



## **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

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### **HIGHLIGHTS**

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- 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 the accession number of PV600050.



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

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

<sup>1</sup>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

<sup>2</sup>Graduate University of Science and Technology, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Nghia Do Ward, Ha Noi, Vietnam

<sup>3</sup>Institute of Life Sciences, Vietnam Academy of Science and Technology, 9/621 Vo Nguyen Giap Street, Linh Xuan Ward, Ho Chi Minh City, Vietnam

<sup>4</sup>Institute of Advanced Technology, Vietnam Academy of Science and Technology, No. 1D, TL29 Street, An Phu Dong Ward, Ho Chi Minh City, Vietnam

<sup>5</sup>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

<sup>6</sup>Vietnam National University Ho Chi Minh City, Linh Xuan Ward, Ho Chi Minh City, Vietnam

<sup>7</sup>Faculty of Pharmacy, Nguyen Tat Thanh University, No. 300A Nguyen Tat Thanh Street, Xom Chieu Ward, Ho Chi Minh City, 700000 Vietnam

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**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

\*Corresponding author: [vanhongthien@iuh.edu.v](mailto:vanhongthien@iuh.edu.v)

## 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 centres of diversification. In Vietnam, the genus is taxonomically well represented, with 29 species currently recognised and distributed primarily in montane tropical forests from northern regions to the central highlands as well as southern regions (Pham 2000a; 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 recognised 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 characterised by trifoliolate or pedate leaves, an evergreen habit with simultaneous presence of old and emerging foliage, and a spadix appendix that is typically exerted, often bearing neuter flowers and apical bristles. Inflorescences and leaves arise separately from the rhizome and may form a pseudostem; the receptacle is cream-coloured 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 and Z. F. Xu, *A. omkoiense* Gusman, *A. petelotii* K. Krause, *A. petiolulatum* Hook. f., *A. pingbianense* H. Li, *A. rostratum* V. D. Nguyen and P. C. Boyce, *A. tridens* Z. X. Ma, T. S. Hoang and 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 2000b; 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 and 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 the sect. *Anomala* 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 an elevation of approximately 1,700 m, where it inhabits the humid understory of tropical evergreen forests. Despite its recent description, no detailed anatomical or palynological studies, nor a DNA barcode, have been published for this taxon. 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.

## 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, 1,800 m in elevation, about 12°02'52.8"N 108°26'22.3"E. The voucher specimens, Luu\_01A and Luu\_01B, were deposited at the Herbarium of the University of Science, Vietnam National University, Ho Chi Minh City (PHH) (Thiers 2025).

## Identification of the 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 (2000b), Nguyen (2000), Nguyen and Boyce (2005), Nguyen (2007), Bruggeman et al. (2013), Hoang et al. (2015), Van et al. (2016), Nguyen (2017), Le et al. (2020), and 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  $(CH_3CO)_2O$  in a test tube, heating in a water bath for 10 min, followed by centrifugation at 2,000 round per min (rpm for 5 min 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 min, centrifuged, and washed with 30% alcohol 2 to 3 times. The alkalisation process involved adding 2 mL of 10% potassium hydroxide (KOH) solution, heating for 5 min, followed by sequential washing with distilled water, hydrochloric acid (ClH N/60), and distilled water until reaching a pH value of 7.0. For optical microscopy (Olympus BX53), pollen samples were stained with Safranin for 2 min, 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 internal transcribed spacer (ITS) 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  $\mu$ L PCR reaction mixture contained 12.5  $\mu$ L master mix (Phu Sa Company, Vietnam), 1.25  $\mu$ L of each primer (10  $\mu$ M, forward and reverse), 9.5  $\mu$ L of deionised water, and 0.5  $\mu$ L of DNA template. The thermal cycling conditions included an initial denaturation at 95°C for 5 min, followed by 35 cycles of 94°C for 1 min, 55°C for 1 min and 30 sec (primer annealing)

and 72°C for 1 min and 30 sec (extension). The PCR products were purified and sequenced using an ABI 3500 Genetic Analyser (Applied Biosystems™ 3500 XL Genetic Analyser) at the Nam Khoa BioTek (Vietnam). The obtained ITS sequence was processed and analysed 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 were used in this study.

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

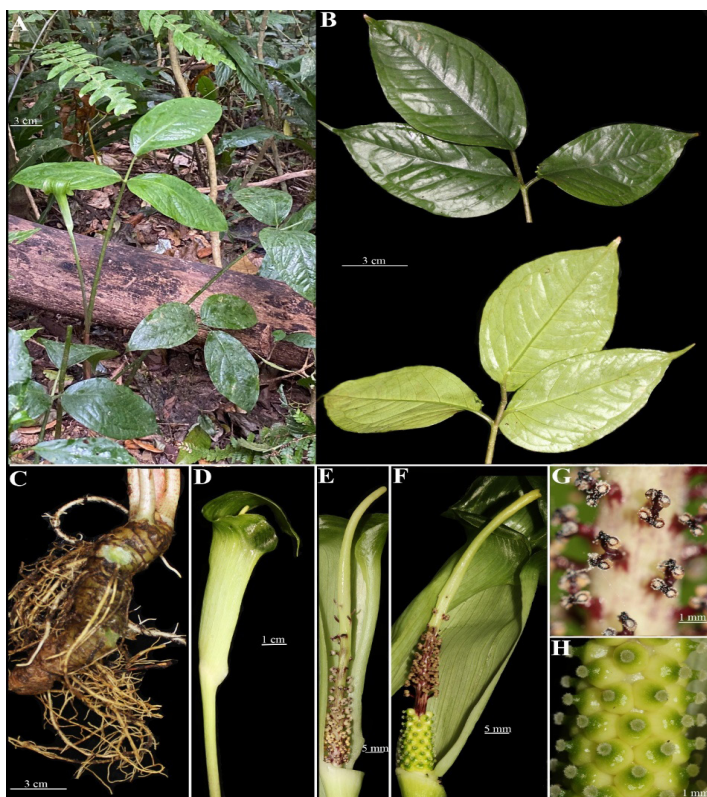
## RESULTS

### Taxonomic Treatment

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

Herb evergreen, 40 cm–60 cm tall; rhizome cylindrical, subterranean, 9 cm–12 cm long, 2 cm–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 cm–25 cm long, green to brown. Leaf blade trifoliate; 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; peduncle arises from pseudostem, upper part free; spathe tube green, cylindrical, 6 cm–8 cm long, 1 cm–1.2 cm in width; spathe limb curved over mouth, ovate, 3.5 cm–4.5 cm long, 2 cm–3 cm wide, green, translucent with a caudate tip ca. 1 cm. Bisexual spadix 7 cm–9 cm long; female part 1.2 cm–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 cm–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 cm–2 cm long, green, naked, cylindrical, apex round. *Male spadix* 7 cm–9 cm long; male zone 3.5 cm–4.5 cm long, synandrium and *appendix* similar to those of bisexual spadix.



**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 by Huyen Trang Luu).

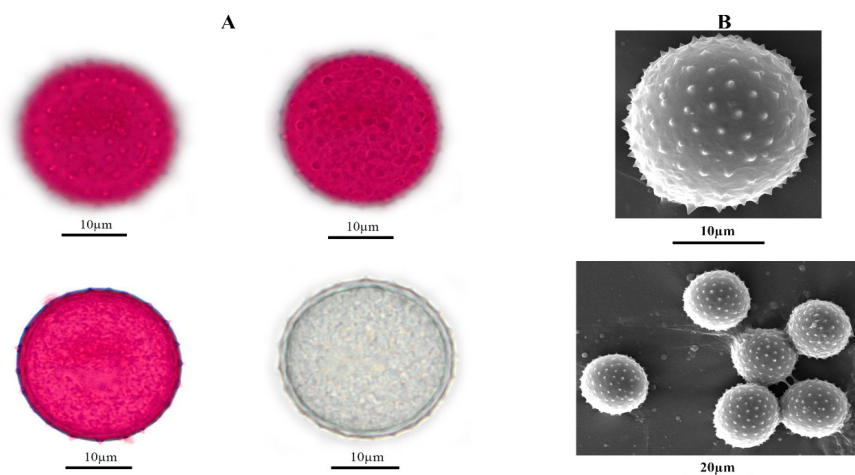
**Type:** VIETNAM. Lam Dong Province, Lac Duong District, Bidoup-Nui Ba National Park, Langbiang Mountain, H. T. Van 06 (holotype SGN!, isotypes SGN! & PHH!).

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

## Pollen Structure

The pollen grains of *Arisaema langbiangense* (Fig. 2) are monads, exhibiting radial symmetry and isopolarity. They are spheroidal in shape, as indicated by nearly equal polar and equatorial axes (24  $\mu\text{m}$ –29  $\mu\text{m}$ ), 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  $\mu\text{m}$ –2.5  $\mu\text{m}$ . 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.

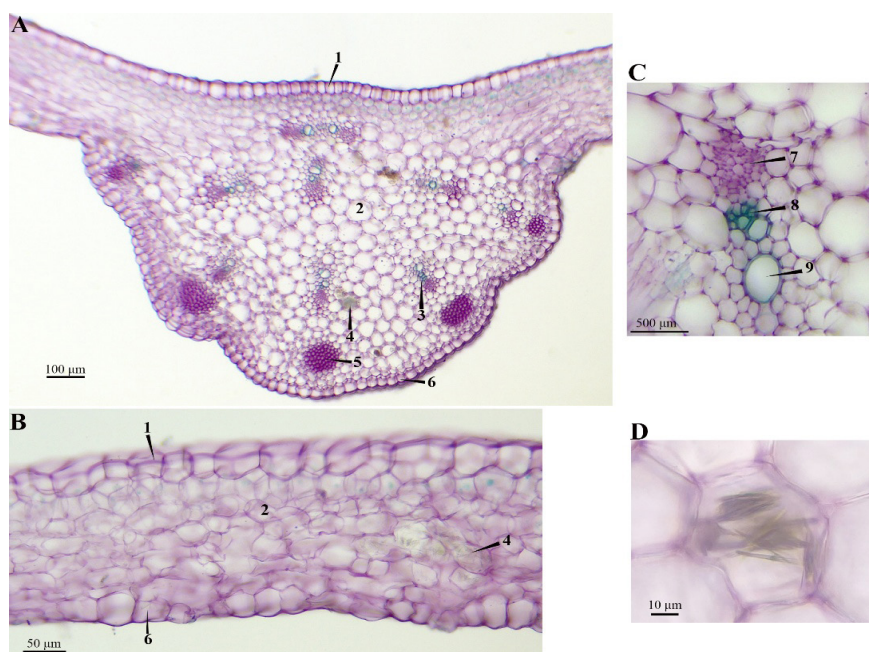


**FIGURE 2:** Pollen grains of *Arisaema langbiangense* (A) observed under light microscopy; and (B) scanning electron microscopy.

## Micro-Morphological Traits

### Leaves (Fig. 3)

The *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.



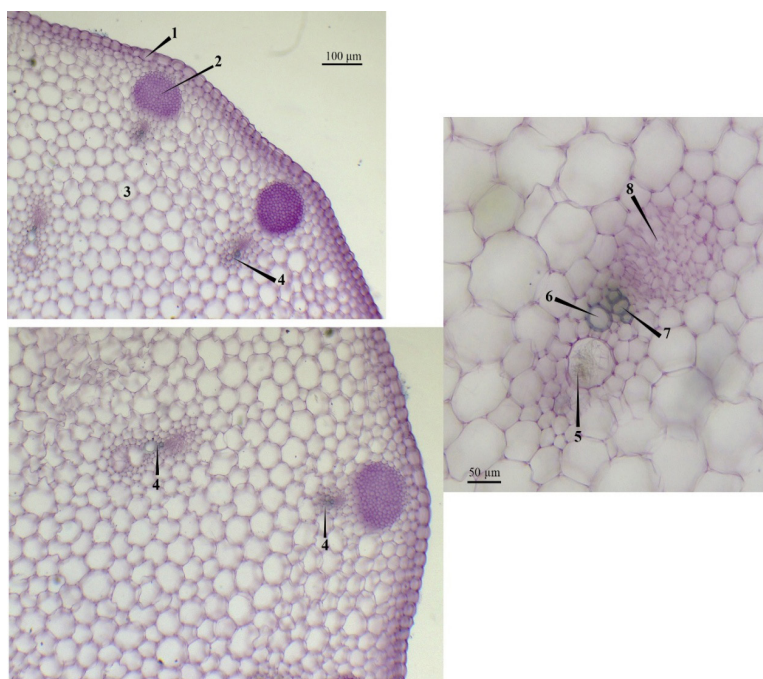
**FIGURE 3:** The cross-section of leaves. (A) Midrib; (B) Lamina; (C) Vascular bundle; (D) Needle-shaped calcium oxalate crystals.

Notes: 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.

**Lamina:** Upper epidermis and lower epidermis consist of a single layer of polygonal cells with cellulose walls, lower surface with numerous stomata. Mesophyll has a 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.

### **Petiole (Fig. 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 centre. Each xylem bundle has 2–5 protoxylem vessels and 1–2 metaxylem vessels, polygonal in shape, centrifugally differentiated.

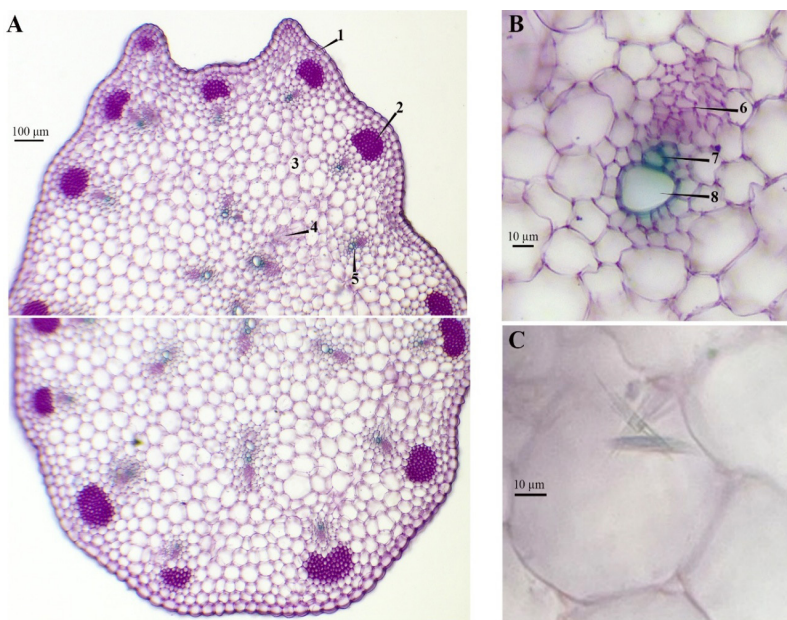


**FIGURE 4:** The cross-section of the petiole.

**Notes:** 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 (Fig. 5)

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



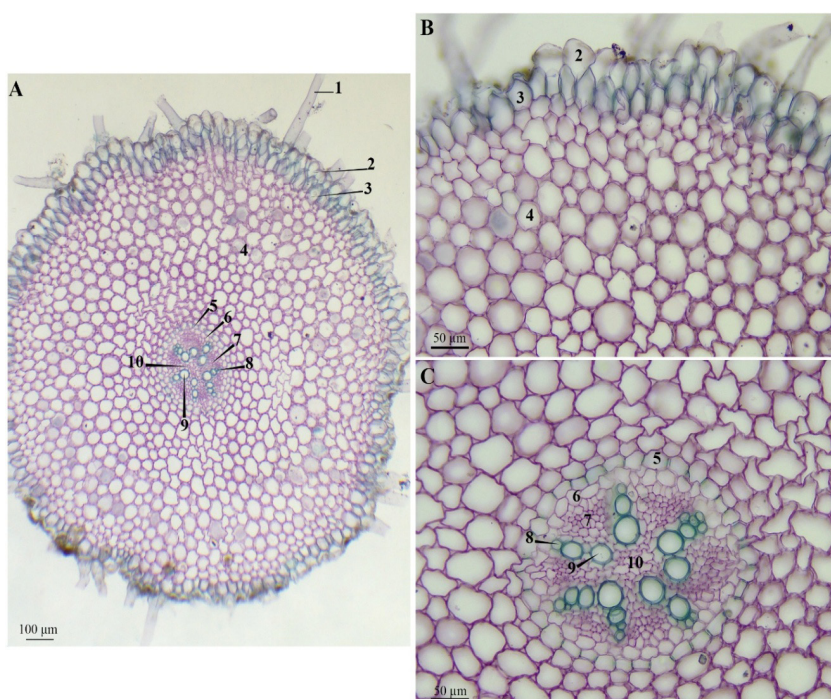
**FIGURE 5:** The cross-section of the petiolule. (A) Whole section; (B) Vascular bundle; (C) Cell with needle-shaped calcium oxalate crystals.

Notes: 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 (Fig. 6)

The cross-section is nearly circular, divided into two zones: the cortical area occupies 2/3 of the cross-section radius and the stele occupies 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 with phellem-impregnated walls. Outer cortical parenchyma has 14–16 layers of polygonal cells arranged randomly, leaving small intercellular spaces; the innermost 2–3 cell layers are arranged in concentric rings. Endodermis with Casparian strip. Stele: Pericycle consists of a single layer of polygonal cells with cellulose walls,

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.



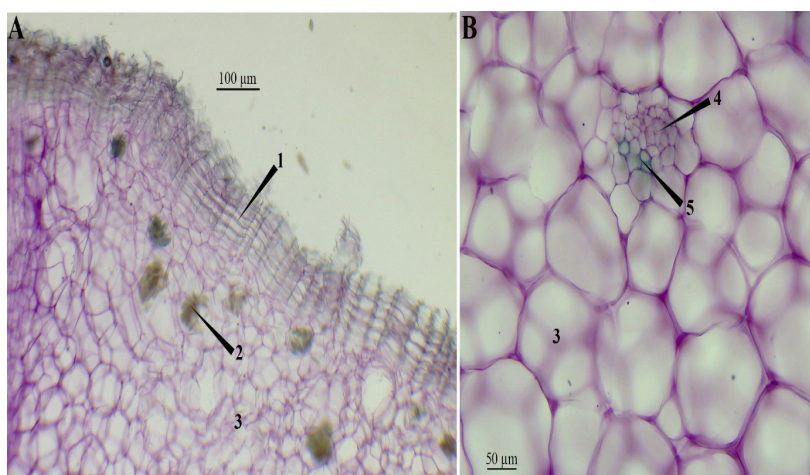
**FIGURE 6:** The cross-section of the root. (A) Whole section; (B) Cortex; (C) Stele.

*Notes:* 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 (Fig. 7)**

The cross-section has no distinction between cortex and stele. Phellem has 8–10 layers of rectangular cells, phellem-impregnated walls, arranged

radially, the outermost layer usually torn. Parenchyma is arranged closely, leaving small intercellular spaces, containing numerous cells with needle-shaped calcium oxalate crystals; vascular bundles are few and small, scattered in the parenchyma zone.

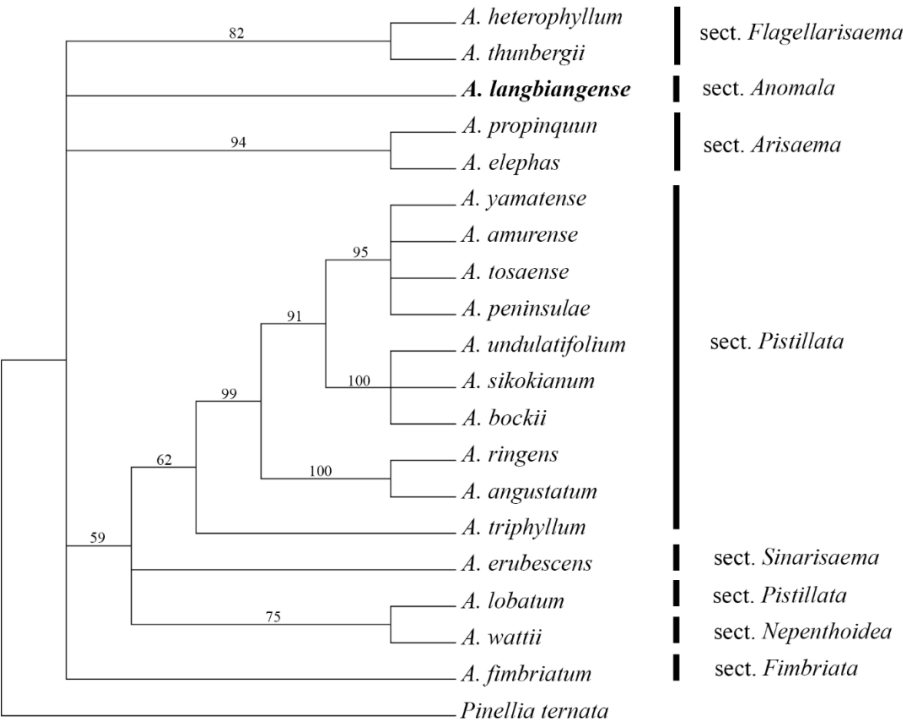


**FIGURE 7:** The cross-section of the rhizome. (A) Cortex; (B) Stele.

Notes: 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 the ITS region of *A. langbiangense* was 446 bps. This sequence was deposited in the NCBI database with the accession number PV600050. The ITS-based phylogenetic tree reconstructed in this study (Fig. 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 the 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 specialised 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*, *A. amurense*, *A. heterophyllum*, *A. propinquum*, *A. leschenaultii*, *A. jacquemontii* and *A. 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; Le et al. 2025). These needle-like crystals contribute 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 oedema (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-morphological 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 sizes.

The rhizomes of *A. langbiangense* and *A. pierreanum* show marked differences in their structural organisation. *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 centre (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 organisation, vascular arrangement and crystal morphology, provide robust diagnostic characters for species identification within *Arisaema*.

The inappropriate 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; 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 minimise 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 µm–29 µm) is larger than that of 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. langbianense* (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. langbianense*, 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 and Murata (2004) used the *trnL-trnF* region to conduct the phylogenetic tree of 77 *Arisaema* species (Renner & Zhang 2004). Similarly, Renner *et al.* (2004) performed the phylogenetic analyses of 81 species of *Arisaema*, 9 of *Typhonium*, and 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*, *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. langbianense*, 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.

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## AUTHORS' CONTRIBUTIONS

Huyen Trang Luu: Contributed to study design, conducted the experiments, performed the statistical analyses and prepared the manuscript.

Hong Quan Bui: Collected the samples during field trips.

Dinh Thach Bui: Collected the samples during field trips.

Hong Truong Luu: Carried out the experiments examining pollen structure and identified the scientific name of the studied species.

Tran Quoc Trung Nguyen: Carried out the experiments examining pollen structure.

Nga Nguyen-Phi: Identified the scientific name of the studied species.

Thu Trang Le-thi: Conducted the anatomical analysis experiments.

Hong Thien Van: Contributed to study design, conducted the experiments, performed the statistical analyses, prepared the manuscript, identified the scientific name of the studied species, and handled all editorial and reviewer queries.

All authors reviewed and approved the final manuscript.

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