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TAXONOMIC STUDY OF THE GENUS *Psychotria* L. (Rubiaceae) IN VIETNAM

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GENERAL INFORMATION

1. The urgency of the dissertation

The Rubiaceae, commonly known as the coffee family, is one of the largest families of flowering plants, comprising an estimated 13,143 species in 611 genera worldwide. Among them, *Psychotria* L. is the most speciose genus, with approximately 1,645 to 2,000 species, predominantly distributed in tropical and subtropical regions of the Americas, Africa, and Asia. Situated in the tropical monsoon climate zone and influenced by the convergence of the Sino-Himalayan and Malesian floristic regions, Vietnam harbors a highly diverse and rich flora. As such, botanical research in Vietnam has long been a source of inspiration for both national and international scientists. In Vietnam, *Psychotria* remains poorly understood and is often difficult to distinguish from other genera within the Rubiaceae. To date, no comprehensive taxonomic revision of the genus has been undertaken for the country. Existing literature is often outdated, with simplified descriptions and inconsistencies in nomenclature, highlighting the need for taxonomic updates, incorporation of new data, and reassessment of species diversity. Furthermore, anatomical data on the genus *Psychotria* in Vietnam are extremely limited and have not received sufficient attention.

Given these considerations, a systematic and integrative taxonomic study of *Psychotria* based on morphological, anatomical, and molecular characteristics is essential. This study aims to provide a scientific and theoretical basis for the taxonomy of *Psychotria* in Vietnam. It also seeks to contribute to the development of a comprehensive species inventory, support future research on the conservation of rare and endangered species, promote the sustainable use and valorization of medicinal resources, and ultimately contribute to the compilation of the Flora of Vietnam in general, and the Rubiaceae in particular. To this end, we conducted the study entitled: **“Taxonomic study of the genus *Psychotria* L. (Rubiaceae) in Vietnam.”**

2. Objectives of the study

- Determine the species composition as well as the biological and ecological characteristics of species belonging to the genus *Psychotria* L. in Vietnam.

- Develop a systematic and comprehensive identification key for the species of *Psychotria* L. occurring in Vietnam.

3. Research scopes

- Conduct a comprehensive review of the genus *Psychotria*.

- Perform field investigations and collect specimens of *Psychotria* species from various habitats across Vietnam.

- Classify *Psychotria* species based on morphological characteristics and conduct preliminary anatomical studies on selected species in Vietnam.

- Apply molecular methods to support the taxonomic classification of *Psychotria* species.

- Develop an identification key and investigate the biological characteristics of *Psychotria* species occurring in Vietnam.

4. The scientific and practical basis of the thesis

Scientific significance: The results of this dissertation contribute to expanding and refining the taxonomic knowledge of the genus *Psychotria* L. in Vietnam, laying a foundation for the future compilation of the *Flora of Vietnam* with respect to the Rubiaceae family in general, and the genus *Psychotria* in particular.

Practical significance: The findings provide a scientific basis for educational and training purposes, and serve as valuable references for applications in agriculture, forestry, ecology, biodiversity conservation, pharmacology, and the sustainable utilization of biological resources.

5. Scope of the study

This study focuses on the taxa of the genus *Psychotria* in Vietnam, based on an integration of existing herbarium specimens preserved in national and international herbaria, combined with additional field collections conducted in selected national parks and nature reserves across

Vietnam. The research encompasses morphological, molecular, and anatomical investigations of these taxa.

Chapter 1. Overview

1.1. The global research status of the genus *Psychotria* L.

1.1.1. The taxonomic position of the genus *Psychotria* L. within the family Rubiaceae.

The genus *Psychotria* L. was first described in 1759 by Carl Linnaeus—widely regarded as the father of plant taxonomy—in the 10th edition of *Systema Naturae* (Editio Decima 2, 1759: 929), based on a single type species, *Psychotria asiatica* L. In 1789, Antoine Laurent de Jussieu, the first botanist to systematically organize plant genera into distinct families, described and established the family Rubiaceae and placed the genus *Psychotria* within it. His classification was based on a set of morphological characteristics, including: two cotyledons, sympetalous corolla, inferior ovary, free stamens, syncarpous fruit, bilocular inferior ovary, five stamens, and opposite leaves. Since then, the taxonomic position and classification of the genus *Psychotria* L. have attracted considerable attention from various authors and have been subject to differing interpretations and revisions.

1.1.1.1. Perspectives on the suprageneric classification of the genus Psychotria L.

- First viewpoint: Placement of the genus Psychotria L. within the tribe Coffeaceae

In 1830, Augustin Pyramus De Candolle proposed a classification system for the order Rubiaceae, in which he divided the group into 13 major tribes. Within the tribe Coffeaceae (now correctly referred to as *Coffeae*), he further subdivided it into two subtribes—*Coffeeae* and *Cephaelideae*—based on differences in floral morphology. The genus *Psychotria* L., comprising 177 species described by De Candolle himself, was placed within the subtribe *Coffeeae* of the tribe *Coffeae*. In 1834, R. Wight and W. Arnott proposed a classification in which the order Rubiaceae was divided into eight tribes: *Cinchonaceae*, *Gardeniaceae*, *Hedyotideae*, *Guettardaceae*,

Paederieae, *Coffeaceae*, *Spermacoceae*, and *Stellateae*. In their system, the genus *Psychotria* L. was also placed within the tribe *Coffeaceae*.

- Second viewpoint: Placement of the genus Psychotria L. within the tribe Psychotrieae

Schlechtendal (1829) was the first to establish the tribe *Psychotrieae*, placing the genus *Psychotria* L. within it. In 1840, Endlicher briefly described the genus and classified it under the subtribe *Coffeeae*, within the tribe *Psychotrieae*, suborder *Coffeaceae*, of the order *Rubiaceae* (Ordo. Rubiaceae). Later, in 1846, Lindley renamed the order *Rubiaceae* as *Cinchonaeae* (Ordo. Cinchonaeae), and placed *Psychotria* in the tribe *Psychotridae*. In 1873, Bentham and Hooker divided the order *Rubiaceae* into three series, further subdividing them into five subseries and 25 tribes. The genus *Psychotria* was distinguished from 26 other genera and placed in Series C (unitegmic ovules), Subseries 2, Tribe *Psychotrieae*. In Africa, Hiern (1877) classified the Rubiaceae into two groups based on the number of ovules per ovary: Group A and Group B. *Psychotria* was placed in Tribe *Psychotrieae*. In 1882, Hooker again used ovule number as a criterion to divide the order *Rubiaceae* into three series—A, B, and C—with subseries α and β, resulting in a total of 16 tribes. Within this framework, *Psychotria* was placed in Tribe *Psychotrieae*, Series C, Subseries β. In 1891, Schumann proposed one of the most foundational systems for Rubiaceae classification. He placed *Psychotria* in subfamily *Coffeoideae*, tribe *Psychotriinae*, and subtribe *Psychotrieae*. However, these earlier views that placed *Psychotria* within the subfamily *Coffeoideae* have largely been rejected by modern botanists. Instead, *Psychotria* is now widely accepted to belong to the tribe *Psychotrieae* within subfamily *Rubioideae*. For example, Bremekamp (1934, 1966) divided the Rubiaceae into eight subfamilies and placed *Psychotria* in Tribe *Psychotrieae* of subfamily *Rubioideae*. Verdcourt (1958) accepted three of Bremekamp's subfamilies—*Cinchonoideae*, *Rubioideae*, and *Guettardoideae*—and similarly placed *Psychotria* in *Psychotrieae*, within *Rubioideae*. Robbrecht (1988) proposed a revised system in which

Rubiaceae was divided into four subfamilies: *Cinchonoideae* (14 tribes), *Ixoroideae* (6 tribes), *Rubioideae* (18 tribes), and *Antirheoideae* (8 tribes). He placed *Psychotria* in the tribe *Psychotrieae* of the subfamily *Rubioideae*. Later, Takhtajan (2009) classified the Rubiaceae (within the order *Rubiales* or *Gentianales*) into three subfamilies: *Rubioideae*, *Ixoroideae*, and *Cinchonoideae*, placing *Psychotria* in Tribe *Psychotrieae* under *Rubioideae*. The most recent study by Razafimandimbison & Rydin (2024) restructured the family Rubiaceae into two major subfamilies: *Dialypentalanthoideae* (38 tribes) and *Rubioideae* (30 tribes). In this updated system, *Psychotria* is placed in Tribe *Psychotrieae*, within the *Psychotrieae alliance* of subfamily *Rubioideae*.

1.2.1.2. Perspectives on the infrageneric classification of the genus Psychotria L.

In addition to varying views on the suprageneric classification of *Psychotria* L., numerous botanists have proposed infrageneric systems to better reflect its diversity across regions. Hiern (1877) divided the genus into five subgenera: *Tetramerae*, *Paniculatae*, *Confertiflorae*, *Bracteatae*, and *Chasalia*. Hooker (1882) grouped 52 Indian species based on seed morphology, while Müller Argoviensis (1895) classified about 250 American species into 12 groups using reproductive traits. Petit (1964, 1966) focused on African species, distinguishing subgenera by the presence of bacterial leaf nodules: *Subgen. Psychotria* (nodules absent) and *Subgen. Tetramerae* (nodules present). Steyermark (1972) separated Neotropical *Psychotria* into two subgenera based on morphology and geography: *Psychotria* and *Heteropsychotria*. Recent molecular analyses (Razafimandimbison et al., 2014) revealed *Psychotria* s.l. is polyphyletic, with genera such as *Amaracarpus*, *Calycosia*, *Camptopus*, *Dolianthus*, *Hydnophytum*, *Grumilea*, and several WIOR taxa nested within it. The authors suggested recognizing *Psychotria* as a broadly defined genus with unresolved boundaries. Building on these findings, Lachenaud (2019) advised against formally dividing African *Psychotria*, citing the lack of clear

monophyletic groups despite morphological diversity and minimal gene flow among continents.

Beyond taxonomic studies, floristic surveys have documented species richness of *Psychotria* in various regions: 59 species in New Caledonia, 76 in Fiji, 23 in Samoa, 18 in Micronesia, and 11 in Hawaii. Other records include 7 species in Tahiti (Welsh, 1998), 61 in Mexico and Central America (Hamilton, 1989), 18 in China (Chen & Taylor, 2011), 42 in the Malay Peninsula (Ridley, 1922), 15 in Singapore (Wong et al., 2019), 4 in Taiwan (Yang, 1998), 5 in Japan (Yamazaki, 2013), and 95 in the Philippines (Sohmer & Davis, 2007).

1.2. Research status of the Genus *Psychotria* L. in Vietnam

Taxonomic studies of the genus *Psychotria* in Vietnam date back to the early 20th century, with Pitard (1924) being among the first to classify and describe species from Vietnam and Indochina. In *Flore générale de l'Indo-Chine*, he proposed two identification keys—based on floral/fruit and vegetative characters—and described 26 species, including 17 from Vietnam, laying the groundwork for future research. Nguyen Tien Ban (1997) highlighted Rubiaceae as one of Vietnam's most diverse families, with 430 species in 90 genera, and estimated around 25 *Psychotria* species. Pham Hoang Ho (2000) listed 30 species and one variety under tribe *Psychotrieae*. Tran Ngoc Ninh (2005) revised the count to 26 species and one variety, while Vo Van Chi (2007) reported 29 species. Later, Vo Van Chi (2018) provided ethnobotanical and medicinal data on nine *Psychotria* species, including their morphology, distribution, and traditional uses.

Selection of the Taxonomic system for the genus *Psychotria* L. in Vietnam

The choice of a classification system is a critical issue in taxonomy. Based on the integration of both morphological and molecular data of *Psychotria* taxa in Vietnam, and through comparative analysis of previously published global taxonomic systems of the genus, we recognize the complexity involved in the classification of this group. Each geographic

region tends to exhibit unique species assemblages, and consequently, taxonomic treatments—particularly at the infrageneric level—often differ. For the suprageneric placement of *Psychotria*, we adopt the system proposed by Razafimandimbison & Rydin (2024), in which the genus is assigned to the tribe *Psychotrieae* within the subfamily *Rubioideae*. Regarding the infrageneric classification, we follow the framework of Razafimandimbison et al. (2014) in combination with the perspective of Lachenaud (2019), which we consider most appropriate for the current state of knowledge. Accordingly, we adopt this system as the foundation for organizing the *Psychotria* taxa occurring in Vietnam.

Chapter 2. MATERIALS AND METHODOLOGY

2.1. Materials

The *Psychotria* taxa in Vietnam were studied based on both naturally occurring populations and herbarium specimens preserved in national and international herbaria. In total, approximately 1,009 specimens were examined and analyzed from across the country.

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 *Psychotria*, to compile information, shape the research scope, and select the classification system for the genus *Psychotira* in the study area.

2.2.2. Field works and specimens' collection

A total of 33 field surveys were conducted across 27 out of 63 provinces and cities, representing various ecological regions of Vietnam, from October 2021 to October 2024. Each lasting from 15 to 20 days.

Photographing specimens: Specimens will be photographed using a Canon 750D with Canon Sigma 17-50mm f/2.8 EX DC HSM OS and 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° ethanol in the field and later dried in the laboratory. Like other plant families, accurate identification of species in the genus *Psychotria* 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 "QB," 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.

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

Anatomy sample collection

Leaf samples, as well as primary and secondary stem samples of the same species, were collected and preserved in 70° ethanol or 5‰ formalin for long-term anatomical study.

2.2.3. Laboratory works

Specimen processing and preservation: Collected specimens were processed and dried at 65°C, then stored at the VNM herbarium for further studies.

Species description and identification: Species descriptions were based on direct measurements and scale-referenced photographs of living specimens in the field. These data were cross-referenced with specialized botanical literature and herbarium specimens housed in both national and international herbaria.

Morphological character analysis: A matrix of morphological traits of *Psychotria* species in Vietnam was constructed and analyzed using

PAUP* software under the Parsimony method, with 1000 bootstrap replicates. Characters were numerically coded (0–6), and missing or unknown data were denoted by "?".

Total genomic DNA extraction and DNA barcoding: Total genomic DNA was extracted using the GeneJET Plant Genomic DNA Purification Mini Kit. DNA quality was checked by 0.8% agarose gel electrophoresis in 0.5× TAE buffer. DNA was considered sufficiently pure if the OD260/280 ratio ranged from 1.6 to 2.0. Genetic regions were screened including chloroplast gene *matK* and nuclear ribosomal regions *ITS*, *ITS1*, and *ITS2*. Suitable primer pairs for each gene region were selected and used for PCR amplification. The best PCR products for *matK* and *ITS* regions were then sequenced bidirectionally (forward and reverse) using the Sanger method at Macrogen Inc. (South Korea). After sequencing, raw reads were edited and aligned. DNA sequences were stored in FASTA format for subsequent analyses. Phylogenetic trees were reconstructed using MEGA 7.0 (Molecular Evolutionary Genetics Analysis), applying the Maximum Likelihood method with 1000 bootstrap replicates.

Anatomical sectioning and staining: Fresh plant materials were manually sectioned using a razor blade. A double-staining technique developed by Trần Công Khánh (1981) was applied. Microscopic observations and imaging were conducted to examine detailed structures of epidermal and stomatal cells.

Anatomical character analysis: Anatomical features were encoded in a binary matrix (0: absence, 1: presence) and analyzed using PAST software. Cluster analysis was performed using UPGMA based on the Jaccard similarity index, with 1000 bootstrap replicates.

Data synthesis and analysis: Phylogenetic trees derived from morphological, anatomical, and molecular data were compiled and compared. The similarities and differences among species were analyzed to elucidate the interspecific relationships within the genus *Psychotria* in Vietnam.

Chapter 3. Results and discussion

3.1. Morphological diversity of the genus *Psychotria* L. in Vietnam

The checklist of *Psychotria* species in Vietnam is shown at **Table 1.**

Table 1. Checklist of *Psychotria* L. species in Vietnam

STT	Tên khoa học	Tên tiếng Việt
	***Psychotria* L.**	
1	*Psychotria adenophylla* Wall.	Lấu tuyến
2	*Psychotria asiatica* L.	Lấu đỏ
3	*Psychotria balansae* Pit.	Lấu Balansa
4	*Psychotria baviensis* Pit.	Lấu Ba Vì
5	*Psychotria bodenii* Wernham	Lấu Bo-đen
6	*Psychotria bonii* Pit.	Lấu Bon
7	*Psychotria cambodiana* Pierre ex Pit.	Lấu Cam-bốt
8	*Psychotria cephalophora* Merr. (5)	Lấu mang đầu
9	*Psychotria condorensis* Pierre ex Pit.	Lấu Côn Đảo
10	*Psychotria fleuryi* Pit.	Lấu Fleury
11	***Psychotria hainanensis* H.L.Li (3)**	Lấu Hải Nam
12	*Psychotria harmandiana* (Pit.) Turner	Lấu Harmand
13	***Psychotria henryi* H.Lév. (4)**	Lấu Henry
14	***Psychotria honbaensis* Bao, Vuong, Tagane & V.S. Dang (1)**	Lấu Hòn Bà
15	*Psychotria langbianensis* Wernham	Lấu Langbian
16	*Psychotria laui* Merr.	Lấu Lau
17	***Psychotria magnifruta* Bao, Đinh, V.S.Dang & Tagane, sp.nov., in.prep. (1)**	Lấu quả to
18	*Psychotria mekongensis* Pit. (4)	Lấu Mê-kông
19	***Psychotria monticola* Kurz (3)**	Lấu núi
20	*Psychotria morindoides* Hutch. (5)	Lấu nhàu

21	***Psychotria nuibamontana* Yahara & Tagane ex Bao, sp.nov., in.prep (1)**	Lấu Núi Bà
22	***Psychotria ngotphamii* Bao, Tagane, Yahara & V.S Dang (1)**	Lấu Phạm Văn Ngọt
23	*Psychotria oligoneura* Pierre ex Pit.	Lấu ít gân
24	***Psychotria phuquocensis* Bao,Vuong & V.S.Dang (1)**	Lấu Phú Quốc
25	*Psychotria poilanei* Pit.	Lấu Poilane
26	*Psychotria prainii* H.Lév.	Lấu xiêm
27	*Psychotria pseudoixora* Pit.	Lấu trang
28	***Psychotria quangtriensis* Bao & T.A.Le, stat.nov., in.prep. (2)**	Lấu Quảng Trị
29	*Psychotria sarmentosa* Bl.	Lấu leo
30	*Psychotria serpens* L.	Lấu bò
31	***Psychotria serpens* var. *membranacea* Pit., stat.nov., in.prep. (2)**	Lấu leo lá mỏng
32	*Psychotria silvestris* Pit. nom.nud.	Lấu rừng
33	***Psychotria* sp1.**	Lấu
34	*Psychotria tonkinensis* Pit.	Lấu Bắc Bộ
35	***Psychtoria tutcheri* Dunn. (3)**	Lấu Tut-cher
36	***Psychotria yunnanensis* Hutch. (4)**	Lấu Vân Nam
	***Eumachia* DC.**	
1	***Eumachia chasaliifolia* (Pit.) Bao & T.A., com.nov.. (2)**	Hoàng thác diệp lá mỏng

(1): new species (2): new combination

(3): new record (4): confirm to occur in Vietnam (5): distribution doubtful

3.1.1. Habit: Species of the genus *Psychotria* L. in Vietnam exhibit considerable growth form diversity, ranging from herbs, low shrubs, and climbers growing on tree trunks or rocky surfaces, to predominantly medium-sized shrubs or small trees reaching heights of 1–5 meters.

3.1.2. Leaf: All species of the genus *Psychotria* L. in Vietnam bear simple leaves, arranged oppositely or in decussate pairs. Leaf pairs are equal in size. The leaf blades are thick and morphologically diverse, most commonly ovate, broadly ovate, narrowly ovate, elliptic, elliptic-oblong, oblong obovate, or oblanceolate in shape.

3.1.3. Petiole: Stipules are morphologically diverse, ranging from triangular to ovate in shape, with apices that may be acute, obtuse, or shallowly to deeply bifid. The outer surface of the stipules is usually glabrous or densely pubescent, puberulent, the margins entire or ciliolate.

3.1.4. Inflorescence: The inflorescences of *Psychotria* L. are cymose and morphologically diverse: cymose to paniculiform; congested-cymose; thyrsiform; cymose-paniculiform; capitate to subcapitate.

3.1.5. Flower: The flowers exhibit variable coloration, most commonly white or creamy white, and less frequently greenish white, pale green, yellowish, yellow, or pink..

+ Calyx: The calyx is actinomorphic. In most species, the calyx is green to yellowish green, rarely reddish-brown, yellow, or white. The calyx tube vanishingly short. The calyx 5 lobes, rarely 4 or 6, which may be triangular, narrowly triangular (symmetrical or asymmetrical), narrowly lanceolate. In some species, the lobes are almost absent or vestigial. The apex is usually acute; the margins are entire or bear short to long hairs.

+ Corolla: The corolla is actinomorphic and distinctly differentiated into a tubular portion and corolla lobes. Corolla color is typically white to creamy white, rarely green, yellow, red, or pink. The corolla tube cup-shaped, funnel-shaped, or cylindrical. The corolla tube is generally glabrous on both surfaces, though sometimes pubescent to puberulent externally, densely puberulous to villous inside at the throat. The corolla lobes 5, rarely 4 or 6, arranged in valvate aestivation. The lobes are triangular to narrowly triangular, with acute apices that may be thickened or not; margins are entire; surfaces are usually glabrous or sparsely pubescent externally. The lobes are often recurved or reflexed at full anthesis.

3.1.6. Stamen: 5 stamens, occasionally 4 or 6, mostly inserted at the throat of the corolla tube (except in *P. condorensis*), alternating with the corolla lobes. The filaments are short to long and slender. The anthers are dorsifixed, bilocular.

3.1.7. Ovary: Cup-shaped or funnel-shaped, and inferior. It consists of two locules, each containing a single anatropous ovule with basal placentation. The style is solitary, glabrous or pubescent, and may be included within or exserted from the corolla throat. The stigma is bilobed, with lobes that may be flared or not, and are typically glabrous or covered with fine hairs. Stigma morphology varies, ranging from clavate and flattened-lanceolate to subulate forms.

3.1.8. Fruit: The fruit is a drupe, globose to subglobose, ovoid, obovoid, ellipsoid. Immature fruits are green, turning red, orange, white, or black upon ripening.

3.1.9. Pyrene: The pyrenes are formed by the lignification and thickening of the endocarp. There are typically two pyrenes per fruit, though occasionally only one develops fully. Pyrenes are generally hemispherical, hemi-ellipsoid, hemi-ovoid, narrowly hemi-ovoid, or inversely hemi-ovoid. In fruits containing a single developed pyrene, it may be globose, subglobose, ovoid, or ellipsoid. Each pyrene is dorsally convex and ventrally flattened. The dorsal surface often bears longitudinal grooves or undulating ridges, which fall into three main categories: shallow, deep, or nearly flat. The ventral surface may present a shallow median groove or lack a groove entirely.

3.1.10. Seed: In transverse section, the seed is typically hemispherical due to the presence of longitudinal ridges and grooves on both the dorsal and ventral surfaces. Depending on the morphology of these surface structures, several seed types can be distinguished: Flattened-convex seeds with densely arranged alternating ridges and grooves on both dorsal and ventral surfaces; endosperm highly folded: flattened-convex seeds with 5–6 ridges and 4–5 irregular grooves on the dorsal side, and two shallow grooves on the ventral

side; endosperm folded; flattened-convex seeds with 5–6 ridges and 4–5 deep or shallow grooves on the dorsal surface, and a single I-shaped, V-shaped, T-shaped groove ventrally; endosperm folded; flattened-convex seeds with both dorsal and ventral surfaces smooth; endosperm copious.

3.1.11. Germination slit: In Vietnamese species of *Psychotria*, the germination slit is consistently located on the ventral surface of the pyrene, at the apical end corresponding to the fruit's stalk. The slit exhibits various morphologies, ranging from clearly prominent and protruding to inconspicuous or barely visible.

3.1.12. Pigment solubility in ethanol: In most surveyed Vietnamese *Psychotria* species, the seed coat contains red pigments that are soluble in 70° ethanol (soluble colored pigments, SCPs). Upon immersion in the same solution, not only the seed coat but also the stem and leaves of these species typically turn reddish-brown.

3.2. Results of phylogenetic tree construction based on Morphological characters

In the morphology-based phylogenetic tree, Vietnamese *Psychotria* species show clear morphological clustering, with initial signs of differentiation among taxa. Nine distinct groups were identified within the *Psychotria* clade, each supported by relatively high bootstrap values (57–100%).

3.2. Anatomical Structure of *Psychotria* Species in Vietnam

3.2.1. Leaf anatomical characteristics

The midrib anatomy of *Psychotria* species in Vietnam displays typical dicotyledonous structure, comprising (adaxial to abaxial): upper epidermis, collenchyma, palisade parenchyma, vascular bundle, spongy parenchyma, lower collenchyma, and lower epidermis. Vascular bundles may include sclerenchyma and raphide bundles of calcium oxalate. Lateral veins share a similar transverse anatomy. The leaf blade is dorsiventral, and the petiole anatomy (from upper to lower surface) includes epidermis, collenchyma, parenchyma, and vascular bundle, with occasional sclerenchyma and calcium oxalate crystals.

3.2.2. Stem anatomical characteristics.

The primary stem structure of *Psychotria* species reflects typical dicotyledonous anatomy, comprising two main regions: the cortex and the stele. The cortex consists of the epidermis, collenchyma, cortical parenchyma, and endodermis, while the stele includes the pericycle, vascular tissues (xylem and phloem), and pith parenchyma. Occasionally, raphide bundles of calcium oxalate or sclerenchymatous cells are observed in transverse sections. Secondary growth arises through the activity of the cork cambium (phellogen–cortex) and vascular cambium.

3.2.3. Anatomical similarities among *Psychotria* Species

Anatomical similarity among species was assessed using UPGMA clustering based on the Jaccard similarity index with 1,000 bootstrap replications. Similarity coefficients ranged from 0.27 to 1.00. *Eumachia montana* clustered closely with *Chassalia curviflora*, forming a distinct group supported by a moderate bootstrap value of 66%. The remaining the *Psychotria* species and *Eumachia ovoidea* were divided into three main groups: **Group I** included species lacking non-glandular trichomes on both leaves and stems; **Group II** comprised species with multicellular non-glandular trichomes on either leaves or stems; and **Group III** consisted of species bearing unicellular non-glandular trichomes on either organ.

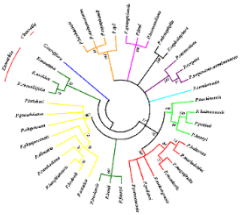

Figure 1. Phylogenetic tree based on morphological characters of species belonging to the genera *Psychotria*, *Eumachia*, and *Chassalia* in Vietnam.

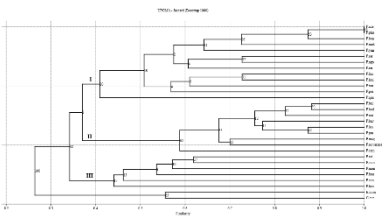

Figure 2. Dendrogram of phylogenetic relationships among species of *Psychotria*, *Eumachia*, and *Chassalia* constructed using UPGMA

3.3. Results of phylogenetic tree construction based on molecular characteristics

3.3.1. Extraction and quality assessment of total genomic DNA

The results of total genomic DNA extraction indicated that the purity of DNA, as measured by the OD260/OD280 ratio using a Nanodrop spectrophotometer, ranged from 1.65 to 2.01, with DNA concentrations varying from 103 to 192 ng/µL.

3.3.2. Screening of several DNA barcode regions for genetic analysis and evaluation in the genus *Psychotria* L.

Based on the amplification efficiency after performing PCR on *Psychotria* samples, two genetic regions were selected for this study: the chloroplast gene region *matK*, using primer pair *mat*K-F and *mat*K-R, and the nuclear ribosomal region *ITS2*, using primer pair ITS-p5 (18S) and ITS-p2 (5.8S).

3.3.3. Sequencing of selected DNA barcode regions for genetic analysis and evaluation within the genus *Psychotria* L.

3.3.3.1. Phylogenetic analysis results based on two individual sequence regions (matK and ITS2).

- matK gene region (Maturase K):

The aligned sequences of the *matK* gene region among the studied species had a total length of 910 base pairs (bp), of which 116 out of 910 positions were variable, and 794 positions were conserved.

- ITS gene region (Internal Transcribed Spacer):

The aligned sequences of the *ITS2* region had a total length of 303 bp, with 168 conserved positions and 135 variable positions identified among the studied species.

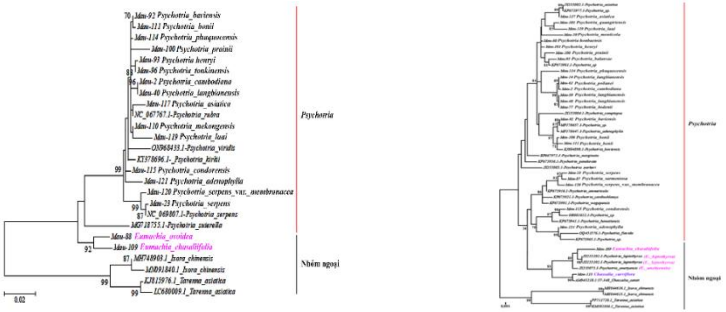

Figure 3. Phylogenetic tree constructed for species in the genus *Psychotria* based on *matK* sequences.

Figure 4. Phylogenetic tree constructed for species in the genus *Psychotria* based on *ITS2* sequences.

3.3.3.2. Phylogenetic analysis based on combined sequence regions (matK–ITS2)

- *mat*K-ITS2: The combined analysis of the *matK* and *ITS2* regions revealed a clear genetic separation between species of *Psychotria* and *Eumachia*. This separation is consistent with the results obtained when combining chloroplast and nuclear DNA regions, supporting the distinction between these genera.

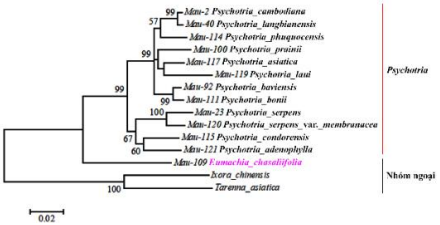

Figure 5. Phylogenetic tree of *Psychotria* species constructed based on combined matK and ITS2 sequence data.

3.4. Key to the species of *Psychotria* L. in Vietnam

1A. Bacterial-nodules in leaves, stipules apex entire, laxly inflorescences.. 2

1B. Non bacterial-nodules in leaves, stipules apex entire or 2-lobed, errect inflorescences 5

2A. Stiuples apex triangular, outer surfaces without two long hairs from the base to apex, 2 mm long 3

2B. Stipules apex ovate-triangular to rounded, outer surfaces have two long hairs from the base to apex, 2 mm long..... 4

3A. Penducle terete with two board longitudinal wings, yellow flowers, stigma lancelolate, unflared to flared ..... ***P. honbaensis***

3B. Penducle terete, pinkish flowers, stigma linear, unflared ..... ***P.* sp1**

4A. Twigs glabrous, leaves blade 2.5–6.5 × 1–2.5 cm long, abaxial surface glabrous, inflorescences glabrous, white flowers, stigma clavate, flared.....

..... ***P. ngotphamii***

4B. Swigs puberulent to tomentose, leaves blade 6–10 x 2–4 cm long, abaxial surface purberulent, inflorescences puberulent, whitish yellow flowers, stigma linear, unflared ..... ***P. nuibamontana***

5A. Climbing, white fruits 6

5B. Herbs, low shrubs, shrubs or small trees, fruits other than white 8

6A. Young twigs glabrous, connate stipules, apex triangular, white flowers. ..... ***P. sarmentosa***

6B. Young twigs puberulent, free stipules, apex triangular or 2-lobed, white to whitish yellow flowers 7

7A. Stipules glabrous, apex triangular or short 2-lobed ..... ***P. serpens***

7B. Stipules puberulent to tomentose, apex triangular or long 2-lobed..... ..... ***P. serpens* var. *membranacea***

8A. Bracts fused into an involucre 9

8B. Bracts not forming an involucre..... 11

9A. Herbs, twigs puberulent, stipules apex 2-lobed, infloresences purbescent, flowres 4-merous ..... ***P. harmandiana***

9B. Shrubs 1–3 m, twigs glabrous, stipules apex triangular, inflorescences glabrous, flowers 5-merous 10

10A. Bracts 1 cm long, marginally irregular, flowers glabrous, calyx lobes margin densely hirsute, white corolla lobes, tubes cup-shaped, fruits broadly ovoid ..... ***P. laui***

10B. Bracts 8 mm long, marginally entire, sparely ciliolate, flowers glabrous, calyx lobes margin entire, pinkish corolla lobes, tubes funnel-shape, fruits ovoid ..... ***P.quangtriensis***

11A. Black fruits when ripe, seeds ventrally flattened, dorsally convex, rugose, with obscure grooves; endosperm strongly ruminate..... 12

11B. Orange to red fruits when ripe, seeds ventrally flattened, dorsally convex, rarely grooved, slightly rugose; endosperm full or slightly ruminate 13

12A. Compact cymose inflorescences with short axes, peduncle 4–8 cm long .... ***P. adenophylla***

12B. Compact cymose inflorescences like capitate, sessile to subsessile

peduncle..... ***P. cephalophora***
13A. Pyrenes dorsal-ventral, without grooves or ridges; seeds with abundant endosperm..... 14
13B. Pyrenes dorsal-ventral, with grooves or ridges; seeds with ruminate endosperm..... 16
14A. Low shrub; leaves obovate; flowers 4–5-merous; calyx lobes linear-oblong, 3–4 mm long..... ***P. baviensis***
14B. Shrub or small tree; leaves ovate or broadly ovate; flowers 5-merous; calyx lobes triangular, 2–3 mm long 15
15A. Stipules 5–7 mm long, broad-margined, apex shallowly 2-lobed; fruit ca. 1 cm long..... ***P. fleuryi***
15B. Stipules 5–8 mm long, apex deeply 2-lobed; fruit ca. 5 mm long .....
..... ***P. bonii***
16A. Flowers tubular, stamens inserted on the corolla tube; fruits orange when ripe. ..... ***P. condorensis***
16B. Flowers cup-shaped or tubular; stamens usually inserted at the corolla throat; fruits red when ripe 17
17A. Compact cymose inflorescences..... 18
17B. Cymose to congest-cymose ingflorescences..... 21
18A. Calyx lobes linear to linear-oblong..... 19
18B. Calyx lobes triangular..... 20
19A. Twigs, abaxial leaves surface, stipules puberulent ..... ***P. prainii***
19B. Twigs, abaxial leaves surface, stipules glabrous..... ***P. hainanensis***
20A. Young twigs pubescent, leaves blade elliptic to narrowly ovate, 4–15,5 × 2–5 cm long, adaxial surface glabrous to pubercent, abaxial leaves surface puberulent. ..... ***P. henryi***
20B. Young twigs glabrous, laeves blade elliptic to broadly ovate, 7–19 × 4–7 cm long, glabrous on both sides ..... ***P. tonkinensis***
21A. Flowers heterostylous; cymose inflorescences; stamens not clustered in a ring at the corolla throat; style short or long..... 22
21B. Flowers monomorphic; congest-cymose inflorescences; stamens clustered in a ring at the corolla throat; style short..... 30
22A. Stipules apex triangular 23

22B. Stipules apex 2-lobed 27
23A. Peduncle shorter or equal than 1,5 cm long 24
23B. Penduncle longer than 1,5 cm long 25
24A. Young stems pubescent; abaxial laeves surface pubescent; Domatia large, hairy; inflorescences sessile or with short peduncle ca. 0.3 cm; flowers creamy white; corolla tube cup-shaped; flowers heterostylous; fruits globose or subglobose ..... ***P. asiatica***
24B. Young stems glabrous; abaxial surface glabrous; Domatia small, glabrous; inflorescence peduncle 0.6–1.5 cm long; flowers green; corolla tube cup-shaped; flowers monomorphic; fruits ellipsoid .... ***P. phuquocensis***
25A. Abaxial leaves surface puberulent ..... ***P. silvestris***
25B. Abaxial leaves surface glabrous 26
26A. Leaf blade narrowly elliptic; inflorescence peduncle 2–3 cm long; secondary axes ca. 1.5 cm long ..... ***P. pseudo-ixora***
26B. Leaf blade elliptic; inflorescence peduncle 4–5 cm long; secondary axes 3.5–4 cm long ..... ***P. oligoneura***
27A. Stipules triangular, 6–8 mm long ..... ***P. tutcheri***
27B. Stipules obovate, 1 cm long 28
28A. Leaves with puberulent to tomentose on both surfaces. ***P. cambodiana***
28B. Leaves with puberulent on adaxial surface 29
29A. Stems, petioles, and outer surface of stipules sparsely puberulent to tomentose; heterostylous flowes ..... ***P. bodenii***
29B. Stems, petioles, and outer surface of stipules densely soft-hairy; flowers monomorphic ..... ***P. langbianensis***
30A. Young twigs glabrous; abaxial surface surface glabrous 31
30B. Young twigs puberulent to tomentose; abaxial surface surface pubescent to tomentose 33
31A. Inflorescences puberulent ..... ***P. mekongensis***
31B. Inflorescences glabrous 32
32A. Calyx lobes obscure; corolla tube cup-shaped ..... ***P. poilanei***
32B. Calyx lobes triangular; corolla tube tubular ..... ***P. yunnanensis***
33A Secondary axes 2–5 cm long; bracts large, ovate ..... ***P. monticola***
33B. Secondary axes less than 2 cm long; bracts triangular. 34

34A. Ovary outer surface pubescent.....***P. magnifructa***
34B. Ovary outer surface glabrous 35
35A. Leaves blade 16–30 × 7–14 cm; secondary veins 15–22 pairs, prominent abaxially, brochidodromous, ending 3–5 mm from margin; calyx lobes obscure ..... ***P. balansae***
35B. Leaves blade 11–21 × 4–10 cm; secondary veins 10–12 pairs, prominent abaxially, sub-brochidodromous; calyx lobes triangular. .....
.....***P. morindoides***

Discussion

This study successfully distinguished species of the three genera, contributing valuable data for future research and the development of the *Flora of Vietnam*. Anatomical analyses provided a general overview of *Psychotria* and related genera in Vietnam. For the first time, most *Psychotria* species and related taxa were assessed for molecular diversity. Among the three markers used, *mat*K showed the highest resolution, despite a lower amplification rate. Most results clearly separated *Eumachia* species, except those based on *rbc*L and *rbc*L–ITS2, which were less effective.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

Based on the morphological, anatomical, and molecular data of *Psychotria* L. species in Vietnam, this dissertation draws the following key conclusions:

1. General morphological characteristics of *Psychotria* L. were described. A morphology-based phylogenetic tree was constructed for 35 species and 1 varieties in Vietnam.

2. Anatomical features were analyzed, and a phylogenetic tree was constructed using UPGMA clustering with Jaccard similarity index for 26 species and 1 varieties. Anatomical images of stems and leaves were also provided.

3. Phylogenetic trees were inferred from individual and combined molecular markers (*matK*, *ITS2*), helping resolve taxonomic relationships among Vietnamese species.

4. A total of 35 species and one variety of this genus are recognized in Vietnam, including five species newly described for science (*Psychotria ngotphamii* Bao, Tagane, Yahara & V.S. Dang; *P. phuquocensis* Bao, Vuong & V.S. Dang; *P. honbaensis* Bao, Vuong, Tagane & V.S. Dang; *P. magnifructa* Bao, Dinh, V.S. Dang & Tagane; and *P. nuibamontana* Tagane & Yahara ex Bao) and one potentially new species (*Psychotria* sp.1). Two species, *P. hainanensis* H.L. Li and *P. monticola* Kurz, are newly recorded for Vietnam. The occurrence of *P. henryi* H.Lév., *P. yunnanensis* Hutch., and *P. tutcheri* Dunn is confirmed. Lectotypes are designated for the following species: *P. balansae* Pit., *P. baviensis* (Drake) Pit., *P. cambodiana* Pierre ex Pit., *P. condorensis* Pierre ex Pit., *P. fleuryi* Pit., *P. lecomtei* Pit., *P. monticola* Kurz, *P. oligoneura* Pierre ex Pit., *P. poilanei* Pit., *P. rhodotricha* Pit., *P. thorelii* Pit., *P. pseudo-ixora* Pit., *P. tonkinensis* Pit., and *P. tutcheri* Dunn. Evidence is provided to support the reduction of *P. rhodotricha* Pit. and *P. thorelii* Pit. to synonyms of *P. prainii* H.Lév., and *P. lecomtei* Pit. to a synonym of *P. bodenii* Wernham. The distinction between *P. laui* Merr. & F.P. Metcalf and *Cephaelis lecomtei* Pit. is clarified, and a new name, *Psychotria quangtriensis* Bao & T.A. Le, is proposed for the latter species. The varietal name *Psychotria sarmentosa* var. *membranacea* (Pit.) P.H. Ho is revised as *P. serpens* var. *membranacea* Pit. In addition, *P. chasaliifolia* Pit. is transferred to the genus *Eumachia*, as *Eumachia chasaliifolia* Bao & T.A. Le.

5. A dichotomous identification key (based on external and internal morphology) and full taxonomic treatment were provided for all Vietnamese taxa of *Psychotria* L., including nomenclature, type specimens, morphological and anatomical descriptions, biological and ecological notes, uses, conservation value, photographic plates, and distribution maps.

RECOMMENDATIONS

The results of this study provide a foundation for future basic and applied research. However, some limitations remain, including the incomplete sampling of *Psychotria* species in Vietnam and the lack of

comprehensive molecular sequencing and anatomical descriptions for all taxa. Therefore, further efforts are recommended to expand specimen collection, increase species coverage, and incorporate additional molecular markers to clarify evolutionary relationships among *Psychotria* and related genera. Future research should also focus on the medicinal potential and conservation of rare and endemic species in Vietnam. Simultaneously, the research is oriented toward the development of an online electronic taxonomic key, providing a foundation for future studies on the medicinal potential of species within the genus, as well as for the conservation of several rare and endemic species in Vietnam.

THE NEW CONTRIBUTIONS OF THE THESIS

This is the first systematic and comprehensive taxonomic revision of the genus *Psychotria* in Vietnam, improving upon previous fragmented or outdated studies. It represents the first attempt to integrate traditional morphological and anatomical approaches with modern molecular techniques to provide an updated and well-supported classification of *Psychotria* taxa in the country. The study provides new evidence distinguishing *Psychotria laui* Merr. & F.P.Metcalf from *Cephaelis lecomtei* Pit., leading to the proposal of a new name for the latter. It also presents evidence supporting the synonymization of two species under *Psychotria prainii* H. Lév., and one species under *Psychotria bodenii* Wernham, along with a taxonomic correction of one variety within the genus.

Lectotypes have been designated for eight species of *Psychotria*, and the presence of *Psychotria silvestris* Pit. in Vietnam has been confirmed, although the name is treated as a *nomen invalidum*.

A comprehensive morphological identification key has been developed for all known *Psychotria* taxa in Vietnam.

The study also describes five new species to science—*Psychotria ngotphamii* Bao, Tagane, Yahara & V.S.Dang; *Psychotria phuquocensis* Bao, Vuong & V.S.Dang; *Psychotria honbaensis* Bao, Vuong, Tagane & V.S.Dang; *Psychotria magnifructa* Bao, Dinh, V.S.Dang & Tagane (in prep.); and *Psychotria nuibamontana* Tagane & Yahara ex Bao (in prep Additionally, two species, *Psychotria hainanensis* H.L.Li; *Psychotria monticola* Kurz, are newly recorded for the flora of Vietnam. The occurrence of *Psychotria henryi* H.Lév., *P. yunnanensis* Hutch., and *P. tutcheri* Dunn in Vietnam is confirmed.

LIST OF PUBLICATIONS ARISING FROM THE DISSERTATION

1. **Quoc Bao Nguyen**; Van Toan Em Quach; Huu Duc Huynh; Quoc Trong Pham; Ba Vuong Truong; Tetsukazu Yahara; Shuichiro Tagane; Van-Son Dang. (2023). A new species of *Psychotria* (Rubiaceae) from Bidoup - Nui Ba National Park, Vietnam. Phytotaxa 618, 2: 188–194. DOI: 10.11646/phytotaxa.618.2.8.

2. **Nguyễn Quốc Bảo**, Phạm Văn Ngọt, Quách Văn Toàn Em, Trương Bá Vương, Phạm Quốc Trọng, Đặng Văn Sơn. (2023). Đặc điểm hình thái, giải phẫu và phân bố của loài Lấu tuyến (*Psychotria adenophylla* Wall.) ở các đảo vùng Nam bộ, Việt Nam. Tạp chí Khoa học Đại học Thủ Dầu Một 6, 67: 24–33. DOI: 10.37550/tdmu.VJS/2023.06.484.

3. Van Toan Em Quach, Van Son Dang, Van Ngot Pham, Ba Vuong Truong, Ngoc Minh Trung Nguyen, Quoc Trong Pham, **Quoc Bao Nguyen**. (2024) *Psychotria phuquocensis* Bao, Vuong & V.S.Dang, a new species of Rubiaceae from Phu Quoc National Park, southern Vietnam. Taiwania 69, 3: 336–371. DOI: 10.6165/tai.2024.69.366.

4. **Nguyễn Quốc Bảo**, Đặng Văn Sơn, Phạm Văn Ngọt, Nguyễn Ngọc Minh Trung, Trương Bá Vương, Phạm Quốc Trọng, Quách Văn Toàn Em. (2024). Nghiên cứu đặc điểm thực vật học và bước đầu khảo sát thành phần hóa học loài lấu (*Psychotria sarmentosa* var. *membranacea* P.H.Hô) thuộc họ Cà phê (Rubiaceae). Tạp chí Khoa học trường Đại học Sư Phạm thành phố Hồ Chí Minh 21, 5: 814–826. DOI: 0.54607/hcmue.js.21.5.4182(2024).

5. **Nguyễn Quốc Bảo**, Đặng Văn Sơn, Quách Văn Toàn Em, Phạm Văn Ngọt. (2024). Khẳng định loài Lấu Henry (*Psychotria henryi* H.Lév.) và Lấu Vân Nam (*Psychotria yunnanensis* Hutch.) phân bố ở Việt Nam. Tạp chí Khoa học trường Đại học Sư Phạm thành phố Hồ Chí Minh 21, 11: 2126–2134. DOI: 10.54607/hcmue.js.21.11.4363(2024).

6. **Quoc Bao Nguyen**, Van Ngot Pham, Van Toan Em Quach, Ba Vuong Truong, Van Son Dang. (2024). *Lectotypication*, morpho-anatomical traits and initial chemical analysis of *Psychotria condorensis* Pierre ex Pitard (Rubiaceae, *Psychotria*): A study on an endemic species from Con Dao National Park, Ba Ria-Vung Tau Province, Vietnam. Taiwania 69(3): 435–444. DOI: 10.6165/tai.2024.69.435.

7. **Quoc Bao Nguyen**, Ba Vuong Truong, Quoc Cuong Nguyen, Shuichiro Tagane, Van Ngot Pham, Van Toan Em Quach, Huu Duc Huynh, Quoc Trong Pham, Van-Son Dang. (2024). *Psychotria honbaensis*, a new species of *Psychotria* (Rubiaceae) with bacterial nodules from Hon Ba Nature Reserve, southern Vietnam [đã chấp nhận từ tạp chí Phytotaxa].

8. Quoc Bao Nguyen, Ba Vuong Truong, Shuichiro Tagane, Tuan Anh Le, Van Ngot Pham, Van Toan Em Quach, Van Huong Bui, Tran Vy Nguyen. (2025). Taxonomic notes of the genus *Eumachia* DC. in the flora of Vietnam and description a new species. Taiwania. 70(2): 262–274. DOI: 10.6165/tai.2025.70.262.