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Chromosome-level assemblies of the endemic Korean species *Abeliophyllum distichum* and *Forsythia ovata*

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***Abeliophyllum distichum* and *Forsythia ovata* are closely related species endemic to Korea and are highly valued as ornamental shrubs in the Oleaceae family. A combination of PacBio and Illumina sequencing with Hi-C scaffolding technologies was employed to develop chromosome-level genome assemblies of these species. The assembled genome sizes are 795.72 Mb for *A. distichum* and 1,108.53 Mb for *F. ovata*. The assemblies exhibit scaffold N50 lengths of 53.12 Mb and 68.97 Mb, with minimal gaps measuring 323.40 kb and 149.00 kb, and 97.71% and 98.82% BUSCO scores for Embryophyta single-copy orthologs, respectively, indicating high contiguity and completeness. The genomes contain 485.24 Mb and 691.68 Mb of repetitive sequences, 4,926 and 7,175 full-length long terminal repeat retrotransposons, and 49,414 and 57,587 protein-coding genes, respectively. The 14 pseudochromosomes encompass 93.80% of the *A. distichum* genome and 89.11% of the *F. ovata* genome, thereby demonstrating one-to-one chromosome-level collinearity. These high-quality genome assemblies serve as invaluable resources for genetic and breeding studies, facilitating a deeper understanding of the evolutionary history of these distinctive species.**

Background & Summary

The Oleaceae family is comprised of 28 genera across five tribes, with approximately 700 species of temperate and tropical shrubs, trees, and occasionally lianas[1]. Many species from the genera *Forsythia*, *Fraxinus*, *Jasminum*, *Ligustrum*, and *Syringa* are widely cultivated for purposes of ornamentation, fragrance, and timber, while olive trees from the genus *Olea* are valued for their fruit and oil production. In the past decade, chromosome-level genome assemblies have been developed for seven Oleaceae species, including *Olea europaea*[2–4], *Fraxinus excelsior*[5], *Osmanthus fragrans*[6], *Fraxinus pennsylvanica*[7], and *Syringa oblata*[8–10], which belong to the tribe Oleeae; *Jasminum sambac*[11,12] in tribe Jasmineae; and *Forsythia suspensa*[13] in tribe Forsythieae. These genome assemblies provide substantial insights into the organization and expression of genes associated with a number of important biological processes, including oil biosynthesis[2], fragrance production[11], disease resistance[5], and the synthesis of medicinal compounds[13]. A comparison of these genomes suggests that a hexaploidization event likely occurred in the Oleaceae approximately 53–61 million years ago (MYA)[14]. Moreover, species within the tribe Oleeae underwent a shared whole-genome duplication (WGD) approximately 28 MYA[2,9]. It is postulated that this duplication event resulted in a doubling of chromosome pairs (n = 23) in these species, in contrast to the tribe Jasmineae (n = 13 for *J. sambac*) and Forsythieae (n = 14 for *F. suspensa*). Nevertheless, the precise nature of this WGD, whether autopolyploid or allopolyploid, and the basic chromosome number of the family remain unknown.

Endemic plants are defined as those that grow naturally within restricted habitats. A total of 373 endemic plant taxa, representing approximately 9.5% of the native flora, have been documented in the checklist of

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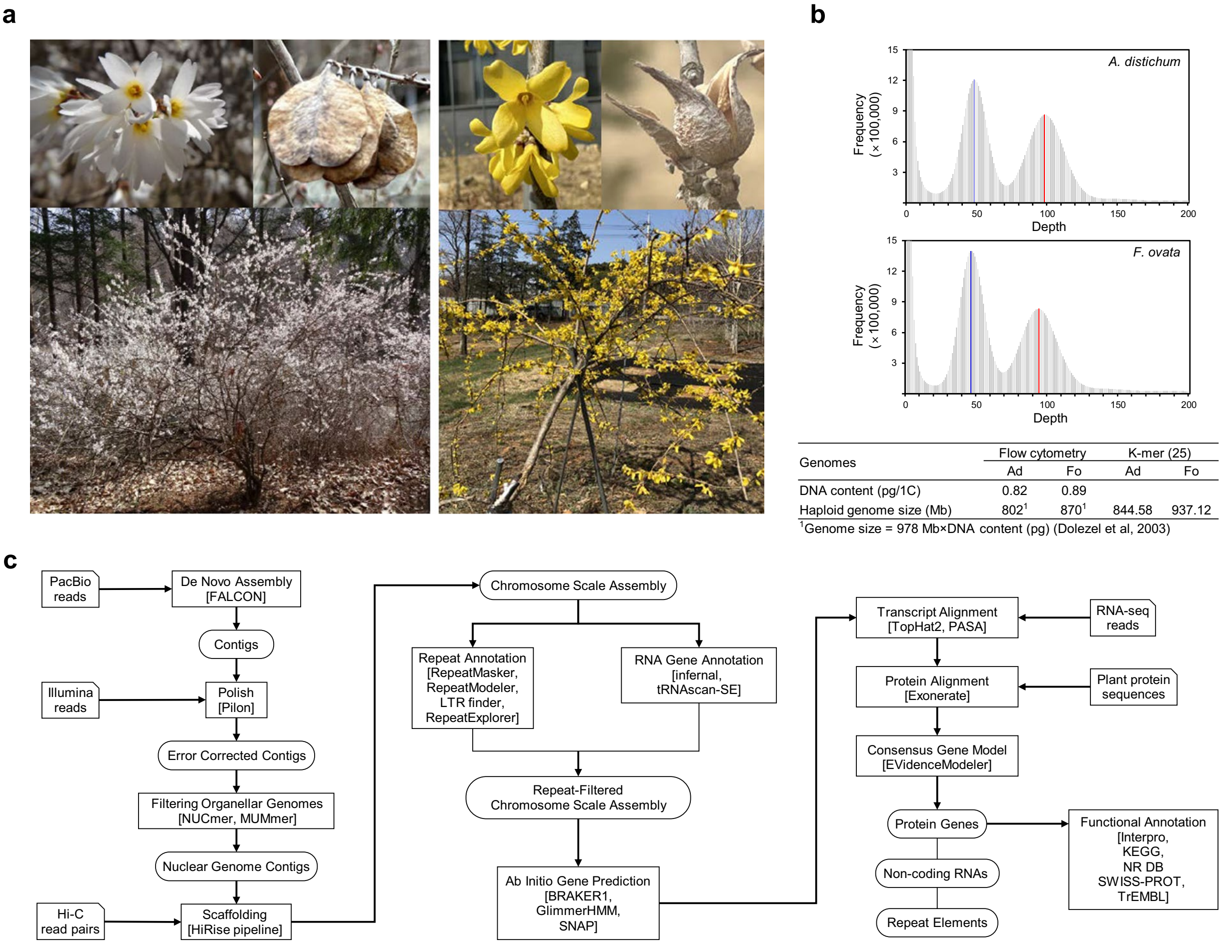
a
b
15
12
9
6
3
Frequency
(× 100,000)
0
50
100
150
200
Depth
A. distichum
F. ovata
c
PacBio reads
De Novo Assembly [FALCON]
Contigs
Illumina reads
Polish [Pilon]
Error Corrected Contigs
Filtering Organellar Genomes [NUCmer, MUMmer]
Nuclear Genome Contigs
Hi-C read pairs
Scaffolding [HiRise pipeline]
Chromosome Scale Assembly
Repeat Annotation [RepeatMasker, RepeatModeler, LTR finder, RepeatExplorer]
RNA Gene Annotation [infernal, tRNAscan-SE]
Repeat-Filtered Chromosome Scale Assembly
Ab Initio Gene Prediction [BRAKER1, GlimmerHMM, SNAP]
Transcript Alignment [TopHat2, PASA]
RNA-seq reads
Protein Alignment [Exonerate]
Plant protein sequences
Consensus Gene Model [EVidenceModeler]
Protein Genes
Non-coding RNAs
Repeat Elements
Functional Annotation [Interpro, KEGG, NR DB SWISS-PROT, TrEMBL]

Genomes	Flow cytometry		K-mer (25)	
	Ad	Fo	Ad	Fo
DNA content (pg/1C)	0.82	0.89		
Haploid genome size (Mb)	802[1]	870[1]	844.58	937.12

[1]Genome size = 978 Mb×DNA content (pg) (Dolezel et al, 2003)

Fig. 1 Reference accessions of *Abeliophyllum distichum* and *Forsythia ovata* used in this study. (**a**) Photographs of *A. distichum* (left) and *F. ovata* (right) flowers, fruits, and trees, taken between March and April 2023. (**b**) Estimation of genome sizes for *A. distichum* (Ad) and *F. ovata* (Fo) based on K-mer 25 distributions. The blue and red peaks correspond to heterozygous and homozygous reads, respectively. The lower panel depicts the estimated haploid genome size derived from homozygous K-mer peaks and flow cytometry analyses. (**c**) Workflow of genome assembly and annotation.

endemic plants on the Korean Peninsula. The list includes *Abeliophyllum distichum*, *Forsythia koreana*, *Forsythia nakaii*, and *Forsythia ovata*, which belong to the tribe Forsythieae[15]. *Abeliophyllum distichum*, the sole species in the monotypic genus *Abeliophyllum*, is distinguished by its white flowers and winged samara-type fruits (Fig. 1a). The genus *Forsythia* comprises 11–13 species, with a predominantly Eastern Asian distribution and one species native to southeastern Europe. *Forsythia* species are characterized by their yellow flowers and capsule fruits (Fig. 1a). Chromosome cytology indicates that both *Abeliophyllum* and *Forsythia* exhibit the same chromosome number ($n = 14$)[16]. The classification of *Abeliophyllum* as a sister group to *Forsythia* within the tribe Forsythieae is supported by studies of pollen morphology[17] and molecular phylogeny using nuclear and chloroplast genes[18,19]. It has been postulated that these species may represent the basal genome structure of the common ancestor of the Oleaceae family, given their relatively small and compact genomes (ca. 1 Gb or less) with 14 chromosomes.

This study aimed to develop high-contiguity assemblies for the genomes of *A. distichum* and *F. ovata* by employing a combination of PacBio and Illumina sequencing and Hi-C scaffolding technologies. We employed RNA sequencing (RNA-seq) across various tissues and conducted whole-genome methylation profiling to annotate the assemblies and identify heterochromatic chromosome regions. Furthermore, we carried out a genome-to-genome comparison with other sequenced Oleaceae genomes. The findings from this study provide valuable resources for biology research, conservation efforts, and breeding programs for these species, as well as for evolutionary studies within the Oleaceae family.

Methods

Plant materials and nucleic acid extraction. We selected the *A. distichum* accession KNKB198505000391 and *F. ovata* accession KNKB202402200001 (Fig. 1a) at the Korea National Arboretum for genome sequencing. Leaf tissues were collected in April and May following a two-day dark treatment period. High-molecular-weight genomic DNA was extracted using established nuclear isolation methods that are suitable for long-read

Type	Species	Library	Platform	Insert size (bp)	Raw data (Gb)	Clean data (Gb)	Sequence coverage (×)[a]
Genome	*A. distichum*	Illumina	HiSeqX	550	98.41	83.45	98.76
		Hi-C	HiSeqX	350 to 1,000	—	40.70	48.16
		PacBio	Sequel	20,000	—	98.14	116.14
	F. ovata	Illumina	NovaSeq6000	550	104.74	96.32	102.79
		Hi-C	HiSeqX	350 to 1,000	—	56.09	59.86
		PacBio	Sequel	20,000	—	84.38	90.05
Methylome	*A. distichum*	BS[b]	HiSeq2000	200	53.32	45.79	54.19
	F. ovata	BS[b]	HiSeq2000	200	53.40	45.89	48.98
Transcriptome	*A. distichum*	mRNA-seq[c]	NextSeq	350	74.40	53.85	63.73
	F. ovata	mRNA-seq[c]	NovaSeq6000	350	65.60	59.14	63.11

Table 1. Statistics of sequencing data used in the genome assemblies of *Abeliophyllum distichum* and *Forsythia ovata*. [a]Sequence coverage was calculated using the genome sizes of *A. distichum* and *F. ovata*, measured at 844.58 and 937.12 Mb, respectively. [b]BS, bisulfite sequencing. [c]mRNA-seq, mRNA-sequencing.

sequencing[20–22]. Total RNA was isolated from leaf, petal, carpel, and stamen tissues collected in April, using the cetyltrimethylammonium bromide method[23]. The RNA was subsequently used for messenger RNA (mRNA) purification and library construction. The quality and quantity of the nucleic acids were evaluated using an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) and a Qubit Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA).

Library construction, genome sequencing, and genome size estimation.

Library construction and sequencing were conducted by Macrogen (Seoul, Korea). For Illumina short-read sequencing, paired-end (PE) sequencing libraries with an insert size of 550 bp were generated using the Illumina TruSeq Nano DNA Kit (Illumina, San Diego, CA, USA). Single-molecule real-time DNA sequencing libraries with an insert size of 20 kb were constructed for PacBio Sequel sequencing using the SMRTbell Prep Kit 3.0 (Pacific Biosciences, Menlo Park, CA, USA). For whole-genome bisulfite sequencing (BS), fragmented DNA sized at 200 bp was treated with bisulfite using the EZ DNA Methylation-Gold Kit (Zymo Research, Irvine, CA, USA), followed by library construction using the Accel-NGS Methyl-Seq DNA Library Kit (Swift Biosciences, Inc., Ann Arbor, MI, USA). For chromosome conformation capture, leaf tissues were provided to Dovetail Genomics (Santa Cruz, CA, USA), which constructed and sequenced Hi-C libraries. RNA libraries were prepared using the Illumina TruSeq RNA v2 Kit (Illumina). As a result, we obtained a total of 203.15 Gb of Illumina raw reads, 182.52 Gb of filtered PacBio subreads, 96.79 Gb of filtered Hi-C reads, 106.72 Gb of raw BS reads, and 140.00 Gb of raw mRNA data (Table 1). Illumina raw reads with a minimum Phred quality score of 20 (Q20) were processed using Trimmomatic v0.36[24] to filter out adapter contamination and low-quality regions. Polymerase chain reaction (PCR) duplicates were removed with Picard v2.2.4 (https://broadinstitute.github.io/picard) using the default parameters. The average read and N50 lengths of the filtered PacBio subreads were 17.61 and 25.47 kb, respectively.

The genome sizes of each species were estimated through the application of both K-mer analysis and flow cytometry. The frequency distribution of K-mer analysis using Illumina PE reads and JELLYFISH v2.1.3 software[25] with a K-mer size of 25 revealed two distinct peaks at coverages of 48 × and 98 × for *A. distichum* and 46 × and 94 × for *F. ovata*, corresponding to heterozygous and homozygous reads, respectively (Fig. 1b). The maximum haploid genome sizes were estimated to be 844.58 Mb for *A. distichum* and 937.12 Mb for *F. ovata*, based on homozygous reads. Flow cytometry, conducted with a CyFlow Space system (Partec BmbH, Münster, Germany) and using diploid *Raphanus sativus* cv. WK10039 (1 C = 0.6 pg) as a reference, indicated that the haploid genome sizes of these species were slightly smaller.

Genome assembly and scaffolding.

A schematic workflow of the genome assembly and annotation processes is presented in Fig. 1c. Initially PacBio subreads were corrected with CANU v2.1.1[26] and pre-assembled into contigs using the FALCON assembler v2.1.4[27], with parameters set as follows: length cutoff, 10 kb; max difference, 80; max coverage, 80; and minimum coverage, 2. The initial haplotype-fused contigs were refined using Pilon[28] for gap filling and sequence error correction, with the parameters–fix bases,–gaps, and–diploid. Mitochondrial and plastid sequences were filtered out from the contigs using organellar genome sequences (GenBank accessions MW645067, MF407178, and MF407183) and NUCmer from the MUMmer 3 package[29]. Subsequently, the resulting contigs were then aligned with the filtered PE reads from the Hi-C libraries using the Dovetail HiRise scaffolding pipeline[30] to produce a scaffold-level assembly. The total length of the assembled genome was 795.72 Mb for *A. distichum*, with a contig N50 of 373.06 kb and scaffold N50 of 53.12 Mb, and 1,108.53 Mb for *F. ovata*, with a contig N50 of 1.19 Mb and scaffold N50 of 68.97 Mb. The longest 14 pseudochromosomes of *A. distichum* and *F. ovata*, ranging from 40.08 to 70.22 Mb and 56.21 to 86.46 Mb, comprised 93.04% and 87.43% of the initial contig sequences, respectively (Table 2 and S1). The alignment of homologous pseudochromosomes of the *A. distichum* and *F. ovata* assemblies using MUMmer[29] demonstrated global collinear synteny across the genome, with some mismatched regions due to inversion (Fig. 2a).

The completeness of the genome assemblies was assessed using orthologous gene analysis with BUSCO v5.4.3[31] in conjunction with OrthoDB v10[32]. The BUSCO analysis indicated that approximately 96.86% to 98.54% of Eudicots genes (comprising 2,326 single-copy orthologs) and 97.71% to 98.82% of Embryophyta

Assembly		*A. distichum*	*F. ovata*
Contig	Number	4,321	1,831
	Total length (bp)	801,868,764	1,129,748,862
	N50 number	610	302
	N50 length (bp)	373,057	1,187,720
Scaffold	Number	879	145
	Total length (bp)	795,717,758	1,108,525,499
	Gaps (bp)	323,400	149,000
	N50 number	7	8
	N50 length (bp)	53,115,898	68,970,119
	Repetitive sequences	485,237,308	691,680,161
	Protein-coding genes	49,414	57,587
	Average size (bp)	3,128	5,027
	Number of exon per gene	4.14	4.65
	Average exon size (bp)	254	231
	Average intron size (bp)	660	1,084
	Gene density (kb/gene)	16.10	19.25
	RNA genes	4,820	6,071
Chromosome	Number	14	14
	Total length (bp)	746,393,055	987,838,149
	Gaps (bp)	323,400	149,000
	GC contents (%)	33.88	33.83
	Repetitive sequences (bp)	358,459,201	512,990,529
	Protein-coding genes	45,792	48,283
	Gene density (kb/gene)	16.30	20.50
	RNA genes	3,814	5,250

Table 2. Genome assembly statistics for *A. distichum* and *F. ovata*.

genes (comprising 1,614 single-copy orthologs) were complete, while only 0.82% to 2.15% of Eudicots genes and 0.31% to1.55% of Embryophyta genes were missing (Table S2). The genome completeness estimates for the *A. distichum* and *F. ovata* assemblies were comparable to those of *J. sambac* and *F. excelsior*, and higher than those of other Oleaceae assemblies, such as those of *S. oblata* and *O. europaea*, reported thus far. By applying cutoffs of 1E^{-10} and >50% coverage, a BLASTN comparison of the assemblies against full-length transcript unigenes generated from mRNA-seq data using Trinity v2.2.0[33] with the default parameters, along with a coding region search using TransDecoder v5.5.0 (https://github.com/TransDecoder/TransDecoder), revealed that each genome assembly recovered >99% of the gene space, indicating high-quality assemblies (Table S3).

Annotation of repetitive sequences and non-coding RNAs. The genome assemblies were masked for repetitive sequences using RepeatMasker v4.0.5 (http://www.repeatmasker.org) and RepeatModeler v1.0.8 (http://www.repeatmasker.org). Retrotransposons (RTs) were identified using LTR_FINDER v1.05[34]. The genomes of *A. distichum* and *F. ovata* contained 485.24 Mb and 691.68 Mb of repetitive sequences, respectively, which corresponds to repetitive sequence ratios of 60.98% and 62.40% (Table 2). The most prevalent classes of repetitive sequences were RTs, accounting for 44.06% and 50.17% of the total, and DNA transposable elements (TEs), comprising 12.00% and 15.86%. The predominant RTs were Ty3/Gypsy and Ty1/Copia, while MuLE-MuDR and hAT-Ac were the most abundant DNA TEs (Table S4). Full-length long terminal repeat RTs (FL-LTR-RTs) were identified from the repetitive sequences using BLASTX searches (cutoffs of 1E^{-10} and >70% coverage) against mobile genetic elements in RepeatExplorer2[35]. A total of 12,101 FL-LTR-RTs, with an average length of approximately 8 kb, were identified (Table 3). The OTA-Tat family was the most prevalent among Ty3/Gypsy FL-LTR-RTs in both genomes, whereas the Tork and SIRE families were the most abundant among Ty1/Copia FL-LTR-RTs in *A. distichum* and *F. ovata*, respectively. Tandem arrays of the telomere sequence (5′-TTTAGGG-3′) and modified units were identified at the ends of several pseudochromosomes (1, 4, 5, 7, 11, 12, 13, and 14 of *A. distichum* and 1, 5, 11, and 14 of *F. ovata*). However, the telomeres of other chromosomes remained unidentified. The investigation of repetitive sequence-enriched heterochromatic regions was conducted using whole-genome methylation data. A total of 44.3 Gb of quality-filtered Illumina BS sequences were aligned to the genome assemblies using BSMAP v2.9[36] with the default parameters. Only those reads that were uniquely mapped were selected, and any PCR duplicates were removed using SAMBAMBA v0.5.9[37]. The methylation ratio at each cytosine site was extracted and partitioned based on context (CG, CHG, and CHH). The mean cytosine methylation levels for each chromosome were calculated using a 100-kb sliding window. The distribution of repetitive sequence-enriched heterochromatic regions and other repetitive sequences are illustrated in Fig. 2b.

Non-coding RNAs (ncRNAs), including microRNAs (miRNAs), small nuclear RNAs (snRNAs), and other RNAs, were identified using Infernal v1.1.4[38]. Ribosomal RNAs (rRNAs) were searched with BLASTN, while transfer RNAs (tRNAs) were predicted using tRNAscanSE[39]. We identified 4,820 ncRNA sequences for

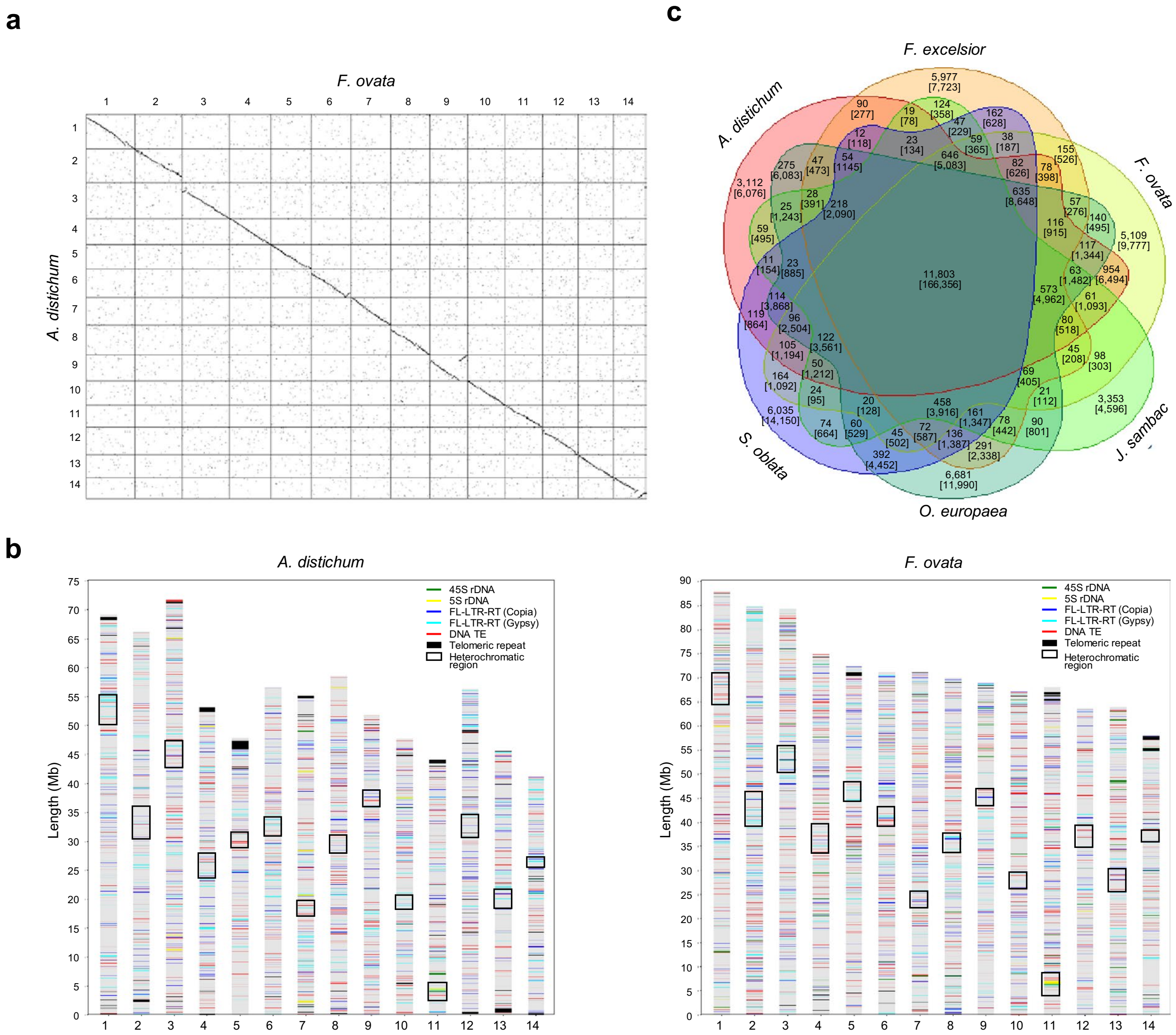

Fig. 2 Chromosomal synteny, repetitive sequence distribution, and gene families in *A. distichum* and *F. ovata*. (**a**) Dot plot comparison of *A. distichum* and *F. ovata* pseudochromosome assemblies. Dots closest to the diagonal line reflect collinearity between the two assemblies. (**b**) Distributions of various repetitive sequences, including rDNA, FL-LTR-RTs, DNA TEs, and telomeric repeats. Black boxes indicate the location of repetitive sequence-enriched heterochromatic regions, and colored lines represent different types of repetitive sequences. (**c**) Venn diagram showing unique and shared gene families among six sequenced genomes of the Oleaceae family. The numbers of gene families and genes (in brackets) for each group are provided. The NCBI and China National Center for Bioinformation accession numbers are as follows: *F. excelsior*, GCA_900149125; *J. sambac*, GWHAZHY00000000; *O. europaea*, GCF_002742605; *S. oblata*, GWHBHRY00000000.

A. distichum and 6,071 for *F. ovata*. This encompasses 1,453 and 1,939 rRNAs, 727 and 953 tRNAs, 254 and 324 miRNAs, 2,285 and 2,705 snRNAs, and 101 and 150 other RNA sequences, respectively (Tables S5 and S6).

Prediction of protein-coding genes and functional annotation. We employed a combination of *ab initio* and evidence-based gene prediction methods for the identification of protein-coding genes. *Ab initio* predictions were conducted on the repetitive sequence-masked genome assemblies using BRAKER1 v1.8[40], GlimmerHMM v3.0.2[41], and SNAP[42], with parameters trained on matrices from *Arabidopsis thaliana* and *O. europaea*. Genes with a coding sequence (CDS) of less than 300 bp, incomplete coding regions, or top matches to transposon-encoded proteins were excluded. For evidence-based gene prediction, we aligned approximately 112.99 Gb of quality-filtered Illumina PE mRNA-seq reads to the genome assemblies using TopHat2[43] with parameters set to–max-intron-length 100000 and–microexon-search. Gene predictions were further refined using the PASA v2.0.2 package[44]. Additionally, gene models from *A. thaliana* (TAIR 10, GCF_000001735), *O europaea* (GCF_002742605), and *F. excelsior* (GCA_900149125) were aligned to the genome assemblies using Exonerate v2.2.0[45]. Finally, the *ab initio* gene models, transcript alignments, and protein alignments were integrated into consensus gene model sets using EVidenceModeler[44]. This process yielded the prediction of 49,414 protein-coding genes for *A. distichum* and 57,587 for *F. ovata*. The protein-coding genes in *A. distichum* averaged

FL-LTR-RT family			***A. distichum***	***F. ovata***
Ty1/Copia	Ale	Number	741	993
		Length (bp)	4,019,949	5,598,449
	Tork	Number	636	962
		Length (bp)	4,133,536	6,281,533
	TAR	Number	491	590
		Length (bp)	3,265,422	4,099,906
	SIRE	Number	332	674
		Length (bp)	3,334,862	7,047,172
	Other elements	Number	724	864
		Length (bp)	5,547,945	6,377,486
	Total	Number	2,924	4,083
		Length (bp)	20,301,714	29,404,546
		% Genome	2.40	3.14
Ty3/Gypsy	OTA-Tat	Number	897	1,283
		Length (bp)	9,789,929	14,573,227
	OTA-Athila	Number	646	977
		Length (bp)	7,408,191	11,661,401
	CRM	Number	163	248
		Length (bp)	1,308,303	1,807,119
	Tekay	Number	134	287
		Length (bp)	1,321,282	2,727,045
	Other elements	Number	162	297
		Length (bp)	983,957	1,764,382
	Total	Number	2,002	3,092
		Length (bp)	20,811,662	32,533,174
		% Genome	2.46	3.47

Table 3. Statistics of annotated FL-LTR-RTs in the *A. distichum* and *F. ovata* genomes.

3,128 bp in length, shorter than those in *F. ovata*, which averaged 5,027 bp. *A. distichum* exhibited fewer exons per gene (4.14 vs. 4.65) and shorter introns (660 bp vs. 1,084 bp) compared to *F. ovata* (Table 2). The average gene density was higher in *A. distichum* (one per 16.10 kb) compared to *F. ovata* (one per 19.25 kb) and other Oleaceae species (one per 18.46–20.98 kb), indicating that *A. distichum* had the most compact organization of gene space among sequenced Oleaceae genomes (Table S7).

All predicted protein-coding genes were functionally annotated using the SwissProt and TrEMBL databases from UniProt (www.ebi.ac.uk/uniprot) and the nucleotide databases of the National Center for Biotechnology Information (NCBI) using BLASTP with cutoffs of 1E^{-10} and >70% coverage. Protein motifs, domains, and Gene Ontology (GO) annotations were identified using the InterPro database (www.ebi.ac.uk/interpro). Metabolic pathways were annotated using the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database (www.genome.jp/kegg/pathway.html). Overall, 44,576 (90.21%) of the genes in *A. distichum* and 50,378 (87.48%) of the genes in *F. ovata* were successfully annotated, while the remaining 4,838 (9.79%) and 7,209 (12.52%) genes were classified as uncharacterized or hypothetical (Table 4). Transcriptional evidence was found for 98.05% of the annotated genes in *A. distichum* and 93.34% in *F. ovata*. For each gene model, the average trimmed mean of M (TMM) value was calculated from the mRNA-seq reads mapped to the CDS using STAR v2.7.9a[46] with the default parameters.

A comparison of the annotated genes between *A. distichum* and *F. ovata*, conducted through an all-against-all BLASTP analysis, revealed that 43,363 genes (87.75%) in *A. distichum* were found to correspond to 54,440 genes (94.54%) in *F. ovata*. There were 6,052 genes specific to *A. distichum* and 3,147 specific to *F. ovata*. Of these, 1,896 (3.84%) and 2,554 (4.44%) genes were classified as uncharacterized or hypothetical. A six-way comparison of genes from *A. distichum* and *F. ovata* with those from four other Oleaceae species (*F. excelsior*, *J. sambac*, *O. europaea*, and *S. oblata*) using OrthoFinder[47] yielded 11,803 gene families (166,356 genes) that were shared across all six species. A total of 3,112 gene families (6,076 genes) were identified as unique to *A. distichum*, while *F. ovata* exhibited 5,109 unique gene families (9,777 genes) (Fig. 2c).

Data Records

All sequencing data and genome assemblies generated in this study can be retrieved from the NCBI database via Bioproject IDs PRJNA1086675 for *A. distichum* and PRJNA1086660 for *F. ovata*. The sequence read data are accessible via the NCBI Sequence Read Archive, with accession numbers SRP495333 for *A. distichum*[48] and SRP495335 for *F. ovata*[49]. The assembled genomes have been deposited in the NCBI GenBank database under the accession numbers JBFOLK000000000.1 for *A. distichum*[50] and JBFOLJ000000000.1 for *F. ovata*[51]. Additionally, the genome assemblies and annotation data are available on figshare[52].

		A. distichum		*F. ovata*	
Annotation		**Number**	**Ratio (%)**	**Number**	**Ratio (%)**
Database	RefSeq	42,540	86.09	45,340	78.73
	TrEMBL	42,420	85.85	49,695	86.30
	InterPro	32,793	66.36	41,751	72.50
	GO	32,427	65.62	43,982	76.37
	SwissProt	26,774	54.18	31,720	55.08
	KEGG Pathway	46,318	93.73	52,056	90.40
	A. thaliana	30,938	62.61	38,210	66.35
	C. canephora	32,561	65.89	40,481	70.30
	F. excelsior	35,230	71.30	43,647	75.79
	O. europaea	42,783	86.58	41,987	72.91
BUSCO eudicot	Complete	2,253	96.86	2,292	98.54
	Fragmented	23	0.99	15	0.64
	Missing	50	2.15	19	0.82
Annotated gene		44,576	90.21	50,378	87.48
Uncharacterized gene		3,873	7.84	3,375	5.86
Hypothetical gene		965	1.95	3,834	6.66
Total		49,414	100	57,587	100

Table 4. Summary of functional annotation for the *A. distichum* and *F. ovata* genomes.

Technical Validation

Nucleic acid quality and quantity evaluation. For each species, nucleic acids were extracted from plant tissues of a single accession. The quality and integrity of the nucleic acids were evaluated using an Agilent 2100 Bioanalyzer (Agilent). The concentrations of nucleic acids were measured using a Qubit Fluorometer. DNA samples intended for PacBio sequencing underwent quality control using an Agilent Femto pulse system, with criteria that required >20% of the DNA to be greater than 40 kb in size. RNA samples were prepared from two biological replicates across four tissues at the same time point.

Quality control of raw sequence data. To obtain clean sequence reads for downstream analysis, we implemented a series of quality control procedures. We removed sequence reads with a quality score below Q20 or with more than 10% unidentified nucleotides (N). Adapter sequences and low-quality regions with a quality score below Q20 were trimmed from the reads, and PCR duplicates were removed. The sequence errors in the PacBio reads were corrected using the CANU v2.2.1[26]. Additionally, PacBio subreads and Hi-C reads were quality-filtered by Macrogen and Dovetail Genomics, respectively.

Evaluation of assembled genomes. We evaluated the completeness and quality of genome assemblies by matching transcriptome data and publicly available eukaryotic orthologous genes. First, the transcript unigenes of *A. distichum* and *F. ovata*, which were assembled from the mRNA-seq data were aligned with the genome assemblies. The results indicated that >99% of the transcriptome unigenes matched the genome assemblies, suggesting sufficient coverage of the gene space. Second, a BUSCO completeness assessment was conducted using single-copy orthologous sets of plants. The BUSCO results showed that 97.71%–98.82% of Embryophyta genes and 96.86%–98.54% of Eudicots genes were complete, whereas only 1.46%–3.14% of Eudicots genes and 1.18%–2.29% of Embryophyta genes were fragmented or missing. These findings collectively demonstrate that the genome assemblies of *A. distichum* and *F. ovata* possess high quality, integrity, and annotation completeness.

Code availability

The software and parameters used in this study are described in the Methods section. No specific custom codes or scripts were utilized. Data processing was conducted according to the manuals and protocols provided with the respective software.

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Author contributions

J.H.M. planned the projects, designed the research, analyzed data, and wrote the manuscript. H.J. and A.C. performed the experiments, assemble the genomes, analyzed data, and participated in manuscript preparation. H.J.K. and J.H.K. planned the projects and participated in manuscript preparation. H.K., S.H.J., S.M.H., H.J.Y. and D.K.K. participated in plant sampling, sequencing, data analysis, and manuscript preparation.

Competing interests

The authors declare no competing interests.

Additional information

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