, 90% of the tested pollen grains were germinated. A sudden decline in germination percentage was noted after 6 and 12 hours with 58 and 38% germination, respectively (Image 1).

Pollen-Ovule ratio

A flower contains three to four anthers, and approximately 4,929.05 ±1829.18 pollen grains are present per anther. Hence, the pollen count per flower was calculated as 14,757.27 ±5487.38. Each flower has 9.4±2.95 ovules and an estimated pollen-ovule ratio of 1,573:1.

Table 1. Reproductive biology of *Salacia gambleana* and *Salacia oblonga*.

Floral characters	*Salacia gambleana*	*Salacia oblonga*
Flowering period	February–March	Throughout
Inflorescence type	Axillary umbel	Axillary umbel
No. of inflorescence per branch (n=26)	42.69±25.68	15.34±8.69
No. of flowers per inflorescence (n=26)	11.5±5.33	5.5±1.87
Flower type	Actinomorphic, Hermaphrodite	Actinomorphic, Hermaphrodite
Flower nature	Protogynous	Protogynous
Flower colour	Green	Greenish-yellow
Flower opening time	0430–0530 h	0130–03.30 h
Anther dehiscence time	0500–0515 h	0430–0530 h
Anther dehiscence mode	Horizontal slit	Longitudinal slit
Odour	Present	Absent
Nectar	Present	Present
Number of anther per flower (n= 18)	3 or 4	3 or 4
Pollen per anther (n= 18)	277.10±165.70	4929.05±1829.18
Mean number of pollen grain per flower (n= 18)	834.56±510.50	14787.27±5487.38
Mean number of ovule per flower (n= 30)	4.45±1.36	9.4±2.95
Pollen:Ovule ratio	187.54:1	1573.11:1
Pollen shape	Triangular	Triangular
Pollen diameter (n= 15)	21.264µm±2.27µm	20.41µm±4.92µm
Pollen tube length (after 2 hours)	83.77±38.68µm	103.37±62.11µm

Mode of pollination and insect interaction

Flowers are dull in appearance and are not fragrant; however, the floral nectars attract the ants such as *Tetraponera* sp., *O. smaragdina*, and *Anoploilepis gracilipes* during flowering. Ants forage 18±6 minutes during a visit. Maximum foraging was observed at 1030–1230 h. Ant movement caused pollen deposition on to own stigma, facilitating self-pollination (Image 1). The developing fruits were often damaged, and the seeds were foraged by caterpillars. The adult could not be collected for identification.

DISCUSSION AND CONCLUSION

As plant rarity is often directly related to the ecology and biology of the species, knowledge on reproductive phenological and biological functions is a prerequisite to unravel the complexities of rarity and for effective conservation and management of the species (Reveal 1981; Rathcke & Lacey 1985; Kempel et al. 2020). The absence of an efficient pollination mechanism has been determined as the main disadvantage in both the species studied. The low pollen-ovule ratio promoted cross-pollination through insects; however, insect visits during flowering were extremely low. Floral characteristics such as petal size, colour and nectar production have a significant role in the reproductive success of plants (Kudon & Whiegham 1998). *Salacia* species generally have small and dull flowers (*S. oblonga* 0.36±0.05 cm; *S. gambleana* 0.55±0.09 cm) and comparatively less nectar production.

The role of ants as pollinators was assumed by their relative abundance compared to other insects (Gómez et al. 1996). Various floral signals, especially nectar characteristics and floral scent, play a crucial role in attracting ants. Ants were the common pollinators, facilitating facultative autogamy in the species. The ants foraging in different flowers of the same plant enabled geitonogamy (Rostás & Tautz 2010). Among the ant species, the weaver ant (*O. smaragdina)* was found to be a pollination limiting factor as it acts as a key predator of some pollinators. It affects the behavior of other flower visitors and thus the plant's reproductive success. Observations of Tsuji et al. (2004) on the fruit orchard of *Nephelium lappaceum* suggested that the presence of *O. smaragdina* nest on the plant lowered flower-visiting rates of flying insects involved in pollination. The presence of weaver ants might be one of the reasons for the absence of other pollinators. In this study, an abundance of *O. smaragdina* ants and the absence of other floral visitors were also observed. Subin et al. (2018) reported that in *Salacia fruticosa*, 70–80% of mature fruits were found infested, and the seeds were consumed by the caterpillars of the butterfly *Bindahara moorei.* A similar infestation was also recorded in *Salacia gambleana* and *S. oblonga* where 40–50% of immature fruits were damaged by caterpillars of *B. moorei.* Insect infestation of fruits and seeds and its impact on seedling bank and subsequent rarity have been reported in many threatened plants in the Western Ghats (Jose et al. 2004, 2016). The observations and results of the present study are expected to aid future studies involving the populations of the *Salacia* species and develop suitable measures for their in situ and ex situ conservation.

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Image 1. A–E—*Salacia gambleana*: A—Flowering twig, single flower in inset | B—Oecophylla smaragdina colonies | C—*O. smaragdina* in flower | D—Pollen germination | E—Fruit showing larvae. F–J—*Salacia oblonga*: F—Flowering twig, single flower in inset | G—Pollen germination | H—Insect incidence in flower bud | I—*O. smaragdina* in flower | J—Cut opened fruit showing larval incidence. © Subin K. & Sarath T.V.

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Appendix 1. Floral part measurements.

Floral parts	*Salacia gambleana*	*Salacia oblonga*
Bud	0.55±0.09 mm	0.36±0.05 mm
Pedicel	0.40±0.07 mm	0.05±0.008 mm
Petal	0.19±0.002 mm	0.26±0.04 mm
Sepal	0.09±0.005 mm	0.17±0.025 mm
Pistil	0.14±0.05 mm	0.19±0.006 mm
Stamen	0.28±0.07 mm	0.20±0.01 mm
Anther	0.08±0.02 mm	0.06±0.025 mm
Filament	0.24±0.07 mm	0.14±0.029 mm

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