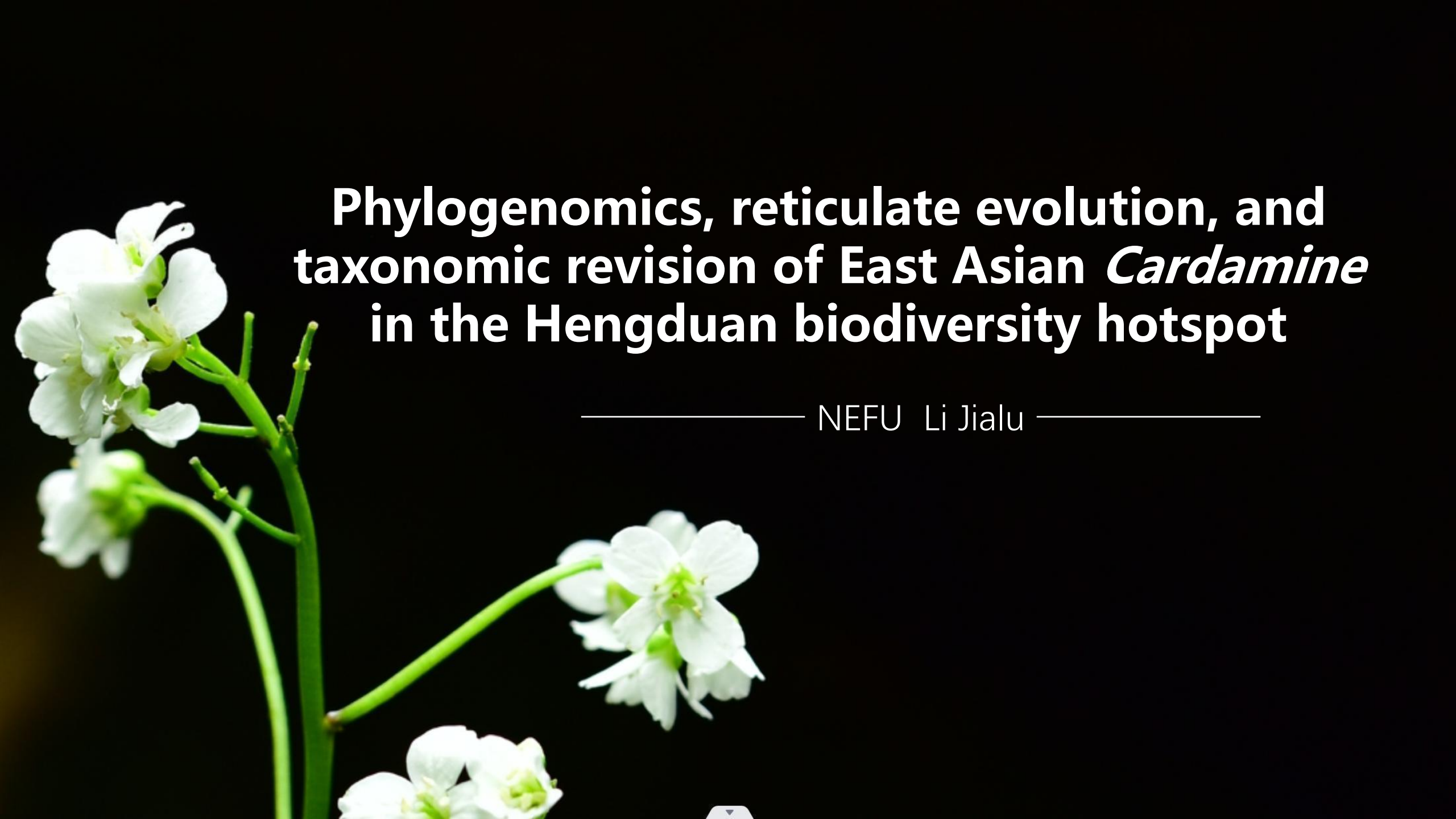

# Phylogenomics, reticulate evolution, and taxonomic revision of East Asian *Cardamine* in the Hengduan biodiversity hotspot

NEFU Li Jialu

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**2. Materials and Methods**

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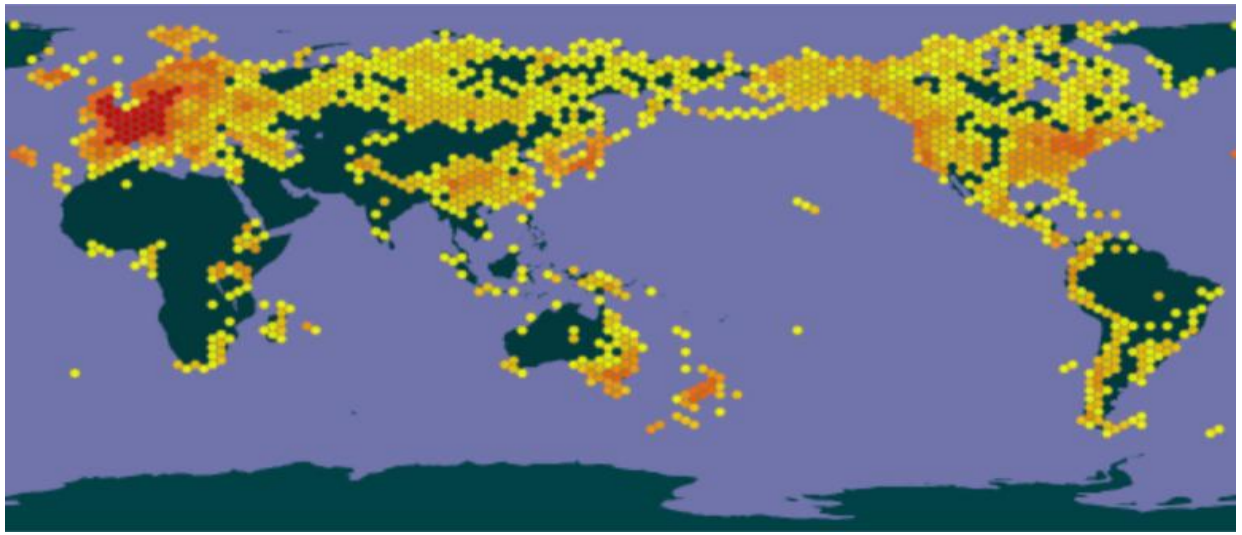
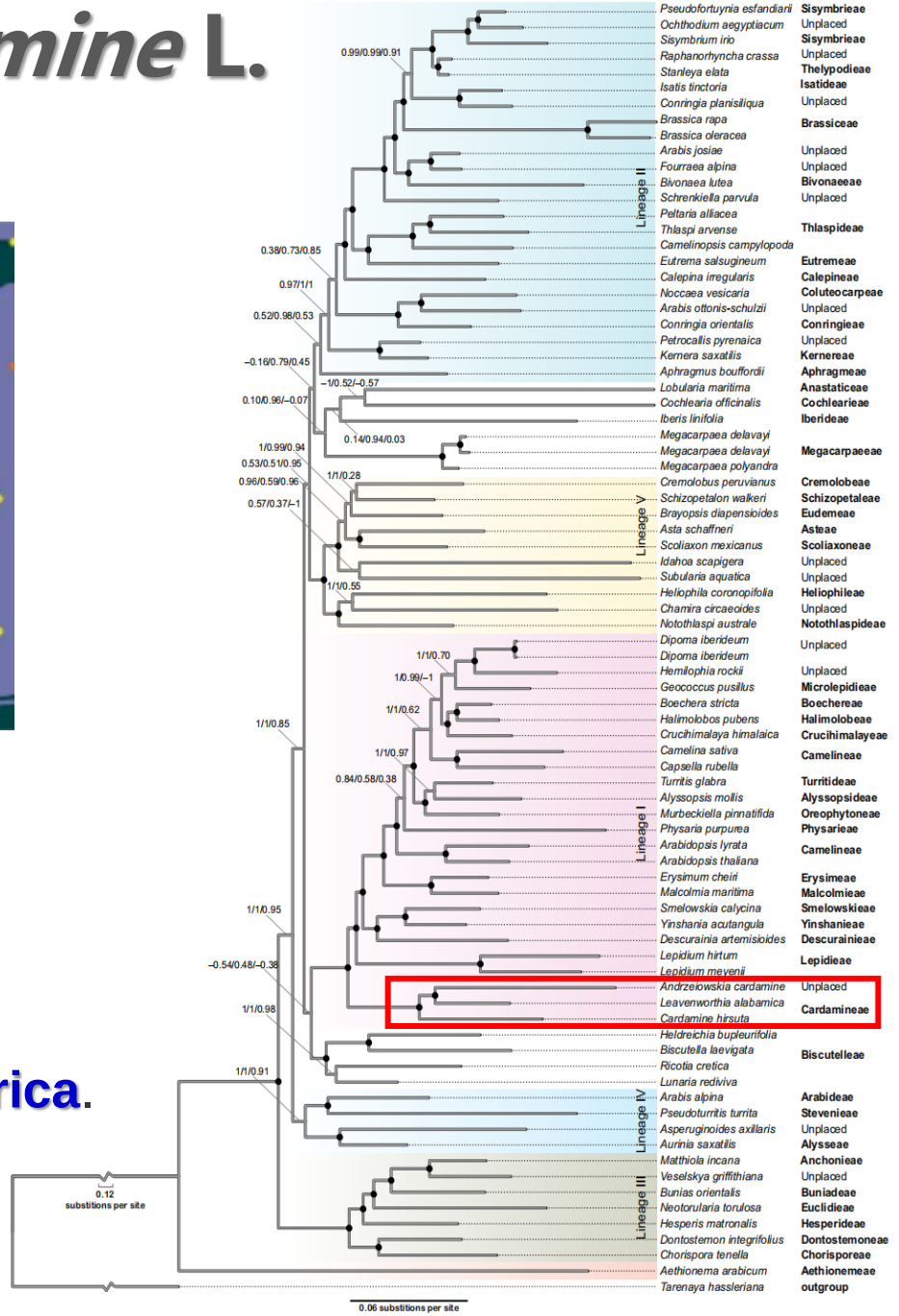
# Background: Overview of *Cardamine* L.

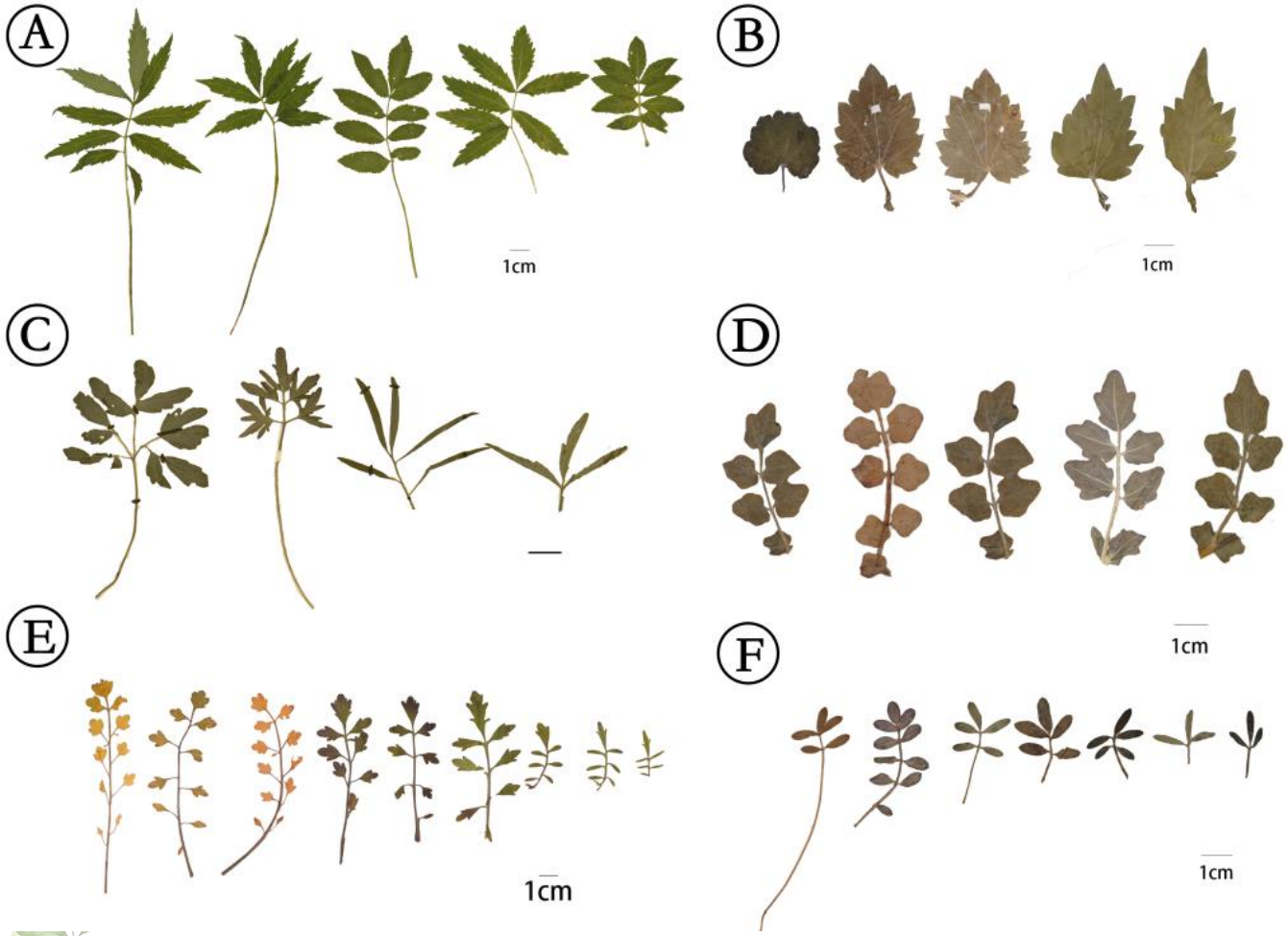


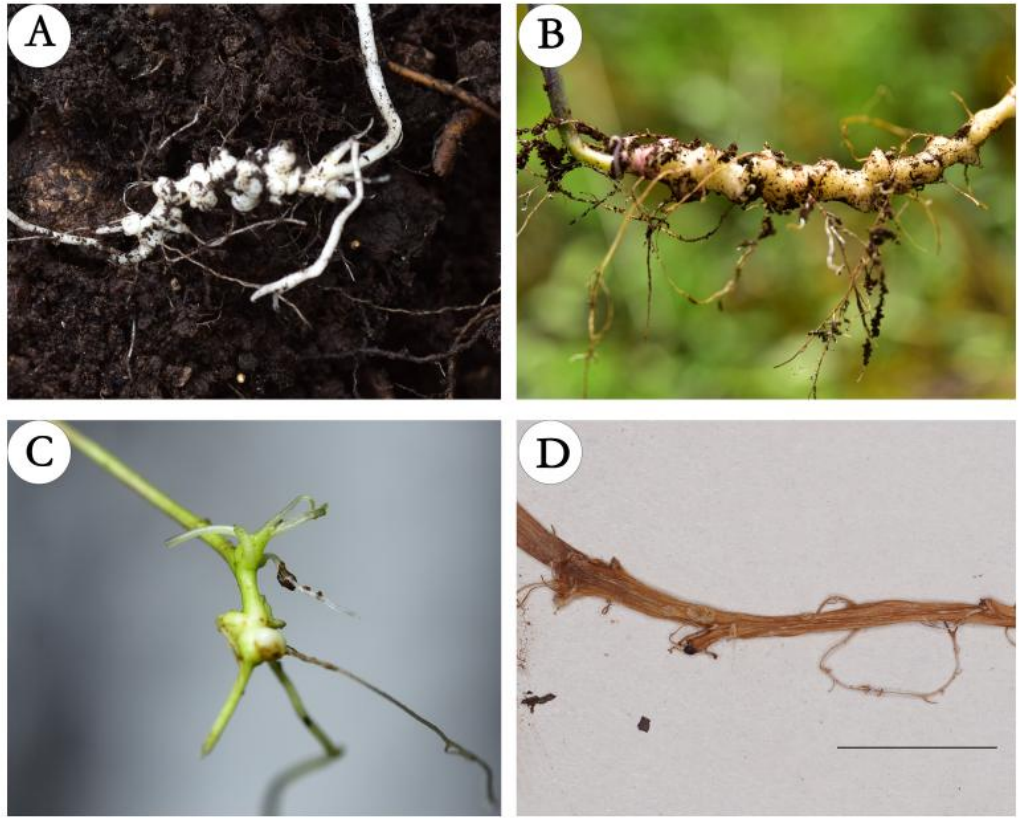
- Brassicaceae, Cardamineae
- 280 species worldwide (Brassibase, 2024).
- Three diversity centers: **Mediterranean**, East Asia (Himalaya-Hengduan Mountains, **HHM**), **North America**.
- 72 species in East Asia, 60 of which are endemic



# Background: Morphological Characteristics

Annual, biennial or perennial plants, usually with rhizomes. Leaves simple or compound.



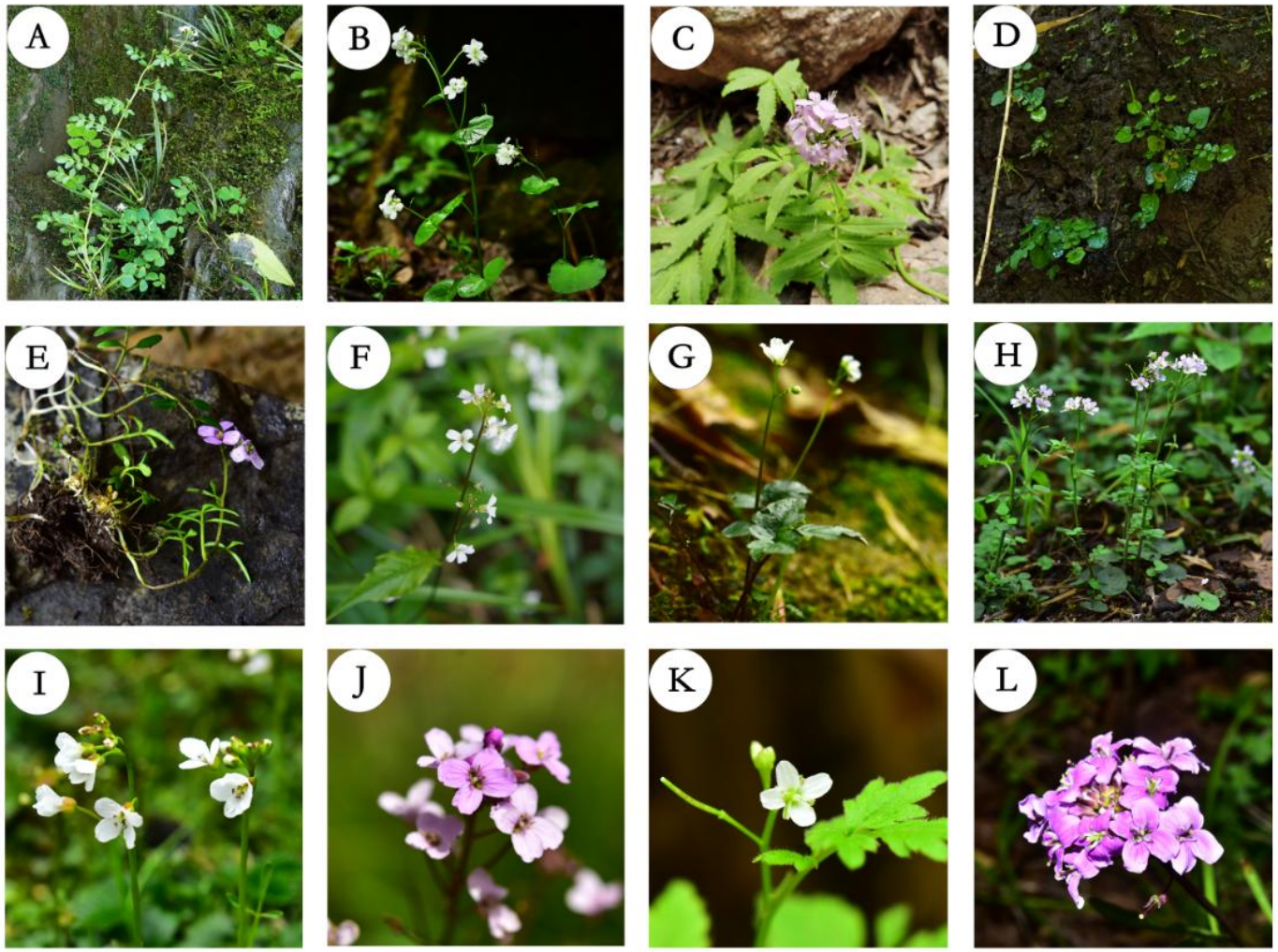



# Background: Morphological Characteristics

- Flowers: white to purple
- Siliques: linear and flat, dehiscing elastically acropetally
- Habitats: moist or wet areas, mixed forests, roadside clearings, mountain slopes







# Background: Taxonomic History

| Taxonomists | Linnaeus | Bentham & Hooker | Prantl | O. E. Schulz | 吴征镒 | Al-Shehbaz |
|---|---|---|---|---|---|---|
| Years | 1753 | 1862 | 1887 | 1903-1936 | 2003 | 1980s to present |
| Main Views | Established *Cardamine* and *Dentaria* | Three subgenera: *Dentaria, Pteroneuron,* and *Kardanoglyphos* | Three sections：sect. *Cardaminella*、sect. *Eucardamine*、sect. *Pterygospermum* | Divided into 13 sections recognized the indepen-dent status of *Loxostemon* | Did not recognize sect. *Lygophyllum* | Proposed a broad-sense *Cardamine* incorporated *Loxostemon* into *Cardamine* |
| Floras | *Species Plantarum* | *Genera plantarum* | | *Flora Reipublicae Popularis Sinicae*<br>*Fl. USSR* | *The Families and Genera of Angiosperms in China* | *Flora of China*<br>*Flora of Pan-Himalaya* |

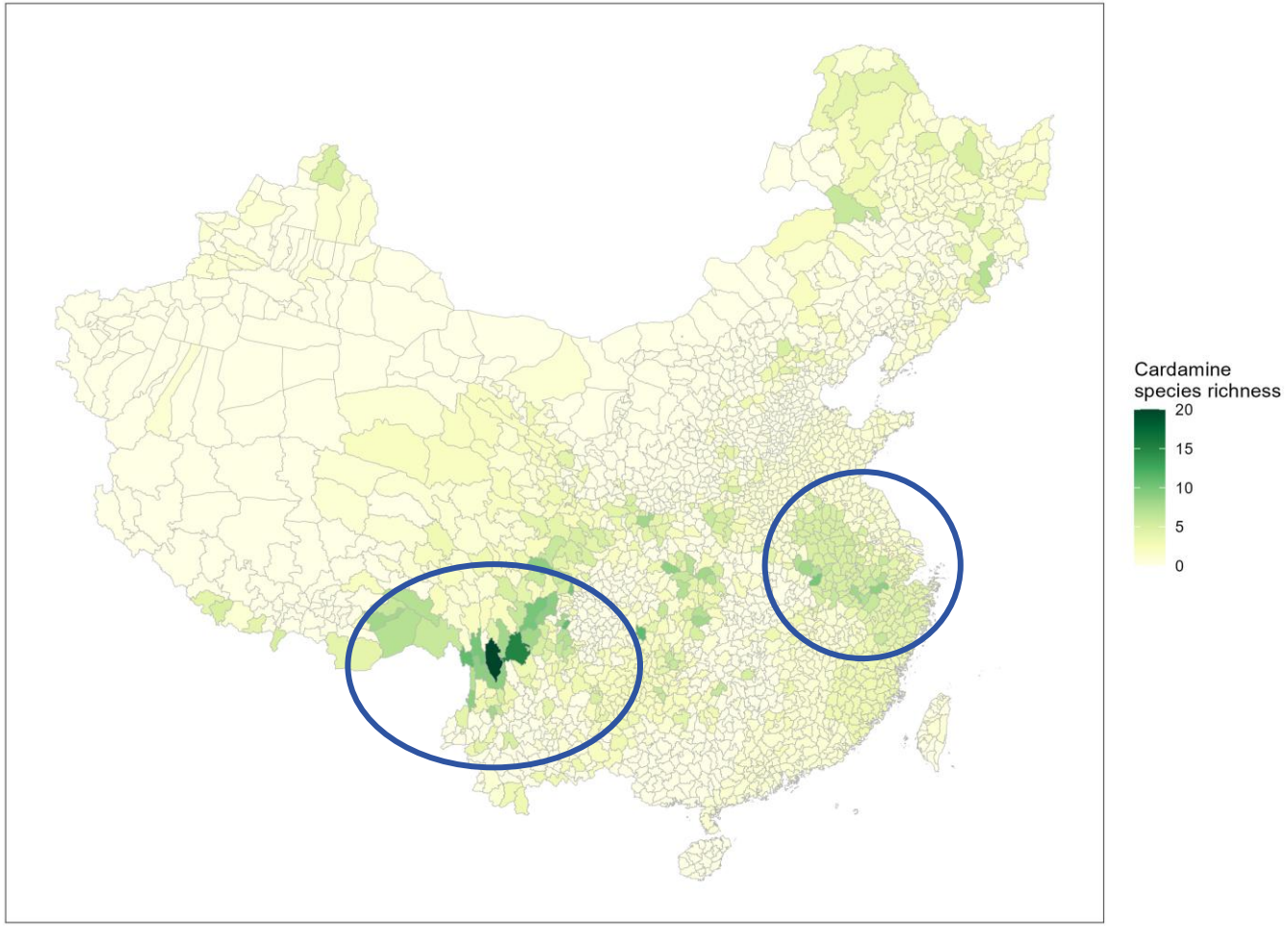
# Background: Taxonomic History

| | | | | |
|---|---|---|---|---|
| **1862** | Hooker & Thomson | Established *Loxostemon* | Type: *L. pulchellus* | |
| **1981-1983** | 蓝永珍，周太炎<br>(Lan Yongzhen, Zhou Taiyan) | Published new species and new combinations | 10 species | *Flora Reipublicae Popularis Sinicae* |
| **2001-2016** | Al-Shehbaz | Incorporated *Loxostemon* into *Cardamine s. l.* | 7 species | *Flora of China*<br>*Flora of Pan-Himalaya* |
| **2003** | 吴征镒等<br>(Wu Zhengyi et al.) | *Loxostemon* is a specialized branch of *Cardamine* and should be retained | 7 species | *The Families and Genera of Angiosperms in China* |

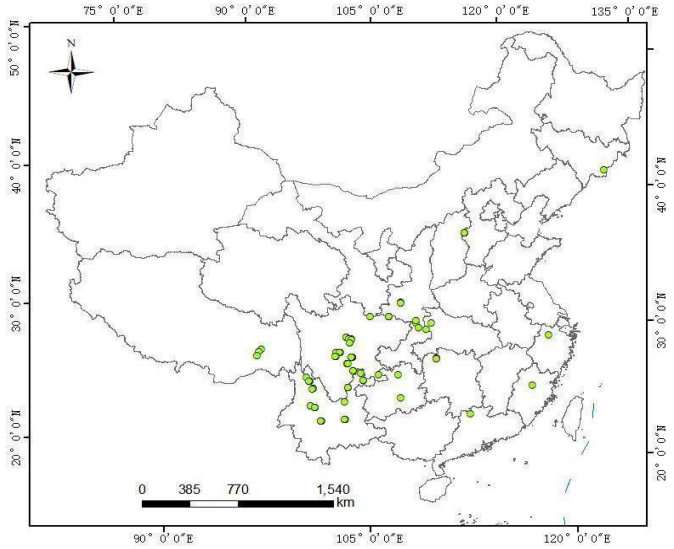
The above treatments are based on biogeography and morphology, lacking evidence from molecular systematics.

# Background: Endemism

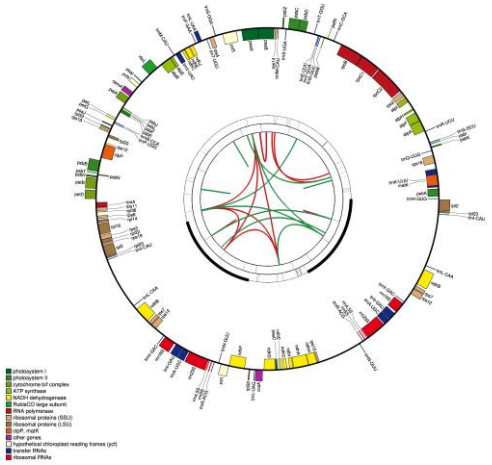
- High endemism: 60 endemic species, including East Asian endemics and HHM endemics (27 sp.)
- Is the exceptional endemism of Hengduan-Himalayan Cardamine primarily driven by recent rapid in situ radiation (the “cradle” effect)?
- Have incomplete lineage sorting (ILS) and historical gene flow/hybridization jointly shaped the reticulate evolutionary history of East Asian *Cardamine*?

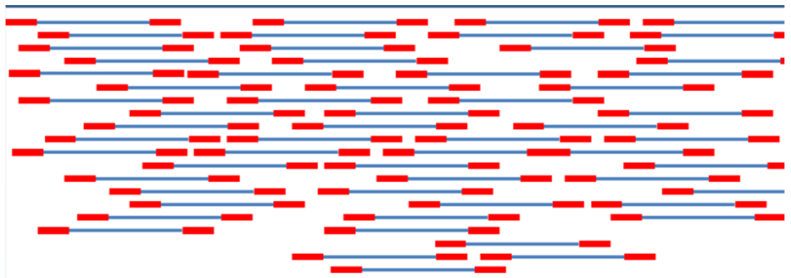


# Materials and Methods







- ✓ Expanded taxon sampling

  50 spp. (45 Cardamine + 5 Loxostemon) · 59 individuals

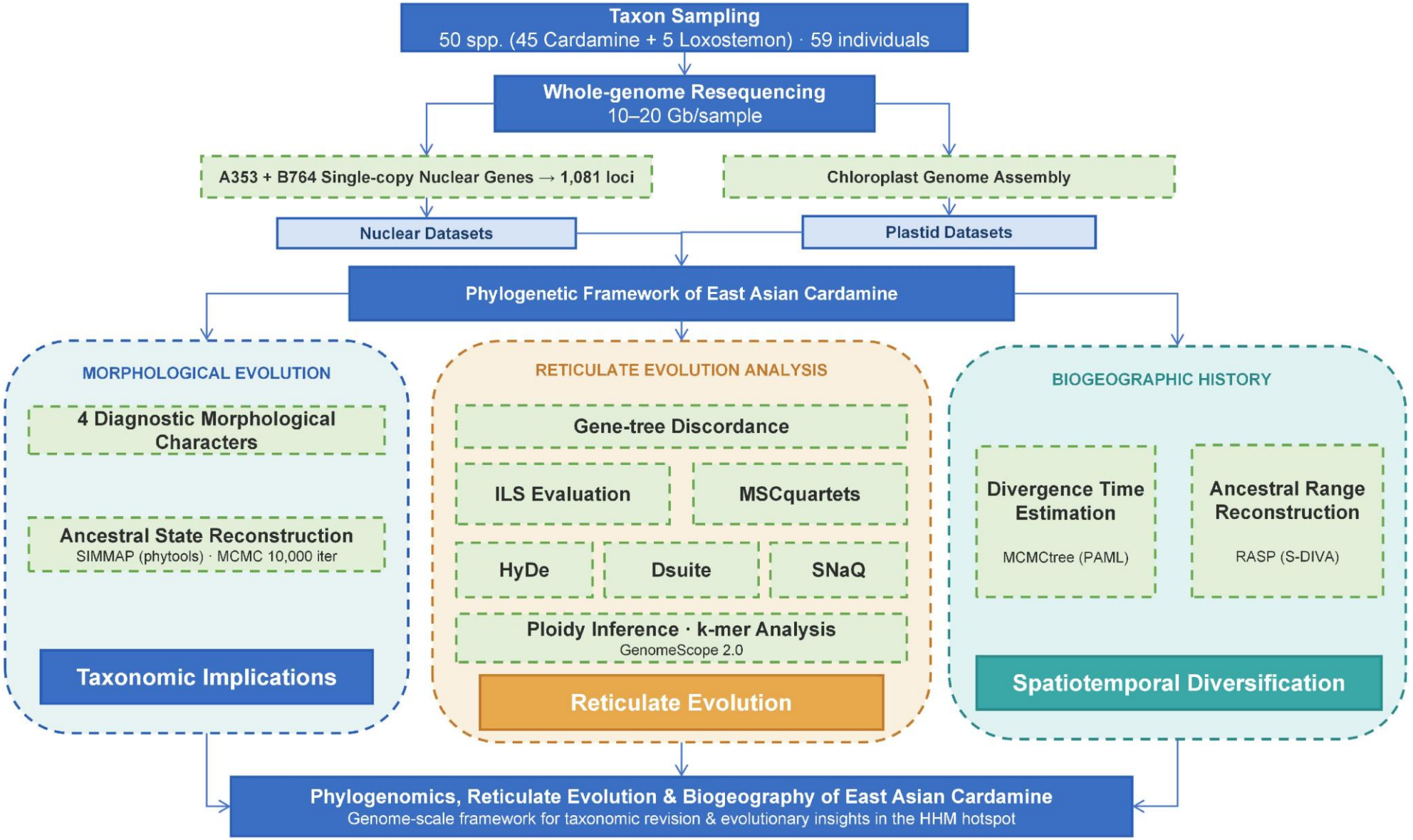
- ✓ Phylogenomic approach:

  Chloroplast genomes

  Angiosperms353 (**A353**) universal single- or low-copy nuclear genes

  Brassicaceae-specific single-copy nuclear genes (**B764**)

# Materials and Methods



# Result and Discussion

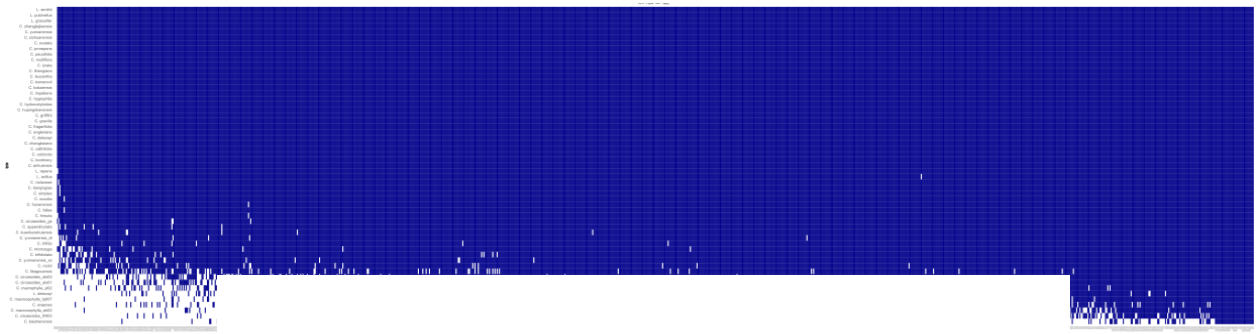
## Phylogenetic analyses based on different datasets

Nuclear Gene Assembly

Gene recovery rate: 12.59%–99.72% across species

B764 dataset: 728 genes, 84–14,118 bp

A353 dataset: 350 genes, 60–3,505 bp



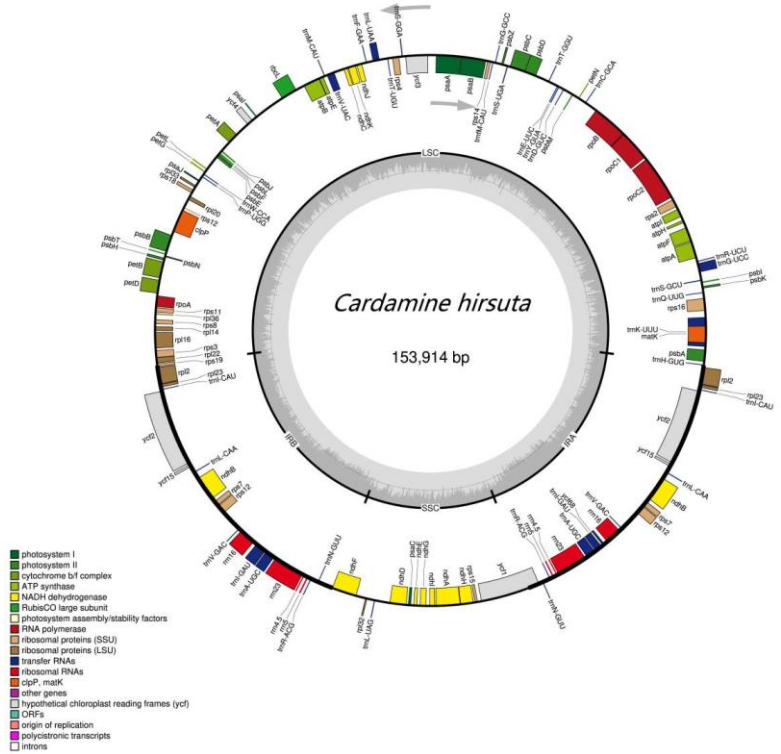
Phylogenetic Datasets

Dataset I: 59 individuals, 530 genes, 90% matrix completeness

Dataset II: 50 species, 877 genes, 90% matrix completeness

Dataset III: 45 species, 933 genes, 100% matrix completeness

Chloroplast Gene Assembly: 62 sequences assembled, 130 genes annotated



# Result and Discussion

## Phylogenetic analyses based on different datasets

Phylogenetic Results

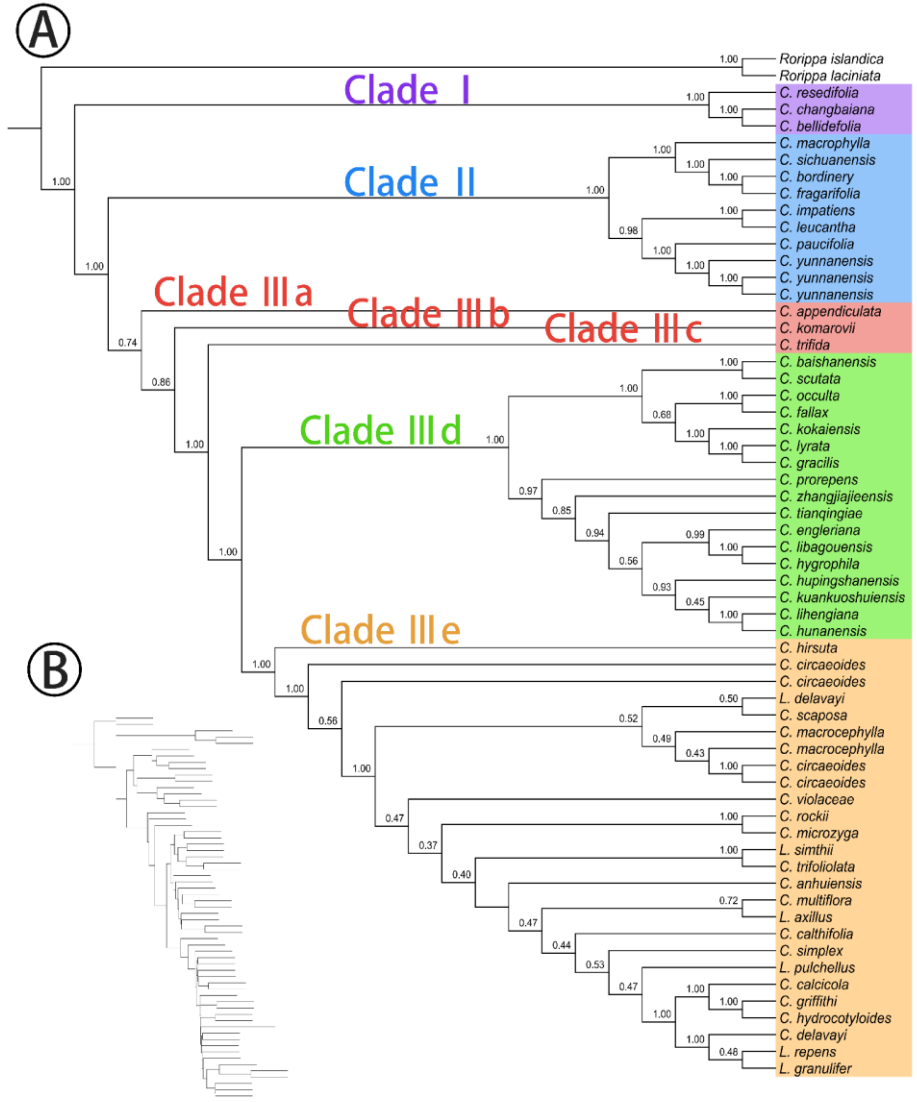
- *Loxostemon* is nested within *Cardamine*.
- 3 major clades with highly congruent topologies.

Clade I – basal lineage; includes sect. *Cardaminella*

Clade II – includes sect. *Macrophyllum*; comprises two well-supported subclades.

Clade III – highest species diversity

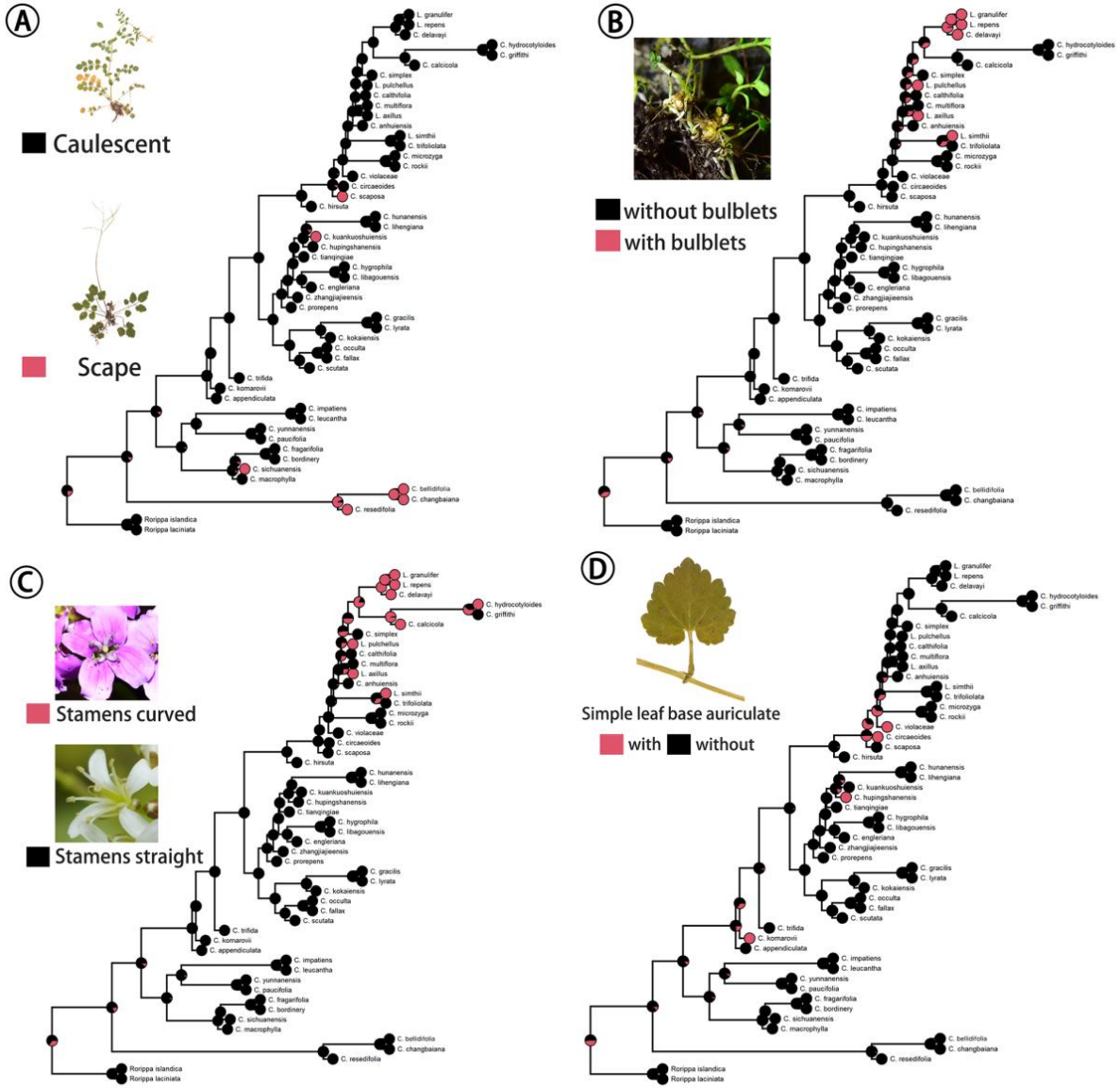
- Subclade IIId – distributed in eastern China and Japan.
- Subclade IIIe – contains all sampled *Loxostemon* and most Himalayan-Hengduan endemic *Cardamine*



# Result and Discussion

## Ancestral State Reconstruction

- *Loxostemon*: curved filaments; bulbils/small-scale buds on rhizomes → multiple independent origins within Clade IIIe → not monophyletic; genus not supported.
- Sect. *Giraldiella* & sect. *Lygophyllum*: leafless flowering stems with all basal leaves and simple amplexicaul leaves, respectively, each arose multiple times independently → neither section is supported.
- Traditional infrageneric groups are not natural and require revision within *Cardamine*.

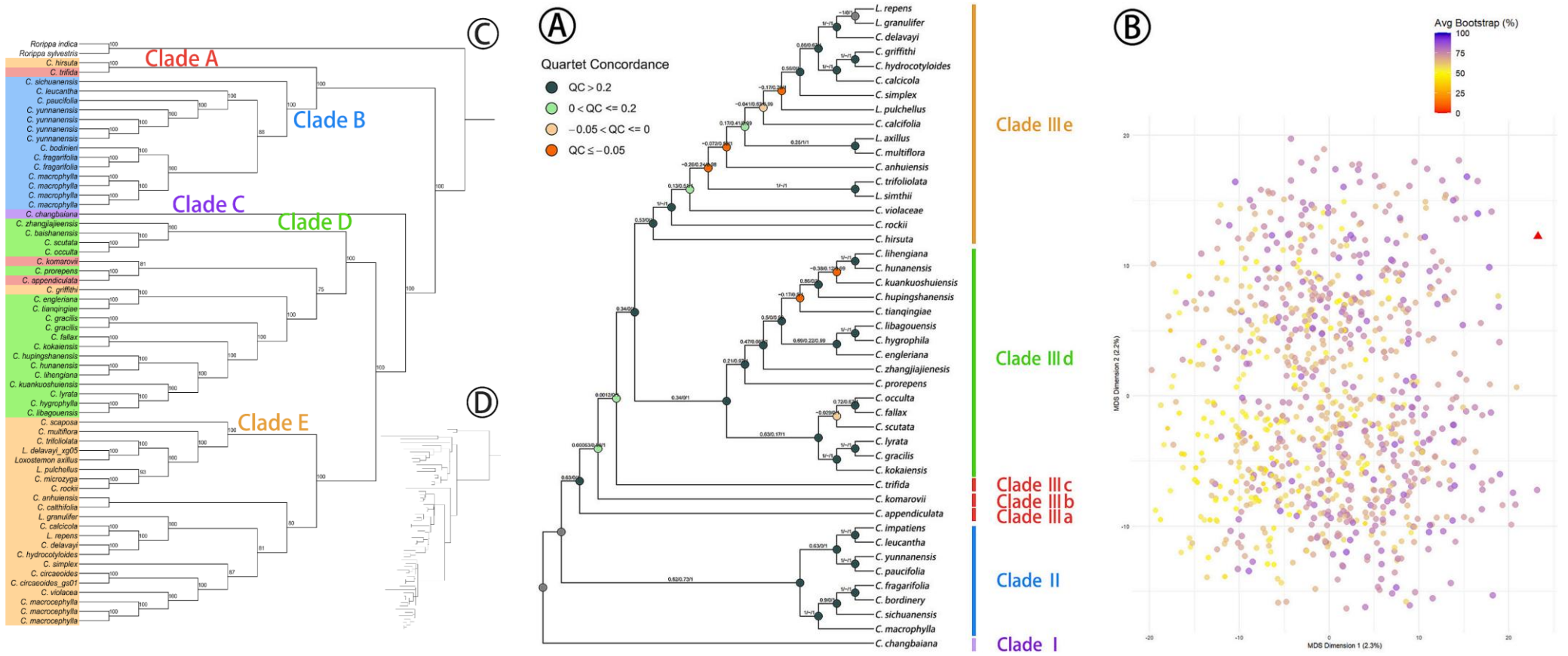


# Section Summary

- *Cardamine* is not monophyletic. Both molecular phylogenetic and morphological evidence support merging *Loxostemon* into *Cardamine*.
- Molecular phylogenetic and morphological evidence do not support recognizing sect. *Lygophyllum* and sect. *Giraldiella*. However, their phylogenetic positions cannot yet be determined because of limited sampling.
- East Asian *Cardamine* can be mainly divided into three major clades.

# Result and Discussion

**Significant discordance among phylogenetic signals.**




Plastid phylogenomic analyses and gene-tree discordance estimates (RF distances, Quartet Sampling) reveal extensive conflict among the nuclear gene trees, the species tree, and the plastid tree.

This discordance is non-random and concentrated at terminal branches, likely reflecting incomplete lineage sorting, hybridization, and/or chloroplast capture.

# Result and Discussion

## Coalescent simulations

Coalescent simulations

- Simulated 10,000 gene trees
- Low ILS: θ ≈ 0.01 (empirical estimate)
- High ILS: θ = 0.1

Distribution of the simulated gene trees is similar to empirical gene trees

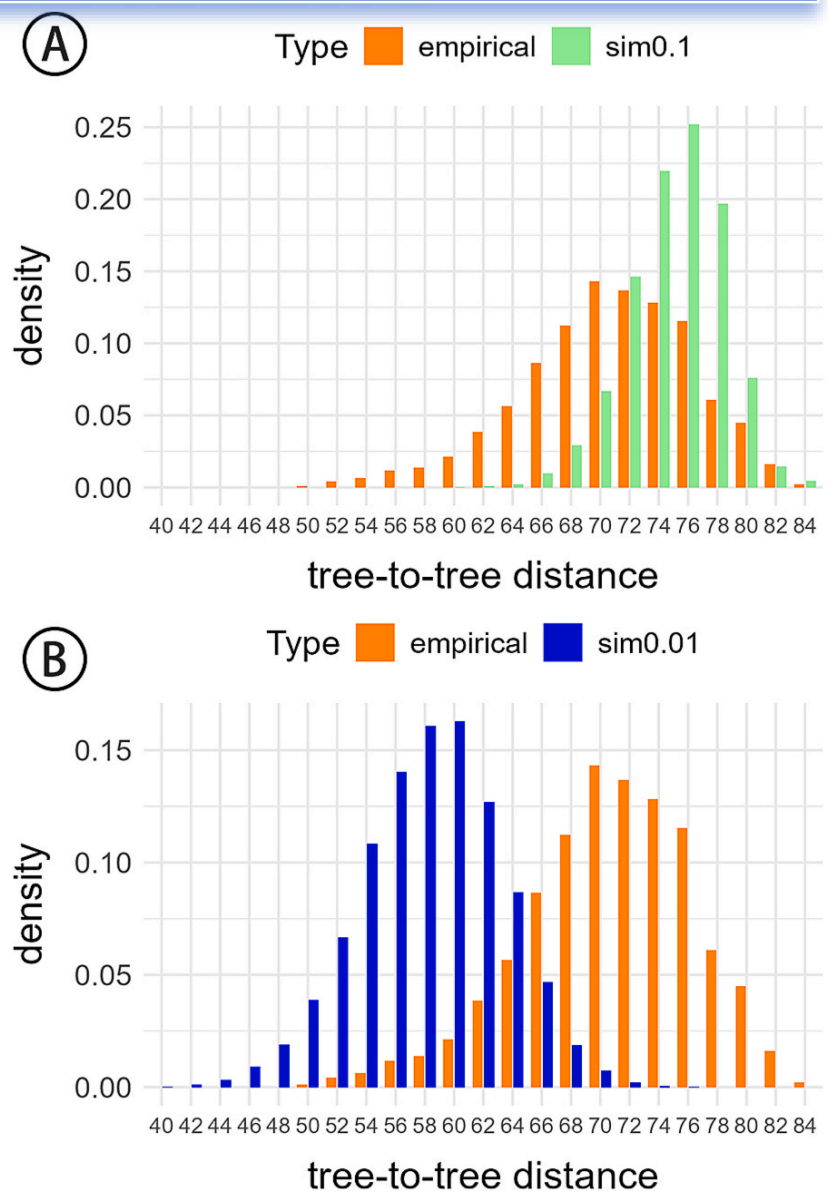
↓

ILS can explain most of the conflict among gene trees.

Distribution of the empirical gene trees is similar to high ILS

↓

**ILS alone cannot fully explain the discordance among the phylogenetic signals.**



# Result and Discussion

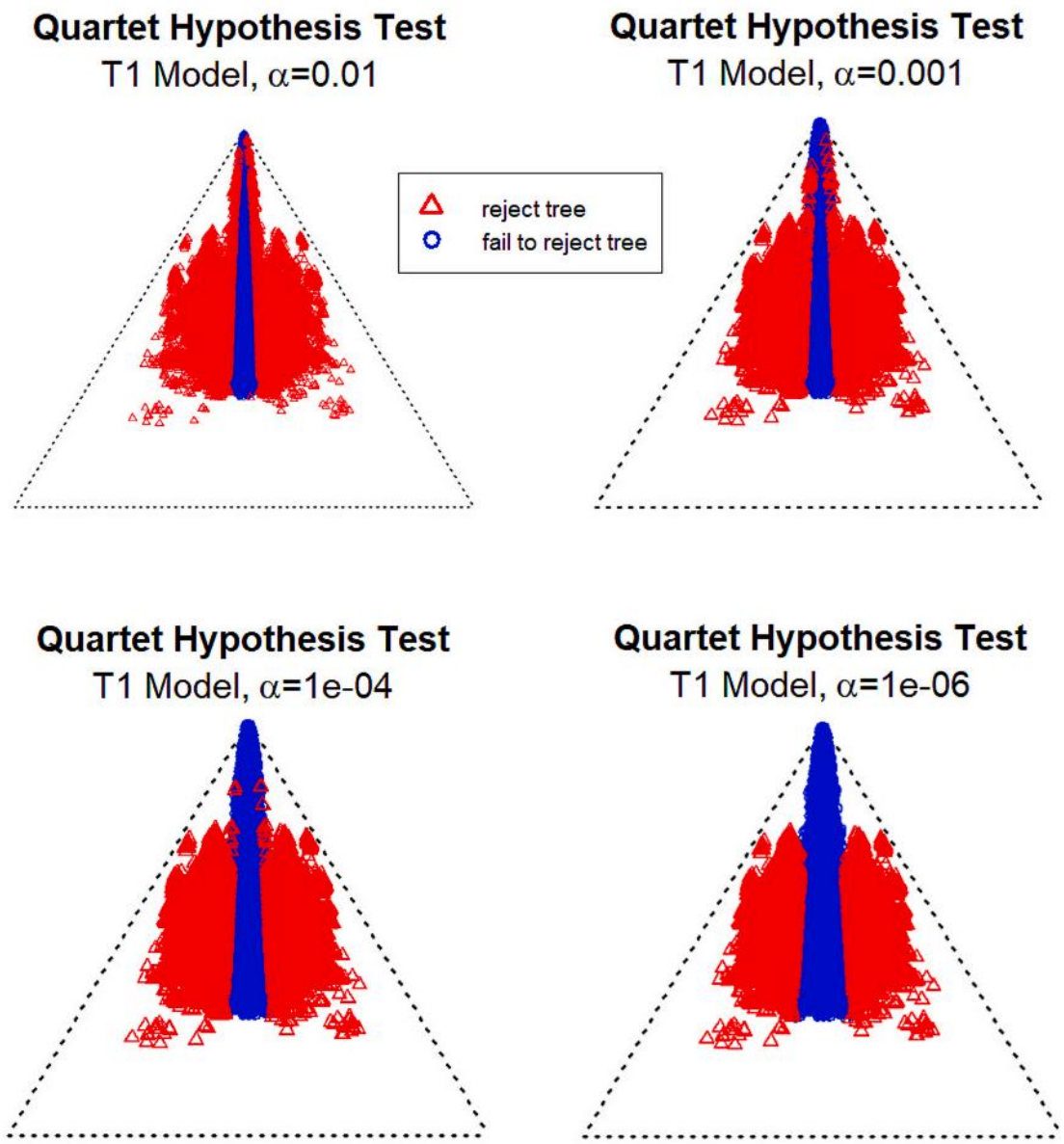
## MSCquartets

- MSCquartets (Dataset 3) at Different Rejection Thresholds, T1 Model

α = 0.01, 0.001, 1e-4, and 1e-6

- Rejected Trees

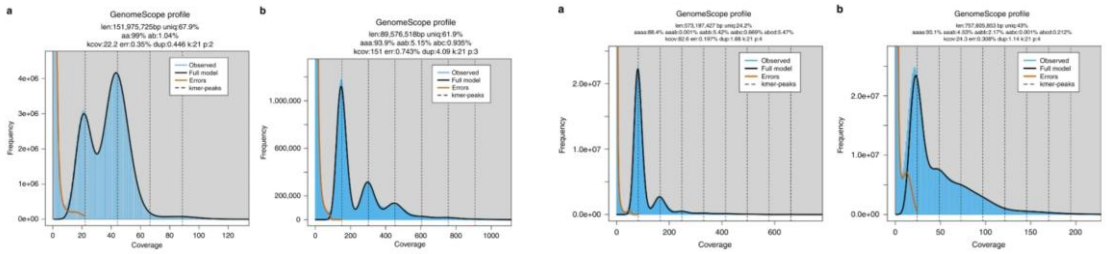
Gene-tree estimation error or more complex biological processes, such as **hybridization and gene flow.**



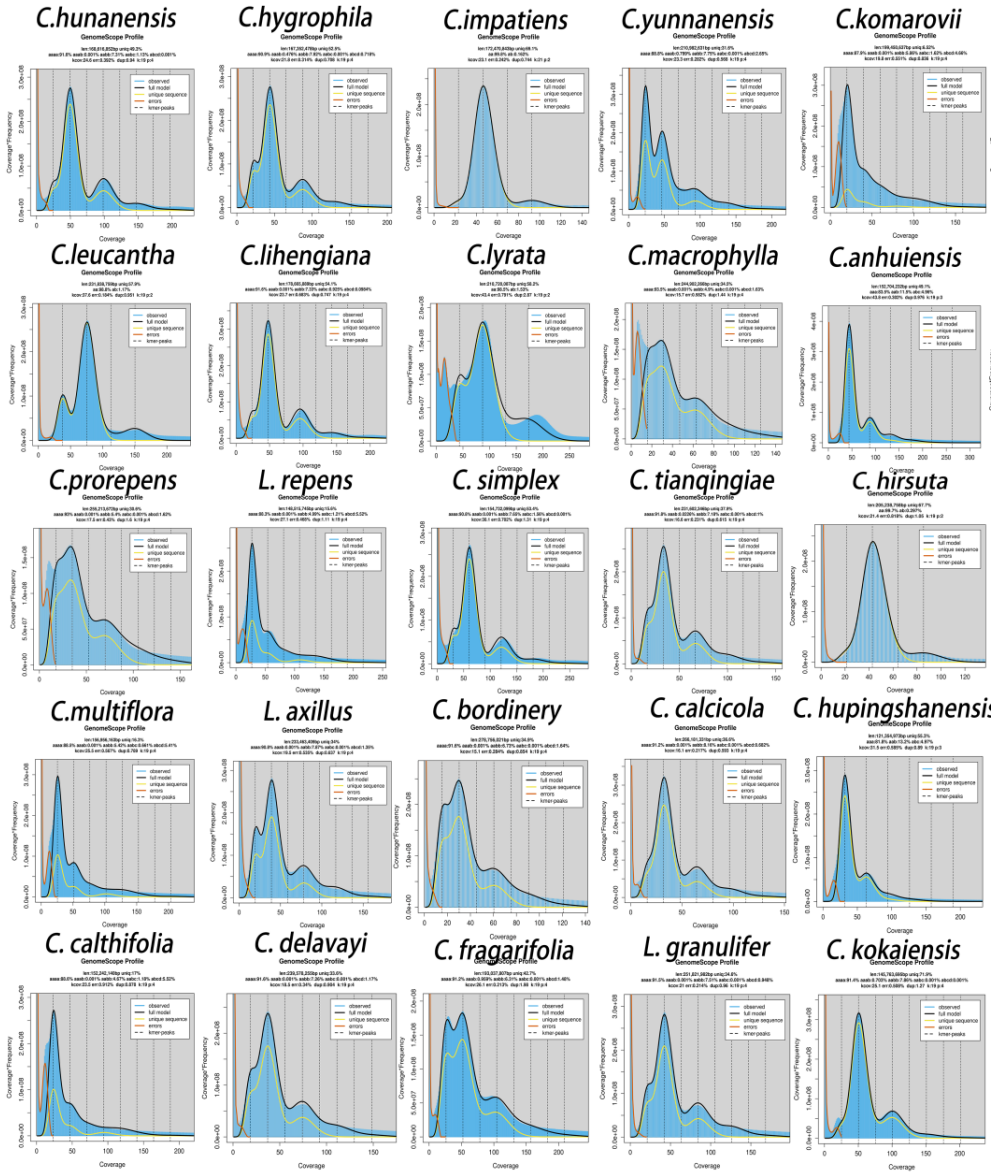
# Result and Discussion

48 species analyzed; 27 with model error < 1%:

- Diploids: *C. leucantha, C. impatiens, C. hirsuta, C. lyrata*
- Triploids: *C. hupingshanensis, C. anhuiensis*
- Hexaploid: *C. trifida*
- Tetraploids: remaining 20 taxa
- In tetraploids, allopolyploid models (aabb/abcd) fit better than autopolyploid models (aaab), suggesting a **hybrid origin of the tetraploid taxa**.

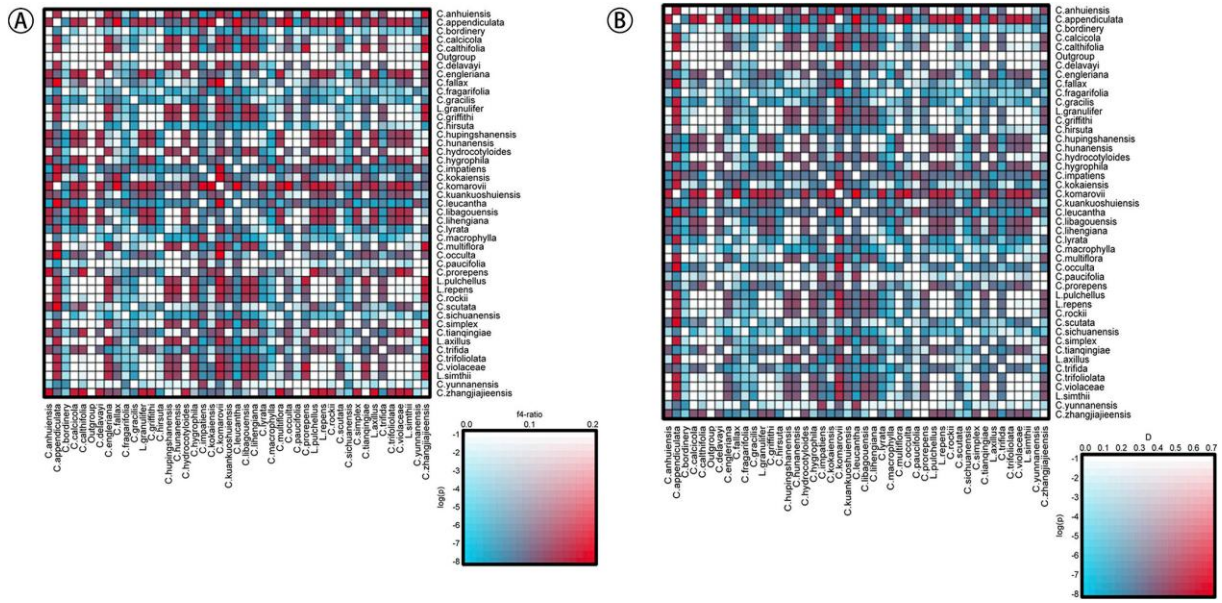


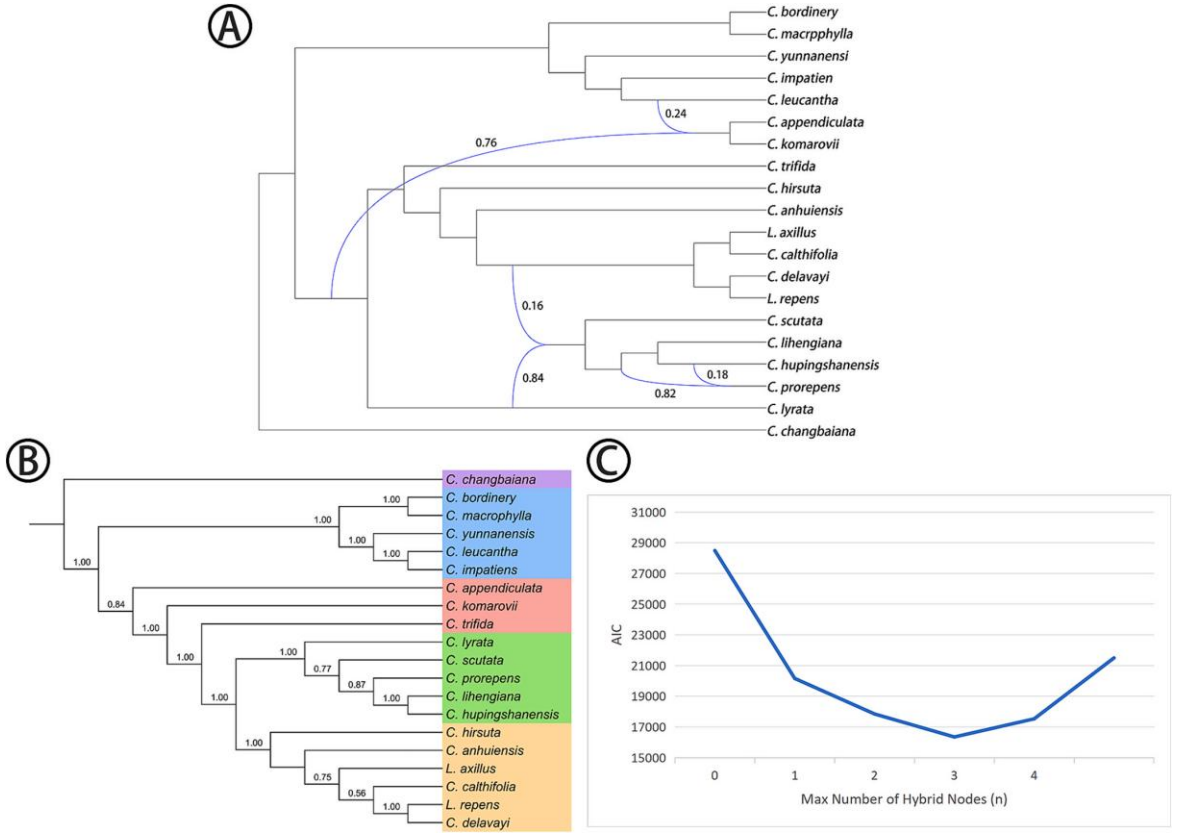
Diploids | Triploids | Tetraploids allopolyploid | Tetraploids autopolyploid



# Background: Overview of *Cardamine* L.

HyDe and ABBA-BABA analyses detected widespread gene flow among extant species; nearly all species showed evidence of introgression.SNaQ inferred three hybridization events



# Section Summary

- Significant phylogenetic conflict is evident among nuclear gene trees, the coalescent species tree, and the plastome tree
- Although incomplete lineage sorting (ILS) is detected, ILS alone cannot fully explain the observed signal conflict.
- Ploidy evidence and HyDe/ABBA-BABA (D-statistic and f4-ratio) analyses indicate widespread hybridization and interspecific gene flow among East Asian Cardamine, and SNaQ analysis supports hybridization events at both basal and internal nodes of the phylogeny.



**The reticulate evolution of East Asian Cardamine is shaped jointly by ILS, hybridization, gene flow, and polyploidization**

# Background: Overview of *Cardamine* L.

1. HHM lineage divergence coincides with Qinghai-Tibetan Plateau uplift

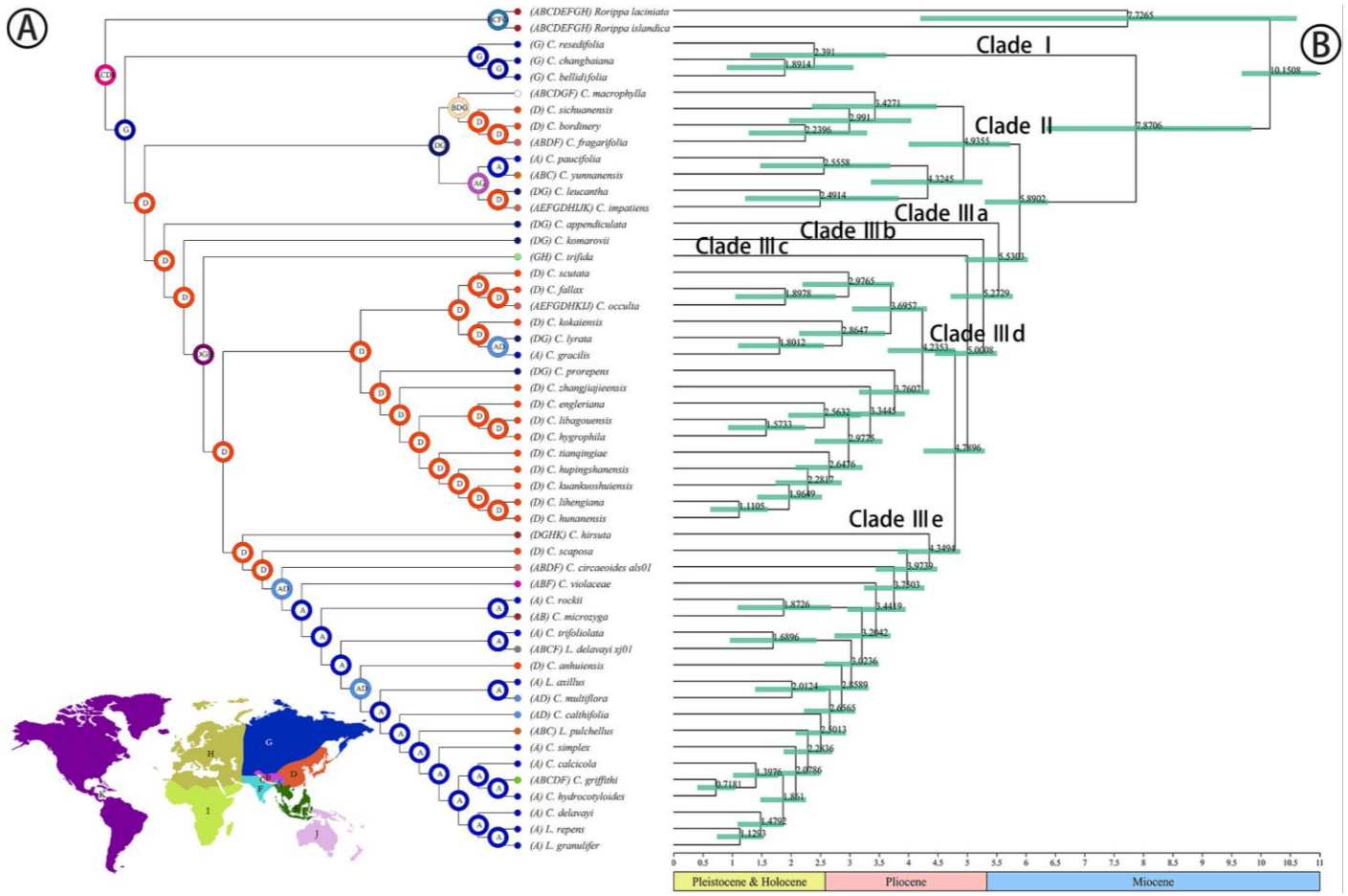
- Clade IIIe radiated 4.34–1.86 Ma, synchronous with Qingzang movements A/B/C.

2. East Asian *Cardamine* endemism is mainly in situ, driven by hybridization

- Most endemics arose locally within Clade IIIe; hybridization and polyploidization are key.

3. HHM region is both a cradle and a species pump

- It generates diversity and exports lineages to the Sino-Japanese Floristic subkingdom.



# Key Conclusions

- Established the first genome-scale phylogenomic framework for East Asian *Cardamine* using complete plastomes and 1,081 single-copy nuclear genes.
- *Loxostemon* is nested within *Cardamine*; repeated evolution of diagnostic traits supports treating it as a synonym.
- Evolutionary history of East Asian *Cardamine*—especially the core Hengduan-Himalayan lineage—was shaped by incomplete lineage sorting and recurrent hybridization.
- The core Hengduan-Himalayan lineage originated in the late Pliocene and underwent rapid in situ diversification, coinciding with Qinghai-Tibet Plateau uplift and associated environmental changes.

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## Phylogenomics, reticulate evolution, and taxonomic revision of East Asian Cardamine (Brassicaceae) in the Hengduan biodiversity hotspot

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ABSTRACT

The genus *Cardamine* (Brassicaceae) exhibits remarkable species diversity and endemism in the Hengduan-Himalayan Mountains (HHM) of East Asia; however, its phylogenetic relationships and evolutionary history remain unclear. Here, we conducted a phylogenomic analysis of 50 species, including 45 East Asian *Cardamine* taxa and five *Loxostemon* species, based on complete chloroplast genomes and 1,081 single-copy nuclear genes. Our results indicate that: (1) *Cardamine* is not monophyletic, with *Loxostemon* nested within the genus; moreover, the diagnostic morphological characters traditionally used to delimit Loxostemon have evolved repeatedly and independently, supporting its synonymization under Cardamine. (2) Divergence time estimation and ancestral range reconstruction suggest that the core HHM clade originated approximately 4.34 Ma and rapidly radiated between 4.34 and 1.86 Ma, temporally coincident with major uplift events of the Qinghai-Tibet plateau (QTP). Integrative analyses of gene tree discordance, hybridization signals, and ploidy variation further demonstrate that this rapid diversification was driven by reticulate evolution involving incomplete lineage sorting and frequent hybridization. This study provides the first genome-scale phylogeny framework for East Asian *Cardamine*, highlights the central role of hybridization in generating plant diversity in the HHM, and offers a robust molecular foundation for future taxonomic revision.

# Future Research Directions

- Focus: Eurasian biogeography of *Cardamine*
- Goal: Test the Mediterranean-origin hypothesis and resolve the dispersal route(s) from the Mediterranean to East Asia
- Key alternatives to distinguish:
  - West-to-east dispersal along the Eurasian forest belt
  - Eastward dispersal via high mountains of West and Central Asia
- Approach:
  - Expand taxon sampling with critical materials from West and Central Asia





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# Thank you!