REPRODUCTIVE BIOLOGY OF A RARE, FLY-POLLINATED ORCHID, *BULBOPHYLLUM TIANGUII*, IN SOUTH CHINA

JIANG, H. D.[1#] – CHEN, N.[1,2#] – PENG, L. H.[1] – YANG, Z.[1,2] – YANG, Y. S.[1] – CHAI, S. F.[1*] – ZOU, R.[1] – CHEN, Z. Y.[1*] – XIONG, Z. C.[1] – WEI, X.[1*]

[1] *Guangxi Key Laboratory of Plant Functional Phytochemicals and Sustainable Utilization, Guangxi Institute of Botany, Guangxi Zhuang Autonomous Region and Chinese Academy of Sciences, Guilin, Guangxi 541006, China*

[2] *College of Tourism and Landscape Architecture, Guilin University of Technology, Guilin, Guangxi 541006, China*

[#] *These authors contributed equally.*

**Corresponding authors*
e-mail: sfchai@163.com; chenzongyou@gxib.cn; weixiao@gxib.cn

(Received 11th Sep 2024; accepted 7th Jan 2025)

Abstract. *Bulbophyllum tianguii*, an endangered orchid species, is predominantly distributed on the surface of karst stone mountains or the stems of humus trees in South China. To better understand the reproductive biology of the *B. tianguii*, we investigated the breeding system and pollination biology in natural populations. The flowering period of the *B. tianguii* population extended from late March to early April, and anthesis of a single flower usually endured 5-6 days. Floral scent analysis indicated that 2-methyl-1-butanol and leaf alcohol were the major compounds produced by *B. tianguii*. The breeding system was self-compatible and facultatively out-crossing, requiring pollinators. The only pollinator identified for *B. tianguii* was *Sarcophaga carnaria*, a species of fly. However, its visiting frequency is extremely low (0.005 visits/flower/h). The hinged labellum of these flowers functioned as a fulcrum. When pollinators entered the flower, they were propelled in a way that ensured contact with the column area. It plays a key role in the pollen dispersal. Natural fruit set for *B. tianguii* were merely 2.5% and 2% in the natural populations. Low reproductive rates in *B. tianguii* were found to be a consequence of few species of pollinators and their low visiting frequency. Pollen limation may be a crucial factor underlying the endangered of *B. tianguii*. Artificial pollination and the release of pollinators represent effective strategies for increasing the fruit yield to *B. tianguii*.

Keywords: *Bulbophyllum tianguii, pollination, breeding system, self-compatibility, Orchidaceae*

Introduction

The majority of orchids possess pollination systems, such as deceptive pollination, which promote outcrossing (Johnson et al., 2003; Jersáková et al., 2006). These plants exhibit an extensive array of floral designs and displays that optimize outcrossing (Jin et al., 2012). A significant proportion of orchids display pollination specificity, with approximately 67% of orchid have only one recorded pollinator (Scopece et al., 2010; Pansarin et al., 2016; Zhang et al., 2024). Consequently, these species with pollination specificity are often pollinator-limited (Scopece et al., 2010). Multiple factors can lead to such pollination limitations, including a lack of pollinator diversity or low visitation frequency (Tremblay et al., 2005; Kolanowska et al., 2021). Orchids tend to exhibit a low natural fruit set (Tremblay et al., 2005). Notably, self-compatible orchids exhibit an extremely low fruit set (Liu et al., 2013; Shi et al., 2024).

Comprising more than 2000 species, *Bulbophyllum* is the largest genus of Orchidaceae and the third largest genus of flowering plants (Frodin, 2004; WCSP, 2018). The genus

DOI: http://dx.doi.org/10.15666/aeer/2302_30533066

Bulbophyllum (Orchidaceae) is distinguished by its precisely balanced, see-saw-like lip (labellum). This lip is connected to the base of the floral column via a minute, resilient hinge (Ridley, 1980; Chen et al., 2011; Wittaya et al., 2019). The flowers of Orchid emit a pungent, foul-smelling scent, which serves to attract flies (Van der Pijl & Dodson, 1969). The genus *Bulbophyllum* is well known to display flowers that are mainly adapted to fly-pollination (Nishida et al., 2004; Laurence et al., 2011; Zhang et al., 2024). In some cases, *Bulbophyllum* plants utilize scent as a reward to attract pollinators (Tan and Nishida, 2007). Floral morphological and scent serve as crucial cues for attracting pollinators (Tan et al., 2021). For instance, the flowers of *Bulbophyllum nipondhii* lack nectar and emit a faint scent resembling decaying wood. Their elastically hinged lip functions as a seesaw balance. When a pollinator enters the flower, the lip tips, propelling the insect against the column (Wittaya et al., 2019). In the case of *Bulbophyllum vinaceum*, as the fly moves towards the base of the lip while lapping up compounds, it crosses the point of imbalance, causing the lip to snap back to its nornal closed position (Tan et al., 2006). The flowers of *Bulbophyllum cheiri*, with the aid of its specialized hinged see-saw lip (labellum), temporarily traps a fruit fly pollinator between its lip and column (Tan et al., 2002). Moreover, *Bulbopphyllum funingense* neither produces a strong odor nor nectar. Instead, it secretes a substance froms its labellum that attracts flies (Zhang et al., 2024). These findings collectively demonstrate that both flower morphology and odor play a vital role in attracting pollinators for *Bulbophyllum* plants.

Studies on plant reproductive biology usually focus on breeding system and pollination ecology (Zhang, 2004; Wittaya et al., 2019). The breeding system of certain *Bulbophyllum* plants have been reported to be self-compatible (Barba et al., 1999; Laurence et al., 2011; Gamisch et al., 2014). For instance, some case with *B. variegatum* (Laurence et al., 2011), *B. ipanemense* and *B. weddellii* (Borba et al., 1999). However, understanding of the reproductive biology of its constituent species remains limited. *Bulbophyllum tianguii* K. Y. Lang & D. Luo is a species within the Orchidaceae family. It was first discovered in 2007 in the Yachang Orchidaceae National Nature Reserve in Guangxi, China (Lang and Luo, 2007). This species blooms in the spring and is mainly distributed on the surfaces of karst rocky mountains or on humus trees (Luo et al., 2020). Subsequent to its initial discovery, additional occurrences of this species have been identified in the Mulun National Nature Reserve in Guangxi and the Wangmo Sutie Nature Reserve in Guizhou. Despite some research on the reproductive ecology of *Bulbophyllum* plants, they are still lacking compared to the abundant species of *Bulbophyllum* plants.

With the deterioration of the natural environment and the destruction caused by human activities, wild resources of orchids have been overexploited (Wei et al., 2022), posing a great threat to the survival of the *B. tianguii* and urgently requiring protection. To protect *B. tianguii* from further degradation, conservation management is required. A necessary component of such management is an understanding of *B. tianguii* reproductive biology. Studies of reproductive biology have proved critical to improved conservation of *Bulbophyllum* populations (Laurence et al., 2011; Wittaya et al., 2019). It is particularly important to study the characteristics of the breeding system of the wild germplasm resources of *B. tianguii* in order to better protect them. Therefore, research on the reproductive ecology of the *B. tianguii* is of great significance for its protection. This study mainly investigated the reproductive biology of the *B. tianguii*. We mainly explore the following two issues: (1) What are the pollinator of the *B. tianguii*? And what attracts pollinator? (2) What is the breeding system of *B. tianguii* in natural populations?

Materials and methods

Study site and species

This study site was in karst mountains of the Yachang Orchidaceae National Nature Reserve (24°44′16″-24°53′58″N, 106°11′31″-106°27′4″E), located in Leye County, Baise City, Guangxi Zhuang Autonomous Region, South China. This area lies within the subtropical monsoon climate zone of western Guangxi. It features an average annual temperature of 16.3°C, an average annual rainfall of 1,216.9 mm, and an average annual sunshine duration of 1,698.7 h. *Bulbophyllum tianguii* is distributed in the Yachang Nature Reserve at an altitude ranging from 950 to 1,821 m. Four populations of *B. tianguii* have been found in the reserve, comprising a total of 840 stems. Most of these plants are clonal and show an aggregated distribution (Chai et al., 2022). This study was conducted in the largest known population of *B. tianguii* (Top of Lao wuji Tiankeng, TLWJTK), while a controlled trial was conducted in the second largest population (Middle of Lao wuji Tiankeng, MLWJTK) for artificial bagging pollination and observation of pollinating visitors. The main accompanying plants in these habitats were *Laurocerasus spinulosa*, *Platycarya strobilacea*, *Choerospondias axillaris*, *Carpinus pubescens*, *Rhaphiolepis indica*, *Rhamnus lamprophylla*, *Myrsine semiserrata* and *Pyrrosia calvata* (*Fig. 1*).

*B. tianguii*is an epiphytic herb. Its pseudobulb is ovate conical or narrowly ovate, with 1.5- to 2.5-cm-long. Leaves are oblong, with 7-to 15-cm-long, and 1.5-to 2-cm wide. The petals acuminate and aristate at apex (arista 1.5 mm long), and 2 fleshy cilicte ridges on the disc of the lip. The labellum is ovate-lanceolate, 6.5 mm long and 3.5mm wide (Lang & Luo, 2007). This species is predominantly found beneath the evergreen deciduous broad-leaved mixed forest with a canopy coverage of 40%-80%. *B. tianguii* is usually attached to the rocks on the upper part of the karst rocky mountain or near mountain tops, as well as on the steep stone walls at the top of the Tiankengs. It is mostly distributed on the semi-sunny slope. The root soil is deciducous humus soil, and a few are soilless. Some individuals can also be attached to the stems of humus -rich trees or tree trunks, and often co-occur with bryophytes (Chai et al., 2022).

***Figure 1.** The characteristics of the habitat of Bulbophyllum tianguii A. Bulbophyllum tianguii grows in a karst habitat; B-C. Bulbophyllum tianguii grow in tree habitats*

Flowering phenology and morphology

During the flowering period in both 2021 and 2022, 100 inflorescences were selected at random and the numbers of flowers in each inflorescence were counted. Then, 40 flowers in 20 inflorescences were selected at random for measures of nectar volume and sugar concentration with a 10-μL microsampler and a hand-held temperature-compensated refractometer (Zhou et al., 2016). Flower longevity was assessed using unpollinated flowers (30 flowers from 15 inflorescences of 15 plants) and hand-pollinated flowers (40 flowers from 20 inflorescences of 20 plants), which were monitored from the time of opening until they had withered.

Floral scent analyses

Flora volatile compounds were analyzed through solid-phase micro extraction (SPME) and GC/MS analyses (Zang and Pawliszyn, 1993; Micheneau et al., 2008). Two different SPME fibers were used: a black Carboxen/Polydimethylsiloxane (CAR/PDMS) fiber (length 1 cm, film thickness 75 μm) recommended for volatile compounds of low molecular weight and a gray StableFlex Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) fiber (length 2 cm, film thickness 50/30 μm) indicated for flavors (volatiles and semivolatiles). The fibers were conditioned priod use according to supplier’s prescriptions, 1 h at 300 ℃ for CAR/PDMS, and 1 h at 270 ℃ for DVB/CAR/PDMS. Before the first daily analysis the fibers were conditioned for 10 min.

Briefly, bud-stage field specimens were collected from the study site and transported to the laboratory. They were placed in a glass-bell, sealed at the large extremity with cotton wool and at the other end by the SPME-fiber. Flower headspace capture was performed for 6 h during the optimal period of fragrance emission. Blank (i.e. exposed fiber without flower) were run to establish a base line. This experiment was performed three times with the same flower to ensure the results were reproducible. Analyses were performed using a Hewlett Packard 6890N gas chromatograph (GC), coupled directly to a Hewlett Packard 5937N mass spectrometer (MS). Compounds were desorbed from the fiber in the GC injector (splitless injection mode) at 250 ℃ and separated on a capillary SPB-5 nonpolar column with helium as carrier gas. The GC oven was programmed to increase temperature from 60 to 230 ℃ at 4 ℃/min, following by a stabilization at 230 ℃ during 40 min. Mass spectra were produced with a current ionization of 70 eV, in a scan range of m/z 30–550. Compounds were identified by comparison of their retention indices and their mass spectral fragmentation and stored on the MS “Nist 2002 ” and “Wiley 7” libraries.

Breeding system

To further evaluate the breeding system of *B. tianguii* scientifically, both the TLWJTK and MLWJTK population were tested with 5 different types of treatments, as follows. 200 flower buds and 100 plants were randomly selected in TLWJTK and MLWJTK, respectively. For these plants, a nylon net bag with 0.5 × 0.5 mm gas was placed on these plants prior to flowering to prevent insect entry into the flowers. The following tests were performed: (1) Open pollination, (control): the flowers were tagged and left open so that natural pollinators could access them. (2) Apomixis: the flowers were bagged and emasculated before anthesis. (3) Autogamy: the flowers were bagged and isolated from visiting organisms. (4) Geitonogamy: the emasculated and bagged flowers were pollinated by hand using pollen from other flowers on the same plant. (5) Xenogamy (artificial outcrossing within the population): the emasculated and bagged flowers were pollinated by hand using pollen from a few flowers from differents in the same population. The flowers used in these tests were marked with tags, and fruit set was recorded at the end of flowering after 1 month, when the seeds were mature but the fruits had not dehisced.

Pollinator observations

Pollinator observations took place from March 26-30 in 2021-2022 in both the TLWJTK and MLWJTK populations. Flowers (80 flowers from 40 inflorescences across 40 plants) were selected at random and observed from 08:00 to 18:00 on sunny or cloudy (but not rainy) days for a total of 50 h. To minimize the potential for disturbances caused by observe movement or odors, all flowers were monitored for a distance of 2 m (Wittaya et al., 2019). Given the small size of these flowers, insect visitation was observed with a digital camera equipped (eos750d-s18-135mm; cannon). Pollinator visitation behaviors, visitation frequency, single flower residence time, and behavior after flower visitation were observed. After observations were complete, insect nets were used to catch pollinators during their visits to these flowers, and specimens were evaluated for species identification. To test for any potential nocturnal pollinators, 40 flowers from 20 inflorescences, were randomly selected and bagged during the day (8:00-18:00), unbagged them at night (18:00-6:00), and assessing pollinia removal and deposition in the early morning on the following day.

To evaluate the morphological consistency between floral and pollinator traits, both flower traits and pollinator size were measured. For flowers, measures traits included the distance between the column and labellum (access height), the width of the labellum (access width), and the distance from the column foot to the operculum (access length). For pollinators, the thorax height, thorax width and body length were measured (Chen and Gao, 2011; Wittaya et al., 2019).

Statistical analyses

Significance of differences between fruit set in the hand pollination treatments was test using a simple χ^2 -test. Differences in flower traits and pollinator body structures were compared with t-test. SPSS 23.0 for Windows was used for all analyses, and figures were constructed in Adobe Photoshop CS6.

Results

Flowering phenology and morphology

The flowering period for *B. tianguii* took place from late March to early April. Inflorescences extended from the base of the pseudobulbs, with each inflorescence exhibiting 1-3 flowers (mean: 2.10 ± 0.40; N = 100) (*Fig. 2A-D*). These flowers were pale yellow with purplish-brown veins. Petalis anguste ovatis, 6.0-7.5 mm longis, 3.5 mm latis, apice acuminatis et arista 1.5 mm longa instructis. Labello ovato-lanceolato viridifusco, ca.6.5 mm longo, basi 3.5 mm lato, et disco cristis duobus carnosis ciliatis armato (hinged structure) (*Fig. 2F*) (Lang and Luo, 2007). Unpollinated flowers generally lasted 5-7 days, with a mean (±SD) of 5.72 (±0.75 days, n = 30). In all

40 hand-pollinated flowers, wilting within was observed 2-3 days. Fruits ripened within 6 months after pollination (*Fig. 2E*). No floral nectar was produced by these flowers, but under sunny conditions, a secretion was produced on the labellum.

Figure 2. *The flowering dynamics, fruiting, and floral traits of Bulbophyllum tianguii. A. Bud intumescence stage; B. Initial flowering stage; C. Full blooming stage; D. Late flowering stage; E. Fruits; F. Flower morphological features. s. Middle sepal; an. Pollen cluster; co. Column; ml. Labellum; pe. Petal; la. Lateral sepals*

Floral scent composition

Analyses of the floral scent composition of *B. tianguii* led to the identification of 7 compounds (*Fig. 3; Table 1*). Among these, the major compounds were 2-methyl-1-butanol and leaf alcohol.

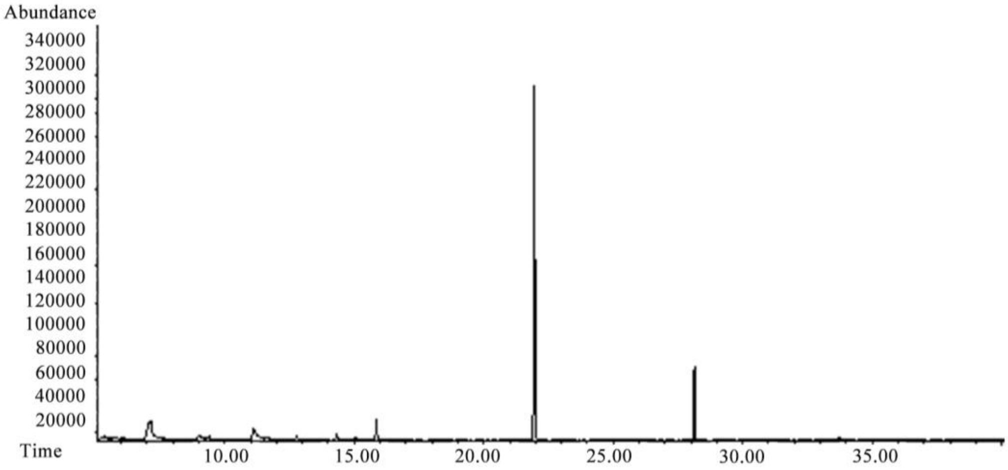

Figure 3. *Mass spectrum analysis of Bulbophyllum tianguii by GC-MS flowers in full bloom*

***Table 1.** Average relative amounts (%) of floral scent compounds in Bulbophyllum tianguii inflorescences*

No.	Compound	CAS	Relative amount ± S.E. (%)
1	2-Methyl-1-butanol	137-32-6	52.91 ± 2.5
2	2-Methyl-4-pentenal	5187-71-3	11.00 ± 1.0
3	Leaf alcohol	928-96-1	20.93 ± 1.21
4	Methacrolein	78-85-3	2.19 ± 0.12
5	1,7-Heptanediol	629-30-1	4.93 ± 0.32
6	3,6,6-trimethyl-bicyclic (3.1.1) heptane-2-ene	4889-83-2	4.85 ± 0.21
7	Camphene	79-92-5	3.19 ± 0.31

Breeding system

The results of the diverse bagging experiments on *B. tianguii* are in *Table 2*. Emasculated and bagged flowers failed to produced fruits and dropped shortly after pollination, indicative of the absence of agamospermy in *B. tianguii*. In the TLWJTK and MLWJTK population, flowers subjected to autogamy treatment did not set fruit. Consequently, the possibility of autonomous self-pollination was excluded. The fruit set of the open pollination was 2.5% in the TLWJTK population and 2% in the MLWJTK population. This indicate that open pollination fruit set was very low. The fruit set of both geitonogamy and xenogamy pollination treatments were relatively hige, 50 and 55% in the TLWJTK population and 37.5 and 40.9% in the MLWJTK population, respectively, which further suggested that *B. tianguii* is self-compatible. Meanwhlie, the fruit set in both the geitonogamy and xenogamy pollination treatments were significant higher than that of the natural contron in both the TLWJTK ($\chi^2 = 57.243$, $p < 0.001$ for geitonogamy vs. control; $\chi^2 = 66.261$, $p < 0.001$ for xenogamy vs. control) and MLWJTK ($\chi^2 = 40.005$, $p < 0.001$ for geitonogamy vs. control; $\chi^2 = 45.060$, $p < 0.001$ for xenogamy vs. control) population, demonstrating that pollen limitation existed at both sites.

***Table 2.** Fruit set of Bulbophyllum tianguii under different pollination treatments*

Treatment	TLWJTK				MLWJTK		
	Flower	Fruits	Fruit set (%)		Flower	Fruits	Fruit set (%)
(1) Open pollination	40	1	2.5		50	1	2
(2) Apomixis	20	0	0		20	0	0
(3) Autogamy	20	0	0		30	0	0
(4) Geitonogamy	20	10	50		16	6	37.5
(5) Xenogamy (artificial outcrossing within the population)	20	11	55		22	9	40.9

Observation of pollinator behaviors

In both the TLWJTK and MLWJTK populations, the only identified pollinator species was the *Sarcophaga carnaria* (*Fig. 4*). All flowers that were bagged during the diurnal period (8:00-18:00) but then unbagged during the nocturnal period (18:00-8:00) remained intact, indicating that no nocturnal pollinator visited flowers of *B. tianguii* outside of our observation time. Pollinator activities was most frequent during 8:00-10:00 and 16:00-18:00 periods. When the *S. carnaria* individuals visit these flowers, they generally land directly on the labellum, and advanced towards the labellum base. However, upon reaching a critical balance point, the weight of the *S. carnaria* causes the upward tipping of the hinged labellum. Its back is pressed toward the column, and the pollen clusters on its back falls right on the slime stigma to complete pollination. When the *S. carnaria* begins to retreat from the flower, its back presses on the operculum and thus forces it open, exposing the viscidium and causing pollen clusters adherence to its back. On average, pollinator visited flowers 20 times over the 50 hour period (80 flowers) in both sites, respectively. The frequency of flower visits was 0.005 visits/flower/h, carried pollen clusters 5 times, and the residence time for a single flower visit was 11.56 ± 3.51 s ($n = 20$) at both sites.

No significant differences were noted between flower access height and *S. carnaria* thorax height ($t = 0.133$, $P > 0.05$). However, there were significant differences between flower access width and *S. carnaria* thorax width ($t = 14.214$, $P < 0.01$), as well as between flower access length and *S. carnaria* body length ($t = -7.351$, $P < 0.01$) (*Table 3*).

***Table 3.** Analyses of the body structural characteristics of visiting pollinators and floral traits (means ± SD)*

Traits/species	Samples	Access/thorax height (cm)	Access/thorax width (cm)	Access/body length (mm)
Floral traits	N = 8	0.474 ± 0.06	0.821 ± 0.02	0.885 ± 0.02
Sarcophaga carnaria	N = 6	0.468 ± 0.05	0.454 ± 0.04	1.235 ± 0.08
Significance of difference	-	ns	*	*

*$P < 0.05$ within the same column, ns not significant

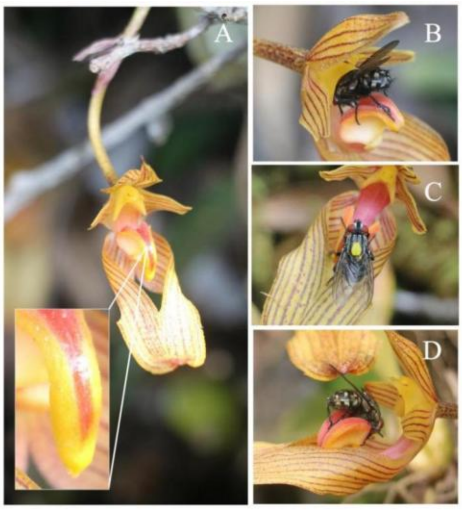

***Figure 4.** Flower characteristics, labellum secretions, and pollinator visitation. An image of the flower with a magnified inset image of the labellum secretions; B. The pollinator, Sarcophaga carnaria, enters the flower; C. Sarcophaga carnaria exits the flower carrying pollen; D. Sarcophaga carnaria disseminates this pollen to other flowers*

Discussion

Floral characteristics and pollinator attraction

The flowers of *B. tianguii* exhibit an average longevity of 5-6 days. However, this longevity declines significantly following pollination. Specifically, the petals often wither within 2-3 days after pollination, indicating that flower senscence is accelerated post-pollination. The characteristics of the flower, such as its shape and odor, play a decisive role in attracting pollinators (Zhang, 2003; Chen et al., 2015; Tan et al., 2021). In the genus *Bulbophyllum*, the majority of species have been reported to be pollinated by flies (Laurence et al., 2011; Zhang et al., 2024). In both the TLWJTK and MLWJTK populations of *B. tianguii*, the only pollinator was the *S. carnaria*, and its visiting frequency is extremely low. Similar observations have been made in other *Bulbophyllum* species, such as *Bulbophyllum variegatum* (Laurence et al., 2011), and *Bulbophyllum nipondhii* (Wittaya et al., 2019). Movable labellum or balanced labellum hinges are thought to be the result of parallel evolution adapted to fly pollination, as found in *Bulbophyllinae* and *Pleurothallidinae* (Van der Pijl and Dodson, 1969; Christensen, 1994). In the majority of *Bulbophyllum* species, the flower features a mobile labellum with its base adjacent to the column. As Tan et al. (2002) reported regarding the fruit fly Bactrocera papayae pollinating *Bulbophyllum cheiri*, when a foraging pollinator tries to enter the floral channal, it has to exert significant pressure on the base of the labellum flap, subsequently making contact with the column and touching it again upon withdrawal. During the pollination process of *B. tianguii*, the “hinge structure” of the labellum plays an crucial role. The *S. carnaria* sucks on the secretions on the labellum, and its weight is just enough for the labellum to be pressed down and rebound. Its back carries the pollen clusters just over the column, allowing it to complete cross-pollination.

Many Bulbophyllum species are known to attract fly pollinators through the emission a foul odor (Van der Pijl and Dodson, 1969; Laurence et al., 2011). However, others emit strong aromatic odors (Tan and Nishida, 2000; Tan et al., 2002). Zingerone has been reported to be responsible for attracting of fruit flies Bactrocera by *Bulbophyllum baileyi* (Tan and Nishida, 2007). Methyl eugenol and raspberry ketone-sensitive Bactrocera species are attracted to flowers of *Bulbophyllum* patens that release zingerone, which is the largest volatile component in the floral fragrance (Tan and Nishida, 2000). Furthermore, *Amorphophallus konjac* can release a volatile mixture dominated mainly by oligosulphides, which may represent a strategy for attracting pollinators (Chen et al., 2015). In this study, we identified 2-methyl-1-butanol and leaf alcohol as the predominant compounds in *B. tianguii*. These compounds may play a crucial role in attracting pollinators and facilitating pollination processes.

Breeding system

The breeding system represents the interplay between the internal genetic mechanisms of the plants and the external enviroment. It plays a pivotal role in plant evolution and trait variation processes (Grant, 1971). Understanding the breeding system of a species helps clarify the evolutionary characteristics and life-history traits shaped by diverse genetic and ecological factor that affect allogamy and autogamy (Escaravage et al., 1997; He et al., 2024). In this study, both geitonogamy and xenogamy treatments led to fruit set, indicating that the breeding system is self-compatible and facultative outcrossing. The results is consistennt with the findings in other Orchidaceae plants, such as *Bulbophyllum variegatum* (Laurence et al., 2011), *Cypripedium japonicum* (Liu et al., 2013), and *Cymbidium aloifolium* (Shi et al., 2024). However, contrasting results have been study for other plants within the *Bulbophyllum* genus. For instance, *B. ambrosia* (Chen et al., 2011), *B. bicolor* (Hu et al., 2017) and *B. funingense* (Zhang et al., 2024) are characterized by self-incompatibility. Such differences are likely rooted in genetic factors and merit futher investigation. Supplementary ecological and phylogenetic studies will be essential for a comprehensive understanding of the evolution of breeding systems within this genus.

Hand-pollination has been shown to significantly enhance fruit set, indicating that the number of pollinators is insufficient or the pollination efficiency is low under natural conditions (Kenji et al., 2014). For *B. funingense*, the natural fruit set were 1.70 ± 4.31% and 6.63 ± 5.29%, respectively (Zhang et al., 2024). In the case of *B. nipondhii*, the natural fruit set ranged from 4.2 to 6.7% (Wittaya et al., 2019). In this study, *B. tianguii* the natural fruit sets were 2.5% and 2%, respectively. This indicates that *B. tianguii* exhibits low fruit set under natural conditions. A survey encompassing 258 angiosperm species revealed that 62% of them experienced pollen limitation at some times or in specific locations (Burd, 1994). In this study, the natural fruit set was significantly lower than the out-crossing, indicating the existence of pollen limitation. This finding implies that there is severe pollen limitation in the wild populations of *B. tianguii*. Such pollen limitation might be one of the contributing factors to the endangerment of this species. The results were consistent with the field observations of pollinators. The only pollinator of *B. tianguii* is the *S. carnaria* and the visiting frequency is extremely low (0.005 visits/flower/h) at both sites. Few species of pollinators and a low visiting frequency were the primary reasons for the low reproductive rates in *B. tianguii*. Field investigation have found that very few seedlings are produced from seeds. Firstly, the seeds of this species are extremely small, with incomplete embryo development, and they are unable to store sufficient nutrients to support germination. Additionally, seed germination also relies on symbiotic fungi to supply nutrients (Liang et al., 2022). Secondly, the fruit pods of this species mature in early October, during which the dry weather makes it difficult to provide suitable conditions for seed germination (Chai et al., 2022).

Reproductive compensation and protective strategies

Plants often evolve a set of reproductive compensation mechanisms. These mechanisms play a crucial role in guaranteeing the unhindered progress of reproduction and the survival of offspring (Bingham et al., 1998). *B. tianguii* can engage in both sexual and asexual reproduction, the former of which can be readily limited by factors including flowering, fruit set, and pollinator activity. When sexual reproduction is impeded, asexual reproduction can consolidate the existing living space, improve survival competitiveness, and maintain the ability of population reproduction (Cao et al., 2022).

Human activities have affected the living environment of many species, making them endangered and in urgent need of protection (Liu et al., 2020a). Extensive excavation and habitat destruction has driven a sharp drop decrease in the availability of wild plant resources, and efforts to understand plant breeding system and pollinator diversity are vital to the formulation of more effective conservation and management approaches given the importance of pollinators to many plant populations (Havens, 1999; Kerans et al., 1998; Liu et al., 2020b). Pollination systems are often related to plant rarity given the importance of insects for the cross-pollination necessary for seed production and fruiting in many cases (Laurence et al., 2011). When devising protection measures for endangered plant resources, relevant departments and institutions should pay attention to the relationship between plants and pollinators (Ashworth et al., 2004; Sun et al., 2006; Kacper et al., 2021). They should protect its original habitat community, maximize the protection of the wild resources of the *B. tianguii*. Additionally, under natural conditions, large scale cross-pollination of wild *B. tianguii* can be carried out to enhance its reproductive efficiency, thereby improving the overall fitness of the offspring. Seeds and rhizomes from different *B. tianguii* populations should be harvested to support artificial reproduction efforts, supporting appropriate preparations for the reintroduction and retablishment of these plants in their natural habitats.

Conclusions

The study indicates that the breeding system of *B. tianguii* is self-compatibility and facultative out-crossing, which requires pollinators. The only pollinator identified for *B. tianguii* was *S. carnaria*. However, the visiting frequency of *S. carnaria* is extremely low. Floral scent analysis indicated that 2-methyl-1-butanol and leaf alcohol were the major compounds produced by *B. tianguii*. Additionally, the hinge structure of the labellar in *B. tianguii* has been found to be crucial in the dissemination of pollinia. The natural fruit set of *B. tianguii* are 2.5% in the TLWJTK population and 2% in the MLWJTK population, respectively. Few species of pollinators and low visiting frequency were the mainly reasons for the low reproduction rates in *B. tianguii*. Pollen limation may be a crucial factor underlying the endangered of *B. tianguii*. Artificial pollination and the release of pollinators represent effective strategies for increasing the fruit yield to *B. tianguii*. Furthermore, appropriate reintroduction programs should be carried out to expand the population size of *B. tianguii*, thereby enhancing its chances of long-term survival and recovery.

Acknowledgments. This study was funded by Guangxi Forestry Administration Project (GUI Lin ke Zi [2021] No. 28), the Key Research and Development Program of Guangxi (GuiKeAB22080097, GuikeAB21196018), the National Natural Science Foundation of China (32060248, 31860169), the Fund of Guangxi Key Laboratory of Plant Functional Phytochemicals and Sustainable Utilization (ZRJJ2023-6), the “Cassia Scholars” Innovation team launch project (CQZ-C-1902).

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