BioOne COMPLETE

A molecular phylogenetic study of the Ephedra distachya / E. sinica complex in Eurasia

Authors: Kakiuchi, Nobuko, Mikage, Masayuki, Ickert-Bond, Stefanie, Maier-Stolte, Maria, and Freitag, Helmut

Source: Willdenowia, 41(2) : 203-215

Published By: Botanic Garden and Botanical Museum Berlin (BGBM)

URL: https://doi.org/10.3372/wi.41.41201

BioOne Complete (complete.BioOne.org) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/terms-of-use.

Usage of BioOne Complete content is strictly limited to personal, educational, and non - commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

Downloaded From: https://bioone.org/journals/Willdenowia on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

NOBUKO KAKIUCHI[1,2*], MASAYUKI MIKAGE[2], STEFANIE ICKERT-BOND[3], MARIA MAIER-STOLTE[4], HELMUT FREITAG[4**]

A molecular phylogenetic study of the *Ephedra distachya / E. sinica* complex in Eurasia

Abstract

Kakiuchi N., Mikage M., Ickert-Bond S., Maier-Stolte M. & Freitag H.: A molecular phylogenetic study of the *Ephedra distachya / E. sinica* complex in Eurasia. – Willdenowia 41: 203–215. December 2011. – Online ISSN 1868-6397; © 2011 BGBM Berlin-Dahlem.
Stable URL: http://dx.doi.org/10.3372/wi.41.41201

This study provides new information on phylogenetic relationships in the *Ephedra distachya* / *E. sinica* complex (*Ephedraceae,* gymnosperms) based on sequence variation and morphology-based delimitation of individual species. Altogether we have included 50 samples from this complex and closely related species with 39 samples sequenced for the first time. Our sampling scheme provides a much broader sampling both in respect of the number of species as well as the number of samples per species than has been accomplished in previous studies. Sequences for *E. dahurica* and *E. pseudodistachya* are given here for the first time. We sequenced the nuclear ribosomal internal transcribed spacer regions (nr ITS1+ITS2) as well as the chloroplast intergenic spacer between *rnL* and *trnF* (cp *trnL-F*), but only ITS1 was sufficiently informative for phylogeny reconstruction. Our data show, for the first time, (1) a well supported *E. strobilacea / E. sarcocarpa / E. transitoria* clade as sister to the "L" or "Asia 2" clade, furthermore (2) a distinct *E. distachya* clade, (3) a composite *E. dahurica* clade that also includes *E. sinica* and a fraction of *E. intermedia,* (4) a well supported *E. regeliana* clade and (5) an unresolved agglomeration of *E. intermedia, E. przewalskii, E. lomatolepis* and *E. pseudodistachya.* Most noteworthy is the support derived from the molecular data for (1) the separation of the hitherto doubtful *E. pseudodistachya* from *E. distachya* and *E. dahurica* and (2) the high degree of sequence similarity of *E. sinica* and *E. dahurica.* Based on morphological, molecular, ecological and biogeographical evidence, *E. sinica* is reduced to a subspecies of *E. dahurica.*

Additional key words: gymnosperms, *Ephedra dahurica, Ephedra intermedia, Ephedra pseudodistachya, Ephedra strobilacea,* Ma-huang, nr ITS, morphology, phylogeny, cp *trnL-F.*

Introduction

The *Ephedra distachya* L. */ E. sinica* Stapf complex is defined here somewhat arbitrarily as a group of morphologically similar and closely related species that additionally also include *E. dahurica* Turcz., *E. lomatolepis* Schrenk and *E. pseudodistachya* Pachom. Taxa in this complex share the same habit, range from 10–40 cm in size, bear 2-seeded female cones which become fleshy at maturity, generally have a rather short micropylar tube as well as paired leaves (Fig. 1). Species delimitation in this complex is difficult, particularly in the vegetative stage which dominates in the field and in the majority of herbarium specimens. The centre of distribution is the steppe and semidesert area of Eurasia, further extending from the Atlantic and Mediterranean to the eastern margin of the continent. Recent molecular work (Long & al. 2004;

1 Faculty of Pharmaceutical Sciences, Kyusyu University of Health and Welfare, 1714-1 Yoshinotyo, Nobeoka, 882-8508 Japan; *e-mail: kakiuchi@phoenix.ac.jp (first author for correspondence).
2 Graduate School of Natural Science and Technology, Kanazawa University, Kana-zawa, 920-1192 Japan.
3 Department of Biology and Wildlife & University Museum Herbarium (ALA), University of Alaska, 907 Yukon Drive, Fairbanks, AK 99775-6960, USA.
4 Institute of Biology, Section Systematics and Morphology of Plants, University of Kassel, 34132 Kassel, Germany; **e-mail: hfreitag@uni-kassel.de (second author for correspondence).

Downloaded From: https://bioone.org/journals/Willdenowia on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Ickert-Bond & Wojciechowski 2004; Huang & al. 2005; Ickert-Bond & al. 2009; Rydin & Korall 2009; Rydin & al. 2010; Ickert-Bond & Rydin 2011) has shown that species in the *E. distachya / E. sinica* complex belong to the "L" or "Asia 2" clade, one of three Asian clades in *Ephedra*. However, *E. pseudodistachya* and *E. dahurica* are here included in a phylogenetic study for the first time. These two taxa and their taxonomic rank are particularly difficult (see below) as is evident from the differing treatments in checklists and floristic accounts of China (as, e.g. Fu & al. 1999, Yang 1993, 2002), Russia (as, e.g. Czerepanov 1995, Peschkova 2005, Galanin 2008) and Mongolia (Grubov 1982; Freitag & Maier-Stolte 2009).

One member of the group, *Ephedra sinica,* is of outstanding pharmaceutical importance. Its aerial parts have been used as a staple crude drug since ancient times. In the Shen-nong-ben-cao-jing, one of the oldest herbals, written in Chinese Han Dynasty (1st century AD), is described under its today's name "Ma-huang" as a remedy for perspiration, to cure a feverous state, for cough suppression and relief from chills and fevers (Tang 2002). Thanks to its analgesic, antipyretic and antitussive effects, *Ephedra* is still a constitutive part in traditional medicinal prescriptions in China and Japan, and their active ingredients, ephedrine alkaloids, are contained in modern pharmaceuticals against colds worldwide. Besides *E. sinica, E. equisetina* Bunge and *E. intermedia* Schrenk & C. A. Mey. are considered the most useful for crude drugs and are prescribed in both Chinese and Japanese pharmacopoeias (Chinese Pharmacopoeia Commission 2005; Japanese Ministry of Health 2006). Among them, *E. sinica* dominates the crude drug market in both countries due to its relatively rich natural resources.

The species of the *Ephedra distachya / E. sinica* complex and related taxa

In the following we introduce the species under consideration in the *Ephedra distachya* / *E. sinica* complex and its relatives.

***Ephedra distachya* —** This species occupies a very wide area ranging from Spain and western France, scattered through the Alps and along the northern Mediterranean coast to eastern Europe and the Near East, from where it continues to western Central Asia and southwestern Siberia (Freitag & Maier-Stolte 1994: map 3; Peschkova 2005: fig. 4). Plants from the dry Rhône valley in the Swiss Alps, differing from typical *E. distachya* by a long and twisted micropylar tube, were described as *E. helvetica* C. A. Mey. Apart from the characteristics of the microphylar tube, they are otherwise identical with typical *E. distachya* and are now generally classified as *E. distachya* subsp. *helvetica* (C. A. Mey.) Asch. & Graebn. (e.g. Freitag & Maier-Stolte 1993, Govaerts 2010a, b). More recently, Nouviant (1993, 1997) described populations from the Italian and the French Alps as *E. negrii* Nouviant. They differ from the Swiss *E. helvetica* by shorter micropylar tubes, thus bridging the gap to typical *E. distachya* (Fig. 1C).

Small plants with delicate twigs and often single-seeded cones from Siberia were described as *Ephedra monostachya* L., but monographers (Meyer 1846; Stapf 1889) have shown that Linné's *E. monostachya* was an admixture of dwarfed *E. distachya* and of *E. monosperma* Gmel. ex C. A. Mey. Nevertheless, while excluding *E. monosperma,* Riedl (1967) and Jarvis (2007) maintained *E. monostachya* at the subspecies level and Nouviant (1998) at the species level claiming that the taxon replaces the northern Mediterranean *E. distachya* from southeastern Central Europe to Siberia.

Doubtful species closely allied or belonging to *Ephedra distachya* are *E. aurantiaca* Takht. & Pachom. from the Caucasus, which differs by having orange to yellow coloured female cones (Takhtajan & Pachomova 1967), and *E. vvedenskyi* Pachom. (1968) from the Kopet Dagh region southeast of the Caspian Sea, which approaches *E. intermedia* with its coarser stature and a slightly elongated micropylar tube. However, *E. aurantiaca* and *E. vvedenskyi* are maintained as distinct species in the account of Govaerts (2010a, b).

***Ephedra pseudodistachya* —** The species grows in eastern Kazakhstan, southern Siberia and northern Mongolia (Peschkova 2005: fig. 4). It can be distinguished from *E. distachya* by the reddish-brownish base of leaf sheaths, and a more delicate habit, with thinner twigs and being usually only 5–20 cm high. Until its description by Pachomova (1968) the respective populations from the former USSR were included in *E. distachya,* while those from Mongolia were classified as *E. sinica* or *E. sinica* var. *pumila* Florin (Florin 1933; Pachomova 1971; Grubov 1982; Gubanov 1996). In Chinese floras (e.g. Liu 1985, Cheng 1978, Fu & al. 1999) the plants were named *E. distachya,* although in Fu & al. (1999) the conspecificity with true *E. distachya* was questioned, but *E. sinica* var. *pumila* was suggested to occur in the country. Even in Russia *E. pseudodistachya* was not unambiguously accepted, e.g. Czerepanov (1995) placed it in synonymy of *E. dahurica.* At the beginning of our study we also were not convinced that the separation of *E. pseudodistachya* and *E. distachya* is justified.

***Ephedra lomatolepis* —** The species is restricted to an area from western Kazakhstan and Uzbekistan to northwestern China (Xinjiang province) and southwestern Mongolia (Freitag & Maier-Stolte 1994: map 7). *E. lomatolepis* differs from all other species of the complex by the wide membranous margins of the bracts in the female cones, an olive-green colour of the stems and leaves partly arranged in whorls of three. However, in areas of overlap, such as along the lower reaches of the Syrdar'ya river in western Kazakhstan where both species have been seen growing side by side, it was difficult to separate them in the vegetative stage.

Downloaded From: https://bioone.org/journals/Willdenowia on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

***Ephedra sinica* —** The species is distributed from central China (Gansu province) and Mongolia eastward up to the Gulf of Bohai (Hebei province) in northeastern China (Cheng 1978; Fu & al. 1999), with an extension to southeastern Siberia (Peschkova 2005: fig. 3). Peculiar characters of *E. sinica* are comparatively long leaves (up to 7 mm), whitish leaf sheaths, flattened basal internodes and completely glabrous twigs. According to the literature, *E. sinica* appeared to be an easily recognisable species, until we started to identify collections from Mongolia and southeastern Siberia, which forced us (Freitag & Maier-Stolte 2009) informally to reduce *E. sinica* to a subspecies of *E. dahurica.* It is noteworthy, that Stapf (1927) made the original description of *E. sinica* from raw material imported to the United States of America from China. In 1999, the Chinese Government proclaimed that *Ephedra* resources in the territory need protection against desertification and over-collecting. According to its significance as a medicinal plant, *E. sinica* and related species are of special interest.

***Ephedra dahurica* —** The species has been described by Turczaninow (1854: 421) from the Transbaikal region of Siberia. It was mistaken by Stapf (1884) and Florin (1933) as *E. monosperma* J. G. Gmel. ex C. A. Mey. and neglected until its revival by Siplivinskiy (1973). *E. dahurica* is widely sympatric with *E. sinica* in southeastern Siberia and northeastern Mongolia (Peschkova 2005: fig. 2; Galanin 2008). According to the description given by Peschkova (2005), *E. dahurica* differs from *E. sinica* by slightly shorter leaves united basally into a brownish sheath and by the twig surface, which is more or less rough due to the presence of short but hard papillae. *E. dahurica* was included by Galanin (2008) as a subspecies of *E. sinica.* Subspecies rank for both taxa was also proposed by Freitag & Maier-Stolte (2009) under the older name *E. dahurica.*

***Related species* —** Additional species of the "L" (or "Asia 2") clade (Rydin & al. 2010) that have not been dealt with in detail in this study because of their clear morphological separation are:

Ephedra intermedia Schrenk & C. A. Mey.: coarse subshrub, usually 30– 80 cm high, female cones with very long, screw-like micropylar tube; semideserts from Iran to China.

Ephedra przewalskii Stapf: delicate to robust subshrub, usually 30–80 cm high, brown, with adherent sand grains, leaves usually in 3s, female cones at maturity with completely membranous bracts; sand deserts from Uzbekistan to Mongolia and China.

Ephedra regeliana Florin: prostrate subshrub, usually 2–5 cm high; alpine steppes of Central Asian high mountains.

Here also belong the three stout Irano-Turanian species *Ephedra strobilacea* Bunge, *E. sarcocarpa* Aitch. & Hemsl. and *E. transitoria* Riedl.

Our study aims to determine whether the mostly weak morphological differentiation in the species complex, in particular regarding the species pairs *Ephedra sinica / E. dahurica* and *E. distachya / E. pseudodistachya* can be supported by molecular data. In order to get most meaningful results, we sampled throughout the distributional areas of the individual species as complete as possible. Besides, we expected to get insights into the genetic variability of widely distributed *Ephedra* species along geographical gradients and to resolve phylogenetic relationships in the species group.

From previous phylogenetic studies in *Ephedra* (e.g. Ickert-Bond 2004, Rydin & Korall 2009, Rydin & al 2010) we were aware of the comparatively low amount of sequence divergence in *Ephedra* and particularly among more closely related groups. Nevertheless, from the topology of the hitherto published phylogenies (the most refined in Rydin & al. 2010) we expected that by means of extended sampling and inclusion of species which were not sequenced yet we could get additional support for taxonomic decisions made based on morphology.

The morphologically well-separated species of the "L" clade (Rydin & al. 2010) are also included in our study in order to elucidate their relationships to the species under particular consideration. However, these species are represented by exemplary samples only. Furthermore, to show the position of the *Ephedra distachya / E. sinica* group among the other Eurasian *Ephedra* clades, selected species from all clades are likewise included in our analysis.

Material and methods

***Plant material* —** Field surveys of *Ephedra dahurica* in southeastern Siberia and *E. sinica* in Mongolia were conducted in July and August 2006, respectively, by the first two authors (Kakiuchi & Mikage 2007; Mikage 2009). Helmut Freitag studied and collected *E. pseudodistachya* and *E. dahurica* in southern and southeastern Siberia in August and September 2003, as well as *E. lomatolepis* and *E. distachya* in various earlier field campaigns in other parts of Eurasia. The specimens were deposited in the herbaria of Kanazawa University (KANP) and Kassel University (KAS). Additionally, a few specimens from other herbaria and selected GenBank sequences with reliable identification and traceable provenance information were included. Herbarium abbreviation follow Thiers (2008+). Taxonomic identifications were made and/or verified by D. Chimitov (Ulan-Ude), H. Freitag, M. Maier-Stolte and M. Mikage except for the GenBank material. In Appendix 1 we provide a complete list of the 59 samples of *Ephedra* included in this study, with geographic origin, voucher information and GenBank accession numbers for all sequences. Several samples from adjoining locations had identical sequences and only one sequence was submitted for Genbank registration. Thus the 59 sequences are in fact based on a total of 80 vouch-

Downloaded From: https://bioone.org/journals/Willdenowia on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use



Fig. 1. A–C: *Ephedra distachya* subsp. *helvetica* – A: with mature female cones; Suisse, Valais, Tourbillon hill near Sion, on S exposed rocky outcrops (with *Sedum album*); phot. H. Freitag, August 1990; B: shoot with male cones; Italy, S Tyrol, Vinschgau, S-exposed slopes above Schlanders; phot. H. Freitag, July 1990; C: shoot with immature female cones, the comparatively short micropylar tubes correspond to *E. "negrii"*; France, Alpes de Hautes Provence, Larange-Montéglin; phot. E. Zippel, May 2005. – D: *E. dahurica* with mature female cones; the distorted twigs are artefacts due to unknown injury that also occur in other species; Russia, Buryatia, c. 100 km S Ulan-Ude, in *Stipa krylovii* steppe near Sulfat'noe Ozero; phot. H. Freitag, September 2003.

ers. In Fig. 2 only the samples of the *E. distachya / E. sinica* group sequenced here for the first time are given. Molecular sequencing and initial phylogenetic analyses were carried out at Kanazawa University, while additional Bayesian inference and Maximum Likelihood analyses were completed at the University of Alaska Fairbanks. Morphological comparisons were done in Kassel. Ecological data are based on field observations made by H. Freitag, unless explicitly citing other sources.

DNA preparation and PCR amplification — Dry twigs were cut into 2 mm long pieces, frozen in liquid nitrogen and ground into powder. Using a DNeasy Plant Mini Kit (Qiagen), DNA was extracted according to the manufacturer's protocol. Total DNA was used as a template for amplifying the nuclear ribosomal internal transcribed spacer regions (nr ITS1 and ITS2) and the chloroplast intergenic spacer region between *trnL* and *trnF* (cp *trnL-F)* by PCR. The primers were designed based on 18S

Downloaded From: https://bioone.org/journals/Willdenowia on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

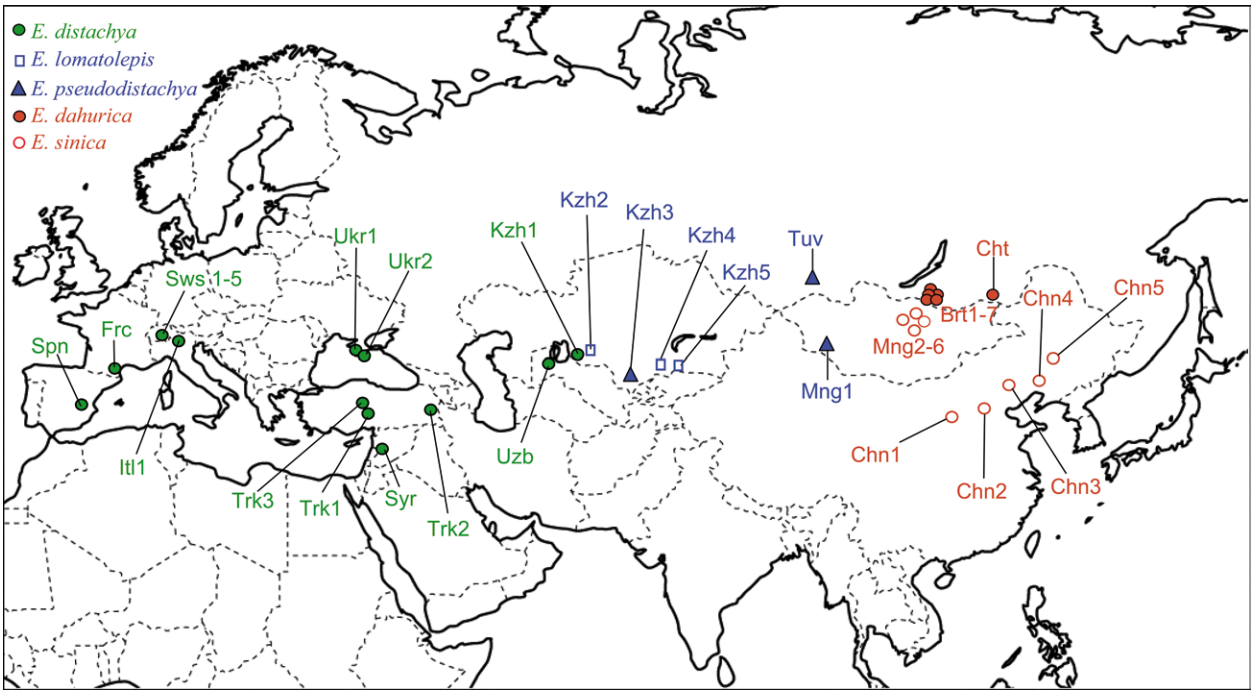


Fig. 2. Distribution map of new sequences (nr ITS1+ITS2 and cp *trnL-F*) generated for accessions of the *Ephedra distachya / E. sinica* complex in the molecular analysis.

and 26S nuclear ribosomal DNA from Genbank (Eph-1F:D38242, Eph-1R:U90708) and previous works (5.8S-R, 5.8S-F: Long & al 2004, Aco-1F, Aco-2R: Taberlet & al. 1991). The primer sets of Eph-1F (GAC GTC GCG AGA AGT TCA TT) / 5.8S-R (CGG GAT TCT GCA ATT CAC AC) and 5.8S-F (GAA CGT AGC GAA ATG CGA TA) / Eph-1R (GTA AGT TTC TCT TCC TCC GC) were used for the amplification of ITS1 and ITS2, respectively. Primers, Aco-1F (CGA AAR CGG TAG ACG CTA CG) and Aco-2R (ATT TGA ACT GGT GAC ACG AG), were used to amplify the *trnL-F* region. Standard PCR was performed in 25 µl reaction mixture containing 2.5 µl of 10× PCR buffer for KOD-Plus, 0.2 mM each of dNTP, 1 mM $MgSO_4$, 0.4 M of each primer, approximately 100 ng of the DNA sample and 0.5 units of KOD-Plus DNA polymerase (Toyobo). PCR was carried out as follows: hot start at 94 °C for 2 min, 30 cycles of denaturation at 94 °C for 15 sec, annealing at 55 °C for 30 sec and elongation at 68 °C for 45 sec, and a final elongation at 68 °C for 5 min. Three microliters of the PCR product was used for agarose gel electrophoresis and the remaining product was purified using the QIA quick PCR Purification Kit (Qiagen).

Sequencing — The purified PCR products were sequenced using a BigDye Terminator Cycle Sequencing Kit (Applied Biosystems) on an ABI PRISM 310 Genetic Analyzer (Applied Biosystems). The primers, Eph-1F, 5.8S-R, Eph-1R and 5.8S-F, were used for the sequencing reaction of the ITS 1 and 2 regions, and Aco-1F and Aco-2R were used for sequencing the *trnL-F* region.

Phylogenetic analysis — The nuclear ribosomal (nr) ITS2 sequences were invariable across all the taxa sampled here and we thus only included the nr ITS1 sequences in our analysis. In the cp *trnL-F* sequences we also detected only very few nucleotide changes among the species under consideration. They were likewise omitted. In several DNA samples multiple banding patterns indicating a putative hybrid origin of the sample or its ancestors were found. Subsequent cloning results were inconclusive and these samples were later excluded. Sequence alignment was done manually in MacClade 4.08 (Maddison & Maddison 2005). We scored gap characters using modified complex-indel-coding (Simmons & Ochoterena 2000; Müller 2006; Simmons & al. 2007). A total of 17 parsimony informative complex-indel-coding gap characters were scored from unambiguously aligned regions. Maximum likelihood tree searches (ML) and ML bootstrapping were performed using RAxML 7.2.7 (Stamatakis & al. 2008; Pfeiffer & Stamatakis 2010). RAxML searches were performed using the general time-reversible (GTR) model with among-site rate heterogeneity modelled by a gamma distribution with 25 rate categories, which sufficiently account for rate heterogeneity (Stamatakis & al. 2008). Non-parametric bootstrapping was used as implemented in the rapid bootstrap option of RAxML on the CIPRES portal using 500 replicates. The trees were rooted with *Ephedra foeminea* Forssk. and *E. fragilis* Desf., members of the most basal clade of the genus in recent phylogenetic analyses (Ickert-Bond & al. 2009; Rydin & Korall 2009). We also included several accessions of the two other Asian *Ephe-*

Downloaded From: https://bioone.org/journals/Willdenowia on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

dra clades (Long & al. 2004; Ickert-Bond & al. 2009; Rydin & Korall 2009).

Bayesian inference using the GTR + G model plus a proportion of invariable sites was performed using MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003). Markov chain Monte Carlo (MCMC) runs started from independent random trees were repeated twice and extended for 1 million generations, with trees sampled every 1000 generation. We used the default priors in MrBayes, namely a flat Dirichlet prior for the relative nucleotide frequencies and rate parameters, a discrete uniform prior for topologies, and an exponential distribution (mean 1.0) for the gamma-shape parameter and branch lengths. Stationarity of the Bayesian MCMC runs was assessed by checking that (1) the standard deviations of split frequencies were less than 0.01; (2) the log probabilities of the data given the parameter values fluctuated within narrow limits; (3) that the convergence diagnostic (the potential scale reduction factor [PSRF] given by MrBayes) approached 1 and (4) by examining the plot provided by MrBayes of the generation number versus the log probability of the data. Trees sampled prior to convergence were discarded as burn-in (2500 trees) and a majority rule consensus tree was constructed from the remaining trees with clade credibility values (posterior probabilities; PP).

Results

The DNA sequence matrix covering 57 accessions from 16 taxa yielded a reasonably well-resolved phylogram (Fig. 3). The outgroup taxa *Ephedra fragilis* and *E. foeminea* (Outgroup, Fig. 3) are sister to an unambiguously supported clade of the three accessions of *E. likiangensis* Florin (ML BS 100 % / PP 1.00). This clade is in turn

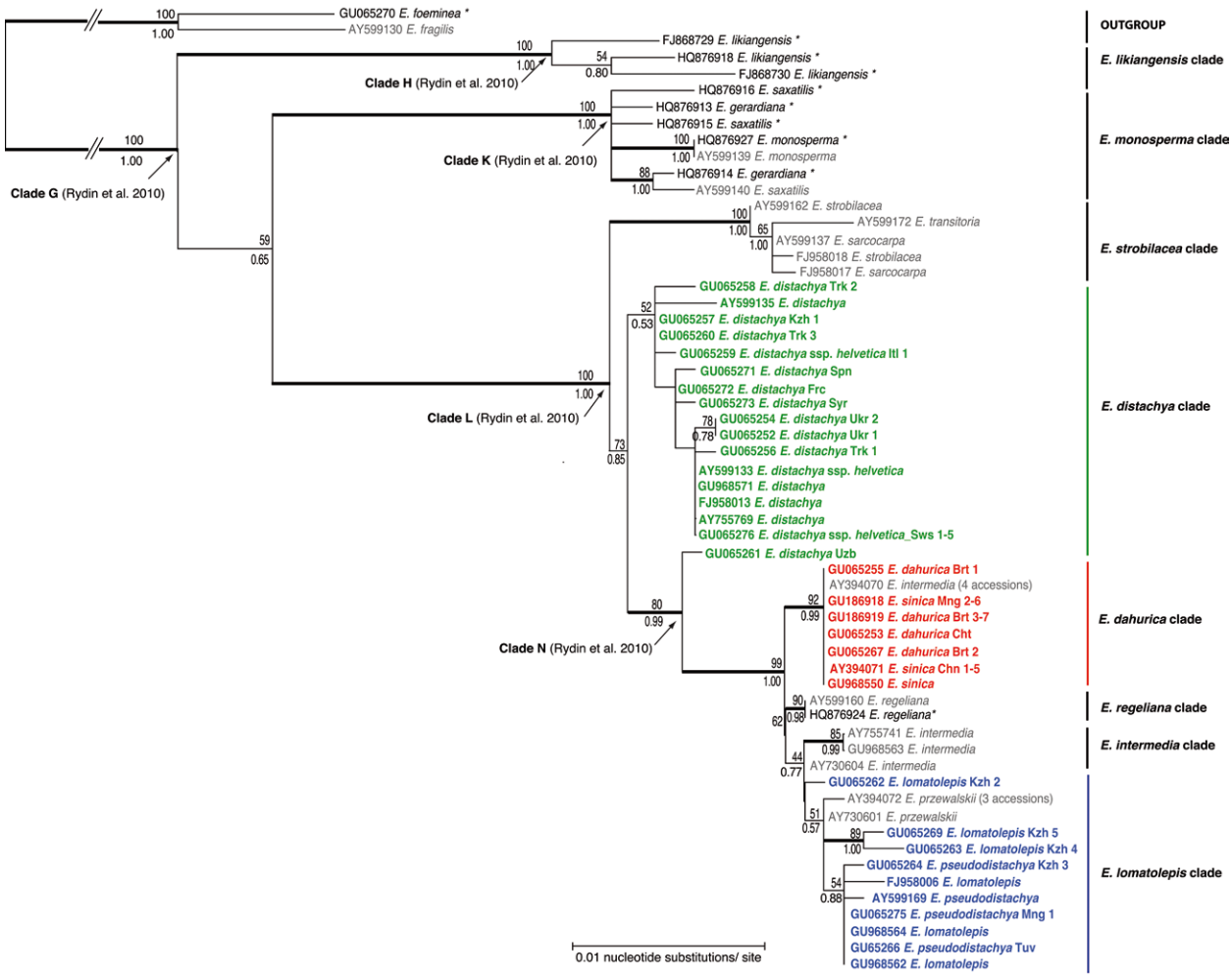


Fig. 3. Maximum likelihood tree for *Ephedra* obtained from nr ITS1 sequences. Likelihood bootstrap values are indicated above branches, Bayesian posterior probability values below branches, and the geographic origin of each accession follows species names. Our own sequences from species of the *E. distachya / E. sinica* group are marked by a geographical abbreviation, those from related species by an asterisk, while data from GenBank is shown in gray font (*E. dahurica* Brt 3–7 represents five accessions with identical ITS1 sequence from Buryatia; *E. sinica* Chn 1–5 represent five accessions from China and *E. sinica* Mng 3–7 represents five accessions from Mongolia with identical ITS1 sequences; GenBank *E. intermedia* AY394070 represents four accessions with identical ITS1 sequences). Branches with hash marks have been shortened to allow for enlargement of ingroup relationships.

Downloaded From: https://bioone.org/journals/Willdenowia on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

marginally well supported (ML BS 59 % / PP 0.65) to be the sister of the remaining taxa that form two unambiguously supported clades (ML BS 100 % / PP 1.00). The first of these two highly supported clades (*E. monosperma* clade, Fig. 3) is comprised of *E. monosperma, E. saxatilis* (Stapf) Royle ex Florin and *E. gerardiana* Wall. ex Florin. The second, larger clade comprises three subclades: The first, unambiguously supported clade (*E. strobilacea* clade, Fig. 3) consists of *E. transitoria, E. strobilacea* and *E. sarcocarpa* (BS 100 % / PP 1.00). The second, marginally supported clade (*E. distachya* clade, Fig. 3) comprises all 20 accessions of *E. distachya* except for the easternmost accession of *E. distachya* from Uzbekistan (ML BS 52 % / PP 0.53). The individual *E. distachya* samples differ from each other by up to three nucleotide changes. The third subclade shows a basally branching *E. distachya* (GU065261) from Uzbekistan as sister to the remaining species. Within the remaining species a polytomy shows a well supported clade made up of 7 accessions of *E. dahurica* representing 8 samples and 3 accessions of *E. sinica* (representing 11 samples) that have almost identical sequences. These 10 sequences of *E. dahurica* and *E. sinica* are placed in a well supported clade (ML BS 92 % / PP 0.99) together with a sequence of *E. intermedia* (AY394070, representing 4 accessions). Another small well supported clade (*E. regeliana* clade, Fig. 3) consists of the two accessions of *E. regeliana* (ML BS 90 % / PP 0.98). Relationships between the remaining accessions of *E. intermedia, E. lomatolepis, E. przewalskii* and *E. pseudistachya* are not well supported and the taxa cannot be reliably placed, except for two accessions of *E. intermedia,* which are highly supported in a clade (*E. intermedia* clade, Fig. 3, ML BS 85 % / PP 0.99), as well as another well supported clade of two accessions of *E. lomatolepis* (BS 89 % / PP 1.00). Three additional accessions of *E. lomatolepis* group together with four accessions of *E. pseudodistachya* in a weakly to moderately supported clade (Fig. 3, ML BS 54 % / 0.88 PP).

Discussion

Relationship of the *Ephedra distachya / E. sinica* group to other species of the "L" clade

The phylogenetic analysis corroborates the monophyly of the "L" clade (Rydin & al. 2010, identical with the second Asia clade in Rydin & Korall 2009) that was previously sampled by Ickert-Bond & al. (2009), Rydin & Korall (2009), Rydin & al. (2010) and Ickert-Bond & Rydin (2011). Likewise its position related to the "K" clade (= first Asia clade, respectively), which widely corresponds to our *Ephedra monosperma* clade, and the "H" clade (= China clade), here named *E. likiangensis* clade, remains basically unchanged (Fig. 3). An interesting and well supported difference (see Results) is the clear separation of the *E. strobilacea* clade. While in earlier analyses *E. strobilacea*, *E. sarcocarpa* and *E. transitoria* formed a subclade together with *E. distachya* in the "M" clade (Rydin & al. 2010), in our phylogeny they appear as a distinct clade at the base of the "L" clade. The distinct position of these taxa is further supported by sharing a very stout habit and inhabiting extremely arid semideserts of the Irano-Turanian region. It is interesting to note that the two accessions of *E. strobilacea* are not resolved as monophyletic, rather one of the accessions (AY599162) appears basally to the remainder, possibly due to the fact that it belongs to the morphologically very similar *E. microbracteata* Ghareman (≡ *E. strobilacea* subsp. *microbracteata* (Ghareman) Freitag & Maier-St.).

Previously, the "N" clade has been shown to include only *Ephedra distachya, E. sinica* and *E. lomatolepis,* but now we show conclusively that *E. pseudodistachya* and *E. dahurica* also belong here (Fig. 3). However, as in phylogenies published before, the morphologically defined *Ephedra distachya / E. sinica* group is not monophyletic because *E. intermedia*, *E. regeliana* and *E. przewalskii* Stapf, which on morphological grounds are well separated, remain nested among *E. sinica, E. dahurica, E. pseudodistachya* and *E. lomatolepis.* On the other hand, *E. fedtschenkoi* Paulsen, which is shown as a member of the "N" clade in Rydin & Korall (2009), is according to our own sequences (not shown) from material collected near the type locality, instead well supported as a member of the "M" clade. The material analysed by Rydin & Korall (2009) is from a cultivated plant of unknown provenance and likely misidentified.

Ephedra intermedia — This is the most puzzling species because the different accessions included in this study are placed in three different clades with two of them unambiguously supported and one (AY394070, representing three different vouchers from Xinjiang province, Long & al. 2005) having the same ITS signature as *E. sinica* and *E. dahurica*. Our results indicate that *E. intermedia* is polyphyletic. Rydin & al. (2010) already detected that their four accessions of *E. intermedia* were placed in two different clades. However, at this point no conclusions can be drawn because the hitherto analysed accessions cover only small sections of the vast distributional area (western Iran to central China). It is noteworthy that seven segregate species have been described by different authors, often from the same or adjoining areas for *E. intermedia: E. glauca* Regel (1880), *E. heterosperma* Nikitin (1957), *E. microsperma* Nikitin (1957), *E. ferganensis* Nikitin (1957), *E. rituensis* Yang & al. (2003), *E. sumlingensis* Sharma & Uniyal (2009), *E. kardangensis* Sharma & al. (2010), *E. khurikensis* Sharma & al. (2010). Except for *E. glauca,* none of these species are generally accepted. Further studies are required to disentangle the conflicting morphological and molecular diversity of the *E. intermedia* species complex.

Ephedra regeliana — The two accessions of *E. regeliana* unambiguously group together (Fig. 3). They are equally well separated from the related Central Asian species by the ITS signature as by their morphology and ecology.

Downloaded From: https://bioone.org/journals/Willdenowia on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Ephedra przewalskii — The two accessions sequenced here are included in a weakly supported clade (ML BS 51 %, PP 0.57) together with *E. intermedia, E. lomatolepis* and *E. pseudodistachya,* but their position within this clade is not well supported, contrary to the unmistakable morphological characters, particularly the distinct membranous bracts.

Relationships among the taxa of the *Ephedra distachya* / *E. sinica* group

***Ephedra distachya* —** We included 17 sequences representing 21 accessions, by far the broadest sampling ever analysed not only of *E. distachya* but of any species of the genus. 16 sequences (20 accessions) form a marginally supported clade (ML BS 52 %, PP 0.53), while one sample from western Uzbekistan appears well supported as the basally diverging species in the *E. dahurica* clade, separated from all other *E. distachya* accessions. Sequences included for *E. distachya* show the highest degree of variation in the ITS1 sequences studied. It is noteworthy, that the *E. distachya* sequences sampled come from a vast distributional area, which extends over several floristic regions with rather diverse climatic conditions. However, a detailed look at the site conditions reveals fundamental similarities. From eastern Europe to western Siberia, *E. distachya* is a component of zonal dry steppes and semi-desert communities, prefers coarse-textured soils and is often associated with species such as *Stipa capillata* L., *Artemisia campestris* L., *Kochia prostrata* (L.) Schrad., *Krascheninnikowia ceratoides* (L.) Gueldenst. and *Salsola laricina* Pall. The isolated occurrences in Central Europe are typical azonal islands on skeletal soils with impoverished steppe vegetation. In the northern Mediterranean as well as along the Atlantic coast *E. distachya* grows in sand dunes or on dry pebble layers. This distributional pattern can best be explained by extensive plant migrations followed by partial extinctions caused by climatic fluctuations during the Pleistocene (Frenzel 1992; Lang 1994).

Interestingly, no trend towards geographical clustering of mutations is recognisable (see Fig. 3). Instead, sometimes samples from widely separated locations group together (e.g. GU065257 from Kazakhstan and GU065272 from southern France), while on the other hand samples from adjoining places differ more conspicuously in their sequences (e.g. the three Turkish samples). Even the three samples of the morphologically distinct *Ephedra distachya* subsp. *helvetica,* which include the most doubtful *E. negrii* (our sample Itl1), do not group together though probably it represents the youngest taxon of the genus. As the area of the Alps was completely covered by ice during long intervals of the Pleistocene, most likely the endemic subspecies has originated from re-migrating subspecies *distachya* only after the retreat of the glaciers that happened c. 15 000 years ago. Detailed molecular studies, which include a broad sampling of the populations in and around the Alps are underway by E. Zippel (Berlin Botanic Garden and Botanical Museum).

The morphologically doubtful subdivision of *Ephedra distachya* into a Mediterranean subsp. *distachya* and an eastern European to Siberian subsp. *monostachya* (L.) Riedl (see p. 204) is not supported by our data. An eastern segregate of *E. distachya* could be presumed from the phylogeny by Rydin & al. (2010), where a collection made by F. K. Karo (no. 236) differs considerably from the otherwise rather homogeneous cluster. The sample is cited as having been collected in southeastern Russia, but at that time Karo collected in southeastern Siberia, mainly in Dahuriya (Chaudhri & al. 1972), far outside of the area of *E. distachya*. Most likely that sample belongs to *E. dahurica,* which otherwise is omitted from their sampling. *E. vvedenskyi* and *E. aurantiaca* (see p. 204) were not included in our sampling. However, a GenBank accession (GU968571, Rydin & al. 2010) under *E. distachya* (Fig. 3) could well represent *E. vvedenskyi* as it was taken from its core area, Turkmenistan. It agrees with other samples of *E. distachya* from the Ukraine.

After all, at least one definite conclusion can be drawn from our results: *Ephedra pseudodistachya* and *E. dahurica,* which are morphologically more similar to *E. distachya,* are clearly confirmed as distinct from *E. distachya* based on their differing molecular signatures. From the localities cited by the pre-Linnean botanists Amman (1739) and Gmelin (1747) from Siberia it is evident that they have lumped together all three species that later were named *E. monostachya,* but by chance unfortunately only one specimen of *E. distachya* was sent to Linné.

***Ephedra sinica* and *E. dahurica* —** The 19 accessions of *E. sinica* and *E. dahurica* used in this study showed extremely low sequence divergence and only 7 distinct sequences are included (several samples had identical sequences, Fig. 3), but form a well supported clade (*E. dahurica* clade, Fig. 3). Unexpectedly they are joined by one sequence of *E. intermedia* that represents 4 accessions with identical nucleotide sequences from Xinjiang province in western China beyond the known area of *E. sinica* and *E. dahurica* (see p. 205). The three taxa have almost identical ITS1 sequences except for one Chita accession, which differs in one base position (Fig. 3). This low sequence divergence contrasts with the intraspecific sequence variation found in *E. distachya*. It is even more surprising when considering that the 19 specimens of the *E. dahurica* / *E. sinica* group span a rather large area from near Lake Baikal in Buryatia to eastern China.

These molecular results are somewhat disappointing because they do not give any support to the morphologically based discrimination of the three species. The deviating *Ephedra intermedia* accession AY394070 can be clearly distinguished from *E. sinica* and *E. dahurica,* but not from other populations of *E. intermedia,* by the corkscrew-like bent micropylar tube, a mutation that parallels *E. distachya* subsp. *helvetica*, and furthermore

Downloaded From: https://bioone.org/journals/Willdenowia on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

by the stout habit and the distinct geographical distribution. The taxonomy of *E. sinica* and *E. dahurica* are further complicated. Though the differential characters are well expressed in the authentic material, the plants in the field and in herbarium collections from Mongolia and southeastern Siberia show all transitions in length of leaves, colour of leaf sheaths and structure of twig surface, so that many plants could be assigned to the one or the other species only statistically. At first, when comparing the type material, we expected that their different habit could serve as a good additional character for identification. While *E. dahurica* (one specimen in LE) shows a typical chamaephytic habit, with annual twigs arising from much stronger and woody perennial branches, the very many original specimens (lectoypification in prep. by Freitag & Maier-Stolte) of *E. sinica* (F, GH, K, LE, US) are almost hemicryptophytic, with perennial stems more or less missing and the annual twigs arising in dense bunches at the level of the soil surface. However, in the field (Buryatia) both types occur in adjoining habitats: the subshrubby plants on rocky slopes with *Caragana pygmaea* (L.) DC. and *Artemisia gmelinii* Web. ex Stechm. and the hemicryptophytic plants on gravelly lower slopes and in plains covered by steppes with dominating *Stipa krylovii* Roshev. Obviously the rocky slopes offer a better water supply and the steep topography protects the plants better against browsing by goat and sheep. Intermediates between both growth forms are common.

For similar reasons Galanin (2008) already reduced both taxa to subspecies. However, his new combination *Ephedra sinica* subsp. *dahurica* (Turcz.) Galanin is formally incorrect because *E. dahurica* is the earlier validly published name. Therefore, if the two taxa are considered as subspecies, they must be named *E. dahurica* subsp. *dahurica* and *E. dahurica* subsp. *sinica* (Stapf) Freitag & Maier-St., respectively. Anyhow, *E. dahurica* seems to be more or less restricted to southeastern Siberia and northern Mongolia, whereas *E. sinica* is common in the steppe areas of eastern and northeastern China. More detailed morphological studies in the area of subsp. *sinica* are warranted.

It has been shown that also the ephedrine content varies considerably in *Ephedra sinica* s.l., from 1.2–1.3 % for Mongolia, 0.76–0.83 % for Inner Mongolia and 0.45 % for Buryatia (Wang & al. 2010).

Our results somewhat contrast with those of Rydin & al. (2010). Out of their 4 samples of *Ephedra sinica,* No. 151 from Inner Mongolia takes a separate position, which could reflect either intraspecific variation not observed in our larger sampling, or incorrect identification.

***Ephedra lomatolepis* —** Our phylogenetic analysis unfortunately does not resolve the relationships in the composite clade consisting of *E. lomatolepis, E. pseudodistachya, E. przewalskii* and one accession of *E. intermedia.* Only one small subclade made up of two samples of *E. lomatolepis* is well supported and its placement among several accessions of *E. przewalskii* confirms the close morphological affinity to that species. However, three other accessions of *E. lomatolepis* cluster together in a weakly supported subclade with the morphologically differing *E. pseudodistachya,* with which it is widely sympatric, and one accession even with *E. intermedia*. We still hesitate to conclude that *E. lomatolepis* is a polyphyletic taxon because the danger of misidentifications is apparent, particularly in the information taken from GenBank. In contrast to our results, the five samples of *E. lomatolepis* included by Rydin & al. (2010) cluster together and appear as sister to *E. przewalskii*. However, these authors did not sample *E. pseudodistachya.*

***Ephedra pseudodistachya* —** The four samples of *E. pseudodistachya* appear indistinguishable from *E. lomatolepis* in the respective heterogeneous clade. Nevertheless, a most interesting result of our study is the distinct position of *E. pseudodistachya* as compared to *E. distachya* and *E. dahurica.* By that, the ITS data support its status as a distinct species, which so far is not generally accepted by taxonomists. The three species are morphologically and ecologically very close to each other. *E. pseudodistachya* was collected from stony slopes in steppe areas with dwarf shrubland communities of *Spiraea hypericifolia* M. Bieb., *S. media* F. Schmidt, *Berberis sibirica* Hort. ex Schult. f. and *Stipa capillata* L. in Tuva and from extremely dry rocky slopes in southern Kazakhstan.

Outlook

It is noteworthy that the low amount of nr ITS1 sequence divergence and the almost complete lack of variation in ITS2 and cp *trnL-F* sequences corroborates findings and conclusions in other studies that species of the *Ephedra distachya* / *E. sinica* complex are comparatively young. Probably they have evolved only from the late Miocene and early Pliocene onwards (e.g. Ickert-Bond & al. 2009). This is also reflected by the comparatively small amount of morphological variation, but contrasts with the biogeographical and ecological specialisation in the Eurasian *Ephedra* species.

Because of the generally low or missing sequence divergence in traditionally used molecular markers, the search for more highly variable regions should be intensified. However, particular attention also should be given to unambiguously identified plant material in order to prevent an increase of confusion in GenBank data. For the time being, the taxonomy of the respective groups can be improved by further morphological studies only. With regard to the "N" clade this is warranted especially in case of *Ephedra intermedia,* but also in the eastern populations of *E. distachya* (W Kazakhstan and Uzbekistan) as well as in *E. pseudodistachya* and *E. lomatolepis* in Central Asia.

Downloaded From: https://bioone.org/journals/Willdenowia on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Acknowledgements

This study was supported by a Grant-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology of Japan. We gratefully acknowledge also the funding of field work by the Deutsche Forschungsgemeinschaft (DFG): Kazakhstan 1992, grant Fr 438; Caspian Lowland 1998, grant Fr 222/14-1; Uzbekistan 2000, grant We 1830/2-1; S Siberia 2003, grant We 1839/2-3, and by the National Science Foundation (USA), grant NSF-0629657. We like to thank Dr Dava Chimitov for support during the field work in Buryatia, Ms Miyuki Sakai, Mr Yuhki Abiru and Mr Jordan Metzgar for technical assistance and Dr Elke Zippel for providing the image reproduced in Fig. 1C. Finally, we want to thank two reviewers for their helpful comments.

Note added in proof — Only after having submitted the manuscript and finished the calculations, we became aware of the article bei Kitani & al., Genetic diversity of *Ephedra* plants in Mongolia inferred from Internal Transcribed Spacer sequences of nuclear ribosomal DNA, Biol. Pharm. Bull. 34: 717–726. 2011. Therefore, unfortunately, their additional sequences could not be included.

References

Amann J. 1739: Stirpium rariorum in imperio Rutheno sponte provenientium icones et descriptiones collectae **1.** – Petropoli: Typographia Academiae Scientiarum.

Chaudhri M. N., Vegter I. H. & Wal C. M. de 1972: Index herbariorum. Part 2, Collectors 3. – Regnum Veg. **86.**

Cheng C. Y. 1978: *Ephedraceae.* – Pp. 468–489 in: Cheng W. C. & Fu L. K. (ed.), Flora Reipublicae Popularis Sinicae **7.** – Beijing: Science Press.

Chinese Pharmacopoeia Commission (ed.) 2005: Pharmacopoeia of the People's Republic of China **1.** – Beijing: People's Medical Publ. House.

Czerepanov S. K. 1995: Vascular plants of Russia and adjacent states (the former USSR). – Cambridge: Cambridge University.

Florin C. R. 1933: Über einige neue oder wenig bekannte asiatische *Ephedra*-Arten der Sektion *Pseudobaccatae* Stapf. – Kongl. Svenska Vetenskapsakad. Handl., ser. 3, **12(1):** 1–49.

Freitag H. & Maier-Stolte M. 1993: *Ephedra*. – P. 49 in: Tutin T. G., Burges N. A., Chater A. O., Edmondson J. R., Heywood V. H., Moore D. M., Valentine D. H., Walters S. M. & Webb D. A. (ed.), Flora europaea, ed. 2, **1.** – Cambridge: Cambridge University.

Freitag H. & Maier-Stolte M. 1994: *Ephedraceae*. – Pp. 5–16, 39–52 in: Browicz K. (ed), Chorology of trees and shrubs in Southwest Asia and adjacent regions **10.** – Poznan: Polish Scientific Publishers.

Freitag H. & Maier-Stolte M. 2009: *Ephedra*. – In: FloraGreif. Virtual flora of Mongolia. – Greifswald University: Published at http://greif.uni-greifwald.de/floragreif

Frenzel B. 1992: Atlas of the palaeoclimates and palaeoenvironments of the northern hemisphere. Late Pleistocene–Holocene. – Budapest: Hungarian Academy of Sciences; Jena: G. Fischer.

Fu L. G., Yu Y. F. & Riedl H. 1999: *Ephedraceae*. – Pp. 97–103 in: Wu, Z. Y. & Raven P. H. (ed.), Flora of China **4.** – Beijing: Science Press & St Louis: Missouri Botanical Garden.

Galanin A. V. 2008: *Ephedra*. – Pp. 74 in: Galanin A. V., Belikovich A. B. & Khrbko O. B. (ed.), Flora of Dahuria **1.** – Vladivostok: Dal'nauka.

Gmelin J. G. 1747: Flora sibirica sive historia plantarum Siberiae **1.** – St. Petersburg: Academia Scientiarum.

Govaerts R. 2010a: World Checklist of *Ephedraceae.* – Royal Botanic Gardens, Kew: published at http://apps.kew.org/wcsp/home.do.

Govaerts R. 2010b: *Ephedraceae.* – In: Euro+Med PlantBase. – Published at http://ww2.bgbm.org/EuroPlusMed/query.asp.

Grubov V. I. 1982: Opredelitel' sosudistykh rastenii Mongolii. – Leningrad: Nauka [Engl. ed.: 2000, Key to the vascular plants of Mongolia. Enfield & Plymouth: Science Publishers].

Gubanov I. A. 1996: Konspekt flory vneshnej Mongolii (sosudistye rasteniya) – Moskva: Valang.

Huang J., Giannasi D. E. & Price R. A. 2005: Phylogenetic relationships in *Ephedra (Ephedraceae)* inferred from chloroplast and nuclear DNA sequences. – Molec. Phylogen. Evol. **35:** 48–59.

Ickert-Bond S. M. & Rydin C. 2011: Micromorphology of the seed envelope of *Ephedra (Gnetales)* and its relevance for the timing of evolutionary events. – Int. J. Pl. Sci. **172:** 36–48.

Ickert-Bond S. M. & Wojciechowski M. F. 2004: Phylogenetic relationship in *Ephedra (Gnetales)*: evidence from nuclear and chloroplast DNA sequence data. – Syst. Bot. **29:** 834–849.

Ickert-Bond S. M., Rydin C. & Renner S. S. 2009: A fossil-calibrated relaxed clock for *Ephedra* indicates an Oligocene age for the divergence of Asian and New World clades and Miocene dispersal into South America. – J. Syst. Evol. **47:** 444–456.

Japanese Ministry of Health, Labour and Welfare 2006: Japanese Pharmacopoeia, ed. 15. – Published at http://www.mhlw.go.jp/index.shtml

Jarvis C. 2007: Order out of chaos: Linnean plant names and their types. – London: The Linnean Society & The Natural History Museum.

Kakiuchi N. & Mikage M. 2007: Collaborative survey of *Ephedra* plants in Buryatia, Russia. – Newslett. Himalayan Bot. **39:** 1–8.

Lang G. 1994: Quartäre Vegetationsgeschichte Europas: Methoden und Ergebnisse. – Stuttgart: G. Fischer.

Liu Y. 1985: Flora in desertis Reipublicae Populorum Sinarum **1.** – Beijing: Science Press [in Chinese].

Downloaded From: https://bioone.org/journals/Willdenowia on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Long C. F., Kakiuchi N., Takahashi A., Komatsu K., Cai S. Q. & Mikage M. 2004: Phylogenetic analysis of the DNA sequence of the non-coding region of nuclear ribosomal DNA and chloroplast of *Ephedra* plants in China. – Pl. Med. **70:** 1080–1085.

Maddison D. R. & Maddison W. P. 2005: MacClade v. 4.08. – Sunderland, Mass.: Sinauer Associates.

Meyer C. A. 1846: Versuch einer Monographie der Gattung *Ephedra* – St. Petersburg: Druckerei der Kaiserlichen Akademie der Wissenschaften.

Mikage M. 2009: Report of achievement for Grant-in-Aids for Project No. 17405014 from the Ministry of Education, Culture, Sport Science and Technology of Japan. – Published at http://kaken.nii.ac.jp/ja/p/17405014

Müller K. 2006: Incorporating information from length-mutational events into phylogenetic analysis. – Molec. Phylogen. Evol. **38:** 667–676.

Nikitin V. A. 1957: *Ephedraceae* – Pp. 503–504 in: Ovchinnikow P. N. (ed.), Flora Tadzhikskoi SSR **1.** – Moskva & Leningrad: Izd. Akedamii Nauk SSSR.

Nouviant J. 1993: *Ephedra negrii.*– Pp. 65 in: Kerguelen M., Index synonymique de la Flore de France. – Paris: Muséum National d'Histoire Naturelle.

Nouviant J. 1997: Recherches sur *Ephedra* en Europe III: Entre *Ephedra distachya* et *helvetica.* – Bull. Murith. Soc. Valais, Sci. Nat. **115:** 68–75.

Nouviant J: 1998: Recherches sur *Ephedra* in Europe V: Typification de *E. monostachya* et clé de détermination des *Ephedra* européens. – Bull. Murith. Soc. Valais, Sci. Nat. **116:** 81–87.

Pachomova M. G. 1968: *Ephedra vvedenskyi* Pachom. sp. nov. – Pp. 198–199 in: Kovalevskaya S. S. (ed.), Conspectus florae Asiae Mediae **1.** – Tashkent: Academia Scientiarum USSR.

Pachomova M. G. 1971: *Ephedra*. – Pp. 25–33 in: Grubov V. I., Macenko A. E. & Pakhomova M. G. (ed.), Plantae Asiae Centralis **6.** – Leningrad: Nauka.

Peschkova G. A. 2005: Synopsis of the Siberian *Ephedra* species *(Ephedraceae)*. – Bot. Zhurn. **90:** 423–426. [in Russian].

Pfeiffer W. & Stamatakis A. 2010: Hybrid MPI/Pthreads parallelization of the RAxML Phylogenetics Code. – In: Ninth IEEE International Workshop on High Performance Computational Biology (HiCOMB 2010) Atlanta. – Published on the CIPRES Science Gateway at http: www.phylo.org/subsections/portal/

Regel E. 1880: *Ephedra glauca* Rgl. – Trudy Imp. St.-Peterburgsk. Bot. Sada **6:** 484.

Riedl R. 1967: Die Gattung *Ephedra* in Europa. – Sci. Pharm. **36:** 225–228.

Ronquist F. & Huelsenbeck J. P. 2003: MrBayes 3.0: Bayesian phylogenetic inference using mixed models. – Bioinformatics **19:** 1572–1574.

Rydin C. & Korall P. 2009: Evolutionary relationships in *Ephedra (Gnetales)*, with implications for seed plant phylogeny. – Int. J. Pl. Sci. **170:** 1031–1043.

Rydin C., Khodabandeh A. & Endress P. K. 2010: The female reproductive unit of *Ephedra (Gnetales)*: comparative morphology and evolutionary perspectives. – Bot. J. Linn. Soc. **163:** 387–430.

Sharma P. & Uniyal P. L. 2009: *Ephedra sumlingensis (Ephedraceae)* Sharma & Uniyal a new species from Himachal Pradesh, India. – Bull. Bot. Surv. India **50:** 179–182.

Sharma P., Uniyal P. L. & Hammer O. 2010: Two new species of *Ephedra (Ephedraceae)* from the western Himalayan Region. – Syst. Bot. **35:** 730–735.

Simmons M. P. & Ochoterena H. 2000: Gaps as characters in sequence-based phylogenetic analyses. – Syst. Biol. **49:** 369–381.

Simmons M. P., Müller K. & Norton A. P. 2007: The relative performance of indel coding methods in simulations. – Molec. Phylogen. Evol. **44:** 724–740

Siplivinskiy V. N. 1973: Chto takoe *Ephedra dahurica* Turcz.? [What is *Ephedra dahurica* Turcz.?]. – Novosti Sist. Vyssh. Rast. **1973:** 355–357.

Stamatakis A., Hoover P. & Rougemont J. 2008: A fast bootstrapping algorithm for the RAxML Web-Servers. – Syst. Biol. **57:** 758–771.

Stapf O. 1889: Die Arten der Gattung *Ephedra*. – Denkschr. Kaiserl. Akad. Wiss., Wien, Math.-Naturwiss. Kl. **56:** 1–112.

Stapf O. 1927: Ma huang of China (*Epheda sinica* Stapf). – Bull. Misc. Inf. Kew. **3:** 133–134.

Taberlet P., Gielly F., Pautou G. & Boubet J. 1991: Universal primers for amplification of three non-coding region of chloroplast DNA. – Pl. Molec. Biol. **17:** 1105–1109.

Takhtajan A. L. & Pachomova M. G. 1967: *Ephedra aurantiaca* Takht. & Pachom. sp. n. – Bot. Mater. Gerb. Inst. Bot. Akad. Nauk Uzbeksk. S.S.R. **18:** 53.

Tang S. W. [ed. by Shang Z. J., based on the 12 century edition by Cheng A.] 2002: Daguan Bencao. – Hefei: Anhui Academic Publisher [in Chinese].

Thiers B. 2008+ [continuously updated]: Index herbariorum: A global directory of public herbaria and associated staff. – New York Botanical Garden: published at http://sweetgum.nybg.org/ih/.

Turczaninow N. S. 1854: Flora baicalensi-dahurica [17]. – Bull. Soc. Imp. Naturalistes Moscou **27:** 353–422.

Wang L., Kakiuchi N. & Mikage M. 2010: Studies on *Ephedra* plants in Asia **6.** Geographical changes of anatomical features and alkaloid content of *Ephedra sinica*. – J. Nat. Med. **64:** 63–69.

Yang C. Y. 1993: *Ephedraceae*. – Pp. 87–109 in: Yang C. Y. (ed.), Flora xinjiangensis **1.** – Wulumuqi: Xinjiang Science, Technology and Hygiene Publishing.

Yang Y. 2002: Classification of Chinese *Ephedra* plants and discussion on their difference. – Doctoral thesis, Graduate School of the Chinese Academy of Science. [in Chinese].

Yang Y., Fu D. & Zhu G. 2003: A new species of *Ephedra (Ephedraceae)* from China. – Novon **13:** 153–155.

Downloaded From: https://bioone.org/journals/Willdenowia on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Appendix 1. List of taxa sampled with voucher and GenBank accession numbers

Full voucher data include collector and collecting number, collecting date, collection locality and, in brackets, herbarium code (following Thiers 2008+) and herbarium accession number, if any. The last part of an entry are the GenBank accession numbers, referring to the ITS1 and *trnL-F* sequences (for Brt 3–7, Chn 1–5 and Mng 3–7 only one accession was submitted to GenBank since the data were identical for each of the respective accessions). Data for own sequences from taxa of the *Ephedra distachya / E. sinica* group are preceded by a geographical abbreviation. Data for sequences (ITS1) taken from GenBank are preceded by an asterisk.

Ephedra dahurica Turcz.: Brt 1, *H. Freitag 33.121* (KAS), 3.9.2003, Russia, Buryatia, Gusinoozersk, GU065255, GU111509; Brt 2, *H. Freitag 33.119* (KAS), 3.9.2003, Russia, Buryatia, c. 95 km SW Ulan-Ude, GU065267, GU111521; Brt 3, *M. Mikage & al.* (KANP 060804337), 4.8.2006, Russia, Buryatia, suburb of Ulan-Ude, GU186919, GU186921; Brt 4, 5, *M. Mikage & al*. (KANP 060809358, KANP 060809361), 9.8.2006, Russia, Buryatia, Tugnuiskii Steppe, GU186919, GU186921; Brt 6, 7, *M. Mikage & al.* (KANP 060810366, KANP 060809370), 10.8.2006, Russia, Buryatia, Khoshun-Uzur near Ulan-Ude; Cht, *H. Freitag 33.130* (KAS), 11.9.2003, Russia, Chita dist., Daurya Biosphere reserve, GU065253, GU111507. — ***E. distachya*** L. subsp. ***distachya****:* Spn, *H. Freitag s.n.* (KAS), 30.5.1963, E Spain, Dehesa de Valencia, GU065271, GU111524; Frc, *P. König s.n.* (KAS), 26.8.1981, S France, Roussillon, St. Cyprien Plage, GU065272, GU111525; Trk 1, *H. Freitag 28.772* (KAS), 5.10.1997, Turkey, C Anatolia, Konya prov., GU065256, GU111510; Trk 2, *M. Nydegger 45.473* (KAS), 19.5.1990, Turkey, E Anatolia, near Aralik, GU065258, GU111512; Trk 3, *H. Freitag 28.834* (KAS), 8.10.1997, Turkey, Ankara prov., E side of Tuz Gölü Lake, GU065260, GU111514; Ukr 1, *H. Freitag 33.259* (KAS), 12.10.2003, Ukraine, S Crimea, Ayudag Mt., GU065252, GU111506; Ukr 2, *H. Freitag 33.231* (KAS), 9.10.2003, Ukraine, NE Crimea, Arabatskaya Strel'ka, GU065254, GU111508; * Ukraine, *Pidopliczka, s.n.* (UPS), 1925, FJ958013; Syr, *H. Freitag 30.144* (KAS), 17.10.2001, Syria, 40 km NE Damascus, GU065273, GU111526; Uzb, *W. Wucherer 646* (KAS), 1.10.1994, Uzbekistan, Aral Lake, cliff on W side, GU065261, GU111515; Kzh 1, *H. Freitag 26.506* (KAS), 26.9.1992, Kazakhstan, Kzyl-Orda dist., 54 km E Novokazalinsk, GU065257, GU111511; * *W. Wucherer 453* (KAS), S Kazakhstan, coast of Aral Lake, AY599135; * *Kolakovsky 3651* (S), 1951, Turkmenistan, GU968571; *(Rydin) 03-684* (S), AY755769. — ***E. distachya*** subsp. ***helvetica*** (C. A. Mey.) Asch. & Graebn.: Itl 1 (*"E. negrii Nouviant"*), *H. Freitag 26.678* (KAS), 22.5.1993, N Italy, S Tyrol, Vinschgau, GU065259, GU111513; Sws 1-5, *N. Kakiuchi & al. 1-5* (KANP 08080801-3, 08080805-6), 8.8.2008, Switzerland, Valais, Sion Castle, GU065276, GU111529; * *H. Freitag 20.332* (KAS), 4.8.1990, Switzerland, Wallis, Mont Orges, AY599133. — ***E. gerardiana*** Wall. ex Florin: *G. & S. Miehe 3.329* (herb. Miehe), 9.7.1990, Pakistan, Yasin, HQ876913; *H. Freitag 34.019* (KAS), 3.9.2004, India, Himachal Pradesh, HQ 876914. — ***E. intermedia*** Schrenk & C. A. Mey.: * *Bartolomew 8.211* (MO), 2001, China, Xinjiang, GU968563; * *C. Long & al.* (KANP 03071510), 17.7.2003, China, Xinjiang, Guhe Zhen, AY730604; * *C. Long & al.* (KANP 02309, 02341, 02363, 02364), 23.7.2003, China, Xinjiang, Aerjin Mountain, AY394070; * [*Rydin*] *03-925* (S), AY755741. — ***E. likiangensis*** Florin: *M. Mikage & al. 707063* (KANP), 22.7.2007, China, Sichuan, FJ868729; *M. Mikage & al. 707026* (KANP), 16.6.2007, China, Yunnan, FJ868730; *G. & S. Miehe 04-165-17* (herb. Miehe), 20.9.2004, China, SE Xizang, HQ876918. — ***E. lomatolepis*** Schrenk: Kzh 2, *H. Freitag & S. Rilke 26.507* (KAS), 26.9.1992, Kazakhstan, Kzyl-Orda dist., 54 km E Novokazalinsk, GU065262, GU111516; Kzh 4, *H. Freitag & S. Rilke 26.343a* (KAS), 18.9.1992, Kazakhstan, Dzhambul dist., 74 km ESE Ulanbel', GU065263, GU111517; Kzh 5, *H. Freitag & S. Rilke 26.355* (KAS), 24.8.1992, Kazakhstan, Dzhambul dist., 72 km SE Ulanbel', GU065269, GU111522; * *I. O. Baitulin & al.* (UPS), 1997, Kazakhstan, FJ958006; * *I. M. Krasnoborov 192* (MO), 1995, Siberia, Novosibirsk, GU968564; * *A. Bosshard & al. 803.24* (Z), 1989, Pakistan, GU968562. — ***E. monosperma*** J. G. Gmel. ex C. A. Mey.: *H. Freitag 33.031* (KAS), 22.8.2003, Buryatia, 30 km S Kyzyl beside track towards Duz Khol, HQ876927; * *H. Hurka & B. Neuffer 12.142* (KAS, OSBU), Mongolia, Gobi Altai, AY599139. ***E. przewalskii*** Stapf: * *C. Long & al.* (KANP 02321, 02326, 02340), 9.7.2003, China, Xinjiang, AY394072; * *C. Long & al.* (KANP 03071131), 13.–22.7.2003, China, Xinjiang, AY730601. — ***E. pseudodistachya*** Pachom.: Kzh 3, *H. Freitag 27.000* (KAS), 1.5.1994, Kazakhstan, Chimkent dist., Alimtau, GU065264, GU111518; Tuv, *H. Freitag 33.002* (KAS), 20.8.2003, Russia, C Tuva, near Kyzyl, GU065266, GU111520; Mng 1, *W. Hilbig & Z. Schamsran s.n.* (HAL), 14.8.1976, Mongolia, Altai dist., E border of Schargain Gobi, GU065275, GU111528; * *H. Hurka & B. Neuffer 10.242* (OSBU), Mongolia, Hovd Aimak, AY599169. — ***E. regeliana*** Florin: * *U. Wündisch 956* (KAS), 6.8.1998, China, SW Xinjiang, AY599160; *G. Miehe 5.318* (herb. Miehe), 23.7.1991, W China, Xinjiang, upper Gez Gorge, HQ876924. — ***E. sarcocarpa*** Aitch. & Hemsl: * *H. Freitag 13.988* (KAS), 2.8.1976, Iran, Kavir desert near Mobarakiyeh, AY599137; * *K. H. Rechinger & al. 2.786* (S), 14.4.1948, Iran, prov. Semnan, inter Veramin et Semnan, FJ958017. — ***E. saxatilis*** (Stapf) Royle ex Florin: *U. Wündisch 1.090* (KAS), 9.8.1995, Nepal, Langtang valley, HQ876916; *G. Miehe 95-18-11* (herb. Miehe), 4.9.1995, China, SE Xizang, AY599140; *R. Singh & P. Radha* (KAS), 6.7.2004, India, Uttanranchal, Deoban, HQ876915. — ***E. sinica*** Stapf: Mng 2, *M. Mikage & al.*

Downloaded From: https://bioone.org/journals/Willdenowia on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

(KANP 20531001), 29.7.2005, Mongolia, Tov dist., Lun, GU186918, GU186920; Mng 3, *M. Mikage & al.* (KANP 20531062), 6.8.2005, Mongolia, Tov dist., Baruun haraa; Mng 4, *M. Mikage & al.* (KANP 20531081), 8.8.2005, Mongolia, Tov dist., Lun; Mng 5, 6, *M. Mikage & al.* (KANP 20531083, KANP 20531085), 8.8.2005, Mongolia, Tov dist., Hustayn; Chn 1, *M. Mikage & al.* (KANP 2109), 5.6.2002, China, Inner Mongolia, Eerdousi, AY394071, AY423430; Chn 2, *M. Mikage & al.* (KANP 2133), 8.6.2002, China, Shangxi prov., Datone; Chn 3, *M. Mikage & al.* (KANP 2139), 10.6.2002, China, Hebei prov. Chengde; Chn 4, *M. Mikage & al* (KANP 2143), 1.6.2002, China, Inner Mongolia, Chifeng; Chn 5, *M. Mikage & al.* (KANP 2145), 11.6.2002, China, Inner Mongolia, Tongliao; * *J. Eriksson 05-9020* (S), 1926, China, Inner Mongolia, GU968550. — ***E. strobilacea*** Bunge: * *K. H. Rechinger & al. 2.703* (S), 21.4.1948, Iran, Semnan and Yesd prov., between Nain and Aghda, FJ 958018; * *K. H. Rechinger 27.161* (US), 26.3.1965, Iran, Kerman, near Fahraj, AY599162. — ***E. transitoria*** Riedl: * *H. Freitag 30.123* (KAS), 12.10.2001, Jordan, Shaumari Wildlife Res., AY599172.

Outgroup: ***Ephedra foeminea*** Forssk.: *H. Freitag 19.807* (KAS), 20.9.1989, Greece, Kephallonia Is., GU065270, GU111523. — ***E. fragilis*** Desf.: * *H. Freitag 27.237* (KAS), 24.9.1995, SE Spain, AY 599130.

Downloaded From: https://bioone.org/journals/Willdenowia on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use