

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our [Editorial Policies](#) and the [Editorial Policy Checklist](#).

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

- | | |
|-------------------------------------|---|
| n/a | Confirmed |
| ☐ | ☒ The exact sample size (<i>n</i>) for each experimental group/condition, given as a discrete number and unit of measurement |
| ☒ | ☐ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| ☐ | ☒ The statistical test(s) used AND whether they are one- or two-sided
<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
| ☐ | ☒ A description of all covariates tested |
| ☐ | ☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons |
| ☒ | ☐ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals) |
| ☒ | ☐ For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted
<i>Give P values as exact values whenever suitable.</i> |
| ☒ | ☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |
| ☒ | ☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
| ☐ | ☒ Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i>), indicating how they were calculated |

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection	No software was used for data collection.
Data analysis	RNA Sequencing and Transcriptome Assembly 1. Fastp: v0.23.2 2. SOAPdenovo-Trans: v1.03 3. Trinity: v2.8.5 4. EvidentialGene Pipeline: Not versioned, includes trformat.pl and tr2aacds.pl 5. BUSCO: v5.4.3 6. kallisto: v0.50.1 Synonymous Substitution Rates and Age Distributions 7. ksrates: v1.1.1 8. BLASTP: v2.11.0+ 9. MCL: v14-137 10. MUSCLE: v3.8.1551 11. PAML: v4.9j (CODEML function) 12. FastTree: v2.1.11 13. i-ADHoRe: v3.0.01 14. CD-HIT: v4.8.1 15. wgd v2 Phylogenetic Analysis

15. OrthoFinder: v2.5.4
 16. MAFFT: v7.475
 17. Trimal: v1.4.1
 18. IQ-TREE: v1.6.12
 19. ASTRAL-Pro2: Not versioned
 20. Mcmctree (from PAML): v4.9j
- Gene Tree Reconciliation and WGD Analysis
21. Whale: v2.0.3
 22. PRANK: v.150803
 23. MrBayes: v3.2.6
 24. ALEobserve: Not versioned
 25. Diamond: v2.0.5.143
- Structural and Functional Analysis
26. Seaview: v5.0.5
 27. alphafold: Not versioned (server-based)
 28. DALI: Not versioned (web-based)
 29. Foldseek: Not versioned
- Cell Wall and Biochemical Analyses
30. CoNekT Framework: Not versioned
 31. CASA lignin: Protocol-based (not a specific software)
 32. Gas Chromatography–Mass Spectrometry: Not versioned
- Carbohydrate and Cell Wall Protein Analysis
33. dbCAN2 Pipeline: Not versioned
 34. Cytoscape: v3.10.1
- Statistical and Enrichment Analyses
35. scipy (Python package): Not versioned
 36. ProScanArray Express: Not versioned
- Visualization
37. MEGAX: v1.0
 38. iTOL: Not versioned

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

The raw sequencing data is available at E-MTAB-13848, while the CDS and protein sequences are found at <https://doi.org/10.6084/m9.figshare.26347330>. The co-expression networks are available at <https://conekt.sbs.ntu.edu.sg/species/>.

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender	N.A.
Reporting on race, ethnicity, or other socially relevant groupings	N.A.
Population characteristics	N.A.
Recruitment	N.A.
Ethics oversight	N.A.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](https://www.nature.com/documents/nr-reporting-summary-flat.pdf)

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	1. Fern Sampling: We aimed to collect at least three samples for each major organ, aiming to have 20 samples / species • 22 ferns from 22 families were sampled across Singapore. • Three biological replicates were taken for each organ, capturing variance in developmental stages and morphological characteristics. 2. Additional Data: • 7 sequenced genomes included. • 79 assembled transcriptomes from the 1000 Plant Transcriptomes Initiative (1KP). 3. Transcriptomic Analyses: • RNA sequencing was conducted for 22 species, with paired-end reads of 150 bp and sequencing depth of 60–70 million reads per sample. 4. Single-Copy Gene Families: • For phylogenomic analysis, 140, 112, 107, and 57 single-copy gene families were constructed depending on dataset composition.
Data exclusions	Data exclusions in the study include: 1. Low-Quality RNA-Seq Reads: • Removed during preprocessing using Fastp to ensure quality for downstream analysis. 2. Redundant or Low-Quality Transcripts: • Removed using the EvidentialGene Pipeline and filtered by transcripts per million (TPM) scores, with transcripts scoring <1 excluded. • Transcripts with GC content <40% or >60% were removed. 3. Non-Fern Sequences: • Assembled transcriptomes were BLASTed against NCBI, and sequences with >70% identity and an e-value <10⁻¹⁰ to non-fern entries were excluded. 4. KS-Age Analysis: • Paralogs and orthologs with sequence anomalies (e.g., sequence length not divisible by 3, invalid codons, or in-frame stop codons) were excluded. 5. Large Gene Families: • Gene families with more than 200 members were filtered out during clustering to reduce noise in phylogenomic analyses. 6. Incomplete Transcriptomes: • Hypothetical Whole-Genome Duplications (WGDs) with retention rates below 0.05 were excluded from analyses due to incompleteness of transcriptome assemblies. 7. Phylogenetic Tree Construction: • Gene trees with bootstrap support values below 50% were excluded from species tree inference. 8. Structural Analyses: • Orthogroups with less than 10 species representatives were excluded from sequence similarity and structural predictions. 9. Functional Annotation: • Genes not meeting the required co-expression thresholds or specificity values for organ-specific classifications were excluded. 10. Potential Contamination: • Non-fern sequences or orthogroups failing checks for expected clades in gain/loss analyses were filtered out.
Replication	Replication in the study is addressed as follows: 1. Biological Replicates: • For each of the 22 ferns, three biological replicates were collected for each organ. This approach ensures variability in developmental stages and morphological characteristics is captured and reduces the impact of random experimental noise. 2. Technical Replicates: • Quality control and RNA library preparation steps involved technical replicates for validation, including: • Repeated quantifications using Qubit. All samples that were recommended by Novogene for sequencing passed QC. • Sequencing depth (~60–70 million reads per sample) sufficient to minimize stochastic sampling errors. 3. Bootstrap Analyses: • For phylogenetic analyses: • Gene trees were generated with 1000 bootstrap replicates using IQ-TREE. • KS-age distribution estimates were subjected to 200 iterations of bootstrap with replacements. 4. Validation via External Datasets: • 79 transcriptomes from the 1000 Plant Transcriptomes Initiative (1KP) and 7 sequenced genomes provided additional validation and replication for evolutionary and phylogenomic analyses. 5. Multiple Analytical Pipelines: • Replication in transcriptome assembly and filtering was ensured by using SOAPdenovo-Trans and Trinity, followed by consolidation and validation with the EvidentialGene Pipeline. 6. Cross-Species Validation: • Organ-specific gene expression and orthogroup analyses were conducted across multiple fern species to replicate findings and establish conserved patterns.
Randomization	N.A. We were not performing statistical analyses or comparing treatments.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

- | | |
|-------------------------------------|--|
| n/a | Included in the study |
| ☒ | ☒ Antibodies |
| ☒ | ☐ Eukaryotic cell lines |
| ☒ | ☐ Palaeontology and archaeology |
| ☒ | ☐ Animals and other organisms |
| ☒ | ☐ Clinical data |
| ☒ | ☐ Dual use research of concern |
| ☐ | ☒ Plants |

Methods

- | | |
|-------------------------------------|---|
| n/a | Included in the study |
| ☒ | ☐ ChIP-seq |
| ☒ | ☐ Flow cytometry |
| ☒ | ☐ MRI-based neuroimaging |

Dual use research of concern

Policy information about [dual use research of concern](#)

Hazards

Could the accidental, deliberate or reckless misuse of agents or technologies generated in the work, or the application of information presented in the manuscript, pose a threat to:

- | | |
|-------------------------------------|---|
| No | Yes |
| ☒ | ☐ Public health |
| ☒ | ☐ National security |
| ☒ | ☐ Crops and/or livestock |
| ☒ | ☐ Ecosystems |
| ☒ | ☐ Any other significant area |

Experiments of concern

Does the work involve any of these experiments of concern:

- | | |
|-------------------------------------|--|
| No | Yes |
| ☒ | ☐ Demonstrate how to render a vaccine ineffective |
| ☒ | ☐ Confer resistance to therapeutically useful antibiotics or antiviral agents |
| ☒ | ☐ Enhance the virulence of a pathogen or render a nonpathogen virulent |
| ☒ | ☐ Increase transmissibility of a pathogen |
| ☒ | ☐ Alter the host range of a pathogen |
| ☒ | ☐ Enable evasion of diagnostic/detection modalities |
| ☒ | ☐ Enable the weaponization of a biological agent or toxin |
| ☒ | ☐ Any other potentially harmful combination of experiments and agents |

Plants

Seed stocks	Ferns are not seed plants. We did not work with spores. The plants were collected in their natural environment or the Singapore Botanical Garden
Novel plant genotypes	No new genotypes were produced
Authentication	We used vouchers (see supplemental table) to document the collected plants.