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Cover: Digital illustration of *Impatiens chamchumroonii* in Krita by Dupati Poojitha.

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ARTICLE

Flower bud growth, mortality rate, and population structure of *Sapria himalayana* Griffith f. *albovinosa* Banziger & Hansen (Rafflesiaceae) in a subtropical forest, northeastern India

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Abstract: *Sapria himalayana* Griff. f. *albovinosa* Banziger & Hansen (Rafflesiaceae) is rare and endemic to northeastern Thailand, Vietnam, and Manipur, with a short flowering season ranging from late October to late November, due to which its detailed phenology is poorly understood. To protect this valuable taxon from extinction threats, monitoring the growth of flower buds is crucial. The objectives of this study were to analyse the growth of flower buds at various developmental stages, the mortality rate, and the population structure of *Sapria himalayana* f. *albovinosa*. The buds were monitored, vertically photographed, and measurements of the plant buds and flowers were recorded for every observation. The present study resulted in the flower bud growth having six different developmental stages, namely the copule, copule-bract transition, bract, bract-perigone transition (BPT), perigone, and anthesis stage, with a diameter range of 0.3–2.3 cm, 2.4–4.0 cm, 4.1–5.5 cm, 5.6–6.1 cm, 6.2–8.1 cm, and 16–20 cm, respectively. The population was dominated by the copule stage in the initial observation, while it was dominated by the perigone and anthesis stages in the final observation, which indicated that the optimal flowering season was from late October to late November. A total of 58 flower buds were recorded, out of which 24.13% of flower buds were dead without reaching maturity due to fungal infections and were injured due to anthropogenic interventions. Hence, the information on the growth of buds, flower development stages and their mortality rate is vital for taxonomic studies, field monitoring, and conservation purposes.

Keywords: Anthesis, anthropogenic, bract, conservation, copule, crucial, endemic, extinction, perigone, rare.

Maringa: *Sapria himalayana* Griffith f. *albovinosa* Banziger & Hansen (Rafflesiaceae) tou ung paar a beimak taangni Northeastern Thailand, Vietnam and Manipur bi khyum heirou tomcha paar. Heirou paar ayei paar nei kheen heiri beimakni thum hoilei. Kheen heiri Kumthil tangla rou youbi Bilwa tangla bil tomcha paar. Ngam heini paar ayei maram ra thimi rapni langaidui muchangmak. Ayei paar a beimakni taangni pha ngam hoibini paar aya lhaipang arou mitmak nanung kalthung ngaakthung nei tloubam khat khoi. Heitounei ngamrou paar ayei kat um younei kheen rou youbini paar kim kham bil thi thung silthung nei tloubam heirou paar ayei khi nei changchap heirou paar ayei mashing (population) thi nei tloubam a namyerwa asiyei thilhak thi thut nei che arou shershang thutlei. Paar heiyei paarna bi heirou photo kou kanlhonni paar heiyei tang mandi (diameter in cm) namyerwa markhe bi langaidui thilhakur. Asiyei thilhak thi thut yei kheen heirou paar heiyei kat um yei kheen youbi kim kham bil heirou tangkak lailai paar heiyei tang malnamak thruktam mupha. Heirap heiri copule, copule-bract transition, bract, bract-perigone transition, perigone heirou anthesis stages with a diameter range of 0.3-2.3 cm, 2.4-4.0 cm, 4.1-5.5 cm, 5.6-6.1 cm, 6.2-8.1 cm and 16-20 cm, respectively. Paar heiyei mashing thithut youri nei tangla heirou ri copule stage ni duichi, heitoubini dongwai rou tangla heirou ri perigone heirou anthesis stages ya khintam ni duichi. Heiyei mukna ri paar heiri Kumthil tangla rou youbi Bilwa tangla bil matachani paar hei paarnei kheen hoilei. Bi heirou ri paar mashing somnga-le-chot (58) paar muphalei, heiyei rilla saruk 24.13% paarshomakni hikur. Paar hei mapung phamakni paarshomakni hikur heiyei ngamri rikwai dawurbi chulwurnei hoilei heirou lhaipang thimi ni paar leina bi heirou manshok nei ngam khat khi hoilei. Thakrei minlhangkur heirap namyer wani ngam hoibini beimak taangei pha paar awa lhangaidui thilhak ngakthung-silthung bini paar aya lhaipangpal bi arou mitheimak kim heimaknung beimak lungkhoi tloubam khat hoilei.

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सत्यमेव जयते

DHANAMANJURI UNIVERSITY

INTRODUCTION

Sapria himalayana Griffith f. *albovinosa* Banziger & Hansen (Banziger et al. 2000), a new form of the Hermit's Spittoon, is rare and endemic to the northeastern Thailand and Vietnam Lang Biang plateau, but a new distribution is recorded from India at the evergreen sub-tropical forest of Machi Village, Tengnoupal District, Manipur (Maring & Pinokiyo 2024). It is differentiated from the typical form in having white dotted warts more or less evenly distributed on the entire wine-red perigone lobe instead of having sulphur-yellow dotted warts on blood-red perigone lobes (Banziger et al. 2000). All members of the genus *Sapria* are distinctive and narrowly host-specific plants belonging to the family Rafflesiaceae. The typical form of *Sapria himalayana* is rare throughout its range from northeastern India, southwestern China, Thailand, Myanmar, to Vietnam. Being a member of the holoparasitic plant family, Rafflesiaceae, and growing on various species of *Tetrastigma* (Vitaceae), it is a little-understood species (Wu & Raven 2003; Nikolov & Davis 2017; Tran et al. 2018; Tanaka et al. 2019; Syiemiong et al. 2022). In India, the typical species of *Sapria* was first reported from the tropical wet evergreen forests of the Mishmi Hills of Lohit District by William Griffith in 1847 and later from the Aka Hills in Kameng District by Norman Loftus Bor in 1938 (Andreas & Jis 2014; Borah & Ghosh 2018). This species is the largest root parasitic angiosperm, having a host-specific relationship with the plant genus *Tetrastigma* of the Vitaceae family.

Tetrastigma bracteolatum (Wallich) Planchon and *T. serrulatum* (Roxb.) Planchon have been reported as the host plant of *Sapria* in Namdapha Park (Arunachalam et al. 2004; Borah & Ghosh 2018). The members of the Rafflesiaceae have a reduced vegetative body among all angiosperms (Nikolov et al. 2014). *Sapria* is well distinguished from the other two genera of Rafflesiaceae, *Rafflesia* R.Br. ex Thomson and *Rhizanthes* Dumort by the presence of 10 perianth lobes in two whorls (Meijer 1997; Nikolov & Davis 2017; Tanaka et al. 2019).

At present, the genus *Sapria* consists of four species, viz., *S. himalayana* Griffith. which has the widest distribution range among them (Wu & Raven 2003; Bendiksby et al. 2010; Ahmad et al. 2020; Syiemiong et al. 2022), with one form, *S. himalayana* Griffith f. *albovinosa* Banziger & Hansen (Banziger et al. 2000), endemic to northeastern Thailand, *S. poilanei* Gagnep, endemic to Cambodia (Gagnepian 1941), *S. ram* H Bänziger & B Hansen, endemic to Thailand (Banziger & Hansen 1997), and *S. myanmerensis* Nob. Tanaka, Nagam, Tagane & M.M. Aung, endemic to Myanmar (Tanaka et al. 2019). *Sapria himalayana* has lost about 44% of conserved genes in Eurosids that are enriched for functions like photosynthesis, plastid organisation, defence, stress response, and nutrient assimilation. It has also gained some genes from its hosts through horizontal gene transfer, showing extreme genome remodelling under parasitism (Cai et al. 2021).

In Rafflesiaceae, previous studies on the bud development, growth rate, mortality, flower phenology, life history and autecology have been conducted for several species of *Rafflesia*, where only a few were found for *Sapria* and none for *Rhizanthes*. Hidayati et al. (2006) and Nais (2001) studied *Rafflesia patma*, *R. keithii*, and *R. pricei* for their similar rapid later-stage bud growth and low bloom success. Sofiyanti et al. (2007) reconstructed the life cycle of *Rafflesia hasseltii*, which was redrawn from Nais (2001). Galindon et al. (2016) and Tolod et al. (2020) reported a new species of *Rafflesia*, namely *R. consueloae* and studied its first flower and fruit development and life history. Susatya (2020) reported the growth of the flower bud, life history and population structure of *R. arnoldii*. Recent work by Rambey et al. (2023) reported on the population and ecology of the endangered *R. meijeri* in Indonesia. Also, Wee et al. (2024) reported the bud development, flower phenology and life history of *R. cantleyi*. The earlier works on ecology, status, and conservation of *Sapria himalayana* Griffith. was done by Elliot (1992). Also, Arunachalam et al. (2004) reported on the population and conservation of *S. himalayana* Griffith. in Namdapha National Park, Arunachal Pradesh, India.

Currently, there is little information regarding the studies on the flower bud growth of *S. himalayana* Griffith f. *albovinosa* Banziger & Hansen (2000), even though it is essential for conservation purposes. The main objectives of this study were to observe the flower bud growth at different developmental stages of *S. himalayana* f. *albovinosa* and the change of its population structure with respect to the growth of the flower bud.

MATERIALS AND METHODS

Study area

The study was conducted in the subtropical evergreen rainforest of Machi Village, situated under the jurisdiction of Tengnoupal District, Manipur, northeastern India. The site is located at a latitude of 24.504^{O} N and a longitude of 94.143^{O} E with an elevation of about 1,477 m. Machi Village is about 62 km away from the capital city, Imphal, of the state of Manipur. The motorable road to the village is in poor condition, with several potholes and uneven surfaces and is also prone to landslides during the monsoon season, which could completely block the way. The present research site is located in a remote area of the village, also there is no motorable road to the site as shown in Image 1. The village is situated in the Indo-Myanmar biodiversity hotspot, which is a region rich in floral and faunal diversity, and harbours numerous interesting and endangered species. The vicinity of the village is inhabited by the Maring Tribe, one of the indigenous tribes of Manipur, which has rich ethnic cultures and traditions. The people of the village practice jhumming cultivation as their main source of income. The entire region of the village crossing the International Indo-Myanmar Road harbours the tropical rainforest to sub-tropical evergreen, and deciduous forests. It is also an ideal habitat for various rare and endangered carnivores and birds.

Field data collection

The present study was carried out from July to November 2024 at the study site. *Sapria himalayana* f. *albovinosa* were found at the community evergreen forest of Machi Village, Manipur, and were monitored, and the measurements of the plant buds and flowers were recorded for every observation at the study site. The observation was made at two-week interval for five months. The flower buds of *Sapria himalayana* f. *albovinosa* are found only on the roots of the host plant, *Tetrastigma*, unlike many species of *Rafflesia* found on both roots and climbing stems of the hosts. They are covered by the litter of the forest floors, making it difficult to observe at first sight. All the buds were discovered through thorough searching, scrutinising, and by removing forest litter in search of other buds whenever a bud was first detected at the study site. As such, this may prove to be an inevitable study limitation and may underestimate the actual population. While there were cases in which some undetected below-ground buds escaped the initial observation, when they grew bigger and surfaced above ground later, they were eventually added to the monitoring system (Wee et al. 2024). The diameters of *Sapria* buds and flowers were measured by the widest diameter length (Elliot 1992). The observation of this study was limited to the visible structure of bud and flower developmental stages of *Sapria himalayana* f. *albovinosa* (Susatya 2020). Each bud and flower was vertically photographed, and its diameter was measured at every observation (Nais 2001; Kamal et al. 2022). The

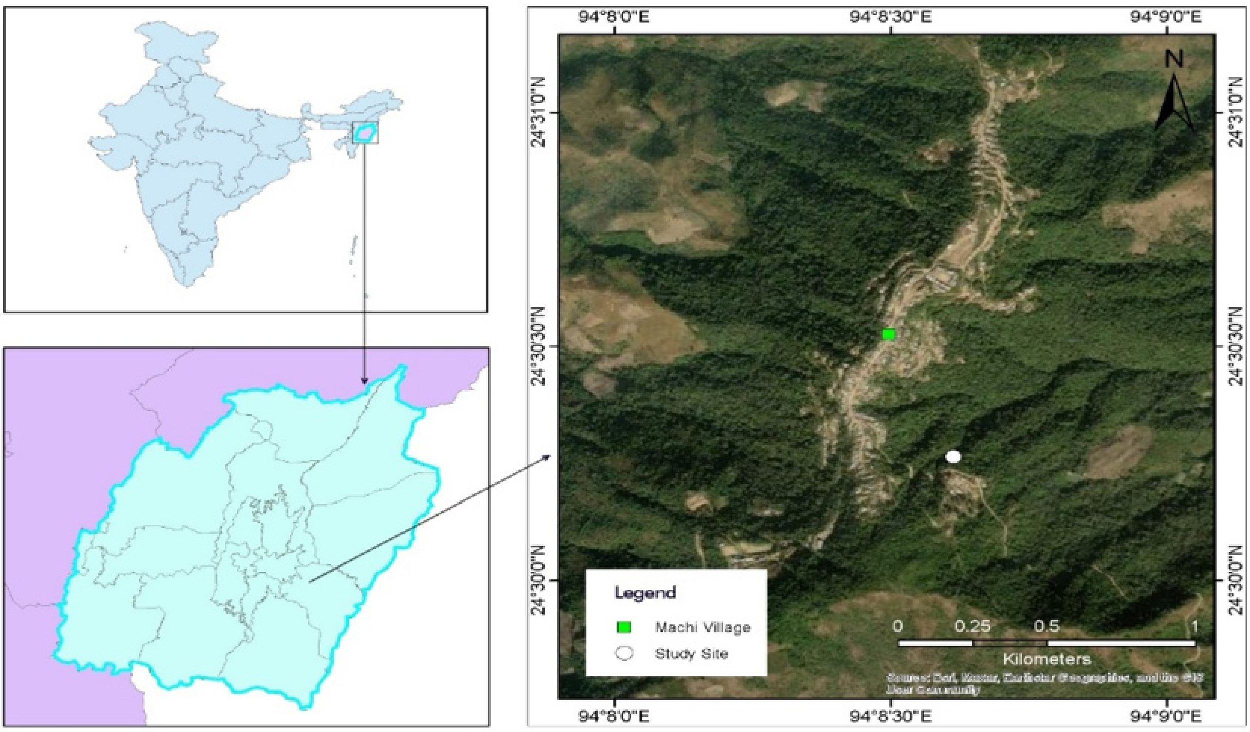

Image 1. A map showing the study area in the sub-tropical evergreen forest of Machi Village, Manipur, northeastern India. The white circle represents the location of *Sapria himalayana* f. *albovinosa*.

bud development was then categorized into different size classes for further analysis, following the methods by Nais (2001), Susatya (2020), and Tolod et al. (2020). The dead buds of all bud diameter sizes were recorded for every observation. Then the mortality rate of the *Sapria* buds was calculated by the formula given below (Nowak et al. 2004).

$$\textit{Mortality rate} = \frac{\textit{Total number of dead buds}}{\textit{Total number of buds recorded}} \times 100$$

The abiotic parameters, such as air temperature, wind speed, humidity, soil temperature, soil moisture, soil pH, and light intensity, at the present study site were recorded using a thermometer and a 4-in-1 soil tester.

Statistical analysis

A non-linear regression analysis was conducted to obtain the exponential growth model equation and coefficient of determination (R^2) for the observed bud diameters to demonstrate a J-shaped bud growth curve. Also, correlation analysis was performed to examine the relationships between environmental factors such as ambient temperature, humidity, wind speed, soil pH, soil temperature, light intensity, and mortality of the buds for a five-month observation. A chi-square test was conducted to assess the bud stage distribution shifts across time of the observation. All statistical analyses were performed using SPSS version 26.

RESULTS AND DISCUSSIONS

Flower bud growth of *Sapria himalayana* f. *albovinosa*

The present study showed that the life cycle of *Sapria himalayana* f. *albovinosa* is complex, as most members of Rafflesiaceae have two parts – the invisible and visible parts (Hidayati et al. 2006; Nais 2001; Kamal et al. 2022). The invisible part includes the inoculation and germination of *Sapria*'s seed occurring inside its host plant roots, whereas the visible part is the emergence of the flower bud, mature bud, and anthesis. The visible part consists of several flower buds developing and is also the only plant structure that is exposed to external environmental factors. Therefore, flower buds at different sizes exhibited different growth rates and developmental stages. The observation limited to only the visible parts of the life cycle resulted in six different development stages of the flower, which were then categorized into the copule, copule-bract transition (CBT), bract, bract-perigone transition (BPT), perigone and anthesis stages (Image 2). Copule, bract and perigone stages were defined by 80–100% of the images of vertically photographed bud respectively covered by copule, bract, and perigone structures. A bud was categorized into CBT, if it grew between copule and bract stages, and the coverage of the images of the photographed bud by the bract reached 40% to 80%. Meanwhile, a bud was grouped into BPT, if it grew between the bract and the perigone stages, with coverage of the images of the photographed bud by the perigone reaching 40% to 80%. Any bud with less than 40% of the coverage by either bract or perigone was also categorised into either copule or bract stages (Susatya 2020). The anthesis stage occurred once during the observation at the bud's diameter range of 16–20 cm. The observation resulted that the diameter range of copule, CBT, bract, BPT, and perigone stages respectively were 0.3–2.3 cm, 2.4–4.0 cm, 4.1–5.5 cm, 5.6–6.1 cm, and 6.2–8.1 cm (Table 1). The growth development of *Sapria's* bud was not in a discrete pattern, where one stage was replaced completely by the next stage. The same growth development was also observed in the typical species of *Sapria* (Elliot 1992). It consisted of a series of overlapping development stages, where before one stage was complete, the following stage had already developed. It was a basic reason why transition stages were introduced in this research. The first visible structure was copule, which was basically the bark of the host plant root covering the actual *Sapria* structure. The first visible structure of *S. himalayana* f. *albovinosa* at Machi had the diameter range of 0.3–2.3 cm (Table. 1). The start of the development of the inner structures of *Sapria* was still unknown though it is needed to be studied whether all the inner structures had been developed in the copule stage or not. The inner structures in the species of *Rafflesia* had already developed in the copule stage while observing its dead bud of 6 cm diameter (Susatya 2020). As the bud grew, the upper copule started to crack to allow the first true structure of the *Sapria* or bract to be visible. Bract was originally pastel

Table 1. The range of diameters of buds and flowers according to its stages.

Name of bud stages	Range of bud diameter (cm)
Copule	0.3–2.3 cm
Copule-bract transition (CBT)	2.4–4.0 cm
Bract	4.1–5.5 cm
Bract perigone transition (BPT)	5.6–6.1 cm
Perigone	6.2–8.1 cm
Anthesis	16–20 cm

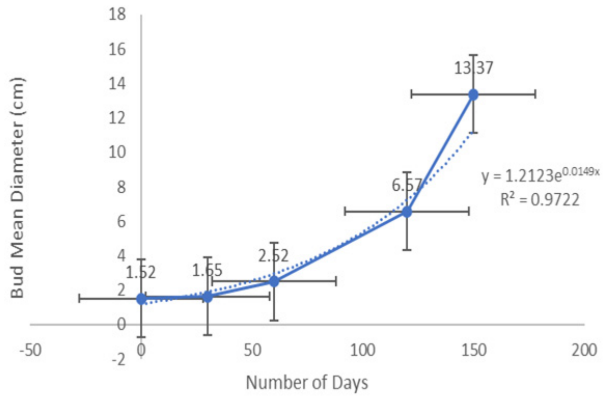

Figure 1. Growth curve of *Sapria himalayana* f. *albovinosa* during the five-month observation.

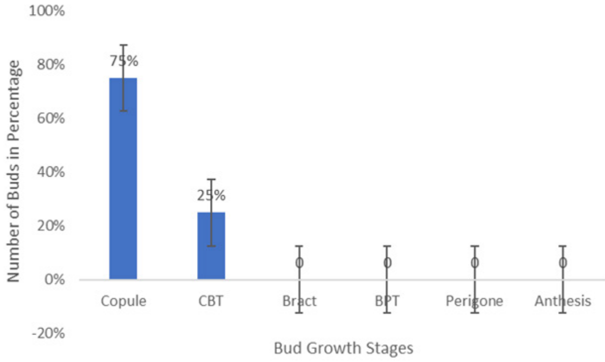

Figure 2. The population structure of *Sapria himalayana* f. *albovinosa* in August.

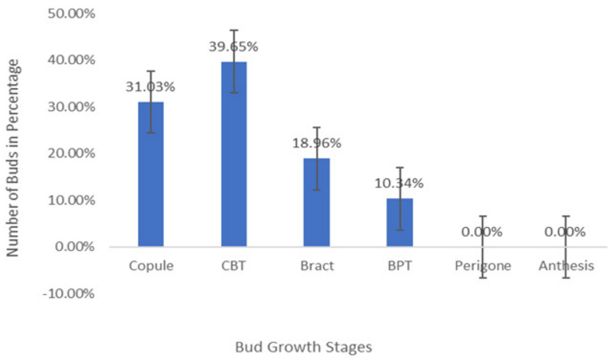

Figure 3. The population structure of *Sapria himalayana* f. *albovinosa* in September.

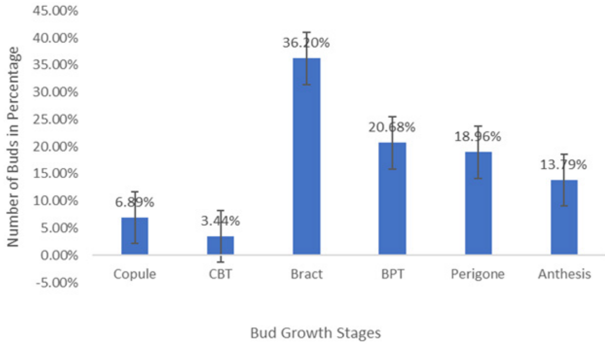

Figure 4. The population structure of *Sapria himalayana* f. *albovinosa* in October.

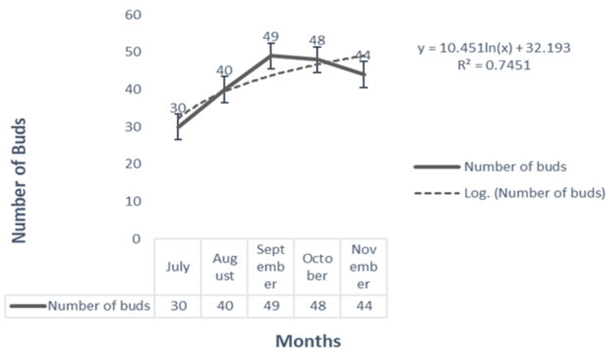

Figure 5. Population growth curve showing number of buds with respect to the month of observation.

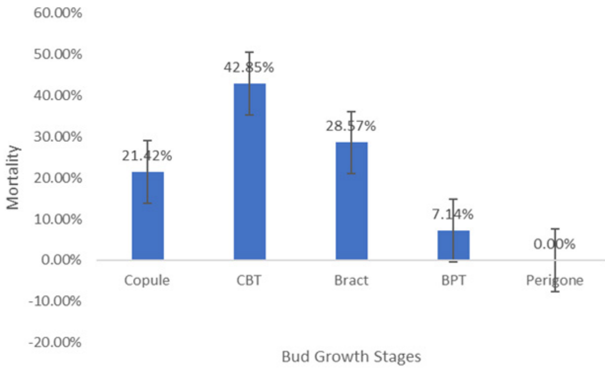

Figure 6. The bud mortality rate of *Sapria himalayana* f. *albovinosa* according to its growth development stage.

pink and white colour, but eventually turned black as it grew older. The bract was gradually replaced by a pale pink perigone stage consisting of buds with a diameter range of 6.2–8.1 cm (Image 2).The bract consisted of two series of five imbricate and whorled scales (Elliot 1992). The pinkish perigone lobes of the bud indicated anthesis to occur within 3–4 days. The field observation showed that when the upper layer of the perigone lobe was slightly raised, then the anthesis would take place within 2–3 days, and lasted between 4–5 days. All flower structures decomposed within a month after flowering. The column was the only female structure that did not decompose and further developed into mature fruit (Elliot 1992). As the flowers opened, they emitted an

Image 2. The flower bud development stages of *Sapria himalayana* f. *albovinosa*: A—copule stage | B—copule-bract transition stage (CBT), copule (cp) gradually replaced by bracts (br) | C—bract stage, a stage where a visible bud is a bract, a similar structure to a sepal | D—bract-perigone transition stage (BPT), a stage where bracts are still largely recognized (br) and gradually replaced by perigone (pr) | E—perigone stage, a visible bud at this stage is all covered by the perigone, a similar structure to a petal | F—Anthesis stage, a flower fully bloomed. © K. Shamran Maring.

odour similar to that of rotting meat, which lasted for 2–3 days. Within 3–4 days after opening, flowers started to darken and eventually turned black. As the flowers turned black, all the plant structures shrank. The base of male flowers and their attachment to the host shriveled rapidly and eventually detached from their host roots. In female flowers, the perigone tube and lobes, diaphragm and disk shriveled as in males, but the column, ovary and surrounding tissues at the base of the column remained alive (Elliot 1992). The base of the perigone tube swelled and remained white externally for about two months after flower opening. This structure constituted the fruit of *S. himalayana* f. *albovinosa*, though a detailed study on the fruits and seeds dispersal is required. The growth rate of flower buds at earlier stages was found to be very slow, while the buds at older stages showed higher growth rates. Therefore, the growth curve of the flower bud showed a typical J-shaped growth curve or an exponential growth curve (Figure 1).

The non-linear regression equation $y = 1.2123\ e^{0.0149x}$ and $R^2 = 0.9722$ was obtained by plotting the bud mean diameter across the number of observed days. The bud diameter grows by about 1.49% of its current size in every additional day. This means the bud diameter doubles roughly every 46–47 days under the observed growth pattern. Therefore, on the initial day of observation, the bud starts at about 1.5 cm in diameter, then, by about 150 days, they grow to above 13 cm in diameter, matching the measured data points (Figure 1).

The present study showed that *S. himalayana* Griffith f. *albovinosa* found at Machi comparatively has crateriform and bilobed or multilobed ramenta apices in male and female flowers, respectively, while the Vietnamese taxon is also bilobed or multilobed (female) or crateriform (male) (Maring & Pinokiyo 2024). The diameter of the crest disk and aperture of the diaphragm for the Machi individuals are 3.4–3.5 cm and 1.9–2.0 cm, respectively. The *S. himalayana* Griffith f. *albovinosa* from Machi Village has a comparatively larger floral span (13–20 cm diameter) than the *S. himalayana* Griffith f. *albovinosa* (11–16 cm diameter) (Banziger et al. 2000), *S. myanmerensis* (10 cm) (Tanaka et al. 2019), *S. poilanei*

Table 2. The abiotic factors of *Sapria himalayana* f. *albovinosa* in Machi evergreen rainforest, Manipur.

Abiotic factors	July	August	September	October	November
Mean air temperature (°C)	23	23	22	20	19
Mean humidity (%)	80	84	86	72	67
Mean wind speed (m/s)	1.60	1.66	1.66	1.66	1.66
Mean soil temperature (°C)	19	24	21	20	18
Mean soil pH	6.5	6.5	6.0	5.5	5.5
Mean soil moisture	Wet+	Wet	Wet	Normal	Dry
Mean light intensity (lux)	90	90	100	100	100

(6.5–12 cm), and *S. ram* (5.5–11 cm) (Banziger & Hansen 1997).

Population structure of *Sapria himalayana* f. *albovinosa*

The population status of *S. himalayana* f. *albovinosa* is very small when compared to the other higher plants. Also, the population of this endemic infraspecific taxon is much smaller than that of the typical species of *Sapria* (Elliot 1992). The initial observation in the month of July showed that the total population is 100% dominated by the copula stage. At the second observation in August, the population structure was 75% copule and 25% copule bract transition (CBT) Stage (Figure 2). Meanwhile, buds at CBT were fewer due to the changes in the flower bud development stage. Larger stages, such as bract and BPT, were observed respectively with 18.96% and 10.34% in September (Figure 3). Within three months, the population structure was significantly changed due to the mortality, new recruitment, and growth of buds from one stage to the next growth development stages. The population structure of this period was shifted toward bract and BPT. During the next observation, in the month of October, the population structure interestingly exhibited all six bud stages, where the bract stage dominated it with 36.20% (Figure 4). The pattern of population structure appeared to be opposite to the initial one, where the perigone and anthesis stages were exhibited with 18.96% and 13.79%, respectively. In the last month's observations, the population structure was dominated by full bloom/anthesis and after-blooming flowers. The flower buds were found at their full bloom stage in October to December, but the optimal flowering season is from late October to late November. During the initial period of research, the number of flower buds increased gradually due to the new recruitment of the buds, while the population size decreased in the later months of observation. This condition could be due to insufficient nutrients for the population of the flower bud to maintain its viability and complete its life cycle (Figure 5). A chi-square test revealed a significant shift in bud stage distribution across time (X^2 = 202.997, df = 20, p = 2.87 x 10^{-32}), this indicates that the proportion of buds at different developmental stages varied markedly with time and did not remain constant over time.

In the five-month observation, a total of 58 flower buds were recorded, out of which 28 buds were recruits and 14 buds died without reaching maturity. The causes of the bud mortality were fungal infections and injuries from anthropogenic interventions, where the injured parts of flower buds were immediately followed by a rotting process that led to the bud mortality. All losses occurred at buds belonging to the copule, CBT, bract, and BPT with copule-bract transition (CBT) stage showing the highest mortality rate of 42.85% (Figure 6). The population structure of *S. himalayana* f. *albovinosa* showed a mortality rate of 24.13%, which is much lower when compared to the mortality rate 40% of the typical species (Elliot 1992). Although the mortality rate is much lower than that of the typical species of *Sapria*, the total population status of this endemic taxon is very small, indicating an alarming signal concerning the future population of *S. himalayana* f. *albovinosa*.

Abiotic factors of *Sapria himalayana* f. *albovinosa* habitat

The ambient temperature of the study site was found to decrease from 23°C (July) to 19°C (November),which is due to the onset of the winter season. The air humidity ranges 67–86 %, and the light intensity was found to be very low, ranging 90–100 lux, probably be caused by the dense canopy cover (Table 2). The high air humidity and low light intensity play a vital role in the existence of *S. himalayana* f. *albovinosa* because the plant preferably grows on the understory of forest floors. Also, in the present study, the flower buds showed higher mortality rates as the light intensity increased. There is a moderately strong positive correlation between light intensity and bud mortality, with a correlation value

(r) = 0.6123, indicating that higher mortality rates are generally associated with higher light intensity. Whereas, the soil pH showed a moderate negative correlation (r = -0.326) with the bud mortality. In Table 2, the soil pH ranges 5.5–6.5, and it is classified as acidic, while the soil temperature ranges 18–24 °C. The soil moisture showed very wet conditions in July, which could be due to the monsoon season, while the soil was dry in November, which could be due to the winter season. Therefore, the abiotic parameters of the environment play an important role in the growth of flower buds as well as in the population dynamics of this rare endemic taxon.

CONCLUSION

The flower bud growth of *Sapria himalayana* f. *albovinosa* has six developmental stages consisting of the copule, CBT, bract, BPT, perigone, and anthesis stages. More detailed studies on the growth rate, mortality rate and population structure of this rare parasitic plant are required to determine the fate of the young flower buds and to estimate its complete life cycle. The findings from this study are useful in intensifying the knowledge of this rare parasitic plant that is becoming vulnerable and is on the brink of extinction. Due to its rarity, ongoing habitat loss, vandalism of existing colonies, and high degree of host specificity, various conservation actions are required to protect this taxon. Hence, the information on the growth of buds, different flower bud developmental stages, and population status is vital for taxonomic studies, field monitoring, and conservation purposes.

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