Genome sequencing and comparative analysis of *Ficus benghalensis* and *Ficus religiosa* species reveal evolutionary mechanisms of longevity

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Introduction

- *Ficus benghalensis* (Indian banyan tree) and *Ficus religiosa* (Peepal) belong to Moraceae plant family
- Keystone species in tropical ecosystem with well-developed morphological characteristics
- Show significantly long average lifespan (>500 years)
- Medicinal and religious importance

*Ficus benghalensis**

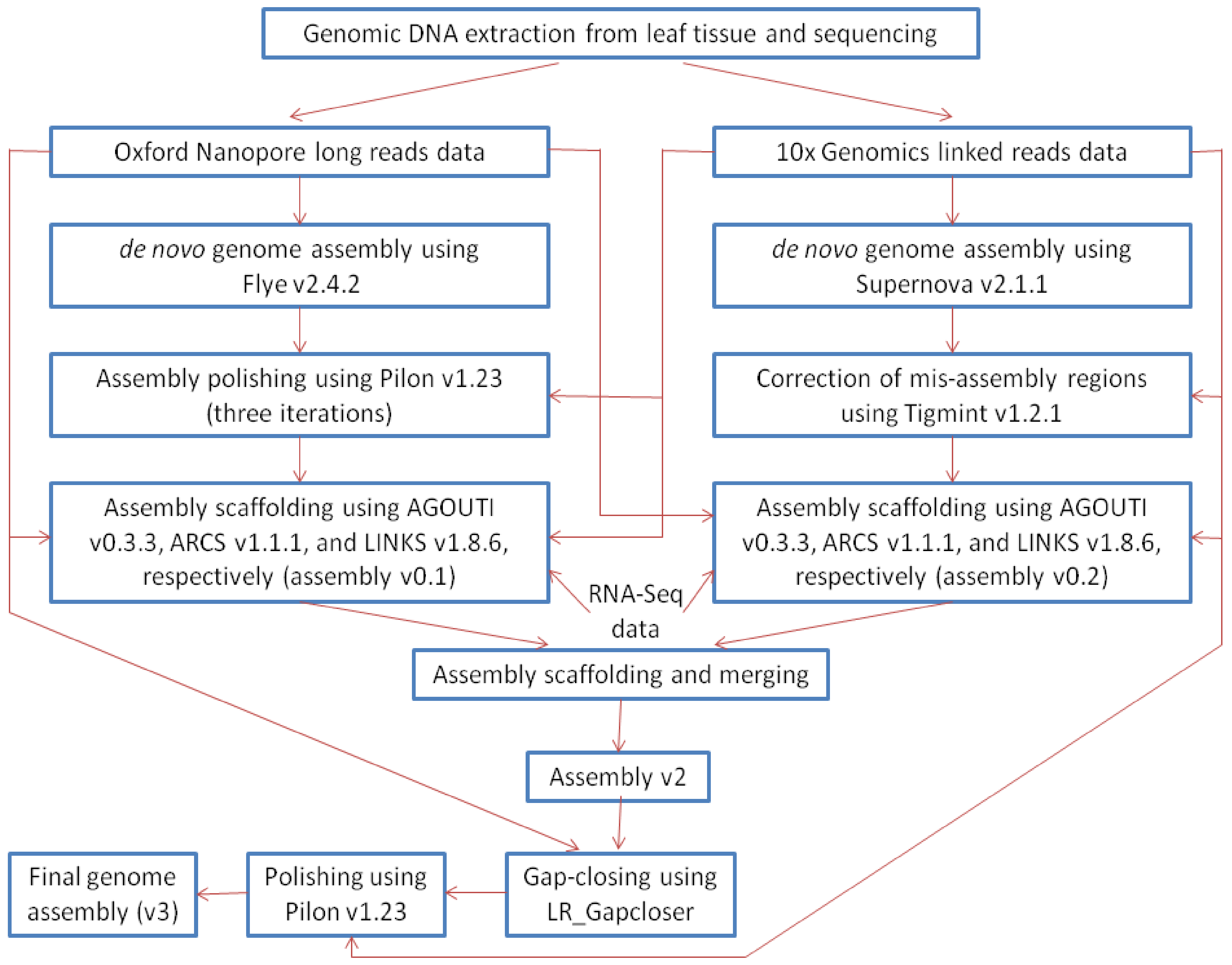
*Ficus religiosa**

(*Picture source: Internet)

Objectives

- To perform whole genome sequencing using Nanopore long reads and 10x Genomics linked reads
- To understand the genomic basis of longevity by performing comparative analysis with other short-lived trees

Study Design



Genomic DNA extraction from leaf tissue and sequencing

Oxford Nanopore long reads data

de novo genome assembly using Flye v2.4.2

Assembly polishing using Pilon v1.23 (three iterations)

Assembly scaffolding using AGOUTI v0.3.3, ARCS v1.1.1, and LINKS v1.8.6, respectively (assembly v0.1)

10x Genomics linked reads data

de novo genome assembly using Supernova v2.1.1

Correction of mis-assembly regions using Tigmint v1.2.1

Assembly scaffolding using AGOUTI v0.3.3, ARCS v1.1.1, and LINKS v1.8.6, respectively (assembly v0.2)

RNA-Seq data

Assembly scaffolding and merging

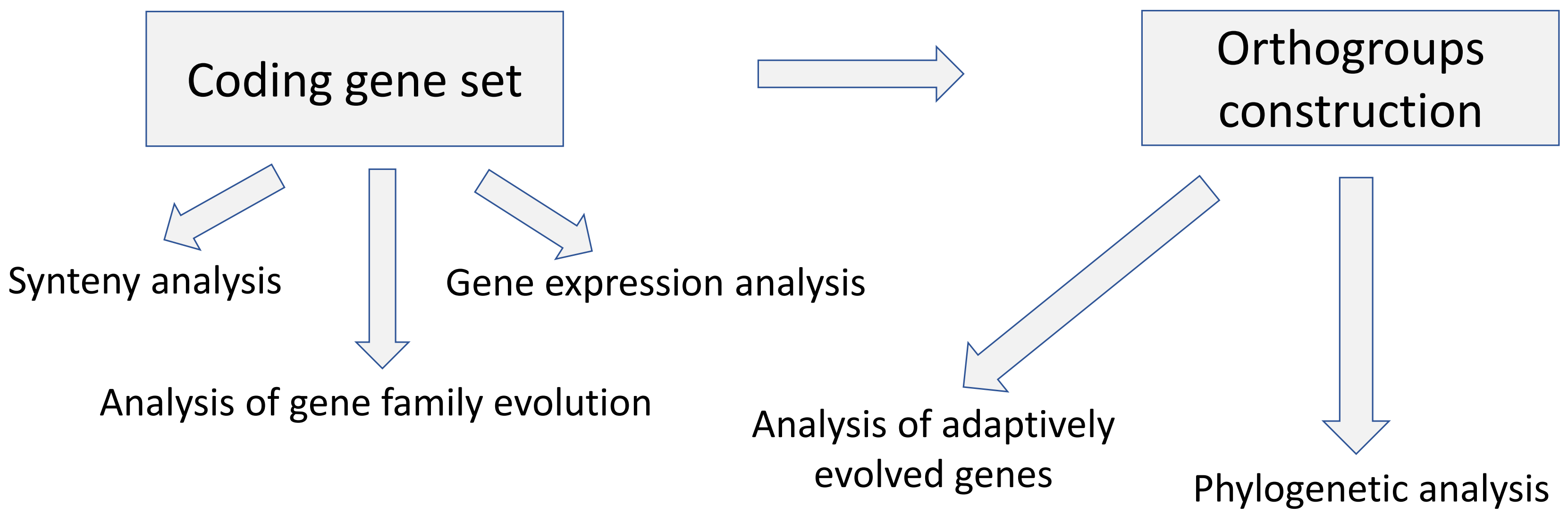
Assembly v2

Gap-closing using LR_Gapcloser

Polishing using Pilon v1.23

Final genome assembly (v3)

Figure 1: Workflow for genome assembly



Coding gene set

Orthogroups construction

Synteny analysis

Gene expression analysis

Analysis of gene family evolution

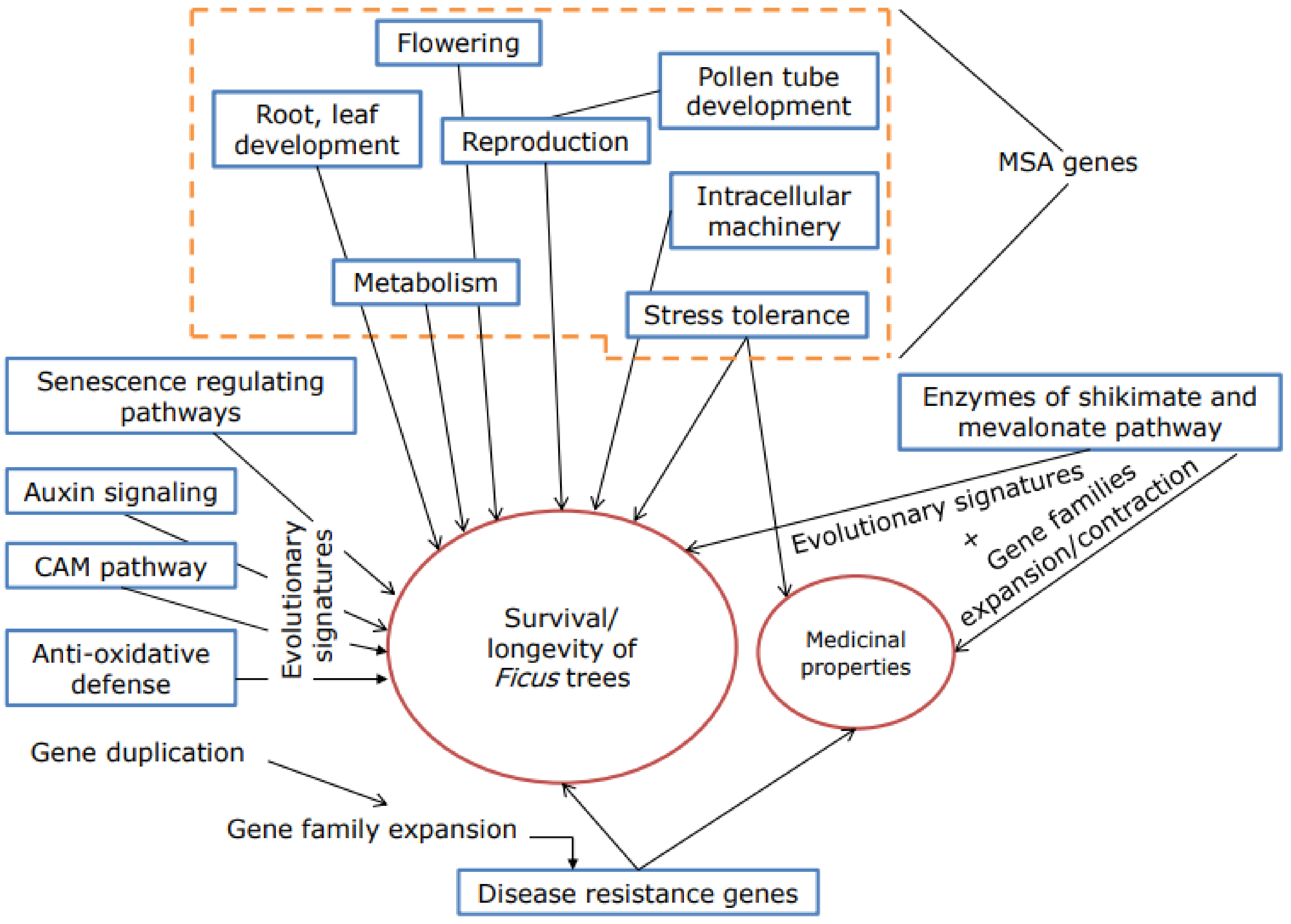
Analysis of adaptively evolved genes

Phylogenetic analysis

Figure 2: Workflow for genome annotation

Results

- Oxford Nanopore long read genomic libraries were prepared using SQK-LSK-109 kit followed by sequencing on MinION Mk1B instrument to generate 9 Gb (23.6X coverage) and 7.2 Gb (21.3X coverage) data from *F. benghalensis* and *F. religiosa* leaves, respectively
- More than 100X linked read data were also generated for both species
- With the help of Nanopore long reads, *F. benghalensis* (392.89 Mbp) and *F. religiosa* (332.97 Mbp) genomes were assembled with N50 values of 486.9 Kb and 553.4 Kb, respectively
- ~25,000 high-confidence coding genes were predicted for both the species
- Both species showed one population bottleneck event ~0.8 Million years ago
- 503 inter-species collinear blocks were identified between *F. benghalensis* and *F. religiosa* genome
- Phylogenetic position was resolved with respect to 50 other Angiosperms



Flowering

Root, leaf development

Reproduction

Pollen tube development

Intracellular machinery

Metabolism

Stress tolerance

MSA genes

Senescence regulating pathways

Auxin signaling

CAM pathway

Anti-oxidative defense

Evolutionary signatures

Enzymes of shikimate and mevalonate pathway

Evolutionary signatures + Gene families expansion/contraction

Survival/ longevity of *Ficus* trees

Medicinal properties

Gene duplication

Gene family expansion

Disease resistance genes

Figure 3: Evolutionary significant biological processes responsible for *Ficus* longevity

- Comparative evolutionary analyses revealed adaptive evolution in genes involved in major cellular mechanisms associated with long-time survival
- Plant organ developmental genes, genes involved in auxin signaling, senescence pathways, stress tolerance were evolved
- Genes involved in shikimate and mevalonate pathways responsible for fig-wasp interactions showed evolutionary signatures
- Disease-resistance gene families were highly expanded (including R-proteins), and showed higher gene expression

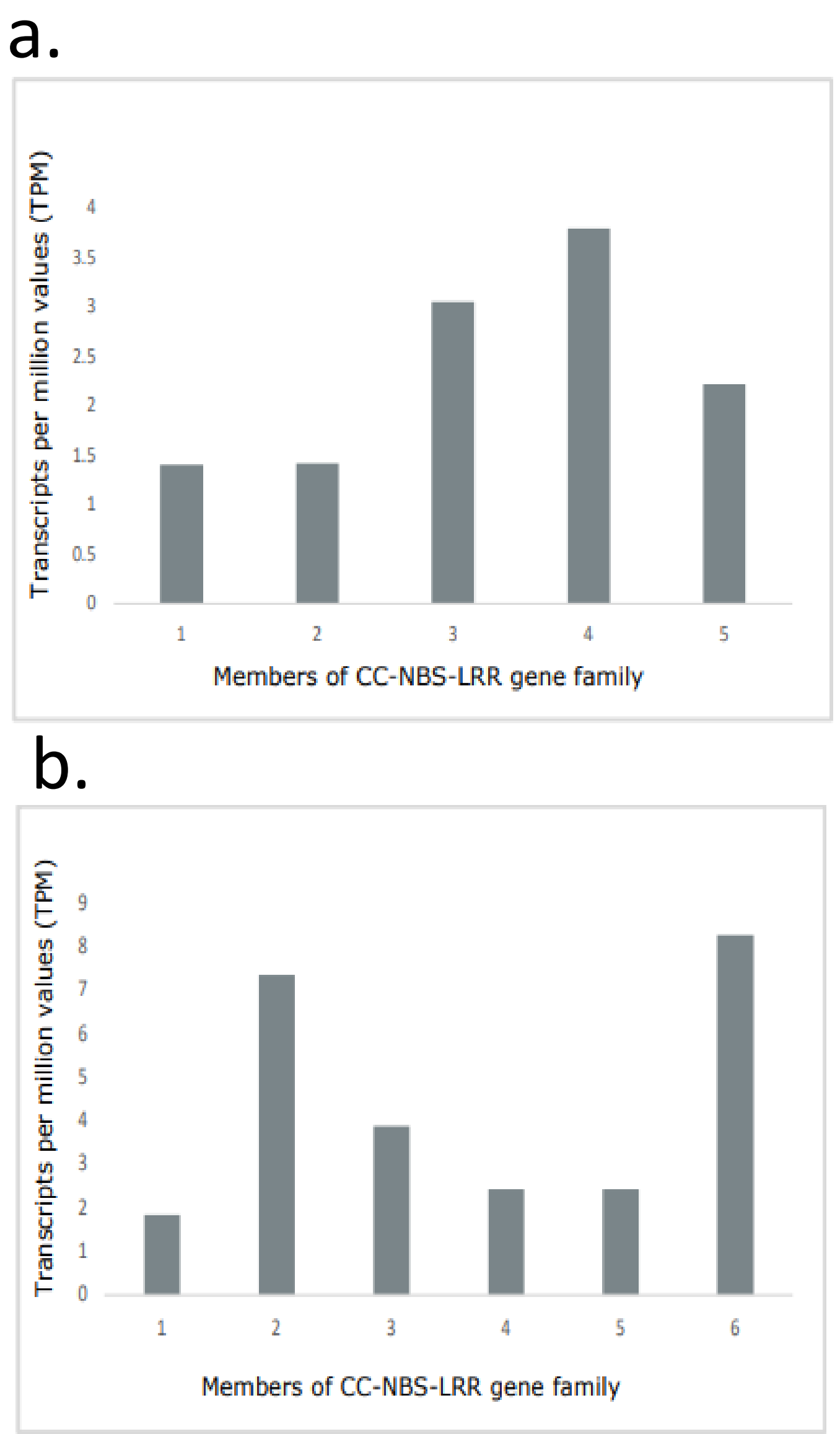


Figure 4: Expression levels of disease-resistance gene family in - **a**. *F. benghalensis*, **b**. *F. religiosa*

Summary of the Study

- ❑ Long reads obtained from Oxford Nanopore sequencing helped to assemble these *Ficus* genomes with ~50% repeat content, and >1.6% heterozygosity
- ❑ Comparative evolutionary analyses including short-lived plants and other *Ficus* species showed adaptive evolution in key mechanisms required for well-developed morphology, and long-time survival of these species

Other Major Genomes Sequenced by Our Group using Nanopore Sequencing

Aloe vera

Curcuma longa

Tinospora cordifolia

Pavo cristatus

(*Picture source: Internet)

References: i) Chakraborty, A., Mahajan, S., Bisht, M. S., & Sharma, V. K. (2022). Genome sequencing and comparative analysis of *Ficus benghalensis* and *Ficus religiosa* species reveal evolutionary mechanisms of longevity. *Iscience*, *25*(10), 105100. https://doi.org/10.1016/j.isci.2022.105100
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