

Relationships and generic delimitation of Eurasian genera of the subtribe Asterinae (Astereae, Asteraceae) using molecular phylogeny of ITS

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Abstract: The subtribe Asterinae (Astereae, Asteraceae) includes highly variable, often polyploid species. Recent findings based on molecular methods led to revision of its volume. However, most of these studies lacked species from Eurasia, where a lot of previous taxonomic treatments of the subtribe exist. In this study we used molecular phylogenetics methods with internal transcribed spacer (ITS) as a marker to resolve evolutionary relations between representatives of the subtribe Asterinae from Siberia, Kazakhstan, and the European part of Russia. Our reconstruction revealed that a clade including all Asterinae species is paraphyletic. Inside this clade, there are species with unresolved basal positions, for example *Erigeron flaccidus* and its relatives. Moreover, several well-supported groups exist: group of the genera *Galatella*, *Crinitaria*, *Linosyris*, and *Tripolium*; group of species of North American origin; and three related groups of Eurasian species: typical Eurasian asters, Heteropappus group (genera *Heteropappus*, *Kalimeris*), and Asterothamnus group (genera *Asterothamnus*, *Rhinactinidia*). The results obtained confirm previous findings with notable exceptions and add important phylogenetic data for 15 previously unstudied species, 11 of which are narrow Asian endemics. Conclusions of this study clarify relations between species and genera within this subtribe. Further molecular phylogenetics analyses of the remaining Eurasian species are needed for taxonomic revision and segregation of Asterinae into several subtribes.

Key words: Asteraceae, Eurasian Asterinae, generic delimitation, ITS, molecular phylogeny

1. Introduction

Species of the subtribe Asterinae (Cass.) Dumort. (tribe Astereae, family Asteraceae) are characterized by a high level of morphological plasticity and polyploidy, and a wide distribution range. According to Nesom and Robinson (2007), this subtribe includes 13 genera and about 270 species. However, many open taxonomic questions remain on species diagnosis, generic delimitation, and even the volume of the subtribe itself.

Brouillet et al. (2009) analyzed the phylogeny of the tribe Astereae worldwide and summarized all studies published before 2009. As a result, some of the genera previously belonging to the subtribe Asterinae Cass. were transferred to other subtribes, for example the large genera *Erigeron* L. (circa 400 species of North American origin) and *Symphotrichum* Nees (92 species, mostly growing in the New World). Unfortunately, their study focused on North America and Africa, but lacked Eurasian Asterinae—no more than 5%–8% of all species growing in

Europe and Asia were included in the construction and analysis of phylogenetic trees.

A comprehensive list of circa 200 Eurasian species of the subtribe Asterinae Cass. is available only from large regional floral summaries (covering, for example, territories of Europe, the ex-Soviet Union, the European part of Russia, Iran, Kazakhstan, China, Central and Middle Asia, or Siberia) (Botschantzev, 1959; Tamamschyan, 1959; Tzvelev, 1959, 1990, 1994; Terekhova, 1965; Grierson, 1975, 1982; Merxmuller et al., 1976; Ling et al., 1985; Ito and Soejima, 1995; Korolyuk, 2007) where treatments for different genera were done at different times by different authors mostly using only morphological criteria. These factors raise the need for a complete revision of the subtribe for the vast territories of Eurasia.

Studies of interspecific and intergeneric relations in Asian Asterinae were restricted to morphometric and cytological approaches until recently. When molecular data were used, some groups with only margins of habitats

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in Russia were studied (Ito et al., 1998). In a recent study, Li et al. (2012) performed phylogenetic analysis of internal and external transcribed spacers (ITS and ETS) of nuclear ribosomal DNA and plastid trnL-F sequences was performed for 62 species of Eurasian *Aster* L. s. l. (sensu lato) and several related genera. The material for this study was obtained from the vast territory of China, which partially filled in the gap in phylogeny of Asian asters and clarified the borders between generic segregates delimited from the genus *Aster* s.l.

We aim to study evolutionary relationships of subtribe Asterinae species (sensu authors of old Eurasian literature, before Nesom (1994a, 1994b), Nesom and Robinson (2007)) of Siberia, Kazakhstan, and the European part of Russia using molecular systematics. Our goal is to expand existing phylogenies with species previously unstudied by molecular methods, to examine intraspecific variation of species with wide distribution, and review and critically analyze existing approaches to genus and subtribe delimitation. As a phylogenetic marker we chose the internal transcribed spacer (ITS) located between 18S and 5.8S and 28S RNA genes in ribosomal gene cluster, which allows for sufficient resolution and extensive comparison with previous effort. However, the revision of current suprageneric taxonomy of Eurasian Asterinae will only be possible with the addition of phylogenetic data on the species that still remain unstudied.

2. Materials and methods

2.1. Taxon sampling

Generic circumscriptions and nomenclature of Eurasian Astereae Cass. are treated by Nesom (1994b), Tzvelev (1994), Korolyuk (1999, 2007), and Nesom and Robinson (2007).

Phylogenetic studies of this taxonomic level usually include one sample per species, but we depart from this practice for some species to account for their 1) wide geographical distribution and 2) morphological variability, which some authors treat as narrow taxon borders.

A total of 40 voucher specimens were collected from Russia and Kazakhstan and examined for sequence variations in ITS (GenBank accession numbers are given in the Table). The voucher specimens of all accessions were identified and deposited in the Herbarium of the Central Siberian Botanical Garden SB RAS (NS) (see the Table). In total, we analyzed 28 species of the subtribe Asterinae: 10 species of *Galatella* Call. s.l., 3 species each of the genera *Heteropappus* Less., *Erigeron* L., and *Aster* L.; 2 species of *Asterothamnus* Novopokr.; one species per genus of *Phalacrocoma* Cass., *Eurybia* (Cass.) Gray S. F. (= *Aster sibirica* L.), *Kalimeris* Cass., *Brachyactis* Ledeb., *Tripolium* Nees., and *Rhinactinidia* Novopokr.; the monotype genus *Arctogeron* DC. (*A. gramineum* (L.) DC.) from Russia,

Mongolia, and Kazakhstan; and one outgroup species, *Eupatorium cannabinum* L. For 15 species ITS sequences are studied for the first time.

To supplement this dataset, 62 previously published ITS sequences were downloaded from GenBank (Noyes and Reiseberg, 1999; Fiz et al., 2002; Brouillet et al., 2009; Karaman-Castro and Urbatsch, 2009; Li et al., 2012), including 58 samples of the tribe Astereae and 3 samples of 3 other tribes.

All sequence samples used in this study are summarized in the Table.

2.2. DNA extraction, polymerase chain reaction (PCR), and sequencing

Silica gel-dried leaf material was frozen in liquid nitrogen and ground to a powder. Total DNA was extracted using the 2× CTAB method (Doyle and Doyle, 1987). The extracted DNA was dissolved in 100 µL of TE buffer and used for polymerase chain reaction (PCR). The primers used to amplify DNA fragment, containing ITS-1 5.8S RNA gene and ITS-2, were ITS5 GAAAGTAAAAGTCGTAACAAGG and ITS4 TCCTCCGCTTATTGATATGC (White et al., 1990). Primers were synthesized by Beagle Co Ltd (St. Petersburg, Russia). The PCR was conducted in a total reaction volume of 50 µL, containing 0.2 mM dNTP mixture, 1.5 U of Taq polymerase (Beagle Co Ltd), enzyme buffer provided by this company, 5 pmol of each primer, and 1 µL of DNA. The PCR was performed under the following conditions: 94 °C – 5 min, (15 seq at 94 °C, 30 seq at 55 °C, 40 seq at 72 °C) 40 cycles, 72 °C – 5 min.

Fragments were separated in 1% agarose gel on 1× TAE buffer. A 100 bp + 1.5 kb ladder (Sibenzyme, Novosibirsk, Russia) was used as molecular weight marker. Specific PCR products were isolated from the gel, filtered through glass wool (LLC “Sigma-Aldrich Rus”, Moscow, Russia), ethanol precipitated, dissolved in water, and sequenced in both directions using the standard methods of the BigDye™ Deoxy Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA, USA) using the same primers as above on an ABI 3100 Genetic Analyzer (Applied Biosystems).

2.3. Sequence alignment and phylogenetic analysis

The data matrix for ITS comprised a total of 102 accessions from 52 species of 25 genera. Data were aligned using the PRANK algorithm (Löytynoja and Goldman, 2005), which was shown to outperform other algorithms in highly gapped alignment as in case of ITS (Nagy et al., 2012). The resulting alignment is 742 bp in length, of which only 311 are conserved. Mean pairwise distance within the tribe is 0.113. A total of 138 indels were coded to be used in Bayesian inference (BI) with the simple algorithm published in Simmons and Ochoterena (2000) as implemented in SeqState software (Muller, 2005).

Table. Taxon sampling, voucher specimens, and GenBank accessions.

GenBank	Taxonomy*	Location, voucher
KJ711879	<i>Arctogeron gramineum</i> (L.) DC. (= <i>Aster gramineus</i> (L.) Kom.)	Russia, Khakassia, Chalgis-Tag village, Korolyuk A., Korolyuk E., NS
KJ711873	<i>Aster alpinus</i>	Kazakhstan, piedmonts of Ulytau Mts., Korolyuk A., Korolyuk E., NS
KJ711889	<i>Aster alpinus</i>	Russia, Altai Republic, terrace of Ustyd river, Korolyuk E., NS
KJ711903	<i>Aster maackii</i>	Russia, Primorsky Krai, Peter the Great Bay, cape Gamov, Shaulo D., NS
KJ711907	<i>Aster tataricus</i>	Russia, Chita Oblast, Borzja village, Adun-Chalon Mts., Korolyuk A., NS
KJ711904	<i>Asterothamnus poliifolius</i>	Mongolia, Khovd aimak, Mankhan somon, Korolyuk A., NS
KJ711881	<i>Asterothamnus heteropappoides</i>	Russia, Tuva Republic, ridge Uyuk, Artemov I., Shaulo D., NS
KJ711891	<i>Brachyactis ciliata</i> (Ledeb.) Ledeb. (= <i>Symphytotrichum ciliatum</i> (Ledeb.) G.L.Nesom)	Russia, Altai Krai, Severka village, 140 m, Korolyuk E., NS
KJ711868	<i>Erigeron altaicus</i>	Russia, Altai Republic, Dzhazator village, 1807 m, Korolyuk E., NS
KJ711877	<i>Erigeron altaicus</i>	Russia, Altai Republic, Dzhazator village, 1807 m, Korolyuk E., NS
KJ711901	<i>Erigeron elongatus</i>	Russia, Altai Republic, Koksu river, 1640 m, Korolyuk E., NS
KJ711906	<i>Erigeron elongatus</i>	Russia, Altai Republic, headwater of Multa river, 2305 m, Artemov I., NS
KJ711875	<i>Erigeron flaccidus</i>	Russia, Altai Republic, Dzhazator village, 1807 m, Korolyuk E., NS
KJ711900	<i>Eupatorium cannabinum</i>	Russia, Krasnodar Krai, Sukko village, Korolyuk A., Korolyuk E., NS
KJ711874	<i>Eurybia sibirica</i>	Russia, Magadan Oblast, Gadkya village, Ovchinnikova S., Khan I., NS
KJ711905	<i>Galatella altaica</i>	Kazakhstan, East Kazakhstan Oblast, Ardynka village, Korolyuk A., NS
KJ711880	<i>Galatella angustissima</i>	Russia, Novosibirsk Oblast, Evsino village, 200 m, Korolyuk E., NS
KJ711882	<i>Galatella biflora</i>	Russia, Novosibirsk, 155 m, Korolyuk E., NS
KJ711883	<i>Galatella biflora</i>	Russia, Novosibirsk Oblast, Evsino village, 200 m, Korolyuk E., NS
KJ711870	<i>Galatella biflora</i>	Russia, Altai Republic, Dzhazator village, 1807 m, Korolyuk E., NS
KJ711867	<i>Galatella divaricata</i>	Kazakhstan, piedmonts of Ulytau Mts., Korolyuk A., NS
KJ711898	<i>Galatella dracunculoides</i>	Russia, Krasnodar Krai, Sukko village, Korolyuk A., Korolyuk E., NS
KJ711878	<i>Galatella hauptii</i>	Russia, Altai Republic, 110 km road Kosch-Agach - Dzhazator, 1894 m, Korolyuk E., NS
KJ711899	<i>Galatella linosyris</i>	Russia, Krasnodar Krai, Sukko village, Korolyuk A., Korolyuk E., NS
KJ711886	<i>Galatella punctata</i>	Russia, Novosibirsk Oblast, Bugotakskie hills, Korolyuk E., NS
KJ711894	<i>Galatella tatarica</i>	Kazakhstan, Dzhayrem village, Korolyuk A., Korolyuk E., NS
KJ711902	<i>Galatella tatarica</i>	Kazakhstan, East-Kazakhstan Oblast, Kurchum ridge, Lomonosova M., NS
KJ711872	<i>Galatella villosa</i>	Kazakhstan, piedmonts of Ulytau Mts., Korolyuk A., Korolyuk E., NS
KJ711895	<i>Galatella villosa</i>	Kazakhstan, piedmonts of Ulytau Mts., Korolyuk A., Korolyuk E., NS
KJ711876	<i>Heteropappus altaicus</i>	Russia, Altai Republic, confluence of Chuya and Katun rivers, Korolyuk E., NS
KJ711887	<i>Heteropappus altaicus</i>	Russia, Chita Oblast, Dahurian Nature Reserve, Teeli gorge, Korolyuk A., Korolyuk E., NS
KJ711871	<i>Heteropappus altaicus</i>	Russia, Altai Republic, Kupchehen village, Korolyuk E., NS
KJ711884	<i>Heteropappus biennis</i>	Russia, Khakassia, Chernoe Ozero village, Cheremuschkina V., NS
KJ711888	<i>Heteropappus biennis</i>	Mongolia, Central Aimak, Bonuur sumon, Korolyuk E., NS

Table. (Continued).

GenBank	Taxonomy*	Location, voucher
KJ711885	<i>Heteropappus medius</i>	Russia, Altai Republic, Cherga village, 650 m, Korolyuk E., NS
KJ711890	<i>Kalimeris integrifolia</i> Turcz. (= <i>Aster pekinensis</i> Hance)	Russia, Chita Oblast, Solnechnaya village, Korolyuk A., NS
KJ711869	<i>Phalacrocoma strigosum</i>	Russia, Altayskiy Krai, Talmenka village, Korolyuk E., NS
KJ711893	<i>Rhinactinidia eremophila</i>	Russia, Tuva Republic, Tsagan-Shibetu ridge, Koge-Dove pass, Artemov I., NS
KJ711892	<i>Rhinactinidia eremophila</i> (Bunge) Novopokr. ex Botsch. subsp. <i>eremophila</i> Koroljuk	Russia, Altai Republic, terrace of Ustyd river, Korolyuk E., NS
KJ711896	<i>Tripolium vulgare</i>	Russia, Krasnodar Krai, Sukko village, Korolyuk A., Korolyuk E., NS
KJ711897	<i>Tripolium vulgare</i>	Russia, Rostov-na-Donu, Korolyuk E., NS
AF046939	<i>Achillea millefolium</i>	J. L. Hudson, Seedsman, Noyes R. D.
JN543817	<i>Aster alpinus</i>	China, Wulumuqi City, 2320 m
JN543709	<i>Aster altaicus</i> var. <i>millefolius</i>	China, Zhang County, 600 m
JN543742	<i>Aster amellus</i>	Bulgaria, Shumen, 400 m
JN543841	<i>Aster asteroides</i>	China, Daocheng County, 2780 m
JN543805	<i>Aster baccharoides</i>	China, Zhuhai City, 100 m
JN543838	<i>Aster brachytrichus</i>	China, Ichang City, 2800 m
JN543712	<i>Aster crenatifolius</i>	China, Maerkang City, 3200 m
JN543847	<i>Aster diplostephioides</i>	China, Jiuzhai County, 2600 m
JN543802	<i>Aster falcifolius</i>	China, Mt. Huping, 400 m
JN543739	<i>Aster fastigiatus</i>	China, Daqin City, 150 m
JN543844	<i>Aster flaccidus</i>	China, Wulumuqi City, 3700 m
JN315928	<i>Aster gramineus</i>	China, Wulanhaote City, 300 m
JN543721	<i>Aster incisus</i>	China, Tonghua County, 560 m
JN543715	<i>Aster indicus</i>	China, Changsha City, 80 m
JN543718	<i>Aster integrifolius</i>	China, Mudanjiang City, 360 m
JN543724	<i>Aster limoniifolius</i>	China, Wulumuqi City, 1800 m
JN543745	<i>Aster maackii</i>	China, Yichun City, 200 m
JN543730	<i>Aster piccolii</i>	China, Mei County, 300 m
JN543763	<i>Aster poliothamnus</i>	China, Zhang County, 500 m
JN543811	<i>Aster sampsonii</i>	China, Mt. Mang, 1100 m
JN315934	<i>Aster scaber</i>	China, Anshan City, 350 m
JN543766	<i>Aster sikuensis</i>	China, Lueyang County, 300 m
JN543748	<i>Aster tataricus</i>	China, Xinglong County, 400 m
JN543706	<i>Aster verticillatus</i>	China, Mt. Emei, 1200 m
JN543853	<i>Aster yunnanensis</i>	China, Kangding City, 3500 m
JN315930	<i>Asterothamnus centraliasiaticus</i>	China, Yinchuan City, 1630 m

Table. (Continued).

GenBank	Taxonomy*	Location, voucher
JN315929	<i>Asterothamnus fruticosus</i>	China, Wulumuqi City, 950 m
AF494007	<i>Bellis perennis</i>	Turkey, Balaban
AF046975	<i>Boltonia asteroides</i>	USA, Missouri, Henderson N. C.
AB196596	<i>Brachyscome decipiens</i>	-
JN315941	<i>Calendula officinalis</i>	China, Changsha, cultivated
JN315931	<i>Callistephus chinensis</i>	China, Anshan City, 340 m
DQ478984	<i>Canadanthus modestus</i>	-
AF046945	<i>Chiliotrichum diffusum</i>	Chile, Cape Horn Island, Prance G. T.
AF046987	<i>Conyza canadensis</i>	USA, Missouri, Ochs C.
DQ478987	<i>Crinitaria linosyris</i>	Austria, Seiller s.n (F)
AF046949	<i>Crinitaria linosyris</i>	Russia, Saratov, Skvortsov A. K.
JN315932	<i>Crinitina linosyris</i>	Bulgaria, Shumen, 400 m
AF046966	<i>Doellingeria umbellata</i>	USA, Michigan, Schmidt H. H.
JN315924	<i>Erigeron annuus</i>	China, Changsha City, 40 m
DQ478976	<i>Erigeron bellidiastrum</i>	-
JN315925	<i>Erigeron breviscapus</i>	China, Lijiang City, 2500 m
AF046988	<i>Erigeron uniflorus</i>	Sweden, Nancyf, Noyes R. D.
DQ479041	<i>Eucephalus glabratus</i>	USA, California, Siskiyou Co., Scott Mountains, Karaman V.
AY772421	<i>Eurybia sibirica</i>	-
FJ457937	<i>Felicia filifolia</i>	-
JN315935	<i>Galatella dahurica</i>	China, Mt. Aër, Nei Mongol, 400 m
JN315933	<i>Galatella villosa</i>	Bulgaria, Shumen, 400 m
JN315920	<i>Grangea maderaspatana</i>	China, Zhaoqing City, 200 m
DQ478979	<i>Grindelia nana</i>	-
AF046960	<i>Kalimeris integrifolia</i>	China, Jiangsu, Wei W.
DQ478988	<i>Kalimeris pinnatifida</i>	USA: S. Carolina: Columbia, Porter s.n.
DQ479031	<i>Madagaster madagascariensis</i>	Madagascar, Central Madagascar, Mt. Ibity, 1700 m, DuPuy B.
DQ479033	<i>Olearia ramulosa</i>	Australia, Victoria, Grampians National Park Area, 370 m, King R. M.
FJ457927	<i>Printzia polifolia</i>	-
JN543727	<i>Rhinactinidia eremophila</i>	China, Wulumuqi City, 2620 m
JN543703	<i>Sheareria nana</i>	China, Changsha City, 30m
JN315926	<i>Symphyotrichum novi belgii</i>	China, Beijing, cultivated
AF046979	<i>Symphyotrichum oblongifolium</i>	USA, Missouri, Brant A. E.
JN315927	<i>Symphyotrichum subulatum</i>	China, Changsha City, 40 m
JN315937	<i>Tripolium vulgare</i>	Bulgaria, Larna, 1 m

*Species authorship is given only for samples deposited in GenBank under synonymous names indicated inside brackets.

The optimal substitution model GTR+G+I was chosen with jModelTest 2.1.4 (Darriba et al., 2012). BI was run in MrBayes 3.2.2 (Ronquist and Huelsenbeck, 2003). Run parameters were as follows: indels treated as binary characters (presence/absence) with empirical frequencies, 4 MCMC chains, MCMC chain length 10 million generations, sampling every 1000 generations, burn-in 25%. Maximum likelihood (ML) phylogenetic analysis was run in raxmlGUI (Silvestro and Michalak, 2012) with 1000 bootstrap replicates.

3. Results

We used molecular phylogenetic analysis of an ITS sequence to reveal the evolutionary relationship among the genera placed in the subtribe Asterinae. A recent study

by Nagy et al. (2012) showed that usage of insertion and deletion (indel) information in DNA alignment leads to higher resolution power of phylogenetic analyses. Indels are better resolved with specialized alignment algorithms like MAFFT and PRANK. As ITS is a rapidly evolving noncoding sequence where indels occur frequently, we made use of 138 indels coded from the alignment.

A general view of the tree is represented in Figure 1a. We used 3 out-group species representing Eurasian tribes of the family Asteraceae: tribe Eupatorieae Cass. – *Eupatorium cannabinum* L., tribe Calenduleae Cass. – *Calendula officinalis* L., tribe Anthemideae Cass. – *Achillea millefolium* L.

Our analysis, in accordance with previous studies, indicates that the tribe Astereae is monophyletic; most

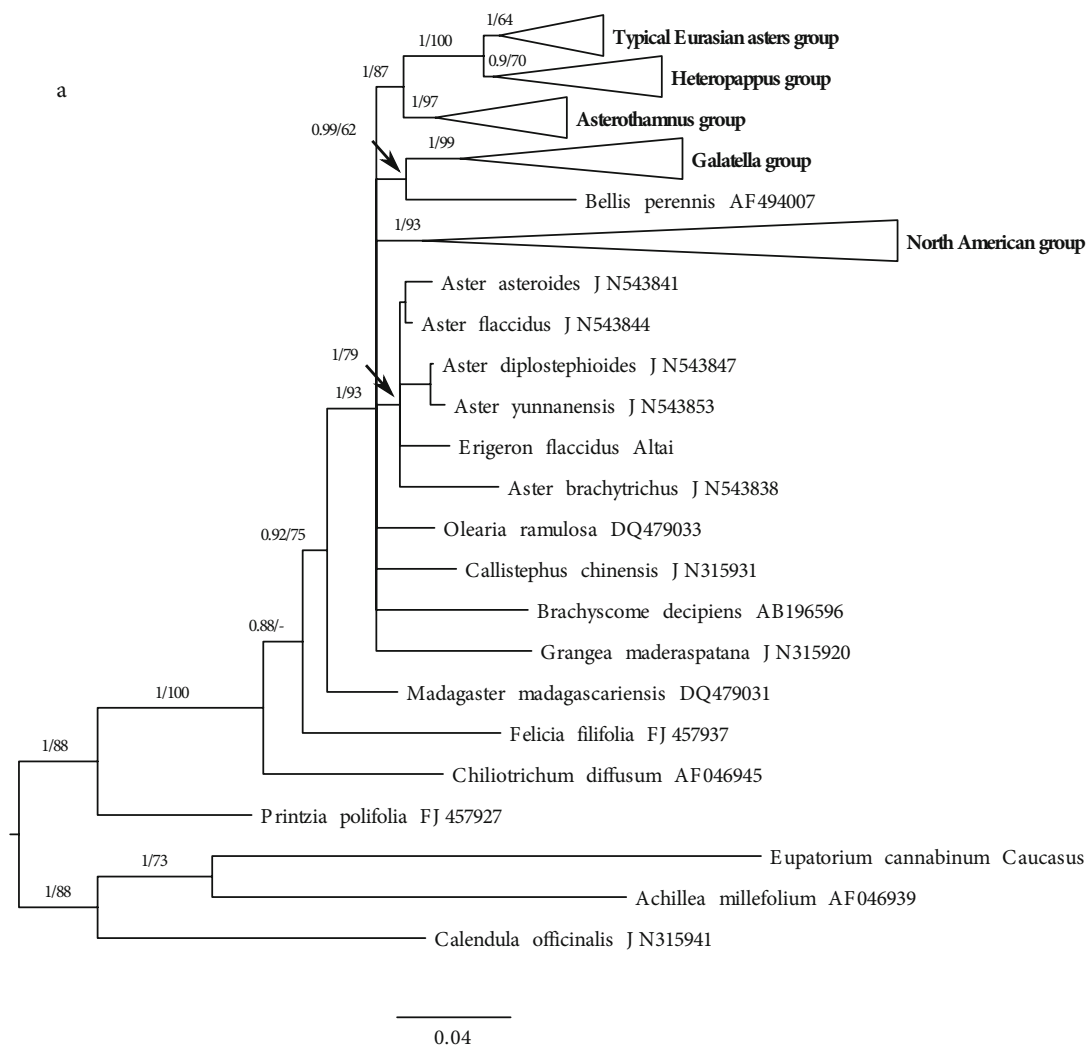


Figure 1. Phylogenetic tree constructed with Bayesian inference (BI) analysis of internal transcribed spacer sequences; 40 specimens sequenced by us have additional information on geographic origin, 62 sequences downloaded from GenBank – accession numbers. Above branches indicate posterior probabilities from BI and bootstrap support percent from maximum likelihood analysis. Values are substituted by ‘-’ if posterior probability is <0.9 and bootstrap support is <50%.

(a) Overview of the tree, five groups of species are collapsed.

basal divergence in the tribe corresponds to *Printzia poliifolia* (L.) Hutch. – South African species, which, according to Noyes and Rieseberg (1999), speaks in favor of the African origin of the tribe.

The monophyletic clade that includes all Eurasian species of the tribe Astereae was previously reported (Noyes and Rieseberg, 1999; Karaman-Castro and Urbatsch, 2009; Li et al., 2012). Inside this clade there are several well-supported groups (Figure 1a). The Galatella group and North American species group are located at basal divergence (Figure 1b), while the three groups with Eurasian origin are more closely related: *Astherotamnus* (Rhinactinidia) group, *Heteropappus* group, and typical Eurasian asters (Figure 1c).

Among the species not belonging to these groups there are several species with basal position in our clade (Figure 1b). They represent several subtribes with different geographical distribution: *Grangea maderaspatana* (L.) Poir. – Southeast Asian species, subtribe Grangeninae; *Bellis perennis* L. – European and Southwest Asian, subtribe Bellidinae; Australian subtribes are represented by *Olearia ramulosa* (Labill.) Benth. – subtribe Hinterhuberinae, *Brachyscome decipiens* Hook. f. – subtribe *Brachiscominae*; *Callistephus chinensis* (L.) Nees – Chinese-Japanese species, remains in subtribe Asterinae. These observations indicate that the subtribe Asterinae is polyphyletic and thus requires a critical revision.

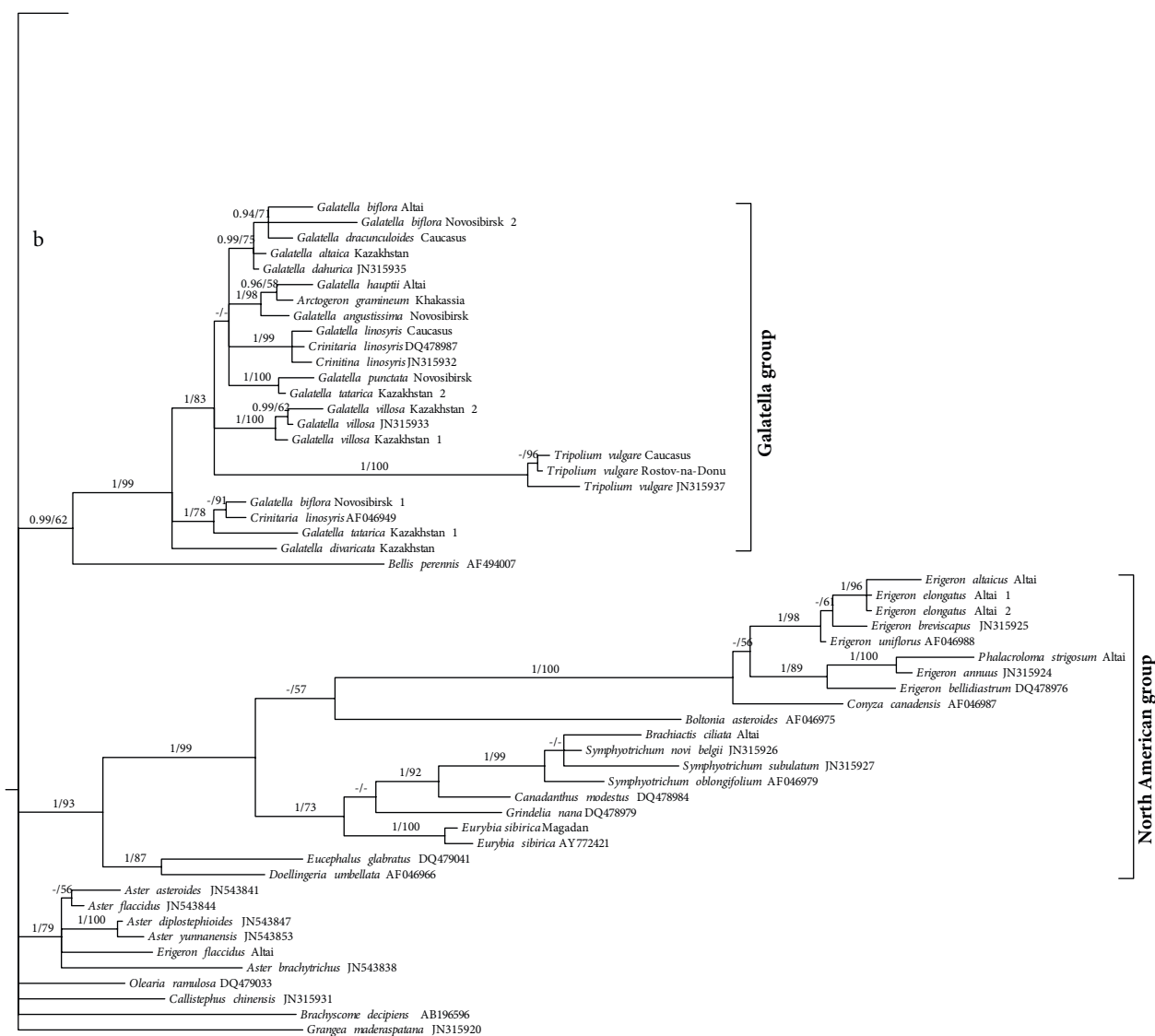


Figure 1. (Continued).

(b) Expanded basal clade, Galatella and North American groups are indicated.

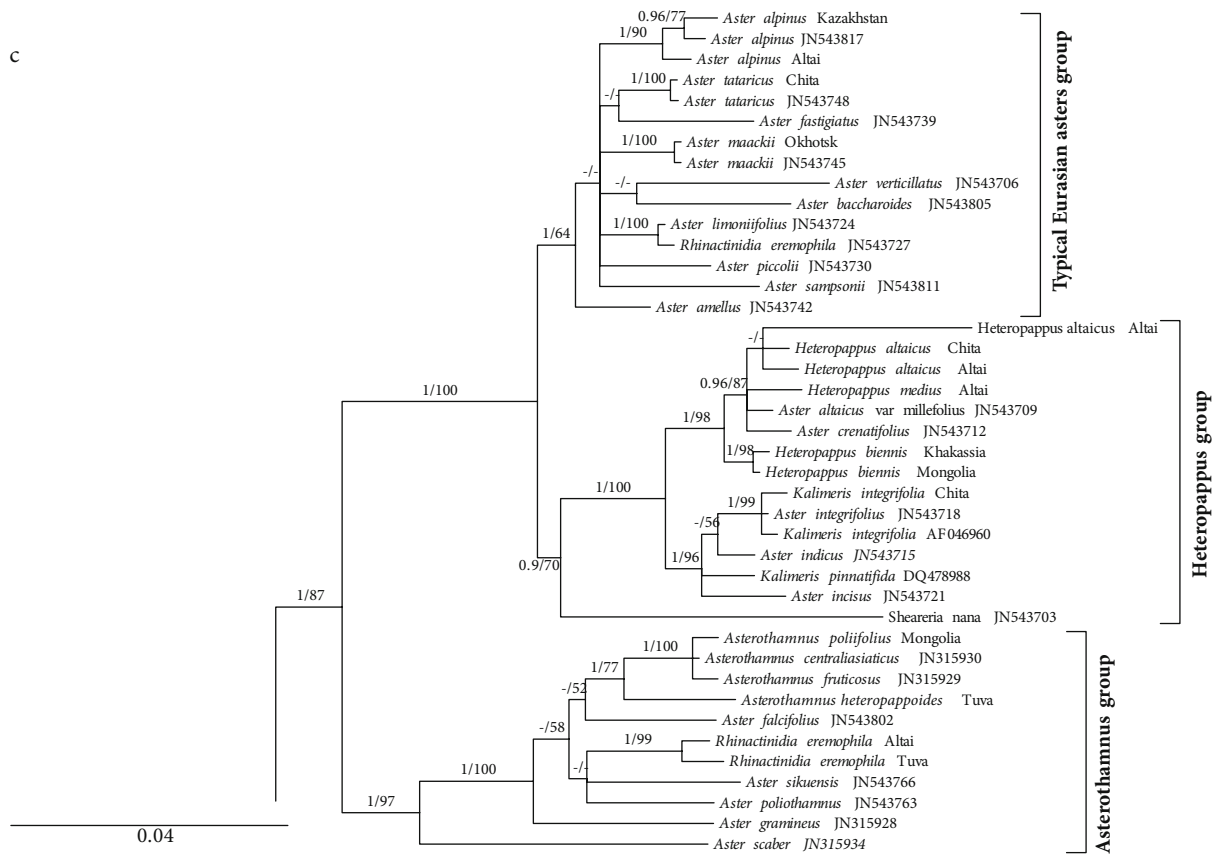


Figure 1. (Continued).

(c) Continuation of (b), 3 groups of Eurasian asters are indicated.

3.1. Galatella group

This group retains basal position in our analysis and includes three closely related genera (*Galatella*, *Crinitaria*, and *Linosyris*), the genus *Tripolium*, and *Arctogeron gramineum* (L.) DC.

The genus *Galatella* Cass. includes circa 35–45 species of perennial rhizome herbs. It is widely distributed in Southern and Middle Europe and a significant part of Asia. There are several tendencies for treatment of relations between this genus and the closely related genera *Crinitaria* Cass. and *Linosyris* Cass.: 1) these three genera are placed in *Aster* L. s.l. (Merxmuller et al., 1976); 2) all three genera are independent (Ledebour, 1844–1846; Terekhova, 1965; Grierson, 1982; Korolyuk, 1999, 2007; Chen et al., 2011); 3) *Galatella* Cass. s.l. is an independent genus, while *Crinitaria* and *Linosyris* are its subgenera (Novopokrovskiy, 1948, 1949) or sections (Tzvelev, 1994); 4) *Galatella* and *Linosyris* are separate genera and *Crinitaria* is included in *Linosyris* as a subsection (Tzvelev, 1959).

The main diagnostic feature of *Galatella* is infertility of all marginal florets in capitula. Sections within the genus differ by involucre width (from broad obconical to

narrow cylindrical), and presence or absence of ray florets and glandular trichomes. Axillary traits: carpological features (disc florets with oblong lanceolate hirtulose achenes, with length equal to pappus) and type of secretory structures, $n = 9$; $2n = 18, 36$. In 9 species, belonging to all sections (or subgenera in synonymum) the cuticle of the exocarp is not sculptured and original large capitate glands on small mono- or bicellular pedicles are found on the achenes (Korolyuk, 1997; Korolyuk et al., 1998). Given the auxiliary morphological traits, the genus *Galatella* differs significantly from the genus *Aster* s. str. (sensu stricto), while the genera *Crinitaria* and *Linosyris* likely belong to *Galatella* as sections.

We obtained ITS sequence data for 10 species (14 samples) of *Galatella* s.l. from Russia, Mongolia, and Kazakhstan belonging to all sections and added 5 published ITS sequences for this genus to our analysis. In the resulting tree all species of the group form a monophyletic clade with high support (Figure 1b).

We included the following species from 4 sections:

Sect. *Crinitaria* (Cass.) Kem.-Nataadze. (capitula homogama, ray florets are not present, narrow cylindrical or obconical involucre, bracts are thin-coriaceous and/

or arachnoid-tomentose): *G. tatarica* (Less.) Novopokr. (= *Crinitaria tatarica* (Less.) Novopokr.), *G. villosa* (L.) Rchb.f. (= *Crinitaria villosa* (L.) Cass.) (Figure 2a). Both species grow in salt-marshy steppes or steppe in the south part of European Russia and Central Asia.

Sect. *Galatella* Tzvelev (involucrum widely obconical, ray florets are present and not fertile, except for *G. biflora* (L.) Nees, both surfaces of leaves covered with dotted glands): *G. altaica* Tzvelev (narrow endemic of Altai and Soongorica mountains, Figure 2b), *G. biflora* (widespread Asian steppe species, Figure 2c), *G. dahurica* DC. (Asian steppe species widely spread from Ural Mountains to Russian Far East, North China, and Mongolia), *G. dracunculoides* (Lam.) Nees (steppe species, distributed in the European part of Russia, Figure 2d), *G. punctata* (Waldst. et Kit.) Nees (widespread Eurasian forest and steppe species, Figure 2e).

Sect. *Fastigatea* Novopokr. (capitula homogama, narrow conical involucrum, ray florets are present): *G. angustissima* (Tausch) Novopokr. (widespread Eurasian steppe species, Figure 2f), *G. hauptii* (Ledeb.) Lindl. (narrow endemic in mountains of Altai, Sayan and North Kazakhstan), *G. divaricata* (Fisch. ex Bieb.) Novopokr. (Central Asia).

Sect. *Eulinosyris* Novopokr. (capitula homogama, all florets are tubular, bisexual, narrow conical involucrum, herbaceous bracts of involucrum): *G. linosyris* (L.) Rchb.f. (= *Linosyris vulgaris* Cass. ex Less., Europe, Figure 2g).

It is interesting that no clades corresponding to sections were found in our analysis. In contrast, different samples of the same species can be more divergent than samples from different sections (see *G. biflora*, *G. linosyris*, *G. tatarica*), which may indicate recent speciation, ongoing hybridization, or inconsistent species treatment within the group. There are no subgroups correlating with morphology and thus with limits of sections.

Tripolium Nees. is a monotypic genus, with type *T. vulgare* Nees (Figure 2h). It is distributed throughout Eurasia (except for polar and tropical regions) and is an invasive species in North America (Nesom, 1994b). Some authors keep it inside *Aster* s.l. as sect. *Tripolium* (Nees) Benth. (Ito and Soejima, 1995). The species' morphology is extremely variable; different forms were sometimes considered as different species. These highly specialized halophyte plants are superficially similar to North American species of *Symphyotrichum* Nees. sect. *Oxytripolium* (DC.) G.L.Nesom (annual duration, glabrous and succulent leaves, strongly accrescent pappus). However, the characteristics of phyllaries, achenes, and chromosome number ($n = 9$) separate *Tripolium* from them as well as from typical *Aster* s. str. Among all Asian asters, Nesom (1994b) set these plants close to *Galatella* and *Crinitaria* and placed them right inside the *Galatella* group. Comparative analysis of the achene

sculpturing pattern indicates that specimens of *Tripolium* and *Galatella* form a reliable group with glandular trichomes on the achene surface and with a glabrous cuticular surface, but the morphology and topology of terpenoid structures place samples of *Tripolium vulgare* closer to ones of the genus *Erigeron* (Korolyuk, 1999).

Our phylogenetic analysis indicates that *Tripolium* falls within the *Galatella* group. Li et al. (2012) made the same observation, suggesting placing *Tripolium* inside the genus *Galatella*. However, we argue that it should be kept separate, as it forms an isolated clade: mean pairwise distance within *Tripolium* is 0.005, within *Galatella* – 0.028, between *Tripolium* and *Galatella* – 0.086. Moreover, these genera differ significantly by morphology and ecology.

Arctogeron gramineum (L.) DC. (Figure 3a) is the only representative of the genus *Arctogeron*, which is sometimes included in *Aster* s.l. Morphologically it differs significantly from other Asian representatives of the subtribe Asterineae by its linear leaves, shifted flowering phase, morphology of seeds, and secretory structures. It grows in Central and Eastern Siberia, northeastern China, and Mongolia, being dominant and codominant in steppe communities.

Li et al. (2012) for the first time analyzed *Arctogeron* with molecular phylogeny methods and placed it close to *Asterothamnus* species. However, in our tree it is located inside the *Galatella* group. Due to this discordance, a further check is needed to clarify the position of this species.

3.2. Relationship between Eurasian and North American asters

American researchers (Noyes and Rieseberg, 1999; Nesom, 1994a, 1994b; Nesom and Robinson, 2007; Vaezi and Brouillet, 2009) argue that indigenous North American species of the subtribe Asterinae are well distinguished from Eurasian ones. As a result, Nesom and Robinson (2007) segregated an independent subtribe Conyzinae Benth. & Hook.f. forming a monophyletic clade with North American origin. This subtribe included the genera *Erigeron*, *Conyza* Less., and 6 others. According to molecular phylogenetic data, basal species in the North American clade are *Eucephalus glabratus* (Greene) Greene and *Doellingeria umbellata* (Miller) Nees, both typical North American species.

We analyzed 10 specimens of 7 species belonging to different genera related to the North American clade: *Erigeron*, *Phalacrolooma* Cass., *Eurybia* (Cass.) Gray S. F., *Brachyactis* Ledeb., and *Aster*. Among them there are several native Asian species, which are traditionally considered inside groups with American origin. While in some cases our results agree with this treatment, there are several exceptions, the most notable being *Erigeron flaccidus* (Bunge) Botsch. (Figure 3b), which, in contrast



Figure 2. Galatella group: (a) *Galatella villosa*, (b) *G. altaica*, (c) *G. biflora*, (d) *G. dracunculoides*, (e) *G. punctata*, (f) *G. angustissima*, (g) *G. linosyris*, (h) *Tripolium vulgare*.

to other *Erigeron* species, does not belong to the North American clade and occupies basal phylogenetic position.

In the genus *Erigeron* we analyzed *E. altaicus* Popov (Figure 3c), *E. elongatus* Ledeb. (Figure 3d), and *E. flaccidus*.

Erigeron elongatus and *E. altaicus* (4 samples analyzed in our study) grow only in Asia. Phylogenetically and morphologically they are close to other *Erigeron* species including Eurasian *E. uniflorus* L. and Chinese *E. breviscapus* (Vaniot) Hand.-Mazz. All these species form a monophyletic group in the North American clade.

Erigeron flaccidus grows in high mountains from Afghanistan through Central Asia to South Siberia, North China, and the Himalayas. Russian researchers include this species in the genus *Erigeron* because its ligulate florets are arranged in many circles (Botschantzev, 1959; Korolyuk, 1999). However, auxiliary morphological traits of *E. flaccidus* are characteristic for the genus *Aster*: it has plate ligulate marginal florets; it belongs to a group of species with glandular trichomes on achenes (in contrast to other *Erigeron* species) and sculptured achene surface. The results of our analysis suggest that this species does not belong to the North American group and rather reproduces the result of Li et al. (2012), falling into a basal group of the subtribe Asterinae grouping together with several species of genus *Aster* sect. *Alpigeni* Nees.:

- *Aster asteroides* (DC.) Kuntze (= *Heterochaeta asteroides* DC., = *Erigeron heterochaeta* (Benth. ex C.B.Clarke) Botsch.). This species is vicarious with *E. flaccidus*, the border between them is the Zaysan basin in Central Asia (Botschantzev, 1959).
- Closely related and presumably ancient Indo-Chinese alpic species *Aster diplostephioides* (DC.) Benth. ex C.B.Clarke (= *Erigeron diplostephioides* (DC.) Botsch.), *Aster yunnanensis* Franchet, and *Aster brachytrichus* Franchet.

We studied 4 species distributed throughout both Eurasia and North America: *Phalacroloma strigosum* (Muehl. ex Willd.) Tzvel. (Figure 3e), *Brachyactis ciliata* (Ledeb.) Ledeb. (Figure 3f), *Eurybia sibirica* (L.) G.L.Nesom (Figure 3g), and *Aster alpinus* L. (Figure 3h). Based on our analysis, only *Aster alpinus* belongs to typical Eurasian asters rather than the North American clade; this will be discussed later. Let us now focus on the other 3 species.

Phalacroloma strigosum (= *Erigeron strigosus* Muehl. ex Willd.) is a ruderal annual plant. It is considered in the genus *Erigeron* sect. *Phalacroloma* (Cass.) Torr. et Gray or in a separate genus *Phalacroloma* Cass. or *Stenactis* Cass. This species originates from North America, but is also reported as invasive in European Russia (Botschantzev, 1959; Tzvelev, 1994) and in the Russian Far East (Barkalov, 1992). During recent decades this species invaded Siberia

(Lashchinskiy et al., 2010). In our tree it lies in the North American clade within *Erigeron* close to *E. annuus* (L.) Pers (= *Ph. annuum* Dumort.), which is also invasive in Eurasia. Its sister clade includes the above-mentioned group—*E. altaicus*, *E. elongatus* and the annual *Conyza canadensis* L. (= *Erigeron canadensis* (L.) Cronq.), cosmopolite species indigenous to North America and invasive in Asia.

Brachyactis ciliata is a halophyte species. In Asia it grows in South Siberia, Middle Asia, Mongolia, China, and Japan. This species is a type for the genus *Brachyactis* described by Ledebour in *Flora Rossica* (1844–1846). For a long time species of what nowadays is called the genus *Brachyactis* were included in different genera and the genus itself was not recognized as independent. Some taxonomists treated the genus *Brachyactis* as closely related to *Erigeron* subgenus *Trimorpha* (Cass.) DC. based on flower morphology. Some taxonomists included species of *Brachyactis* s. str. inside *Aster* subgenus *Oxytripolium* (DC.) A.Gray or *Aster* subgenus *Conyzopsis* (Torr. & A.Gray) A.Gray based on the absence of ligulate florets and small number of disk florets. Nesom (1994a) transferred *Brachyactis* s. str. as the section *Conyzopsis* (Torr. & A.Gray) G.L.Nesom in the large genus *Symphyotrichum* Nees. (type species – basionym *Aster novii-belgii* L.), where he included a total of 96 species in 8 sections. The species *Brachyactis ciliata* (Ledeb.) Ledeb. (= *Symphyotrichum ciliatum* (Ledeb.) G.L.Nesom) was chosen as a lectotype of *Symphyotrichum* sect. *Conyzopsis* (Torr. et Gray) G.L.Nesom. Nesom argues that it is one of the sections with significant genetic isolation, which is supported by the lack of hybridization with species of other sections. Species of *Symphyotrichum* section *Conyzopsis* are usually annual plants with a taproot characterized by unlobed leaves and narrowly paniculated capitulescence, n = 7.

In our tree *Brachyactis ciliata* falls within the North American clade together with North American *Symphyotrichum* species from different sections by Nesom (1994b): *S. novii-belgii* (L.) G.L.Nesom, *S. subulatum* (Michx.) G.L.Nesom, *S. oblongifolium* (Nutt.) G.L.Nesom. Thus, although Asian taxonomists still consider *Brachyactis ciliata* in *Brachyactis*, we shall agree with Nesom and include *B. ciliata* in the genus *Symphyotrichum* as *S. ciliatum*.

Eurybia sibirica (L.) G.L.Nesom was first observed in Siberia by Turczaninow (1842–1856) as *Aster sibiricus* L. Traditionally for Siberia, this species is still considered in the genus *Aster*. Nesom (1994b) moved this species together with several others to the genus *Eurybia* (Cass.) Gray S. F. The latter treatment is supported by our ITS data: this species lies in a monophyletic group with no internal structure together with American species of the genus *Symphyotrichum*, *Canadanthus modestus* (Lindley) G.L.Nesom, and *Grindelia nana* Nutt.



Figure 3. (a) Species with contradictory position *Arctogeron gramineum*. (b) Basal species *Erigeron flaccidus*. North American group: (c) *Erigeron altaicus*, (d) *E. elongatus*, (e) *Phalacrocoma strigosum*, (f) *Brachiactis ciliata*, (g) *Eurybia sibirica*. (h) Typical Eurasian asters group: *Aster alpinus*.

Thus, based on our results, we can speculate that both *Brachyactis ciliata* and *Eurybia sibirica* came to Asia from the American continent. The same is true for all representatives of *Erigeron* (except *E. flaccidus*) and *Phalacrocoma strigosum*.

3.3. Typical Eurasian asters group

The group of typical Eurasian asters consists of several species including the type species for the genus *Aster* s. str. – *A. amellus* L., distributed west of the Ural Mountains. We studied 3 species of this group, *Aster alpinus* L. (Figure 3h), *A. tataricus* L., and *A. maackii* L., and found them to be closely related. Li et al. (2012) came to the same conclusion and also included in this group *A. fastigiatus* Fisch. (= *Turczaninowia fastigiata* (Fisch.) DC.). The specific morphology of inflorescences allows some authors to treat this species in a separate monotypic genus, *Turczaninowia* DC.; however, ITS data do not confirm the isolated position of this species.

Aster alpinus is a species with a disjunctive distribution in Eurasian–West-American boreal-mountainous continental habitats. We previously postulated that *A. alpinus* is close to *A. amellus* based on additional features: character and morphology of terpenoid-containing structures, main chromosome number ($n = 9$, $2n = 18$, 36 , 54), and composition of coumarin compounds (Korolyuk, 1999). Phylogenetic results confirm this assumption.

Aster tataricus grows in Southeast Siberia, Mongolia, Korea, China, and Japan. A close species, *A. maackii*, is distributed in China and the Russian Far East. These species both have characteristic traits of typical *Aster* s. str. and are morphologically and phylogenetically similar to *A. amellus*.

The Eurasian aster clade also includes species belonging to *Aster* s. str. section *Aster* (about 60 species, 40 of which are endemic to China; by sensu Chen et al., (2011)). We included several of them in our analysis: narrowly endemic Chinese species *Aster baccaroides* (Benth.) Steetz, *A. sampsonii* (Hance) Hemsley, and *A. piccolii* J.D. Hooker (different authors included this species to small genera *Asteromoea* Blume, *Gymnaster* Kitam., *Kalimeris* (Cass.) Cass.), and a widely distributed South Asian species *Aster verticillatus* (Reinwardt) Brouillet, Sempl & Y.L.Chen.

According to Li et al. (2012), this group also includes two closely related species: *Aster limoniifolius* Fedtch. (= *Rhinactinidia limoniifolia* (Less.) Novopokr. ex Botsch.) and *Rh. eremophila* (Bunge) Novopokr. ex Botsch. (= *Aster eremophilus* Bunge). In our study we analyzed 2 samples of *Rh. eremophila* and found them in a separate clade close to *Asterothamnus* (*Asterothamnus* group, discussed below). Our suggestion is that samples studied by Li et al. (2012) belong to typical asters, rather than to *Rhinactinidia* (see also discussion in *Asterothamnus* group section).

3.4. Heteropappus group

Species of the Asian genus *Heteropappus* Less. grow in Siberia, Middle Asia, Mongolia, China, North India, and Japan. Its representatives are perennial, annual, or biennial herbs, $n = 9$, $2n = 18$, 36 . Lessing segregated this genus from *Aster* s.l. based on “heteropappia” (pappi are shorter on the marginal achenes and longer on the disc achenes). It is interesting that heteropappia is gradually lost in the sea–continent direction, which leads to discordant interpretations of genus volume, status, and species content. Either all intracontinental and coastal species are included in *Aster* L. (sect. *Pseudokalimeris* Kitam.) (Ito et al., 1995; Chen et al., 2011), or some species are left in *Aster*, and some are separated in *Heteropappus* (Grubov, 1982), or both continental and coastal species are considered as the independent genus *Heteropappus* (Tamamschyan, 1959; Ling et al., 1985; Ito et al., 1998; Korolyuk, 1999, 2007), or some species are included in the genus *Calimeris* Nees sect. *Asteromeris* Turcz. (Turczaninow, 1842–1856).

Of the 4 species growing in Siberia, we studied 3 belonging to the intracontinental group: *Heteropappus altaicus* (Willd.) Novopokr. (Middle Asia, South Siberia except for arctic regions, India, China, Mongolia; Figure 4a), *H. medius* (Kryl.) Tamamsch. (narrow endemic of Altai mountains), and *H. biennis* (Ledeb.) Tamamsch. ex Grub. (Middle and West Siberia, Russian Far East, Mongolia, China; Figure 4b). They form a reliably monophyletic clade with out-group *Sheareria nana* S. Moore. This clade also includes Chinese *Aster crenatifolius* Handel-Mazetti (= *Heteropappus crenatifolius* (Handel-Mazetti) Grierson).

Our results indicate that the *Heteropappus* group also includes eastern species belonging to the group *Kalimeris* Cass., which is a small East-Asian genus with about 14 species, $n = 9$. The main diagnostic feature consolidating the group is a very short or inconspicuous pappus. There is an ongoing discussion about the genus independency and volume (Gu and Hoch, 1997). Two species grow in Siberia on the northwestern border of their habitat: *Kalimeris integrifolia* Turcz. (= *Aster integrifolius* Franch., = *Boltonia integrifolia* Benth. et Hook.) and *K. incisa* (Fisch.) DC. (= *Aster incisus* Fisch., = *Asteromoea incisa* (Fisch.) Koidz., = *Boltonia incisa* (Fisch.) Benth. – type of genus). Species with more southern distribution also fall within this group: *Aster indicus* L. (= *Kalimeris indica* (L.) Sci. Bip.), *K. pinatifida* (Maxim.) Kitam. (Li et al., 2012). Our results indicate that all these species form a monophyletic clade sister to the genus *Heteropappus*.

3.5. Asterothamnus group

This group is the last of the three related clades of Eurasian origin. In our tree it includes the monophyletic genera *Asterothamnus* Novopokr. and *Rhinactinidia* Novopokr. as well as several representatives of *Aster* s. l. from China.



Figure 4. Heteropappus group: (a) *Heteropappus altaicus*, (b) *H. biennis*. Asterothamnus group: (c) *Asterothamnus heteropappoides*, (d) *A. polifolius*, (e) *Rhinactinidia eremophila*.

Nesom (1994b) recognized the genus *Asterothamnus* as independent and based on morphology forms the *Asterothamnus* group, with such genera as *Asterothamnus*, *Kemulariella* Tamamsch., *Krylovia* Schischk. (= *Rhinactinidia* Novopokr.), *Psychogeton* Boiss., and *Arctogeron*. Our previous studies of morphology and topography of terpenoid structures (Korolyuk, 1999), external morphological and carpological traits, and achene surface ultrasculpture (Korolyuk, 1997) revealed

significant differences between these genera. Furthermore, we showed that these species are independent from *Aster* s.l. and proposed that all these species growing in specific climate of Central Asia are unified by convergent morphology. According to our current phylogenetic reconstruction the *Asterothamnus* group is a sister clade to typical Eurasian asters. It is monophyletic and includes the genera *Asterothamnus* and *Rhinactinidia* and some Asian species of *Aster* (*A. falcifolius* Hand.-Mazz., *A. sikuensis*

W.W.Sm. & Farrer, *A. poliothamnus* Diels, *A. scaber* Thunb.) and *Aster gramineus* (= *Arctogeron gramineum* (L.) DC.), the species with controversial phylogenetic position (see discussion at end of Galatella group section). The genera *Kemulariella* and *Psychrogeton* remain unstudied and their evolutionary relations are yet to be clarified.

Asterothamnus has 7 species (type – *A. alyssoides* (Turcz.) Novopokr) endemic to Asia. Its representatives are typical semishrubs with xeromorphic structure of organs, narrow elongated capitula, and small ecological amplitude. We studied 2 representatives of the genus: *A. heteropappoides* Novopokr. (Figure 4c) and *A. poliifolius* Novopokr. (Figure 4d), both growing in desert steppes on rocky slopes in Tuva and West Mongolia. We also included data from Li et al. (2012) for closely related species: *A. fruticosus* (Winkler) Novopokr. and *A. centrali-asiaticus* Novopokr. from Mongolia and Central China. In the resulting tree all *Asterothamnus* species form a single monophyletic clade with high statistical support.

Rhinactinidia, another Asian endemic genus, was segregated from the genus *Aster* by Lessing under the name *Rhinactina* based on zygomorphy of tubular disk florets, but, as Novopokrovskiy mentioned, this trait “does not withstand any criticism” (1950). For a long time the name and the volume of the genus remained controversial (Novopokrovskiy, 1950; Tamamschyan, 1959; Ling et al., 1985; Botschantzev, 1986; Nesom, 1994b; Korolyuk, 2007; Nesom and Robinson, 2007). In a previous study we discovered additional diagnostic traits for this genus; based on the morphology and topography of terpenoid-containing structure samples of *Rhinactinidia* segregate from typical asters as well as from other species of the tribe, as only this species has glandular epiderm and lacks glandular trichomes on achenes (Korolyuk et al., 1998).

The genus includes 3 species growing in mountainous regions on dry, mostly rocky substrates. Two species—*Rh. limoniifolia* (Less.) Novopokr. ex Botsch. and *Rh. popovii* (Botsch.) Tamamsch.—are endemic to Middle Asia and Mongolia. *Rh. eremophila* (Bunge) Novopokr. ex Botsch. (Figure 4e) is a rare species of mountains in South Siberia, North Mongolia, and North Kazakhstan. Moreover, according to our previous results, these species differ from asters by the life form type (herbaceous cushions), which also might be an important diagnostic trait (Cheremuschkina et al., 2011). We studied *Rh. eremophila* and, beyond any doubt, it falls within the *Asterothamnus* group. However, its position does not replicate the results of Li et al. (2012), where it lies within typical Eurasian asters. Thus, while our Chinese colleagues suggest that *Rhinactinidia* should be treated as a series of the section *Aster*, we argue that it is a separate genus close to *Asterothamnus*.

4. Discussion

At the present moment there is no complete monographic work dedicated to Eurasian species of subtribe Asterinae representatives growing in Eurasia. Taxonomists studying this subtribe either integrate more species in *Aster* L. s.l. or delimit many genetic segregates or genera, often well separated geographically. Traditionally, for the vast territory of European and Asian parts of Russia *Aster* is treated s. str. (26 species for Asian Russia by Tamamschyan (1959)) and many small typical Asian genera are excluded: *Tripolium* (1 species), *Kemulariella* (6 spp.), *Heteropappus* (12 spp.), *Kalimeris* (2 spp.), *Doellingeria* Nees. (1 sp.), *Turczaninowia* DC. (monotypic), *Rhinactinidia* (2 spp.), *Asterothamnus* (7 spp.) and *Arctogeron* (monotypic) (Tamamschyan, 1959), *Galatella* (35–45 spp., sensu by Tzvelev (1959, 1990, 1994)). The genus *Erigeron* (72 spp. sensu by Botschantzev (1959)) was always treated as separate.

In many cases questions of delimitation of species and genera remain open. We addressed this problem using molecular phylogenetic methods, focusing on the subtribe species from Siberia and adjacent regions. In general our results confirm the findings of other authors (Noyes and Rieseberg, 1999; Fiz et al., 2002; Karaman-Castro and Urbatsch, 2009; Li et al., 2012). For 15 species, we obtained molecular phylogenetic data for the first time. Eleven of these species are narrow Asian endemics.

We used full length ITS as a DNA marker, which is a common practice in plant phylogeny. It is variable enough to get a good resolution on a generic level, the one we are interested in. As ITS is a noncoding region, indel mutations occur frequently. We made use of these data, as it was shown to increase the resolution power of subsequent analysis (Nagy et al., 2012).

The resulting tree revealed that the tribe Astereae is monophyletic and most basal positions are occupied by species from the southern hemisphere. However, species of the subtribe Asterinae do not form a monophyletic clade, but form a cluster together with representatives of other subtribes (e.g., Belledinae – *Bellis perennis*, Brachyscominae – *Brachyscome decipiens*; Grangeinae – *Grangea maderaspatana*) (see Figure 1a). This group is well supported in our phylogeny and has the out-group species *Madagaster madagascariensis*. Inside this cluster, along with some species retaining unresolved basal position we detected several subgroups with Eurasian species (see Figures 1b and 1c):

The Galatella group is characterized by high intraspecific variation level, which overlaps differences between species and even subgenera/sections. Our data do not confirm delimitation of the genera *Linosyris* and *Crinitaria*. The genus *Tripolium* is quite distinctive, in contrast to other representatives of the group.

The group of North American species is also well supported. Its Eurasian representatives include *Phalacrocoma strigosum* (invasive since the 20th century), *Brachyactis ciliata*, and *Eurybia sibirica*. We assume that these last two species might also have come to Asia from the North American continent. *Erigeron elongatus* and *E. altaicus* are distributed only in Asia and possess morphological traits typical for this genus. According to phylogenetic data these species definitely belong to *Erigeron* and might originate from North American ancestors. At the same time, *E. flaccidus* (Bunge) Botsch is also an Asian species, but it lies in an independent clade with basal position in the tribe; this position is also confirmed by auxiliary traits. Thus we propose to revise its generic affiliation.

Three groups of Eurasian species are close to each other: the *Astherotamnus* group (Central Asian species of *Astherotamnus* and *Rhinactinidia*), the typical Eurasian asters group (with type species for *Aster* s. str.), and the *Heteropappus* group (East Asian and Asian species of *Heteropappus* and *Kalimeris*). Thus we assume that the above-mentioned small genera of Asian species should be treated as independent, but not in the genus *Aster* s.l. More studies on other endemic Asian representatives of the subtribe Asterinae are needed to clarify the taxonomy of this group at the generic level.

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- Our study complemented the previous research in a sense that what is called the subtribe Asterinae in the Russian literature indeed corresponds to several groups: Galatella, North American, and Eurasian groups, *Erigeron flaccidus*, and related species. However, here we, similarly to Li et al. (2012), cannot treat these groups at exact taxonomic levels and revise the status and volume of the subtribe Asterinae for Eurasia, as was previously done for North America (Nesom and Robinson, 2007). The reasons are the morphological heterogeneity of groups discovered and lack of molecular phylogenetics data on circa 100 Eurasian species that might occupy key evolutionary positions. Thus, molecular phylogenetics analysis of all Eurasian Asterinae species and further taxonomic revision with morphological key generation of the group are important objectives for future research.

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