Molecular Phylogeny and Biogeography of *Adenocaulon* Highlight the Biogeographic Links between New World and Old World

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Adenocaulon (Asteraceae) is a small genus with only five species but has a broad amphi-Pacific distribution pattern with three species distributed disjunctly in South America, Central America, and North America and two endemic species spanning from eastern Asia to the Himalayas. To trace the biogeographic pattern of the genus, we reconstructed its phylogenetic relationships and diversification history based on one nuclear and eight plastid gene regions. Our results showed that *Adenocaulon* is monophyletic and may have originated in Central America during the Miocene, dispersed into North America and finally reached the Himalayas via the Bering Land Bridge. The hypothesized trajectory implies that long-distance dispersal may have played an important role in the formation of the distribution of this group of species. This hypothesis seems to have gained support from the special morphological structure of fruits of the genus.

Keywords: *Adenocaulon*, Asteraceae, East Asia, America, herbaceous flora

INTRODUCTION

The genus *Adenocaulon* Hook., consisting of five species, is a small genus in the angiosperm family Asteraceae, and has a broad amphi-Pacific distribution (Bittmann, 1990). Two species are endemic to East Asia while the other three occur in North and South America. Among them, *Adenocaulon nepalense* Bittmann is native to Bhutan, Nepal and India, and *Adenocaulon himalaicum* Edgew is distributed from the Himalayas to northeastern Asia and Japan (Bittmann, 1990; Funk and Hind, 2016). In the New World, *Adenocaulon bicolor* Hook. is distributed in North America, *Adenocaulon lyratum* S. F. Blake in Guatemala and Chiapas of Mexico, and *Adenocaulon chilense* Less. in Chile and Argentina (**Figure 1**; Funk and Hind, 2016). Thus the five species generally present a disjunct distribution pattern, with no species of *Adenocaulon* present in Africa, the Mediterranean, tropical Asia or Oceania.

Historically the taxonomic position of *Adenocaulon* has undergone extensive circumscription. Jensen and Kim (1996) placed it in the tribe Mutisieae, Katinas (2000) placed it in Cichorioideae, and Kim et al. (2002) transferred the genus to the Nassauvieae. A recent molecular phylogenetic study strongly supports the two American species, *A. bicolor* and *A. chilense*, forming a single lineage within Mutisieae (Panero and Funk, 2008), with the relationship of *Adenocaulon* to the
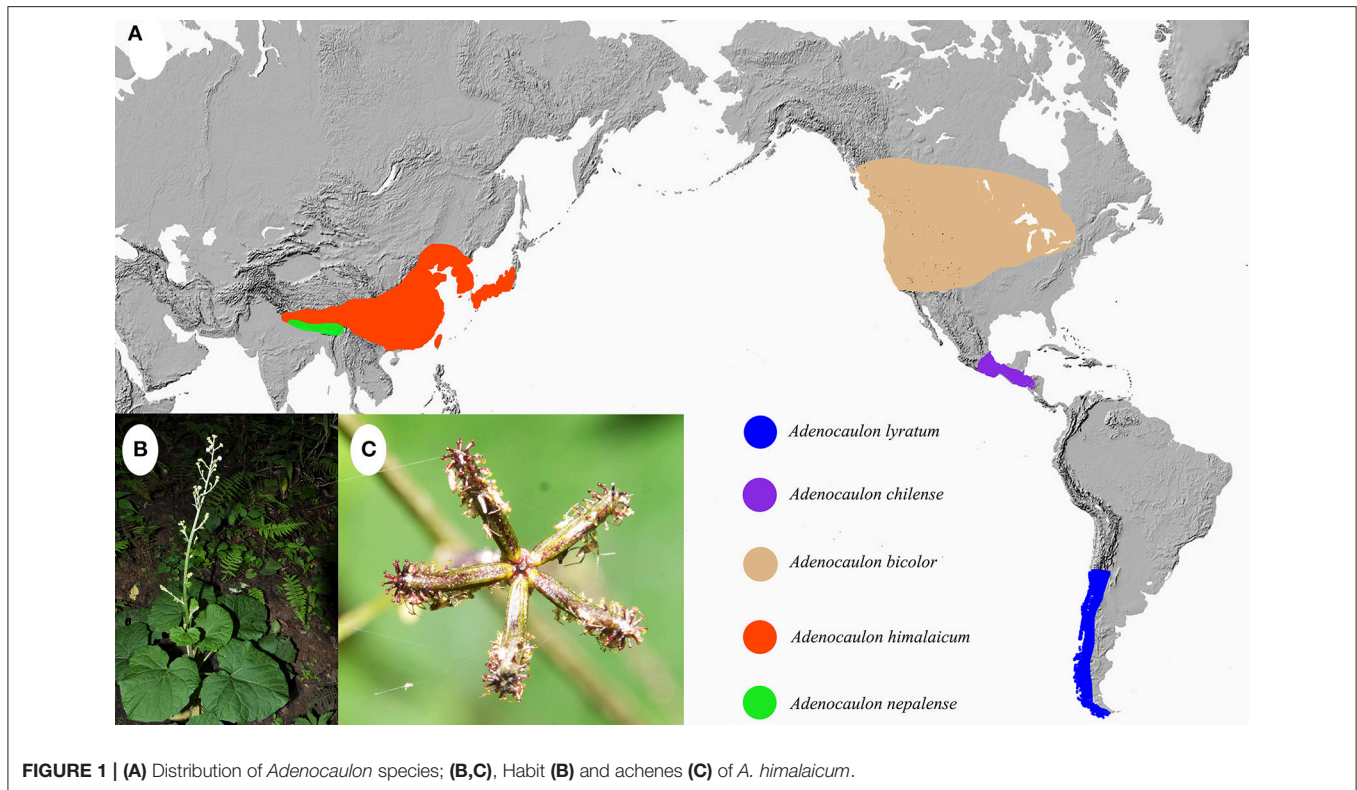
FIGURE 1 | (A) Distribution of *Adenocaulon* species; **(B,C)**, Habit **(B)** and achenes **(C)** of *A. himalaicum*.

other genera in Mutisieae being unresolved. The phylogenetic relationship and geographic pattern of the genus are still unclear. The tribe Mutisieae, as well as other members of the early diverging branches of Asteraceae (e.g., Barnadesioideae and Mutisioideae *s.l.*), has a predominant center of diversity in South America, which is believed to be the most possible ancestral area of these lineages (Katinas et al., 2009; Ortiz et al., 2009).

A distinct feature of members of Asteraceae is one-seeded cypselae fruit. Further, the majority of extant Asteraceae species have a modified calyx above the inferior ovary structure called a pappus, allowing for seed dispersal by wind and biological agents over great distances (Katinas et al., 2013). However, this specialized morphological trait is not evident in *Adenocaulon*. Members of this genus usually grow in under-stories of temperate moist forests (Katinas, 2000), which may well be a good case study of the biogeographic link between the Old World (specifically East Asia) and the New World by inferring the dispersal mechanism and biogeographic patterns across the Pan-Pacific areas.

Although the East Asian and North, Central and South American disjunct pattern is not unusual in angiosperms, and in fact has been investigated in several studies (e.g., Wen et al., 2010), the species-rich family Asteraceae provides a good system to probe the biogeographic pattern of taxa with distinct distribution patterns around the globe (Panero and Funk, 2008). Specifically members of *Adenocaulon* may provide an excellent opportunity to examine species adaptation in a recent diversification. Fossil and phylogenetic evidence demonstrates that Asteraceae may be of South American origin and underwent a major diversification event, followed by an African explosion (DeVore and Stuessy, 1995; Funk et al., 2005; Barreda et al., 2010; Stuessy, 2010) and subsequently moved to various continents (Nie et al., 2016). However, this model does not apply to the small genus *Adenocaulon*.

The goal of this study is to elucidate the phylogenetic relationships within the genus and to infer its historical biogeography utilizing chloroplast and nuclear DNA in conjunction with fossil evidence. Knowing details about how and when the genus originated and dispersed can provide insight into understanding the plausible biogeographic affinity between East Asia and America. Our study would shed new lights on the formation of intercontinental disjunction of plants.

MATERIALS AND METHODS

Taxa Sampling and Data Collection

Ten samples (accessions) were used for each of the 5 species of *Adenocaulon* (**Table 1**). Based on the results from previous phylogenetic analyses of Asteraceae (Panero and Funk, 2008; Ortiz et al., 2009), additional samples were used from eight genera that are closely related to *Adenocaulon* (*Gerbera, Leibnitzia, Chaptalia, Trichocline, Brachyclados, Pachylaena, Mutisia,* and *Chaetanthera* of the tribe Mutisieae of the subfamily Mutisioideae) and 15 distantly related genera of the subfamily (*Trixis, Dolichlasium, Jungia, Perezia, Nassauvia, Acourtia, Leucheria, Proustia,* and *Lophopappus* in the tribe Nassauvieae of the subfamily Mutisioideae; *Onoseris, Urmenetea, Lycoseris, Aphyllocladus, Gypothamnium,* and *Plazia* from the tribe

TABLE 1 | Species names, localities, voucher deposition, and GenBank accession numbers used in the present study.

Taxa	Voucher No.	Country	Herbarium	ITS	matK	ndhF	rbcL	rpoB	trnL-F	psbA-trnH	rpl32-trnL	rpl32-ndhF
Acicarpha tribuloides				EU841174	EU841362	AJ429129	EU841135		EU547626	EU841312		
Acourtia turbinata	Panero 2891	Mexico	TEX.	FJ979684	EU385317	EU385126	EU384940	EU385412	EU385032		FJ979732	FJ979784
Adenocaulon bicolor		USA	TEX.	FJ979672	EU385320	EU385129	EU384943	EU385415	EU385035		FJ979722	FJ979773
Adenocaulon chilense	Simon 382	Argentina	US.	FJ979674	EU385319	EU385128	EU384942	EU385414	EU385034		FJ979724	FJ979775
Adenocaulon himalaicum	dt35	China	KUN	MG710419	MG710438	MG710431	MG710457	MG710426	MG710451	MG710467		
Adenocaulon himalaicum	dt36	China	KUN	MG710420	MG710439	MG710432	MG710458	MG710427	MG710452	MG710468	MG710445	MG710464
Adenocaulon himalaicum	dt060	China	KUN	MG710421	MG710440	MG710433	MG710459	MG710428	MG710453	MG710469	MG710446	MG710465
Adenocaulon himalaicum	dt061	China	KUN	MG710422	MG710441	MG710434	MG710460	MG710429	MG710454	MG710470	MG710447	MG710466
Adenocaulon himalaicum	dt062	China	KUN	MG710423	MG710442	MG710435	MG710461	MG710430	MG710455	MG710471	MG710448	
Adenocaulon himalaicum	dt163	Korea	KUN	MG710478	MG710482	MG710480	MG710475	MG710474	MG710477	MG710479	MG710476	MG710481
Adenocaulon lyratum				FJ979673							FJ979723	FJ979774
Adenocaulon nepalense	dt701	Bhutan	KUN	MG710424	MG710443	MG710436	MG710462			MG710472	MG710449	
Adenocaulon nepalense	dt702	Bhutan	KUN	MG710425	MG710444	MG710437	MG710463		MG710456	MG710473	MG710450	
Aphyllocladus spartioides	Simon 508	Argentina	US.		EU385323	EU385132	EU384946	EU385418	EU385038			
Barnadesia lehmannii		Argentina	US.	AF412877	EU841321	L39394	EU841100	EU385422	EU547621	EU841272		
Brachyclados caespitosus	Bonifacino 459	Argentina	US.		EU385329	EU385137	EU384951	EU385424	EU385044			
Chaetanthera pentacaenoides	Bonifacino 293	Argentina	US.	DQ355904	EU385334	EU385142	EU384956	EU385429	EU385049			
Chaptalia nutans	Panero 2002-19	USA	TEX.	GU126772	EU385335	EU385143	EU384957	EU385430	EU385050		GU126751	
Chimantaea humilis					EU385336	EU385144	EU384958	EU385431	EU385051			
Chuquiraga spinosa	Simon 522	Argentina	US.	EU841150	EU385338	EU385146	EU384960	EU385433	EU385053	EU841285		
Dasyphyllum reticulatum			US.	AF412862	EU385342	EU385150	AY874428	EU385437	EU385057			
Dinoseris salicifolia					EU385346	EU385154	EU384967	EU385441	EU385061			
Dolichlasium lagascae	Simon 811	Argentina	US.	EF530259	EU385347	EU385155	EU384968	EU385442	EU385062			
Doniophyton anomalum		Argentina	US.	EU841164	EU385348	EU385156	EU384969	EU385443	EU385063	EU841297		
Duidaea pinifolia					EU385349	EU385157	EU384970	EU385444	EU385064			
Gerbera piloselloides		S.Africa	US.	GU126788	EU385355	EU385163	EU384975	EU385450	GU817941	GU818379	GU126765	
Gongylolepis benthamiana				KF989515	KF989843	EU385167	EU384980	EU385454	KF989621	KF989932		
Gypothamnium pinifolium				EU729342	KM192116	EU729346	EU736105	KM191884	EU729338			
Hyaloseris cinerea				KF989512	KF989840	KF989728	EU384989	EU385463	KF989618	KF989929		
Jungia polita	Simon 292	Argentina	US.	EF530233	EU385370	EU385178	EU384991	EU385465	EU385084			
Leibnitzia anandria				GU724275	HM989740	L39402	GQ436437			GQ435075	GU126756	
Leucheria thermarum	Simon 383	Chile	US.	EF530254	EU385371	EU385179	EU384992	EU385466	EU385085	EU841306	FJ979725	
Lophopappus cuneatus	Simon 563	Argentina	US.	FJ979676	EU385374	EU385182	EU384995	EU385469	EU385088		FJ979676	FJ979777
Lycoseris triplinervia					KM192117	KM192106	KM192095	KM191885	KM191907			
Mutisia kurtzii			US.	EF530235	EU841353	AF233835				EU841303	GU126763	
Nassauvia pygmaea	Bonifacino 179	Argentina	US.	EU239267	EU385377	EU385186	EU384999	EU385473	EU385092	EU841309	FJ979736	FJ979788
Onoseris liicifolia				KF989529	KF989857	KF989745	GQ890341	GQ890344	KF989634	KF989945		
Pachylaena atriplicifolia	Simon 684	Argentina	US.	EF530250	EU385383	EU385192	EU385005	EU385479	EU385098			

(Continued)

TABLE 1 | Continued

Taxa	Voucher No.	Country	Herbarium	ITS	matK	ndhF	rbcL	rpoB	trnL-F	psbA-trnH	rpl32-trnL	rpl32-ndhF
Perezia purpurata	Simon 594	Argentina	US.	EF530239	EU385385	EU385194	EU385007	EU385481	EU385100	EU841307	FJ979693	FJ979744
Plazia daphnoides	Simon 536	Argentina	US.	EF530226	EU385388	EU385197	EU385010	EU385484	EU385103	EU841301		
Proustia cuneifolia	Simon 511	Argentina	US.	EU841168	EU385390	EU385199	EU385012	EU385486	EU385105	EU841300		
Schlechtendalia luzulifolia				KF989506	KF989833	L39395	EU841125	KM191889	KF989612	KF989935		
Stenopadus talaumifolius				KF989518	KF989846	EU385207	EU385019	EU385494	KF989624			
Stifftia chrysantha				KF989519	EU385399	EU385208	EU385020	EU385495	JF920296	JF920291		
Stomatochaeta condensata					EU385401	EU385210	EU385021	EU385497	EU385116			
Tricholine boecheri	Bonifacino 142	Argentina	US.	GU126792	EU385404	EU385213	EU385024	EU385500	EU385119		GU126767	
Trixis divaricata	Santos 2659	Brazil	TEX.		EU385405	EU385214	EU385025	EU385501	EU385120			
Urmenetea atacamensis				EF530231	GQ890334	GQ890340	GQ890343	GQ890346	EF530277			
Wunderlichia mirabilis				DQ414742	EU385408	EU385217	EU385028	EU385504	DQ414744	KF989938		

Onoserideae of the subfamily Mutisioideae). We also included nine species from Wunderlichioideae and five species from Barnadesioideae. We used *Acicarpha spathulata* (Calyceraceae) to serve as the outgroup, following Panero and Funk (2008) (**Table 1**).

DNA Extraction, Amplification, and Sequencing

Total genomic DNA was isolated from silica-dried leaf materials and herbarium specimens using a Universal Genomic DNA Extraction Kit (Takara, Dalian, China). Nine gene regions, including eight plastid DNA regions (*ndhF, matK, rpoB, rbcL, trnL-F, rpl32-trnL, rpl32-ndhF,* and *psbA-trnH*) and the nuclear ITS, were employed. According to Shaw et al. (2007) and Timme et al. (2007), both *rpl32-trnL* and *rpl32-ndhF* have faster molecular evolution rate than other chloroplast regions and can provide adequate resolution in phylogenetic reconstructions of Asteraceae. The ITS data were generated from single amplifications using primers ITS4 and ITS5 (White et al., 1990). Primers used for amplification and sequencing were tabe and tabf for *trnL-F* region (Taberlet et al., 1991), Z1 and 1204R for *rbcL* (Zurawski et al., 1981), and *psb*A_F and *trn*H_R for *psbA-trnH* intergenic spacer (Sang et al., 1997). The primer sets for *ndhF, matK,* and *rpoB* regions were from Panero and Funk (2008), while the primers for *rpl*32-*trn*L and *rpl*32-*ndh*F were from Shaw et al. (2007). Amplified products were purified using a Qiaquick gel extraction kit (Qiagen, Inc., Valencia, California, USA) and sequenced in both directions by an ABI 3730 automated sequencer (Applied Biosystems, Foster City, California, USA). The resulting sequences were edited using Sequencher (version 4.1.4) and aligned with MUSCLE (version 3.6; Edgar, 2004), followed by manual adjustment in Se-Al (v2.0a11; Rambaut, 2002). All sequences generated for this study were deposited in GenBank under accession numbers shown in **Table 1**.

Phylogenetic Analysis

The nuclear and the plastid datasets were analyzed both separately and simultaneously using maximum parsimony (MP), Bayesian inference (BI), and maximum likelihood (ML). The topologies of the data sets were compared with each other to detect any incongruence. Because no significant incongruence was observed, we chose to combine the nrDNA and cpDNA data sets. For the maximum parsimony analysis we used PAUP/(version 4.0b10; Swofford, 2003). All characters were weighted equally and unordered. Heuristic searches were conducted using 100 random-taxon-addition replicates with tree bisection-reconnection (TBR) branch swapping, using MulTrees option in effect, and a maximum of 10,000 trees. Bootstrap analyses (1,000 pseudoreplicates) were conducted to examine the relative level of support for individual clades on the cladograms of each search (Felsenstein, 1985).

Models of nucleotide substitution were selected based on the Akaike Information Criterion (AIC) as determined by MrModelTest (version 2.3; Nylander, 2004). Maximum-likelihood searches and bootstrap analyses were performed on the XSEDE online computing cluster accessed via the CIPRES Science Gateway (Miller et al., 2010) using RAxML-HPC2

(version 7.4.2; Stamatakis et al., 2008). Data were partitioned into gene regions, allowing for independent parameter estimates on each partition, with branch length estimates optimized across all gene regions. The GTR + Γ model was employed for all analyses.

Bayesian inference was conducted using MrBayes (version 3.2.2, Ronquist and Huelsenbeck, 2003). The Markov chain Monte Carlo (MCMC) algorithm was run for 10,000,000 generations with one cold and three heated chains, starting from random trees and sampling one out of every 1,000 generations. The burn-in and convergence diagnostics were graphically assessed using AWTY (Nylander et al., 2008). The burn-in trees were excluded, and the remaining trees were assumed to be representative of the posterior probability (PP) distribution.

Divergence Time Estimation

The combined nuclear and plastid sequences were used to estimate the divergence time of *Adenocaulon* using BEAST (version 1.8.0; Drummond and Rambaut, 2007). BEAST employs a Bayesian MCMC approach to co-estimate topology, substitution rates and node ages (Drummond et al., 2002). BEAUti was used to set criteria for the analysis, in which we applied a general time reversible (GTR) nucleotide-substitution model with Gamma + Invariant sites, gamma shape distribution (with four categories) and proportion of invariant sites. A Yule tree prior model was implemented in the analysis, with rate variation across branches assumed to be uncorrelated and lognormally distributed (Drummond et al., 2006). Posterior distributions of parameters were approximated using two independent MCMC analyses of 50,000,000 generations (sampling once for every 5,000 generations) after a 10% burn-in for each. Convergence of the chains was checked using Tracer (version 1.5; Rambaut and Drummond, 2007).

For the fossil calibrations, we followed the strategy adopted by Nie et al. (2016). The first calibration point was the *Raiguenrayun cura* Barreda, Katinas, Passalia & Palazzesi capitulescence from the Patagonia described as belonging to the crown Asteraceae and dated by radiometric methods at 47.5 Ma (von Nickisch-Rosenegk et al., 1999). This fossil was used as a minimum age constraint for the split between Barnadesioideae and the rest of family and with an applied lognormal prior distribution with an offset of 47.5 Ma, a mean of 1.0 and a standard deviation of 0.7. As a minimum age constraint for the crown group of the subfamily Barnadesioideae, we used the fossil pollen *Quilembaypollis* sp., from the latest Oligocene/earliest Miocene, dated at 23 Ma (Chiang et al., 1998). Following Bergh and Linder (2009), the 95% confidence interval for this prior lies between 16.9 and 44.1 Ma with the mean at 22.3 Ma.

Biogeographic Analyses

We compiled distribution data for the *Adenocaulon* species and assigned the included taxa to respective ranges. Considering the areas of endemism in *Adenocaulon* and tectonic history of continents, we used four areas for the *Adenocaulon* taxa: (A) South America, (B) Central America, (C) North America, and (D) East Asia. Each sample was assigned to its respective area according to its contemporary distribution range.

Biogeographic inferences were obtained by applying both statistical dispersal–vicariance analysis (S-DIVA) and dispersal-extinction-cladogenesis (DEC) model calculated by Lagrange (Ree and Smith, 2008) implemented in RASP (version 3.0) using the default settings (Yu et al., 2010). A subset of 1,000 randomly selected trees from the posterior distribution output of BEAST was used and the maximum number of individual unit areas was set to two. The probability of dispersal between areas was maintained as equal.

RESULTS

Phylogenetic Analyses

The incongruence length difference and Shimodaira and Hasegawa (SH) tests failed to reveal significant incongruence, allowing the individual datasets to be combined. There were 12,416 characters of combined data set, of which 1,571 were variable, and 1,266 parsimony informative sites. In general, the combined data matrix provided greater resolution and stronger support for phylogenetic relationships than did the individual datasets (**Figure 2**).

The results from MP and BI analysis, as well as ML analysis, strongly support *Adenocaulon* being a monophyletic clade (PP = 100, BP = 100, PL = 100). Within *Adenocaulon*, the Central American species, *A. lyratum*, diverged first, followed by *A. chilense* being sister to a clade including the remaining species from North America and East Asia albeit with low support (PP = 64, BP = 54, PL = 85). The two species found in East Asia, *A. himalaicum* and *A. nepalense*, form a clade with high support values (PP = 100, BP = 93, PL =98), which is sister to *A. bicolor* (North America) (**Figure 2**).

Biogeographic Analysis

The chronogram and results of divergence-time estimation based on the nrDNA and cpDNA data showed that the crown age of *Adenocaulon* was estimated at 9.05 Ma with a 95% highest posterior density (HPD) of 4.73–14.99 Ma. The stem age, that is the divergence time between *Adenocaulon* and its close relatives, was estimated at 14.74 Ma (with 95% HPD: 9.6–23.7 Ma). The split between the South American species *A. chilense* from the remaining species of *Adenocaulon* was at 6.92 Ma (95%HPD: 3.55–12.05 Ma), whilst the disjunction between North American *A. bicolor* and East Asian species at 4.79 Ma (95% HPD: 2.11–8.68) (**Figure 3**). S-DIVA analysis demonstrated that *Adenocaulon* may have originated in the tropical America and then dispersed to North America and East Asia (**Figure 3**).

DISCUSSION

### Phylogenetic Relationship of *Adenocaulon* with Other Genera within Mutisieae

Our study showed that *Adenocaulon* is monophyletic within the Mutisieae (PP = 100, BP = 100, PL = 100) and is sister to a clade including *Gerbera*, *Leibnitzia*, *Chaptalia*, *Trichocline*, and *Brachyclados* (**Figure 2**). The genus is characterized by
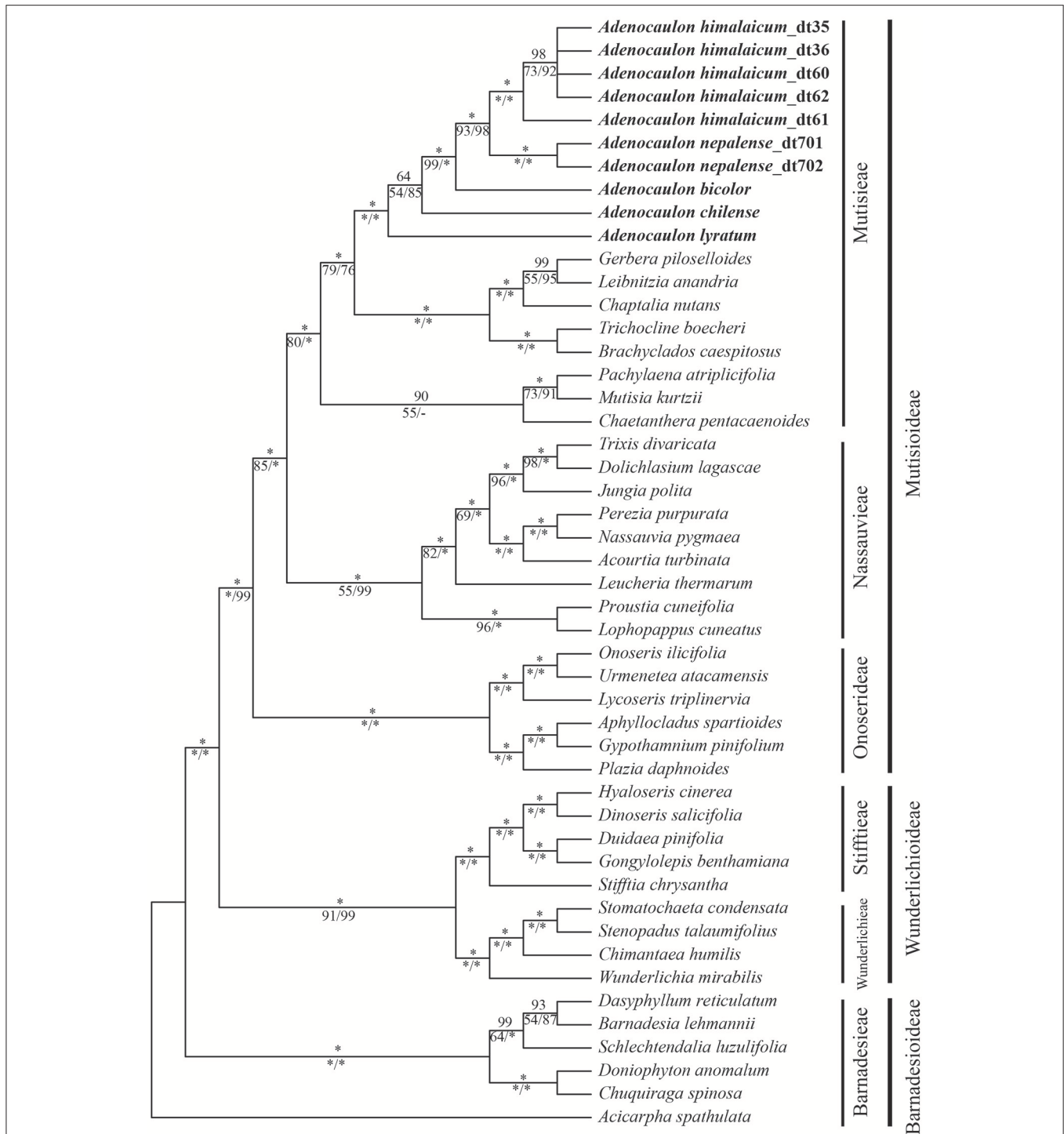
FIGURE 2 | Phylogeny of the basal grade of the Asteraceae, including Mutisioideae, Wunderlichioideae and Barnadesioideae, based on eight chloroplast DNA regions (*ndhF*, *matK*, *rpoB*, *rbcL*, *trnL-F*, *rpl*32-*trn*L, *rpl*32-*ndhF*, and *psbA-trnH*) and nuclear ITS. Numbers above the nodes are Bayesian posterior probabilities and below the nodes are bootstrap values obtained from MP analysis and posterior probabilities support values from ML analysis. "*" indicates full support for the respective analysis.

a distinct set of synapomorphy including basal constriction of anther appendages, small anthers, disciform capitula, and typical glandular achenes without pappus (Bittmann, 1990).

The placement of *Adenocaulon* has long been disputed among numerous taxonomists, going as far as calling it an anomalous genus, and being unplaced in the tribe (Bremer,
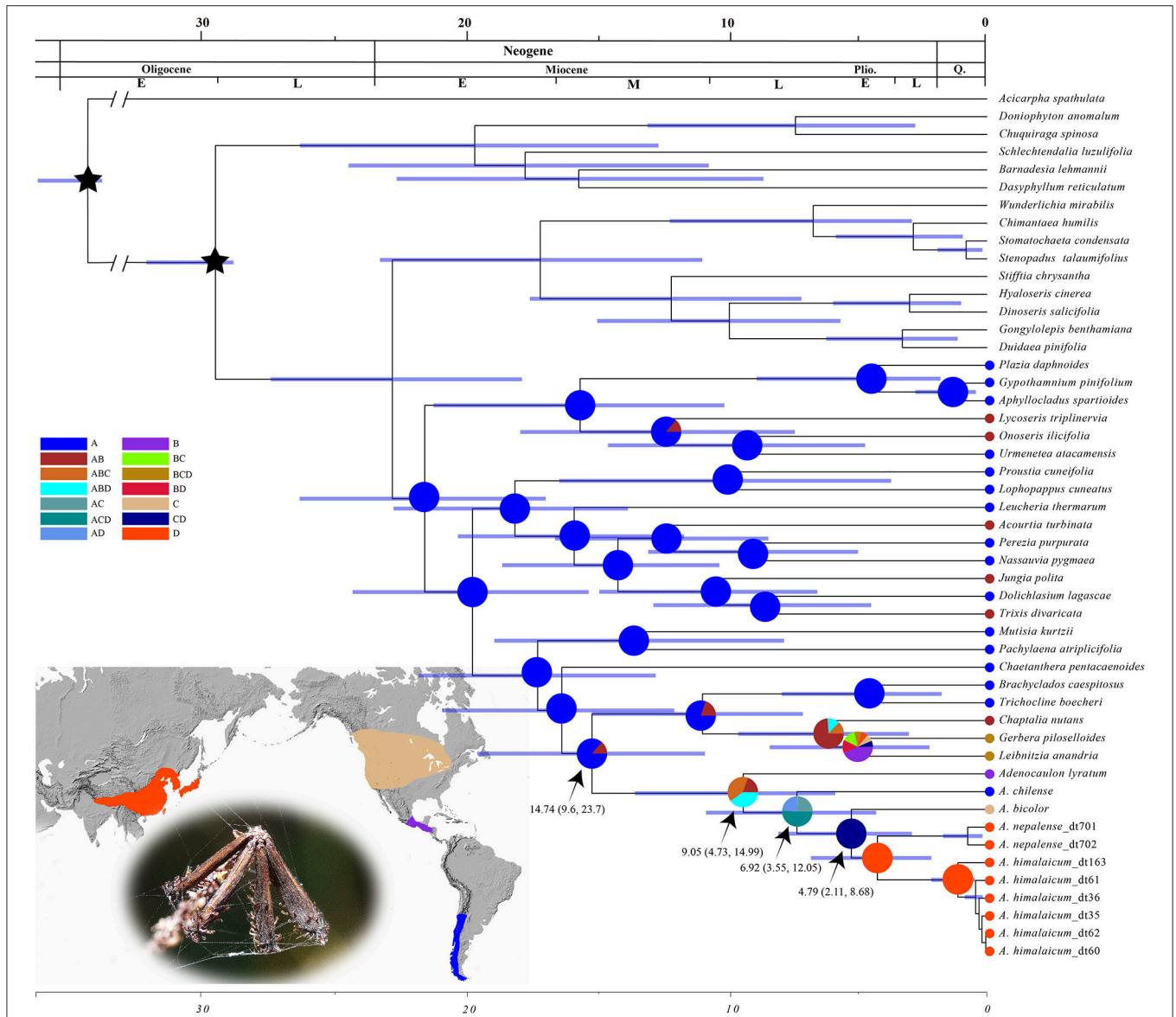
FIGURE 3 | The results of BEAST analysis and RASP ancestral area reconstruction analysis based on combined chloroplast DNA and nrlTS. Blue bars represent the 95% highest posterior density intervals for node ages. Fossil calibrations are marked with black stars. Four areas of endemism are defined: A (blue), South America; B (purple), Central America; C (yellow), North America; D (red), eastern Asia. Pie charts show probabilities of ancestral area reconstructions.

1994). Based on our broad analysis of the early diverging clades of Asteraceae, *Adenocaulon* is confirmed to belong to the tribe Mutisieae (Mutisioideae) and to be sister to the *Gerbera*-complex (PP = 100, BP = 79, PL = 76) (**Figure 2**).

Our results indicate that the Central American *A. lyratum* diverged early. This species differs from the other four species in a stem winged by decurrently leaf bases, lyrate-pinnatifid sessile leaves and distinct club-shaped achene. Representatives of *A. chilense* from southern Chile and Argentina show petiolate entire leaves in a rosulate order. The pistillate flowers have a distinct bilabiate corolla and the scabrate pollen exhibit

two layers of columellae with a larger inner one. The other three species from the Northern Hemisphere form a clade with high support. They share common features, e.g., leaves concentrated at the base, leaves triangular with cordate leaf base, heart- or kidney-shaped, with dentate or sinuate margin. Within this clade, the North American *A. bicolor* is sister to a clade with the two Asian species *A. bicolor* and *A. himalaicum*; these two species can be easily distinguished from each other by the glandular hairs: the former being pin-shaped, occurring on both vegetative and synflorescence branches, whereas the latter is nail-shaped but restricted to the synflorescence branches (Bittmann, 1990). *A. nepalense* is

a recently described new species (Bittmann, 1990) endemic to Bhutan, Nepal, and India, and having distinct features such as winged by decurrently leaf-based stems and the bearing whip hairs achenes.

The Origin and Early Dispersal of *Adenocaulon*

Our biogeographic reconstruction suggests that *Adenocaulon* may have originated in Central America and then dispersed to North America and South America during the Miocene (14.74 Ma, 95% HPD: 9.6–23.7) (**Figure 3**). The dispersal of biota between Central America, North America and South America (commonly called the Great American Biotic Interchange) is one of the most significant events in neotropical biogeography. The Great American Biotic Interchange was caused by the closure of the Isthmus of Panama ca. 3 Ma (Burnham and Graham, 1999; Cody et al., 2010), resulting in the exchange of plants and animals between the two once separated continents. However, some authors suggest that an earlier land connection made possible the exchange of biota within the Americas. A land bridge may have existed between North and South Americas 3–7 Ma (Bermingham and Martin, 1998). Moreover, some plants could have successfully crossed the Isthmus of Panama before it closed (Cody et al., 2010; Bacon et al., 2015). Here, we propose that *Adenocaulon* may have dispersed into South America from Central America during the Miocene via the land bridge link. Hypotheses of long distance dispersal events have gained popularity in the last decade as a way of explaining many disjunct distributions of plant lineages (Givnish et al., 2004; Popp et al., 2011; Nie et al., 2013).

Dispersal of *Adenocaulon* may have been enhanced by birds due to its special fruit structures. The prominent glandular hairs on the cypselae are very sticky and cling readily to fabrics, fur, and feathers. The intercontinental disjunction within the range of *Adenocaulon* is possibly a result of dispersal of cypselae on the feathers of birds. The bird dispersal pattern may also account for the intracontinental disjunction of *A. bicolor* (in the Pacific Northwest, the Black Hills, and the northern Great Lakes region). Thus, we do not postulate vicariance as a factor that shaped the disjunct pattern of *Adenocaulon*.

The Intercontinental Dispersal within Northern Hemisphere

The intercontinental dispersal of plants in the Northern Hemisphere has been a focus of biogeography for a long time (Donoghue et al., 2001; Donoghue and Smith, 2004). During the Cenozoic, two routes for plant dispersal between the New World and the Old World were the Bering Land Bridge and the North Atlantic Land Bridge (Milne and Abbott, 2002; Wen et al., 2010). These two links have played significant roles in the formation of the modern flora of the Northern Hemisphere but happened in different geologic

times (Tiffney, 1985a,b). The Bering Land Bridge is assumed to have existed from the Paleocene to Miocene (Tiffney and Manchester, 2001) but later this dispersal route was blocked due to global cooling (Milne, 2006). We suggest that *Adenocaulon* most likely migrated from North America to East Asia via the Bering Land Bridge. The estimated divergence time between the North American and East Asian groups is ca. 11.91 Ma, which coincided the cooling of Northern Hemisphere in which the continuous distribution of the Boreotropical flora was disrupted (Tiffney and Manchester, 2001). Our results suggest *Adenocaulon* may have originated in Central America, dispersed into North America and further into East Asia via the Bering Land Bridge, and finally reached the Himalayas.

Role of Fruits in *Adenocaulon* Dispersibility

Our results suggest that the biogeographic history of *Adenocaulon* species have been influenced by dispersal events, which could be facilitated by their unusual floral features. The pappus and the fruit trichome can prevent desiccation, and other characteristics of the family lack in *Adenocaulon*. Instead, the fruits of *Adenocaulon* are covered by glands with a sticky secretion. An umbrella-like "infructescence" emerges from the otherwise short plants usually embedded in other under-story vascular plants or mosses, and exposes the sticky fruits. Pervious researches indicated that viscid fruits can adhere by bird feathers (Carlquist, 1967, 1983). Also, the possibility that birds could have been the dispersal vectors for *Adenocaulon* fruits was proposed by Bittmann (1990) to explain some of the extant distributions of the genus. Alternatively, successful over-water dispersal events could be postulated for *Adenocaulon*; the oily fruit cover is suggested to aid in flotation, as was the case for other families. Thus, the absence of pappus in *Adenocaulon* does not mean that this genus lacks mechanisms for dispersal. The sticky fruits, with the "infructescence," can act as an alternative dispersal mechanism involving several potential dispersal vectors.

AUTHOR CONTRIBUTIONS

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