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Integrating Morphology and Chloroplast Genomics: A New East Asian Species of *Aletris* (Nartheciaceae) With Insights Into Regional Phylogeny and Evolution

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ABSTRACT

The genus *Aletris* L. (Nartheciaceae) encompasses approximately 21–24 species distributed in East Asia and North America, yet taxonomic ambiguity persists due to overlapping morphological traits among closely related species. During fieldwork in southeastern Xizang, China, a morphologically distinct candidate species, *Aletris medogensis*, was discovered. To validate its taxonomic status and explore evolutionary relationships within East Asian *Aletris*, we integrated detailed morphological observation with comparative chloroplast phylogenomics. The newly proposed species is characterized by creeping stolons, narrow leaves, and glandular-pubescent inflorescences. Comparative analysis of 14 East Asian *Aletris* complete chloroplast genomes revealed a conserved quadripartite structure with species-specific variations, including pseudogenization of *ycf1*, loss of *rrn4.5*, and shifts in IR boundaries. Phylogenomic analyses strongly supported *A. medogensis* as a distinct species closely related to *A. alpestris*. We identified 18 hypervariable regions as potential molecular markers and detected signals of positive selection in genes *ccsA*, *cemA*, and *rps12*, suggesting adaptive evolution. This study confirms the recognition of *A. medogensis* as a new species endemic to the eastern Himalayas and demonstrates the utility of chloroplast genomics in resolving taxonomic complexity and understanding evolutionary mechanisms in *Aletris*.

1 | Background

The genus *Aletris* L. was established by Linnaeus (1753), with *Aletris farinosa* L. from southeastern North America designated as the type species. It was traditionally placed in the family Liliaceae Juss. (Ambrose 1980; Wu et al. 2003), but molecular phylogenetic studies have since shown closer relationships

to the genera *Lophiola*, *Metanarthecium*, *Narthecium*, and *Nietneria*, leading to their reclassification into Nartheciaceae by Caddick et al. (2002). *Aletris* is the largest genus in this family, with approximately 21–24 species found in East Asia and North America (Zhao et al. 2012). In China, 15 species and one variety have been recorded, nine of which are endemic, primarily distributed in the central and southwestern regions.

Abbreviations: BI, Bayesian inference; cp, chloroplast; DD, data deficient; gDNA, genomic DNA; IR, inverted repeat; LSC, large single copy; LSR, long sequence repeat; ML, maximum likelihood; PCG, protein-coding gene; PIP, parsimony informative site; SNP, single nucleotide polymorphism site; SSC, small single copy; SSR, simple sequence repeat; SVS, singleton variable site.

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Aletris species typically grow on mountain slopes, roadsides, and shrublands. They prefer moist, shaded environments but can tolerate drought and adapt to various conditions (Yang et al. 2024; Akahori et al. 1971). These perennial herbs possess fibrous roots and basal rosettes of grass-like leaves (lanceolate to linear). A simple, erect scape bears a terminal raceme of bisexual flowers. Each flower has a bract and a smaller bracteole on the pedicel, a 6-lobed perianth (white to golden-orange; cylindrical to campanulate) with rough abaxial surfaces. Six short stamens insert on the perianth tube, and the 3-lobed stigma yields a beaked, loculicidal capsule enclosed by the persistent perianth (Liang and Turland 2000; Nong et al. 2024). Many *Aletris* species are of ecological and medicinal importance, traditionally used to treat coughs, hemoptysis, dysmenorrhea, and pulmonary abscesses (Li et al. 2014; Challinor et al. 2015).

During a 2024 field investigation in southeastern Xizang, we encountered an unusual *Aletris* species growing on moss-covered rocks along streams (Figure 1). It resembled *A. pauciflora* var. *pauciflora* (Klotzsch) Hand.-Mazz. (Brotherus and Handel-Mazzetti 1936), *A. pauciflora* var. *khasiana* F. T. Wang & Tang (Wang and Tang 1978), and *A. alpestris* Diels (Adolf 1905) in having rosette-like clustered leaves, perianth glandular, with lobes shorter than or equal to the perianth, and the bract and bracteole borne at the apex of the pedicel. However, it differed in its rhizomes horizontally creeping from the base of the plant, narrower linear leaves, inflorescences with glandular hairs, and shorter pedicels, bracts, and bracteoles. We propose it as a new species, *Aletris medogensis* W.B.Ju, Y.L.Qiu & Bo Xu, based on morphological traits and molecular phylogenetic analysis derived from the chloroplast (cp) genome.



FIGURE 1 | Habit of *Aletris medogensis* on moss-covered rocks along streams (photographed by Wen-Bin Ju).

In recent years, comparative chloroplast genome analysis has emerged as an essential tool in plant phylogenetics and species identification due to its highly conserved structure, maternal inheritance, and relatively slow evolutionary rate (Zhao et al. 2019; Dopp et al. 2021; Lu et al. 2016). Repeat sequences, inverted repeat (IR) boundaries shift, and nucleotide diversity sites within the chloroplast genome can reveal hypervariable regions and develop molecular markers to evaluate genomic evolutionary history and facilitate species delimitation (Keller et al. 2017; Sabir et al. 2014). Furthermore, the chloroplast genome is not merely a neutral phylogenetic marker; it plays a fundamental role in photosynthesis, stress response, and other essential metabolic pathways. Analyzing selective pressures on chloroplast genes can therefore reveal signatures of adaptive evolution directly linked to environmental factors, such as light intensity, temperature, and water availability, which are particularly relevant for species inhabiting specific niches like the montane ecosystems of the Himalayas (Dong et al. 2018; Rui et al. 2025).

In the genus *Aletris*, although traditional morphological classification has amassed considerable knowledge, taxonomic controversies persist among some closely related species due to overlapping morphological traits and limited molecular data (Zhao et al. 2012; Liang and Turland 2000; Nong et al. 2024). In this study, we first described the morphological traits of *Aletris medogensis* in detail and presented its complete chloroplast (cp) genome for the first time. Then, comparative genomics and phylogenomic analyses were conducted by integrating the previously published cp genome data of East Asian *Aletris* species and related genera in Nartheciaceae, with the following objectives: (1) assess the validity of the newly proposed species status for *Aletris medogensis*; (2) characterize the global structural features and investigate variations in repeat elements among cp genomes; (3) identify highly variable regions suitable for species identification and phylogenetic studies; (4) reconstruct a robust phylogeny of East Asian *Aletris* species and its relatives within Nartheciaceae; and (5) investigate adaptive evolution patterns of cp genes in *Aletris*. This study confirms the distinct taxonomic status of *A. medogensis*, reveals the genetic differentiation of East Asian *Aletris* cp genomes, and sheds light on the evolutionary history and adaptive mechanisms within Nartheciaceae. This integrative framework offers new perspectives for taxonomic studies and biodiversity conservation in complex plant groups.

2 | Materials and Methods

2.1 | Sampling and Morphological Comparison

Specimens of *Aletris medogensis* were collected from Hanmi to Xiaoyandong, opposite Beibeng Township, Medog County, Nyingchi City, Xizang, China. The voucher specimen was deposited in the Herbarium of the Chengdu Institute of Biology (CDBI), Chinese Academy of Sciences (<https://cib.cas.cn/zzjg/zcbm/bbg/>, Bo Xu, xubo@cib.ac.cn), under the accession number CDBI0298322 (Figure S1). Specimens of related species were examined from herbaria PE, HNWP, WUK, SZ, and SM for comparative analysis. Morphological data, including leaves/bract-like leaves length and width, scape size, inflorescence length, and floral parts (Table 1), were precisely quantified using ImageJ v1.48 (Schneider et al. 2012).

TABLE 1 | Morphological comparison of *Alettris medogensis* and related taxa.

Character	<i>A. medogensis</i>	<i>A. alpestris</i>	<i>A. pauciflora</i> var. <i>pauciflora</i>	<i>A. pauciflora</i> var. <i>khasiana</i>
Plant	Tillering with horizontally creeping stolons	None	None	None
Leaves	2.5–9 cm × 0.45–0.9 mm, few and slender, linear	1.5–8 cm × 2–4 mm, numerous, densely tufted, linear-lanceolate	5–25 cm × 2–8 mm, few and laxly tufted, linear-lanceolate to linear	5–10 cm × 5–7.5 mm, linear-lanceolate to linear
Scape size	3.5–16 cm × 0.7–1 mm	7–20 cm × 0.5–1 mm	3.5–40 cm × 1.5–2 mm (6.5–8.5 cm × 1.5–2 mm)	7–15 cm × 2–2.5 mm
Scape hairy	Glabrous, glandular are sparse near the inflorescence	Sparsely puberulent	Densely puberulent	Densely puberulent
Bract-like leaves	0.2–0.6 cm long	Less than 1 cm long	1.5–5.0 cm long	1.5–5.0 cm long
Inflorescence	raceme 1.5–2.5 cm, densely 10–14-flowered, densely glandular hairs	Raceme 1–4 cm, laxly 4–10-flowered, rachis puberulent	Raceme 1–5 cm, densely to laxly 4–6-flowered, rachis pubescent	Raceme 1–5 cm, densely to laxly 18–20-flowered, rachis pubescent
Bract and bracteole	1.0–2.0 mm long, shorter than flower	1.5–4.0 mm long, shorter than flower	3–20 mm long, one of them 1–4 × flower length	3.5–7.0 mm long, subequaling or slightly exceeding the flower
Pedicel	Less than 0.5 mm long	2–4 mm long	1–12 mm long	1–12 mm long
Perianth	Glabrous, lobes ovate, parted nearly to 1/2 of its length	Glabrous but often densely papillose, lobes lanceolate, parted nearly to 1/2 of its length	Glabrous, lobes oblong-ovate to lanceolate, parted nearly to 1/4 of its length	Glabrous, lobes oblong-ovate to lanceolate, parted nearly to 1/4 of its length
Capsule	Apex abruptly narrowed	Apex abruptly narrowed	Apex of valves gradually narrowed	Apex of valves gradually narrowed

2.2 | Sequencing, Assembly, Annotation, and Comparison

The total genomic DNA (gDNA) was extracted from the dried leaves of *Aletris medogensis* using a modified CTAB method (Porebski et al. 1997). The gDNA was enzymatically fragmented into 200–500bp fragments, followed by end-repair, poly-A tail addition, and adapter ligation. After PCR amplification, a small-fragment DNA library was constructed and sequenced on the Illumina NovaSeq 6000 platform (Illumina, San Diego, CA, USA) in PE150 mode. To obtain high-quality sequences, fastp v0.23.2 (Chen et al. 2018) was used to filter out all low-quality reads, including those with adapters, more than 20% of bases having Phred quality < 5, and reads with > 10% N content. The chloroplast (cp) genome was assembled using GetOrganelle v1.7.7.1 (Jin et al. 2020) and annotated using PGA (Qu et al. 2019), with *Aletris pauciflora* var. *pauciflora* as a reference. A Python script (Appendix S1) was used to examine gene length and count, as well as the start and stop codons. In addition, Geneious Prime 8 software (<https://www.geneious.com>) was employed to determine the pseudogenes. The final circular map was drawn using OGDRAW v1.3.1 (Greiner et al. 2019). In addition, the genomic

data of other closely related species within Nartheciaceae, including 13 *Aletris*, 1 *Metanarthecium*, 1 Nartheciaceae, and 1 *Dioscorea* accession (Table 2), were also downloaded and curated. Specifically, genomes with problematic annotations were systematically re-annotated using our standardized PGA protocol to ensure data uniformity.

To compare the contraction and expansion of inverted repeat regions among the 14 cp genomes of *Aletris* and its closely related genera, we identified and visualized boundaries of large single copy (LSC), small single copy (SSC), and IRa/IRb regions in the 17 cp genomes using IRscope (Amiryousefi et al. 2018). Furthermore, to explore whether there were additional alterations, such as gene rearrangements or inversions, a synteny analysis of the *Aletris* cp genomes was performed utilizing the mVISTA program (Mayor et al. 2000).

2.3 | Repeat Sequence Analysis

Simple sequence repeats (SSRs) in *Aletris medogensis* and the 13 published *Aletris* accessions (Table 2) were identified using

TABLE 2 | Characteristics of 17 Nartheciaceae complete chloroplast genomes, including 1 newly generated accession (*Aletris medogensis*) and the previously published 16 accessions.

Species name	Size (bp)				GC content (%) total (LSC/SSC/IR)	No. of genes (PCGs/tRNA/rRNA)	GenBank accession
	Total	Large single-copy region (LSC)	Small single-copy region (SSC)	Inverted repeat (IR)			
<i>Aletris medogensis</i>	154,557	83,194	18,167	26,598	37.38 (35.26/31.18/42.82)	131 (85/38/8)	PV472299
<i>Aletris megalantha</i>	154,704	83,265	18,113	26,663	37.44 (35.33/31.27/42.82)	131 (85/38/8)	MW080684
<i>Aletris fauriei</i> ^a	154,440	83,501	18,183	26,378	37.5 (35.45/31.31/42.88)	131 (85/38/8)	NC_033412
<i>Aletris spicata</i>	154,999	83,511	18,160	26,664	37.48 (35.4/31.27/42.84)	131 (85/38/8)	NC_033411
<i>Aletris gracilis</i>	154,201	83,018	17,855	26,664	37.45 (32.44/31.26/42.84)	131 (85/38/8)	PP988376
<i>Aletris stenoloba</i>	154,993	83,505	18,160	26,664	37.48 (34.01/31.28/42.84)	131 (85/38/8)	PP988377
<i>Aletris scopulorum</i>	154,167	82,706	18,151	26,655	37.49 (32.93/31.22/42.84)	131 (85/38/8)	PP988375
<i>Aletris pedicellata</i>	154,204	83,021	17,855	26,664	37.45 (32.44/31.26/42.83)	131 (85/38/8)	PP988374
<i>Aletris pauciflora</i> var. <i>khasiana</i> ^a	154,309	83,076	23,519	23,857	37.42 (32.42/33.44/43.11)	130 (85/38/7)	PP988373
<i>Aletris pauciflora</i> var. <i>pauciflora</i>	154,205	83,022	17,855	26,664	37.45 (32.44/31.26/42.84)	131 (85/38/8)	PP988372
<i>Aletris laxiflora</i>	154,468	83,043	18,003	26,711	37.47 (31.31/31.32/42.86)	131 (85/38/8)	PP988370
<i>Aletris glandulifera</i>	155,143	83,583	18,248	26,656	37.39 (32.86/31.17/42.83)	131 (85/38/8)	PP988368
<i>Aletris glabra</i>	154,201	83,018	17,855	26,664	37.45 (32.44/31.25/42.84)	131 (85/38/8)	PP988367
<i>Aletris alpestris</i>	154,551	83,158	18,093	26,650	37.51 (33.69/31.37/42.85)	131 (85/38/8)	PP988366
<i>Metanarthecium luteoviride</i>	153,777	82,852	17,777	26,574	37.38 (35.17/31.19/54.77)	130 (84/38/8)	KT895904
<i>Narthecium ossifragum</i> ^a	155,312	84,051	18,135	26,563	37.57 (35.47/31.63/39.59)	130 (85/37/8)	OY986978
<i>Dioscorea futschauensis</i>	153,946	83,979	18,909	25,529	37.21 (35.04/31.21/42.99)	124 (86/38/0)	NC_039808

^aWe re-annotated the genome based on the sequences from NCBI.

MISA software (Beier et al. 2017), with threshold settings of 10 repeat units for mononucleotides, 6 for dinucleotides, 5 for trinucleotides, 4 for tetranucleotides, and 3 for penta- and hexanucleotides. Then, long sequence repeats (LSRs), including forward (F), reverse (R), palindrome (P), and complementary (C) repeats were detected within the LSC, IRa/b, and SSC regions of the *Alettris* chloroplast genomes using the REPuter program (Kurtz et al. 2001) with a maximum number of computed repeats of 5000 and minimal repeat size of 30 bp.

2.4 | Molecular Marker Identification

The initial alignment of the 13 *Alettris* complete chloroplast genomes was conducted using MAFFT v7 (Kato and Standley 2013). Subsequent identification of hypervariable loci with potential phylogenetic utility was detected based on a sliding-window analysis method to quantify nucleotide diversity (π) in *Alettris*, with a window length of 600 bp and a step size of 200 bp. A Python script (Appendix S2) was developed for identifying hypervariable regions. Adjacent windows with a top 5% π value and several single nucleotide polymorphism sites (SNPs) > 25 were concatenated into contiguous hypervariable regions. The final characterization of these regions included quantitative assessments of SNPs, singleton variable sites (SVSSs), parsimony informative sites (PIPs), and mean π values of contiguous hypervariable regions.

2.5 | Phylogenetic Relationship Reconstruction

The complete chloroplast genome sequences of *A. medogensis* and 16 published species were retrieved from GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>; Table 2) and aligned with MAFFT. After removing poorly aligned regions using Gblocks v0.91b (Castresana 2000), the best-fit nucleotide substitution model (TVM+I+G) was determined by jModelTest v2.1.10 (Darriba et al. 2012). Maximum likelihood (ML) and Bayesian inference (BI) trees were then constructed using IQ-tree v2.1.4 (Nguyen et al. 2015) and MrBayes v3.2.7a (Ronquist et al. 2012), respectively, based on the best-fit model. *Dioscorea futschauensis* was set as the outgroup. Finally, the resulting trees were visualized and further landscaped in FigTree v1.4.4 (<https://github.com/rambaut/figtree/releases/tag/v1.4.4>).

2.6 | Analysis of Selective Pressure

Given the central role of the chloroplast in photosynthesis and its interaction with environmental stressors, signatures of positive selection in its genes can provide insights into molecular adaptations at the intersection of metabolism and ecology. To this end, we calculated the nonsynonymous (d_N) and synonymous (d_S) substitution rates (ω) for each PCG. In general, $\omega > 1$ indicates that the gene is under positive selection, which may drive species adaptation; $\omega = 1$ implies neutral evolution, where no selection is acting; and $\omega < 1$ reflects negative or purifying selection, with lower values signifying stronger selective constraints (Wang et al. 2010).

We first retrieved the PCGs shared by 17 Nartheciaceae species via CPStools v2.0.2 (Huang et al. 2024) and translated them

into amino acid sequences. Homologous amino acid sequences in 17 cp genomes were then aligned using ParaAT v2.0 (Zhang et al. 2012), and the corresponding CDS alignments (codon-based) were generated with the Epa2nal.pl script from ParaAT. Finally, KaKs_Calculator v3.0 (Wang et al. 2010) software was employed to calculate ω values for all PCGs in each species pair.

3 | Results

3.1 | Morphological Analyses

Morphological observations of both fresh samples from the field and dried herbarium specimens revealed that the new species exhibited morphological affinities with *Alettris alpestris*, *A. pauciflora* var. *pauciflora*, and *A. pauciflora* var. *khasiana*. The new species shares several characteristics with *A. alpestris*, including features of the plant, inflorescence structure, involucre bracts, perigone segments, corolla tube, and ovary. It also shows similarities to *A. pauciflora* var. *pauciflora* and *A. pauciflora* var. *khasiana* in leaf shape, stamens, and lobes. However, the new species is morphologically distinct from three taxa, characterized by its narrower leaves, obtuse apex, basal division, the inflorescence axis being densely covered with glandular trichomes, and two bracts that are both shorter than the flowers and unequal in length. Detailed morphological comparisons are provided in Table 1.

3.2 | Characteristics of the *Alettris* Chloroplast Genomes

This study generated and deposited a new chloroplast genome of *Alettris medogensis* in GenBank (accession no. PV472299), along with 13 published genomes (two of which were re-annotated) for comparison (Table 2). The results indicated that the whole cp genomes of *Alettris* ranged from 154,167 (*A. scopulorum*) to 155,143 bp (*A. glandulifera*), exhibiting a typical quadripartite structure consisting of two IR regions (IRa and IRb) of 23,857–26,711 bp, an LSC region of 82,706–83,583 bp, and an SSC region of 17,855–23,519 bp (Table 2). The GC contents of the *Alettris* cp genomes were similar (37.38%–37.51%), with the IRs showing the highest GC levels (42.82%–43.11%), followed by the LSC (31.31%–35.45%) and SSC regions (31.17%–33.44%; Table 2).

The cp genomes of *Alettris* are highly similar in terms of gene content, with most encoding 131 genes, including 85 protein-coding genes (PCGs), 38 tRNA genes, and 8 rRNA genes (all located in the IRs; Table 2; Figure 2). Two species had few missing genes and/or pseudogenes (Table 3). Specifically, a *rrn4.5* gene is missing in *Alettris pauciflora* var. *khasiana* and an additional *ycf1* gene is a pseudogene in *A. fauriei* (Table 2). Among the 85 PCGs, 73 were unique, and six (*ndhB*, *rpl2*, *rpl23*, *rps7*, *rps12*, and *ycf2*) were duplicated due to their location in the IRs. Likewise, 22 of the tRNA genes are unique, while eight tRNA genes (*trnA-UGC*, *trnH-GUG*, *trnI-CAU*, *trnI-GAU*, *trnL-CAA*, *trnN-GUU*, *trnR-ACG*, and *trnV-GAC*) and all four rRNA genes (*rrn23*, *rrn16*, *rrn5*, and *rrn4.5*) were duplicated. Additionally, a total of 18 genes were found to contain introns across the 14 cp genomes, with nine PCGs (*atpF*, *ndhA*, *ndhB*, *petB*, *petD*, *rpl2*, *rpl16*, *rpoC1*, and *rps16*) and six tRNA genes (*trnA-UGC*,

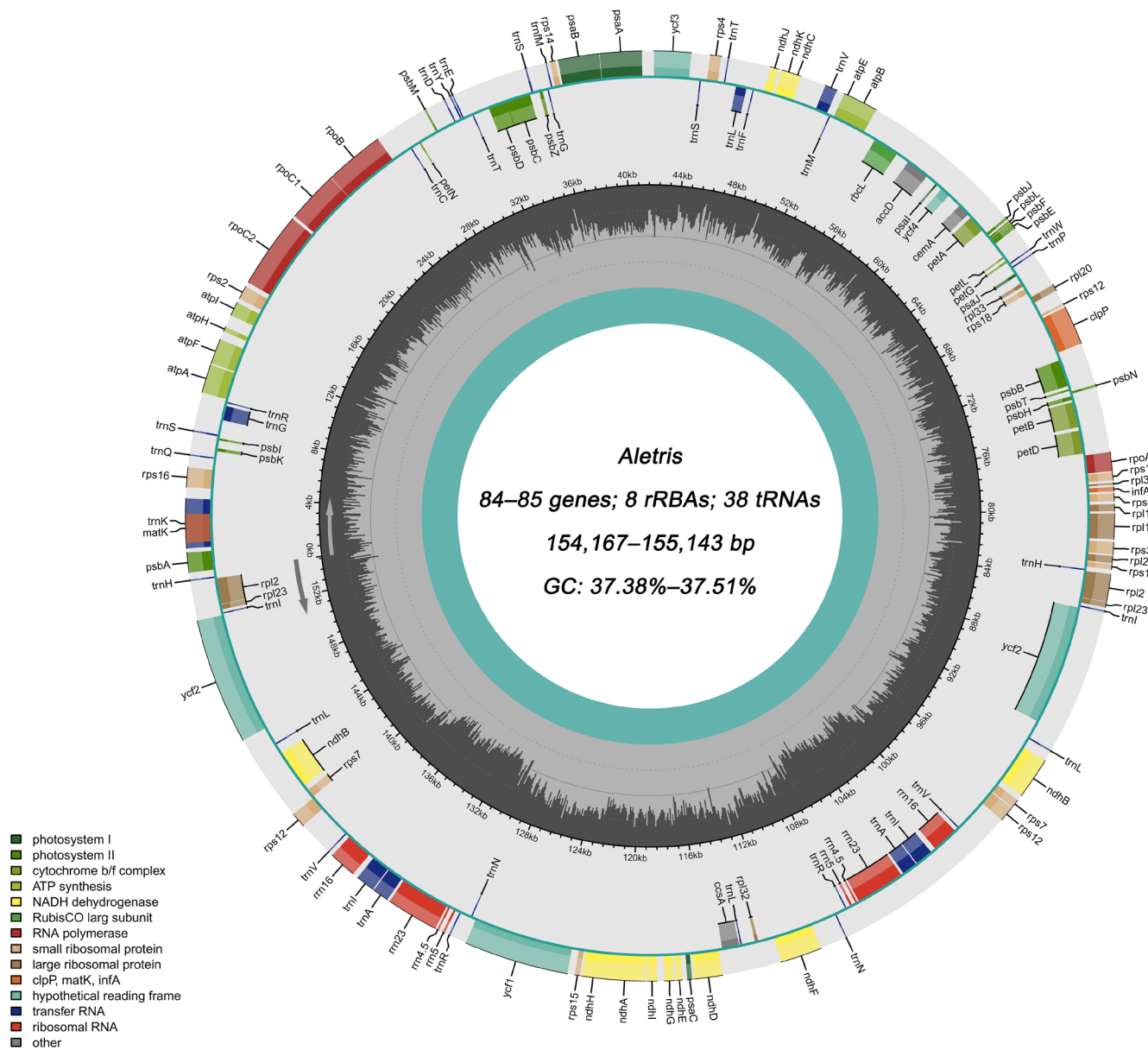


FIGURE 2 | The chloroplast genome map of East Asian *Aletris* species. The outer circle shows distribution of genes (different colors represent different roles). The arrows indicate the transcription directions of the genes inside and outside of the circle. The gray inner circle represents the GC content.

trnG-UCC, *trnI-GAU*, *trnK-UUU*, *trnL-UAA*, *trnV-UAC*) containing one intron, while only three PCGs (*rps12*, *ycf3*, and *clpP*) contained two introns (Table 3).

3.3 | Codon Usage of *Aletris* cp Genomes

The RSCU analysis revealed a similar codon usage pattern among the 14 *Aletris* species, with each containing all 64 codons that encode 20 amino acids (Figure 3; Table S1). In these genomes, leucine exhibited the highest number (29,369) of codons, while cysteine was the least abundant, with 3288 codons (Figure 3; Table S2). The highest mean RSCU value was identified as UUA (1.88), and the lowest was AGC (mean RSCU=0.28), which encode leucine and serine, respectively

(Figure 3; Table S2). Furthermore, 31 codons were found with a mean RSCU of >1, of which 29 were A/U-ending codons; 33 codons were found with a mean RSCU of ≤1, of which 30 were G/C-ending codons. This suggests strong codon usage bias favoring A/U-ending codons over G/C-ending codons in *Aletris* chloroplast genomes (Figure 3; Table S2).

3.4 | SSRs and Long Repeat Sequences of *Aletris* Cp Genomes

The number of SSRs in the 14 *Aletris* species varied from 45 in *A. scopulorum* to 75 in *A. megalantha*, in which mononucleotide SSRs were the most abundant, followed by tetra- and di-nucleotide SSRs (Figure 4a; Table S3). Among the

TABLE 3 | Summary of gene contents present in the *Aletris* chloroplast genomes.

Category	Gene group	Gene name
Photosynthesis	Subunits of photosystem I	<i>psaA, psaB, psaC, psaI, psaJ</i>
	Subunits of photosystem II	<i>psbA, psbB, psbC, psbD, psbE, psbF, psbH, psbI, psbJ, psbK, psbL, psbM, psbN, psbT, psbZ</i>
	Subunits of NADH dehydrogenase	<i>ndhA</i> (1), <i>ndhB</i> (1) (×2), <i>ndhC, ndhD, ndhE, ndhF, ndhG, ndhH, ndhI, ndhJ, ndhK</i>
	Subunits of cytochrome <i>b/f</i> complex	<i>petA, petB</i> (1), <i>petD</i> (1), <i>petG, petL, petN</i>
	Subunits of ATP synthase	<i>atpA, atpB, atpE, atpF</i> (1), <i>atpH, atpI</i>
	Large subunit of rubisco	<i>rbcL</i>
Self-replication	Proteins of large ribosomal subunit	<i>rpl14, rpl16</i> (1), <i>rpl2</i> (1) (×2), <i>rpl20, rpl22, rpl23</i> (×2), <i>rpl32, rpl33, rpl36</i>
	Proteins of small ribosomal subunit	<i>rps11, rps12</i> (2) (×2), <i>rps14, rps15, rps16</i> (1), <i>rps18, rps19, rps2, rps3, rps4, rps7</i> (×2), <i>rps8</i>
	Subunits of RNA polymerase	<i>rpoA, rpoB, rpoC1</i> (1), <i>rpoC2</i>
	Ribosomal RNAs	<i>rrn16</i> (×2), <i>rrn23</i> (×2), <i>rrn5</i> (×2), <i>rrn4.5</i> (×2) ^a
	Transfer RNAs	<i>trnA-UGC</i> (1) (×2), <i>trnC-GCA, trnD-GUC, trnE-UUC, trnF-GAA, trnG-GCC, trnG-UCC</i> (1), <i>trnH-GUG</i> (×2), <i>trnI-CAU</i> (×2), <i>trnI-GAU</i> (×2), <i>trnK-UUU</i> (1), <i>trnL-CAA</i> (×2), <i>trnL-UAA</i> (1), <i>trnL-UAG, trnM-CAU, trnN-GUU</i> (×2), <i>trnP-UGG, trnQ-UUG, trnR-ACG</i> (×2), <i>trnR-UCU, trnS-GCU, trnS-GGA, trnS-UGA, trnT-GGU, trnT-UGU, trnV-GAC</i> (×2), <i>trnV-UAC</i> (1), <i>trnW-CCA, trnY-GUA, trnM-CAU</i>
Other genes	Maturase	<i>matK</i>
	Protease	<i>clpP</i> (2)
	Envelope membrane protein	<i>cemA</i>
	Acetyl-CoA carboxylase	<i>accD</i>
	c-type cytochrome synthesis gene	<i>ccsA</i>
	Translation initiation factor	<i>infA</i>
	Conserved hypothetical chloroplast reading frames	<i>ycf1</i> ^b , <i>ycf2</i> (×2), <i>ycf3</i> (2), <i>ycf4</i>

Note: (1) Genes with one intron; (2) genes with two introns; (×2) genes with two copies.

^aOne of the *rrn4.5* genes is missing in *Aletris pauciflora* var. *hasiana*.

^bAn additional *ycf1* pseudogene in *Aletris fauriei*.

motifs in the SSRs, A/T were the most frequently occurring motifs, followed by AT/AT and TTTCA/TGAAA motifs (Figure 4b; Table S4). In addition, most of the SSRs were located in the LSC (35–59) and SSC (6–12) regions, and very few were located in the IRA/IRb (1/1–4/4; Table S3). REPuter identified 34–45 repeat sequences with a length > 30 bp, of which palindromic (17–28) and forward (14–21) repeat sequences were most abundant, while complement (0–2) and reverse (0–1) repeat sequences were very rare and almost nonexistent in many species (Figure 4c; Table S5). Most of the repeat sequences (21–26) were located in gene regions of the 14 cp genomes, followed by intergenic regions (7–16, IGS) and introns (4–6; Figure 4d; Table S5). In addition, most of the repeat sequences were less than 50 bp, and a few were larger than 100 bp (Table S6). Notably, *A. pauciflora* var. *hasiana*

had a palindrome repeat sequence with a length of 2820 bp (Table S6).

3.5 | Variance Analysis of the IR Boundaries

We compared the IR boundaries among 17 *Nartheciaceae* cp genomes, including *Dioscorea futschauensis*, *Metanarthecium luteoviride*, *Narthecium ossifragum*, and the 14 *Aletris* accessions mentioned above (Table 2), observing minor variations in gene content, structure, and order at the quadripartite borders (Figure 5; Figure S2). The JLA (IRA-LSC) and JSA (IRA-SSC) boundaries were highly conserved in all 14 *Aletris* cp genomes, with the former positioned between *psbA* and *trnH*, and the latter cut through the *ycf1* gene. The distance from the

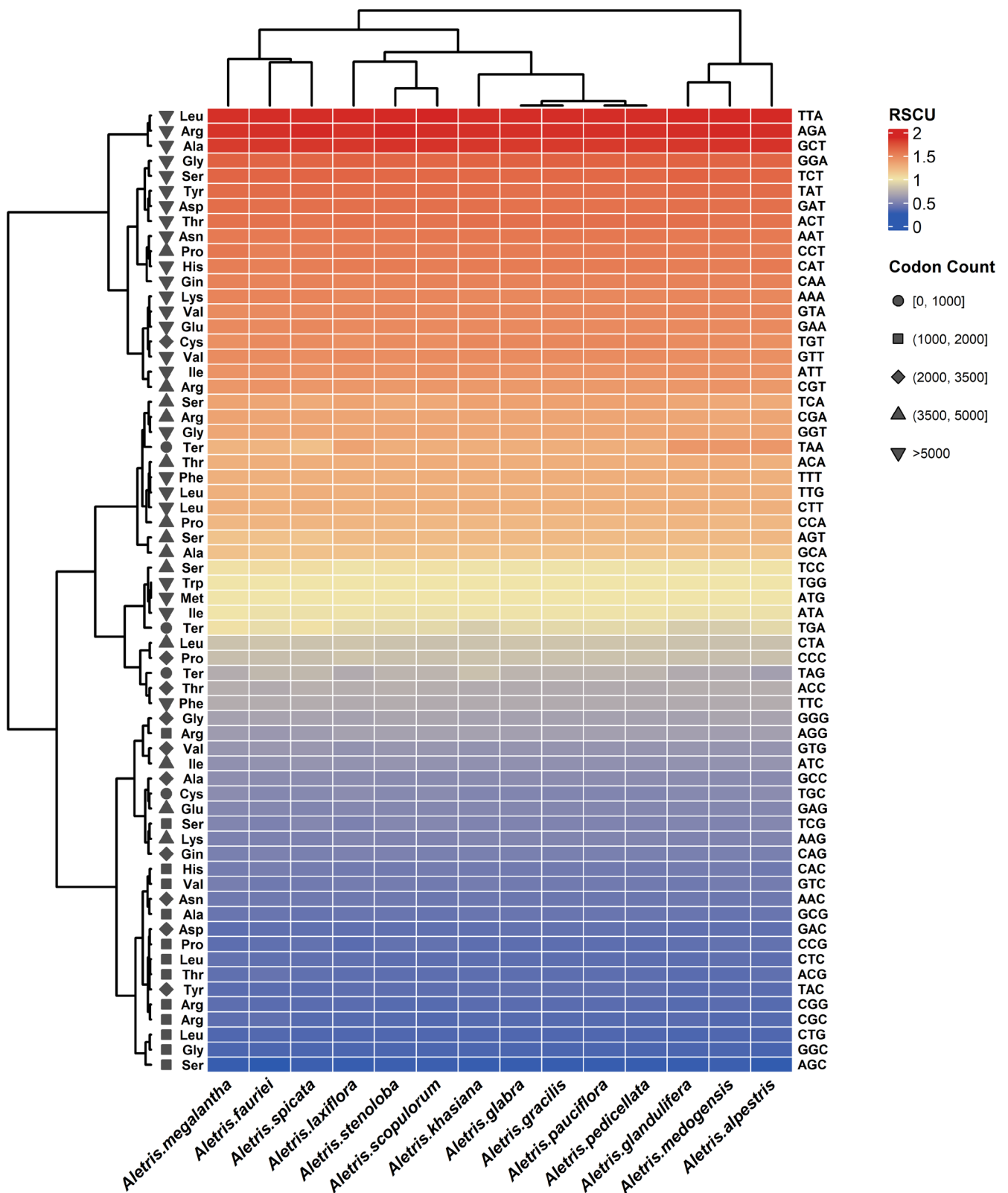


FIGURE 3 | Heatmap of RSCU values among *Aletris* species. The color gradient from blue to red represents the range of RSCU values and different graphics on the left side represent different quantity ranges.

JLA boundary to *psbA* was consistently 138bp, whereas that to *trnH* varied from 56 to 249bp (Figure 5). The JLB (IRb-LSC) boundaries cut through *rps19* in most *Aletris* species, with 104bp of *rps19* extending into the IRb, while the JLA boundary of *A. fauriei* lay 77bp away from *rps19* due to the contraction

of IRb. Similarly, the *ndhF* gene extended 2–16bp into the JSB (IRb-SSC) boundaries in most *Aletris* species except *A. fauriei*, where *ndhF* extended 153bp into IRb because of IR expansion (Figure 5). Differences in the expansion/contraction of the IRs were also found in other Nartheciaceae species; only 2 and

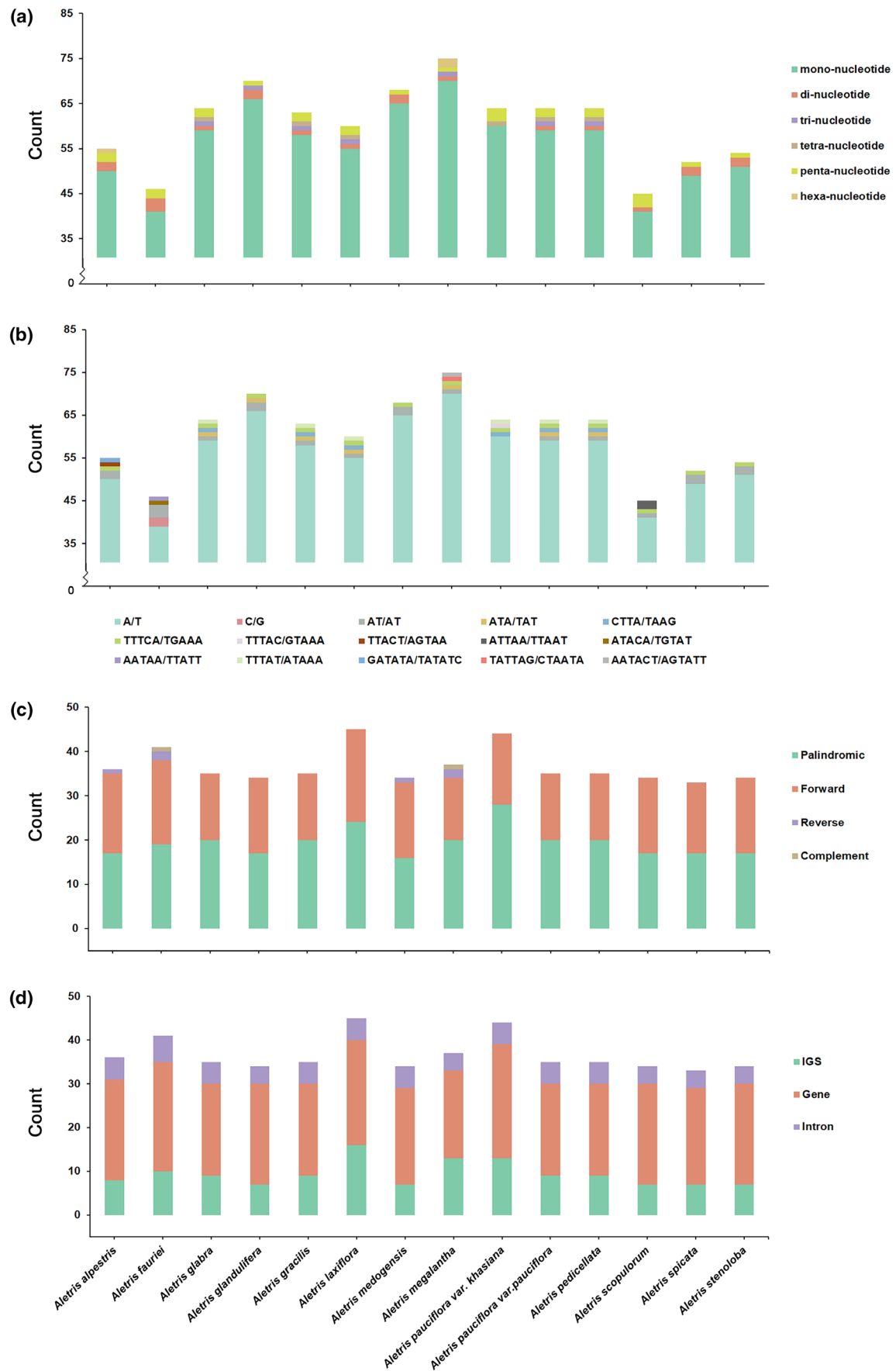


FIGURE 4 | Legend on next page.

FIGURE 4 | Patterns of simple sequence repeats [SSRs (a, b)] and long sequence repeats [LSRs (c, d)] for the 14 *Alettris* chloroplast genomes. (a) Number of motifs and their abundance of SSRs in each species. (b) Type of motifs and their abundance of SSRs in each species. (c) Type and abundance of LSRs in each species. (d) Number of LSRs at different locations in each genome.

28bp of *rps19* extended into the IRb boundary in *Dioscorea futschauensis* and *Narthecium ossifragum*, respectively. In addition, an inversion event involving the *ndhF* and *ycf1* genes in the *Dioscorea futschauensis* cp genome occurred, with the former now extending 6bp into the JSA, and the latter was cut through by the JSB boundary.

3.6 | Candidate Molecular Markers Identification

Using sliding window analysis, 18 hypervariable regions were identified as potential East Asia-specific molecular markers for *Alettris* taxonomy, primarily occurring in the LSC and SSC regions and ranging from 523 to 2133bp (Figure 6; Table 4). Among them, 11 intergenic spacers and 2 gene regions are located in the LSC region, 2 intergenic spacers and 1 gene are located in the SSC region, and the remaining intergenic spacer of *trnN-GUU* and *rrn4.5* (*trnN-GUU-rrn4.5*) is positioned in the IRa region (Figure 6). Notably, the *ccsA-ndhD* intergenic spacer was the longest (2133bp) and contained the greatest number of parsimony informative sites (PIP, 49) as well as the highest Pi value (0.020851; Table 4). In addition, the *ycf1*, *petA*, and *rpoC2* genes also displayed sufficient variability and could be utilized as molecular markers for species identification and genetic studies within *Alettris* (Figure 6).

3.7 | Phylogenetic Relationship of *Alettris*

We found that the relationships among all 14 East Asian *Alettris* species were completely congruent between ML and BI analyses, forming a strongly supported monophyletic group (BS=100%, PP=1.0) that was sister to the outgroup taxa: *Dioscorea futschauensis*, *Metanarthecium luteoviride*, and *Narthecium ossifragum* (BS=100%, PP=1.0; Figure 7). While the BI analysis fully resolved the outgroup relationships, with *Metanarthecium luteoviride* and *Narthecium ossifragum* forming a sister group that was then sister to *Dioscorea futschauensis* (PP=1.0), the ML analysis left them as an unresolved polytomy (Figure 7).

Within *Alettris* species, three evolutionary clades emerged with strong support (BS=100%, PP=1): *A. fauriei* occupies an isolated position as the earliest-diverging lineage (Clade III), sister to the remaining species, which can be further subdivided into two additional clades (Figure 7). The new species, *A. medogensis*, clustered as a sister group to a subclade consisting of *A. spicata*, *A. stenoloba*, *A. alpestris*, and *A. scopulorum*, and together as a sister to *A. glandulifera*, forming Clade II. Clade I united *A. gracilis*, *A. glabra*, *A. pauciflora*, *A. pedicellata*, *A. pauciflora* var. *khasiana*, *A. laxiflora*, and *A. megalantha*. Critically, *A. medogensis* exhibited stronger phylogenetic affinity to *A. alpestris* in Clade II than to its morphological relatives *A. pauciflora* var. *pauciflora* and *A. pauciflora* var. *khasiana* in Clade I (Figure 7), revealing significant morphology–molecular discordance.

3.8 | Selective Pressure of cp Genes in Nartheciaceae

A total of 77 PCGs shared among 17 Nartheciaceae cp genomes were analyzed for selective pressure, and most of them were subjected to purifying selection ($K_a/K_s < 1$; Figure 8; Table S7). By calculating the K_a/K_s ratios for homologous genes within species pairs, only *ccsA* in *Alettris scopulorum*, *cemA* in *A. medogensis* and *A. stenoloba*, *rps12* in *A. spicata* and *A. fauriei*, *psbK* in *Dioscorea futschauensis*, and *rpl2* in *Narthecium ossifragum* showed signatures of positive selection ($K_a/K_s > 1$). These findings suggest that some mutations were positively favored by selection in specific species. Furthermore, eight genes (*atpE*, *matK*, *ndhF*, *cemA*, *atpF*, *ccsA*, *rps12*, and *ycf1*) evolved faster in most *Alettris* species than in the other three Nartheciaceae species branches (Figure 8; Table S7), potentially reflecting species-specific adaptations.

4 | Discussion

The discovery of *Alettris medogensis* as a new species (Figure 1), supported by both morphological distinctiveness and chloroplast genome analysis, highlights the importance of integrating traditional taxonomy with modern molecular tools. This study not only expands known endemism in the eastern Himalayas but also provides critical insights into the evolutionary dynamics and genomic features of Nartheciaceae.

4.1 | Chloroplast Genome Evolution in *Alettris*

Consistent with other angiosperms (Sugiura 1992; Daniell et al. 2016), the comparative analysis of the 14 East Asian *Alettris* cp genomes revealed a conserved quadripartite structure with minor variations in size (154,167–155,143bp) and gene content (Table 2). Various elements influencing chloroplast structure and function, such as the contraction and expansion of the IR regions, gene insertions, deletions, duplications, inversions, and alterations in introns, have been reported in many species (Tsudzuki et al. 1992; Lin et al. 2012; Li et al. 2020). Notably, the pseudogenization of the *ycf1* gene in *A. fauriei* and the loss of *rrn4.5* in *A. pauciflora* var. *khasiana* are of functional interest. The former is significant given its role in plastid protein import and stress responses, while the latter might reduce ribosomal redundancy in stable riparian niches, potentially enhancing DNA repair capacity in high-UV montane habitats (Dong et al. 2015; Wei et al. 2024). Additionally, the IR boundary dynamics also observed here—particularly the expansion of *ndhF* into IRb and the contraction of *rps19* from IRb in *A. fauriei* (Figure 5)—reflect species-specific genomic rearrangements. Such IR contractions/expansions can alter gene dosage and disrupt operon integrity, potentially affecting photosynthetic efficiency (Kim and Lee 2005; Zoschke and Bock 2018).

Inverted Repeats

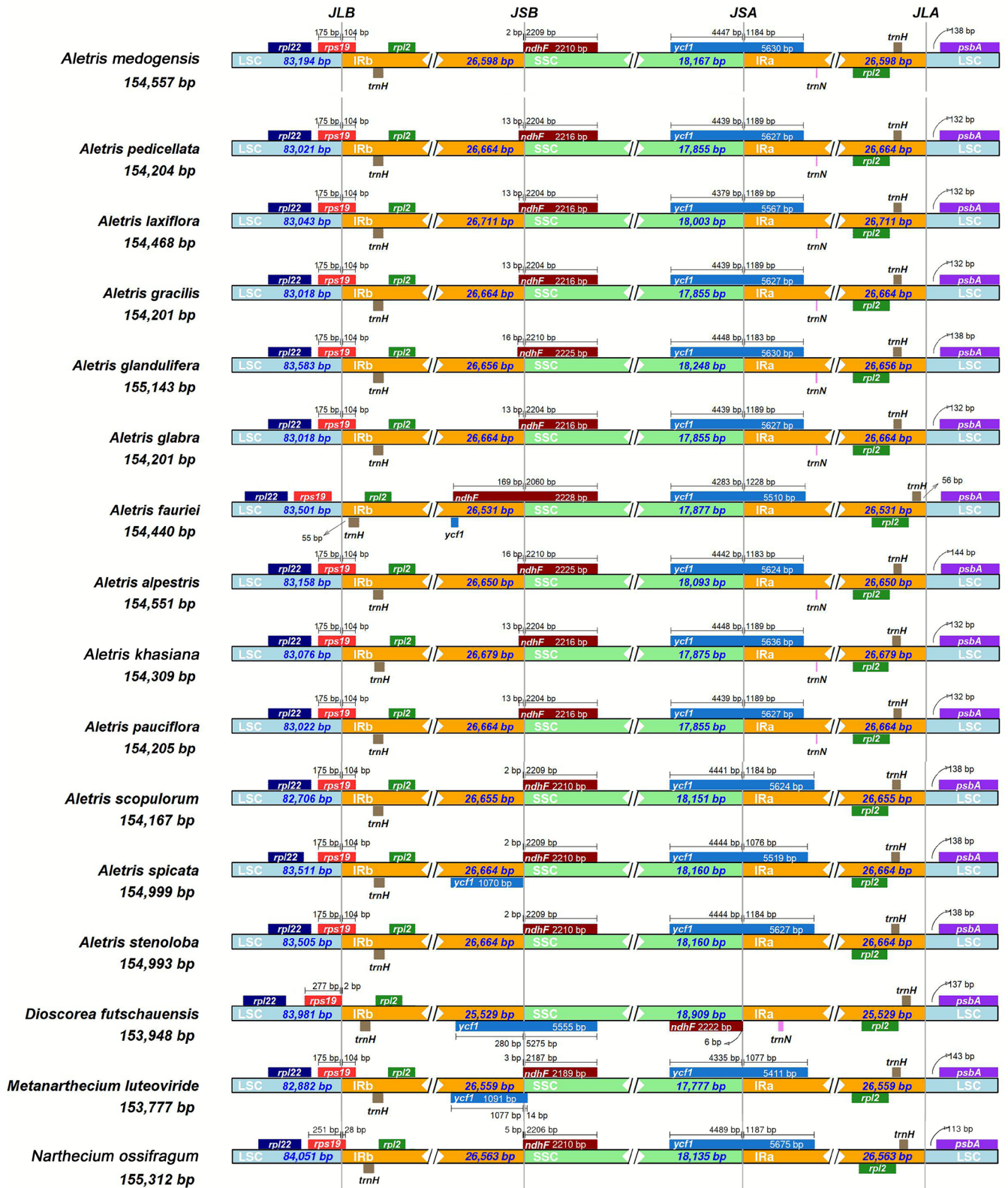


FIGURE 5 | Comparison of LSC, SSC, and IR region boundaries in 17 Nartheciaceae chloroplast genomes.

The strong codon usage bias favoring A/U-ending codons (Figure 3) aligns with angiosperm-wide trends but shows intensified bias in Himalayan species (Liu and Xue 2005; Smarda

et al. 2014). This likely optimizes translational efficiency under low-temperature stress, as A/U-rich transcripts fold less stably, facilitating ribosome binding in cold environments (Chiba

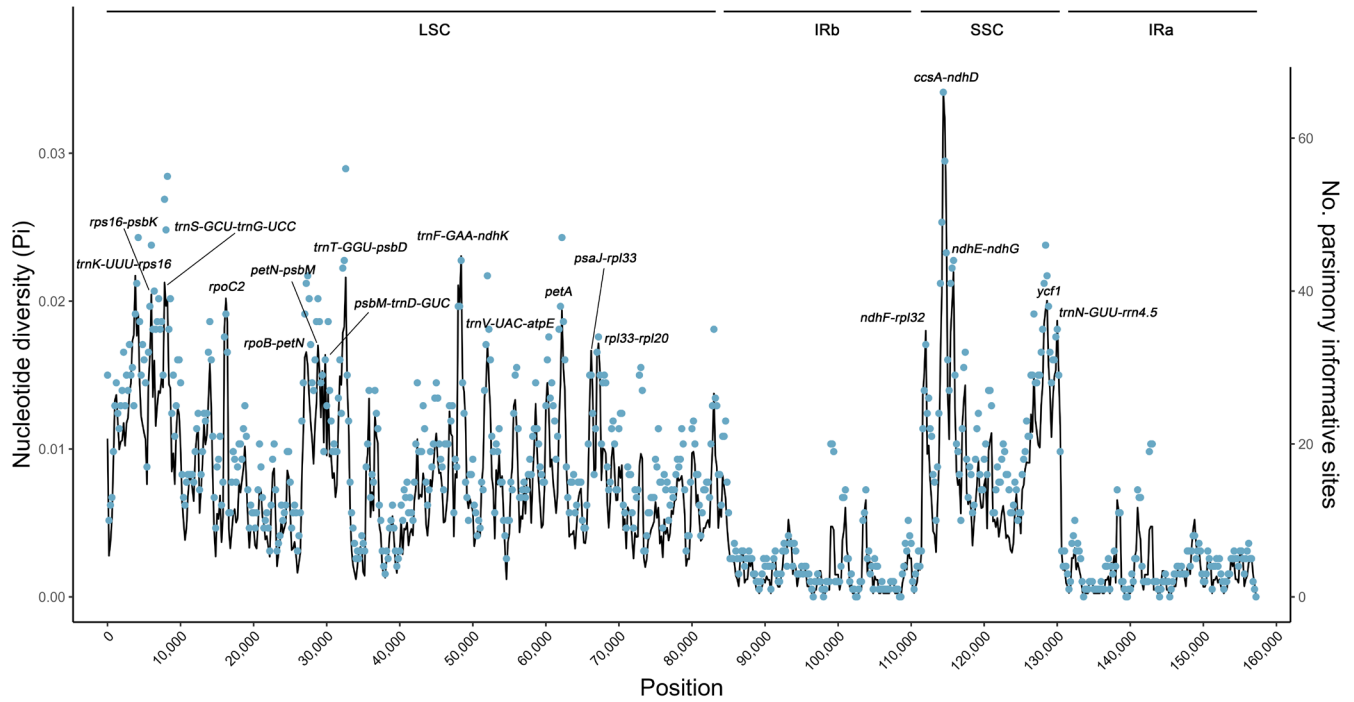


FIGURE 6 | Nucleotide diversity (Pi, black line, vertical left axis) and number of single nucleotide polymorphism sites (blue dots, vertical right axis) of 14 *Aletris* cp genomes based on sliding window analysis. The window length is 600 bp and the step size is 200 bp. The horizontal axis indicates the position of the midpoint of a window. The 18 regions with high diversity (top 5%) are indicated above the peaks.

TABLE 4 | Hypervariable regions identified among the 14 cp genomes of *Aletris*.

Start	End	Length	# SNP	# SVS	# PIP	Pi	Gene name
3400	4745	1346	78	50	22	0.016959	<i>trnK-UUU-rps16</i>
5800	6578	779	48	32	14	0.016218	<i>rps16-psbK</i>
7800	8759	960	78	56	18	0.020378	<i>trnS-GCU-trnG-UCC</i>
16,000	16,993	994	42	23	13	0.012838	<i>rpoC2</i>
27,000	27,789	790	54	37	14	0.01674	<i>rpoB-petN</i>
28,800	29,361	562	38	26	12	0.017218	<i>petN-psbM</i>
29,800	30,389	590	31	11	20	0.016653	<i>psbM-trnD-GUC</i>
32,200	33,185	986	64	45	13	0.016593	<i>trnT-GGU-psbD</i>
48,000	48,892	893	51	32	18	0.016814	<i>trnF-GAA-ndhK</i>
52,000	52,586	587	42	28	12	0.017529	<i>trnV-UAC-atpE</i>
62,200	62,744	545	39	30	8	0.015932	<i>petA</i>
66,200	66,722	523	26	14	11	0.016847	<i>psaJ-rpl33</i>
67,000	67,779	780	39	23	14	0.01477	<i>rpl33-rpl20</i>
112,000	112,596	597	33	15	17	0.018093	<i>ndhF-rpl32</i>
114,200	116,332	2133	154	104	49	0.020851	<i>ccsA-ndhD</i>
117,400	117,996	597	32	18	14	0.014381	<i>ndhE-ndhG</i>
128,200	129,199	1000	68	42	26	0.018297	<i>ycf1</i>
130,000	130,599	600	35	17	18	0.018681	<i>trnN-GUU-rn4.5</i>

Note: The start and end positions are referred to the alignment sequences of 14 *Aletris* species.

Abbreviations: # PIP, number of parsimony informative sites; # SNP, number of single nucleotide polymorphism sites; # SVS, number of singleton variable sites.

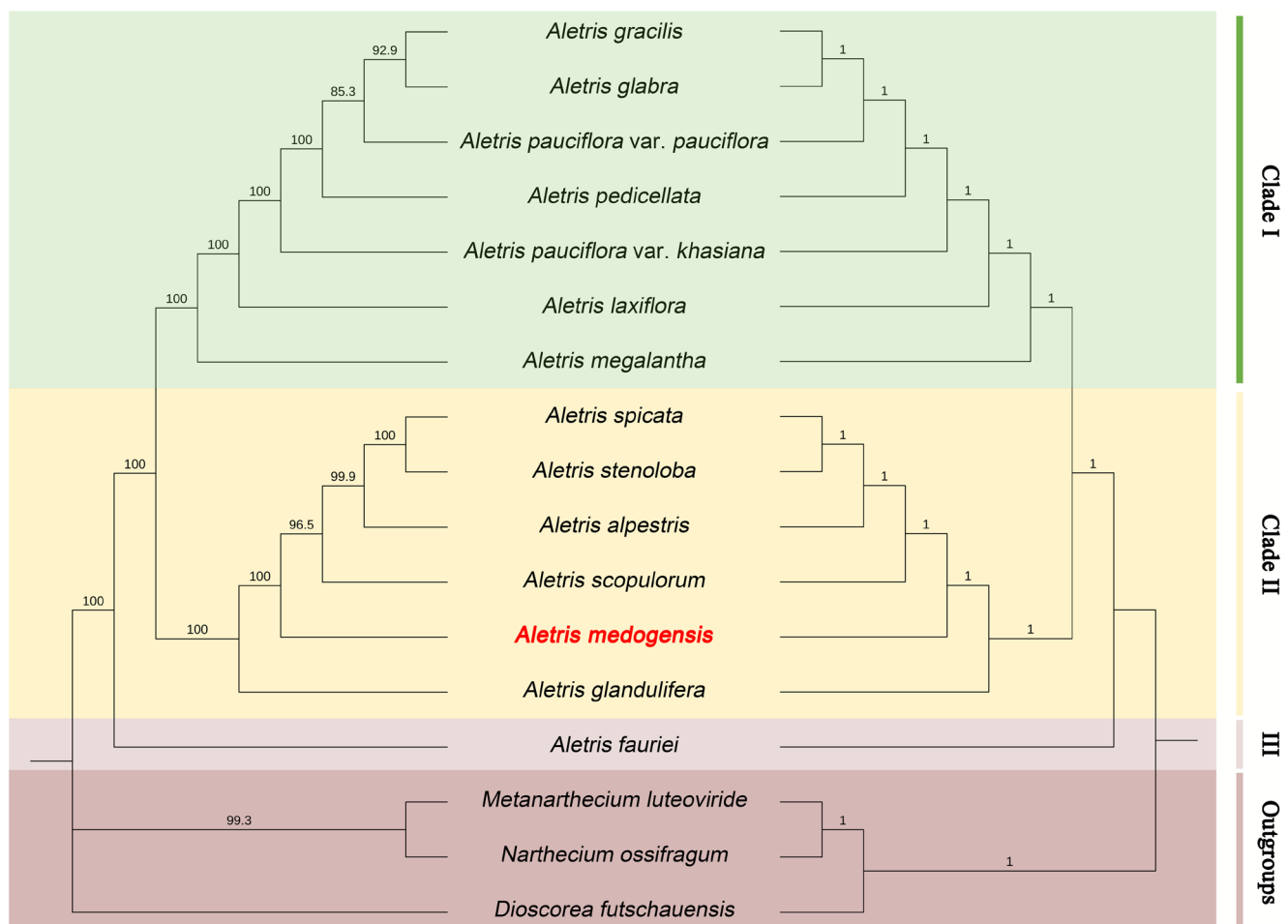


FIGURE 7 | Phylogenetic tree inferred from Maximum Likelihood (ML, on the left) and Bayesian Inference (BI, on the right) methods for East Asian *Aletris* species and their closely related species based on whole chloroplast genomes. The numbers above branches indicate ML bootstrap support (BS) and Bayesian posterior probabilities (PP) respectively.

et al. 2013; Krasileva et al. 2017). Additionally, the excess of SSRs and LSRs in intergenic regions (Figure 4d) suggests their role in regulating genome plasticity; the 2820bp palindromic repeat in *A. pauciflora* var. *khasiana* (Table S6) could promote recombination-driven innovation, potentially accelerating adaptation to microhabitat heterogeneity (Wicker et al. 2007; Oliver et al. 2013).

4.2 | Phylogenetic Implications for *Aletris* Taxonomy

Although *A. medogensis* possesses a unique combination of morphological characters not found in its morphologically similar relatives (e.g., *A. alpestris*, *A. pauciflora*), morphological characteristics alone may be insufficient to resolve species boundaries, where subtle differences in floral structures and vegetative traits often overlap among taxa (Table 1). Our chloroplast phylogenomic analysis resolves long-standing controversies in East Asian *Aletris* systematics. First, *Metanarthecium luteoviride* formed a clade with *Narthecium ossifragum* (PP=1, Figure 7), providing robust genomic evidence to exclude it from *Aletris*—a conclusion consistent with Zhao et al. (2012) but contradicting fragment-based studies (Merckx et al. 2008; Fuse et al. 2012). Second, our

data confirm that *A. pauciflora* var. *khasiana* and *A. pauciflora* var. *pauciflora* are phylogenetically distinct (Figure 7), validating Zhao et al.'s (2012) proposal to recognize the former as a separate species. Critically, the whole cp genome tree provided robust molecular evidence to corroborate the distinctiveness of *Aletris medogensis* by demonstrating that it was phylogenetically distant from the two varieties of *A. pauciflora* (which were nested within Clade I), and that, despite belonging to the same major clade (Clade II), it was not closely related to *A. alpestris*, which formed a separate subclade (Figure 7). This discordance between morphological affinity and phylogenetic placement underscores the necessity of molecular data to clarify evolutionary relationships in taxonomically challenging groups (Redwan et al. 2015).

While our integrative evidence from morphology and chloroplast phylogenomics robustly supports the recognition of *A. medogensis* as a distinct species, phylogenetic inferences based solely on plastid data may not always reflect the true species tree because of evolutionary processes such as incomplete lineage sorting and introgression, due to its uniparental inheritance. Therefore, the employment of nuclear markers, or a combination of nuclear and plastid data, is strongly advocated in future studies to further validate and refine the phylogenetic relationships proposed here.

constraints of essential photosynthetic and ribosomal genes. However, positive selection ($\omega > 1$) was detected in *ccsA* (*A. scopulorum*), *cemA* (*A. medogensis* and *A. stenoloba*), and *rps12* (*A. spicata* and *A. fauriei*). These genes, involved in cytochrome synthesis, chloroplast envelope stability, and ribosomal function (Xie and Merchant 1996; Ogawa et al. 1994; Ramundo et al. 2013), have also been found to undergo positive selection in other taxa (e.g., *Ficus*, *Populus*) (Zhang et al. 2022; Shi et al. 2025). Their accelerated evolution might reflect species-specific adaptations to environmental stressors like high-altitude habitats or pathogen resistance (Zhang et al. 2020). The faster evolutionary rates of *matK* and *ndhF* in *Aletris* than in other Nartheciaceae genera further highlight lineage-specific selective regimes, possibly linked to ecological diversification (Barthet and Hilu 2007; Zhao et al. 2017).

4.5 | Conservation Implications

Despite its restricted distribution in Medog County, *A. medogensis* is currently classified as data deficient (DD) due to insufficient population data. The species' reliance on moss-covered riparian habitats makes it vulnerable to climate change, which can manifest as altered stream flow regimes and the desiccation of its required microclimate, and to direct anthropogenic disturbances such as tourism development and infrastructure construction along its fragile streamside habitat (Xu et al. 2009; Zhang and Yan 2023). In-depth surveys are needed across similar ecosystems in the Eastern Himalayas to assess the true distribution and conservation status of this endemic lineage, allowing for essential morphometric comparisons across populations to validate a suite of stable, diagnostic traits. Additionally, the genomic resources generated here, including hypervariable markers and cp genome annotations, will facilitate monitoring efforts and inform conservation strategies for this endemic lineage.

5 | Conclusions

The integration of morphology, chloroplast genomics, and phylogenomics effectively resolves taxonomic conflicts in *Aletris* and reveals evolutionary mechanisms underlying species diversification. This work enhances our understanding of Himalayan plant diversity and provides a framework for future studies on cryptic species in evolving ecosystems.

6 | Taxonomic Treatment

Aletris medogensis W.B.Ju, Y.L.Qiu & Bo Xu, sp. nov.

6.1 | Type

CHINA. Xizang: Motuo County, Beibeng Xiang, from Hanmi to Xiaoyandong on the opposite side, ca. 2931 m, 29°23'25.24", 95°06'00.75", occurring on moss-covered rocks along riparian zones. June 9, 2024, Ju Wen-Bin, Li Jiang-Tao, Zeng Li-Ting,

and Deng Hui-Wen YLZB12018 (holotype: CDBI0298322; Figure S1).

6.2 | Diagnosis

Aletris medogensis is morphologically most similar to *Aletris alpestris*, but it can be distinguished from the latter in having narrower leaves (0.45–0.9 mm wide vs. 1–2.5 mm wide) and an obtuse apex (vs. an acuminate apex); the flower pedicels are extremely short, the rachis has densely glandular hairs (vs. sparsely puberulent), the two bracts are unequal in length and shorter than the flowers (vs. one of them 1–4 × flower length). Lobes are obtuse to rounded at the apex (vs. the apex being obtuse to acute).

6.3 | Description

Perennial herb, tillering with developing creeping stolons. Roots are usually fibrous. Leaves in basal rosette, occasionally, leaf primordia split from the basal region, giving rise to new leaves, linear, 2.5–9 cm × 0.45–0.9 mm, apex obtuse, glabrous. Scape 3.5–16 cm × 0.7–1 mm, glandular are sparse near the inflorescence and progressively become glabrous toward the lower part, bract-like leaves 0.2–0.6 cm long in the middle and lower part, lanceolate or ovate-lanceolate, apex acute. Raceme 1.5–2.5 cm, densely 10–14-flowered, pedicels extremely short, rachis densely glandular hairs; bract and bracteole lanceolate, 2–4 mm, borne at the apex of the pedicel, shorter than the flower. Perianth white, glabrous, split to the middle; tube campanulate; lobes recurved or erect, ovate, apex obtuse to rounded, 1.2–2 × 0.8–1.5 mm. Filaments of stamens adnate to perianth, 0.5 mm, anthers elliptical, 0.5 mm, the style abruptly constricted into a short style, stigma not or only slightly thickened, capitate. Ovary ovate, ridged. Fruits capsular, 3-locular (Figures 9 and 10).

6.4 | Phenology

Flowering from June to July, fruiting from August to September.

6.5 | Etymology

Located in southeastern Xizang Autonomous Region, Medog (Pinyin spelling “motuo”) County is one of the biodiversity hotspots in China, which has rich plant diversity in the Eastern Himalaya (Qiu et al. 2022). The new species, *Aletris medogensis*, is found in this region and is named after the geographic location. Its Chinese name, mo tuo fen tiao er cai (墨脱粉条儿菜).

6.6 | Distribution and Ecology

This new species is currently known only from the opposite bank of Hanmi to the Xiaoyandong direction in Motuo County, Nyingchi City, Xizang (Tibet), China, at an elevation of 2200–2400 m. *Aletris medogensis* grows on moss-covered rocks along streams in moist environments.

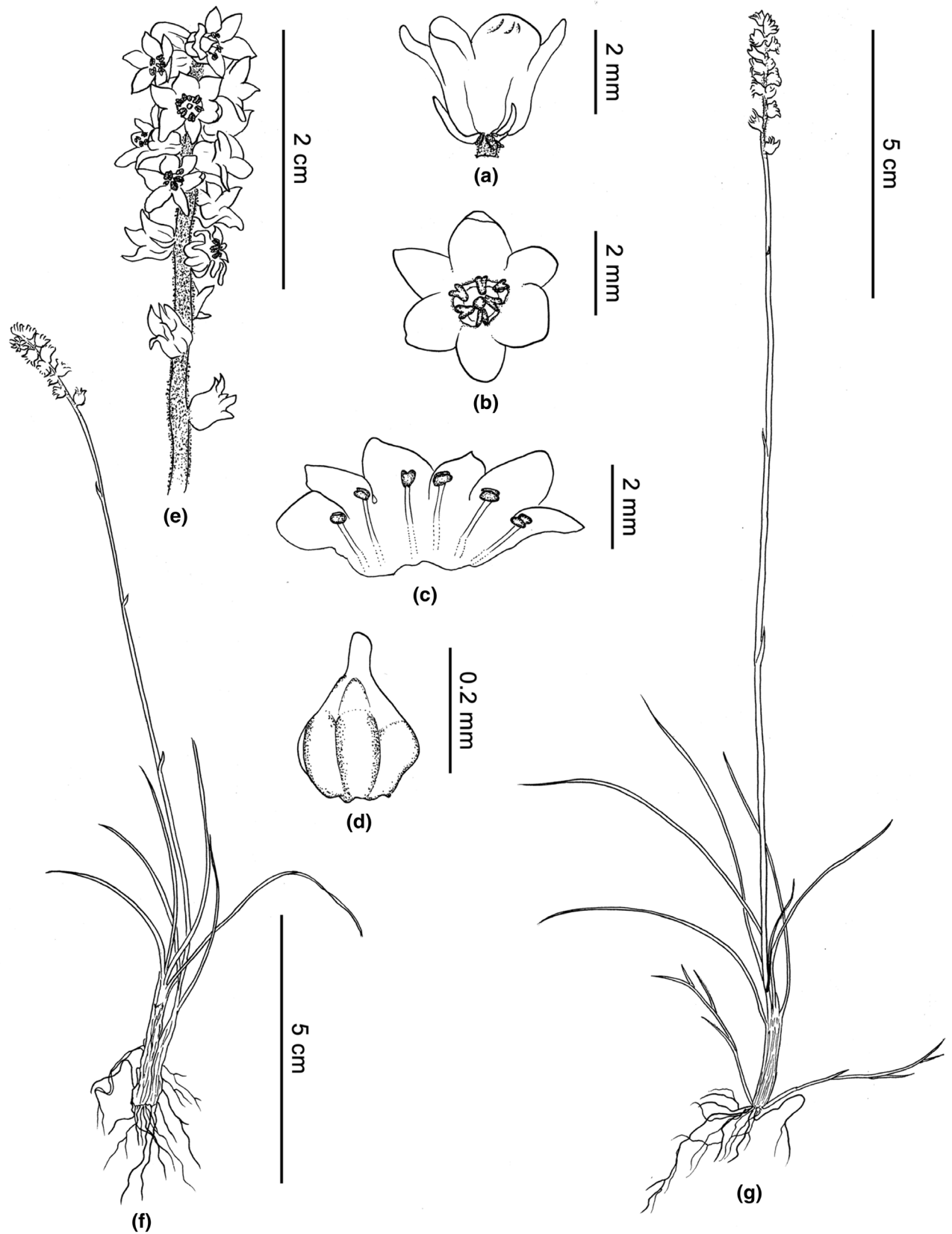


FIGURE 9 | Line drawing of *Aletris medogensis*. Lateral (a) and front (b) views of the flower. (c) Stamens. (d) Ovary and stigma. (e) Inflorescence. (f, g) Plants.

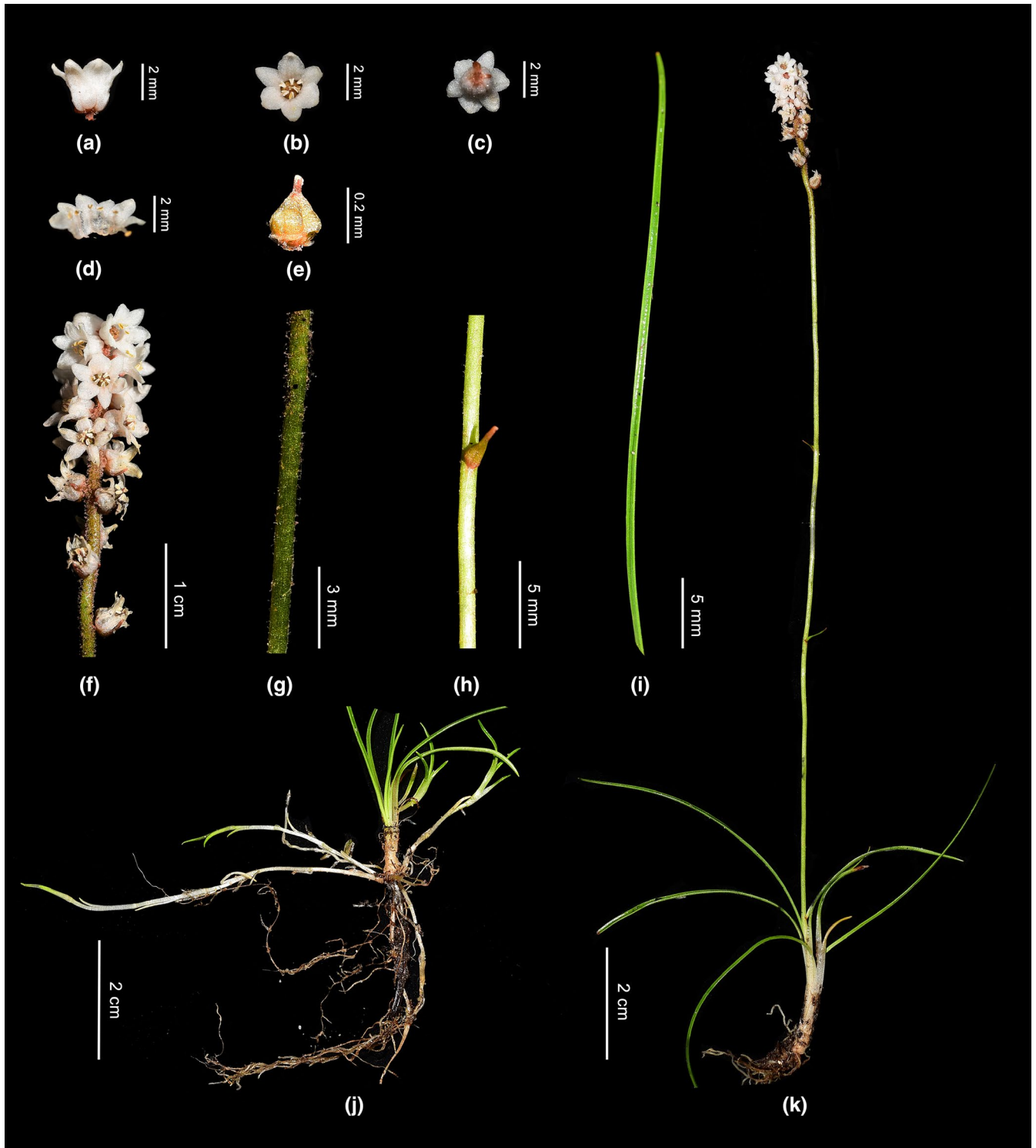


FIGURE 10 | *Aletris medogensis*. Lateral (a), front (b), and back (c) views of the flower. (d) Stamens. (e) Ovary and stigma. (f) Inflorescence. (g) Scape. (h) Bracteate leaf. (i) Leaf. (k) Roots. (l) Plant.

6.7 | Conservation Status

Currently known from a small population at the type locality, approximately 150 mature individuals have been observed. The data available for this new species are still insufficient to assess its conservation status. According to the IUCN 2023 Red List criteria (International Union for Conservation of Nature 2023), the species is classified as Data Deficient (DD). Further collection

and monitoring efforts are required to enable more conclusive assessments of the species' rarity and vulnerability.

Author Contributions

Xiong Li: data curation (lead), software (equal), visualization (equal), writing – original draft (lead), writing – review and editing (lead). **Yong-Ling Qiu:** data curation (equal), software (equal), writing – original

draft (equal), writing – review and editing (supporting). **Jiang-Tao Li:** investigation (equal), writing – review and editing (supporting). **Bo Xu:** writing – original draft (supporting), writing – review and editing (supporting). **Qi Yu:** writing – review and editing (supporting). **Wen-Bin Ju:** investigation (lead), project administration (lead), resources (lead), writing – review and editing (equal).

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Ethics Statement

This study's material collections and experimental research followed the relevant institutional, national, and international guidelines and legislation.

Consent

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The chloroplast genome of *Aletris medogensis* has been deposited into the National Center for Biotechnology Information (NCBI) database with accession number PV472299.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Appendix S1–S2:** ece372654-sup-0001-AppendixS1-S2.docx. **Figures S1–S2:** ece372654-sup-0002-FigureS1-S2.docx. **Tables S1–S7:** ece372654-sup-0003-TableS1-S7.xlsx.