Biogeographische Beziehungen zwischen den Alpen, dem Kaukasus und den asiatischen Hochgebirgen

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Inhaltsverzeichnis:

Einleitung

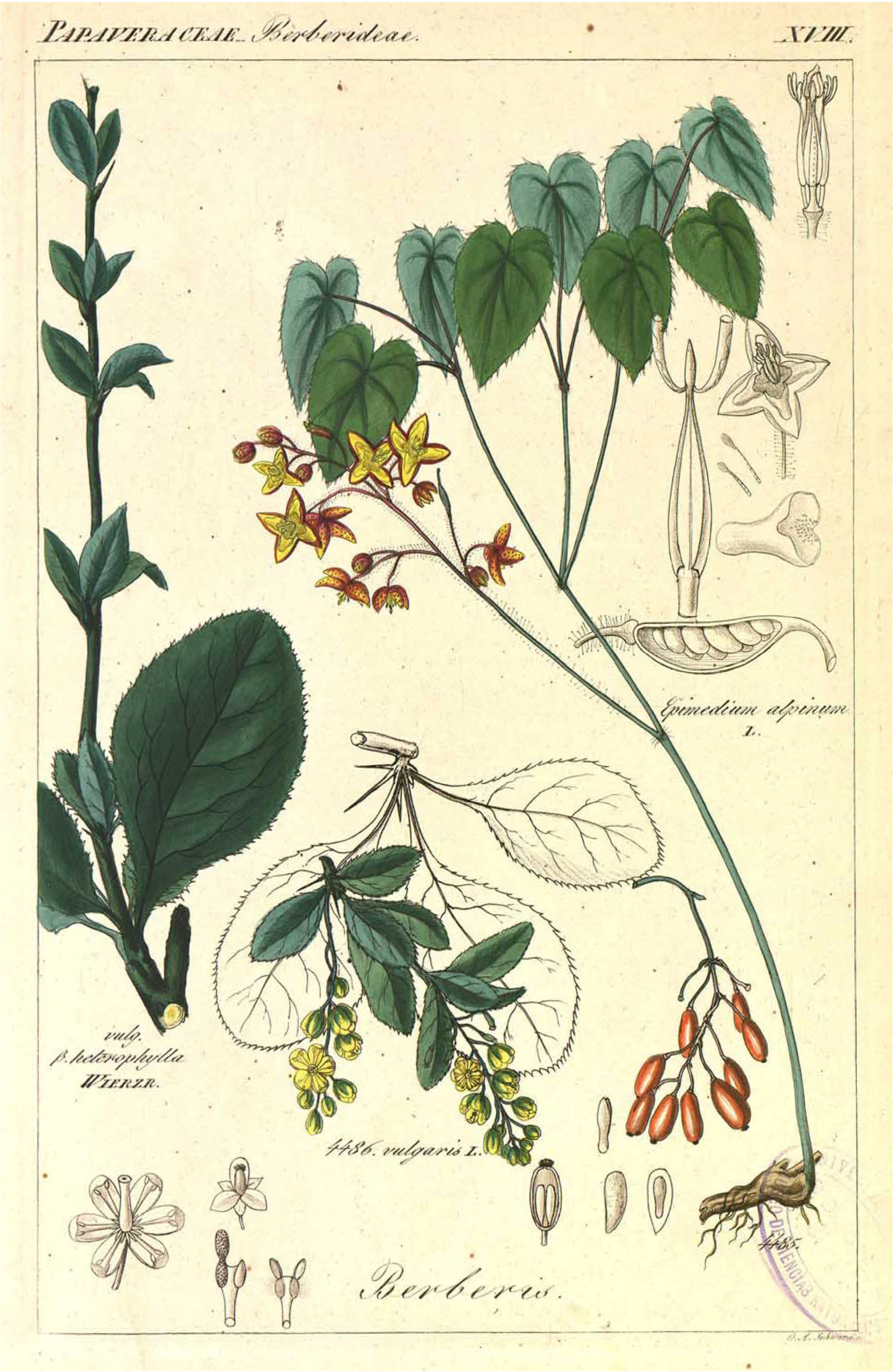
Mit der 2004 von Aeschimann et al. vorgelegten *Flora Alpina* wird zum ersten Mal, seit dem Beginn der wissenschaftlichen Erforschung der Alpen im 18. Jahrhundert, der gesamte Alpenraum vollständig floristisch erfasst. Der Alpenbogen ist ein Zentrum an Biodiversität mitten in Europa und beherbergt über 4000 Gefäßpflanzenarten, was einem guten Drittel der Biodiversität Europas entspricht. Eine ähnlich hohe Diversität findet sich in Europa nur auf dem Balkan und der Iberischen Halbinsel (Aeschimann et al. 2004). Viele Arten der Alpen kommen auch in anderen mittel- und südeuropäischen Gebirgen vor, die Ozenda (1988) als das Europäische Alpine System mit den Alpen zusammenfasst. Dieses umfasst neben den Alpen den Apennin, die Dinariden, das Balkangebirge inkl. der Rhodopen, das Zentralmassiv, den Jura, die Karpaten inkl. der Tatra, Korsika, die Pyrenäen, den Schwarzwald und die Vogesen (Ozenda 1988, Aeschimann et al. 2004).

Die Alpen und einige der anderen Gebirge des Alpinen Systems erfuhren ihre Haupthebung vor 10 bis 2 Millionen Jahren (Ager 1975), d. h. in diesem Zeitraum sind die Habitate entstanden, in denen wir heute diese große Diversität beobachten. Die Alpenflora muss sich also in den letzten 10 Millionen Jahren entwickelt haben und zwar entweder durch Evolution der Tertiärflora Europas (Gams 1933, siehe auch Mai 1995) oder durch Zuwanderung aus anderen Gebieten. Schon seit der Mitte des 19. Jahrhunderts haben sich Autoren mit der Herkunft der Alpenflora beschäftigt. Dies waren vor allem Hooker (1861), Christ (1867), Ball (1879), Engler (1879, 1916), Jerosch (1903) spätere Brockmann-Jerosch (1908), Diels (1910), Braun-Blanquet (1923), Gams (1933) und Scharfetter (1938). Sie taten dies mit floristischen Vergleichen und dem Wissen ihrer Zeit über die Verwandtschaft der Taxa. Zusammen mit den damals bekannten Daten über die Geologie und die Klimaveränderungen vergangener Zeiträume kamen sie zu dem Schluss, dass beide Prozesse, Evolution von Taxa im Gebiet der Alpen und Einwanderungen von Taxa aus anderen Räumen, zusammen gespielt haben mussten, um die heutige Vielfalt der Flora erklären zu können.

Christ (1867) war laut Brockmann-Jerosch (1908) der erste, der Ähnlichkeiten zwischen den Floren der Alpen und der asiatischen Gebirge erkannte. In diesem Zusammenhang häufig zitierte Gattungen (z. B. Ozenda 1988) sind *Gentiana* L., *Pedicularis* L., *Primula* L. und *Saussurea* DC. Die ungleiche Verteilung der Diversität mit höheren Artenzahlen in Asien und geringeren Artenzahlen in Europa wurde als eindeutiger Hinweis für die Einwanderung von Taxa aus dem asiatischen Raum in die Alpenregion gewertet. Die Verbindung nach Asien ist über zwei mögliche Routen denkbar, eine nördliche und eine südliche. Das Europäische Alpine System ist durch eine Reihe von Gebirgszügen, von West nach Ost, dem Taurus, Kaukasus, Elburs, Kopet-Dag, Hindukusch, Pamir, Tien-Schan, Karakorum mit dem Himalaya, dem Altai und dem Tibetischen Hochland verbunden. Dies stellt eine mögliche südliche Verbindungsroute dar. Die andere Möglichkeit einer nördlichen, borealen, arktischen oder subarktischen Verbindung drängt sich schon bei Jerosch (1903) und Brockmann-Jerosch (1908) auf. Sie untersucht die schweizerische Alpenflora floristisch-geographisch und beschreibt als arktisch-altaisches Element Taxa, die außer in den Alpen disjunkt in der Arktis und in den nordasiatischen Gebirgen vorkommen. Das entspricht 22,5 % der schweizerischen Alpenflora und ist ein Hinweis auf eine nördliche Verbindung. Aber auch Brockman Jerosch findet ein alpin-altaisches Element, das nur in den Alpen und den nordasiatischen Gebirgen vorkommt und in der Arktis fehlt. In der schweizerischen Alpenflora sind das 4,8 % der Flora und spricht für eine südliche Verbindung dieser Taxa nach Asien. Scharfetter (1938) übernimmt Brockman-Jeroschs geographische Elemente für die Ostalpen und erweitert sie um zahlreiche Beispiele. Eine vollständige Bearbeitung der ostalpinen Flora gibt er allerdings nicht.

Ein floristischer Austausch zwischen dem Europäischen Alpinen System und den Asiatischen Gebirgen ist spätestens ab dem Holozän nicht mehr möglich. Nach Norden hin sind die Alpen durch die Wälder Mittel- und Osteuropas von der Arktis getrennt. Im Süden und nach Osten hin trennen die mediterrane und irano-turanische Vegetation die wichtigen Gebirgszüge in Richtung Osten. In beiden Gebieten gibt es keine Habitate für Pflanzen mit alpiner oder montaner Ökologie. In der Vergangenheit, insbesondere während der Klimaschwankungen des Plio- und Pleistozäns, ist jedoch ein Austausch auf beiden Routen denkbar.

Die Beziehungen zwischen Taxa oder Populationen in den Alpen und innerhalb des Europäischen Alpinen Systems wurden in letzter Zeit mit modernen molekularen Methoden phylogenetisch und phylogeographisch untersucht (z. B. Comes und Kadereit 2003, Schönswetter und Tribsch 2005, Mráz et al. 2007, Kropf et al. 2009). Die Beziehungen des Europäischen Alpinen Systems zu den asiatischen Gebirgen sind hingegen nicht gut untersucht. Dabei bietet der Fortschritt im floristischen Wissen und bei den Verwandtschaftsanalysen, der seit dem letzten und vorletzten Jahrhundert gemacht wurde, eine neue Basis für die Betrachtung der biogeographischen Beziehungen der Alpen zu anderen Gebirgssystemen. Schon Scharfetter (1938) hebt die Bedeutung der „morphologisch-phylogenetischen Methode“ für die Beantwortung der Frage nach der „Heimat“, dem Entstehungsort der Alpenpflanzen, hervor. Die heutigen molekularen Phylogenien bieten neue Einsichten und besser gestützte Hypothesen denn je zuvor. Zusätzlich ermöglichen Molekulare Uhren in vielen Verwandtschaftskreisen mit schlechter paläontologischer Datenlage eine zeitliche Einordnung wichtiger Diversifizierungsereignisse und erweitern so die Sicht auf vergangene Prozesse der Arealbildung. Insgesamt ist die Datengrundlage für biogeographische Fragestellungen breiter geworden und die Frage der Beziehung der Alpenflora zum Kaukasus und den asiatischen Hochgebirgen kann neu gestellt werden. Ob der Kaukasus als Gebirge auf der südlichen Route dabei eine Rolle spielt, oder ob eine nördliche Verbindung nach Asien einen größeren Einfluss hatte, soll durch neue molekulare Phylogenien und aktuelle floristische Vergleiche überprüft werden. Der Auswahl der Taxa kommt hierbei eine besondere Bedeutung zu, da diese über ihre Areale die Untersuchungsgebiete repräsentieren. In zwei der folgenden Artikel werden molekulare Phylogenien von Taxa, die disjunkt in den Alpen, dem Kaukasus und den asiatischen Gebirgen vorkommen, erstellt und diskutiert. Das sind im ersten Artikel *Epimedium* L. (Berberidaceae, Tafel 1) und im zweiten Artikel *Atropa* L. und *Scopolia* Jacq. aus der eurasiatischen Tribus der Hyoscyameae (Solanaceae, Tafeln 2-5). Die molekularen Phylogenien lassen Rückschlüsse auf die Abfolge der Arealbildung in diesen Gruppen zu. Außerdem wurden molekulare Uhren angewandt, um die Arealbildung auch zeitlich einzuordnen. Der dritte Artikel arbeitet aktuelle floristische und phylogenetische Kenntnisse auf. Ein klassischer floristischer Vergleich von Taxa, die in den Alpen, den asiatischen Gebirgen und auf den potentiellen Verbindungsrouten vorkommen, bildet die Grundlage für eine Literaturrecherche nach molekularen Phylogenien für die Fragestellung interessanter Taxa. Diese werden biogeographisch ausgewertet und interpretiert und schließen die Ergebnisse der beiden ersten Artikel ein.

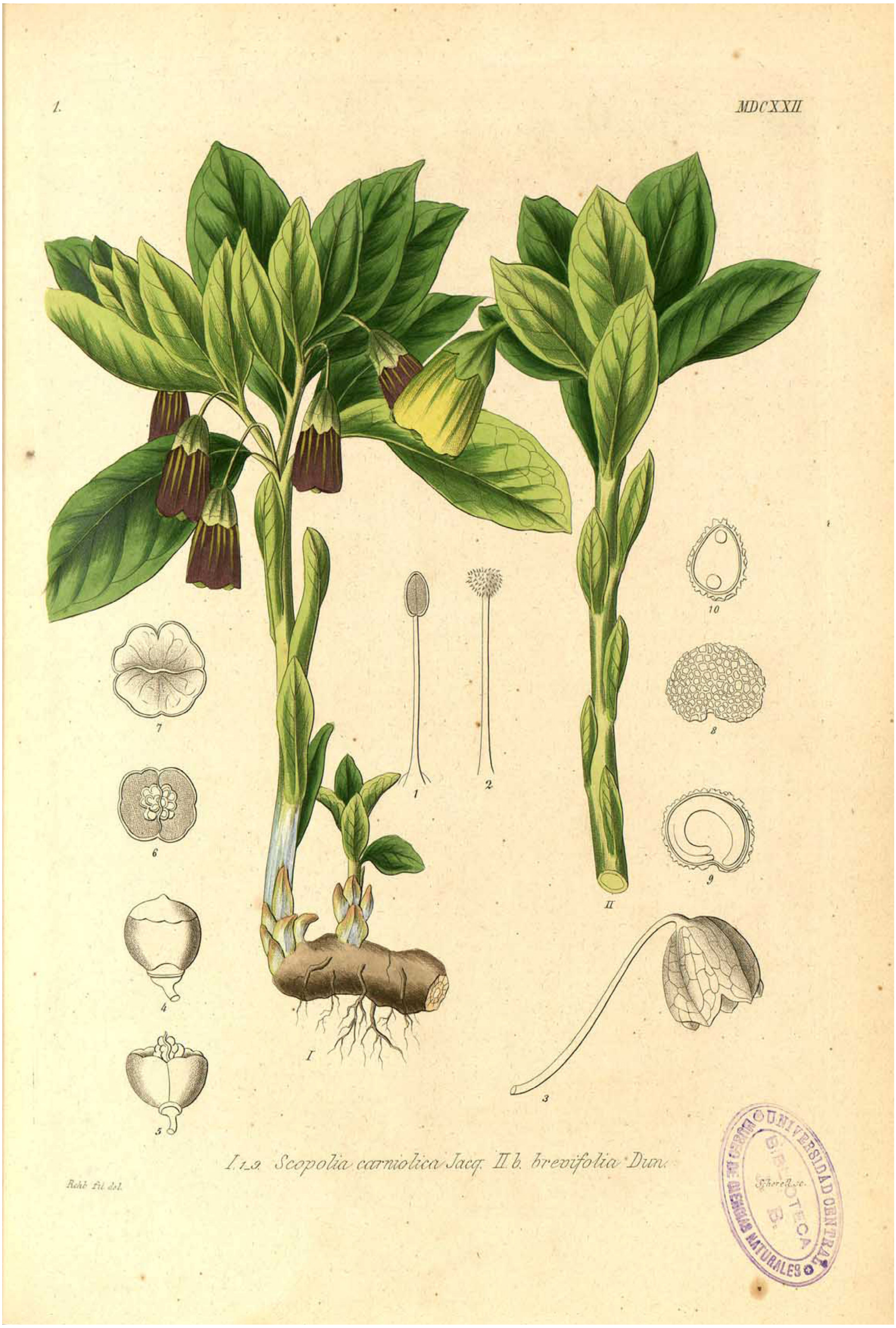


Tafel 1. *Epimedium alpinum* L. und andere Berberidaceae (Reichenbach 1838-1839).

Tafel 2. *Atropa belladonna* L. (Jacquin 1776).

Tafel 3. *Hyoscyamus niger* L. (Sims 1823).

Tafel 4. *Physochlaina praealta* Miers (Hooker und Smith 1851).



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Tafel 5. *Scopolia carniolica* Jacq. (Reichenbach 1861-1862).

1. Artikel: Phylogeny and Biogeography of *Epimedium/Vancouveria*

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Phylogeny and Biogeography of *Epimedium*/*Vancouveria* (Berberidaceae): Western North American - East Asian Disjunctions, the Origin of European Mountain Plant Taxa, and East Asian Species Diversity

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Abstract

Using ITS and *atpB-rbcL* spacer sequences of 38 (of 55) species of the highly disjunct Eurasian/North African *Epimedium* and all three species of its western North American sister genus *Vancouveria,* we reconstructed the phylogeny of these two genera and dated major splits with a molecular clock approach. *Epimedium* was found to be monophyletic with a stem age dated to between 9.7 and 7.4 million years ago (My). Within *Epimedium*, almost all sections as recognized in the most recent classification of the genus were found to be monophyletic but subg. *Epimedium* was found to be paraphyletic in relation to subg. *Rhizophyllum*. Range formation in Eurasia proceeded as follows: in a first step, the western Himalayan part of the generic distribution area (sect. *Polyphyllon*) was separated from the remainder, followed by a split between the Chinese distribution area (sect. *Diphyllon*) and the remainder, the separation of the highly disjunct range of *E. pinnatum* from the Caucasus plus *E. perralderianum* from North Africa (subg. *Rhizophyllum*) and the remainder, and in a last step the separation of the European *E. alpinum* plus the Turkish *E. pubigerum* from the range of the genus in Japan, Korea, northeastern China and Far Eastern Russia (sects. *Epimedium* and *Macroceras*). These results imply that the European mountain species are not most closely related to taxa in mountain areas towards the east (e.g., Caucasus, Himalayas) but rather to taxa in the Far East. Accordingly, in *Epimedium* the link between western Eurasia and eastern Asia apparently was not through intervening mountain regions but probably through a more northerly deciduous forest belt which does not exist any longer. The largest number of species of *Epimedium* is found in China (sect. *Diphyllon*: 44 species mainly in Hubei, Sichuan and Guizhou provinces). The age of this clade was dated to 0.52 to 0.4 My. This implies that the diversification of this group probably is the result of frequent range shifts in the Quaternary.

Keywords: *atp*B-*rbc*L spacer, DIVA, ITS, molecular clock, Quaternary diversification

2. Artikel: Phylogeny and Biogeography of Hyoscyameae

Manuscript working title:

Phylogeny and Biogeography of the Hyoscyameae (Solanaceae): European – East Asian Disjunctions and the Origin of European Mountain Plant Taxa

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Abstract

It has long been claimed that Asian high mountain areas constitute an important source for European mountain floras. The connection between these areas might have been through intervening mountain regions such as the Himalayas, Hindu Kush, Elburs and Caucasus, or along a more northern route. To address this question, we have analysed plant groups with a disjunct distribution in Eurasia (Europe, Caucasus and East Asia). Their phylogeny is expected to reveal the sequence of range splits in Eurasia, and to answer the question whether the European representatives are closely related to taxa in the Caucasus than to eastern representatives or not. If yes, this is interpreted as evidence in favour of a mountain route from Asia into Europe.

The Hyoscyameae, a tribe of Solanaceae, consisting of *Atropa*, *Scopolia*, *Hyoscyamus*, *Physochlaina*, *Atropanthe*, *Anisodus* and *Prezewalskia* is a well-supported monophyletic clade. Whereas the latter three genera have a rather narrow distribution area and are endemic to W China and the Himalayas, the remaining genera are widely or disjunctly distributed in Asia and reach into Europe and even N Africa. Several taxa of Hyoscyameae have a montane or alpine ecology and are associated with mountain ranges.

We reconstructed the phylogeny of the Hyoscyameae using nuclear and plastid markers. All genera were found to be monophyletic, but relationships among them are not fully supported. In the *Prezewalskia*/*Physochlaina*/*Scopolia* clade first branches are mainly found in W China and C Asia and the close relationship between European/Caucasian and Japanese *Scopolia* taxa supports the assumption of a northern connection between the European mountains and E Asia. This is congruent with results obtained by us in *Epimedium* (Berberidaceae). In *Atropa*, *A. baetica* from N Africa/Southwest Europe is sister to the remainder of the genus which is distributed across Europe, in the Caucasus and in C Asia. The Himalayan *A. acuminatum* can not be distinguished from the European *A. belladonna* on the DNA-sequence level, and both are part of a polytomy together with Caucasian and C Asian taxa. Although these latter results do not allow us to draw any conclusions about the relationships and possible origin of most of the European material of *Atropa*, the sister group relationship of *A. baetica* to the rest of the genus may imply that much in contrast to our findings in the *Prezewalskia*/*Physochlaina*/*Scopolia*-clade, range formation in *Atropa* proceeded from west to east.

Keywords: ITS, *ndhF/trnLF*, BEAST, Bayesian relaxed molecular clock.

Introduction

Hyoscyameae Endl. is a herbaceous, mostly perennial tribe of Solanoideae (Solanaceae) and comprises 7-8 genera and about 40 species (Lu and Zhang 1986, D'Arcy and Zhang 1992, Olmstead and Bohs 2007, Olmstead et al. 2008). It is characterized by the possession of a circumscissile capsule (pyxidium) that is present in all genera except *Atropa* and by their secondary chemistry (Tétényi 1987).

The original circumscription of Hyoscyameae by Endlicher (1839) included only *Hyoscyamus*, *Anisodus* and *Scopolia*. Later on Miers (1849) and Bentham (1876) included *Physochlaina* together with various other genera. In *Die natürlichen Pflanzenfamilien* Wettstein (1895) included *Przewalskia* in the subtribe Hyoscyaminae, now only together with *Hyoscyamus, Scopolia* (incl. *Anisodus*) and *Physochlaina*. All these he included in the tribe Solaneae incl. *Atropa* and *Mandragora*. Also Tétényi (1987) grouped *Atropa* and *Mandragora* together with Hyoscyameae but in the rank of a subfamily as Atropoideae which he based on chemical compounds, aestivation of corolla lobes, and their Eurasian biogeography. For a comprehensive history of the classification of the Hyoscyameae see Hoare and Knapp (1997). D'Arcy (1991) and Hunziker (2001) did not include *Atropa* and *Mandragora* in Hyoscyameae but molecular studies clearly place *Atropa* into the well supported monophyletic Hyoscyameae to which *Mandragora* is not closely related (Olmstead and Sweere 1994, Olmstead et al. 1999, Yuan et al. 2006, Olmstead et al. 2008 and references therein). The classifications of Hunziker (2001) and Olmstead and Bohs (2007) are shown in Table 1.

TABLE 1. Classification and species number of the tribe Hyoscyameae and the outgroup taxa used in the Hyoscyameae data sets (see Materials and Methods) according to Hunziker (2001) and Olmstead and Bohs (2007).

Hunziker (2001)	**Olmstead and Bohs (2007)**
subfam. **Solanoideae**	subfam. **Solanoideae**
tribe Mandragoreae: *Mandragora* L.	*Mandragora*, not assigned to a more inclusive clade in the subfam.
	"**Atropina**", clade name without rank
tribe Lycieae: *Lycium* L.	*Lycium* of "Lyciina", clade without rank
tribe Jaboroseae: *Jaborosa* JUSS.	*Jabrorosa*, not assigned to a more inclusive clade in "Atropina"
tribe **Atropeae**: *Atropa* L., 2 species	
tribe **Hyoscyameae**:	tribe **Hyoscyameae**:
Anisodus LINK, 4 species	*Anisodus*, 4 species
	Archihyoscyamus A.M.LU, 1 species
	Atropa, 3 species
Atropanthe PASCHER, 1 species	*Atropanthe*, 1 species
Hyoscyamus L., ~23 species incl. *Archihyoscyamus*	*Hyoscyamus*, ~20 species
Physochlaina G. DON, ~8 species	*Physochlaina*, 11 species
Przewalskia MAXIM., 1 species	*Przewalskia*, 1 species
Scopolia JACQ., 2 species	*Scopolia*, 2 species

The distribution of Hyoscyameae is shown in Fig. 1. and shortly summarized here form Weinert (1972), Meusel et al. (1978), Zhang et. al. (1994) and Hunziker (2001). *Atropa* is disjunctly distributed from Morocco/southern Spain, where *Atropa baetica* Willk. occurs, over Europe, the Caucasus and C Asia to the western Himalaya/Karakorum. In all these latter areas different species (*A. belladonna*, *A. caucasica*, *A. komarovii* and *A. acuminata*) have been described, but Hunziker (2001) all includes them in *A. belladonna*. *Atropa* mainly occurs in mountainous regions of up to 2700 m and is the only Hyoscyameae with a berry and a spreading calyx, whereas the remaining genera have a pyxidium hidden in the tubular part of the calyx. The two species of *Scopolia* also show a disjunct distribution with *S. carniolica* in Europe/the Caucasus and *S. japonica* [incl. *S. parviflora* (Dunn) Nakai and *S. lutescens* Y.N.Lee] in E Asia. Both occur at elevations of up to 1700 m. *Hyoscyamus* is the most widespread genus and occurs from Madeira, Canary Islands and N Africa in the west, to Europe, SW Asia, India and China in the east. It achieved the greatest biodiversity in the tribe with up to 23 annual, biennial and perennial species. Its centre of diversity is SW Asia (Meusel et al. 1978). It can occur in lowlands and as a weed but also reaches elevations of 2000 m in the Atlas and up to 3000 m in the Himalayas (Hunziker 2001). *Physochlaina* is also a widespread member of Hyoscyameae with eight to eleven species but its centre of diversity clearly is more eastern, and it ranges westwards only to the Caucasus. *Physochlaina* species mainly occur in valleys and mountain forests between 800 and 4500 m. The four species of *Anisodus* are distributed in the Himalayas and China at high altitudes of 2000-4500 m. The monotypic *Atropanthe* is endemic to southern C China at elevations between 1400 and 3000 m. *Przewalskia* is also monotypic and *P. tangutica* is endemic to cold and dry habitats of the Tibetan plateau at 3200 to 5000 m. It has also been reported in Northern Sikkim (India) at elevations of 5200-5400 m (Sanjappa and Raju 1989).

Although different characters have been exploited in several taxa of Hyoscyameae (cytological studies: Tu et al. 2005; leaf epidermis: Yang et al. 2000; flower ontogeny: Yang et al. 2002a, 2002b; seed morphology: Zhang et al. 2005; a morphological phylogeny: Hoare and Knapp 1997), and a molecular phylogeny of Solanaceae (Olmstead et al. 2008) based on two nuclear markers included 15 accessions, the phylogenetic relationships within the tribe and its biogeography have not been fully explored.

The sister group of Hyoscyameae in Olmstead et al.'s (2008) "Atropina" clade consisting of the "Lycieae", *Nolana*, *Sclerophylax*, *Jaborosa* and *Latua* (all mostly of temperate South America) is not clear, but the whole "Atropina" clade seems to be sister to or at least one of the first branching groups in Solanoideae.

Solanaceae are mainly distributed in the New World and probably originated there. Only eight to nine rather derived lineages dispersed into the Old World (Olmstead et al. 2008). Hyoscyameae are one of them and only occur in Eurasia and N Africa. With around 40 species they are rather diverse in contrast to the other Old World clades of Solanoideae

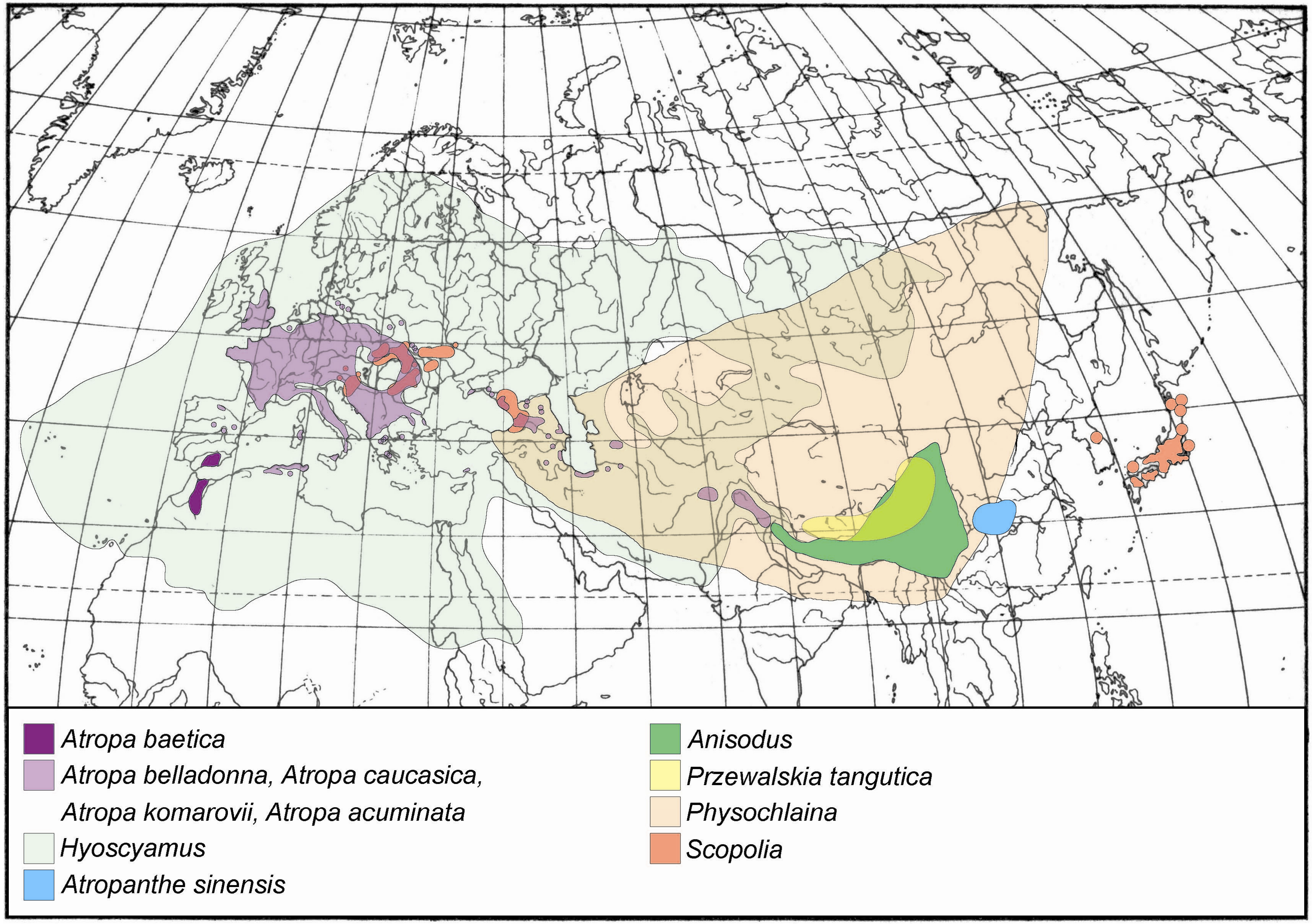

FIG. 1. Distribution map of members of Hyoscyameae drawn after Meusel et al. (1978), Hoare and Knapp (1997), Lu and Zhang (1986) and Zhang et al. (1994).

[one clade of *Lycium* (three species) as part of "Atropina", *Mandragora* (two species), *Physalis alkekengi*, a clade of Witaninae (20 species in SW Asia) and one of *Lycianthes* (20 species in SW Asia). The distribution of the genera of Hyoscyameae shown in Fig. 1 and summarized above, raises questions about the origin of the widespread disjunct ranges of *Scopolia* and *Atropa*, the widespread but more continuously distributed *Hyoscyamus* and *Physochlaina*, and the more narrowly distributed *Przewalskia*, *Atropanthe* and *Anisodus*. Understanding the pattern and the time frame of this diversification can help to address the question of the links between the flora of Asian mountain ranges and the European Alpine system. Asian high mountain ranges have long been claimed to be an important source of European alpine/montane plant taxa (e.g., Christ 1867; Engler 1879; Diels 1910; Gams 1933; Ozenda 1988; Ellenberg 1996) because of the uneven distribution of species diversity of Eurasian plant taxa with much higher diversity in Asia than in Europe. A successive sister group relationship from eastern taxa to groups distributed further to the west would imply a mountain route from the Himalayas, Hindu Kush, Elburs, Caucasus and Taurus towards Europe. In contrast, a close relationship of European representatives to taxa in E Asia would imply a more northern route. See also Kadereit et al. (2008), where a list of genera spanning this geographical range is provided and molecular phylogenetic literature was screened for these area relationships.

Materials and Methods

Taxon Sampling and Markers used. Altogether 14 (-18) different species of all seven genera of Hyoscyameae were included in this study. *Atropa* and *Scopolia* accessions cover the geographical distribution of these two genera and their taxonomic subdivision. As a nuclear marker the internal transcribed spacer (ITS) was used. As chloroplast marker the *atpB-rbcL* spacer was sequenced but showed very little sequence variation that resulted in poorly resolved trees (data not considered further). Therefore not all taxa were sequenced, but *ndhF* and *trnLF* sequence data (both cp-DNA) were kindly provided by RG Olmstead (Olmstead et al. 2008). A Partition Homogeneity Test (Farris et al. 1994; implemented in PAUP* 4.0b10; Swofford 2003) with 100 homogeneity replicates, 10 random addition sequences, tree-bisection-reconnection (TBR) branch swapping on best only and MULTREES on was performed to test whether the nuclear ITS data can be combined with the cp *ndhF*/*trnLF* data. No significant incongruence ($p > 0.01$) between the data sets was detected and the combined data matrix was used in addition to the nuclear ITS data. Taxa of the combined matrix are a subset of the ITS dataset and in some cases sequences of closely related species of a genus (e.g. in *Hyoscyamus*) were joined to create a full length sequence. For composition, GenBank accession numbers and voucher information of these two Hyoscyameae data sets: ITS alone and ITS/*ndhF*/*trnLF* see Table 2.

DNA Extraction, Amplification and Sequencing. Total genomic DNAs were extracted from silica-dried leaves or from herbarium material using NucleoSpin plant DNA extraction kits (Macherey-Nagel, Düren, Germany) following the manufacturer's protocol. The standard 25 µl PCR reaction mix consisted of 2 mM $MgCl_2$, 200 µM dNTPs, 1 pM primer, 0.025 U/µL *Taq* polymerase and 1-2 µL of DNA extract in ddH_2O and the reaction buffer as provided by the manufacturer of the polymerase. PCR reactions were carried out in a Biometra T3 or a PTC 100^{TM} MJ Research thermocycler using the program: 60 sec at 94°C, followed by 35 cycles of 18 sec at 94°C, 30 sec at 52°C, 60 sec at 72°C and a post-treatment of 78 sec at 52°C and 8 min at 72°C for ITS and the *atpB*-*rbcL* spacer. ITS was amplified using primers 18 S (5'-CCT TMT CAT YTA GAG GAA GGA G-3') and 28S (5'-CCG CTT ATT KAT ATG CTT AAA-3' [Muir and Schlötterer 1999]). In some cases it was necessary to amplify two overlapping fragments with the primer pairs ITS A (5'-GGA AGG AGA AGT CGT AAC AAG G-3'), ITS C (5'-GCA ATT CAC ACC AAG TAT CGC-3') and ITS B (5'-CTT TTC CTC CGC TTA TTG ATA TG-3'), ITS D (5'-CTC TCG GCA ACG GAT ATC TCG-3' [Blattner 1999]). The primers *atpB*-*rbcL* f (5'-GAA GTA GTA GGA TTG ATT CTC-3') and *atpB*-*rbcL* r (5'-CAA CAC TTG CTT TAG TCT CTG-3' [Xu et al. 2000]) were used for amplification of the *atpB*-*rbcL* spacer. PCR products were checked on 0.8% agarose gels and purified directly using the Macherey-Nagel PCR purification kit. Purified PCR products were cycle-sequenced with the ABI Prism Dye Terminator Cycle Sequencing Ready Reaction Kit (BD 3.0 in 10 µl reactions) by Perkin Elmer (Waltham, Massachusetts, USA) using the PCR primers listed above and following the manufacturer's protocol. Products were purified and analysed by GENterprise (Mainz, Germany). Forward and reverse sequences were manually edited and merged into consensus sequences using Sequencer^{TM} 4.1.2 (GeneCodes Corp., Ann Arbor, Michigan, USA), and aligned manually in MacClade 4.01 (Maddison and Maddison 2000).

Phylogenetic Analysis and Molecular Clock. Maximum Parsimony (MP) analyses were performed using PAUP* with 1,000 replicated heuristic searches using the same settings as described above for the Partition Homogeneity Test. Branch support was assessed with 100 bootstrap (BS) replicates with 10 random taxon additions each and TBR and MULTREES on.

For Maximum Likelihood (ML) the appropriate model of DNA substitution for the inference of phylogenetic relationships under ML was estimated using Modeltest 3.06 (Posada and Crandall 1998). For the ITS data set the GRT+G model (Rodriquez et al. 1990, Tavaré 1996) selected by the Akaike Information Criterion (AIC; Posada and Buckley 2004) was chosen [settings: Lset Base=(0.1840 0.3330 0.3028) Nst=6 Rmat=(1.0995 1.9129 1.1149 0.5202 3.7540) Rates=gamma Shape=0.3138 Pinvar=0;]. For the combined ITS and *ndhF*/*trnLF* data set the GTR+I+G model selected by the AIC was used [settings: Lset Base=(0.2814 0.1919 0.2011) Nst=6 Rmat=(1.4719 1.7323 0.3566 1.4077 2.7562) Rates=gamma Shape=0.7078 Pinvar=0.6635;]. Maximum Likelihood heuristic searches and BS branch support were performed in PAUP* with 100 replicated heuristic searches and the same settings as in the analyses before.

For the estimation of divergence times a clock enforced tree was calculated with the same ML settings. The program Modeltest (Posada and Crandall 1998) was used to perform a likelihood ratio test (Felsenstein 1988) comparing the log-likelihood scores of the tree with and without enforcing a molecular clock. There was a significant difference between the two scores at the 0.01 level indicating that a molecular clock was rejected. Nevertheless ages of the nodes were estimated with published ITS rates (Kay et al. 2006) of herbaceous plants. The slowest rate (*Saxifraga* L., 1.72×10^{-9} substitutions/site/year, Vargas et al. 1999) and a fast rate (*Soldanella* L., 8.34×10^{-9} substitutions/site/year, Zhang et al. 2001) were used and time estimates for nodes numbered in Fig. 2 are shown in Table 4.

Bayesian Evolutionary Analysis by Sampling Trees v1.4.8 (BEAST) by Drummond and Rambaut (2007) was used to simultaneously estimate a tree topology and divergence times for the ITS data set. BEAST can calculate a relaxed molecular clock with a mean substitution rate and a standard deviation provided as a prior distribution (Drummond et al. 2007). From Kay et al. (2006) the mean substitution rate of 4.125×10^{-9} substitutions/site/year and the standard deviation of 1.8078×10^{-9} substitutions/site/year were calculated using all rates of herbaceous taxa between the slow *Saxifraga* and the fast *Soldanella* rate. The BEAST.xml input file (available from the corresponding author on request) was created with the Bayesian Evolutionary Analysis Utility v1.4.8 (BEAUti; implemented in BEAST, Drummond and Rambaut 2007). The Hyoscyameae were defined as monophyletic in order to set a root. The substitution model parameters were the same as in the ML analysis and the gamma distribution was modelled with four categories. The relaxed Bayesian clock was implemented with rates for each branch drawn independently from a lognormal distribution (Drummond et al. 2006). A birth and death prior was set for the branch lengths and the mean rate was set as described above. Other priors were in default settings and the Monte Carlo Markov Chain (MCMC; Drummond et al. 2002) was initiated on a random starting tree. The first runs were used to examine the MCMC performance and operators were adjusted as suggested by the output analysis. The final run was performed with 10,000,000 iterations, a burn in of 10% and a sample frequency of 1,000. After assessing convergence in Tracer v1.4.1 (Rambaut and Drummond 2007) as described in the BEAST manual (Drummond et al. 2007), the maximum clade credibility tree was summarized in TreeAnnotator v1.4.8 (Drummond and Rambaut 2007) with a posterior probability (post. prob.) limit of 0.5 and summarizing mean node heights. This was visualized in FigTree v1.2.2 (Rambaut 2006) and is shown in Fig. 3.

A second molecular clock approach based on fossil and published group ages of Solanales was performed with BEAST in order to obtain another age estimate for Hyoscyameae in a broader context and independent of ITS rates. Therefore a subset of the *ndhF*/*trnLF* Solanaceae dataset of Olmstead et al. (2008) was arranged, representing Hyoscyameae, the Atropina clade, main branches of other Solanoideae and the remaining groups of Solanaceae as well as Convolvulaceae as sister to Solanaceae (Stevens 2001 onwards) and Montiniaceae as outgroup (see Table 3). In BEAUTi appropriate taxon groups to define calibration nodes and for setting prior distributions for these nodes were defined. The grouping Solanaceae/Convolvulaceae was constrained as monophyletic in order to set the root at the split of this vs. Montiniaceae. Age estimates obtained from molecular studies indicate that Solanales are 86-73 Million years (My) old (Wikström et al. 2001, Magallon and Castillo 2009), and so the root was set to a normal distributed prior with a mean of 80 My and a standard deviation of 4 My covering this range. A fossil in Convolvulaceae (lower Eocene, Müller 1981) was set with a log normal prior distribution and a zero offset of 55.8 My. For Solanaceae rapid diversification in the late Cretaceous/early Tertiary has clear molecular and dating evidence (Magallon et al. 1999, Magallon and Sanderson 2001, Knapp 2002, Magallon and Castillo 2009) so that a log normal prior distribution with a zero offset of 65 My was fixed. Instead of setting hard upper and lower bounds normal and log normal prior distributions better account for uncertainty by allowing times outside the most likely age boundaries by giving them low but non-zero probabilities (Bergh and Linder 2008). The zero offset sets a strict minimum age of a group. So these settings correspond to the findings that Solanales are around 80 My old, Solanaceae already existed in the lower Tertiary and Convolvulaceae in the lower Eocene. For this dataset seven BEAST runs of 10,000,000 generations and a sample frequency of 1000 were performed and combined with LogCombiner v1.4.8 (Drummond and Rambaut 2007) to reach satisfactory Effective Sample

Sizes (ESS). The rest of the settings and calculation procedure was the same as in the Hyoscyameae dataset described above. The tree is shown in Fig. 5.

TABLE 2. Composition of the Hyoscyameae ITS dataset. Source and voucher information of plant material and GenBank accession numbers. Taxa that are included in the **combined ITS/*trnL*/*ndhLF* data set** are in bold letters. The accession numbers for the trnL/ndhLF sequences are given in Table 3. Where different species of a genus were combined (see Fig. 4) the pairs are marked (^, $^{+}$, $^{\#}$, *, °) in Table 2 and 3.

Taxon	Source of seeds or plant material	Collection number, Herbarium or GenBank accession number.
Anisodus carniolicoides (C. Y. Wu & C. Chen) D'Arcy & Zhi Y. Zhang	IPK Gatersleben	C. Uhink S18 MJG
Anisodus luridus Link & Otto	China, Xizang, SE Tibet	B. Dickoré 10281, MJG
Anisodus luridus (sub. Nom. *Scopolia straminifolia*)	BG Regensburg, Nepal, Kyapra to Ghunsa 3300 m	C. Uhink S13, MJG
Anisodus luridus (sub. Nom. *Anisodus tanguticus*)	China, Yunnan, Meili Xue Shan	Raab-Straube/Smalla 783, B 3536; C. Uhink S45, MJG
Anisodus luridus (sub nom. *Scopolia straminifolia*)	BG Dahlem	C. Uhink S11, MJG
Anisodus tanguticus (Maxim.) Pascher	China, Sichuan, E Tibet	B. Dickoré 8325, MJG
Atropa _cuminate Royle	Nepal Prov. Bagmati Patan, 1330 m (BG Liege)	C. Uhink A20, MJG
Atropa acuminata	BG St. Gallen	C. Uhink A13, MJG
***Atropa baetica* Willk.^**	Jaeu Subide al Pico Cabaunas, Siena de Cazorla,	P. Sandez Gomer and F. Akcacaz sn., MUB 22888
Atropa baetica	Cuenca, Torre Vigia de Ceno Gordo, Entre las Majadas y El Mosquillo,	Jose M. Henauz sn., MUB 42636
Atropa belladonna L.	Anastie Fatu, lasi, Orasul Solca, Romania	C. Uhink A18, MJG
Atropa belladonna	BG Palermo Territiri di Piano Battaglia-Madonie 9-000	C. Uhink A31, MJG
Atropa belladonna	BG Münster D NRW Menden Lendringen, 115 m NN, TK 25 4513	C. Uhink A46, MJG
Atropa belladonna	Lama del Peking am Parco Nazionale della Majella, Palena, Italy 900 m	C. Uhink A17, MJG
Atropa caucasica Kreyer	IPK Gatersleben,	C. Uhink A39, MJG
Atropa caucasica	Bakony mountains, Bakonybèl village, Ungarn	C. Uhink A54, MJG
Atropa caucasica	Armenien	V. Avetisian 144916, ERE
*Atropa komarovi*i Blin. & Shal.	IPK Gatersleben, Kasachstan,	C. Uhink A38, MJG

TABLE 2 continued

Atropa komarovii	C Asia, Mt. Turm, W Kopet-Dagh) Khozly Gorge, Taschkent, Usbekistan	C. Uhink A27, MJG
Atropanthe sinensis (Hemsl.) Pascher	BG Alpinus Viotte, Trento, Italy	C. Uhink S48, MJG
Atropanthe sinensis (sub. nom. *Scopolia japonica*)	BG Bern	C. Uhink S8, MJG
Atropanthe sinensis	BG Mainz	C. Uhink S52, MJG
Atropanthe sinensis	BG Göttingen	C. Uhink S27, MJG
***Hyoscyamus aureus* L.**[#]	Side, Studentenexkursion Inst. f. Spez. Botanik, Südtürkei 2008,	D3-4, MJG
***Hyoscyamus niger* L.**[+]	BG Mainz	C. Uhink Hyo1, MJG
Hyoscyamus niger *	Mt. Tianshan, Tianchi Lake, Urumtschi, W China,	JW Kadereit sn., MJG
Jaborosa integrifolia Lam.		AY028130, AY028148
Lycium australe F. Muell.		AY028131, AY028149
Lycium barbarum L.		AF455747
Mandragora officinarum L.	BG Mainz	C. Uhink M1, MJG
Physochlaina orientalis (M. Bieb.) G. Don	BG Mainz	C. Uhink Phy1, MJG
***Physochlaina praealta* Miers°**	China, Xizang, SE Tibet	Dickoré 10441, MJG
Przewalskia tangutica Maxim.	China, Xizang, S Tibet	Dickoré 9353, MJG
Scopolia carniolica Jacq.	BG Mainz	C. Uhink S50, MJG
Scopolia carniolica	BG Lublin, Bieszczady, Zasan, Polen	C. Uhink S38, MJG
Scopolia carniolica	Slovenien: Kocevje, Jama pod Krenom	Hellmann s.n., B 3573
Scopolia carniolica	BG Cluj-Napoca	C. Uhink S2, MJG
Scopolia carniolica (sub. var. breviflora)	BG Mainz	C. Uhink S51, MJG
Scopolia carniolica (sub. var. carniolica)	BG Edinburg	E 19642567
Scopolia japonica Maxim. (sub. nom. Lutescens)		AY478394, AY478408
Scopolia japonica (sub. nom. Lutescens)		AY478395, AY478409
Scopolia japonica (sub. nom. Parviflora)		AY478393, AY478407
Scopolia japonica (sub. nom. Parvoflora)		AY478390, AY478404
Scopolia japonica (sub. nom. Parvoflora)		AY478391, AY478405
Scopolia japonica (sub. nom. Parvoflora)		AY478392, AY478406

TABLE 3. Composition of Solanales *trnL*/*ndhLF* data set and GenBank accession numbers. Taxa were chosen from the Solanaceae phylogeny of Olmstead et al. (2008). Taxa that are included in the **combined ITS/*trnL*/*ndhLF* data set** are in bold letters. Where different species of a genus were combined (see Fig. 4) the pairs are marked (^, $^{+}$, $^{\#}$, *, °) in Table 2 and 3.

Taxon	**GenBank accession numbers, *ndhF***	**GenBank accessi numbers, *trnLF***
Montiniaceae		
Montinia caryophyllacea THUNB.	AF130178	AY206764
Convolvulaceae		
Convolvulus arvensis L.	AJ236243	AY101102
Dinetus truncatus (KURZ) STAPLES	AY936343	AY101162
Evolvulus glomeratus NEES & C. MART.	AY936341	AY101121
Ipomoea batatas (L.) LAM.	AF130177	AY101071
Solanaceae groups indicated after Olmstead et al. (2008)		
Schizanthus		
Schizanthus pinnatus RUIZ & PAV.	U08929	AY206766
Goetzeoideae		
Goetzea elegans WYDL.	AY206746	AY206760
Metternichia principes J. C. MIKAN	AY206748	AY206729 & AY206763
Duckeodendron		
Duckeodendron cestroides KUHLM.	AY206743	AY206725 & AY206757
Cestroideae		
Browallia eludens VAN DEVENDER & P. D. JENKINS	EU580865	EU580964
Cestrum tomentosum L. F.	EU126009	EU580977
Salpiglossis sinuata RUIZ & PAV.	U08928	AY206730 & AY206765
Vestia lycioides WILLD.	AY206751	AY206769
Benthamielleae		
Combera paradoxa SANDWITH	EU580873	EU580980
Petunieae		
Hunzikeria texana (TORR.) D'ARCY	EU580886	EU580993
Nierembergia hippomanica MIERS	EU580917	EU581033
Petunia axillaris (LAM.) BRITTON	U08926	AY098702
Schwenckieae		
Schwenckia glabrata KUNTH	EU580942	EU581057
Nicotianoideae		
Anthocercis angustifolia F. MUELL.	AY098704	AY098671
Cyphanthera anthocercidea HAEGI	AY098716	AY098684
Nicotiana acuminata (GRAHAM) HOOK.	U08923	AY098696
Nicotiana glauca GRAHAM	AJ585910	AJ577414

Table 3 continued

Solanoideae		
Capsicum minutiflorum (Rusby) Hunz.	DQ667543	EU580970
Datura stramonium L.	U08917	EU580984
Mandragora caulescens C. B. Clarke	EU580911	EU581023 (as *M. chinghaiensis*)
Mandragora officinarum L.	U08922	EU581024
Physalis peruviana L.	EU580928	EU581044
Solanum dulcamara L.	U47419	AY266231
Withania somnifera (L.) Dunal	EU580952	EU581069
Witheringia meiantha (Donn. Sm.) Hunz.	EU126020	EU581072
Solanoideae-Atropina-Clade		
Grabowskia duplicata Arn.	EU580883	DQ124555
Jaborosa squarrosa (Miers) Hunz. & Barboza	EU126013	EU581004
Lycium barbarum L.	EU580907	AB036572 & AB036601
Lycium cestroides Schltdl.	U08920	AB036578 & AB036607
Nolana linearifolia Phil.	EU580918	EU581034
Solanoideae-Atropina-Clade Hyoscyameae		
Anisodus luridus Link & Otto	EU580856	EU580955
Anisodus tanguticus (Maxim.) Pascher	EU580857	EU580956
Atropa belladonna L.^	EU580859	EU580959
Atropanthe sinensis (Hemsl.) Pascher	EU580860	EU580960
***Hyoscyamus albus* L.**[+]	EU580887	EU580994
Hyoscyamus boveanus* Asch. & Schweinf.	EU580888	EU580995
Hyoscyamus desertorum (Asch. ex Boiss.) Täckh.	EU580889	EU580996
***Hyoscyamus muticus* L.**[#]	EU580890	EU580997
Hyoscyamus pusillus L.	EU580891	EU580998
***Physochlaina infundibularis* Kuang°**	EU580930	EU581046
Physochlaina orientalis (M. Bieb.) G. Don	EU580931	EU581047
Przewalskia tangutica Maxim.	EU580935	EU581050
Scopolia carniolica Jacq.	EU580944	EU581060
Scopolia japonica Maxim.	EU580945	EU581061

Results

Phylogenetic Analysis. The ITS data set comprised 45 taxa including the outgroup that consisted of three other species of the Atropina clade of Olmstead et al. (2008) and *Mandragora officinarum*. It was 687 base pairs (bp) long, of which 231 were variable and 161 parsimony informative.

The MP analysis resulted in 4160 shortest trees of 467 steps (CI = 0.71, RI = 0.91). The ML analysis resulted in only one best tree and is shown in Fig. 2. The topologies of the strict MP consensus tree, the ML tree and the BEAST tree (Fig. 3) are identical with respect to the major groups. In the following branches will be categorized as not supported [ML- and MP-BS <70%, posterior probability (post. prob.)] <0.85), weekly supported (ML- and MP-BS of 70-79%, post. prob. of 0.85-0.89), moderately supported (ML- and MP-BS of 80-89%, post. prob. of 0.9-0.94) and highly supported (ML- and MP-BS >90%, BEAST post. prob. >0.95). Hyoscyameae are moderately (88% MP-BS) to highly (98% ML-BS) supported as monophyletic. In the BEAST analysis the ingroup was set to be monophyletic (see Materials and Methods) in order to root the tree and this causes a posterior probability (post. prob.) of 1 for this grouping. The first split in the trees is between *Hyoscyamus*/*Atropa* (71% MP-BS) and the rest of Hyoscyameae (76% MP-BS, 0.89 BEAST post. prob.), but these groupings are only weakly or not supported. *Hyoscyamus* and *Atropa* are both highly supported in all analyses and *Atropa beatica* is highly supported as sister to the other *Atropa* accessions. Within these, *Atropa* accessions from Kazakhstan, Uzbekistan and Armenia form a group only in the BEAST analysis (0.93 BEAST post. prop.) but are in a polytomy with the rest of the *Atropa* accessions that form a highly supported group in all analyses. In the other Hyoscyameae *Atropanthe* and *Anisodus* (both highly supported) form a trichotomy with a highly supported clade consisting of *Przewalskia*, *Physochlaina* and *Scopolia*. Therein, *Physochlaina* and *Scopolia* are weakly supported as sister in MP and highly supported in the BEAST analyses but not supported by ML. *Scopolia* accessions from Japan are a strongly supported group in all three analyses but the European/Caucasian *Scopolia* are only weakly or not supported.

The combined data set of ITS and the cp-Data comprised 17 taxa and 3758 characters of which 464 were variable and 221 parsimony informative.

The MP and ML analyses resulted both in one best tree only (MP 737 steps, CI = 0.78, RI = 0.71) and share their topology with the BEAST tree that is shown in Fig. 4. Hyoscyameae are defined as monophyletic in the BEAST analysis but highly supported in MP and ML analyses and *Atropa* (MP- and ML-BS 100%, post. prob. is not given for this node, see above) is supported sister (ML-BS 76%, BEAST post. prob. 0.96) to the rest of the tribe in the ML and BEAST analyses. *Hyoscyamus* (MP- and ML-BS 100%, BEAST post. prob. 1) again is sister to the remaining Hyoscyameae, but this relationship is only supported in the BEAST analysis (post. prob. 0.96). *Anisodus* and *Atropanthe* are not supported, successive sisters to a clade of *Przewalskia*/*Physochlaina*/*Scopolia* that appears highly

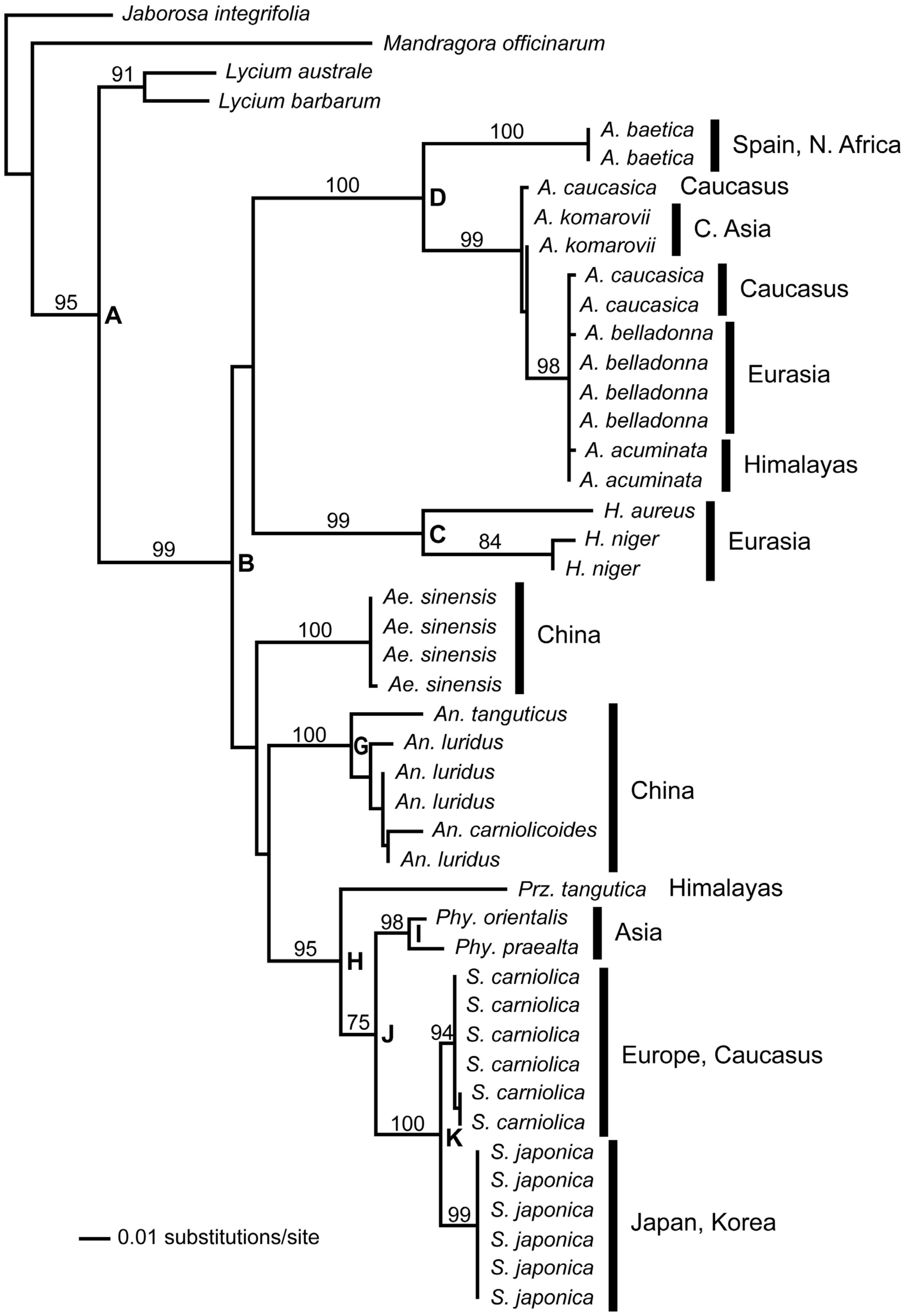

FIG. 2. ML phylogram of the Hyoscyameae ITS dataset. Bootstrap branch support (>70%) is indicated above branches and nodes are named according to Table 4. The Distribution of the taxa is also shown in Fig. 1.

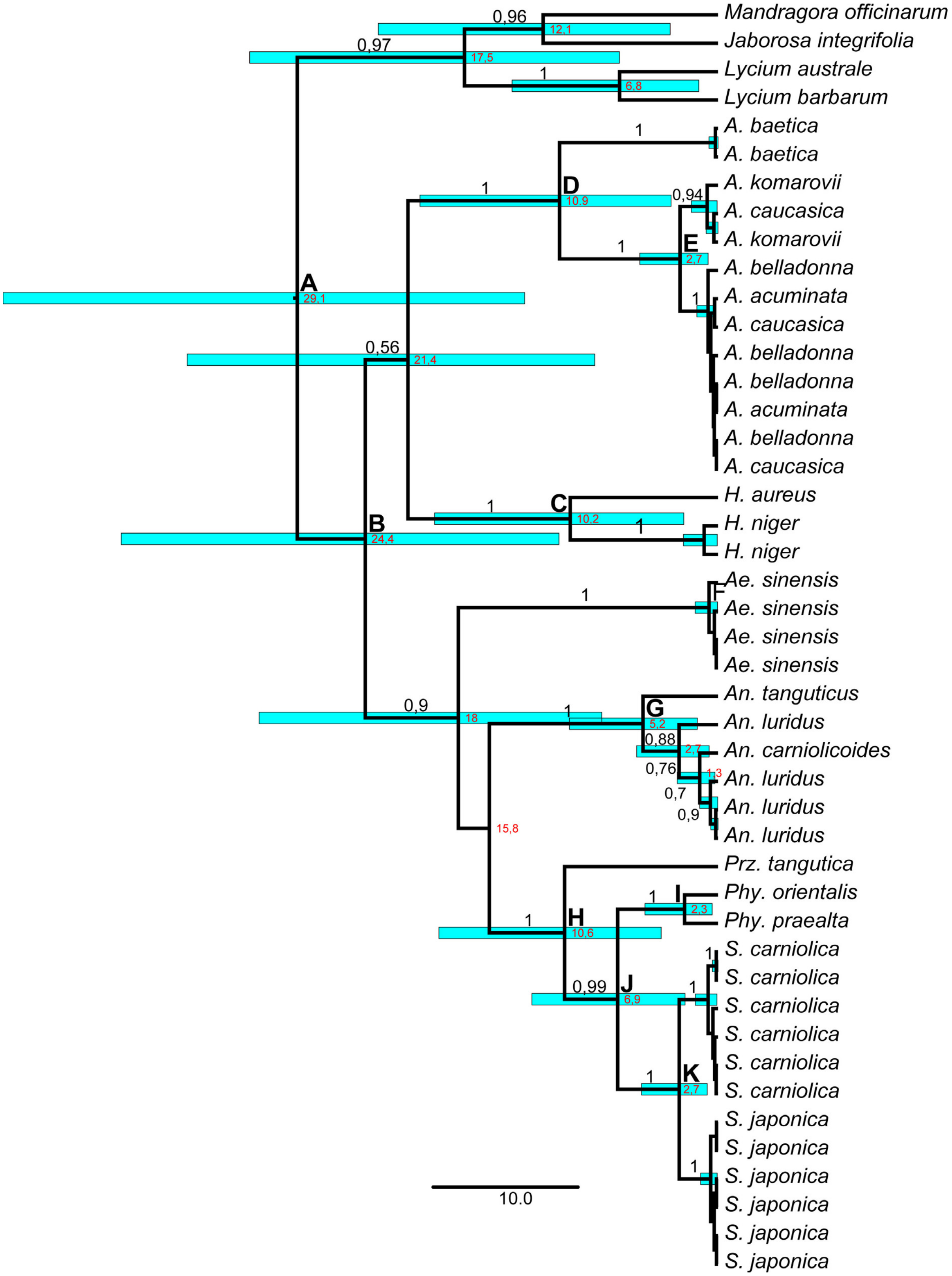

FIG. 3. BEAST chronogram of the Hyoscyameae ITS dataset. Posterior probabilities of the clades are indicated above the branches and node ages in My are given in the node bars showing the 95% posterior density distribution. Nodes are named according to Table 4 and Fig. 2. Node bars are only given for branches with a post. prob > 0.5. Scale axix is in My.

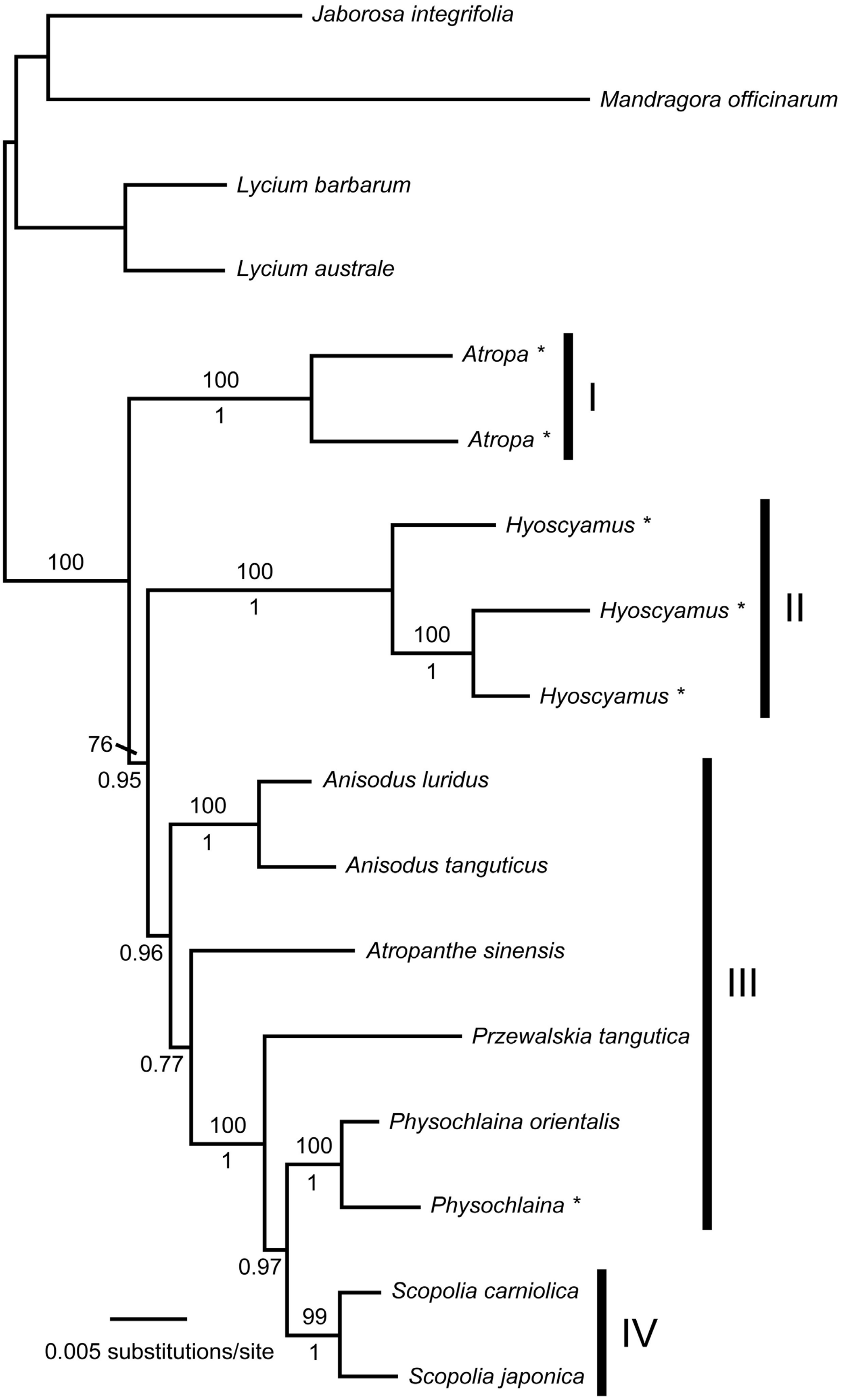

FIG. 4. ML phylogram of the combined ITS/*trnL*/*ndhLF* data set. Bootstrap branch support (>70%) is indicated above and BEAST posterior probabilities below branches. Taxa of this data set where the sequences are composed of two closely related species are named by the genus only and marked with an asterisk).

FIG. 5. BEAST chronogram of the Solanales *trnL/ndhLF* data set. Posterior probabilities of the clades are indicated above the branches and node ages in My are given in the node bars showing the 95% posterior density distribution. Node bars are only given for branches with a post. prob > 0.5. Scale axix is in My.

supported in all three analyses. Therein *Physochlaina* and *Scopolia* are resolved as sister groups, but only the BEAST analysis supports this relationship with a post. prob. of 0.97.

Molecular clock. Age estimates obtained from the ITS clock-enforced ML tree topology and from the mean ages of the BEAST analysis of the ITS data are summarized in Table 4. The BEAST tree of the ITS data set is shown in Fig. 3 with mean ages indicated at the nodes and the 95% posterior densities as node bars.

The second, age-based clock analysis with the *ndhF*/*trnLF* dataset of Solanales, shown in Fig. 5, resulted in a stem age for Hyoscyameae of 25,6 My and a crown age of 19.5 My were *Atropa* clearly splits first and is well supported sister to the remaining Hyoscyameae. *Anisodus* and *Hyoscyamus* are rapidly splitting without support at medium node ages of 16.5 and 15.1 My, the latter together with the well supported (BEAST post. prob. 0.96) rest of the tribe. In this group *Atropanthe* is sister to *Przewalskia*/*Physochlaina*/*Scopolia* (post. prob. 1; node age 12.4 My) and diversification of this clade starts at a mean age of 9.4 My.

TABLE 4. Comparison of ML clock-enforced age estimates calculated with a low (*Saxifraga*, 1.72 x 10^{-9} substitutions/side/year) and a high (*Soldanella*, 8.34 x 10^{-9} substitutions/side/year) ITS substitution rate with relaxed clock mean age estimates calculated in BEAST. The nodes are indicated in the ITS ML phylogram of Hyoscyameae in Fig. 2 and in the BEAST chronogram in Fig. 3: **A** stem age of Hyoscyameae, **B** basal polytomy in Hyoscyameae, **C** crown age of *Hyoscyamus* **D** split of *A. baetica* vs. remainder *Atropa* species, **G** crown age of *Anisodus*, **H** split of *Przewalskia* vs. *Physochlaina*/*Scopolia*, **J** split *Physochlaina* vs. *Scopolia*, **K** split Japanese *Scopolia* vs. European/Caucasian *Scopolia*.

node ages in My	**A**	**B**	**C**	**D**	E	F	**G**	**H**	I	**J**	**K**
ML high ITS rate	66.8	58.7	27.9	29	5.2*	0.6	12.2	26.7	5.8	17.4	5.2
ML low ITS rate	13.7	12	5.8	6	1.1*	0.1	2.5	5.5	1.2	3.7	1.1
ML mean ITS rate	40.3	35.4	16.9	17.5	3.2*	0.4	7.4	16.1	3.5	10.6	3.2
BEAST mean ITS rate	29.1	24.4	10.2	10.9	2.6	0.6	5.2	10.6	2.3	6.9	2.7

* This node does not appear in the ML phylogram shown in Fig. 2 but in the ML chronogram (not shown).

Discussion

Phylogeny and Classification. Hyoscyameae incl. *Atropa* are monophyletic according to the chosen outgroup taxa with high branch supports (Figs. 2, 4 and 5). This has also been shown by other molecular phylogenetic analyses (Olmstead et al. 2008 and references therein) and is further supported by a shared retroposon insertion in Hyoscyameae (Yuan et al. 2006) and the shared occurrence of tropine (Tétényi 1987). Seed characters also support the inclusion of *Atropa* in Hyoscyameae (Zhang et al. 2005). All genera of Hyoscyameae are highly supported and monophyletic in our analyses, as far as they are represented (Figs. 2-4).

Within Hyoscyameae the backbone of the phylogeny is not supported (no BS values >70%) in the ML phylogeny of the ITS data set (Fig. 2), and in a strict view *Atropa*, *Hyoscyamus*, *Atropanthe*, *Anisodus* and a clade of *Przewalskia*, *Physochlaina* and *Scopolia* together form a basal polytomy. In the BEAST analysis of this data set (Fig. 3) another group appears with a post. prob. of 0.9: *Atropanthe* and *Anisodus* group together with the *Przewalskia*/*Physochlaina*/*Scopolia* clade (see below). The combination of the ITS with the cp data (Fig. 4) yielded a better but still not fully resolved basal branching pattern of Hyoscyameae. The groupings that appear using the different datasets and tree construction methods will be discussed and compared with other characters that have been studied in Hyoscyameae (morphological cladistics: Hoare and Knapp 1997; karyology: Hunziker 2001,Tu et al. 2005, Yuan et al. 2006; seed surface: Zhang et al. 2005; pollen surface: Zhang and Lu 1984, Lu and Zhang 1986; leaf epidermis: Yang et al. 2000, floral ontogeny: Yang et al. 2002a, 2002b; secondary compounds: Tétényi 1987) in order to search for additional characters supporting weak branches of the molecular phylogenies.

In the combined dataset (Fig. 4) and in the Solanaceae cp dataset (Fig. 5) ***Atropa*** splits off first as weakly to highly supported sister to the remaining Hyoscyameae. This relationship is also supported by fruit morphology. The majority of the Atropina clade of Olmstead et al. (2008) has berries like *Atropa*. The pyxidium of most Hyoscyameae (unique in Solanaceae) that is hidden in the tubular part of the enlarged/inflated calyx, is derived from an immature berry (Knapp 2002). The most parsimonious explanation is that the fruit type berry is the plesiomorphic state and the pyxidium arose once and is a synapomorphic character state of Hyoscyameae without *Atropa*. Both classifications shown in Table 1 can reflect this relationship. Either *Atropa* in a separate tribe Atropeae is sister to Hyoscyameae excl. *Atropa*, as suggested by Hunziker (2001), or *Atropa* can be included in Hyoscyameae as suggested by Olmstead and Bohs (2007). Species number in *Atropa* is contentious (Hunziker 2001, two species; Hoare und Knapp 1997, three species; D'Arcy 1991 five species). ITS variation can not distinguish more than two distinct lineages in our sampling (Figs. 2 and 3).

Hyoscyamus is the next branching clade from the remaining Hyoscyameae sensu Hunziker in the combined dataset (Fig. 4) but this relationship is only supported in the BEAST analysis (post. prob. of 0.96). *Hyoscyamus* has a zygomorphic corolla, whereas the remaining Hyoscyameae have actinomorphic to slightly unequal corollas (Hunziker 2001).

Leaf margins of *Hyoscyamus* species are variously incised, versus entire leaf margins in the rest of Hyoscyameae (Hoare and Knapp 1997). These morphological features describe synapomorphies of *Hyoscyamus* and are plesiomorphies when *Atropa* is considered the most basal clade of the tribe and the symplesiomorphies (actinomorphic corollas and entire leave margins) can not be used to further support the group of ***Anisodus*/*Atropanthe*/*Przewalskia*/ *Physochlaina*** and ***Scopolia***. This group finds moderate support in the BEAST analysis of the ITS data set (Fig. 3). All species except *Physochlaina* possess solitary flowers, which could be interpreted as a synapomorphy for this clade. Other morphological, chemical or karyological characters supporting the group were not found.

Within this clade ***Atropanthe*** shows an affinity to the *Przewalskia*/*Physochlaina*/*Scopolia* group in the results of the Solanaceae cp data set (Fig. 5, 0,96 post. prob.) and in the combined matrix (Fig. 4, not supported), but these findings are not congruent with the results obtained from the ITS data set. No other characters could be found to further elucidate the position of *Atropanthe*.

The position of ***Anisodus*** also remains unclear. It shares the absence of 3-tricloyloxitropane that all other Hyoscyameae possess with *Atropanthe*, but the absence of this chemical compound is probably not a good character state for phylogenetic interpretation.

Only one group that is well supported in all analyses and data sets can be observed. It consists of ***Przewalskia*, *Physochlaina*** and ***Scopolia***. Zhang et al. (2005) suggested a close relationship of these taxa using seed surface characters. Within this group, ***Physochlaina*/*Scopolia*** are weakly to highly supported sister in our molecular ITS data (Figs. 2, 3) but results of the Solanaceae cp data set (Fig. 5) do not confirm this. In the resulting tree of the combined matrix (Fig. 4) this sister group relationship appears not supported in the ML analysis, but with a high BEAST post. prob. of 0,9. However, Zhang et al. (2005) described seed characters that *Scopolia* and *Physochlaina* share and which support their sister group relationship. In the Solanaceae cp data set *Scopolia japonica* groups together with *Przewalskia* and *Scopolia* is not monophyletic. However *Scopolia* clearly is monophyletic in the results of our ITS and the combined data set with high branch support and the two species are morphologically and ecologically very similar so that their close relationship has never been in doubt. Hybridization or introgression that could result in different signals of nuclear and plastid data (Sang and Zhong 2000, Barber et al. 2007, Kim and Donoghue 2008) seem quite unlikely because the geographical ranges of *Przewalskia* and *Scopolia* are separated and their ecological requirements are so different that sympatry is inconceivable. The small sample size in the cp data where only one accession of each *Scopolia* species is present might offer an explanation for this.

Karyological studies by Tu et al. (2005) are differently interpreted by Yuan et al. (2006) with respect to the base numbers of chromosomes and ploidy levels. Yuan et al. (2006) interpreted the chromosome numbers of 2n = 48 for *Anisodus*, *Atropanthe* and *Scopolia*, 2n = 42 for *Physochlaina*, 2n = 44 for *Przewalskia* and 2n = 28/34/68 for *Hyoscyamus* with a base number of x = 12 as predominantly tetraploid, and *Atropa* with 2n = 72/60 as hexaploid. Together with their finding of a retroposon insertion that is only present in two of three copies of the "waxy" gene of *Atropa*, they interpreted *Atropa* as an ancient hybrid of a tetraploid and a diploid, extinct parent. Tu et al. (2005) always set the base chromosome number to the least common denominator, x = 6 in *Atropa*, *Anisodus*, *Atropanthe* and *Scopolia*, x = 7 in *Physochlaina*, x = 11 in *Przewalskia* and x =14/17 in *Hyoscyamus* and achieved various ploidy levels of up to dodecaploid in *Atropa*. Both studies do not mention various other chromosome counts in the group reported by Hunziker (2001). These do not always fit the base numbers proposed by Tu et al. (2005). Olmstead and Sweere (1994), Olmstead and Bohs (2007) and Olmstead et al. (2008), name and confirm a monophyletic x = 12 clade consisting of Solanoideae plus other groups of Solanaceae and Hyoscyameae are part of this clade. Therefore a base number of x = 12 for Hyoscyameae is probably most reasonable with chromosome changes and genome mutations having occurred in the evolution of the group. Anyhow, a phylogenetic signal could not be found in chromosome numbers.

Results from the study of other character complexes like leaf epidermis (Yang et al. 2000), floral ontogeny (Yang et al. 2002a, 2002b) and pollen surface (Lu 1989, Lu and Zhang 1989) have been screened but are not concordant with our molecular phylogenies or their taxon sampling is not comprehensive for the tribe. The characters used by Hoare and Knapp (1997) in their morphological cladistic analysis resulted in totally different groupings and mostly were uninformative on our tree topology. The weak support of the base of the molecular phylogeny of Hyoscyameae and the paucity of other characters might be due to rapid early diversification (Maddison 1989, Hoelzer and Melnick 1994, Walsh et al. 1999, Jackman et al. 1999, Fishbein et al. 2001)of the five main groups: *Atropa*, *Hyoscyamus*, *Anisodus*, *Atropanthe* and *Przewalskia*/*Physochlaina*/ *Scopolia* that happened about 5 My after the ancestor of the group had reached Eurasia in the mid Oligocene (Fig 3 and Table 4) and spread over a wide area.

Molecular clock analysis. Age estimates via published ITS rates are summarized in Table 4. The strict molecular clock calculated with ML resulted in slightly older mean age estimates than the relaxed BEAST analysis (see also Fig. 3). The results of the latter are very close to the age estimates obtained from the second age-based approach (Fig. 5) and therefore we prefer the younger estimates recovered by the BEAST analysis. Overall, the age estimates obtained for Hyoscyameae by both timing approaches are similar in scale of geological ages and when error bars are considered.

Biogeographical implications. ORIGIN OF HYOSCYAMEAE. The exclusively Eurasian and N African Hyoscyameae clearly belong to the Atropina clade of Olmstead et al. (2008) which has its origin in the New World, and mainly occurs in temperate S America. Only one other lineage of Atropina, one group of *Lycium*, reached the Old World and Australia by long-distance dispersal (Fukuda et al. 2001). The majority of species of this clade occurs in S Africa (~20 species) which is proposed as the area of origin of the Eurasian (~10 species) and the Australian (one species) taxa (Fukuda et al. 2001, Levin and Miller 2005, Levin et al. 2007). *Lycium* reached Eurasia around 14 My ago in the mid Miocene (Fukuda et al. 2001). Although our mean age estimates for the stem age of Hyoscyameae are older, about 29 My (BEAST mean ITS rates; Table 4) and about 26 My (BEAST cp data, age-based; Fig. 5), an intercontinental long-distance dispersal from S America to Eurasia also has to be postulated. This event took place in the Oligocene. That Hyoscyameae took the same route as *Lycium* from S America to S Africa and then to Eurasia is not very likely, because no related lineages can be found in S Africa. Also a route over N America and Beringia to Asia is not likely because Hyoscyameae are absent from N America and all lineages of Atropina (except Hyoscyameae) are of S American origin, and if N America was reached by Atropina, these taxa are derived (Olmstead et al. 2008). So most likely the ancestor of Hyoscyameae reached Eurasia in a rare long-distance dispersal event from S America.

The basal splits of the molecular phylogeny of the Hyoscyameae are not well resolved and only few other characters could be found to further support these relations. Therefore biogeographical implications for the whole tribe can only be drawn carefully. Nevertheless a series of vicariance events is the most plausible explanation for the overall pattern of the recent distribution (Fig. 6). Subsequent diversification of some groups and range expansion of a few taxa obscure the pattern of vicariance and resulted in the partly overlapping distribution areas observed today (Fig. 1).

DIVERSIFICATION OF HYOSCYAMEAE – A SEQUENCE OF VICARIANCE EVENTS. The first split of *Atropa* vs. the remaining Hyoscyameae can be interpreted as a west vs. east split in Eurasia (Fig. 6, I), because *Atropa* has a western centre of diversity and those taxa that reach the Himalayas are derived (Figs. 2 and 3). In contrast to that, the remaining Hyoscyameae have a more eastern distribution. Even *Hyoscyamus* with its wide range clearly has its centre of diversity in SW to SC Asia where most of the perennial species occur. Only the short-lived annual and perennial species spread widely into the Mediterranean area and into S Sibiria (Meusel et al. 1978). The second split (Fig. 6, II) would be *Hyoscyamus* vs. the remaining genera of Hyoscyameae (*Anisodus*, *Atropanthe*, *Przewalskia*, *Physochlaina* and *Scopolia*; Fig. 4). This indicates a further the range formation in the east, with *Anisodus*, *Atropanthe, Przewalskia* and *Physochlaina* clearly distributed in the Himalayas and China with only *Ph. orientalis* ranging westwards to the Caucasus and *Ph. physaloides* northwards to Mongolia and S Sibiria (Meusel et al. 1978, Lu 1986, Lu and Zhang 1986, Hunziker 2001). The following split (Fig. 6, III) separates this Chinese/Himalayan distribution area from the highly disjunct range of *Scopolia* in Japan/Korea and SE Europe/Caucasus (Fig. 6, IV). The reasons and time frames of the different intrageneric differentiations are discussed in the following.

WIDESPREAD AND DISJUNCT – THE RELATIONSHIPS OF THE EUROPEAN *SCOPOLIA* AND *ATROPA* SPECIES. The sequence of the latter two splits (III and IV) are consistent to the pattern we found in *Epimedium* (Berberidaceae; Zhang et al. 2007) where Chinese representatives split off earlier in the phylogeny than the also disjunctly distributed European/Caucasian representatives separated from the Far East *Epimedium* species. Therefore a northern connection between the species of Europe/Caucasus (and N Africa in *Epimedium*) and the species from the Far East must have existed longer than a connection to the C Chinese representatives. *Epimedium* and *Scopolia* are very similar in their ecological requirements. Both are elements of the understory of broad-leaved deciduous forests in temperate mountain regions (Meusel et al. 1978), so this connection must have been a more or less continuous forest belt that does not exist any longer. Molecular dating placed the split between Europe/Caucasus and Far East in *Epimedium* to the Pliocene/Pleistocene, whereas the mean age of the split of *Scopolia* species in our dating of Hyoscyameae is around 2.7 My in the Pliocene (node K in Fig. 3 and Table 4). Both studies strongly suggest fragmentation of a once continuous forest belt in northern Eurasia to be the reason for the present disjunct distribution of these taxa (see also Stearn 2002 for *Epimedium*). For *Scopolia* this explanation was already offered by Lu and Zhang (1986). A steady decline in global temperatures and a drying trend in higher latitudes that started in the Miocene/Pliocene (Willis and McElwain 2002) are likely to have caused this fragmentation. Concerning the origin of European mountain plant taxa the area relationships found by us, with closer relations of European/Caucasian taxa to E Asian taxa than to more neighbouring representatives, clearly imply a biogeographical connection of W Eurasia to E Asia not via southern intervening mountain ranges but via a more northern route.

Atropa, similar in ecology to *Scopolia* and *Epimedium*, occurs in submediterranean mountains from N Africa/S Spain over Europe and reaches eastwards to the Caucasus, Hindu Kush and West Himalayas (Fig. 1). The overall distribution pattern is very similar to *Epimedium* in W Eurasia (see Zhang et al. 2007), but area formation/biogeography seems to differ in the two genera. In *Atropa* the first split is that of *A. baetica* in the very SW of the generic distribution range (Fig. 6, I'). Subsequent area formation is not entirely clear from our phylogeny. In the topologies shown in Figures 2 and 3 the W Himalayan accessions, together with the European and part of the Caucasian material are not resolved. This group is either sister (Fig. 3) to accessions from C Asia (east of the Caspian Sea) and the other accession from the Caucasus or in a polytomy (Fig. 2) with these. Our molecular dating of *Atropa* implies an age of more than 20 My for *Atropa* and sets the first split of its range to around 11 My ago. This is in concordance with the onset of the Mediterranean climate that emerged in the middle of the Miocene, when seasonal contrasts in temperature regime began. These led to fragmentation of forest vegetation on the Iberian Peninsula (Thompson 2005) and consequently the range of *Atropa* was split. Today *A. baetica* only occurs in forests at elevations of 1500-2000 m in SE Spain and Morocco and *A. belladonna* occurs only sparsely in N Spain. In *Epimedium* a rather opposite pattern can be observed. First the W Himalayan region separated from the genus range followed by the N African and Caucasian range separating from the highly disjunct ranges in Europe/Turkey and the Far East. The age of *Epimedium* was calculated not older than 10 My and the first split of the range formation was around 7 My ago (Zhang et al. 2007) indicating a range formation from east to west. Although in *Atropa* no direction is implied by the phylogenetic relationships, the unresolved position of the W Himalayan accessions in a terminal position of the tree and the first split in *Atropa* in the very SW of its generic range rather imply a range formation proceeding from west to east as opposed to the pattern observed in *Epimedium*. Summarizing the above, the similarities observed in the distribution pattern of Eurasian *Epimedium* and *Atropa* are not caused by the same biogeographical events. Concerning the origin of the European material of *Atropa* our results indicate a Mediterranean rather than an Asian origin.

ASIAN DIVERSITY OF HYOSCYAMEAE – ECOLOGICALLY DIVERSE TAXA VS. HIGH MOUNTAIN SPECIALISTS The widespread *Hyoscyamus* has, according to the sequence of range splits suggested above, its origin in its centre of diversity in SW and SC Asia. For

Hyoscyamus no molecular phylogeny is available and our sampling is insufficient. *Hyoscyamus* species occurring in the centre of diversity are perennials like the other members of Hyoscyameae (Meusel et al. 1978). The short annual/biennial habit is the derived character state and must have evolved later in the history of the genus. These short-lived species in *Hyoscyamus* are also the most widespread and the wide distribution of the genus observed today might be a rather recent phenomenon. In general *Hyoscyamus* is more xerophytic and light-demanding than most of the other Hyoscyameae. In our molecular clock analysis *Hyoscyamus* has a stem age of around 15-24 My and the split of the included species is calculated to around 10 My ago (node C in Fig. 3 and Table 4, Fig. 5) which falls into the time period of the onset of Mediterranean climate in the Miocene (Thompson 2005). Tropical vegetation began to disappear and the recent Mediterranean flora assembled. Much in contrast to *Atropa*, *Scopolia* and *Epimedium* that seem to have maintained their ecology over time, *Hyoscyamus* diverged into new open habitats and today has the broadest ecological range in the tribe.

Physochlaina has its centre of diversity in the Himalayas and China and only one species spreads westwards to the Caucasus and one northwards to Mongolia and S Siberia. Similar to *Hyoscyamus*, *Physochlaina* shows a wider adaptability to environmental conditions (Lu and Zhang 1986). Only two species were represented in our molecular phylogeny, and no others were available. Lu and Zhang (1986) suggested that species that have similar ecological requirements to *Scopolia* and occur in warm and moist broad-leaved forests to be ancestral in *Physochlaina*, while species that are adapted to more arid conditions and higher altitudes to be derived. In this context the centre of origin would be in SW China as also suggested by our scenario of sequential range splits in the tribe, and the more western and more northern taxa would have expanded to these areas. The mean stem group age of *Physochlaina* is 9 to 7 My (node J in Fig. 3 and Table 4, Fig. 5) and so the subsequent diversification of the genus corresponds to the late uplift phase of the Asian mountain ranges that began 50-40 My ago but experienced a significant increase at about 20-8 My ago (Harrison et al. 1992 for Himalaya, Li and Fang 1999, Tapponnier et al. 2001).

The uplift of the Himalayas and the Tibetan Plateau also triggered the evolution of the remaining representatives of the tribe as has been suggested earlier by Lu and Zhang (1986), but in contrast to *Physochlaina* and *Hyoscyamus*, *Anisodus* has only four species and *Atropanthe* and *Przewalskia* are both monotypic. *Anisodus* and *Przewalskia* occur at rather high altitudes whereas *Atropanthe sinensis* inhabits warmer and moister habitats at lower altitudes, hence being more comparable to *Scopolia*. The origin of *Atropanthe* and *Anisodus* reaches back to the mid Miocene and *Przewalskia* is a bit younger (late Miocene). These genera have not expanded their range and are restricted to their Himalayan habitats.

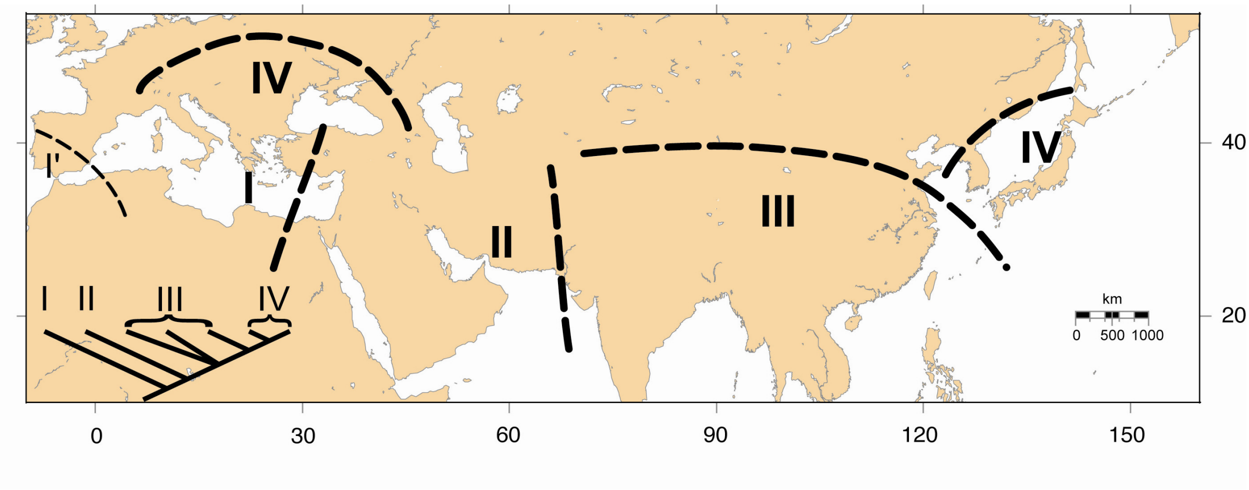

FIG. 6. Sequence of range formation in Hyoscyameae. Roman figures in the map and on the simplified tree refer to the clades indicated in Fig. 4. I□' indicates the split of *Atropa baetica* vs. remaining *Atropa*. Map created with online map creation (Weinelt 1996 onwards)

In summary: The common ancestor of Hyoscyameae reached Eurasia in the Oligocene via long-distance dispersal from S America. In the Oligocene the climate of Eurasia was more tropical and the ancestor of Hyoscyameae expanded its range. From the Mid Miocene onwards the climate became cooler and drier. This resulted in range fragmentation of Hyoscyameae. The origin and the centre of diversity of Atropina is temperate S America. Hyoscyameae that have maintained their ecological preferences today inhabit moist broad-leaved deciduous forests (*Atropa*, *Scopolia*, *Atropanthe* and parts of *Physochlaina*) and can be interpreted relict. Taxa that broadened their ecological spectrum towards Mediterranean climates experienced diversification and range expansion like *Hyoscyamus* and parts of *Physochlaina*. Other adapted to rising mountain ranges and changed their ecology accordingly. They became ecological specialists in smaller areas like *Przewalskia* and *Anisodus*.

A comparable pattern is found in *Mandragora* (two species are well defined, Hunziker 2001). *Mandragora officinarum* Land and *M. caulescens* C.B. Clarke show an east-west geographical and an altitudinal partition. Whereas *M. officinarum* is distributed in the Mediterranean and SW Asia at lower altitudes, *M. caulescens* occurs in the Himalayas, Burma and W China at high altitudes (Zhang et al. 1994, Hunziker 2001). This West-East split between the two species is dated to the Miocene in our Solanales data set (Fig. 5) and like the pattern in Hyoscyameae corresponds to the onset of the Mediterranean climate (Thompson 2005) and to the main uplift of the Eurasian Mountain system (Harrison et al. 1992).

Concerning the origin of the European Hyoscyameae our results indicate a northern connection to E Asia for *Scopolia* and a Mediterranean origin of *Atropa* where contact to Asia was through southern intervening mountain ranges.

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3. Artikel: Alpine-Asian relationships

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Asian relationships of the flora of the European Alps

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Abstract

Background: It has long been recognised that the Alps have strong biogeographical links to Asia.

Aims: We investigate here whether the two areas are connected either through southern (mountain chains between the Alps and the Himalayas) or through northern regions.

Methods: We compiled the geographical distribution of plant genera growing both in the Alps and the Himalayas, and outside these two regions. This compilation was used to search for molecular phylogenetic literature which was screened for area relationships informative for our purposes.

Results: Of 933 genera growing in the Alps, 653 were considered further. Of the 429 genera growing in the Alps and the Himalayas, 218 grow in both northern and southern intervening areas, 203 only in southern intervening areas, three only in northern intervening areas, and five in the Alps and Himalayas but not in between. *Epimedium*, *Primula* sect. *Auricula* and *Scopolia* can be considered examples for a northern connection. *Brachypodium*, Poaceae subtribe Loliinae, *Bupleurum*, *Doronicum* and *Atropa* are examples for a southern connection. In the latter group centres of diversity are clearly located in the Mediterranean area and occurrences further east appear to be derived.

Conclusions: Although our database is very narrow, we predict that ideas about the biogeographical composition of the flora of the Alps made by several early authors will be confirmed. It will be found to contain a northern element originating from Asia via a northern connection, and a southern element originating from the Mediterranean area or southwest Asia.

Keywords: Alps; area relationships; biogeography; Himalayas; molecular phylogeny

Zusammenfassung

Hintergrund: Biogeographische Verbindungen der Alpen nach Asien wurden schon im 19. Jahrhundert durch floristische Vergleiche entdeckt.

Ziele: In den vorliegenden drei Artikeln wird untersucht, ob diese Verbindung entlang einer nördlichen, arktisch-borealen Route oder entlang einer südlichen Route über dazwischen liegende Gebirge wie den Kaukasus bestand.

Methoden: Molekulare Phylogenien von *Epimedium* (Berberidaceae) und der Tribus der Hyoscyameae (Solanaceae), deren Vertreter disjunkt in den Alpen, dem Kaukasus und in asiatischen Gebirgen vorkommen, wurden mit nukleären und plastidären Markern erstellt und biogeographisch ausgewertet. Zur Datierung der Diversifizierungsereignisse und Arealbildung diente eine molekulare Uhr. Ein aktueller floristischer Vergleich von Gattungen, die in den Alpen, den asiatischen Gebirgen und in Gebieten entlang der potentiellen Routen vorkommen, wurde unternommen und ausgewertet. Daran anschließend wurde nach molekularphylogenetischer Literatur für interessante Gruppen aus diesem Vergleich recherchiert, um diese biogeographisch bezüglich der Fragestellung zu interpretieren.

Ergebnisse: Von 429 Gattungen, die in den Alpen und im Himalaya vorkommen, wachsen 218 entlang der nördlichen und der südlichen Route. 203 kommen nur entlang der südlichen und drei nur entlang der nördlichen Route vor. Fünf kommen nur in den Alpen und im Himalaya vor. *Epimedium*, *Scopolia*/*Physochlaina* aus den Hyoscyameae und *Primula* sect. *Auricula* sind Beispiele für eine nördliche biogeographische Verbindung zwischen den Alpen und Asien. Diese bestand zumindest in den ersten beiden Fällen wahrscheinlich aus einem durchgängigen Laubwaldgürtel, der durch das abkühlende und trockener werdende Klima im Pliozän und Pleistozän fragmentiert wurde und heute nicht mehr besteht. Es handelt sich also um ein Vikarianzmuster und weniger um eine Migrationsroute. Die größere Diversität dieser Guppen, die in Asien beobachtet werden kann ist sekundär entstanden. *Atropa* aus den Hyoscyameae, *Brachypodium*, die Subtribus Loliinae aus den Poaceae, *Bupleurum* und *Doronicum* sind Beispiele für eine südliche Verbindung. Hier liegen die Diversitätszentren im Mediterraneum und die Vorkommen im Osten sind abgeleitet.

Schlussfolgerungen: Die Datengrundlage aus der Literaturrecherche ist nicht sehr breit und die meisten Phylogenien waren für die Fragestellung nicht aussagekräftig. Die historischen Erkenntnisse und die Ideen über den Zusammenhang der Alpenflora mit der asiatischer Gebirge werden grundsätzlich bestärkt. Die Flora der Alpen enthält ein Element

das über eine nördliche Verbindung noch lange mit asiatischen Gebirgen in Kontakt stand und ein südliches Element, das aus dem Mittelmeerraum bzw. aus SW Asien stammt. Die Beispiele für eine nördliche Verbindung sprechen allerdings nicht für eine Migration von Asien nach Europa sondern zeigen ein Vikarianzmuster auf.

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