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Taxonomic study of the genus *Bulbophyllum* Thouars (Orchidaceae) in the Central Highlands based on morphological and molecular approaches.

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LIST OF THE PUBLICATIONS RELATED TO THE DISSERTATION

- Truong Ba Vuong, Van Son Dang, Hop Tran, Tatiana V. Maisak, Leonid V. Averyanov. 2020. *Bulbophyllum sridithii* (Orchidaceae), new species from Vietnam. Taiwania 65(4): 473-477.

- Truong Ba Vuong, Leonid V. Averyanov, Roland Amsler, Van Canh Nguyen, Hop Tran, Tatiana V. Maisak & Van Son Dang. 2020. New species, *Bulbophyllum trongquyetii* (Orchidaceae) from Vietnam. Phytotaxa 464 (4): 293-298.

- Pham Phuoc Dien, Leonid Averyanov, Dang Van Son, Nguyen Dinh Hiep, Tatiana Maisak, Truong Quang Tam, Dang Minh Quan & **Truong Ba Vuong**. 2021. A new species and new record of *Bulbophyllum* (Orchidaceae) from Vietnam. Phytotaxa 522 (1): 068–072.

- Dang Minh Quan, Leonid V. Averyanov, Tatiana V. Maisak, Bui Van Huong, Dang Van Son, Truong Quang Tam, **Truong Ba Vuong**. 2021. New taxa of *Bulbophyllum* (Orchidaceae) in the Flora of Vietnam. Taiwania 66(2): 258–266.

- Minh Tam Thao Ngoc Duong, Van Son Dang, Leonid V. Averyanov, Quang Tam Truong, Roland Amsler, **Truong Ba Vuong**. 2021. Two new species of *Bulbophyllum* sect. *Racemosae* (Orchidaceae) from Vietnam. Nordic Journal of Botany 39 (9): 1 – 6.

- Van Canh Nguyen, Leonid V. Averyanov, Tatiana V. Maisak, Thi Lien Thuong Nguyen, Van Khuong Nguyen, Thi Thanh Dat Pham, Danh Duc Nguyen, Van Son Dang, Warapong Aromyen & **Truong Ba Vuong**.2023. The review of *Bulbophyllum* section *Tripudianthes* (Orchidaceae) in the flora of Vietnam with the description of new species, *B. viridipallidum*. Phytotaxa 598 (1): 059–068.

- Minh Quan Dang, Leonid V. Averyanov, Tatiana V. Maisak, Quoc Bao Nguyen, Van Huong Bui, Bao Ngan Tu, Van Canh Nguyen & **Truong Ba Vuong**. 2023. *Bulbophyllum sondangii* (Orchidaceae), a new species from Da Lat Plateau, southern Vietnam. Phytotaxa 589 (2): 203–208.

GENERAL INFORMATION

The urgency of the dissertation

The Orchid family (Orchidaceae Juss.) plays a significant role in the angiosperms, comprising approximately 739 genera and over 32,096 species. In nature, orchids are often found in rugged terrains with year-round temperate climates. They represent a critical and sensitive component of native flora and serve as bioindicators of primary forests.

The genus *Bulbophyllum* Thouars, the largest genus in the Orchidaceae family, includes over 2,168 accepted species and ranks as one of the most species-rich genera globally, second only to *Astragalus*, which contains more than 3,000 species. According to 2019 data, the genus *Bulbophyllum* in Vietnam comprised about 146 species. By 2024, scientists had described and published 35 new species and new records of *Bulbophyllum* for Vietnam, raising the total number of species in this genus to 181.

Species within the genus *Bulbophyllum* are widely distributed across various habitats, with many species possessing not only scientific value but also ornamental and medicinal significance. However, research on the Orchidaceae family in general, and the genus *Bulbophyllum* in particular, remains limited, incomplete, and taxonomically imprecise in Vietnam. In some sections, species exhibit highly similar morphological characteristics, differing only in a few minor traits. This has led to numerous taxonomic errors, misapplied nomenclature, and a lack of reference specimens for study.

The Central Highlands, comprising the provinces of Kon Tum, Gia Lai, Đắk Lắk, Đắk Nông, and Lâm Đồng, is recognized as one of Vietnam's biodiversity hotspots. It is home to six national parks—Chư Mom Ray, Kon Ka Kinh, Yok Đôn, Chư Yang Sin, Bidoup-Núi Bà, and Tà Đùng—as well as several nature reserves, such as Ngọc Linh, Kon Chư Răng, Ea Sô, Nam Kar, and Nam Nung. Despite extensive studies on biodiversity and plant resources in this region, comprehensive documentation of Orchidaceae species, particularly those within the genus *Bulbophyllum*, remains

inadequate. Moreover, *Bulbophyllum* species face significant threats, including widespread harvesting and trade for ornamental purposes, traditional medicine, and export. These activities often occur openly in markets, along roadsides, and on social media platforms, exacerbating the risk of extinction for many species.

Therefore, conducting a comprehensive investigation and study of the genus *Bulbophyllum* within the Orchidaceae family in the Central Highlands using morphological and molecular approaches is an essential task. This effort not only enhances our understanding of the biodiversity in the Central Highlands but also supports the conservation and sustainable use of these valuable natural resources. For those reasons, we undertook the project titled: **"Taxonomic study of the genus *Bulbophyllum* Thouars (Orchidaceae) in the Central Highlands based on morphological and molecular approaches."**

Objectives of the study

- Determine the species composition, briefly ecological notes, and distribution of species in the genus *Bulbophyllum* Thouars in the Central Highlands.

- Complete the classification and establish a systematic and comprehensive identification key for the genus *Bulbophyllum* in the Central Highlands, providing a basic information for compiling the Flora of Vietnam for this plant genus and improving research and training in related fields.

Research scopes

- Field surveys and collect specimens of species in the genus *Bulbophyllum* from various habitats in the Central Highlands.

- Classify the species in the genus *Bulbophyllum* using morphological methods.

- Classify the species in the genus *Bulbophyllum* using molecular methods.

- Create a key for identifying taxa in the genus *Bulbophyllum* in the study area.

- Document the resource value of the genus *Bulbophyllum* in the study area.

The scientific and practical basis of the thesis

- The results of the thesis are fundamental documents on taxonomy for the genus *Bulbophyllum* in the Central Highlands, serving research and education in botany in Vietnam.

- It serves as the database for compiling the flora of Vietnam, specifically for the genus *Bulbophyllum* and the Orchidaceae family in general.

- This is an important step for conducting in-depth studies and proposing conservation measures for the genus *Bulbophyllum* as well as the entire Orchidaceae family.

- The research results of the thesis provide a database serving applied science fields, biological resources, biodiversity, and educational training.

Chapter 1. Overview

1.1. The global research status of the genus *Bulbophyllum*

The genus *Bulbophyllum* was described by Louis-Marie Aubert Du Petit-Thouars in 1822 based on the type species *Bulbophyllum nutans* (Thouars) Thouars in his work *Histoire particulière des plantes Orchidées recueillies sur les trois îles australes d'Afrique, de France, de Bourbon et de Madagascar*. However, prior to this, several genera that later became synonyms of *Bulbophyllum*, such as *Phyllorkis* Thouars, were described in 1809 in the journal *Nouveau Bulletin des Sciences*, with the type species *Phyllorkis nutans* Thouars (later classified as *B. nutans*).

Notably, *Cirrhopetalum* is a section that has received the most attention when studying the genus *Bulbophyllum*. This genus has experienced the most mergers and separations due to differing opinions, and finally, Reichenbach (1861) merged *Cirrhopetalum* into *Bulbophyllum* and reclassified it as a section within the genus (*Bulbophyllum section*

Cirrhopetalum (Lindl.) Rchb.). The genus *Cirrhopetalum* was firstly described by Lindley in 1830 based on the type species *Cirrhopetalum thouarsii* Lindl., which was originally described as *Bulbophyllum longiflorum* by Thouars in 1822. In this work, Lindley proposed and described six species: *Cirrhopetalum thouarsii* Lindl., *C. roxburghii* Lindl., *C. vaginatum* Lindl., *C. blumii* Lindl., *C. wallichii* Lindl., and *C. macraei* Lindl. The main characteristics that led Lindley to separate the genus *Cirrhopetalum* were: (1) the base of the lateral sepals' margin turning inward, (2) the apical part of lateral sepals much longer than dorsal sepal, and (3) the inflorescence with man flowers and spreading. Later, in 1842, in the journal *Edwards's Botanical Register*, Lindley described *C. medusa* Lindl. However, by 1961, Reichenbach merged this species into *Bulbophyllum section Cirrhopetalum*. Over time, *B. medusa* (Lindl.) Rchb.f. was transferred to *section Desmosanthes* (Bl.) J.J. Sm. In 1843, Lindley updated and categorized 21 species into *section Cirrhopetalum*, dividing them into two groups: (1) the group with many flowers: *C. vaginatum* Lindl., *C. medusa* Lindl., *C. caespitosum* Wall. ex Lindl., *C. cornutum* Lindl., *C. maculosum* Lindl., *C. chinense* Lindl., *C. thouarsii* Lindl., *C. wallichii* Lindl., *C. roxburghii* Lindl., *C. macraei* Lindl., *C. picturatum* Lodd., *C. auratum* Lindl., *C. cumingii* Lindl., *C. nutans* Lindl., *C. fimbriatum* Lindl., *C. elongatum* (Blume) Lindl., *C. capitatum* (Blume) Lindl.; and (2) the group with single flowers: *C. antenniferum* Lindl., *C. maxillare* Lindl., *C. blumii* Lindl., *C. compressum* Lindl.

In 1861, Reichenbach transferred numerous species from various genera into the genus *Bulbophyllum* and organized them into three major groups, referred to as sub-genera: (1) Uniflora (comprising six subgroups) includes species with solitary flowers (among which are two species from *Cirrhopetalum* that have solitary flowers); (2) Spicata seu Racemosae includes species with racemose inflorescences (divided into two subgroups: "Rachis filiformis" and "Rachis foliiformis" (*Megaclinium*)); (3) Umbellata

consists of the section *Cirrhopetalum* (with multiple flowers) and the section *Brachyantha*, totaling 85 species.

Hooker (1890), in his work *The Flora of British India*, maintained the distinction between the genera *Cirrhopetalum* and *Bulbophyllum*, noting the distinguishing features of *Cirrhopetalum*: morphology resembling *Bulbophyllum* but differing in the circular arrangement of flowers, the short dorsal sepal, and significantly elongated lateral sepals. He recorded 34 species in this genus. Additionally, Hooker mentioned that *Bulbophyllum* comprises four sections: *Epicranthes*, *Oxysepala*, *Eu-Bulbophyllum*, and *Ione* (*Ione* later synonymized with the genus *Sunipia*). He also established a new genus, *Henosis*.

Holttum (1957), in his work *Orchids of Malaya*, summarized the key characteristics of the genus *Cirrhopetalum* as follows: (1) Flowers are clustered at the apex of the rachis inflorescence and spread out in a fan-like (sub-umbellate) arrangement. (2) The dorsal sepal and petals have long hairs along their margins. (3) The lateral sepals are much longer than the dorsal sepal, twisted at the base, the upper margins to connate and, occasionally, the lower margins to form "a tube" structure.

In 1994, Garay and collaborators continued to recognize *Cirrhopetalum* as a distinct genus from *Bulbophyllum* and divided it into three sections:

- *Cirrhopetalum* section *Wallichii* (e.g., *C. wallichii*),
- *Cirrhopetalum* section *Recurvae* (e.g., *C. curtisii*),
- *Cirrhopetalum* section *Cirrhopetalum* (e.g., *B. longiflorum*).

In 1973, Seidenfaden, in his work *Notes on the Genus Cirrhopetalum*, rejected the separation of *Cirrhopetalum* as an independent genus and transferred 214 species of *Cirrhopetalum* into *Bulbophyllum*. By 1992, in *The Orchids of Indochina*, Seidenfaden recorded and recognized 86 orchid species, of which the genus *Bulbophyllum* accounted for 34 species.

In India, Schuiteman et al. (2022), in their work *A Checklist of Orchidaceae of India*, recorded and listed 136 species of the genus *Bulbophyllum*.

In China, according to the *Flora of China*, Volume 25 (2009), focusing on Orchidaceae, the genus *Bulbophyllum* included 103 species across 18 sections. From 2011 to mid-2024, the number of *Bulbophyllum* species recognized in China increased to 164.

For Cambodia, the total number of *Bulbophyllum* species remains incomplete. In 2016, Averyanov et al. reported four new records of *Bulbophyllum* for Cambodia. Later, in 2018, they added one more species to the records for this country.

In Laos, Gale et al. (2018), in their work *A Guide to Orchids of Laos*, documented 102 species of *Bulbophyllum*. Subsequently, Averyanov et al. (2018) described one new species and added seven new records of *Bulbophyllum* for the flora of Laos. By 2019, a new species for science, *B. pachypodum* Aver., was described in Laos, bringing the total number of *Bulbophyllum* species in the country to 111.

In Thailand, Seidenfaden, in his work *Orchid Genera in Thailand VIII* (1979), provided detailed notes on *Bulbophyllum*, including taxonomic characteristics and illustrations for 150 species distributed in Thailand. In 2017, Vermeulen et al. discovered and described a new section, *Physometra* J.J.Vermeulen, Suksathan & Watthana, based on the new species *B. physometrum* from northern Thailand. Between 2019 and 2024, the number of *Bulbophyllum* species recognized in Thailand reached approximately 163.

In Myanmar, Ormerod et al. (2021) documented 1,037 orchid species from 152 genera, including 122 species of *Bulbophyllum*. This is the most comprehensive update on Orchidaceae and *Bulbophyllum* species for the country.

In Borneo, Vermeulen et al. (2015) recorded and described 288 species, of which 284 were accompanied by photographs or illustrations.

1.2. Research status of the Genus *Bulbophyllum* in Vietnam

1.2.1. *Bulbophyllum* studies in Vietnam

Studies on orchids in Vietnam began early, with significant contributions from Gagnepain and Guillaumin (1934) in their work *"Flore générale de l'Indo-Chine."* The authors documented 411 species belonging to 82 genera of orchids in Indochina, including the genus *Bulbophyllum*. For Vietnam, *Bulbophyllum* was recorded with 21 species, and *Cirrhopetalum* with 18 species. Later, all species in *Cirrhopetalum* were transferred to *Bulbophyllum*. However, two species—*Bulbophyllum proboscideum* (Gagnep.) Seidenf. & Smitinand and *Bulbophyllum vaginatum* (Lindl.) Rchb.f.—have yet to be recorded for Vietnam.

In 1992, Seidenfaden, in *"The Orchids of Indochina,"* recorded 800 species from 140 genera of orchids in Indochina, with 72 species of *Bulbophyllum* in Vietnam.

In 1998, Trần Hợp, in *"Phong Lan Việt Nam,"* briefly described 1,153 orchid species from 137 genera, including 73 species of *Bulbophyllum*.

In 2000, Phạm Hoàng Hộ, in *"Cây cỏ Việt Nam, quyển 3,"* provided brief descriptions and illustrations of 800 orchid species across 90 genera in Vietnam, documenting 75 species of *Bulbophyllum* alongside species in the genera *Sunipia*, *Trias*, and *Monomeria*.

In 2005, the publication *"Danh lục các loài thực vật Việt Nam,"* edited by Nguyễn Tiến Bân, listed 71 species of *Bulbophyllum*.

In *"Lan hoang dã Phú Quốc - hướng dẫn định danh ngoài thực địa"* (2009), Lý Thọ documented 99 orchid species across 53 genera in Phú Quốc National Park, including 10 species of *Bulbophyllum*. In 2019, Trương Bá Vương and collaborators reported a new record for Vietnam from Phú Quốc National Park: *Bulbophyllum tenuifolium* (Blume) Lindl.

Leonid V. Averyanov has made substantial contributions to orchid research in Vietnam. In 2003, Averyanov and Averyanova, in their *"Updated Checklist of the Orchids of Vietnam,"* reported approximately 897 orchid

species across 152 genera, with 96 species of *Bulbophyllum*. In 2013, Averyanov and collaborators, in *"The Orchids of Cuc Phuong National Park: Illustrated Guide,"* documented 13 species and one variety of *Bulbophyllum* in Cuc Phuong National Park.

In 2015, Averyanov, colleagues and contributors, in *"New Data on Orchid Diversity of Vietnam 2011–2015,"* recorded 1,184 orchid species from 167 genera in Vietnam, including 108 species of *Bulbophyllum*.

According to the author's updates, by 2019, Vietnam had approximately 146 species of *Bulbophyllum*. By 2023, the number had increased to around 167 species.

1.2.2. *Bulbophyllum* studies in Center Highlands

In the Central Highlands of Vietnam, information about the genus *Bulbophyllum* remains limited. Only a few species have been recorded in the plant checklists of National Parks and Nature Reserves. However, this region is known for its rich and diverse orchid flora, including the genus *Bulbophyllum*.

According to the *"Updated Checklist of the Orchids of Vietnam"* by Averyanov and Averyanova, 70 species of *Bulbophyllum* were recorded in the Central Highlands.

Thus, the genus *Bulbophyllum* in Vietnam, and particularly in the Central Highlands, holds significant scientific value. New species and records continue to be discovered by researchers.

Therefore, conducting thorough surveys and in-depth studies of *Bulbophyllum* in the Central Highlands is essential and practical for conservation efforts and the sustainable utilization of this valuable natural resource, both now and in the future.

Chapter 2. MATERIALS AND METHODOLOGY

2.1. Materials

The study material includes all taxa belonging to the genus *Bulbophyllum* (Orchidaceae) in the Central Highlands. The author used a

total of 920 specimens, with 220 specimens collected from the studied areas and 700 specimens from the Online Virtual Herbarium.

2.2. Methodology

2.2.1. Inherited method

Documenting and analyzing data from scientific works in books and specialized journals, as well as from different survey results and evaluations related to the genus *Bulbophyllum*, to compile information, shape the research scope, and select the classification system for the genus *Bulbophyllum* in the study area.

2.2.2. Field works and specimens' collection.

The surveys were conducted from June 2020 to May 2023, consisting of 12 sampling fieldworks to areas in the Central Highlands, each lasting from 15 to 20 days.

Photographing specimens: Specimens will be photographed using a Canon 700D with a Canon EF-S 60mm f/2.8 Macro USM lens. Detailed imagines of the plant and flowers will be taken to create a photographic collection for identification purposes.

Field specimen processing: Specimen collection takes place during the field surveys. The collected specimens are preserved in 70^0 ethanol in the field and later dried in the laboratory. Like other plant families, accurate identification of species in the genus *Bulbophyllum* requires fully opening flowers and fruits. Therefore, collected specimens should include all parts such as flowers, fruits, pseudobulbs, and leaves to facilitate easier identification. Specimens from the same host plant are assigned the same sample number "BV," while specimens from different host plants but of the same species are assigned different numbers. Information such as coordinates, location, time, collector, altitude, and habitat conditions, as well as local and scientific names (if known), flower color, and scent, are carefully recorded in the field notebook. For *Bulbophyllum* species that have not flowered and are brought from the forest, they are grown in cooperation with

orchid gardens in the Central Highlands. Once they flower, they will be processed for study.

DNA sample collection: Fresh leaf samples are used for DNA analysis and stored in Silica Gel.

2.2.3. Laboratory works

Processing and drying: Beside the specimens preserved in alcohol, the remaining specimens are processed and dried.

Species description: The species description is based on measured data and photographs taken with a ruler from fresh specimens in the field.

Scientific name identifies: The scientific name is determined by comparing morphological characteristics and classifying taxa within the genus *Bulbophyllum*. Identification is based on taxonomic literature concerning the Orchidaceae family and related *Bulbophyllum* species. Additionally, specimens in domestic plant museums and digitized museums worldwide are consulted for comparison.

Species distribution: Based on published data related to *Bulbophyllum* species, a distribution map is created. Additionally, specimens from museums in Vietnam (VNM, VNMN, LE…) and digitized museums worldwide, such as B, BM, KEW, MNHN, L, W… are consulted to determine the species' distribution in various provinces of Vietnam and around the world.

DNA Data Processing: Twenty-three species representing 23 sections in the study area are selected for DNA extraction using the CWBIO DNA extraction kit following the protocol. The sequences are aligned using MAFFT v.7.429 according to Katoh and Standley (2013). The aligned sequence matrix is used to construct a phylogenetic tree using the Maximum Likelihood (ML) method. ML analysis is performed with RAxML by Stamatakis et al. (2008) using the GTRGAMMAI model to identify the best-fit tree. The phylogenetic tree is constructed on the CIPRES Science Gateway (http://www.phylo.org) with 1000 bootstrap replications for

confidence estimation. The FigTree software is used to view the phylogenetic tree (http://tree.bio.ed.ac.uk/software/figtree/).

Along with 73 *Bulbophyllum* species and 2 species from the *Dendrobium* genus from Genbank, a phylogenetic tree is also constructed.

Chapter 3. RESULTS

3.1. Choosing a Classification System

The author used the taxonomic system of Gravendeel et al. (2014), Chase et al. (2015) and some taxonomic changes in section from Vermeulen (2015)

Family: Lan (Orchidaceae)

Subfamily: Epidendroideae

Tribe: Malaxideae

Subtribe: Dendrobiinae

Genus: *Bulbophyllum*

– with 66 sections from Asian region

3.2. General morphology characters of Bulbophyllum in Center Highlands of Vietnam

3.2.1. **Habit**: There are two main habits of species of genus *Bulbophyllum* e.g. epiphytic (commonly observe) and lithophytic.

3.2.2. **Pseudobublb**: The pseudobulbs of the genus *Bulbophyllum* are diverse in shape from ovate, broadly ovate to nearly globose, narrowly ovate, elliptic, or conoid; erect or oblique, or nearly flat. The size of pseudobulb from 5 mm (species of section *Macrocaulia*) up to 7 cm (*Bulbophyllum striatulum*).

3.2.3. **Leaf**: from 1 to 2, elliptic, ovate to broadly ovate, oblong or semiterete (case of *B. semiteretifolium*), apex rounded, obtuse, acute or slightly retuse.

3.2.4. **Inflorescence**: The inflorescence arises from the base of the pseudobulbs and can be classified into three types (1) Raceme with many flowers, either closely spaced or laxly arranged; (2) Sub-umbellate raceme:

characterized by a very short rachis, differing from a true umbel as the flowers do not originate from a single point; (3) Single flower, rarely two (e.g., *B. ambrosia*, which typically has one flower but occasionally produces two or three).

3.2.5. **Sepals**: some types of sepals (1) Dorsal sepal with entire margin, ciliate or villose hairs on margin, apex acute to acuminate or caudate, occasionally with single seta in middle; (2) Lateral sepals as long as or longer than dorsal sepal; lateral sepals connate either upper margin or both or remain separate.

3.2.6. **Petals**: The petals margin are entire, ciliate, villose hairs, erose or hairs with appendages. The surface can be smooth or papillose.

3.2.7. **Labellum**: The labellum is a specially modified third petal. It is attached to the apex of the column foot by an appendage and is movable. The labellum can be simple or have auricles, with a surface that is either hairy, papillose, or smooth, and may feature keels or grooves.

3.2.8. **Column and column foot**: The column is a unique floral structure formed by the fusion of the stamens, style, and stigma, with or without wings on the lower margin. The wings can be rounded, triangular, or rhombic. The column foot is mostly forward-pointing or upcurved, with the apex sometimes bilobed.

3.2.9. **Stelidia**: Stelidia is an important morphological characteristic. Different sections exhibit different types of stelidia. Stelidia can be prominent or not, elongated or small, triangular, subulate, pointing forward or downward, and may have a small tooth on the upper margin.

3.4. Key to the sections of *Bulbophyllum* in the study area.

Based on the classification system of Gravendeel et al. (2014), Chase et al. (2015), and some section changes from Vermeulen (2015), combined with the results of DNA sequence analysis of 23 species (representing 23

sections), we have arranged 97 species belonging to 23 sections according to the following key:

The tribe *Dendrobieae* includes two genera: *Dendrobium* and *Bulbophyllum*. The taxa within the genus *Bulbophyllum* are identified based on relevant taxonomic references such as Averyanov (1994), Phạm Hoàng Hộ (2000), Seidenfaden (1973, 1979, 1992), Vermeulen (2015), and publications in related taxonomic journals..

1a Pseudobulb with two leaf.....2

1b Pseudobulb with single leaf.....3

2a Flowers open in succession, outer surface of sepals glabrous .....
.....*Blepharistes*

2b Flowers open in same time, outer surface of sepals with hairs or papillose-hispid ..... *Lemniscata*

3a Inflorescence with more than two flowers (rarely one), raceme or raceme sub-umbellate.....4

3b Inflorescence with single flower.....19

4a Raceme inflorescence 5

4b Raceme sub-umbellate inflorescence 11

5a Flower with hairs in outer surface or margin of sepals, petals margin hairy, lip with hair or not .....*Hirtula*

5b Flower glabrous 6

6a Pseudobulb small in compare with plant size 7

6b Pseudobulb bigger, obvious.....9

7a Flower from one to two (rarely three), stelidia unclear .....*Aeschymanthoides*

7b Flower many, stelidia obviously 8

8a Many flowers, yellow to white flowers (rarely 2 in case of *B. pauciflorum*), raceme, laxly flower .....*Stachysanthes*

8b Densely arranged brown-red flowers in ovate, or orbicular, or laxly .....*Brachystachyae*

9a Lip with auricles at base .....*Racemosae*
9b Lip without auricles 10
10a Inflorescence pendulous, or slightly arching .....*Biseta*
10b Inflorescence erect or oblique..... *Reptantia*
11a Margin of dorsal sepal or petals glabrous 12
11b Margin of dorsal sepal erose, hairy or not, margin of petals erose,cilaye or with long hairs.....14
12a Dorsal sepals and petals with single seta in middle, largely stelidia. .....*Macrostelydia*
12b Dorsal sepals and petals without seta, stelidia small 13
13a Lateral sepals as long as or three tims longer, not twisted at base .....*Desmosanthes*
13b Lateral sepals longer dorsal sepal from 3 to 6 times, twisted at base ..... *Brachyantha*
14a Dorsal sepal with entire, erose margin, petals with erose or hairy 15
14b Dorsal sepal and petals with long hairs 16
15a Lateral sepals separate, as long as or 2 times longer dorsal sepal, not twisted at base.....*Eublepharon*
15b Lateral sepals twisted at base and connate in upper and lower margin, 2–5 longer than dorsal sepal .....*Rhytionanthos*
16a Lateral sepals separate, not elongated.....*Acrochaene*
16b Lateral sepals connate, apical elongated.....17
17a Stelidia with small tooth on inner surface.....*Cirrhopetalum* (s.str.)
17b Stelidia without tooth on inner surface.....18
18a Lip 10–15 mm ..... *Cirrhopetaloides*
18b Lip not more than 5 mm ..... *Ephippium*
19a Creeping rhizome.....20
19b Rhizome pendoulous 21
20a Small plants, flower less than 10 mm ..... *Macrocaulia*
20b Bigger plants, flower more than 10 mm 22

21a Petals with appendages on aped..... *Epicrianthes*

21b Petals without appendages.....*Oxysepala*

22a Pollinaria 4, not equally, inner ½ outer, diagram at base of pedicel same as above ..... *Leopardinae*

22b Pollinaria 4, same size, diagram at base of pedicel bigger than above .....*Sestochilos*

List of species in sections:

***Bulbophyllum* section *Acrohaene* (Lindl.) J.J.Verm. & de Vogel**

There are two species in the study area: *B. sigaldiae*, *B. dayanum*.

***Bulbophyllum* section *Aeschymanthoides* Carr**

There are two species in the study area: *B. hainnanense*, *B. hymenanthum*

***Bulbophyllum* section *Biseta* J.J. Verm.**

There is one species in the study area: *B. sonii*

***Bulbophyllum* section *Blepharistes* J.J.Verm., Schuit. & de Vogel**

There is one species in the study area: *B. blepharistes*.

***Bulbophyllum* section *Brachyantha* Rchb.f.**

There are seven species in the study area: *B. thydoi*, *B. elatum*, *B. umbellatum*, *B. spathulatum*, *B. retusiusculum*, *B. manhdatii*, *B. trongquyetii*.

***Bulbophyllum* section *Brachystachyae* Benth. & Hook.f.**

There are seven species in the study area: *B. khasyanum*, *B. repens*, *B. gracilipes*, *B. apiferum*, *B. poilanei*, *B. bidoupense*, *B. alcicorne*.

***Bulbophyllum* section *Cirrhopetaloides* Garay, Hamer & Siegerist**

There are three species and two varieties in the study area: *B. wendlandianum*, *B. putidum*, *B. frostii*, *B. blaosense* var. *blaoense*, *B. blaoense* var. *flavescences*.

***Bulbophyllum* section *Cirrhopetalum s.str.* (Lindl.) Reichenbach**

There are two species in the study area: *B. annamense, B. longiflorum*.

***Bulbophyllum* section *Desmosanthes* (Blume) J.J.Sm.**

There are 12 species in the study area: *B. semiteretifolium*, *B. odoratissimum*, *B. tixieri*, *B. boulbetii*, *B. pinicola*, *B. astelidum, B. ngoclinhensis*, *B. sutepense*, *B. corallinum*, *B. echinulus, B. evrardii*, *B. stenobulbon*.

***Bulbophyllum* section *Epicrianthes* (Blume) Hook.f.**

There is one species in the study area: *B. abbrevilabium*

***Bulbophyllum* section *Ephippium* (Blume) Schltr.**

There are four species in the study area: *B. sridithii*, *B. pecten-veneris*, *B. lepidum, B. andersonii*, *B. sondangii*.

***Bulbophyllum* section *Eublepharon* J.J.Verm., Schuit. & de Vogel**

There is one species in the study area: *B. scabratum* Rchb.f.

***Bulbophyllum* section *Hirtula* Ridl.**

There are ten species in the study area: *B. clipeibulbum*, *B. neglectipetalum*, *B. penicillium*, *B. spadiciflorum*, *B. dasystachys*, *B. scaphiforme*, *B. nigrescens*, *B. phitamii*, *B. nigripetalum*, *B. secundum*.

***Bulbophyllum* section *Lemniscata* Pfitz.**

There are eight species in the study area: *B. lemniscatoides*, *B. polliculosum*, *B. wallichii*, *B. refractum*, *B. viridipallidum*, *B. hirtum*, *B. averyanovii*, *B. chelicerum*.

***Bulbophyllum* section *Leopardinae* Benth. & Hook.f.**

There are three species in the study area: *B. ambrosia*, *B. psittacoglossum*, *B. pectinatum*.

***Bulbophyllum* section *Macrocaulia* (Blume) Aver.**

There are four species in the study area: *B. ovalifolium* (*B. bryoides*), *B. catenarium*, *B. ignevenosum*, *B. Physocoryphum*.

***Bulbophyllum* section *Macrostelydia* Garay, Hammer & Siegerist.**

There is one species in the study area: *B. delitescens*.

***Bulbophyllum* section *Oxysepala* Benth. & Hook.f.**

There are two species in the study area: *B. Clandestinum, B. tortuosum*.

***Bulbophyllum* section *Racemosae* Benth. & Hook.f.**

There are eight species in the study area: *B. dissitiflorum*, *B. vulinhae*, *B. reclusum*, *B. rufinum, B. orientale*, *B. gunnarri*, *B. careyanum*, *B. striatulum*.

***Bulbophyllum* section *Rhytionanthos* (Garay, Hamer & Siegerist) J.J. Verm.**

There are one species and one variety in the study area: *B. salmoneum*, *B. taeniophyllum* var. *denticulatoalatum*.

***Bulbophyllum* section *Reptantia* J.J. Verm.**

There are two species in the study area: *B. luanii*, *B. reptans*.

***Bulbophyllum* section *Sestochilos* (Breda) Benth. & Hook. f.**

There are eight species in the study area: *B. affine*, *B. pteroglossum*, *B. coweniorum*, *B. macranthum*, *B. hiepii*, *B. orectopetalum*, *B. capillipes*, *B. siamense*.

***Bulbophyllum* section *Stachysanthes* (Blume) Aver.**

There are three species in the study area: *B. pauciflorum*, *B. gibbosum*, *B. apodum*.

3.4. The genetic relationship among species within the genus *Bulbophyllum* in the study area based on molecular data

The size of the ITS gene region for the 23 species in 23 sections is 835 bp. Based on ITS region data of the genus *Bulbophyllum* studied in the Central Highlands, a phylogenetic tree was constructed using the Maximum Likelihood method, with the following observations made by the PhD student:

The independent clade containing the section *Reptantia* has a bootstrap value of 100%, consistent with the study by Thawara et al.

The clade consisting of the sections *Hirtula*, *Stachysanthes*, *Aeschymanthoides*, and *Brachystachyae* is closely related to each other phylogenetically, with a bootstrap value greater than 70%. The molecular proximity between *Hirtula* and *Stachysanthes* aligns with the study by Thawara et al. At the same time, in the morphological classification key, these four sections share common traits, such as having racemose inflorescences. The pseudobulbs of species in *Hirtula* are conical, ranging

from slightly flattened to shield-shaped, while the pseudobulbs of the remaining three sections (*Stachysanthes*, *Aeschymanthoides*, *Brachystachyae*) are small and less distinct compared to the plant.

The clade consisting of the sections *Oxysepala* and *Epicranthes* has a bootstrap value of 92%, and they also show morphological similarity in the classification key. Species in these sections from the Central Highlands typically have a similar growth habit, with pendulous pseudobulbs and solitary flowers.

The clade consisting of the sections *Lemniscata* and *Racemosae* shows a close molecular relationship with a bootstrap value of 70%, which is consistent with the studies of Hu et al. and Thawara et al. Furthermore, according to previous research by Averyanov (1994), Seidenfaden (1979, 1992), the section *Tripudianthes*, which includes species present in the Central Highlands such as *B. kanburiense* and *B. refractum*, was considered a separate section. However, Vermeulen later merged it into the section *Lemniscata*, which aligns with the molecular results of this dissertation.

The clade consisting of the sections *Biseta* and *Acrohaene* shows a close molecular relationship with a bootstrap value of 85%. However, they do not exhibit a clear separation, as *B. sonii* (section *Biseta*) is closely related to *B. sigaldea* (section *Acrohaene*). On the other hand, morphologically, these two sections can be clearly distinguished by the presence of auricles on the lip of *Acrohaene*, which are absent in *Biseta*.

The research results indicate that most species are grouped based on morphological classification and molecular markers. However, some species within the same section are morphologically similar but do not show molecular similarity in the phylogenetic tree, suggesting significant variation in the ITS gene region among these species. This separation also occurs in the study by Hu et al. (2020). Therefore, these results provide an initial database and serve as a foundation for the classification system of the genus

Bulbophyllum.

3.5. **Resource value**

3.5.1. Medicinal value

According to Võ Văn Chi in the book *"Từ điển cây thuốc Việt Nam"* (Vietnamese Medicinal Plants Dictionary), Volume 1, the author mentioned several *Bulbophyllum* species used as medicinal plants:

- *Bulbophyllum reptans*: The whole plant is used to treat bronchitis, cough, tuberculosis, coughing up blood, chronic gastritis, and is applied externally for injuries and fractures.
- *Bulbophyllum odoratissimum*: Used to treat pulmonary tuberculosis, coughing up blood, chronic bronchitis, chronic sore throat, cold-related pain, irregular menstruation, and fractures.
- *Bulbophyllum ambrosia*: Used to treat hepatitis.

In recent years, the populations of these three species have significantly declined due to overharvesting by local people and the excessive purchasing by traders for medicinal use, leading to the rapid shrinkage of their populations.

According to the book *Orchids as Aphrodisiac, Medicine or Food* by Eng Soon Teoh, some *Bulbophyllum* species in the Central Highlands are used for medicinal purposes, including:

- Used as tonics: *Bulbophyllum retusiusculum*, *B. rufinum*.
- For liver diseases: *B. lepidum*.
- For treating burns: *B. siamense*.

3.5.2. Ornamental value

Most species of *Bulbophyllum* are cultivated as ornamental plants in orchid collections. The species with raceme sub-umbellate inflorescence are favored by collectors due to their unique flower structure, with inflorescences that spread like an umbrella or form long inflorescence with many flowers. In addition to sections with small flowers such as *Macrocaulia*, *Epicranthes*, and *Oxysepala*, sections like *Acrohaene*,

Cirrhopetalum (s.str.), *Brachyantha*, *Racemosae*, *Sestochilos*, *Hirtula*, and *Lemniscata* are widely cultivated in collections because of their umbellate-liked inflorescences, large flowers, and diverse colors. Moreover, the unique pseudobulb shapes, ranging from flattened to almost flat and growing closely together in sections like *Hirtula* and *Lemniscata*, attract attention and are collected.

3.6. **Conservation**: According to CITES (Notification No. 25/TB-CTVN dated February 17, 2023), species of the genus *Bulbophyllum* are listed in CITES Appendix II. Based on the IUCN Red List, there are 4 species: *B. polliculosum* (DD), *B. abbrevilabium* (LC), *B. coweniorum* (DD), and *B. evrardii* (EN). According to Decree No. 84/202/NĐ-CP dated September 22, 2021, which amends and supplements Decree 06/2019/NĐ-CP on the management of endangered, rare forest plants and animals and the implementation of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), species of the genus *Bulbophyllum* are listed in Appendix II-A. According to the Vietnam Red List in 2007, there are 4 species in the study area: *Bulbophyllum astelidum* (EN B1+2b,c), *B. averyanovii* (EN B1+2b,c), *B. ngoclinhensis* (EN B1+2b,c), *B. tixieri* (EN B1+2b,c). Among these, the species *B. averyanovii* and *B. ngoclinhensis* have not been encountered or collected from the wild nor found in any orchid collections.

3.7. **The geographical distribution of the genus *Bulbophyllum* in the Central Highlands.**

3.7.1. Geographical distribution according to global geographic regions

3.7.1.1. Vietnam Endemic species

In study area there 25 endemic species: *B. annamense, B. astelidum, B. chelicerum, B. bidoupense, B. boulbetii, B. clipeibulbum, B. evrardii, B. frostii, B. gunnarii, B. hiepii, B. neglectipetalum, B. phitamii, B. poilanei, B. ngoclinhensis*, ***B. manhdatii***, *B. sonii*, *B. thydoi*, *B. semiteretifolium*, *B. spadiciflorum*, ***B. sondangii***, ***B. sridithii***, B. tixieri, ***B. trongquyetii***, ***B.***

viridipallida, ***B. vulinhae***, It accounts for 22.1% of the total number of species in the study area, including 6 species newly described to science in this study (in bold).

3.7.1.2. Indochina region

These species are only distributed in the mainland region of Indochina mainland (included Myanmar, Thailand, Laos, Cambodia and Vietnam) total 12 species: *B. apiferum, B. alcicorne, B. coweniorum, B. dasystachys, B. luanii, B. nigrescens, B. physocoryphum, B. polliculosum, B. reclusum, B. salmoneum, B. sigaldiae, B. wendlandianum*, 12.5% in total.

3.7.1.3. Himalayan region

There 15 species: *B. capillipes, B. careyanum, B. dissitiflorum, B. elatum, B. gracilipes, B. hirtum, B. khasyanum, B. oriantale, B. penicillium, B. pteroglossum, B. repens, B. rufinum, B. secundum, B. spathulatum, B. wallichii*, It accounts for 15.6%, distributed from the Indian region and parts of India, Bhutan, Nepal along the Himalayan range, through southwestern China, and extending to the regions of Myanmar, Thailand, Laos, Cambodia, and Vietnam. However, there are some species from the Indochina region and southwestern China (not extending to the Himalayas), including 7 species: *B. corallinum*, *B. echinulus*, *B. nigripetalum*, *B. psittacoglossum*, *B. pinicola*, *B. scaphiforme*, and *B. sutepense*, which account for 7.3%. The distribution of these species is in Indochina (including Myanmar) and southwestern China, not extending through the Himalayan region (Bhutan, Nepal, or India).

3.7.1.4. Malesian region

This region includes species such as *B. abbrevilabium*, *B. apiferum*, *B. ignevenosum*, *B. lemniscatoides*, *B. putidum*, and *B. gibbosum*, which are distributed in the Malay Peninsula, the island regions of Indonesia, the Philippines, and the areas of Myanmar, Thailand, Laos, Cambodia, and Vietnam.

3.8.1.5. Sino Japananese region

Only single species, *B. hainanense*, is found in the Hainan Island region and the Central Highlands (Khánh Hòa) of Vietnam, accounting for 1%. This species is located in the Sino-Japanese geographical region, but its distribution has not been recorded in Taiwan or Japan.

3.7.1.6. Widely distribution secies

Species distribute in two regions: *Bulbophyllum pecten-veneris*, *B. catenarium, B. lepidum, B. ovalifolium, B. pauciflorum, B. apodum, B. clandestinum, B. blepharistes, B. dayanum, B. hymenanthum, B. macranthum, B. orectopetalum, B. siamense, B. refractum, B. tortuosum, B. affine, B. pectinatum, B. umbellatum, B. ambrosia, B. andersonii, B. delitescens, B. odoratissimum, B. reptans, B. scabratum, B. stenobulbon, B. retusiusculum, B. longiflorum*.

3.7.2. The geographical distribution of the genus *Bulbophyllum* in the Central Highlands and other regions of Vietnam.

Among the 97 species recorded in the Central Highlands, 43 species are endemic, found exclusively in the study area; 13 species are recorded in the northern mountainous regions (found in Sơn La, Điện Biên, Lai Châu, Lào Cai, Hà Giang, Cao Bằng, and Bắc Kạn); 7 species are recorded in the midland northern mountainous region (found in Hanoi, Hòa Bình, Phú Thọ, Vĩnh Phúc); 7 species are recorded in the North Central region (found in Thanh Hóa, Nghệ An, Quảng Bình, Quảng Trị, and Huế); 21 species are recorded in the Central South region (found in Da Nang, Quảng Nam, Bình Định, Phú Yên, Khánh Hòa, Ninh Thuận, and Bình Thuận); 3 species are also recorded in the Southeastern region (Tây Ninh and Đồng Nai), and 3 species in the Southwestern region (Phú Quốc).

CONCLUSIONS AND RECOMMENDATIONS

Conclusion

The dissertation collected 220 herbarium specimens belonging to 97 species of *Bulbophyllum* in the Central Highlands of Vietnam.

The dissertation provided detailed descriptions, taxonomic treatment, identification, statistics, and classification for 97 *Bulbophyllum* species in the region, including six new species and one new variety to science.

In terms of molecular study, the dissertation was generated for 23 species. The results showed that most species clustered into groups consistent with both morphological classification and molecular markers, thus supporting the accuracy and scientific value of traditional taxonomy. However, some morphologically similar species within the same section did not group together in the phylogenetic tree, indicating that additional genetic regions should be incorporated to better resolve the taxonomic positions of *Bulbophyllum* species in the Central Highlands.

Based on morphological characteristics, *Bulbophyllum* species in the Central Highlands were classified into respective sections. Identification keys to sections and to species within each section were also constructed.

Seven medicinally valuable species were recorded from related literature sources. The study area harbors four species listed in the Vietnam Red Data Book and four species listed in the IUCN Red List. Most *Bulbophyllum* species are cultivated as ornamentals, especially those in the sections *Acrochaene*, *Cirrhopetalum* (s.str.), *Brachyantha*, *Racemosae*, *Sestochilos*, *Hirtula*, and *Lemniscata*.

Recommendations

The *Bulbophyllum* genus in the Central Highlands and in Vietnam, in general, is a plant genus of significant value not only in daily life and resources but also in contributing to scientific knowledge. This dissertation has surveyed the *Bulbophyllum* species in the Central Highlands. Therefore, we recommend expanding the research area to collect more samples for species inventory, increasing the sample size for phylogenetic analysis, and continuing research to evaluate and analyze additional gene regions to clarify the phylogenetic relationships among taxa in the *Bulbophyllum* genus.

Furthermore, more studies on species diversity, distribution, and taxonomic arrangement are needed to complete the database for this genus as well as the Orchidaceae family, with the aim of creating a comprehensive flora for orchids in Vietnam.

The new contributions of the thesis

- This is the first species inventory and classification of the genus *Bulbophyllum* for the Central Highlands, based on an approach combining morphological and molecular methods.
- Six new species have been discovered and described as new for science: *B. manhdatii, B. sondangii, B. sridithii, B. trongquyetii, B. viridipallida, B. vulinhae*, and one new variety *B. blaoense* var. *flavescences*.