

Studies of Vietnamese Pteridophyte Flora 3

Cheng-Wei Chen^{1,2,3}, Van Truong Do⁴, Quang Cuong Truong⁵, Viet Dai Dang⁶, Hong Truong Luu⁶, Yi-Shan Chao³, Yao-Moan Huang⁷, Kuo-Fang Chung²

¹ Biodiversity Program, Taiwan International Graduate Program, Academia Sinica and National Taiwan Normal University, Taipei 115, Taiwan

² Biodiversity Research Center, Academia Sinica, Taipei 115, Taiwan

³ Department of Life Science, National Taiwan Normal University, Taipei 106, Taiwan

⁴ Vietnam National Museum of Nature, Vietnam Academy of Science and Technology, Hanoi, Vietnam

⁵ International Center for Tropical Highlands Ecosystems Research of Bidoup - Nui Ba National Park, Lam Dong, Vietnam

⁶ Institute of Advanced Technology, Vietnam Academy of Science and Technology, Ho Chi Minh City, Vietnam

⁷ Taiwan Forestry Research Institute, Taipei 100, Taiwan

Corresponding authors: Cheng-Wei Chen (wade0504@gmail.com); Kuo-Fang Chung (bochung@gate.sinica.edu.tw)

Abstract

This is the third paper in a series dedicated to updating the knowledge of the Vietnamese pteridophyte flora. Based on recent collections, we first reported three new national records of ferns: *Haplopteris yakushimensis*, *Lindsaea kohkongensis*, and *Pteris pseudowulaiensis*. Secondly, we conducted phylogenetic analyses to investigate the placements of *Lindsaea kohkongensis* and *Leptochilus poilanei*, each based on three plastid DNA markers. Our results revealed that *Lindsaea kohkongensis* is sister to *L. ensifolia*, while *Leptochilus poilanei* is embedded within *L. cantoniensis*. We discussed these results in the context of systematics. Lastly, we reported chromosome numbers for 20 fern species in Vietnam. For seven of these species, including *Gymnosphaera salletii*, *Lepisorus spicatus*, *Leptochilus poilanei*, *Pteridrys costularis*, *Pteris latipinna*, *Pyrrosia eberhardtii*, and *Tectaria setulosa*, these counts were recorded for the first time. Additionally, three new cytotypes were identified for *Diplazium doederleinii*, *Pteris esquirolii*, and *Tectaria harlandii*. This study underscores the need for more diverse data, including DNA sequences, chromosome numbers, and reproductive modes, to be collected and integrated into systematic studies and taxonomic treatments to enhance our understanding of Vietnam's pteridophyte flora.

Key words: Chromosome number, cytology, Indochina, new records, phylogeny



Academic editor: Joel Nitta

Received: 9 November 2024

Accepted: 24 March 2025

Published: 17 April 2025

Citation: Chen C-W, Do VT, Truong QC, Dang VD, Luu HT, Chao Y-S, Huang Y-M, Chung K-F (2025) Studies of Vietnamese Pteridophyte Flora 3. PhytoKeys 255: 143–169. <https://doi.org/10.3897/phytokeys.255.141395>

Copyright: © Cheng-Wei Chen et al.

This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0).

Introduction

This marks the third paper in our series on Vietnam's pterido-flora (Chen et al. 2021; 2023). This series of studies aims to provide updated knowledge of the country's pteridophyte flora, based on recent expeditions as well as the studies of herbarium specimens and relevant literature. Here, we reported three new additions to Vietnam's flora: *Haplopteris yakushimensis* C.W.Chen & Ebihara (Fig. 1), *Lindsaea kohkongensis* I.C.Hwang, M.O.Moon & B.Y.Sun (Fig. 2), and *Pteris pseudowulaiensis* Y.S.Chao (Fig. 3) based on our new collections.

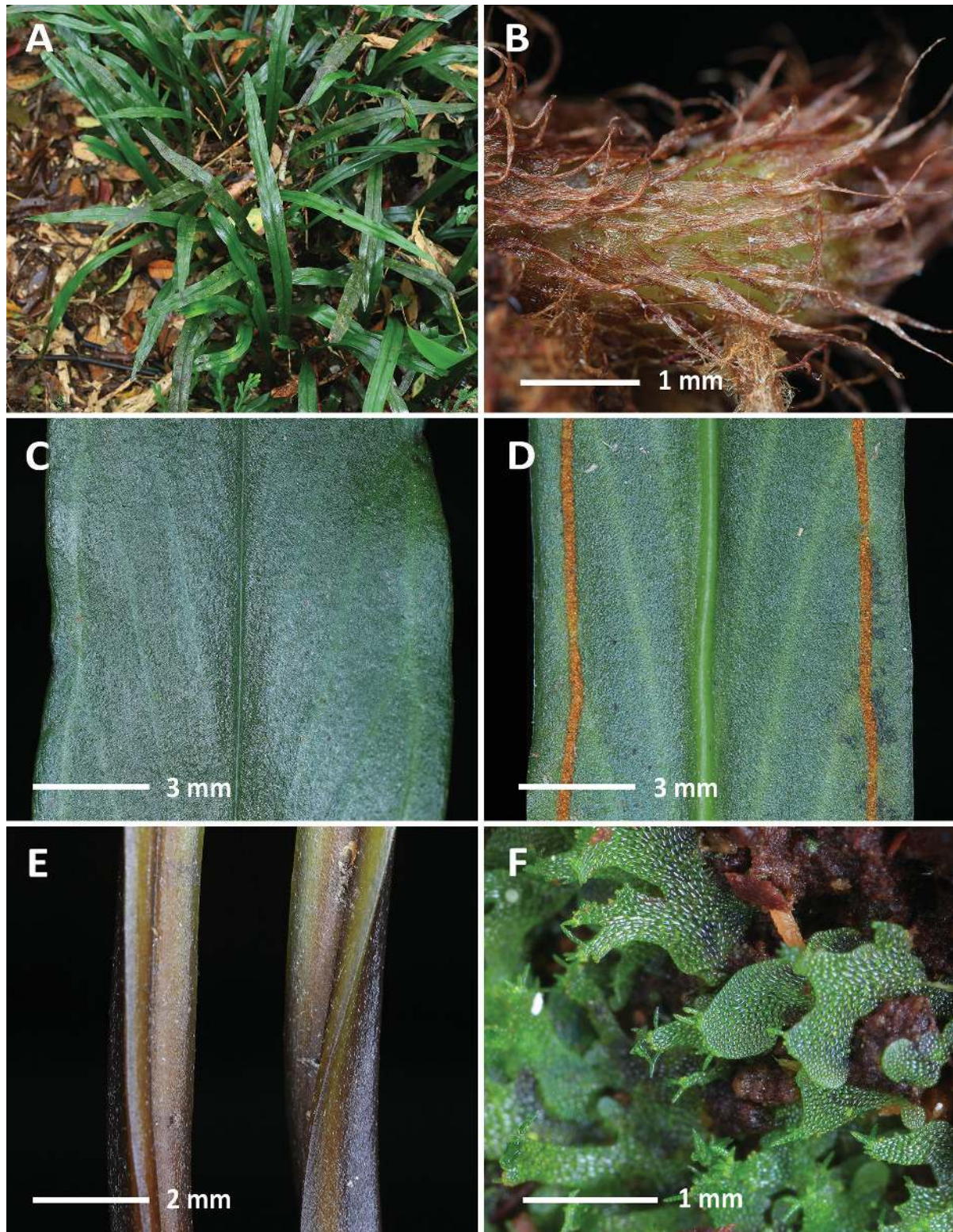


Figure 1. *Haplopteris yakushimensis* C.W.Chen & Ebihara (Chen Wade6952) **A** habit **B** rhizome scales **C** adaxial lamina **D** abaxial lamina **E** stipes **F** gametophytes. Photographed by C.-W. Chen.

Additionally, we performed phylogenetic analyses to elucidate the placements of *Lindsaea kohkongensis* (Fig. 2) and *Leptochilus poilanei* (C.Chr. & Tardieu) Liang Zhang & Li Bing Zhang (Fig. 4). In 2018, we collected an unknown *Lindsaea* specimen from Phu Quoc Island of southern Vietnam. This specimen closely

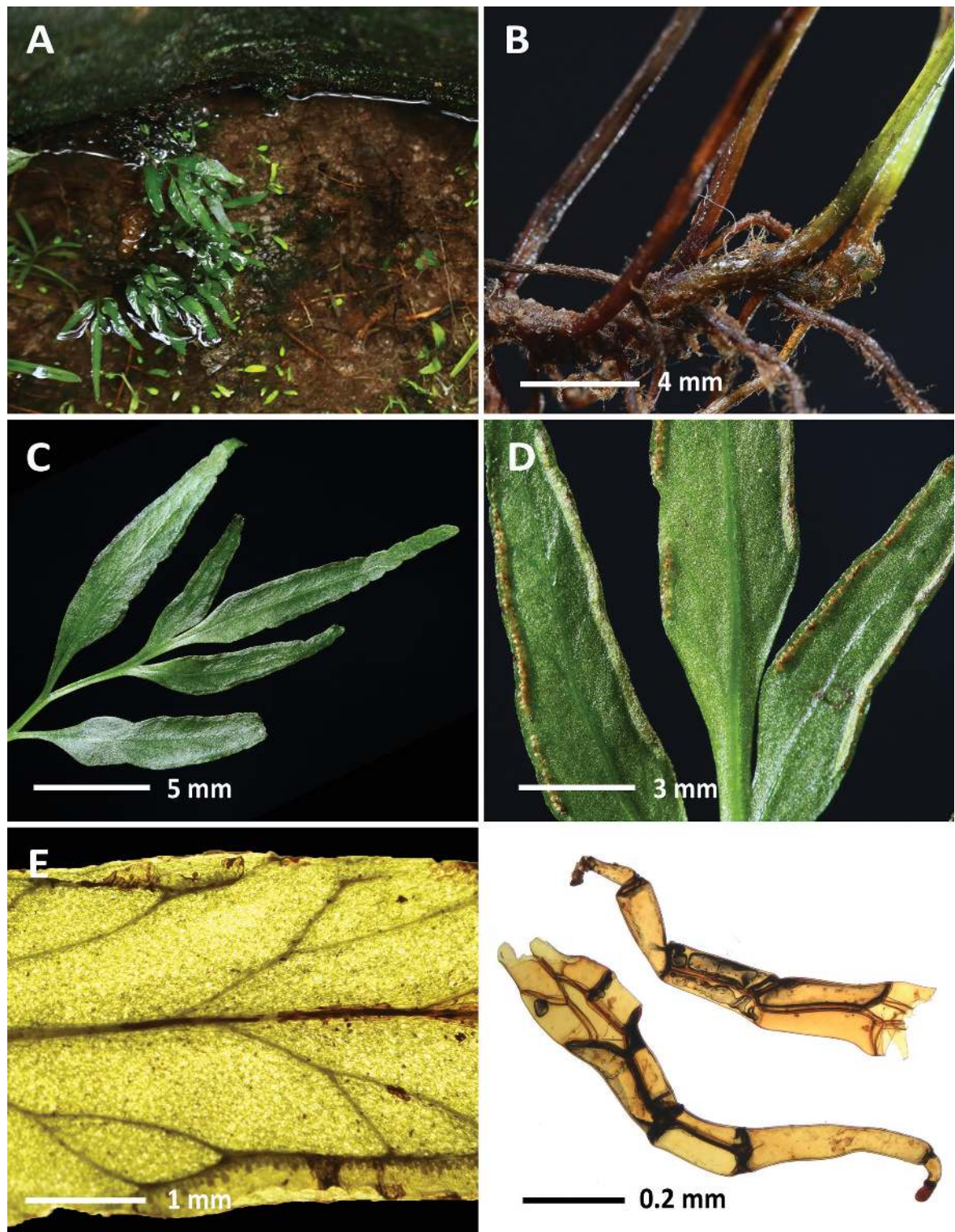


Figure 2. *Lindsaea kohkongensis* I.C.Hwang, M.O.Moon & B.Y.Sun (Chen Wade5034) **A** habit **B** rhizome **C** adaxial lamina **D** abaxial lamina **E** venation **F** rhizome scales. Photographed by C.-W. Chen.

resembles *L. ensifolia* Sw. but being much smaller and with an unusual subaquatic habit. Specimens from Cambodia and Malaysia with the same morphology had recently been described as *Lindsaea kohkongensis* (Yun et al. 2023) but without molecular data. Here, we conducted a phylogenetic analysis to test whether *L. kohkongensis* is a distinct species or an eco-form of *L. ensifolia*.

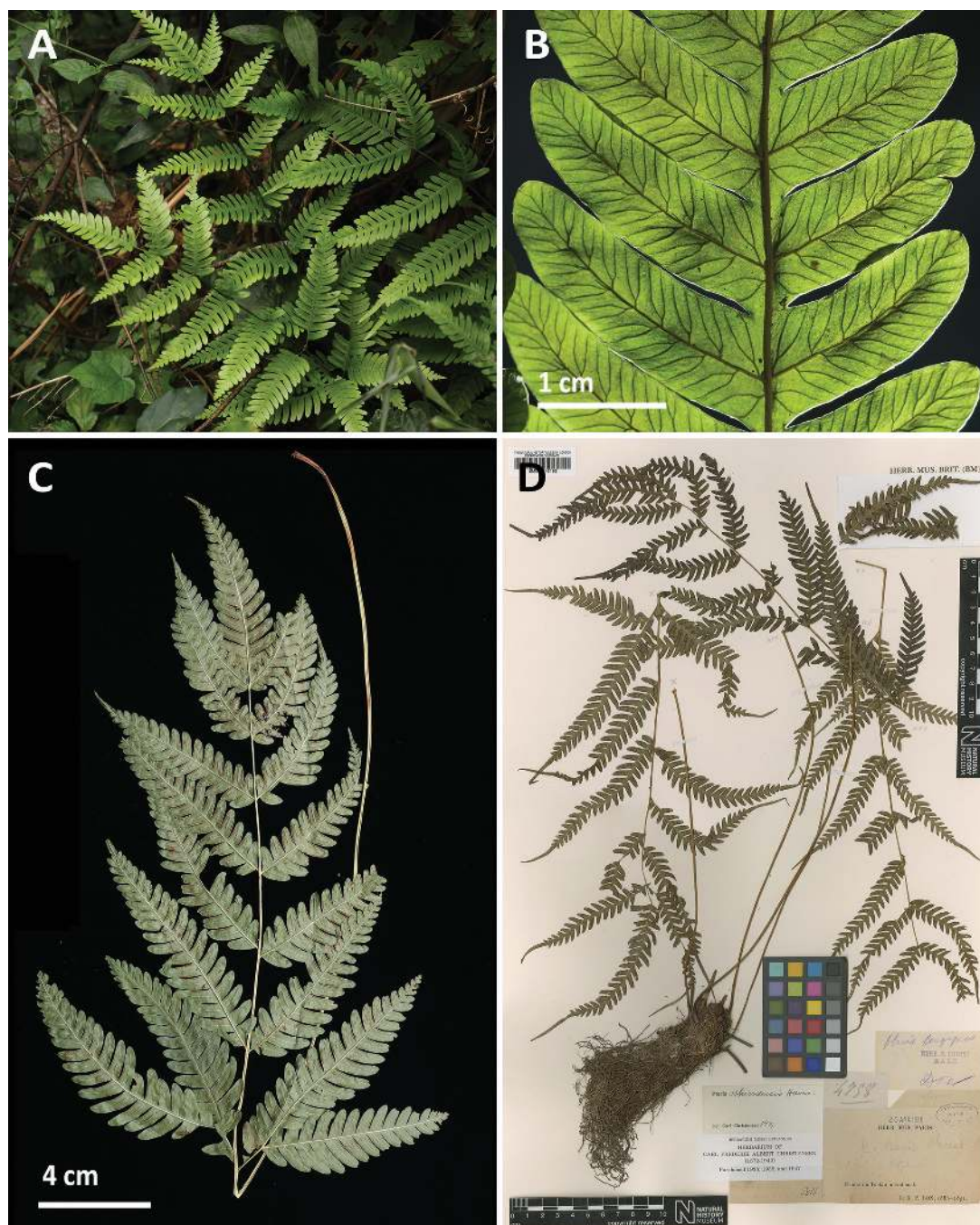


Figure 3. Comparison of *Pteris pseudowulaiensis* C.M.Kuo (A, B, C, Chao 3509 and 3517) and *P. oshimensis* Hieron. (D, Bon 4758) **A** habit **B** lamina (backlight) **C** specimen **D** specimen, CC BY © The Trustees of the Natural History Museum, London. Photographed by C.-W. Chen and Y.-S. Chao.

Leptochilus poilanei was described by Christensen and Tardieu-Blot (1939) under the genus *Colysis* ($\equiv$ *Leptochilus*), based on specimens collected from Annam, which is now part of central Vietnam. Following its initial description, the species remained largely unstudied until Zhang et al. (2018) reclassified it under *Leptochilus*. To our knowledge, this species is only known from its type collection, and our new collection (Fig. 4) from Nui Chua National Park represents the second collection of this species. It can be readily distinguished from other congeneric species in Vietnam by its strongly dimorphic fronds: fertile fronds are mostly linear and simple (rarely tripartite), while sterile ones are simple to pinnatifid.

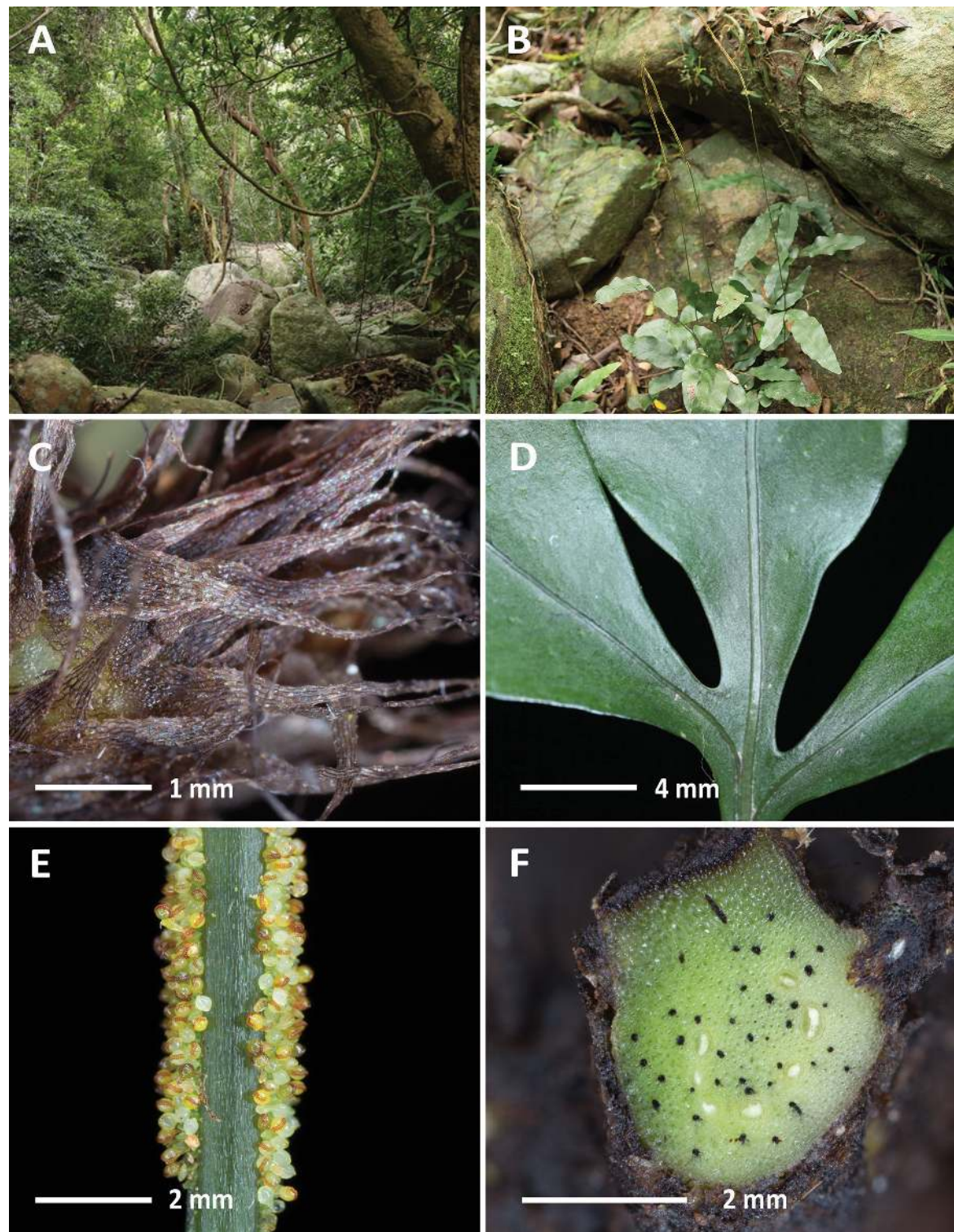


Figure 4. *Leptochilus poilanei* (C.Chr. & Tardieu) Liang Zhang & Li Bing Zhang (*Chen Wade6804*) **A** habitat **B** habit **C** rhizome scales **D** sterile frond **E** fertile frond **F** cross section of rhizome. Photographed by C.-W. Chen.

Lastly, we presented new chromosome counts of Vietnamese fern species and explored the implications of our findings in the context of systematics. Chromosome numbers have long been recognized as important information in plant systematics (Rice et al. 2015). This significance is particularly pronounced for ferns, as they exhibit the highest frequency of polyploid speciation

among vascular plants (Wood et al. 2009). Despite the importance of chromosome number data, research on Vietnamese ferns in this regard has been limited. To date, no systematic survey has been conducted. Instead, available data remains sporadic and dispersed throughout various articles, often in the context of new species' descriptions (e.g., Chen et al. 2023), new species records (e.g., Ding et al. 2013), or cytological studies focused on specific taxa, including specimens collected from Vietnam (e.g., Nayak and Singh 1989). By reporting the chromosome numbers of Vietnamese fern species and demonstrating how these data can be integrated into systematic studies, we aim to raise awareness of chromosome studies among local botanists, thereby encouraging further related research in the future.

Materials and methods

Specimen identification and distribution

We conducted field expeditions in Bidoup Nui-Ba National Park, Cuc Phuong National Park, Phia Oac-Phia Den National Park, Phu Quoc National park, and Nui Chua National Park during 2018–2023. To ensure the correct identifications of our newly collected specimens, we compared them with herbarium specimens, relevant literature, including published papers, checklist, and flora from neighboring countries (see notes under each species), and original protologues and types through the Biodiversity Heritage Library (<https://www.biodiversitylibrary.org/>) and JSTOR Global Plants (<https://plants.jstor.org/>). To confirm the known distribution of each species, we searched the names against World Ferns (Hasler 1994–2024) and GBIF (<https://www.gbif.org/>) and manually examined the available specimen images.

Cytological observations

To determine chromosome numbers, root tips were collected either from the field or transplanted plants in greenhouses. These root tips were treated with a 1:1 mixture of hydroxyquinoline and cycloheximide (Sigma-Aldrich, USA) for approximately 16 hours at 18 °C. Following this, the root tips were fixed in a 3:1 mixture of 95% ethanol and 45% acetic acid for about 12 hours at room temperature. Subsequently, the root tips were macerated using a 1:1 mixture of cellulase (Yakult, Japan) and pectolyase (Sigma-Aldrich, USA) for 1 hour at 37 °C. Finally, the treated root tips were squashed in 2% acetocarmine and observed under a microscope (Zeiss Axio Imager A1, Germany). To determine the ploidy of our observed chromosome numbers, we consulted two online databases, Chromosome Counts Database (CCDB, Rice et al. 2015) and Index to Plant Chromosome Numbers (IPCN, Goldblatt and Lowry 2011), and considered the lowest sporophytic counts known for a genus as the diploid.

Furthermore, we counted the spore numbers per sporangium and observed the spore shape regularity to determine the reproductive modes whenever possible. We also compared the spore sizes of *Leptochilus cantoniensis* (Baker) Ching and *L. poilanei* by measuring 30 spores from each species.

Molecular phylogenetic analysis

To elucidate the phylogenetic placement of *Lindsaea kohkongensis* and *Leptochilus poilanei*, we conducted two phylogenetic analyses each based on three chloroplast markers including *matK*, *trnH-psbA*, and *trnL-F* for *Lindsaea* and *rbcL*, *rps4-trnS*, and *trnL-F* for *Leptochilus*. These markers were chosen to integrate our newly generated sequences into previous studies (Lehtonen et al. 2010; Zhang et al. 2019; Chen et al. 2020a; Fujiwara et al. 2023). We extracted genomic DNA from fresh fronds using Qiagen DNeasy Plant Mini Kit (Hilden, Germany), following the manufacturer's protocol. We conducted PCR to amplify the five DNA markers using the primers listed in Table 1.

For *Lindsaea*, we included the sequences of 26 specimens as listed in Appendix 1: Table A1. Among these, the sequences of 14 specimens were downloaded from GenBank. To test the monophyly of *L. ensifolia*, this sampling included 1) *L. ensifolia* from a broad geographic range, including Bangladesh, Brunei, Cambodia, India, Malaysia, Nepal, Solomon Islands, Thailand, and Vietnam; and 2) closely related species identified by Lehtonen et al. (2010). For *Leptochilus*, in addition to our newly sequenced *L. poilanei*, we download the sequences of another 21 specimens from GenBank. In total, 22 specimens representing 19 species were included in the phylogenetic analysis as listed in Appendix 1: Table A2. This sampling covered all the major clades found in previous studies (Zhang et al. 2019; Chen et al. 2020a).

For our newly generated sequences, we first manually inspected raw reads, removing any ambiguous bases using BioEdit (Hall 1999). Subsequently, we aligned the sequences of each marker using MUSCLE (Edgar 2004) with default settings. These individual marker alignments were then concatenated into a single alignment, because chloroplast genome is non-recombining and therefore has a single evolutionary history. We then conducted maximum likelihood (ML) analyses using IQTREE (Minh et al. 2020) each with five partitions (three codon positions for *matK* and *rbcL*, and *rps4-trnS*, *trnH-psbA* and *trnL-F*, each treated as a single partition for simplification although they contain partly coding regions). The best-fit model for each partition and the best-fit partition scheme were determined by ModelFinder (Lanfear et al. 2012; Kalyaanamoorthy et al. 2017) as implemented in IQTREE (Minh et al. 2020). To assess branch support, we performed 1000 ultrafast bootstrap replicates using UFBoot (Hoang et al. 2018). The two concatenated alignments and the resulting phylogenetic trees are available on the Dryad Digital Repository (Chen et al. 2024).

Table 1. PCR Primers used in this study.

Region	Name	Sequence 5' to 3'	Reference
<i>matK</i>	FERN <i>matK</i> fEDR	ATTCATTCRATRTTTTATTHTGGARGAYAGATT	Kuo et al. 2011
<i>matK</i>	DeLin <i>matK</i> rNRD	CTACGCAAYSCATCYCGATT	Kuo et al. 2011
<i>rbcL</i>	1F	ATGTCAACCACAAACAGAAAC	Fay et al. 1997
<i>rbcL</i>	1379R	TCACAAGCAGCAGCTAGTTCAGGACTC	Wolf et al. 1999
<i>rps4-trnS</i>	<i>rps5</i>	ATGTCCCGTTATCGAGGACCT	Nadot et al. 1995
<i>rps4-trnS</i>	<i>trnSR</i>	TACCGAGGGTTTCAATC	Smith and Cranfill 2002
<i>trnH-psbA</i>	<i>trnH</i>	CGCGCATGGTGGATTCAATCC	Tate and Simpson 2003
<i>trnH-psbA</i>	<i>psbA3'f</i>	GTTATGCATGAACGTAATGCTC	Sang et al. 1997
<i>trnL-F</i>	FernL1lr1	GGYAATCCTGAGCCAAATC	Li et al. 2009
<i>trnL-F</i>	F	ATTTGAACCTGGTGACACGAG	Taberlet et al. 1991

Results

Phylogenetic placement of *Lindsaea kohkongensis*

The concatenated three-marker alignment contains 1832 bp including 85 parsimony informative sites and 39.2% missing data (17,973 bp). ModelFinder merged all the five partitions into one alignment with the best-fit model identified as K3Pu+F+R2. The phylogram generated by the ML analysis based on the concatenated alignment is shown in Fig. 5. The inferred species relationships closely align with the phylogeny reconstructed by Lehtonen et al. (2010) although the nodes are poorly supported in general. However, *Lindsaea kohkongensis* is strongly supported as the sister group to a clade comprising all the *L. ensifolia* specimens included in this analysis.

Phylogenetic placement of *Leptochilus poilanei*

The concatenated three-marker alignment contains 3331 bp including 248 parsimony informative sites and 17.8% missing data (13077 bp). ModelFinder subset the alignment into two partitions, the first including first and third codon position of *rbcL*, and the second including the second codon position of *rbcL* along with

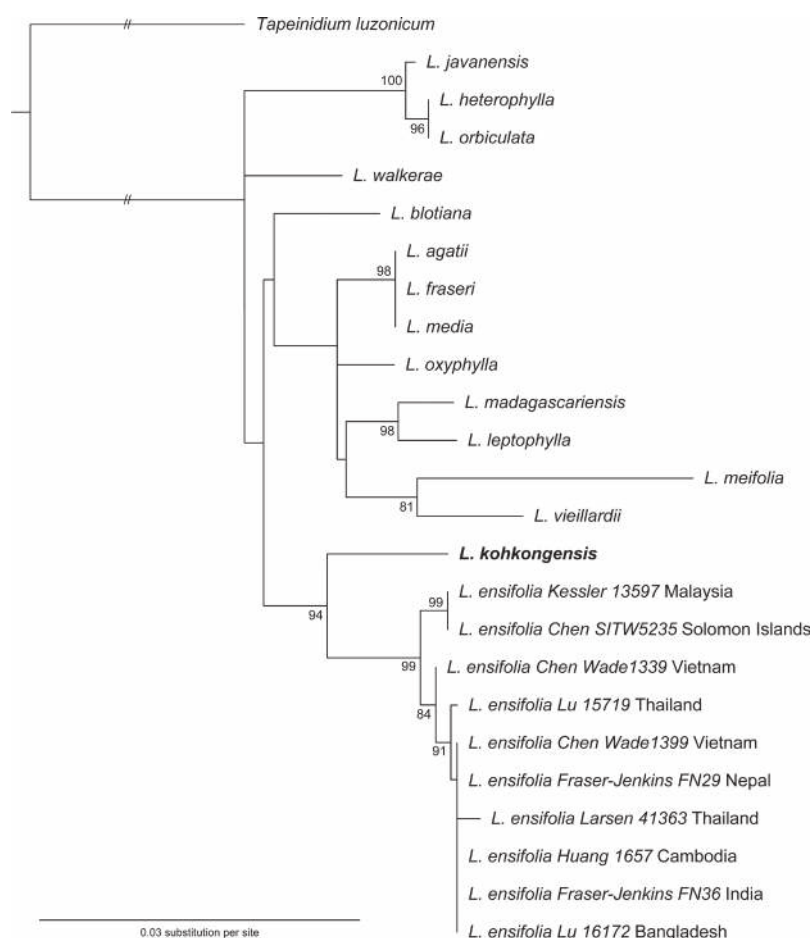


Figure 5. Phylogram of *Lindsaea* reconstructed from the maximum likelihood analysis of the concatenated plastid dataset (*matK*, *trnH-psbA*, and *trnL-F*). The branch length to the outgroup *Tapeinidium* is not to scale for better visualization. Support values below 80 are not shown on the nodes. *L. kohkongensis* is indicated in bold.

rps4-trnS and *trnL-F*. The best-fit models determined for the two partitions were TNe+I+G4 and K3Pu+F+R2, respectively. The phylogram generated by the ML analysis based on the concatenated alignment is shown in Fig. 6. The inferred species relationships are similar to previous studies (Zhang et al. 2019; Chen et al. 2020a) as expected, although poorly supported in general. *Leptochilus poilanei* is nested in a highly supported clade containing three specimens of *L. cantoniensis*.

Both *L. cantoniensis* (based on Hsu 3399) and *L. poilanei* (based on Chen Wade6804) produce 64 spores in each sporangium with normal morphology. The spore size of *L. cantoniensis* and *L. poilanei* is 44.3 ± 3.4 and 63.0 ± 4.9 μm , respectively. The spores of *L. poilanei* are significantly larger than those of *L. cantoniensis* (t-test, $p < 0.001$).

Chromosome number of 20 species

We successfully counted the chromosome numbers of 20 species, each determined by multiple cells. Their chromosome numbers and ploidy levels inferred from the known lowest base number are shown in Table 2. Figs 7–13 illustrated representative cells for all the 20 species. Among these, chromosome numbers were recorded for the first time for seven species: *Gymnosphaera salletii* (Tardieu & C.Chr.) S.Y.Dong, *Lepisorus spicatus* (L.f.) Li Wang, *Leptochilus poilanei*, *Pteridrys costularis* Li Bing Zhang, Liang Zhang, N.T.Lu & X.M.Zhou, *Pteris latipinna* Y.S.Chao & W.L.Chiou, *Pyrrosia eberhardtii* (Christ) Ching, and *Tectaria setulosa* (Baker) Holttum. Furthermore, we reported the new cytotypes for three species: *Diplazium doederleinii* (Luer) Makino, *Pteris esquirolii* H.Christ, and *Tectaria harlandii* (Hook.) C.M.Kuo.

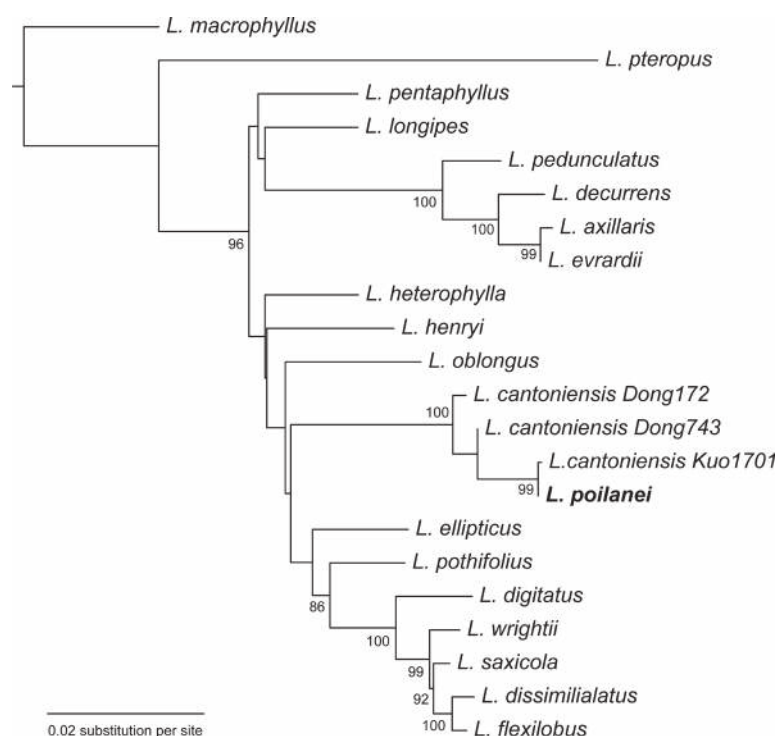


Figure 6. Phylogram of *Leptochilus* reconstructed from the maximum likelihood analysis of the concatenated plastid dataset (*rbcL*, *rps4-trnS*, and *trnL-F*). Support values below 80 are not shown on the nodes. *L. poilanei* is indicated in bold.

Table 2. Plant materials used in this study for chromosome counting, their inferred ploidy, spores number in each sporangium (s/s), and corresponding figures. Asterisks “*” indicate newly reported species. Hash marks “#” indicate newly reported cytotypes. Em-dashes “—” indicate missing data.

Taxa	Voucher	Chromosome no.	Ploidy	s/s	Figure
<i>Asplenium normale</i> D.Don	Chen Wade5121	2n = 72	2x	64	7A
<i>Asplenium tenerum</i> G.Forst.	Chen Wade5342	2n = 144	4x	64	7B
<i>Ctenitis eatonii</i> (Baker) Ching	Chen Wade6815	2n = 82	2x	—	7C
<i>Didymochlaena truncatula</i> (Sw.) J.Sm	Chen Wade5817	2n = 82	2x	64	8A
<i>Diplazium doederleinii</i> (Lueress.) Makino#	Chao 3524	2n = 82	2x	64	8B
<i>Diplazium donianum</i> (Mett.) Tardieu	Chen Wade6853	2n = 123	3x	—	8C
<i>Gryothrix simplex</i> (Hook.) S.E.Fawc. & A.R.Sm	Chen Wade6879	2n = 72	2x	64	9A
<i>Gymnosphaera salletii</i> (Tardieu & C.Chr.) S.Y.Dong*	Chen Wade6586	2n = 138	2x	64	9B
<i>Lepisorus spicatus</i> (L.f.) Li Wang*	Chen Wade6598	2n = 70	2x	—	9C
<i>Leptochilus poilanei</i> (C.Chr. & Tardieu) Liang Zhang & Li Bing Zhang*	Chen Wade6804	2n = 144	4x	64	10A
<i>Pleocnemia winitii</i> Holttum	Chao 3534	2n = 82	2x	64	10B
<i>Polystichum biaristatum</i> (Blume) T.Moore	Chen Wade5070	2n = 82	2x	64	10C
<i>Pteridrys costularis</i> Li Bing Zhang, Liang Zhang, N.T.Lu & X.M.Zhou*	Chen Wade6819	2n = 82	2x	—	11A
<i>Pteris cadieri</i> H.Christ	Chen Wade5737, Chao 3501	2n = 87	3x	32	11B, 11C
<i>Pteris esquirolii</i> H.Christ#	Chao 3511	2n = 58	2x	64	12A
<i>Pteris latipinna</i> Y.S.Chao & W.L.Chiou*	Chao 3526	2n = 58	2x	32	12B
<i>Pteris pseudowulaiensis</i> C.M.Kuo	Chao 3515	2n = 58	2x	32	12C
<i>Pyrrosia eberhardtii</i> (Christ) Ching*	Chen Wade5607	2n = 74	2x	—	13A
<i>Tectaria harlandii</i> (Hook.) C.M.Kuo#	Chen Wade6874	2n = 80	2x	—	13B
<i>Tectaria setulosa</i> (Baker) Holttum *	Chen Wade6852	2n = 80	2x	64	13C

Discussion

Systematic uniqueness of *Lindsaea kohkongensis*

When we collected this species from Phu Quoc island in 2018, we were hesitant to describe it. Morphologically, it closely resembles *L. ensifolia*, leading us to question if it might be an eco-form of the latter. In this study, we conducted a phylogenetic analysis based on three chloroplast regions, sampling *L. ensifolia* from a wide geographic distribution throughout Asia and the Pacific Islands. The results indicate that *L. kohkongensis* is sister to *L. ensifolia* rather than being embedded within it (Fig. 6). On one hand, this suggests that *L. kohkongensis* can be recognized as a distinct species based on the principle of monophyly. On the other hand, it could also be classified within a broadly circumscribed *L. ensifolia*. To our knowledge, *L. ensifolia* typically grows terrestrially in a diverse habitat from swampy forests, dipterocarp forests, to exposed grasslands (Kramer 1971; personal observation). In contrast, *L. kohkongensis* is exclusively found in shaded valleys as lithophytes and is frequently submerged in water (Yun et al. 2023; personal observation). This distinction suggests an ecological speciation event that needs to be tested in the future.

While identifying our specimen from Phu Quoc island, we came across a rheophyte form of *L. ensifolia* described as *L. ensifolia* var. *rheophila* K.Iwats from Sumatra (Iwatsuki 1977). After examining the type materials deposited in KYO, we concluded that this is the same species as *L. kohkongensis* and should be treated as a synonym of it. As noted by Kramer (1967), *L. ensifolia* is one of the most widespread and variable species of the genus. Apart from var. *rheophila*, few other

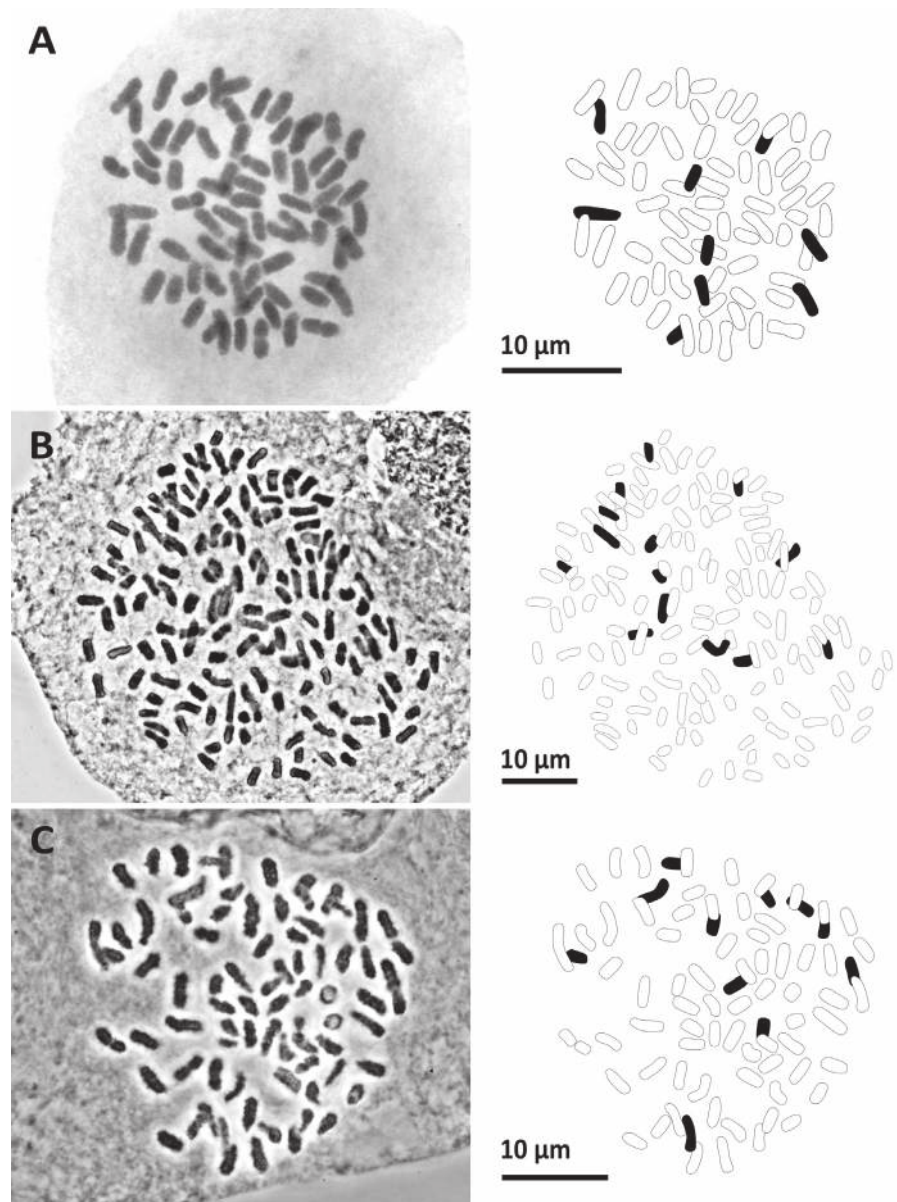


Figure 7. Mitotic chromosomes with explanatory illustrations **A** *Asplenium normale* ($2n = 72$) **B** *Asplenium tenerum* ($2n = 144$) **C** *Ctenitis eatonii* ($2n = 82$).

subspecies or varieties have been published, such as subsp. *coriacea* (Alderw.) K.U.Kramer from Bornean swamp forests. Furthermore, ploidy variations (diploid, triploid, and tetraploid) and different reproductive modes (sexual and apomixis) have been observed in *L. ensifolia* (e.g., Lin et al. 1996). Although we here tentatively recognize *L. kohkongensis* as a distinct species, the variability observed in *L. ensifolia* underscores the need for a systematic study of this complex species.

Hybrid origin hypothesis of *Leptochilus poilanei*

Our phylogenetic analysis based on three plastid markers unambiguously resolves *L. poilanei* within a clade that includes three specimens of *L. cantoniensis* (Fig. 6). This result is unexpected, considering the clear morphological distinctions between these two species. Specifically, fully developed sterile fronds of *L. poilanei* are pinnatifid, while those of *L. cantoniensis* remain consistently simple (Fig. 14).

Our examination of the reproductive mode indicates that both species undergo sexual reproduction, as evidenced by the production of 64 well-formed spores in each sporangium. The mitotic chromosome count for *L. poilanei* is $2n = 144$ (Fig. 10A), suggesting it is a tetraploid, given the base number of the genus is $x = 36$ (Rice et al. 2015). Although the chromosome number of *L. cantoniensis* is presently unknown, its spores are significantly smaller than those of *L. poilanei* (44.3 ± 3.4 vs. $63.0 \pm 4.9 \mu\text{m}$). Considering the widely found correlation between ploidy and spore size in ferns (Barrington et al. 1986), we propose that *L. cantoniensis* is likely a diploid species.

Building on the evidence from our study, we propose that *L. poilanei* has a hybrid origin. We suggest that *L. poilanei* originated from hybridization between *L. cantoniensis* (as the maternal parent, considering chloroplast inheritance in ferns, e.g., Gastony and Yatskievych 1992) and an unidentified diploid species, followed by polyploidization. The close similarity in chloroplast DNA markers between *L. cantoniensis* and *L. poilanei* suggests a relatively recent hybridization event. This hypothesis gains further support from the overlapping distribution of *L. cantoniensis* and *L. poilanei* in central Vietnam. While the identity of the other parent remains elusive, we anticipate it is likely a species with pinnatifid fronds, given that *L. poilanei* exhibits this characteristic. Future studies employing bi-parentally inherited nuclear markers and comprehensive sampling of the genus in Vietnam are essential to test our hypothesis.

New findings of chromosome number

In the following paragraphs, we discussed the systematic implications of our new finding (i.e., new species counts or new cytotypes) species by species, following alphabetical order.

***Diplazium doederleinii* (Luer.) Makino— $2n = 82$, sexual diploid, Fig. 8B**

Previous studies reported triploid ($2n = 123$) and tetraploid ($2n = 164$) specimens from Japan (Takamiya et al. 2001). Our discovery of a diploid specimen from Vietnam provides further support for the long-observed cytogeographic pattern: diploids are typically found in warmer tropics, whereas polyploids are prevalent in cooler temperate regions (e.g. Chang et al. 2013). As demonstrated by Takamiya et al. (2001), there is a little genetic divergence between triploids and tetraploids. Future studies could incorporate the newly discovered diploid into genetic analyses to further investigate the origins of polyploids.

***Gymnosphaera salletii* (Tardieu & C.Ch.) S.Y.Dong— $2n = 138$, sexual diploid, Fig. 9B**

The first chromosome count reported for the species. This Vietnam endemic species was recently reevaluated by Li et al. (2024), who described two new species previously been misidentified as *G. salletii*. *Gymnosphaera* is predominantly a paleotropical genus, comprising approximately 46 species (Dong and Zuo 2018). All four previously reported species with chromosome counts in the genus are diploid, namely *G. capensis* (L.f.) S.Y.Dong, *G. gigantea* (Wall. ex Hook.) S.Y.Dong, *G. khasyana* (T.Moore ex Kuhn) Ching, and *G. podophylla*

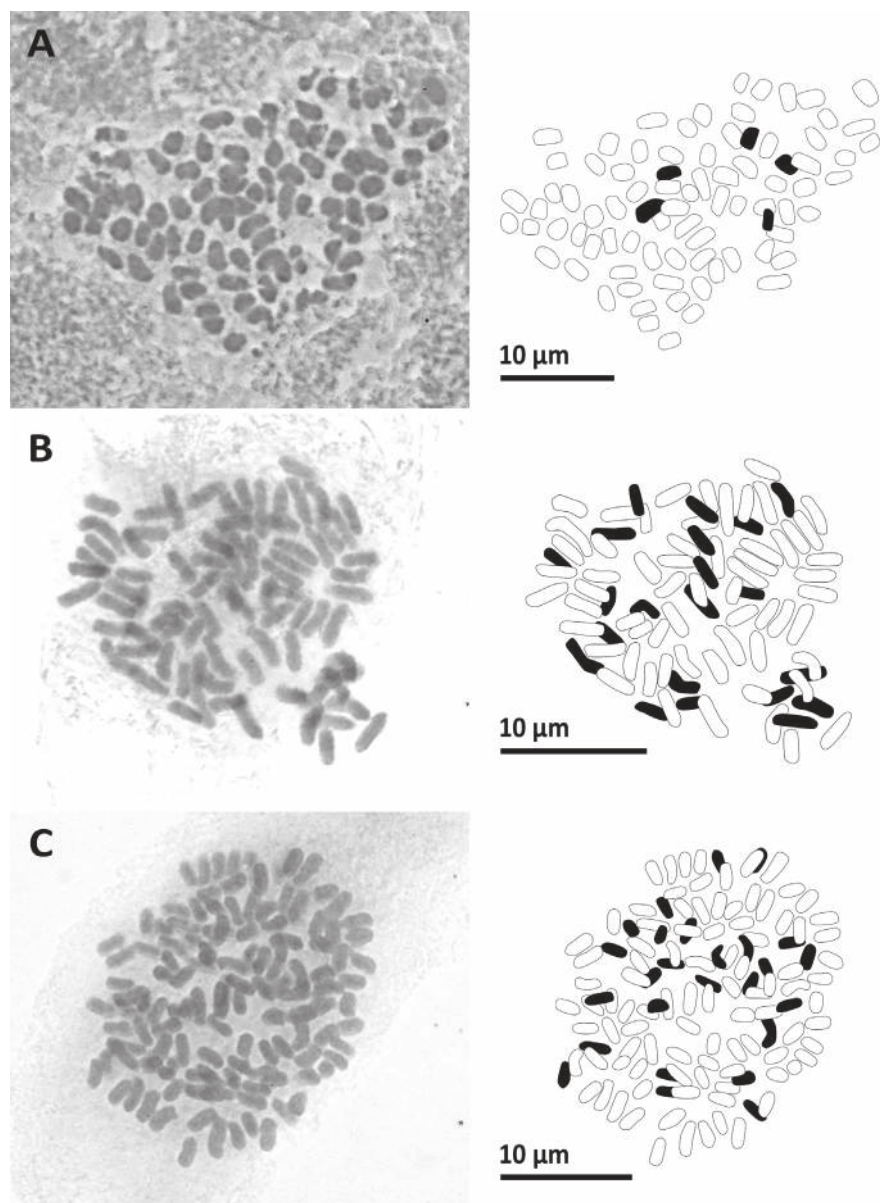


Figure 8. Mitotic chromosomes with explanatory illustrations **A** *Didymochlaena truncatula* ($2n = 82$) **B** *Diplazium doederleinii* ($2n = 82$) **C** *Diplazium donianum* ($2n = 123$).

(Hook.) Copel. (Manton and Sledge 1954; Löve et al. 1977; Kato 1999). Notably, there is a correlation between geographic distribution and the basic chromosome number: all Asian species exhibit $x = 69$, while African species have $x = 70$.

***Lepisorus spicatus* (L.f.) Li Wang— $2n = 70$, diploid, Fig. 9C**

The first chromosome count reported for the species. Found across the paleotropics, this species has often been confused with *L. mucronatus* (Fée) Li Wang. Following Hovenkamp (1998), we identify the species by the presence of entire rhizome scales with hyaline margins. Following Zhao et al. (2020), this species belongs to section *Belvisia* (Mirb.) C.F. Zhao, R.Wei & X.C. Zhang, a small clade comprising eight species (Hovenkamp and Franken 1993). *Lepisorus mucronatus* is the only other species in this section with known chromosome numbers and has been determined as tetraploid ($2n = 140$) from Australia (Tindale and Roy 2002).

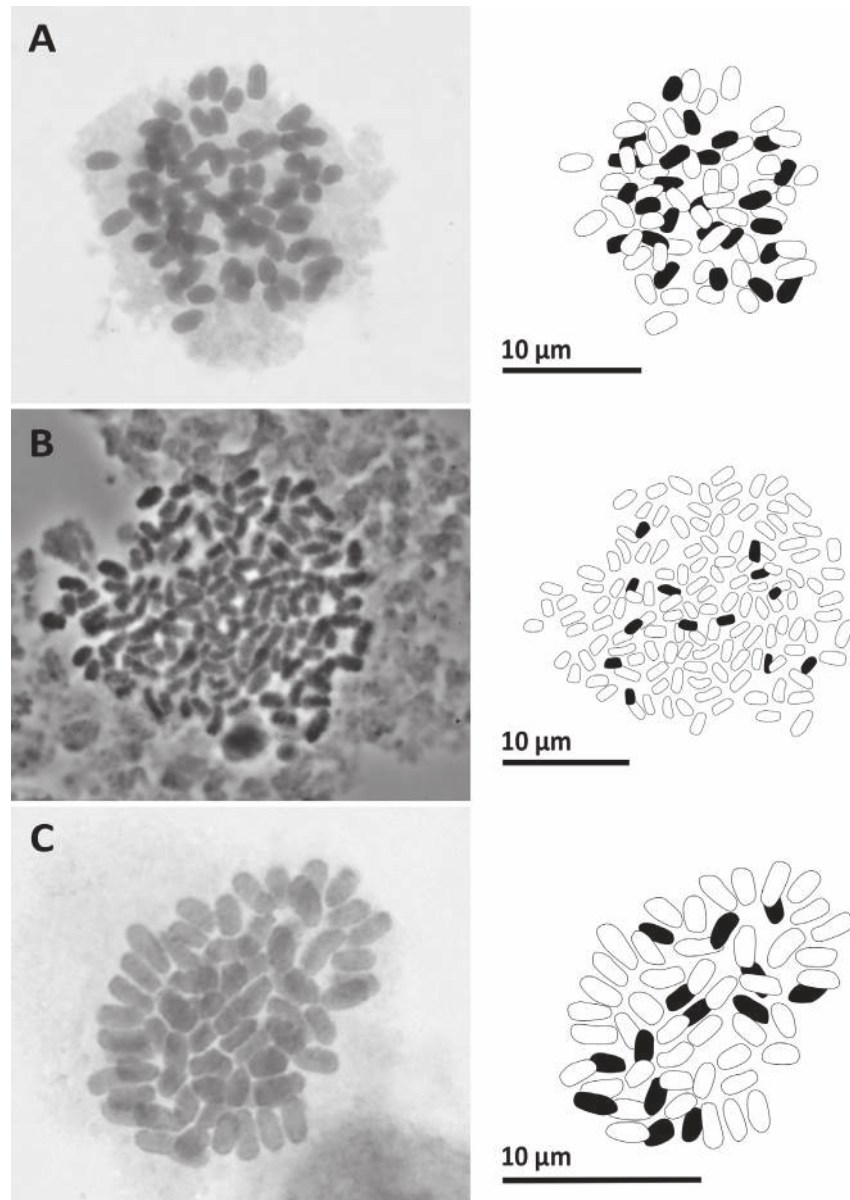


Figure 9. Mitotic chromosomes with explanatory illustrations **A** *Gypsothrix simplex* ($2n = 72$) **B** *Gymnosphaera salletii* ($2n = 138$) **C** *Lepisorus spicatus* ($2n = 70$).

***Leptochilus poilanei* (C.Chr. & Tardieu) Liang Zhang & Li Bing Zhang— $2n = 144$, sexual tetraploid, Fig. 10A**

As discussed in previous sections, we hypothesize that this Vietnam endemic species originated from a hybridization between *L. cantoniensis* and an unidentified diploid species, followed by polyploidization. *Leptochilus* is predominantly a tropical Asian genus with approximately 51 species (Zhang et al. 2024). Prior to this study, chromosome numbers were known for only four species including: *L. axillaris* (Cav.) Kaulf., *L. decurrens* Blume, *L. ellipticus* (Thunb.) Noot., and *L. pothifolius* (Buch.-Ham. ex D.Don) Fraser-Jenk. (e.g., Mitui 1968; Löve et al. 1977; Manickam and Irudayaraj 1989). Among these four species, *L. decurrens* is the only one reported to exist in both diploids and tetraploids, while the other three species are known only as diploids.

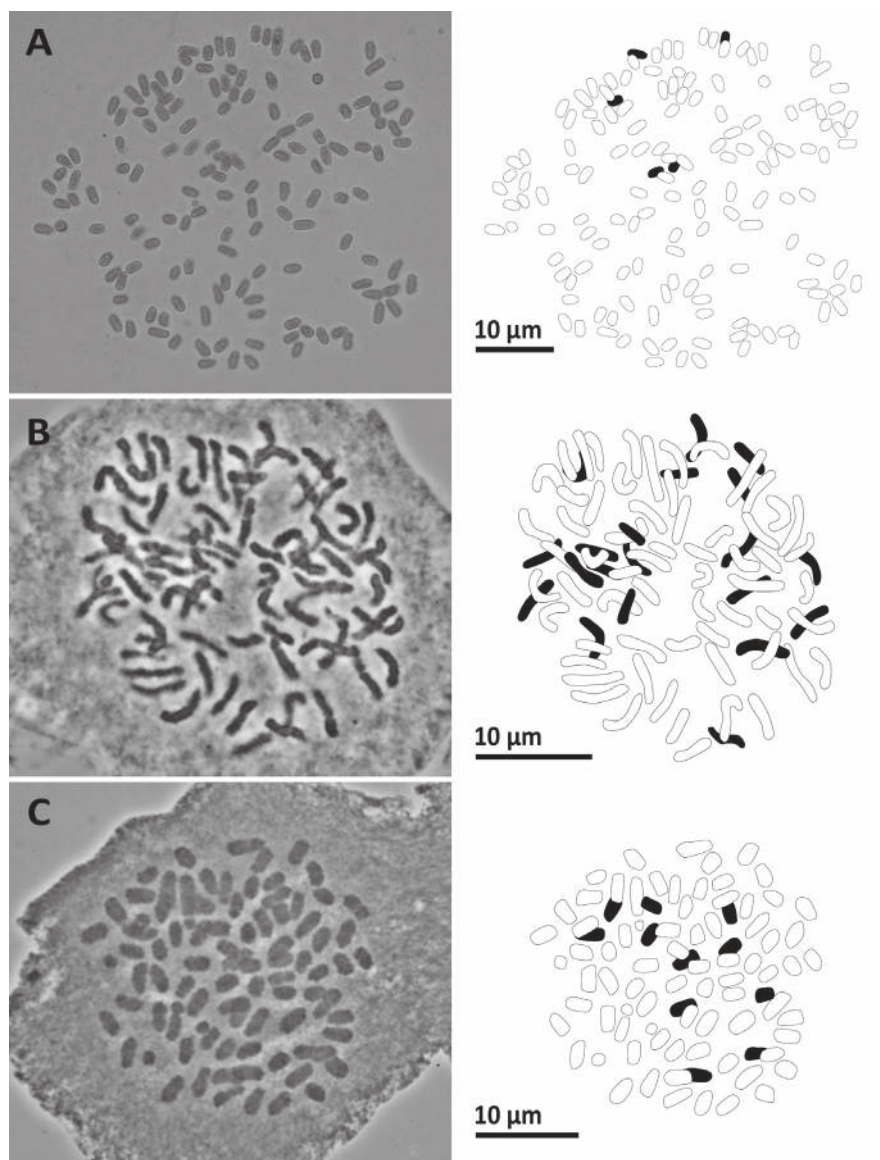


Figure 10. Mitotic chromosomes with explanatory illustrations **A** *Leptochilus poilanei* ($2n = 144$) **B** *Pleocnemia winitii* ($2n = 82$) **C** *Polystichum biaristatum* ($2n = 82$).

***Pteridrys costularis* Li Bing Zhang, Liang Zhang, N.T.Lu & X.M.Zhou— $2n = 82$, diploid, Fig. 11A**

The first chromosome count reported for the species. *Pteridrys* is a tropical Asian genus with ca. 22 species, and 11 of them are recorded in Vietnam (Zhou and Zhang 2019). So far, all the four species (the other three are *P. australis* Ching ex C.Chr. & Ching, *P. cnemidaria* (Christ) C.Chr.et.Ching, and *P. syrmatica* (Willd.) C.Chr. & Ching) with reported chromosome numbers are diploids (Manton 1954; Tsai and Shieh 1985; Bidin and Go 1995).

***Pteris esquirolii* H.Christ— $2n = 58$, sexual diploid, Fig. 12A**

This species is reported from southern China, Taiwan, and Vietnam (Chao 2019). Prior to this study, the only reported chromosome count was $2n = \text{ca. } 90$

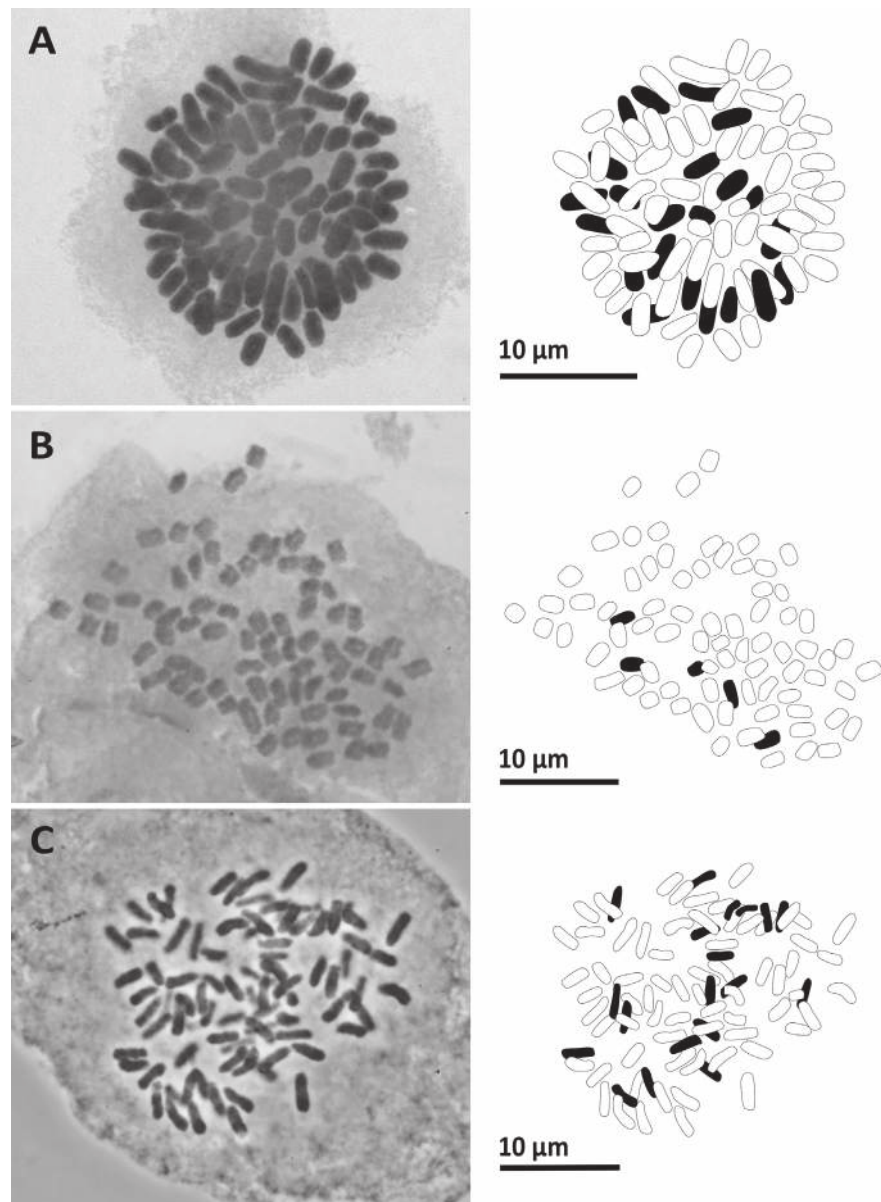


Figure 11. Mitotic chromosomes with explanatory illustrations **A** *Pteridrys costularis* ($2n = 82$) **B** *Pteris cadieri* ($2n = 87$) **C** *Pteris cadieri* ($2n = 87$).

(presumably an apogamous triploid due to the count of 32 spores per sporangium) from southern China (Lin et al. 2002). Although Chao (2019) found very little genetic divergence among populations from China and Taiwan, it may be worthwhile to include the Vietnamese population in future analyses, given their different ploidy level and reproductive mode. This is particularly important considering the prevalence of reticulate evolution observed in the genus (e.g., Chao et al. 2012, 2022).

***Pteris latipinna* Y.S.Chao & W.L.Chiou— $2n = 58$, apogamous diploid, Fig. 12B**

This is the first chromosome count reported for this species. This species was firstly described from Taiwan (Chao et al. 2017) and later reported from China and Vietnam (Chen et al. 2020b). Our chromosome counts support the hypothesis proposed by Chao et al. (2022) that *P. latipinna* is a diploid,

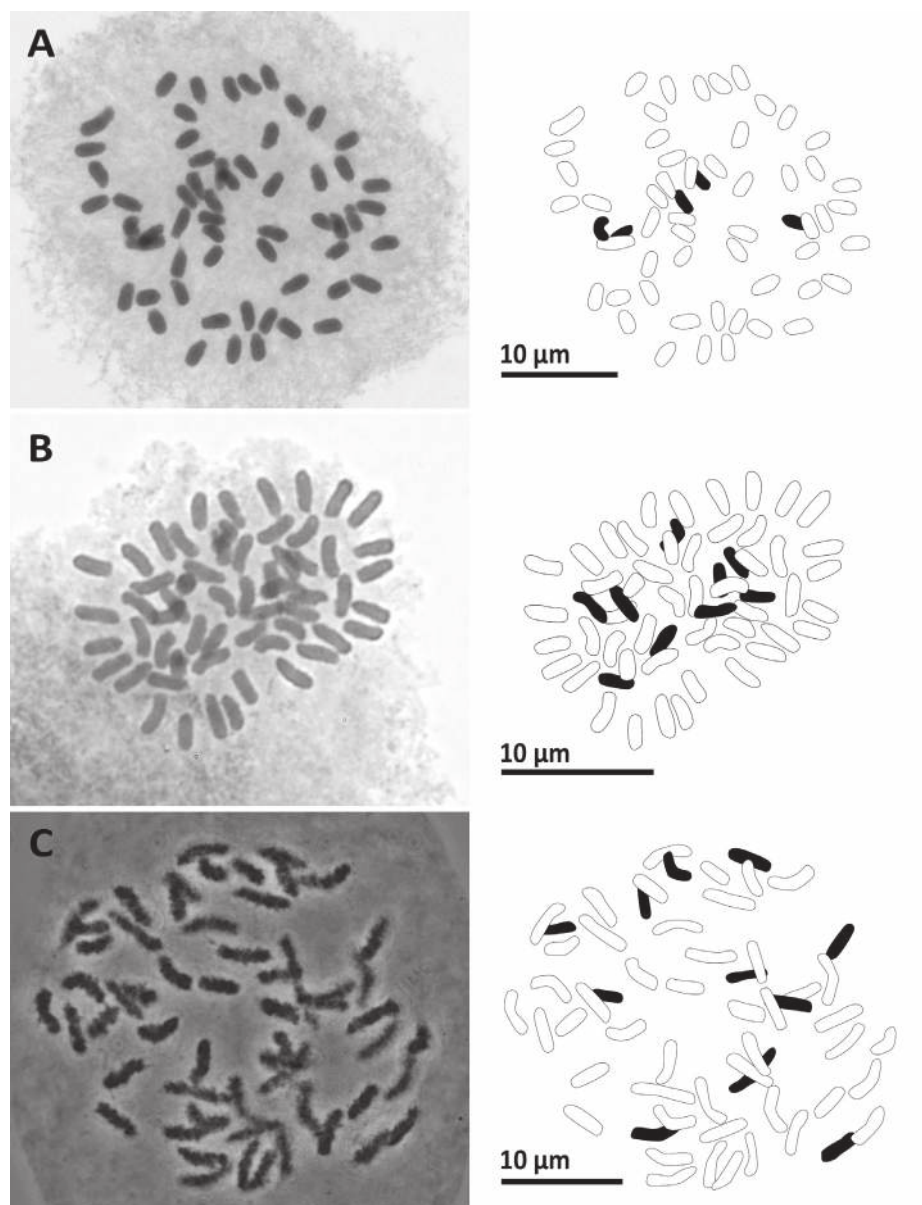


Figure 12. Mitotic chromosomes with explanatory illustrations **A** *Pteris esquirolii* ($2n = 58$) **B** *Pteris latipinna* ($2n = 58$) **C** *Pteris pseudowulaiensis* ($2n = 58$).

as indicated by flow cytometry. Our spore number counts also confirm that *P. latipinna* is reproduced through apogamous reproduction, which is common for the genus (Walker 1962).

***Pyrrosia eberhardtii* (Christ) Ching— $2n = 74$, diploid, Fig. 13A**

The first chromosome count reported for the species. This species is recorded in southern China, Thailand, and Vietnam, with its type locality in Vietnam. It has sometimes been grouped under a broadly defined *P. lingua* (Thunb.) Farw. alongside other closely related species such as *P. oblonga* Ching and *P. heteractis* (Mett.) Ching. (Hovenkamp 1986). This broader classification of *P. lingua* is further supported by Zhou et al. (2017), showing a close relationship among these species. The chromosome numbers of *P. lingua* have been reported from China and Japan, and both are diploids (e.g., Takei 1969; Kato 1999).

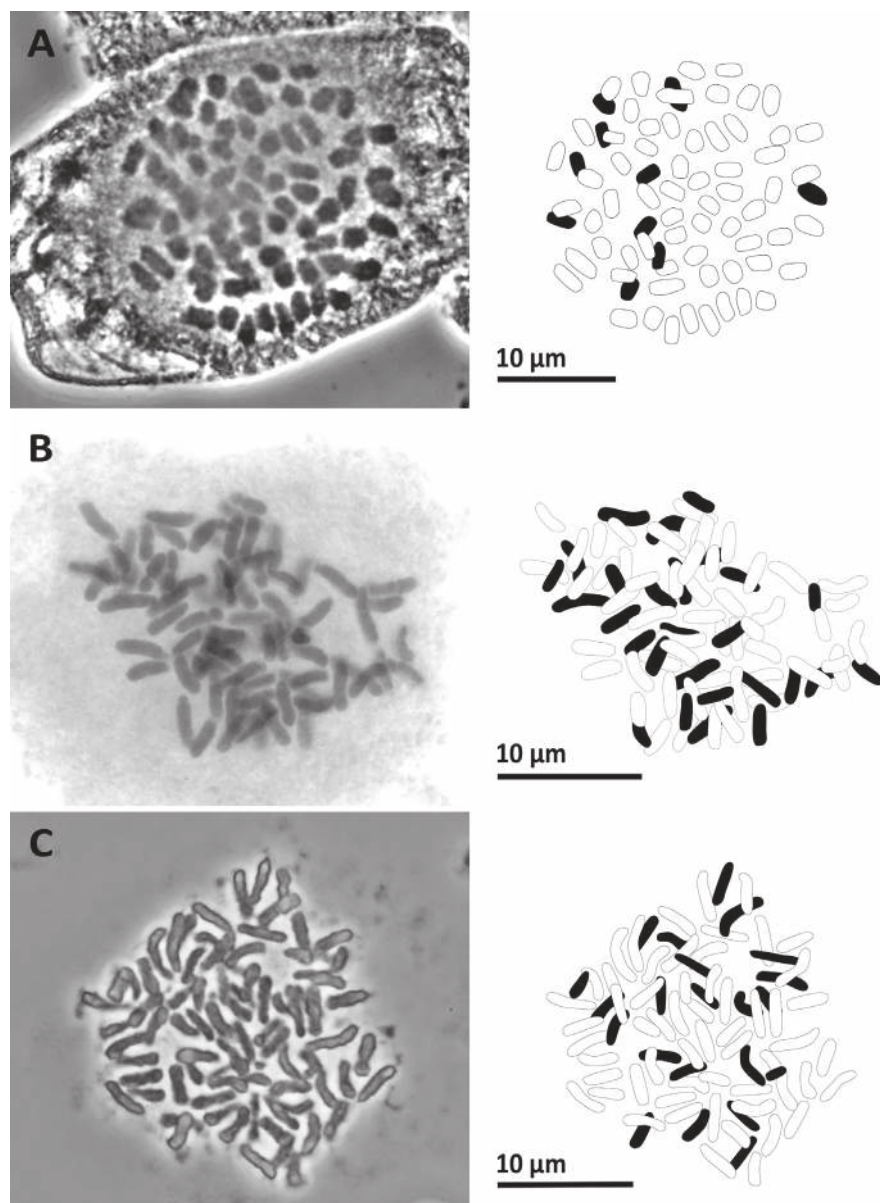


Figure 13. Mitotic chromosomes with explanatory illustrations **A** *Pyrrosia eberhardtii* ($2n = 74$) **B** *Tectaria harlandii* ($2n = 80$) **C** *Tectaria setulosa* ($2n = 80$).

***Tectaria harlandii* (Hook.) C.M.Kuo— $2n = 80$, diploid, Fig. 13B**

This species has been documented in the Ryukyu Islands, southern China, northern Vietnam, and Taiwan. Tetraploid populations ($n = 80$) have been reported in the Ryukyu Islands (as *Hemigramma decurrens* (Hook.) Copel., Mitui 1976) and Taiwan (Tsai and Shieh 1985). In our study, we provide the first record of a diploid form Vietnam. Notably, this species has been proposed as the maternal parent of the triploid ($2n = 120$) sterile hybrid species *T. × hongkongensis* (Zhao and Dong 2016), with *T. zeilanica* (Houtt.) Sledge as the paternal parent. It is reasonable to assume that *T. × hongkongensis* originated from the hybridization between diploid and tetraploid parents. Given that both diploids and tetraploids are now confirmed in these two parental species (Manton and Sledge 1954; Tsai and Shieh 1985), it would be interesting to investigate whether there is a bias in the direction of hybridization based on ploidy level.

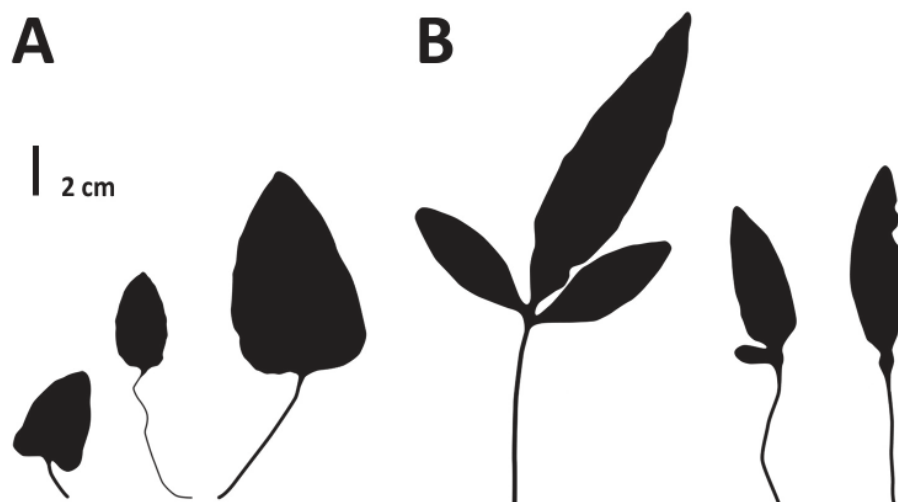


Figure 14. Comparison of the sterile fronds between *Leptochilus cantoniensis* and *L. poilanei* **A** *L. cantoniensis*, based on Kuo 1701 (TAIF [509328], left), Cadière 158 (MICH [1191289], middle), and s.c., s.n. (K [000959730], right) **B** *L. poilanei*, based on Poilane 5373 (BM [000036782], all the three fronds).

***Tectaria setulosa* (Baker) Holttum— $2n = 80$, sexual diploid, Fig. 13C**

The first chromosome count reported for the species. Initially described from Ba Vi mountain range in northern Vietnam, this species has since been documented in southern China, Indochina, and Peninsular Malaysia. Additionally, a variety *raciborskii* (Alderw.) Holttum has been further identified extending to Java without known ploidy.

New records from Vietnam

***Haplopteris yakushimensis* C.W.Chen & Ebihara, Phytotaxa 156(4): 232. 2014.**
Fig. 1

Type. JAPAN. • Kagoshima Pref., Yakushima Island, Nakabase River, 20 Aug 1982, Nakaike s.n. (holotype TNS [VS-45666!]).

Distribution and ecology. This species was previously recorded only in Japan and Taiwan (Chen et al. 2014, TPG 2019). In Vietnam, *H. yakushimensis* is found in damp evergreen broadleaf forests at elevations of 1200–1360 m, where it grows on rocks with thick compost near streams.

Specimens examined. VIETNAM. • Cao Bang Province: Phia-Oac Phia-Den National Park, 6 Dec 2013, Zhang et al. 6755 (MO, TAIF [499240!], VNMN). Phia-Oac Phia-Den National Park, Nguyen Binh District, Thanh Cong Ward, 22.589539°N, 105.880403°E, 1353 m, 8 Nov 2023, Chen Wade6952 (TAIF!, VNMN!). • Ha Giang Province: Vi Xuyen District, Cao Bo Ward, 22.767500°N, 104.880394°E, 1200–1360 m, 12 Sep 2000, Harder et al. 5514 (UC [1763099!]).

Note. *Haplopteris*, a fern genus comprising approximately 40 species, is primarily found in tropical Africa, Asia, and the Pacific Islands (Schuettpeitz et al. 2016). In Vietnam, eight *Haplopteris* species were previously recorded: *H. angustifolia* (Blume) E.H.Crane, *H. doniana* (Mett. ex Hieron.) E.H.Crane, *H. elongata* (Sw.) E.H.Crane, *H. ensata* (Christ) C.W.Chen & S.Linds., *H. ensiformis* (Sw.) E.H.Crane, *H. flexuosa* (Fée) E.H.Crane, *H. hainanensis* (C.Chr. ex Ching) E.H.Crane, and

H. sikkimensis (Kuhn) E.H.Crane (Chen et al. 2023). *Haplopteris yakushimensis* can be distinguished from these species by having fronds broader than 1 cm wide, costae that are grooved on the adaxial side and raised on the abaxial side, and the submarginal (ca. 1–2 mm away from the margins) sori lines.

Recent studies have identified two genetically distinct yet morphologically indistinguishable lineages in *H. yakushimensis* (Kuo et al. 2017; TPG 2019). With the discovery of new populations in Vietnam, we are currently working to clarify these lineages using an integrated approach that includes reproductive biology, genome size estimation, and nuclear markers. The results of this research will be published in a separate paper.

***Lindsaea kohkongensis* I.C.Hwang, M.O.Moon & B.Y.Sun, Korean J. Pl. Taxon. 53(4): 289. 2023.**

Fig. 2

Type. CAMBODIA • Koh Kong, Thma Bang District, near Chamnar village, 22 Dec 2013, Sun et al. C5520 (holotype HIBR; isotypes HIBR, KB).

Distribution and ecology. This species was recently described from Cambodia and Malaysia. In Vietnam, it grows along a valley in damp tropical forests as lithophytes and is frequently submerged in water.

Specimens examined. VIETNAM. • Kien Giang Province: Phu Quoc district, Phu Quoc National Park, 6 Mar 2018, Chen Wade5034 (TAIF [514046!]).

Note. Morphologically, this species closely resembles *Lindsaea ensifolia*. Although *L. kohkongensis* is usually a much smaller species than *L. ensifolia*, we did not identify any qualitative trait to distinguish them. Currently, subaquatic habitat preference appears to be the most reliable characteristic for differentiating this species from *L. ensifolia*. Yun et al. (2023) described *L. kohkongensis* as having free venation, in contrast to the anastomosing venation found in *L. ensifolia*. However, this is not true. As demonstrated in Fig. 3G of their paper and Fig. 2F of the current study, the veins of *L. kohkongensis* are connected near the lamina margin, forming areoles in fertile fronds.

***Pteris pseudowulaiensis* Y.S.Chao, Taiwania 66(3): 314. 2021.**

Fig. 3

Type. TAIWAN • New Taipei, Mt. Pataoerh, 600–700 m, 29 Apr 2016, Hsu 8437 (holotype TAIF [497137!]; isotype TAIF [497138!]).

Distribution and ecology. This species, recently described from Taiwan, was initially reported from southern China and Taiwan (Chao et al. 2021). In Vietnam, *P. pseudowulaiensis* was discovered in relatively dry evergreen broadleaf forests at lowland areas of Cuc Phuong National Park.

Specimens examined. VIETNAM • Ninh Binh Province: Cuc Phuong National Park, Muong Khu trail, Y.-S. Chao 3509, 3515, 3517, 3531 (TAIF!, VNMN!).

Note. This species belongs to the *Pteris fauriei* Hieron. complex, a taxonomically challenging group due to its reticulate evolution involving hybridization, polyploidization, and apomixis (Chao et al. 2022). In Vietnam,

five species from this complex have been recorded, namely *P. arisanensis* Tagawa (Chao et al. 2022), *P. kawabatae* Sa.Kurata (Chao et al. 2021), *P. latipinna* Y.S.Chao & W.L.Chiou (Chen et al. 2020b), *P. oshimensis* Hieron. (Liao et al. 2013), and *P. pseudowulaiensis*. Morphologically, *P. pseudowulaiensis* closely resembles *P. oshimensis* but differs by having broader pinnae, measuring 2–3.5 cm compared to less than 2 cm in *P. oshimensis*. According to Chao et al. (2022), *P. pseudowulaiensis* is an apogamous diploid originating from hybridization of *P. wulaiensis* C.M.Kuo (a species reported from Japan and Taiwan) and another unknown species.

Acknowledgements

We extend our gratitude to Hoang Thi Nha, Nong Minh Hoan, Ly Ton Senh, Trong Tin Tran Vo, and Cong Ty Tu for their assistance during fieldwork. Our sincere thanks also go to the directors of Bidoup Nui-Ba National Park, Cuc Phuong National Park, Phia Oac-Phia Den National Park, Phu Quoc National Park, and Nui Chua National Park, as well as the Vietnam Academy of Science and Technology, for granting us the collection permits. We thank Li-Bing Zhang for sharing his duplicated specimen of *Haplopteris yakushimensis* with us. We are indebted to the curator and staff of the BM herbarium for granting us access to their specimen.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

Field work in Nui Chua National Park was supported by the Vietnam Academy of Science and Technology (UDNGDP.01/24-25). Funding for this study was provided by the National Science and Technology Council of Taiwan (NSTC 112-2621-B-054-001-MY3).

Author contributions

CWC conceptualizes the study, conducts the analysis, and writes the original draft. CWC, YMH, YSC collect the data. YMH and KFC provide lab equipment. All authors conduct field work and revise the manuscript.

Author ORCIDs

Cheng-Wei Chen  <https://orcid.org/0000-0001-9709-6739>

Van Truong Do  <https://orcid.org/0000-0002-0585-5513>

Hong Truong Luu  <https://orcid.org/0000-0002-7036-7081>

Yi-Shan Chao  <https://orcid.org/0000-0001-7433-5987>

Kuo-Fang Chung  <https://orcid.org/0000-0003-3628-2567>

Data availability

All of the data that support the findings of this study are available in the main text.

References

- Barrington DS, Paris CA, Ranker TA (1986) Systematic inferences from spore and stomate size in the ferns. *American Fern Journal* 76: 149–159. <https://doi.org/10.2307/1547723>
- Bidin AA, Go R (1995) Chromosome base numbers for *Tectaria* and allied genera in peninsular Malaysia. *Natural History Research (Chiba)* 3: 115–122.
- Chang Y, Li J, Lu S, Schneider H (2013) Species diversity and reticulate evolution in the *Asplenium normale* complex (Aspleniaceae) in China and adjacent areas. *Taxon* 62: 673–687. <https://doi.org/10.12705/624.6>
- Chao YS (2019) New record of *Pteris esquirolii* in Taiwan, with two similar new species, *Pteris rugosifolia* and *Pteris subesquirolii*. *Systematic Botany* 44: 274–281. <https://doi.org/10.1600/036364419X15562052252072>
- Chao YS, Dong SY, Chiang YC, Liu HY, Chiou WL (2012) Extreme multiple reticulate origins of the *Pteris cadieri* complex (Pteridaceae). *International Journal of Molecular Sciences* 13: 4523–4544. <https://doi.org/10.3390/ijms13044523>
- Chao YS, Ebihara A, Chiou WL, Huang YM (2017) *Pteris latipinna* sp. nov. (Pteridaceae), a new species segregated from *Pteris fauriei*. *PhytoKeys* 85: 95–108. <https://doi.org/10.3897/phytokeys.85.14884>
- Chao YS, Chiou WL, Ebihara A, Hsu TC, Chang YH, Lin CY (2021) Taxonomic and nomenclatural novelties in the *Pteris fauriei* group (Pteridaceae). *Taiwania* 66: 307–316.
- Chao YS, Ebihara A, Chiou WL, Tsai JM, Huang YW, Ranker TA (2022) Reticulate evolution in the *Pteris fauriei* group (Pteridaceae). *Scientific Reports* 12: 9145. <https://doi.org/10.1038/s41598-022-11390-7>
- Chen CW, Ebihara A, Chiou WL, Li CW (2014) *Haplopteris yakushimensis* (Pteridaceae, Vittarioideae), a new species from Yakushima Island, Japan. *Phytotaxa* 156: 229–234. <https://doi.org/10.11646/phytotaxa.156.4.5>
- Chen CC, Hyvönen J, Schneider H (2020a) Exploring phylogeny of the microsoroid ferns (Polypodiaceae) based on six plastid DNA markers. *Molecular Phylogenetics and Evolution* 143: 106665. <https://doi.org/10.1016/j.ympev.2019.106665>
- Chen CW, Hsu TC, Chao YS, Lu PF, Li CW, Tram NKT, Nguyen ILPT, Dang MT, Luu HT, Parris B (2020b) A newly recorded genus and twelve newly recorded species of ferns for Vietnam from Lang Biang Plateau. *Phytotaxa* 443: 121–143. <https://doi.org/10.11646/phytotaxa.443.2.1>
- Chen CW, Ebihara A, Cheng KY, Hsu TC, Huang YM, Dang VD, Luu HT, Le VS, Li CW (2021) Studies of Vietnamese pteridophyte flora 1. *Systematic Botany* 46: 573–581. <https://doi.org/10.1600/036364421X16312067913507>
- Chen CW, Hsu TC, Doan HS, Do VH, Luu HT, Le VS, Liu YC, Li CW, Huang YM, Chung KF (2023) Studies of Vietnamese pteridophyte flora 2. *Systematic Botany* 48: 159–172. <https://doi.org/10.1600/036364423X16847773873161>
- Chen CW, Do VT, Truong QC, Dang VD, Luu HT, Chao YS, Huang YM, Chung KF (2024) Studies of Vietnamese Pteridophyte Flora 3 [Dataset]. *Dryad*. <https://doi.org/10.5061/dryad.4j0zpc8mz>
- Christensen C, Tardieu-Blot ML (1939) Les fougères d'Indochine. XV. Dipteroideae, XVI. Polypodioideae, XVII. Elaphoglossoideae. *Notulae Systematicae* 8: 175–210.
- Ding HH, Wang P, Dong SY (2013) *Dryopsis* (Dryopteridaceae), a fern genus new to Vietnam. *Taiwania* 58: 80–84.

- Dong SY, Zuo ZY (2018) On the recognition of *Gymnosphaera* as a distinct genus in Cyatheaceae. *Annals of the Missouri Botanical Garden* 103: 1–23. <https://doi.org/10.3417/2017049>
- Edgar RC (2004) MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* 32: 1792–1797. <https://doi.org/10.1093/nar/gkh340>
- Fay MF, Swensen SM, Chase MW (1997) Taxonomic affinities of *Medusagyne oppositifolia* (Medusagynaceae). *Kew Bulletin* 52: 111–120. <https://doi.org/10.2307/4117844>
- Fujiwara T, Quang BH, Tagane S, Murakami N, Oguri E (2023) *Leptochilus ornithopus* (Polypodiaceae), a new hemiepiphytic fern species from central highlands of Vietnam. *Phytotaxa* 584: 149–160. <https://doi.org/10.11646/phytotaxa.584.3.2>
- Gastony GJ, Yatskievych G (1992) Maternal inheritance of the chloroplast and mitochondrial genomes in cheilanthoid ferns. *American Journal of Botany* 79: 716–722. <https://doi.org/10.1002/j.1537-2197.1992.tb14613.x>
- Goldblatt P, Lowry PP (2011) The Index to Plant Chromosome Numbers (IPCN): Three decades of publication by the Missouri Botanical Garden come to an end. *Annals of the Missouri Botanical Garden* 98: 226–227. <https://doi.org/10.3417/2011027>
- Hall TA (1999) BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41: 95–98.
- Hassler M (1994–2024) World ferns. Synonymic checklist and distribution of ferns and lycophytes of the world. <https://www.worldplants.de/ferns/> [last accessed 9 Nov 2024]
- Hoang DT, Chernomor O, Von Haeseler A, Minh BQ, Vinh LS (2018) UFBoot2: Improving the ultrafast bootstrap approximation. *Molecular Biology and Evolution* 35: 518–522. <https://doi.org/10.1093/molbev/msx281>
- Hovenkamp PH (1986) A monograph of the fern genus *Pyrrosia* (Polypodiaceae). *Leiden Botanical Series* 9: 1–280. https://doi.org/10.1163/9789004628090_003
- Hovenkamp PH (1998) Polypodiaceae. *Flora Malesiana-Series 2. Pteridophyta* 3: 1–234.
- Hovenkamp PH, Franken NAP (1993) An account of the fern genus *Belvisia* Mirbel (Polypodiaceae). *Blumea* 37: 511–527.
- Iwatsuki K (1977) Notes on Asiatic pteridophytes 1. *Acta Phytotaxonomica et Geobotanica* 28: 160–166.
- Kalyanamoorthy S, Minh BQ, Wong TK, Von Haeseler A, Jermini LS (2017) ModelFinder: Fast model selection for accurate phylogenetic estimates. *Nature Methods* 14: 587–589. <https://doi.org/10.1038/nmeth.4285>
- Kato M (1999) A cytotoxic study of Hainan (S. China) pteridophytes with notes on polyploidy and apogamy of Chinese species. In: Zhang XC, Shing KH (Eds) *Ching Memorial Volume*. China Forestry Publishing House, Beijing, 1–19.
- Kramer KU (1967) The lindsaeoid ferns of the Old World III. Notes on *Lindsaea* and *Spheonomeris* in the Flora Malesiana area. *Blumea* 15: 557–574.
- Kramer KU (1971) *Lindsaea*-group. *Flora Malesiana-Series 2. Pteridophyta* 1: 177–254.
- Kuo LY, Li FW, Chiou WL, Wang CN (2011) First insights into fern *matK* phylogeny. *Molecular Phylogenetics and Evolution* 59: 556–566. <https://doi.org/10.1016/j.ympev.2011.03.010>
- Kuo LY, Chen CW, Shinohara W, Ebihara A, Kudoh H, Sato H, Huang YM, Chiou WL (2017) Not only in the temperate zone: Independent gametophytes of two vittarioid ferns (Pteridaceae, Polypodiales) in East Asian subtropics. *Journal of Plant Research* 130: 255–262. <https://doi.org/10.1007/s10265-016-0897-x>

- Lanfear R, Calcott B, Ho SY, Guindon S (2012) PartitionFinder: Combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution* 29: 1695–1701. <https://doi.org/10.1093/molbev/mss020>
- Lehtonen S, Tuomisto H, Rouhan G, Christenhusz MJ (2010) Phylogenetics and classification of the pantropical fern family Lindsaeaceae. *Botanical Journal of the Linnean Society* 163: 305–359. <https://doi.org/10.1111/j.1095-8339.2010.01063.x>
- Li FW, Tan BC, Buchbender V, Moran RC, Rouhan G, Wang CN, Quandt D (2009) Identifying a mysterious aquatic fern gametophyte. *Plant Systematics and Evolution* 281: 77–86. <https://doi.org/10.1007/s00606-009-0188-2>
- Li SH, Zuo ZY, Chen CW, Pham VT, Luu HT, Dong SY (2024) Three out of one: Revising the species delimitation of the tree fern *Gymnosphaera salletii* (Cyatheaceae), with particular reference to the foliar nectary. *Botanical Journal of the Linnean Society* 204: 63–75. <https://doi.org/10.1093/botlinnean/boad036>
- Liao W, Ding M, Wu ZY, Prado J, Gilbert MG (2013) Pteris. In: Wu ZY, Raven PH, Hong DY (Eds) *Flora of China*. Science Press, Missouri Botanical Garden Press, Beijing, St. Louis, 181–256.
- Lin SJ, Iwatsuki K, Kato M (1996) Cytotaxonomic study of ferns from China, I. Species of Yunnan. *Shokubutsu Kenkyu Zasshi* 71: 214–222.
- Lin SJ, Zhuang HR, Iwatsuki K, Lu HS (2002) Cytotaxonomic study of ferns from China II. Species of Fujian. *Shokubutsu Kenkyu Zasshi* 77: 129–138.
- Löve A, Löve D, Pichi Sermolli REG (1977) *Cytotaxonomical Atlas of the Pteridophyta*. Cramer, Liechtenstein.
- Manickam VS, Irudayaraj V (1989) Corrections to the cytology of ferns of the western Ghats. *Indian Fern Journal* 6: 131–132.
- Manton I (1954) Cytology of One Hundred Ferns. Appendix in *Revised Flora of Malaya*, vol. 2: Ferns, Holttum RE. Government Printing Office, Singapore.
- Manton I, Sledge WA (1954) Observations on the cytology and taxonomy of the pteridophyte flora of Ceylon. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* 238: 127–185. <https://doi.org/10.1098/rstb.1954.0008>
- Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, Von Haeseler A, Lanfear R (2020) IQ-TREE 2: New models and efficient methods for phylogenetic inference in the genomic era. *Molecular Biology and Evolution* 37: 1530–1534. <https://doi.org/10.1093/molbev/msaa015>
- Mitui K (1968) Chromosomes and speciation in ferns. *Science Reports of the Tokyo Kyoiku Daigaku B* 13: 185–333.
- Mitui K (1976) Chromosome numbers of some ferns in the Rykyu Islands. *Shokubutsu Kenkyu Zasshi* 51: 33–41.
- Nadot S, Bittar G, Carter L, Lacroix R, Lejeune B (1995) A phylogenetic analysis of monocotyledons based on the chloroplast gene *rps4*, using parsimony and a new numerical phenetics method. *Molecular Phylogenetics and Evolution* 4: 257–282. <https://doi.org/10.1006/mpev.1995.1024>
- Nayak SK, Singh PK (1989) Cytological studies in the genus *Azolla*. *Cytologia* 54: 275–286. <https://doi.org/10.1508/cytologia.54.275>
- Rice A, Glick L, Abadi S, Einhorn M, Kopelman NM, Salman-Minkov A, Mayzel J, Chay O, Mayrose I (2015) The Chromosome Counts Database (CCDB) – a community resource of plant chromosome numbers. *The New Phytologist* 206: 19–26. <https://doi.org/10.1111/nph.13191> [last accessed 4 Nov 2024]

- Sang T, Crawford DJ, Stuessy TF (1997) Chloroplast DNA phylogeny, reticulate evolution, and biogeography of *Paeonia* (Paeoniaceae). *American Journal of Botany* 84: 1120–1136. <https://doi.org/10.2307/2446155>
- Schuettpelz E, Chen CW, Kessler M, Pinson JB, Johnson B, Davila A, Cochran AT, Huiet L, Pryer KM (2016) A revised generic classification of vittarioid ferns (Pteridaceae) based on molecular, micromorphological, and geographic data. *Taxon* 65: 708–722. <https://doi.org/10.12705/654.2>
- Smith AR, Cranfill R (2002) Intrafamilial relationships of the thelypteroid ferns (Thelypteridaceae). *American Fern Journal* 92: 131–149. [https://doi.org/10.1640/0002-8444\(2002\)092\[0131:IROTTF\]2.0.CO;2](https://doi.org/10.1640/0002-8444(2002)092[0131:IROTTF]2.0.CO;2)
- Taberlet P, Gielly L, Pautou G, Bouvet J (1991) Universal primers for amplification of three noncoding regions of chloroplast DNA. *Plant Molecular Biology* 17: 1105–1109. <https://doi.org/10.1007/BF00037152>
- Takamiya M, Ohta N, Yatabe Y, Murakami N (2001) Cytological, morphological, genetic, and molecular phylogenetic studies on intraspecific differentiations within *Diplazium doederleinii* (Woodsiaceae: Pteridophyta). *International Journal of Plant Sciences* 162: 625–636. <https://doi.org/10.1086/320144>
- Takei M (1969) Karyological studies in Polypodiaceae I. Karyotypes of a few species of the genus *Lemmaphyllum* and *Pyrrosia* in Japan. *The Botanical Magazine* 82: 482–487. <https://doi.org/10.15281/jplantres1887.82.482>
- Tate JA, Simpson BB (2003) Paraphyly of *Tarasa* (Malvaceae) and diverse origins of the polyploid species. *Systematic Botany* 28: 723–737.
- Tindale MD, Roy SK (2002) A cytotaxonomic survey of the Pteridophyta of Australia. *Australian Systematic Botany* 15: 839–937. <https://doi.org/10.1071/SB00034>
- TPG (2019) Updating Taiwanese pteridophyte checklist: A new phylogenetic classification. *Taiwania* 64: 367–395.
- Tsai JL, Shieh WC (1985) A cytotaxonomic survey of the fern family Aspidiaceae (sensu Copeland) in Taiwan. *Journal of Science and Engineering* 22: 121–144.
- Walker TG (1962) Cytology and evolution in the fern genus *Pteris* L. *Evolution; International Journal of Organic Evolution* 16: 27–43. <https://doi.org/10.2307/2406264>
- Wolf PG, Sipes SD, White MR, Martines ML, Pryer KM, Smith AR, Ueda K (1999) Phylogenetic relationships of the enigmatic fern families Hymenophylloidsaceae and Lophosoriaceae: Evidence from *rbcL* nucleotide sequences. *Plant Systematics and Evolution* 219: 263–270. <https://doi.org/10.1007/BF00985583>
- Wood TE, Takebayashi N, Barker MS, Mayrose I, Greenspoon PB, Rieseberg LH (2009) The frequency of polyploid speciation in vascular plants. *Proceedings of the National Academy of Sciences of the United States of America* 106: 13875–13879. <https://doi.org/10.1073/pnas.0811575106>
- Yun N, Moon MO, Sun BY, Hwang IC (2023) *Lindsaea kohkongensis* (Lindsaeaceae), a new species from Cambodia and Malaysia. *Korean Journal of Plant Taxonomy* 53: 288–293. <https://doi.org/10.11110/kjpt.2023.53.4.288>
- Zhang L, Guo L, Zhang LB (2018) New combinations in the fern genus *Leptochilus* (Polypodiaceae). *Phytotaxa* 374: 172–176. <https://doi.org/10.11646/phytotaxa.374.2.10>
- Zhang L, Lu TN, Zhou XM, Chen DK, Knapp R, Zhou L, Gao XF, Zhang LB (2019) A plastid phylogeny of the Old World fern genus *Leptochilus* (Polypodiaceae): Implications for cryptic speciation and progressive colonization from lower to higher latitudes. *Molecular Phylogenetics and Evolution* 134: 311–322. <https://doi.org/10.1016/j.ympev.2019.01.013>

- Zhang L, Lu TN, Zhou XM, Zhou Z, Thepkayson K, Luong TT, Zhang LB (2024) Exploring the diversity of the java fern genus *Leptochilus* (Polypodiaceae) in the Indo-Burma biodiversity hotspot. *Phytotaxa* 659: 213–235. <https://doi.org/10.11646/phytotaxa.659.3.1>
- Zhao HG, Dong SY (2016) A new hybrid of *Tectaria* (Tectariaceae) from southern China. *Phytotaxa* 266: 213–218. <https://doi.org/10.11646/phytotaxa.266.3.5>
- Zhao CF, Wei R, Zhang XC, Xiang QP (2020) Backbone phylogeny of *Lepisorus* (Polypodiaceae) and a novel infrageneric classification based on the total evidence from plastid and morphological data. *Cladistics* 36: 235–258. <https://doi.org/10.1111/cla.12403>
- Zhou XM, Zhang LB (2019) A monograph of the fern genus *Pteridrys* (Pteridryaceae). *Systematic Botany* 44: 243–273. <https://doi.org/10.1600/036364419X15562052252153>
- Zhou XM, Zhang L, Chen CW, Li CX, Huang YM, Chen DK, Lu TN, Cicuzza D, Knapp R, Luong TT, Nitta JH (2017) A plastid phylogeny and character evolution of the Old World fern genus *Pyrrosia* (Polypodiaceae) with the description of a new genus: *Hovenkampia* (Polypodiaceae). *Molecular Phylogenetics and Evolution* 114: 271–294. <https://doi.org/10.1016/j.ympev.2017.06.020>

Appendix 1

Table A1. Specimens of *Lindsaea* used for molecular phylogenetic analysis in this study. New sequences generated in this study are indicated in bold. En-dashes “–” indicate missing data.

Taxa	Voucher	Herbarium	Country	Matk	Trnh-Psba	Trnl-F
<i>Lindsaea agatii</i> (Brack.) Lehtonen & Tuomisto	Chen SITW5698	TAIF [481048]	Solomon Islands	PQ149394	PQ149410	PQ149412
<i>Lindsaea blotiana</i> K.U.Kramer	Rakotondrainibe 6350	P	Madagascar	–	GU478510	GU478813
<i>Lindsaea ensifolia</i> Sw.	Fraser-Jenkins FN29	TAIF [357318]	Nepal	–	PQ149405	–
<i>Lindsaea ensifolia</i> Sw.	Fraser-Jenkins FN36	TAIF [357830]	India	–	PQ149404	–
<i>Lindsaea ensifolia</i> Sw.	Huang 1657	TAIF [307881]	Cambodia	–	PQ149407	–
<i>Lindsaea ensifolia</i> Sw.	Kessler 13597	UC [1951608]	Malaysia	–	GU478521	–
<i>Lindsaea ensifolia</i> Sw.	Larsen 41363	AAU	Thailand	–	GU478522	GU478853
<i>Lindsaea ensifolia</i> Sw.	Lu 15719	TAIF [398238]	Thailand	PQ149396	PQ149403	PQ149416
<i>Lindsaea ensifolia</i> Sw.	Lu 16172	TAIF [296864]	Bangladesh	–	PQ149408	–
<i>Lindsaea ensifolia</i> Sw.	Chen SITW5235	TAIF [480899]	Solomon Islands	PQ149399	PQ149402	PQ149415
<i>Lindsaea ensifolia</i> Sw.	Chen Wade1339	TAIF [411090]	Vietnam	PQ149398	PQ149401	PQ149414
<i>Lindsaea ensifolia</i> Sw.	Chen Wade1399	TAIF [411162]	Vietnam	PQ149397	PQ149406	PQ149417
<i>Lindsaea fraseri</i> Hook.	Streimann 8951	L	Australia	–	FJ360908	FJ360998
<i>Lindsaea heterophylla</i> Dryand.	Kramer 8285	Z	China	–	KF652043	KF652056
<i>Lindsaea javanensis</i> Blume	Chen Wade4151	TAIF [458804]	Vietnam	PQ149393	PQ149409	PQ149411
<i>Lindsaea kohkongensis</i> I.C.Hwang, M.O.Moon & B.Y.Sun	Chen Wade5034	TAIF [514046]	Vietnam	PQ149395	PQ149400	PQ149413
<i>Lindsaea leptophylla</i> Baker	Raharimalala 2017	MO	Madagascar	–	GU478509	GU478819
<i>Lindsaea madagascariensis</i> Baker	Rakotondrainibe 6349	P	Madagascar	–	GU478512	GU478815
<i>Lindsaea media</i> R.Br.	van der Werff 11655	MO	Australia	–	GU478516	GU478843
<i>Lindsaea meifolia</i> (Kunth) Mett. ex Kuhn	Liesner 7062	MO	Venezuela	–	GU478478	GU478783
<i>Lindsaea orbiculata</i> (Lam.) Mett. ex Kuhn	Averyanov et al. VH4814	AAU	Vietnam	–	FJ360922	FJ361013
<i>Lindsaea oxyphylla</i> Baker	Gautier 2662	P	Madagascar	–	GU478508	GU478816
<i>Lindsaea vieillardii</i> Mett.	McKee 5304	U	New Caledonia	–	GU478496	GU478844
<i>Lindsaea walkerae</i> Hook.	Bostock 638	Z	Australia	–	GU478517	GU478841
<i>Tapeinidium luzonicum</i> (Hook.) K.U.Kramer	Kessler 13610	GOET	Malaysia	–	GU478444	GU478751

Table A2. Specimens of *Leptochilus* used for molecular phylogenetic analysis in this study. New sequences generated in this study are indicated in bold.

Taxa	Voucher	Herbarium	Country	Rbcl	Rps4-Trns	TrnI-F
<i>Leptochilus axillaris</i> (Cav.) Kaulf.	Wu 2462	KUN	Laos	JX103701	JX103743	JX103785
<i>Leptochilus cantoniensis</i> (Baker) Ching	Dong 743	PE	China	EU482945	EU482995	EU483041
<i>Leptochilus cantoniensis</i> (Baker) Ching	Dong 172	PE	China	EU482946	EU482996	EU483042
<i>Leptochilus cantoniensis</i> (Baker) Ching	Kuo 1701	TAIF [509328]	China	MT137055	MH665095	MH665162
<i>Leptochilus decurrens</i> Blume	Chen Wade1769	TAIF [388951]	Indonesia	MH768462	MH768527	MH768586
<i>Leptochilus digitatus</i> (Baker) Noot.	Chao 1556	TAIF (432480)	Vietnam	MH665034	MH665097	MH665164
<i>Leptochilus dissimilialatus</i> (Bonap.) Liang Zhang & Li Bing Zhang	Zhang et al. 6362	CDBI, MO, VNMN	Vietnam	MH768419	MH768481	MH768547
<i>Leptochilus ellipticus</i> (Thunb.) Noot.	Chen Wade3656	TAIF [440115]	Japan	MH768444	MH768508	MH768572
<i>Leptochilus evrardii</i> (Tardieu) Liang Zhang & Li Bing Zhang	Zhang et al. 8633	CDBI, MO	Vietnam	MH768461	MH768526	MH768585
<i>Leptochilus flexilobus</i> (Christ) Liang Zhang & Li Bing Zhang	Chen Wade2518	TAIF [440142]	China	MH665042	MH665106	MH665173
<i>Leptochilus henryi</i> (Baker) X.C.Zhang	Zhang et al. 9289	CDBI	China	MH768459	MH768524	MH768583
<i>Leptochilus heterophyllus</i> (S.K.Wu & P.K.Lôc) comb. ined.	Wu WP-135	KUN	Vietnam	JX103688	JX103730	JX103772
<i>Leptochilus longipes</i> (Ching) X.C.Zhang	Chen Wade4102	TAIF [458794]	Vietnam	MH665049	MH665114	MH665181
<i>Leptochilus macrophyllus</i> (Blume) Noot.	Chen Wade1962	TAIF [388897]	Indonesia	MH768449	MH768513	MH768575
<i>Leptochilus oblongus</i> Li Bing Zhang, Liang Zhang & N.T.Lu	Zhang et al. 6299	CDBI, MO, VNMN	Vietnam	MH768429	MH768491	MH768557
<i>Leptochilus pedunculatus</i> (Hook. & Grev.) Fraser-Jenk.	Chen Wade1334	TAIF [410768]	Vietnam	MH051168	MH113467	MH113500
<i>Leptochilus pentaphyllus</i> (Baker) Liang Zhang & Li Bing Zhang	Zhang 1777	KUN	China	MH768474	MH768539	MH768599
<i>Leptochilus poilanei</i> (C.Chr. & Tardieu) Liang Zhang & Li Bing Zhang	Chen Wade6804	TAIF	Vietnam	PQ149390	PQ149391	PQ149392
<i>Leptochilus pothifolius</i> (Buch.-Ham. ex D.Don) Fraser-Jenk.	Chen Wade2519	TAIF [440141]	China	MH665056	MH665122	MH665189
<i>Leptochilus pteropus</i> (Blume) Fraser-Jenk.	Chen 1010	H	Taiwan	MH051176	MH113475	MH113508
<i>Leptochilus saxicola</i> (H.G.Zhou & Hua Li) Liang Zhang & Li Bing Zhang	Wei 2017	KUN	China	MH768471	MH768536	MH768595
<i>Leptochilus wrightii</i> (Hook.) X.C.Zhang	Chen 1087	H	Taiwan	MH051170	MH113469	MH113502