TROPICAL LIFE SCIENCES RESEARCH

# Morphological, Palynological, and Molecular Insights of *Arisaema langbiangense*: An Endemic Species from Langbiang Plateau, Southern Vietnam

**Authors:**

Huyen Trang Luu, Hong Quan Bui, Dinh Thach Bui, Hong Truong Luu, Tran Quoc Trung Nguyen, Nga Nguyen-Phi, Thu Trang Le-thi and Hong Thien Van

***Correspondence:** vanhongthien@iuh.edu.vn

**Submitted:** 10 May 2025; **Accepted:** 15 January 2026; **Early view:** 12 February 2026

**To cite this article:** Huyen Trang Luu, Hong Quan Bui, Dinh Thach Bui, Hong Truong Luu, Tran Quoc Trung Nguyen, Nga Nguyen-Phi, Thu Trang Le-thi and Hong Thien Van. (in press). Morphological, palynological, and molecular insights of *Arisaema langbiangense*: An endemic species from Langbiang Plateau, Southern Vietnam (Early view). *Tropical Life Sciences Research.*

## Highlights

- The anatomical characteristics such as the leaf, petiole, petiolule, root, and rhizome of *A. langbiangense* are described for the first time.
- Pollen grains of the studied plant are large, spheroidal, inaperturate monads with a tectate-echinate exine densely covered in conical spines, consistent with insect-mediated pollination.
- The ITS sequence of *A. langbiangense* is successfully sequenced and deposited in the NCBI database for the first time under accession number of PV600050.

**EARLY VIEW**

# Morphological, Palynological, and Molecular Insights of *Arisaema langbiangense*: An Endemic Species from Langbiang Plateau, Southern Vietnam

[1,2]Huyen Trang Luu, [1]Hong Quan Bui, [2,3]Dinh Thach Bui, [2,4]Hong Truong Luu, [4]Tran Quoc Trung Nguyen, [5,6]Nga Nguyen-Phi, [7]Thu Trang Le-thi and [1]Hong Thien Van*

[1]Institute of Biotechnology and Food Technology, Industrial University of Ho Chi Minh City, No. 12 Nguyen Van Bao Street, Hanh Thong Ward, Ho Chi Minh City, Vietnam
[2]Graduate University of Science and Technology, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Nghia Do Ward, Ha Noi, Vietnam
[3]Institute of Life Sciences, Vietnam Academy of Science and Technology, 9/621 Vo Nguyen Giap Street, Linh Xuan Ward, Ho Chí Minh City, Vietnam
[4]Institute of Advanced Technology, Vietnam Academy of Science and Technology, No. 1D, TL29 Street, An Phu Dong Ward, Ho Chi Minh City, Vietnam
[5]Faculty of Biology and Biotechnology, University of Science, Ho Chi Minh City, No. 227 Nguyen Van Cu Street, Cho Quan Ward, Ho Chi Minh City, Vietnam
[6]Vietnam National University Ho Chi Minh City, Linh Xuan Ward, Ho Chi Minh City, Vietnam
[7]Faculty of Pharmacy, Nguyen Tat Thanh University, No. 300A Nguyen Tat Thanh Street, Xom Chieu Ward, Ho Chi Minh City, Vietnam, 700000

*Corresponding author: vanhongthien@iuh.edu.vn

Running title: Micro-morphological, Pollen Characteristics and DNA Barcode of *A. langbiangense*

**Submitted:** 10 May 2025; **Accepted:** 15 January 2026; **Early view:** 12 February 2026
**To cite this article:** Huyen Trang Luu, Hong Quan Bui, Dinh Thach Bui, Hong Truong Luu, Tran Quoc Trung Nguyen, Nga Nguyen-Phi, Thu Trang Le-thi and Hong Thien Van. (in press). Morphological, palynological, and molecular insights of *Arisaema langbiangense*: An endemic species from Langbiang Plateau, Southern Vietnam (Early view). *Tropical Life Sciences Research.*

**Abstract:** *Arisaema langbiangense* is an endemic species first described in Vietnam in 2016, with limited research to date. This study provides the first integrated analysis of its anatomical, palynological, and molecular characteristics to facilitate species identification and future taxonomic work. Anatomically, the leaf, petiole, petiolule, root, and rhizome exhibit distinctive features, including adaxial-abaxial polarity in leaves, homogeneous mesophyll, consistent xylem-phloem orientation, abundant needle-shaped calcium oxalate crystals, and scattered vascular bundles in the rhizome. Pollen grains are large, spheroidal, inaperturate monads with a tectate-echinate exine densely covered in conical spines, consistent with insect-mediated pollination and similar to other *Arisaema* species. The ITS region (446 bp) of *A. langbiangense*, newly sequenced and deposited in GenBank, represents the first available ITS region of *A. langbiangense* for section *Anomala*. Phylogenetic analysis based on ITS data places the species in a distinct clade, highlighting its genetic uniqueness and supporting sectional relationships consistent with previous plastid-based studies. These findings contribute valuable insights into the taxonomy and evolutionary placement of this poorly known species.

**Keywords**: Anatomy, *Arisaema langbiangense*, Pollen Structure, ITS, Southern Vietnam

## INTRODUCTION

The family Araceae comprises a morphologically diverse assemblage of monocotyledonous flowering plants, among which *Arisaema* Mart. stands out as one of its largest genera, encompassing approximately 200 described species (Gusman & Gusman 2006;Ohi-Toma et al. 2016). These species are predominantly distributed across the temperate and subtropical regions of Asia, extending from the eastern Himalayas to Southeast Asia and Malesia, and secondarily in North America and northeastern Africa (Mayo et al. 1997; Gusman & Gusman 2006). The genus *Arisaema* exhibits high levels of endemism and morphological diversity, especially in the Sino-Himalayan and Sino-Japanese floristic regions, which are considered its main centers of diversification. In Vietnam, the genus is taxonomically well represented, with 29 species currently recognized and distributed primarily in montane tropical forests from northern regions to the central highlands as well as southern regions (Pham 2000;Nguyen 2000; Nguyen & Boyce 2005; Nguyen 2007;Bruggeman et al. 2013;Luu et al. 2013;Luu et al. 2014;Hoang et al. 2015; Van et al. 2016;Nguyen 2017; Le et al. 2020;Luu et al. 2020;Luu et al. 2022;Ma et al. 2024).

Among the 15 currently recognized sections of *Arisaema* (Ohi-Toma et al. 2016), the section *Anomala* is of particular interest due to its distinct morphological characteristics and biogeographical range. This section is typified by *Arisaema anomalum* Hemsl., and includes approximately 29 species distributed mainly in tropical and subtropical regions of East and

Southeast Asia (Gusman & Gusman, 2006;Bruggeman et al. 2013). Members of *Arisaema* sect. *Anomala* are characterized by trifoliolate or pedate leaves, evergreen habit with simultaneous presence of old and emerging foliage, and a spadix appendix that is typically exserted, often bearing neuter flowers and apical bristles. Inflorescences and leaves arise separately from the rhizome and may form a pseudostem; the receptacle is cream-colored and flowering may occur year-round (Gusman & Gusman 2006). In Vietnam, this section is represented by at least 13 species, including *A. balansae* Engl., *A. claviforme* Brugg., J.Ponert, Rybková & Vuong, *A. garrettii* Gagnep., *A. langbiangense* Luu, Nguyen-Phi & H.T.Van*, A. lihengianum* J.Murata & S.K.Wu, *A. menglaense* Y.H.Ji, H.Li & Z.F.Xu*, A. omkoiense* Gusman, *A. petelotii* K.Krause, *A. petiolulatum* Hook.f., *A. pingbianense* H.Li, *A. rostratum* V.D.Nguyen & P.C.Boyce, *A. tridens* Z.X.Ma, T.S.Hoang & X.X.Zhou*,* and *A. victoriae* V.D.Nguyen, which are mainly found in montane evergreen forests and often restricted to narrow geographical ranges (Pham 2000; Nguyen 2000; Nguyen & Boyce 2005; Nguyen 2007; Bruggeman et al. 2013; Hoang et al. 2015; Van et al. 2016; Nguyen 2017; Le et al. 2020; Ma et al. 2024).

*Arisaema langbiangense* Luu, Nguyen-Phi & H.T.Van was recently described from Langbiang Mountain, Bidoup-Nui Ba National Park, Lam Dong Province, southern Vietnam (Van et al. 2016). This species belongs to sect. *Anomala* and was discovered during a botanical survey in 2014 (Van et al. 2016). It is morphologically allied to species such as *A. claviforme*, *A. garrettii*, and *A. petiolulatum*, yet can be readily distinguished by a combination of unique traits: green rhizome flesh (a first report within the genus), a conspicuous pseudostem, a forward-bent spadix appendix, and obovoid ovaries with five to six lobes (Van et al. 2016). Thus far, *A. langbiangense* is known only from its type locality at approximately 1700 m elevation, where it inhabits the humid understory of tropical evergreen forests. Despite its recent description, no detailed anatomical or palynological studies as well as DNA barcode of this taxon have been published. Given the systematic significance of floral micromorphology and pollen structure in *Arisaema* taxonomy (Gusman & Gusman 2006), a comprehensive investigation into the morphological, anatomical, palynological, and molecular features of *A. langbiangense* is essential for a more robust understanding of its taxonomic position within sect. *Anomala* and the genus as a whole.

## MATERIALS AND METHODS

The specimens of *A. langbiangense* were collected from Langbiang Mountain, Bidoup-Nui Ba National Park, Lac Duong District, Lam Dong Province, Vietnam, 1800 m in elevation, about 12°02'52.8"N 108°26'22.3"E. The voucher specimens, Luu_01A and Luu_01B, was deposited at the Herbarium of University of Science, Vietnam National University, Ho Chi Minh City (PHH) (Thiers, 2025).

## Identification of scientific name

The sampling and preparation of plant specimens were performed in accordance with the guidelines from the Royal Botanic Gardens, Kew (Bridson & Forman 1999). The scientific name of the studied species was identified using the comparative morphology method. Accordingly, the reproductive and vegetative characteristics of *A. langbiangense* were compared with those of other *Arisaema* species reported in the previous studies conducted by Pham (2000), Nguyen (2000), Nguyen & Boyce (2005), Nguyen (2007), Bruggeman et al. (2013), Hoang et al. (2015), Van et al. (2016), Nguyen (2017), Le et al. (2020), Ma et al. (2024).

## Plant sample preparation for anatomical observations

Thin sections of the root, leaf, petiole, petiolule, and rhizome of *A. langbiangense* were prepared and treated with sodium hypochlorite solution for bleaching. The samples were then double-stained with iodine green and carmine. After several washes with distilled water, the specimens were preserved in a 10% glycerol solution (Van et al. 2024). Observations of these specimens were made using an Olympus BX53 Digital Upright Microscope.

## Pollen preparation for observations

The pollen sample preparation followed a modified version of Erdtman's method (Erdtman 1943). Initially, the sample underwent dehydration by combining pollen with 2 ml sulfuric acid ($H_2SO_4$), 26 ml acetic acid ($CH_3COOH$), and 6 ml acetic anhydride $(CHCO)_2O$ in a test tube, heating in a water bath for 10 minutes, followed by centrifugation at 2000 rpm for 5 minutes and supernatant discarded. For acetolysis, 28 ml acetic acid, 4 ml acetic anhydride, and 8 ml sulfuric acid were added to the residue, heated for 10 minutes, centrifuged, and washed with 30% alcohol 2-3 times. The alkalization process involved adding 2 ml of 10% KOH solution, heating for 5 minutes, followed by sequential washing with distilled water, hydrochloric acid (ClH N/60), and distilled water until reaching pH value of 7.0. For optical microscopy (Olympus BX53), pollen samples were stained with Safranin for 2 minutes, then centrifuged, and the supernatant was discarded before mounting. For scanning electron microscopy (SEM; Tescan Vega), pollen grains were dried using a critical point dryer to prevent structural collapse, and were subsequently sputter-coated with a thin layer of gold to enhance surface conductivity before imaging.

### DNA extraction, PCR amplification, and sequence analysis

The total DNA of *A. langbiangense* was extracted using a modified CTAB 2X method (Aboul-Maaty et al. 2019). The primer pair used for ITS (internal transcribed spacer) region amplification consists of the following sequences: ITS1: TCCGTAGGTGAACCTGCGG/ITS4: TCCTCCGCTTATTGATATGC (White et al. 1990). This region was amplified using a Mastercycler PCR system (Eppendorf, Germany). Each 25 µL PCR reaction mixture contained 12.5 µL master mix (Phu Sa Company, Vietnam), 1.25 µL of each primer (10 µM, forward and reverse), 9.5 µL of deionized water, and 0.5 µL of DNA template. The thermal cycling conditions included an initial denaturation at 95°C for 5 minutes, followed by 35 cycles of 94°C for 1 minute, 55°C for 1 minute and 30 seconds (primer annealing), and 72°C for 1 minute and 30 seconds (extension). The PCR products were purified and sequenced using an ABI 3500 Genetic Analyzer (Applied Biosystems™ 3500 XL Genetic Analyzer) at the Nam Khoa BioTek (Vietnam). The obtained ITS sequence was processed and analyzed with FinchTV and Seaview software. The ITS sequence of *A. langbiangense* alignments with those of other *Arisaema* species from the NCBI database (Table 1) were performed using BioEdit and ClustalX2. Finally, PAUP* (* Phylogenetic Analysis Using PAUP) (Swofford 2002) was used to plot the phylogenetic tree.

**Table 1.** The ITS sequences of the *Arisaema* species from the NCBI database used in this study.

| Scientific names | Accession numbers | Scientific names | Accession numbers |
|---|---|---|---|
| *A. heterophyllum* | KT634029 | *A. amurense* | MZ675656 |
| *A. thunbergii* | KT634007 | *A. elephas* | MH807940 |
| *A. triphyllum* | KF977433 | *A. ringens* | KC846130 |
| *A. fimbriatum* | KR534427 | *A. angustatum* | AF291914 |
| *A. erubescens* | JF975897 | *A. tortuosum* | PQ676515 |
| *A. lobatum* | MH711210 | *A. bockii* | MH807939 |
| *A. wattii* | AF469031 | *A. sikokianum* | AB513178 |
| *A. propinquum* | MW828311 | *A. yamatense* | LC704923 |
| *A. peninsulae* | KT634039 | *Pinellia ternata* | PQ163938 |
| *A. undulatifolium* | AB611695 | | |

## RESULTS

### Taxonomic Treatment

***Arisaema langbiangense*** Luu, Nguyen-phi & H.T.Van (Figure 1)

Herb evergreen, 40–60 cm tall; rhizome cylindrical, subterranean, 9–12 cm long, 2–3 cm in diameter, green flesh, grey outside. Leaves 1–3; petiole and petiolule connecting into pseudostem at lower part, free part of the petiole, 20–25 cm long, green to brown. Leaf blade trifoliolate; central leaflet with petiolule ca. 2 cm long, ovate, base rounded, pale green under side, dark green above, apex acuminate; midrib prominent abaxially, impressed adaxially, lateral veins diverging from the midrib and collective vein at ca 3 mm from margin; lateral leaflets similar to central leaflet but asymmetrical with petiolule shorter than those of central leaflet, ca. 1 cm long. Inflorescence solitary; pedunce arises from pseudostem, upper part free; spathe tube green, cylindrical, 6–8 cm long, 1–1.2 cm in wide; spathe limb curved over mouth, ovate, 3.5–4.5 cm long, 2–3 cm wide, green, translucent with a caudate tip ca. 1 cm. Bisexual spadix 7–9 cm long; female part 1.2–1.5 cm long, ovaries green, obovoid, ca. 2 mm in diameter, densely arranged; stigma emerging from a thick style, ca. 1 mm long; male part 1.5–2 cm long, synandrium 2–3 stamens, loosely arranged; thecae cream with purple top, horseshoe-shaped, dehiscent by dark purple round pore at anthesis; *appendix* slightly exserted from tube mouth, 1.5–2 cm long, green, naked, cylindrical, apex round. *Male spadix* 7–9 cm long; *male zone* 3.5–4.5 cm long, synandrium and *appendix* similar to those of bisexual spadix.

***Type*:** VIETNAM. Lam Dong Province, Lac Duong District, Bidoup-Nui Ba National Park, Langbiang Mountain, H.T.Van 06 (holotype SGN!, isotypes SGN! & PHH!).

***Distribution*:** The studied species has been only found at the type location.

**Studied specimens:** VIETNAM. Lam Dong Province, Lac Duong District, Bidoup-Nui Ba National Park, Langbiang Mountain, Luu 01_A and Luu 01_B (PHH), 9 February 2025.

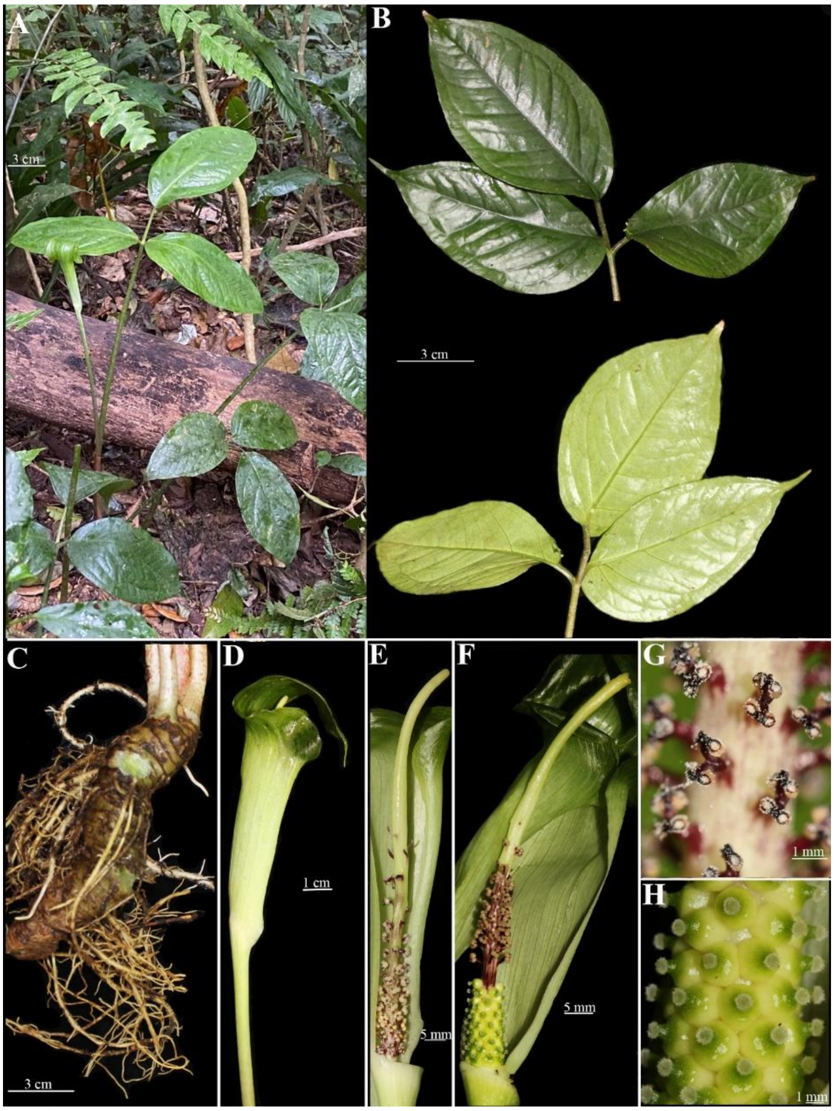

**Figure 1.** *Arisaema langbiangense* Luu, Nguyen-phi & H.T.Van. A: the species in habitat, B: leaflets, C: rhizome, D: spathe, E: male spadix, F: bisexual spadix, G: Synandria, H: ovaries. Photos: Huyen Trang Luu.

### Pollen structure

The pollen grains of *Arisaema langbiangense* (Figure 2) are monads, exhibiting radial symmetry and isopolarity. They are spheroidal in shape, as indicated by nearly equal polar and equatorial axes (24–29 µm), with a P/E ratio ranging from 0.9 to 1.0. The grains are inaperturate, lacking visible apertures. The exine is tectate, with a continuous tectum, and has a thickness of approximately 2–2.5 µm. The surface is ornamented with evenly distributed conical spines, forming a typical echinate pattern. Under light microscopy, the pollen grains appear spherical with a red-stained exine (using safranin), and a clearly distinguishable two-layered wall. Scanning electron microscopy (SEM) confirms the absence of apertures and reveals a smooth surface densely covered with conical spines, without signs of collapse or degradation.

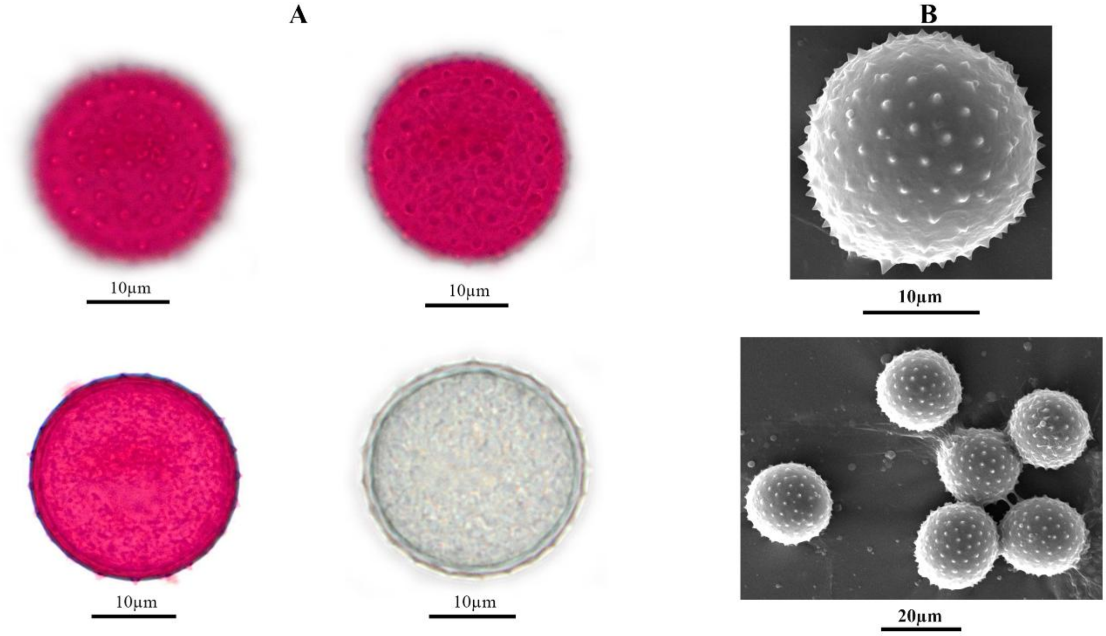

**Figure 2**: Pollen grains of *Arisaema langbiangense* observed under light microscopy (A) and scanning electron microscopy (B)

## Micro-morphological traits

### **Leaves** (Figure 3)

*Midrib* is flat or slightly concave on the upper surface, convex on the lower surface. Upper epidermis and lower epidermis consist of a single layer of polygonal cells. Angular collenchyma forms clusters below the lower epidermis, with polygonal cells, cellulose walls, and irregular sizes. Parenchyma with small polygonal or intercellular spaces, polygonal cells, cellulose walls, 2-3 layers of parenchyma cells under the upper epidermis contain abundant chloroplasts. Vascular bundles with xylem above phloem, arranged in 2-3 rows in the parenchyma zone, the bottom row with the smallest vascular bundles located just above each collenchyma cluster. Each xylem bundle has 2-5 protoxylem vessels and 1-2 metaxylem vessels, polygonal in shape.

*Lamina*: Upper epidermis and lower epidermis consist of a single layer of polygonal cells with cellulose walls, lower surface with numerous stomata. Mesophyll has homogeneous structure with only one type of parenchyma consisting of irregular polygonal cells forming small intercellular spaces. The parenchyma zone contains numerous cells with needle-shaped calcium oxalate crystals.

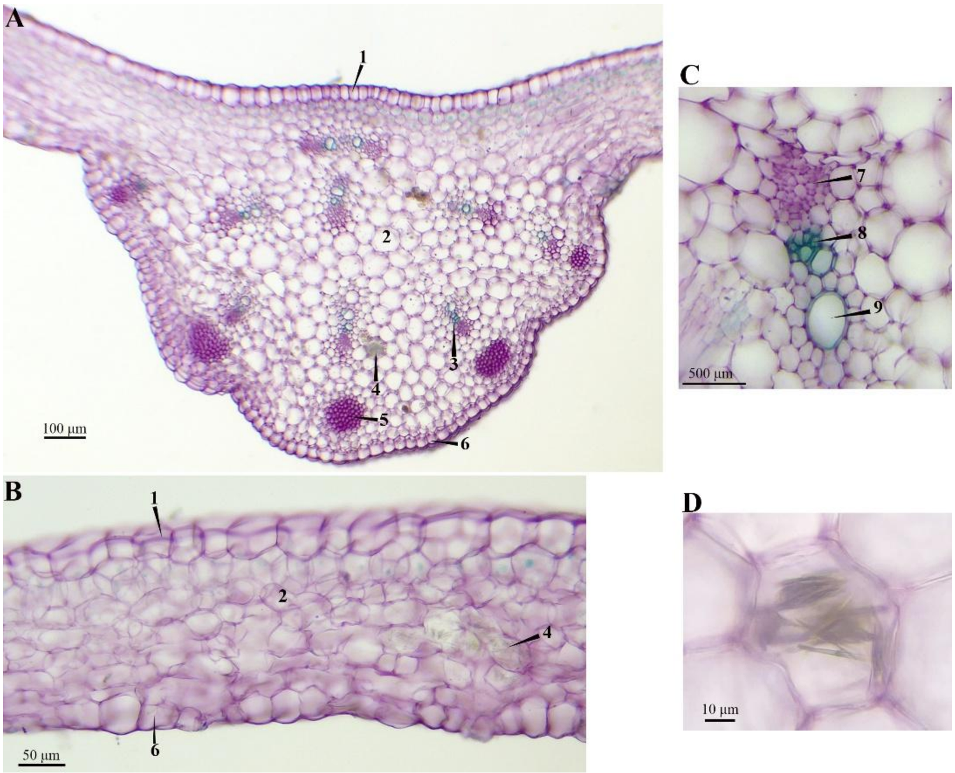

**Figure 3.** The cross-section of leaves. A: midrib, B: lamina, C: vascular bundle, D: needle-shaped calcium oxalate crystals. 1: upper epidermis, 2: parenchyma, 3: vascular bundle, 4: cell with needle-shaped calcium oxalate crystals, 5: angular collenchyma, 6: lower epidermis, 7: phloem, 8: protoxylem, 9: metaxylem.

**Petiole** (Figure 4)

The cross-section is circular or oval. Epidermis consists of a single layer of polygonal cells with cellulose walls. Angular collenchyma forms clusters below the epidermis. Parenchyma forms different shapes creating small polygonal or intercellular spaces. Vascular bundles with phloem above xylem, arranged irregularly in multiple rings, the outermost ring has the smallest vascular bundles located just below each collenchyma cluster, bundle size increases towards the center. Each xylem bundle has 2-5 protoxylem vessels and 1-2 metaxylem vessels, polygonal in shape, centrifugally differentiated.

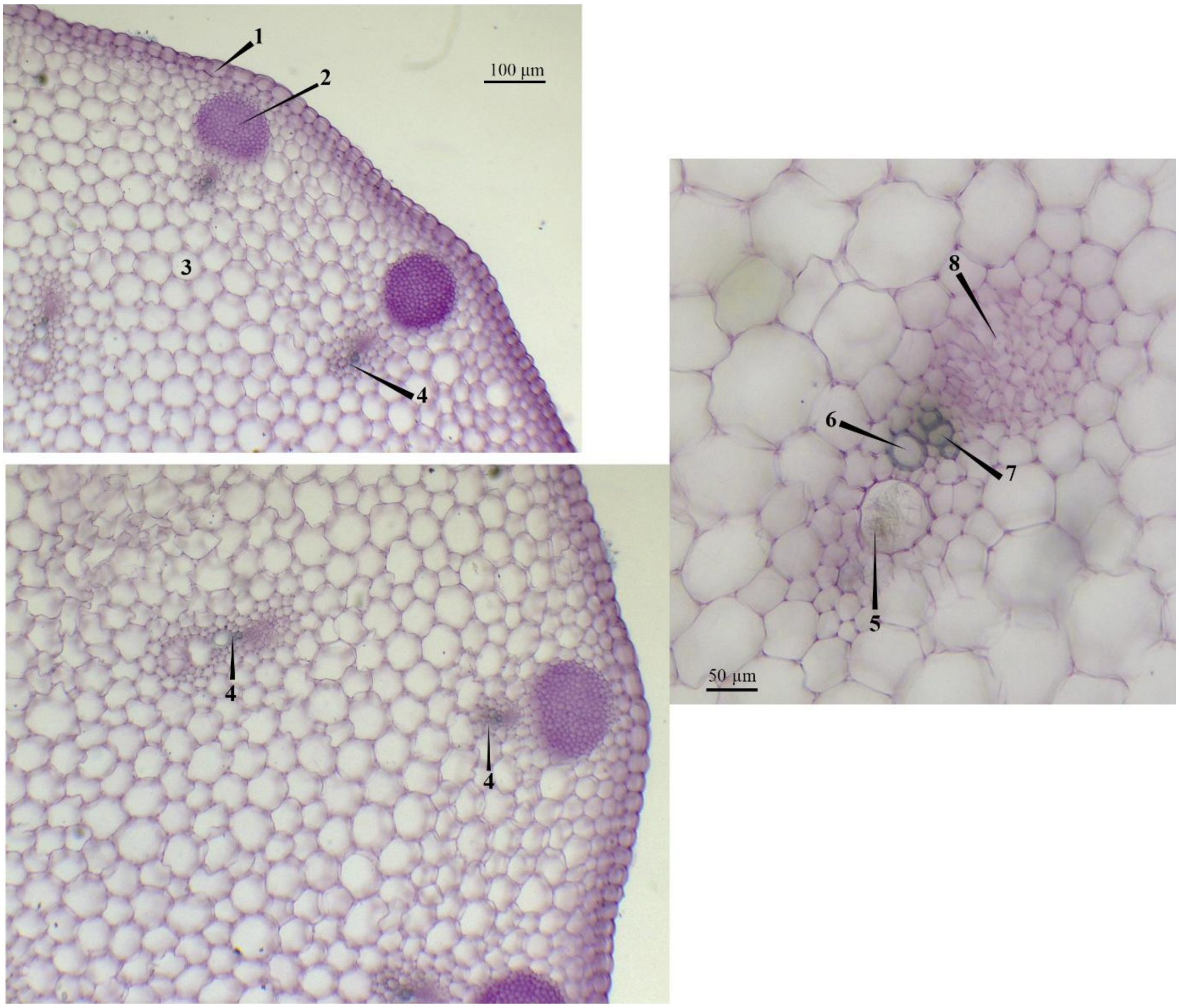

**Figure 4.** The cross-section of petiole. 1: epidermis, 2: angular collenchyma, 3: parenchyma, 4: vascular bundle, 5: cell with needle-shaped calcium oxalate crystals, 6: metaxylem, 7: protoxylem, 8: phloem.

**Petiolule** (Figure 5)

The cross-section shows shallow concave upper surface, structure similar to petiole. The parenchyma zone of petiolule, petiole, and leaf contains numerous cells with needle-shaped calcium oxalate crystals.

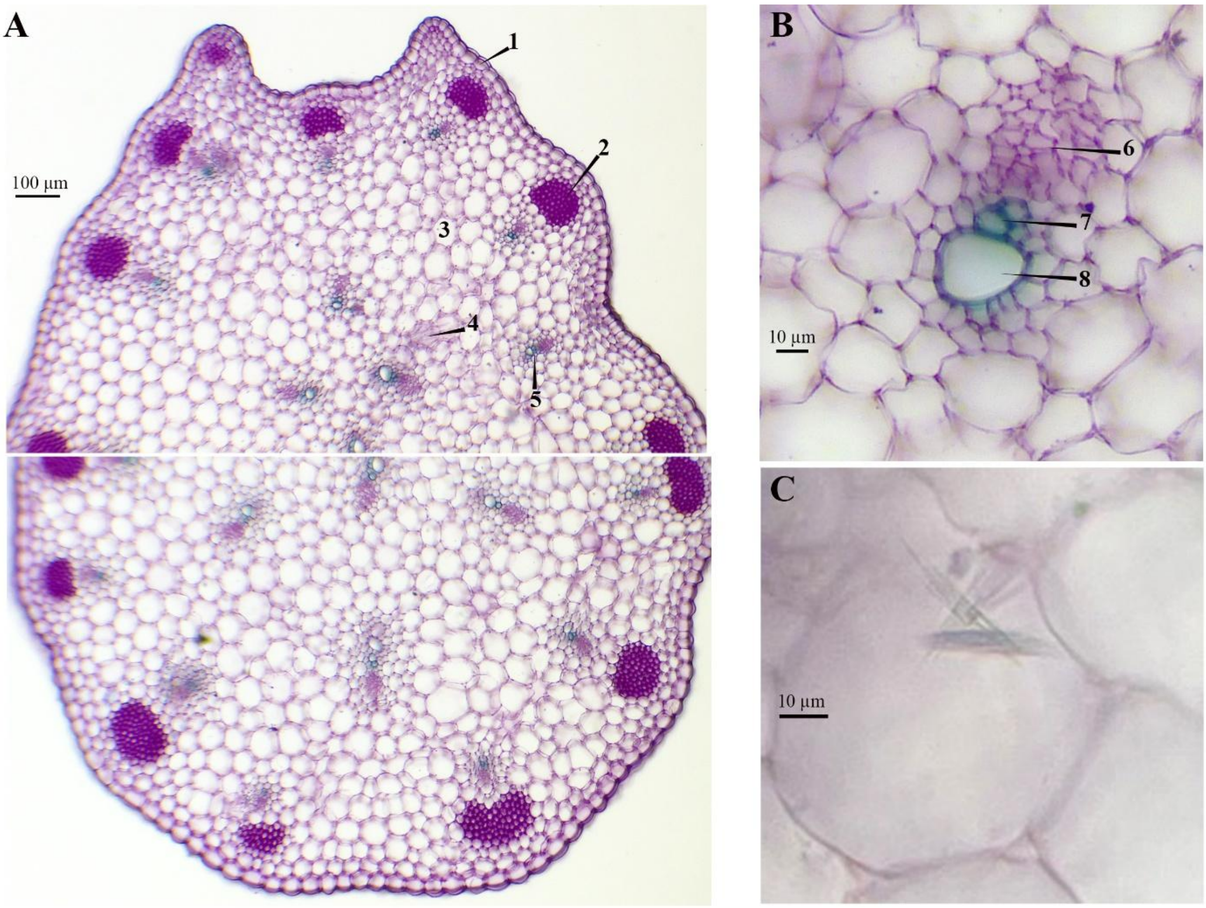

**Figure 5.** The cross-section of petiolule. A: whole section, B: vascular bundle, C: cell with needle-shaped calcium oxalate crystals. 1: epidermis, 2: angular collenchyma, 3: parenchyma, 4: cell with needle-shaped calcium oxalate crystals, 5: vascular bundle, 6: phloem, 7: protoxylem, 8: metaxylem.

**Root** (Figure 6)

The cross-section is nearly circular, divided into two zones, cortical area occupying 2/3 of the cross-section radius, stele occupying 1/3 of the cross-section radius. Cortical area: Piliferous layer consists of a single layer of polygonal cells, irregular size, thin cellulose walls or impregnated with phellem, with numerous root hairs. Exodermis consists of a single layer of polygonal cells, phellem-impregnated walls. Outer cortical parenchyma has 14-16 layers of polygonal cells arranged randomly leaving small intercellular spaces, innermost 2-3 cell layers arranged in concentric rings. Endodermis with casparian strip. Stele: Pericycle consists of a single layer of polygonal cells with cellulose walls, fairly uniform size, alternating with endodermis. The vascular system comprises 5-7 phloem bundles alternating with 5-7 protoxylem bundles in a ring, separated by medullary rays. Phloem bundles consist of polygonal cells arranged randomly in clusters. Protoxylem bundles consist of 2-5 xylem vessels, polygonal in shape, radially differentiated. 5-6 metaxylem vessels lie below primary phloem and protoxylem. Medullary ray consists of a single row of horizontally flattened polygonal cells with cellulose walls. Medullary parenchyma has 2-3 layers of polygonal cells with cellulose walls, closely arranged.

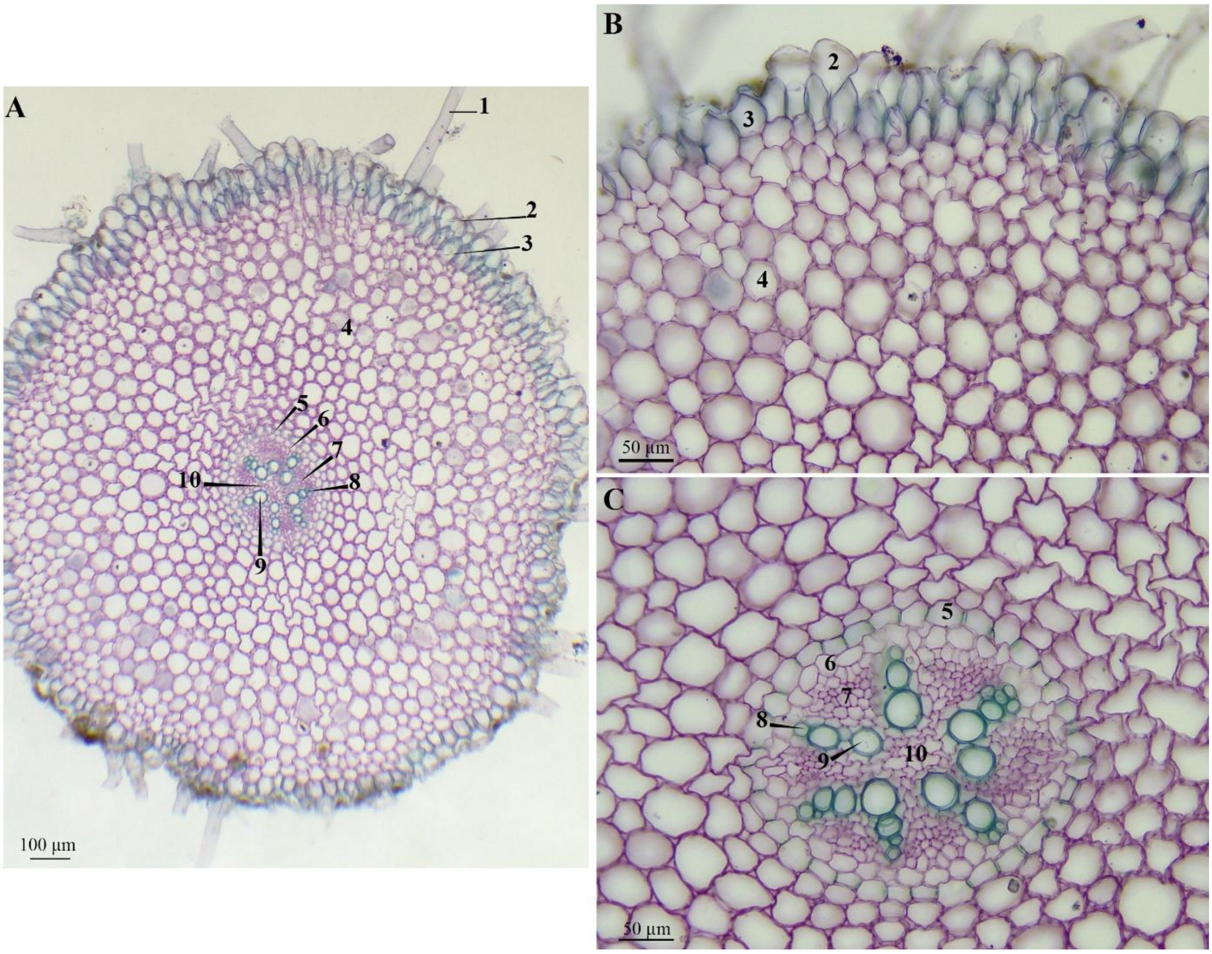

**Figure 6.** The cross-section of root. A: whole section, B: cortex, C: stele. 1: root hair, 2: piliferous layer, 3: exodermis, 4: cortical parenchyma, 5: endodermis with casparian *strip, 6:* pericycle, 7: phloem, 8: protoxylem, 9: metaxylem, 10: medullary parenchyma.

**Rhizome** (Figure 7)

The cross-section has no distinction between cortex and stele. Phellem has 8-10 layers of rectangular cells, phellem-impregnated walls, arranged radially, outermost layer usually torn. Parenchyma arranged closely or leaving small intercellular spaces, containing numerous cells with needle-shaped calcium oxalate crystals; vascular bundles are few and small, scattered in the parenchyma zone.

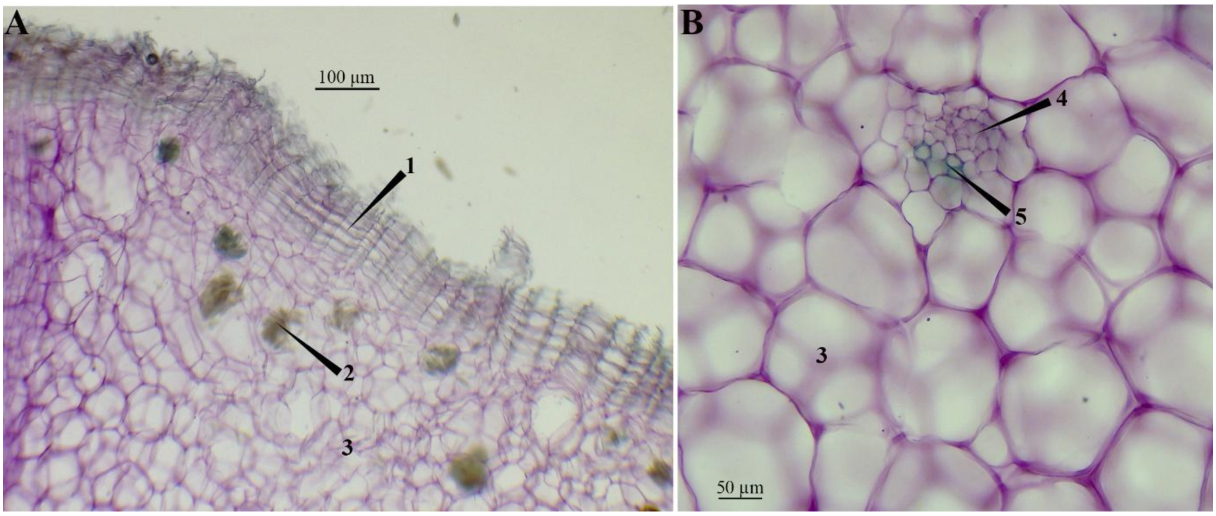

**Figure 7.** The cross-section of rhizome. A: cortex, B: stele. 1: cork, 2: cell with needle-shaped calcium oxalate crystals, 3: parenchyma, 4: phloem, 5: xylem.

### Phylogenetic analysis of *Arisaema langbiangense*

The final length of *ITS* region of *A. langbiangense* were 446 bps. This sequences was deposited in NCBI database with the accession numbers of PV600050. The ITS-based phylogenetic tree reconstructed in this study (Figure 8) reveals seven well-supported sections within the genus *Arisaema*: *Flagellarisaema*, *Arisaema*, *Pistillata*, *Sinarisaena, Nepenthoidea, Fimbriata,* and *Anomala*. Within this framework, *A. langbiangense* forms a distinct and isolated clade, not clustering with any other species included in the tree. This separation is not necessarily indicative of deep evolutionary divergence but is largely attributed to the current lack of ITS sequences for species of section *Anomala* in public databases such as GenBank. To our knowledge, *A. langbiangense* represents the first publicly available ITS sequence for section *Anomala*. Its inclusion therefore marks a critical advancement in filling a significant gap in nuclear phylogenetic data for the genus. A similar pattern of isolated placement is observed for *A. fimbriatum*, the sole representative of section *Fimbriata*, which also appears as a clade containing only one species.

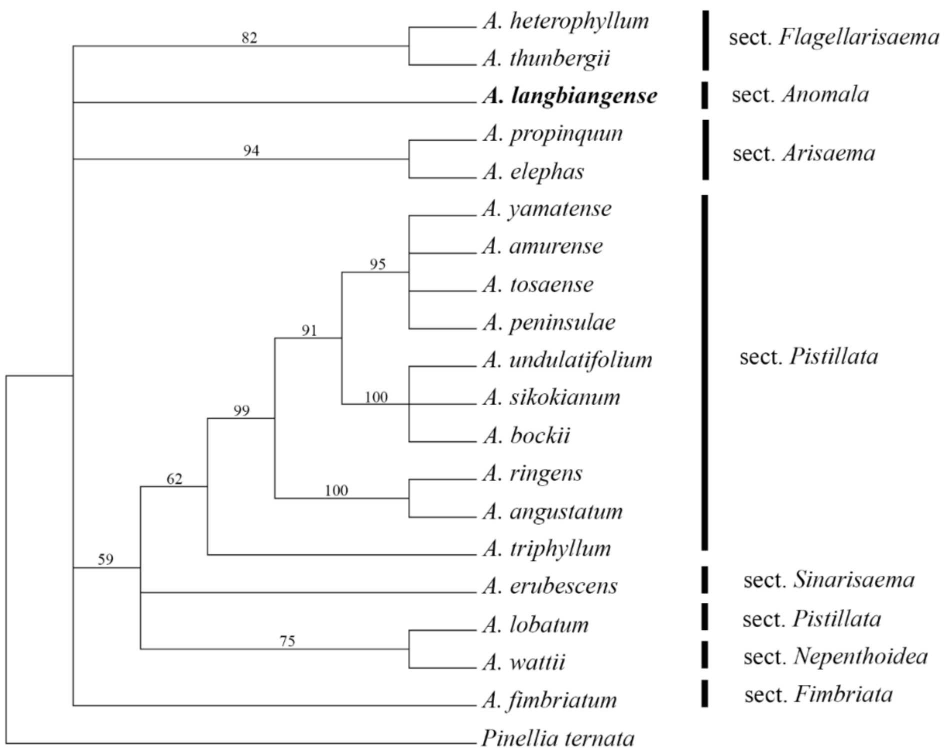

**Figure 8.** One of the most parsimonious trees inferred from ITS sequences illustrates the genetic relationships between *Arisaema langbiangense* (indicated in bold) and other *Arisaema* species obtained from the NCBI database. The bootstrap values of 50% or more are shown above the nodes.

## DISCUSSION

Calcium oxalate crystals are ubiquitous in the plant kingdom, particularly within the family Araceae, where they serve multiple physiological and ecological roles. In Araceae, these crystals occur in various forms, including raphides, druses, prisms, and crystal sand, and are typically found in specialized cells called idioblasts across organs such as leaves, stems, roots, and inflorescences (Franceschi & Horner 1980). In *Arisaema*, these crystals are primarily raphides, with druses and crystal sand also reported (Saadi & Mondal 2011). Their presence has been documented in species including *Arisaema erubescens*, *Arisaema amurense*, *Arisaema heterophyllum*, *Arisaema propinquum*, *Arisaema leschenaultii*, *Arisaema jacquemontii*, and *Arisaema pierreanum*, with high concentrations in tubers and inflorescences (Genuaf & Hillson 1985;Selvakumari 2015;Mir et al. 2020;Barabé et al. 2004;Qi et al. 2021;H. T. Le et al. 2025). These needle-like crystals contributes to toxicity by mucosal irritation, swelling, and hoarseness; and extracts from *A. erubescens* can be lethal to *Oncomelania hupensis* snails (Qi et al. 2021). *A. triphyllum* raphides similarly induce oral edema (Jadhav &

Gugloth 2019), while extracellular crystals on inflorescences, such as in *A. erubescens*, deter florivores (Coté & Gibernau 2012). These findings underscore the ecological and physiological significance of calcium oxalate crystals in Araceae generally and in *Arisaema* particularly.

Recently, Le *et al.* (2025) provided the details of the micro-morphilogical characteristics of *Arisaema pierreanum* collected from southern Vietnam. The anatomical comparison of *A. langbiangense* and *A. pierreanum* reveals both shared and distinct features. Accordingly, both species share fundamental similarities, particularly in the presence of calcium oxalate crystals in nearly all plant parts. However, several distinctive anatomical features differentiate these two species. The roots of both species exhibit a circular cross-section with distinct cortical and pith regions. In *A. langbiangense*, the outer cortical parenchyma consists of 14-16 layers of randomly arranged polygonal cells that create small intercellular spaces, while the innermost 2-3 layers are arranged in concentric rings. In contrast, *A. pierreanum* shows a clearly differentiated inner cortical region with 8-9 layers of rectangular cells arranged in radial rows (Le et al. 2025). This radial arrangement in *A. pierreanum* differs significantly from the more random arrangement seen in *A. langbiangense*. Another notable difference appears in the stele region, where *A. langbiangense* possesses 5-7 phloem bundles alternating with 5-7 protoxylem bundles arranged in a ring. *A. pierreanum*, however, contains 7-8 phloem bundles alternating with 7-8 protoxylem bundles (Le et al. 2025). Additionally, the pericycle in *A. langbiangense* consists of a single layer of relatively uniform polygonal cells with cellulose walls, whereas in *A. pierreanum*, the pericycle includes 1-2 layers of polygonal cells with irregular size.

The rhizomes of *A. langbiangense* and *A. pierreanum* show marked differences in their structural organization. *A. langbiangense* lacks a clear distinction between cortex and stele, while *A. pierreanum* exhibits a well-defined separation with the cortical area being smaller than the stele (Le et al. 2025). The phellem layer in *A. langbiangense* comprises 8-10 layers of rectangular cells with phellem-impregnated walls arranged radially, whereas *A. pierreanum* has only 3-4 layers of flattened rectangular cells. Furthermore, calcium oxalate crystal distribution differs between the two species. While *A. langbiangense* contains numerous cells with needle-shaped calcium oxalate crystals, *A. pierreanum* possesses both needle-shaped and distinctive spiny-shaped calcium oxalate crystals (Le et al. 2025), presenting a diagnostic characteristic for species identification. In the leaf midrib, *A. langbiangense* is flat or slightly concave on the upper surface and convex on the lower surface. *A. pierreanum* shows a similar pattern but is specifically described as "slightly concave on the upper side, convex on the lower side" (Le et al. 2025). Both species have a single layer of polygonal cells forming the upper and lower epidermis. The vascular arrangement in the midrib also differs between species. In *A. langbiangense*, vascular bundles are arranged in 2-3 rows within the parenchyma zone, with the bottom row containing the smallest vascular bundles positioned just above each collenchyma cluster. Conversely, *A. pierreanum* has vascular bundles haphazardly arranged throughout the medullary parenchyma, with bundle size gradually increasing toward the center (Le et al. 2025).

Expanded comparison with the micro-morphological traits of *Arisaema leschenaultii* (Selvakumari 2015) and *Arisaema propinquum* (Mir et al. 2020) reveals differences in anatomical structures among the four discussed species. Comparatively, *A. leschenaultii* features a lacunate root cortex and amphivasal corm vascular bundles, with only needle-shaped calcium oxalate crystals, while *A. propinquum* exhibits schizogenous cortical cavities in the rhizome and prismatic crystals, distinguishing it from the needle-shaped crystals of *A. langbiangense* and *A. leschenaultii*. These anatomical differences, particularly in cortical organization, vascular arrangement, and crystal morphology, provide robust diagnostic characters for species identification within *Arisaema*.

The inaperturate pollen with echinate surface ornamentation has also been reported (based on SEM images) in several species of the Araceae family (Hesse et al. 2009;Halbritter et al. 2018). These morphological features are strongly associated with entomophilous (insect-mediated) pollination. The echinate exine may enhance adhesion of pollen grains to insect vectors, particularly beetles and flies, which are commonly involved in pollination within the Araceae (Grayum 1986;Grayum 1990;Hesse 2000). The continuous tectum and relatively thick exine may contribute to the structural integrity and protect the pollen protoplast during transport on insect bodies or within enclosed floral chambers. Additionally, inaperturate pollen may represent an adaptive trait to minimize water loss and mechanical damage in humid, enclosed environments, which are typical of many Araceae inflorescences. These features facilitate efficient pollen transfer via insect vectors, as observed in Taiwanese *Arisaema* species like *A. grapsospadix* and *A. matsudai*, which also exhibit pollen morphology suited for insect pollination (Grayum 1990;Wang 1996).

The pollen of *A. langbiangense* shares significant similarities with other *Arisaema* species, particularly those from Taiwan (Wang 1996). In Taiwan species, *A. thunbergii* ssp. *autumnale* has the largest pollen grains (21 μm) while *A. consanguineum* has the smallest grains (12 μm). The pollen of *A. langbiangense* (24–29 μm) is larger than all Taiwanese species documented in Wang’s study. All *Arisaema* species’ pollens in Wang's study have an inaperturate structure and spines on the exine surface, closely matching the pollen structure of *A. langbiangense* (Wang 1996). Within the Araceae family, genera like *Pinellia* and *Alocasia* also exhibit echinate pollen, whereas others have psilate or verrucate exines, reflecting diverse pollination strategies (Hesse et al. 2009;Halbritter et al. 2018). The distinct palynological profile of *A. langbiangense*, particularly its large size and tectate-echinate exine structure, contributes to our understanding of morphological diversity within *Arisaema* and may offer insights into evolutionary relationships and taxonomic classification within this genus.

Over the past two decades, chloroplast markers have been increasingly used in studies of the genus *Arisaema*. For instance, Zhang & Murata (2004) used *trn*L-*trn*F region to conducted the phylogenetic tree of 77 *Arisaema* species (Renner & Zhang 2004). Similarly, Renner *et al.* (2004) peformed the phylogenetic analyses of 81 species of *Arisaema*, 9 of *Typhonium*, 5 of *Pinellia* based on two chloroplast markers such as *trnL–trnF* and *rpl20–50rps12* (Renner et al. 2004). Notably, Ohi-Toma (2016) used four chloroplast markers, including *trnL–trnF, rpl20–50rps12, psbB–psbH* and *rpoC2–rps2* to conduct the phylogenetic tree of 15 sections of the genus *Arisaema* (Ohi-Toma et al. 2016). Meanwhile, the phylogenetic analyses using the nuclear region (ITS sequence) have so far been limited to only 6 out of the 15 sections, namely *Flagellarisaema*, *Arisaema*, *Pistillata*, *Sinarisaena, Nepenthoidea*, and *Fimbriata* (Begley-Miller et al. 2014;Noh et al. 2018;Tan et al. 2018).

In the present study, we report the ITS sequence of *A. langbiangense*, representing section *Anomala* for the first time. Although our ITS tree does not include all species of section *Anomala* due to the absence of nuclear sequence data, the resulting tree shows clear sectional-level structure and largely conforms with the plastid-based phylogeny of Ohi-Toma et al. (2016), in which section *Anomala* corresponds to Clade VI, a well-supported monophyletic group that includes species such as *A. balansae*, *A. garrettii*, *A. lihengianum*, and *A. petelotii*. Importantly, this study provides the first nuclear sequence data for section *Anomala*, thereby contributing a crucial new resource for future phylogenetic analyses and the expansion of the *Arisaema* molecular database. With continued sampling and sequencing of ITS regions across additional species, these data will enable more comprehensive comparisons between nuclear and plastid phylogenies and improve resolution of relationships at both sectional and species levels.

## CONCLUSION

The present study provides the first integrative assessment of *Arisaema langbiangense* combining micromorphological, anatomical, palynological, and molecular (ITS region) data. Notably, it reports the first ITS sequence for section *Anomala*, establishing a crucial nuclear barcode for future phylogenetic and taxonomic studies. Distinctive anatomical traits, including vascular bundle arrangement and the presence of raphide-containing idioblasts, together with specific pollen ornamentation patterns, offer valuable diagnostic features that support species delimitation within the genus. Phylogenetic analysis of the ITS region further confirms the placement of *A. langbiangense* within section *Anomala* and highlights its genetic distinctiveness from closely related taxa. By integrating morphological and molecular evidence, this study not only clarifies the systematic position of this endemic species but also provides essential data to guide its conservation and management.

## ACKNOWLEDGEMENTS

The authors would like to thank the Ho Chi Minh City Science and Technology Development Fund for supporting this work under project code 47/2024/HĐ-QKHCN 23/9/2024.

## AUTHORS’ CONTRIBUTIONS

Huyen Trang Luu and Hong Thien Van contributed to study design, conducted the experiments, performed the statistical analyses, and prepared the manuscript.
Tran Quoc Trung Nguyen and Hong Truong Luu carried out the experiments examining pollen structure.
Hong Thien Van, Nga Nguyen-Phi and Hong Truong Luu identified the scientific name of the studied species.
Hong Quan Buu and Dinh Thach Bui collected the samples during filed trips.
Thu Trang Le-thi conducted the anatomical analysis experiments.
Hong Thien Van handled all editorial and reviewer queries.

All authors reviewed and approved the final manuscript.

## REFERENCES

Aboul-Maaty, N. A.-F., & Oraby, H. A.-S. (2019). Extraction of high-quality genomic DNA from different plant orders applying a modified CTAB-based method. *Bulletin of the National Research Centre*, *43*(1), 25. https://doi.org/10.1186/s42269-019-0066-1

Barabé, D., Lacroix, C., Chouteau, M., & Gibernau, M. (2004). On the presence of extracellular calcium oxalate crystals on the inflorescences of Araceae. *Botanical Journal of the Linnean Society*, *146*(2), 181–190. https://doi.org/10.1111/j.1095-8339.2004.00318.x

Begley-Miller, D. R., Hipp, A. L., Brown, B. H., Hahn, M., & Rooney, T. P. (2014). White-tailed deer are a biotic filter during community assembly, reducing species and phylogenetic diversity. *AoB PLANTS*, *6*. https://doi.org/10.1093/aobpla/plu030

Bridson, D., & Forman, L. (1999). *The Herbarium Handbook-Third Edition. Royal Botanic Gardens*. Royal Botanic Gardens, Kew, UK.

Bruggeman, P., Vuong, T. B., Rybková, R., & Ponert, J. (2013). *Arisaema claviforme* sp. nov. and a new record of *Arisaema* (Araceae) from Vietnam. *Nordic Journal of Botany*, *31*(5), 556–560. https://doi.org/10.1111/j.1756-1051.2013.00111.x

Coté, G. G., & Gibernau, M. (2012). Distribution of calcium oxalate crystals in floral organs of Araceae in relation to pollination strategy. *American Journal of Botany*, *99*(7), 1231–1242. https://doi.org/10.3732/ajb.1100499

Franceschi, V. R., & Horner, H. T. (1980). Calcium oxalate crystals in plants. *The Botanical Review*, *46*(4), 361–427. https://doi.org/10.1007/BF02860532

Erdtman, G. (1943). Pollen preparations. In Frans Verdoorn (Ed.), *An Introduction to Pollen Analysis: Vol. XII* (pp. 36–44). Chronica Botanica Company.

Genuaf, J. M., & Hillson, C. J. (1985). The Occurrence, Type and Location of Calcium Oxalate Crystals in the Leaves of Fourteen Species of Araceae*. In *Annals of Botany* (Vol. 56).

Grayum, M. (1986). Correlations between pollination biology and pollen morphology in the Araceae, with some implications for angiosperm evolution. *Monographs in Systematic Botany from the Missouri Botanical Garden*, *14*, 313–327.

Grayum, M. (1990). Evolution and phylogeny of the Araceae. *Annals of the Missouri Botanical Garden* , *77*, 628–697.

Gusman, G., & Gusman, L. (2006). *The genus Arisaema. A monograph for botanists and nature lovers, 2nd ed. ARG*. Gärtner Verlag Kommanditgesellschaft, Ruggell.

Halbritter, H., Ulrich, S., Grímsson, F., Weber, M., Hesse, M., Zetter, R., Buchner, R., Svojtka, M., & Frosch-Radivo, A. (2018). *Illustrated Pollen Terminology*. Germany: Springer International Publishing.

Hesse, M. (2000). Pollen wall stratification and pollination. In *Pollen and Pollination* (pp. 1–17). Springer Vienna. https://doi.org/10.1007/978-3-7091-6306-1_1

Hesse, M., Halbritter, H., Zetter, R., Weber, M., Buchner, R., Frosch-Radivo, A., & Ulrich, S. (2009). *Pollen Terminology: An Illustrated Handbook*. Springer.

Hoang, T., Trinh, N., Nguyen, Q., Pham, V., & Pham, Q. (2015). Arisaema lihengianum (Araceae): a new record from Vietnam. *Science Research Reporter*, *5*, 97–99.

Jadhav, D., & Gugloth, R. (2019). Poisoning due to Arisaema triphyllum ingestion. *Indian Journal of Critical Care Medicine*, *23*(5), 242–243.

Le, C., Ha, M., Nguyen, M., Trinh, X., Tran, V., & Nguyen, V. (2020). Arisaema menglaense (Araceae:Arisaemateae) newly recorded for northern Vietnam. *Aroideana*, *43*(3 & 4), 12–21.

Le, H. T., Van, H. T., Nguyen, Q. H., Le-Thi, T. T., & Nguyen-Phi, N. (2025). Morphological and anatomical characteristics and chemical components of Arisaema pierreanum. *Research Journal of Biotechnology*, *20*(5), 18–24. https://doi.org/10.25303/205rjbt18024

Luu, H. T., Nguyen, H. C., Nguyen, T. Q. T., & Nguyen, Q. B. (2022). \(\textit{Arisaema vietnamense}\) (section \(\textit{Nepenthoidea}\), Araceae): a new species from Vietnam. *Academia Journal of Biology*, *44*(1), 1–9. https://doi.org/10.15625/2615-9023/16646

Luu, H. T., Nguyen, Q. D., Nguyen, H. C., Van, H. T., & Nguyen, V. Du. (2020). Arisaema liemiana (Araceae: Arisaemateae), a new species from southern Central of Vietnam. *Phytotaxa*, *468*(2), 214–220. https://doi.org/10.11646/phytotaxa.468.2.5

Luu, H. T., Nguyen, Q. D., Vu, N. L., Vuong, D. H., Kieu, D. T., & Vo, H. S. (2014). *Arisaema chauvanminhii* (Araceae), a New Species from Vietnam. *Annales Botanici Fennici*, *51*(6), 394–398. https://doi.org/10.5735/085.051.0605

Luu, H., Tran, G., Nguyen, T., & Nguyen, V. (2013). Arisaema honbaense (Araceae) - a new species from Vietnam. *Folia Malaysiana*, *14*, 45–50.

Ma, Z., Hoang, T., & Xin-Xin, Z. (2024). Materia Revisionis Arisaemarum: II. Arisaema tridens (Araceae), a new rhizomatous species from Central Vietnam. *Aroideana* , *47*(3), 8–14.

Mayo, S., Bogner, J., & Boyce, P. (1997). *The genera of Araceae*. Royal Botanic Gardens, Kew, England.

Mir, P., Mohi-ud-din, R., Dar, M., & Bader, G. (2020). Pharmacognostical standardization, phytochemical investigation, and anthelminthic activity of Arisaema propinquum Schott rhizomes. *Pharmacognosy Research*, *12*(2), 181. https://doi.org/10.4103/pr.pr_106_19

Nguyen, V. (2000). Two new species of Arisaema from Vietnam. *Aroideana*, *23*, 36–40.

Nguyen, V. (2007). Two new species of the genus Arisaema Mart. (Araceae) described for Flora of Vietnam. *VNU Journal of Science, Natural Sciences and Technology* , *23*, 86–90.

Nguyen, V. (2017). *Flora of Vietnam-Araceae Juss.* Publishing House for Science and Technology, Ha Noi.

Nguyen, V., & Boyce, P. (2005). Two new species of genus Arisaema (Araceae) from Northern Vietnam. *Folia Malaysiana*, *6*((1 & 2)), 35–40.

Noh, P., Han, K., Kim, W., Yang, S., Choi, G., Ko, S., & Moon, B. (2018). Study on Molecular Phylogenetics of Korean Arisaema Species Based on Universal DNA Barcodes. *Korean J. Plant Res*, *31*(1), 37–51.

Ohi-Toma, T., Wu, S., Murata, H., & Murata, J. (2016). An updated genus-wide phylogenetic analysis of *Arisaema* (Araceae) with reference to sections. *Botanical Journal of the Linnean Society*, *182*(1), 100–114. https://doi.org/10.1111/boj.12459

Pham, H. (2000). *Araceae, in: Pham-hoang, H. (Ed.) Cây cỏ Việt Nam: An Illustrated Flora of Vietnam, vol. 3.* . Youth Publishing House, Ho Chi Minh City.

Pham, H. H. (2000). *Cay co Viet Nam, an Illustrated Flora of Vietnam*. Youth Publication.

Qi, C., Wang, J., Wu, X., He, S., Zhang, Q., Wu, J., & Zhao, C. (2021). Botanical, Traditional Use, Phytochemical, and Toxicological of Arisaematis rhizoma. *Evidence-Based Complementary and Alternative Medicine*, *2021*, 1–25. https://doi.org/10.1155/2021/9055574

Renner, S. S., Zhang, L., & Murata, J. (2004). A chloroplast phylogeny of *Arisaema* (Araceae) illustrates Tertiary floristic links between Asia, North America, and East Africa. *American Journal of Botany*, *91*(6), 881–888. https://doi.org/10.3732/ajb.91.6.881

Renner, S. S., & Zhang, L.-B. (2004). Biogeography of the Pistia Clade (Araceae): Based on Chloroplast and Mitochondrial DNA Sequences and Bayesian Divergence Time Inference. *Systematic Biology*, *53*(3), 422–432. https://doi.org/10.1080/10635150490445904

Saadi, S., & Mondal, A. (2011). Studies on the calcium oxalate crystals of some selected aroids (Araceae) in Eastern India. *Journal of Plant Sciences*, *6*(1), 135–142.

Selvakumari, P. (2015). Pharmacognostical standardization of Arisaema leschenaultii Blume. Indian Journal of natural resouces. *Indian Journal of Natural Resouces*, *6*(2), 143–152.

Swofford, D. (2002). *PAUP*: phylogenetic analysis using parsimony (and other methods), version 4.0 beta 10*. Sunderland: Sinauer.

Tan, S., Luo, Y., Hollingsworth, P. M., Burgess, K. S., Xu, K., Li, D., & Gao, L. (2018). DNA barcoding herbaceous and woody plant species at a subalpine forest dynamics plot in Southwest China. *Ecology and Evolution*, *8*(14), 7195–7205. https://doi.org/10.1002/ece3.4254

Thiers B (2025). Index Herbariorum: A global directory of public herbaria and associated staff. http://sweetgum.nybg.org/ih/

Van, H., Phan-thi, D., Nguyen, N., Lam. BBP, Luu, H., Tran, T., & Nguyen-Phi, N. (2024). Morpho-anatomical traits, DNA barcode and phytochemical screening of *Aglaonema cochinchinense*. *Historia Naturalis Bulgarica,* 46 (12), 325–337.

Van, H. T., Nguyen-Phi, N., & Luu, H. T. (2016). A new species of Arisaema (Araceae) from Vietnam. *Phytotaxa*, *277*(1), 90. https://doi.org/10.11646/phytotaxa.277.1.9

Wang, J. (1996). The systematic study of Taiwanese Arisaema (Araceae). *Botanical Bulletin of Academia Sinica*, *37*, 61–87.

White, T.J., Bruns, T., Lee, S. & Taylor, J. (1990). *Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics*. p.315-322. In: M.A. Innis (ed.), PCR Protocol: A Guide to Methods and Amplifications. Academic Press, San Diego, CA, USA.