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# PHYLOGENETIC EVIDENCE CONFIRMS *ADIANTUM MENGLIANENSE* AS A NEW RECORD FOR LAOS WITH THE REDISCOVERY OF *ADIANTUM GINGKOIDES* (PTERIDACEAE)

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ABSTRACT

*Adiantum menglianense* is reported for Laos for the first time, based on a specimen collected in Luang Prabang Province, northern Laos, and supported by molecular evidence. Details on its habitat and distribution, and photographs, are provided. In addition, photographs of natural habitat and the phylogenetic position of *A. gingkoides* C.Chr., an endemic and rare species from Laos, are provided for the first time as the species is rediscovered 95 years after its original collection.

Keywords: karst limestone, maidenhair fern, phylogeny, pteridophyte, *rbcL*

## INTRODUCTION

Pteridophyte studies in Laos have recently gained attention. Between 2021 and 2025, approximately 36 species representing 27 genera and 12 families have been newly documented, including six species endemic to Laos, most of which are associated with limestone habitats (Tagane *et al*. 2021; Vongthavone *et al*., 2021, 2025; Sripeng *et al*., 2024; Zhang *et al*., 2024; Miyazaki *et al*., 2025). Limestone ecosystems in Laos are increasingly recognized as hotspots for endemic pteridophyte species as well as many newly documented species of flowering plants, e.g. *Begonia brunneosquamata* Phonep. & C.W.Lin (Begoniaceae) (Phonepaseuth & Lin, 2025), *Lysionotus calcicola* Phonep., Soulad. & Souvann. (Gesneraceae) (Souvannakhoummane *et al*., 2025), *Tetrastigma fruticosum* Soulad., Phonep. & Tagane (Tagane *et al*., 2025), which are narrowly restricted to karst landscapes. As noted by Lindsay & Middleton (2012–, continuously updated), further exploration of limestone sites is likely to reveal additional locally endemic species, highlighting the importance of continued botanical surveys and conservation efforts in these habitats. Continued identification of herbarium collections and additional botanical surveys in poorly explored regions are likely to reveal further diversity within the fern flora of Laos. Details for each taxon of pteridophyte reported from Laos in past five years are provided in Appendix 1.

The genus *Adiantum* L., commonly known as "maidenhair ferns," has a worldwide distribution and comprises approximately 245 accepted species (POWO, 2025). All species of *Adiantum* share a distinctive morphological feature not found in other fern lineages: their sporangia are borne directly on the reflexed margin of the pinnae, forming a "false

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indusium" or "pseudoindusium" (TAGAWA & IWATSUKI, 1985; ZHANG *ET AL*., 2013). Ten species of this genus have been recorded from Laos, namely *A. capillus-veneris* L., *A. caudatum* L., *A. erylliae* Tardieu & C.Chr., *A. flabellulatum* L., *A. gingkoides* C.Chr., *A. membranifolium* S. Linds. & Suksathan, *A. philippense* L., *A. soboliferum* Wall. ex Hook., *A. stenochlamys* Baker, and *A. zollingeri* Mett. ex Kuhn (LINDSAY & MIDDLETON, 2012–, continuously updated; SRIPENG *ET AL*., 2024). Among them, *A. gingkoides* is the only species endemic to Laos, with little known about its habitat and distribution.

During field collections conducted in northern Laos (Luang Prabang and Ngoi districts, Luang Prabang Province) from August to September 2023, central Laos (Feuang District, Vientiane Province; Thakhek, Gnommalart, and Nakai districts, Khammouane Province) in August 2024; and northern and north-central Vietnam (Phu Tho and Nghe An provinces) in December 2024, several *Adiantum* species were collected, particularly those belonging to the morphologically challenging *A. philippense* complex. DNA barcoding is required to accurately distinguish species within this group.

EBIHARA *ET AL*. (2010) evaluated the utility of *rbcL* and *trnH-psbA* using 733 taxa of pteridophytes and found that these two barcodes are effective for identifying the pteridophyte flora of Japan. WANG *ET AL*. (2016) applied DNA barcodes to identify closely related species of Chinese *Adiantum* and found that *rbcL* had the highest PCR amplification rate (94.3%) and sequencing success rate (90.8%). Therefore, this study utilized *rbcL* as DNA barcoding to identify *Adiantum* species.

## MATERIALS AND METHODS

### DNA Extraction, Amplification and Sequencing

Total DNA was extracted from silica dried leaf material using a 2× CTAB protocol modified from DOYLE & DOYLE (1987). The *rbcL* gene (ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit) was selected for barcoding in this study. This gene serves as a valuable phylogenetic marker for resolving deeper evolutionary relationships because of its relatively slow rate of evolution, and it is the most commonly sequenced plastid region in ferns (NITTA *ET AL*., 2022).

For amplification of the chloroplast *rbcL* gene, I followed HORI *ET AL*. (2018), using primers namely af3 (5′-ATGTCACCACAAACGGAGACTAAAGC-3′) and cr3 (5′-GCGGCAGCCAATTCCGGACTCCA-3′). In addition, *trnL-F* intergenic spacer sequences including *trnL* intron (*trnL-L-F*) primers FernL1Ir1 (5′- GGYAATCCTGAGCCAAATC -3′, LI *ET AL*. 2010) and F (5′- ATTTGAACTGGTGACACGAG -3′, TABERLET *ET AL*., 1991) were also amplified for *A. gingkoides* (accession number PX900790).

Polymerase chain reaction (PCR) amplifications were performed in a 10 µL reaction mixture containing 3.3 µL of $ddH_2O$, 0.6 µL of each forward and reverse primer (10 µM), 5 µL of Prime STAR Max DNA Polymerase (Takara, Kyoto, Japan) and 0.5 µL of DNA template. The PCR profile followed YONEOKA *ET AL*. (2024): 95 °C for 7 min, followed by 35 cycles of 98 °C for 10 s, 58 °C for 15 s, and 72 °C for 8 s, and 72 °C for 7 min. Amplification products were visualized under UV light after electrophoretic separation on a 1.5% agarose TAE gel stained with SYBR Safe gel stain (Applied Biosystems). To remove superfluous dNTPs and primers, PCR products were subsequently purified using ExoSAP-IT (Applied Biosystems) according to the manufacturer's protocols. Sequencing PCRs used the same primers as for amplification, and reaction mixtures for sequencing were prepared using the SuperDye Cycle Sequencing Kit v3.1 (Edge BioSystems, California, USA). The protocol included an initial denaturation at 96 °C for 45 s, followed by 28 cycles of denaturation at 96 °C for 10 s,

primer annealing at 50 °C for 5 s, and primer extension at 60 °C for 2 min. Sequencing PCR products were purified and analyzed at the Makino Herbarium using an AB 3130 Genetic Analyzer (Applied Biosystems).

### Taxon Sampling and Phylogenetic Analyses

A total of 83 sequences were included in this study, 82 of *Adiantum* spp. as ingroup taxa, and *Antrophyum latifolium* Blume (vittarioids) as the outgroup taxon, following Huiet *et al*. (2018). Besides that, 8 sequences of 6 species were newly generated from this study (*A*. *gingkoides* is sequenced for the first time) (See Table 1). The remaining sequences were downloaded from GenBank (see Table 2), including most species in the *A*. *philippense* clade and species that occur in Eurasia (Huiet *et al*., 2018) with additional samples from previous literature (Ebihara *et al*., 2010; Lu *et al*., 2012; Lestari *et al*., 2014; Kuo *et al*., 2016; Wang *et al*., 2016). The sequence alignment was performed with MAFFT (Katoh *et al*., 2019) followed by manually checking and editing in Aliview (Larsson, 2014). A maximum likelihood tree was constructed and analyzed using the IQ-TREE web server (version 1.6.12) (Nguyen *et al*., 2015), with TIMe+I+G4 for *rbcL* model selected according to Bayesian Information Criterion (BIC), ModelFinder (Kalyaanamoorthy *et al*., 2017) and employing one-thousand bootstrap pseudoreplicates via the ultrafast bootstrap (Hoang *et al*., 2018). The tree was visualized using FigTree v.1.4 (Rambaut, 2012).

Table 1. Taxon, country of origin, collection location and voucher specimens of newly sequenced in this study

| Taxon | Country | Locality | Voucher (herbarium code) | GenBank accession number (*rbcL*) |
|---|---|---|---|---|
| *Adiantum gingkoides* C.Chr. | Laos | Gnommalart District, Khammouane Province | Phonepaseuth, P. et al. P097 (FOF) | PX900791 |
| *Adiantum* sp. LPB23025 | Laos | Ngoi District, Luangprabang Province | Phonepaseuth, P. & Vongsavath, V. LPB23025 (FOF) | PX900794 |
| *Adiantum* sp. LPB23018 | Laos | Ngoi District, Luangprabang Province | Phonepaseuth, P. & Vongsavath, V. LPB23018 (FOF) | PX900798 |
| *Adiantum* sp. FE23002 | Laos | Feuang District, Vientiane Province | Phonepaseuth, P. et al. FE23002 (FOF) | PX900793 |
| *Adiantum* sp. FE23005 | Laos | Feuang District, Vientiane Province | Phonepaseuth, P. et al. FE23005 (FOF) | PX900792 |
| *Adiantum* sp. NK028 | Laos | Nakai District, Khammouane Province | Phonepaseuth, P. et al. NK028 (FOF) | PX900795 |
| *Adiantum* sp. PHVN012 | Vietnam | Pu Hoat Nature Reserve, Nghe An Province. | Phonepaseuth, P. et al. PHVN012 (IEBR) | PX900796 |
| *Adiantum* sp. PHVN059 | Vietnam | Pu Hoat Nature Reserve, Nghe An Province. | Phonepaseuth, P. et al. PHVN059 (IEBR) | PX900797 |

Table 2. Ingroup and outgroup taxa sampled in this study. For each collection, the species name, Country of origin, voucher information, and GenBank accession numbers (*rbcL*) are provided.

| Taxon | Country | Voucher (herbarium code) | GenBank number |
|---|---|---|---|
| *A. calcareum* Gardner | Brazil | Hirai 717 (SP) | MH019545 |
| *A. capillus-junonis* Rupr. | China | Sundue 988 (DUKE) | MH019547 |
| *A. capillus-junonis* Rupr. | China (Guangxi) | J.-M. Lu 111 (KUN) | JF935314 |
| *A. capillus-junonis* Rupr. | Japan (Oita Pref.) | 743693 (TNS) | AB574795 |
| *A. capillus-veneris* L. | U.S.A. | Huiet 104 (UC) | KU147273 |
| *A. capillus-veneris* L. | Russia | J. Wen 10360 (US) | JF935345 |
| *A. capillus-veneris* L. | Japan (Oita Pref.) | 777847 (TNS) | AB574796 |
| *A. capillus-veneris* L. | China (Guangxi) | J.-M. Lu 138 (KUN) | JF935320 |
| *A. caudatum* L. | Laos | Sugong WS-1837 (MO) | MH019548 |
| *A. caudatum* L. | China (Hainan) | J.-M. Lu 209 (KUN) | JF935296 |
| *A. caudatum* L. | China (Yunnan) | J.-M. Lu 050 (KUN) | JF935297 |
| *A. caudatum* L. | India | Shyamalima Saikia | PP801084 |
| *A. comorense* Fée | Tanzania | Mwangoka 5695 (MO) | MH019551 |
| *A. davidii* Franch. | China | Kuo 1561 (TAIF) | MH019554 |
| *A. deflectens* Mart. | Brazil | Schuettpelz 1390 (DUKE) | MH019557 |
| *A. delicatulum* Mart | Brazil | Hirai 704 (SP) | MH019558 |
| *A. diaphanum* Blume | Taiwan | 777847 (TNS) | AB574797 |
| *A. diaphanum* Blume | China | Wuzhishan Fern Survey 67 (MO) | MH019560 |
| *A. diaphanum* Blume | China (Hainan) | J.-M. Lu 208 (KUN) | JF935301 |
| *A. edgeworthii* Hook. | Japan (Oita Pref.) | 743692 (TNS) | AB574798 |
| *A. edgeworthii* Hook. | Vietnam | Kuo 1840 (TAIF) | MH019565 |
| *A. flabellulatum* L. | Japan (Okinawa Pref.) | 764008 (TNS) | AB574799 |
| *A. flabellulatum* L. | Taiwan | Schuettpelz 1016A (DUKE) | MH0147275 |
| *A. flabellulatum* L. | Taiwan | Kuo 3377 (TAIF) | PP559815 |
| *A. flabellulatum* L. | China (Guizhou) | J.-M. Lu 192 (KUN) | JF935315 |
| *A. flabellulatum* L. | China (Guangdong) | J.-M. Lu 450 (KUN) | JF935295 |
| *A. flabellulatum* L. | China (Hainan) | J. Wen 6585 (US) | JF935325 |
| *A. formosanum* Tagawa | Taiwan | Li 1402 (DUKE) | MH019571 |
| *A. formosum* R.Br. | New Zealand | J. Wen 10779 (US) | JF935352 |
| *A. formosum* R.Br. | - | Cultivated, Smith s.n. (UC) | KC984520 |
| *A. gravesii* Hance | China | Zhang 376 (MO) | MH019579 |
| *A. gravesii* Hance | China (Guangxi) | J.-M. Lu 129 (KUN) | JF935312 |
| *A. gravesii* Hance | China (Guangdong) | J.-M. Lu 451 (KUN) | JF935317 |
| *A. hispidulum* Sw. | Taiwan | Schuettpelz 1168A (DUKE) | MH019580 |
| *A. hosei* Baker | Malaysia | Beaman 10302 (UC) | MH019582 |
| *A. induratum* H.Christ. | Vietnam | WP 963 (MO) | MH019585 |
| *A. induratum* H.Christ. | China (Hainan) | J.-M. Lu 210 (KUN) | JF935309 |
| *A. induratum* H.Christ. | - | isolate WFH008 | KT426956 |
| *A. incisum* Forssk. | Tanzania | Gereau 6840 (MO) | MH019584 |
| *A. madagascariense* H.Rosend. | Madagascar | Trigui 531 (MO) | MH019594 |
| *A. malesianum* J.Ghatak | Malaysia | Schuettpelz 666 (DUKE) | MH019596 |
| *A. malesianum* J.Ghatak | China (Guangdong) | L.Huiet 111 (UC) | EF452132 |
| *A. mariesii* Baker | China | Wade 2493 (TAIF) | MH019597 |
| *A. mariesii* Baker | China (Guangxi) | J.-M. Lu 120 (KUN) | JF935302 |
| *A. menglianense* Y.Y.Qian | Taiwan | MO Awan-1 (TAIF) | KU945417 |
| *A. menglianense* Y.Y.Qian | - | Yan12415 | KP637234 |
| *A. menglianense* Y.Y.Qian | - | Yan12409 | KP637233 |

Table 2 (continued).

| Taxon | Country | Voucher (herbarium code) | GenBank number |
|---|---|---|---|
| *A. menglianense* Y.Y.Qian | China (Guangxi) | J.-M. Lu 114 (KUN) | JF935311 |
| *A. mettenii* Kuhn | Tanzania | Massawe 364 (MO) | MH019602 |
| *A. monochlamys* D.C.Eaton | Taiwan | Li 427 (TAIF) | MH019603 |
| *A. monochlamys* D.C.Eaton | Japan (Tokyo Pref.) | 764001 (TNS) | AB574800 |
| *A. myriosorum* Baker | China | Kuo 2128 (TAIF) | MH019605 |
| *A. ogasawarense* Tagawa | Japan (Tokyo Pref.) | 774843 (TNS) | AB574801 |
| *A. pedatum* L. | U.S.A. | Rothfels 3839 (DUKE) | MH019612 |
| *A. pedatum* L. | - | Huiet 117 (UC DNA voucher) | U05602 |
| *A. pedatum* L. | Japan (Shizuoka Pref.) | 764338 (TNS) | AB574802 |
| *A. phanerophlebium* (Bak.) C.Chr. | Madagascar | J. Kluge 8039 (Z) | MH019615 |
| *A. philippense* L. | Costa Rica | Rothfels 2655 (DUKE) | MH019616 |
| *A. philippense* L. | Indonesia | WN153 | LC004122 |
| *A. philippense* L. | Indonesia | WN142 | LC004111 |
| *A. philippense* L. | Indonesia | J. Wen 10206 (US) | JF935340 |
| *A. philippense* L. | Philippines (Luzon) | J. Wen 8257 (US) | JF935330 |
| *A. philippense* L. | China (Yunnan) | J.-M. Lu 001 (KUN) | JF935331 |
| *A. philippense* L. | China (Yunnan) | Y.-X. Zhang 009 (KUN) | JF935299 |
| *A. poiretii* Wikstr. | Mexico | Windham 513 (DUKE) | MH019620 |
| *A. schweinfurthii* Kuhn | Tanzania | Gereau 5966 (MO) | MH019630 |
| *A. soboliferum* Wall. ex Hook. | Laos | S.K.Wu 294 (MO) | MH019635 |
| *A. soboliferum* Wall. ex Hook. | - | isolate WFH038 | KT427017 |
| *A. soboliferum* Wall. ex Hook. | - | isolate Sunhang17076 | KT427016 |
| *A. stenochlamys* Baker | Malaysia | Schuettpelz 900A (DUKE) | MH019636 |
| *A. taiwanianum* Tagawa | Taiwan | Schuettpelz 1131A (DUKE) | MH019637 |
| *A. thongthamii* Suksanthan | Thailand | Suksathan 3303 (QBG) | MH019639 |
| *A. rhizophytum* Schrad. | Brazil | Fiaschi 3568 (SPF) | MH019626 |
| *Antrophyum latifolium* Blume | Papua New Guinea | Ranker 1774 (COLO) | EF452138 |

## RESULTS

### Phylogenetic Analyses

The aligned dataset contained 83 sequences and 1,309 nucleotide sites, of which 973 (74.3%) were constant and 278 (21.2%) were parsimony informative. The ML tree (Fig. 1) shows the phylogenetic relationships of eight newly collected specimens of *Adiantum* spp. Six specimens of *Adiantum* from Laos were resolved within the "philippense clade" and were distributed among several strong supported subclades (UFBS = 95–100). *Adiantum* sp. LPB23018 was placed in a strongly supported subclade together with accessions identified as *A. menglianense* from China and Taiwan (UFBS = 95), and was clearly separated from the clade containing *A. philippense* sensu stricto (UFBS = 94). Two Laotian specimens (*Adiantum* sp. NK028, *Adiantum* sp. FE23002) clustered with accessions of *A. philippense*, whereas *Adiantum* sp. LPB23025 and *Adiantum* sp. FE23005 were placed with *A. caudatum* and *A. soboliferum*, respectively, each with strong bootstrap support (UFBS = 98–100). *Adiantum gingkoides* C.Chr. formed a strongly supported sister relationship with *A. capillus-junonis* Rupr. in the ML tree with strong bootstrap support (UFBS = 100) within

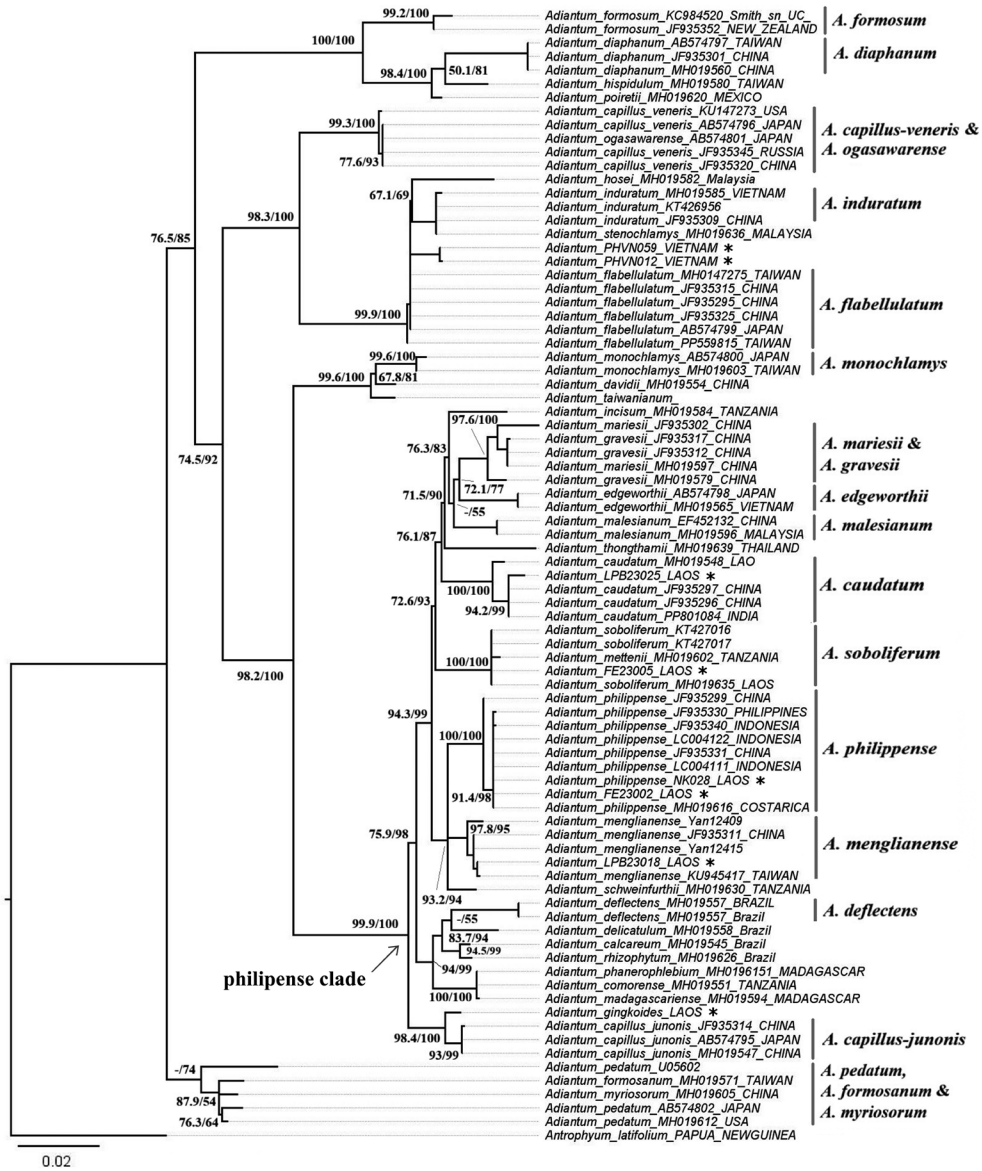


Figure 1. Maximum likelihood trees of species of *Adiantum* based on *rbcL* sequences. Species shown with asterisk are newly sequences in this study. Bootstrap values including Shimodaira-Hasegawa-like approximate likelihood ratio test (SH-aLRT) and Ultrafast bootstraps (UFBS) are shown at each branch (SH-aLRT/UFBS) when both values exceed 50%. Values greater than 80% for SH-aLRT and 95% for UFBS are considered as strong support.

the philippense clade and was distant from morphologically similar taxa such as *A. gravesii*. The two Vietnamese specimens of *Adiantum* cf. *flabellulatum* (PHVN059, PHVN012 were resolved within a broader clade including *A. flabellulatum*, and *A. hosei*, *A. induratum*, *A. stenochlamys* clade.

## Taxonomic Account

***Adiantum menglianense*** Y.Y.Qian [Pteridaceae] (Fig. 2)

Acta Bot. Austro Sin. 8: 37–38 (1992).

Type: China. Yunnan: Menglian, 900–1,400 m, 23 December 1984, *Qian 801* (lectotype: HITBC-81381, designated by Kuo *et al*. 2016; isolectotypes: HITBC-81380, HITBC-109908).

This species is placed in the *Adiantum philippense* complex (Kuo *et al*., 2016). First described from Yunnan Province, South China (Qian, 1992), and also confirmed to occur in Taiwan with phylogenetic analysis, determined by reproductive mode and ploidy level, in which 64 spores per sporangium and sexual diploids were found (Kuo *et al*., 2016). This species has more dissected fertile pinnules than *A. philippense* L. with narrower false indusia 2–6 mm (vs. *A. philippense* subsp. *philippense* (2–12 mm) (Qian, 1992; Kuo *et al*., 2016).

Habitat and ecology: This species was found growing on red soil mixed with degraded limestone in a shaded semi-deciduous forest at an altitude of 440–460 m a.s.l. It shares its habitat with *A. caudatum* L. and other flowering plants such as *Begonia luangprabangensis* Phonep., and *B.* aff. *demissa* Craib (Begoniaceae), *Microchirita* sp. (Gesneriaceae).

Distribution: China (Yunnan Province), Laos (Luang Prabang Province), Taiwan.

Specimens examined: Laos. Luang Prabang Province, Ngoi District, Nongkhiaw village, on the walking trail to Nangnone karst viewpoint. Around point 20°34′33″N, 102°36′30″E, 440–460 m a.s.l. 23 August 2023, *Phonepaseuth, P. & Vongsavath, V. LPB23018* (FOF). Same Province, Luang Prabang District, Long Lan mountain, along trail from Ban Den Savang to Ban Long Vai, 750 m a.s.l., 24 November 2017, *Hsu, T.C. 9982* (TAIF [TAIF540938, digital image!]).

***Adiantum gingkoides*** C.Chr. [Pteridaceae] (Fig. 3)

Bull. Mus. Natl. Hist. Nat., Sér. 2, 6: 100 (1934).

Type: Laos. Thakhek district, Tham Village, on the limestone walls of a very humid cave, December 1930. *Colani, M. 4139* (holotype BM, isotype NY [NY00127470, digital image!], P [P00607853, P00607854, digital image!], UC [UC74445, digital image!], US [US00134480, digital image!]).

This species has fronds resembling those of *Ginkgo biloba* L. (Ginkgoaceae), but noted misspelling of epithet (Lindsay & Middleton, 2012–, continuously updated). This species differs from closely related species in having a darker and narrower indusium, non-proliferous leaves, shorter petioles, and margin of the pinnae not being incised between the sori (Christensen, 1934). The type specimens were collected from "Village de Tham, Common Prov." in December 1930 by the French archaeologist Madeleine Colani. Based on the map of villages and schools provided by the Ministry of Education and Sports of Laos (MOES, 2024), this locality is presumed to be the Elephant Cave, a notable large cave in present day Thakhek District, Khammouane Province, Laos. Additional evidence indicates that Colani was working in Thakhek district, Khammoune Province during October 1930 to studied vestiges of Bacsonian-Hoabinhian, for the Neolithic traces (Genovese, 2015: 60). In January 1931, Colani collected another set of *A. gingkoides* specimens, together with *Antrophyum* species, in "Village de Mahaxay, Mahaxay District" (see specimens examined), which corresponded with her documented activities during that month (Genovese, 2023:

152). Since Colani was not a plant taxonomist, she may have passed these specimens to the French botanist and entomologist Paul Alfred Pételot, who then assigned herbarium numbers. Consequently, some of the type specimens collected by her are cited under Pételot's name rather than Colani's.

This species was rediscovered and photographed by me on 18 August 2019, in a field survey in Gnommalart District, Khammouane Province, at the time an immature plant (without spores, Fig. 3E). A subsequent collection from the same locality was made three years later (Fig. 3F–H).

Based on morphology notes provided by CHRISTENSEN (1934), this species is closely related to *A. leveillei* Christ (currently treated as *A. gravesii* Hance) and *A. semiorbiculatum* Bonap., but differs in having fewer and larger pinnae, shorter pinna petioles, and non-glaucous fronds. Examination of newly collected specimens, together with comparison to the type material, line drawings and published descriptions (CHRISTENSEN, 1934; TARDIEU-BLOT & CHRISTENSEN, 1938; LINDSAY & MIDDLETON, 2012–, continuously updated) confirm the overall match with *Adiantum gingkoides*.

Habitat and ecology: This species grows on humid limestone walls and near cave entrances from altitudes of 150 m to 240 m a.s.l. It shares its habitat with *Argostemma* spp. (Rubiaceae), *Impatiens* sp. (Balsaminaceae), *Begonia murina* Craib (Begoniaceae), *Microchirita hamosa* (R.Br.) Yin Z.Wang and *Paraboea khotamiae* Phonep. & Souvann. (Gesneriaceae).

Distribution: Endemic to Laos, currently known from four localities in three districts (Thakhek, Gnommalart, and Mahaxay) of Khammouane Province.

Proposed conservation status: This species was found only in Khammouane Province, Central Laos. Numbers of individuals from 20 to 100 were found in new localities (Pha Kataiythong karst area, and Ban Mouang karst area, respectively). The Area of Occupancy (AOO) is calculated at 16 km$^2$ and the Extent of Occurrence (EOO) at 349.98 km$^2$ using GeoCat (BACHMAN *ET AL*., 2011). Suitable habitat for this species extends into Phou Hin Poun National Park. However, several limestone karsts outside the national park are being destroyed for cement production and road construction. Based on these data, the species is preliminarily assessed as Endangered (EN, B1ab(iii); B2ab(iii); D).

Specimens examined: LAOS. Khammoune Province, Gnommalart District (new district record), Pha Kataithong, 17°33′09″N, 105°05′50″E, at altitude 225 m a.s.l., 16 September 2022, *Phonepaseuth, P. et al. P097* (FOF); Same district, Ban Muang karst limestones, 17°32′37″N, 105°03′55″E, at altitude 230–240 m a.s.l., 5 September 2024, *Tagane et al. Z1675* (FOF, KAG [KAG188338, digital image!]). Mahaxay District, Mahaxay village, on limestone rock in humid tall forest, at 160 m a.s.l., 5 January 1931, *Colani, M. 4252* (P [P01294135, digital image!], MICH [MICH1592761, digital image!]). ibid., at 150 m a.s.l., 22 January 1931, *Colani, M. 4256* (P[P01462195, digital image!], MICH [MICH1592760, digital image!]).

## DISCUSSION

### Taxonomic Interpretation of Phylogenetic Results

Adiantum menglianense *as a new record for Laos*

The Laotian specimen LPB23018 was placed with strong support within the clade containing reference sequences of *Adiantum menglianense* from China and Taiwan, and was clearly separated from *A. philippense* sensu stricto. While this molecular result alone is

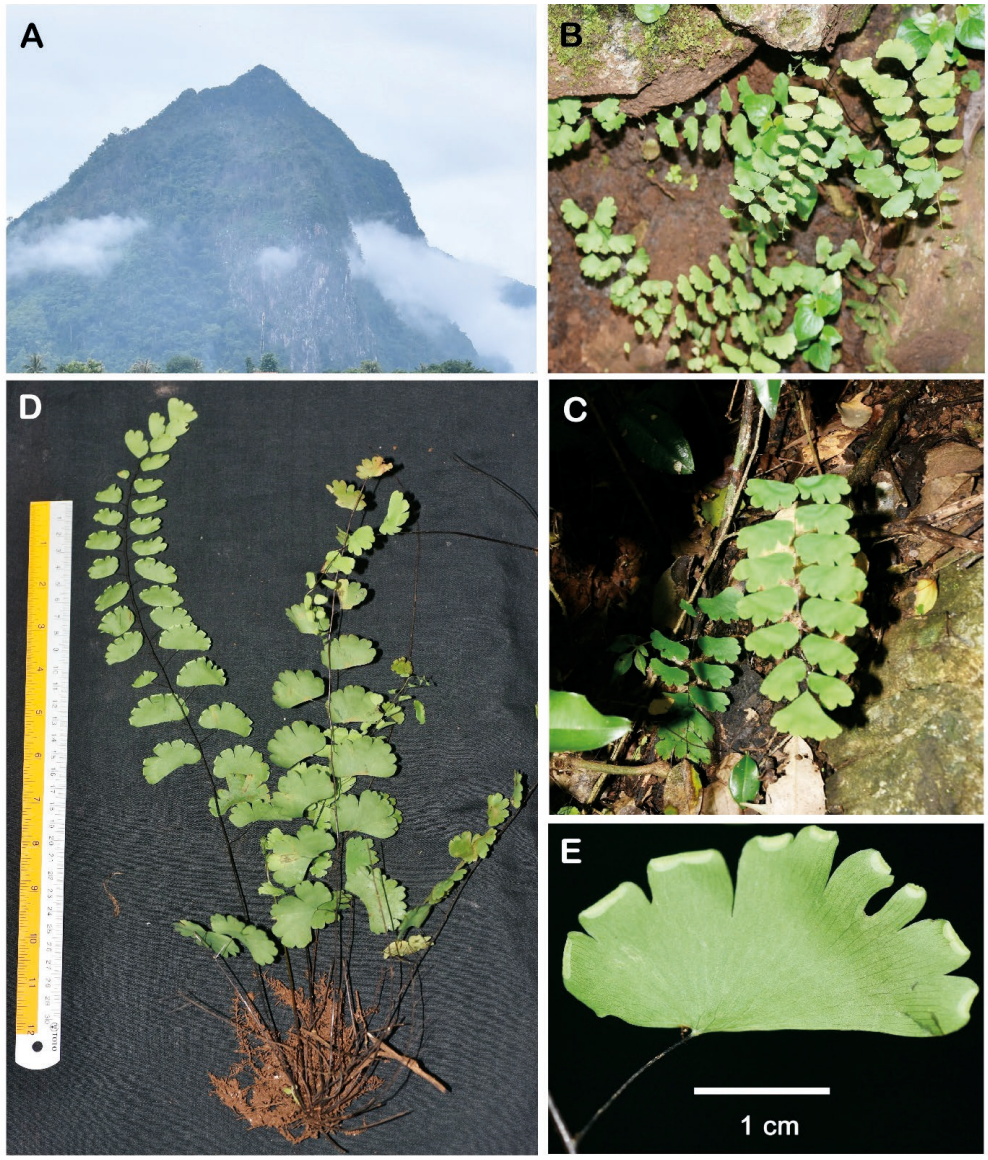


Figure 2. *Adiantum menglianense* Y.Y.Qian. A, habitat; B–C, plant habit in habitat; D, whole plant; E, portion of lower pinna surface showing false indusia. Photos by Phongphayboun Phonepaseuth (taken on 23 August 2023 at Nangnone karst viewpoint, Ngoi District, Luang Prabang Province).

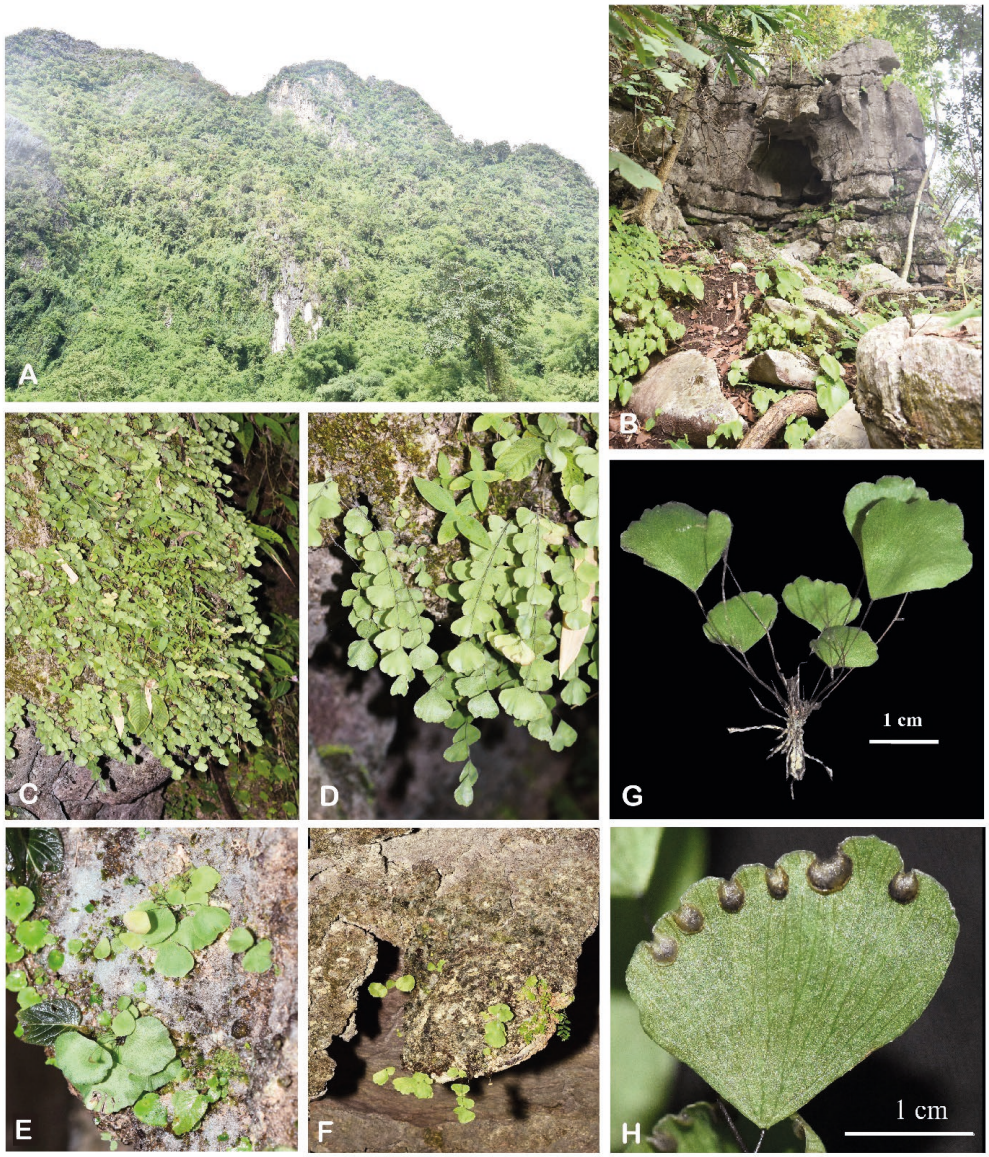


Figure 3. *Adiantum gingkoides* C.Chr. A–B, habitat; C–F, habit in natural habitat (C, on wet limestone wall; D–F, on caves entrance); G, whole plant; H, portion of lower pinna surface showing false indusia. Photos by Phongphayboun Phonepaseuth (A–D taken on 5 September 2024 in Ban Muang karst limestones, Gnommalart District, Khammoun Province; E, taken on 18 August 2019; F–H, on 16 September 2022 in Pha Kataithong, Gnommalart District, Khammoun Province).

insufficient to confirm species identity, morphological examination of the Laotian material provides congruent evidence.

The specimens exhibit diagnostic characters consistent with *A. menglianense*, including more deeply dissected fertile pinnules (5–10 segments) and narrower false indusia (1.8–5.8 mm), which differ from those of *A. philippense* as documented by Qian (1992) and Kuo *et al.* (2016). The agreement between molecular placement and morphological characters supports the interpretation that the Laotian population represents *A. menglianense*. On this basis, the species is here recognized as newly recorded for the fern flora of Laos.

*Rediscovery and phylogenetic position of* Adiantum gingkoides

*Adiantum gingkoides*, a species endemic to Laos and not collected since its original discovery in 1930–1931, was rediscovered during recent field surveys in Khammouane Province. Morphological examination of newly collected specimens, together with comparison to type material, historical descriptions, and published illustrations (Christensen, 1934; Tardieu-Blot & Christensen, 1938; Lindsay & Middleton, 2012–, continuously updated), confirms their close correspondence with *A. gingkoides*.

Phylogenetically, *A. gingkoides* was resolved within the philippense clade and formed a strongly supported sister relationship with *A. capillus-junonis*. This placement contrasts with earlier assumptions of close affinity with morphologically similar species such as *A. gravesii*. The combined morphological and molecular evidence supports the continued recognition of *A. gingkoides* as a distinct species and clarifies its evolutionary relationships within *Adiantum*.

In several cases, including specimens provisionally identified as *Adiantum* cf. *flabellulatum*, molecular data alone were insufficient to resolve species identity, indicating that detailed morphological examination remains necessary for confident identification.

## DNA Barcoding and Species Identification in the Laotian Fern Flora

DNA barcoding has become an essential tool for resolving species boundaries in taxonomically challenging fern groups, particularly within *Adiantum* (Lestari *et al.*, 2014; Kuo *et al.*, 2016; Wang *et al.*, 2016; Huiet *et al.*, 2018). In this study, molecular evidence was essential for confirming the presence of *A. menglianense* in Laos for the first time, as well as for clarifying the phylogenetic position of the endemic *A. gingkoides*, a species not observed since its original collection 95 years ago. These results highlight how genetic data provide reliable diagnostic characters where morphology alone is insufficient, and demonstrate the value of integrating DNA barcodes into taxonomic research on the fern flora of Laos.

Among 332 fern and lycophyte specimens collected from 2022 to 2024, 34 specimens (approximately 10%) were initially identified as unrecorded or unknown from Laos. These were mainly collected from limestone areas in northern and central Laos, as well as from regions with limited prior sampling in southern Laos, e.g. Dak Cheung Plateau (Phonepseuth *et al.*, 2024). Species identification and DNA barcoding of these specimens are currently ongoing. This work represents an important first step toward establishing a genetic database of fern species in Laos to support future research and conservation efforts.

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Appendix 1. Pteridophyte taxa recently documented from Laos (2021–2025).

| Family | Taxon | Region | Province | Life form and habitat | Distribution | References |
|---|---|---|---|---|---|---|
| ATHYRIACEAE | *Cornopteris opaca* (D.Don) Tagawa | South | Champasak | Terrestrial on wet ground in hill evergreen forest | India, Bhutan, Nepal, China, Japan, Thailand, Laos, Vietnam, Indonesia | Tagane *et al.* (2021) |
| | *Diplazium platychlamys* C.Chr. | South | Champasak | Terrestrial in hill evergreen forest | Laos and Vietnam | Tagane *et al.* (2021) |
| ASPLENIACEAE | *Asplenium perakense* C.G.Matthew & Christ | South | Champasak | Lithophytic on semi-shaded mossy moist rocks in wet evergreen forest | Thailand, Laos, and Malaysia | Vongthavone *et al.* (2021) |
| | *Asplenium simonsianum* Hook. | South | Champasak | Epiphytic on moss-covered tree trunks in wet evergreen forest | India, China, Thailand, and Laos | Vongthavone *et al.* (2021) |
| | *Asplenium tenerum* G.Forst. | South | Champasak | Lithophytic on semi-shaded rocks and Epiphyte on tree trunks in dense montane conifer forest | India, Sri Lanka, Japan (Ogasawara), Korea, Laos, Myanmar, Vietnam, Cambodia, Malaysia, Indonesia, Philippines, Pacific islands | Tagane *et al.* (2021) |
| | *Hymenasplenium cheilosorum* (Kunze ex Mett.) Tagawa | South | Champasak | Terrestrial on wet ground in hill evergreen forest | India, Bhutan, Nepal, Sri Lanka, China, Japan, Myanmar, Thailand, Laos, Vietnam, Malaysia, Indonesia, Philippines | Tagane *et al.* (2021) |

Appendix 1 (continued).

| Family | Taxon | Region | Province | Life form and habitat | Distribution | References |
|---|---|---|---|---|---|---|
| DENNSTAEDTIACEAE | *Histiopteris incisa* (Thunb.) J.Sm. | South | Champasak | Terrestrial on exposed and humus-rich slopes in wet evergreen forest | Pantropical and Laos | VONGTHAVONE *ET AL.* (2021) |
| | *Microlepia calvescens* (Hook.) C.Presl | North | Oudomxay | Terrestrial on reddish soil site on road side, forest margins, secondary forest | India, E. Himalaya, S. China, Upper Myanmar, Thailand, Laos, Vietnam Indonesia, and Taiwan | MIYAZAKI *ET AL.* (2025) |
| DRYOPTERIDACEAE | *Arachniodes pseudoassamica* Ching | South | Champasak | Terrestrial in semi-shaded under-story in wet evergreen forest | China, and Laos | VONGTHAVONE *ET AL.* (2021) |
| | *Polystichum attenuatum* Tagawa & K.Iwats. | South | Champasak | Terrestrial on humus-rich ground in wet evergreen forest | India, Myanmar, China, Taiwan, Thailand, Laos, and Vietnam | VONGTHAVONE *ET AL.* (2021) |
| HYMENOPHYLLACEAE | *Crepidomanes christii* (Copel.) Copel. | South | Champasak | Epiphytic on tree trunks in lowland evergreen forest | India, Thailand, Laos, Malaysia, Indonesia, and Philippines | VONGTHAVONE *ET AL.* (2021) |
| HYPODEMATIACEAE | *Leucostegia truncata* (D.Don) Fraser-Jenk. | South | Champasak | Epiphytic on moss-covered bed-rock in wet evergreen forest | India, Bhutan, Nepal, Sri Lanka, China, Taiwan, Myanmar, Thailand, Laos, Vietnam, Cambodia, Malaysia, Indonesia, Philippines, and New Guinea | VONGTHAVONE *ET AL.* (2021) |

Appendix 1 (continued).

| Family | Taxon | Region | Province | Life form and habitat | Distribution | References |
|---|---|---|---|---|---|---|
| LINDSAEACEAE | *Lindsaea orbiculata* (Lam.) Mett. ex Kuhn | North | Oudomxay | Terrestrial on reddish soil site on road cutting, half shade | NE India, S China, Myanmar Laos, Thailand, Cambodia, W & C Malesia, Taiwan, S Japan | MIYAZAKI *ET AL.* (2025) |
| LYCOPODIACEAE | *Palhinhaea crassifolia* (Spring) Fraser-Jenk. & Kholia | North | Oudomxay | Terrestrial on reddish soil site on sunny roadside | Laos, India, SE Asia | MIYAZAKI *ET AL.* (2025) |
| PLAGIOGYRIACEAE | *Plagiogyria adnata* (Blume) Bedd. | South | Champasak | Terrestrial in primary dense montane conifer forest, near streams | India, China, Japan, Myanmar, Thailand, Laos, Vietnam, Malaysia (Borneo, Peninsula), Indonesia (Java, Sumatra), Philippines (Luzon) | TAGANE *ET AL.* (2021) |
| POLYPODIACEAE | *Drynaria deuansavanhii* Vongthavone & Tagane | Central | Kham-mouane | Lithophytic on limestone karst/ Epiphyte on tree trunk. | Endemic to Laos | VONGTHAVONE *ET AL.* (2025) |
| | *Grammitis barathrophylla* (Baker) Christenh. | South | Champasak | Epiphytic on lower part of mossy tree trunks in wet evergreen forest | China, Thailand, Laos, Vietnam, Cambodia, Malaysia, Indonesia, Philippines, and New Guinea. | VONGTHAVONE *ET AL.* (2021) |
| | *Lepisorus henryi* (Hieron. ex C.Chr.) Li Wang | South | Champasak | Epiphytic on tree trunks in wet evergreen forests | India, Bhutan, Nepal, China, Thailand, Laos, and Vietnam | VONGTHAVONE *ET AL.* (2021) |

Appendix 1 (continued).

| Family | Taxon | Region | Province | Life form and habitat | Distribution | References |
|---|---|---|---|---|---|---|
| | *Lepisorus spicatus* (L.f.) Li Wang | South | Champasak | Epiphytic on tree trunks in wet evergreen forests | Tropical Asia and Laos. | Vongthavone *et al.* (2021) |
| | *Leptochilus bolikhamsaiensis* Liang Zhang, Thepkaysone & Z.Zhou | Central | Bolikhamxai | Lithophytic on limestone rocks in disturbed rainforest | Endemic to Laos | Zhang *et al.* (2024) |
| | *Leptochilus hemionitideus* (Wall. ex C.Presl) Noot. | South | Champasak | Terrestrial on wet ground in hill evergreen forest | India, Bhutan, Nepal, China, Japan, Thailand, and Laos | Tagane *et al.* (2021) |
| | *Leptochilus khammouanensis* Liang Zhang, Thepkaysone & Z.Zhou | Central | Khammouane | Lithophytic on limestone rock in disturbed primary forests | Endemic to Laos | Zhang *et al.* (2024) |
| | *Leptochilus luangprabangensis* Liang Zhang, Thepkaysone & Z.Zhou | North | Luang Prabang | Hemiepiphytic on a small tree trunk, the roots extended into the soil below, in limestone mountain | Endemic to Laos | Zhang *et al.* (2024) |
| | *Leptochilus wusugongii* Liang Zhang & Li Bing Zhang | North | Luang Prabang | Lithophytic on limestone in the primary forest | Endemic to Laos | Zhang *et al.* (2024) |
| | *Microsorum steerei* (Harr.) Ching | Central | Khammouane | Epiphytic on tree trunk in limestone karst forest | China, Laos, Vietnam, and Taiwan. | Vongthavone *et al.* (2025) |
| | *Pyrrosia flocculosa* (D.Don) Ching | Central | Khammouane | Epiphytic on tree trunk in limestone karst forest | India, Nepal, Bhutan, Bangladesh, China, Myanmar, Thailand, Laos, and Vietnam | Vongthavone *et al.* (2025) |
| | *Selliguea oxyloba* (Wall. ex Kunze) Fraser-Jenk. | South | Champasak | Lithophytic on exposed bedrocks beside open grassland or epiphytic on moss-covered tree trunks | India, Nepal, Myanmar, China, Thailand, Laos, and Vietnam | Vongthavone *et al.* (2021) |

Appendix 1 (continued).

| Family | Taxon | Region | Province | Life form and habitat | Distribution | References |
|---|---|---|---|---|---|---|
| | *Tricholepidium normale* (D.Don) Ching | South | Champasak | Epiphytic on mossy tree trunk in wet evergreen forest | India, Bhutan, Nepal, Myanmar, China, Japan, Thailand, Laos, Vietnam, Malaysia, and Indonesia. | Vongthavone *et al.* (2021) |
| PTERIDACEAE | *Adiantum membranifolium* S.Linds. & Suksathan | Central | Khammouane | Lithophytic on limestone karst/ wall | Laos and Thailand | Sripeng *et al.* (2024) |
| | *Adiantum menglianense* Y.Y.Qian | North | Luang Prabang | Terrestrial in limestone karst forest | China, Laos, and Taiwan | This study |
| | *Antrophyum formosanum* Hieron. | Central | Khammouane | Epiphytic on lower tree trunk, in wet evergreen forests | Laos, Taiwan, and Japan | Vongthavone *et al.* (2025) |
| | *Hemionitis fragilis* (Hook.) Christenh. | Central | Khammouane | Lithophytic on limestone karst | Africa, Europe, and Asia (China, Myanmar, Thailand, Laos and Vietnam) | Vongthavone *et al.* (2025) |
| | *Hemionitis khammouanensis* Vongthavone & Phonep. | Central | Khammouane | Lithophytic on limestone karst | Endemic to Laos | Vongthavone *et al.* (2025) |
| | *Pteris taiwanensis* Ching | North | Oudomxay | Terrestrial on the side of stream, edge of forest | Assam, Nepal, East Himalaya, Laos, and Taiwan | Miyazaki *et al.* (2025) |
| THELYPTERIDACEAE | *Amblovenatum decorum* (Domin) S.Linds. & D.J.Middleton | Central | Khammouane | Terrestrial near Waterfall | Africa, Asia (Sri Lanka, Thailand, Laos, Vietnam, Cambodia, Malesia) and Australia | Vongthavone *et al.* (2025) |
| | *Metathelypteris laxa* (Franch. & Sav.) Ching | North | Oudomxay | Terrestrial in forest margin in secondary forest | China, Thailand, Laos, Japan, S. Korea, and Taiwan | Miyazaki *et al.* (2025) |