# Phylogenetic relationships and generic delimitation of Eurasian Aster (Asteraceae: Astereae) inferred from ITS, ETS and *trnL-F* sequence data

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Received: 18 September 2011 Returned for revision: 7 November 2011 Accepted: 13 February 2012 Published electronically: 19 April 2012

• *Background and Aims* The classification and phylogeny of Eurasian (EA) *Aster* (Asterinae, Astereae, Asteraceae) remain poorly resolved. Some taxonomists adopt a broad definition of EA *Aster*, whereas others favour a narrow generic concept. The present study aims to delimit EA *Aster sensu stricto* (*s.s.*), elucidate the phylogenetic relationships of EA *Aster s.s.* and segregate genera.

• *Methods* The internal and external transcribed spacers of nuclear ribosomal DNA and the plastid DNA *trnL-F* region were used to reconstruct the phylogeny of EA *Aster* through maximum parsimony and Bayesian analyses. • *Key Results* The analyses strongly support an *Aster* clade including the genera *Sheareria*, *Rhynchospermum*, *Kalimeris* (excluding *Kalimeris longipetiolata*), *Heteropappus*, *Miyamayomena*, *Turczaninowia*, *Rhinactinidia*, eastern Asian *Doellingeria*, *Asterothamnus* and *Arctogeron*. Many well-recognized species of Chinese *Aster s.s.* lie outside of the *Aster* clade.

• Conclusions The results reveal that EA Aster s.s. is both paraphyletic and polyphyletic. Sheareria, Rhynchospermum, Kalimeris (excluding K. longipetiolata), Heteropappus, Miyamayomena, Turczaninowia, Rhinactinidia, eastern Asian Doellingeria, Asterothamnus and Arctogeron should be included in Aster, whereas many species of Chinese Aster s.s. should be excluded. The recircumscribed Aster should be divided into two subgenera and nine sections. Kalimeris longipetiolata, Aster batangensis, A. ser. Albescentes, A. series Hersileoides, a two-species group composed of A. senecioides and A. fuscescens, and a six-species group including A. asteroides, should be elevated to generic level. With the Aster clade, they belong to the Australasian lineages. The generic status of Callistephus should be maintained. Whether Galatella (including Crinitina) and Tripolium should remain as genera or be merged into a single genus remains to be determined. In addition, the taxonomic status of A. auriculatus and the A. pycnophyllus–A. panduratus clade remains unresolved, and the systematic position of some segregates of EA Aster requires further study.

Key words: Asteraceae, Astereae, ETS, Eurasian Aster, generic delimitation, infrageneric classification, ITS, molecular phylogeny, *trnL-F*.

# INTRODUCTION

Aster sensu lato (s.l.; Asterinae, Astereae, Asteraceae) has been a taxonomic dumping ground for large numbers of morphologically similar but distantly related taxa (Noves and Rieseberg, 1999; Dorn, 2003). Aster s.l. occurs mainly in the Northern Hemisphere in both Eurasia (EA) and North America (NA) and is estimated to comprise 250-1000 species (Ling et al., 1985; Nesom, 1994b; Ito and Soejima, 1995; Noves and Rieseberg, 1999). Based primarily on achene morphology and cytology, Nesom (1994b) segregated NA Aster species from Aster s.l. and redistributed them among generic segregates Symphyotrichum, Doellingeria, Eucephalus, etc. At the same time, he kept the remainder, about 180 species, as Aster sensu stricto (s.s.), typified by A. amellus. Consequently, Aster, containing approx. 180 species, is restricted to the Northern Hemisphere of the Old World. Internal transcribed spacer (ITS) sequence phylogenetic data (Noyes and Rieseberg, 1999) support the viewpoint of Nesom (1994b) that a fundamental difference exists between NA and EA *Aster*. Furthermore, ITS data indicate that EA *Aster* is nested in the Southern Hemisphere grade and does not form a monophyletic group (Noyes and Rieseberg, 1999, Brouillet *et al.*, 2001, 2009b; Fiz *et al.*, 2002), and African *Aster* should be separated from *Aster s.s.* (Brouillet *et al.*, 2009b). The classification and phylogeny of EA *Aster* have remained poorly resolved, however, because of insufficient sampling in these studies.

The circumscription of EA Aster has confused botanists for several decades. Many taxonomists have adopted a broad definition of EA Aster. In Flora Europaea, Merxmüller et al. (1976) maintained Doellingeria, Galatella, Crinitaria (the name Crinitaria is a synonym of Galatella and species that are considered part of Crinitaria should be included in Crinitina) and Tripolium in Aster. Similarly, Grieson (1975) accepted Aster s.l. in Flora of Turkey and the East Aegean Islands because he did not recognize Kemulariella and Tripolium as segregate genera. In Flora of Japan, Ito and

© The Author 2012. Published by Oxford University Press on behalf of the Annals of Botany Company. All rights reserved. For Permissions, please email: journals.permissions@oup.com Soejima (1995) merged *Tripolium* as section *Tripolium* into *Aster*, placed *Heteropappus* in section *Pseudocalimeris*, included *Kalimeris* within section *Asteromoea*, and associated *Doellingeria* and *Miyamayomena* into section *Teretiachaenium*.

Other taxonomists have favoured a narrow generic concept of EA Aster and have recognized small genera endemic to eastern Asia. Tamamschyan (1959) segregated two new genera (Kemulariella and Conyzanthus) from Aster and recognized many small genera such as Doellingeria, Kalimeris, Asterothamnus, Krylovia, Turczaninowia, Galatella, Linosyris (= Crinitina) and Tripolium. Czerepanov (1995) followed Tamamschyan (1959) except that he placed Galatella and Linosyris under the genus name Crinitaria (= Crinitina). Nesom (1994a, b) made Aster largely equal to EA Aster s.s. and EA Aster s.l. almost equal to sub-tribe Asterinae Dumort.

Ling et al. (1985) treated Chinese Asterinae in the narrow sense of Aster, recognizing generic status for Gymnaster (= Miyamayomena), Kalimeris, Callistephus, Heteropappus, Doellingeria, Turczaninowia, Krylovia (= Rhinactinidia), Asterothamnus. Galatella. Linosvris (= Crinitina).Arctogeron and Tripolium. These treatments were followed completely for floras of Chinese provinces (e.g. Zhuang, 2004; Lin, 2007). Despite this, Chinese Aster s.s. remains a large genus with approx. 100 species, of which 75 are endemic to China (Fu, 1983; Ling et al., 1985; Chen, 1988, 1990; Zhu and Min, 1990; Li and Liu, 2002; Li and Zhang, 2004). Therefore, China, especially south-western China (the Qinghai-Tibetan and Yunnan-Guizhou Plateaux and Sichuan Province), is the diversity centre of Aster, as it is for many genera (Huang, 2011).

Molecular markers, especially ITS and the external transcribed spacer (ETS) of 35S ribosomal DNA, have frequently been used to investigate phylogenetic relationships in Astereae (e.g. Noyes and Rieseberg, 1999; Lowrey et al., 2001; Markos and Baldwin, 2001; Cross et al., 2002; Fiz et al., 2002; Roberts, 2002; Lowell et al., 2003; Urbatsch and Roberts, 2003; Urbatsch et al., 2003; Roberts and Urbatsch, 2004; Karaman, 2006; Selliah and Brouillet, 2008; Andrus et al., 2009; Brouillet et al., 2009a,b; Karaman-Castro and Urbatsch, 2009; Vaezi and Brouillet, 2009). Molecular evidence implies that neither EA Aster s.l. nor EA Aster s.s. is monophyletic (Gu et al., 1994; Ito et al., 1995, 1998; Xiang and Semple, 1996; Noves and Rieseberg, 1999; Fiz et al., 2002), but only a few species of EA Aster have been included in previous analyses. Although molecular data support a close relationship among Kalimeris, Heteropappus, Miyamayomena, Sheareria, Rhynchospermum and Aster s.s. (Ito et al., 1995, 1998; Noyes and Rieseberg, 1999; Fiz et al., 2002; Gao et al., 2009), the phylogenetic relationships among these genera are unresolved owing to limited taxon sampling of EA Aster s.s. Recently, 27 species of EA Aster s.l. were included in a phylogenetic analysis of Aster s.l. (Brouillet et al. 2009b), but no statistical support was presented for the clades of the ITS phylogenetic tree. To date, no molecular data have been provided for Turczaninowia, Krylovia, Asterothamnus and Arctogeron, and, in particular, Chinese Aster s.s. has not been phylogenetically studied using DNA sequences even though it represents the overwhelming

majority of EA *Aster s.s.* Thus, a reliable phylogenetic analysis based on extensive taxon sampling is essential to determine the inter- and intrageneric relationships of EA *Aster*.

Principally based on nuclear ribosomal DNA (nrDNA) ITS, ETS and plastid *trnL-F* sequence data of *Sheareria nana*, *Rhynchospermum verticillatum* and 62 species of EA *Aster s.l.*, the present study aims to (1) reconstruct the phylogeny of EA *Aster s.l.*; (2) redelimit the genus *Aster* and discuss its infrageneric classification; and (3) discuss the systematic position of EA *Aster* segregates.

# MATERIALS AND METHODS

Generic circumscriptions and nomenclature of Astereae follow Nesom and Robinson (2007) except for *Turczaninowia*, which follows Ling *et al.* (1985), and *Crinitina*, which is substituted for *Crinitaria* (a synonym of *Galatella*). The name *Aster setchuenensis* follows the International Plant Names Index. The division of phylogenetic lineages of Astereae refers to Brouillet *et al.* (2009*b*). Voucher DBY9206 was deposited in the Wenzhou University Herbarium (WZU) and the others in the Hunan Normal University Herbarium (HNNU; see the Appendix).

# Taxon sampling

Seventy-six species of Astereae and three outgroup species were collected from China and Bulgaria and examined for sequence variations in nrDNA ITS, ETS and plastid DNA trnL-F (GenBank accession numbers are given in the Appendix). The vouchers of all accessions were identified using published keys and compared with herbarium specimens in the Institute of Botany, Chinese Academy of Sciences Herbarium (PE), Northwest Agriculture and Forestry University Herbarium (WUK), Sichuan University Herbarium (SZ), Chengdu Institute of Biology Herbarium (CDBI), HNNU, Herbarium of Kunming Institute of Botany, the Chinese Academy of Sciences (KUN), the Herbarium of the South China Botanical Garden, Chinese Academy of Sciences (IBSC), Guangxi Institute of Botany Herbarium (IBK), Institute of Botany, Jiangsu Province and Chinese Academy of Sciences Herbarium Guizhou Academy of Sciences (NAS), Herbarium (HGAS), Central China Normal University Herbarium (CCNU), Wuhan Botanical Garden, Chinese Academy of Sciences Herbarium (HIB), Inner Mongolia University Herbarium (HIMC) and Fudan University Herbarium (FUS). Of the 76 species included in this study (see the Appendix), 41 represent three sections and 20 series of EA Aster s.s. (Ling et al., 1985; Chen, 1988; Li and Liu, 2002), 21 represent 12 segregate genera of EA Aster s.l., four generic groups of Nesom's (1994b) Asterinae, and two recently recognized close relatives of EA Aster s.s. (S. nana and R. verticillatum; Fiz et al., 2002; Brouillet et al. 2009b; Gao et al., 2009). The data matrix for ITS comprises 110 accessions from 48 genera and 110 species of tribe Astereae (see the Appendix). Seventy-six accessions were newly sequenced, and the remaining 34 were obtained from GenBank (Appendix). Of the 110 accessions, 41 species belong to EA Aster s.s., 21 species are 12 separate genera of EA Aster s.l., one is Astereae incertae sedis, three are members of

Bellidinae or Grangeinae, and 44 represent the six phylogenetic lineages of Astereae (Brouillet et al. 2009b). These phylogenetic lineages of Astereae are the early diverging lineages (e.g. Madagaster madagascariensis, Felicia filifolia and Printzia polifolia), the palaeo-South American clade (e.g. Chiliotrichum diffusum), the New Zealand clade (e.g. Olearia covenyi), the Australasian lineages, the South American lineages (e.g. Baccharis neglecta) and the NA lineage (e.g. Conyza sumatrensis and Symphyotrichum subulatum). Brouillet et al. (2009b) divided the Australasian lineages into seven genus or species groups, whereas ten genus or species groups are, in fact, included in the depiction of the grouping (fig. 37.1 C in Brouillet et al. 2009b). In the current analysis, 19 species (Appendix) were sampled to represent these ten groups. Because Brouillet et al. (2009b) consider Olearia s.s. to be a sister to EA Aster, five species were sampled to represent sub-clades of the *Olearia s.s.* clade.

In the combined matrix of ITS, ETS and trnL-F, 78 accessions from 25 genera and 78 species of tribe Astereae were included (Appendix). Seventy-six accessions were newly sequenced, and the remaining two were obtained from GenBank (Appendix). Of the 78 accessions (Appendix), 41 belong to EA Aster s.s., 21 belong to 12 segregate genera of EA Aster s.l., one is Astereae incertae sedis, and three are members of Bellidinae or Grangeinae. The remaining 12 accessions represent three phylogenetic lineages of tribe Astereae (Brouillet et al. 2009b), the palaeo-South American clade (e.g. C. diffusum), the Australasian lineages (e.g. two Myriactis spp.) and the NA lineage (seven species such as C. sumatrensis and S. subulatum). In all analyses, Chrysanthemum coronarium and Dendranthema indicum of tribe Anthemideae and Calendula officinalis of tribe Calenduleae were selected as outgroups for the rooting of the phylogenetic trees (Appendix) because in molecular phylogenetic analyses Anthemideae and Astereae are sisters, and Calenduleae is a sister to tribes Gnaphalieae, Anthemideae and Astereae (Panero and Funk, 2008; Garcia et al., 2010).

# DNA extraction, polymerase chain reaction (PCR) and sequencing

Total genomic DNA was isolated from fresh leaf material or silica gel-dried leaves using a modified cetyltrimethylammonium bromide procedure (Doyle and Doyle, 1987). Amplification and sequencing were performed using the primers ITS1 and ITS4 (White *et al.*, 1990) for the ITS region, Ast-8 (Markos and Baldwin, 2001) and 18S-ETS (Baldwin and Markos, 1998) for the ETS region, and c and f (Taberlet *et al.*, 1991) for the plastid DNA *trnL-F* region ( $trnL^{UAA}$ - $trnL^{UAA}$ - $trnF^{GAA}$ ).

The PCR mixture contained 1  $\mu$ L (50–100 ng) of sample DNA, 2 × 2  $\mu$ L of primer (10 pmol), 5  $\mu$ L of 10 × PCR buffer, 3  $\mu$ L of Mg<sup>2+</sup> (25 mM), 0.8  $\mu$ L of deoxyribonucleotide triphosphate (each 25 mM), 0.5  $\mu$ L of *Taq* DNA polymerase (5 U  $\mu$ L<sup>-1</sup>) and sterile water for a final volume of 50  $\mu$ L. The PCR parameters were as follows: initial denaturation for 4 min at 95 °C followed by 30 cycles of denaturation (95 °C, 1 min), annealing (56 °C, 40 s) and extension (72 °C, 1 min), and a final extension of 10 min at 72 °C.

PCR products were purified using a UNIQ-10 Spin Column PCR Product Purification Kit (Sangon Biotech Co., Ltd, Shanghai, China) following the manufacturer's instructions. Sequencing reactions were performed in both directions by Sangon Biotech Co., Ltd.

#### Sequence alignment and phylogenetic analysis

Boundaries of the ITS, ETS and trnL-F regions were determined through comparison with previously published sequences of tribe Astereae (Noyes and Rieseberg, 1999; Liu et al., 2002; Urbatsch et al., 2003). All DNA sequences were aligned initially using Clustal X1.83 (Jeanmougin et al., 1998) and then adjusted manually in BioEdit (Hall, 1999). The ITS region was analysed separately and in a combined data set with the ETS and *trnL-F* regions. The incongruence length difference test (Farris et al. 1994) was carried out to test the homogeneity between data sets using PAUP\* version 4.0b10 with 1000 replicates. Maximum parsimony (MP) and Bayesian inference (BI) methods were performed for the data sets using PAUP\* version 4.0b10 (Swofford, 2001) and MrBayes version  $3 \cdot 1 \cdot 2$ (Ronguist and Huelsenbeck, 2003), respectively. In the MP analysis, characters were equally weighted and treated as unordered, gaps were treated as missing data, and a heuristic search was implemented with 1000 random additional sequence replicates and sub-tree pruning-regrafting branch swapping. Bootstrap analyses based on 1000 replicates with ten random additions per replicate were used to estimate the confidence of the clades. The MaxTrees setting in PAUP\* was set to 5000 for the searches and bootstrap tests. For BI analysis of the ITS region and combined data set, the best-fitting model of each sequence partition (ITS1, ITS2, 5.8S, ETS, trnL-F intron, exon, the internal guide sequence) was determined using MrModeltest 2.2 (Nylander, 2004). The SYM + G model was chosen for the 5.8S region, and the GTR + I + G model for the ITS1, ITS2 and ETS regions. The GTR + G model was chosen for the intron and the internal guide sequence partitions of the *trnL-F* region and the K80 model for the exon partition. The Markov chain Monte Carlo algorithm was run for 1 000 000 generations, resulting in an overall sampling of 10 000 trees. The first 3000 trees were discarded as a conservation burn-in, and the remaining trees were used to construct the 50 % majority rule consensus tree.

### RESULTS

#### Characterization of nucleotide data

The aligned ITS sequence matrix of 110 taxa contained 689 base pairs, of which 394 were variable and 315 were potentially parsimony informative. Pair-wise distance within ingroup varied from 0 to 18.7 % (average = 6.7 %). The incongruence length difference test indicated that the data sets were not significantly heterogeneous (P = 0.01). Therefore, a combined analysis of the three regions was performed using PAUP\* and MrBayes. The combined data set of 78 taxa consisted of 2313 positions, with 641 potentially parsimony-informative characters and 283 phylogenetically uninformative variable



FIG. 1. The 50 % majority rule consensus tree from the Bayesian analysis of nuclear ribosomal DNA internal transcribed spacer sequences. (A) Bayesian posterior probabilities ( $\geq$ 0.89) and bootstrap values ( $\geq$ 50 %) are indicated above the branches; '-' indicates that Bayesian posterior probabilities are <0.89 or bootstrap percentages are <50 %. Some clades are indicated by numbers below the branch. Abbreviations: *A., Aster; C., Chrysanthemum; S., Symphyotrichum.* Triangles, '*Kalimeris* group'; squares, *Doellingeria*; circles, '*Galatella* group'. (B) The *Aster* clade (continued part of A). Bayesian posterior probabilities are <50 %) are indicated above the branches; '-' indicates that Bayesian posterior probabilities ( $\geq$ 0.89) or bootstrap values ( $\geq$ 50 %) are indicated above the branches; '-' indicates that Bayesian posterior probabilities are <0.89 or bootstrap percentages are <50 %. Some clades are indicated above the branches; '-' indicates that Bayesian posterior probabilities are <0.89 or bootstrap percentages are <50 %. Some clades are indicated above the branches; '-' indicates that Bayesian posterior probabilities are <0.89 or bootstrap percentages are <50 %. Some clades are indicated by numbers below the branch. Abbreviations: *A., Aster; As., Asterothamnus; H., Heteropapus; M., Miyamayomena; R., Rhynchospermum.* See key for symbols. Some clades are indicated by numbers below the branch. Abbreviations of the lineages are identical to those given in the Appendix and are shown on the right side of the taxa. The labelled species are discussed in groups in the text.



#### Fig. 1. Continued

characters. Pairwise distance between sequences varied from 0.1 to 11.7% (average = 4.6%).

#### Phylogenetic analyses

For convenience, some clades were numbered (Figs 1A, B and 2). Phylogenetic analyses using ITS and combined data sets yielded generally consistent phylogenetic trees (Bayesian trees; see Figs 1A, B and 2), although the BI and MP analyses based on the combined data generated trees with higher bootstrap support (BS) and Bayesian posterior probability (PP), and some clades (e.g. 17 and 18; Fig. 2) of the combined tree were unresolved in the ITS trees (Fig. 1A). The Aster clade (clade 8; Figs 1 and 2) with A. amellus (the type species of Aster) was strongly supported (PP = 0.99 in Fig. 1; PP = 1.00 and BS = 98 in Fig. 2) by the ITS and combined data set analyses. Sheareria, Rhynchospermum and some EA Aster segregates such as Heteropappus, Kalimeris (excluding section Cordifolium), Miyamayomena, Turczaninowia, Rhinactinidia, Arctogeron, Asterothamnus and eastern Asian Doellingeria were deeply nested within the Aster clade (clade 8), whereas other segregates (e.g. *Callistephus*, Galatella, Crinitina, Tripolium and K. longipetiolata) and 17 species of Aster s.s. (e.g. A. nitidus, A. asteroides and A. panduratus) occurred in other clades and showed close (clade 7; Figs 1A and 2), remote (e.g. clade 18 in Fig. 1A; clade 22 in Fig. 2) or unresolved (e.g. clade 9-16; Fig. 1A) relationships with the Aster clade. Callistephus, K. longipetiolata, two *Myriactis* spp. and 15 *Aster* spp. formed a moderately supported clade (clade 18; PP = 100; Fig. 2) that was unresolved in the ITS tree (Fig. 1A). *Tripolium, Galatella* and *Crinitina* constituted a well-supported clade (clade 22: PP = 1.00, BS = 92 in Fig. 1A; PP = 1.00, BS = 95 in Fig. 2) sister to *Bellis perennis*, but this relationship was weakly supported (clade 23: PP = 0.89 in Fig. 1A; PP = 0.91, BS = 57 in Fig. 2). The monophyly of the NA clade (clade 20) was moderately to strongly supported in both phylogenetic analyses (PP = 0.95 in Fig. 1; PP = 1.00, BS = 100 in Fig. 2).

#### DISCUSSION

### Relationship between EA Aster and NA asters

In this study, the ITS and combined data set analyses (Figs 1 and 2) clearly indicate that the *Aster* clade (clade 8 in Figs 1A and 2) is strongly supported (PP = 0.99 in Fig. 1; PP = 1.00, BS = 98 in Fig. 2) in an unresolved Astereae polytomy (Fig. 1A) or is embedded within clade 19 which includes *Myriactis* (sub-tribe Lagenophorinae) of the Australian lineages (see Fig. 2), whereas NA Astereae forms a moderately to strongly supported clade (clade 20: PP = 0.95 in Fig. 1; PP = 1.00, BS = 100 in Fig. 2). Therefore, EA *Aster* has no close relationship to NA asters. These results support the viewpoint of Nesom (1994b) that a fundamental difference exists between NA and EA *Aster*; they do not support the opinion of Xiang and Semple (1996) that *Aster s.s.* comprises not only EA taxa but also the segregate genus *Eurybia* and that



FIG. 2. The 50 % majority rule consensus tree from the Bayesian analysis of the combined data set (nuclear ribosomal DNA internal and external transcribed spacer sequences and plastid genome DNA *trnL-F* sequences). Bayesian posterior probabilities ( $\geq$ 0.89) and bootstrap values ( $\geq$ 50 %) are indicated above the branches; '-' indicates that Bayesian posterior probabilities are <0.89 or bootstrap percentages are <50 %. Some clades are indicated by numbers below the branch. Abbreviations: *A., Aster, As., Asterothamnus; C., Chrysanthemum; H., Heteropappus; M., Miyamayomena; R., Rhynchospermum; S., Symphyotrichum.* Abbreviations of the lineages are identical to those given in the Appendix and are shown on the right side of the taxa. Some species are labelled with the symbols shown in Fig. 1.

EA *Aster* is derived from NA *Aster*. *Aster alpinus*, distributed in both EA and NA, is deeply nested within the EA *Aster* clade (clade 8; see Figs 1B and 2), which implies that this species originated in EA and dispersed to NA.

#### Relationship between EA Aster and Australasian lineages

According to Brouillet et al. (2009b), Australasian lineages are part of a large polytomy at the crown of Astereae. Although our data sets did not include a large sample of Australasian taxa, the ITS tree (Fig. 1) included 19 sampled species that represented the ten genus or species groups of Australasian lineages of Brouillet et al. (2009b). The ITS tree (Fig. 1A) showed that the Aster clade (clade 8) is a clade of the large polytomy of the crown of Astereae, but it does not group with any of the Australian (e.g. Olearia astroloba and Remva kauaiensis), Hawaiian (Tetramolopium humile) or Asian (Myriactis) species of the Australasian lineages. Brouillet et al. (2009b) have proposed that EA Aster s.s. was a sister to the Australasian Olearia s.s. and had Australasian ancestors. Three species (including the generic type Olearia tomentosa) of the Australasian Olearia s.s. (Brouillet et al. 2009b) constitute a clade (Fig. 1A) but not a sister to the Aster clade. The phylogenetic tree from the combined data set, which is more resolved, includes only a few species (Myriactis and Callistephus) of the Australasian lineages. In the combined tree (Fig. 2), clade 17 (PP = 0.99, BS = 54) with the Aster clade (clade 8) is a sister to clade 18 (PP =1.00) that includes Myriactis and Callistephus (representatives of the Australasian lineages) and clades 17 and 18 group further into clade 19 (PP = 0.95) which might correspond to the Australasian lineages. Therefore, EA Aster (clade 8; Fig. 2) and some of its segregates (clades 9, 10, 12-16, and Aster spp. of clade 11; Fig. 2) belong to the Australasian lineages. A more extensive taxon sampling of Australasian Astereae for an analysis of combined DNA sequences is needed to study the origin of both EA Aster and its segregates.

#### Status of the 'Rhynchospermum group'

According to Nesom (1994*a*) and Nesom and Robinson (2007), two monotypic genera, *Sheareria* and *Rhynchospermum*, belong to the *Rhynchospermum* group of sub-tribe Lagenophorinae. The present study shows that these genera are well nested within the *Aster* clade, however, and not closely related to each other (Figs 1B and 2).

Sheareria. Endemic to China, this was first placed in tribe Astereae and later transferred to tribe Heliantheae (Hoffmann, 1890). Chen (1979) recognized it as belonging to sub-tribe Milleriinae of Heliantheae. Robinson (1981) redelimited Heliantheae and considered *Sheareria* to be a member of Astereae. Li *et al.* (2008) provided micromorphological, anatomical and cytological evidence for moving the genus from Heliantheae to Astereae but did not determine its systematic position within tribe Astereae. Nesom (1994*a*) and Nesom and Robinson (2007) placed *Sheareria* in sub-tribe Lagenophorinae, but Nesom (1994*a*) doubted a natural alignment with Lagenophorinae. Gao *et al.* (2009) used an ITS data set to show that *Sheareria* formed a strongly supported clade with *Kalimeris integrifolia* and *A. amellus* rather than with *Myriactis humilis*, a species of Lagenophorinae, which implies that *Sheareria* should be transferred from Lagenophorinae to Asterinae. Both the ITS (Fig. 1B) and the combined (Fig. 2) trees show that *Sheareria* is well nested within the *Aster* clade. *Sheareria nana* differs noticeably from other species of the *Aster* clade owing to its somewhat reduced leaves (bract-like, linear) and assimilating branches, solitary head with only 5–8 florets, functionally staminate disc flowers and epappose and glabrous achenes. *Sheareria nana* forms a single-species subclade (clade 2) of clade 8 in all analyses (Figs 1B and 2), and it could be designated as a section of *Aster*.

Rhynchospermum. The monotypic genus Rhynchospermum is distributed in eastern and southeastern Asia (Nesom and Robinson, 2007). Ling et al. (1985) included the genus in subtribe Bellidinae, and Zhang and Bremer (1993) placed it into their 'Bellis group' with Bellis and Bellium, whereas Nesom (1994a) and Nesom and Robinson (2007) assigned it to the Rhynchospermum group of sub-tribe Lagenophorinae. A previous phylogenetic analysis of ITS (Fiz et al., 2002) suggested that Rhynchospermum is related to neither Bellis nor Myriactis (sub-tribe Lagenophorinae) but to A. amellus and K. integrifolia. Brouillet et al. (2009b) also showed Rhynchospermum nested within Aster s.s., which is supported by our ITS and combined data sets (see Figs 1B and 2). In the phylogenetic trees (Figs 1B and 2) R. verticillatum is nested within the Aster clade (clade 8) and belongs to a clade (PP = 0.94 in Fig. 1B; PP = 1.00 in Fig. 2) with three species of series Turbinati of section Aster (see the Appendix). Although Rhynchospermum has some unique characters, such as a caducous pappus and biseriate pistillate ray florets with a short ligule, our results (Figs 1B and 2) suggest that it should be merged in Aster s.s. and placed in series Turbinati.

# Status of the 'Kalimeris group'

Nesom (1994a, b, 2000) has suggested that the Kalimeris group is composed of five small genera: Boltonia, Callistephus, Heteropappus, Kalimeris and Miyamayomena. This arrangement is unsupported by previous reports and the present study.

Kalimeris. This is native to eastern Asia, and one of its diagnostic characters is short pappi. Its complex taxonomic history has been reviewed in detail by Gu and Hoch (1997). Kalimeris shares several floral and achene characters with the small NA genus Boltonia, which led Bentham (1861, 1873) to place Kalimeris in Boltonia as one of three sections. Tamamschyan (1959), Ling et al. (1985) and Nesom (1994b, 2000) retained Kalimeris as a segregate genus, however. Gu and Hoch (1997) made a detailed comparison of the achenes and pappi of Boltonia and Kalimeris and concluded that their similarity was rather superficial. Based on ITS data, Fiz et al. (2002) and Brouillet et al. (2009b) demonstrated that Kalimeris and Boltonia are in divergent clades. Our phylogenetic analyses show that Kalimeris and Boltonia belong to different, strongly supported clades (clades 8 and 20, respectively; Fig. 1), supporting the view that no close relationship exists between Kalimeris and Boltonia.

Kalimeris was sub-divided into two sections by Kitamura (1937): Kalimeris and Cordifolium. Section Cordifolium has cordiform leaves with long petioles, two or three series of subequal phyllaries and cylindrical achenes with 4-7 ribs. The section includes two species, K. miaueliana, endemic to Japan, and K. longipetiolata, endemic to China (Kitamura, 1937; Ling et al., 1985). Gu and Hoch (1997) excluded section Cordifolium from Kalimeris and left it as part of Aster, and Ito and Soejima (1995) merged the section within Aster section Aster, although restriction fragment length polymorphisms (RFLPs) of plastid DNA supported a close relationship between K. miqueliana and Doellingeria scaber (Ito 1995. 1998). Nesom (1993)al., transferred et K. longipetiolata to Doellingeria, as D. longipetiolata, but the present results show that it is related to neither NA Doellingeria species nor Asian Doellingeria species. In the ITS tree, K. longipetiolata occupies an unresolved position (clade 14 in Fig. 1B) within the big polytomy, and in the combined tree it belongs to a polytomy (clade 18 in Fig. 2) with two Myriactis spp. and many other species of the Australasian lineages. Kalimeris longipetiolata should be treated as a new monotypic genus and be placed with the Australasian lineages.

Kalimeris (excluding section Cordifolium) has been recognized as having a close relationship with EA Aster s.s. and Heteropappus according to morphological comparisons (Gu and Hoch, 1997), cytological studies (Huziwara, 1950; Tara, 1972, 1973), RFLPs of plastid DNA (Ito et al., 1995, 1998) and ITS data (Noyes and Rieseberg, 1999; Fiz et al., 2002; Brouillet et al. 2009b). The taxonomic status of Kalimeris remains to be determined, however (Gu and Hoch, 1997). In the two trees in our study, three Kalimeris spp. (excluding section Cordifolium) are well nested in the Aster clade and form a highly supported clade (PP = 1.00 and BS = 99 in Fig. 1B; PP = 1.00 and BS = 100 in Fig. 2). Kalimeris (excluding section Cordifolium) is characterized by laterally compressed achenes with short pappus bristles no longer than the length of the corolla tube (Gu and Hoch, 1997) and S-type chromosomes (Li, 2006). Thus, Kalimeris (excluding section Cordifolium) is monophyletic, and treating Kalimeris as series Kalimeris of Aster is reasonable.

In the phylogenetic trees (Figs 1 and 2) *Kalimeris* is nested in clade 1 with *A. ageratoides* and *Heteropappus*, whereas *Miyamayomena* belongs to clade 3 with *A. amellus*. Natural hybridizations between *Kalimeris* and *A. ovatus* (formerly *A. ageratoides* subsp. *ovatus*; Tara, 1972, 1989), between *Kalimeris* and *A. ageratoides* (Li, 2006), and between *Kalimeris* and *Heteropappus* (Tara, 1973) support a close relationship with the *A. ageratoides* complex and *Heteropappus*, as do morphological studies (Gu and Hoch, 1997). Hu (1967) transferred a few species of *Aster*, including *A. smithianus*, to *Kalimeris* based on their short pappi, whereas our analyses showed that *A. smithianus* is not closely related to *Kalimeris* (Figs 1 and 2).

Miyamayomena. This was separated from *Kalimeris* and initially named *Gymnaster* (Kitamura, 1937, 1982; Chen, 1986). It is characterized by a lack of pappi (Kitamura, 1937, 1982; Ling *et al.*, 1985; Chen, 1986). Although there are only five species (Chen, 1986), *Miyamayomena* is as variable morphologically

as the large genus Aster and may in fact be an artificial assemblage (Gu and Hoch, 1997). Ito and Soeiima (1995) treated M. savatieri, the generic type, as a species of Aster section Teretiachaenium which also includes A. scaber (= D. scaber). In the phylogenetic trees based on RFLPs of plastid DNA, two species of Miyamayomena did not form a clade: M. koraiensis was nested in the Aster clade, and M. savatieri was a sister to the Aster clade. Therefore, Miyamayomena could be polyphyletic (Ito et al., 1994, 1998). Our analyses (Figs 1B and 2) show that three Chinese Miyamayomena spp., M. piccolii, M. *simplex* and *M. angustifolius*, are nested within the *Aster* clade (clade 8) and should be merged into Aster. These species belong to different clades, implying that a lack of pappi is not a homologous synapomorphy and that *Miyamayomena* is not monophyletic. Mivamavomena angustifolius (clade 4) is sister to clade 5 (Figs 1B and 2) and might be designed as a section of Aster. Miyamayomena piccolii and M. simplex form a strongly to weakly supported clade (PP = 0.99 in Fig. 1B; PP = 1.00 and BS = 84 in Fig. 2) embedded within the A. amellus clade (clade 3 in Figs 1B and 2) and might be treated as a series of section Aster, whereas the taxonomic positions of Mivamavomena koraiensis and M. savatieri. endemic to Japan and North Korea, respectively, remain to be determined.

Heteropappus. In 1832 the genus Heteropappus was established and the type species, H. hispidus, was transferred from Aster (Lessing, 1832). Heteropappus includes approx. 30 species distributed in eastern and central Asia and the Himalayan region (Ling et al., 1985). The genus is characterized by its two series of sub-equal herbaceous phyllaries and dimorphic pappi (shorter on the ray achenes and longer on the disc achenes: Ling et al., 1985; Gu and Hoch, 1997). Some species such as *H. altaicus* have a monomorphic pappus, so Grierson (1964) redefined Heteropappus by the unequal corolla lobes of the disc florets. Zygomorphic disc florets are also found in some species of Aster and Kalimeris, however. RFLPs of plastid DNA show that *H. hispidus* is embedded in Aster (Ito et al., 1998), implying that Heteropappus should be included in Aster. Our analyses (Figs 1 and 2) also strongly support the placement of Heteropappus in Aster. The two sampled species, representing two sections, form a highly to weakly supported (PP = 1.00 and BS = 79in Fig. 1B; PP = 1.00 and BS = 100 in Fig. 2) sub-clade of the A. ageratoides clade (clade 1), which might indicate that Heteropappus should be treated as a series of section Ageratoides (corresponding to clade 1).

Callistephus. This is a monotypic genus native to China. Based on its double pappus and unique involucre (outer bracts foliaceous and innermost white scarious), it was distinguished from *Aster* in 1817 by Cassini (Ling *et al.*, 1985; Nesom, 2000). *Heteropappus hispidus* was placed in *Callistephus* by de Candolle as *Callistephus biennis* (Nesom, 2000), implying that *Callistephus* and *Heteropappus* might be related to each other. Zhang and Bremer (1993) suggested that *Callistephus*, *Gymnaster*, *Heteropappus* and *Kalimeris* are closely related to each other and to *Aster*. Nesom (1994b) thought that *Callistephus* is similar to some species of *Myriactis* (sub-tribe Lagenophorinae) in habit and tendency toward pappus reduction, but he placed *Callistephus* within the *Kalimeris* group given the similar morphology of leaves, receptacles, disc corollas, and papillate collecting appendages of the style branches, the arrangement of the capitulum and the tendency toward pappus reduction (Nesom, 1994*a*, *b*, 2000). Our analyses (Figs 1 and 2) reveal that *Callistephus* has no close relationships with the other four genera of the *Kalimeris* group or with *Myriactis*. In the combined tree (Fig. 2), *Callistephus* and *Myriactis* occur in the same polytomy (clade 18) that is part of the Australasian lineages, which is concordant with the result of Brouillet *et al.* (2009*b*) that placed *Callistephus* in the large Australasian polytomy. We suggest that *Callistephus* maintain its generic status.

# Status of Turczaninowia

Turczaninowia fastigiata is native to north-eastern Asia (Tamamschyan, 1959; Ling et al., 1985; Ito and Soejima, 1995) and is characterized by its dense vestiture and small heads (with flowers and fruits reduced correspondingly) in a compact capitulescence. Turczaninowia fastigiata was originally published as Aster fastigiatus in 1812 (Ling et al., 1985) and was segregated as the monotypic genus Turczaninowia by de Candolle in 1836 (Nesom, 1994b). Tamamschyan (1959), Ling et al. (1985) and Bremer (1994) followed de Candolle's treatment, whereas Nesom (1994b) and Nesom and Robinson (2007) supported the inclusion of the species in Aster, and Ito and Soejima (1995) placed this species in Aster section Aster. The ovarian sterility of some of the inner disc flowers of this species and the triangular collecting appendages of its style branches are considered hallmarks of a possible close relationship with Galatella (Ling et al., 1985; Nesom, 1994b). Our phylogenetic trees (Figs 1 and 2) suggest that T. fastigiata does not merit generic rank or have a close relationship to Galatella; rather it should be transferred to Aster section Aster.

# Status of Doellingeria

Nees established Doellingeria in 1832, typified by D. umbellata. Bentham (1873) advocated a conglomerated Aster and included Doellingeria within a larger Aster. Some botanists continued to recognize Doellingeria as a distinct genus, however. Its phylogenetic position is equivocal. Zhang and Bremer (1993) placed Doellingeria in the Aster group. Nesom classified it first in sub-tribe Solidagininae (Nesom, 1993), then in sub-tribe Symphyotrichinae (Nesom, 1994a) or in sub-tribe Asterinae (Nesom, 1994b), and recently as an unplaced genus of Astereae (Nesom and Robinson, 2007). Doellingeria includes 11 species, of which three are NA and eight are eastern Asian species (Nesom, 1993, 1994b). RFLPs of plastid DNA show that eastern Asian Doellingeria is embedded in Aster s.s. (Ito et al., 1994), and hybridization between eastern Asian Doellingeria and Aster has been reported (Saito et al., 2007), whereas ITS data support an earlybranching position of NA Doellingeria (represented by D. umbellatus) in the NA Astereae clade (Noves and Rieseberg, 1999; Brouillet et al., 2001). In our trees (Figs 1 and 2) NA Doellingeria belongs to the NA clade (clade 20; Fig. 1A), and eastern Asian Doellingeria (represented by

*Doellingeria scaber*) is embedded in clade 8 (the *Aster* clade; Figs 1B and 2), which implies that *Doellingeria* is biphyletic and that eastern Asian *Doellingeria* should be moved from *Doellingeria* (which is typified by NA *D. umbellatus*) to *Aster*. Ito and Soejima (1995) placed eastern Asian *Doellingeria* and *Miyamayomena* together in *Aster* section *Teretiachaenium*. Our analyses (Figs 1B and 2) show that eastern Asian *Doellingeria* and *Miyamayomena* belong to different sub-clades (clades 6 and 7, respectively) of the *Aster* clade (clade 8), however. In clade 7 (Figs 1B and 2) eastern *Asian Doellingeria* is a sister to a clade with *Arctogeron*, *Asterothamnus* and three species of *Aster s.s.*, showing that it diverged early in *Aster* evolution and suggesting that eastern Asian *Doellingeria* should be treated as an independent section of *Aster*.

# Status of Aster segregates of the 'Asterothamnus group'

Nesom (1994a, b) set up an Asterothamnus group consisting of five small genera, Asterothamnus, Krylovia (= Rhinactinidia), Arctogeron, Kemulariella and Psychrogeton, of which the first four are segregates of Aster. The Asterothamnus group occurs primarily in central Asia and is characterized by a woody stem base, caespitose habit, sessile-glandular and tomentose stems and leaves, few or solitary heads and strongly coiling rays (Nesom, 1994b). Most of these features may be convergent characters resulting from adaptive modification under harsh environmental conditions (drought or cold), however. Our samples were limited to Asterothamnus, Rhinactinidia and Arctogeron (Appendix) because Kemulariella and Psychrogeton materials were unavailable.

Asterothamnus. This was segregated from Aster in 1950 by Novopokrovskiy, and its generic status has been accepted by several authors (Tamamschyan, 1959; Ling et al., 1985; Zhang and Bremer; 1993; Bremer, 1994; Nesom, 1994b; Czerepanov, 1995; Nesom and Robinson, 2007). The genus comprises seven species endemic to deserts and desert steppes in central Asia (Ling et al., 1985; Zhao, 1996). Asterothamnus has distinctive characters: it is a strongly branching sub-shrub with a woody rhizome, linear or narrowly elliptic leaves, densely or thinly tomentose stems and leaves and solitary or few heads in a loose corymb, reflecting adaptation to drought. In our phylogenetic trees (Figs 1B and 2) Asterothamnus belongs to the Aster clade (clade 8) and should be treated as a member of Aster. The two species sampled form a well-supported sub-clade (PP = 1.00 and BS = 100) that is nested in clade 7 with A. sikuensis, A. poliothamnus, A. falcifolius, Arctogeron and eastern Asian Doellingeria in both phylogenetic analyses (Figs 1B and 2). Asterothamnus is obviously different in morphology from the other members of clade 7 and should be regarded as a section of Aster.

Arctogeron gramineum. This is the only species of *Arctogeron* and is distributed in north-eastern China, Mongolia and eastern Russia. It occurs on dry mountain slopes or stony slopes and displays characters linked to drought adaptation such as low-growing and mat-forming habit and linear-subulate leaves. The species was originally described in

1753 by Linnaeus as a member of *Erigeron* and then established as a separate genus in 1836 by de Candolle and transferred to *Aster* in 1907 by Komarov (reviewed by Ling *et al.*, 1985). Like *Asterothamnus*, *Arctogeron* belongs to the *Aster* clade (clade 8; Figs 1B and 2) and should be treated as a member of *Aster*. It is well nested in clade 7 (Figs 1B and 2) and should be treated as a monotypic section of *Aster*.

Rhinactinidia. This is a genus of four species native to central Asia and Siberia (Ling et al., 1985; Czerepanov, 1995). It was established as a genus in 1831 by Lessing and was later included in Aster s.l. (Ling et al., 1985). Its generic status is currently generally accepted (Tamamschyan, 1959; Ling et al., 1985; Zhang and Bremer, 1993; Bremer, 1994; Nesom, 1994b; Czerepanov, 1995; Nesom and Robinson, 2007). Nesom (1994b) suggested that Asterothamnus and Krylovia (= Rhinactinidia) are closely related in terms of similarities such as keeled phyllaries, a coiling-reflexing disc corolla, and two-veined achenes with glandular surfaces. Rhinactinidia is considered different from Aster in its diagnostic characters and zygomorphic disc corollas (Ling et al., 1985), but these features can also be found in Aster s.s. Our study shows (Figs 1B and 2) that Rhinactinidia is well nested within the Aster clade, belongs to the A. amellus clade (clade 3) and has no close relationship with Asterothamnus. Two samples of Rhinactinidia form a wellsupported clade (PP = 1.00 and BS = 100 in Figs 1B and 2), and Rhinactinidia should be treated as a series of section Aster.

# Status of the 'Galatella group'

According to Nesom (1994a, b), the Galatella group of Asterinae s.s. includes three genera, Galatella (approx. 30 species), Crinitina (13 species) and Tripolium (a monotypic genus). These genera have been treated as three sections of Aster (Galatella, Linosyris and Tripolium, respectively) by some botanists but as segregate genera in other studies (reviewed by Ling et al., 1985; Nesom, 1994b). Furthermore, Nesom was indecisive about whether Galatella and Crinitina might belong in Solidagininae (Nesom, 1991) or whether they are more closely related to typical Aster (Nesom, 1994b). Based on ITS data, Fiz et al. (2002) and Brouillet et al. (2009b) found that Galatella and Crinitina form a well-supported clade, and a few studies have shown that Galatella or Crinitina are weakly related to Bellidinae rather than to Aster (Noyes and Rieseberg, 1999; Fiz et al., 2002; Karaman, 2006). Our phylogenetic analyses (Figs 1 and 2) show that Galatella, Crinitina and Tripolium constitute a well-supported clade (clade 22: PP = 1.00 and BS = 92 in Fig. 1A; PP = 1.00 and BS = 95 in Fig. 2). Furthermore, in the combined analysis (Fig. 2), Crinitina linosyris, Crinitina villosa and Galatella dahurica form a well-supported clade (clade 21: PP = 1.00, BS = 97), which would support the merger of Crinitina into Galatella. Whether Tripolium deserves generic status or whether the three genera should be merged into a single genus remains to be determined. If the latter is reasonable, the oldest name would have to be used for the genus, i.e. Galatella. In our analyses the Galatella-Crinitina-Tripolium clade (clade 22; Figs 1A and 2) is closely related to neither the Aster clade nor Solidago

*decurrens* (a representative of sub-tribe Solidagininae). Similarities between the *Galatella* group and *Aster* in leaves, disc style branches, achenes and heads (Nesom, 1994*b*) are superficial and have developed in parallel, and the *Galatella* group should be separated from *Aster*. The trees show a moderate to weak relationship (clade 23: PP = 0.89 in Fig. 1A; PP = 0.91 and BS = 57 in Fig. 2) between the three genera of the *Galatella* group and *Bellis*, which is consistent with the conclusions of Fiz *et al.* (2002). The systematic position of the *Galatella* group remains unresolved.

### Redelimitation of Aster

According to our data, all existing generic delimitations of Aster are problematic. The EA Aster as delimited by some botanists (e.g. Ling et al., 1985; Nesom, 1994b; Nesom and Robinson, 2007) is paraphyletic because it excludes some of the descendants of the most recent common ancestor. Therefore, monophyletic Aster should include such genera as Sheareria, Rhynchospermum, Kalimeris (excluding K. longipetiolata), Heteropappus, Mivamavomena, Rhinactinidia, Turczaninowia, Asterothamnus, Arctogeron and eastern Asian Doellingeria. Conversely, EA Aster as delimited by other botanists (e.g. Merxmüller et al., 1976; Ito and Soejima, 1995) is polyphyletic because it includes morphologically similar but distantly related taxa. Callistephus, Galatella, Crinitina and Tripolium should be excluded from Aster. The Aster clade (clade 8) is strongly supported in both the ITS tree (PP = 0.99; Fig. 1B) and the combined tree (PP = 1.00, BS = 98; Fig. 2), so the Aster clade is the recircumscribed genus Aster. Molecular data (Figs 1 and 2) revealed, however, that many Chinese Aster spp. should be excluded from Aster, although their status as Aster species, except for series Albescentes, has not been doubted. Of 41 sampled species of Aster s.s. (Ling et al., 1985; Chen, 1988; Ito and Soejima, 1995; Li and Liu, 2002), 17 should be removed from the genus.

Series Hersileoides (Aster section Orthomeris, sensu Ling et al., 1985) is endemic to western China and consists of two restricted species, A. hersileoides and A. nitidus (Ling et al., 1985; Yin et al., 2010). They are characterized by a shrubby habit, solitary capitula at the apex of branches, membranous receptacular bracts and a short outer pappus. A karyotypic study of these species (Yin et al., 2010) showed that they are diploid and have shorter chromosomes and higher asymmetry of karyotype than that with A. ageratoides. Our study demonstrates that the series is a well-supported monophyletic group (clade 9: PP = 0.99 and BS = 98 in Fig. 1A; PP =1.00 and BS = 100 in Fig. 2). Although the systematic position of the series has never been questioned, the ITS phylogenetic tree (Fig. 1A) shows that clade 9, series Hersileoides, is not closely related to clade 8, the Aster clade, and in the combined tree (Fig. 2) the sister relationship between clades 8 and 9 is only weakly supported (BS = 54), even though the Bayesian PP is high (0.99). Therefore, the series should be removed from Aster, and it might be reasonable to elevate the series to a generic level in sub-tribe Asterinae.

Aster albescens var. albescens, A. argyropholis and A. lavanduliifolius are representative of series Albescentes. Western China is the centre of diversity of this series, with six of the seven species being endemic to the region (the

exception being A. albescens which is distributed from western China to the southern Himalavas: Ling et al., 1985; Chen, 1988). Ling et al. (1985) established the series and placed it within Aster section Orthomeris. The series differs from others in the section with its shrubby habit, pinnate primary lateral leaf veins, relatively small heads, small rays and fourto six-veined, sub-cylindric achenes. Our studies (Figs 1A and 2) demonstrate that series Albescentes is a well-supported monophyletic taxon (clade 12: PP = 1.00 and BS = 98 in Fig. 1A: PP = 1.00 and BS = 100 in Fig. 2) and should be removed from Aster. Nesom (1994b) suggested that series Albescentes is closely related to the NA group, in which it would be positioned near NA Doellingeria. The present results provide no evidence to support this relationship, however. On the contrary, series Albescentes occurs in a polytomy (clade 18: PP = 1.00 in Fig. 2) with *Myriactis* and other segregates of Aster s.s., implying that series Albescentes may belong to the Australasian lineages rather than to the NA clade (clade 20; Fig. 2). In the ITS analysis the series occurs at an unresolved position within a polytomy (Fig. 1A) in Astereae. Its systematics requires further investigation; however, series Albescentes should undoubtedly be removed from Aster and be considered for generic rank.

According to Ling *et al.* (1985), *A. auriculatus* and *A. panduratus* belong to section *Aster* series *Auriculati*, and *Aster pycnophyllus* belongs to section *Orthomeris* series *Sikkimenses*. In the trees (Figs 1A and 2), the three species are well nested in a clade with *Myriactis* (clade 11: PP = 1.00 and BS = 97 in Fig.1A; PP = 1.00 and BS = 100 Fig. 2) and distantly related to *Aster*, suggesting that they should be removed from *Aster*. Although *Myriactis* is quite different from these three species with its two- to multiple-seriate ray florets, male disc florets and glandular collar, they do not form a sub-clade sister to *Myriactis*. Therefore, the relationships among the three species and *Myriactis* require further study.

Of the 15 sampled species of Aster section Alpigenia (Appendix), seven fall in the Aster clade, and the other eight fall outside it (Figs 1A and 2). Of these eight species, six (A. asteroides, A. brachytrichus, A. diplostephioides, A. flaccidus, A. setchuenensis and A. yunnanensis) form a wellsupported clade (clade 10: PP = 0.99 in Fig. 1A; PP = 1.00and BS = 98 in Fig. 2), implying that these species might become a new genus. The systematic position of this group is unresolved, however. In the ITS tree (Fig. 1A) clade 10 falls within a big polytomy, and in the combined tree (Fig. 2) it belongs to clade 18, a polytomy, with Myriactis. Aster senecioides, the sole member of a monotypic series of section Alpigenia, forms a strongly supported clade (clade 15: PP = 1.00 and BS = 93 in Fig. 1; PP = 1.00 and BS =100 in Fig. 2) with A. fuscescens, also the sole member of a monotypic series of section Aster (Ling et al., 1985). These two species are at an unresolved position within the big polytomy in the ITS tree (Fig. 1A) and belong to a polytomy (clade 18; Fig. 2) in the combined tree. Clade 15 might be treated as a separate genus. Similarly, in the ITS tree (Fig. 1A), A. batangensis (clade 16) occupies an unresolved position within the big polytomy of EA Astereae, and, in the combined tree (Fig. 2), clade 16 belongs to clade 18. Our phylogenetic trees (Figs. 1A and 2) show that A. batangensis seems to deserve the status of a monotypic genus. Thus, A. series

*Hersileoides*, *A*. series *Albescentes*, a six-species group including *A. asteroides*, a group composed of *A. senecioides* and *A. fuscescens*, and *A. batangensis* should be elevated to generic level, and, together with the *Aster* clade, placed with the Australasian lineages.

Nesom (2000) stated that Aster, even in its more restricted morphological definition, still encompasses a great deal of variation, and the description remains correspondingly general. Herein, Aster is expanded to include some segregates of Aster s.l. and other genera, making Aster more complex in some morphological features. For example, treating Sheareria as a section of Aster adds to Aster some new characters such as bract-like leaves, assimilating branches, small heads with only 5-8 florets, and functionally staminate disc flowers. Arctogeron brings to Aster such new features as caespitose herbs, narrow grass-like leaves with a scabrous ciliate margin and densely silvery pubescent cypselas. The high morphological diversity implies that Aster has undergone an evolutionary radiation since it originated. Aster displays a broad morphological variability in pappi (e.g. pappi are one- to four-seriate or absent, short or long, persistent or caducous) that, as mentioned above, has been used as a diagnostic character in delimiting some genera. Pappi are absent in clades 2 and 4 and in the M. piccolii-M. simplex clade of clade 3 (Figs 1B and 2), which implies that the disappearance of a pappus has evolved independently at least three times in Aster. Kalimeris, A. smithianus, A. dolichopodus and A. souliei share reduced pappi but occur in different sub-clades (Figs 1B and 2), suggesting convergent evolution toward pappus shortening. Dimorphic pappi (different lengths of pappi between ray and disc florets) are a diagnostic feature of Heteropappus, but dimorphic pappi are also found in A. homochlamydeus (W.-P. Li, unpubl. res.), which is another example of convergent evolution of pappi. Furthermore, no evolutionary relationships occur among dimorphic pappi, short pappi and absent pappi, i.e. no evolutionary series from dimorphic pappi to epappi exists. According to Ling et al. (1985), series Turbinati is characterized by four- to sevenseriate phyllaries, whereas our phylogenetic trees (Figs 1 and 2) show that three species (A. turbinatus, A. baccharoides and A. sampsonii), A. jishouensis (series Turbinati), with multiseriate phyllaries, and R. verticillatum, with two- to threeseriate phyllaries, form a clade, and this clade is not closely related to another species with multiseriate phyllaries, A. sampsonii. Therefore, the multiseriate phyllaries feature has arisen more than once in Aster. Noticeably, six species in one sub-clade of clade 7 (Figs 1B and 2) share a more or less shrubby habit, and clade 9 (sister to clade 8 in Fig. 2) is also characterized by a shrubby habit, which might mean that shrubby habit may represent a symplesiomorphy in clades 8 and 9. Whether EA Aster, with predominantly herbaceous perennials, originated from a woody ancestor is worth considering. Nonetheless, in clade 6 (Figs. 1B and 2), the shrubby habit of A. baccharoides and A. smithianus seems to be a convergence because these species occur within clades 1 and 3, respectively (Figs 1B and 2), and are not closely related to each other, and each of them is the only shrub in its clade. The morphology of Aster is so complex that further tracing of important morphological characters in the phylogenetic trees is necessary to reveal their phylogenetic significance.

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# Infrageneric classification of Aster

After extensive changes in the generic delimitation of Aster, its infrageneric systematics should be reconstructed. Three infrageneric taxonomic schemes of EA Aster s.s. have been described. First, Ling et al. (1985) divided Chinese Aster s.s. into three sections [Aster, Orthomeris (a name based on an NA type in genus Oclemena) and Alpigenia] and 27 series. Next, Ito and Soejima (1995) recognized five sections of Japanese Aster: Tripolium (a monotypic section), *Pseudocalimeris* (largely equal to the genus *Heteropappus*). Teretiachaenium (including the taxa of Miyamayomena and eastern Asian Doellingeria), Asteromoea (similar to Kalimeris) and Aster (largely equal to Aster s.s.). Finally, Nesom (1994b) divided Aster into four sections and taxa incertae sedis. The former includes sections Aster, Alpigeni (including sub-sections Homochaeta, Heterochaeta and Senecioides), Ageratoides and Calimeridei, and the latter is a six-species group. The current study supports none of these taxonomic systems, however.

We suggest clade 8 (Figs 1B and 2) as the genus Aster and clades 6 and 7 (Figs 1 and 2) as two subgenera of Aster. In clade 6, each of four sub-clades (clades 1-4; Figs 1B and 2) could be treated as a section. As mentioned above, M. angustifolius (clade 4; Figs 1B and 2) and S. nana (clade 2; Figs 1B and 2) may be treated as monotypic sections. Clade 1 is well supported in all analyses (PP = 1.00 and BS = 99 in Fig. 1B; PP = 1.00 and PP = 100 in Fig. 2) and could be regarded as section Ageratoides, typified by A. ageratoides (Nesom, 1994b). Section Ageratoides includes all taxa of section Pseudocalimeris and section Asteromoea and some members of section Teretiachaenium and section Aster (sensu Ito and Soejima, 1995); it corresponds more or less to sections Ageratoides of Nesom (1994b) and Orthomeris of Ling et al. (1985). Clade 3 is well supported only by BI (PP = 0.96 in Fig. 1B; PP = 1.00 in Fig. 2) but not by MP analysis. It might be treated as section Aster, typified by A. amellus. Although all previous schemes have recognized section Aster, their circumscriptions differ from ours. Some members (e.g. A. dolichopodus, A. mangshanensis, A. smithianus and A. vestitus) of section Aster of Ling et al. (1985) are nested in clade 1 (section Ageratoides) rather than in clade 3 (section Aster), and some members of section Orthomeris (e.g. A. sampsonii, A. turbinatus, A. baccharoides and A. jishouensis; Ling et al., 1985; Li and Liu, 2002) are nested in clade 3 rather than in clade 1. In fact, Nesom (1994b) agreed with Ling et al. (1985) in the circumscription of section Aster. As mentioned above, in Flora of Japan (Ito and Soejima, 1995) section Aster has a much wider definition than ours. Some species of section Alpigenia in the classifications of Ling et al. (1985) and Nesom (1994b) belong to clade 1 (section Ageratoides) or clade 3 (section Aster), and the others occur outside of the Aster clade, suggesting that section Alpigenia should be abandoned.

Clade 7, the other sub-clade of clade 8, is moderately to well supported (PP = 0.99 and BS = 79 in Fig. 1B; PP = 1.00 and BS = 92 in Fig. 2) and could be treated as the other subgenus of *Aster*. The subgenus consists of three segregates (eastern Asian *Doellingeria, Asterothamnus* and *Arctogeron*) of *Aster s.l.* and three species (*A. falcifolius, A. poliothamnus* and

A. sikuensis) of Aster s.s. As discussed above, eastern Asian Doellingeria, Asterothamnus and Arctogeron should be treated as three different sections. According to Ling et al. (1985), A. falcifolius is the only member of series Falcifolii of section Orthomeris, and A. poliothamnus and A. sikuensis belong to series Vestiti of section Aster. These three species have more or less woody stems that are similar to those of the other taxa of clade 7, Asterothamnus and Arctogeron. Aster falcifolius is characterized by solitary flowers and bracteole leaves that become denser until grading into phyllaries. It should be raised to the sectional level. Aster poliothamnus and A. sikuensis share some features, such as four- to fiveseriate phyllaries and the absence of rhizomes, and form a strongly to weakly supported clade (PP = 1.00, BS = 71; Fig. 2). These two species may deserve the status of a section. As a result, the subgenus (clade 7: Figs 1B and 2) would comprise five sections.

According to Ling *et al.* (1985), the recircumscribed *Aster* has seven series with two or more species included in our analyses. None of these is monophyletic, however. All three species of series *Vestiti* (*A. vestitus, A. poliothamnus* and *A. sikuensis*; Ling *et al.*, 1985) were sampled and occur in clades 6 and 7 (see Figs 1B and 2), and they should be placed in different subgenera. *Aster alpinus, A. handelii, A. heterolepis* and *A. oreophilus* are assigned to series *Alpigenia* (Ling *et al.*, 1985) but occur in four clades of section *Ageratoides* (clade 1; Figs 1B and 2) and section *Aster* (clade 3; Figs 1B and 2). Although *A. fanjingshanicus, A. tongolensis* and *A. souliei* of series *Tongolenses* (Ling *et al.*, 1985) belong to clade 1, they are not closely related to one another (Figs 1B and 2). All of the series (*sensu* Ling *et al.*, 1985) of *Aster* must be re-evaluated.

More than half the species of *Aster* (Tamamschyan, 1959; Grieson, 1975; Merxmüller and Schreiber, 1976; Ling *et al.*, 1985; Czerepanov, 1995; Ito and Soejima, 1995) are not included in our study; therefore, a more extensive taxon sampling of molecular sequence data is necessary for a full phylogenetic reconstruction of *Aster*. Because more than half of the sampled species of section *Alpigenia* (*sensu* Ling *et al.*, 1985) should be excluded from *Aster*, it is particularly important to collect molecular data for all the species. Because the combined analysis shows better resolution than that of the ITS phylogeny in *Aster s.l.*, the combined data for the Australasian lineages are needed to resolve the origin and systematic position of *Aster* and its segregates.

# ACKNOWLEDGEMENTS

We thank the referees for their useful comments, suggestions and questions; Professor De-Yuan Hong, Liang-Bi Chen and Qiner Yang for their generous concern and help; the management departments of many of China's National Nature Reserves; the faculty of the natural sciences departments at Shumen University; Professors Gong-Xi Chen and Dun-Yan Tan for field assistance; the curators of the PE, WUK, SZ, CDBI, HNNU, KUN, IBSC, IBK, NAS, HGAS, CCNU, HIB, HIMC and FUS herbaria for access to specimens; Professor Dong-Ping Li for kindly offering research facilities for our use; Professor Xiang Hu, Professor Ping Zhang, Ming Tang and Feng-Ming Qian for technical assistance; and Professor Chengqi Ao for providing the leaves of *M. angustifolius*. This study was financed by the National Natural Science Foundation of China (grant nos 30470131 and 39899400) and by the Scientific Research Fund of Hunan Provincial Education Department (grant no. 08A046).

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### APPENDIX

Present taxonomy*	Phylogenetic lineages and infrageneric classification of $Aster^{\dagger}$	Numbers, locations and altitudes of vouchers <sup>‡</sup>	GenBank accession number <sup>§</sup>		
			ITS	ETS	trnL-F
Unplaced taxa					
Doellingeria umbellata	NA	_	AF046966	NA	
Eurybia sibirica	NA	_	AY772421	AY772435	GU480699
Nannoglottis delavayi	BL	_	AY017167	NA	
Sub-tribe					
Homochrominae					
Felicia filifolia	BL	_	FJ457937	NA	
Sub-tribe					
Hinterhuberinae					
Celmisia mackaui	NZ	_	AF422115	NA	
Chiliotrichum diffusum	PSA	_	AF046945	DQ479128	AF452501

Taxa sampled, phylogenetic lineages, vouchers and GenBank accessions.

Present taxonomy*	Phylogenetic lineages and infrageneric classification of $Aster^{\dagger}$	Numbers, locations and altitudes of vouchers <sup>‡</sup>	GenBank accession number <sup>§</sup>		
			ITS	ETS	trnL-F
Madagaster madagascariensis	BL	-	DQ479031	NA	
Mairia hirsuta	BL	_	FJ457929	NA	
Olearia astroloba	AL	-	AF497646	NA	
Olearia ballii	AL	_	AF497662	NA	
Olearia calcarea	AL	_	AF497663	NA	
Olearia ciliata	AL	_	AF497667	NA	
Olearia cordata	AI	_	AF497668	NA	
Olearia covenni	NZ		ΔΕ/07711	NΔ	
Olearia rudis	ΔΙ		ΔΕ/07677	NΔ	
Olearia tomentosa			ΔΕ/07650	NΔ	
Oritrophium hieracioides	PSA	_	DO479116	NA	
Pleurophyllum hookeri	NZ		HQ479110	NΔ	
Printzia polifolia	BL	_	FI457027	NA	
Pteronia camphorata vor	BL	-	DO470118	NA	
camphorata	BL	_	DQ4/9118	INA	
Remya kauaiensis Sub-tribe Brachyscominae	AL		AF497684	NA	
Brachyscoma viaidula	A I		DO478004	NA	
Calotis hispidula	AL	-	AB106507	NA	
Sub triba Pallidinaa	AL	-	AD190397		
Pallis paramis	PE	IWD1002008; Changeha oultivated	IN215019	INI215042	INI215904
Sub triba Grangainaa	BE	LWF1005008, Changsha, cultivated	JIN313916	JIN313942	JIN313094
Grangea maderaspatana	GR	LWP0802034; Zhaoqing City,	JN315920	JN315944	JN315896
Dichrocephala auriculata Sub-tribe	GR	LWP0708234; Dali City, 2300 m	JN315919	JN315943	JN315895
Lagenophorinae					
Myriactis nepalensis	AL	LWP0509002; Kunming City, 2300 m	JN315921	JN315945	JN315897
Myriactis wightii	AL	LWP0509010; Kunming City, 2200 m	JN315922	JN315946	JN315898
Rhynchospermum verticillatum	AL	LWP0607065; Mt. Emei, 1200 m	JN543706	JN543707	JN543708
Sheareria nana Sub-tribe Baccharidinae	AL	LWP0701001; Changsha City, 30m	JN543703	JN543704	JN543705
Baccharis neglecta	SA	-	U97604	NA	
Camptaera gracilis	ΔI	_	AF247069	NΔ	
Kinnistia suaedifolia	AI	_	AF497660	NA	
Minuria integerrima			AF0/6057	NΔ	
Minuria macrorhiza			AF247076		
Tatramolonium humila	AL	-	DO470040	ΝA	
var. humile	AL	_	DQ479040	11/2	
Arctogeron gramingum	SEA	I WD0606014, Wyleshaota City	IN315029	IN315052	IN315004
Arciogeron gramineum	SEA	300 m	JIN313928	JIN313932	JIN313904
Aster	Eurasian Aster s.s.		115 105 10	77.75.405.40	D
Aster amellus	Section Aster series Amelli	LWP0408002; Shumen, Bulgaria, 400 m	JN543742	JN543743	JN543744
Aster maackii	Section Aster series Macrocephali	LWP0609043; Yichun City, 200 m	JN543745	JN543746	JN543747
Aster tataricus	Section Aster series Macrocephali	LWP0108018; Xinglong County, 400 m	JN543748	JN543749	JN543750
Aster fuscescens	Section Aster series Fuscescentes	YGS1007021; Gongshan County, 2500 m	JN543751	JN543752	JN543753
Aster auriculatus	Section Aster series Auriculati	LWP0509059; Yongsheng County, 2200 m	JN543754	JN543755	JN543756
Aster panduratus	Section Aster series Auriculati	LWP1012067; Guiyang City, 1100 m	JN543757	JN543758	JN543759
Aster mangshanensis	Section Aster series Auriculati	LWP0511034; Mt. Mang, 1670 m	JN543760	JN543761	JN543762
Aster poliothamnus	Section Aster series Vestiti	LWP0506001; Zhang County, 500 m	JN543763	JN543764	JN543765
Aster sikuensis	Section Aster series Vestiti	LWP0510025; Lueyang County, 300 m	JN543766	JN543767	JN543768

APPENDIX 1. Continued

Continued

Present taxonomy*	Phylogenetic lineages and infrageneric classification of $Aster^{\dagger}$	Numbers, locations and altitudes of vouchers <sup>‡</sup>	GenBank accession number <sup>§</sup>		
			ITS	ETS	trnL-F
Aster vestitus Aster taliangshanensis	Section Aster series Vestiti Section Aster series	LWP0509023; Lijiang City, 2610 m LWP0607056; Xichang City,	JN543769 JN543772	JN543770 JN543773	JN543771 JN543774
Aster dolichopodus	Section Aster series Smithiani	2800 m LWP0409060; Maerkang City,	JN543775	JN543776	JN543777
Aster smithianus	Section Aster series Smithiani	2500 m LWP0508034; Maerkang City, 2600 m	JN543778	JN543779	JN543780
Aster ageratoides var. lasiocladus	Section Orthomeris series Ageratoides	LWP0112018; Changsha City, 110 m	JN543781	JN543782	JN543783
Aster homochlamydeus	Section Orthomeris series Ageratoides	LWP0508004; Li County, 2600 m	JN543784	JN543785	JN543786
Aster hersileoides	Section Orthomeris series Hersileoides	LWP0807002; Li County, 2100 m	JN543787	JN543788	JN543789
Aster nitidus	Section Orthomeris series Hersileoides	LWP0505007; Nanchuan County, 660 m	JN543790	JN543791	JN543792
Aster albescens var. albescens	Section Orthomeris series Albescentes	LWP0508123; Baoxing County, 2010 m	JN543862	JN543863	JN543864
Aster argyropholis	Section Orthomeris series Albescentes	LWP0409045; Maerkang City, 2500 m	JN543793	JN543794	JN543795
Aster lavanduliifolius	Section Orthomeris series Albescentes	LWP0708053; Yajiang county, 2720 m	JN543796	JN543797	JN543798
Aster pycnophyllus	Section Orthomeris series Sikkimenses	LWP0509091; Dali City, 2800 m	JN543799	JN543800	JN543801
Aster falcifolius	Section Orthomeris series Falcifolii	LWP0410050; Mt. Huping, 400 m	JN543802	JN543803	JN543804
Aster baccharoides	Section Orthomeris series Turbinati	LWP0802001; Zhuhai City, 100 m	JN543805	JN543806	JN543807
Aster iishouensis	Section Orthomeris series Turbinati	LWP1012015: Jishou City, 600 m	JN543808	JN543809	JN543810
Aster sampsonii	Section Orthomeris series Turbinati	LWP0511060: Mt. Mang. 1100 m	IN543811	IN543812	IN543813
Aster turbinatus	Section Orthomeris series Turbinati	I WP0110029: Fenghua City 60 m	IN543814	IN543815	IN543816
Aster alpinus	Section Alpinenia series Alpini	LWP0607020; Wulumuqi City, 2320 m	JN543817	JN543818	JN543819
Aster handelii	Section Alpinenia series Alpini	LWP0708174; Zhongdian County, 3400 m	JN543820	JN543821	JN543822
Aster heterolepis	Section Alpinenia series Alpini	LWP0507004; Jiuzhai County, 2600 m	JN543823	JN543824	JN543825
Aster oreophilus	Section Alpinenia series Alpini	LWP0509016; Lijiang City, 3000 m	JN543826	JN543827	JN543828
Aster fanjingshanicus	Section Alpinenia series Tongolensis	LWP0606082; Mt. Fangjing, 2300 m	JN543829	JN543830	JN543831
Aster tongolensis	Section Alpinenia series Tongolensis	LWP0708147; Xiangcheng County, 3300 m	JN543832	JN543833	JN543834
Aster souliei	Section Alpinenia series Tongolensis	LWP0708084; Litang County, 4000 m	JN543835	JN543836	JN543837
Aster brachytrichus	Section Alpinenia series Latibracteati	LWP0607075; Xichang City, 2800 m	JN543838	JN543839	JN543840
Aster asteroides	Section Alpinenia series Asteroides	LWP0708112; Daocheng County, 2780 m	JN543841	JN543842	JN543843
Aster flaccidus	Section Alpinenia series Asteroides	LWP0607026; Wulumuqi City, 3700 m	JN543844	JN543845	JN543846
Aster diplostephioides	Section <i>Alpinenia</i> series <i>Diplostephioides</i>	LWP0507020; Jiuzhai County, 2600 m	JN543847	JN543848	JN543849
Aster setchuenensis	Section <i>Alpinenia</i> series <i>Diplostephioides</i>	LWP0508007; Maerkang City, 2800 m	JN543850	JN543851	JN543852
Aster yunnanensis	Section <i>Alpinenia</i> series <i>Diplostephioides</i>	LWP0508089; Kangding City, 3500 m	JN543853	JN543854	JN543855
Aster senecioides	Section Alpinenia series Senecioides	LWP0708215; Lijiang City, 2800 m	JN543856	JN543857	JN543858
Aster batangensis	Section Alpinenia series Batangenses	LWP0606039; Lijiang City, 2700 m	JN543859	JN543860	JN543861
Asterothamnus centrali-asiaticus	SEA	LWP0607045; Yinchuan City, 1630 m	JN315930	JN315954	JN315906
Asterothamnus fruticosus	SEA	LWP0607005; Wulumuqi City, 950 m	JN315929	JN315953	JN315905
Callistephus chinensis	SEA	LWP0108021; Anshan City, 340 m	JN315931	JN315955	JN315907
Crinitina linosyris	SEA	LWP0408001; Shumen, Bulgaria, 400 m	JN315932	JN315956	JN315908
Crinitina villosa	SEA	LWP0408009; Shumen, Bulgaria, 400 m	JN315933	JN315957	JN315909

# APPENDIX 1. Continued

Continued

Present taxonomy*	Phylogenetic lineages and infrageneric classification of $Aster^{\dagger}$	Numbers, locations and altitudes of vouchers <sup>‡</sup>	GenBank accession number <sup>§</sup>		
			ITS	ETS	trnL-F
Doellingeria scaber	SEA	LWP0108025; Anshan City, 350 m	JN315934	JN315958	JN315910
Galatella dahurica	SEA	LWP0609047; Mt. A'er, Nei Mongol, 400 m	JN315935	JN315959	JN315911
Heteropappus altaicus var. millefolius	SEA	LWP0506010; Zhang County, 600 m	JN543709	JN543710	JN543711
Heteropappus crenatifoliu	SEA	LWP0409037; Maerkang City, 3200 m	JN543712	JN543713	JN543714
Kalimeris indica	SEA	LWP0806017; Changsha City, 80 m	JN543715	JN543716	JN543717
Kalimeris incisa	SEA	LWP0609107; Tonghua County, 560 m	JN543721	JN543722	JN543723
Kalimeris integrifolia	SEA	LWP0609077; Mudanjiang City, 360 m	JN543718	JN543719	JN543720
Kalimeris longipetiolata	SEA	LWP0508104; Baoxing County,	JN315936	JN315960	JN315912
Miyamayomena angustifolius	SEA	DBY9206; Yongjia County. 200 m	JN543736	JN543737	JN543738
Miyamayomena piccolii	SEA	LWP0510055: Mei County 300 m	IN543730	IN543731	IN543732
Miyamayomena simplex	SEA	LWP0508083; Kangding City, 2800 m	JN543733	JN543734	JN543735
Rhinactinidia eremophila	SEA	LWP0607036; Wulumuqi City, 2620 m	JN543727	JN543728	JN543729
Rhinactinidia limoniifolia	SEA	LWP0607012; Wulumuqi City, 1800 m	JN543724	JN543725	JN543726
Tripolium vulgare	SEA	LWP0311001: Varna Bulgaria 1 m	IN315937	IN315961	IN315913
<i>Turczaninowia fastigiata</i> Sub-tribe Solidaginae	SEA	LWP0609030; Daqin City, 150 m	JN543739	JN543740	JN543741
Solidago decurrens	NA	LWP0510116; Lichuan County, 1050 m	JN204176	JN204177	JN204178
Sub-tribe Pentachaetinae					
<i>Pentachaeta aurea</i> Sub-tribe Boltoniinae	NA	-	AF046972	NA	
<i>Boltonia asteroides</i> Sub-tribe	NA	-	AF477632	NA	
Machaerantherinae					
Machaeranthera tanacetifolia	NA	-	AF477661	NA	
Sub-tribe					
Symphyotrichinae					
Symphyotrichum novi-belgii	NA	LWP0606002; Beijing, cultivated.	JN315926	JN315950	JN315902
Symphyotrichum subulatum	NA	LWP1010007; Changsha City, 40 m	JN315927	JN315951	JN315903
Sub-tribe Astranthiinae Astranthium integrifolium	NA	-	AF046984	NA	
Sub-tribe Chrysopsidinae Chrysopsis mariana	NA	_	GQ892729	NA	
Sub-tribe Conyzinae					
Conyza japonica	AIS	LWP0606032; Lijiang City, 2500 m	JN315938	JN315962	JN315914
Conyza sumatrensis	NA	LWP1009002; Changsha City, 35 m	JN315923	JN315947	JN315899
Erigeron annus	NA	LWP1010009; Changsha City, 40 m	JN315924	JN315948	JN315900
<i>Erigeron breviscapus</i> Tribe Anthemideae	NA	LWP0606055; Lijiang City, 2500 m	JN315925	JN315949	JN315901
Chrysanthemum coronarium	OG	LWP1004010; Changsha, cultivated.	JN315939	JN315963	JN315915
Dendranthema indicum Tribe Calenduleae	OG	LWP1012002; Changsha City, 80 m	JN315940	JN315964	JN315916
Calendula officinalis	OG	LWP1004006; Changsha, cultivated.	JN315941	JN315965	JN315917

# APPENDIX 1. Continued

\* Generic circumscriptions and nomenclature of Astereae follow Nesom and Robinson (2007) except *Turczaninowia* which follows Ling *et al.* (1985) and *Crinitina* Soják is substituted for *Crinitaria* Cass. The name *Aster setchuenensis* follows the International Plant Names Index (IPNI).

<sup>†</sup> Phylogenetic lineages: follows Brouillet *et al.* (2009*b*); infrageneric classification of *Aster* follows Ling *et al.* (1985). AIS, Astereae *incertae sedis*; AL, Australasian lineages; BE, Bellidinae; BL, early-branching lineages; ETS, external transcribed spacer; GR, Grangeinae; ITS, internal transcribed spacer; NA, North American lineage; NZ, New Zealand clade; OG, outgroup; PSA, palaeo South American clade; SA, South American lineages; SEA, segregates of Eurasian *Aster s.l.* 

\* Information is omitted for the accessions that were obtained from GenBank. Four species were collected from Bulgaria and the others from China.

§ One or two sequence (ETS, trnL-F) data unavailable.