



Dispersals of Hyoscyameae and Mandragoreae (Solanaceae) from the New World to Eurasia in the early Miocene and their biogeographic diversification within Eurasia

Tieyao Tu^{a,1}, Sergei Volis^b, Michael O. Dillon^c, Hang Sun^{a,*}, Jun Wen^{a,d,**}

^a Key Laboratory of Biodiversity and Biogeography, Kunming Institute of Botany, Chinese Academic of Sciences, Kunming 650204, PR China

^b Department of Life Science, Ben Gurion University of the Negev, Israel

^c Department of Botany, The Field Museum, 1400 South Lake Shore Drive, Chicago, IL, USA

^d Department of Botany, National Museum of Natural History, Smithsonian Institution, Washington, DC, USA

ARTICLE INFO

Article history:

Received 1 June 2010

Revised 8 September 2010

Accepted 9 September 2010

Available online 19 September 2010

Keywords:

Biogeography

Disjunction

Dispersal

Mediterranean

South America

Tibetan Plateau

Vicariance

ABSTRACT

The cosmopolitan Solanaceae contains 21 tribes and has the greatest diversity in South America. Hyoscyameae and Mandragoreae are the only tribes of this family distributed exclusively in Eurasia with two centers of diversity: the Mediterranean–Turanian (MT) region and the Tibetan Plateau (TP). In this study, we examined the origins and biogeographical diversifications of the two tribes based on the phylogenetic framework and chronogram inferred from a combined data set of six plastid DNA regions (the *atpB* gene, the *ndhF* gene, the *rps16-trnK* intergenic spacer, the *rbcl* gene, the *trnC-psbM* region and the *psbA-trnH* intergenic spacer) with two fossil calibration points. Our data suggest that Hyoscyameae and Mandragoreae each forms a monophyletic group independently derived from different New World lineages in the early Miocene. Phylogenetic relationships within both tribes are generally well resolved. All genera of Hyoscyameae are found to be monophyletic and they diversified in middle to late Miocene. At nearly the same time, Mandragoreae split into two clades, corresponding to the MT region and the TP region, respectively. Both the phylogenetic relationships and the estimated ages of Hyoscyameae and Mandragoreae support two independent dispersal events of their ancestors from the New World into Eurasia. After their arrivals in Eurasia, the two tribes diversified primarily in the MT region and in the TP region via multiple biogeographic processes including vicariance, dispersal, recolonization or being preserved as relicts, from the mid Miocene to the late Quaternary.

Published by Elsevier Inc.

1. Introduction

The origin and evolution of disjunct distributions of organisms have long interested biogeographers (Manos and Donoghue, 2001; Raven and Axelrod, 1974; Thorne, 1972; Wen, 1999; Wen and Ickert-Bond, 2009; Wu, 1983). Vicariance became the prevailing explanation for many types of intercontinental disjunctions of plants and animals in the 1970s with the wide acceptance of the plate tectonics theory (Good, 1974; Nelson and Platnick, 1980; Sanmartin, 2003; Wiley, 1988). For example, the classical

* Corresponding author. Address: Key Laboratory of Biodiversity and Biogeography, Kunming Institute of Botany, CAS, Lanhei Road 132#, Kunming 650204, Yunnan, China. Fax: +86 871 5215002.

** Corresponding author at: Department of Botany, National Museum of Natural History, MRC-166/Botany, Smithsonian Institution, PO Box 37012, Washington, DC 20013-7012, USA. Fax: +1 202 786 2563.

E-mail addresses: hsun@mail.kib.ac.cn (H. Sun), wenj@si.edu (J. Wen).

¹ Present address: Key Laboratory of Plant Resources Conservation and Sustainable Utilization, South China Botanical Garden, Chinese Academic of Sciences, Guangzhou 510650, PR China.

trans-Atlantic distributions of many plants have been considered associated with the breakup of Africa and South America (Raven and Axelrod, 1974; Wu et al., 2003). However, these two landmasses separated from each other 100–120 million years ago (mya) (Bauer, 1993; Hay et al., 1999), and vicariance may be appropriate only to interpret the distributions of some anciently diverged plant taxa, such as Annonaceae and Alstroemeriaceae – Luzuriagaceae, that were dated to Cretaceous (Doyle et al., 2004; Vinnersten and Bremer, 2001). Many plant taxa disjunct between Eurasia and North America may represent elements of the once continuous Arcto-Tertiary or boreal floras in the Tertiary (Li, 1952; Tiffney, 1985; Tiffney and Manchester, 2001). Nevertheless, these disjunctions from the breakup of the once continuous Arcto-Tertiary or boreal floras may be restricted to the mid-Tertiary temperate elements (such as *Anemone* of Ranunculaceae and *Viburnum* of Caprifoliaceae) or early Tertiary tropical elements (such as Illiciaceae), which adapted to the climatic conditions in the high-latitude regions of the northern hemisphere (Tiffney and Manchester, 2001; Wen, 1999, 2001). For taxa with tropical affinities and/or younger than Miocene, the vicariance hypothesis may not be plausible.

Recently, several molecular studies based on robust phylogenies and divergence time estimates suggest the importance of long-distance dispersal for some intercontinental disjunctions at the species or the genus level (Dick et al., 2007; Givnish et al., 2004; Nie et al., 2005; Schaefer et al., 2009; Wen and Ickert-Bond, 2009; Yokoyama et al., 1998). Long-distance dispersal between continents can be mediated by migratory animals, extreme meteorological phenomena, ocean currents, floating islands, or winds (Houle, 1998, 1999; Nathan et al., 2008; Renner, 2004; Winkworth et al., 2002). Ocean currents have been suggested for some taxa with trans-oceanic disjunctions, such as *Lathyrus* L. of Fabaceae (Kenicer et al., 2005), *Chrysosplenium* L. of Saxifragaceae (Soltis et al., 2001), and *Ceiba pentandra* L. of Malvaceae (Dick et al., 2007). Taxa with small propagules such as *Metrosideros* Banks ex Gaertn. of Myrtaceae (Wright et al., 2000), orchids (Carlquist, 1996; Winkworth et al., 2002) and ferns (McGlone et al., 2001) may disperse over long-distances via winds. *Lycium* L. (Solanaceae) has been suggested to be dispersed in a single event by birds from the New World to Eurasia followed by spreading into southern Africa (Fukuda et al., 2001; Levin and Miller, 2005). Long-distance dispersal has been also advocated as an explanation for the wide distribution of *Nicotiana* L. (Solanaceae) in the Americas, Australia and Africa (Aoki and Ito, 2000; Clarkson et al., 2004; Mummenhoff and Franzke, 2007).

Solanaceae is a cosmopolitan family with ca. 21 tribes, 100 genera and 2500 species (Hunziker, 2001; Olmstead and Bohs, 2007; Olmstead et al., 2008). This family has a center of diversity in the New World, particularly in South America (Hunziker, 2001). Hyoscyameae and Mandragoreae, however, are largely restricted to Eurasia, with only a few species of both tribes extending to northern Africa. The origins of the two Eurasian solanaceous tribes have been of interest to taxonomists and biogeographers, and different hypotheses were proposed regarding the formations of their current distributions. Lu and Zhang (1986) first analyzed the diversity and distribution of Hyoscyameae and noted that many genera and species occur in the Tibetan Plateau (TP) area, especially the mountains of the southeastern TP. They thus postulated its origin in the mountains of the southeastern TP. The Gondwanian origins of the two tribes, however, were postulated by some authors because most basal extant taxa of Solanaceae occur in the southern hemisphere (Hoare and Knapp, 1997; Symon, 1991; Tu et al., 2005; Ungricht et al., 1998). Based on the molecular phylogeny of Solanaceae and Solanales (Bremer et al., 2002; Olmstead et al., 2008; Stefanovic et al., 2002) and their overall distributions, Olmstead et al. (2008) suggested the New World as the most likely place of origin for the family with eight to nine minimum dispersal events into the Old World, which include the dispersals of Mandragoreae and Hyoscyameae.

Hyoscyameae contains eight genera and Mandragoreae is a monogeneric tribe with five species (Akhani and Ghorbani, 2003; Hoare and Knapp, 1997; Lu, 1997; Olmstead et al., 2008; Ungricht et al., 1998). Members of the two tribes are found throughout Eurasia but their distributions are often interrupted by deserts, steppes and mountains. The five mandrake species occur discontinuously in the Tibetan Plateau, the Turanian region, and the Mediterranean basin. Many genera of Hyoscyameae are isolated either in the Mediterranean–Turanian (MT) region (*Atropa* L., *Archihyoscyamus* A.M. Lu) or in the TP region (*Przewalskia* Maxim., *Anisodus* Link et Otto and *Atropanthe* Pascher). The two species of *Scopolia* are distributed disjunctly between eastern Asia and the Mediterranean basin. Overall two centers of diversity can be identified for both tribes: the MT region and the TP region. These two regions have been considered the most important 'hot spots' of biodiversity in Eurasia (Myers et al., 2000). Besides Hyoscyameae and Mandragoreae, many other unrelated taxa have similar distribution patterns in the two regions (see review by Sun, 2002). It has

been hypothesized that this distribution pattern among different Eurasian taxa could have been resulted from a common geologic or climatic event (Greuter, 1991; Jakob et al., 2007; Petit et al., 2003; Ribera and Blasco-Zumeta, 1998; Steward and Lister, 2001; Wu, 1988). Since the Cretaceous, the most remarkable physical changes of Eurasia were the retreat of Tethys Sea before Tertiary, the collision of the Indian Plate with Eurasia in early Tertiary followed by the uplift of the Himalayas and the aridification of Central Asia in the middle to late Tertiary, the connection and the breakup of Eurasia with North America through the Bering Land Bridge (BLB) and the North Atlantic Land Bridge (NALB) in the early to middle Tertiary, and the repeated advances/retreats of glaciers in the Quaternary (Barron and Peterson, 1989; Butler, 1995; Harrison et al., 1992; Lomolino et al., 2006; Tiffney and Manchester, 2001; Wen, 1999). However, the associations between the environmental changes and the distributions of plants throughout Eurasia have rarely been tested. Moreover, the physical events may not be the only option to explain the plants' distribution in Eurasia.

The explicit estimates of both phylogenies and ages of lineages are of critical importance in inferring the biogeographic histories of Hyoscyameae and Mandragoreae. Previous molecular phylogenetic studies on Solanaceae have shown that the Eurasian Hyoscyameae and Mandragoreae are nested within clades comprising groups endemic to or largely distributed in South America (such as Nolaneae, Lycieae, Physaleae and Solaneae) (Yuan et al., 2006; Olmstead et al., 2008), clearly suggesting the New World origin of these two tribes. However, the lack of information on their divergence times has hindered our understanding on whether the formation of the two intercontinental disjunctions was caused by dispersals from the New World to Eurasia, or by vicariance due to the breakup of the previously continuous distributions resulted from climate changes. More importantly their subsequent diversification within Eurasia needs to be analyzed based on a phylogenetic framework. In this study, we sampled seven of the eight genera from Hyoscyameae and all five species from Mandragoreae, and employed six plastid markers to infer a phylogeny. Using two fossils of Solanaceae as calibration points, we estimated the ages of the Old World–New World disjunctions, and the disjunctions within Eurasia. The phylogeny and the chronogram were then used to test the origins of the two tribes and to examine their biogeographic diversifications within Eurasia.

2. Materials and methods

2.1. Taxon sampling, DNA extraction, PCR, and sequencing

The sampling covered all five species of Mandragoreae and all eight genera of Hyoscyameae except the monotypic *Archihyoscyamus* A.M. Lu, which was previously placed in the genus *Hyoscyamus* (Hoare and Knapp, 1997; Lu, 1997). Due to the close relationships of the two tribes to Nolaneae, Lycieae, Jaboroseae, Physaleae and Solaneae from the subfamily Solanoideae (Olmstead et al., 2008), sequences from 15 samples of these tribes were also included in our phylogenetic analyses. Six plastid markers including the *atpB* gene, the *ndhF* gene, the *rps16-trnK* and the *psbA-trnH* intergenic spacer, the *rbcl* gene and the *trnC-psbM* region, were used to infer a phylogeny. The six markers were newly sequenced in this study or obtained from our recent study (Dillon et al., 2009). Two *Nicotiana* species (*N. sylvestris* Speg.; *N. tomentosiformis* Goodsp.) were used to root the tree. The taxa, the GenBank accession numbers, and the voucher information are presented in Table 1.

Total genomic DNA was extracted from silica gel-dried leaf materials following Tu et al. (2008) or from fresh leaves of transplanted individuals or of plants raised from seeds. The primers and PCR protocols for amplifying the six markers can be found in

Table 1

Samples, Vouchers, Source and GenBank accessions included in the phylogeny reconstruction and in the dating analysis. Numbers in the brackets behind the species are corresponding to the ones in Fig. 1. Accessions of Nijmegen refer to the Solanaceae collections at the University of Nijmegen, Netherlands; herbarium abbreviations are as follows: KUN = Kunming Institute of Botany, China; BUN = Ben-Gurion University of the Negev, Israel; F = Field Museum, USA; A = Arnold Arboretum of Harvard University, USA; IBSC = South China Botanical Garden; US = US National Herbarium of the Smithsonian Institution, USA.

Species	Vouchers or sources	GenBank accession					
		<i>psbA-trnH</i>	<i>rps16-trnK</i>	<i>trnC-psbM</i>	<i>ndhF</i>	<i>rbcl</i>	<i>atpB</i>
<i>Anisodus acutangulus</i> C.Y. Wu and C. Chen	T. Tu 0212 (KUN)	HQ216148	HQ215987	HQ216032	HQ216077	HQ216113	HQ215950
<i>Anisodus carniolicoides</i> (C.Y. Wu and C. Chen) D' Arcy and Zhang	T. Tu 0220 (KUN)	HQ216149	HQ215988	HQ216033	HQ216078	HQ216114	HQ215951
<i>Anisodus luridus</i> Link and Otto	T. Tu 02101 (KUN)	HQ216150	HQ215989	HQ216034	HQ216079	HQ216115	HQ215952
<i>Anisodus tanguticus</i> (Maxim.) Pascher	T. Tu 0280 (KUN)	HQ216151	HQ215990	HQ216035	HQ216080	HQ216116	HQ215953
<i>Atropa belladonna</i> L. (1)	H. Sun 9040 (KUN)	HQ216153	HQ215992	HQ216037	FJ914032	FJ914178	FJ914159
<i>Atropa belladonna</i> L. (2)	V. Cherniak and T. Tu 01 (KUN)	HQ216152	HQ215991	HQ216036	HQ216081	HQ216117	HQ215954
<i>Atropa komarovii</i> Blin. and Shalyt.	S. Volis s.n.	HQ216154	HQ215993	HQ216038	HQ216082	HQ216118	HQ215955
<i>Atropanthe sinensis</i> (Hemsl.) Pascher	T. Tu 02107 (KUN)	HQ216155	HQ215994	HQ216039	HQ216083	HQ216119	HQ215956
<i>Grabowskia boerhaviifolia</i> Schldtl. (1)	M. Dillon 8581 (F)	EU742439	EU742371	EU742507	EU742303	FJ914177	FJ914158
<i>Grabowskia boerhaviifolia</i> Schldtl. (2)	S. Leiva 2724 (F)	HQ216156	HQ215995	HQ216040	HQ216084	HQ216120	HQ215957
<i>Hyoscyamus albus</i> L.	884750066 (Nijmegen)	HQ216158	HQ215997	HQ216042	HQ216086	HQ216122	HQ215959
<i>Hyoscyamus aureus</i> L.	914750009 (Nijmegen)	HQ216157	HQ215996	HQ216041	HQ216085	HQ216121	HQ215958
<i>Hyoscyamus tuberosus</i> L.	974750072 (Nijmegen)	HQ216160	HQ215999	HQ216044	HQ216088	HQ216123	HQ215961
<i>Hyoscyamus niger</i> L. (1)	T. Tu 0270 (KUN)	HQ216161	HQ216000	HQ216045	HQ216089	HQ216124	HQ215962
<i>Hyoscyamus niger</i> L. (2)	T. Tu 0246 (KUN)	HQ216162	HQ216001	HQ216046	HQ216090	HQ216125	HQ215963
<i>Hyoscyamus niger</i> × <i>H. albus</i>	894750186 (Nijmegen)	HQ216159	HQ215998	HQ216043	HQ216087	-	HQ215960
<i>Hyoscyamus pusillus</i> L.	894750188 (Nijmegen)	HQ216163	HQ216002	HQ216047	HQ216091	-	HQ215964
<i>Hyoscyamus turcomanicus</i> Pojark.	904750014 (Nijmegen)	HQ216164	HQ216003	HQ216048	HQ216092	HQ216126	HQ215965
<i>Jaborosa squarrosa</i> (Miers) Hunz. and Barboza	V. Quipuscoa et al. 3400 (F)	HQ216165	HQ216004	HQ216049	HQ216093	HQ216127	HQ215966
<i>Lycianthes</i> sp.	J. Wen et al. Tibet 2111 (US)	HQ216166	HQ216005	HQ216050	FJ914029	FJ914173	FJ914154
<i>Lycium americanum</i> Jacq.	V. Quipuscoa et al. 2862	EU742440	EU742372	EU742508	EU742304	HQ216128	HQ215967
<i>Lycium chinense</i> Mill.	T. Tu 0660 (KUN)	HQ216168	HQ216007	HQ216052	FJ914028	FJ914171	FJ914152
<i>Lycium deserti</i> Phil.	M. Dillon 8545 (F)	EU742443	EU742375	EU742511	EU742305	FJ914172	FJ914153
<i>Mandragora autumnalis</i> Bertol.	S. Volis M-J-S 01	HQ216169	HQ216008	HQ216053	HQ216096	HQ216129	HQ215968
<i>Mandragora caulescens</i> C.B. Clark (1)	J. Zhang and T. Tu 137-9 (KUN)	HQ216170	HQ216009	HQ216054	HQ216097	HQ216130	HQ215969
<i>Mandragora caulescens</i> C.B. Clark (2)	J. Zhang and T. Tu 137-10 (KUN)	-	HQ216010	HQ216055	-	HQ216131	HQ215970
<i>Mandragora caulescens</i> C.B. Clark (3)	L. Yue 13 (KUN)	HQ216171	HQ216011	HQ216056	HQ216098	HQ216132	HQ215971
<i>Mandragora chinghaiensis</i> K.Z. Kuang and A.M. Lu (1)	Y. Duan and T. Tu 01 (KUN)	HQ216172	HQ216013	HQ216058	HQ216099	HQ216134	HQ215973
<i>Mandragora chinghaiensis</i> K.Z. Kuang and A.M. Lu (2)	Boufford et al. 31534 (A)	-	HQ216012	HQ216057	-	HQ216133	HQ215972
<i>Mandragora officinarum</i> L. (1)	S. Volis Ant2 (BUN)	HQ216173	HQ216014	HQ216059	HQ216100	HQ216135	HQ215974
<i>Mandragora officinarum</i> L. (2)	S. Volis Tur1/06 (BUN)	HQ216176	HQ216017	HQ216062	HQ216103	HQ216138	HQ215977
<i>Mandragora officinarum</i> L. (3)	884750044 (Nijmegen)	HQ216174	HQ216015	HQ216060	HQ216101	HQ216136	HQ215975
<i>Mandragora officinarum</i> L. (4)	934750027 (Nijmegen)	HQ216175	HQ216016	HQ216061	HQ216102	HQ216137	HQ215976
<i>Mandragora turcomanica</i> Mizgir.	S. Volis Mt10 (BUN)	HQ216177	HQ216018	HQ216063	HQ216104	HQ216139	HQ215978
<i>Nicotiana tomentosiformis</i> Goodsp.	Yukawa et al., 2006	AB240139	AB240139	AB240139	AB240139	AB240139	AB240139
<i>Nicotiana sylvestris</i> Speg.	Yukawa et al., 2006	AB237912	AB237912	AB237912	AB237912	AB237912	AB237912
<i>Nolana sessiliflora</i> Phil.	M. Dillon 8644 (F)	EU742494	EU742926	EU742562	EU742358	FJ914168	FJ914194
<i>Nolana acuminata</i> Miers ex Dunal	M. Dillon 8100 (F)	EU742443	EU742375	EU742511	EU742307	FJ914163	FJ914144
<i>Phrodus microphyllus</i> Miers	M. Dillon 8643 (F)	EU742442	EU742374	EU742510	EU742306	FJ914176	FJ914157
<i>Physalis peruviana</i> L.(1)	T. Tu 0661 (KUN)	HQ216179	HQ216020	HQ216065	FJ914034	FJ914180	FJ914161
<i>Physalis peruviana</i> L.(2)	S. Volis s.n. (BUN)	HQ216178	HQ216019	HQ216064	FJ914035	FJ914181	FJ914162
<i>Physochlaina orientalis</i> G. Don	924750019 (Nijmegen)	HQ216180	HQ216021	HQ216066	HQ216105	HQ216140	HQ215979
<i>Physochlaina physaloides</i> (L.) G. Don	914750085 (Nijmegen)	HQ216181	HQ216022	HQ216067	HQ216106	HQ216141	HQ215980
<i>Physochlaina praealta</i> (Dcne.) Miers	T. Tu 0724 (KUN)	HQ216182	HQ216023	HQ216068	HQ216107	HQ216142	HQ215981
<i>Przewalskia tangutica</i> Maxim. (1)	T. Tu 0275 (KUN)	HQ216183	HQ216024	HQ216069	HQ216108	HQ216143	HQ215982
<i>Przewalskia tangutica</i> Maxim. (2)	D. Boufford et al. 31702 (A)	HQ216184	HQ216025	HQ216070	HQ216109	HQ216144	HQ215983
<i>Scopolia carniolica</i> Jacq. (1)	V. Cherniak and T. Tu 02 (KUN)	HQ216186	HQ216027	HQ216072	HQ216111	HQ216146	HQ215985
<i>Scopolia carniolica</i> Jacq. (2)	H. Sun 9000 (KUN)	HQ216185	HQ216026	HQ216071	HQ216110	HQ216145	HQ215984
<i>Scopolia japonica</i> Maxim.	KWNU 062582 (IBSC)	HQ216187	HQ216028	HQ216073	HQ216112	HQ216147	HQ215986
<i>Solanum chilense</i> (Dunal) Reiche	M. Dillon8717 (F)	HQ216188	HQ216029	HQ216074	FJ914031	FJ914175	FJ914156
<i>Solanum pennellii</i> Correll	M. Dillon8779A (F)	HQ216189	HQ216030	HQ216075	FJ914030	FJ914174	FJ914155
<i>Withania somnifera</i> (L.) Dunal	T. Tu 0650 (KUN)	HQ216190	HQ216031	HQ216076	FJ914033	FJ914179	FJ914160
<i>Witheringia macrantha</i> (Standl. and C.V. Morton) Hunz.	Olmstead et al. (2008)	-	-	-	EU126019	-	-
<i>Witheringia meiantha</i> (Donn. Sm.) Hunz.	Olmstead et al. (2008)	-	-	-	EU126020	-	-

Tu et al. (2008) and Dillon et al. (2009). The amplification was performed in 25 μ l reaction-mixture volumes using the Bioline Taq polymerase and associated reagents at 2.0 mM MgCl₂ concentration except for *trnC-psbM*, which used 4.0 mM MgCl₂. The amplification profiles were 3 min at 95 °C then 35 cycles of 30 s at 94 °C, 1 min at 50 °C, and 2 min at 72 °C, with a 10-min extension at 72 °C, ending with 4 °C hold. The PCR products were purified by the polyethylene glycol (PEG) precipitation method (Hiraishi et al., 1995). The cycle sequencing reactions were conducted in 10 μ l volumes which contained 0.25 μ l BigDye 3.1, 0.5 μ l primers, 2.0 μ l purified PCR products and 1.75 μ l sequencing buffer. The sequencing reactions were run on an ABI 3730 automated sequencer (Applied Biosystems, Foster City, California, U.S.A.).

2.2. Phylogenetic analyses

Sequencher 4.5 (Gene Codes Corporation, 2005) was used to evaluate chromatograms for base confirmation and to edit contiguous sequences. Sequences were initially aligned with ClustalX version 1.83 (Thompson et al., 1997), followed by manual adjustments using Se-Al v2.0a11 (Rambaut, 2007). The phylogeny reconstruction based on the combined sequences matrix was performed using maximum parsimony (MP) and Bayesian analyses. The parsimony analysis was conducted with PAUP version 4.10b (Swofford, 2003) using heuristic searches with 100 random addition sequences replicates, tree bisection–reconnection (TBR) branch swapping, MulTrees option in effect and character state changes equally weighted. The gaps of the sequences matrix were treated as missing data. The bootstrap percentages (Felsenstein, 1985) for support of the internal nodes were obtained with 1000 replicates. In each replicate, we performed 10 random sequences addition replicates followed by tree bisection–reconnection (TBR) swapping algorithm and saved all trees in each replicate.

Bayesian inference (Rannala and Yang, 1996) was conducted using MrBayes version 3.1.2 (Ronquist and Huelsenbeck, 2003) using the model estimated by Modeltest 3.7 (Posada and Buckley, 2004; Posada and Crandall, 1998). The Markov chain Monte Carlo (MCMC) algorithm was run for 2000,000 generations with four incrementally heated chains, starting from a random tree and sampling one out of every 100 generations. The first 10% trees were discarded as burn-in and the remaining trees were used to construct a Bayesian consensus tree.

2.3. Divergence time estimates

To evaluate the molecular clock assumption for our data, likelihood scores for clock and non-clock models were compared using a likelihood ratio (LR) test (Felsenstein, 1981). The LR was calculated as $2 \times (\ln L_{\text{clock}} - \ln L_{\text{nonclock}})$ and assumed to follow a chi-squared distribution with the number of degrees of freedom (n) equals to the number of terminals minus two. The assumption of rate constancy was rejected in this study because the constrained and unconstrained analyses differed significantly (LR = 167.35, d.f. = 31, $P = 0$). We then used a Bayesian method which allows a relaxed evolutionary model to estimate the divergence time. The Bayesian analysis was conducted using the software BEAST 1.5.4 (Drummond and Rambaut, 2007). The BEAUti was used to set criteria for the analysis. We used the Akaike Information Criterion (AIC) estimated by MODELTEST version 3.7 (Posada and Crandall, 1998) and the Bayes Factor (BF) calculated by TRACER version 1.5 (Rambaut and Drummond, 2007) to determine which nucleotide substitution models best fitted our data for dating. In addition we assumed an uncorrelated lognormal model of rate variation among branches in the tree and employed a Yule prior on the birth rate of new lineages (Drummond et al., 2006). We reduced some taxa that are topologically uncertainty or are densely sampled in

a same clade, which prevented the MCMC from reaching a stationary state. The tree was rooted by constraining the two species of *Nicotiana* to be sister to other species of Solanoideae and no other phylogenetic relationships were constrained. The analyses were completed with a randomly generated starting-tree topology. Two fossil calibrations of Solanaceae were used to place priors on the ages of nodes within the tree. A normally distributed prior for the stem age of *Physalis* was assigned to be 8.6 million years (mys) with a standard deviation [SD] of 1.0 according to the *Physalis*-like seeds found in the bottom of the upper Tortonian in middle Miocene from Silesia of Central Europe (Szafer, 1961). The SD here represents the upper and lower bounds of the geologic epochs from which the fossils were collected. A lognormally distributed calibration prior of 10.5 mys (lognormal mean = 1.0; lognormal SD = 1.0) was assigned to the stem node of *Solanum* based on the *Solanum*-like seeds reported from the European middle to late Miocene (Collinson et al., 1993). The lognormal distribution prior here represents a “hard” minimum constraint for this calibration point. The calibration procedure in this study is similar to Dillon et al. (2009) in dating the *Nolana* L. f., in which a fossil from Convolvaceae instead of from *Solanum* and a data matrix of three genes rather than six genes used here.

The MCMC simulations were run with 10,000,000 iterations and trees were sampled every 1000. The first 10% of the iterations were discarded as burn-in. Log-files were analyzed in TRACER version 1.5 (Rambaut and Drummond, 2007), and the effective sample sizes (ESS) were used to evaluate MCMC convergence within chains. The resulting trees were combined using TreeAnnotator version 1.5.2 (part of the BEAST package), with a burn-in of 1000 trees. Final trees were checked and edited in FigTree version 1.3.1 (Rambaut, 2009). The divergence times are given as the mean and the 95% highest posterior density (HPD) in millions of years. The 95% HPD intervals defines the precision of the estimation.

3. Results

3.1. Phylogenetic analyses

The combined matrix of the six plastid markers from 52 samples comprises 8252 positions, of which 1010 are variable (12.1%) and 683 are parsimony-informative (8.1%). The number of positions contributed by each individual region is as follows: 1091 from *atpB*, 2014 from *ndhF*, 734 from *psbA-trnH*, 1392 from *rbcl*, 936 from *rps16-trnK*, and 2085 from *trnC-psbM*.

The Bayesian analysis produced a tree with a similar topology as the strict consensus of the six parsimony trees. All clades with high bootstrap values (BP) in the parsimony analysis had high posterior probability (PP) values in the Bayesian analysis. The strict consensus topology of the parsimony analysis with BP and PP values for each clade is shown in Fig. 1. Using *Nicotiana* as the outgroup, the monophyly of both Hyoscyameae and Mandragoreae is strongly supported by the parsimony and the Bayesian analyses. Closely related to Hyoscyameae are the South American *Jaborosa* Juss. and a clade consisting of *Nolana*, *Lycium*, *Phrodus* Miers and *Grabowskia* Schltdl., which are centered in or endemic to South America. The genera within Hyoscyameae are each monophyletic.

The Mandragoreae are weakly supported as sister to a clade comprising members from Solaneae, Physaleae and Capsiceae. It was thus not directly related to Hyoscyameae. Within the tribe, two major clades were recovered, one including *M. officinarum* L., *M. autumnalis* Bertol. and *M. turcomanica* Mizgir. from the MT region and the other consisting of *M. caulescens* C.B. Clark and *M. chinghaiensis* K.Z. Kuang and A.M. Lu from the TP region. Accessions from each species clustered as a single clade except for three of *Mandragora caulescens*, which formed a polytomy with *M. chinghaiensis*.

3.2. Divergence times

Results from MODELTEST using the AIC favored the General Time Reversible (GTR) model of nucleotide substitution with gamma-distributed rate heterogeneity among sites and a proportion of invariant sites (GTR + G + I) (Lanave et al., 1984; Yang, 1994). This model was also favored by the criterion of Bayes factors when compared to the HKY model and the GTR model with or without gamma-distributed rate heterogeneity among sites and/or a proportion of invariant sites.

Using a combination of six plastid DNA regions calibrated with two fossil calibration points of *Solanum* and *Physalis*, the mean divergence times of Hyoscyameae and Mandragoreae from their sister groups are estimated to be 17.96 (95% HPD: 10.52–27.20) mya and 20.48 (95% HPD: 12.22–30.21) mya, respectively. The crown age of Hyoscyameae is 12.78 (95% HPD: 7.00–19.38) mya and that of Mandragoreae is 9.82 (95% HPD: 4.40–16.18) mya. All genera of Hyoscyameae became diversified by the end of Miocene. The age of the rosette-forming *Przewalskia* (Hyoscyameae) at 5.75 (95% HPD: 2.35–9.44) mya is ca. three times older than the sympatric rosette-forming *Mandragora chinghaiensis* (Mandragoreae) (1.84 mya, 95% HPD: 0.42–3.68). The narrow endemic *Atropa komarovii* Blin. and Schal. (Hyoscyameae) and *M. turcomanica* (Mandragoreae) near the border of Turkmenistan and Iran were both recently diversified from their close relatives in southwestern Asia and Europe at less than 0.5 mya. The disjunction between the Mediterranean *Scopolia carnolica* Jacq. and the eastern Asian *S. japonica* Maxim. is estimated to be 2.70 (95% HPD: 0.62–5.46) mya. The divergence times for the nodes in the phylogeny are shown in Fig. 2.

4. Discussion

4.1. Phylogenetic relationships

Our combined plastid data support the monophyly of both Hyoscyameae and Mandragoreae as previously reported by Olmstead et al. (2008) and Yuan et al. (2006). A more thorough taxon sampling and the use of more markers in this study resulted in better resolved phylogenies for both tribes.

Atropa has been either excluded from (Lu and Zhang, 1986) or included in Hyoscyameae (Hoare and Knapp, 1997) based on morphology. The difference in its placement is largely due to the fact that *Atropa* has berries not enclosed by fruit calyces, whereas all the other genera in Hyoscyameae have pyxidial, which are enveloped by enlarged fruit calyces and are dry at maturity (Knapp, 2002). In fact, *Atropa* and the pyxidate Hyoscyameae share synapomorphies of aperturate pollen grains and tropine esters (Hoare and Knapp, 1997; Tétényi, 1987; Zhang and Lu, 1984). Since berries are common in the close relatives of the tribe (e.g., Lycieae and *Jaborosa*), pyxidial with inflated fruit calyces in all genera of Hyoscyameae except for *Atropa* most likely represent a derived character state.

Our data reveal a basal-most position for *Atropanthe* in the pyxidate Hyoscyameae (Fig. 1). In the two-gene phylogeny of Olmstead et al. (2008), this position was occupied by *Anisodus*, whereas *Atropanthe* grouped with *Physochlaina*, *Scopolia*, *Przewalskia* and *Hyoscyamus* with weak support (BP = 30). The relationships among *Atropanthe* and other genera of Hyoscyameae were not well resolved in the phylogeny inferred from nuclear granule bound starch synthase I sequence (GBSSI) data (Yuan et al., 2006). *Atropanthe* shows morphological similarities to *Atropa* on declinate stamens and tubular-campanulate corollas, which are twice as long as the calyces. These similarities between the two genera led Chen and Chen (1977) proposed a close relationship for them. These similarities are likely to be symplesiomorphies rather than synapomorphies, because *Atropa* hosts the basal-most position and is not sister to *Atropanthe* in our phylogenetic tree (Fig. 1).

A clade consisting of *Anisodus*, *Hyoscyamus*, *Physochlaina*, *Przewalskia* and *Scopolia* is weakly supported. Nevertheless, each genus in this clade is monophyletic with strong support (Fig. 1). These five genera each can be easily diagnosed by morphology. All four species of *Anisodus* analyzed for the first time here are perennial herbs with the largest campanulate flowers (corolla 2.5–4.5 cm long) in the tribe. Furthermore, the accrescent calyces are greatly enlarged after maturation and loosely or tightly enclosing the fruits. The monophyly of *Hyoscyamus* is supported by its usually sessile solitary flowers and spiny tips of fruit calyces. The clade of the remaining three genera (*Przewalskia*, *Physochlaina* and *Scopolia*) is consistent with the previous analyses (Olmstead et al., 2008; Yuan et al., 2006). The two widely disjunct species of *Scopolia* in eastern Asia and in southeastern Europe form a strongly supported clade. These two species are morphologically similar with minor differences in shape and size of calyx and corolla. In contrast, the two-gene phylogeny of Solanaceae (Olmstead et al., 2008) suggested that the two species of *Scopolia* formed a paraphyletic group with the Tibetan *Przewalskia* (BP = 30). Although it has never been mentioned previously, the sister relationship between *Physochlaina* and *Przewalskia* in this study is congruent with their similarities in cytology (Tu et al., 2005) and their paniculate inflorescences rather than single flowers as in all other genera of the tribe.

Like *Atropa*, *Mandragora* was either included in or excluded from Hyoscyameae in different treatments (Hoare and Knapp, 1997; Lu and Zhang, 1986; Tu et al., 2005). *Mandragora* and *Atropa* share characters of berries with membranaceous calyces not enclosing the fruits, and the presence of belladonnine (Razzakov et al., 1998). Nevertheless, these character states are common in other closely or distantly related solanaceous genera (such as *Physalis* and *Solanum*) as well and are thus potentially not indicative of relationships between the two genera. Moreover, *Mandragora* does not contain tropine, which can be considered a synapomorphy of Hyoscyameae (Hoare and Knapp, 1997). The absence of tropine esters, the inaperturate pollen without tectum and the fibrils observed in the seed coat of *Mandragora* differentiate the genus from members of Hyoscyameae (Diez and Ferguson, 1984; Hoare and Knapp, 1997; Punt and Clarke, 1980; Sandina and Tarasevich, 1982; Zhang and Lu, 1984; Zhang et al., 2005). In this study, a closer relationship of *Mandragora* with Solaneae and Physaleae rather than with Hyoscyameae is supported by our plastid sequence data. This relationship is consistent with previous plastid and nuclear phylogenies of Solanaceae (Olmstead et al., 2008; Yuan et al., 2006).

Within *Mandragora*, the recovery of two major clades comprising species from the MT and from the TP, respectively, is congruent with the cladistic analysis based on morphology (Ungricht et al., 1998). The delimitations of species from each clade in the classification of Ungricht et al. (1998), however, are partly corroborated by our molecular phylogeny. In the MT clade, only two species (*M. officinarum* and *M. turcomanica*) were recognized by Ungricht et al. (1998), and *M. autumnalis* was merged to *M. officinarum*. The sister relationship of *M. autumnalis* to *M. turcomanica* rather than to *M. officinarum* was recovered in this study (Fig. 1). Morphologically, the seeds of *M. officinarum* are less than half the size of those of *M. autumnalis* and *M. turcomanica* (S. Volis, unpublished). Between the latter two species, there is no difference in seed size, whereas the former species has a more vigorous habit and larger leaves (Akhani and Ghorbani, 2003; Mizgireva, 1942, 1978). In the TP clade, *Mandragora chinghaiensis* has rosette leaves and short stems, whereas the stems of *M. caulescens* often elongate to 60 cm long (Kuang and Lu, 1978). Ungricht et al. (1998) stated that the stem height of *M. chinghaiensis* is within the variation range of *M. caulescens* and suggested the former as a synonym of the latter. During our field explorations, we did not find any individuals in two populations of *M. chinghaiensis* having elongated stems like

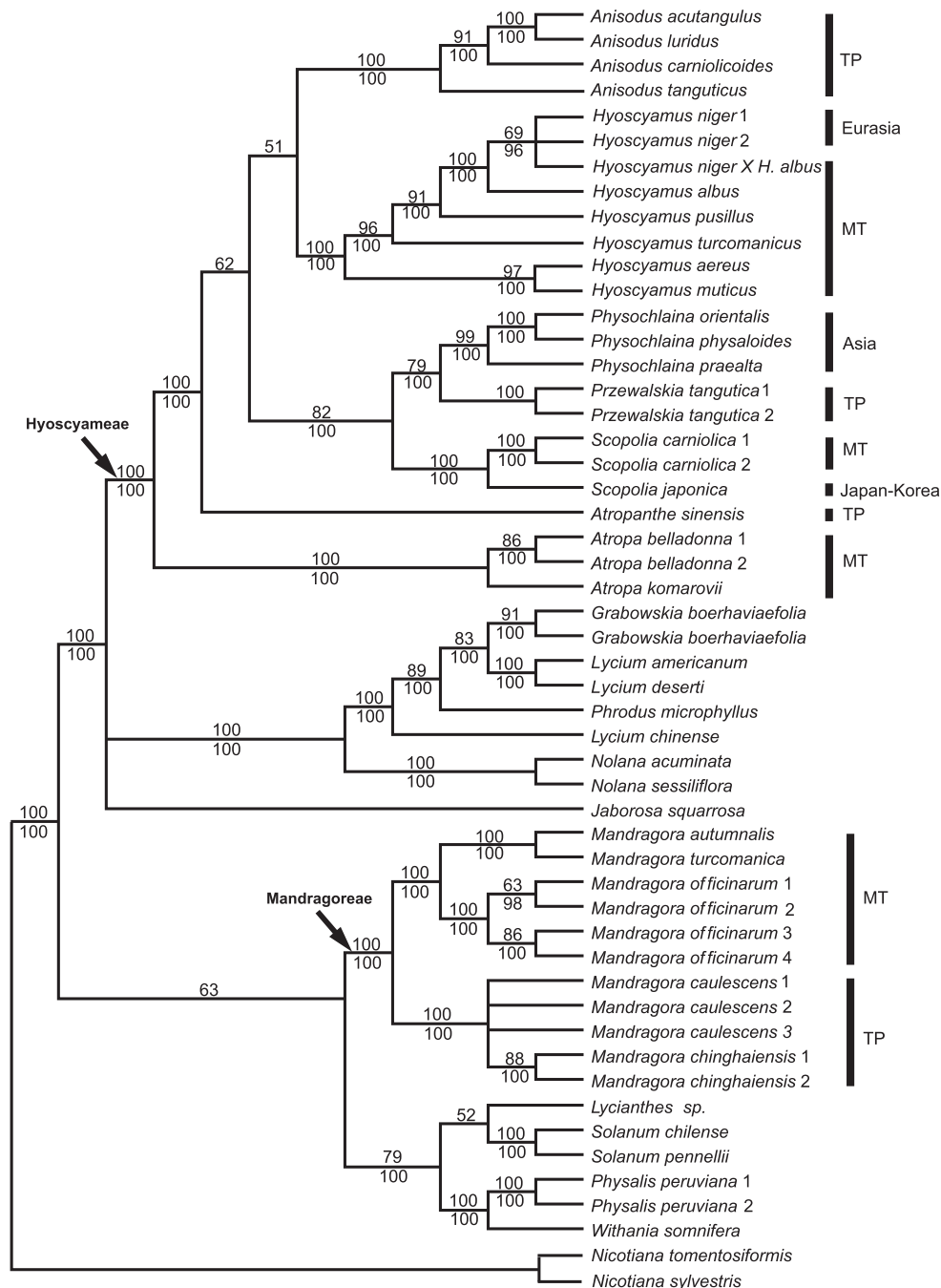


Fig. 1. The strict consensus tree of Hyoscyameae and Mandragoreae based on the combined sequence data of six plastid markers (tree length of 1451 steps; consistency index = 0.78; retention index = 0.91). The tree was rooted with *Nicotiana*. Bootstrap values from 1000 replicates are above the branches and Bayesian posterior probabilities are below the branches. The bootstrap values lower than 50% and the Bayesian posterior probabilities lower than 95% are not shown.

those of *M. caulescens*. In the present study, two accessions of *M. chinghaiensis* from localities separated by about 400 km (km) grouped together. However, it formed a polytomy with three accessions of *M. caulescens* (Fig. 1). Sequences of more variable markers and sampling from more populations are needed to test the delimitations of the two species.

4.2. Dispersals of Hyoscyameae and Mandragoreae from the New World to Eurasia

Our results confirmed the sister relationship between Hyoscyameae and the clade consisting of taxa endemic to South America (e.g. *Nolana* and *Jaborosa*) (Dillon et al., 2007; Hunziker, 2001), or

widespread taxa but having a center of diversity in South America (e.g. Lyceae) (Fukuda et al., 2001; Levin and Miller, 2005). A sister relationship was recovered between Mandragoreae and a clade of Capsiceae, Physaleae and Solaneae centered in the Americas. This relationship needs further warranty by more sequences and expanded taxon sampling because of its weak support (BP = 63; Bayesian PP < 95%). Using two fossils of Solanaceae as calibration points in this study, we dated the split between *Nicotiana* and the clade of the rest taxa at 24.41 mya. Wikström et al. (2001) dated the split between *Nicotiana* and *Petunia* at 23–25 mya in their chronogram of angiosperm. Clarkson et al. (2005) dated the age of the split between *Nicotiana* and its sister, *Symonanthus*, at 15.3 mya by using ITS sequence data and one calibration point.

The split between *Nolana* and *Lycium* inferred by our data is at 10.20 mya, which is very similar to the results of Dillon et al. (2009), who dated it at 10.39 mya by using three plastid DNA regions and two fossil calibration points. The ages of *Nicotiana* and *Nolana* estimated in different studies suggest that our data are generally comparable with previous findings. The ages of Hyoscyameae and Mandragoreae (17.96 mya and 20.48 mya, respectively) are much younger than the breakup of the Gondwanaland (100–120 mya) (Bauer, 1993; Hay et al., 1999) and thus reject the hypothesis of the Gondwanaland vicariance, which assumes that their ancestors occurred in habitable areas of Gondwanaland and migrated to Eurasia from Africa in the late Cretaceous or in the early Tertiary (Hoare and Knapp, 1997; Symon, 1991; Tu et al., 2005). Instead, the ages of the two tribes and their affinities to the tropical or subtropical American elements support the

hypothesis of two independent dispersals of the two tribes from the New World to Eurasia as postulated by Olmstead et al. (2008).

The disjunctions across the tropical Atlantic have been kindly reviewed by Renner (2004) and at least 110 plant genera were found to have the trans-Atlantic disjunctions. By analyzing 11 well studied cases of the 110 disjunctions, Renner (2004) summarized that four of them are correlated with wind, six dispersed via ocean currents and one without clear mechanism hypothesized. If Hyoscyameae and Mandragoreae followed one of these mechanisms of dispersals, ocean currents seem to be more likely because neither the berries of Mandragoreae nor the pyxidia of Hyoscyameae can be dispersed via wind.

Nevertheless, dispersal by animals may be an alternative explanation as it has been demonstrated to be important for the wide distributions of some other solanaceous plants. The fleshy berries

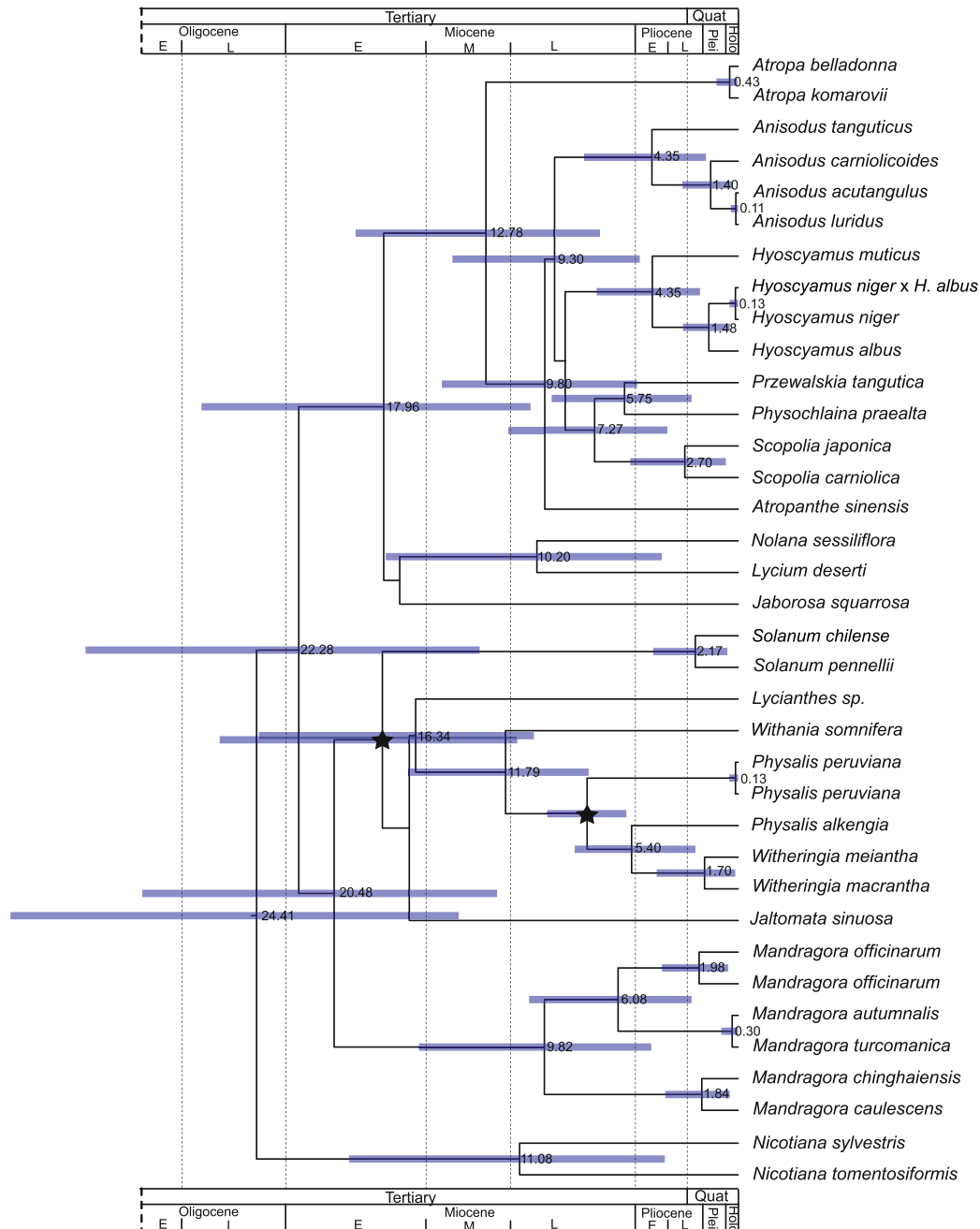


Fig. 2. Bayesian divergence time estimates of Hyoscyameae and Mandragoreae based on the combined sequence data from six plastid gene markers (*ndhF*, *atpB*, *rbcL*, *trnH-trnH*, *rps16-trnK*, *trnC-psbM*). Two fossil calibration points were marked by stars. The blue bars on the nodes indicate 95% posterior credibility intervals.

of Solanaceae are attractive to a wide variety of vertebrates, including birds, bats and small rodents (Cipollini and Levey, 1997a,b,c; Knapp, 2002; Symon, 1979). The phylogeny of *Lycium* suggested that species of the genus in Africa, Eurasia and the Pacific islands were most likely dispersed from the New World (Fukuda et al., 2001; Levin and Miller, 2005). For example, *L. sandwicense* A. Gray occurs on the Pacific islands (Easter, Hawaiian, and the Japanese Daito and Ogasawara) that are 3900 to >10,000 km away from its place of origin in the New World. The presence of *L. sandwicense* on these islands further confirmed the role of long-distance dispersal in this genus (Fukuda et al., 2001). Studies on seed dispersals of another species of *Lycium* on Alegranza of the Canary Islands (ca. 100 km from the Atlantic coast of Africa) revealed that both lizards and birds were responsible for the dispersal of the *L. intricatum* (Nogales et al., 1998, 1999). *Mandragora* in this study meets the syndrome of animal dispersal hypothesis due to its bright orange fleshy berries. The dispersal of *Mandragora* from the Americas to Eurasia may thus be facilitated by animals, probably by birds. Unlike *Mandragora*, most genera of Hyoscyameae bear pyxidial and do not seem to favor the long-distance dispersal through animals at first glance. Nevertheless, the ancestor of Hyoscyameae likely had fleshy berries because its sister groups (e.g., Lycieae and Jaboroseae) and the basal-most *Atropa* in Hyoscyameae bear berries. The intercontinental dispersal of the ancestor of the extant Hyoscyameae may thus be facilitated by animals.

4.3. Disjunctions of Hyoscyameae and Mandragoreae between eastern and western Eurasia

Discontinuous distributions within Eurasia have been reported for many plant genera. One of the distribution patterns is the well known western–eastern Eurasian disjunction. Representatives include *Eremurus* M. Bieb. (Asphodelaceae), *Colutea* L. (Fabaceae), *Psammosilene* W.C. Wu and C.Y. Wu (Caryophyllaceae), *Cotinus* Mill. (Anacardiaceae), *Parapteropyrum* A.J. Li (Polygonaceae), *Ceratostigma* Bunge (Plumbaginaceae), *Sibiraea* Maxim. (Rosaceae) and *Arenaria* L. (Caryophyllaceae) (Sun, 2002; Wu, 2004). Vicariance driven by the uplift of the TP in the Tertiary has been postulated as the major factor on the formation of this disjunct pattern (Axelrod et al., 1998; Sun, 2002; Zhang et al., 2006). This hypothesis has been corroborated by a few molecular phylogenetic studies of plants, including *Helleborus* L. (Ranunculaceae) (Sun et al., 2001) and *Cedrus* Trew (Pinaceae) (Qiao et al., 2007).

Both Hyoscyameae and Mandragoreae show the eastern–western intercontinental disjunct distributions within Eurasia (Fig. 3). This disjunct pattern in Mandragoreae is represented by the two major clades, one from the MT region and the other from the TP region (Fig. 3), and its origin has been generally accepted as a result of the interruption of a once more continuous distribution before the uplift of the TP (Kurbanov, 1994; Proskuryakova and Belyanina, 1985; Tu et al., 2005; Ungricht et al., 1998) and has been cited as an example to show the existence of a Tertiary continuous flora in Eurasia (Sun, 2002; Wu, 2004). The uplift of the TP was mainly in the Miocene (Harrison et al., 1992) and is the major late Tertiary geologic event in Eurasia. It may have greatly affected the distributions of plants within Eurasia. The age of the disjunction in the mandrakes was estimated to be 9.82 mya (95% HPD: 4.40–16.18 mya), thus not conflicting with the Tertiary vicariance hypothesis.

The eastern–western Eurasian disjunction in Hyoscyameae is represented by two species of *Scopolia* with *S. carniolica* in mesophytic forests in the Mediterranean region and *S. japonica* in eastern Asia. The vicariance from a once continuous distribution throughout Eurasia like the one for mandrakes was suggested to explain this disjunction (Hoare and Knapp, 1997; Lu and Zhang, 1986; Olmstead and Palmer, 1992; Olmstead et al., 1992; Tu

et al., 2005). Even though the vicariance can not be rejected in this study, the geologic background for the disjunction of *Scopolia* seems different from that of *Mandragora* based on its much younger estimated age (2.70 mya, 95% HPD: 0.62–5.46 mya). Rather than the uplift of the TP, the Pliocene climatic fluctuations followed by the Quaternary glaciations (Chandler et al., 1994; Combourieu-Nebout et al., 2000; Fauquette and Bertini, 2003; Webb and Bartlein, 1992; Willis et al., 1999) may be a more likely contributing factor for this disjunction. Long-distance dispersal may not be a favored explanation for the disjunction of *Scopolia* because the seeds of the genus fall to the ground by gravity and may be locally dispersed (Olmstead and Palmer, 1992; Hoare and Knapp, 1997; Knapp, 2002).

4.4. Biogeographic diversification in the MT region

Although both Mandragoreae and Hyoscyameae have members in the MT region, they may not share the same biogeographic history. As discussed earlier, the Mediterranean *Mandragora* and *Scopolia* did not diverge from their eastern Asian sisters at the same time. The MT region had experienced complex geologic and climatic changes from early Tertiary to the Quaternary. When ancestors of the two tribes arrived in Eurasia in the early Miocene, this region had high summer rainfalls and was rich in elements with tropical or subtropical affinities, such as Verbenaceae, Bombacaceae, Juglandaceae, Melicaceae, Menispermaceae, Sapindaceae, Sapotaceae and Simaroubaceae (Thompson, 2005). The summer climate became drier later and some of the tropical or subtropical elements, such as *Avicennia* L. (Verbenaceae), *Nyssa* L. (Nyssaceae), *Microtropis* Wall. ex Meissn. (Celastraceae) and *Leea* L. (Leeaceae), became extinct from this region (Thompson, 2005). Ancestors of the two tribes of Solanaceae survived as relicts of the Mediterranean Tertiary flora.

There are three Mediterranean mandrake species occupying different distribution ranges, i.e., *M. officinarum* in the western Mediterranean basin (from Turkey to Spain and Morocco), *M. autumnalis* in the eastern Mediterranean basin (from Israel to Syria) and *M. turcomanica* in the Turanian region (near the border of Turkmenistan and Iran) (Akhani and Ghorbani, 2003; Mizgireva, 1942; Ungricht et al., 1998) (Fig. 3). Our phylogeny recovered a sister relationship between *M. officinarum* from the west and the *M. autumnalis*–*M. turcomanica* clade from the east. The Miocene/Pliocene climatic changes and the Pleistocene glaciation in the Mediterranean are the major factors which had a profound impact on the floristic and faunal components and distributions in this region (Michaux et al., 2005; Ribera and Blasco-Zumeta, 1998; Thompson, 2005). The former factor corresponds better with the scenario of the separation between the western Mediterranean *M. officinarum* from the two eastern species because this differentiation was estimated to be in the late Miocene (Fig. 3). A sister relationship between *M. autumnalis* and *M. turcomanica* suggests a disjunction of *Mandragora* between the eastern Mediterranean basin and the Turanian region. This disjunct pattern has been reported in other taxa and hypothesized to support the vicariance of the Mediterranean flora or fauna (Kadereit and Yaprak, 2008; Ribera and Blasco-Zumeta, 1998; Rosselló et al., 2007). However, the vicariance hypothesis is rejected in this study because the mean divergence time between this species pair was dated to be only 0.30 mya (95% HPD: 0.001–0.85 mya), much younger than the Syrian Desert separating the two regions. Instead the young age of this disjunction favors the dispersals mediated by animals as suggested by Proskuryakova and Belyanina (1985) and Kurbanov (1994).

Atropa komarovii and *A. belladonna* are very similar in morphology with only minor differences in flower color. Their distributions may represent a comparable disjunct pattern to that of *Mandragora*

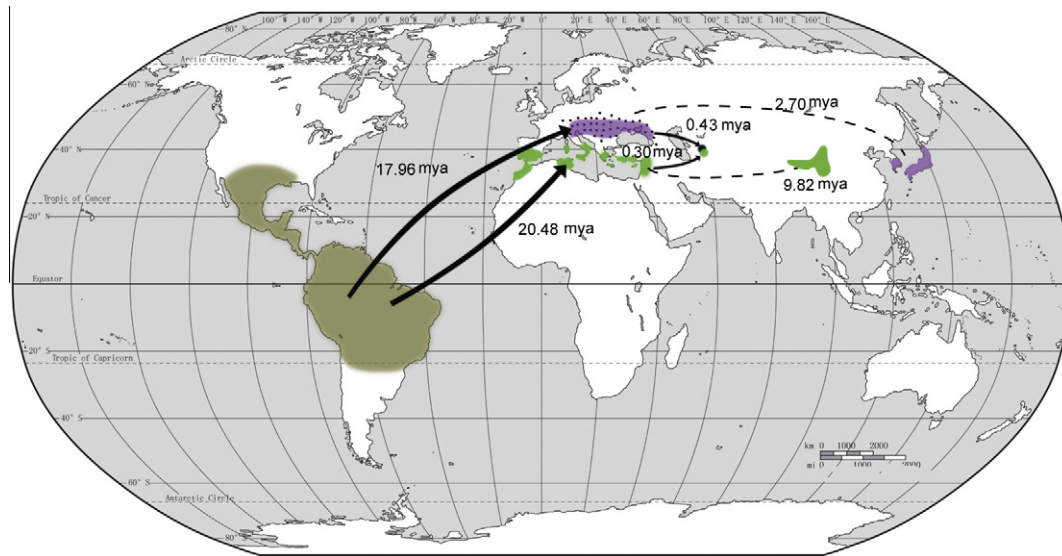


Fig. 3. A schematic map showing the disjunctions of Hyoscyameae and Mandragoreae and their likely origins via two independent dispersal events (arrows) from the New World into Eurasia, the two recent dispersals (arrows) of *Atropa* (areas with black dots) and *Mandragora* (green areas) within the MT region, and the two independent vicariance events (dashed lines) of *Scopolia* (purple areas) and *Mandragora* in the MT region and in eastern Asia, respectively.

autumnalis–*M. turcomanica*, because the Turkmenistan endemic *A. komarovii* is only a few kilometers from the populations of *M. turcomanica* (Kurbanov, 1994). In this study, two accessions of *A. belladonna* were sampled from the Caucasus region (Republic of Georgia and the Russian coast of the Black Sea), an area north of the distributional range of *M. autumnalis*. The stem age of *Atropa* was dated to middle Miocene (12.78 mya, 95% HPD: 7.00–19.38 mya), whereas a Quaternary divergence of 0.42 (95% HPD: 0.02–1.09) mya is estimated for the disjunct pair of *A. belladonna*–*A. komarovii*. This age is as young as that of *M. autumnalis*–*M. turcomanica* and may suggest a similar scenario of dispersal. Like those of *Mandragora*, the fleshy berries of *Atropa* may have facilitated the dispersal of the species eastward, or vice versa.

4.5. Biogeographic diversification on the Tibetan Plateau

Like the MT region, the TP region had undergone dramatic geologic and climatic changes during the Tertiary and the Quaternary. The plateau was warm and humid in the early Tertiary. The majority of the TP arose rapidly in the Miocene and reached the present height before the Pliocene (Harrison et al., 1992; Peng et al., 2006; Wang et al., 2008). In the Pliocene and in the Quaternary, the climate oscillations and the advances/retreats of glaciers may be the most remarkable features in this region. The platform of the present TP has a mean elevation of over 4500 m and is generally dominated by high-elevation cold meadow steppes and desert steppes (Chang, 1981). In the southeastern TP, however, there are a series of mountains separated by deep, narrowly incised river valleys with diverse habitats from the bottoms of the valleys to the peaks of the mountains. Corresponding to the complex climatic and geologic history of the TP, many of the plants there may have experienced four likely biogeographic processes: (1) some Tertiary tropical or subtropical elements went extinct on the Tibetan Plateau; (2) some elements survived in the refugia at the margins of the TP, especially in the mountains of the southeastern TP; (3) some plants in the refugia recolonized the inner platform of the TP after the geologic or ecologic changes; and (4) some adapted to the changed habitats and survived there as relicts.

Although temperate elements are dominant in the present flora, fossils of tropical and subtropical elements have been repeatedly reported in the inner platform of the TP, including *Livistona* R. Br.

(Palmae), *Meliosma* Bl. (Sabiaceae), *Lithocarpus* BL. (Fagaceae), *Myrica* L. (Myricaceae) and *Litseaephyllum* (Corallinaceae) (Guo, 1985; Sun, 2002; Tao, 2000; Wei et al., 1998). The presence of tropical and subtropical elements in the platform of the TP in the strata of early to mid-Tertiary suggests that extinctions may have occurred in a wide range of taxa.

The second scenario may explain the biogeographic diversification of taxa restricted to or centered in the mountains of the southeastern TP, such as *Atropanthe* and *Anisodus*. *Atropanthe* occupies the basal-most position in the pyxidate Hyoscyameae and occurs exclusively in the southeastern margin of the tribe's range in China (Fig. 4A). Its phylogenetic position and its split from its sisters in the late Miocene suggest that it is not a derived genus. The most likely hypothesis for the distribution of *Atropanthe* is that the mountains of the southeastern TP with diverse habitats served as a refugia for this genus during the uplift of the TP in the Miocene. *Anisodus* may represent another example of diversification in the refugia of the southeastern TP. All four species of *Anisodus* grow in the forests of the southeastern TP and partly overlap with *Atropanthe* (Fig. 4B). The four species may have diversified within the refugia. The age of *Anisodus* is very similar to that of *Atropanthe*, supporting their comparable evolutionary history.

Unlike the other three species of *Anisodus* occurring exclusively in forests of the southeastern TP, *A. tanguticus* also extends to the platform of the TP (Fig. 4A). Populations of *A. tanguticus* in the steppes of the TP may be the result of a recent dispersal. During our field exploration, we found populations of *A. tanguticus* near the largest Quaternary glacier remains of the TP, providing direct evidence for a recent spread of the species on the plateau platform. *Hyoscyamus* contains ca. 20 species and has most species in Central Asia and the Near East. *Hyoscyamus niger* has a wider distribution than other species, ranging from the Mediterranean basin to Europe and Siberia (Fig. 4C), and it is the only species of the genus on the Tibetan Plateau. The wide range of *H. niger* and its phylogenetic position argue for a recent biogeographic expansion. The split between *H. niger* and *H. albus* is estimated to be 1.48 (95% HPD: 0.42–2.79) mya, thus supporting the hypothesis of recent biogeographic expansion. The utility of this species as a medicinal plant may have also contributed to the wide range of this species (Hoare and Knapp, 1997; Symon, 1991).

The TP mandrakes are sympatric with *Anisodus*. Different from the *Anisodus* species, the two mandrake species do not overlap in dis-

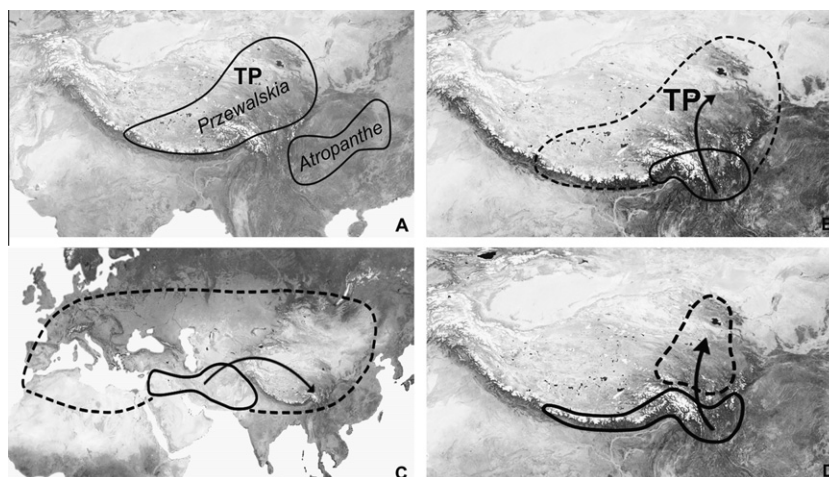


Fig. 4. Geographical distributions of Hyoscyameae and Mandragoreae on the Tibetan Plateau and their hypothesized origins. (A) *Przewalskia* on the TP platform as a relic via adaptation to the changed environments and the preservation of *Atropanthe* in the refugium of southeastern TP; (B) recolonization of *Anisodus tanguticus* (dashed line) from the center of diversity of the genus (solid line) in the southeastern TP to inner TP; (C) recolonization of the genus (solid line) in the MT region; and (D) recolonization of *Mandragora chinghaiensis* (dashed line) in the southeastern TP mountains (solid line) to the TP platform. The distributional ranges were made based on specimen records in KUN, WUK, PE, HNWP, IBSC, CDBI and previous reports (Lu and Zhang, 1986; Hoare and Knapp, 1997; Ungricht et al., 1998).

tribution with each other. *Mandragora caulescens* occurs in forests or shrublands on the mountains of the southeastern TP whereas *M. chinghaiensis* appears on steppes of the inner platform (Fig. 4D). Morphologically, *M. chinghaiensis* has rosette leaves and is different from *M. caulescens* with elongated stems. The morphological differentiation between these two species may have resulted from their adaptation to diverse habitats. The elongated stems of *M. caulescens* may be adaptation for more light under the thick forests or shrublands, whereas the rosette leaves may be more advantageous for *M. chinghaiensis* to avoid strong wind and extra ultraviolet exposure on the open steppes. The age of *M. chinghaiensis* can be dated to the Quaternary, suggesting its likely spread from the mountains of the southeastern TP after adapting to the TP platform. The recent range expansion from the 'hot spot' of the mountains of the southeastern TP has been observed in other plants and birds at the population level. Examples include *Juniperus przewalskii* (Cupressaceae) (Zhang et al., 2005), *Metagentiana striata* (Gentianaceae) (Chen et al., 2008), *Pedicularis longiflora* (Orobanchaceae) (Yang et al., 2008), *Picea crassifolia* (Pinaceae) (Meng et al., 2007), and the red-necked snow finch *Pyrgilauda ruficollis* (Qu et al., 2005). Phylogeographic studies on these species show that populations from the wide range of the platform are dominated by one or a few haplotypes and are mostly nested within diverse haplotypes from the southeastern mountains of the TP.

Besides recolonization of many plants from the edges of the plateau, a few other plants may have survived as relicts on the platform of the TP. The population structure of *Aconitum gymnanthum* (Ranunculaceae) recovered two genetic diversification centers on the platform, suggesting that this cold-tolerant species may have survived in some ice-free places on the TP platform during the Quaternary glaciations (Wang et al., 2009). The scenario may be applicable to interpret the origin of *Przewalskia*. This monotypic genus is endemic to the platform or the alpine zone of the TP and the Himalayas (Fig. 4A). With its unique morphology of rosette leaves and greatly enlarged fruit calyces, *Przewalskia* has been recognized as a classical adaptation to the extremely cold and windy environment with strong ultraviolet exposure (Lu, 1997; Lu and Zhang, 1986). In some places, it grows with *M. chinghaiensis* on the meadows of the TP. Nevertheless, *Przewalskia* may have a different biogeographical history because it never occurs under the montane forests of the southeastern TP and its age is much older than *M. chinghaiensis*

(5.75 mya vs. 1.84 mya). Instead of a recent speciation, *Przewalskia* may have adapted to the uplift of the TP and have survived as a Tertiary relict. Although an old age is inferred for *Przewalskia* in this study, our data are silent on which part of the TP may have served as a refugium for *Przewalskia* and whether a recent spreading played a role on the present distribution of the old genus. A better understanding of the biogeographical history of *Przewalskia* may require information on the phylogeography of the genus at the population level and inferences regarding the ancient distribution pattern of *Physoclaina*, the sister of *Przewalskia* with about 12 species in Central and eastern Asia.

Acknowledgments

The authors thank G.M. van der Weerden, Ki-oung Yoo, Vladimir Cherniak, Yuanwen Duan, Victor Quipuscoa, Yonghong Zhang, and Liangliang Yue for assistance in obtaining plant materials. We thank Lee Weight, Jeffery Hunt, David Boufford, Jipei Yue, Zelong Nie, Ying Meng, Yang Yang, Jianwen Zhang, Zhumei Ren, Lei Xie, Hongli Tian, Yunjuan Zuo, Rong Li, Xinwei Xu, and Pingting Chen for lab or field assistance. The study was supported by grants of the National 973 Project of China (2007CB411601), the National Natural Science Foundation of China (30625004), 40930209 and the US National Science Foundation (DEB-0415573 to Michael Dillon and Jun Wen). Lab work was partially supported by the Laboratory of Analytical Biology at the National Museum of Natural History of the Smithsonian Institution.

References

- Akhani, H., Ghorbani, A.B., 2003. *Mandragora turcomanica* (Solanaceae) in Iran: a new distribution record for an endangered species. *Syst. Biodivers.* 1, 177–180.
- Aoki, S., Ito, M., 2000. Molecular phylogeny of *Nicotiana* (Solanaceae) based on the nucleotide sequence of the *matK* gene. *Plant Biol.* 2, 316–324.
- Axelrod, D.I., Al-Shehbaz, I., Raven, P.H., 1998. History of the modern flora of China. In: Zhang, A., Wu, S. (Eds.), *Floristic Characteristics and Diversity of East Asian Plants*. China Higher Education Press, Beijing, pp. 43–45.
- Barron, E.J., Peterson, W.H., 1989. Model simulation of the Cretaceous ocean circulation. *Science* 244, 684–686.
- Bauer, A.M., 1993. African–South American relationships: a perspective from the *Reptilia*. In: Goldblatt, P. (Ed.), *Biological Relationships Between Africa and South America*. Yale University Press, New Haven, pp. 244–288.
- Bremer, B., Bremer, K., Heidari, N., Erixon, P., Olmstead, R.G., Anderberg, A.A., Kallersjö, M., Barkhordarian, E., 2002. Phylogenetics of Asterids based on 3

- coding and 3 non-coding chloroplast DNA markers and the utility of non-coding DNA at higher taxonomic levels. *Mol. Phylogenet. Evol.* 24, 274–301.
- Butler, R., 1995. When did India hit Asia? *Nature* 373, 20–21.
- Carlquist, S., 1996. Plant dispersal and origin of the Pacific island floras. In: Keast, A., Miller, S.E. (Eds.), *The Origin and Evolution of Pacific Island Biotas, New Guinea to Eastern Polynesia: Patterns and Processes*. SPB Academic, Amsterdam, pp. 153–164.
- Chandler, M., Rind, D., Thompson, R., 1994. Joint investigations of the middle Pliocene climate II: GISS GCM northern hemisphere results. *Glob. Planet. Change* 9, 197–219.
- Chang, D.H.S., 1981. The vegetation zonation of the Tibetan Plateau. *Mt. Res. Dev.* 1, 29–48.
- Chen, C., Chen, C., 1977. On the Chinese genera *Scopolia* Jacq., *Anisodus* Link and Otto and *Atropanthe* Pascher. *Acta Phytotaxon. Sin.* 15, 57–68.
- Chen, S., Wu, G., Zhang, D., Gao, Q., Duan, Y., Zhang, F., Chen, S., 2008. Potential refugium on the Qinghai-Tibet Plateau revealed by the chloroplast DNA phylogeography of the alpine species *Metagentiana striata* (Gentianaceae). *Bot. J. Linn. Soc.* 157, 125–140.
- Cipollini, M.L., Levey, D.J., 1997a. Antifungal activity of *Solanum* fruit glycoalkaloids: implications for frugivory and seed dispersal. *Ecology* 78, 799–809.
- Cipollini, M.L., Levey, D.J., 1997b. Secondary metabolites of fleshy vertebrate-dispersed fruits: adaptive hypotheses and implications for seed dispersal. *Amer. Naturalist* 150, 346–372.
- Cipollini, M.L., Levey, D.J., 1997c. Why are some fruits toxic? Glycoalkaloids in *Solanum* and fruit choice by vertebrates. *Ecology* 78, 782–798.
- Clarkson, J.J., Knapp, S., Garcia, V.F., Olmstead, R.G., Leitch, A.R., Chase, M.W., 2004. Phylogenetic relationships in *Nicotiana* (Solanaceae) inferred from multiple plastid DNA regions. *Mol. Phylogenet. Evol.* 33, 75–90.
- Clarkson, J.J., Lim, K.Y., Kovarik, A., Chase, M.W., Knapp, S., Leitch, A., 2005. Long-term genome diploidization in allopolyploid *Nicotiana* section *Repandae* (Solanaceae). *New Phytol.* 168, 241–252.
- Collinson, M.E., Boulter, M.C., Holmes, P.L., 1993. Magnoliophyta ('Angiospermae'). In: Betton, M.J. (Ed.), *The Fossil Record 2*. Chapman and Hall, London, pp. 809–841.
- Combourieu-Nebout, N., Fauquette, S., Quezel, P., 2000. What was the late Pliocene Mediterranean climate like: a preliminary quantification from vegetation. *Bull. Soc. Geol. Fr.* 171, 271–277.
- Dick, C.W., Bermingham, E., Lemes, M.R., Gribel, R., 2007. Extreme long-distance dispersal of the lowland tropical rainforest tree *Ceiba pentandra* L. (Malvaceae) in Africa and the Neotropics. *Mol. Ecol.* 16, 3039–3049.
- Diez, M.J., Ferguson, I.K., 1984. Pollen morphology of *Mandragora autumnalis* Bertol (Solanaceae). *Pollen et Spores* 26, 151–160.
- Dillon, M.O., Tu, T., Soejima, A., Yi, T., Nie, Z., Tye, A., Wen, J., 2007. Phylogeny of *Nolana* (Nolaneae, Solanoideae, Solanaceae) as inferred from granule-bound starch synthase 1 (GBSSI) sequences. *Taxon* 56, 1000–1011.
- Dillon, M.O., Tu, T., Xie, L., Quipuscoa Silvestre, V., Wen, J., 2009. Biogeographic diversification in *Nolana* (Solanaceae), a ubiquitous member of the Atacama and Peruvian Deserts along the western coast of South America. *J. Syst. Evol.* 47, 457–476.
- Doyle, J.A., Sauquet, H., Scharaschkin, T., Le Thomas, A., 2004. Phylogeny, molecular and fossil dating, and biogeographic history of Annonaceae and Myricaceae (Magnoliales). *Int. J. Plant Sci.* 165, S55–S67.
- Drummond, A.J., Ho, S.Y.W., Phillips, M.J., Rambaut, A., 2006. Relaxed phylogenetics and dating with confidence. *PLoS Biol.* 4, 699–710.
- Drummond, A., Rambaut, A., 2007. BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evol. Biol.* 7, 214.
- Fauquette, S., Bertini, A., 2003. Quantification of the northern Italy Pliocene climate from pollen data: evidence for a very peculiar climate pattern. *Boreas* 32, 361–369.
- Felsenstein, J., 1981. Evolutionary trees from DNA sequences: a maximum-likelihood approach. *J. Mol. Evol.* 17, 368–376.
- Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39, 783–791.
- Fukuda, T., Yokoyama, J., Ohashi, H., 2001. Phylogeny and biogeography of the genus *Lycium* (Solanaceae): inferences from chloroplast DNA sequences. *Mol. Phylogenet. Evol.* 19, 246–258.
- Gene Codes Corporation, 2005. *Seqencher 4.5*. Gene Codes Corporation, Michigan, USA.
- Givnish, T.J., Millam, K.C., Evans, T.M., Hall, J.C., Chris Pires, J., Berry, P.E., Sytsma, K.J., 2004. Ancient vicariance or recent long-distance dispersal? Inferences about phylogeny and South American–African disjunctions in Rapateaceae and Bromeliaceae based on *ndhF* sequence data. *Int. J. Plant Sci.* 165, S35–S54.
- Good, R., 1974. *The Geography of Flowering Plants*, fourth ed. Longman, London.
- Greuter, W., 1991. Botanical diversity, endemism, rarity, and extinction in the Mediterranean area: an analysis based on the published volumes of *Med-Checklist*. *Bot. Chron.* 10, 63–79.
- Guo, S., 1985. Preliminary interpretation of tertiary climate by using megafossil floras in China. *Palaeontol. Cathay* 2, 169–176.
- Harrison, T.M., Copeland, P., Kidd, W.S.F., Yin, A., 1992. Rising Tibet. *Science* 255, 1663–1670.
- Hay, W.W., Deconto, R.M., Wold, C.N., Wilson, K.M., Voigt, S., Schulz, M., Wold, A.R., Dullo, W., Ronov, A.B., Balukhovskiy, A.N., Söding, E., 1999. Alternative global Cretaceous paleogeography. In: Barrera, E., Johnson, C. (Eds.), *Evolution of the Cretaceous Ocean-Climate System*. Geological Society of America, Boulder, pp. 1–47.
- Hiraishi, A., Kamagata, Y., Nakamura, K., 1995. Polymerase chain reaction amplification and restriction fragment length polymorphism analysis of 16S rRNA genes from methanogens. *J. Ferment. Bioeng.* 79, 523–529.
- Hoare, A.L., Knapp, S., 1997. A phylogenetic conspectus of the tribe Hyoscyameae (Solanaceae). *Bull. Nat. Hist. Mus. Lond. (Bot.)* 27, 11–29.
- Houle, A., 1998. Floating islands: a mode of long-distance dispersal for small and medium-sized terrestrial vertebrates. *Divers. Distrib.* 4, 201–216.
- Houle, A., 1999. The origin of platyrrhines: an evaluation of the Antarctic scenario and the floating island model. *Am. J. Phys. Anthropol.* 109, 541–559.
- Hunziker, A.T., 2001. The genera of Solanaceae illustrated, arranged according to a new system. Gantner, Ruggell, Liechtenstein.
- Jakob, S.S., Ihlow, A., Blattner, F.R., 2007. Combined ecological niche modeling and molecular phylogeography revealed the evolutionary history of *Hordeum marinum* (Poaceae) – niche differentiation, loss of genetic diversity, and speciation in Mediterranean Quaternary refugia. *Mol. Ecol.* 16, 1713–1727.
- Kadereit, G., Yaprak, A.E., 2008. *Microcnemum coralloides* (Chenopodiaceae-Salicornioideae): an example of intraspecific East–West disjunctions in the Mediterranean region. *Anales Jard. Bot. Madrid* 65, 415–426.
- Kenicer, G.J., Kajita, T., Pennington, R.T., Murata, J., 2005. Systematics and biogeography of *Lathyrus* (Leguminosae) based on internal transcribed spacer and cpDNA sequence data. *Am. J. Bot.* 92, 1199–1209.
- Knapp, S., 2002. Tobacco to tomatoes: a phylogenetic perspective on fruit diversity in the Solanaceae. *J. Exp. Bot.* 53, 2001–2022.
- Kuang, K., Lu, A., 1978. *Mandragora*. In: Kuang, K., Lu, A. (Eds.), *Flora Reipublicae Popularis Sinicae*. vol. 67. Science Press, Beijing, pp. 137–141, 159–160.
- Kurbanov, D., 1994. Flora of Kopetdagh. In: Fet, V., Atamuradov, K.I. (Eds.), *Biogeography and Ecology of Turkmenistan*. Kluwer Academic Publishers, Dordrecht, Netherlands, pp. 105–126.
- Lanave, C., Preparata, G., Saccone, C., Serio, G., 1984. A new method for calculating evolutionary substitution rates. *J. Mol. Evol.* 20, 86–93.
- Levin, R.A., Miller, J.S., 2005. Relationships within tribe Lycieae (Solanaceae): paraphyly of *Lycium* and multiple origins of gender dimorphism. *Am. J. Bot.* 92, 2044–2053.
- Li, H., 1952. Floristic relationships between eastern Asia and eastern North America. *Trans. Am. Philos. Soc.* 42, 371–429.
- Lomolino, M.V., Riddle, B.R., Brown, J.H., 2006. *Biogeography*, third ed. Sunderland, MA, Sinauer Associates, pp. 135–176.
- Lu, A., 1997. *Archihyoscyamus*: a new genus of Solanaceae from western Asia. *Adansonia* 19, 135–138.
- Lu, A., Zhang, Z., 1986. Studies of the subtribe Hyoscyaminae in China. In: D'arcy, W.G. (Ed.), *Solanaceae: Biology and Systematics*. Columbia University Press, New York, pp. 56–78.
- Manos, P.S., Donoghue, M.J., 2001. Progress in northern hemisphere phylogeography: an introduction. *Int. J. Plant Sci.* 162, S1–S2.
- Mcglone, M.S., Duncan, R.P., Heenan, P.B., 2001. Endemism, species selection and the origin and distribution of the vascular plant flora of New Zealand. *J. Biogeogr.* 28, 199–216.
- Meng, L., Yang, R., Abbott, R.J., Miede, G., Hu, T., Liu, J., 2007. Mitochondrial and chloroplast phylogeography of *Picea crassifolia* Kom (Pinaceae) in the Qinghai-Tibetan Plateau and adjacent highlands. *Mol. Ecol.* 16, 4128–4137.
- Michaux, J., Bellinva, E., Lymberakis, P., 2005. Taxonomy, evolutionary history and biogeography of the broad-toothed field mouse (*Apodemus mystacinus*) in the eastern Mediterranean area based on mitochondrial and nuclear genes. *Biol. J. Linn. Soc.* 85, 53–63.
- Mizgireva, O.F., 1942. A new species of *Mandragora* in Turkmenia. *Trudy Turkmensk. Fil. Akad. Nauk SSSR* 2, 165.
- Mizgireva, O.F., 1978. *Mandragora turcomanica*. *Izv. Akad. Nauk Kazahsk. SSR, Ser. Bot.* 54–55.
- Mummenhoff, K., Franzke, A., 2007. Gone with the bird: late Tertiary and Quaternary intercontinental long-distance dispersal and allopolyploidization in plants. *Syst. Biodivers.* 5, 255–260.
- Myers, N., Mittermeier, R.A., Mittermeier, C.G., da Fonseca, G.A.B., Kent, J., 2000. Biodiversity hotspots for conservation priorities. *Nature* 403, 853–858.
- Nathan, R., Schurr, F.M., Spiegel, O., Steinitz, O., Trakhtenbrot, A., Tsoar, A., 2008. Mechanisms of long-distance seed dispersal. *Trends Ecol. Evol.* 23, 638–647.
- Nelson, G., Platnick, N.L., 1980. A vicariance approach to historical biogeography. *Bioscience* 30, 339–343.
- Nie, Z., Wen, J., Sun, H., Bartholomew, B., 2005. Monophyly of *Kelloggia* Torrey ex Benth. (Rubiaceae) and evolution of its intercontinental disjunction between western North America and eastern Asia. *Am. J. Bot.* 92, 642–652.
- Nogales, M., Delgado, J.D., Medina, F.M., 1998. Shrikes, lizards and *Lycium intricatum* (Solanaceae) fruits: a case of indirect seed dispersal on an oceanic island (Alegranza, Canary Islands). *J. Ecol.* 86, 866–871.
- Nogales, M., Hernandez, E.C., Valdes, F., 1999. Seed dispersal by common ravens *Corvus corax* among island habitats (Cañarian Archipelago). *Ecoscience* 6, 56–61.
- Olmstead, R.G., Bohs, L., 2007. A summary of molecular systematic research in Solanaceae: 1982–2006. *Acta Hort.* 745, 255–268.
- Olmstead, R.G., Bohs, L., Migid, H.A., Santiago-Valentin, E., Garcia, V.F., Collier, S.M., 2008. A molecular phylogeny of the Solanaceae. *Taxon* 57, 1159–1181.
- Olmstead, R.G., Palmer, J.D., 1992. A chloroplast DNA phylogeny of the Solanaceae subfamilial relationships and character evolution. *Ann. Mo. Bot. Gard.* 79, 346–360.
- Olmstead, R.G., Sweere, J.A., Whitley, J.P., Palmer, J.D., 1992. Molecular phylogeny of the Solanaceae taxonomic and evolutionary implications. *Am. J. Bot.* 79, 157.

- Peng, Z., Ho, S.Y.W., Zhang, Y., He, S., 2006. Uplift of the Tibetan Plateau: evidence from divergence times of glyptosternoid catfishes. *Mol. Phylogenet. Evol.* 39, 568–572.
- Petit, R.J., Aguinalgalde, I., de Beaulieu, J., Bittkau, C., Brewer, S., Cheddadi, R., Ennos, R., Fineschi, S., Grivet, D., Lascoux, M., Mohanty, A., Muller-Starck, G., Demesure-Musch, B., Palme, A., Martin, J.P., Rendell, S., Vendramin, G.G., 2003. Glacial refugia: hotspots but not melting pots of genetic diversity. *Science* 300, 1563–1565.
- Posada, D., Crandall, K.A., 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* 14, 817–818.
- Posada, D., Buckley, T.R., 2004. Model selection and model averaging in phylogenetics: advantages of akaike information criterion and Bayesian approaches over likelihood ratio tests. *Syst. Biol.* 53, 793–808.
- Proskuryakova, G.M., Belyanina, N.B., 1985. To the systematics of the genus *Mandragora* and its history. *Biol. Nauki (Moscow)*, 2, 76–86.
- Punt, W., Clarke, G.C.S., 1980. The northwest European pollen flora, II (parts 8–20). Elsevier Scientific Publishing Company, New York, pp. 1–30.
- Qiao, C., Ran, J., Li, Y., Wang, X., 2007. Phylogeny and Biogeography of *Cedrus* (Pinaceae) inferred from sequences of seven paternal chloroplast and maternal mitochondrial DNA regions. *Ann. Bot.* 100, 573–580.
- Qu, Y., Ericson, P.G.P., Lei, F., Li, S., 2005. Postglacial colonization of the Tibetan plateau inferred from the matrilineal genetic structure of the endemic red-necked snow finch, *Pyrgilauda ruficollis*. *Mol. Ecol.* 14, 1767–1781.
- Rambaut, A., 2007. Se-al version 2.0a11. Available from: <<http://tree.bio.ed.ac.uk/software/seal/>>.
- Rambaut, A., 2009. FigTree version 1.3.1. Available from: <<http://tree.bio.ed.ac.uk/software/figtree/>>.
- Rambaut, A., Drummond, A.J., 2007. Tracer version 1.5. Available from: <<http://beast.bio.ed.ac.uk/Tracer>>.
- Rannala, B., Yang, Z., 1996. Probability distribution of molecular evolutionary trees: a new method of phylogenetic inference. *J. Mol. Evol.* 43, 304–311.
- Raven, P.H., Axelrod, D.I., 1974. Angiosperm biogeography and past continental movements. *Ann. Mo. Bot. Gard.* 61, 539–673.
- Razzakov, N.A., Aripova, S.F., Akhmedova, E., Karimov, A., 1998. *Mandragora turcomanica* alkaloids. *Khim. Prir. Soedin.*, 831–832.
- Renner, S., 2004. Plant dispersal across the tropical Atlantic by wind and sea currents. *Int. J. Plant. Sci.* 165, S23–S33.
- Ribera, I., Blasco-Zumeta, J., 1998. Biogeographical links between steppe insects in the Monegros region (Aragón, NE Spain), the eastern Mediterranean, and Central Asia. *J. Biogeogr.* 25, 969–986.
- Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19, 1572–1574.
- Rosselló, J.A., Lázaro, A., Cosín, R., Molins, A., 2007. A phylogeographic split in *Buxus balearica* (Buxaceae) as evidence by nuclear ribosomal markers: when ITS paralogues are welcome. *J. Mol. Evol.* 64, 143–157.
- Sandina, I.B., Tarasevich, V.F., 1982. Some palynological data to the study of the genera *Whitleya*, *Atropanthe*, and *Scopolia* s. Str. (Solanaceae). *Bot. Zhurn.* 67, 146–154.
- Sanmartin, I., 2003. Dispersal vs. Vicariance in the Mediterranean: historical biogeography of the Palearctic Pachydeminae (Coleoptera, Scarabaeoidea). *J. Biogeogr.* 30, 1883–1897.
- Schaefer, H., Heibl, C., Renner, S.S., 2009. Gourds afloat: a dated phylogeny reveals an Asian origin of the gourd family (Cucurbitaceae) and numerous oversea dispersal events. *Proc. Roy. Soc. B (Biol. Sci.)*, 276, 843–851.
- Soltis, D.E., Tago-Nakazawa, M., Xiang, Q., Kawano, S., Murata, J., Wakabayashi, M., Hibsich-Jetter, C., 2001. Phylogenetic relationships and evolution in *Chrysosplenium* (Saxifragaceae) based on *matK* sequence data. *Am. J. Bot.* 88, 883–893.
- Stefanovic, S., Krueger, L., Olmstead, R.G., 2002. Monophyly of the Convolvulaceae and circumscription of their major lineages based on DNA sequences of multiple chloroplast loci. *Am. J. Bot.* 89, 1510–1522.
- Steward, J.R., Lister, A.M., 2001. Cryptic northern refugia and the origins of the modern biota. *Trends Ecol. Evol.* 16, 608–613.
- Sun, H., 2002. Tethys retreat and Himalayas–Hengduan Mountains uplift and their significance on the origin and development of the Sino-Himalayan elements and alpine flora. *Acta Bot. Yunnan.* 24, 273–288.
- Sun, H., Mclewin, W., Fay, M.F., 2001. Molecular phylogeny of *Helleborus* (Ranunculaceae), with an emphasis on the eastern Asian–Mediterranean disjunction. *Taxon* 50, 1001–1018.
- Swofford, D.L., 2003. Paup. Phylogenetic analysis using parsimony (and other methods). Sinauer Associates, Sunderland, Massachusetts.
- Symon, D.E., 1979. Fruit diversity and dispersal in *Solanum* in Australia. *J. Adelaide Bot. Gard.* 1, 321–331.
- Symon, D.E., 1991. Gondwanan elements of the Solanaceae. In: Hawkes, J.G., Lester, R.N., Nee, M., Estrada, N. (Eds.), *Solanaceae III: Taxonomy, chemistry, evolution*. Kew Publishing, Royal Botanic Gardens, Kew, pp. 139–150.
- Szafer, W., 1961. Miocene flora from stare Gliwice in Upper Silesia. *Inst. Geol. Prace.* 33, 1–205.
- Tao, J., 2000. The Evolution of the Late Cretaceous–Cenozoic Floras in China. Science Press, Beijing.
- Tétényi, P., 1987. A chemotaxonomic classification of the Solanaceae. *Ann. Mo. Bot. Gard.* 74, 600–608.
- Thompson, J.D., 2005. Plant evolution in the Mediterranean. Oxford University Press, Oxford.
- Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G., 1997. The Clustal.x windows interface. Flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucl. Acid Res.* 25, 4876–4882.
- Thorne, R.F., 1972. Major disjunctions in the geographic ranges of seed plants. *Quart. Rev. Biol.* 47, 365–411.
- Tiffney, B.H., 1985. Perspectives on the origin of the floristic similarity between eastern Asia and eastern North America. *J. Arnold. Arboretum*, 66, 243–273.
- Tiffney, B.H., Manchester, S.R., 2001. The use of geological and paleontological evidence in evaluating plant phylogeographic hypotheses in the northern hemisphere Tertiary. *Int. J. Plant Sci.* 162, S3–S17.
- Tu, T., Sun, H., Gu, Z., Yue, J., 2005. Cytological studies on the Sino-Himalayan endemic *Anisodus* and four related genera from the tribe Hyoscyameae (Solanaceae) and their systematic and evolutionary implications. *Bot. J. Linn. Soc.* 147, 457–468.
- Tu, T., Dillon, M., Sun, H., Wen, J., 2008. Phylogeny of *Nolana* (Solanaceae) of the Atacama and Peruvian deserts inferred from sequences of four plastid markers and the nuclear *LEAFY* second intron. *Mol. Phylogenet. Evol.* 49, 561–573.
- Ungriht, S., Knapp, S., Press, J.R., 1998. A revision of the genus *Mandragora* (Solanaceae). *Bull. Nat. Hist. Mus. Bot. ser.* 28, 17–40.
- Vinnersten, A., Bremer, K., 2001. Age and biogeography of major clades in Liliales. *Am. J. Bot.* 88, 1695–1703.
- Wang, L., Abbott, R.J., Zheng, W., Chen, P., Wang, Y., Liu, J., 2009. History and evolution of alpine plants endemic to the Qinghai-Tibetan Plateau: *Aconitum gymnantrum* (Ranunculaceae). *Mol. Ecol.* 18, 709–721.
- Wang, C., Zhao, X., Liu, Z., Lippert, P.C., Graham, S.A., Coe, R.S., Yi, H., Zhu, L., Liu, S., Li, Y., 2008. Constraints on the early uplift history of the Tibetan Plateau. *Proc. Nat. Acad. Sci. USA* 105, 4987–4992.
- Webb, T., Bartlein, P.J., 1992. Global changes during the last 3 million years: climatic controls and biotic responses. *Annu. Rev. Ecol. Syst.* 23, 141–173.
- Wei, J., Wang, C., Wan, X., Yi, H., 1998. Variation history of height and vegetation of the Tibet Plateau in Tertiary. *Geoscience* 12, 318–326.
- Wen, J., 1999. Evolution of eastern Asian and eastern North American disjunct distributions in flowering plants. *Annu. Rev. Ecol. Syst.* 30, 421–455.
- Wen, J., 2001. Evolution of eastern Asian–eastern North American biogeographic disjunctions: a few additional issues. *Int. J. Plant Sci.* 162, S117–S122.
- Wen, J., Ickert-Bond, S.M., 2009. Evolution of the Madrean–Tethyan disjunctions and the North and South American amphitropical disjunctions in plants. *J. Syst. Evol.* 47, 331–348.
- Wikström, N., Savolainen, V., Chase, M.W., 2001. Evolution of the angiosperm: calibrating the family tree. *Proc. R. Soc. B*, 268, 2211–2220.
- Wiley, E.O., 1988. Vicariance biogeography. *Annu. Rev. Ecol. Syst.* 19, 513–542.
- Willis, K.J., Kleczkowski, A., Crowhurst, S.J., 1999. 124,000-year periodicity in terrestrial vegetation change during the late Pliocene epoch. *Nature* 397, 685–688.
- Winkworth, R.C., Wagstaff, S.J., Glenny, D., Lockhart, P.J., 2002. Plant dispersal N.E.W.S from New Zealand. *Trends Ecol. Evol.* 17, 514–520.
- Wright, S.D., Yong, C., Dawson, J.W., Whittaker, D.J., Gardner, R.C., 2000. From the cover: riding the ice age El Niño? Pacific biogeography and evolution of *Metrosideros* subg. *Metrosideros* (Myrtaceae) inferred from nuclear ribosomal DNA. *Proc. Nat. Acad. Sci. USA* 97, 4118–4123.
- Wu, Y., 2004. The floristic characteristics in the region of Bayan Har Mountains. *Acta Bot. Yunnan.* 26, 587–603.
- Wu, Z., 1983. On the significance of Pacific intercontinental discontinuity. *Ann. Mo. Bot. Gard.* 70, 577–590.
- Wu, Z., 1988. Hengduan mountains flora and its significance. *J. Jpn. Bot.* 63, 297–311.
- Wu, Z., Lu, A., Tang, Y., Chen, Z., Li, D., 2003. The Families and Genera of Angiosperms in China. Sciences Press, Beijing.
- Yang, Z., 1994. Maximum likelihood phylogenetic estimation from DNA sequences with variable rates over sites: approximate methods. *J. Mol. Evol.* 39, 306–314.
- Yang, F., Li, F., Ding, X., Wang, X., 2008. Extensive population expansion of *Pedicularis longiflora* (Orobanchaceae) on the Qinghai-Tibetan Plateau and its correlation with the Quaternary climate change. *Mol. Ecol.* 17, 5135–5145.
- Yuan, Y., Zhang, Z., Chen, Z., Olmstead, R.G., 2006. Tracking ancient polyploids: a retroposon insertion reveals an extinct diploid ancestor in the polyploid origin of belladonna. *Mol. Biol. Evol.* 23, 2263–2267.
- Yokoyama, J., Suzuki, M., Iwatsuki, K., Hasebe, M., 1998. Molecular phylogeny of *Coriaria*, with special emphasis on the disjunct distribution. *Mol. Phylogenet. Evol.* 14, 11–19.
- Zhang, Q., Chiang, T., George, M., Liu, J., Abbott, R.J., 2005. Phylogeography of the Qinghai-Tibetan Plateau endemic *Juniperus przewalskii* (Cupressaceae) inferred from chloroplast DNA sequence variation. *Mol. Ecol.* 14, 3513–3524.
- Zhang, Z., Fan, L., Yang, J., Hao, X., Gu, Z., 2006. Alkaloid polymorphism and ITS sequences variation on the *Spiraea japonica* complex (Rosaceae) in China: traces of the biological effects of the Himalaya-Tibet Plateau uplift. *Amer. J. Bot.* 93, 762–769.
- Zhang, Z., Lu, A., 1984. Pollen morphology of the subtribe Hyoscyaminae (Solanaceae). *Acta Phytotaxon. Sin.* 22, 175–180.
- Zhang, Z., Yang, D., Lu, A., Knapp, S., 2005. Seed morphology of the tribe Hyoscyameae (Solanaceae). *Taxon* 54, 71–83.