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Authors: Jafari, Farzaneh, Osaloo, Shahrokh Kazempour, and Mozffarian, Valiollah

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FARZANEH JAFARI[1], SHAHROKH KAZEMPOUR OSALOO[1*] & VALIOLLAH MOZFFARIAN[2]

Molecular phylogeny of the tribe *Astereae* (*Asteraceae*) in SW Asia based on nrDNA ITS and cpDNA *psbA-trnH* sequences

Abstract

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In the present study, nucleotide sequences of the nrDNA ITS and the plastid DNA *psbA-trnH* region were used to reconstruct the phylogeny of the tribe *Astereae* in SW Asia using parsimony, Bayesian and likelihood methods. The ITS analysis showed that the SW Asian representatives of the tribe divide into two groups. One group arises just above the African lineage, while the second is part of the large Australasian polytomy at the crown of the tribe. SW Asian *Aster*, *Crinitina*, *Galatella*, *Lachnophyllum* and *Psychrogeton* appear to be non-monophyletic, whereas *Chamaegeron*, Eurasian *Erigeron* and *Myriactis* are monophyletic. *Dichrocephala integrifolia* is allied with S African members of subtribe *Grangeinae*. *Chamaegeron* and *Lachnophyllum gossypinum* are sister taxa and both are allied with the *Bellis* and *Galatella* group. *Aster bachtiaricus* is the earliest diverging branch of a large polytomy at the crown of the tribe. *Psychrogeton* species form three distinct clades. *Heteropappus altaicus* is nested in the *Aster* clade. *Conyzanthus squamatus* is nested within *Symphyotrichum* species.

Additional key words: *Compositae*, *Aster*, *Chamaegeron*, *Galatella*, *Lachnophyllum*, *Psychrogeton*

Introduction

The *Astereae* is the second largest tribe of the *Asteraceae*, with 222 genera and c. 3100 species (Brouillet & al. 2009a). The tribe is characterized by deltate to triangular or lanceolate style appendages (Nesom & Robinson 2007). It is part of the subfamily *Asteroideae* and forms a clade with the *Calenduleae*, *Gnaphalieae* and *Anthemideae*, the last of which is often considered its sister tribe (Brouillet & al. 2009a; Funk & al. 2009).

Based on the recent classification of Nesom & Robinson (2007), members of the tribe present in SW Asia can be classified into eight subtribes: *Asterinae* (including *Aster* L., *Crinitina* Soják, *Galatella* Cass., *Heteropappus* Less., *Psychrogeton* Boiss. and *Tripolium* Nees); *Bellidinae* (*Bellis* L.); *Conyzinae* (*Conyza* Less. and *Erigeron* L.); *Grangeinae* (*Dichrocephala* L'Her. ex DC.); *Homochrominae* (*Chamaegeron* Schrenk and *Lachnophyllum* Bunge); *Lagenophorinae* (*Myriactis* Less.); *Solidagininae* (*Solidago* L.); and *Symphyotrichinae* (*Conyzanthus* Tamamsch.). However, *Neobrachyactis* Brouillet, a new genus of three species (type: *Neobrachyactis roylei* (DC.) Brouillet), was not attributed to a subtribe (Chen & al. 2011). In Flora of China, Chen & al. (2011) recognized *Neobrachyactis* as distinct from *Brachyactis* s.str. (the type of which, *B. ciliata* (Ledeb.) Ledeb., belongs to the North American genus *Symphyotrichum* Nees) in being glandular and having compressed, 2-ribbed achenes.

1 Department of Plant Biology, Faculty of Biological Sciences, Tarbiat Modares University, 14115-154, Tehran, Iran; *e-mail: skosaloo@modares.ac.ir (author for correspondence); ficcaso7@hotmail.com

2 Department of Botany Research Institute of Forests and Rangelands, 3185-116, Tehran, Iran; e-mail: mozaffarian89@yahoo.com

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The first global phylogenetic analysis of the tribe based on nrDNA ITS sequence data was done by Brouillet & al. (2009a). The presence of two S African genera, *Denekia* Thunb. and *Printzia* Cass., at the base of the tree suggested that the tribe originated in Africa. The Chinese genus *Nannoglottis* Maxim. is the second lineage in the tribe. It is followed by dispersals to S Africa, South America, New Zealand and Australia. Next, an African lineage gave rise to Asian and Mediterranean members. Finally, the crown lineage of the *Astereae* includes *Callistephus chinensis* (L.) Nees, Australasian lineages, South American lineages and the North American clade.

Hitherto, many Asian members of the tribe (*Chamaegeron*, *Lachnophyllum*, *Neobrachyactis* and *Psychrogeton*) as well as the cosmopolitan *Conyzanthus* have not been phylogenetically studied using DNA sequences. Only few Eurasian representatives of some genera, especially *Aster* s.str. and segregate genera, were investigated in previous phylogenetic studies (Noyes & Rieseberg 1999; Brouillet & al. 2009a; Li & al. 2012).

The nuclear ribosomal internal transcribed spacer (ITS) and external transcribed spacer (ETS) and plastid sequence data have been used to infer phylogenetic relationships in *Astereae* (Morgan 1997; Noyes & Rieseberg 1999; Noyes 2000; Brouillet & al. 2001; Lowrey & al. 2001; Markos & Baldwin 2001; Cross & al. 2002; Fiz & al. 2002; Liu & al. 2002; Semple & al. 2002; Wagstaff & Breitwieser 2002; Lowell & al. 2003; Morgan 2003; Roberts & Urbatsch 2003, 2004; Urbatsch & al. 2003; Beck & al. 2004; Brouillet & al. 2004; Eastwood & al. 2004; Field & al. 2006; Watanabe & al. 2006; Selliah & Brouillet 2008; Andrus & al. 2009; Brouillet & al. 2009a, 2009b; Karaman-Castro & Urbatsch 2009; Vaezi & Brouillet 2009; Wagstaff & al. 2011; Li & al. 2012; Nakamura & al. 2012; Strijk & al. 2012).

The main goals of this study are (1) to investigate the phylogenetic relationships among SW Asian members of the tribe *Astereae*, and (2) to evaluate the monophyly and relationships of several species-rich genera of the tribe occurring in SW Asia (*Aster*, *Chamaegeron*, *Erigeron* and *Psychrogeton*) mainly based on nrDNA ITS sequence data.

Material and methods

Taxon sampling

The ITS sequences included 142 accessions representing 52 genera and 117 species, with 51 accessions newly sequenced (all from Iran except two from Turkey and one from Kazakhstan) and 91 obtained from GenBank. *Achillea millefolium* L. and *Ursinia nana* DC. (*Anthemideae*), *Anaphalis margaritacea* (L.) Benth. & Hook. f. (*Gnaphalieae*), *Blumea brevipes* (Oliv. & Hiern.) Wild (*Inuleae*) and *Calendula officinalis* L. (*Calenduleae*) were used as outgroups (Noyes & Rieseberg 1999; Funk & al. 2009).

The *psbA-trnH* sequences included 18 accessions representing six genera and 15 species, with 15 accessions newly sequenced (all from Iran) and three obtained from GenBank. *Galatella litvinovii* Novopokr. (*Bellidinae*) was used as an outgroup.

Leaf samples were obtained from specimens in the following herbaria in Iran (herbarium codes according to Thiers 2015+): Ferdowsi University, Mashhad (FUMH), Research Institute of Forests and Rangelands, Tehran (TARI), Tehran University (TUH) and Shahid Bahonar University, Kerman. Respective voucher information and GenBank accession numbers are listed in the Appendix.

DNA extraction, amplification and sequencing

Genomic DNA was extracted using the modified CTAB protocol of Doyle & Doyle (1987). Sodium metabisulfite (1 % w/v) was added to the DNA isolation buffer. The entire nrDNA ITS (ITS1–5.8S–ITS2) region was amplified by polymerase chain reaction (PCR) using either the universal primers AB101 and AB102 (Douzery & al. 1999) or ITS5m (Sang & al. 1995) and ITS4 (White & al. 1990). The *psbA-trnH* region was amplified using the psbA (Sang & al. 1997) and trnH (Tate & Simpson 2003) primers.

The PCR was performed in a 25 µL volume, containing 10 µl deionized water, 10× PCR Buffer with 1.5 mmol/L MgCl2 (Roche Diagnostics, Canada), 10 µmol/L of each dNTP, 0.5 µl of each primer (10 pmol/µl), 2.5 %–5 % DMSO, one unit of *Taq* DNA polymerase and 1 µl template DNA (20–70 ng).

PCR procedures for the nrDNA ITS region were 2:30 min at 94 °C for predenaturation, followed by 35–37 cycles of 94 °C for 1 min, 51–58 °C for 1 min, and 72 °C for 1 min plus a final extension of 72 °C for 7 min. For the *psbA-trnH* region, the PCR conditions were 2:30 min at 94 °C for predenaturation, followed by 35–37 cycles of 94 °C for 45s, 58 °C for 40s, and 72 °C for 1–1:10 min plus a final extension of 72 °C for 7 min. The quality of PCR products was checked by electrophoresis on a 1 % (w/v) agarose gel (using 1X TBE as the gel buffer) stained with ethidium bromide and then visualized under UV light. Amplified products were cleaned. Cleaned products were sequenced using the Bigdye terminator cycle sequencing ready reaction kit (Applied Biosystems, U.S.A.) with the appropriate primers in an ABI Prism 3730xl DNA sequencer (Applied Biosystems, U.S.A.).

Sequence alignment

Sequences were edited using BioEdit v. 7.0.5.3 (Hall 1999). Matrices were aligned initially using MUSCLE (Edgar 2004) and subsequently edited manually. Insertions and deletions (indels) were observed in all alignments and treated as missing data.

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Table 1. Dataset and tree statistics from analyses of nuclear and chloroplast regions.

	nrDNA ITS	cpDNA *psbA-trnH*
Sequences(n)	142	18
CI of MPTs	0.333	0.938
RI of MPTs	0.669	0.960
Number of MPTs	2181	66
Length of MPTs	2371	16

Phylogenetic analyses

Maximum parsimony — Maximum parsimony analyses were conducted using PAUP* version 4.0b10 (Swofford 2002). The heuristic search option was employed with the following options: MulTrees, tree bisection reconnection (TBR) branch swapping with 100 random addition sequence replicates, and a maximum of 10 000 trees retained.

To evaluate clade support, a bootstrap analysis was performed using a full heuristic search with 1000 replicates (Felsenstein 1985), each with simple addition sequence and TBR branch swapping, and a maximum of 100 trees retained per replicate.

Bayesian method — The most appropriate model and parameter estimates for Bayesian analyses were selected using the program MrModeltest version 2.0 (Nylander 2004) based on the Akaike information criterion (AIC) (Posada & Buckley 2004). On the basis of the Modeltest results, datasets were analysed using the GTR+I+G model for nrDNA ITS and F81+G model for *psbA-trnH* sequences.

Bayesian analysis was performed using MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003). The analysis was carried out with 10 million generations for ITS and 4 million generations for *psbA-trnH*, using the Markov chain Monte Carlo search. MrBayes performed two simultaneous analyses starting from different random trees (Nruns=2) each with four Markov chains and trees sampled at every 100 generations. The first 25 % of trees were discarded as the burn-in. The remaining trees were then used to build a 50 % majority rule consensus tree accompanied with posterior probability values. Tree visualization was carried out using Tree ViewX version 0.5.0 (Page 2005).

Maximum likelihood method — Maximum likelihood analyses were performed for the datasets in raxmlGUI v. 1.3. (Silvestro & Michalak 2012). The model employed for each dataset is the same as that for Bayesian analyses. Parametric bootstrap values for maximum likelihood were calculated in raxmlGUI base on 1000 replicates with one search replicate per bootstrap replicate.

Results

Characterization of nucleotide data

The aligned ITS matrix comprises 142 sequences and 678 characters, including 350 (350/678=52 %) potentially parsimony-informative sites and 328 parsimony-uninformative ones.

For the *psbA-trnH* region, the matrix of 18 sequences contains 356 characters, of which 11 are potentially parsimony-informative sites and 345 are parsimony-uninformative. More information about the datasets and tree statistics from analyses of the nuclear and chloroplast regions is summarized in Table 1.

Phylogenetic analyses

ITS phylogeny (Fig. 1A & B)

Maximum parsimony, maximum likelihood and Bayesian methods gave very similar results. We here show only Bayesian trees along with posterior probability (PP) and bootstrap values from both maximum likelihood (LBS) and maximum parsimony (PBS) analyses. The *Astereae* form a monophyletic group (PP=1.00, LBS=93, PBS=93). The S African genus *Printzia* is sister to all other *Astereae*, followed by the Chinese genus *Nannoglottis*, which is sister to the remaining *Astereae* (PP=1.00, LBS=98, PBS=79). The following clades are successively recovered along the spine of the Bayesian tree: (1) the S African *Mairia* Nees and the New Zealand-paleo-South American clade (PP=0.79); (2) the African clade, the subtribe *Homochrominae* including *Commidendrum robustum* (Roxb.) DC., which is sister to *Amellus strigosus* (Thunb.) Less. and *Felicia aethiopica* (Burm. f.) Grau (PP=1.00, LBS=78, PBS=85); and (3) and a clade (PP=1.00, LBS=89, PBS=79) comprising the remaining *Astereae*. Within (3), the African *Conyza gouanii* (L.) Willd. and *Lachnophyllum noeanum* Boiss. are successive sisters to an unresolved polytomy of the African-S Asian subtribe *Grangeinae*, the Mediterranean-Eurasian *Bellidinae* (sensu Brouillet & al.), C Asian taxa including *Chamaegeron* and *Lachnophyllum gossypinum* Bunge, and a large polytomic clade comprising *Aster bachtiaricus* Mozaff. and the Australasian, South American and North American clades.

Dichrocephala integrifolia (L. f.) Kuntze is sister to *Grangea maderaspatana* (L.) Poir., *Nidorella polycephala* DC. and *N. resedifolia* DC. (PP=1.00, LBS=93, PBS=79). *Crinitina*, *Galatella* and *Tripolium* constitute a well-supported clade (PP=1.00, LBS=100, PBS=97), which is sister to *Bellis annua* L. and *B. perennis* L., this relationship being moderately supported (PP=1.00, LBS=54). *Chamaegeron* and *Lachnophyllum gossypinum* form a moderately supported clade (PP=0.95, PBS=66), which is sister to a clade comprising *Bellis* and the *Galatella* group (PP=0.51). *Aster bachtiaricus* is allied with the large polytomy at the crown of the *Astereae*

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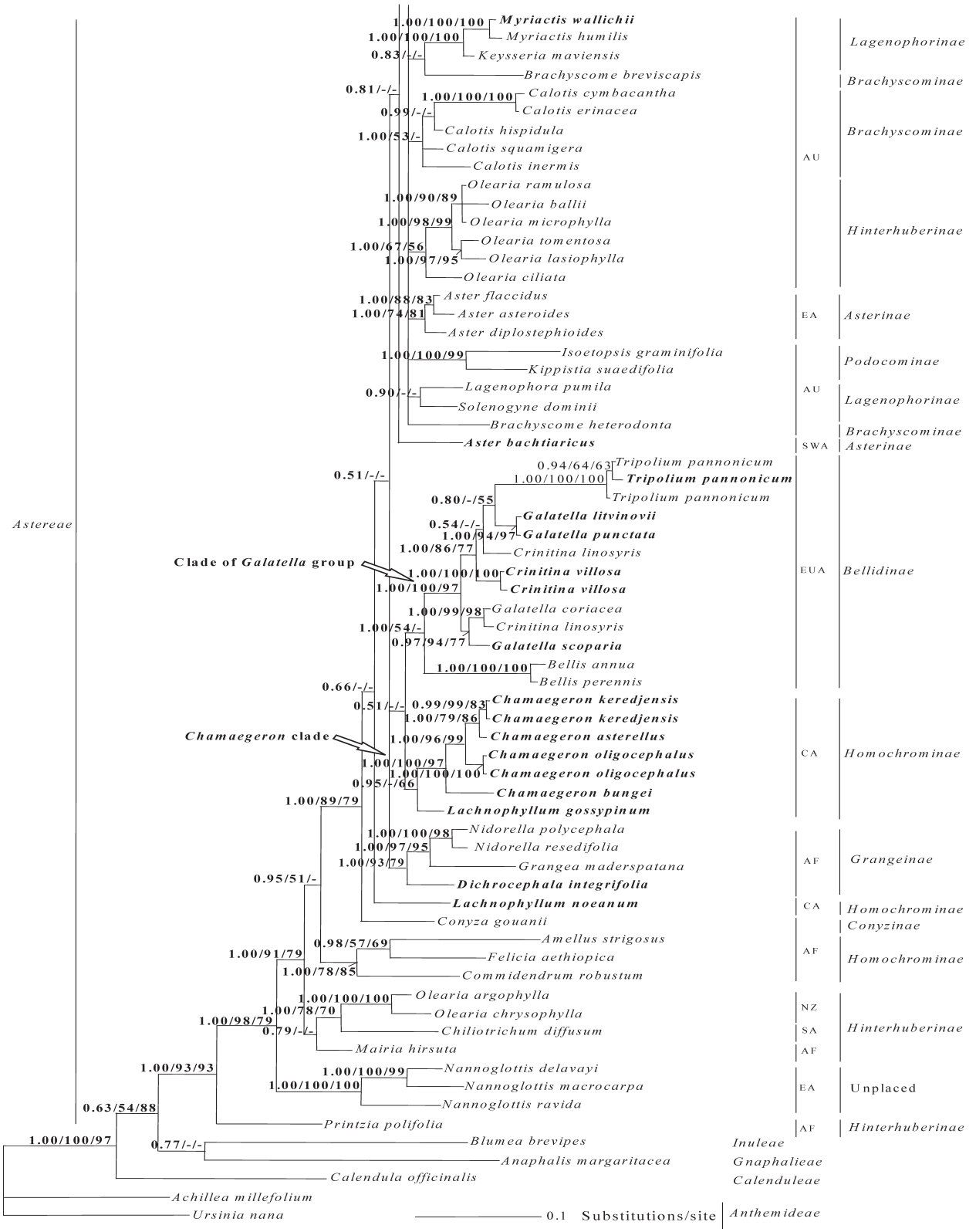


Fig. 1A. Fifty percent majority rule consensus tree resulting from Bayesian analysis of nrDNA ITS dataset. Lower half of tree. Numbers above branches are posterior probability (PP) and bootstrap values from maximum likelihood (LBS) and maximum parsimony (PBS) analyses, respectively. Values <50 % are not shown. Region of origin: AF = Africa; AU = Australia; CA = Central Asia; EA = East Asia; EUA = Eurasia; NA = North America; NAF = North Africa; NZ = New Zealand; SA = South America; SWA = Southwest Asia.

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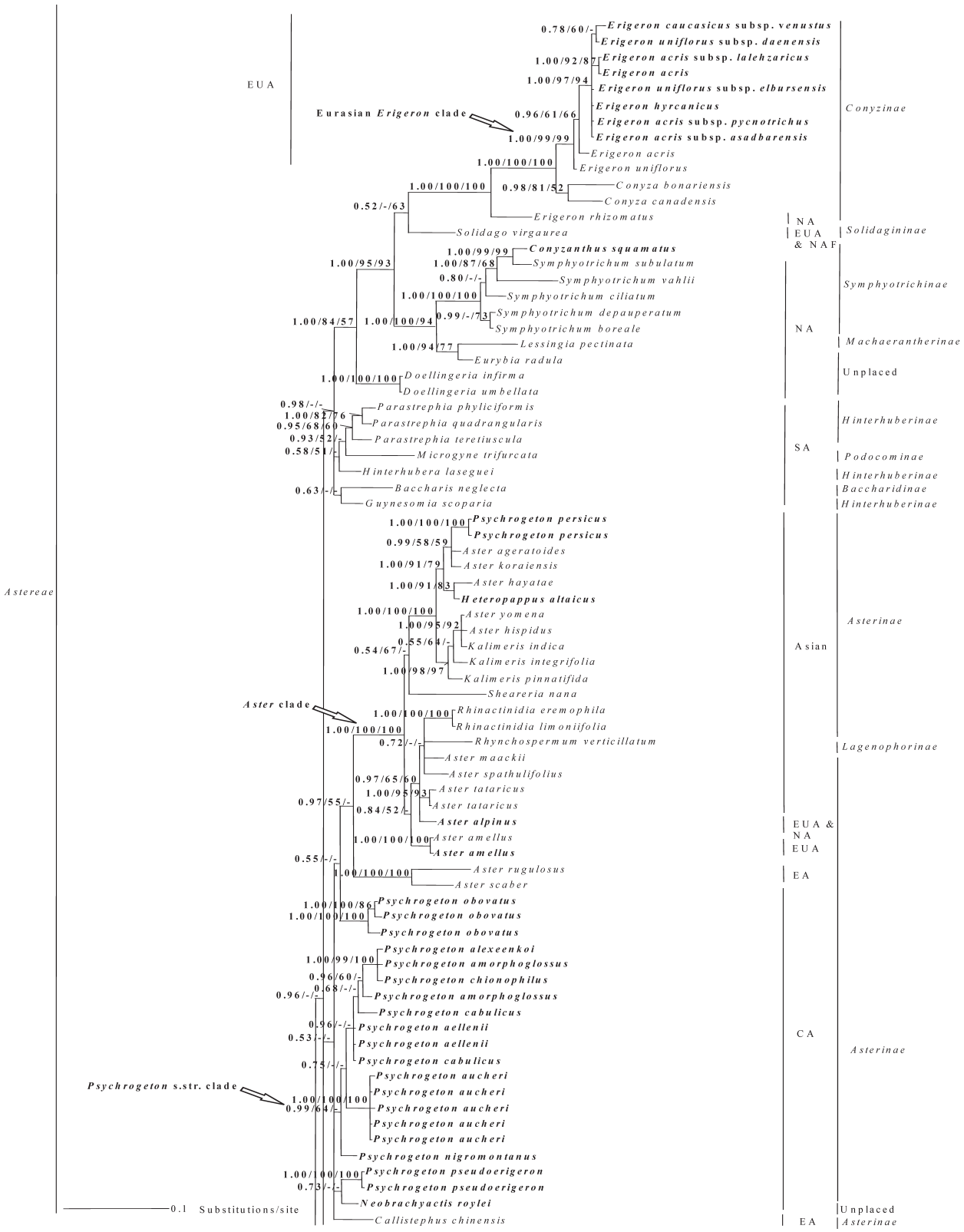


Fig. 1B. Fifty percent majority rule consensus tree resulting from Bayesian analysis of nrDNA ITS dataset. Upper half of tree. For explanation see Fig. 1A.

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(PP=0.96). *Myriactis wallichii* Less. is sister to *M. humilis* Merr. (PP=1.00, LBS=100, PBS=100); they form a strongly supported clade with *Keysseria maviensis* (H. Mann.) Cabrera (PP=1.00, LBS=100, PBS=100). The C Asian clade includes *Psychrogeton* species (excluding *P. obovatus* (Benth.) Grierson, *P. persicus* (Boiss.) Grierson and *P. pseudoerigeron* (Bunge) Novopokr. ex Nevski) and is moderately supported (PP=0.99, LBS=64) by the ITS analyses. The three accessions of *P. obovatus* form a well-supported clade (PP=1.00, LBS=100, PBS=100), which is sister to the *Aster* clade (PP=0.55). The two accessions of *P. pseudoerigeron* are closely related to *Neobrachyactis roylei* (PP=0.73). *Aster ageratoides* Turcz., *A. koraiensis* Nakai and *P. persicus* form an unresolved subclade (PP= 0.99, LBS=58, PBS=59), which is sister to the subclade of *A. hayatae* H. Lév. & Vaniot and *Heteropappus altaicus* (Willd.) Novopokr. *Conyzanthus squamatus* is nested within *Symphyotrichum*. *Erigeron acris* L. and subspecies, *E. caucasicus* subsp. *venustus* (Botsch.) Grierson, *E. hyrcanicus* Bornm. & Vierh. and *E. uniflorus* L. and subspecies form a strongly supported clade (PP=1.00, LBS=99, PBS=99).

***psbA-trnH* phylogeny** (Fig. 2)

Neither the subtribe *Asterinae* sensu Nesom & Robinson (2007) nor sensu Brouillet & al. (2009a) seems to be monophyletic. *Aster*, *Galatella*, *Psychrogeton*, *Solidago* and *Tripolium* form an unresolved clade (PP=0.96, LBS=74, PBS=72). The phylogram includes: (1) *Aster bachtiaricus*; (2) *Psychrogeton nigromontanus* (Boiss. & Buhse) Grierson; (3) the *Erigeron* clade (PP=0.96, LBS=66, PBS=73), comprising *E. annuus* (L.) Pers. and *E. strigosus* Muhl. ex Willd.; (4) the clade comprising *Solidago gigantea* Aiton as sister to *Aster alpinus* L. and *Psychrogeton persicus* (PP=0.71, LBS=66 , PBS=61); (5) the *Psychrogeton* clade (PP=0.99, LBS=83, PBS=83), in which the species occupy unresolved positions; and (6) the *Tripolium–Psychrogeton obovatus* clade (PP=0.69, PBS=66).

Discussion

The nrDNA ITS tree for the *Astereae* in the present study (Fig. 1A & B) is topologically similar to that of Brouillet & al. (2009a). We sequenced and analysed several Asiatic taxa that were not included in that study. In contrast to that study, the Eurasian subtribe *Bellidinae* is sister to the *Chamaegeron–Lachnophyllum gossypinum* clade rather than to the *Grangeineae*. Also, an assemblage of Asian species of *Aster* and allies, the so-called *Aster* clade (Li & al. 2012), is in the large polytomy at the crown of the *Astereae*, but it does not group with any of the Australian or Asian species of the Australasian lineages. This is in agreement with the nrDNA ITS study of Li & al. (2012).

In the current analysis, the SW Asian representatives of the *Astereae* are generally divided into two groups: (1) representatives that are outside the crown lineages and are the first lineage to diverge after the *Grangeinae*, including *Chamaegeron*, *Dichrocephala integrifolia*, the *Galatella* group (or *Bellidinae* of Brouillet & al. 2009a, b), *Lachnophyllum gossypinum* and *L. noeanum*; and (2) representatives that are part of the large polytomy at the crown of the tribe, e.g. *Aster*, *Heteropappus*, *Myriactis*, *Neobrachyactis* and *Psychrogeton* occurring in the Australasian polytomy, and the other representatives, e.g. *Conyzanthus* and *Erigeron* (including two species of *Conyza*) nested in the North American clade. Below we examine the status of these genera within the *Astereae* in further detail.

The first genera derived after the African lineage

Lachnophyllum — This is a small C and SW Asian genus of two species (Nesom & Robinson 2007). The genus is characterized by recurved ray flowers and often lanate, long hairs and stipitate glands. The nrDNA ITS data did not support a close relationship between them. *Lachnophyllum gossypinum* is closely allied with *Chamaegeron* (see below), whereas *L. noeanum* appears in an isolated position above *Conyza gouanii*, as sister to an unresolved polytomy of the African subtribe *Grangeinae*, Mediterranean-Eurasian *Bellidinae* (sensu Brouillet & al. 2009a, 2009b), C Asian taxa including *Chamaegeron* and *Lachnophyllum gossypinum* and the large polytomic clade comprising *Aster bachtiaricus* and the Australasian-Asian, South American and North American clades (PP=0.66).

Chamaegeron — This includes four species distributed in C Asia, Iran, Afghanistan and Pakistan. With the exception of *Chamaegeron oligocephalus* Schrenk, the species are endemic to Iran, i.e. *C. asterellus* (Bornm.) Botsch., *C. bungei* (Boiss.) Botsch. and *C. keredjensis* (Bornm. & Gauba) Grierson. *Chamaegeron* has distinctive characters, is glabrous or pubescent with branching habit and basally connate pappus bristles falling as a unit. The *Chamaegeron* clade is strongly supported in the ITS tree (PP=1.00, LBS=100, PBS=97). *Chamagaeron bungei* is the basal species and is characterized by one series of ray florets and obovate leaves that are covered by hirsute and glandular hairs. It is followed by the clade consisting of *C. asterellus*, *C. keredjensis* and *C. oligocephalus* (PP=1.00, LBS=96, PBS=99). This clade is, in turn, divided into two subclades: (1) a subclade of two accessions of *C. oligocephalus* (PP=1.00, LBS=100, PBS=100); and (2) a subclade including *C. asterellus* as sister to two accessions of *C. keredjensis* (PP=1.00, LBS=79, PBS=86). *Chamaegeron asterellus* and *C. keredjensis* are covered by glandular, villous and hirsute hairs, whereas *C. oligocephalus* is glabrous.

***Galatella* group** — According to Nesom (1994), the *Galatella* group includes *Crinitina*, *Galatella* and *Tripolium*. They constitute a well-supported clade in the ITS

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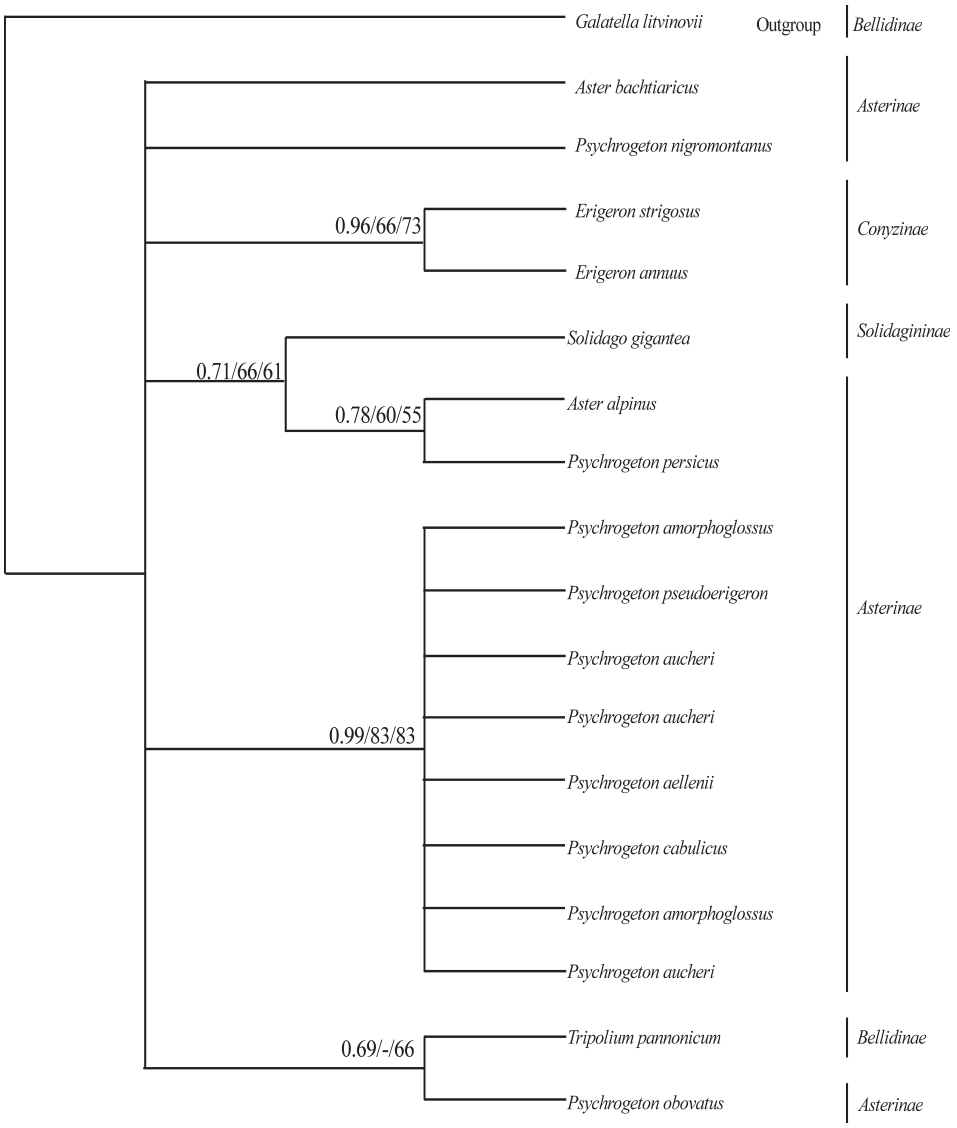


Fig. 2. Fifty percent majority rule consensus tree resulting from Bayesian analysis of plastid *psbA-trnH* dataset. Numbers above branches are posterior probability (PP) and bootstrap values from maximum likelihood (LBS) and maximum parsimony (PBS) analyses, respectively. Values <50 % are not shown.

tree (PP=1.00, LBS=100, PBS=97). The present analysis is in agreement with the previous studies (Fiz & al. 2002; Brouillet & al. 2009a, 2009b; Li & al. 2012) regarding the sister-group relationship of the *Galatella* group with the Euro-Mediterranean genus *Bellis* (*B. annua* and *B. perennis*). Brouillet & al. (2009a) pointed out that *Crinitina*, *Galatella* and *Tripolium* should be included in *Bellidinae* rather than in *Asterinae*, which is also consistent with our analyses. The *Galatella* group comprises two well-supported subclades: (1) a subclade including *G. scoparia* (Kar. & Kir.) Novopokr. from Kazakhstan and two sister taxa, *C. linosyris* (L.) Soják and *G. coriacea* Novopokr.; and (2) a subclade comprising *C. linosyris*, *C. villosa* (L.) Soják, *Galatella litvinovii*, *G. punctata* (Waldst. & Kit.) Nees and *T. pannonicum* (Jacq.) Dobrocz.

Galatella is composed of 30 species (Nesom & Robinson 2007) and, as currently understood, the genus is not monophyletic. *Crinitina* is a small genus of 13 species (Nesom & Robinson 2007). The nrDNA ITS data show that *Crinitina* is also not monophyletic. Two accessions of *C. linosyris* were placed in two different subclades within the group, and were not allied with the congeneric *C. villosa*. It is worth noting that two ITS accessions of *C. linosyris* obtained from GenBank (DQ478987 and AF046949), determined by Karaman-Castro & Urbatsch (2009) and Noyes & Rieseberg (1999), respectively, are completely different from each other and placed in different subclades. Therefore, it appears that one of these accessions may be misidentified. *Crinitina villosa* is similar to *Galatella scoparia* in having yellow discoid capitula. The two accessions of *C. villosa* were not united, however, with *G. scoparia*.

Tripolium is a unispecific genus growing in salt-marshes, salt-marsh meadows and moist meadows across Europe and N Asia to America (Tamamschjan 1959; Nesom 1994). Nesom (1994) and Brouillet & al. (2009a, 2009b) noted the morphological similarities of *Tripolium* (including its distinctly corymboid capitulescence, herbaceous, broadly rounded, multinervate phyllaries, and tendency for raylessness) to *Crinitina* and *Galatella* and suggested that these taxa may be closely related. Our results support their hypothesis.

Dichrocephala — This contains ten species occurring in Africa and tropical countries according to Nesom & Robinson (2007), who defined it as a member of the *Grangeinae*. *Dichrocephala integrifolia* is characterized by lyrate-pinnatifid leaves and achenes without a pappus. It is allied with *Grangea maderaspatana* and the two species of *Nidorella* in the *Grangeinae*. In the present study, the inclusion of *Chamaegeron* and *Lachnophyllum* showed

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that the *Bellidinae* (including the Euro-Mediterranean genus *Bellis* and the Eurasian *Galatella* group) are not sister to the African *Grangeinae*, which disagrees with a previous study (Brouillet & al. 2009b).

Genera belonging to the large crown polytomy of the tribe

Psychrogeton — This includes 20 species from Asia (Grierson 1967; Grierson & Rechinger 1982; Nesom & Robinson 2007). Grierson (1967) recognized *Psychrogeton* as an isolated genus due to the sterile (functionally male) disc achenes. However, two species (*P. chionophilus* (Boiss.) Krasch. and *P. obovatus* represent exceptions in that the disc florets appear to be fertile. Grierson indicated Pamir-Hindu Kush as the likely biodiversity centre of *Psychrogeton*, with dispersal occurring from there to C Asia, Afghanistan, Iran, NE Iraq, E Turkey and NE Syria. Both the ITS and *psbA-trnH* analyses do not support the monophyly of *Psychrogeton*. In the ITS tree, all species except *P. obovatus*, *P. persicus* and *P. pseudoerigeron* formed a clade (*Psychrogeton* s.str.). *Psychrogeton obovatus* is sister to the *Aster* clade. This species is one of the most isolated species of the genus (Grierson & Rechinger 1982). It is characterized by leaflike outer involucral bracts, obovate disc achenes (fertile) and coarsely toothed leaves, which are similar to those of *Aster* species. *Psychrogeton persicus*, represented by two accessions, along with *Aster ageratoides* and *A. koraiensis*, is deeply nested within the *Aster* clade. *Psychrogeron pseudoerigeron* is closely related to *Neobrachyactis roylei*. These species have similar geographical distributions and habitats. However, *Neobrachyactis* differs from *P. pseudoerigeron* in being an annual and having fertile disc florets. In addition, the numerous highly reduced ray florets of *Neobrachyactis* contrast with those of *P. pseudoerigeron*, which are much longer than the pappus. *Psychrogeton* s.str. is composed of three lineages. First, *P. nigromontanus* is sister to the remaining species (PP=0.99, LBS=64). It differs from the other taller species (e.g. *P. aucheri* (DC.) Grierson and *P. pseudoerigeron*) in its numerous obliquely cut tubular female ray florets with the corolla ½–⅔ as long as the style. The second lineage is *P. aucheri*, represented here by five accessions. It is characterized by a 2-seriate pappus and ray-floret corollas as long as the style. The third lineage comprises five species: *P. aellenii* (Rech. f.) Grierson, *P. alexeenkoi* Krasch., *P. amorphoglossus* (Boiss.) Novopokr., *P. cabulicus* Boiss. and *P. chionophilus*. Different accessions of *P. aellenii*, *P. amorphoglossus* and *P. cabulicus* did not cluster together. There were some single-nucleotide polymorphisms between the accessions of these species, maybe caused by hybridization. We analysed two accessions of *P. amorphoglossus* and *P. cabulicus* that had morphological differences (e.g. shape of leaves and density of hairs). The separation of *P. amorphoglossus* accessions in different clades can be evidence of hybridization. Considering the great diversity in chromosome numbers and ploidy levels in the *Astereae* (Ito & al. 1994; Brouillet & al. 2009a), it is possible that there are both diploid and polyploid populations for *P. amorphoglossus* and *P. cabulicus.* One accession of *P. cabulicus* is sister to *P. amorphoglossus* and an unresolved subclade of *P. alexeenkoi*, *P. amorphoglossus* and *P. chionophilus*. The latter three species are caespitose and monocephalous and restricted to C to S Iran.

The *psbA-trnH* analysis (Fig. 2) showed that *P. aellenii*, *P. amorphoglossus*, *P. aucheri*, *P. cabulicus* and *P. pseudoerigeron* form a well-supported clade (PP=0.99, LBS=83, PBS=83). However, *P. nigromontanus* occupies an unresolved position, and *P. obovatus*, as in the ITS tree, is distinct from other *Psychrogeton* species. Chloroplast data show a relationship between *P. persicus* and *Aster alpinus* (PP=0.78, LBS=60, PBS=55).

Asian *Aster* and allies — The ITS tree showed that the *Aster* clade did not group with any of the Australian (e.g. *Brachyscome* Cass., *Calotis* R. Br., *Isoetopsis* Turcz., *Keysseria* Lauterb., *Kippistia* F. Muell. and *Olearia* Moench) or Asian (*Myriactis*) species of the Australasian lineages, in agreement with the study of Li & al. (2012). Of 16 sampled species of *Aster* s.str., *A. asteroides* (DC.) Kuntze, *A. bachtiaricus*, *A. diplostephioides* (DC.) Benth. ex C. B. Clarke and *A. flaccidus* Bunge place in the lower half of the ITS tree (Fig. 1A) while the others place in the upper half of that tree (Fig. 1B). Considering the position of *Kalimeris* (Cass.) Cass., *Heteropappus altaicus* and *Psychrogeton persicus*, which are nested within the Eurasian *Aster* clade, our results confirm the hypothesis of Li & al. (2012) that Eurasian *Aster* is paraphyletic and polyphyletic.

Aster bachtiaricus, a woody perennial plant, is distinct from other *Aster* s.str. in the ITS tree and is isolated within the large polytomy at the crown of the *Astereae* (PP=0.96). It appears to be sister to the vast radiations that occurred in Australasia-Asia and in South and North America. On the *psbA-trnH* tree, *A. bachtiaricus* also has a distinct position and does not ally with *A. alpinus*.

Heteropappus — Molecular data support a close relationship between *Aster* s.str., *Heteropappus*, *Kalimeris*, *Rhinactinidia* Novopokr., *Rhynchospermum* Reinw. and *Sheareria* S. Moore, as has been found in previous studies (Ito & al. 1995, 1998; Noyes & Rieseberg 1999; Fiz & al. 2002; Brouillet & al. 2009a; Gao & al. 2009; Li & al. 2012). Previous studies (Ito & al. 1998; Li & al. 2012; Chen & al. 2011) showed that *Heteropappus* is embedded within *Aster*. Our ITS tree also supports the placement of *Heteropappus* in *Aster*. *Heteropappus altaicus* and *A. hayatae* form a subclade (PP=1.00, LBS=91, PBS=83) that is sister to the unresolved subclade including *A. ageratoides*, *A. koraiensis* and *Psychrogeton persicus* (PP=1.00, LBS=91, PBS=79).

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Eurasian *Erigeron* — Grierson & Rechinger (1982) divided *Erigeron* into *E.* subg. *Erigeron* and *E.* subg. *Trimorpha* (Cass.) Popov. *Erigeron* subg. *Trimorpha* (including *E. acris*) with an intermediate series of eligulate female florets differs from *E.* subg. *Erigeron* (including *E. caucasicus* Steven, *E. hyrcanicus* and *E. uniflorus*) without this kind of intermediate florets. In the ITS tree, *Erigeron* forms a strongly supported clade. *Erigeron acris* subsp. *lalehzaricus* Rech. f. is sister to *E. acris*. *Erigeron caucasicus* subsp. *venustus* and *E. uniflorus* subsp. *daenensis* (Vierh.) Rech. f. form a moderately supported clade, but *E. acris* subsp. *asadbarensis* (Vierh.) Rech. f., *E. acris* subsp. *pycnotrichus* (Vierh.) Grierson, *E. hyrcanicus* and *E. uniflorus* subsp. *elbursensis* (Boiss.) Rech. f. occupy unresolved positions. *Erigeron uniflorus* (AF046988) and *E. acris* (AF118496), obtained from GenBank are successive sisters to the Eurasian *Erigeron* clade. It is noteworthy that some differences exist between the *E. acris* and *E. uniflorus* accessions obtained from GenBank and those from Iran newly sequenced here. Despite the existence of morphological differences, there are no marked differences between the sequences of species that belong to *E.* subg. *Erigeron* and *E.* subg. *Trimorpha*. It has been suggested that a northern migration of *Erigeron* species from North America to Eurasia occurred during the Pleistocene when land bridges may have facilitated intercontinental migration (Huber & Leuchtmann 1992; Noyes 2000). The present study appears to support this hypothesis.

Conyzanthus — Nesom (1994) treated *Conyzanthus squamatus* as *Symphyotrichum squamatum* (Spreng.) G. L. Nesom. Nesom & Robinson (2007) included *Conyzanthus* within *Symphyotrichum* subg. *Symphyotrichum*. Our results confirm Nesom's view. In the ITS tree, *C. squamatus* is well nested with *Symphyotrichum* species. *Symphyotrichum squamatum* is sister to the other species introduced in Asia: *S. subulatum* (Michx.) G. L. Nesom. Considering the position of *S. ciliatum* and that the clade includes *S. squamatum* and *S. subulatum*, it seems that the species of *Symphyotrichum* migrated to Asia at least two distinct times.

Myriactis — The ITS tree shows a strong relationship between the currently analysed *Myriactis wallichii* and the Australian representative, *M. humilis* (PP=1.00, LBS=100, PBS=100). Morphological characters, including campanulate heads and absent pappus, seem to confirm the relationships.

Conclusions

It appears that, due to the level of homoplasy and many parallel mutations in the ITS dataset, we observe polytomies and low resolution in the respective tree. The SW Asian representatives of the *Astereae* used in this study form two main groups in the ITS tree. One is composed of *Lachnophylum*, *Chamaegeron* and the *Galatella* group near the base of the tree; the second comprises *Myriactis*, *Neobrachyactis*, *Psychrogeton*, *Aster*, *Heteropappus* and *Erigeron* as representatives of the large polytomy at the crown of the tree. The current study does not support the monophyly of *Aster*, *Galatella*, *Lachnophyllum* and *Psychrogeton* in their current status. *Aster bachtiaricus* seems to be excluded from *Aster*. More taxon sampling and DNA sequence data, especially of rapidly evolving genes, are definitely needed to get a clear-cut picture of phylogenetic relationships among some members of the tribe, in particular *Psychrogeton*.

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References

Andrus N., Tye A., Nesom G., Bogler D., Lewis C., Noyes R., Jaramillo P. & Francisco-Ortega J. 2009: Phylogenetics of *Darwiniothamnus* (*Asteraceae*: *Astereae*) – molecular evidence for multiple origins in the endemic flora of the Galápagos Islands. – J. Biogeogr. **36:** 1055–1069.

Beck J. B., Nesom G. L., Calie P. J., Baird G. I., Small R. L., Schilling E. E. 2004: Is subtribe *Solidagininae* (*Asteraceae*) monophyletic? – Taxon **53:** 691–698.

Brouillet L., Allen G. A., Semple J. C. & Ito M. 2001: ITS phylogeny of North American asters (*Asteraceae*: *Astereae*). – Botany 2001 Meeting, Albuquerque, N. Mex., Aug. 12–16.

Brouillet L., Anderberg A. A., Nesom G. L., Lowrey T. K. & Urbatsch L. E. 2009b: *Welwitschiella* is a member of the African subtribe *Grangeinae* (*Asteraceae Astereae*): a new phylogenetic position based on *ndhF* and ITS sequence data. – Kew Bull. **64:** 645–660.

Brouillet L., Lowrey T., Urbatsch L., Karaman-Castro V., Sancho G., Wagstaff S. & Semple J. C. 2009a: Phylogeny and evolution of the *Astereae* (*Compositae* or *Asteraceae*). – Pp. 449-490 in: Funk V. A., Susanna A., Stuessy T., Bayer R. (ed.), Systematics, Evolu-

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tion and Biogeography of the *Compositae*. – Vienna: IAPT.

Brouillet L., Urbatsch L. & Roberts R. P. 2004: *Tonestus kingii* and *T. aberrans* are related to *Eurybia* and the *Machaerantherinae* (*Asteraceae*: *Astereae*) based on nrDNA (ITS and ETS) data: reinstatement of *Herrickia* and a new genus. *Triniteurybia*. – Sida **21:** 889–900.

Burgess K. S., Fazekas A. J., Kesanakurti P. R., Graham S. W., Husband B. C., Newmaster S. G., Percy D. M., Hajibabaei M. & Barrett S. C. H. 2011: Discriminating plant species in a local temperate flora using the *rbcL+matK* DNA barcode. – Meth. Ecol. Evol. **2:** 333–340.

Chen Y. L., Chen Y. S., Brouillet L. & Semple J. C. 2011: *Astereae*. – Pp. 545–652 in: Wu Z. Y., Raven P. H. & Hong D. Y. (ed.), Flora of China **20–21** (*Asteraceae*). – Beijing: Science Press; St Louis: Missouri Botanical Garden Press.

Cross E. W., Quinn C. J. & Wagstaff S. J. 2002: Molecular evidence for the polyphyly of *Olearia* (*Astereae*: *Asteraceae*). – Pl. Syst. Evol. **235:** 99–120.

Douzery E. J. P., Pridgeon A. M., Kores P., Linder H. P., Kurzweil H. & Chase M. W. 1999: Molecular phylogenetics of *Diseae* (*Orchidaceae*): a contribution from nuclear ribosomal ITS sequences. – Amer. J. Bot. **86:** 887–899.

Doyle J. J. & Doyle J. L. 1987: A rapid DNA isolation procedure for small quantities of fresh leaf tissue. – Phytochem. Bull. **19:** 11–15.

Eastwood A., Gibby M. & Cronk Q. 2004: Evolution of St Helena arborescent *Astereae* (*Asteraceae*): relationships of the genera *Commidendrum* and *Melanodendron*. – Bot. J. Linn. Soc. **144:** 69–83.

Edgar R. C. 2004: Muscle: multiple sequence alignment with high accuracy and high throughput. – Nucleic Acids Res. **32:** 1792–1797.

Felsenstein J. 1985: Confidence limits on phylogenies: an approach using the bootstrap. – Evolution **38:** 783–791.

Field B. L., Houben A., Timmis J. N. & Leach C. R. 2006: Internal transcribed spacer sequence analyses indicate cytoevolutionary patterns within *Brachycome* Cass. (*Asteraceae*). – Plant Syst. Evol. **259:** 39–51.

Fiz O., Valcarcel V. & Vargas P. 2002: Phylogenetic position of Mediterranean *Astereae* and character evolution of daisies (*Bellis*, *Asteraceae*) inferred from nrDNA ITS sequences. – Mol. Phylogen. Evol. **25:** 157–171.

Funk V. A., Susanna A, Stuessy T. F. & Robinson H. 2009: *Compositae* metatrees: the next generation. – Pp. 747–777 in: Funk VA, Susanna A, Stuessy T., Bayer R. (ed.), Systematics, Evolution, and Biogeography of the *Compositae*. – Vienna: IAPT.

Gao T., Wang W. & Bayer R. J. 2009: Systematic position of the enigmatic genus *Sheareria* (*Asteraceae*) – evidence from molecular, morphological and cytological data. – Taxon **58:** 769–780.

Gao T., Yao H., Song J., Zhu Y., Liu C. & Chen S. 2010: Evaluating the feasibility of using candidate DNA barcodes in discriminating species of the large *Asteraceae* family. – B. M. C. Evol. Biol. **10-324:** 1–7.

Grierson A. 1967: The genus *Psychrogeton* (*Compositae*). – Notes Roy. Bot. Gard. Edinburgh **27:** 101–147.

Grierson A. & Rechinger K. H. 1982: *Compositae* V. *Astereae*. – In: Flora iranica: flora des iranischen Hochlandes und der umrahmenden Gebirge; Persien, Afghanistan, Teile von West-Pakistan, Nord-Iraq, Azerbaidijan, Turkmenistan **154.** – Graz: Akademische Druck- und Verlagsanstalt.

Hall T. A. 1999: BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. – Nucl. Acids Symp. Ser. **41:** 95–98.

Hong S. Y., Cho K. S. & Yoo K. O. 2012: Phylogenetic analysis of Korean native *Aster* plants based on internal transcribed spacer (US) sequences. – Korean J. Hort. Sci. Technol. **30:** 178–184.

Huber W. & Leuchtmann A. 1992: Genetic differentiation of the *Erigeron* species (*Asteraceae*) in the Alps: a case of unusual allozymic uniformity. – Pl. Syst. Evol. **183:** 1–16.

Ito M., Soejima A., Hasebe M. & Watanabe K. 1995: A chloroplast-DNA phylogeny of *Kalimeris* and *Aster*, with reference to generic circumscription. – J. Pl. Res. **108:** 93–96.

Ito M., Soejima A. & Nishino T. 1994: Phylogeny and speciation of Asian *Aster*. – Korean J. Pl. Taxon **24:** 133–143.

Ito M., Soejima A. & Watanabe K. 1998: Phylogenetic relationships of Japanese *Aster* (*Asteraceae*, *Astereae*) sensu lato based on chloroplast-DNA restriction site mutations. – J. Pl. Res. **111:** 217–223.

Karaman-Castro V. & Urbatsch L. E. 2009: Phylogeny of *Hinterhubera* group and related genera (*Hinterhuberinae*: *Astereae*) based on the nrDNA ITS and ETS sequences. – Syst. Bot. **34:** 805–817.

Li W. P., Yang F. S., Jivkova T. & Yin G. S. 2012: Phylogenetic relationships and generic delimitation of Eurasian *Aster* (*Asteraceae*: *Astereae*) inferred from ITS, ETS and trnL-F sequence data. – Ann. Bot. **109:** 1341–1357.

Liu J. Q., Gao T. G., Chen Z. D. & Lu A. M. 2002: Molecular phylogeny and biogeography of the Qinghai–Tibet Plateau endemic *Nannoglottis* (*Asteraceae*). – Molec. Phylogen. Evol. **23:** 307–325.

Lowell E. U., Roberts R. P. & Karaman V. 2003: Phylogenetic evaluation of *Xylothamia*, *Gundlachia*, and related genera (*Asteraceae*, *Astereae*) based on ETS and ITS nrDNA sequence data. – Amer. J. Bot. **90:** 634–649.

Lowrey T. K., Quinn C. J., Taylor R. K., Chan R., Kimball R. T. & De Nardi J. C. 2001: Molecular and mor-

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phological reassessment of relationships within the *Vittadinia* group of *Astereae* (*Asteraceae*). – Amer. J. Bot. **88:** 1279–1289.

Markos S. & Baldwin B. G. 2001: Higher-level relationships and major lineages of *Lessingia* (*Compositae*, *Astereae*) based on nuclear rDNA internal and external transcribed spacer (ITS and ETS) sequences. – Syst. Bot. **26:** 168–183.

Morgan D. R. 1997: Reticulate evolution in *Machaeranthera* (*Asteraceae*). – Syst. Bot. **22:** 599–615.

Morgan D. R. 2003: nrDNA external transcribed spacer (ETS) sequence data, reticulate evolution, and the systematics of *Machaeranthera* (*Asteraceae*). – Syst. Bot. **28:** 179–190.

Nakamura K., Denda T., Kokubugata G., Forster P. I., Wilson G., Peng C. I, Yokota M. 2012: Molecular phylogeography reveals an antitropical distribution and local diversification of *Solenogyne* (*Asteraceae*) in the Ryukyu Archipelago of Japan and Australia. – Bot. J. Linn. Soc. **105:** 197–217.

Nesom G. L. 1994: Review of the taxonomy of *Aster* sensu lato (*Asteraceae*: *Astereae*), emphasizing the new world species. – Phytologia **77:** 141–297.

Nesom G. L. & Robinson H. 2007: *Astereae*. – Pp. 316–376 in: Kadereit J. W., Jeffrey C. (ed.), The families and genera of vascular plants **8**. Flowering Plants. Eudicots. *Asterales*. – Berlin: Springer.

Noyes R. D. 2000: Biogeographical and evolutionary insights on *Erigeron* and allies (*Asteraceae*) from ITS sequence data. – Pl. Syst. Evol. **220:** 93–114.

Noyes R. D. & Rieseberg L. R. 1999: ITS sequence data support a single origin for North American *Astereae* (*Asteraceae*) and reflect deep geographic divisions in *Aster* s.l. – Amer. J. Bot. **86:** 398–412.

Nylander J. A. A. 2004: MrModeltest v2. Program distributed by the author. – Uppsala: Evolutionary Biology Centre, Uppsala University.

Page D. M. 2005: TreeviewX: Tree drawing software for Apple Macintosh and Microsoft Windows, version 0.5.0. – Published at http://darwin.zoology.gla.ac.uk/~rpage/treeviewx/download.html

Posada D. & Buckley T. R. 2004: Model selection and model averaging in phylogenetics: advantages of AIC and Bayesian approaches over likelihood ratio tests. – Syst. Biol. **53:** 793–808.

Roberts R. P. & Urbatsch L. E. 2003: Molecular phylogeny of *Ericameria* (*Asteraceae*, *Astereae*) based on nuclear ribosomal 3´ ETS and ITS nrDNA sequence data. – Taxon **52:** 209–228.

Roberts R. P. & Urbatsch L. E. 2004: Molecular phylogeny of *Chrysothamnus* and related genera (*Asteraceae*, *Astereae*) based on nuclear ribosomal 3´ ETS and ITS nrDNA sequence data. – Syst. Bot. **29:** 199–215.

Ronquist F. & Huelsenbeck J. P. 2003: MrBayes 3: Bayesian phylogenetic inference under mixed models. – Bioinformatics **19:** 1572–1574.

Saito Y., Kokubugata G. & Möller M. 2007: Molecular evidence for a natural hybrid origin of *Doellingeria* ×*sekimotoi* (*Asteraceae*) using ITS and *matK* sequences. – Int. J. Plant Sci. **168:** 469–476.

Sancho G. & Karaman-Castro V. 2008: A phylogenetic study in American *Podocominae* (*Asteraceae*: *Astereae*) based on morphological and molecular data. – Syst. Bot. **33:** 762–775.

Sang T., Crawford D. J. & Stuessy T. F. 1995: Documentation of reticulate evolution in peonies (*Paeonia*) using internal transcribed spacer sequences of nuclear ribosomal DNA: implications for biogeography and concerted evolution. – Proc. Natl. Acad. Sci. U.S.A. **92:** 6813–6817.

Sang T., Crawford D. J. & Stuessy T. F. 1997: Chloroplast DNA phylogeny, reticulate evolution, and biogeography of *Paeonia* (*Paeoniaceae*). – Amer. J. Bot. **84:** 1120–1136.

Schilling E. E., Beck J. B., Calie P. J. & Small R. L. 2008: Molecular analysis of *Solidaster* cv. Lemore, a hybrid goldenrod (*Asteraceae*). – J. Bot. Res. Inst. Texas **2:** 7–18.

Schlaepfer D. R., Edwards P. J., Widmer A. & Billeter R. 2008: Phylogeography of native ploidy levels and invasive tetraploids of Solidago gigantea. – Molec. Ecol. **17:** 5245–5256.

Selliah S. & Brouillet L. 2008: Molecular phylogeny of the North American eurybioid asters (*Asteraceae*, *Astereae*) based on the nuclear ribosomal internal and external transcribed spacers. – Canad. J. Bot. **86:** 901–915.

Semple J. C., Heard S. B. & Brouillet L. 2002: Cultivated and Native Asters of Ontario (*Compositae*: *Astereae*). *Aster* L. (including *Asteromoea* Blume, *Diplactis* Raf. and *Kalimeris* Cass., *Callistephus* Cass., *Galatella* Cass., *Doelllingeria* Nees, *Oclemena* E. L. Greene, *Eurybia* (Cass.) S. F. Gray, *Canadanthus* Nesom, and *Symphyotrichum* Nees (including *Virgulus* Raf.). – Univ. Waterloo Biol. Ser. **41:** 1–134.

Silvestro D. & Michalak I. 2012: raxmlGUI: a graphical front-end for RAxML. – Org. Divers. Evol. **12:** 335–337.

Strijk J. S, Noyes R. D., Strasberg D., Cruaud C., Gavory F., Chase M. W, Abbott R. J, Christophe T. 2012: In and out of Madagascar: dispersal to peripheral islands, insular speciation and diversification of Indian Ocean daisy trees (*Psiadia*, *Asteraceae*) – PLoS ONE **7:** 1–17.

Swofford D. L. 2002: PAUP*: Phylogenetic analysis using parsimony (*and other methods). Ver. 4.0b10. – Sunderland: Sinauer Associates.

Tamamschjan S. G. [inter alios] 1959: *Astereae* Cass. – Pp. 24–290 in: Shishkin B. K. (ed.), Flora SSSR **25**. – Moskva & Leningrad: Izdatelstvo Akademii Nauk SSSR.

Tate J. A. & Simpson B. B. 2003: Paraphyly of *Tarasa* (*Malvaceae*) and diverse origins of the polyploid species. – Syst. Bot. **28:** 723–737.

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Thiers B. 2015+ [continuously updated]: Index herbariorum: a global directory of public herbaria and associated staff. – New York Botanical Garden: published at http://sweetgum.nybg.org/ih/ [accessed 26 Jan 2015].

Urbatsch L. E., Roberts R. P. & Karaman V. 2003: Phylogenetic evaluation of *Xylothamia*, *Gundlachia*, and related genera (*Asteraceae*, *Astereae*) based on ETS and ITS nrDNA sequence data. – Amer. J. Bot. **90:** 634–649.

Vaezi J. & Brouillet L. 2009: Phylogenetic relationships among diploid species of Symphyotrichum (*Asteraceae*: *Astereae*) based on two nuclear markers, ITS and GAPDH. – Molec. Phylogen. Evol. **51:** 540–553.

Wagstaff S. J. & Breitwieser I. 2002: Phylogenetic relationships of New Zealand *Asteraceae* inferred from ITS sequences. – Pl. Syst. Evol. **231:** 203–224.

Wagstaff S. J., Breitwieser I. & Ito M. 2011: Evolution and biogeography of *Pleurophyllum* (*Astereae*, *Asteraceae*), a small genus of megaherbs endemic to the subantarctic islands. – Amer. J. Bot. **98:** 62–75.

Watanabe K., Kosuge K., Shimamura R., Konishi N. & Taniguchi K. 2006: Molecular systematics of Australian *Calotis* (*Asteraceae*: *Astereae*). – Austral. Syst. Bot. **19:** 155–168.

White T. J., Bruns T., Lee S. & Taylor J. 1990: Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. – Pp. 315–322 in: Innis M., Gelfand D., Sninsky J. & White T. (ed.), PCR protocols: a guide to methods and applications. – San Diego: Academic Press.

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Appendix

The taxa included in the nrDNA ITS and *psbA-trnH* analyses are listed in this Appendix. For each sequence, a voucher or the source together with its GenBank accession number are cited. Herbarium codes are according to Thiers (2015+). A dash "–" indicates that a sequence was not available in GenBank.

Taxon	**Voucher** (new sequence in this study)	**Source** (sequence obtained from GenBank)	**GenBank accession no. nrDNA ITS**	**GenBank accession no. cpDNA *psbA-trnH***
Achillea millefolium L.		Noyes & Rieseberg (1999)	AF046939	–
Amellus strigosus (Thunb.) Less.		Noyes & Rieseberg (1999)	AF046942	–
Anaphalis margaritacea (L.) Benth. & Hook. f.		Noyes & Rieseberg (1999)	AF046937	–
Aster ageratoides Turcz.		Hong & al. (2012)	HQ154047	–
Aster alpinus L.	Iran: *Wendelbo & Assadi 27775* (TARI)		LC027372	LC027423
Aster amellus L.	Turkey: *Davis 47617* (TARI)		LC027373	–
Aster amellus		Noyes & Rieseberg (1999)	AF046961	–
Aster asteroides (DC.) Kuntze		Li & al. (2012)	JN543841	–
Aster bachtiaricus Mozaff.	Iran: *Mozaffarian 57812* (TARI)		LC027374	LC027424
Aster diplostephioides (DC.) Benth. ex C. B. Clarke		Li & al. (2012)	JN543847	–
Aster flaccidus Bunge		Li & al. (2012)	JN543844	–
Aster hayatae H. Lév. & Vaniot		Hong & al. (2012)	HQ154041	–
Aster hispidus Thunb.		Hong & al. (2012)	HQ154044	–
Aster koraiensis Nakai		Hong & al. (2012)	HQ154046	–
Aster maackii Regel		Hong & al. (2012)	HQ154042	–
Aster rugulosus Maxim.		Saito & al. (2007)	AB259330	–
Aster scaber Thunb.		Hong & al. (2012)	HQ154049	–
Aster spathulifolius Maxim.		Hong & al. (2012)	HQ154050	–
Aster tataricus L. f.		Chen & Han (unpublished)	FJ980359	–
Aster tataricus		Hong & al. (2012)	HQ154046	–
Aster yomena (Kitam.) Honda		Hong & al. (2012)	HQ154048	–
Baccharis neglecta Britton		Morgan (1997)	U97604	–
Bellis annua L.		Fiz & al. (2002)	AF490579	–
Bellis perennis L.		Noyes & Rieseberg (1999)	AF046950	–
Blumea brevipes (Oliv. & Hiern.) Wild		Noyes & Rieseberg (1999)	AF046936	–
Brachyscome breviscapis C. R. Carter		Shimamura & Watanabe (unpublished)	AB435100	–
Brachyscome heterodonta DC.		Noyes & Rieseberg (1999)	AF046955	–
Calendula officinalis L.		Noyes & Rieseberg (1999)	AF046938	–
Callistephus chinensis (L.) Nees		Brouillet & al. (2009b)	FJ457947	–
Calotis cymbacantha F. Muell.		Watanabe & al. (2006)	AB196610	–
Calotis erinacea Steetz		Watanabe & al. (2006)	AB196609	–
Calotis hispidula (F. Muell.) F. Muell.		Watanabe & al. (2006)	AB196597	–
Calotis inermis Maiden & Betche		Watanabe & al. (2006)	AB196599	–
C*alotis squamigera* C. T. White		Watanabe & al. (2006)	AB196598	–
Chamaegeron asterellus (Bornm.) Botsch.	Iran: *Mirtadzadini 1234* (Shahid Bahonar University of Kerman)		LC027375	–
Chamaegeron bungei (Boiss.) Botsch.	Iran: *Faghihinia & Hojjat 27968* (FUMH)		LC027376	–
Chamaegeron keredjensis (Bornm. & Gauba) Grierson	Iran: *Assadi & Akhani 61185* (TARI)		LC027378	–
Chamaegeron keredjensis	Iran: *Bothmer & Buttler 1927* (TARI)		LC027377	–
Chamaegeron oligocephalus Schrenk	Iran: *Ayatollahi & Zangooei 14922* (FUMH)		LC027380	–

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Chamaegeron oligocephalus	Iran: *Emadzadeh & al. 37036* (FUMH)		LC027379	–
Chiliotrichum diffusum (G. Forst.) Kuntze		Noyes & Rieseberg (1999)	AF046945	–
Commidendrum robustum (Roxb.) DC.		Noyes & Rieseberg (1999)	AF046943	–
Conyza bonariensis (L.) Cronquist		Noyes (2000)	AF118513	–
Conyza canadensis (L.) Cronquist		Noyes & Rieseberg (1999)	AF046987	–
Conyza gouanii (L.) Willd.		Noyes & Rieseberg (1999)	AF046948	–
Conyzanthus squamatus (Spreng.) Tamamsch. (*Symphyotrichum squamatum* (Spreng.) G. L. Nesom)	Iran: *Akramian 38407* (FUMH)		LC027381	–
Crinitina linosyris (L.) Soják		Noyes & Rieseberg (1999)	AF046949	–
Crinitina linosyris		Karaman-Castro & Urbatsch (2009)	DQ478987	–
Crinitina villosa (L.) Soják	Turkey: *Davis & Hedge 32617* (TARI)		LC027382	–
Crinitina villosa	Iran: *Zehzad & Farbodnia 4018* (TARI)		LC027383	–
Dichrocephala integrifolia (L. f.) Kuntze	Iran: *Bazghandi 34389* (FUMH)		LC027384	–
Doellingeria infirma (Michx.) Greene		Selliah & Brouillet (2008)	EU200188	–
Doellingeria umbellata (Mill.) Nees		Noyes & Rieseberg (1999)	AF046966	–
Erigeron acris L.	Iran: *Joharchi & Behroozian 44170* (FUMH)		LC027385	–
Erigeron acris		Noyes (2000)	AF118496	–
Erigeron acris subsp. *asadbarensis* (Vierh.) Rech. f.	Iran: *Assadi & Mozaffarian 33231* (TARI)		LC027386	–
Erigeron acris subsp. *lalehzaricus* Rech. f.	Iran: *Assadi 83177* (TARI)		LC027387	–
Erigeron acris subsp. *pycnotrichus* (Vierh.) Grierson	Iran: *Wendelbo & Assadi 13317* (TARI)		LC027388	–
Erigeron annuus (L.) Pers.		Schlaepfer & al. (2008)	–	EU337691
Erigeron caucasicus subsp. *venustus* (Botsch.) Grierson	Iran: *Mozaffarian 87576* (TARI)		LC027389	–
Erigeron hyrcanicus Bornm. & Vierh.	Iran: *Assadi & Maassoumi 51232* (TARI)		LC027390	–
Erigeron rhizomatus Cronquist		Noyes & Rieseberg (1999)	AF046992	–
Erigeron strigosus Muhl. ex Willd.		Burgess & al. (2011)	–	HQ596691
Erigeron uniflorus L.		Noyes & Rieseberg (1999)	AF046988	–
Erigeron uniflorus subsp. *daenensis* (Vierh.) Rech. f.	Iran: *Mozaffarian 58164* (TARI)		LC027391	–
Erigeron uniflorus subsp. *elbursensis* (Boiss.) Rech. f.	Iran: *Mozaffarian 66259* (TARI)		LC027392	–
Eurybia radula (Aiton) G. L. Nesom		Selliah & Brouillet (2008)	EU200207	–
Felicia aethiopica (Burm. f.) Grau		Noyes & Rieseberg (1999)	AF046941	–
Galatella coriacea Novopokr.		Fiz & al. (2002)	AF494003	–
Galatella litvinovii Novopokr.	Iran: *Zangooei & Arjmandi 39249* (FUMH)		LC027393	LC027425
Galatella punctata (Waldst. & Kit.) Nees	Iran: *Ghahreman & Mozaffarian 17600* (TUH)		LC027394	–
Galatella scoparia (Kar. & Kir.) Novopokr.	Kazakhstan: *Krashninko & al. 3257* (TARI)		LC027395	–
Grangea maderaspatana (L.) Poir.		Noyes & Rieseberg (1999)	AF046951	–
Guynesomia scoparia (Phil.) Bonif. & G. Sancho		Karaman-Castro & Urbatsch (2009)	DQ479035	–
Heteropappus altaicus (Willd.) Novopokr.	Iran: *Joharchi 15545* (FUMH)		LC027396	–
Hinterhubera laseguei Wedd.		Karaman-Castro & Urbatsch (2009)	DQ479015	–
Isoetopsis graminifolia Turcz.		Brouillet & al. (2009b)	GU226433	–
Kalimeris indica (L.) Sch. Bip.		Gao & al. (2010)	GU724288	–

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Kalimeris integrifolia Turcz. ex DC.		Noyes & Rieseberg (1999)	AF046960	–
Kalimeris pinnatifida (Maxim.) Kitam.		Karaman-Castro & Urbatsch (2009)	DQ478988	
Keysseria maviensis (H. Mann.)		Karaman-Castro & Urbatsch (2009)	DQ479036	–
Kippistia suaedifolia F. Muell.		Cross & al. (2002)	AF497660	–
Lachnophyllum gossypinum Bunge	Iran: *Faghihinia 27848* (FUMH)		LC027397	–
Lachnophyllum noeanum Boiss.	Iran: *Fattahi & Mirabdali 2783* (TARI)		LC027398	–
Lagenophora pumila (G. Forst.) Cheeseman		Karaman-Castro & Urbatsch (2009)	DQ479037	–
Lessingia pectinata Greene		Markos & Baldwin (2001)	AF251598	–
Mairia hirsuta DC.		Brouillet & al. (2009b)	FJ457929	–
Microgyne trifurcata Less.		Sancho & Karaman-Castro (2008)	EF151482	–
Myriactis humilis Merr.		Noyes & Rieseberg (1999)	AF046959	–
Myriactis wallichii Less.	Iran: *Zangooei & Alvani 15885* (FUMH)		LC027399	–
Nannoglottis delavayi (Franch.) Y. Ling & Y. L. Chen		Liu & al. (2002)	AY017167	–
Nannoglottis macrocarpa Y. Ling & Y. L. Chen		Liu & al. (2002)	AY017166	–
Nannoglottis ravida (C. Winkl.) Y. L. Chen		Liu & al. (2002)	AY017160	–
Neobrachyactis roylei (DC.) Brouillet	Iran: *Mirtadzadini 1238* (Shahid Bahonar University of Kerman)		LC027400	–
Nidorella polycephala DC.		Karaman-Castro & Urbatsch (2009)	DQ478999	–
Nidorella resedifolia DC.		Noyes & Rieseberg (1999)	AF046952	–
Olearia argophylla (Labill.) F. Muell. ex Benth.		Noyes & Rieseberg (1999)	AF046944	–
Olearia ballii (F. Muell.) Hemsl.		Cross & al. (2002)	AF497662	–
Olearia chrysophylla Benth.		Cross & al. (2002)	AF497710	–
Olearia ciliata (Benth.) Benth.		Cross & al. (2002)	AF497667	–
Olearia lasiophylla Lander		Cross & al. (2002)	AF497652	–
Olearia microphylla (Vent.) Maiden & Betche		Cross & al. (2002)	AF497671	–
Olearia ramulosa (Labill.) Benth.		Cross & al. (2002)	AF497681	–
Olearia tomentosa (J. C. Wendl.) DC.		Cross & al. (2002)	AF497650	–
Parastrephia phyliciformis (Meyen) Cabrera		Karaman-Castro & Urbatsch (2009)	DQ479023	–
Parastrephia quadrangularis (Meyen) Cabrera		Karaman-Castro & Urbatsch (2009)	DQ479024	–
Parastrephia teretiuscula (Kuntze) Cabrera		Karaman-Castro & Urbatsch (2009)	DQ479026	–
Printzia polifolia (L.) Hutch		Brouillet & al. (2009b)	FJ457927	–
Psychrogeton aellenii (Rech. f.) Grierson	Iran: *Hojjat & Zangooei 29239* (FUMH)		LC027401	LC027426
Psychrogeton aellenii	Iran: *Rafeie & Zangooei 27396* (FUMH)		LC027402	–
Psychrogeton alexeenkoi Krasch.	Iran: *Assadi 83200* (TARI)		LC027403	–
Psychrogeton amorphoglossus (Boiss.) Novopokr.	Iran: *Mozaffarian 66260* (TARI)		LC027404	LC027427
Psychrogeton amorphoglossus	Iran: *Mozaffarian & Assadi 40873* (TARI)		LC027405	LC027428
Psychrogeton aucheri (DC.) Grierson	Iran: *Assadi 75131* (TARI)		LC027409	LC027429
Psychrogeton aucheri	Iran: *Joharchi & Znagooei 15737* (FUMH)		LC027406	–
Psychrogeton aucheri	Iran: *Mozaffarian 48524* (TARI)		LC027410	LC027431

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Psychrogeton aucheri	Iran: *Mozaffarian 58130* (TARI)		LC027408	LC027430
Psychrogeton aucheri	Iran: *Mozaffarian 64992* (TARI)		LC027407	–
Psychrogeton cabulicus Boiss.	Iran: *Assadi & Mozaffarian 35757* (TARI)		LC027412	–
Psychrogeton cabulicus	Iran: *Zangooei & Hosseinzadeh 24477* (FUMH)		LC027411	LC027432
Psychrogeton chionophilus (Boiss.) Krasch.	Iran: *Safaian 245* (TARI)		LC027413	–
Psychrogeton nigromontanus (Boiss. & Buhse) Grierson	Iran: *Bothmer & Buttler 1905* (TARI)		LC027414	LC027433
Psychrogeton obovatus (Benth.) Grierson	Iran: *Mirtadzadini 1200* (Shahid Bahonar University of Kerman)		LC027417	–
Psychrogeton obovatus	Iran: *Mozaffarian 42160* (TARI)		LC027415	LC027434
Psychrogeton obovatus	Iran: *Mozaffarian 63877* (TARI)		LC027416	–
Psychrogeton persicus (Boiss.) Grierson	Iran: *Joharchi 33846* (FUMH)		LC027419	–
Psychrogeton persicus	Iran: *Mozaffarian 48643* (TARI)		LC027418	LC027435
Psychrogeton pseudoerigeron (Bunge) Novopokr. ex Nevski	Iran: *Edmonson 1288* (TARI)		LC027420	–
Psychrogeton pseudoerigeron	Iran: *Rafei & al. 2608* (FUMH)		LC027421	LC027436
Rhinactinidia eremophila (Bunge) Novopokr. ex Botsch		Li & al. (2012)	JN543727	–
Rhinactinidia limoniifolia (Less.) Novopokr. ex Botsch		Li & al. (2012)	JN543724	–
Rhynchospermum verticillatum Reinw.		Fiz & al. (2002)	AF494001	–
Sheareria nana S. Moore		Gao & al. (2009)	AY572953	–
Solenogyne dominii L. G. Adams		Nakamura & al. (2012)	AB604758	–
Solidago gigantea Aiton		Schlaepfer & al. (2008)	–	EU337305
Solidago virgaurea L.		Schilling & al. (2008)	EU125378	–
Symphyotrichum boreale (Torr. & A. Gray) Á. Löve & D. Löve		Vaezi & Brouillet (2009)	EU781390	–
Symphyotrichum ciliatum (Ledeb.) G. L. Nesom		Vaezi & Brouillet (2009)	EU781410	–
Symphyotrichum depauperatum (Fernald) G. L. Nesom		Selliah & Brouillet (2008)	EU200226	–
Symphyotrichum subulatum (Michx.) G. L. Nesom		Vaezi & Brouillet (2009)	EU781409	–
Symphyotrichum vahlii (Gaudich.) G. L. Nesom		Eastwood & al. (2004)	AY193804	–
Tripolium pannonicum (Jacq.) Dobrocz.	Iran: *Assadi & Akhani 61625* (TARI)		LC027422	LC027437
Tripolium pannonicum		Brouillet & al. (2009b)	FJ457946	–
Tripolium pannonicum		Hong & al. (2012)	HQ154043	–
Ursinia nana DC.		Noyes & Rieseberg (1999)	AF046940	–

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