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Research Article

Delimitation and phylogeny of *Aletris* (Nartheciaceae) with implications for perianth evolution

[1,2,3]Yi-Min ZHAO [1]Wei WANG [1]Shu-Ren ZHANG*

[1](*State Key Laboratory of Systematic and Evolutionary Botany, Institute of Botany, Chinese Academy of Sciences,* Beijing 100093, China)
[2](*Graduate University of Chinese Academy of Sciences,* Beijing 100049, China)
[3](*Guangxi Botanical Garden of Medicinal Plants,* Nanning 530023, China)

Abstract *Aletris*, containing approximately 21 species, is the largest genus in Nartheciaceae, and is disjunctively distributed in eastern Asia and eastern North America. Its delimitation has been controversial because it is uncertain whether *Metanarthecium* should be included in the genus. Although there are a few molecular phylogenetic studies on *Aletris*, the interspecific relationships within the genus have never been evaluated in a phylogenetic context. Here we used two cpDNA loci, *matK* and *trnL-F*, to delimitate *Aletris* and discuss the phylogeny within the genus. Phylogenetic analyses showed *Metanarthecium* might be distantly related to *Aletris*. This is also supported by morphological, palynological, cytological, and phytochemical data. Therefore, *Metanarthecium* should be excluded from *Aletris*. Within *Aletris*, there are two major clades: *A. farinosa* and *A. lutea* of eastern North America and *A. glabra* of eastern Asia form clade A; and the remaining Asian species form clade B. The Asian clade includes three subclades: subclade I (two varieties of *A. pauciflora*, and *A. glandulifera* and *A. megalantha*), subclade II (three samples of *A. laxiflora*), and subclade III (all other sampled Asian species). Based on phylogenetic relationships, *A. pauciflora* var. *khasiana* deserves a specific status, and *A. gracilipes*, formerly a synonym of *A. laxiflora*, should be reinstated. The reconstruction of the perianth evolution indicates that perianth connate halfway and glabrous on abaxial surface are plesiomorphic for *Aletris* and Nartheciaceae. Farinose-glutinous perianth is a diagnostic character for clade A.
Key words *Aletris*, *matK*, *Metanarthecium*, Nartheciaceae, perianth evolution, phylogeny, *trnL-F*.

Traditionally, *Aletris* was thought to be a member of Liliaceae *s.l.* (e.g., Ambrose, 1980). However, modern research has indicated that the genus and its allies should be removed from Liliaceae s.l. to another family, Nartheciaceae sensu APG III (2009). Molecular phylogenetics has contributed greatly to the delimitation of Nartheciaceae. Based on the combined molecular and morphological data, Caddick et al. (2002a) found that *Aletris*, *Lophiola*, *Metanarthecium*, and *Narthecium* formed a monophyletic clade. Subsequently, Caddick et al. (2002b) formally defined Nartheciaceae, which contains *Aletris*, *Lophiola*, *Metanarthecium*, *Narthecium*, and *Nietneria*.

Within Nartheciaceae, the phylogenetic relationship between *Aletris* and *Metanarthecium* is problematic. Molecular phylogenetic studies by different researchers were contradictory about the relationship of the two genera. The chloroplast DNA *matK* and *rbcL* data revealed that *Metanarthecium* was relatively remotely related to *Aletris* (Fuse & Tamura, 2000; Tamura et al., 2004). However, in the phylogenetic analyses based on chloroplast, mitochondrial, and/or nuclear genes, *Metanarthecium* and *Aletris* formed a clade (Merckx et al., 2006, 2008, 2009; Merckx & Bidartondo, 2008). Merckx et al. (2008) placed *Metanarthecium* in *Aletris*.

Metanarthecium is monotypic, with only one species, *M. luteoviride* Maxim., which is endemic to Japan (Merckx et al., 2008). The taxonomic contention of *Metanarthecium* is the basis of the dispute over the delimitation of *Aletris*. Based on comparison of connate portions of perianth, Franchet (1896) merged *Metanarthecium* with *Aletris*. It was followed by Hara (1967), Akahori et al. (1971), Zomlefer (1997), and Tamura (1998). Numerical analyses also showed that *M. luteoviride* was nested in the two American species of *Aletris* (Ambrose, 1980). However, the chromosome (Satô, 1942) and pollen (Takahashi & Kawano, 1989; Merckx et al., 2008) characters of *M. luteoviride* differ markedly from those of *Aletris*. Because there were some differences between *Aletris* and *Metanarthecium*, many authors treated the latter as a separate genus (e.g., Edward & Browne, 1961; Dahlgren et al., 1985; Takhtajan, 1997; Remizowa et al., 2008).

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* Author for correspondence. E-mail: srzhang@ibcas.ac.cn; Tel.: 86-10-62836087; Fax: 86-10-62590843.

Aletris, the largest genus in Nartheciaceae, contains approximately 21 species (Sullivan, 1973; Liang & Turland, 2000; Wu et al., 2003). The genus displays a disjunctive distribution in eastern Asia and eastern North America (Boufford & Spongberg, 1983). Five species of the genus are distributed in eastern North America (Zomlefer, 1997; Wunderlin, 1998; Weigant, 2002), and the remainders in eastern and southeastern Asia. All of the aforementioned molecular studies concerning *Aletris* only sampled one to several species of the genus. Phylogeny within *Aletris* has not been investigated using molecular data. Perianth characters are traditionally considered the most important in the taxonomy of *Aletris* (Liang & Turland, 2000; Weigant, 2002), however, the evolutionary pattern of perianth characters also need to be evaluated in a phylogenetic context.

In this study, we have broadly sampled *Aletris* species, and aimed to: (i) clarify the delimitation of *Aletris*; (ii) investigate the phylogenetic relationships within *Aletris*; and (iii) discuss the perianth evolution of *Aletris*.

1 Material and methods

1.1 Taxon sampling

We sampled all five genera of the Nartheciaceae, including 41 terminals representing 18 of the 21 species of *Aletris* (16 from China and two from North America), one species each of the monotypic *Metanarthecium* and *Nietneria*, two species of eight of *Narthecium*, and one species of two of *Lophiola*. Based on previous molecular phylogenetic studies (Caddick et al., 2002a; Givnish et al., 2005; Davis et al., 2006), one species of Dioscoreaceae, *Dioscorea* sp., was chosen as the outgroup. Sampled taxa and GenBank accession numbers are shown in Appendix I. Voucher specimens of newly collected materials (39 samples of 16 taxa of *Aletris* and one sample of *Dioscorea*) were deposited in the Herbarium of the Institute of Botany, Chinese Academy of Sciences (PE).

1.2 DNA extraction, polymerase chain reaction amplification, and sequencing

Total DNA was extracted from silica-gel-dried leaf materials using the modified CTAB protocol of Doyle & Doyle (1987). Standard polymerase chain reaction procedures were used to amplify the target DNA regions. The *matK* and *trnL-F* regions were amplified and sequenced using the *matK* primers *matK*-AF and *matK*-8R (Ooi et al., 1995) and *trnL-F* primers c and f (Taberlet et al., 1991), respectively. Polymerase chain reaction products were purified using a gel extraction kit (UNIQ-10; Sangon, Shanghai, China). Sequencing reactions were carried out using the DYEnamic ET Dye Terminator Cycle Sequencing Kit (Amersham Pharmacia Biotech, Piscataway, NJ, USA) following the manufacturer's protocols. A total of 78 sequences of *matK* and *trnL-F* were newly generated.

1.3 Phylogenetic analysis

Sequences were aligned using CLUSTALX version 1.83 (Thompson et al., 1997) then adjusted manually with BioEdit (Hall, 1999). Two ambiguous regions of *trnL-F* were cut off from the sequences prior to phylogenetic analyses. All DNA datasets were analyzed using maximum parsimony (MP), maximum likelihood (ML), Bayesian inference methods in PAUP* version 4.0b10 (Swofford, 2003), PhyML version 2.4.3 (Guindon & Gascuel, 2003), and MrBayes version 3.0b4 (Ronquist & Huelsenbeck, 2003). We visually inspected the individual bootstrap (BS) consensus trees on a node to node basis to test the congruence among the individual DNA datasets by identifying contradictory nodes with >70% BS support.

In the MP analyses, heuristic searches were carried out with tree bisection reconnection branch swapping, one tree held at each step during stepwise addition, MulTrees in effect, steepest descent off, and 1000 replicates of random addition. Gaps were coded as missing data. A BS analysis (Felsenstein, 1985) was run with 1000 BS replicates with 10 random taxon additions and heuristic search options. Each dataset was assigned its own model of nucleotide substitution, as determined by the Akaike information criterion in Modeltest version 3.06 (Posada & Crandall, 1998). The likelihood analysis was carried out using the GTR substitution model with proportion of invariable sites and gamma distribution parameter, determined by Modeltest as above. Nodal support on the ML tree was evaluated by the non-parametric BS (1000 replicates). For Bayesian inference, searches were based on 1 000 000 generations with four chains of the Markov Chain Monte Carlo (MCMC). Runs were started from a random tree and allowed to proceed in parallel while sampling and recording the topology every 100 generations of the MCMC chain. For each run, majority rule (>50%) consensus trees were constructed after removing the "burn-in period" samples (the first 25% of sampled trees). Posterior probability (PP) was used to estimate the nodal robustness.

1.4 Perianth evolution

The reconstruction of perianth evolution in *Aletris* was carried out using the parsimony method with MacClade version 4.06 (Maddison & Maddison, 2003). The tree used in the analysis was generated from the combined *matK* and *trnL-F* data. Each species has one terminal except for *A. pauciflora* (Klotzsch)

Hand.-Mazz., of which monophyly is not supported. Because of the taxonomic significance, two perianth characters were selected: abaxial surface of perianth (tomentose, glabrous, farinose-glutinous, and densely woolly); and connate degree of perianth (half, basally, and almost totally).

2 Results

2.1 Analysis of *trnL-F* data

The aligned *trnL-F* region encompassed 1125 positions after excluding 9 bp of two ambiguous regions. The numbers of variable and parsimony informative sites, tree statistics for the MP analysis, and the models of evolution estimated by Modeltest are shown in Table 1.

The topologies of Bayesian and MP trees are highly congruent with the ML tree (Fig. 1). *Lophiola*, *Nietneria*, and *Narthecium* form a clade with strong support (MP BS = 99%, ML BS = 99%, PP = 100%). *Aletris* and *Metanarthecium* form the other clade with strong support (MP BS = 91%, ML BS = 91%, PP = 98%), where *Metanarthecium* is nested in the former and forms a monophyletic group with *A. glabra* Bureau & Franch. (MP BS = 81%, ML BS = 69%, PP = 99%).

2.2 Analysis of *matK* data

The numbers of variable and parsimony informative sites, tree statistics for the MP analysis, and the models of evolution estimated by Modeltest are shown in Table 1.

The topologies of Bayesian, MP, and ML trees for *matK* are almost congruent (Fig. 2). *Metanarthecium luteoviride* is sister to the *Narthecium-Lophiola* clade (MP BS < 50%, ML BS = 58%, PP = 80%). Monophyletic *Aletris* is strongly supported (MP BS = 100%, ML BS = 100%, PP = 100%).

2.3 Analysis of the combined *matK* and *trnL-F* data

The *trnL-F* sequence of *M. luteoviride* was obtained from a DNA sample (MWC630) in the DNA Bank of Royal Botanic Gardens, Kew (Merckx et al., 2008), whereas its *matK* sequence was generated by Fuse & Tamura (2000) from material collected in Japan. Because the results of the *trnL-F* data conflicted with both the *matK* and non-molecular data (morphological and pollen characters), the material used by Merckx et al. (2008) might be wrongly identified. Therefore, the *trnL-F* sequence of *M. luteoviride* is excluded from the combined analyses.

The trees obtained from MP analysis based on the combined *matK* and *trnL-F* data are almost consistent with those on the *matK* dataset in topology (Figs. 2, 3). *Metanarthecium* was supported as sister to the clade containing *Lophiola*, *Narthecium*, and *Nietneria* (MP BS < 50%, ML BS = 52%, PP = 100%). The monophyly of *Aletris* is moderately to strongly supported (MP BS = 100%, ML BS = 78%, PP = 100%). *Aletris* is divided into two major clades, A and B. Within clade A, *A. glabra* from eastern Asia is sister to two eastern North American species (*A. farinosa* L. and *A. lutea* Small). Clade B, consisting of the remaining Asian species, is divided into three subclades, I, II, and III. Subclade I includes *A. glandulifera* Bureau & Franch., *A. megalantha* F. T. Wang & T. Tang, and two varieties of *A. pauciflora*. Three samples of *A. laxiflora* Bureau & Franch. form subclade II, which is sister to the remainder of *Aletris* (subclade III).

Within *Aletris*, *A. alpestris* Diels, *A. glabra*, and *A. laxiflora* are strongly supported as monophyletic, whereas *A. cinerascens* F. T. Wang & T. Tang, *A. pauciflora*, *A. pedicellata* F. T. Wang & T. Tang, *A. spicata* (Thunb.) Franch., and *A. stenoloba* Franch. are not monophyletic (Fig. 3).

3 Discussion

3.1 Delimitation of *Aletris*

The monophyly of *Aletris* is questionable because its relationship with *Metanarthecium* is unclear. Different phylogenetic studies have reinforced the conflicting relationships between these two genera. The chloroplast *matK* and *rbcL* data revealed that *Metanarthecium* was sister to *Lophiola-Narthecium* clade, and *Aletris* was monophyletic with strong support (Fuse & Tamura, 2000; Tamura et al., 2004). In the morphological tree of Caddick et al. (2002a), *Aletris* was sister to the clade including *Lophiola*, *Metanarthecium*, *Narthecium*, and *Nietneria*. However, in the phylogenetic analyses based on nuclear 18S rRNA gene and mitochondrial *nad1* b-c intron (Merckx et al., 2006) or 18S rDNA gene and plastid *atpB-rbcL* spacer, *trnL* intron, and *trnL-trnF* spacer (Merckx et al., 2008), *Metanarthecium* was sister to, or nested in, *Aletris*.

All sequences of *Metanarthecium* used by Merckx et al. (2006, 2008, 2009) were generated from the DNA sample (MWC630) preserved at the DNA Bank of the Royal Botanic Gardens, Kew (http://data.kew.org/dnabank/), with the voucher "Inoue s.n. ?". In the present analyses of *trnL-F* data, we also used the sequence of Merckx et al. (2008), and we obtained a similar result, in that *Metanarthecium* was nested in *Aletris* (Fig. 1). The result conflicts with that based on *matK* (Fuse & Tamura, 2000), *rbcL* (Caddick et al., 2002a), and combined *matK* and *rbcL* (Tamura

Table 1 Statistics from analyses of two cpDNA loci, *matK* and *trnL-F*, used to delimitate *Aletris*

Dataset	Total length (bp)	No. of variable characters	No. of informative characters	No. of trees	Length of trees	CI	RI	RC	Model
matK	1199	277	114	64	356	0.86	0.88	0.76	TVM+G
trnL-F	1125	136	66	1	158	0.92	0.95	0.87	TVM+G
matK + *trnL-F*	2324	413	180	48	518	0.87	0.90	0.79	TVM+G

CI, Consistency index; RC, Rescaled consistency index; RI, Retention index; TVM+G, Models of evolution estimated by Modeltest.

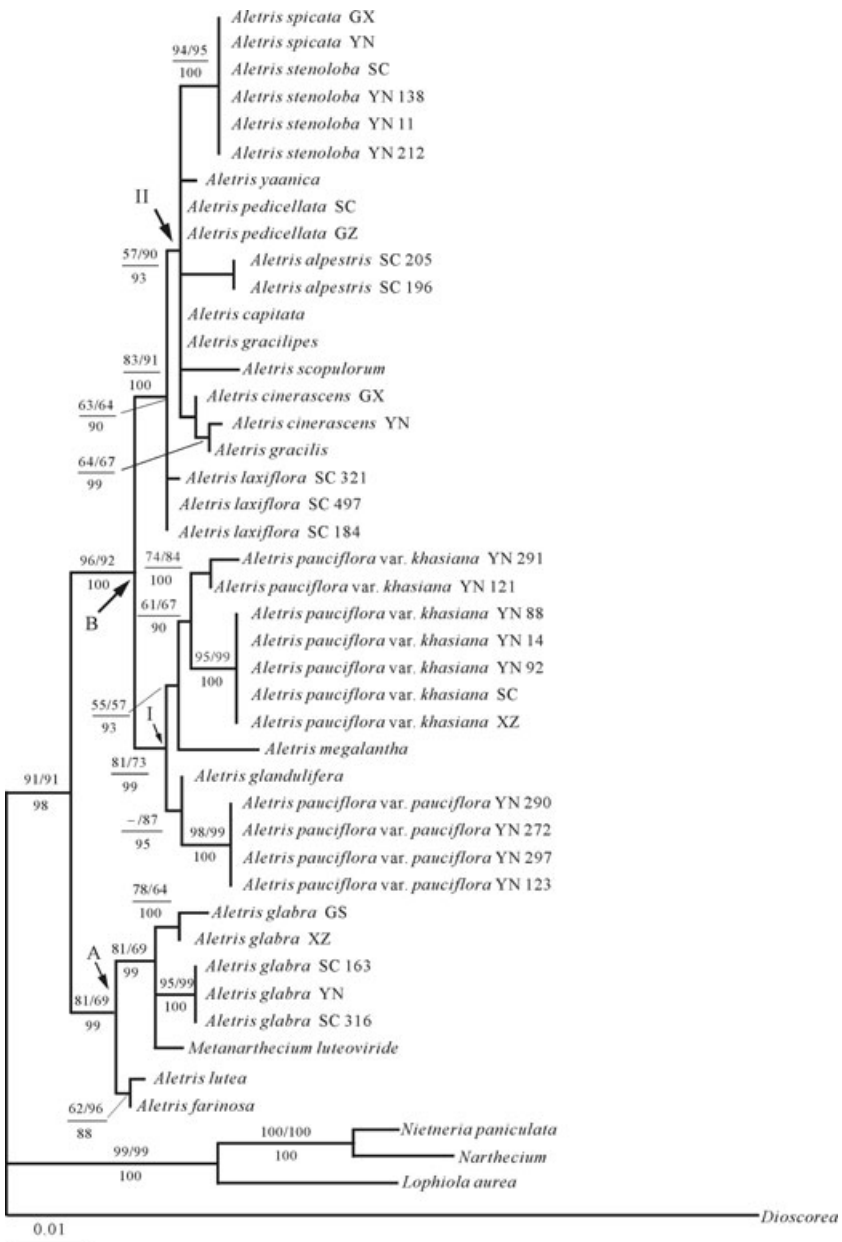

Fig. 1. Maximum likelihood tree inferred from *trnL-F* data. Numbers above branches indicate maximum parsimony/maximum likelihood bootstrap values, and numbers below branches indicate Bayesian posterior probabilities. –, Node not found in the strict consensus tree; GS, Gansu; GX, Guangxi; GZ, Guizhou; SC, Sichuan; XZ, Xizang; YN, Yunnan.

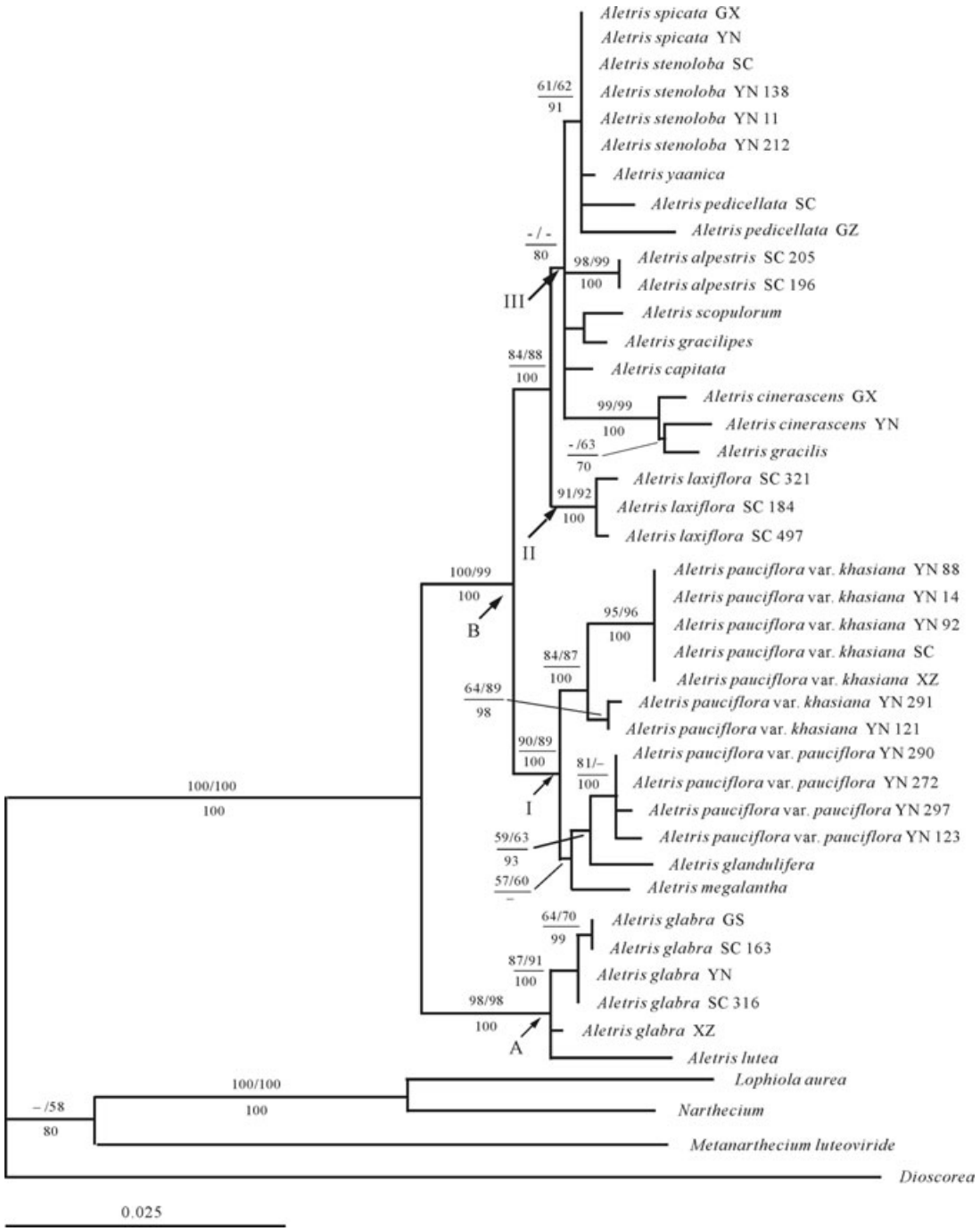

Fig. 2. Maximum likelihood tree inferred from *matK* data. Numbers above branches indicate maximum parsimony/maximum likelihood bootstrap values, and numbers below branches indicate Bayesian posterior probabilities. –, Node not found in the strict consensus tree; GS, Gansu; GX, Guangxi; GZ, Guizhou; SC, Sichuan; XZ, Xizang; YN, Yunnan.

et al., 2004) data. Furthermore, it conflicts with the phylogenetic analyses based on morphological data (Caddick et al., 2002a). *Metanarthecium*, together with *Lophiola*, *Narthecium*, and *Nietneria*, formed a clade being sister to *Aletris* in the morphological tree of Caddick et al. (2002a). Considering such conflict, we doubt the reliability of the DNA sample (MWC630) from the DNA Bank of the Royal Botanic Gardens, Kew.

In our analyses based on *matK* (Fig. 2) and combined *matK* and *trnL-F* (Fig. 3) sequences, the

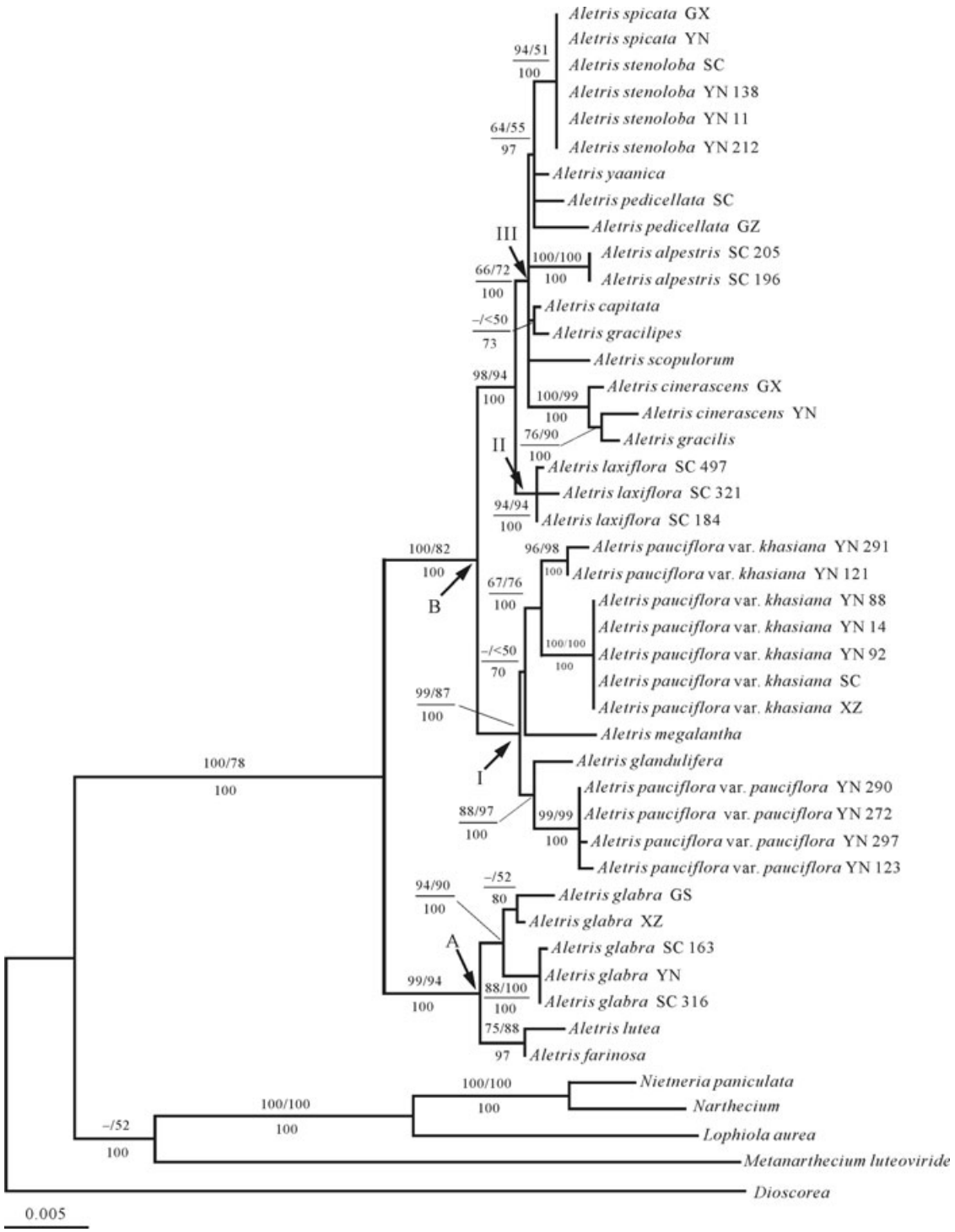

Fig. 3. Maximum likelihood tree inferred from combined *matK* and *trnL-F* data. Numbers above branches indicate maximum parsimony/maximum likelihood bootstrap values, and numbers below branches indicate Bayesian posterior probabilities. –, Node not found in the strict consensus tree; GS, Gansu; GX, Guangxi; GZ, Guizhou; SC, Sichuan; XZ, Xizang; YN, Yunnan.

monophyly of *Aletris* was supported moderately to strongly (*matK*: MP BS = 100%, ML BS = 100%, PP = 100%; combined *matK* and *trnL-F*: MP BS = 100%, ML BS = 78%, PP = 100%), and *Metanarthecium* was sister to *Lophiola-Narthecium* (MP BS < 50%, ML BS = 58%, PP = 80%) or *Lophiola-Narthecium-Nietneria* (MP BS < 50%, ML BS = 52%, PP = 100%) clade.

Metanarthecium was established by Maximowicz (1867), with only one species, *M. luteoviride*. Franchet (1896) merged *Metanarthecium* with *Aletris* based on the fact that *M. luteoviride* and some species of *Aletris* shared the ovaries adnate to perianths basally. By comparing *M. luteoviride* with *A. gracilipes* F. T. Wang & T. Tang, *A. revoluta* (conspecific with *A. laxiflora*), and *A. stenoloba*, Hara (1967) found that they all had perianths connate at base, and then accepted the opinion of Franchet (1896). However, the character that perianth is connate at base has evolved independently at least three times within the Nartheciaceae (Fig. 4: A). On the basis of morphological similarities and a shared basic chromosome number ($x = 13$), some authors also placed *Metanarthecium* in *Aletris* (Tamura, 1998; reviewed in Merckx et al., 2008). However, *Narthecium* and *Nietneria* have a basic chromosome number of $x = 13$ as well (Tamura, 1998). Thus, the basic chromosome number of $x = 13$ may be plesiomorphic for Nartheciaceae.

Metanarthecium differs from *Aletris* in many non-molecular aspects, such as: inflorescence with 2–4 paracladia versus without paracladia (Remizowa et al., 2008); much larger lumina with small projections of pollens versus small lumina without projections (Takahashi & Kawano, 1989; Merckx et al., 2008); two nucleolus in the telophase versus lacking nucleoli (Satô, 1942); and five sapogenins (metagenin, nogiragenin, neonogiragenin, narthogenin, and isonarthogenin) versus three sapogenins (diosgenin, isonarthogenin, and bethogenin) and two sterols (β-sitosterol and stigmasterol) (Akahori et al., 1971). Additionally, *Metanarthecium* is unique amongst Nartheciaceae in possessing almost superior ovaries and sepal nectaries (Remizowa et al., 2008).

Combining molecular and non-molecular (morphological, palynological, cytological, and phytochemical) evidence, it is supported that *Aletris* excluding *Metanarthecium* is monophyletic.

3.2 Phylogeny of *Aletris*

Aletris is divided into two major clades, A and B (Fig. 3). Clade A includes *A. farinosa* and *A. lutea* of eastern North America and *A. glabra* of eastern Asia. The clade is morphologically supported by the abaxial surface of perianth being farinose-glutinous (Fig. 4: B). Clade B includes the remaining Asian species. The most interesting point of the topology is that eastern North American species nest in eastern Asian ones, and sister to *A. glabra*, which is a typical eastern Asian species. This result seems to support the viewpoint of Wu et al. (2003) that the ancestor of North American *Aletris* might be from Asian *Aletris*.

Clade B comprises three subclades. In subclade I, seven samples of *A. pauciflora* var. *khasiana* form a clade being sister to *A. megalantha*, and four samples of *A. pauciflora* var. *pauciflora* form a clade being sister to *A. glandulifera*. Both *A. pauciflora* var. *khasiana* and *A. pauciflora* var. *pauciflora* are monophyletic, but they are not closely related to each other (Figs. 1–3). Although these two varieties share some morphological characters (Wang et al., 1978; Liang & Turland, 2000), *A. pauciflora* var. *khasiana* markedly differs from *A. pauciflora* var. *pauciflora* in having stout scape (vs. slender scape) and many-flowered raceme (vs. fewer-flowered). Therefore, *A. pauciflora* var. *khasiana* may deserve a specific rank. *Aletris gracilipes* was once treated as a synonym of *A. laxiflora* (Liang & Turland, 2000). However, our analyses indicate that the three samples of *A. laxiflora* form a clade with strong support (subclade II: MP BS = 94%, ML BS = 94%, PP = 100%), whereas *A. gracilipes* falls in subclade III (Figs. 1–3). *Aletris gracilipes* has pedicels 6–10-mm long with bracts and bracteoles basally, but *A. laxiflora* has pedicels less than 5 mm with bracts and bracteoles distally (rarely at middle). In addition, bracts of *A. gracilipes* are obviously shorter than pedicels, whereas bracts of *A. laxiflora* are longer than pedicels. Thus, the specific status of *A. gracilipes* should be reinstated.

Subclade III covers nearly 50% of *Aletris* species, distributed through southern Japan to southwestern China. The resolution of the subclade is poor. More samples and DNA markers are needed to construct a robust phylogeny for the subclade. Even so, the relationships among several species are worthy of discussion. Liang & Turland (2000) remarked that *A. stenoloba* and *A. spicata* were so similar that they might be easily confused. In the present study, six samples representing these two species have almost uniform *matK* and *trnL-F* sequences. *Aletris spicata* and *A. stenoloba* might thus be conspecific. *Aletris alpestris* and *A. yaanica* G. H. Yang are highly similar morphologically, and were once thought to be conspecific by the present authors. However, they are not closely related in our phylogenetic trees (Figs. 1–3).

3.3 Evolution of perianth characters in *Aletris*

Traditionally, the characters of perianth were regarded as the most important in the taxonomy of *Aletris* (Wang et al., 1978; Liang & Turland, 2000; Sullivan,

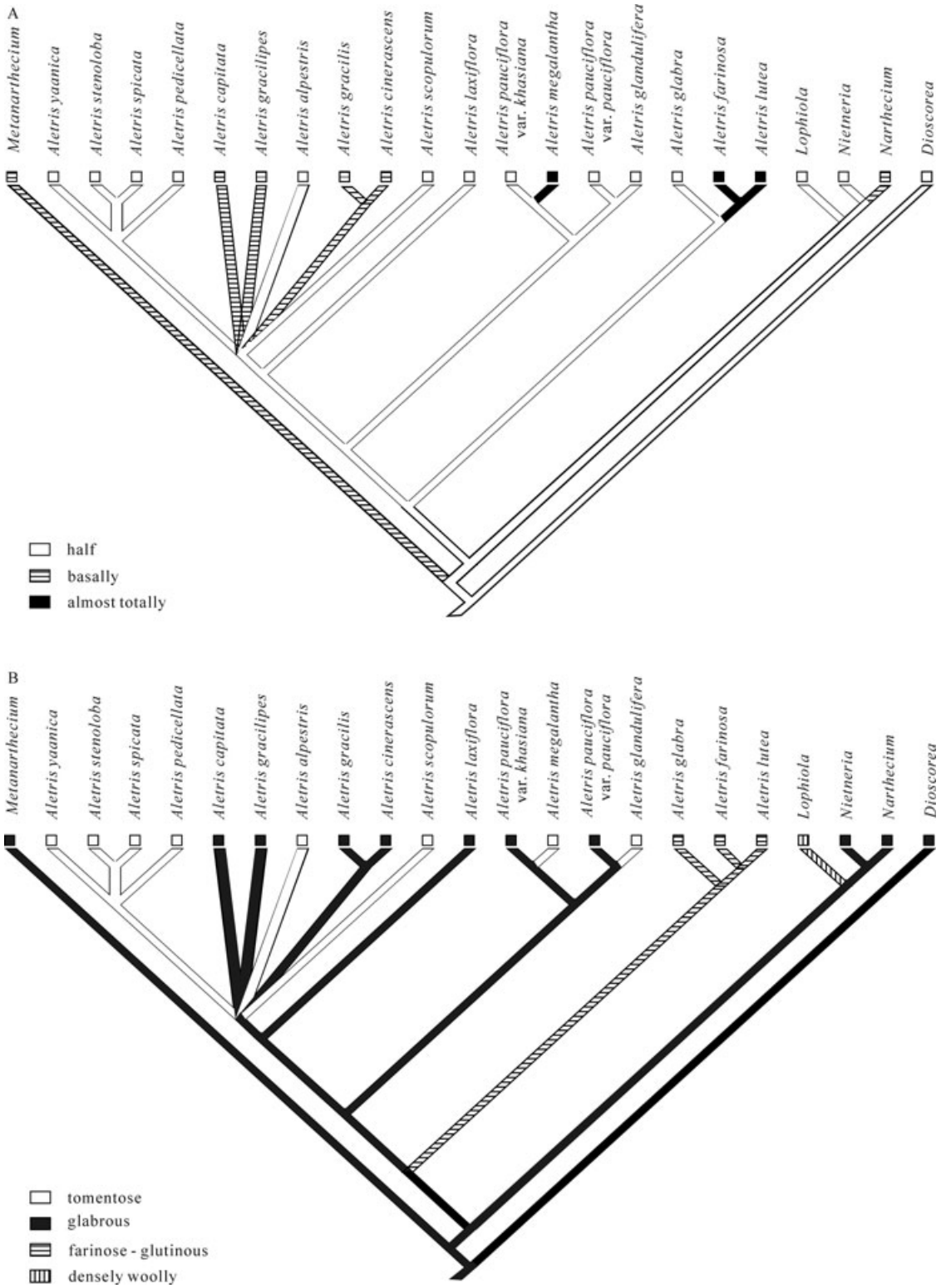

Fig. 4. Parsimony inference of perianth evolution of *Aletris* using MacClade. **A,** Connate degree of perianth. **B,** Abaxial surface of perianth.

2002). In *Aletris*, the perianth is connate basally in various degrees forming a perianth tube, and distally 6-lobed. The evolutionary reconstruction of connate degree of perianths suggests that the perianth with the lobes nearly equal to the tube in length (perianth connate halfway) might be ancestral in *Aletris*, and the perianth with short (perianth almost totally connate) or long lobes (perianth basally connate) might be derived (Fig. 4: A). The long-tubed perianth has evolved independently at least twice, *A. megalantha* of China and *A. farinosa* and *A. lutea* of eastern North America. In fact, all the five species in North America have long perianth tubes with lobes no longer than 0.2 × tube length (Sullivan, 1973; Zomlefer, 1997; Weigant, 2002). The lobes of *A. megalantha* of China are 0.3–0.5 × tube length, whereas the remaining species of eastern Asia show a series of variations in the length of lobes (approximately 0.6–5 × tube length).

Within *Aletris*, the abaxial surface of perianth is glabrous, tomentose, or farinose-glutinous. The reconstruction of the evolution of the abaxial perianth surface character suggests that glabrous surface might be ancestral for *Aletris* as well as for Nartheciaceae (Fig. 4: B). Tomentose perianth has evolved more than twice in *Aletris*. Farinose-glutinous perianth is a diagnostic character for clade A, which includes *A. glabra* of eastern Asia and *A. lutea* and *A. farinosa* of eastern North America.

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Appendix I

Voucher data for taxa sampled for molecular analyses and GenBank accession numbers in the following sequence: species sampled, locality, voucher, GenBank accession numbers for *matK, trnL*, and *trnL-F*.

Nartheciaceae: *Aletris alpestris* Diels, China, Sichuan, Zhang SR 305 (PE), JN086355, JN086386, JN086424; *Aletris alpestris* Diels, China, Sichuan, Zhao YM 196 (PE), JN086366, JN086394, JN086432; *Aletris capitata* F. T. Wang & T. Tang, China, Sichuan, Zhao YM 181 (PE), JN086365, JN086395, JN086433; *Aletris cinerascens* F. T. Wang & T. Tang, China, Guangxi, Zhang SR 336 (PE), JN086369, JN086389, JN086427; *Aletris cinerascens* F. T. Wang & T. Tang, China, Yunnan, Zhao YM 236 (PE), JN086374, JN086415, JN086453; *Aletris farinosa* L., RBG Kew DNA Bank (MWC105), Chase 105 (NCU), no *matK* available, EU186254, EU186242; *Aletris glabra* Bureau & Franch., China, Gansu, Zhang SR 499 (PE), JN086340, JN086396, JN086434; *Aletris glabra* Bureau & Franch., China, Sichuan, Zhang SR 316 (PE), JN086356, JN086385, JN086423; *Aletris glabra* Bureau & Franch., China, Sichuan, Zhao YM 163 (PE), JN086359, JN086411, JN086449; *Aletris glabra* Bureau & Franch., China, Xizang, Zhang SR 470 (PE), JN086341, JN086380, JN086418; *Aletris glabra* Bureau & Franch., China, Yunnan, Zhao YM 150 (PE), JN086377, JN086409, JN086447; *Aletris glandulifera* Bureau & Franch., China, Sichuan, Zhang SR 496 (PE), JN086346, JN086381, JN086419; *Aletris gracilipes* F. T. Wang & T. Tang, China, Sichuan, Zhang SR 555 (PE), JN086368, JN086390, JN086428; *Aletris gracilis* Rendle, China, Xizang, Zhang SR 359 (PE), JN086372, JN086413, JN086451; *Aletris laxiflora* Bureau & Franch., China, Sichuan, Zhang SR 321 (PE), JN086354, JN086387, JN086425; *Aletris laxiflora* Bureau & Franch., China, Sichuan, Zhang SR 497 (PE), JN086342, JN086384, JN086422; *Aletris laxiflora* Bureau & Franch., China, Sichuan, Zhao YM 184 (PE), JN086370, JN086397, JN086435; *Aletris lutea* Small, USA, Florida, W. B. Zomlefer 718 (Herbarium of the

Botanical Gardens, Osaka City University), AB040173, J. Anderson 35 (LV), EU186256, EU186243; *Aletris megalantha* F. T. Wang & T. Tang, China, Yunnan, Zhao YM 225 (PE), JN086373, JN086414, JN086452; *Aletris pauciflora* (Klotzsch) Hand.-Mazz. var. *pauciflora* (Klotzsch) Hand.-Mazz., China, Yunnan, Zhang SR 272 (PE), JN086349, JN086406, JN086444; *Aletris pauciflora* (Klotzsch) Hand.-Mazz. var. *pauciflora* (Klotzsch) Hand.-Mazz., China, Yunnan, Zhang SR 290 (PE), JN086357, JN086407, JN086445; *Aletris pauciflora* (Klotzsch) Hand.-Mazz. var. *pauciflora* (Klotzsch) Hand.-Mazz., China, Yunnan, Zhang SR 297 (PE), JN086350, JN086405, JN086443; *Aletris pauciflora* (Klotzsch) Hand.-Mazz. var. *pauciflora* (Klotzsch) Hand.-Mazz., China, Yunnan, Zhao YM 123 (PE), JN086345, JN086412, JN086450; *Aletris pauciflora* (Klotzsch) Hand.-Mazz. var. *khasiana* (Hook. f.) F. T. Wang & T. Tang, China, Sichuan, Zhang SR 492 (PE), JN086344, JN086382, JN086420; *Aletris pauciflora* (Klotzsch) Hand.-Mazz. var. *khasiana* (Hook. f.) F. T. Wang & T. Tang, China, Xizang, Zhang SR 482 (PE), JN086343, JN086383, JN086421; *Aletris pauciflora* (Klotzsch) Hand.-Mazz. var. *khasiana* (Hook. f.) F. T. Wang & T. Tang, China, Yunnan, Zhang SR 291 (PE), JN086351, JN086404, JN086442; *Aletris pauciflora* (Klotzsch) Hand.-Mazz. var. *khasiana* (Hook. f.) F. T. Wang & T. Tang, China, Yunnan, Zhao YM 14 (PE), JN086361, JN086402, JN086440; *Aletris pauciflora* (Klotzsch) Hand.-Mazz. var. *khasiana* (Hook. f.) F. T. Wang & T. Tang, China, Yunnan, Zhao YM 88 (PE), JN086358, JN086401, JN086439; *Aletris pauciflora* (Klotzsch) Hand.-Mazz. var. *khasiana* (Hook. f.) F. T. Wang & T. Tang, China, Yunnan, Zhao YM 92 (PE), JN086371, JN086410, JN086448; *Aletris pauciflora* (Klotzsch) Hand.-Mazz. var. *khasiana* (Hook. f.) F. T. Wang & T. Tang, China, Yunnan, Zhao YM 121 (PE), JN086363, JN086399, JN086437; *Aletris pedicellata* F. T. Wang & T. Tang, China, Guizhou, Zhao YM 256 (PE), JN086375, JN086416, JN086454; *Aletris pedicellata* F. T. Wang & T. Tang, China, Sichuan, Zhao YM 211 (PE), JN086347, JN086393, JN086431; *Aletris scopulorum* Dunn, China, Hunan, Luo ZC 3 (PE), JN086378, JN086408, JN086446; *Aletris spicata* (Thunb.) Franch., China, Guangxi, Zhang SR 349 (PE), JN086353, JN086388, JN086426; *Aletris spicata* (Thunb.) Franch., China, Yunnan, Zhao YM 117 (PE), JN086362, JN086400, JN086438; *Aletris stenoloba* Franch., China, Sichuan, Zhao YM 210 (PE), JN086367, JN086392, JN086430; *Aletris stenoloba* Franch., China, Yunnan, Zhao YM 11 (PE), JN086360, JN086403, JN086441; *Aletris stenoloba* Franch., China, Yunnan, Zhao YM 138 (PE), JN086364, JN086398, JN086436; *Aletris stenoloba* Franch., China, Yunnan, Zhao YM 212 (PE), JN086376, JN086417, JN086455; *Aletris yaanica* G. H. Yang, China, Sichuan, Zhang SR 542 (PE), JN086348, JN086391, JN086429; *Lophiola aurea* Ker Gawl., USA, Florida, W. B. Zomlefer 719 (Herbarium of the Botanical Gardens, Osaka City University), AB040176, R. Newell 23/8 (LV), EU186248, EU186236; *Metanarthecium luteoviride* Maxim., Japan, Pref. Shiga, M. N. Tamura, J. Yamashita, and S. Fuse 8009 (Herbarium of the Botanical Gardens, Osaka City University), AB040163, no *trnL* or *trnL-F* available; *Narthecium asiaticum* Maxim., Japan, Pref. Shiga, M. N. Tamura, J. Yamashita, and S. Fuse 8012 (Herbarium of the Botanical Gardens, Osaka City University), AB040162, no *trnL* or *trnL-F* available; *Narthecium ossifragum* Huds., Janeway & Castro 7607 (LV), no *matK* available, EU186251, EU186239; *Nietneria paniculata* Steyerm., O. Hokche & P. J. M. Maas 849 (U), no *matK* available, EU186250, EU186238.

Outgroup: Dioscoreaceae: *Dioscorea* sp., China, Sichuan, Zhang SR 494 (PE), JN086352, JN086379.