Systematics of *Clematis* in Nepal, the evolution of tribe *Anemoneae* DC. (Ranunculaceae) and Phylogeography and the Dynamics of Speciation in the Himalaya

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The Nepalese endemic *Clematis phlebantha* L.H.J. Williams.
Stainton #4281, 6th June 1963, Suli Gad, West Nepal.
Photograph A.D. Stainton.
Sibbald Trust care of Royal Botanic Garden Edinburgh.

Declaration

I hereby declare that the work contained in this thesis is my own unless otherwise acknowledged and cited. This thesis has not in in whole or part been previously presented for any degree.

Abstract

The genus *Clematis* L. (Ranunculaceae) was used as a new model group to assess the role of the Himalayan orogeny on generation of biodiversity through investigations of its phylogeny, phylogeography and taxonomy.

Although existing checklists include 28 species of *Clematis* from Nepal, a comprehensive taxonomic revision of available material in herbaria and additional sampling from fieldwork during this study has led to the recognition of 21 species of *Clematis* in Nepal, including one species (*C. kilungensis*) not previously recorded from Nepal.

Exisiting phylogenetic and taxonomic concepts were tested with the addition of new samples from Nepal. The results highlight the shortcomings of the previous studies which were poorly resolved and indicate the need for a thorough revision of the sectional classification. Despite the increased sampling the results are still equivocal due to poor statistical support along the backbone of the phylogeny. Groups of species in well supported terminal clades are broadly comparable with results from previous studies although there are fewer clearly recognisable and well supported clades.

The published dates for the evolution of *Clematis* were tested and the methodology of the previous study critically reappraised. The results indicate that the genus *Clematis* is approximately twice as old as previously reported and evolved in the middle Miocene. The phylogeny also demonstrates that, even allowing for poor support for the relationships between groups of species within *Clematis*, the extant Nepalese species must have multiple independent origins from at least 6 different colonisations. With their occurrence in the Pliocene and Pleistocene, these events are relatively recent in relation to the Himalayan orogeny, and may be linked more to the dispersal ability of *Clematis* than to the direct effects of the orogeny.

Additional Nepalese samples of *Koenigia* and *Meconopsis* were added to exisiting datasets and these were reanalysed. The result from *Clematis*, *Koenigia* and *Meconopsis* were appraised in light of the the geocientific literature and previously published phylogeographic studies to create an overview of the drivers behind speciation in the Himalaya.

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Chapter 1: Introduction

The mega-diverse Himalaya is the world greatest mountain range, home to an estimated 10,000 plant species and nine of the ten tallest peaks. Despite this the Himalaya is a relatively understudied system, with even its origin still unclear. Richardson *et al*. (2012) proposed that collaboration between systematists, palaeobiologists, palaeoclimatologists and geologists with their relatively independent datasets and methodologies would lead to a better understand the abiotic and biotic history of SE Asia. This proposal holds true for any region including the Himalaya where these histories are not well known.

The phlyogeographic literature for the Himalaya is limited but increasing. Studies generally focus on groups that are distributed on the Qinghai-Tibetan Plateau and Hengduan Mountains of SW China as well as the Himalaya (Jabbour & Renner 2011; Zhou *et al*. 2013). Those studies that have specifically examined the evolution of groups in the Himalaya such as *Berberis* (Adhikari 2010) and *Begonia* (Rajbhandary *et al*. 2011) offer insights into the drivers that have led to the extant diversity in the Himalayan mountain chain.

In both previous studies that focused on the Nepal Himalaya dispersal ability was discussed in terms of the evolution of the groups in the mountain range. *Berberis* fruits are bird-dispersed and *Begonia* is splash-dispersed. Choosing a new study group with a different method of dispersal would offer an opportunity to compare and contrast evolutionary history in the Nepal Himalaya. *Clematis* L. (Ranunculaceae) in Nepal is found in almost every district, from the low altitude tropical *Terai* to sub-montane habitats at 4500m. They are woody climbers in Nepal, but can be herbaceous in other parts of their range, and are dispersed by means of wind-blown achenes. This study focuses on Nepalese *Clematis* as a new model group to study phylogeography in the Himalaya

Globally the ca. 300 *Clematis* have a near cosmopolitan distribution but are found mainly in north temperate habitats. Their climatic tolerance, attractive flowers and ease with which they hybridise make *Clematis* a horticulturally important genus and they are widely cultivated in Europe, North America and Japan. While not widely cultivated in Nepal, eight wild species of *Clematis* are used in traditional medicine, as vegetables and as fodder for livestock (Manandhar & Manandhar 2002).

The existing phylogenetic framework for *Clematis* (Miikeda *et al*. 2006; Xie *et al*. 2011), offered the opportunity to study the Himalayan species, which were not included in the previous studies, not only in a regional but also the global context.

One interesting aspect of *Clematis* is the unusual pattern of distribution across in Nepal, with a higher number of recorded species in the west of the country, which is the opposite of many speciose Himalayan groups. Another reason was the existing published phylogenetic framework for *Clematis* that suggested a rapid burst of speciation in the Late Miocene from an evolutionary origin in China (Xie *et al*. 2011).

The previous *Clematis* studies (Miikeda *et al*. 2006; Xie *et al*. 2011) lacked Himalayan representation in the phylogeny with only a single species, *C. alterana,* sampled. Xie *et al*.'s (2011) study used a secondary age constraint to date the phylogeny and was the first to estimate the evolutionary age of *Clematis*. Due to a lack of usable fossil *Clematis* the dating constraint used was a derived date from the Anderson *et al*. (2005) study of evolution of major angiosperms families.

Milne (2009) demonstrated that wider sampling and deep node calibration in groups out with the focus could be used to accurately date phylogenies lacking usable fossils. Therefore reassessing *Clematis* with increased Himalayan sampling in the context of a wider phylogeny of the Ranunculaceae, which does have usable fossils and have been used in published studies (Cheng & Xie 2014), is required.

With the uncertainties surrounding the timings of the Indian plate collision with Asia (Searle *et al*. 1987; Tapponnier *et al*. 2001; Ali & Aitchison 2004; Aitchison *et al*. 2007; Hinsbergen *et al*. 2012), the initiation of the Himalaya uplift (Valdyia 2002; Adlakha *et al.* 2013 for an overview) and the development of the monsoon (Quade *et al*. 1989; Molnar *et al*. 1993; Dettman *et al*. 2001; Clift & Plumb 2011) it is necessary to reassess previously published studies in the light of these uncertainties. To create a broad overview of what is driving speciation in the Himalaya existing datasets will be reanalysed with additional sampling from Nepal and incorporate the results from the new model group of *Clematis*.

Principal aims

- To provide a taxonomic revision of *Clematis* in Nepal, including identification keys, full descriptions and distribution maps.
- Re-evaluate the evolutionary history Ranunculaceae specifically the Anemonodiae.
- Test the origin and diversification of *Clematis* in the Nepal Himalaya.

- Review the drivers of speciation in the Himalaya using *Clematis* as a new model group and adding additional samples to existing *Meconopsis* and *Koenigia* molecular datatsets and reassessing these in-light of the geoscientific literature and in the context of published phylogenetic studies of Himalayan groups.

Chapter 2: Revision of *Clematis* L. (Ranunculaceae) in Nepal

Introduction

The Ranunculaceae is a family of about 60 genera and 1700 species (Mabberley, 2008) and mainly consists of herbaceous plants apart from the woody monotypic North American genus *Xanthorhiza* and the widespread woody climber *Clematis*. Generic delimitation within Ranuculaceae has remained relatively stable through the recent changes brought about by the APG classifications, and the circumscriptions of the largest genera in the family, *Ranunculus* (600 species), *Delphinium* (365), *Thalictrum* (330), *Clematis* (ca. 300), and *Aconitum* (300), are consistent with those of De Jussieu (1789).

Clematis is a cosmopolitan genus with over 300 species globally. The majority of diversity is found in temperate habitats but there are some species of tropical habitats. It is found in Africa, North & South America, Australasia and Europe, but most species are from mainland Asia. The centre of diversity for the genus is China which has 147 species, of which 93 are endemic (Grey-Wilson, 2000; Johnson, 2001, Wang & Bartholomew, 2001).

Clematis is a pre-Linnaean generic name that can be traced back to the Latin *Clematide*, which is still the Italian name for *Clematis*, and is used in Dioscoride’s *Materia Medica* (1547) in which two species are discussed. Carl Linnaeus formalized the use of the name in *Species Plantarum* (Linnaeus, 1753) and published nine species of *Clematis*, dividing the genus into those with a climbing habit (*Scandentes*) and those with upright growth (*Erectae*). At the same time he decribed the closely related genus *Atragene,* and included within it species with staminodes, such as *A. alpina* and *A. zeylanica*. Moench (1794) further divided the genus with the separation of *C. viticella* L. into the new genus *Viticella* Moench, renaming the species *Vitacella deltoidea*.

De Candolle's (1818) treatment of 86 *Clematis* species placed them in four sections, *Flammula* DC., *Viticella* (Moench) DC., *Cheiropsis* DC. and *Atragene* (L.) DC., and he created the genus *Naravelia* DC. for *Atragene zeylanica.* Although *Atragene* had been defined by the presence of staminodes, de Candolle differentiated *Naravelia* from *Clematis* section *Atragene* on the basis of its having only two leaf segments and the presence of tendrils at the apex of the petiole, compared with trisect (trifoliate) leaves in section *Atragene*. However *Naravelia*’s leaf morphology is more accurately described as two basal leaflets with three terminal leaflets modified into tendrils. His *Prodromus* (1824) added four species to the genus and upheld his previous infragreneric classification of *Clematis* and the distinction from *Naravelia*.

David Don's 1825 *Prodromus Florae Nepalensis* (Don, 1825) listed the species found in Nepal and is dealt with in the next section. George Don fil., brother of David, listed 99 species of *Clematis* and largely upheld de Candolle's taxonomy (1818, 1824) for *Clematis* and *Naravelia*. However he differed by treating *Atragene* as a distinct genus not a section within *Clematis* in his *A General History of the Dichlamydeous Plants* (Don, 1831).

Spach (1839) radically altered the taxonomy of *Clematis* by resurrecting the genus *Viticella*, raising de Candolle's section *Cheiropsis* to a new genus of that name, and creating two new genera, *Meclatis* and *Viorna*, as well as maintaining *Atragene* as a distinct genus.

Kuntze (1885) was the first complete monograph of *Clematis*, and in it he reduced the genus to 66 species and redefined much of the existing species as subspecies and varieties of those subspecies. He also included the genus *Naravelia* within *Clematis*. Species distributions were defined using very broad geographic terms such as 'Himalayan', so it is impossible to be sure which taxa were then known to occurr in Nepal. Kuntze also formulated an infrageneric taxonmy based on a modified Linnean view of *Clematis* preferring to reduce the subsequent taxnonomies to synonomy. His classification returned to treating the genus on the species being climbing versus non-climibingand further dividing the climbings species between those with bud-scales and those without. Kuntze subdivided the sections (table 2.1) but did not explicitly name these divisions as subsections, so they could be considered more as an aid to identification than taxonomic units. Although Kuntze's sectional classification has not stood the test of time this was the first monographic treatment that dealt all the species known at that time.

Table 2.1 Kuntze's (1885) infra-generic taxonomy for the genus *Clematis*.

Section	"Subsections"	
Scandentes Eperulatae	a) Styli filiformes	b) Styli brevissimi crasso-subulati haud filiformes

Scandentes Perulatae	a) Filaments glabra	b) Filaments pilose
Escandentes	a) Caudea carpllorum nullae vel abortivae	b) Caudae carpellorum longae barbarae

Three years after Kuntze' monograph Prantl (1888) published a new classification of *Clematis* as he did not agree with the Kuntze's treatment or its conclusions. Prantl's infrageneric classification, with 5 sections and 12 subsections, is a more widely accepted treatment and it has become the baseline taxonomy for subsequent revisionary work.

Schneider (1906) revised *Clematis* to 3 sections and 11 subsections, but only worked on cultivated species.

Handel-Mazzetti (1939) published a classification of *Clematis* that largely following Prantl and Schnider but added the new section *Naravelopsis* in his publication dealing with Chinese Ranunculaceae.

Wang split *Clematis* into three genera; *Archiclematis, Clematis* (with 8 sections and 7 subsections) and *Naravelia* for the account of *Clematis* for *Flora Reipublicae Popularis Sinicae* (Wang 1980).

Keener & Dennis (1982) classified North American and Mexican species of *Clematis* in four subgenera. Despite the regional nature of this study a comphrehensive revision of the infrageneric taxonomy of the genus was clearly considered from the extensive synonomy of genera, subgenera and sections.

Serov (1988) study of the 22 species of *Clematis* in the USSR used nine sections for those species and treated *Atragene* as distinct genus.

Tamura's infrageneric classification recognised 4 subgenera, 15 sections, 9 subsections and 4 series. This account waspublished in Japanese, but it forms the basis of more recent authors' subgeneric circumscriptions (Grey-Wilson, 2000; Johnson, 2001; Wang, 2005).

The recent morphology-based monographs of *Clematis* by Grey-Wilson (2000) treated 297 species in 9 subgenera, 16 sections and 26 subsections. Johnson (2001) recognised 18 sections, 36 subsections covering and 325 species (table 2.2), and Wang & Li's (2005) classification of *Clematis* based on Wang’s extensive monographic work on the genus (Wang, 1998; 2000a; 2000b; 2000c; 2001; 2002; 2003; 2006) comprised four subgenera, 15 sections and numerous subsections and series within them for the 354 species. These three taxonomies generally agree fairly closely with each other in species delimations, but diverge in infrageneric classifications.

Johnson’s (2000) comprehensive treatment of *Clematis* covered 325 species and many cultivated varieties. It is of relevance to this study that Johnson upheld Grey-Wilson’s treatment of *Clematis tibetana* and its infraspecific taxa, and used narrow speciees concepts which distinguished many species with limited morphological differences. Johnson took the decision to treat the *Naravelia* as a section of *Clematis*, but did not make all the necessary nomenclatural changes to transfer all the species to *Clematis*, claiming that he did not want increase nomenclatural complexity. Falck & Lehtonen (2014) resolved the remaining nomenclatural issues surrounding the transfer of *Naravelia* to *Clematis*, although none of the species concerned are found in Nepal.

Table 2.2 Johnson's (2001) Sectional classification for the Genus *Clematis* with geographic distribution of groups and number of species

Section	**Subsection**	**Number of Species**	**Range**
Archiclematis		1	Xizang (Tibet), Nepal
Pseudoanemone		19	Africa
Novae-Zeelandiae		11	New-Zeland
Atragene	*Atragene*	15	Temperate to subartic Northern Hemisphere
	Brachyblasti	3	Central Asia
Naravelia		7	Tropical E Asia
Tubulosae		8	E Asia

Connatae	*Connatae*	45	E, SE & S Asia & Africa
	Henryanaae	1	China, Vietnam
	Aethusifoliae	1	East Asia
Meclatis		14	SE Europe, Cental Asia, W China to N. Korea
Bebaeanthera		4	Nepal, SW China, Japan
Cheiropsis	*Cheiropsis*	2	Mediterranian, Himalaya, SW China, Japan, E, SE Asia and Africa
	Montanae	7	Himalaya, Tibet, China
	Williamsianae	1	Japan
Clematis	*Clematis*	20	Mediterranian, Himalaya, SW China, Japan, E, SE and S Asia.
	Pierotianae	10	E Asia
	Potaninianae	2	W China, Korea
	Africanae	17	Africa
	Dioiciae	21	N and S America
Lasiantha		2	SW USA and adjacent Mexico

Aspidantera	*Aspidantera*	8	Australia
	Papuasicae	6	Malesian islands (Celebes, New Guinea, Solomon Islands)
Naraveliopsis		21	Tropical E Asia
Viorna	*Viorna*	10	S USA and E Mexico
	Crispae	1	S USA
	Integrifoliae	6	Europe, USA
	Baldwinianae	2	S USA (Florida)
	Hirsutissimae	3	N America
	Fuscae	2	N China, Korea, adjacent Russia
Flammula	*Flammula*	12	Widespread in Europe & Asia
	Meyenianae	11	E & SE Asia
	Fasciculiflorae	1	China (Yunnan)
	Chinenses	6	E Asia
	Uncinatae	2	E Asia
	Angustifoliae	1	NE Asia
	Crassifoliae	1	SE Asia
Petrocarpa		1	South Korea
Fruticella	*Fruticella*	5	Central Asia, Mongolia China,

			Korea, adjacent NE Russia
	Phlebanthae	1	Nepal
	Isphanicae	1	Iran, C Asia
	Songaeicae	2	C Asia, W China
Viticella	*Viticella*	5	S Europe, SW Asia
	Floridae	4	Japan
	Lanuginosae	1	Central China
	Patentes	1	N China, Korea, adjacent Russia

Taxonomic History of *Clematis* in Nepal

The first *Clematis* species described from Nepal were collected by Francis Buchanan-Hamilton in 1802. These collections were included in A.P. de Candolle's account of *Clematis* in *Regni Vegetabilis Systematis Naturalis* (1818). The 79 species included the six new species from Buchanan-Hamilton's collections, *Clematis acuminata* DC., *C. buchananiana* DC., *C. grewiiflora* DC., *C. loasifolia* DC., *C. montana* Buch.-Ham. ex DC. and *C. nepalensis* DC. Another Nepalese species, *C. gouriana* Roxb. ex DC., was also included in this account, but it was not known to be present in Nepal at that time. The first specimen of this species collected in Nepal was by Lall Dhwoj in 1928 and then it was collected on several occasions on British Museum led expeditions in the 1950s. The first literature record is from the *Catalogue of Nepalese Vascular Plants* (Malla, 1976).

De Candolle's account of the genus in his *Prodromus* (de Candolle, 1821) listed a total of 90 species, including two additional Nepalese species, *Clematis connata* DC. and *Clematis smilacifolia* Wall. The latter species had been published by Nathaniel Wallich two years previously in *Asiatic Researches*. These species are both based on specimens from Wallich's East India Company herbarium, and were probably collected by Edward Gardener in 1819.

David Don's 1825 *Prodromus Florae Nepalensis* (Don, 1825) was the first complete account of the plants of Nepal and was written while he was working for Aylmer Brouke Lambert. This work used specimens of Buchanan-Hamilton and Gardner, though the latter are all cited as Wallich's collections, which has led to some confusion in later years. Fraser-Jenkins (2006) clarified many such issues relating to the history of the early plant collection in Nepal. Don's account of *Clematis* listed six species in Nepal; *Clematis grewiaeflora*, *C. buchanani, C. loasifolia*, *C. acuminata*, *C. montana* & *C. anemoniflora*.

In the account *Clematis montana* and *C. anemoniflora* are illegitimate superfluous names for *C. napaulensis* and *C.montana* respectively, which are cited as in the synonym of Don's species. The renaming of *C. napaulensis* as *C. montana* does not appear to have been widely followed and has not led to any taxonomic or nomenclatural confusion. However, Don's *Clematis anemoniflora* was maintained by his brother George Don (1831) as a distinct species. Hooker (1855) treated Don's name as illegitimate as Don had cited *C. montana* DC. as a synonym in the protologue and so is placed into synonymy with *C. montana* var. *grandiflora* Hooker. Then Kuntze (1885) wrongly renamed Hooker's *C. montana* var. *grandiflora* as *C. montana* var. *anemoniflora* based on Don's earlier publication date. Finally, Brühl (1896) resurrected *C. montana* var. *anemoniflora* as a variety distinct from *C. montana* var. *grandiflora* suggesting that Kuntze and Hooker were wrong to assume it was a large-flowered variety.

Don also created *C. buchanani* which is treated as an orthographic variant of *C. buchananiana* as it is clear from the account that this is the species that Don was referring to, so again this has not led to taxonomic or nomenclatural confusion. He also tentatively synonomised *C. connata* placing it as a synonym of *C. acuminata* with a question mark.

Joseph Hooker and Thomas Thomson's *Flora Indica* (Hooker and Thomson, 1855) lists eighteen species and six varieties, and includes Nepal in the ranges of five of these species. This publication is also the first mention of the occurrence of *Naravelia zeylanica* in Nepal and they also placed *C. loasiflora* in *species dubiae* as it was based on a sterile specimen and suggested that it must be *C. grewiflora*, *C. wightiana* or C. *buchananiana*. Hooker's *Flora of British India* (Hooker 1872) increased the number of *Clematis* in India to twenty species and six varieties, and although Nepal is not explicitly mentioned in the distributions of any species of *Clematis*, it is in that of *Naravelia zeylanica*.

In 1891 Paul Brühl monographed *Clematis*, and the RBGE holds a bound copy of his hand written manuscript. Although nothing in it is validly published, it is a valuable late 19th century source of information on south Asian and Himalayan *Clematis*. Brühl did, however, publish species *in Annals of the Botanic Garden Calcutta* (Brühl & King 1896), which included the East Himalayan endemic *Clematis montana* var. *tongluensis* Brühl.

Very little was systematic work was carried out on Himalayan *Clematis* until Michio Tamura published his large body of work on the systematics, developmental biology and evolution of the Ranunculaceae (Kitamura 1954; Tamura, 1956, 1962, 1963a, 1963b, 1964, 1965a, 1965b, 1968a, 1968b, 1986, 1987). He published a number of taxa from Nepal, *Clematis alternata* Kitam. & Tamura, *C. bracteolata* Tamura, *C. chrysantha* var. *brevipes* Tamura, *C. orientalis* var. *uniflora* Tamura, and these taxa were described from the large numbers of herbarium specimens collected during Japanese-led expeditions in Nepal from the 1950s onwards. Tamura also raised *C. tongluensis* (Brühl) Tamura to the level of species.

In the *Enumeration of the Flowering Plants of Nepal* (Hara, 1979) Hara listed 22 species of *Clematis* and one species of *Naravelia* from Nepal. This account was based on the specimens from Nepal held at the Natural History Museum, London (BM), but also included information from a selection of literature sources. For example, it included the Nepalese endemic *C. bracteolata* Tamura which is only known from the type collection at the University of Kyoto (KYO) and *C. terniflora* DC., which is a species from E Asia which has a herbarium specimen in KATH from a living plant cultivated in the National Botanical Garden at Godawari, southeast of Kathmandu.

The *Flora of Bhutan* account of *Clematis* (Grierson, 1984) enumerated 13 species, 11 of which are widespread Asian species that also occur in Nepal. One of the other species, *Clematis cadima* Hook.f. and Thomson, may possibly occur in the very south of Nepal as it is a widespread low altitude species which is known from China, Vietnam and SE India. The Bhutan record still requires confirmation, as when checking the herbaria at BM, E and K no new additional records were discovered. Grierson (1984) also maintained *Naravelia* as a distinct genus from Clematis and recorded *Naravelia zeylanica* as growing in the country.

Grey-Wilson (1989) published a revision of *Clematis orientalis* and its allies which is equivalent to section *Meclatis*, and dealt with some of the taxonomic confusion surrounding the yellow-flowered species. He also published *Clematis confusa* Grey-Wilson in 1991. This species was described from a Bhutanese specimen but also cited several Kew specimens that had been collected in Nepal.

Brandenburg (2000) carried out a comprehensive systematic study of *Clematis* section *Meclatis* between 1975 and 1999, and this study was the first cladistic analysis of the genus, although it used morphological rather than molecular characters. Brandenburg’s treatment of Section *Meclatis* differs somewhat from Grey-Wilson’s and the characters he used to differentiate taxa in his key are also different. He stated that *Clematis orientalis* does not occur in Nepal but gave no justification for how this decision had been reached.

Grey-Wilson's (2000) monographic treatment was aimed more at an amateur audience than as a piece of serious taxonomy. However, he upheld his previous taxonomy of *Clematis orientalis* and its allies (Grey-Wilson, 1989) and his publication of *C. confusa* (Grey-Wilson, 1991).

The *Annotated Checklist of the Flowering Plants of Nepal* (Press *et al*. 2000) built on the previous work by Hara (1979) and can be considered the baseline data for taxonomic work on the Flora of Nepal. In this work *Clematis* has 24 species with two subspecies and one variety in Nepal.

The account of *Clematis* in *The Flora of Mustang* (Yonekura, 2008) is the most recent revision of the Nepalese species, though it covers only a subset of these species, and in it Yonekura listed 8 taxa from Mustang district. For his revision he treated the material of *Clematis tibetana* from Mustang as *C. tibetana* subsp. *brevipes,* an intermediate between the NW Himalayan subspecies *tibetana* and the SW Tibetan subspecies *vernayi*. *S*ubspecies *brevipes* is based on Tamura's *C. chrysantha* var. *brevipes* and synonomises Tamura's *C. orientalis* var. *uniflora* as well as Grey-Wilson's treatment of Nepalese *C. tibetana* subsp. *vernayi* varieties, with Yonekura arguing that the continuous variation morphology does not allow the separation of distinct taxonomic entities.

Utilising material held in Japanese herbaria collected on their extensive fieldwork in the area, Yonekura's treatment for the Flora of Mustang also covers seven other taxa: *Clematis barbellata* var. *obtusa*, *C. bracteolata*, *C. grata*, *C. puberula*, *C. tortuosa*, *C. buchananiana*, and *C. rehderiana*. The most questionable treatment in the account is that of *C. bracteolata*; Yonekura modified the protologue description of the species despite not locating the type material in the herbarium of Kyoto University (KYO).

Table 2.3 details the full list of species as recorded in Nepal from *The Annotated Checklist of Flowering plants in Nepal* and the *Flora of Mustang* (Press *et al*. 2000; Yonekura 2006) with their taxonomic position in the the three most recent infrageneric classifications.

Table 2.3 Nepalese *Clematis* species listed (Press *et al*. 2000; Yonekura 2006) before this treatment with their infra-generic positions in the three most recent classifications.

Pre-treatment Taxa in Nepal	**Infrageneric classification**		
	Grey-Wison (2000)	Johnson (2001)	Wang & Li (2005)
Clematis acuminata	Subgenus *Campanella* Section *Campanella*	Section *Connatae*	Subgenus *Viorna* Section *Viorna* Subsection *Connatae* Series *Connatae*
Clematis alternata	Subgenus *Archiclematis*	Section *Archiclematis*	Subgenus *Viorna* Section *Archiclematis*
Clematis barbellata	Subgenus *Campanella* Section *Bebaenanthera*	Section *Bebaenanthera*	Subgenus *Viorna* Section *Viorna* Subsection *Bebaenanthera*
Clematis bracteolata	[Not treated]	Section *Connatae*	N/A
Clematis buchananiana	Subgenus *Campanella* Section *Campanella*	Section *Connatae*	Subgenus *Viorna* Section *Viorna* Subsection *Connatae* Series *Connatae*

Clematis confusa	Subgenus *Campanella* Section *Campanella*	[Not Treated]	[as a var. of *C. connata*]
Clematis connata	Subgenus *Campanella* Section *Campanella*	Section *Connatae*	Subgenus *Viorna* Section *Viorna* Subsection *Connatae* Series *Connatae*
Clematis gouriana	Subgenus *Clematis* Section *Clematis* Subsection *Clematis*	Section *Clematis* Subsection *Clematis*	Subgenus *Clematis* Section *Clematis* Subsection *Clematis* Series *Clematis*
Clematis grata	Subgenus *Clematis* Section *Clematis* Subsection *Clematis*	Section *Clematis* Subsection *Clematis*	Subgenus *Clematis* Section *Clematis* Subsection *Clematis* Series *Clematis*
Clematis graveolens	Subgenus *Campanella* Section *Meclatis*	Section *Meclatis*	Subgenus *Clematis* Section *Brachiata*e Series Wightianae
Clematis grewiiflora	Subgenus *Campanella*	Section *Connatae*	Subgenus *Viorna*

	Section *Campanella*		Section *Viorna* Subsection *Connatae* Series *Connatae*
Clematis kilungensis	Subgenus *Campanella* Section *Campanella*	Section *Connatae*	As a synonym of *C. barbellata*
Clematis montana	Subgenus *Cheiropsis* Section Montanae	Section *Cheiropsis* Subsection Montanae	Subgenus *Cheiropsis* Section *Cheiropsis* Subsection Montanae Series Montanae
Clematis napaulensis	Subgenus *Cheiropsis* Section *Cheiropsis* Subsection *Cheiropsis*	Section *Cheiropsis* Subsection *Cheiropsis*	Subgenus *Cheiropsis* Section *Cheiropsis* Subsection Cirrhosae
Clematis orientalis	Subgenus *Campanella* Section *Meclatis*	Section *Meclatis*	Subgenus *Clematis* Section *Meclatis*
Clematis orientalis var. *uniflora*	[As *C. tibetana* var. *vernayi*]	[as above]	[as *C. tibetana*]
Clematis phlebantha	Subgenus Flammula Section *Fruticella* Subsection *Phlebanthae*	Section *Fruticella* Subsection *Phlebanthae*	Subgenus *Clematis* Section *Clematis* Subsection *Angustifoliae*

			Series *Phlebanthae*
Clematis puberula	Subgenus *Clematis* Section *Clematis* Subsection *Pierotianae*	Section *Clematis* Subsection *Pierotianae*	Subgenus *Clematis* Section *Clematis* Subsection *Clematis* Series *Puberulae*
Clematis rehderiana	Subgenus *Campanella* Section *Campanella*	Section *Connatae*	Subgenus *Viorna* Section *Viorna* Subsection *Connatae* Series *Connatae*
Clematis roylei	Subgenus *Campanella* Section *Campanella*	Section *Connatae*	Subgenus *Viorna* Section *Viorna* Subsection *Connatae* Series *Connatae*
Clematis sikkimensis	[treated as a synonym of *C. acuminata*]	[treated as variety of [C. acuminata]	[= C. *siamensis*.] Subgenus *Viorna* Section *Viorna* Subsection *Connatae* Series *Connatae*

Clematis smilacifolia	Subgenus *Clematis* Section *Narveliopsis*	Section *Narveliopsis*	Subgenus *Clematis* Section *Narvaliopsis*
Clematis staintonii	[not published at this time]	[not published at the time]	Subgenus *Viorna* Section *Viorna* Subsection *Connatae* Series *Connatae*
Clematis terniflora	Subgenus *Flammula* Section *Flammuula* Subsection *Flammula*	Section *Flammuula* Subsection *Flammula*	Subgenus *Clematis* Section *Clematis* Subsection *Rectae* Series Rectae
Clematis tibetana	Subgenus *Campanella* Section *Meclatis*	Section *Meclatis*	Subgenus *Clematis* Section *Meclatis*
Clematis tibetana subsp. *vernayi*	[as above]	[as above]	[as above]
Clematis tibetana subsp. *vernayi* var. *lacinifolia*	[as above]	[as above]	[as above]
Clematis tibetana subsp. *brevipes*	[Basionym of this name treated as a synonym of *Clematis tibetana* subsp. *vernayi*]	[Basionym of this name treated as a synonym of *Clematis tibetana* subsp. *vernayi*]	[as above]

Clematis tongluensis	Subgenus *Cheiropsis* Section *Montanae*	Section *Cheiropsis* Subsection *Montanae*	Subgenus *Cheiropsis* Section *Cheiropsis* Subsection *Montanae* Series *Tongluenses*
Clematis tortuosa	[as *C. buchananiana*]	Section *Connatae*	
Clematis wallichii	N/A	N/A	Subgenus *Cheiropsis* Section *Cheiropsis* Subsection *Montanae* Series *Tongluenses*
Clematis zemuensis	Subgenus *Clematis* Section *Clematis* Subsection *Pierotianae*	Section *Clematis* Subsection *Pierotianae*	Subgenus *Clematis* Section *Clematis* Subsection *Rectae* Series *Zemuenses*
Naravelia zeylanica	[Not treated]	[*Clematis naravelia*] Section *Naravelia*	[not treated]

General morphology of *Clematis*

Clematis are unusual in the Ranunculaceae as it contains plants with woody stems. They are generally lianes but can also be sub-shrubs and occasionally herbaceous.

Clematis have opposite compound leaves, that can be ternate, pinnate or bipinnate. The execption is the Himalayan species *C. alternata* which has alternate simple leaves. Johnson (2001) reported that immature plants from sections *Cheiropsis* and *Connatae* can have an alternate leaves that changes to opposite in mature plants. Leaf and leaflet margins can be entire, toothed or lobed. The level of hairiness on the surface, margins and veins of leaves and leaflets varies from glabrous to densely hairy. There level of leaf hairiness can vary greatly between individuals of the same species. The species that climb do so by means of a twining petiole. Two sections, *Viorna* and *Naravelia*, have species with terminal leaflets reduced to form tendril like structures to aid climbing.

Flowers can be either solitary or grouped to form an inflorescence and can be either axillary or terminal, generally from the current year's growth but not always. Inflorescence are generaly cymose but occasionally panicles. Buds are generally valvate; the sepal margins touch when in bud, or occasionally imbricate; the margins overlap. *Clematis* flowers are generally singular, although monecious flowers are also found in some species. Dioecious species are found generallyin Australasia and North and South America and are often taxonomically grouped to reflect this. Flower shape varies from open with spreading sepals to campanulate, cylindrical or tubular. Flowers may be nodding or erect.

Petals are absent in *Clematis.* Generally, there are 4 petaloid sepals although they can be absent or as many as 8, and they vary in size from large and showy to reduced and inconspicuous. Sepals range in colour from white to yellow, red, purple and blue and shades in between. Stamens are numerous. The filaments are generally flat and linear but are sometimes widened at the apex, middle or base and range from glabrous to hairy. Anthers comprise of two thecae attached to the top of the filaments by a connective, which may or may not protrude. Staminodes, sterile stamen, are found in section *Atragene* and are petaloid and are also present in sections *Naravelia* but these are clubshaped not petaloid.

Styles are ususally plumose in its entirety or in part. Ovaries are glabrous or hairy, they are always separate.

Fruits of *Clematis* are achenes, dry fruit containing a single seed.

Materials and methods

This study examined 1063 herbarium specimens from 56 of Nepal's 75 districts and made field observations of wild populations in Darchula, Rukum and Dolpa Districts. Specimens collected during the Darchula 2012 and the Baglung, Rukum and Dolpa 2014 expeditions are deposited at the Royal Botanic Garden Edinburgh (E), National Herbarium of Nepal (KATH), Tribhuvan University Herbarium (TUCH) and Tokyo University Herbarium (TI).

The herbaria of the Natural History Museum London (BM) and Royal Botanic Gardens, Kew (K) were consulted and specimens sent on loan to E for study. The Nepalese specimens at KATH were also studied, and these filled in gaps in the distribution of taxa especially from the Tarai and Mid-hills which are poorly represented in UK herbaria.

High resolution images of type specimens that could not physically be accessed were viewed on *JSTOR Global Plants* (http://plants.jstor.org.) and the Chinese Virtual Herbarium (http://www.cvh.org.cn/).

Type material and representative specimens of widespread species were consulted to clarify species concepts but most descriptions are based solely on the morphological characters from Nepalese specimens. The exception to this is *Clematis zeylanica*; there are few specimens from Nepal and these are in poor condition and lacking important floral and carpological characters. While vegetative characters are derived from Nepalese material flower and fruit characters were taken from geographically close specimens from India. Several other species that lacked comphrehensive collections from Nepal had flowering and fruiting times supplemented from specimens outside Nepal.

The style and formatting of the following species descriptions follows the Flora of Nepal guidelines for contributors v. 7.0 (2012). For simplicity the species descriptions are in alphabetical order rather than in any taxonomic order. Arranging *Clematis,* which is a small group of species in Nepal, to one of the competeing infra-generic taxonomies of Tamura, Wang, Grey-Wilson or Johnson was deemed to be unnecessary.

Characters were quantifed and recorded from herbarium specimens and field records when collected on field work in 2012 and 2014. These data were added to a species description template generated by following the Flora of Nepal guidelines for contributors v. 7.0 (2012). No statistical analytical methods were used on the morphological data to inform species concepts for the taxonomy chapter.

To generate the key the species were initially grouped based on their phylogenetic position and a guide tree was drawn to aid their separation. Characters that could that distinguish the clades were used as the basis for couplets in the key. The key was generated from the morphology in species descriptions produced in this study. Although some geographic delimitation of species is possible it is not helpful in the context of a key as specimens are often studied at a later date, often with incomplete field recorded locality data.

Provisional IUCN status for the species in Nepal was assessed using the *Guidelines for Using the IUCN Red List Categories and Criteria* version 11 (2014). The georeferenced herbarium specimens in the Flora of Nepal database were used to calculate the Extent of Occurance (EOO) and Area of Occurance (AOO) using the Geospatial Conservation Assessment Tool (GeoCAT; http://geocat.kew.org/). All species are national level assessments with the exception of *Clematis alternana*, *C. kilungensis*, *C. phlebantha* and *C. tibetana* subsp. *brevipes* which are global assessments as these species have restricted distribution that cover Nepal and adjacent Tibet only.

Morphology of *Clematis* in Nepal

***Clematis* L.** Species Plantarum 1: 543. 1753, Gen. Pl. ed. 5, 242, 1754.

Archiclematis Tamura, *Atragene* L., *Naravelia* Adans., *Naravel* Adans. nom. rej.

Woody climbers or rarely subshrubs, perennial, evergreen or deciduous. Leaves rarely alternate, usually opposite or occasionally fascicled, rarely simple. Leaflets usually ternate or pinnate or bipinnate, rarely with terminal leaflets modified to tendrils. Petioles with bases unwinged, or occasionally winged and fused to form a ring around the stem. Inflorescences cymose, sometimes flowers solitary, pedunculate, 2-bracteate or ebracteate. Flowers bisexual, occasionally solitary and axillary, usually in a cymose inflorescence, sometimes solitary or in a fascicle on an axillary shortshoot. Caylx campanulate, cylindrical or open. Sepals 4, petaloid, spreading or erect or ascending. Petals absent. Stamens numerous, rarely outer stamens sterile modified into linear or petaloid staminodes; anthers introse. Carpels numerous, usually pubescent or villous, ovules solitary. Achenes compressed; persistent style usually much elongated, plumose.

About 300 species throughout the world, with 22 species in Nepal, three of which are endemic. At least one non-native species is cultivated in Nepal and the presence one species from the Annotated Checklist (Press *et al.* 2000) is doubtful as it is only known from the type specimens in KYO which have not been seen.

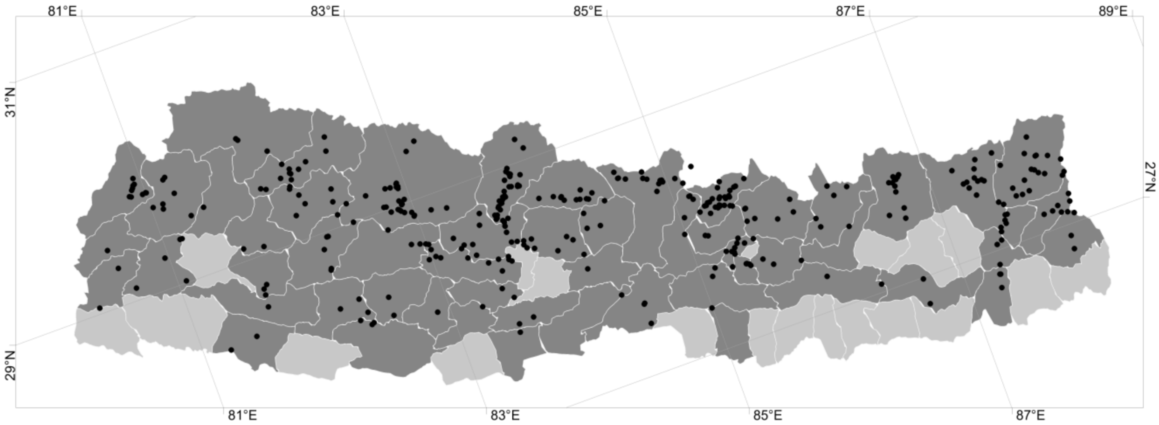


Figure 2.1 Dot map of georeferenced herbarium specimens of *Clematis* from Nepal at BM, E, KATH, K, LINN, P, TI.

There are no records of *Clematis* for twenty of Nepal's 75 districts. It is known that Nepal's Terai belt is undercollected and therefore one of the most under represented areas from Nepal in European Herbaria. The survey of *Clematis* and *Naravelia* held in KATH yielded some specimens not duplicated in E, K or BM. The recorded altitudinal range of *Clematis* in Nepal is 140 to 4850 m and its species are found from the Terai to sub-alpine forest. It is therefore very likely that the gaps in the map above are an artefact of collecting rather than a true reflection of *Clematis*' distribution within Nepal.

It was noted that data points on map did not always occur in the corresponding administrative district. It was found that a combination of factors led to this, including incorrect assignment of administrative districts to locality on the specimen records and also the administrative district name field did not always match the name used in the KML file used to generate the maps.

Key to Species

1a Stamens completely glabrous……………………………………………………….…….2
1b Stamens hairy at least in part…..………………………………………………………..10

2a Flowers in axillary or terminal inflorescences………………….………………………..3
2b. Flowers solitary in leaf axils….…………………………………………………………..8

3a Connective not protruding beyond tip of anther………………………….. ***15. C. puberula***

3b Connective protruding beyond tip of anther……………………………………........…..4

4a Staminodes present. Leaves pinnate, with apical leaflets modified into tendrils………..
…………………………………………………………………………………**21.*C. zeylanica***

4b Staminodes absent. Leaves simple, ternate, pinnate, bipinnate but lacking tendrils……..5

5a Leaves simple. Flowers brown. ……………………………………......**17. *C. smilacifolia***

5b Leaves ternate, pinnate or bipinnate. Flowers white, creamy white, whitish yellow…….6

6a Inflorescence 3--7-flowered cyme. Sepals narrowly ovate or oblong, 10--20 X 5--6 mm
……………………………………………………………….……….**20. *C. zemuensis***

6b Inflorescence 9 to many-flowered. Sepals 5--7 X 1.5--2
mm……………….…………….7

7a Leaflet margins simple or denticulate, sparsely pubescent above, sparsely pilose below…
…………………………………………………………………………….**7. *C. gouriana***

7b Leaflet margins coarsely serrate, glabrous above, sparsley pilose below……….**8. *C. grata***

8a Sub-shrub. Leaflets 0.5 X 1.5 cm, densely silvery tomentose below….**14. *C. phlebantha***

8b Climbing shrub with twining petioles. Leaflets 2.5--7.5 X 2--4 cm, glabrous or sparsely..9

9a Pedicel bracteate. Sepals campanulate…………………………………**13. *C. nepaulensis***

9b Pedicel ebracteate. Sepals spreading, flat……………...………………………………10

10a Sepals oblong to narrowly ovate, 30--60 X 15--20 mm ……………..**19. *C. tongluensis***

10b Sepals oval-elliptic, 10--30 X 20--25 mm………………………………**12. *C. montana***

11a Leaves opposite, ternate or pinnate…………………….……………..……………..12

11b Leaves alternate, simple…………………………………….…………**2. *C. alternata***

12a Flowers solitary, ebracteate, from a fascicle on an axillary shortshoot…………………13

12b Flowers in a cymose inflorescence or if solitary, bracteate, axillary or occasionally
terminal ……………….……………………………………………………………………14

13a Sepals brown red. Sepals obtuse to elliptic, 17 X 15 mm….……………...***3. C. barbellata***

13b Sepals yellow with a brown purple base. Sepals ovate to obovate, 24--28 X 13--16 mm
……………………………………………………………….…….**11. *C. kilungensis***

14a Sepals spreading, ascending or open. Stamens wider at the base than above……..…15
14b Sepals erect. Stamens strap shaped…………………………………….….…..….…16

15a Sepals entire, acute or acuminate tip, reflexed……………………………**18. *C. tibetana***
15b Sepals emarginate, not reflexed………………………………..……**9. *C. graveolens***

16a Petiole bases fused to form a ring around the stem…………………..………………17
16b Petioles bases not fused to form ring around the stem.…………………………………19

17a Inflorescence 3--9-flowered. Sepals creamy yellow…………….……..…**6. *C. connata***
17b Inflorescence 1--9-flowered. Sepals brown…...………………………..…...**5. *C. confusa***

18a Leaflet apex acuminate, distinctly longer on terminal leaflet than laterals. Flowers creamy white.…………………..………………...………………………**1. *C. acuminata***
18b Leaflet apex acute or acuminate or cuspidate or obtuse, if acuminate not distinctly longer on terminal leaflet than lateral. Flowers yellow……………………………..…………...20

19a Stems, petioles, pedicels and sepals glabrate or pubescent or canescent or villose….21
19b Stems, petioles, pedicels and sepals all with dense velutinous hairs…**10. *C. grewiiflora***

20a Leaflets 2--5 X 1.6--3 cm. Bracteoles awl-shaped, villose……………**16. *C. rehderiana***
20b Leaflets 5--10 X 5--10 cm. Bracteoles narrowly ovate usually 3-toothed…………………………………………………………..…**4. *C. buchananiana***

1. *Clematis acuminata* DC. Syst. Nat. 1:148 (1817).

Candolle, Prodr. 1:6 (1824). Don, D., Prodr. fl. nepal. 192 (1825). Don, G., Gen.Sys. 1:6 (1831). Hooker, J.D. & Thomson, Fl. ind. 11 (1855). Hooker, J.D. Fl. Brit. India 1:5 (1875). Kuntze, Ver Bot. Vereins. Prov. Brandenburg 26:167 (1885). Brühl, Ann. Roy. Bot. Gard. (Calcutta) 5:74-75 (1896). Gupta, Bull. Lucknow Natl. Bot. Gard. 54: Pl.1 (1961). Kapoor, Bull. Lucknow Natl. Bot. Gard. 78:8-9 (1962). Malla, Bull. Dept. Med. Pl. Nep. 2: 3 (1969). Hara, Enum. Fl. Pl. Nepal 2:14 (1979). Grierson, Fl. Bhutan 1(2):289 (1984). Annot. Checkl. Fl. Pl. Nepal 252 (2000). Grey-Wilson, *Clematis* the Genus, 150-151 (2000). Annot. Checkl. Fl. Pl. Nepal: 253 (2000). Johnson, The Genus *Clematis*: 291 (2001).

Type: Nepal, Narainhetty, Buch.-Ham. *s.n.*, (31/Dec/1802) [both annotated as *C. trinervis*]. Lectotype: (BM) !,[BM000521575] designated here. Syntype (LINN) ! [LINN-HS974-21]

Synonomy: *Clematis trinervis* Buch.-Ham. ex DC., Syst. Nat. 1:148 (1817) [cited in synonymy as *ined.*] - Type: As above.

Clematis acuminata var. *wallichii* Hook. f. & Thomson, Fl. Brit. India 1: 6 (1872). - Type: [No specimen cited in protologue.]

Description: Climber. Stems ribbed, pubescent or sparsely pubescent. Leaves ternate, petiole to 5 cm. Leaflets ovate to narrowly ovate, 3 or 5-lobed, 5--12 X 1.5--3 cm, base rounded or cordate, apex acuminate, distinctly longer on terminal leaflet than lateral leaflets, margin serrulate at middle,otherwise entire, glabrescent below, sparsely pubescent above. Inflorescence axillary, flowers 1--3(--9)-flowered cyme; peduncles 1--4 cm; bracts leaf-like, entire or 3-lobed, to 5 mm. Flowers creamy white, campanulate. Pedicels 2.5--3 cm; bracteoles small, awl-shaped. Sepals 4, oblong to ovate, 10--15 X 0.3--0.4 mm, erect, recurved tip, apex acute, sparsely pubescent inside and outside. Stamens 5--8 mm; filaments linear, pilose; anthers to 2.5 mm long; connective pilose, not protruding. Achenes ovate to almost round, 6 X 4 mm, flat with a distinct rim, pubescent; style persistent, plumose.

Distribution: Nepal,W Himalaya, E Himalya, Assam-Burma, E Asia.

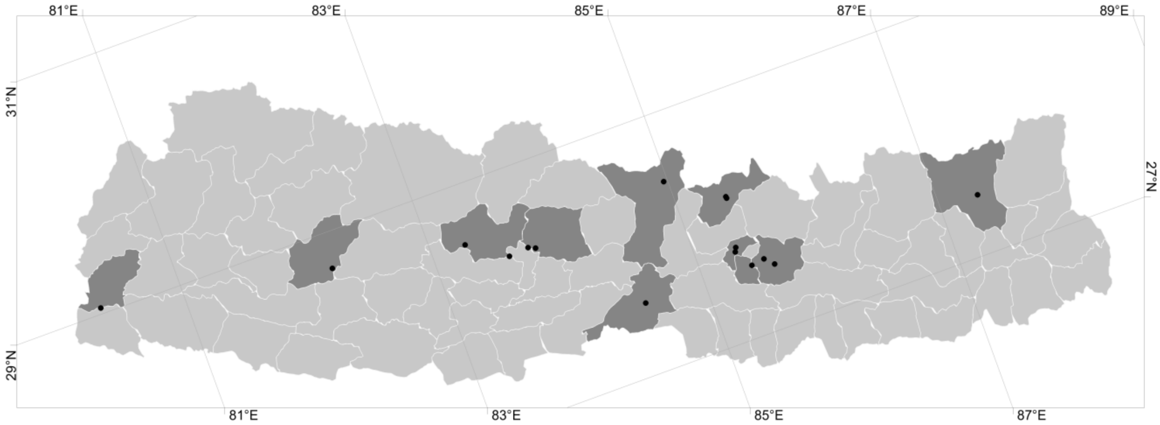


Figure 2.2 Distribution of *Clematis acuminata* DC. in Nepal

Specimens seen: Eastern, Shakya, P.R. 9549 (KATH); Central, Buchanan-Hamilton, F. (LINN); Joshi, D.P. & Rajbhandari, K.R. 75635 (KATH); Stainton, J.D.A. 8228 (E); Manandhar, N.P. 3233 (KATH); Pradhan, P. 4284 (KATH); Nicolson, D.H. 2472 (KATH); Tabata, H., Rajbhandari, K.R., Tsuchiya, K. & Konno, Y. 7565 (KATH); **Western**, Metz, J.J. 9241 (KATH); Vickery, A.R. 437 (BM); **Mid Western**, Vickery, A.R. 437 (KATH); Joshi, L. 9495214 (KATH); Dawson, G. 272 (BM); Mikage, M., Yoshimitsu, M., Kaneda, A., Mouri, C., Tatsukawa, S., Asada, Y. & Senoo, M. 9964091; **Far Western**, Rajbhandari, K.R., Bhattarai, S., Sharma, I. & Joshi, R. 7048 (KATH)

Altitudinal Range: 200-3000 m.

Ecology: Climbing shrubs or small trees in forests, on hillsides and in river gullys.

Flowering Time: October-December.

Fruiting Time: January-May.

National IUCN Status (Provisional): Data Deficient (DD).

Clematis acuminata in Nepal has an EOO of 37,800 km^2 suggesting Near Threatened (NT) and an AOO of 64 km^2 suggesting Endangered (EN), however there are no data on population or range fluctuations.

Notes: *Clematis acuminata* is a widespread species found along the Himalaya from NW India to western China and south into Burma, with a number of intraspecific taxa described. The only variety that occurs in Nepal is the typical variety *Clematis acuminata* var. *acuminata*, which extends from NW India as far east as Bhutan. The other varieties are distributed in the eastern part of the species' range.

Clematis sikkimensis (Hook.f. & Thomson) Drumm. ex Burkill has sometimes been placed into synonymy as a variety of *Clematis acuminata*, but in prepatory work for the *Flora of China* Wang (2001) synonomised it under *C. siamensis* J.R.Drumm. & Craib. *Clematis sikkimensis* was first listed in Nepal by Malla (1969) and was subsequently included Hara *et al.* (1979) and Press *et al.* (2000). This was based on the identification of Pradhan 4284 (KATH), as *C. acuminata var. sikkimensis*, but redetermined during this revision as *C. acuminata* var. *acuminata*. Wang (2000) cited one specimen of *C. siamensis* from Nepal, Banerji 1379 (GH) collected in Charikot, but this was also found to be *C. acuminata*. However, it should be noted that Joshi 9495214 (KATH) Ghorka and Joshi & Rajbhandari 75635 (KATH) which were collected northwest of Kathmandu both have inflorescences with nine flowers, which is considerably more than in other specimens, for which 1--3 flowers is typical. Inflorescences of *C. siamensis* have seven to many flowers. Wang and Bartholemew (2001) differentiated *C. siamensis* from *C. acuminata* by its ovate to elliptic leaflets with an acuminate apex compared with broadly lanceolate leaflets with a long caudate apex. Johnson (2001) differentiated *C. siamensis* from *C. acuminata* subsp. *sikkimensis* as the former is restricted to Thailand and has longer conical flowers.

Although no specimens were cited by Hooker and Thomson (1875) for *Clematis acuminata* var. *wallichii*, the type at the BM of *Clematis acuminata* [BM000521575] was annotated by Kuntze as *Clematis acuminata* var. *wallichii*.

The type specimen, BM000521575, at the Natural History Museum, London (BM) was determined by K. K. Shrestha as the holotype. This is specimen was chosen as lectotype here.

Figure 2.3 ***Clematis acuminata*****. Stainton #8228, 15th December 1980, south of Panaoti, Mahabharat lekh, Dhankuta District. Photographer A.D. Stainton. Sibbald Trust care of Royal Botanic Garden Edinburgh.**

2. *Clematis alternata* Kitam. & Tamura Acta Phytotax. Geobot. 15(5): 129 (1954).

Hara, Enum. Fl. Pl. Nepal 2:14 (1979). Kapoor, Bull. Lucknow Natl. Bot. Gard. 124:18 (1966). Hara, Enum. Fl. Pl. Nepal 2:14 (1979). Grey-Wilson, *Clematis* the Genus: 130-131. (2000). Annot. Checkl. Fl. Pl. Nepal: 252 (2000). Johnson, The Genus *Clematis*: 140 (2001). Wang & Bartholomew Fl. China. 6: 386 (2001).

Type: Nepal. Nakao *s.n.* 2200 m Gumrun to Satari. [Holotype and isotype KYO - not seen]

Synonymy: *Archiclematis alternata* (Kitamura & Tamura) Tamura. *Sci. Rep. Coll. Gen. Educ. Osaka Univ. 16(2): 31 1967*.

Description: Climber. Stems ribbed, pubescence reflexed or appressed becoming canescent at the nodes. Leaves alternate, petiole to 5 cm, simple or 3 or 5-lobed, 5--10 X 4--7 cm, base cordate, apex acute to short acuminate, margin serrulate, sparsely pubescent above, pubescent to pilose below. Inflorescence axillary, flowers solitary or in a 3-flowered cymule; peduncle 5--8 cm; bracts ca. 1 cm with 3 teeth towards apex. Flowers deep red, cylindrical. Pedicels to 6 cm, pubescent; bracteoles awl-like, ca. 0.5 cm. Sepals 4, oblong to ovate, 25 X 5--10 mm, erect, tip recurved, apex acute, sparsely pubescent inside and out, margin dense tomentum. Stamens 1--3 cm; filaments flat, wider and with pilose hairs towards the base; anthers 2.5--3 mm, connective glabrous, acutely protruding or not protruding. Achenes not seen.

Distribution: Nepal, Tibetan Plateau.

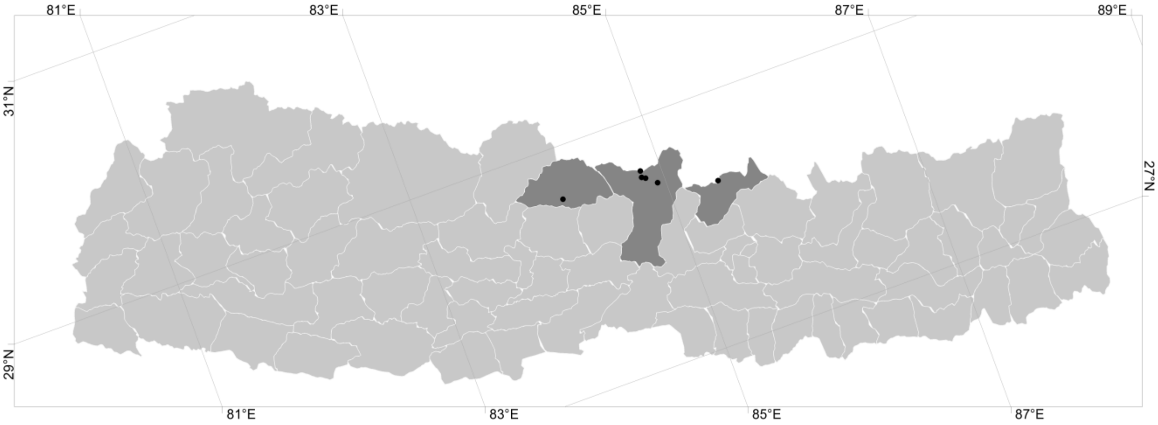


Figure 2.4 Distribution of *Clematis alternata* Kitamura & Tamura in Nepal

Specimens examined: Central, Polunin, O.V. 847 (BM); **Western**, Manaslu 08 20815060 (E); Manaslu 08 20816057 (E); Stainton, J.D.A. 3909 (BM); Stainton, J.D.A. 8865 (BM).

Altitudinal range: 1500-3400 m

Ecology: Grows through small shrubs and trees on forested rocky slopes.

Flowering Time: July.

Fruiting Time: August.

Global IUCN Status (Provisional): Data Deficient (DD).

Clematis alterana has an EOO of 1,036 km^2 suggesting Endangered (EN) and an AOO of 24 km^2 suggesting Endangered (EN), however there are no data on population or range fluctuations.

Notes: This species is endemic to a small area of northern central Nepal and the adjacent Tibetan Plateau.

Tamura (1967) created the monotypic genus *Archiclematis* for this species, presumably believing that the alternate leaf arrangement was a primitive characteristic and used it to separate this species from the rest of *Clematis* in which the leaf arrangement is generally opposite. Johnson (2001) considered that *Clematis alternata* merited a section based on vegetative differences but conceded that it was florisitically very like Section *Connatae* which consists of *C. connata* and its allies.

Phylogenetic reconstructions by Miikeda (2006), Xie *et al.* (2011) and this study reveal that *C. alternata* is a derived lineage, not ancestral and there are no grounds for recognising the genus *Archiclematis*. Its position in the phylogeny from this study suggests that placement in a section on its own is not merited either and it should be part of a more widely circumscribed Section *Connatae*.

Figure 2.5 ***Clematis alternata*****. Manasalu 08 20815060. Top: Flowering stem. Bottom: Flower detail.**

3. *Clematis barbellata* Edgew. Trans. Linn. Soc. London 20(1): 25. 1846.

Hooker, Bot. Mag. t.4759 (1854). Hooker, J.D. Fl. Brit. India 1:3 (1872). Kuntze, Ver. Bot. Vereins. Prov. Brandenburg 26:159 (1885). Gupta, Bull. Lucknow Natl. Bot. Gard. 54: Pl. 5 (1961). Kapoor, Bull. Lucknow Natl. Bot. Gard. 78:12-16 (1962). Hara, Enum. Fl. Pl. Nepal 2:14 (1979). Riedl & Nasir, Fl. Pakistan 193: 95 (1991). Grey-Wilson, *Clematis* the Genus: 150-151. (2000). Annot. Checkl. Fl. Pl. Nepal: 252 (2000). Johnson, The Genus *Clematis*: 140 (2001). Wang, Acta Phytotax. Sin. 39(1):16 (2001). Yonekura, Fl. Mustang 69-70 (2008).

Type: India: Himachal Pradesh, [Chur] Choor, M.P. Edgeworth 9. 1844, Himalaya, 9000 ft Lectotype: K [K000075702]! designated here.**Synonymy**:

Clematis barbellata var. *obtusa* Kitam. & Tamura, Fauna Fl. Nepal Himalaya 127 (1955) *non* Wang Acta Phytotax. Sin. 39(1):39 (2001). Type: Nakao [195], Pisang 24 May 1953 [KYO], Paratype: Nakao *s.n.* Tukucha 25 April 1953 [KYO]. [Although neither specimen was seen during this revision Wang (2000) noted that he had seen the type and the number is 195, although this is not cited in the protologue]

Clematis japonica Kunze *non* Thunb. In Ver. Bot. Vereins. Prov. Brandenburg 26:159 (1885) [No specimen seen].

Description: Climber. Stems round, slightly ridged. Leaves ternate, emerging from fascicles of small hairy leaf buds clustered at nodes, petiole to 8 cm. Leaflets ovate to narrowly ovate, more or less 3-lobed, terminal leaflet 2--5(--6.5) X 1--3 cm, lateral leaflets (1.3-)-2--4.5 X (0.9-)-1.2--2 cm, base rounded, apex acute or cupidate, margin coarsely serrate, glabrate or sparsely pubescent to pubescent above and below. Inflorescence a fascicle of 1, 2 or occassionally 4 flowers on an axillary shortshoot. Flowers dull purple to brown, openly campanulate, pedicel to 10 cm, villose when young becoming sparsely villose when mature; bracteoles small trident-like emerging from fascicle. Sepals 4, obtuse to elliptic, 17 X 15 mm, ascending, tip recurved, apex acute to rounded, densely pubescent inside and out, margin densely tomentose, Stamens 15--20 mm; filaments flattened, pubescent; anthers 2--3 mm; connective densely pubscent at tip, not protruding. Achenes ovate or obovate, 3--4 X 2--3 mm, indistinct rim, glabrous to glabrate; style peristent, golden, to 4 cm, plumose.

Distribution: Nepal, W Himalaya.

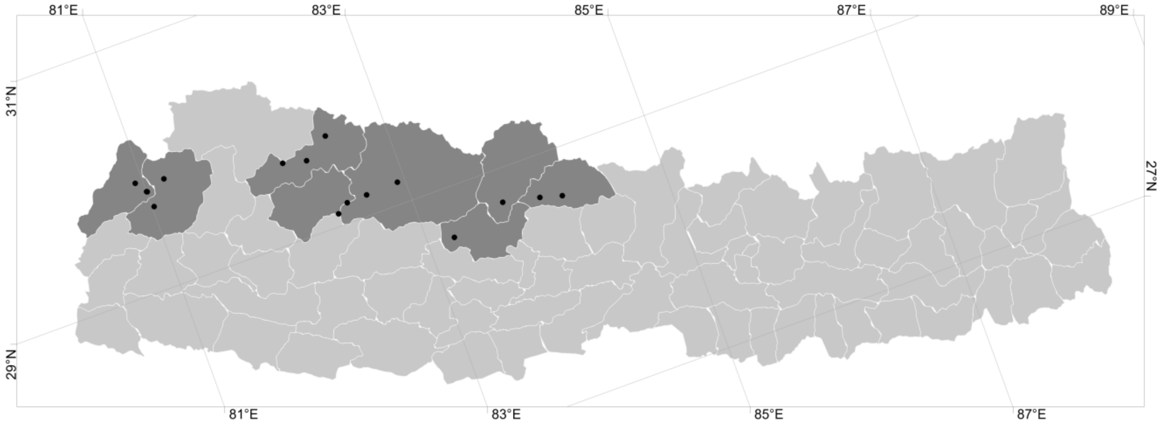


Figure 2.6 Distribution of *Clematis barbellata* Edgew. in Nepal

Specimens examined: Western, Stainton, J.D.A., Sykes, W.R. & Williams, L.H.J. 775 (BM); Stainton, J.D.A., Sykes, W.R. & Williams, L.H.J. 3045 (BM) (P) Ross, N., Ghimire, M.K., Merello, M., Joshi, L., Koirala, B., Lo, S., Poudel, A. & Sharma, S. 63 (E); Ross, N., Ghimire, M.K., Merello, M., Joshi, L., Koirala, B., Lo, S., Poudel, A. & Sharma, S. 95 (KATH); **Mid Western,** Dobremez, J.F. & Manandhar, N.P. 2859 (KATH); Stainton, J.D.A. 4258 (BM) (E) (KATH);Polunin, O.V., Sykes, W.R. & Williams, L.H.J. 1101 (BM); Polunin, O.V., Sykes, W.R. & Williams, L.H.J. 4349 (BM); Shakya, P.R. & Roy, B. 5535 (KATH); Shakya, P.R. & Roy, B. 5660 (KATH); **Far Western,** Darchula 2012 1217031 (E) (KATH) (TI) (TUCH); Shrestha, T.B. 4227 (KATH); Stainton, J.D.A. 4945 (BM);Dobremez, J.F. 2070 (KATH); Dobremez, J.F. 2247 (BM) (KATH)

Altitudinal range: 2500-3600 m.

Ecology: Climbing on shrubs and small trees in secondary, riverine and mixed forests.

Flowering Time: May-June.

Fruiting Time: July-September.

National IUCN Status (Provisional): Data Deficient (DD).

Clematis barbellata in Nepal has an EOO of 16,996 km^2 suggesting Vulnerable (VU) and an AOO of 60 km^2 suggesting Endangered (EN), however there are no data on population or range fluctuations.

Notes: Despite *Clematis barbellata* being a fairly distinct element in the west Himalayan flora it has had a chequered taxonomic history. It was placed into synonymy by Kuntze and treated as *Clematis japonica*, citing Thunberg’s poor description of *C. japonica* as the reason. However, although there are similarities in their flower shape and colour, the key difference in anther morphology and the large disjunct between these species are enough to separate them.

Two collections from Mustang were given varietal rank by Kitamura & Tamura (1955) as *Clematis barbellata* var. *obtusa*. The description was completely inadequate as the new variety was distinguished from the typical variety by having sepals that are elliptic-ovate, not acuminate and externally villose. Wang (2001) and Yonekura (2008) both upheld this variety in their respective accounts but apparently neither were able to locate the holotype. Yonekura did, however, cite the paratype from KYO.

Looking at specimens from the range of *Clematis barbellata*, [chiefly E and K, but also KATH and BM,] *Clematis barbellata* appears to exhibit a continuous variation in shortening and blunting of the sepal tip and an increase in pubescence on the sepals and leaflets from west to east. *Clematis barbellata* var. *obtusa* is therefore reduced to synonymy.

The inadequate description of *Clematis barbellata* var. *obtusa* also resulted in Wang (2001) incorrectly synonymising *Clematis kilungensis* W. T. Wang & M. Y. Fang with it, based on the obtuse sepals of that species. *C. kilungensis* flowers are yellow with a purple base and the sepals are ovate to obovate and 24 to 28 mm long by 13 to 16 mm wide, whereas *C. barbellata* flowers are dull purple to brown and the sepals are obtuse-elliptic and 17mm long by 15 mm wide.

Johnson (2001) mistakenly cited *Clematis nepalensis* Royle (non. DC.), Ill. Bot. Himal. Mts. [Royle] 1:51 (1839) as a synonym of *C. barbellata*. In Royle’s account he cited de Candolle as author and the Wallich numerical list specimen 4680. The specimen at E corresponding to Wallich’s List number 4680A, from Nepal, is *Clematis napaulensis* DC. Royle also cited David Don’s *C. montana* D.Don which is a superfluous name for *C. napaulensis*. DC.

4. *Clematis buchananiana* DC. Syst Nat. 1:140 (1818).

Candolle, Prodr. 1:4 (1824). Don, D., Prodr. fl. nepal. 191 (1825). Don, G. Gen.Sys. 1:5 (1831). Hooker & Thomson Fl. Indica 1:10 (1855). Hooker, Fl. Brit. India 1:6 (1872). Kuntze, Verh. Bot. Vereins Prov. Brandenburg 26:131 (1885) Gupta, Bull. Lucknow Natl. Bot. Gard. 80: Pl. 25 (1961). Kapoor, Bull. Lucknow Natl. Bot. Gard. 124:23-26 (1963). Hara, Enum. Fl. Pl. Nepal 2:14 (1979). Grierson, Fl. Bhutan 1(2):289 (1984). Riedl & Nasir.

Fl. Pakistan 193:89-91 (1991). Annot. Checkl. Fl. Pl. Nepal 252 (2000). Grey-Wilson. *Clematis* the Genus: 143. (2000). Wang, Acta. Phytotax. Sin. 38(4): 333 (2000). Johnson, The Genus *Clematis*: 296 (2001). Wang & Bartholomew, Fl. China 6:378 (2001). Yonekura, Fl. Mustang, Nepal. 71-72 (2008).

Types: Buch.-Ham. *s.n.* Narain Hetty 30th Oct. 1802. Lectoypes: BM, [BM000521576]! designated here, Syntype: (LINN), 26th Oct 1802 [LINN-HS974-24]! as *C. buchaniana*.

Synonymy: *Clematis buchaniana* DC. Prodr. 1:4 (1824). [*orth*. *var*.]

Clematis buchanani D.Don Prodr. Fl. Nepal. 191 (1835). [*orth*. *var*.]

Clematis tortuosa Wall. Numer. List #4675. 1831. *nom. nud.* Types: Bangladesh, Sillet. Francis De Silva *s.n.*, Numer. List #4675. K! [K000075706], E! [E00438587 & E00438586].

Clematis buchananiana DC. var. *rugosa* Hook.f & Thoms. Fl. Brit. India 1:6 (1872). Wall. Cat. 4677.

Clematis buchananiana DC. var. *vitifoia* Wall. ex Hook.f. & Thoms. Flora Indica 1:11 (1855). Type: Nepal: Gossain Than, Aug 1821 Numer. List #4676, K! [K000675182]

Clematis buchananiana var. *tortuosa* Hook.f & Thoms. Fl. Brit. India 1:6 (1872). Types: Bangladesh, Sillet. Francis De Silva *s.n.*, Numer. List #4675. K! [K000075706], E! [E00438587 & E00438586].

Clematis tortuosa Wall. & C.E.C Fisch. Bull. Misc. Inform. Kew (1929) (1): 4 (1929). Types: Bangladesh, Sillet. Francis De Silva *s.n.*, Numer. List #4675. K! [K000075706], E! [E00438587 & E00438586].

Clematis staintonii – W.T.Wang., Acta Phytotax. Sin. 38 (4): 333 fig. 4:1-3. (2000). Type: Nepal, Lete (south of Tukcha).Stainton, Sykes & Williams 1659. Holotype P! [P00125453], Isotype BM! [BM000833348]

Clematis vitifolia Wall., Numer. List #4676. 1831. n*om. nud.* Type: Nepal: Gossain Than, Aug 1821 Numer. List #4676, K! [K000675182]

Description: Climber. Stems ribbed, glabrate or pubescent to canescent. Leaves ternate or pinnate, (3 or)5 or 7 leaflets, petiole to 8 cm. Leaflets ovate, entire or shallowly 3 or 5-lobed, 5--10 X 5--10 cm, base cuspidate, apex cuspidate to obtuse, margin coarsely serrate, glabrate to pubescent to pilose above and below. Inflorescence axillary, 3--9-flowered cyme;

peduncle 4.5--7 cm, bracts entire, 3-lobed or ternate, sessile, irregularly toothed to serrulate, ca. 1 cm long, sparsely pubescent. Flowers yellow, campanulate. Pedicels 3--5 cm; bracteoles small, narrowly ovate usually 3-toothed. Sepals 4, ovate to obovate, 15--30 X 2--10 mm, erect, tip recurved, apex obtuse or acute or mucronate, sparsely pubescent to glabrescent inside, tomentose outside. Stamens to 10 mm; filaments 6--7 mm, narrow linear, densely pubescent at base otherwise pubescent or glabrous; anthers 3--4 mm, connective barely protruding. Achenes rhombic, 2--4 X 1--2 mm, densely pubescent, flattened with indistinct rim; style persistent, to 4 cm, plumose.

Distribution: Nepal, W Himalaya, E Himalaya, S Asia, E Asia, Assam-Burma, SE Asia.

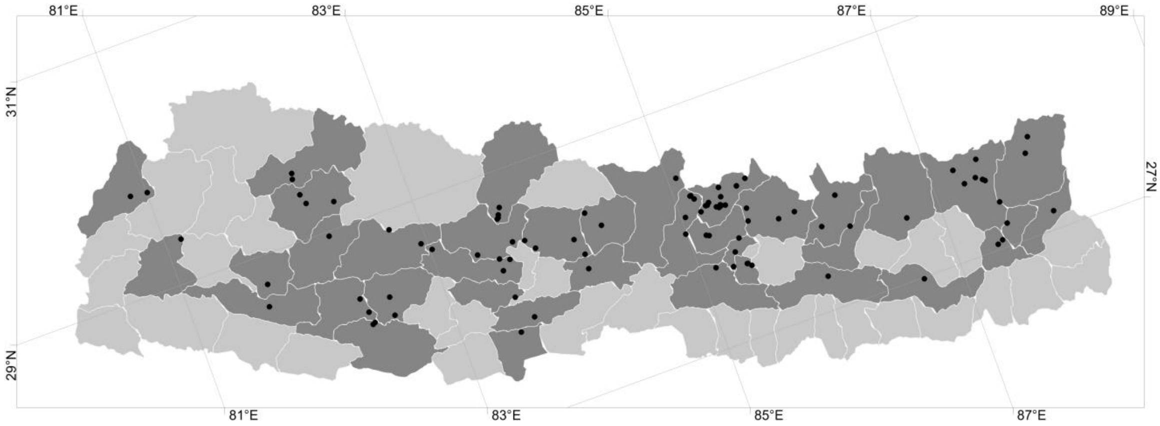


Figure 2.7 Distribution of *Clematis buchananiana* DC. in Nepal

Specimens examined: Eastern, Hara, H., Kanai, H., Kurosawa, S. & Murata, G. 63044335 (BM) (KATH); The 2nd Botanical Expedition to East Himalaya 1963 6304332 (E); Beer, L.W. 25620 (BM); Beer, L.W., Lancaster, C.R. & Morris, D. 10116 (BM); Beer, L.W., Lancaster, C.R. & Morris, D. 12280 (BM); EMAK 1029 (E); EMAK 189 (E); EMAK 398 (E) (KATH); EMAK 856 (E); EMAK 893 (E); Grey-Wilson, C., Sinnott, M., Zmartzy, S., Long, D.G., McBeath, R., Noltie, H.J., Subedi, M.N. & Crawford, S. 58 (E) (K) (KATH); Pradham, P., Rajbhandari, K.R. & Nirola, R. 274 (KATH); LKSRD 355; LKSRD 41; The 2nd Botanical Expedition to East Himalaya 1963 6304336 (KATH); Brough, M.A. 30 (BM); Stainton, J.D.A. 1040 (BM) (KATH); Stainton, J.D.A. 6604 (BM); Shakya, P.R. & Rajbhandari, K.R. 3474 (KATH); Shakya, P.R. & Rajbhandari, K.R. 3484 (KATH); **Central,** Manandhar, N.P. 12937 (KATH); Manandhar, N.P. 13070 (KATH); Manandhar, N.P. 13111 (KATH); Miyamoto, F., Rajbhandari, K.R., Akiyama, S., Amano, M., Ikeda, H. & Tsukaya, H. 9430088 (KATH); Amatya, M.M. & Bhajracharya, T.K. 13936 (KATH); Buchanan-Hamilton, F. (LINN); Nicolson, A.C. 2903 (KATH); Bowes-Lyon, S.A. 27 (BM);

Manandhar, N.P. 3129 (KATH); [no collector] 9743 (KATH); Amatya, M.M. & Bhajracharya, T.K. 7318 (KATH); Manandhar, N.P. et al. 10731 (KATH); Pradhan, P. & Thapa, R. 6753 (KATH); Pradhan, P. 4314 (KATH); Schilling, A.D. 1098 (K); Schilling, A.D. 706 (KATH); Schilling, A.D. 712 (KATH); Bailey, F.M. 34 (BM); de Haas, J.H. 2325 (BM); Dobremez, J.F. 1002 (KATH); Dobremez, J.F. 585 (BM); Halliwell, B. 140 (K); Kurmi, P.P. & Bhatt, G.D. 200387 (KATH); Manandhar, N.P. et al. 431 (KATH); Miyamoto, F., Rajbhandari, K.R., Akiyama, S., Amano, M., Ikeda, H. & Tsukaya, H. 9400031 (BM); Miyamoto, F., Rajbhandari, K.R., Akiyama, S., Amano, M., Ikeda, H. & Tsukaya, H. 9400031 (KATH); Miyamoto, F., Rajbhandari, K.R., Akiyama, S., Amano, M., Ikeda, H. & Tsukaya, H. 9420177 (KATH); Polunin, O.V. 1451 (BM); Polunin, O.V. 1625 (BM) (E); Polunin, O.V. 585 (BM); Rajbhandary, S.B. & Manandhar, N.P. 9296 (KATH); Saiju, H.K. & Amatya, P.M. 5877 (KATH); Shakya, P.R. & Adhikari, M.K. 723 (KATH); Shrestha, I. & Joshi, N. 3292001 (KATH); Stainton, J.D.A., Sykes, W.R. & Williams, L.H.J. 6378 (BM); Takayama, H., Arai, K., Hatta, H., Hoshino, T., Miyamoto, F., Subedi, M.N. & Takatsuki, S. 9220379 (BM); Bhattarai, N.K. 86307 (KATH); Bhattarai, N.K. 86372 (KATH); Bhattarai, N.K. 87219 (KATH); de Haas, J.H. 2119 (BM); Zimmermann, A. 1930 (BM); Banerjee, R.N. & Shrestha, T.B. 2797 (KATH); Rajbhandari, K.R. & Roy, B. 1990 (KATH); Dhwoj, L. 350 (BM) (E); Stainton, J.D.A. 8238 (E); Manandhar, N.P. 14013 (KATH); Manandhar, N.P. 14114 (KATH);Manandhar, N.P. 12667 (KATH); Shakya, P.R.; **Western,** Manandhar, N.P. 92591 (KATH); Manandhar, N.P. 97791 (KATH); Stainton, J.D.A., Sykes, W.R. & Williams, L.H.J. 3442 (BM); Stainton, J.D.A., Sykes, W.R. & Williams, L.H.J. 9010 (BM) (E); Stainton, J.D.A., Sykes, W.R. & Williams, L.H.J. 1659 (BM) (P); Stainton, J.D.A., Sykes, W.R. & Williams, L.H.J. 7488 (BM) (E); Stainton, J.D.A., Sykes, W.R. & Williams, L.H.J. 7927 (BM); Manandhar, N.P. & Acharya, S.K. 27192 (KATH); Manandhar, N.P. 108091 (KATH); Mikage, M., Anjiki, N., Kondo, N., Lacoul, P. & Yonekura, K. 9550155 (BM); Mikage, M., Hirano, R., Takahashi, A. & Yonekura, K. 9684234 (BM); Gardner, P.C. 1283 (BM); Manandhar, N.P. & Acharya, S.K. 9692 (KATH); Mikage, M., Yoshimitsu, M., Kaneda, A., Mouri, C., Tatsukawa, S., Asada, Y. & Senoo, M. 9964094 (KATH); Mikage, M., Yoshimitsu, M., Kaneda, A., Mouri, C., Tatsukawa, S., Asada, Y. & Senoo, M. 9964095 (KATH); Shakya, P.R. & Shrestha, T.B. 10624 (KATH); Stainton, J.D.A., Sykes, W.R. & Williams, L.H.J. 9052 (E) (KATH); Bhattarai, N.K. 85523 (KATH); Stainton, J.D.A., Sykes, W.R. & Williams, L.H.J. 5962 (BM); Manandhar, N.P. 9667 (KATH); Joshi, D.P. & Amatya, M.M. 741389 (KATH); Manandhar, N.P. & Regmi, P.M. 159 (KATH); Joshi, D.P. & Amatya, M.M. 731080 (KATH); Joshi, D.P. & Amatya, M.M. 741411 (KATH); Joshi, D.P. & Amatya, M.M. 73960

(KATH); **Mid Western,** Manandhar, N.P. 36191 (KATH); Polunin, O.V., Sykes, W.R. & Williams, L.H.J. 5595 (BM) (E); Adhikari, S.R. & Manandhar, N.P. 9186 (KATH); Malla, S.B. 14141 (KATH); Manandhar, N.P. & Joshi, D.P. 6897 (KATH); Polunin, O.V., Sykes, W.R. & Williams, L.H.J. 4934 (BM); Shakya, P.R. & Roy, B. 5876 (KATH); Shinozaki, K. (KATH); Shrestha, T.B. & Manandhar, N.P. 245 (KATH); Manandhar, N.P. & Regmi, P.M. 233 (KATH); Bhattarai, N.K. & Baral, S.R. 88503 (KATH); Bhattarai, N.K. & Baral, S.R. 88511 (KATH); Joshi, D.P. & Amatya, M.M. W34082 (KATH); Manandhar, N.P. & Bhattarai, N.K. 8646 (KATH); BRD A130 (E) (KATH) (TI) (TUCH); Shrestha, T.B. 24 (BM); Shrestha, T.B. 24 (KATH); Joshi, D.P. & Amatya, M.M. W28082 (KATH); Kurmi, P.P. KB110 (KATH); **Far Western,** Darchula 2012 1217010 (E) (KATH) (TI) (TUCH); Shakya, P.R., Adhikari, M.K. & Subedi, M.N. 8005 (KATH); Bista, M.S. & Joshi, D.P. 243 (KATH); Itoh, K. & Rajbhandary, S. 1261 (KATH); Yadar, L.B. 7 (KATH)

Altitudinal Range: 900-3900 m.

Ecology: Climbing over shrubs and small trees in *Quercus* forest, *Abies* and *Larix* forest, *Rhododendron barbatum* on degraded hillsides.

Flowering Time: July-October.

Fruiting Time: November-February.

National IUCN Status (Provisional): Data Deficient (DD).

Clematis buchananiana in Nepal has an EOO of 89,333 km^2 suggesting Least Concern (LC) and an AOO of 356 km^2 suggesting Endangered (EN), however there are no data on population or range fluctuations.

Notes: *Clematis staintonii* was originally described as being very close to *C. buchananiana* by Wang (2000) and differing only in being generally smaller. Yonekura (2006) in the Flora of Mustang treated *C. staintonii* as a synonym of *Clematis tortuosa*.

Clematis tortuosa was originally described by Fischer (1929) who distinguished it from *Clematis buchananiana* by its lighter hairs, different leaf shape and fewer flowers on the inflorescence. Although the original description did not hint at why the eptithet ‘tortuosa’ was used, Gupta (1961) illustrated *C. tortuosa* with a twisted anther, and this is possibly the origin of the name. It should however be noted that twisted anthers can be observed in specimens of many related species.

Grey-Wilson (2000) followed Hooker and Thomson's treatment of *C. tortuosa* as a variety of *C. buchananiana* which is distinguished by its glabrous leaflets. Johnson (2001) treated *C. tortuosa* as a distinct species based on pubescence and occasionally twisted anthers. Yonekura (2008) also treated *C. tortuosa* as a distinct species but justified this by the difference in the shape of the calyx.

There is therefore, little consistency in the characters that have been used to define *C. buchananiana* and its related taxa. The species that are included in the phylogeny (see chapter 3) form a polytomy, suggesting that they are phylogenetically very close, and in the absence of strong molecular and morphological evidence to the contrary it was decided to treat *C. staintonii* and *C. tortuosa* as synonyms of *C. buchananiana*.

A specimen at Kew [K000675183] which is with annotated Wall. Cat. 4675 and a later annotation of '= Wall Cat. 4676' is not considered to be the type of *C. vitifolia* as the catalogue number for that name looks like a much later annotation.

5. *Clematis confusa* Grey-Wilson Kew Mag. 8 (4): 161 (1991).

Annot. Checkl. Fl. Pl. Nepal 252 (2000). Grey-Wilson, *Clematis* the Genus: 145. (2000). Wang, Acta Phytotax. Sin. 39:14 (2001).

Type: Bhutan: Paro Chu. Between Drukgye Dzong and Sharna Sampa. Rushforth, K. D., #KR0765. Holotype: K! [K000075721], Isotypes: K! [K000075722], E! [E00438585]

Synonym: *Clematis connata* var. *confusa* (Grey-Wilson) W.T.Wang. *Acta Phytotax. Sin. 39: 14 2001*.

Description: Climber. Stems ribbed, glabrate. Leaves ternate or pinnate with 3 or 5 leaflets, petiole to 15 cm, glabrate to very sparsely pubescent; petiole bases fused to form a ring around the stem to 6mm wide. Leaflets ovate, shallowly 3-lobed, teminal leaflet occasionally deeply 3-lobed, 4.5--11 X 2.4--9 cm, base cordate, apex acuminate, margin coarsely serrate, glabrate to very sparsely pubescent above and below. Inflorescence axillary, cyme 1-5-flowered; peduncle to 6 cm, bracts leaf-like, falcate, 2 teeth towards base. Flowers brown, campanulate. Pedicels to 3.8 cm, ebracteolate. Sepals 4, ovate, 12--20 X 5--7 mm, erect, tip recurved, appressed golden pubescence outside, lanate inside, margin tomentose. Stamens to 15 mm; filaments lanate, to 11mm; anthers 3.5--4 mm, connective lanate, apex acute, pubescent. Achenes not seen.

Distribution: Nepal, E Himalaya.

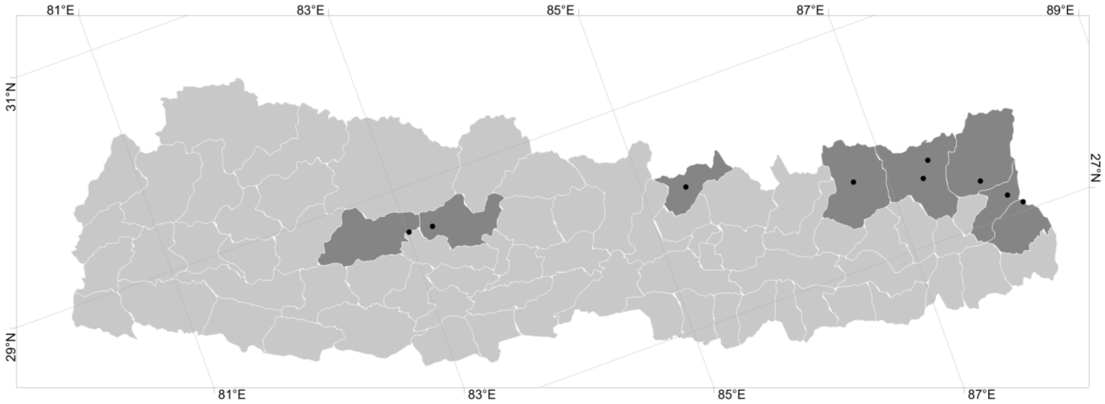


Figure 2.8 Distribution of *Clematis confusa* Grey-Wilson in Nepal.

Specimens examined: Eastern, DNEP3 BX300 (E), (KATH); LKSRC 222 (E); LKSRD 349 (E); Grey-Wilson, C., Sinnott, M., Zmartzy, S., Long, D.G., McBeath, R., Noltie, H.J., Subedi, M.N. & Crawford, S. 1148 (K). Stainton, J.D.A. 1444 (BM) (E) (KATH); **Central**: Banerjee, R.N. 461 (KATH). **Mid Western**: BRD A119. **Western:** Stainton, J.D.A., Sykes, W.R. & Williams, L.H.J. 4471 (BM).

Altitudinal Range: 2580-3600 m.

Ecology: Climbing on small shrubs in open forests, degraded secondary forests.

Flowering Time: September-October.

Fruiting Time: October.

Global IUCN Status (Provisional): Data Deficient (DD).

Clematis confusa has an EOO of 19,312 km^2 suggesting Vulnerable (VU) and an AOO of 44 km^2 suggesting Endangered (EN), however there are no data on population or range fluctuations.

Notes: The discovery of a new population of *Clematis confusa* in Rukum district during fieldwork in 2014 has increased the rage of this species which was previously known from Eastern Nepal, Sikkim and West Bhutan.

Clematis confusa is only known from a few localities in Nepal, Lachen in Sikkim and adjacent western Bhutan. Vegetatively this species is easily confused with *Clematis connata*. *Clematis confusa* differs in its floral morphology by having 1 to 9 brown flowers in its inflorecence, while *C. connata* has up to 9 pale yellow to white flowers. It is therefore possible that *C. confusa* has been under-recorded because it has been misidentified as *C. connata* when sterile.

This species was not included in Johnson's monograph (2001) although it had been published nine years previously.

Wang (2001) reduced *Clematis confusa* to synonymy as part of the preparation for the *Flora of China* account. However, I agree with Grey-Wilson's original assertion that *C. confusa* is distinct from *C. connata* because of the differences in their flowers. The phylogenetic analysis also suggests that the two species are distinct.

While the vegetation morphology of the mid-western specimen of *C. confusa* is larger and the connective is more sparsley lanate than that if the Eastern Nepalese specimens and Type material, it does not nessecitate and distinct taxon. The disjunct in the distribution probably suggests that *C. confusa* is undercollected or mis-identified when the specimens are sterile.

The fuiting time is taken from the Bhutanese isotype at E.

An image of what is probably this species was collected in Thangba VDC, Rolpa District by Shanta Budha Magar and Prabin Bhandari who are to deposit specimens in TUCH and KATH.

This species is in cultivation in the UK under the Chris Chadwell collection number CC5478. The exact origin of this material is not known, and it appears to have been collected and exported from Nepal without permits.

6. *Clematis connata* DC. Prodr. 1:4 (1824).

Don, G., Gen.Sys. 1:5 (1831). Hooker, J. D. & Thomson, Fl. Indica 1:11 (1855). Hooker, Fl. Brit. India 1:6 (1872). Kuntze, Verh. Bot. Vereins Prov. Brandenburg 26:131 (1885). Gupta, Bull. Lucknow Natl. Bot. Gard. 80: Pl. 26 (1963). Kapoor, Bull. Lucknow Natl. Bot. Gard. 124:26-30(1966). Hara, Enum. Fl. Pl. Nepal 2:14 (1979). Grierson, Fl. Bhutan 1(2):290 (1984). Riedl & Nasir. Fl. Pakistan 193:91 (1991). Annot. Checkl. Fl. Pl. Nepal 252 (2000). Grey-Wilson, *Clematis* the Genus: 138. (2000). Johnson, The Genus *Clematis*: 298-299 (2001). Wang & Bartholomew, Fl. China 6:380 (2001). Wang, Acta. Phytotax. Sin. 39(1): 1 (2001).

Type: Nepal, Wallich Numer. List #4679. Lectotype: BM! [BM000521577] designated here. Syntypes E! [E00507832 & E00507831].

Synonymy: *Clematis venosa* Royle. Ill. Bot. Himal. Mts. 51. (1839). Type: India [Or Pakistan]. NW India. Royle *s.n.* Isotype K! [K000075701]
Clematis amplexicaulis Edgew. Trans. Linn. Soc. London. 20(1):24 1846 [1851 published 29 Aug 1846 - T.L.2] Type: Edgeworth *s.n.* 1844 nr Dhanaulti K! [K000075699]
Clematis velutina Edgew. Trans. Linn. Soc. London. 20(1):25 1846 [1851 published 29 Aug 1846 - T.L.2] Type: Edgeworth 5 1844 nr Simla K! [K000075700]
Clematis buchananiana subsp. *connata* (DC.) Kuntze. Verh. Bot. Vereins Prov. Brandenburg 26:131 (1885).
Clematis buchananiana subsp. *connata* var. *latipes* Kuntze. Verh. Bot. Vereins Prov. Brandenburg 26:131 (1885). [no specimen cited in protologue]
Clematis coriigera H.Lév. Repertorium Specierum Novarum Regni Vegetablis. 12:281 (1913). Type: E.E. Maire. Sept 1912. [ex herb. Lev.] E! Holotype[E00081543] Isotype [E00081544].
Clematis gracilis Edgew. Transactions of the Linnean Society of London. 20(1):24 1846 [1851 published 29 Aug 1846 - T.L.2] Type: Edgeworth 6 1844 nr Shioli K! [K000075698]
Clematis buchananiana Buch.-Ham. ex DC. var. *trullifera* Franch. Pl. dalavay. 3 (1889). Delavay 20 Jan 1887 #3127. Holotype: P! [P00145083]. Isotype: K! [K000677958]
Clematis trullifera (Franch.) Finet & Gagnep. Bulletin de la Société Botanique de France. 50:547 (1904).
Clematis connata var. *trullifera* (Franch.) W.T.Wang Acta Phytotaxonomica Sinica. 36(2): 170 (1998).
Clematis connata var. *lanceolata S.N.*Biswas. Indian Journal of Forestry. 8(1):61 (1985). Type: India, [Uttarakhand] Tehri Garwhal: Kaudia. Sahni 21545 Holotype (DD) [Not Seen]
Clematis nutans Royle var. *pseudoconnata* Kuntze Verh. Bot. Vereins Prov. Brandenburg 26:131 (1885). Type: Nepal, Wallich Numer. List #4679. Cited by Kuntze as *Clematis buchananiana* v. *connata* (Wallich, mus. brit.) [BM000521577] *nom. illeg. superfl.*

Description: Climber. Stems ribbed, pubescent to pilose. Leaves pinnate, 5 or 7 leaflets, petiole to 8 cm, sparsely pubescent to pilose, petiole bases fused to form a ring around the stem to 6 mm wide. Leaflets ovate to narrowly elliptic, 2--14 X 1--10 cm, bases rounded to truncate, apex acute to short acuminate, margins coarsely serrate but irregular, sparsely pubescent above and below when young becoming glabrate when mature. Inflorescences axillary, cyme to 3--9-flowered, peduncle 10--20 cm, bracts leaf-like, entire to shallowly 3-lobed, obovate to ovate or falcate, toothed to irregularly toothed, pubescent. Flowers pale yellow to white, campaulate. Pedicel to 3--5 cm, golden pubescent to velutinus; bracteoles leaf-like, entire to shallowly 3-lobed,obovate to ovate or falcate, toothed to irregularly toothed, pubescent or glabrate. Sepals 4, narrowly ovate, 15--30 X 2--8 mm, erect, tip recurved, apex acute to obtuse, pubescent to velutinous outside, margin tomentose, pubescent inside but glabrous toward base. Stamens to 12 mm; filaments pubescent, villous at the middle, glabrescent at the apex, 7--12 mm; anthers 3--5 mm, connective glabrous, acutely protruding or not. Achenes obovate or elliptic almost orbicular, 3--4 X 2.5 mm, densely silky hairy; persistent style to 3.5 cm, plumose.

Distribution: Nepal, W Himalaya, E Himalaya, Tibetan Plateau, E Asia.

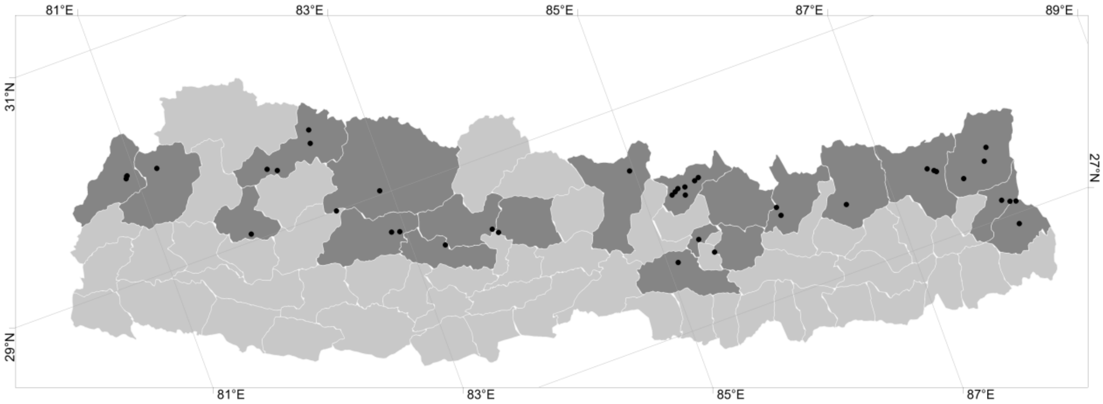


Figure 2.9 Distribution of *Clematis connata* DC. in Nepal

Specimens examined: **Eastern**, Beer, L.W. 9500 (BM); EMAK 870 (E); Pradhan, P., Manandhar, N.P. & Amatya, N. 1099 (KATH); Hara, H., Kanai, H., Kurosawa, S., Murata, G., Togashi, M. & Tuyama, T. 6304322 (KATH); Hara, H., Kanai, H., Kurosawa, S., Murata, G., Togashi, M. & Tuyama, T. 6304330 (BM); Hara, H., Kanai, H., Kurosawa, S., Murata, G., Togashi, M. & Tuyama, T. 6304330 (KATH); Hara, H., Kanai, H., Kurosawa, S., Murata, G., Togashi, M. & Tuyama, T. 6304331 (KATH);Beer, L.W. 25673 (BM); Beer, L.W., Lancaster, C.R. & Morris, D. 148 (BM); Grey-Wilson, C. et al. 4502 (K); Grey-Wilson, C., Sinnott, M., Zmartzy, S., Long, D.G., McBeath, R., Noltie, H.J., Subedi, M.N. & Crawford, S. 1148 (E); Grey-Wilson, C., Zmartzy, S., Sinnott, M., Long, D.G., McBeath, R., Noltie, H.J. & Subedi, M.N. 287 (E) (KATH); Hara, H., Kanai, H., Kurosawa, S., Murata, G. & Togashi, M. 6304328 (KATH); Hara, H., Kurosawa, S. & Tuyama, T. 6204325 (KATH). **Central**, Buchanan-Hamilton, F. (LINN); Joshi, D.P. & Rajbhandari, K.R. 75|812 (KATH);

Kanai, H. 11139 (KATH); Manandhar, R.K. 10752 (KATH); Bhattarai, N.K. 861055 (KATH); Bhattarai, N.K. 86950 (KATH); Maine, A. 440 (BM); Miyamoto, F., Rajbhandari, K.R., Akiyama, S., Amano, M., Ikeda, H. & Tsukaya, H. 9410035 (BM); Stainton, J.D.A. 5130 (BM); Kanai, H., Chuma, C. & Nagano, T. 674041 (KATH); Banerjee, R.N., Shrestha, T.B. & Upadhyay, A.V. 2740 (KATH); Banerjee, R.N., Shrestha, T.B. & Upadhyay, A.V. 2797 (KATH); Shah, S.B. 182 (KATH); **Western**, Manandhar, N.P. & Acharya, S.K. 511-92 (KATH); Malla, S.B., Shrestha, J. & Rajbhandari 13821 (KATH); Suzuki, M., Acharya, N., Fujii, N., Joshi, L., Kajita, T., Kondo, N., Mikage, M., Noshiro, S. & Yoda, K. 9455200 (BM); Shrestha, T.B. & Bista, M.S. 1709 (KATH); **Mid Western**, BRD A289 (E) (KATH) (TI) (TUCH); Minaki, M., Joshi, K.K., Kadota, Y., Sugita, H., Takahashi, A., Tsuda, S., Yagi, H. & Yonebayashi, C. 9109217 (BM); Minaki, M., Joshi, K.K., Kadota, Y., Sugita, H., Takahashi, A., Tsuda, S., Yagi, H. & Yonebayashi, C. 9109223 (BM); Shrestha, T.B. & Manandhar, N.P. 9114 (KATH); Manandhar, N.P. & Joshi, D.P. 7076 (KATH); Polunin, O.V., Sykes, W.R. & Williams, L.H.J. 5296 (BM) (E); Shakya, P.R., Subedi, M.N. & Uprety, R.K. 9771 (KATH); Shrestha, T.B. & Manandhar, N.P. 209 (KATH); Shrestha, T.B. & Manandhar, N.P. 225 (KATH); Stainton, J.D.A., Sykes, W.R. & Williams, L.H.J. 4714 (BM) (E); **Far Western**, Darchula 2012 1217026 (E) (KATH) (TI) (TUCH); Darchula 2012 1217027 (E) (KATH) (TI) (TUCH); Darchula 2012 1217028 (E) (KATH) (TI) (TUCH); Shakya, P.R., Adhikari, M.K. & Subedi, M.N. 8282 (KATH)

Altitudinal Range: 1350-3400 m.

Ecology: Climbing through shrubs and on trees in *Abies*, *Rhodendron*, *Betula* forest.

Flowering Time: July-October.

Fruiting Time: September-October.

National IUCN Status (Provisional): Data Deficient (DD).

Clematis connata has an EOO of 69,510 km^2 suggesting Least Concern (LC) and an AOO of 164 km^2 suggesting Endangered (EN), however there are no data on population or range fluctuations.

Notes: This species is widespread species throughout the Himalaya. Sterile specimens can be mis-identified with *Clematis confusa* because of the characteristic winged petiole bases for these species. However in flower *C. connata* is easily distinguished from these species by its pale yellow to white flowers.

The original description by de Candolle (1824) did not cite a specimen number, only ‘Wallich Nepalia’. Wallich’s Numerical List (Wallich, 1828) gives specimens of this name under 4679 citing that they were in the herbarium 1824 and Nepalia 1821.

K.K. Shrestha annotated the BM specimen as holotype, which is now designated a lectotype here.

Figure 2.10 ***Clematis connata*** **BRD A289. Top: immature flowering stem. Bottom: Unopened flower.**

7. Clematis gouriana Roxb. ex DC. Syst. Nat. 1: 138–139. 1818[1817]

Roxburgh, Hort Bengal. 43 (1814). Candolle, Prodr. 1:9(1824). Don, G., Gen.Sys. 1:4 (1831). Roxburgh, Fl. ind. Ed 1832 2:670-671 (1832). Hooker, J. D. & Thomson, Fl. Indica 1:8 (1855). Hooker Fl. Brit. India 1:4 (1872). Kuntze Verh. Bot. Vereins Prov. Brandenburg 26:100 (1885). Kapoor, 78:22-29 (1962). Bull. Lucknow Natl. Bot. Gard., Gupta, in Bull. Lucknow Natl. Bot. Gard. 54: pl.40 (1961). Gupta, in Bull. Lucknow Natl. Bot. Gard. 80: pl.27 (1966). Hara, Enum. Fl. Pl. Nepal 2:14 (1979). Grierson, Fl. Bhutan 1(2):290 (1984). Riedl & Nasir, Fl. Pakistan 193:86-88 (1991). Annot. Checkl. Fl. Pl. Nepal 252 (2000). Grey-Wilson. *Clematis* the Genus: 38-39 (2000). Johnson, The Genus *Clematis*: 428 (2001). Wang & Bartholomew, Fl. China 6:349 (2001). Wang, Actca Phytotax. Sin. 41(1):37-38.

Type: Roxburgh, Calcutta 1809. (G!) [G00358187], (K) ! [K000675151]

Synonym: *Clematis gouriana* Roxb. Hort. Bengal. 43 (1814) nom. nud.

Clematis cana Wall. Numer. List # 4672 (1828) nom. nud.

Clematis vitalba subsp. *gouriana* (Roxb.) Kuntze. Verh. Bot. Vereins Prov. Brandenburg 26:100 (1885). [as *C. vitalba* subsp. *gauriana*].

Clematis martini H.Lév. Fl. Kouy-Tchéou 3:53 (1915) Types: China, Hao-Kiang, Esquirol, J. H. [Rev. Père] 576, Aug. 1905. E! [E00081569] & China, Pien-Yang to So-Fou Cavalerie, P. J., 2662 Nov. 1905 E! [E00081568]

Description: Climber. Stems ribbed, sparsely pubescent, almost canescent at nodes. Leaves pinnate occasionaly basal leaflets bipinnate, 5 (or 9), petiole flattened at base. Leaftlets, broad ovate to narrowly ovate, 3--10 X 1--4.5 cm, base rounded or cordate, apex acute to mucronate, simple or denticulate margin, sparsely pubescent above, sparse pubescent below, pubescent on veins. Inflorescence an axillary or terminal cyme, 9 to many-flowered; bracts leaf-like, simple or 3-lobed or ternate. Flowers white to creamy white, open. Sepals 4(--5), oblong or obovate, 5--7 X 1.5--2 mm,spreading, apex obtuse,,glabrate or pubescent inside, pubescent outside, tomentose margin. Pedicles 7--17 mm, pubescent; bracteoles small, awl-shaped, c. 2mm, glabrescent or sparsely pubescent. Stamens to 9 mm; filaments linear, 2--7 mm, glabrous; anthers 1--1.5 mm glabrous, connective acutely protruding. Achenes oblong or narrowly ovate, 4 X 1 mm, pubescent, distinct narrow rim; persistent style to 6 cm, plumose.

Distribution: Nepal, W Himalaya, E Himalaya, Assam-Burma, S Asia, E Asia, SE Asia.

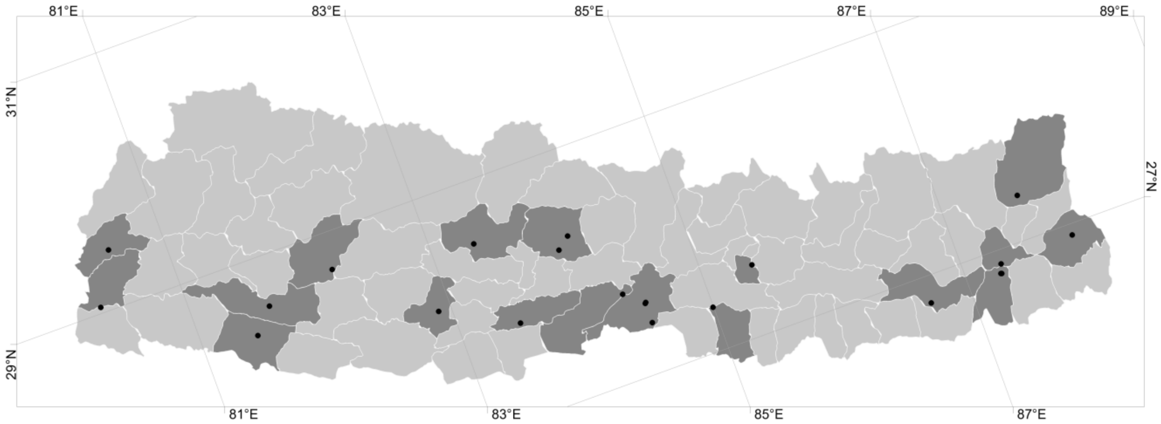


Figure 2.11 Distribution of *Clematis gouriana* Roxb. ex DC

Specimens Examined: **Eastern**, Dhankuta Pradham, P., Manandhar, N.P. & Amatya, N. 737 (KATH); Shrestha, T.B. & Bhattacharya, T.K. 72286 (KATH); Sunsari, Stainton, J.D.A. 1804 (BM (E) (KATH); Taplejung, Kanai, H., Murata, G. & Togashi, M. 6300440 (BM); Kanai, H., Murata, G., Togashi, M. & Tuyama, T. 6300440 (KATH); Udyapur, Stainton, J.D.A. 6651 (BM); **Central**, Patan, Manandhar, R.K. et al. 7439 (KATH); Dolkha, Shakya, P.R. & Ohsawa, M. 1014 (KATH); Bara, DNEP2 A157; Chitwan, DNEP2 A84; Joshi, D.P., Bajracharya, I. & Kayastha, R. 753390 (KATH); Manandhar, N.P. 13844 (KATH); Manandhar, N.P. 13881 (KATH); Pendry, C.A., Shrestha, K.K., Miller, A.G., Giri, A., Dahal, S., Pandey, N., Pullan, M.R., Shrestha, S., Shrestha, K.K. & Shakya, L.R. A157 (KATH); Pendry, C.A., Shrestha, K.K., Miller, A.G., Giri, A., Dahal, S., Pandey, N., Pullan, M.R., Shrestha, S., Shrestha, K.K. & Shakya, L.R. a84 (KATH); Suzuki, M., Acharya, N., Kurosawa, T., Lacoul, P., Mikage, M., Takahashi, A. & Yoda, K. 9611011 (BM); Tabata, H., Rajbhandari, K.R., Tsuchiya, K. & Konno, Y. 7565 (BM); **Western**, Baglung, Manandhar, N.P. & Acharya, S.K. 59392 (KATH); Myagdi, Stainton, J.D.A., Sykes, W.R. & Williams, L.H.J. 9192 (BM); Kaski, Stainton, J.D.A. 6088 (BM); Stainton, J.D.A., Sykes, W.R. & Williams, L.H.J. 8959 (BM); Gulmi, Joshi, D.P. & Amatya, M.M. 731099 (KATH); Nawalparasi, Mikage, M., Acharya, N., Kurosawa, T. & Takahashi, A. 9611048 (BM); Palpa, Stainton, J.D.A., Sykes, W.R. & Williams, L.H.J. 8803 (BM) (E); **Mid Western**, Bardia, Kurmi, P.P. 623 (KATH); Sharma, L.R. 5003 (KATH); Shukla, R.N. 5110 (KATH); Jajarkot, Polunin, O.V., Sykes, W.R. & Williams, L.H.J. 5718 (BM); Surkhet, Manandhar, N.P. 30791 (KATH); Pyuthan, Bhattarai, N.K. & Baral, S.R. 88327 (KATH); Rolpa, Bhattacharya, T.K. & Shrivastava, V.K. 84-8528 (KATH); Manandhar, N.P. & Bhattarai, N.K. 8789 (KATH); **Far Western**, Baitadi; Kattel, L.P. 157 (KATH); Dadeldhura, Rajbhandari, K.R., Bhattarai, S., Sharma, I. & Joshi, R. 7048 (KATH)

Altitudinal Range: 140-2100 m.

Ecology: Climbing through shrubs in mixed *Bombax* forests; riverine forest with *Pinus roxburghii*, *Shorea robusta*, *Lagerstroemia parvifolia;* distrubed riverine forest with *Trewia nudiflora* and *Litsea salicifolia*.

Flowering Time: October-November.

Fruiting Time: November-January.

National IUCN Status (Provisional): Data Deficient (DD).

Clematis gouriana has an EOO of 66,850 km^2 suggesting Least Concern (LC) and an AOO of 84 km^2 suggesting Endangered (EN), however there are no data on population or range fluctuations.

Notes: This species was first described from material collected in the ruins of the old Bengal capital of Gour, root of the epithet, in 1800 by Henry Creighton.

Clematis gouriana is similar to the European *Clematis vitalba* in terms of morphology and is in effect the Asian equivalent and was deemed by Kuntze to be a subspecies of *C. vitalba*.

This is a widespread species which occurs across much of southern Asia. As it is a species of sub-tropical to warm temperate forests it is probably under-collected in Nepal and will be found more widely than currently recorded.

Wang (2003) in his revision of *Clematis* sect. *Clematis* cited that he had not seen the Type at BM, but later annotated the G specimen as Holotype.

8. *Clematis grata* Wall. Pl. Asiat. Rar. 1:83.t.98. 1830.

Royle, Ill. Bot. Himal. Mts. 1:51 (1839). Hooker, J. D. & Thomson, Fl. Indica 1:7 (1855). Hooker Fl. Brit. India 1:4 (1872). Kuntze Verh. Bot. Vereins Prov. Brandenburg 26:100 (1885). Gupta, Bull. Lucknow Natl. Bot. Gard. 54 pl.40 (1961) Kapoor, Bull. Lucknow Natl. Bot. Gard. 78:22 (1962). Hara, Enum. Fl. Pl. Nepal 2:14 (1979). Riedl & Nasir. Fl. Pakistan 193:88-89 (1991). Wang, Acta. Phytotax. Sin. 36:155 (1998). Annot. Checkl. Fl. Pl. Nepal

252 (2000). Wang & Bartholomew, Fl. China. 6:350 (2001). Johnson, Clematis 430-432 (2001). Grey-Wilson. *Clematis* the Genus: 39-40 (2000). Yonekura,Fl. Mustang 70-71 (2008).
Type: India: Kumaon, Wall. Numer. List #4668B [Robert Blinkworth]. Isosyntype (E) ! [E00505136]; Sirmore, Wall. Numer. List #4668D, [Dr. Govan], Isosyntype: (E!) [E00505137].

Synonyms: *Clematis grata* Wall. Num. List. 4668. (1828). *nom nud*.

Clematis vitalba subsp. *grata* (Wall.) Kuntze, Verh. Bot. Vereins Prov. Brandenburg 26:100 (1885).

Clematis cordata Royle, Ill. Bot. Himal. 51 (1839). [No specimen seen]

Description: Climber. Stem ribbed, pubescent, canescent at the nodes. Leaves pinnate, 5 leaflets, petiole to 5cm. Leaflets ovate, 4.5--7 X 4.5--5 cm, base rounded, apex acute to shortly acuminate, margin coarsely serrate, glabrate above, pubescent on veins, sparsely pilose below, denser on veins. Inflorescence axillary, cyme, 9 to many flowered; peduncle to 13 cm; bracts simple, 3-lobed or petiolate ternate, with 1-2 teeth, ca. 1cm, densely pubescent. Flowers white, open. Pedicel to 4 cm, densely pubescent to canescent; bracteoles linear or awl-like to 1mm, occasionally 1-2 teeth. Sepals 4, ovate to obovate, 5 X 2mm, spreading becoming reflexed, apex acute, glabrous inside, densely pubescent outside. Stamens to 5 mm, entirely glabrous; filaments linear to 4 mm; anthers ca. 1 mm, connective acutely protruding. Achenes ovate almost round, 2--2.5 X 2 mm, pubescent, distinct rim; persistent style to 3 cm.

Distribution: Nepal, W Himalaya.

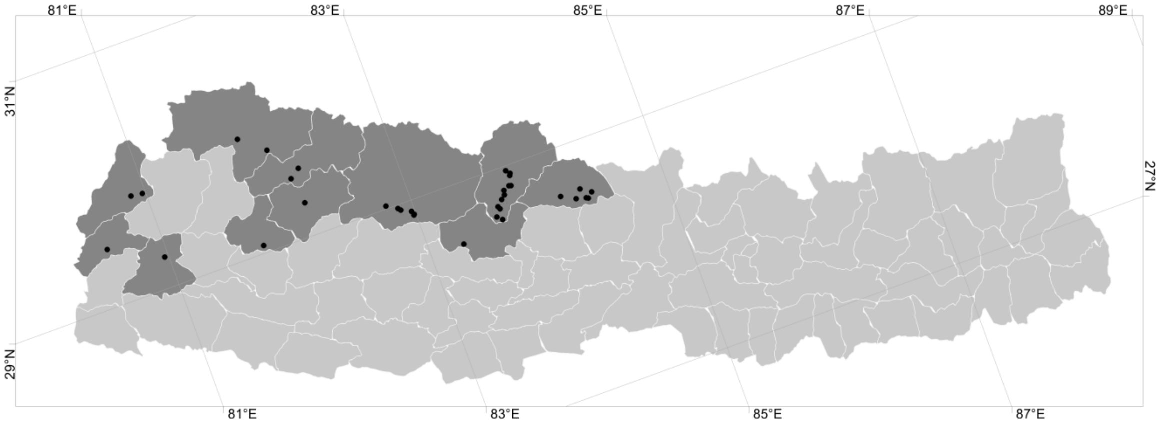


Figure 2.12 Distribution of *Clematis grata* Wall. in Nepal

Specimens Examined: **Western,** Hoshino, T., Amano, M., Koba, H., Miyoshi, N., Rajbhandari, K.R., Sato, M., Shrestha, P. & Takatsuki, S. 9666109 (BM) (E); Manandhar, V.K. 010 (KATH); Mikage, M., Anjiki, N., Kondo, N., Lacoul, P. & Yonekura, K.9550348 (BM) (KATH); Shrestha, T.B. & Bista, M.S. 2079 (KATH); Shrestha, T.B. & Bista, M.S. 2198 (KATH); Shrestha, T.B. & Bista, M.S. 2334 (KATH); Shrestha, T.B. 5461 (KATH); Stainton, J.D.A. 5580 (BM); Stainton, J.D.A., Sykes, W.R. & Williams, L.H.J. 1495 (BM) (P); Stainton, J.D.A., Sykes, W.R. & Williams, L.H.J. 1495 (P); Subedi, M.N. 0040078 (KATH); Subedi, M.N. 2862002 (KATH); Subedi, M.N. 952002 (KATH); Polunin, O.V., Sykes, W.R. & Williams, L.H.J. 5102 (E); Dobremez, J.F. 684 (BM); Joshi, D.P. & Amatya, M.M. 73450 (KATH); Manandhar, N.P. 9793 (KATH); Manandhar, N.P. 9954 (KATH); Manaslu 08 20812289 (E); Mikage, M., Acharya, N., Kurosawa, T., Lacoul, P., Takahashi, A. & Yoda, K. 9460329 (BM); BRD A198 (E) (KATH) (TI) (TUCH); **Mid Western**, BRD A208 (E) (KATH) (TI) (TUCH); Einarsson, S., Skärby, L. & Wetterhall, B. 2669 (BM); Einarsson, S., Skärby, L. & Wetterhall, B. 2669 (KATH); Minaki, M., Joshi, K.K., Kadota, Y., Sugita, H., Takahashi, A., Tsuda, S., Yagi, H. & Yonebayashi, C. 9109339 (BM); Shrestha, K.K. SP044A (KATH); Tabata, H., Keshab R. & Tsuchiya, K. 3402 (BM);JRS B157 (E) (KATH); Malla, S.B. 14207 (KATH); Itoh, K. & Rajbhandary, S. 1174 (KATH); Manandhar, N.P. & Joshi, D.P. 6789 (KATH); Shakya, P.R., Subedi, M.N. & Uprety, R.K. 8482 (KATH); Malla, K.J., Roy, B. & Watanabe, T. 1185 (KATH);Polunin, O.V., Sykes, W.R. & Williams, L.H.J. 5102 (BM); Shakya, P.R., Subedi, M.N. & Uprety, R.K. 8774 (KATH); **Far Western,** Sharma, I., Joshi, R., Uprety, R. & Pandey, J. 587; Amatya, M.M. & Regmi, P.M. W69082 (KATH); Amatya, M.M. & Regmi, P.M. W69882 (KATH); Shakya, P.R., Adhikari, M.K. & Subedi, M.N. 7925 (KATH); Shakya, P.R., Adhikari, M.K. & Subedi, M.N. 7939 (KATH);Sharma, I., Joshi, R., Uprety, R. & Pandey, J. 507 (KATH)

Altitudinal Range: 1300-3800 m.

Ecology: Climbing over shrubs. Open forests, degraded hillsides near settlements.

Flowering Time: June-September.

Fruiting Time: July-December.

National IUCN Status (Provisional): Data Deficient (DD).

Clematis grata has an EOO of 26,502 km^2 suggesting Near Threatened (NT) and an AOO of 136 km^2 suggesting Endangered (EN), however there are no data on population or range fluctuations.

Notes: There is an excellent plate of *Clematis grata* in *Plantae Asiaticae Rariores*. Wallich cited two collections in the protologue Dr. Govan collection from Sirmore and a Robert Blinkworth collection from Kumaon. The Wallich Catalogue also lists two additional collections of *C. grata* not mentioned by Wallich in the protologe: 4668A, H.Bl. [specimen at K! [K000675150] and 4668C from Srinaghur specimen at E ! [E00505128]

This species is a western Himalayan element which does not occur to the east of the Stainton Line. Although Stainton (1972) described *C. grata* as a western Himalayan element, he mentions varieties distributed in China and E Asia but does not specify what they are. Wang (1993) transferred *Clematis grata* var. *argentilucida* (H.Lév.& Vaniot) Rehder to *Clematis apiifolia* var. *argentilucida* (H.Lév.& Vaniot) W.T.Wang, *Clematis grata*. var. *lobulata* Rehder & E. H. Wilson to *Clematis gratopsis* W.T.Wang, *Clematis grata* var. *grandidentata* Rehder & E. H. Wilson *to Clematis grandidentata* (Rehder & E. H. Wilson) W. T. Wang var. *grandidentata* and *Clematis grata* var. *likiangensis* Rehder to *Clematis grandidentata* (Rehder & E. H. Wilson) W. T. Wang var. *likiangensis* (Rehder) W.T. Wang

During fieldwork in 2014 this species was observed to be very locally abundant along the Thuli Bheri Nadi in Dopla district. The flowers have a distinctive synthetic soapy scent when in full sun.

Specimens from India were use to supplement the range of the fruiting time for this species.

9. *Clematis graveolens* Lindl. J. Hort. Soc. London 1:307 (1846)

Hooker, J.D. & Thomson, Fl. ind. 9 (1855). Hooker, J.D. Fl. Brit. India 1:4 (1875). Kuntze, Ver Bot. Vereins. Prov. Brandenburg 26:124 (1885).Gupta, Bull. Lucknow Natl. Bot. Gard. 54 pl.11 (1961) Kapoor, Bull. Lucknow Natl. Bot. Gard. 78:34 (1962). Hara, Enum. Fl. Pl. Nepal 2:15 (1979). Grey-Wilson, Kew Bull. 44(1):33-60 (1989). Riedl & Nasir, Fl. Pakistan 193:92-93 (1991). Annot. Checkl. Fl. Pl. Nepal 252 (2000). Grey-Wilson, *Clematis* the Genus 166. (2000). Johnson, The Genus *Clematis* 335 (2001). Wang, *Acta Phytotax.* Sin. 42:326 (2004).

Type: Cultivated Specimen. Wm Munro *s.n.* (CAMB-Lindley Herb.) [NOT SEEN]

Synonymy: *Clematis orientalis* subsp. *graveolens* (Lindl.) Kuntze. Verh. Bot. Vereins Prov. Brandenburg 26: 124 1885.

Clematis orientalis subsp. *graveolens* var. *aitchisoni* Verh. Bot. Vereins Prov. Brandenburg 26: 124 1885. Types: Aitchison Kurum Valley 614, 718 (P!) [P00140441].

Clematis orientalis subsp. *graveolens* var. *subtripinnata* Kuntze. Verh. Bot. Vereins Prov. Brandenburg 26: 124 1885. Type: India: Kunwar, Falconer 6. (K!) [K000675164].

Clematis parvifolia Edgew. *Trans. Linn. Soc. London 20: 25 (1851) Type: India [?]:* Kundau and Bearvallies, NW Himalaya. *Edgeworth 1051 (K)! [K000675163].*

Description: Climber. Stems ribbed, glabrate, pubescent at the nodes. Leaves bipinnate, 5 or 7 leaflets, petiole 3.5--5 cm, glabrate to sparsely pubescent. Leaflets elliptic or narrowly ovate, 10--35 X 5--14 mm, 1--3 small lobes near base, glabrate above, glabrate or sparsely pubescent below. Inflorescence axillary occassionally terminal, solitary or cymule 3-flowered; peduncle to 9 cm; bracts leaf-like but smaller, entire or 3-lobed. Flowers yellow, open. Pedicels to 10 cm; ebracteolate. Sepals 4, elliptic, 11--15 X 5--10 mm, spreading or reflexed, tip recurved or not, apextruncated or emarginate, glabrate outside with tomentose margin, pubescent to languinose inside with glabrate margin. Stamens to 12 mm entirely glabrous or sparsely pilose; filaments to 10 mm; anthers c. 2mm. Achenes ovate to obovate, 2--3 X 1--2 mm, flat, distinct rim, canescent; peristent style to 4 cm, plumose.

Distribution: Nepal, W Himalaya.

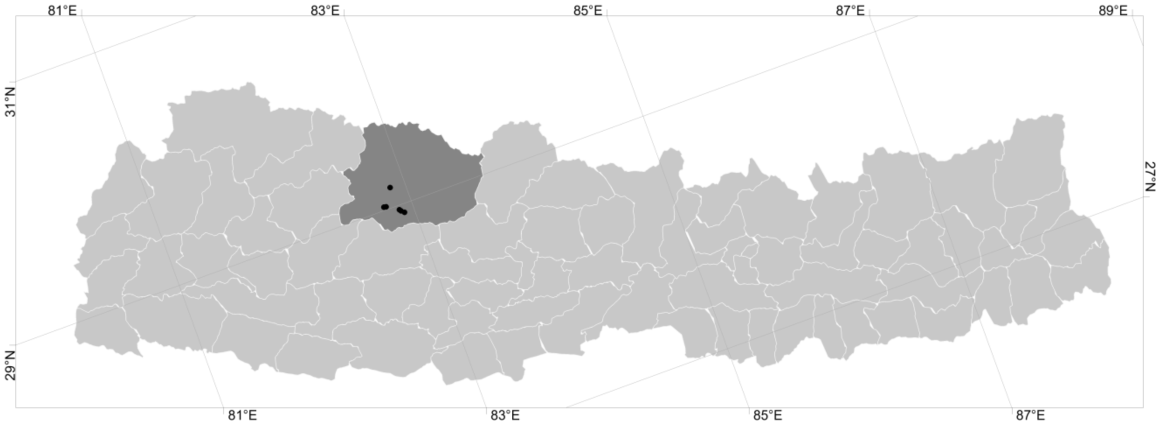


Figure 2.13 Distribution of *Clematis graveolens* Lindl. in Nepal

Specimens examined: Mid Western, BRD A216 (E) (KATH) (TI) (TUCH); BRD A220 (E) (KATH) (TI) (TUCH); Einarsson, S., Skärby, L. & Wetterhall, B. 1673 (BM); Einarsson, S., Skärby, L. & Wetterhall, B. 1673 (KATH); Polunin, O.V., Sykes, W.R. & Williams, L.H.J. 3339 (BM) (E); Shakya, P.R. & Roy, B. 5914 (KATH); Shrestha, K.K. & Ghimire, S.K. (KATH); Shrestha, T.B. 5266 (BM) (KATH)

Altitudinal Range: 2000-3200 m.

Ecology: Trailing down river banks, over rocks and shrubs.

Flowering Time: August-September.

Fruiting Time: August-December.

National IUCN Status (Provisional): Data Deficient (DD).

Clematis graveolens has an EOO of 155 km^2 suggesting Endangered (EN) and an AOO of 24 km^2 suggesting Endangered (EN), however there are no data on population or range fluctuations.

Notes: In Nepal this species is known only from Dolpa District. Then appears again 600 km west in NW India in the hills around Shimla and occurs as far west as Afghanistan. As this is a fairly distinct species with a large distribution in the west Himalaya it is possible that the disjunct is merely an artefact of collection and that is also occurs in the rest of the Nepalese transhimalaya and the bordering Indian states.

Clematis graveolens is a readily identifiable among the Nepalese species because the apex of the sepals are emarginate, and it has very small leaflets. Indeed, foliage alone is enough to distinguish sterile specimens from all other Nepalese species.

Wang (2004) excluded *C. graveolens* from section *Meclatis,* and placed it in section Brachiatae claiming that its floral morophology is “identical” to *C. brachiata* and allies. However, the two samples *of C. graveolens* fall with in a well supported clade consisting of species from Section *Meclatis* not *Brachiatae* (see chapter 3), so his conclusion is not supported by this study.

Fruiting times have been supplemented from Indian specimens as only BRD A216 and A220 had evidence of achenes.

Figure 2.15 *Clematis grata* BRD A220. Top: Leaf. Bottom. Sepals with characteristic notched apex.

10. *Clematis grewiiflora* DC. Syst.Nat. 1:140 (1817)

Candolle, Prodr. 1:4 (1824). Don, D., Prodr. fl. nepal. 191. (1825). Don, G., Gen.Sys. 1:5 (1831). Hook.f & Thomson Fl. indica 1:10 (1855). Hooker, J.D. Fl. Brit. India 1:6 (1875). Hooker, Bot. Mag. t.6369 (1879). Kuntze, Verh. Bot. Vereins Prov. Brandenburg 26: 130 (1885). Hara, Enum. Fl. Pl. Nepal 2:15 (1979). Gupta, Bull. Lucknow Natl. Bot. Gard. 80: Pl. 28 &29 (1963). Kapoor, Bull. Lucknow Natl. Bot. Gard. 124: 30-33 (1966). Grierson, Fl. Bhutan 1(2):290 (1984). Annot. Checkl. Fl. Pl. Nepal 252 (2000). Grew-Wilson, *Clematis* the Genus. 143 (2000). Johnson, The Genus *Clematis*. 301-302 (2001). Wang & Bartholomew, Fl. China. 6: 378 (2001)

Type: Nepal, Harain [Narain] Hetty. Buchanan- Hamilton *s.n.* Lectotype: (BM ex Herb. Lam.)! [BM000521578] designated here, Syntype: (LINN)! [LINN-HS 974.22.1].

Synonyms: *Clematis loasiflora* DC. Syst. Nat. 1:140 (1818). Type: Nepal, Narainhetty. Buchanan-Hamilton, *s.n.* (26 Oct 1802). (LINN)! [LINN-HS974-27]

Clematis buchananiana subsp. *grewiiflora* Kuntze Verh. Bot. Vereins Prov. Brandenburg 26: 130 (1885).

Description: Climber. Stem ribbed, covered in golden brown velvety hair. Leaves ternate or pinnate, 5 leaflets. Leaflets ovate to broadly ovate, entire or 3 or 5-lobed, 1.5--10 X 0.5--10 cm, tip acute or acuminate sometimes mucronate, margin irregulate mucronate teeth, golden brown or white velutinous to densely white or golden brown villous above, abaxial dense golden villous hair below. Inflorescence axillary, (1--)3--7-flowered cyme; peduncle to 25 mm; bracts ovate or linear, often 3-lobed, velutinous. Flowers yellow, campanulate. Sepals 4, 18--30 X 11 mm, oblong narrowly ovate, erect, tip reflexed, apex acute or truncate, golden brown velutinous inside and out. Stamens to 20 mm; filaments linear with long silky hair, shorter to base; anthers to 5mm, connective glabrous, acutely protruding or not. Achenes ovate or obovate 5 X 3 mm, flattened, adpressed hairy; persistent style to 4 cm, plumose.

Distribution: Nepal, W Himalaya, E Himalaya, Tibetan Plateau.

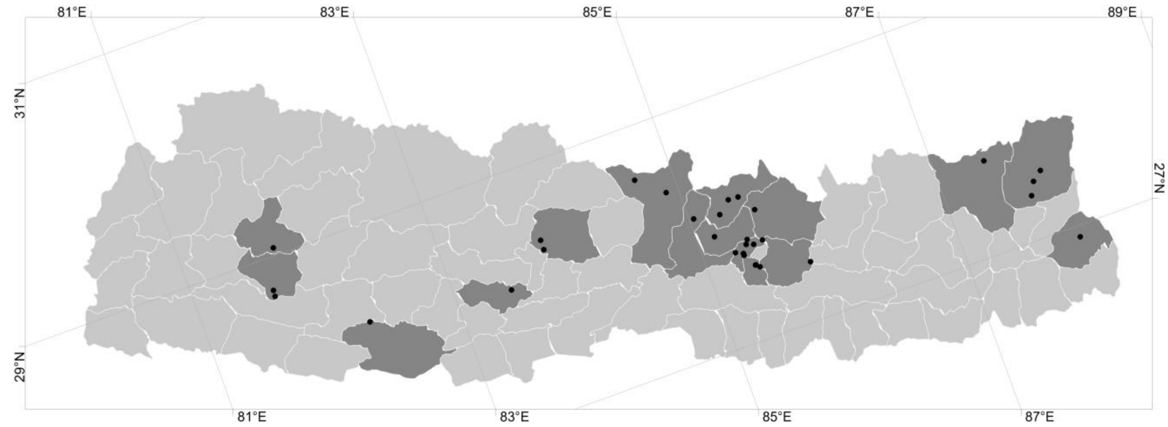


Figure 2.16 Distribution of *Clematis grewiiflora* DC. in Nepal

Specimens Examined: Eastern, Sankhuwasabha, Beer, L.W. 12281 (BM); Norkett, A.H. 8125 (BM); Ilam, Upadhyay, A.V. 10128 (KATH); Taplejung; Brough, M.A. 203 (BM); Hara, H., Kanai, H., Kurosawa, S., Murata, G., Togashi, M. & Tuyama, T. 6304318 (K); Hara, H., Kanai, H., Kurosawa, S., Murata, G., Togashi, M. & Tuyama, T. 6304319 (BM); Upadhyay, A.V. 1613 (KATH); **Central**, Dhading, Manandhar, N.P. 266 (KATH); Kathmandu, Amatya, M.M. 12791 (KATH); Buchanan-Hamilton, F. (LINN) [LINN-HS974-22-1]; Joshi, D.P. & Rajbhandari, K.R. 75617 (KATH); Joshi, D.P. & Rajbhandari, K.R. 75706 (KATH); Joshi, D.P. & Rajbhandari, K.R. 75823 (KATH); Samam & Shrestha, R. 652 (KATH); Shrestha, T.B. & Shakya, P.R. 11623 (KATH); Shrestha, T.B. & Upadhya 631 (KATH); Wallich, N. *s.n.* (K); Wallich, N. *s.n.*(LINN) [LINN-HS974-22-2]; Kavre Palanchok, Kanai, H. & Shakya, P.R. 674899 (KATH); Manandhar, N.P. 12221 (KATH); Nuwakot; Bowes-Lyon, S.A. 17 (BM); Patan; Malla, S.B. & Rajbhandary, S.B. 213 (KATH); Schilling, A.D. 712 (KATH); Schilling, A.D. 734 (K); Rasuwa, Joshi, D.P. 2263 (KATH); Sindupalchok, Bhattarai, N.K. 86372 (KATH); **Western**, Gorkha, Gardner, P.C. 360 (BM); Manaslu 08 20812158 (E); Kaski, Dawson, G. 273 (BM); Schilling, A.D. 2449 (BM), Gulmi, Joshi, D.P. & Amatya, M.M. 46482 (KATH); **Mid Western**, Dailekh, Bhattacharya, T.K. & Kurmi, P.P. 5743 (KATH); Malla, K.J. & Sharma, P.R. 1559 (KATH); Malla, K.J. 1683 (KATH); Dolpa, Shrestha, K.K. & Ghimire, S.K. SP128 (KATH); Kalikot, Manandhar, N.P. & Bhattarai, N.K. 9120 (KATH); Dang, Polunin, O.V., Sykes, W.R. & Williams, L.H.J. 3667 (BM) (E); Rolpa, Bhattacharya & Shrivastava 8485/30 (KATH);

Altitudinal Range: 1100-2800 m.

Ecology: Climbing over shrubs.

Flowering Time: November-December.

Fruting Time: December-March.

National IUCN Status (Provisional): Data Deficient (DD).

Clematis grewiiflora has an EOO of 47,808 km^2 suggesting Least Concern (LC) and an AOO of 112 km^2 suggesting Endangered (EN), however there are no data on population or range fluctuations.

Notes: *Clematis grewiiflora* is readily distinguished from closely related species by the very dense white or golden tomentum on its stems, leaves and sepals.

Hooker (1855) was the first to question whether *Clematis loasiflora* is a valid species, noting that the orginal description was based on sterile material. Grey-Wilson (2000) and Johnson (2001) also treated it as distinct from *C. grewiiflora*. Johnson cited living material collected by Arno Kasvi, Turku University's Botanical Gardens, from 2020 m in Nepal, but with no further locality data and Ludlow and Sherriff 6718 from SE Tibet as representative of *C. loasiflora*. Both authors distinguished the two species on the density and colour of the indumentum and the [slight] altitudinal difference in between *C. grewiiflora* and *C. loasiflora* from Nepal, Sikkim Bhutan, SE Tibet. However the separation of the two is not maintained here because of the absence of type material *C. loasiflora* for comparison and the wide range of variation found in the indumentum of specimens of *C. grewiiflora*.

The twisted anther character which was used to delimit *C. buchananiana* and *C. tortuosa* (Gupta 1961, Johnson 2001) is evident on several specimens.

A specimen Man08 20812158 has a recorded altitude of 3190 m, which is very high for this species but the identification has not been confirmed as the only specimen is located in TI and is currently not available on the Society of Himalayan Botany online database.

Flowering and fruiting times were supplemented with data from Indian, Bhutanese and Chinese specimens.

11. *Clematis kilungensis* W.T.Wang & M.Y.Fang Fl. Reipubl. Popul. Sin. 28: 355 (1980).

Grey-Wilson, *Clematis* the Genus: 145. (2000). Johnson, The Genus *Clematis*: 304 (2001). Wang, Acta Phytotax. Sin. 39(1):39 (2001). Wang & Bartholomew Fl. China. 6: 383 (2001).

Type: CHINA: XIANG. Tibet Medicinal Plant Expedition, 332, 17 Jun 1972. Kilung [Gyirong Xian] [Holotype PE00934711! and Isotype PE00934712!]

Synonym: *Clematis barbellata* var. *obtusa* Kitam. & Tamura. *sensu* Wang *pro parte* Acta Phytotax. Sin. 39(1):39 (2001).

Description: Climber. Stems shallowly ribbed. Leaves ternate, emerging from fasicles of small hairy leaf buds clustered at nodes. Leaflets ovate, 2.5--4.5 X 1.8--2.4 cm, base rounded, apex acute to shortly acuminate, margin coarsely serrate, glabrate above, pilose on veins, sparsely pilose below, denser on veins. Inflorescence a fascicle 1-flowered on an axillary on shortshoot; peduncles 4.5--7 cm; bracts ternate, sessile, with 1 or 2 teeth, ca. 1 cm, densely lanuginose. Flowers yellow with brown purple base, campanulate. Pedicels ebracteolate. Sepals 4, ovate to obovate, 24--28 X 13--16 mm, ascending, tip recurved, apex acute, sparsely pubescent above and below margin densely villose. Stamens to 12 mm; filaments to 9 mm, densely languinose; anthers to 3 mm, connective glabrate or languinose. Achenes not seen.

Distribution: Nepal, Tibetan Plateau.

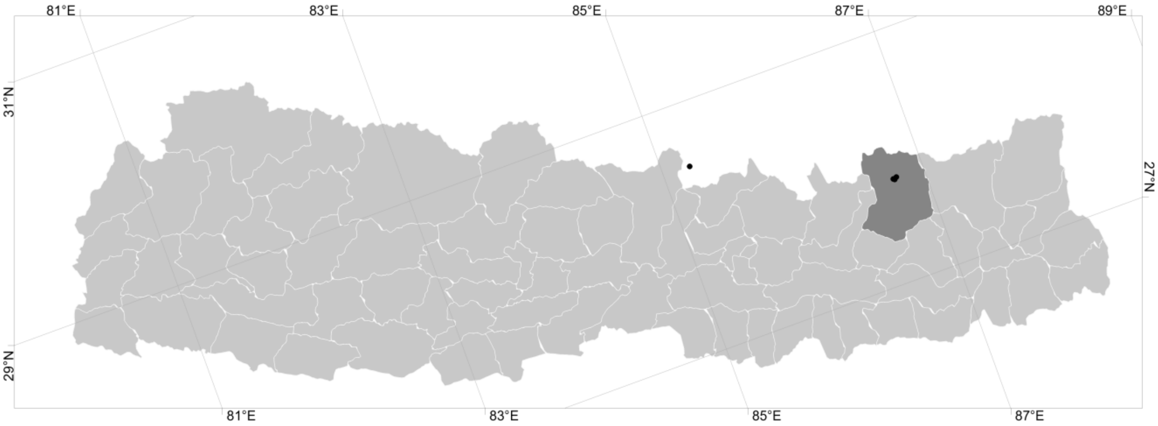


Figure 2.17 Distribution of *Clematis kilungensis* W.T.Wang & M.Y.Fang in Nepal [and locality of Holotype in Tibet]

Specimens examined: **Eastern,** DNEP1 261 (E); DNEP1 261 (KATH); No collector [cultivated] (K); Stonor, C.R. 52 (K); Zimmermann, A. 495 (BM). [**CHINA**. Tibet Medicinal Plant Expedition, 332, Kilung [Gyirong Xian] (PE)! [HOLOTYPE & ISOTYPE]]

Altitudinal range: ca. 3400 m [3700 m in Gyirong Xian].

Ecology: *Abies spectabilis* and *Pinus wallichiana* forest.

Flowering Time: May-June.

Fruiting Time: Unknown.

Global IUCN Status (Provisional): Data Deficient (DD).

Clematis kilungensis has an EOO of 188 km^2 suggesting Endangered (EN) and an AOO of 16 km^2 suggesting Endangered (EN), however there are no data on population or range fluctuations.

Notes: This species is endemic to Solukhumbu District of Nepal and Gyirong Xian to the north of Langtang on the Tibetan plateau, the species' type locality. Kilung is a derivation of Kyirong which is an orthographic variant of the name which is also now spelt as Gyirong.

In its protologue *Clematis kilungensis* is described in comparison with two Chinese species, *C. pseudopogonandra* and *C. pogonandra*. It differs from the former in its trifoliate leaves and from latter in its leaflets which have a serrate margin, 1 or 2 teeth and pubescence on the inner and outer surfaces of the sepals.

As noted previously, the inadequate description of *C. barbellata* var. *obtusa* also resulted in Wang (2001) incorrectly synonymising *C. kilungensis* W. T. Wang & M. Y. Fang based on the obtuse sepals of that taxon. The type material in PE looks very similar to the Zimmerman and DNEP collections from Nepal and although habit of these specimens is similar to *C. barbellata*, the floral morphology is clearly different. *C. kilungensis* flowers are yellow with a purple base and sepals which are ovate to obovate and 24--28 mm long by 13--16 mm wide, whilst *C. barbellata* flowers are dull purple to brown and the sepals are obtuse-elliptic and about 17 mm long by 15 mm wide.

All Nepalese material of this species was collected during May while in flower, as was the type material from Tibet.

12. ***Clematis montana* Buch.-Ham. ex DC.** Syst. Nat 1:164 (1817)

Candolle, Prodr. 1:9 (1824). Don, D., Prodr. fl. nepal. 192 (1825). Wallich. Pl. As. Rar. 3 t.217 (1830). Hooker & Thomson Fl. Indica 1:6 (1855). Hooker, Fl. Brit. India 1:2 (1872). Kuntze, Ver Bot. Vereins. Prov. Brandenburg 26:141-142 (1885). Brühl. Ann. Roy. Bot. Gard. (Calcutta) 5: 72-73 (1896). Gupta, Bull. Lucknow Natl. Bot. Gard. 54: Pl.34-35 (1961). Kapoor, Bull. Lucknow Natl. Bot. Gard. 124:42-53 (1966). Hara, Enum. Fl. Pl.

Nepal 2:15 (1979). Grierson, Fl. Bhutan, 286 (1984). Riedl & Nasir. Fl. Pakistan 193:86 (1991). Annot. Checkl. Fl. Pl. Nepal: 252 (2000). Grey-Wilson, *Clematis* the Genus: 150-151. (2000). Wang Acta. Phytotax. Sin. 38(4): 306-307. (2000). Johnson, The Genus *Clematis*: 294-295 (2001). Wang, Acta. Phytotax. Sin. 39(1): 2-3 (2001). Wang & Bartholomew, Fl. China. 6:342 (2001). Wang, Acta. Phytotax. Sin. 40(3): 206-213 & 223-224 (2002).

Type: Buch.-Ham. *s.n.* in Herb. Lambert. Lectotype [BM?] [BM000541171] designated here.

Synonymy: *Anemone curta* Wall. Numer. List. #4690 Nom. nud. Type: (E)! [E00505108]

Clematis pundata Wall. Nom. nud. Numer. List. #4682 nom. nud. Type: (E)! [E00505111]

Clematis anemoniflora D.Don Prod. fl. nepal. 192 (1825). *nom. illeg.*; *non*. Buch.-Ham. ex DC. (1817)

Clematis montana var. *grandiflora* Hook. *Bot. Mag. 70: t. 4061 1844*.

Clematis montana subsp. *normalis* var. *edentata* Kuntze *Verh. Bot. Vereins Prov. Brandenburg 26: 141 1885*. Type: Wallich, herb. Bot. Berol [presumably lost Type from B]

Clematis montana subsp. *normalis* var. *incisa* Kuntze Verh. Bot. Vereins Prov. Brandenburg 26: (1885).

Clematis montana subsp. *normalis* var. *pubescens* Kuntze *Verh. Bot. Vereins Prov. Brandenburg 26: 141 1885*. Types: Kumaon, herb. Bot. Berol [presumably lost Type from B], Manipur (K) [Not seen]

Clematis montana subsp. *normalis* var. *angustifolia* Kuntze Verh. Bot. Vereins Prov. Brandenburg 26: 141 (1885). Type: Kumaon 2300 m [Not Seen]

Clematis montana subsp. *normalis* var. *brevifolia* Kuntze Verh. Bot. Ver. Brand. 26:141 (1885). Lectotype. Hooker, J.D. sn. Sikkim. Jungh. 1849-07-23 (K!) (Wang 2000)

Clematis montana subsp. *normalis* var. *anemoniflora* (D.Don) Kuntze. Verh. Bot. Vereins Prov. Brandenburg 26: 141 (1885). [citing the type of Hookers *Clematis montana* var. *grandiflora*.]

Clematis montana subsp. *normalis* var. *obtusisepala* Kuntze Verh. Bot. Vereins Prov. Brandenburg 26: 142 (1885). [No specimens cited.]

Clematis montana subsp. *normalis* var. *flavida* Kuntze Verh. Bot. Vereins Prov. Brandenburg 26: 142 (1885). Type: ex Hk. F. & Th. (cfr. subsp. {}) [Not Seen.]

Clematis montana subsp. *normalis* var. *rubens* Kuntze Verh. Bot. Vereins Prov. Brandenburg 26: 142 (1885). Type: ex Hk. F. & Th. [Not Seen.]

Clematis montana subsp. *normalis* var. *nutantiflora* Kuntze Verh. Bot. Vereins Prov. Brandenburg 26: 142 (1885). Type: Kumaon, Khasia herb. Bot. Berol [presumably lost Type from B]

Clematis montana subsp. *normalis* var. *uniflora* Kuntze Verh. Bot. Vereins Prov. Brandenburg 26: 142 (1885). Type: Khasia herb. Bot. Berol [presumably lost Type from B]

Clematis montana subsp. *normalis* var. *brevipedunculata* Kuntze Verh. Bot. Vereins Prov. Brandenburg 26: 142 (1885). Type: Himalaya, herb. Bot. Berol [presumably lost Type from B].

Clematis montana subsp. *praecox* Kuntze. Verh. Bot. Ver. Brand. 26:142. (1885.) Type: Sikkim, mus Bot. berol. [presumably destroyed at B]

Clematis montana subsp. *praecox* var. *herbacea* Kuntze. Verh. Bot. Ver. Brand. 26:142. (1885.) [No specimens cited.]

Clematis montana var. *praecox* (Kuntze) Brühl, Ann. Bot. Garden. Calcutta 5(2): 73. 1896.

Clematis montana var. *anemoniflora* (D.Don pro sp., non Kuntze) Brühl, Ann. Bot. Gard. Calcutta 5(2):72-74 (1896).

Clematis tripartita W.T.Wang *Acta Phytotax. Sin. 38: 500 2000*. Locality: China: Xizang: Nyalam Xian, 7 km south of city, on slopes, 4000 m, 9 June 1990 Collector and Number: Sino-Japan Exped. T450 Institution(s): HT: PE [Not Seen]

Clematis wallichii W.T.Wang *Acta Phytotax. Sin. 39: 2 2001*. Type Robert Blinkworth *s.n.* Wallich Cat. 4681B Kamaon (BM) ! [BM000581918] ISOTYPE . Wallich 4681B (E) ! [E00505110] [E00505112]

Description: Climber. Stems ribbed, sparsely pubescent when immature. Leaves ternate, clustered in fascicles at bud. Leaflets, variable, ovate or elliptitic or narrowly ovate, 0.9--2.5

X 0.5--1.5 cm, base rounded or almost cuneate, acute or acuminate tip, simple or irregularly dentate margin, glabrate above and below. Inflorescence a fascicle of 1, 2 or occassionally 4 flowers on an axillary shortshoot; ebracteate. Flowers white or occasionally slighty pink, open. Pedicels to 10 cm; ebractolate. Sepals 4, oval to elliptic, 18--30 X 20--25 mm, spreading, tip reflexed, apex obtuse or cuspidate or occasionally emarginate, glabrous to glabrate inside and outside. Stamens to 7.5 mm, glabrous; filaments linear to 4 mm; anthers to 3.5 mm, connective not protruding. Achenes rounded to elliptic, 3--5 X 3--4 mm, glabrous, distinct rim, persistent style to 4 cm, plumose.

Distribution: Nepal, W Himalaya, E Himalaya, Tibetan Plateau, Assam-Burma, E Asia SW Asia.

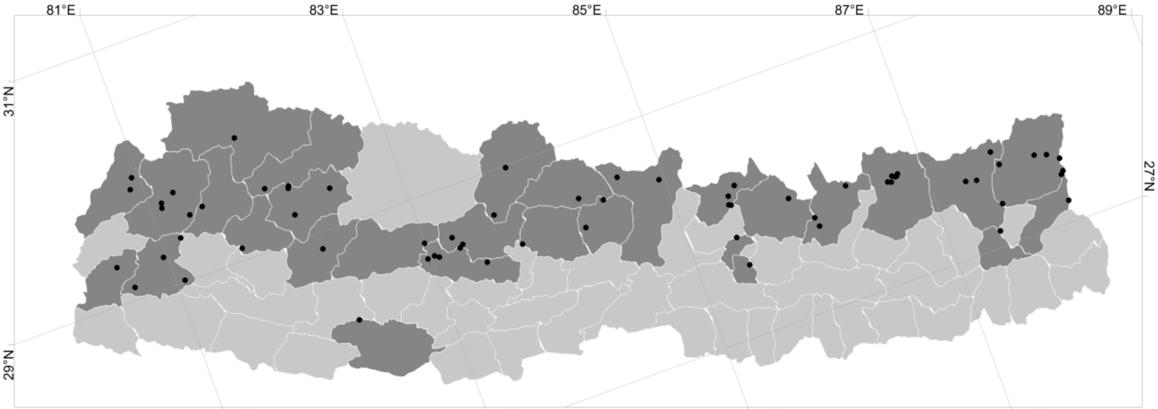


Figure 2.18 Distribution of *Clematis montana* Buch.-Ham. ex DC. in Nepal

Specimens Examined: Eastern, Dhankuta, Lancaster, C.R. 6 (BM); Sankhuwasabha, Beer, L.W. 25349 (BM); Beer, L.W., Lancaster, C.R. & Morris, D. 8389 (BM); Kanai, H., Ohashi, H., Iwatsuki, K., Ohba, H., Iwatsuki, Z. & Shakya, P.R. 723360 (BM); Ohba, H., Akiyama, S., Ikeda, H., Kikuchi, T., Noshiro, S., Omori, Y., Subedi, M.N. & Wakabayashi, M. 9154109 (BM); Stainton, J.D.A. 180 (BM); Stainton, J.D.A. 180 (E); Stainton, J.D.A. 261 (BM); Stainton, J.D.A. 361 (E); The 5th Botanical Expedition to Himalaya 1972 723360 (E); Panchthar, Adhikari, B., Poudel, R.C., Kuwar, R.M., Humagain, K., Pandey, J. & Khatri Chhetri, N.B. 53; Noshiro, S., Akiyama, S. & Acharya, N. 9263231 (BM); Taplejung, Beer, L.W. 8320 (BM) (E); Beer, L.W., Lancaster, C.R. & Morris, D. 10790 (BM); Kanai, H., Ohashi, H., Iwatsuki, K., Ohba, H., Iwatsuki, Z. & Shakya, P.R. 720563 (BM); Noshiro, S., Akiyama, S. & Acharya, N. 9263197 (BM); Shrestha, T.B. 15745 (KATH); The 5th Botanical Expedition to Himalaya 1972 720563 (E); Solu Khumbu, de Haas, J.H. 2972

(BM); Dhwoj, L. 0603 (BM); DNEP1 245 (E); DNEP1 51 (E); DNEP3 AY81 (E); Stonor, C.R. 29 (K); Zimmermann, A. 502 (BM); **Central**, Kathmandu, Nicolson, D.H. 3350 (BM); Patan, Schilling, A.D. 782 (K); Rasuwa, Bailey, F.M. (BM); Bowes-Lyon, S.A. 57 (BM); Buchanan-Hamilton, F. (LINN) [LINN-HS974-25]; Fell, K.R. 25 (BM); Halliwell, B. 116 (K); Manandar 7004 (KATH); Polunin, O.V. 497 (BM) (E); Polunin, O.V. 547 (BM); Samam & Bista, M.S. 13111 (KATH); Schilling, A.D., Sayers, C.D. & Bista, M.S. 400 (K); Schilling, A.D., Sayers, C.D. & Bista, M.S. 400 (KATH); Sindupalchok, Bhattarai, N.K. 90391 (KATH); EKSIN 218 (E); Dolkha, Dhwoj, L. 0668 (BM); Sharma, I., Subedi, M.N. & Kurmi, P.P. 1 (KATH); **Western**, Mustang, Miehe, S., Miehe, G. & Koch, K. 01-083-08 (BM); Stainton, J.D.A., Sykes, W.R. & Williams, L.H.J. 955 (BM) (P); Myagdi, Einarsson, S., Skärby, L. & Wetterhall, B. 62 (BM); Stainton, J.D.A., Sykes, W.R. & Williams, L.H.J. 2594 (BM) (P); Stainton, J.D.A., Sykes, W.R. & Williams, L.H.J. 9098 (BM); Vickery, A.R. 455 (BM); Gorkha, Gardner, P.C. 1007 (BM); Gardner, P.C. 436 (BM); Gardner, P.C. 447 (BM); Gardner, P.C. 515 (BM); Gardner, P.C. 516 (BM); Manaslu 08 20816085 (E); Kaski, Dawson, G. 517 (BM); Lamjung, Stainton, J.D.A., Sykes, W.R. & Williams, L.H.J. 5118 (BM); Manang, Joshi, D.P. & Amatya, M.M. 73/504; **Mid Western**, Jajarkot, Polunin, O.V., Sykes, W.R. & Williams, L.H.J. 1853 (BM), Humla, JRS B161 (E) (KATH; Jumla, JRS B59 (E) (KATH); Polunin, O.V., Sykes, W.R. & Williams, L.H.J. 2067 (BM); Polunin, O.V., Sykes, W.R. & Williams, L.H.J. 955 (BM); Shakya, P.R. & Joshi, D.P. 525 (KATH); Shakya, P.R. & Roy, B. 5429 (KATH); Shakya, P.R. & Roy, B. 5812; Kalikot, Polunin, O.V., Sykes, W.R. & Williams, L.H.J. 1937 (BM) (E); Mugu, Polunin, O.V., Sykes, W.R. & Williams, L.H.J. 849 (BM); Polunin, O.V., Sykes, W.R. & Williams, L.H.J. 872 (BM); Dang, Polunin, O.V., Sykes, W.R. & Williams, L.H.J. 3677 (BM) (E); Baglung, Boesi, A. & Cardi, F. N32 (P); Dobremez, J.F. 113 (BM); Stainton, J.D.A., Sykes, W.R. & Williams, L.H.J. 207 (BM) (E); Stainton, J.D.A., Sykes, W.R. & Williams, L.H.J. 2626 (BM); Stainton, J.D.A., Sykes, W.R. & Williams, L.H.J. 2626 (P); **Far Western**, Dadeldhura, Pradhan, P., Uprety, R.K., Pradhan, N. & Dabadi, N. 1042 (KATH); Darchula, Darchula 2012 1217029 (E) (KATH) (TI) (TUCH); Darchula 2012 1217057 (E) (KATH) (TI) (TUCH); Bajhang, Bajhang09 20911093 (E); Dobremez, J.F. 2011 (BM); Dobremez, J.F. 2185 (BM); Bajura, Suzuki, M., Hatta, H., Kurosaki, N., Mikage, M., Miyamoto, F., Rajbhandari, K.R., Takayama, H. & Terada, K. 9192408 (BM); Doti, Dobremez, J.F. 1829 (BM); Dobremez, J.F. 1829 (KATH); Dobremez, J.F. 1934 (KATH); Dobremez, J.F. 1959 (BM); Pradhan, P., Uprety, R.K., Pradhan, N. & Dabadi, N. 1189 (KATH)

Altitudinal range: 1300-4900 m.

Ecology: Climbing over trees and shrubs in forests and shrubs (*Rhododendron lepidotum*) in more open situations.

Flowering Time: April-August.

Fruiting Time: June-Sepember.

National IUCN Status (Provisional): Data Deficient (DD).

Clematis montana has an EOO of 88,288 km^2 suggesting Least Concern (LC) and an AOO of 260 km^2 suggesting Endangered (EN), however there are no data on population or range fluctuations.

Notes: This is a widespread and variable taxon whose distribution stretches from Aghanistan in the west to mainland China and Taiwan in the east. A full revision of all the variation across the range would need to be undertaken to put the differences in morphology in Nepal in context. It was therefore decided to take the broad view and treat all the morphological diversity within a single species.

David Don's *Clematis anemoniflora* was maintained by his brother George Don (1831) as a distinct species on the basis of the pedicel lacking a bract, but this is not evident on the BM specimen. Hooker (1855) treated Don's name as illegitimate as it cited *C. montana* as a synonym in the protologue and places into synonymy it with *C. montana* var. *grandiflora*. However, the BM specimen annotated as *C. anemoniflora* has smaller sepals than the Buchanan-Hamilton specimen of *C. montana* in the herbarium of the Linnaean Society (LINN), dated 11th April 1802, Chitlong, which is probably the large flowered variety *C. montana* var. *grandiflora* Hooker referred to in Botanical Magazine (1844)

Kuntze (1885) wrongly renamed Hooker's *C. montana* var. *grandiflora* as *C. montana* var. *anemoniflora* based on Don's earlier publication date. In 1896 Brühl resurrected *C. montana* var. *anemoniflora* as a distinct variety suggesting that Kuntze and Hooker were wrong to assume it was a large-flowered variety, as the sepals on the BM specimen are obovate, 1.5--3 cm with a rounded tip.

Grey-Wilson (2000) distinguished early flowering material with smaller flowers that emerge before the leaves and occurring in E Nepal, Sikkim, Bhutan & S Tibet as *C. montana* subsp. *praecox*. He also recognised *Clematis montana* var. *grandiflora* for larger flowered individuals found throughout the species' range.

Wang (2000) created *C. tripartita* W.T.Wang based on *Schilling, Saywo and Bista 400* at Kew and differentiated it from *C. montana* by its smaller, 3-lobed leaflets, narrowly obovate sepals and densely puberulous ovaries.

Clematis wallichii was also described by Wang (2000) and is known only from the type which is a specimen from the Wallich Herbarium apparently collected in Nepal. However, the number cited is actually a Robert Blinkworth specimen from Kumoan in North India.

Most of the diversity described appears to be nothing more than size variants of *C. montana*. Broad sampling from across the entire range would be required to determine whether there is sufficient genetic diversity to justify maintaining the complicated taxonomy of these taxa. In the molecular phylogeny the five samples of *C. montana*, two from Xizang in China and three from Nepal, form a well supported clade.

13. ***Clematis napaulensis* DC.** Syst. Nat. 1: 164 (1817)

Candolle, Prodr. 1:9(1824). Don, D., Prodr. fl. nepal. 192 (1825). Don, G., Gen.Sys. 1:9 (1831). Royle, Ill. Bot. Himal. Mts. 1:51 (1839). Hooker & Thomson Fl. Indica 1:6 (1855). Hooker, Fl. Brit. India 1:2 (1872). Gupta, Bull. Lucknow Natl. Bot. Gard. 54: Pl.14 (1961). Kapoor, Bull. Lucknow Natl. Bot. Gard. 78: 40-41 (1961), Kapoor, Bull. Lucknow Natl. Bot. Gard. 124:77-78 (1966). Hara, Enum. Fl. Pl. Nepal 2:15 (1979). Annot. Checkl. Fl. Pl. Nepal: 252 (2000). Grey-Wilson. *Clematis* the Genus: 93. (2000). Johnson, The Genus *Clematis*: 382 (2001). Wang & Bartholomew, Fl China. 6:346 (2001). Wang, Acta. Phytotax. Sin. 40(3):239-240 (2002).

Type: Nepal: Buchanan-Hamilton *s.n.* Lectotype: (BM)! [BM000521580] designated here. Syntypes: Nepal: Narainhetty, Buchanan-Hamilton *s.n.* 2/2/1803, (LINN)! [LINN-HS974-19], Buchanan-Hamilton *s.n.* 3/2/1803 (LINN)! [LINN-HS974-20].

Synonymy: *Clematis montana* D.Don Prodr. fl. nepal. 192 (1825), *nom. illeg.*; *non*. Buch.-Ham. ex DC. (1817)

Clematis nepalensis Royle (non. DC.), Ill. Bot. Himal. Mts. 1:51 (1839). *ortho*. *var*.

Clematis nipalensis DC. ex Hooker & Thomson, Fl. Indica 1:6 (1855). *ortho*. *var*.

Clematis cirrhosa var. *nepalensis* (DC.) Kuntze *Verh. Bot. Vereins Prov. Brandenburg 26:* 143 (1885).

Clematis cirrhosa subsp. *heterophylla* Kuntze, *Verh. Bot. Vereins Prov. Brandenburg 26: 143 (1885)*. Types: India: Kumaon, Strachey and Winterbottom (K), Madden (K). [Not Seen]

Clematis cirrhosa var. *nepalensis* forma. *angustifoliola* Kuntze *Verh. Bot. Vereins Prov. Brandenburg 26:* 143 (1885) [could be a subvariety] [No specimens cited]

Clematis forrestii W.W.Sm. Notes Roy. Bot. Gard. Edinburgh 8:183 (1914) Type: China, Yunnan, open situations on western flank of Shuili-Salwin divide, 25º 20' N, 7000-8000 ft, Dec. 1912. Forrest, G. 9398 (E)! [E00117181] (K)! [K000675100], Same locality, Feb 1913, Forrest, G. 9526 (E)! [E00346537] (K)! [K000675099]

Description: Climber. Stems ribbed, glabrate when immature becoming glabrous. Leaves ternate, opposite on immature stems verticillate from fascicles of leaf bud on mature stems, petiole 2--6 cm. Leaflets narrowly ovate, 2 or 3-lobed, 10 X 3.5 cm, base rounded or cuneate or cordate, apex acute or cuspidate, margin entire or with few teeth, glabrous or glabrate above and below. Inflorescence a fascicle of 1 to 10 flowers on an axillary shortshoot; ; peduncle to 1.5 cm; bracteoles 2 fused, cupulate. Flowers greenish creamy yellow, campanulate. Sepals 4, oblong, 1--2 X 0.5--1 cm, erect, tip recurved to strongly recurved, apex acute, glabrate inside, velutinous outside. Stamens 1--3 cm; filaments wider at base, glabrous; anthers 2.5--3 mm, connective not protruding.Achenes ovate to almost round, 6 X 4 mm, flat with distinct rim, pubescent, presistent style to 4.5 cm, plumose.

Distribution: Nepal, E Himalaya, E Asia.

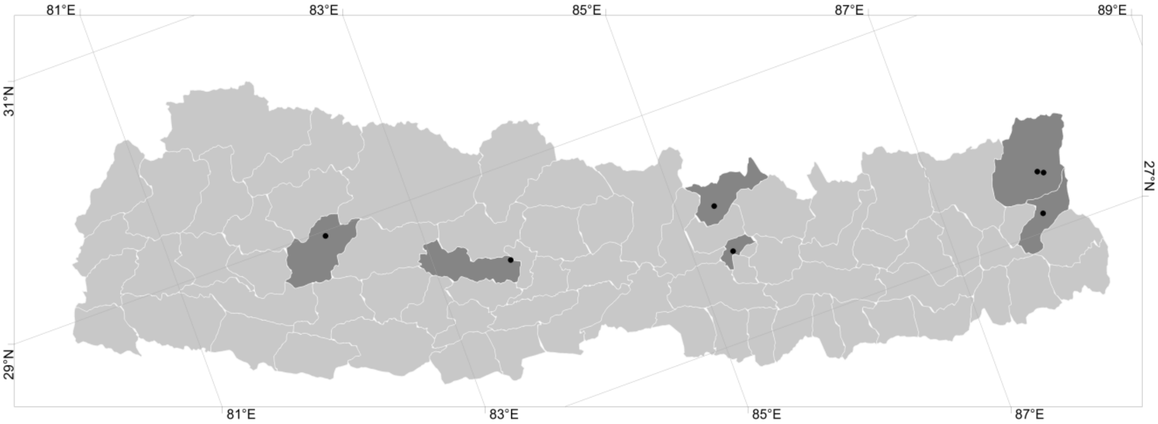


Figure 2.19 Distribution of *Clematis napaulensis* DC. in Nepal

Specimens examined: **Eastern**, Panchthar, Hooker, J.D. (P); Taplejung, Hara, H., Kanai, H., Murata, G., Togashi, M. & Tuyama, T. 6300463 (KATH); Upadhyay, A.V. 1601

(KATH); **Central**, Kathmandu, Buchanan-Hamilton, F. (LINN) [LINN-HS974-19]; Buchanan-Hamilton, F. (LINN) [LINN-HS974-20]; Rasuwa, Bowes-Lyon, S.A. 20 (BM); Bowes-Lyon, S.A. 20 (KATH);); **Western**, Baglung, Joshi, D.P. & Amatya, M.M. 731111 (KATH); Joshi, D.P. & Amatya, M.M. 731127 (KATH); Togashi, M. & Tuyama, T. 73-1111 (KATH); **Mid Western**, Jajarkot, Polunin, O.V., Sykes, W.R. & Williams, L.H.J. 3870 (BM

Altitudinal range: 1500-3400 m.

Ecology: Grows through small shrubs and trees in forests.

Flowering Time: October-February.

Fruiting Time: February-March.

National IUCN Status (Provisional): Data Deficient (DD).

Clematis napaulensis has an EOO of 21,782 km^2 suggesting Near Threatened (NT) and an AOO of 28 km^2 suggesting Endangered (EN), however there are no data on population or range fluctuations.

Notes: This species is possibly under recorded in Nepal from the fragmentary distribution record generated from herbarium specimens. In cultivation it is reported to be a late summer deciduous species (Johnson 2001) if this also happens in its native range, coupled with the late flowering time it may be that this species has been overlooked during fieldwork.

Clematis napaulensis and *C. cirrhosa* L. with its five varieties are the only members of section *Cheriopsis* DC. They have a disjunct distribution with *C. cirrhosa* and varieties occurring in the Mediterranean basin and C. *napaulensis* occurring from Nepal eastwards into SW China.

This species was introduced into horticulture by George Forrest following his 1912-3 expedition to Yunnan. *Forrest 9526* was described as *Clematis forrestii* by William Wright Smith and seed from the collection was grown on and distributed.

14. *Clematis phlebantha* L.H.J.Williams Journal Royal Horticultural Society London. 93:345 (1968)

Brickell, Bot Mag. 178 t.574 (1970). Hara, Enum. Fl. Pl. Nepal 2:15 (1979). Grey-Wilson. *Clematis* the Genus: 130-131. (2000), Annot. Checkl. Fl. Pl. Nepal: 253 (2000). Johnson, The Genus *Clematis*: 662 (2001).

Type: [West Nepal] Suli Gad. Latitude: 29° 0' N, Longitude: 82° 55' E. Stainton 4281.

Holotype BM ! [BM000521581], Isotypes (A)! [A00286220], (E)! [E00438582], (K)! [K000675180], (KATH)! [KATH001120], (US)! [US00103674].

Description: Sub-shrub. Stems, villous when immature, glabrous when mature. Leaves pinnate, (5 or)7(or 9) leaflets, petiole to 10 mm, villous. Leaflets obdeltoid, 0.5 X 1.5 cm, 3 or 5-lobed, silvery villous above, white lanate below. Inflorescence terminal or axillary, solitary, 1(or 3)-flowered. Flowers white, open. Pedicels to 8 cm, lanate; bracteoles absent or leaf-like, unequally 2-lobed or 3-lobed, villose. Sepals 5--7, obovate, 10--20 X 5--10 mm, spreading, apex cuspidate, villous outside thinning to the margins, glabrous inside. Stamens to 7 mm, glabrous; filaments 3--5 mm, linear; anthers 1.5--2 mm, connective not protruding. Achenes elliptic, 5 X 2.5 mm, villous, persistent style to 3cm, plumose.

Distribution: Nepal.

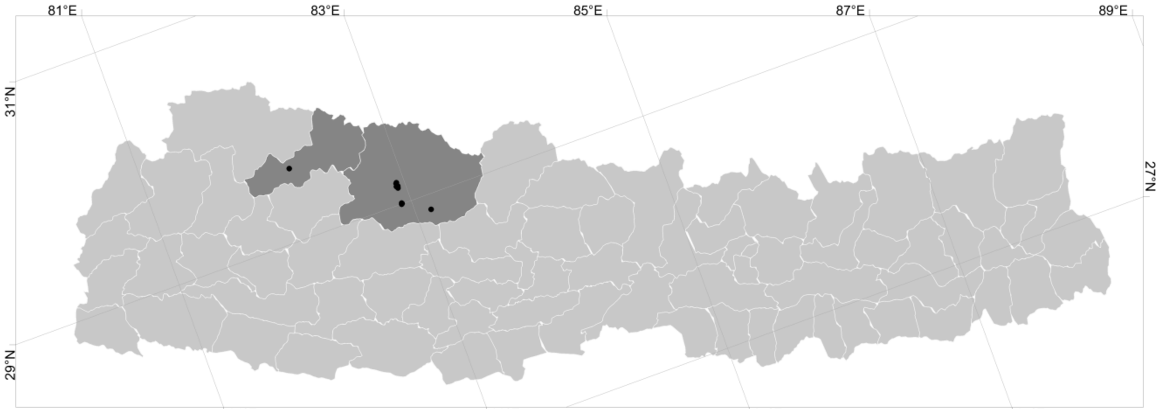


Figure 2.20 Distribution of *Clematis phlebantha* L.H.J.Williams in Nepal

Specimens examined: **Mid Western**, BRD A262 (E) (KATH) (TI) (TUCH); BRD B151 (E) (KATH) (TI) (TUCH); Einarsson, S., Skärby, L. & Wetterhall, B. 3136 (BM); Minaki, M., Joshi, K.K., Kadota, Y., Sugita, H., Takahashi, A., Tsuda, S., Yagi, H. & Yonebayashi, C. 9109271 (BM); Polunin, O.V., Sykes, W.R. & Williams, L.H.J. 1096 (BM) (E) (K); Polunin, O.V., Sykes, W.R. & Williams, L.H.J. 2152 (BM) (E); Polunin, O.V., Sykes, W.R. &

Williams, L.H.J. 3436 (BM) (K); Rajbhandari, K.R. & Malla, K.J. 6737 (KATH); Shakya, P.R. & Roy, B. 5933 (KATH); Shakya, P.R. & Roy, B. 6021 (KATH); Shrestha, T.B. 5316 (BM) (KATH); Stainton, J.D.A. 4270 (BM) (K); Stainton, J.D.A. 4281 (E) (G); Mugu, Polunin, O.V., Sykes, W.R. & Williams, L.H.J. 4943 (BM)

Altitudinal range: 2500-3400 m.

Ecology: Grows on open slopes, in rock crevices, hot dry sheer cliff faces.

Flowering Time: June-July.

Fruiting Time: August-September.

Global IUCN Status (Provisional): Data Deficient (DD).

Clematis phlebantha has an EOO of 995 km^2 suggesting Endangered (EN) and an AOO of 32 km^2 suggesting Endangered (EN), however there are no data on population or range fluctuations.

Notes: This species is endemic to Nepal and has so far only been recorded along the Suli Gad and the cliff at the southern end of Phoksumdo Tal in Dolpa District and on the Barbung Khola in Mugu District. It is easily distinguished from all other Nepalese *Clematis* species as it is a sub-shrub rather than a climber, with small leaflets covered with silvery villous hairs above and dense white lanate hairs below.

Plants grown from Polunin, Sykes and Williams 3436 received an RHS award of Merit in 1964, but the species did not persist in cultivation.

Figure 2.21 *Clematis phlebantha* BRD A262. Top Left: Sub-shrub habit. Top Right: Short fruiting stem. Bottom Achenes.

15. *Clematis puberula* Hook.f & Thomson. *Fl. Brit. India* 1:4 (1872)

Kuntze. *Verh. Bot. Vereins Prov. Brandenburg 26:* 148 (1885). Gupta, Bull. Lucknow Natl. Bot. Gard. 54: Pl.15 (1961). Kapoor, Bull. Lucknow Natl. Bot. Gard. 78: 42-44 (1961), Hara, Enum. Fl. Pl. Nepal 2:15 (1979). Grierson Fl. Bhutan 290. (1984). Annot. Checkl. Fl. Pl. Nepal: 253 (2000). Grey-Wilson. *Clematis* the Genus: 45. (2000), Wang, Acta. Phytotax. Sin. 38(6): 406 (2000). Johnson, The Genus *Clematis*: 448-449 (2001). Wang & Bartholomew Fl. China. 6: 353 (2001). Yonekura, Fl.Mustang 71 (2008).

Type: Bangladesh: Khasia, Nurtiong. Hooker, J.D. & Thomson, T. *s.n.*, 1 Oct 1850 Lectotype: (K) ! [K000675153]. Syntypes: Bangladesh: Khasia, Hooker, J.D. & Thomson, T. *s.n.*, (K)! [K000675153], (E)! [E00504279]. [These two specimens lacking the collections date and the Nurtiong locality of the lectoype.]

Synonymy: *Clematis parviloba* subsp. *puberula* (Hook. f. & Thomson) Kuntze *Verh. Bot. Vereins Prov. Brandenburg 26:* 148 (1885)

Description: Climber. Stems ribbed, pubescent, densest at nodes. Leaves pinnate, biternate or bipinnate with lower segment pairs ternate, petiole to 20 cm, flattened at base, pubescent. Leaflets ovate or narrowly ovate, entire or shallowly 2 or 3-lobed, 0.5--6.5 X 2.5(--3.5) cm, apex acute to cuspidate, base rounded, margin entire or with few teeth; petiolules to 3.5 cm, pubescent. Inflorescence axillary, cyme 3--15-flowered; peduncle 2--3 cm, glabrous to pubescent; bracts narrowly ovate to narrowly obovate, to 5 mm, apex acute, margin entire, pubescent above and below. Flowers pale yellow to creamy white, open. Pedicel, 1--2 cm sparsely pubescent to pubescent; bracteoles small, narrowly ovate to ovate, to 3 mm, pubescent. Sepals 4(or 5) oblong, spathulate, 8--15 X 2--3(--5) mm wide, spreading, apex acute, villose outside, margin tomentose, glabrate inside. Stamens 3--9.5 mm; filaments to 7 mm, narrowly linear, flat, glabrous; anthers ca. 1mm, oblong, connective not protruding. Achenes not seen.

Distribution: Nepal, E Himalaya, Assam-Burma, Tibetan Plateau, E Asia.

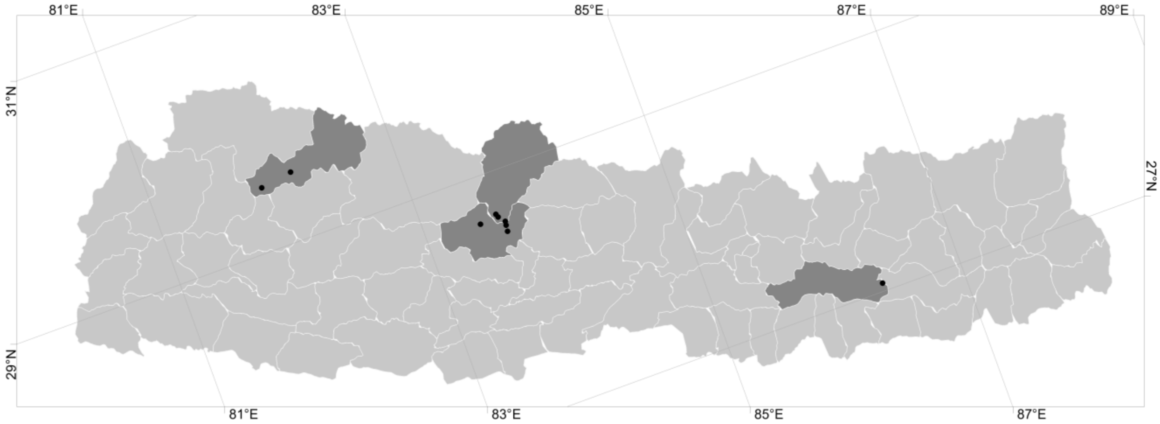


Figure 2.22 Distribution of *Clematis puberula* Hook.f & Thomson in Nepal

Specimens examined: **Central,** Rajbhandari, K.R. & Roy, B. 2187 (KATH); Zimmermann, A. 2105 (BM); **Western,** Manandhar, N.P. 10247 (KATH); Mikage, M., Acharya, N., Kurosawa, T., Lacoul, P., Takahashi, A. & Yoda, K. 9460469 (BM); Stainton, J.D.A., Sykes, W.R. & Williams, L.H.J. 7708 (BM) (E); Stainton, J.D.A., Sykes, W.R. & Williams, L.H.J. 7724 (BM) (E) (KATH); Joshi, D.P., Bhattarai, N.K., Dabadi, N. & Pradhan, N. 9385 (KATH); Manandhar, N.P. & Acharya, S.K. 24292 (KATH); Mikage, M., Anjiki, N., Kondo, N., Lacoul, P. & Yonekura, K. 9550207 (BM) (KATH); Tabata, H., Rajbhandari, K.R., Tsuchiya, K. & Konno, Y. 6126 (BM); **Mid Western,** Polunin, O.V. & Sykes, W.R. 3051 (BM) (E).

Altitudinal range: 2200-3030 m.

Ecology: Found growing over shrubs and trees branches in open situations.

Flowering Time: August-December.

Fruiting Time: September-December.

National IUCN Status (Provisional): Data Deficient (DD).

Clematis puberula has an EOO of 6,375 km^2 suggesting Vulnerable (VU) and an AOO of 36 km^2 suggesting Endangered (EN), however there are no data on population or range fluctuations.

Notes: Although the specimens listed here are clearly *C. puberula* it is odd that neither Grey-Wilson nor Johnson cited this species as growing in Nepal.

Four varieties are recognised in the *Flora of China* account (Wang 2000b and Wang & Bartholomew 2001) with the Nepalese specimens belonging to *C. puberula* var. *puberula*.

Clematis puberula was originally described as having a range from Simla to Gharwal in the NW of India and the Khasia Hills in NE India. Again given the large geographic area of this species' range it is likely that the currently recorded fragmented distribution in Nepal is a result of under collection.

Fruiting times were taken from specimens from India, Bhutan and China as very few Nepalese collections are in fruit.

16. *Clematis rehderiana* Craib. Bull. Misc. Inform. Kew 1914: 150 1914.

Hara, Enum. Fl. Pl. Nepal 2:15 (1979). Annot. Checkl. Fl. Pl. Nepal 253 (2000). Grey-Wilson. *Clematis* the Genus: 139 & 144-145. (2000), *Johnson. The Genus Clematis 316 (2001),* Wang & Bartholemew Fl. China 6:380 (2000), *Yonekura Fl. Mustang. 72 (2008).*

Type: Cultivated: Lemoine 211-04 Holotype: (K) ! [K000075697].

Paratypes: China: Sichuan, Tachienlu. Wilson, E. H. 3120 (K)! [K000075713], 3120a, (K)! [K000075715], 3120b. (K)! [K000075713], [Soulié 450, Pratt 592 (*pro parte*) also listed by Craib but not seen.]

Synonymy: *Clematis nutans* Royle var. *thyrsiodes* Rehder & Wilson. [Variety of a *nom. illeg*. homonym.]

Clematis veitchiana Craib. Bull. Misc. Inform. Kew 1914: 151 1914. Type: Cultivated: Veitch, 641-10. K! [K000075696]

Clematis roylei Rehder var. *roylei sensu* Johnson *The Genus Clematis 316 (2001), pro parte* [Material from Nepal.]

Clematis roylei Rehder *sensu* Grey-Wilson *Clematis* the Genus: 139 (2000), *pro parte* [Material from Nepal]

Description: Climber. Stems ribbed, sparsely pubescent denser at the nodes. Leaves pinnate 5 or 7 leaflets, petiole pubescent. Leaflets ovate, 3-lobed, 2--5 X 1.6--3 cm, apex acute, margin serrate, villose above and below. Inflorescence axillary, (5--)9--12-flowered cyme; peduncle to 13 cm; bracts ovate or rhombic, 3-lobed, 10--30 X 5--25 mm, villose. Flowers pale yellow, campanulate. Pedicel to 3 cm; bracteoles awl-shaped, villose. Sepals 4, narrowly ovate, 14--20 X 0.5 mm, erect, tip recurved, apex acute, pubescent to villose inside and outside, margin tomentose. Stamens to 15 mm villose; filaments linear with a widend base, villose; anthers 2--2.5 mm, connective protruding, acute. Achenes not seen.

Distribution: Nepal, E Asia, Tibetan Plateau.

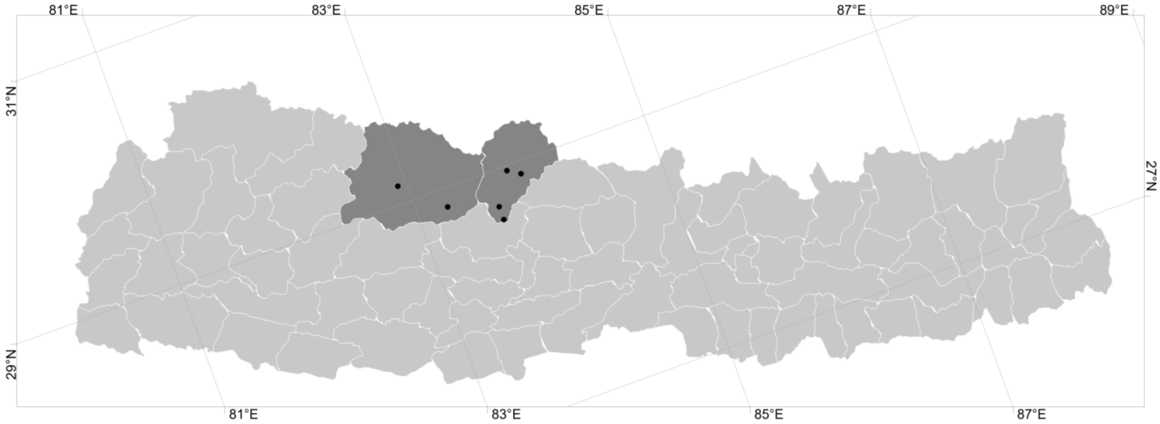


Figure 2.23 Distribution of *Clematis rehderiana* Craib in Nepal

Specimens examined: Western, Joshi, D.P. & Dhungana, S.K. 763928 (KATH); Stainton, J.D.A., Sykes, W.R. & Williams, L.H.J. 7241 (BM) (E) (KATH); **Mid Western,** BRD A259; Stainton, J.D.A. 4415 (BM) (KATH); Stainton, J.D.A., Sykes, W.R. & Williams, L.H.J. 7267 (E) (KATH)

Altitudinal range: 2800-4200 m.

Ecology: Climbing of small trees and shrubs along field margins and on south facing open slopes and remnant forest patches in *Caragana* scrub.

Flowering Time: July-September.

Fruiting Time: September.

National IUCN Status (Provisional): Data Deficient (DD).

Clematis rehderiana has an EOO of 2,332 km^2 suggesting Endangered (EN) and an AOO of 24 km^2 suggesting Endangered (EN), however there are no data on population or range fluctuations.

Notes: This species in Nepal has been been described as both *Clematis roylei* and *C. rehderiana* in previous works. Johnson (2001) does not cite either as species growing in Nepal. Grey-Wilson (2000) & Gupta (1967) called this species *C. roylei* stating that it is often confused with *C. rehderiana* and Yonekura (2008) in the Flora of Mustang called this species *C. rehderiana*.

The stamens of Indian *C. roylei* have hairs only on the lower half of the filament, whereas Chinese *C. rehderiana* have hairs along the full length of the filament and along the connective between the anthers, a character shared with the Nepalese material. The Nepalese material also shares pinnate leaflet arrangement with *C. rehderiana* compared with ternate or biternate leaflets found on Indian *C. roylei*. These characters are summeised in table 2.4

Table 2.4 Character comparisons for *Clematis roylei*, *C. rehderiana* and Nepalese material that has been determined as both of these species.

Species	Leaflet arrangement	Stamen pubescence
Clematis roylei	Ternate to biternate	Lower half of filaments only
Clematis rehderiana	Pinnate	Full length of stamens
Nepalese Material	Pinnate	Full length of stamens

The location of this population in the trans-Himalayan region is disjunct from both the eastern, main, distribution of *C. rehderiana* and that of *Clematis roylei* in NW India. Further investigation into the true relationships between these three species is required to ascertain the status of this this species in Nepal

Fruiting time for this species was taken from Chinese specimens all the Nepalese material is in flower.

Figure 2.24 *Clematis rehderiana*. BRD 259 Top: Inflorescence. Bottom: Flowering branch.

17. ***Clematis smilacifolia*** Wall. Asiat. Res. 13:402 (1820).

Candolle, Prodr. 1:10 (1824). Don, G., Gen.Sys. 1:6 (1831). Gupta, 54: pl.13 (1961) Bull. Lucknow Natl. Bot. Gard. (1961), Kapoor, *Bull. Lucknow Natl. Bot. Gard.* 78:49 (1962), Hara, Enum. Fl. Pl. Nepal 2:16 (1979). Grierson Fl. Bhutan 1(2):288 (1984). Grey-Wilson. *Clematis* the Genus: 139. (2000), Annot. Checkl. Fl. Pl. Nepal: 253 (2000). Wang, Acta. Phytotax. Sin. 38(6): 414 (2000). Johnson, The Genus *Clematis*: 522 (2001). Wang & Bartholomew Fl. China 6: 368 (2001).

Types: India: Meghalaya Mt. Sillet, [Mount Sillet], FD [Francis De Silva], Wallich, #4683. Syntypes: (K)! [K000675139], & (E)! [E00438577, E00438578, E00438579, E00438580]

Synonymy: *Clematis subpeltata* Wall. Pl. Asiat. Rar. 1:19 (1829). Type: Myanmar: Wallich #4684A (E!) [E00433995, E00433996].

Clematis inversa Griff. Not. Pl. Asiat. 4:700 (1854) [specimen not seen.].

Clematis smilacifolia var. *subpeltata* (Wall.) Kuntze. Verh. Bot. Vereins Prov. Brandenburg. 26:121 (1881).

Clematis loureiroana var. *subpeltata* (Wall.) Hand.-Mazz. Acta Hoti Gothob. 12:219 (1939).

Description. Climber. Stems glabrous to glabrate. Leaves simple, ovate to deltoid-ovate, 8--11.5 X 6--9 cm, leathery, , base cordate to subcordate, margin entire, apex obtuse to acute, glabrous above and below, petiole 6--12.5 cm, glabrate. Inflorescence axillary, a 3--7-flowered cyme; peduncle 5--11 cm, glabrate; bracts linear, 2.5--2.7 cm, glabrate. Flowers brown, open. Pedicels to 4 cm, glabrate; ebracteolate. Sepals 4, linear, 15--25 X 4--5 mm, reflexed, tip strongly recurved, apex obtuse, sparsely pubescent to sparsely villose inside and outside, margin tomentose. Stamen to 25 mm glabrous; filaments 15-20 mm; anthers 3--4 mm, connective distinctly protruding to 4 mm. Achenes ca. 1 X 0.5 cm, glabrate to sparsely villose, distinct rim; persitent style to 4 cm plumose.

Distribution: Nepal, S Asia, Assam-Burma, SE Asia, Tibetan Plateau, E Asia E Himalaya.

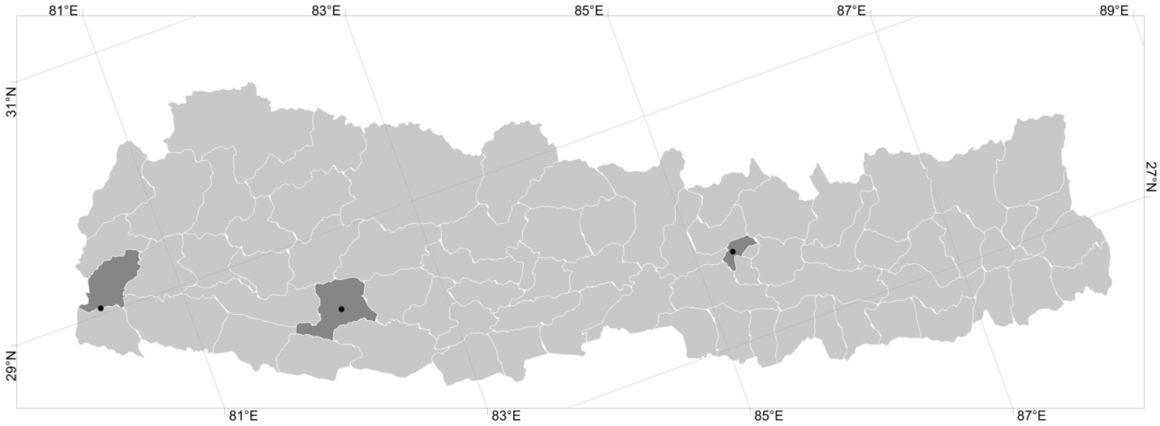


Figure 2.25 Distribution of *Clematis smilacifolia* Wall. in Nepal

Specimens examined: Central, Itoh, K., Samam & Shrestha 1402 (KATH) !; **Mid Western,** Manandhar, N.P. & Regmi, P.M. 226 (KATH) !; **Far Western,** Rajbhandari, K.R., Bhattarai, S., Sharma, I. & Joshi, R. 7046 (KATH) !

Altitudinal range: 700-1500 m.

Ecology: [At these altitudes in the West of Nepal this species is likely to be found in subtropical semi-evergreen forest types.]

Flowering Time: November-December.

Fruiting Time: December-March.

National IUCN Status (Provisional): Data Deficient (DD).

Clematis smilacifolia has an EOO of 5,732 km^2 suggesting Vulnerable (VU) and an AOO of 12 km^2 suggesting Endangered (EN), however there are no data on population or range fluctuations.

Notes: *Clematis smilacifolia* has a wide distribution from Uttar Pradesh in NW India to the islands of Malesia. It is unclear whether it is uncommon in Nepal or merely under-collected. Although the species includes several varieties, all the collections seen from Nepal so far are *C. smilacifolia var. smilacifolia*.

This incomplete description was taken from digitised herbarium specimens from KATH as there is no Nepalese representation of this species in BM, E or K. The KATH specimens also lack any additional notes about the habitats they were collected from.

Flowering and fruiting times were augmented with specimen data from Chinese specimens.

18. ***Clematis tibetana*** **Kuntze** Verh. Bot. Vereins Brandenburg 172 (1885).

Wang, Acta Phytotax. Sin. 36:164 (1996). Johnson, The Genus Clematis 356 (2000). Hara, Enum. Fl. Pl. Nepal 2:16 (1979). Grey-Wilson, The Plantsman 7(4):198-199 (1986). Grey-Wilson, Kew Bull. 44(1):46-49 (1986). Annot. Checkl. Fl. Pl. Nepal 253 (2000). Wang, Acta Phytotac. Sin. 44(4):401-436 (2006). Wang & Bartholemew, Fl China 6:365 (2000). Yonekura,Fl. Mustang 71-72 (2008).

Types: India: Milam[?], Kumaon 11,500ft. Strachy and Winterbottom 3. Syntypes: K! [K000675129], BM! [BM000541175].

Synonymy: *Clematis tibetana* var. *normalis* Kuntze Verh. Bot. Vereins Brandenburg. 172 (1885).

Clematis tibetana var. *debilis* Kuntze Verh. Bot. Vereins Brandenburg. 172 (1885) [No specimens cited].

Description: Climber. Stems ribbed, glabrate to sparsely pilose. Leaves ternate, pinnate or bipinnate, petiole 3--7 cm, sparsely pilose. Leaflets (3 or)5 or 7(or 13), ovate, 2--4-lobed, 2--4 X 1--2(--2.5) cm, base cuneate to rounded, apex acute, margin entire to laciniate, glabrous above and below. Inflorescence axillary, solitary or cymule, 1 or 3-flowered; peduncle 2--4 cm sparsely pilose; bracts ternate or simple with 3 lobes, sparsely pilose. Flowers yellow, sometimes mottled with brown, campanulate or openly campanualate. Pedicels 4--8 cm sparsely pilose; ebracteolate. Sepals 4, oblong, 15--20 X 4--7 mm, ascending, tip recurved or incurved, apex acute or acuminate, pilose to canescent outside, glabrate to sparsely pubescent inside, margin tomentose. Stamens to 6 mm, sparsely pilose; filaments to 4 mm; anthers 1.7--4 mm, connective not protruding. Achenes obovate, 3--3.5 X 1.5--2 mm; persistent style 3--4 cm, plumose.

18a. ***Clematis tibetana*** **Kuntze subsp.** ***brevipes*** **(Tamura) Yonek.** Fl. of Mustang. 73-74 (2008).

Tamura *Acta Phytotax. Geobot. 23: 30 (1968), Tamura Acta Phytotax. Geobot. 37: 156 (1986).* Grey-Wilson, Kew Bull. 44:47 (1989).

Type: Namikawa 124, Nepal: Pijehl, Sept. 1958 (KYO). [not seen]

Synonyms: *Clematis chrysantha* Ulbr.var. *brevipes* Tamura Acta Phytotax. Geobot. *23: 30 (1968).*

Clematis chrysantha var. *paucidentata* Tamura Acta Phyt. Geobot. 23: 29 & 30 (1968) [type not seen].

Clematis orientalis auct. non L. Bull. Dept. Med. Pl. Nepal. 7:33 (1979).

Clematis orientalis L. var. *uniflora* Tamura *Acta Phytotax. Geobot. 37: 156 (1986). [Types]*

Clematis tibetana subsp. *vernayi* (C.E.C.Fisch.) Grey-Wilson *var. laciniifolia Grey-Wilson. Kew Bull. 44: 48 (1989).* [type] Nepal: Yara Stainton Sykes, Williams 2130 E! [E00531106]

Clematis tibetana subsp. *vernayi* (C.E.C.Fisch.) Grey-Wilson var. *vernayi pro parte quoad* specimens *ex* Nepal. *Kew Bull. 44: 47 (1989).*

Clematis tibetana Kuntze var. *tibetana pro parte quoad* specimens *ex* Nepal. *Acta Phytotax. Sin.* 44(4):419 (2006).

Clematis *tibetana* var. *vernayi* (C.E.C.Fisch) W.T.Wang. *Acta. Phytotax. Sin.* 36:164 (1998). *pro parte quoad* specimens *ex* Nepal.

Description: Sepals recurved at tip. Anthers 1.8--2.5 mm.

Distribution: Nepal.

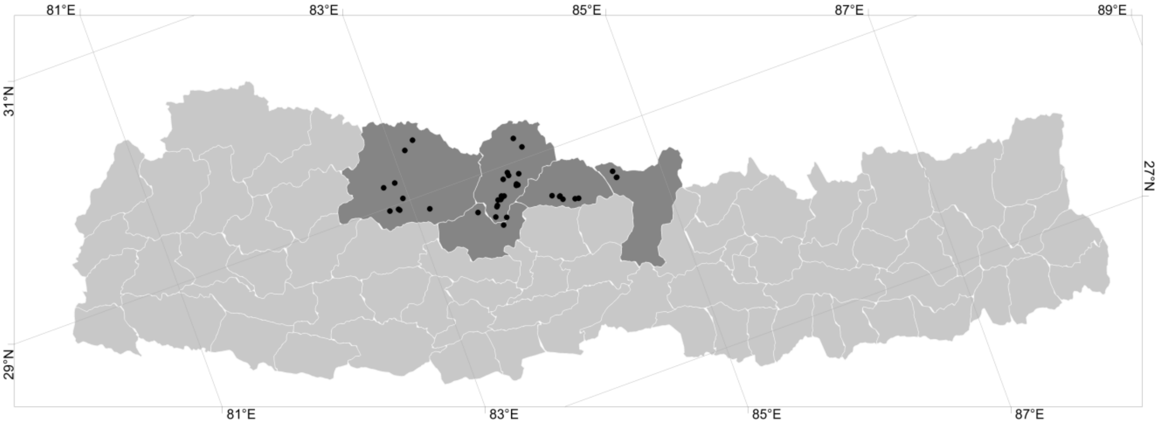


Figure 2.26 Distibution of *Clematis tibetana* Kuntze subsp. *brevipes* (Tamura) Yonek. in Nepal.

Specimens seen: **Western,** Herbal Garden, Kanazawa University, Japan Expedition to W. Nepal (1995) 9550291 (E); EA 45 (E); Hoshino, T., Amano, M., Koba, H., Miyoshi, N., Rajbhandari, K.R., Sato, M., Shrestha, P. & Takatsuki, S. 9662083 (BM); Iokawa, Y., Subedi, M.N., Takahashi Y. & Kano, K. 20020024 (E); Joshi, D.P., Bhattarai, N.K., Dabadi, N. & Pradhan, N. 14885 (KATH); Lange, H.J. 34 (K); Malla, S.B., Shrestha, J. & Rajbhandari 13850 (KATH); Malla, S.B., Shrestha, J. & Rajbhandari 138589 (KATH); Malla, S.B., Shrestha, J. & Rajbhandari 13859 (KATH); Manandhar, N.P. 10178 (KATH); Miehe, S., Miehe, G. & Koch, K. 01-005-04 (BM); Miehe, S., Miehe, G. & Koch, K. 0110013 (BM); Mikage, M., Anjiki, N., Kondo, N. & Lacoul, R. 9550269 (BM); Noshiro, S., Amano, M., Kurosawa, T. & Subedi, M.N. 20103019 (E); Shakya, P.R., Shrestha, I. & Shakya, S. 10523 (KATH); Shrestha, T.B. & Bista, M.S. 2431 (KATH); Stainton, J.D.A., Sykes, W.R. & Williams, L.H.J. 1375 (BM) (E) (KATH); Stainton, J.D.A., Sykes, W.R. & Williams, L.H.J. 1395 (E); Stainton, J.D.A., Sykes, W.R. & Williams, L.H.J. 1396 (BM); Stainton, J.D.A., Sykes, W.R. & Williams, L.H.J. 1423 (BM) (E) (KATH); Stainton, J.D.A., Sykes, W.R. & Williams, L.H.J. 2019 (BM) (E) (KATH);Stainton, J.D.A., Sykes, W.R. & Williams, L.H.J. 2019 (E); Stainton, J.D.A., Sykes, W.R. & Williams, L.H.J. 2019 (KATH); Stainton, J.D.A., Sykes, W.R. & Williams, L.H.J. 2130 (BM) (E); Stainton, J.D.A., Sykes, W.R. & Williams, L.H.J. 5645 (BM); Stainton, J.D.A., Sykes, W.R. & Williams, L.H.J. 8024 (BM) (KATH); Subedi, M.N. 0040014 (KATH); Subedi, M.N. 1852002 (KATH); Subedi, M.N. 80064 (KATH); Tabata, H., Rajbhandari, K.R., Tsuchiya, K. & Konno, Y. 6214 (KATH); Stainton, J.D.A., Sykes, W.R. & Williams, L.H.J. 7543 (BM) (E) (KATH); Manaslu 08 20811160 (E); Manaslu 08 20812190 (E); Manaslu 08 20815142 (E); Joshi, D.P. & Amatya, M.M. 73505 (KATH); Joshi, D.P. & Amatya, M.M. 73525 (KATH); Joshi, D.P. & Amatya, M.M. 73586 (KATH); Lowndes, D.G. L1078 (BM); Manandhar, N.P. 9829 (KATH); Manandhar, N.P. 9895 (KATH); McBeath, R. 1564 (E); Sykes, W.R. WR 182/96 (E); **Mid Western,** BRD A219; Einarsson, S., Skärby, L. & Wetterhall, B. 3118 (BM); Einarsson, S., Skärby, L. & Wetterhall, B. 3142 (BM); Grey-Wilson, C. & Phillips, B. 693 (K); Grey-Wilson, C. & Phillips, B. 751 (K); Grey-Wilson, C. 534 (K); Minaki, M., Joshi, K.K., Kadota, Y., Sugita, H., Takahashi, A., Tsuda, S., Yagi, H. & Yonebayashi, C. 9109277 (BM); Polunin, O.V., Sykes, W.R. & Williams, L.H.J. 1086 (BM); Polunin, O.V., Sykes, W.R. & Williams, L.H.J. 2314 (BM) (E); Polunin, O.V., Sykes, W.R. & Williams, L.H.J. 3439 (BM); Shrestha, K.K. & Ghimire, S.K. SP002 (KATH); Shrestha, T.B. 5269 (KATH); Shrestha, T.B. 5315 (KATH)

Altitudinal range: 1700-4000 m.

Ecology: Generally open, dry habitats, often at degraded sites. Growing over rocks and climbing over small shrubs and *Artemisia* at stream sides and riverbanks.

Flowering Time: June-September.

Fruiting Time: July-September.

Global IUCN Status (Provisional): Data Deficient (DD).

Clematis tibetana subsp. *brevipes* has an EOO of 10,117 km^2 suggesting Vulnerable (VU) and an AOO of 136 km^2 suggesting Endangered (EN), however there are no data on population or range fluctuations.

Figure 2.27 *Clematis tibetana* subsp. *brevipes.* Manasalu 08 215142. Top: habit. Bottom: flowering stem.

18b. ***Clematis tibetana* Kuntze subsp. *vernayi* (C.E.C.Fisch.) Grey-Wilson** *Kew. Bull.* 44: 48 1989.

Grey-Wilson. *Clematis* the Genus: 169. (2000), Annot. Checkl. Fl. Pl. Nepal: 253 (2000). Johnson, The Genus *Clematis*: 357 (2001). Wang., W.T. Acta. Phytotax. Sin. 36:164 (1998). Wang, W.T. (2006) Acta. Phytotax. Sin. 44(4): 418-421. (2006). Grey-Wilson in Kew Bull. 44(1)47-49. Grey-Wilson in The Plantsman 7(4):198 (1986)

Type: China: Xizang, 12 miles west of Gangtse.C.S. Cutting & A.S. Vernay 57 22/8/1935. Holotype: (K)![K000675130]. Isotypes: (K) ! [K000675132], (A)! [A00038366].

Synonyms: *Clematis vernayi* C.E.C.Fisch. Kew Bull. 1937: 95. (1937).

Clematis tibetana var. vernayi (C.E.C.Fisch) W.T.Wang. Acta. Phytotax. Sin. 36:164 (1998).

Description: Sepals incurved at tip. Anthers 2.4--4 mm.

Distribution: Nepal, Tibetan Plateau.

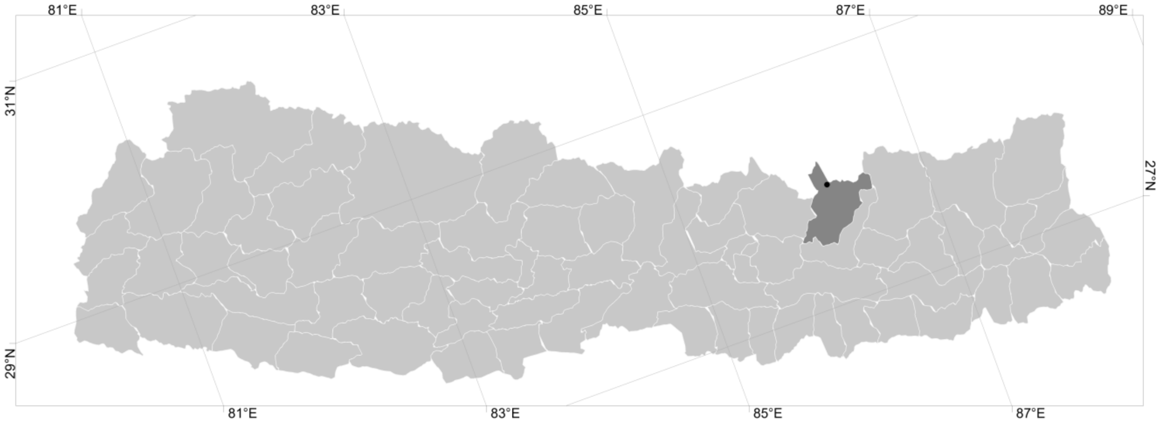


Figure 2.28 Distribution of *Clematis tibetana* Kuntze subsp. *vernayi* (C.E.C.Fisch.) Grey-Wilson in Nepal

Specimens seen: **Eastern**, Hingston. R.W.G. 84.(K!).

Altitudinal range: 4000 m [1700-4500 m in Tibet].

Ecology: Dry habitats growing over rocks on hillsides and river banks and walls around settlements.

Flowering Time: July.

Fruiting Time: July-August.

National IUCN Status (Provisional): Data Deficient (DD).

Clematis tibetana subsp. *vernayi* only known from a single collection in Nepal.

Notes: Hingston 84 was collected during the 1924 Everest expedition which approached the mountain from Tibet. However Hingston, who was the expedition doctor, noted that they crossed the border into Nepal in the Rangshar valley, so it is believed that this specimen was collected in Nepal.

Yonekura (2008) considered *Clematis tibetana* subsp. *brevipes* to be intermediate between *C. tibetana* subsp. *tibetana* (distributed in NW India and adjacent Tibet) and *C. tibetana* subsp. *vernayi* (from the Tibetan Plateau around Lhasa), citing various intermediate characters (Ohba, Iokawa & Sharma, 2008). There are very few collections of *Clematis tibetana* subsp. *tibetana*, making it difficult to adequetly assess the morphological differences between the three subspecies of *C. tibetana*. The three subspecies appear to have distinct distributions, but morphologically there are only slight differences in anther size, and sepal shape and size to differentiate them. Grey-Wilson's separation of the Nepalese specimens into either *C. tibetana* subsp. *vernayi* var. *vernayi* or *C. tibetana* subsp. *vernayi* var. *laciniifolia* is not accepted as there is considerable variation in leaflet morphology among specimens from Nepal and no clear distinction can be made.

Climatic data from the subspecies' areas of distribution suggest that *C. tibetana* subsp. *vernayi* and *C. tibetana* subsp. *brevipes* have different ecological requirements, summerised in table 2.5.

Table 2.5 Comparitive climatic differences for *Clematis tibetana* varieties from extracted herbarium records using Worldclim data in GIS.

	Monthly Temperature Range	Precipitation in Coldest Quarter	Precipitation Seasonality (Coefficient of Variation)
C. tibetana subsp. *vernayi*	12-15˚C	<10 mm	100-150
C. tibetana subsp. *brevipes*	9-12 ˚C	50-150 mm	50-100

Clematis tibetana subsp. *brevipes* specimens from Mustang collected by Stainton, Sykes and Williams during 1954 were misidentified as *C. orientalis* by L. H. J. Williams and a duplicate was deposited in KATH and the species was subsequently listed in the *Catalogue of Nepalese Vascular Plants* (Malla 1976) in the *Bulletin of the Department of Medicinal Plants*. As a result almost all the specimens in KATH of *C. tibetana* subsp. *brevipes* taxa were subsequently identified as *C. orientalis*.

The earliest reference to *Clematis tangutica* in Nepal is from Grey-Wilson's collections from Dolpa district during 1973 and mentioned in his published expedition report (Grey- Wilson, 1974) .His collections from that expedition are deposited in K and are annotationsedby Grey-Wilson as *C. tangutica*. The second annotations dated 1978 are again by Grey-Wilson but he changed the name to *C. vernayi.*

Wang W.T. (2006) identified several Nepalese specimens as *C. tibetana* var. *tibetana*: Polunin, Sykes and Williams 1086 (S, US) Barbung Khola, Lowndes 1078 (G) Marsyandi Valley, Stainton, Sykes, Williams 1395 (GH) 5646 (G) Muktinath, Shrestha 5315 (US) Ringmo. He considered these distinct from *Clematis tibetana* subsp. *vernayi* but did give his reasons. Duplicates of these collections in BM, E or KATH have been identified as *C. tibetana* var. *brevipes* during this revison.

Wang was first to identified *Hingston 48* (K), as *C. tibetana* subsp. *vernayi*. Species distribution modelling for this subspecies, covered in chapter 4, highlighted a small area south of the border with Tibet where there is a 50% or greater chance of it occurring.

Fruiting times were taken from Tibetean specimens at BM & E.

19. ***Clematis tongluensis* (Brühl) Tamura** Acta Phytotax. Geobot. 19: 77 1962.

Brühl. Ann. Roy. Bot. Gard. (Calcutta) 5:74 (1896). Gupta, Bull. Lucknow Natl. Bot. Gard. 80: Pl.38 (1963). Kapoor, Bull. Lucknow Natl. Bot. Gard. 124:73-74 (1966), Hara, Enum. Fl. Pl. Nepal 2:16 (1979). Grierson Fl. Bhutan 1(2):286 (1984)**.** Annot. Checkl. Fl. Pl. Nepal: 253 (2000). Grey-Wilson. *Clematis* the Genus: 80-81. (2000). Johnson, The Genus *Clematis*: 405 (2001). Wang, Acta Phytotax. Sin. 40:227-228 (2002).

Types: Tonglu (T.T. [Thomas Thomson], Gamble, K[ing]'s Collector), Rangbul K[ing]'s collector, Gantuk, K[ing]'s Collector. [Not seen]

[There are no duplicates of the specimens cited in the protologue in K, BM or E. However, the protologue does have an illustration which may serve as a type if these specimens cannot be located in CAL.]

Synonyms: *Clematis montana* var. *tongluensis* Brühl *Ann. Roy. Bot. Gard. (Calcutta) 5(2): 74, pl. 103 (1896).*

Clematis montana subsp. *sinchungica* Kuntze Verh. Bot. Vereins Prov. Brandenburg 26: 141 (1885) Types: India: Sikkim, Sinching Hooker. Anderson (B). [Presumably lost. No duplicates found at K, E or BM.]

Clematis montana var. *intermedia* Brühl Ann. Roy. Bot. Gard. (Calcutta) 5(2): 74 1896. Type: India: Sikkim, Tonglu. King’s Collector [not seen].

Description: Climber. Stems ribbed, glabrate to sparsely pubescent. Leaves ternate, petiole 3--7.5 cm, sparsely pubescent. Leaflets oblong-ovate or ovate-narrowly ovate, 2-3-lobed, 2.5--7.5 X 2--4 cm, base cuneate to rounded, apex acute to short acuminate, margin coarsely serrate, sparsely pubescent above and below but denser on veins. Inflorescence axillary, flowers solitary; peduncle sparsely pubescent; ebracteate. Flowers white, open. Sepals 4, oblong to narrowly ovate, 30--60 X 15--20 mm, spreading, tip slightly recurved, apex acute, sparsely pilose inside, glabrate outside, margin fimbrate. Stamens to 12 mm, glabrous; filaments 6--8 mm; anthers 3--4 mm, connective acutely protruding. Achenes 2--3 X 2--3mm, pubescent, persisent style to 3.5 cm, plumose

Distribution: Nepal, E Himalaya.

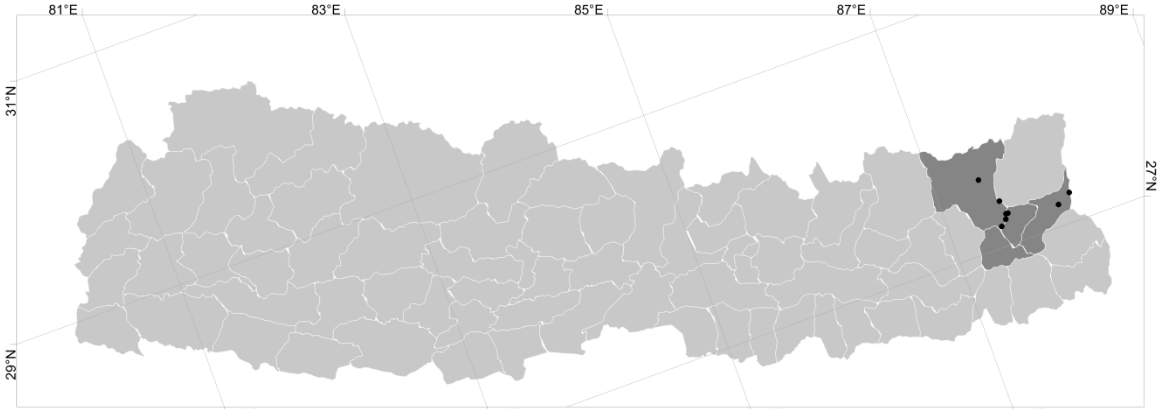


Figure 2.29 Distribution of *Clematis tongluensis* (Brühl) Tamura in Nepal

Specimens examined: Eastern, Ohba, H., Akiyama, S., Ikeda, H., Kikuchi, T., Noshiro, S., Omori, Y., Subedi, M.N. & Wakabayashi, M. 9154039 (BM); Beer, L.W., Lancaster, C.R. & Morris, D. 10132 (BM); Beer, L.W., Lancaster, C.R. & Morris, D. 10134 (BM); Stainton, J.D.A. 809; Grey-Wilson, C., Sinnott, M., Zmartzy, S., Long, D.G., McBeath, R., Noltie, H.J., Subedi, M.N. & Crawford, S. 86 (E) (K) (KATH); Omori, Y., Acharya, N., Fujikawa, K., Munemasa, M., Okada, M., Ree, R.H., Tateno, M. & Thapa, N. 9900047 (E); 4th Botanical Expedition to the Eastern Himalaya 69980 (E); Shrestha, T.B. & Joshi, D.P. 136 (BM)

Altitudinal range: 2580-3030 m.

Ecology: Climbing through shrubs in *Quercus semecarpifolia* - *Rhododendron* forest.

Flowering Time: July-August.

Fruiting Time: August-October.

National IUCN Status (Provisional): Data Deficient (DD).

Clematis tongluensis has an EOO of 1,576 km^2 suggesting Endangered (EN) and an AOO of 32 km^2 suggesting Endangered (EN), however there are no data on population or range fluctuations.

Notes: This species is an E Himalayan endemic found only in E Nepal, Sikkim and Bhutan.

Clematis tongluensis is superficially very similar to *C. montana* but it is readily distinguished by its larger, strap like sepals. In addition it flowers on short stems from the current year's growth and not from the fascicled leaf bundles at axils like *C. montana*.

20. *Clematis zemuensis* W.W.Sm. Rec. Bot. Surv. India iv. 166 (1911).

Gupta, Bull. Lucknow Natl. Bot. Gard. 54: pl.21 (1961), Kapoor, Bull. Lucknow Natl. Bot. Gard. 78:63-64 (1962). Hara, Enum. Fl. Pl. Nepal 2:16 (1979). Grierson Fl. Bhutan. 1(2):291 (1984). Grey-Wilson, *Clematis* the Genus:44. (2000), Annot. Checkl. Fl. Pl. Nepal 253 (2000). Johnson, The Genus *Clematis*: 450 (2001).

Type: India: Sikkim, Lower Zemu, 9000ft, 20 Jul. 1909. Smith & Cave 2671. Syntype: (K)! [K000675184].

Description: Climber. Stem ribbed, glabrate to pubescent.Leaves clusted around nodes, pinnate, bipinnate or biternate, 5 or 7 or 9 leaflets, petiole to 2cm, pubescent. Leaflets ovate or narrowly ovate, 2 or 3-lobed, 1--3 X 0.5--2 cm, base rounded or oblique, apex acute to cuspidate or shortly acuminate, margin irregularly serrate, pubescent to pilose above, densely pubescent to pilose below, denser on midvein, petiolule to 1.5 cm, pubescent. Inflorescence axillary, 3--7-flowered cyme, pubescent; peduncle to 10 cm, pubescent to canescent; bracts to 0.5 cm, pubescent to canescent. Flowers yellowish white, open. Pedicels 2--5 cm, pubescent; bracteoles to 5 mm long, linear margin toothed, pubescent. Sepals 4 (or 6), narrowly ovate or oblong, 10--20 X 5--6 mm, spreading, tip slightly reflexed, apex acute to cuspidate or mucronacte, pilose outside, margin tomentose, glabrous inside. Stamens 5--7 mm glabrous; filaments linear 2--3 mm, flat; anthers to 4 mm, connective acutely protruding. Achenes not seen.

Distribution: Nepal, E Himalaya.

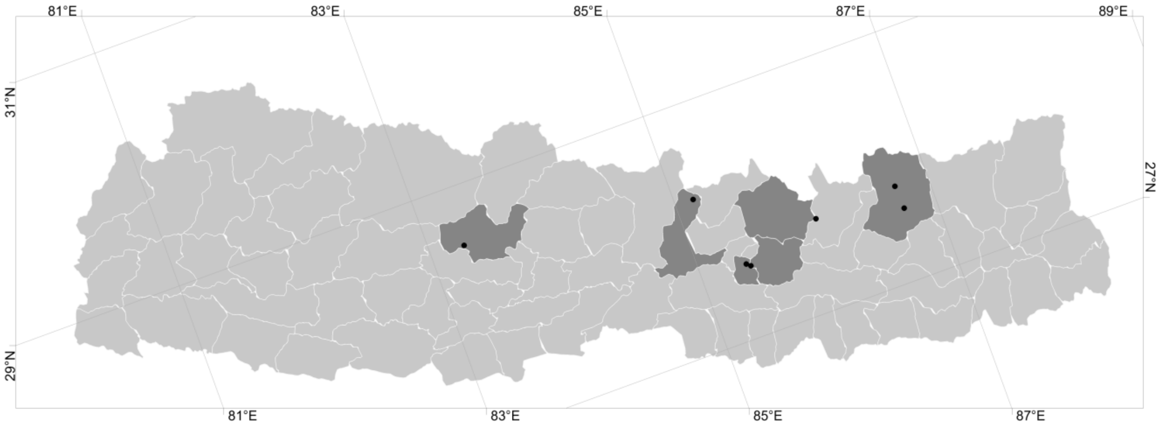


Figure 2.30 Distribution of *Clematis zemuensis* W.W.Sm. in Nepal.

Specimens examined: **Eastern,** DNEP3 BY19 (E); DNEP3 BY19 (KATH); **Central,** Ganesh Himal, Central Nepal Expedition (1994) 9420175 (E); [no collector] 10592 (KATH); Hara, H., Iwatsuki, K., Ohba, H. & Iwatsuki, Z. 723354 (BM) (E); The 3rd Botanical Expedition to Eastern Himalaya 1967 673134 (E); **Central,** Shrestha & Adhikari 14608 (KATH); **Western,** Stainton, J.D.A., Sykes, W.R. & Williams, L.H.J. 2514 (BM); Stainton, J.D.A., Sykes, W.R. & Williams, L.H.J. 3514 (E)

Altitudinal range: 1600-3000 m.

Ecology: Growing over *Juniperus* and *Rhododendron* on open hillsides.

Flowering Time: July-August.

Fruiting Time: Unknown.

National IUCN Status (Provisional): Data Deficient (DD).

Clematis zemuensis has an EOO of 12,795 km^2 suggesting Vulnerable (VU) and an AOO of 28 km^2 suggesting Endangered (EN), however there are no data on population or range fluctuations.

Notes: This species is an E Himalayan endemic only known from Nepal, Sikkim and Bhutan.

Neither Grey-Wilson (2000) nor Johnson (2001) recorded this species as occurring in Nepal. The paucity of collections suggests that it is not a common species. Again this may well be an under collected species in Nepal.

The only fruiting specimens from Nepal are a Dhwoj collection dated 1930. There are no other specimens from the range of *C. zemuensis* that are in fruit.

21. Clematis zeylanica **(L.) Poir.** Encycl. [J. Lamarck et al.] Suppl. 2. 296. 1811 [23 Oct 1811]

Candolle, Syst. Nat. 1:167 (1818). Candolle, Prod. 1:10 (1824). Don, G., Gen.Sys. 1:6 (1831). Hooker & Thomson Fl. Indica 1:3 (1855). Hooker, Fl. Brit. Ind 1:7 (1872). Kuntze, Verh. Bot. Vereins Brandenburg 26:121 (1885). Hara, Enum. Fl. Pl. Nepal 2:18 (1979). Annot. Checkl. Fl. Pl. Nepal: 255 (2000). Johnson, The Genus *Clematis*: 263 (2001). Wang & Bartholomew, Fl. China. 6:387 (2001).

Type: Lectotype : Herb. Hermann 2: 64; 3: 41; 4: 55, No. 226 (BM) [Designated Dassanayake in Dassanayake & Clayton (ed.), *Revised Handb. Fl. Ceylon* 10: 353 (1996)] [Not Seen].

Original material: Ind.[ia]: zeylanica certe. JES [annotated by James Edward Smith]. (LINN)! [Linn 711.6].

Synonymy: *Atragene zeylanica* Linnaeus, Sp. Pl. 1:542. (1753).

Naravelia zeylanica (L.) DC. Syst. Nat. 1:167 (1817).

Naravelia pilulifera Hance var. *yunnanensis* Y.Fei, *Acta Bot. Yunnan. 19(4): 406 406 (1997).*

Description: Climber. Stem shallowly ribbed, glabrate to sparsely pubescent. Leaves pinnate, leaflets 5, petiole 5.5--7 cm, sparsely pubescent to sparsely pilose. Basal pair of leaflets leaf-like, ovate, 6--11 × 6--8 cm, base rounded to cuneate, apex shortly acuminate, margin entire, sparsely pilose above, denser on veins, glabrate below, sparsely pilose on veins, petiolule 1--2 cm, sparsely pilose, apical 3-leaflets modified into tendrils. Inflorescences axillary, to 39-flowered cyme, to 25 cm, pubescent to pilose, bracts leaf-like 1--4.5 X 0.4--3 cm, glabrate above, sparsely pilose below. Flowers yellowish white, open. Pedicel to 2 cm; bracteoles minute, deltoid, pilose. Sepals 4, ovate, 6--10 X 3--4 mm, spreading, tip not reflexed, apex acute, villous outside, margin tomentose, glabrous inside, margin tomentose. Staminodes to 10 mm X 0.8--1 mm, clavate, obtuse apex, glabrous; stamens narrowly ovate, 4.5--5 X 0.5--1 mm glabrous, flat; filaments to 1 mm; anthers to 3.5 mm, connective protruding to 0.5 mm, acute. Achenes fusiform, 6--10 X 0.8--1 mm, tortuose, distinct rim, glabrous; persistent style to 3 cm, velutinous.

Distribution: Nepal, E Himalaya, Assam-Burma, S Asia, E Asia.

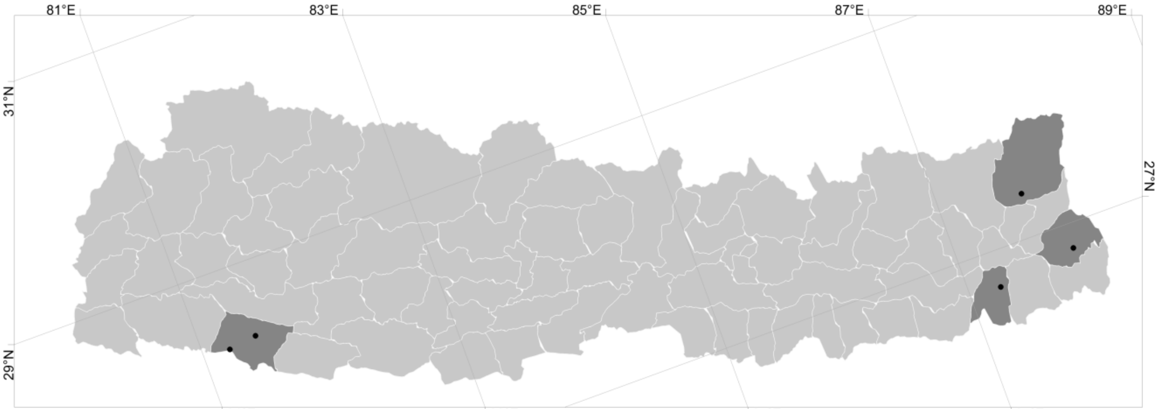


Figure 2.31 Distribution of *Clematis zeylanica* (L.) Poir. in Nepal

Specimens examined: **Eastern**, Bhajracharya, T.K. 17790 (KATH); Hara, H., Kanai, H., Kurosawa, S., Murata, G. & Togashi, M. 6300437 (BM); Hara, H., Kanai, H., Kurosawa, S., Murata, G. & Togashi, M. 6300437 (K); Upadhyay, A.V. 1340 (BM); **Mid Western,** Bhattacharya, T.K. 3647 (KATH); Kurmi, P.P. 10009 (KATH); Sharma, P.R. 3725 (KATH); Shukla, R.N. 5116 (KATH)

Altitudinal range: 150 m-290 m.

Ecology: Tropical Terai Forest; Sal forest.

Flowering Time: October.

Fruiting Time: November-January.

National IUCN Status (Provisional): Data Deficient (DD).

Clematis zeylanica has an EOO of 30,038 km^2 suggesting Near Threatened (NT) and an AOO of 20 km^2 suggesting Endangered (EN), however there are no data on population or range fluctuations.

Notes: There are very few collections of this species and the exisiting specimens are generally poor and lacking good flowers and mature fruit, making it difficult to describe it well from Nepalese material. Flower and fruit characters were taken from the following Indian material at E: Buchanan-Hamilton 1253-1, 1253-2; Salanha, 15563, Cave *s.n.* (3.1.1922). The flowering time was also taken from flowering specimens from India and therefore may be an inaccurate reflection of the actual flowering time in Nepal.

There are duplicates of a single collection at BM, E & K and five collections from Nepal in KATH. It is however a widespread species occuring from NW India into SE Asia and it is likely that it will occur in most of Nepal's poorly-collected Terai belt.

Clematis zeylanica is readily distinguished from all other species in Nepal by its terminal leaflets which are modified into tendrils, though according to Johnson (2001) tendrils are not present on immature plants. The five collections in KATH had all previously been misidentified as *C. montana*, despite clear differences in the leaves and inflorescences between the two species.

Johnson (2001) placed into synonymy the genus *Naravelia* with *Clematis*. Xie (2011) suggested that the molecular data did not support *Naravelia* being retained as a distinct genus, supporting Johnson's earlier morphological assessment. However, Johnson failed to create *nomen nova*. for two SE Asian species whose epithets had already been used in *Clematis*. This was resolved by Fack & Lehtonen (2014).

Uncertain Taxa

Clematis bracteolata Tamura Acta Phytotax. Geobot. 23(1-2):28 1968.

JOHNSON, The Genus *Clematis*: 295 (2001). Yonakura in: OHBA et al., Fl. Mustang [Nepal]: 70. (2008)

Type: Nepal: Mustang district, Sangda 3700 m. Mishioka & Namikawa 558, 27th July 1958. (KYO) Photograph!

Notes: *Clematis bracteolata* is endemic to Nepal and known only from the type collection in KYO. Yonekura (2008) noted that he was unable to locate this specimen when writing the account for the *Flora of Mustang*.

This species was described as having solitary flowers and differentiated from other species in having two ternate bracteoles midway on the peduncle.

Tamura likened *Clematis bracteolata* to *C. pseudo-pogonandra*. Johnson (2001) and Yonekura (2008) appear to have simply modified the original description. Based on the poor quality digital image of the specimen available from The Kyoto University Museum website (http://www.museum.kyoto-u.ac.jp), I believe it is closer to *C. tibetana* subsp. *brevipes* which can have solitary flowers and can also have bracteoles that are ternate. However due to the poor quality of the digital image and until further material can be gathered and the type material is refound at KYO it is not possible to make a definitive decision about the identity of this specimen.

Cultivated Taxa ***Clematis terniflora*** **-** cultivated at Godawari botanic garden.

Chapter 3: The evolution of tribe *Anemoneae* DC. (Ranunculaceae)

Introduction

The Ranunculaceae Juss. is a cosmopolitan, but mainly temperate, plant family of 62 genera and ca. 2500 species (Mabberley 2008). Within the order Ranunculales Berchtold & J. Presl its sister family is the Berberidaceae Juss., and several phylogenetic studies have found strong support for this relationship (Kadereit *et al.* 1995; Hoot *et al.* 1999; Wang, *et al.* 2009).

The generally accepted subfamily structure of the Ranunculaceae follows Tamura (1993) and consists of five subfamilies; *Helleboroideae*, *Hydrastidoideae*, *Isopyroideae*, *Ranunculoideae* and *Thalictroideae*. The molecular relationships in the Ranunculaceae have been studied most recently by Cai *et al.* (2010) who used *rbc*L data to assess the subfamilial relationships and suggested a revised classification of five subfamilies: *Coptoideae*, *Helleboroideae*, *Hydrastidoideae*, *Ranunculoideae* and *Thalictroideae*..The APG website (Stevens 2001 onwards) for the Ranunculaceae claims to follow Tamura (1993) but appears to follow its own classification of *Glaucidioideae* (one species), *Hydrastidoideae* (one species), *Coptoideae* (17 species), *Thalictroideae* (450 species) and *Ranunculoideae* (2000+ species). The APG treatment placed into synonymy *Helleboroideae* with *Ranunculoideae* and *Isopyroideae* with *Coptoideae*. The treatment also included the monotypic *Glaucidioideae* which have previously been placed in a monotypic family Glaucidiaceae by Tamura (1972) because of its affinities to both the Ranunculaceae and the Papaveraceae and was not part of the Cai et al. (2010) study.

The *Ranunculoideae*, the largest of the subfamilies, is further subdivided into a differing number of tribes depending on the taxonomy followed. Tamura (1993) recognised three tribes, *Adonideae*, *Anemoneae* and *Ranunculeae*, while Mabberley (2008) recognised seven, *Helleboreae*, *Actaeaea*, *Nigelleae*, *Delphinieae*, *Adonideae*, *Anemoneae* and *Ranunculeae*. *Ranunculoideae* has been studied extensively using molecular data. Many of the studies have tested the monophyly of speciose genera and how they relate to smaller allied genera.

Emadzade *et al.* (2010) concluded that the tribe *Ranunculeae* should be circumscribed to maintain the monophyly of a speciose and broadly-defined *Ranunculus* (ca. 600 species, Mabberley 2008) by including within it several formerly accepted genera - *Batrachium* (DC.) Grey, *Aphanostemma* A.St.-Hil. and *Gampsoceras* Steven. This revised circumscription maintains several small genera: *Arcteranthis* Greene, *Beckwithia* Jeps, *Callianthemoides* Tamura, *Ceratocephala* Moench, *Coptidium* Nyman, *Cyrtorhyncha* Nutt., *Ficaria* Schaeff., *Halerpestes* Greene, *Hamadryas* Comm. ex Juss., *Krapfia* DC., *Kumlienia* Greene, *Laccopetalum* Ulbr., *Myosurus* L., *Oxygraphis* Bunge, *Paroxygraphis* W.W.Sm., *Peltocalathos* Tamura and *Trautvetteria* Fisch. & C.A.Mey.

Jabbour & Renner (2012) studied the relationships of speciose genera in tribe Delphinieae Schrödinger, *Aconitum* L. (ca. 300 species, Mabberley 2008) *Delphinium* L. (ca. 320 species, Mabberley 2008) and the less speciose *Consolida* (ca. 40 species (Mabberley 2008) and *Aconitella* (ca. 10 species, generally placed into synonymy with *Delphinium*, Mabberley 2008). They showed *Aconitum* to be nested in *Delphinium sensu lato* but proposed the synonymisation of *Consolida* and *Aconitella* with *Delphinium* and the separation of the basal lineage of *Delphinium* (*Staphisagria*) to maintain the monophyly of *Delphinium* and *Aconitum*. This approach maintains the nomenclatural stability of the ca. 300 species of *Aconitum*.

The genera of the tribe Anemoneae DC. have also been reasonably well studied. It was shown that the moderately speciose *Anemone* (ca. 150 species (Mabberley 2008) should be more widely circumscribed to include related genera *Barneoudia* Gay, *Hepatica* Mill., *Knowltonia* Salisb., *Oreithales* Schltdl., and *Pulsatilla* Mill. (Ehrendorfer & Samuel 2001; Schuettpelz *et al.* 2002; Meyer *et al.*2010; Hoot *et al.* 2012; Zhang *et al.* 2015b). These studies support previous taxonomic decisions based on morphology and chromosome numbers to synonymise these genera with *Anemone* (Dutton *et al.* 1997). In the case of Chinese species of *Pulsatilla* (Jiang *et al.* 2015) the taxonomic decisions were directly based on the results of these molecular studies.

Miikeda *et al.* (2006) studied the relationships in *Clematis* L. (ca. 300 species Mabberley 2008) and related genera and found *Archiclematis* (Tamura) Tamura, *Naravelia* DC. and *Clematopsis* Bojor ex Hutch. were all nested in *Clematis*, a finding supported by Xie *et al.* (2011). These molecular results in turn supported taxonomic decisions that had been made about the placement of these genera in *Clematis* based on morphology: *Clematopsis* (Tamura 1987; Grey-Wilson 2000), *Archiclematis* (Grey-Wilson 2000; Johnson 2001; Wang & Bartholemew 2006) *and Naravelia* (Johnson 2001).

While the phylogenetic relationships have been assessed and taxonomic relationships of these genera have been tested through monophyly, studies investigating the evolution of these groups using molecular dating techniques are still lacking. *Ranunculus* has been dated using methods which use a mixture of criteria to constrain nodes e.g. secondary calibration, the fossil record, age calculations based on allelic frequency and the geological age of an island (Emadzade & Hörandl 2011; Hörandl & Emadzade 2011). *Clematis* (Xie *et al*. 2011) and *Delphinium* (Jabbour & Renner 2012) also both been investigated using secondary dating methods to constrain nodes (Xie *et al.* 2011; Jabbour & Renner 2012).

There is little agreement in the timing of the events of the evolutionary history of the Ranunculaceae. The estimated evolulutionary age of divergence of the Ranunculaceae and the Berberidaceae range from 104 Ma (million years ago) (Anderson *et al.* 2005) to 67 Ma (Bell *et al.* 2010), while the crown of the Ranunculaceae ranges from 87 Ma (Anderson *et al.* 2005) to 55 Ma (Bell *et al.* 2010).

Table 3.1 Estimated evolutionary ages of the divergence of the Ranunculaceae & the Berberidaceae and the crown node indicating the diversification of the extant diversity in the Ranunculaceae from published studies.

	Berberidaceae - Ranunculaceae split Ma	Crown of Ranunculaceae Ma
Nonparametric rate smoothing (NPRS) (Anderson *et al.* 2005)	104	87
DelTran (Wikiström *et al.* 2001)	100	85
Penalized Likelihood (PL) (Anderson *et al.* 2005)	90	73
ML (Wikiström *et al.* 2001)	87	72
ACCtran (Wikiström *et al.* 2001)	84	65
Penalized Likelihood (PL) uncorrelated lognormal (UCLN) (Magallón *et al.* 2015)	80.28 (68.17-95.84)	-
Lognormal Clock Mean (95% HDP) (Bell *et al.* 2010)	72 (58-87)	59 (44-73)
Exponential Clock Mean (95% HDP) (Bell *et al.* 2010)	67 (54-80)	55 (41-66)

In terms of calibration points, the most robust study of evolutionary ages across the angiosperms is that of Magallón *et al.* (2015), which used 137 fossil-calibrated nodes in a phylogeny representing 87% of the families reconstructed and used 5 plastid and nuclear markers. The study is the most comprehensive picture of key timings in angiosperm evolution to date.

Fossils and dating

Using fossil calibrated molecular phylogenies is the key approach to correlating biological evolution with geological, paleoclimatic and other historical events and processes that have shaped extant biodiversity. However, it is an evolving field and its methodologies continue to develop as more data become available. Justifying the use of fossils and correctly assigning them to extant groups is essential to minimise error in analyses (Parham 2012).

There are few unequivocal fossils belonging to the Ranunculaceae and thus few are available to be used in dating studies. Pigg & Devore (2005) noted that in comparison to other families in the Ranunculales, such as the Menispermaceae and the Berberidiaceae, the Ranunculaceae's fossil record is almost non-existent before the Oligocene and relatively poor from the Miocene onwards. The reasons given for this include the primarily herbaceous habit of plants in the Ranunculaceae, the lower preservation potential of leaves, flowers and pollen versus trees in the woodland environment and the perceived preference for habitats not conducive to fossilisation, such as high altitude areas.

Some studies have suggested that genera are fossil-poor such as those in *Ranunculus* (Paun 2005, Emadzade & Hörandl 2011; specifically pre-quaternary fossils), *Aconitum* (Jian *et al.* 2014) and *Anemone* (Meyer *et al.* 2010). When fossils do exist in the tribe *Ranunculeae* DC they are excluded from use because it is difficult to attribute them to genera. (Emadzade & Hörandl 2011) or to extant groups within genera (e.g. *Delphinium*, Jabbour & Renner 2012; and *Clematis*, Xie *et al.* 2011). However, fossil Ranunculaceae have been used in Basal Eudicot studies (Anderson *et al.* 2005) and in studies across the Angiosperms (Magallón 2015). Fossils have also been used in genus-level studies such as those on *Caltha* (Cheng & Xie 2014) and *Myosurus* (Emadzade & Hörandl 2011).

Recently there was the discovery of the fossil Ranunculaceae taxon *Leefructus*, from early Cretaceous deposits (125.8 - 122.6 Ma) in China and assigned to the stem Ranunculaceae by

Sun *et al.* (2011). There is, however, some controversy over the authenticity of the fossil highlighted by Zhou (2014), but no details are given. There is no evidence of its use in dated molecular studies at this time. If this fossil is confirmed it has the possibility to significantly alter the age of the Ranunculaceae, and the Ranunculales.

Although not discussed by Cheng & Xie (2014), their study of the evolution of *Caltha* which used a fossil-calibrated approach reported the root age of their analysis to be 123.4 Ma (95% HPD: 94.5-156.4). This is in line with the fossil reported by Sun *et al.* (2011) and substantially older than the reported ages for the crown of Ranunculaceae (Wikistrom *et al.* 2001; Anderson *et al.* 2005; Bell *et al.* 2010; Magallón *et al*. 2015). Two Ranunculaceae fossils were used to calibrate the phylogeny of *Caltha* (Cheng & Xie 2014). *Eocaltha zoophilila* Rodriguez-de la Rosa, Cevallos-Rerriz & Silva-Pineda, a fossil from the late cretaceous (71-81 Ma), is believed to be a close relation to *Caltha* with the shared morphology of adaptations for aquatic seed dispersal. The crown of *Cimicifugia* was calibrated with a normally distributed mean of 54 Ma to correspond to the *Paleoactea* Pigg & DeVore from the late Paleocene (51-57 Ma).

Emadzade & Hörandl (2011) used a mixture of calibration techniques to date evolutionary events in the tribe *Ranunculeae*. The oldest calibration point used was the split between *Ranunculus* and *Clematis* which was set at 46.6 Ma using the date estimated by Anderson *et al.* (2005). A node corresponding to the stem of *Myosurus* was calibrated using fossil achenes from the Oligocene with a minimum age of 23 Ma. Achenes of *Myosurus* have distinctive morphological and anatomical features; the outer face of the achene is planar and has a terminal beak, so the determination of this fossil is regarded as reliable. In the *Ranunculus* clade, the divergence time between *R. carpaticola* and *R. notabilis* was set to 0.914 Ma, based on the genetic distance from allelic frequencies of allozymes. Finally *R. caprarum*, the only endemic buttercup of the Juan Fernández Archipelago, was constrained using the maximum age of emergence of the island archipelago. The node where this species diverged from the mainland linage was set to 2 Ma, the maximum age of the island. The authors reasoned the islands' geological history is not complicated and also that there has not been a radiation of species from the initial colonisation, *R. caprarum* being the sole *Ranunculus* on the archipelago.

Fossils have not yet been used to date molecular phylogenies of *Clematis* (Xie *et al.* 2011) as they are unattributable to extant groups. Pigg & Devore (2005) include two *Clematis* fossils in a list of Ranunculaceae fossils. A fossil achene found in Germany and assigned to the fossil taxon *Clematis panos* Heer (Weyland 1937) was dated to the Oligocene (23-33.9 Ma). The other putative fossil assigned to *Clematis*, based on its leaf morphology and found in British Columbia, dates to the early-middle Eocene (ca. 55-40 Ma). Reid & Reid (1915) detailed the paleo-flora of the Ruvarian and Teglian plant-beds from the Dutch-German border dating from the Pliocene (2.58 - 5.33 Ma) which includes fossil achenes of *Clematis* and *Anemone*. The *Clematis* achenes were likened to the extant European *Clematis vitalba* by the authors but were identified by Reid & Reid (1915) as Himalayan *C. grata* based on a greater similarity in achene morphology.

Correct placement of fossil taxa on a phylogeny is essential in order to minimise errors. Assigning fossils to incorrect nodes will inevitably lead to distortions in the estimation of the age of evolutionary events (Parham *et al*. 2011). Fossil ages cannot be considered definitive, but are the best current estimate and are thus liable to change with better understanding of the stratigraphy in which they were found. Justifying the use of fossils and correctly assigning them to extant groups is an essential premise for any analysis, to provide a best estimate (Parham *et al.* 2011). Emadzade & Hörandl (2011) is a good example of well-justified fossil use – they demonstrated the morphological similarity of their cited fossil to extant *Myosurus*, give an indication of their confidence and referred back to previous literature (Tamura 1995; Emadzade *et al.* 2010).

Milne (2009) demonstrated that for groups lacking fossil evidence it is possible to estimate maximum age ranges by using deep fossils to calibrate phylogenies. The study also showed that increased taxon sampling would likely improve the accuracy of estimated diversification ages.

In the case of groups in the Ranunculaceae for which there are few fossils, such as *Clematis* and *Anemone*, the ability to estimate their ages using calibrated points further back in their history is at the moment the only way to proceed. Xie *et al.* (2011) dated *Clematis* using a single secondary calibration, based on the Anderson *et al.*'s (2005) study, which used fourteen calibrated nodes in comparison to Magallón *et al.*'s (2015) 137. Thus Magallón *et al.* (2015) provides a more robust estimate. In the case of *Anemone* there are no dated molecular phylogenies due to a lack of fossils (Meyer *et al.* 2010).

As Parham *et al.* (2011) noted, the refinement and revision of geochronological literature must be taken into account when calibrating analyses. In this case as revised estimated divergence dates become available, such as the Magallón *et al* (2015) study, datasets that have used secondary calibration points with ages are different should be reanalysed.

Dated phylogeny of *Clematis*

Previous genus level studies into the history of *Clematis* by Miikeda *et al.* (2006) and Xie *et al.* (2011) have highlighted its relatively recent evolution. The Miikeda *et al.* (2006) study sampled 32 taxa, approximately 10% of the currently accepted species, covering the taxonomic diversity of *Clematis*. Their study inferred that *Clematis* had undergone recent and rapid speciation because the monophyletic clade containing *Clematis* was well differentiated but had short branch lengths. They also suggested that some clades had distributions that spanned several continents, which are the result of long distance dispersal through *Clematis*’s wind-dispersed achenes. Xie *et al.* (2011) expanded the taxon sampling to 75 taxa, approximately 25% of the currently accepted species. This study had a heavy focus on species covered in the Flora of China where approximately 30% of global diversity of *Clematis* is found. The existing sampling of 25% of the described diversity while covering much of the taxonomic diversity has poor representation of non-Chinese species. For instance only a single Himalayan species (*Clematis alternata*) has been sampled and used in both studies.

The Xie *et al*. (2011) study also used an *a priori* calibrated dated molecular phylogeny approach to estimate that the stem age of *Clematis* originated in the Oligocene, 25.99 Ma (95% HPD: 9.19-43.77 Ma) and a crown age from the Miocene, 7.81 Ma (95% HPD: 3.99-13.14 Ma). Their findings of a long stem to the *Clematis* clade and relatively recent crown age in conjunction with the lack of resolution at the base of the clade supported Miikeda *et al.*’s inference that *Clematis* has undergone recent and rapid speciation, but they did not discuss the possible reasons for this long root branch length between *Clematis* and the sister genera.

To evaluate the age of *Clematis*, Xie *et al.* used a secondary calibration point at the crown node of their dataset, constraining this to 46.59 Ma - the divergence of *Ranunculus* from *Clematis* and other genera, cited by Anderson *et al.* (2005). The authors held back from a from describing a detailed historical biogeographical reconstruction of the genus, presumably due to the poorly supported and unresolved relationship between major clades; but they did suggest that the major driver of speciation in the genus was global climate change during the Neogene and beginning of the Quaternary.

A fossilised achene from Germany was attributed to the fossil taxon *Clematis panos* Herr by Weyland (1937), but is unfortunately un-attributable to extant groups in the genus because of the relatively poorly preserved character features of the fossil achenes and the achenes of extant *Clematis*. The fossil achene is, in my opinion, probably not that of a *Clematis*. While it is does share characteristics with Anemonoid achenes it lacks the distinctive plumose, persistent style found on many of the achenes of tribe *Anemoneae* (*Clematis, Anemoclema* and *Pulsatilla* part of *Anemone sensu lato*).

If this fossil achene is correctly attributed to *Clematis*, then the Oligocene date (23-33.9 Ma) for the fossil would likely make the estimated stem age for *Clematis* reported by Xie *et al.* questionable because of the large confidence interval associated with the 25.99 Ma (95% HPD: 9.19-43.77 Ma) date. This is also true for the possible Clematis leaf fossil from the Eocene (40 - 55 Ma). Both fossils may also substantially alter the crown date of 7.81 Ma (95%HPD: 3.99-13.14 Ma) reported in that study.

As the Xie *et al.* did not use fossil calibration the study relied on a secondary date of divergence citing Anderson *et al.* (2005), arguing that it is a robust date because Anderson *et al.* 'used multiple fossil calibration points and the terminal dates were probably underestimates'. However after reviewing the Anderson *et al.* paper it is unclear how Xie *et al*. derived the 45.59 Ma date from this study. The only dates reported in the paper and supplementary material relate to the crown and stem ages for the whole family, not the time at which *Ranunculus* and *Clematis* diverged. This date was also cited and used as a secondary calibration point in a study (Emadzade & Hörandl 2011) focusing on *Ranunculus* and again it was not clear how this date was derived.

In both previous studies, *Clematis* formed a poorly supported grouping with <50% bootstrap support (Miikeda *et al.* 2006) and > 70% MB value from Maximum Likelihood (Xie *et al.* 2011). Neither study referred directly the lack of support along the backbone of the *Clematis* clade and the impact that this would have on the inferences they drew on the evolutionary history with in the genus.

In the course of this research, the uncertainties over dating in Ranunculaceae studies became apparent and the sequencing of new Himalayan specimens of *Clematis* presented the opportunity to revise and reassess the evolutionary history the Ranunculaceae, specifically the *Anemoneae* DC., in order to the robustly re-evaluate the evolutionary history of *Clematis.*

Aims and Objectives

Aim

To re-assess the evolutionary history of the Ranunculaceae with a particular focus on dating the tribe *Anemoneae* in order to provide a wider context for a more detailed analysis of *Clematis* (chapter 4).

Objectives

- Reanalyse the existing molecular dataset for the Ranunculaceae for which we have complete data (ITS and *atpB-rbcL* regions), building on the work of Miikeda *et al.* (2006) and Xie *et al.* (2011).

- Incorporate Nepalese *Clematis* into the existing phylogenetic framework with these regions

- Calibrate analysis with multiple fossils and a secondary calibration point to explore family-level divergence events

Materials and methods

Taxon sampling

Thirty-two accessions of *Clematis* were sampled from collections gathered in the field on Flora of Nepal fieldwork and held at the Royal Botanic Garden Edinburgh (RBGE). A further ten samples collected during fieldwork to Nepal in (2012) and eleven from fieldwork in 2014 were also included. Five samples collected by other RBGE projects in Oman, Russia, France, Bhutan and USA were also included.

The intention was to have the largest complete dataset of accessions from the tribe *Anemoneae* and related outgroups possible. Based on gene regions used in previous studies and available sequences in Genbank this would require both the nuclear DNA (nDNA) internal transcribed spacer region (ITS) and the chloroplast (cpDNA) *aptB-rbcL* spacer. Seventeen of the 32 new, Nepalese and other accessions (Table 3.2) were successfully amplified for both those regions. The existing ITS and *atpB-rbcL* sequence data for all *Anemone*, related genera and outgroups from other genera in the Ranunculaceae were downloaded from Genbank (Table 3.3).

Table 3.2 Amplified *Clematis* accessions from collections at RBGE. With their unique EDNA number, identification and Collection information

EDNA number	Taxon Name	Collection	Country of Origin
EDNA13-0034028	*Clematis connata*	Darchula(2012)1217028	Nepal
EDNA13-0034032	*Clematis montana*	EKSIN 218	Nepal
EDNA13-0034035	*Clematis tibetana* subsp. *brevipes*	Manaslu 08 20815142	Nepal
EDNA13-0034037	*Clematis alternata*	Manaslu 08 20816057	Nepal
EDNA13-0034038	*Clematis grewiiflora*	Manaslu 08 20812158	Nepal
EDNA13-0034041	*Clematis montana*	Manaslu 08 20816085	Nepal
EDNA13-0034043	*Clematis ladakiana*	Chadwell 81	Pakistan
EDNA13-0034044	*Clematis alpina*	Schof s.n.	France
EDNA13-0034047	*Clematis montana*	EMAK 399	Nepal
EDNA14-0036908	*Clematis* sp nov 1.	GAK 8802	Oman

EDNA14-0036910	*Clematis* sp nov 2.	BRD A119	Nepal
EDNA14-0036912	*Clematis grata*	BRD A198	Nepal
EDNA14-0036913	*Clematis graveolens*	BRD A216	Nepal
EDNA14-0036914	*Clematis tibetana* subsp. *brevipes*	BRD A219	Nepal
EDNA14-0036915	*Clematis graveolens*	BRD A220	Nepal
EDNA14-0036917	*Clematis phlebantha*	BRD A262	Nepal
EDNA14-0036920	*Clematis grata*	BRD A208	Nepal

Table 3.3 GenBank accessions used in the *Anemoneae* dataset

Name	***atp*B-*rbc*L**	**ITS**	**Voucher**	**Reference**
Aconitum rockii	KF233820.1	KF233840.1	isolate X109	Cheng and Xie (2014)
Actaea dahurica	KF233828.1	KF233848.1	isolate XWT041	Cheng and Xie (2014)
Actaea vaginata	KF233827.1	KF233847.1	isolate X110	Cheng and Xie (2014)
Adonis brevistyla	KF233829.1	KF233849.1	isolate X144	Cheng and Xie (2014)
Anemoclema glaucifolium	KJ819821.1	KJ819802.1	Q. E. Yang 3104	Zhang *et al.* (2015)
Anemoclema glaucifolium	KJ819822.1	KJ819801.1	Q. E. Yang 2649	Zhang *et al.* (2015)

Anemone americana	AY055407.1	AY055386.1		Schuettpelz *et al.* (2002)
	JF810701.1	JF810691.1	isolate3185_3191	Hoot *et al.* (2012)
Anemone antucensis	AF311735.1	AY056049.1		Ehrendorfer and Samuel (2001)
Anemone begoniifolia	KJ819830.1	KJ819810.1	Y. Zhang 91	Zhang *et al.* (2015) (2015)
Anemone blanda	AY055422.1	AY055402.1		Schuettpelz *et al.* (2002)
Anemone bracteata	JF810702.1	JF810692.1	isolate 426	Hoot,S.B *et al.* (2012)
Anemone caffra	AY055420.1	AY055399.1		Schuettpelz *et al.* (2002)
Anemone canadensis	AY055408.1	AY055387.1		Schuettpelz *et al.* (2002)
Anemone caroliniana	AY055423.1	AY055403.1		Schuettpelz *et al.* (2002)
Anemone cernua var. koreana	GU732566.1	GU732647.1	Han&Lee062583	Xie *et al.* (2011)
Anemone crassifolia	AY055419.1	AY055398.1		Schuettpelz *et al.* (2002)
Anemone davidii	KJ819831.1	KJ819811.1	Y. Zhang 93	Zhang *et al.* (2015)

Anemone demissa	AY055413.1	AY055392.1		Schuettpelz *et al.* (2002)
Anemone demissa	KJ819819.1	KJ819799.1	J. Cai *et al.* 13PXB025	Zhang *et al.* (2015) (2015)
Anemone drummondii	AY055424.1	AY055404.1		Schuettpelz *et al.* (2002)
Anemone fanninii	JF810695.1	JF810685.1	isolate 491	Hoot *et al.* (2012)
Anemone flaccida	AB115462.1	AY055391.1		Miikeda *et al.* (2006)
Anemone flaccida	AY055412.1	KJ819809.1		Schuettpelz *et al.* (2002)
Anemone flaccida	KJ819829.1	AB120212.1	Y. Zhang 90	Zhang *et al.* (2015)
Anemone griffithii	KJ819818.1	KJ819798.1	J. Cai *et al.* 13CS7679	Zhang *et al.* (2015)
Anemone hokouensis	KJ819828.1	KJ819808.1	Y. Zhang & M. Tang 89	Zhang *et al.* (2015)
Anemone hortensis	FJ639847.1	KJ819805.1	isloate 62	Meyer *et al.* (2010)
Anemone howellii	KJ819825.1	AY055397.1	M. Tang & J. P. Luo	Zhang *et al.* (2015)
Anemone hupehensis	AY055418.1	KJ819793.1		Schuettpelz *et al.* (2002)
Anemone imbricata	KJ819813.1	AY055390.1	J. Cai *et al.* 13CS6946	Zhang *et al.* (2015)

Anemone keiskeana	AY055411.1	AY055401.1		Schuettpelz *et al.* (2002)
Anemone knowltonia	AY055421.1	AY055405.1		Schuettpelz *et al.* (2002)
Anemone multifida	AY055425.1	AY055393.1		Schuettpelz *et al.* (2002)
Anemone narcissiflora	AY055414.1	JF422886.1		Schuettpelz *et al.* (2002)
Anemone nemorosa	AF386091.1	AY055394.1		Ehrendorfer and Samuel (2001)
Anemone obtusiloba	AY055415.1	KJ819797.1		Schuettpelz *et al.* (2002)
Anemone obtusiloba	KJ819815.1	KJ819795.1	J. Cai *et al.* 13CS6953	Zhang *et al.* (2015)
Anemone obtusiloba	KJ819817.1	AY055400.1	J. Cai *et al.* 13CS7032	Zhang *et al.* (2015)
Anemone occidentalis	AY055426.1	KJ819812.1		Schuettpelz *et al.* (2002)
Anemone orthocarpa	KJ819832.1	FJ639888.1	Y. Zhang & M. Tang 76	Zhang *et al.* (2015)
Anemone pavonina	FJ639852.1	JF810686.1	Isolate 63	Meyer *et al.* (2010)
Anemone quinquefolia	JF810696.1	FJ639889.1	isolate 28	Hoot *et al.* (2012)
Anemone ranuculoides	FJ639853.1	AY055388.1	isloate 55	Meyer *et al.* (2010)

Anemone richardsonii	AY055409.1	KF233842.1		Schuettpelz *et al.* (2002)
Anemone rivularis	AY055417.1	KF233842.1		Schuettpelz *et al.* (2002)
Anemone rivularis	GU732490.1	AY055396.1	Tibet761	Xie *et al.* (2011)
Anemone rivularis	GU732490.1	GU732571.1	Tibet7612	Xie *et al.* (2011)
Anemone rivularis	KF233822.1	GU732571.1	isolate X91	Cheng and Xie (2014)
Anemone rivularis	KF233822.1	KJ819807.1	isolate X912	Cheng and Xie (2014)
Anemone rivularis	KJ819827.1	KJ819796.1	Y. Zhang & M. Tang 88	Zhang *et al.* (2015)
Anemone rupicola	KJ819816.1	KJ819804.1	J. Cai *et al.* 13CS7010	Zhang *et al.* (2015)
Anemone sumatrana	KJ819824.1	KJ819803.1	Tran Van Tien s.n.	Zhang *et al.* (2015)
Anemone sylvestris	KJ819823.1	AY055389.1	Y. Liu 178	Zhang *et al.* (2015)
Anemone tenuicaulis	AY055410.1	JF810689.1		Schuettpelz *et al.* (2002)
Anemone tenuifolia var. tenuifolia	JF810699.1	JF810690.1	isolate 2310	Hoot *et al.* 92014
Anemone thomsonii	JF810700.1	KJ819800.1	isolate 2513	Hoot *et al.* (2014)

Anemone tomentosa	KJ819820.1	FJ639905.1	J. P. Luo & L. Wang	Zhang *et al.* (2015)
Anemone transsilvanica	FJ639869.1	AY055395.1		Meyer *et al.* (2010)
Anemone trullifolia	AY055416.1	KJ819794.1		Schuettpelz *et al.* (2002)
Anemone trullifolia var. linearis	KJ819814.1	FJ639898.1	J. Cai *et al.*	Zhang *et al.* (2015)
Anemone virginiana	FJ639862.1	KJ819806.1		Meyer *et al.* (2010)
Anemone xingyiensis	KJ819826.1	FJ639882.1	Y. Zhang & M. Tang 77	Zhang *et al.* (2015)
Asteropyrum cavaleriei	KF233826.1	KF233846.1	isolate Zhu s.n.	Cheng and Xie (2014)
Batrachium pekinense	KF233824.1	KF233844.1	isolate X2010017	Cheng and Xie (2014)
Callianthemum anemonoides	AY365400.1	AY365390.1		Schuettpelz and Hoot (2004)
Callianthemum coriandrifolium	AY365401.1	AY365393.1		Schuettpelz and Hoot (2004)
Caltha palustris	KF233830.1	KF233853.1	isolate X200405044	Cheng and Xie (2014)
Caltha palustris var. barthei	KF233831.1	KF233852.1	isolate X134	Cheng and Xie (2014)

Caltha palustris var. umbrosa	KF233832.1	KF233851.1	isolate X72	Cheng and Xie (2014)
Caltha palustris var. sibirica	KF233833.1	KF233850.1	isolate X2010288	Cheng and Xie (2014)
Caltha rubriflora	KF233834.1	KF233855.1	isolate D94	Cheng and Xie (2014)
Caltha rubriflora	KF233835.1	KF233854.1	isolate D95	Cheng and Xie (2014)
Caltha scaposa	KF233838.1	KF233859.1	isolate D91	Cheng and Xie (2014)
Caltha scaposa	KF233839.1	KF233858.1	isolate D99	Cheng and Xie (2014)
Caltha sinogracilis	KF233836.1	KF233857.1	isolate D90	Cheng and Xie (2014)
Caltha sinogracilis	KF233837.1	KF233856.1	isolate D96	Cheng and Xie (2014)
Clematis acerifolia	GU732491.1	GU732572.1	Shi051	Xie *et al.* (2011)
Clematis aethusifolia	GU732492.1	GU732573.1	Xie2006031	Xie *et al.* (2011)
Clematis afoliata	AB115443.1	AB120193.1		Miikeda *et al.* (2006)
Clematis akebioides	GU732493.1	GU732574.1	Xie2004025	Xie *et al.* (2011)
Clematis alpina	GU732494.1	GU732576.1	Horandl9517	Xie *et al.* (2011)

Clematis alpina	GU732495.1	GU732575.1	Wen10381	Xie *et al.* (2011)
Clematis alternata	AB115440.1	AB120190.1		Miikeda *et al.* (2006)
Clematis angustifolia	AB115449.1	AB120199.1		Miikeda *et al.* (2006)
Clematis apiifolia	AB115430.1	GU732577.1		Miikeda *et al.* (2006)
Clematis apiifolia	GU732496.1	AB120180.1	Xie2006094	Xie *et al.* (2011)
Clematis armandii	GU732497.1	GU732578.1	Xie2006050	Xie *et al.* (2011)
Clematis brachiata	GU732498.1	GU732582.1	Luke11695	Xie *et al.* (2011)
Clematis brachiata	GU732499.1	GU732581.1	Wen11065	Xie *et al.* (2011)
Clematis brachiata	GU732500.1	GU732580.1	Wen11077	Xie *et al.* (2011)
Clematis brachiata	GU732501.1	GU732579.1	Wen9472	Xie *et al.* (2011)
Clematis brachyura	AB115454.1	AB120204.1		Miikeda *et al.* (2006)
Clematis brevicaudata	GU732502.1	GU732583.1	Xie2006026	Xie *et al.* (2011)
Clematis cf. insidiosa	GU732563.1	GU732644.1	Wen 9513	Xie *et al.* (2011)

Clematis chinensis	GU732503.1	GU732584.1	Xie2006055	Xie *et al.* (2011)
Clematis chrysocoma	GU732504.1	GU732587.1	Tibet1605	Xie *et al.* (2011)
Clematis chrysocoma	GU732505.1	GU732586.1	Tibet979	Xie *et al.* (2011)
Clematis chrysocoma	GU732506.1	GU732585.1	Xie2004DL32	Xie *et al.* (2011)
Clematis confusa	GU732507.1	GU732588.1	Tibet891	Xie *et al.* (2011)
Clematis crassifolia	AB115444.1	AB120194.1		Miikeda *et al.* (2006)
Clematis crispa	GU732508.1	GU732589.1	Wen10019	Xie *et al.* (2011)
Clematis delavayi	AB115452.1	GU732590.1		Miikeda *et al.* (2006)
Clematis delavayi	GU732509.1	AB120202.1	Tibet1256	Xie *et al.* (2011)
Clematis drummondii	GU732510.1	GU732591.1	Wen9735	Xie *et al.* (2011)
Clematis eichleri	AB115459.1	AB120209.1		Miikeda *et al.* (2006)
Clematis erectisepala	GU732511.1	GU732592.1	Xie2003YJ01	Xie *et al.* (2011)
Clematis fasciculiflora	AB115453.1	AB120203.1		Miikeda *et al.* (2006)

Clematis finetiana	GU732512.1	GU732593.1	Xie2006052	Xie *et al.* (2011)
Clematis florida	AB115436.1	AB120186.1		Miikeda *et al.* (2006)
Clematis fusca	AB115429.1	GU732594.1		Miikeda *et al.* (2006)
Clematis fusca	GU732513.1	AB120179.1	Xie2006004	Xie *et al.* (2011)
Clematis gentianoides	AB115460.1	AB120210.1		Miikeda *et al.* (2006)
Clematis glycinoides	GU732514.1	GU732595.1	Mcpherson622	Xie *et al.* (2011)
Clematis heracleifolia	GU732515.1	GU732596.1	Xie & Shi 2003H03	Xie *et al.* (2011)
Clematis hexapetala	AY055406.1	AY055385.1		Schuettpelz *et al.* (2002)
Clematis hexapetala	GU732516.1	GU732597.1	Xie2003143	Xie *et al.* (2011)
Clematis integrifolia	GU732517.1	GU732599.1	Horandl9512	Xie *et al.* (2011)
Clematis integrifolia	GU732518.1	GU732598.1	Wen10380	Xie *et al.* (2011)
Clematis intricata	KF233823.1	KF233843.1	isolate X2010722	Cheng and Xie (2014)
Clematis japonica	AB115437.1	AB120187.1		Miikeda *et al.* (2006)

Clematis lasiandra	AB115435.1	GU732600.1		Miikeda *et al.* (2006)
Clematis lasiandra	GU732519.1	AB120185.1	Xie2004082	Xie *et al.* (2011)
Clematis lasiantha	AB115450.1	GU732602.1		Miikeda *et al.* (2006)
Clematis lasiantha	GU732520.1	GU732601.1	Janeway9366	Xie *et al.* (2011)
Clematis lasiantha	GU732521.1	AB120200.1	Janeway9369	Xie *et al.* (2011)
Clematis leschenaultiana	GU732522.1	GU732603.1	Xie2004001	Xie *et al.* (2011)
Clematis ligusticifolia	AB115451.1	GU732605.1		Miikeda *et al.* (2006)
Clematis ligusticifolia	GU732523.1	GU732604.1	Castro s.n.	Xie *et al.* (2011)
Clematis ligusticifolia	GU732524.1	AB120201.1	Janeway9513	Xie *et al.* (2011)
Clematis linearifolia	GU732525.1	GU732606.1	Li *et al.* 35	Xie *et al.* (2011)
Clematis loureiroana	GU732526.1	GU732607.1	Tibet 2157	Xie *et al.* (2011)
Clematis meyeniana	GU732528.1	GU732609.1	Xie2003099	Xie *et al.* (2011)
Clematis montana	GU732529.1	GU732611.1	Tibet1226	Xie *et al.* (2011)

Clematis montana	GU732530.1	GU732610.1	Xie2004027	Xie *et al.* (2011)
Clematis nobilis	AB115456.1	AB120206.1		Miikeda *et al.* (2006)
Clematis ochotensis	AB115432.1	AB120182.1		Miikeda *et al.* (2006)
Clematis orientalis	AB115446.1	GU732613.1		Miikeda *et al.* (2006)
Clematis orientalis	GU732531.1	GU732612.1	Li 13	Xie *et al.* (2011)
Clematis orientalis	GU732532.1	AB120196.1	Li 14	Xie *et al.* (2011)
Clematis patens	AB115434.1	AB120184.1		Miikeda *et al.* (2006)
Clematis peterae	GU732533.1	GU732615.1	Li4918	Xie *et al.* (2011)
Clematis peterae	GU732534.1	GU732614.1	Xie2004002	Xie *et al.* (2011)
Clematis pierotii	AB115441.1	AB120191.1		Miikeda *et al.* (2006)
Clematis pinnata	GU732535.1	GU732616.1	Xie2004BT01	Xie *et al.* (2011)
Clematis pitcheri	GU732536.1	GU732617.1	Wen9723	Xie *et al.* (2011)
Clematis pogonandra	GU732537.1	GU732618.1	Xie2004024	Xie *et al.* (2011)

Clematis potaninii	AB115448.1	GU732619.1		Miikeda *et al.* (2006)
Clematis potaninii	GU732538.1	AB120198.1	Xie2004023	Xie *et al.* (2011)
Clematis pubescens	GU732539.1	GU732620.1	Li *et al.* 200714	Xie *et al.* (2011)
Clematis ranunculoides	GU732540.1	GU732621.1	Xie2004ZD25	Xie *et al.* (2011)
Clematis recta	GU732541.1	GU732623.1	Horandl9514	Xie *et al.* (2011)
Clematis recta	GU732542.1	GU732622.1	Wen10382	Xie *et al.* (2011)
Clematis rehderiana	GU732543.1	GU732624.1	Xie2004DQ11	Xie *et al.* (2011)
Clematis reticulata	GU732544.1	GU732625.1	Wen9780	Xie *et al.* (2011)
Clematis rutoides	GU732545.1	GU732628.1	Wen9489	Xie *et al.* (2011)
Clematis rutoides	GU732546.1	GU732627.1	Wen9548	Xie *et al.* (2011)
Clematis rutoides	GU732547.1	GU732626.1	Wen9553	Xie *et al.* (2011)
Clematis serratifolia	AB115455.1	AB120205.1		Miikeda *et al.* (2006)
Clematis siamensis	GU732548.1	GU732629.1	Xie2004TM09	Xie *et al.* (2011)

Clematis sibirica	GU732549.1	GU732630.1	Xie2004XL029	Xie *et al.* (2011)
Clematis sp.	GU732564.1	GU732645.1	Wen 9476	Xie *et al.* (2011)
Clematis stans	AB115438.1	AB120188.1		Miikeda *et al.* (2006)
Clematis strigillosa	GU732550.1	GU732631.1	Wen9691	Xie *et al.* (2011)
Clematis taiwaniana	GU732551.1	GU732632.1	Wen9385	Xie *et al.* (2011)
Clematis tangutica	AB115445.1	GU732633.1		Miikeda *et al.* (2006)
Clematis tangutica	GU732552.1	AB120195.1	Tibet57	Xie *et al.* (2011)
Clematis tashiroi	AB115442.1	AB120192.1		Miikeda *et al.* (2006)
Clematis terniflora	AB115433.1	GU732608.1		Miikeda *et al.* (2006)
Clematis terniflora var. mandshurica	GU732527.1	AB120183.1	Wen10383	Xie *et al.* (2011)
Clematis texensis	AB115447.1	GU732634.1		Miikeda *et al.* (2006)
Clematis texensis	GU732553.1	AB120197.1	Wen9749	Xie *et al.* (2011)
Clematis tibetana	GU732554.1	GU732636.1	Tibet396	Xie *et al.* (2011)

Clematis tibetana	GU732555.1	GU732635.1	Tibet917	Xie *et al.* (2011)
Clematis uncinata var. ovatifolia	AB115439.1	GU732637.1		Miikeda *et al.* (2006)
Clematis uncinata	GU732556.1	AB120189.1	Xie2004007	Xie *et al.* (2011)
Clematis villosa	AB115461.1	AB120211.1		Miikeda *et al.* (2006)
Clematis virginiana	GU732557.1	GU732640.1	Wen10002	Xie *et al.* (2011)
Clematis virginiana	GU732558.1	GU732639.1	Wen10387	Xie *et al.* (2011)
Clematis virginiana	GU732559.1	GU732638.1	Wen10494	Xie *et al.* (2011)
Clematis vitalba	AB115457.1	GU732643.1		Miikeda *et al.* (2006)
Clematis vitalba	GU732560.1	GU732642.1	Horandl9513	Xie *et al.* (2011)
Clematis vitalba	GU732561.1	GU732641.1	Wen10354	Xie *et al.* (2011)
Clematis vitalba	GU732562.1	AB120207.1	Wen10373	Xie *et al.* (2011)
Clematis williamsii	AB115431.1	AB120181.1		Miikeda *et al.* (2006)
Delphinium pachycentrum	KF233821.1	KF233841.1	isolate X263	Cheng and Xie (2014)

Helleborus orientalis	AY365416.1	AY365384.1		Schuettpelz and Hoot (2004)
Hepatica acutiloba	AM267300.1	AM267285.1		Pfosser *et al.* (2011)
Hepatica transsilvatica	FJ639869.1	FJ639905.1		Meyer *et al.* (2010)
Hepatica nobilis	FJ639868.1	FJ639904.1	isloate 472	Meyer *et al.* (2010)
Knowltonia sp.	AB115465.1	AB120215.1	OM073	Miikeda *et al.* (2006)
Naravelia laurifolia	AB115458.1	GU732646.1		Miikeda *et al.* (2006)
Naravelia laurifolia	GU732565.1	AB120208.1	Wen10182	Xie *et al.* (2011)
Pulsatilla chinensis	GU732569.1	GU732650.1	Xie2006024	Xie *et al.* (2011)
Pulsatilla dahurica	GU732567.1	GU732648.1	Xie2006025	Xie *et al.* (2011)
Pulsatilla sp.	GU732570.1	GU732651.1	Xie 2007001	Xie *et al.* (2011)
Pulsatilla turczaninovii	GU732568.1	GU732649.1	Xie2006027	Xie *et al.* (2011)
Ranunculus potaninii	KF233825.1	KF233845.1	isolate X278	Cheng and Xie (2014) (2013)

Trollius yunnanensis	AY365417	HQ440206.1	Wang *et al.* (2010)

Extraction, PCR and sequencing.

Total genomic DNA (tDNA) was extracted from silica-gel dried leaf material using the CTAB method (Doyle & Doyle 1987). This extraction method resulted in low concentration and poor quality total genomic DNA, DNeasy Plant mini Kits (Qiagen Ltd, Crawley, West Sussex, UK) were then used to re-extract samples following the manufacturer's protocol.

For PCR reactions nDNA and cpDNA primer selection followed Xie *et al.* (2011) utilising *Clematis*-specific cpDNA primers developed by Miikeda *et al.* (2006: table 3) and universal ITS primers from White *et al.* 1990.

Table 3.4 Primers used to amplify *Clematis* in this study

Primer	5' to 3'-end	Reference
ITS		
ITS5P	GGAAGGAGAAGTCGTAACAAG	White *et al.* 1990
ITS8P	CACGCTTCTCCAGACTACA	
Or		
AB101	ACGAATTCAGGTGAAGTGTTCG	Sun *et al.* 1994
AB102	TAGAATTCCCCGGTTCGCTCGCCGTTAC	
*atp*B-*rbc*L		
atpBF	AGTAGTAGGATTGATTCTCA	Miikeda *et al.*2006
rbclR	CAACACTTGCTTCTAGTCTCT	

The PCR was carried out on DNA Engine Tetrad 2 Peltier Thermal Cycler by Bio-Rad. The 25 µl reactions were modified if the standard method was ineffective (Table 3.5):

Table 3.5 PCR recipes used to amplify DNA regions in PCR reactions

First PCR		Modified		Problematic	
Reagents	Volume (µl)	Reagents	Volume µl	Reagents	Volume µl
10 x NH$_4$ reaction buffer	2.5	10 x NH$_4$ reaction buffer	2.5	10 x NH$_4$reaction buffer	2.5
dNTPs (0.2mM)	2.5	dNTPs (0.2mM)	2.5	dNTPs (0.2mM)	2.5
MgCl$_2$ (50mM)	1.25	MgCl$_2$ (50mM)	1.25	MgCl$_2$ (50mM)	1.25
Forward Primer (10µM)	0.75	Forward Primer (10µM)	0.75	Forward Primer (10µM)	0.75
Reverse Primer (10µM)	0.75	Reverse Primer (10µM)	0.75	Reverse Primer (10µM)	0.75
Taq (5U/µl)	0.2	Taq (5U/µl)	0.2	Taq (5U/µl)	0.2
CES	5	TBT-PAR	10	TBT-Par	10
Distilled H$_2$O	11.05	Distilled H$_2$O	6.05	Distilled H$_2$O	5.05
Template DNA	1	Template DNA	1	Template DNA	2

The thermal cycler for ITS was set to follow White *et al.* (1990) (Table 3.6).

Table 3.6 ITS PCR protocol

Stage	Temperature	Time	Cycles
Initial Denature	94°C	3min	1
Denature	94°C	1min	

Annealing	55°C	1min	30
Extension	72°C	90sec	
Final Extension	72°C	5min	1
Termination	10°C	forever	1

Initially, for the *atp*B-*rbc*L spacer, the thermal cycler was set to follow the protocol in Miikeda *et al.* (2006) but this yielded no results. Reagents were reordered and the following additives were tried; BSA, CES and TBT-par. Primers were reordered and the volume of template DNA was doubled. The PCR temperature protocol (Table 3.7) from Chiang *et al*. (1998) as published for use with a universal *atpB-rbcL* primer set was used in place of that of Miikeda *et al*. (2006). The major difference was a change in annealing temperature from 60°C to 49°C.

Table 3.7 Modified *atp*B-*rbc*L PCR protocol

Stage	Temperature	Time	Cycles
Initial Denature	94°C	3min	1
Denature	94°C	1min 30sec	35
Annealing	49°C	1min 30sec	
Extension	72°C	5min	
Final Extension	72°C	10min	1
Termination	10°C	forever	1

The quality and quantity of PCR product was assessed using 1% agarose gel. Samples of successfully amplified ITS with clearly visible bands were purified using ExoSAP-IT (USB Corporation) following the manufacturer’s protocol. Initially the cpDNA bands were faint, so extension and annealing times were increased by 15 seconds each and an additional 5 cycles added to the PCR protocol, as suggested by troubleshooting protocols in the RBGE molecular laboratory handbook, before clearly visible bands were purified.

10 µl sequencing reactions were carried out using DNA Engine Tetrad 2 Peltier Thermal Cycler by Bio-Rad using the standard Bigdye reaction protocol: Bigdye 0.5 µl, 5 x Sequencing Buffer 2 µl, Primer 0.32 µl, Distilled H_2O 5.68 µl, Template DNA 1.5 µl. Product to be analysed were sent to the GenePool service at University of Edinburgh.

Analysis

Sequence Data

New forward and reverse sequences fragments were combined, aligned and edited in Sequencher^{TM} (V. 4.5, Genetic Codes Corporation, Ann Arbor, Michigan, US).

All downloaded and newly generated ITS and *atpB-rbcL* sequence data were aligned automatically using MAFFT version 7, Multiple alignment program for amino acid or nucleotide sequences, through the Computational Biology Research Centres' online portal (http://mafft.cbrca.jp/alignment/server/) then checked, edited manually and where possible minimising changes, using Mesquite (Maddison & Maddison 2011, 2015).

Phylogenetic analyses were carried out using Bayesian methods. Bayesian analyses were carried out using Markov Chain Monte Carlo (MCMC) methods in MrBayes v.3.2.1. (Huelsenbeck & Ronquist 2001, Ronquist & Huelsenbeck, 2003, Ronquist *et al.* 2009). Nucleotide substitution models (Table 3.8) were tested using Akaike information criteria (AIC) in JModeltest2 v.2.1.7 (Darriba *et al.* 2012, Guindon & Gascuel 2003). Models implemented in the phylogenetic analyses that were suggested differed depending on the regions selected for analyses. Two parallel independent runs, of ten million generations were sampled every 100th generation resulting in 100,000 trees and 25% were discarded as 'burn-in'. Tracer v.1.5 was used to assess the convergence to stationarity of the results.

Table 3.8 Jmodeltest suggested models of evolution that were incorporated into the phylogenetic analyses

	Nuclear (nrITS)	**Chloroplast (atpb-rbcl)**
Number of Character	685	1334

Model	GTR+I+G	F81+G
Frequency of A	0.3522	0.3569
Frequency of C	0.1345	0.1348
Frequency of G	0.1398	0.1393
Frequency of T	0.3735	0.3690
p-inv	1.0120	0.6160
gamma	0.20	n/a

ITS and *atpB-rbcL* trees were compared clade by clade for incongruence in the topologies of the tree outputs before the combined analysis was performed.

As Bayesian support values are known to have the effect of inflating support for relationships (Huselsenbeck & Ronquist 2001; Cummings *et al*. 2003; Erixon *et al*. 2003; Simmons *et al*. 2004), a conservative approach to support values was taken, with strong support deemed to be 0.95 pp. or greater, moderate support 0.9 - 0.95 pp. and poor support less than 0.9 pp..

Divergence time estimations

Divergence times were estimated using a Bayesian approach implemented in BEAST v.1.7.4 (Drummond & Rambaud, 2012) for the combined ITS & *atp*B-*rbc*L dataset. The three nodes in the Ranunculaceae tree were constrained with the following priors: the crown of *Caltha* / *Helleborus* was constrained with a normal distribution and a mean of 77.5 Ma that corresponds to *Eocaltha zoophilila* Rodriguez-de la Rosa, Cevallos-Rerriz & Silva-Pineda. The crown of *Cimicifugia* was calibrated with a normally distributed mean of 54 Ma to correspond to the *Paleoactea* Pigg & DeVore from the late Paleocene (51-57 Ma). These calibration points were modified from Cheng & Xie (2014). The third calibration point constrained the age for the Ranunculaceae with a normally distributed mean of 87 Ma based on Anderson *et al*. (2005) for one set of analyses and a normally distributed mean 80.3 Ma based on Magallón *et al*. (2015) for another set of analyses.

For the BEAST analyses the Birth and Death incomplete sampling tree prior was selected because of incomplete coverage across the genera selected (Stadler, 2009). The analysis implemented a relaxed clock with uncorrelated log-normal model of rate variation (Drummond *et al.* 2006). The GTR+G+I model suggested by JModelTest was implemented. The input file for the analysis was generated using BEAUti v.1.7.4 (Drummond & Rambaut, 2012).

Two independent runs of 100 million generations were done for the two different sets.

The two separate runs from each analysis were checked visually using Tracer v.1.6 (Rambaut *et al.* 2013) for convergence to stationarity and that effective sample size (ESS) values of greater the 200 had been achieved. The two independent runs for each analysis we combined using LogCombiner v.1.7.4 (Drummond *et al.* 2012). A maximum clade credibility (MCC) tree was generated from the sampled trees using TreeAnnotator v.1.7.4 (Drummond *et al.* 2012), with mean ages, 95% highest posterior probability (HPD) intervals and posterior probabilities calculated.

Visualisation of Trees

Phlyogenetic and Divergence Time estimation trees were visualised using FigTree v.1.4 (Rambaut, 2012)

Results

Phylogenetic Relationships

The major clades were well supported with no incongruence, but there were minor incongruences in the topology of smaller derived clades and individual lineages (Figures 3.1, 3.2, 3.3a, 3.3b, 3.3c).

The tribe *Anemoneae* form a monophyletic group in the nDNA, cpDNA and Combined Bayesian analyses (1.0 pp.), sister to a monophyletic clade of the accessions from the *Helleboreae, Coptoideae, Adonideae* and *Ranunculeae* in the ITS and combined analyses

(1.0 pp.). The cpDNA tree has poor support (<0.90 pp.) and the relationship of the *Anemoneae* to the other tribes in the Ranunculaceae is unresolved.

In tribe *Anemoneae*, *Anemone sensu lato* is sister to *Clematis sensu lato* and *Anemoclema* with strong support in the nDNA, cpDNA and combined analyses (1.0 pp.). *Clematis sensu lato* is sister to *Anemoclema* with strong support in the nDNA and combined (1.0 pp.) but poorly supported in cpDNA analysis (0.87 pp.).

Clematis sensu lato is monophyletic and strongly supported in the nDNA (0.99 pp.), cpDNA and combined analyses (1.0 pp.). *Anemone sensu lato* is also monophyletic but with moderate support in the nDNA (0.92 pp.) analysis and poor support in the cpDNA analysis (0.71 pp.), but is strongly supported in the combined analyses (1.0 pp.).

There were minor incongruences in the topology of the *Anemone sensu lato* clade between analyses.

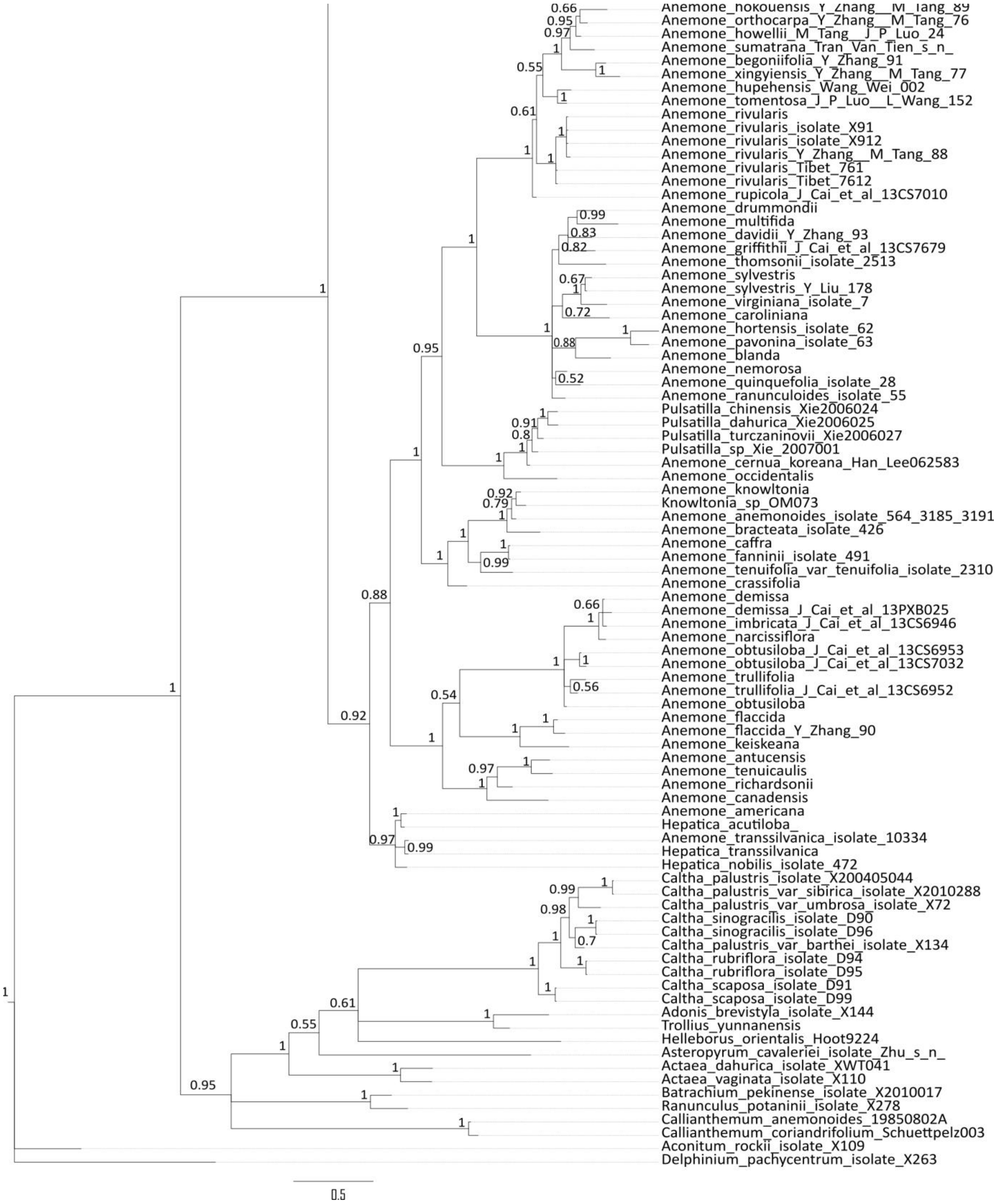
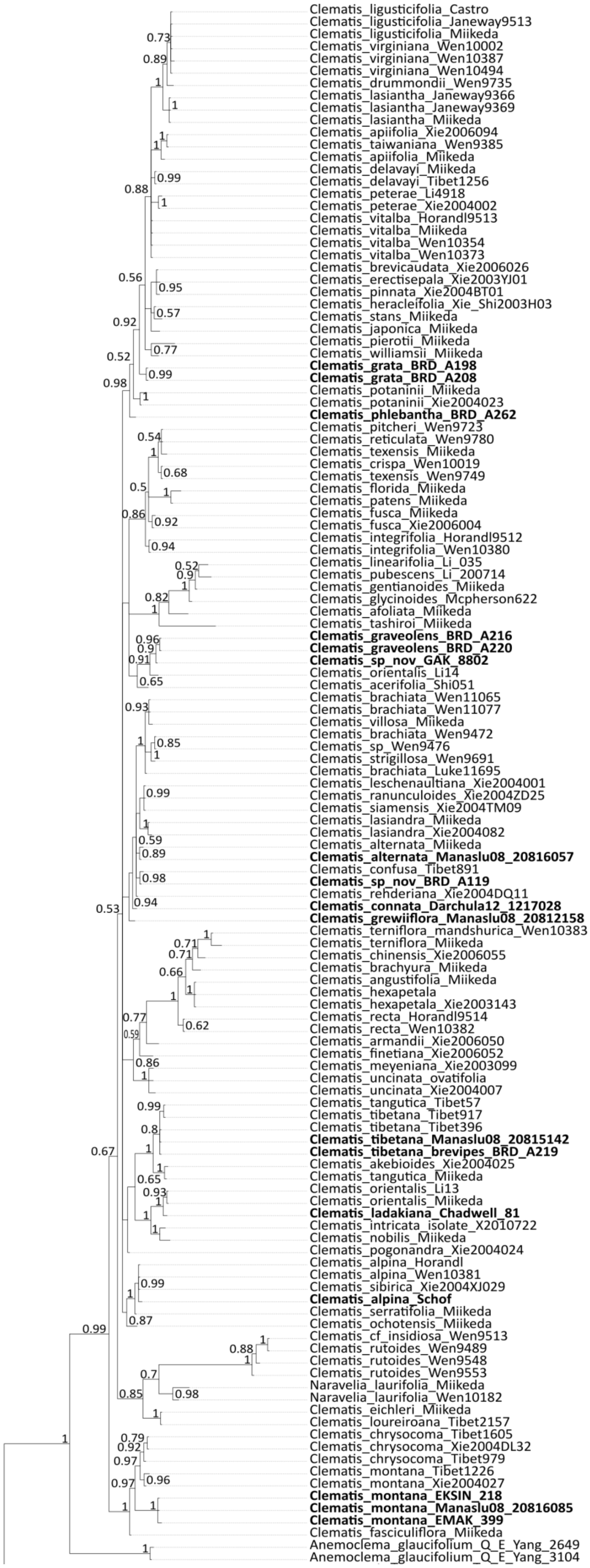


Figure 3.1 MrBayes consensus tree based on the *Anemoneae* nDNA (ITS) dataset. The numbers at nodes correspond to the posterior probabilities (pp.).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Clematis_virginiana_Wen10002
0.89
Clematis_virginiana_Wen10387
Clematis_virginiana_Wen10494
1
Clematis_drummondii_Wen9735
Clematis_lasiantha_Janeway9366
1
Clematis_lasiantha_Janeway9369
Clematis_lasiantha_Miikeda
1
Clematis_apiifolia_Xie2006094
1
Clematis_taiwaniana_Wen9385
Clematis_apiifolia_Miikeda
Clematis_delavayi_Miikeda
0.99
Clematis_delavayi_Tibet1256
0.88
Clematis_peterae_Li4918
1
Clematis_peterae_Xie2004002
Clematis_vitalba_Horandl9513
Clematis_vitalba_Miikeda
Clematis_vitalba_Wen10354
Clematis_vitalba_Wen10373
Clematis_brevicaudata_Xie2006026
0.56
Clematis_erectisepala_Xie2003YJ01
0.95
Clematis_pinnata_Xie2004BT01
Clematis_heracleifolia_Xie_Shi2003H03
0.57
Clematis_stans_Miikeda
0.92
Clematis_japonica_Miikeda
Clematis_pierotii_Miikeda
0.77
0.52
Clematis_williamsii_Miikeda
Clematis_grata_BRD_A198
0.99
0.98
Clematis_grata_BRD_A208
Clematis_potaninii_Miikeda
1
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Clematis_phlebantha_BRD_A262
Clematis_pitcheri_Wen9723
0.54
Clematis_reticulata_Wen9780
1
Clematis_texensis_Miikeda
Clematis_crispa_Wen10019
0.68
Clematis_texensis_Wen9749
0.5
Clematis_florida_Miikeda
1
Clematis_patens_Miikeda
0.86
Clematis_fusca_Miikeda
0.92
Clematis_fusca_Xie2006004
Clematis_integrifolia_Horandl9512
0.94
Clematis_integrifolia_Wen10380
0.52
Clematis_linearifolia_Li_035
0.9
Clematis_pubescens_Li_200714
1
Clematis_gentianoides_Miikeda
0.82
Clematis_glycinoides_Mcpherson622
1
Clematis_afoliata_Miikeda
Clematis_tashiroi_Miikeda
0.96
Clematis_graveolens_BRD_A216
0.9
Clematis_graveolens_BRD_A220
0.91
Clematis_sp_nov_GAK_8802
Clematis_orientalis_Li14
0.65
Clematis_acerifolia_Shi051
Clematis_brachiata_Wen11065
0.93
Clematis_brachiata_Wen11077
Clematis_villosa_Miikeda
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1
0.85
Clematis_sp_Wen9476
1
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Clematis_brachiata_Luke11695
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0.99
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Clematis_siamensis_Xie2004TM09
1
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0.59
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0.89
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Clematis_confusa_Tibet891
0.98
Clematis_sp_nov_BRD_A119
Clematis_rehderiana_Xie2004DQ11
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0.53
Clematis_connata_Darchula12_1217028
Clematis_grewiiflora_Manaslu08_20812158
1
Clematis_terniflora_mandshurica_Wen10383
0.71
Clematis_terniflora_Miikeda
0.71
Clematis_chinensis_Xie2006055
Clematis_brachyura_Miikeda
0.66
Clematis_angustifolia_Miikeda
1
Clematis_hexapetala
1
Clematis_hexapetala_Xie2003143
0.77
Clematis_recta_Horandl9514
0.62
Clematis_recta_Wen10382
0.59
Clematis_armandii_Xie2006050
Clematis_finetiana_Xie2006052
0.86
Clematis_meyeniana_Xie2003099
1
Clematis_uncinata_ovatifolia
Clematis_uncinata_Xie2004007
0.99
Clematis_tangutica_Tibet57
Clematis_tibetana_Tibet917
0.8
Clematis_tibetana_Tibet396
Clematis_tibetana_Manaslu08_20815142
1
0.67
Clematis_tibetana_brevipes_BRD_A219
1
Clematis_akebioides_Xie2004025
0.65
Clematis_tangutica_Miikeda
0.93
Clematis_orientalis_Li13
1
Clematis_orientalis_Miikeda
1
Clematis_ladakiana_Chadwell_81
1
Clematis_intricata_isolate_X2010722
Clematis_nobilis_Miikeda
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0.99
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1
Clematis_alpina_Schof
Clematis_serratifolia_Miikeda
0.87
Clematis_ochotensis_Miikeda
0.99
1
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0.88
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1
Clematis_rutoides_Wen9548
0.7
Clematis_rutoides_Wen9553
Naravelia_laurifolia_Miikeda
0.85
0.98
Naravelia_laurifolia_Wen10182
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1
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1
0.79
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0.92
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0.97
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1
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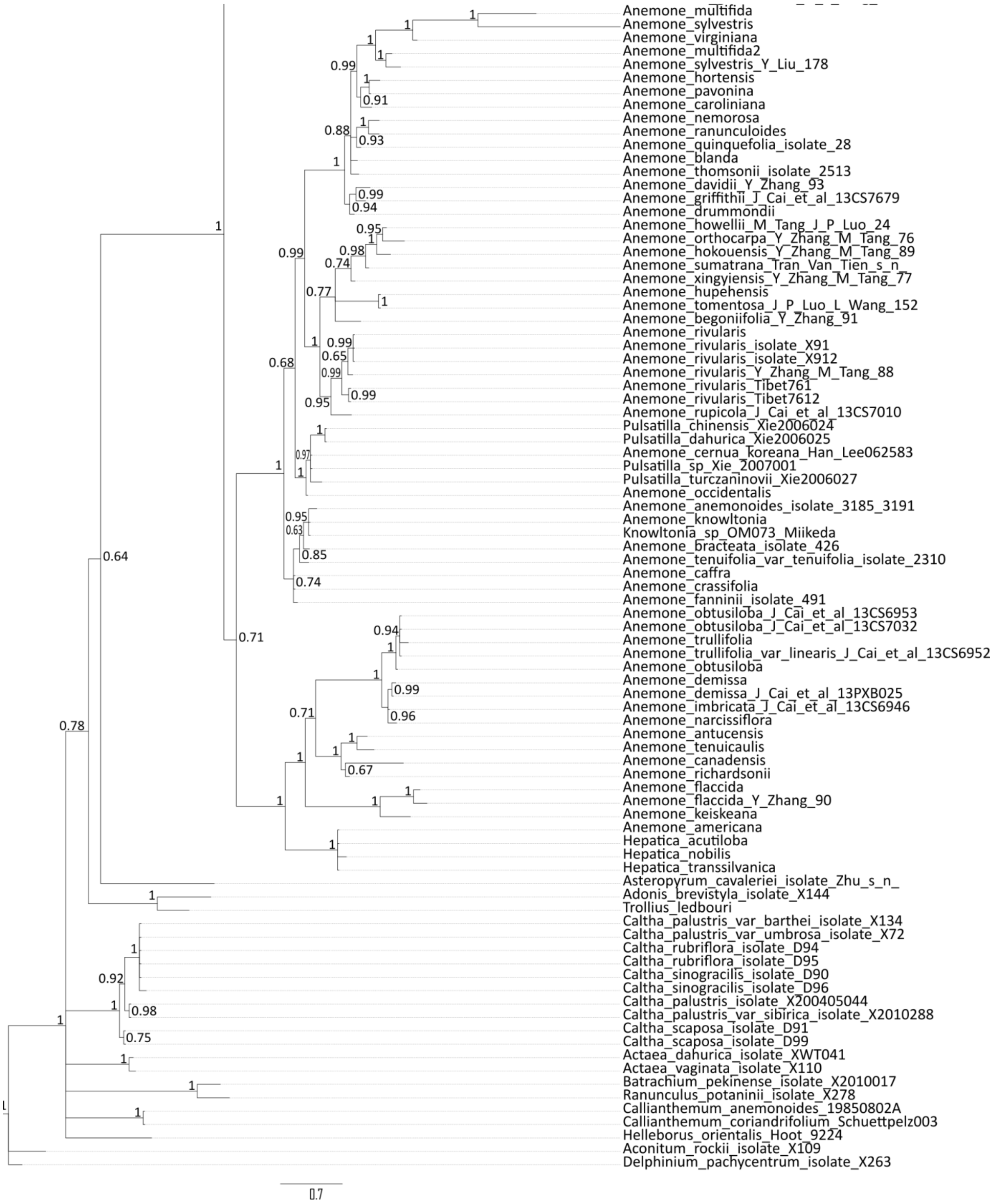
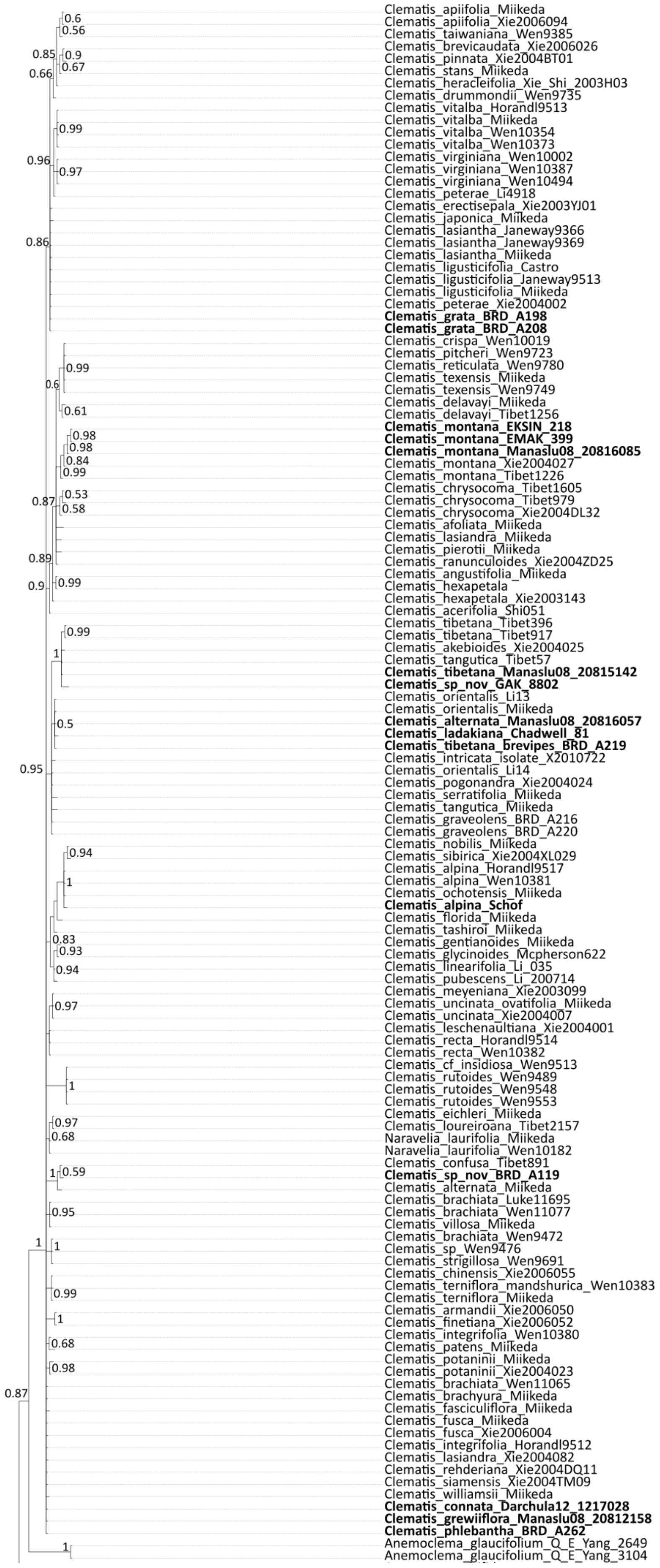


Figure 3.2 MrBayes consensus tree based on cpDNA (*atp*B-*rbc*L spacer) dataset. The numbers at nodes correspond to the posterior probabilities (pp.)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Clematis_crispa_Wen10019
Clematis_pitcheri_Wen9723
Clematis_reticulata_Wen9780
Clematis_texensis_Miikeda
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Clematis_delavayi_Tibet1256
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Clematis_pierotii_Miikeda
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Clematis_tibetana_Manaslu08_20815142
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Clematis_orientalis_Miikeda
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Clematis_pogonandra_Xie2004024
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Clematis_tangutica_Miikeda
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Clematis_ochotensis_Miikeda
Clematis_alpina_Schof
Clematis_florida_Miikeda
Clematis_tashiroi_Miikeda
Clematis_gentianoides_Miikeda
Clematis_glycinoides_Mcpherson622
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Clematis_pubescens_Li_200714
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Clematis_uncinata_ovatifolia_Miikeda
Clematis_uncinata_Xie2004007
Clematis_leschenaultiana_Xie2004001
Clematis_recta_Horandl9514
Clematis_recta_Wen10382
Clematis_cf_insidiosa_Wen9513
Clematis_rutoides_Wen9489
Clematis_rutoides_Wen9548
Clematis_rutoides_Wen9553
Clematis_eichleri_Miikeda
Clematis_loureiroana_Tibet2157
Naravelia_laurifolia_Miikeda
Naravelia_laurifolia_Wen10182
Clematis_confusa_Tibet891
Clematis_sp_nov_BRD_A119
Clematis_alternata_Miikeda
Clematis_brachiata_Luke11695
Clematis_brachiata_Wen11077
Clematis_villosa_Miikeda
Clematis_brachiata_Wen9472
Clematis_sp_Wen9476
Clematis_strigillosa_Wen9691
Clematis_chinensis_Xie2006055
Clematis_terniflora_mandshurica_Wen10383
Clematis_terniflora_Miikeda
Clematis_armandii_Xie2006050
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Clematis_phlebantha_BRD_A262
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0.56
0.85
0.9
0.67
0.66
0.99
0.96
0.97
0.86
0.99
0.6
0.61
0.98
0.98
0.84
0.99
0.53
0.87
0.58
0.89
0.99
0.9
0.99
1
0.5
0.95
0.94
1
0.83
0.93
0.94
0.97
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0.87
1

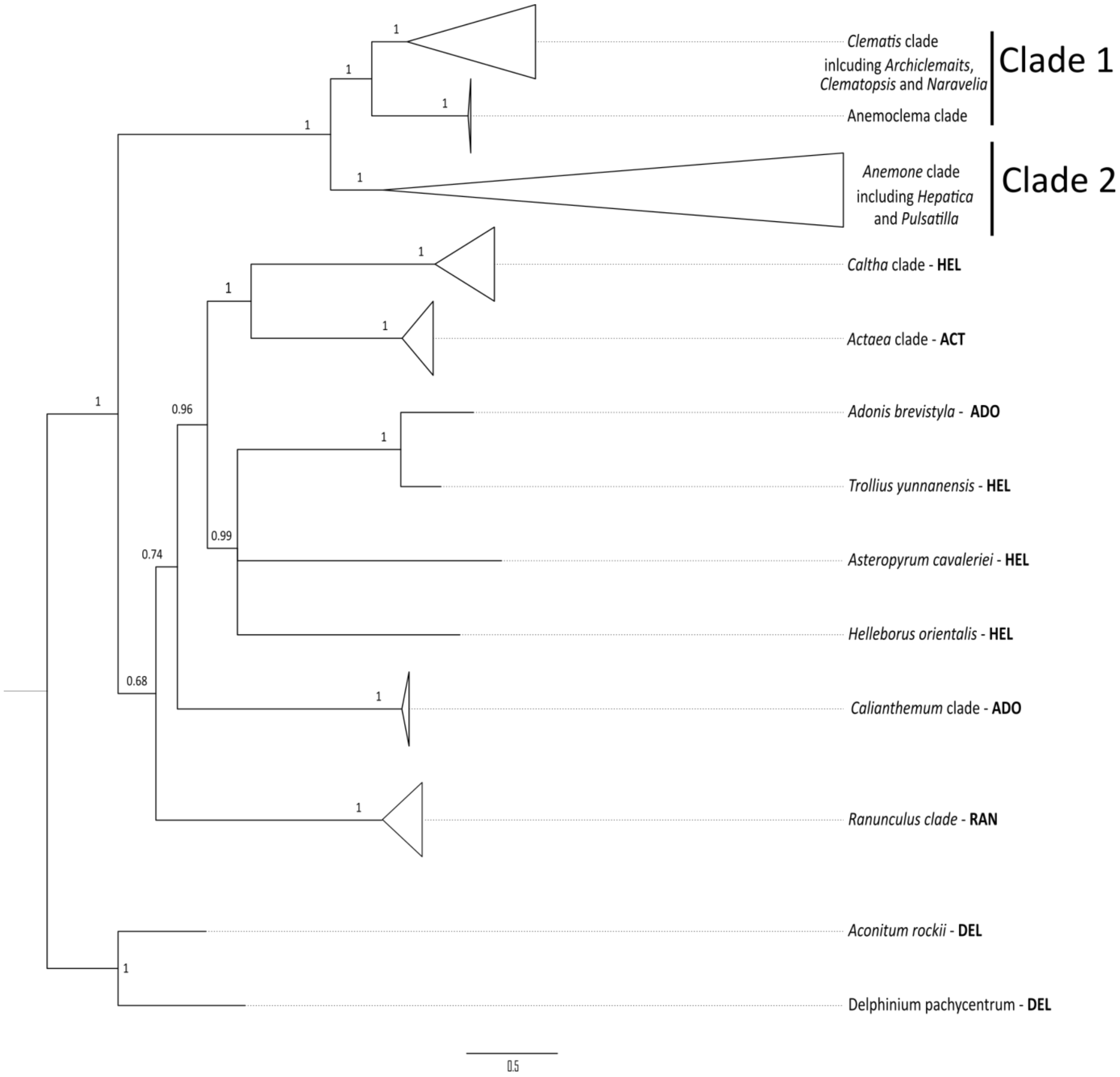


Figure 3.3a Overview tree of the relationships in MrBayes consensus tree from combined nDNA (ITS) & cpDNA (atpB-rbcl spacer) dataset. The numbers at nodes correspond to posterior probabilities (pp.). Outgroups have 3 letter codes corresponding to the tribe in the subfamily Ranunculoideae following Tamura (1993). HEL - Helleboreae, DEL - Delphinieae, RAN - Ranunculeae, ADO - Adonideae, COP - Coptoideae. Clades 1 and 2 are expanded in Figures 3.3b and 3.3.c.

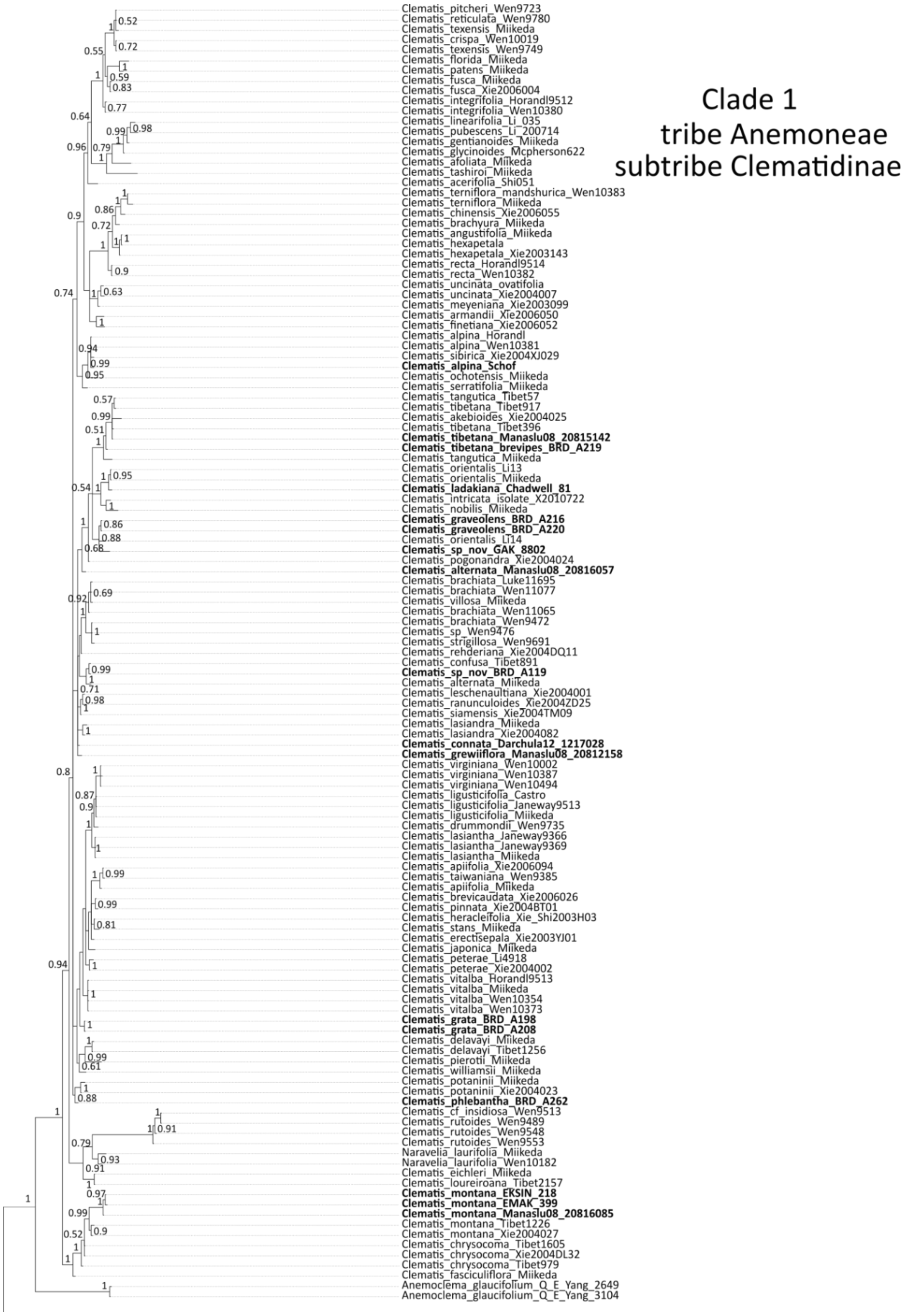


Figure 3.3b Clade 1 corresponding to subtribe *Clematidinae* generated from the MrBayes Consensus tree from combined nDNA (ITS) & cpDNA (*atp*B-*rbc*L spacer) dataset. Numbers at nodes correspond to posterior probabilities (pp.)

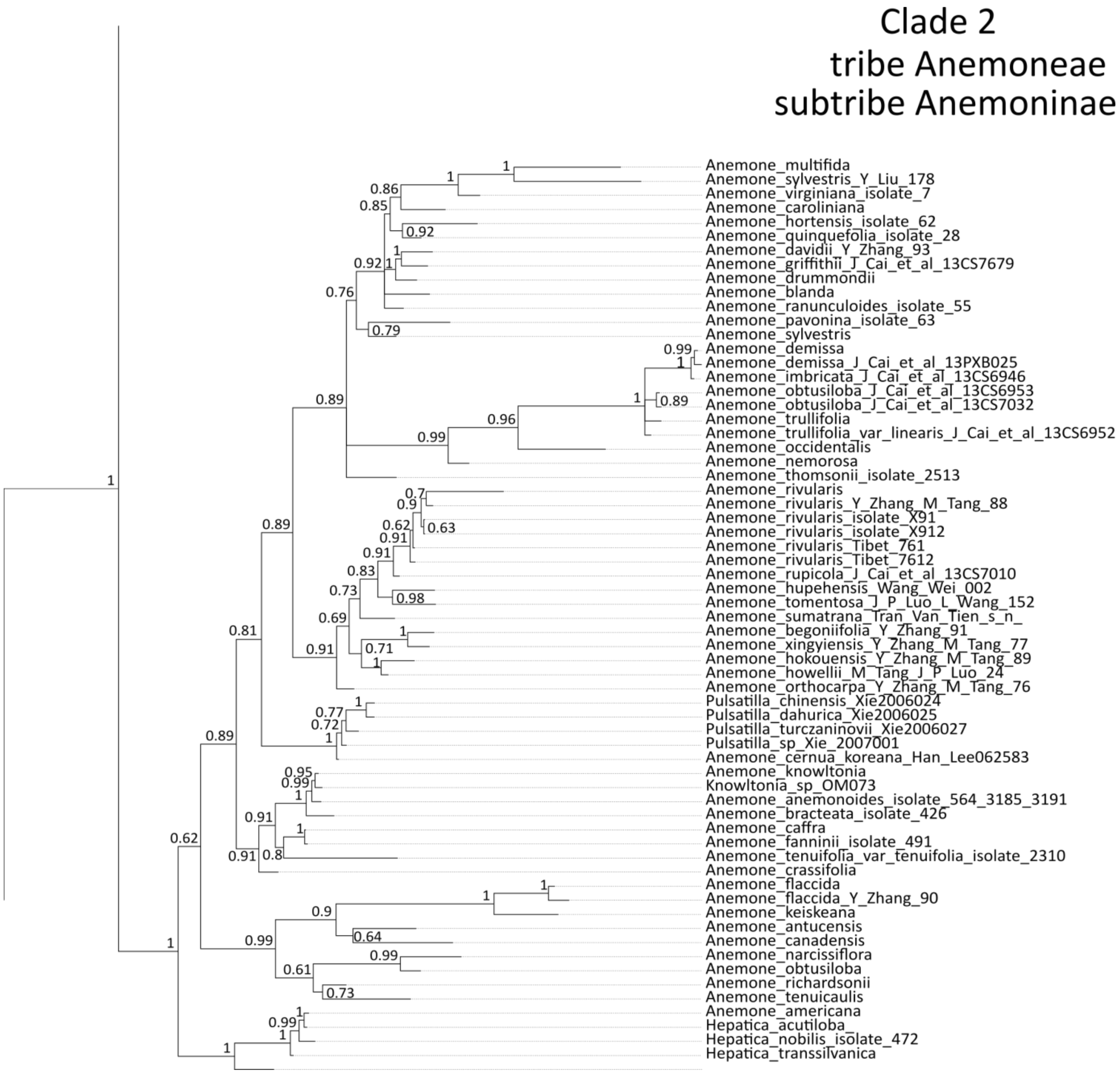


Figure 3.3c Clade 2 corresponding to subtribe *Anemoninae* generated from the MrBayes Consensus tree from combined nDNA (ITS) & cpDNA (*atp*B-*rbc*L spacer) dataset. Numbers at nodes corresond to posterior probabilities (pp.)

Divergence time estimate results

The major clades (table 3.9) in the Anderson and Magallón constrained analyses had strong support: *Anemoneae* (1.0 pp & 0.99 pp. respectively), *Anemoninae* (0.97 pp. & 0.99 pp. respectively), *Clematidinae* (1.0 pp. & 0.99 respectively), *Clematis* (1.0 pp. & 0.99 pp. respectively) and were congruent with the topology of the MrBayes consensus nDNA, cpDNA and combined region trees.

Table 3.9 Evolutionary age estimates (Ma) of major clades and support values generated from the BEAST Analyses (pp value is the likelihood support for the clade, 95%HPD estimated age range only shown when support for a clade (pp) is >= 0.95).

	Anderson constrained (Figure 3.4)	**Magallón constrained (Figure 3.5)**
Ranunculus/ *Anemone*-*Clematis* split	75.52 Ma 0.44 pp.	68.61 Ma 0.46 pp.
Anemoninae - *Clematinidae* Split	54.88 Ma (95% HPD: 40.8-70.45 Ma) 1.0 pp.	49.33 Ma (95% HPD: 37.37-60.44 Ma) 0.99 pp.
Anemone Stem	As above	As above
Anemone Crown	47.62 Ma (95% HPD: 34.63 - 63.42 Ma) 0.97 pp.	43.88 Ma (95% HPD: 32.71-55.02 Ma) 0.95 pp.

Clematis Stem age	30.18 Ma (95% HPD: 16.35-47.83 Ma) 1.0 pp.	26.69 Ma (95% HPD: 14.63-42.72 Ma) 0.99 pp.
Clematis Crown age	16.88 Ma (95% HPD: 9.79-26.07 Ma) 1.0 pp.	15.01 Ma (95% HPD: 9.02-22.95 Ma) 0.99 pp.

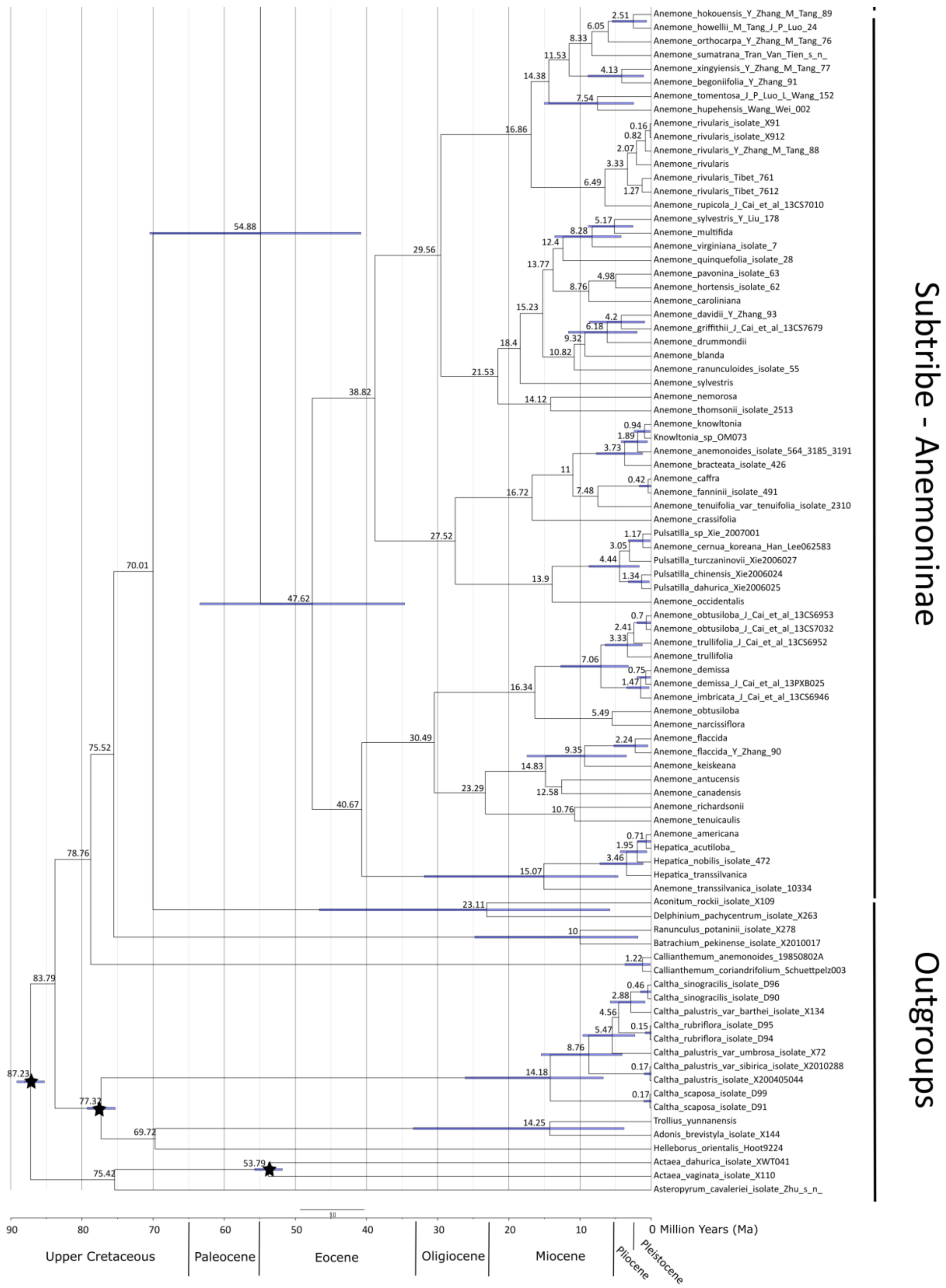
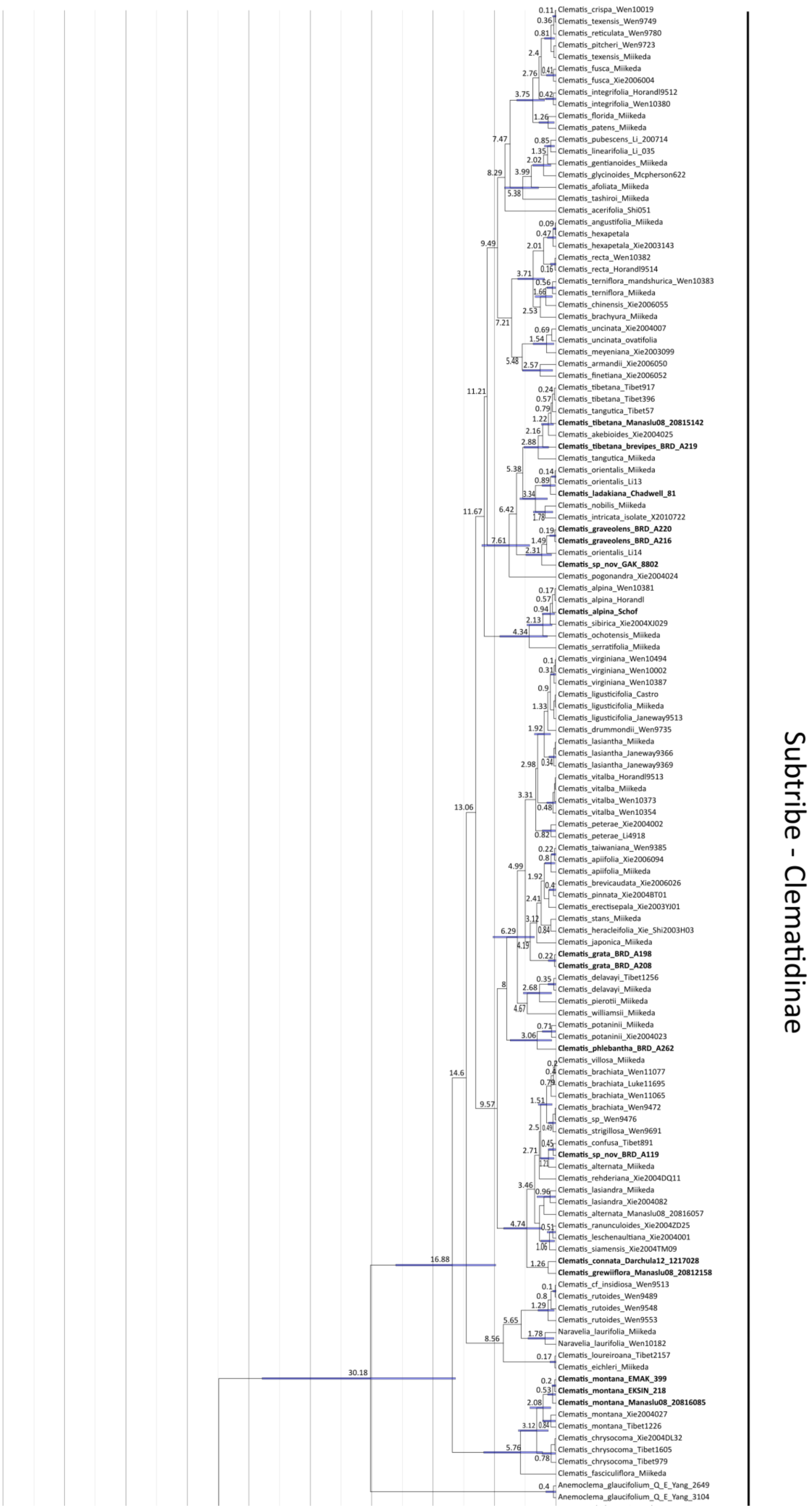


Figure 3.4 Maximum Clade Credibility tree generated from the Anderson *et al*. (2005) constrained BEAST analysis for the combined nDNA (ITS) and cpDNA (*atp*B-*rbc*L) dataset. The numbers correspond to divergence age estimates (Ma), bars represent 95% confifdence intervals. Stars indicate the *a priori* age constrained nodes, 87 Ma mean age of the Ranunculaceae (Anderson *et al.* 2005); 77.5 Ma, *Eocaltha zoophilila* fossil on the crown of *Caltha*; 54 Ma, *Paleoactea* fossil on the crown of *Cimicifugia*.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0.41 Clematis_fusca_Miikeda
2.76 Clematis_fusca_Xie2006004
3.75 0.42 Clematis_integrifolia_Horandl9512
Clematis_integrifolia_Wen10380
1.26 Clematis_florida_Miikeda
Clematis_patens_Miikeda
7.47 0.85 Clematis_pubescens_Li_200714
1.35 Clematis_linearifolia_Li_035
2.02 Clematis_gentianoides_Miikeda
8.29 3.99 Clematis_glycinoides_Mcpherson622
Clematis_afoliata_Miikeda
5.38 Clematis_tashiroi_Miikeda
Clematis_acerifolia_Shi051
0.09 Clematis_angustifolia_Miikeda
0.47 Clematis_hexapetala
9.49 2.01 Clematis_hexapetala_Xie2003143
Clematis_recta_Wen10382
0.16 Clematis_recta_Horandl9514
3.71 0.56 Clematis_terniflora_mandshurica_Wen10383
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2.53 Clematis_chinensis_Xie2006055
Clematis_brachyura_Miikeda
7.21 0.69 Clematis_uncinata_Xie2004007
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Clematis_meyeniana_Xie2003099
5.48 2.57 Clematis_armandii_Xie2006050
Clematis_finetiana_Xie2006052
11.21 0.24 Clematis_tibetana_Tibet917
0.57 Clematis_tibetana_Tibet396
0.79 Clematis_tangutica_Tibet57
1.22 Clematis_tibetana_Manaslu08_20815142
2.16 Clematis_akebioides_Xie2004025
2.88 Clematis_tibetana_brevipes_BRD_A219
Clematis_tangutica_Miikeda
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3.34 Clematis_ladakiana_Chadwell_81
Clematis_nobilis_Miikeda
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7.61 1.49 Clematis_graveolens_BRD_A216
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Clematis_pogonandra_Xie2004024
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Clematis_serratifolia_Miikeda
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0.31 Clematis_virginiana_Wen10002
Clematis_virginiana_Wen10387
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Clematis_lasiantha_Janeway9366
2.98 0.34 Clematis_lasiantha_Janeway9369
Clematis_vitalba_Horandl9513
Clematis_vitalba_Miikeda
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Clematis_peterae_Xie2004002
0.82 Clematis_peterae_Li4918
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Clematis_grata_BRD_A208
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Clematis_sp_Wen9476
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1.29 Clematis_rutoides_Wen9548
5.65 Clematis_rutoides_Wen9553
1.78 Naravelia_laurifolia_Miikeda
8.56 Naravelia_laurifolia_Wen10182
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Clematis_eichleri_Miikeda
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Clematis_montana_Xie2004027
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Subtribe - Clematidinae

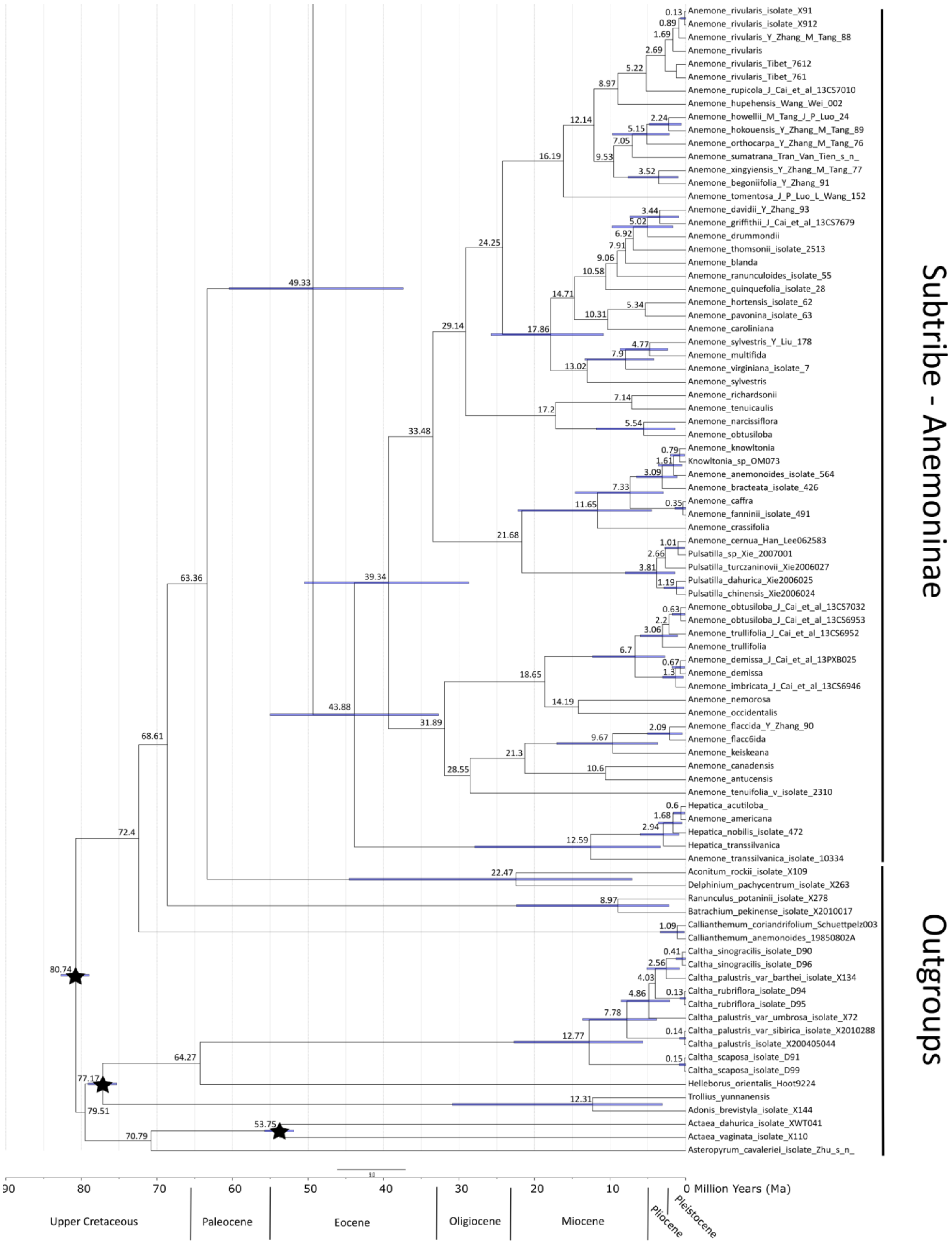
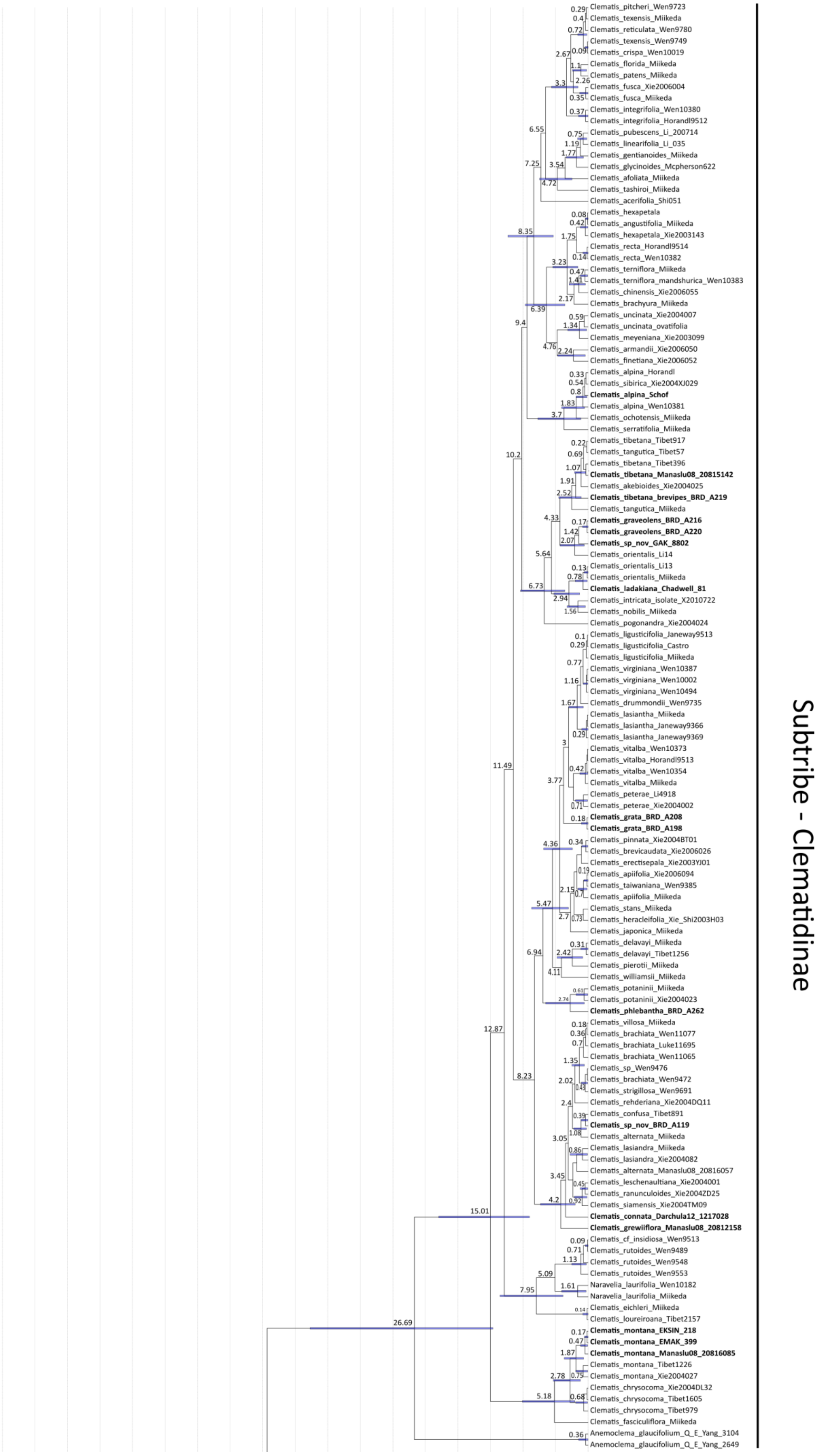


Figure 3.5 Maximum Clade Credibility tree generated from the Magallón *et al*. (2015) BEAST analysis using the combined nDNA (ITS) and cpDNA (*atp*B-*rbc*L) combined data. Numbers are divergence time estimates (Ma), bars represent 95% confifdence intervals. Stars are the a priori age constrained nodes, 80.74 Ma mean age of Ranunculaceae (Magallón *et al.* 2015); 77.5 Ma, *Eocaltha zoophilila* fossil on the crown of *Caltha*: 54 Ma, *Paleoactea* fossil on the crown of *Cimicifugia*.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0.29
0.22
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0.14
0.13
0.1
0.09
0.08
Subtribe - Clematidinae

Discussion

Support across the trees

There is strong congruence between the major clades *Anemoneae*, *Cleminideae*, *Clematis sensu lato*, *Anemone sensu lato* and the outgroups in the two analyses. In *Clematis sensu lato* the lack of resolution in the cpDNA tree makes it difficult to assess congruence between the nDNA and cpDNA.

Phylogenetic Relationships – across the whole group

Tribe *Anemoneae*

This study looks at the relationships across tribe Anemoneae and shows that there is good support for this grouping of genera within the Ranunculaceae. It also supports the inclusion of *Knowltonia*, *Hepatica* and *Pulsatilla* in *Anemone* as previously reported in several molecular studies (Ehrendorfer and Samuel 2001, Schuettpelz *et al.* 2002, Meyer *et al.* 2010, Hoot *et al.* 2012, Zhang *et al.* 2015b) and the phylogenetic placement of *Archiclematis*, *Naravelia* and *Clematopsis* in the *Clematis* clade by Miikeda *et al.* (2006) and Xie *et al.* (2011). These results suggests that the genera of the Anemoneae should be reduced in number and more broadly circumscribed, with *Knowltonia*, *Hepatica* and *Pulsatilla* placed into synonymy with *Anemone sensu lato* and *Naravelia* placed into synonymy with *Clematis*. The placement of *Anemoclema* as a well-supported sister clade to *Clematis sensu lato* supports the findings of Zhang *et al.* (2015b).

Zhang *et al.* (2015b) suggested that *Anemoclema* should be recognised as a second genus in the subtribe *Clematidinae* and sister to the subtribe *Anemoninae*. While there is a strong signal in the phylogeny to support the distinction between subtribe *Clematidinae* and subtribe *Anemoninae* as proposed by Zhang *et al.* (2015b), the taxonomic reduction of ten genera to three, supported by the monophylly of *Clematis* and *Anemone* make this taxonomic rank somewhat superfluous.

Subtribe *Anemoninae* (*Anemone sensu lato*)

There is some minor incongruence between the major clades in the separate ITS and *atp*B-*rbc*L, and the combined analysis but the major clades are congruent with the combined tree reported in Zhang *et al*. (2015). Some of the derived relationships and placement of taxa are not congruent with Zhang *et al*. (2015) and Hoot *et al*. (2012). The support values along the backbone of the clade are poor, at less than 0.90 pp., with the exception of the *Hepatica* clade which is sister to the rest of the *Anemone* clade.

The placement of Himalayan *Anemone obtusiloba* in this analysis is paraphyletic, with the NW Indian accession in a different clade from the two Chinese accessions. The NW Indian accession had been used in *Anemone* studies up to and including Hoot *et al*. 2012 but was omitted in favour the two Chinese origin accession in the Zhang *et al*. (2015) study of *Anemone* section *Begoniifolia*. The sequence from the NW Indian accession is different from that of the two Chinese accessions and probably accounts for its omission from the most recent study. The voucher from NW India was collected in the Indian state of Himachal Pradesh and is in E , while the two Chinese vouchers are from Zeyu and Markam counties in SE Tibet and it has not been possible to compare their morphologies. The type collection of *Anemone obtusiloba* is a Wallich specimen from ‘Gosaingsthan’ in Nepal, published by David Don in his 1825 *Prodromus*. The recent revision of *Anemone* section *Himalayicae* (Ziman *et al*. 2007), which lectotypified the Wallich specimen from the BM herbarium, gives a large geographical range for this species, from Afghanistan through the Himalaya to the mountains of SW China and north to Gansu and Shanxi. With such a large distribution the possibility of *Anemone obtusiloba* containing cryptic species should be investigated.

Subtribe Clematidinae (*Clematis sensu lato* and *Anemoclema*)

The phylogenetic relationships in the genus Clematis will discussed in chapter 4 using an expanded dataset.

Divergence time estimates

The age of tribe *Anemoneae*

The estimated mean age of divergence between subtribe *Clematidinae* (*Clematis* & *Anemoclema* clade) and subtribe *Anemoninae* (the *Anemone* clade) dates to the Eocene (33.9-55.8 Ma) according to both the Anderson (54.88 Ma - 95% HPD: 40.8-70.45 Ma) and Magallón (49.33 Ma - 95% HPD: 37.37-60.44 Ma) constrained analyses, however the 95% confidence intervals are large, covering 30 Ma and 23 Ma respectively. Taking the confidence intervals into account this increases the range from the late Upper Cretaceous to the mid-late Eocene.

The fossilised Anemonoid achene (Weyland 1937) that is dated to the Oligocene (23-33.9 Ma) does not conflict with the Upper Cretaceous - Eocene (37.37 - 70.45 Ma) timeframe estimated for the evolution of tribe *Anemoneae* from these analyses. The putative *Clematis* leaf fossil dating to the early-middle Eocene (40.4-55.8 Ma) is much older than the estimated crown ages of *Clematis* (9-26 Ma) and only just overlaps with the *Clematis* stem ages (14-47 Ma) and would likely alter those dates if it was attributable to a group and used in future analyses. Other fossils attributable to *Anemoneae* are from the Pliocene and are much younger than the estimated divergence events.

These dates are in-line with Magallón *et al.*'s (2015) study which demonstrated that most plant families having their origins in an Upper Cretaceous - Eocene. As the Ranunculaceae is an early diverging lineage in Eudicots it is reasonable to relate the continuing lineage creation in the Ranunculaceae to the same processes that have led to the more recently evolved families. However, Magallón *et al.* (2015) stated that there is currently no conclusive evidence of a relationship between the global tectonic changes, higher global temperatures and their findings of a concentration of family origins.

The age of subtribe *Anemoninae*

Although there now a considerable molecular dataset available for *Anemone* and associated analysis of the phylogenetic relationships this is the only known attempt to produce a dated phylogeny, possibly due to the lack of fossils to constrain dates within the phylogeny. These reported dates are the first for the genus, but what follows is a broad discussion and not a comprehensive analysis as *Anemone* is the sister group to *Clematis* which is the main focus of this study.

The crown age of *Anemone sensu lato* dates from 47.62 Ma (34.63-64.42 Ma) according to Anderson and 43.89 Ma (32.71-55.05 Ma) according to Magallón. The mean ages from both analyses fall in the mid-Eocene, with the confidence interval of Anderson reaching back to the Paleocene.

The Himalayan clade of *Anemone* - subsection *Himalayicae* dates to 7.6 Ma (95% HPD: 3.2-12.70 Ma) in the Anderson constrained analysis, and to 6.7 Ma (2.74-12.31 Ma) in the Magallón constrained analysis. This excludes the NW Indian accession of *A. obtusiloba* whose placement differs between the trees. These mean dates are from the late Miocene to the Pliocene/Pleistocene boundary.

Subsections *Rivulareae* and *Rupicolae* both contain Himalayan species but the relationships are poorly supported. The estimated evolutionary dates from the Anderson and Magallón constrained analyses are during the early-middle Miocene at 16.86 Ma and 12 Ma, respectively. With the subsequent diversifications beginning in the late Miocene at 6.49 Ma from the Anderson constrained analysis or 5.22 Ma from the Magallón constrained analysis.

These dates are tentative due to lack fossil evidence, but following Milne (2009) using a deep-dated phylogeny approach and with increased sampling, taking into account as much diversity as is available in the *Anemoneae*, these dates represent the first estimate.

The age of subtribe *Clematidinae*

The mean stem age of *Clematis* from the Anderson constrained analysis was 30.18 Ma (95% HPD: 16.35-47.83 Ma) and from the Magallón constrained analysis was 26.69 Ma (95% HPD: 14.63-42.72 Ma). These are broadly in-line with the Xie *et al.* (2011) study who reported a stem age of 25.99 Ma (95% HPD: 9.19.-43.77 Ma). One aim of these analyses was to reassess the divergence age of *Ranunculus* and *Clematis* that has been used in the Xie *et al.* (2011) *Clematis* and the Emadzade & Horandl (2011) *Ranunculus* studies. As stated earlier, after reviewing the Anderson *et al.* (2005) paper it is unclear how these authors interpreted the 45.59 Ma date. The corresponding node from this study is 75.52 Ma & 68.61 Ma in the Anderson and Magallón constrained analyses respectively, a substantial difference of 25 - 30 Ma. However both these nodes are poorly supported (0.44 pp.) and (0.46 pp.) respectively, so these ages must be treated with some caution.

These mean stem ages are however not directly comparable as *Anemoclema* was not available for the Xie *et al.* (2011) study. The comparable node for the stem age is the split between subtribe *Clematidinae* (*Clematis* & *Anemoclema* clade) and subtribe *Anemoninae* (*Anemone senu lato*) and those mean ages are 54.88 Ma (95% HPD: 40.80-70.45 Ma) and 49.33 Ma (95% HPD: 37.37-60.44 Ma) for the Anderson and Magallón constrained analyses respectively. These dates are approximately twice the reported age of the Xie *et al.* study.

The mean crown age of *Clematis* from the Anderson constrained analysis was 16.88 Ma (95% HPD: 9.79-26.07 Ma) and from the Magallón constrained analysis was 15.01 Ma (95% HPD: 9.02-22.95 Ma). These dates are almost twice as old as that reported by Xie *et al.* (2011) who reported 7.82 Ma (95% HPD: 3.99-13.14 Ma). Despite the differences in reported mean ages and confidence intervals the extant diversity appears to have been generated in the Miocene, with only the oldest date in the Anderson constrained analysis falling in the Oligocene.

The doubling of the ages of the nodes is attributed to the more robust dating technique and the Xie *et al*. (2011) study. This study used two fossil calibrated nodes and one secondary calibrated point based of the evolutionary age of the Ranunculaceae based on results of multiple fossil calibrated nodes compared to a single node, dubious secondary calibration node.

However the use of the *Anemoclema* lineage as the ‘correct’ sister group to *Clematis* has had the effect of reducing the long stem age reported by Xie *et al.* (2011) which they attributed to rapid speciation of the genus. The more than doubling of the crown age estimates in the two analyses in comparison to the reported age by Xie *et al*. is also indicative that the burst that has led to the extant diversity of *Clematis* might not been so rapid.

Conclusion

For the first time the evolutionary age of *Anemone sensu lato* has been estimated. The mean ages from the analyses place this event in the mid-Eocene 43.89 to 47.62 Ma.

The other main finding presented here is that the evolution of extant diversity of *Clematis* is older than previously reported, with estimated evolutionary dates of 16.88 Ma (95% HPD: 9.79-26.07 Ma) or 15.01 Ma (95% HPD: 9.02-22.95 Ma), compared with 7.82 Ma (95% HPD: 3.99-13.14 Ma) reported by Xie *et al*. (2011). All three analyses point to evolutionary events occurring in the Miocene. The doubling in age is primarily due to the selection of priors when dating the phylogeny. In this study, two fossil calibrated nodes plus a secondary calibrated node were used in each of the analyses. This is compared to the Xie *et al*. study which used a single secondary calibrated node derived from Anderson *et al*. (2005), of which no critical appraisal appears to have taken place previously.

This reanalysis of the evolutionary events in the Ranunculaceae focusing on tribe *Anemoneae* using multiple fossils and constraining the age of the family using different dates, readily available in published studies, has given different results for the *Clematis-Anemone* split, 75.52 Ma & 68.61 Ma, which will used to further test the *Clematis* phylogeny in chapter 4.

Chapter 4: The Phylogeography of *Clematis* L. (Ranunculaceae) in Nepal

Introduction

The phylogeny of the genus has been studied in some detail by Miikeda *et al*. (2006) and Xie *et al*. (2011), between them covering the taxonomic and morphological diversity of the genus and about 25% of the described species-level diversity. Miikeda sampled all of Tamura's (1993), taxonomic groupings and found that the phylogeny was not consistent with existing infrageneric taxonomies As a result the characters used for infrageneric classification did not correlate with the major clades in their study. The lack of phylogenetic support for the existing morphology-based taxonomies was also reinforced by Xie *et al*. (2011) who tested the taxonomies of Tamura (1993) and Wang & Li (2005), but did not test the taxonomies of Grey-Wilson (2000) or Johnson (2001).

The two studies did not test the taxonomy of Johnson (2001) despite it being the most recent and most comprehensive monograph of *Clematis*. It could be because of the regional nature of these studies or because Tamura's classification was the mostly widely followed and Wang (Wang & Li, 2005) had extensively monographed Asian *Clematis* (see chapter 2) as part of the preparation for *the Flora of China*. The sampling by Miikeda *et al*. and Xie *et al.* did however cover all of Johnson's (2001) sectional classification, but was missing representation from eight small and often monotypic subsections.

The evolutionary history of *Clematis* was discussed only briefly by Miikeda *et al*. (2006) who attributed the short branch lengths of the *Clematis* lineages in the neighbour-joining tree to relatively recent evolution. Xie *et al*. (2011) tested this hypothesis using a secondary calibrated node in their phylogeny to estimate evolutionary divergence dates. Xie's study did discover several well-supported, derived clades that formed geographical groupings corresponding strongly to African, Madagascan and North American species. Despite their assertion that their study was not biased towards the Asian species, some of their findings may be products of their sampling and their conclusions around the geographical groupings of some clades are incorrect. For example, a clade corresponding to the *Meclatis* group that they describe as a N to SW Chinese group is a product of their sampling. While they did sample *C. orientalis* from Asia they did not sample accessions from as far west as Turkey and the Greek Islands where this species also grows. A clade corresponding to the section *Cheiropsis* subsection *Montanae* that they describe as species found in the mountains of SW

China is another product of their sampling bias towards China. They sampled two accessions of *C. montana* from Tibet but this species occurs westward along the Himalayan chain with records in Pakistan and possibly the Hindu Kush in Afghanistan.

Neither Miikeda *et al*. (2006) nor Xie *et al*. (2011) attempted a historical *geographical* reconstruction of the genus, although Xie *et al*. stated that the most likely origin of the genus would be China.

The increased sampling in the Xie *et al*. study had a heavy focus on species covered in the *Flora of China* where approximately 30% of global diversity of *Clematis* is found. The study used an *a priori* calibrated dated molecular phylogeny approach to estimate that the stem age of *Clematis* originated in the Oligocene, 25.99 Ma (95% HPD: 9.19-43.77 Ma) and a crown age from the Miocene, 7.81 Ma (95% HPD: 3.99-13.14 Ma). Their findings of a long stem to the *Clematis* clade and relatively recent crown age, in conjunction with the lack of resolution at the base of the clade, supported Miikeda *et al*.'s inference that *Clematis* has undergone recent and rapid speciation. They did not discuss the possible reasons for this long branch between *Clematis* and the sister genera.

Other studies focused on *Clematis* in Asia (Zhang, 2012 & Zhang et.al 2013) have been at the population level. The population structures in both these studies focused on the N China and date to the Pleistocene and Holocene. These events are related much more to recent climate change and periods of glaciation.

The current global framework for the evolution of *Clematis* has regional focus on E and N Asia, justified as China is the centre of diversity. The previous published research has been used as a basis for this PhD research to revaluate the previous work with geographically extended sampling and to use *Clematis* as a model organism to exploring the generation of diversity in the Himalaya.

Aims

To determine the phylogenetic position of Nepalese *Clematis* in the context of an expanded dataset and revised dating estimates in order to better understand biogeographical patterns in the Himalaya and relationships among *Clematis*. The objectives are to:

- Reanalyse the molecular sequence data of Miikeda *et al*. (2006) and Xie *et al*. (2011) with newly sequenced accessions from Nepal to increase Himalayan representation in the phylogeny.

- Test the geographical origin of *Clematis* as proposed by Xie *et al*. by extending the geographical sampling in *Clematis*.
- Test the geographical delimitations of SW Chinese mountainous [section] *Cheiropsis* clade and the N to SW Chinese [section] *Meclatis* clade as proposed by Xie *et al*. (2011).
- Test the taxonomies of Grey-Wilson (2000) and Johnson (2001) against the phylogenetic evidence.
- Assess the dates of the *Clematis* fossils, none of which were used as constraints in the analyses, with the results from the dated molecular phylogenies.

Material and Methods

Phylogenetics

The extraction and PCR methods are the same as chapter 3. Three additional accessions of Nepalese *Clematis* (Table 4.1) not used in the *Anemoneae* dataset because of missing *atp*B-*rbc*L data were incorporated in to this dataset. Sequences of cpDNA *rpo*B-*trn*C region were downloaded from Genbank (Table 4.2) for all accessions of *Clematis* from Xie *et al*. and Miikeda *et al*. which had been used in the *Anemoneae* dataset in chapter 3.

Outgroup selection followed Xie *et al*. but included the two accessions of *Anemoclema glaucifolia*, which was found to be the sister lineage to *Clematis* by Zhang *et al*. (2015) and confirmed by the *Anemoneae* analyses in chapter 3.

Phylogenetic analyses followed the methodology described in chapter 3.

The *rpo*B-*trn*C dataset was aligned automatically using MAFFT version 7, Multiple alignment program for amino acid or nucleotide sequences, through the Computational Biology Research Center's online portal (http://mafft.cbrc.jp/alignment/server/) then checked and edited manually using Mesquite (Maddison & Maddison 2011, 2015).

Aligned sequence data were analysed using JModeltest2 (Darriba *et al*., 2012, Guindon & Gascuel, 2003) to determine the best-fit models of nucleotide substitution through statistical selection. See Table 4.3 below for model selection. Corrected Akalike information criterion (AIC) was tested.

Phylogenetic analyses were carried out in MrBayes v.3.2 (Huelsenbeck and Ronquist, 2001, Ronquist and Huelsenbeck 2003), with models suggested by the jModeltest analyses. Two parallel runs consisting of one cold and three heated chains were run for 20 million generations. The chains were sampled every thousandth generation and resulted in 200,000 sampled trees from each chain with 25% discarded, the default burn-in for MrBayes (Ronquist *et al*., 2009).

The nuclear (nDNA) region (ITS) and the combined Chloroplast (cpDNA) regions (*atp*B-*rbc*L, *rpo*B-*trn*C) were analysed independently to look for incongruence in the topology of the trees, and finally a combined analysis for all three regions.

Table 4.1 *Clematis* accessions used in these analyses. Accessions in bold are those that were not used in Chapter 3 because of the missing *atp*B-*rbc*L region data.

EDNA number	**Taxon name**	**Collection**	**ITS**	***atp*B-*rbc*L**	***rpo*B-*trn*C**
EDNA13-0034028	*Clematis connata*	Darchula 2012 1217028	✓	✓	✓
EDNA13-0034031	***Clematis buchananiana***	Darchula 2012 1217010	✓		✓
EDNA13-0034032	*Clematis montana*	EKSIN 218	✓	✓	✓
EDNA13-0034035	*Clematis tibetana bervipes*	Manaslu 08 20815142	✓	✓	
EDNA13-0034037	*Clematis alternata*	Manaslu 08 20816057	✓	✓	
EDNA13-0034038	*Clematis grewiiflora*	Manaslu 08 20812158	✓	✓	✓
EDNA13-0034041	*Clematis montana*	Manaslu 08 20816085	✓	✓	
EDNA13-0034043	*Clematis ladakiana*	Chadwell 81	✓	✓	
EDNA13-0034044	*Clematis alpina*	Schof s.n.	✓	✓	✓
EDNA13-0034047	*Clematis montana*	EMAK 399	✓	✓	✓

EDNA14-0036908	*Clematis* sp.	GAK 8802	✓	✓	
EDNA14-0036910	*Clematis* sp. nov.	BRD A119	✓	✓	✓
EDNA14-0036912	*Clematis grata*	BRD A198	✓	✓	✓
EDNA14-0036913	*Clematis graveolens*	BRD A216	✓	✓	✓
EDNA14-0036914	*Clematis tibetana brevipes*	BRD A219	✓	✓	✓
EDNA14-0036915	*Clematis graveolens*	BRD A220	✓	✓	✓
EDNA14-0036917	*Clematis phlebantha*	BRD A262	✓	✓	✓
EDNA14-0036918	***Clematis connata***	BRD A289	✓		✓
EDNA14-0036919	***Clematis phlebantha***	BRD B151	✓		✓
EDNA14-0036920	*Clematis grata*	BRD A208	✓	✓	✓

Table 4.2 Genbank accession details of *Clematis* and outgroup with the additional rpoB-trnC region used in these analyses.

Name	**rpoB-*trn*C**	**Voucher**	**Reference**
Anemone cernua	AB116910.1		Miikeda *et al.* (2006)
Anemone cernua var. koreana	GU732809.1	Han&Lee062583	Xie *et al.* (2011)
Anemone flaccida	AB116909.1		Miikeda *et al.* (2006)
Anemone rivularis	GU732733.1	Tibet761	Xie *et al.* (2011)
Clematis acerifolia	GU732734.1	Shi051	Xie *et al.* (2011)
Clematis aethusifolia	GU732735.1	Xie2006031	Xie *et al.* (2011)
Clematis afoliata	AB116890.1		Miikeda *et al.* (2006)

Clematis akebioides	GU732736.1	Xie2004025	Xie *et al*. (2011)
Clematis alpina	GU732738.1	Wen10381	Xie *et al*. (2011)
Clematis alpina	GU732737.1	Horandl9517	Xie *et al*. (2011)
Clematis alternata	AB116887.1		Miikeda *et al*. (2006)
Clematis angustifolia	AB116896.1		Miikeda *et al*. (2006)
Clematis apiifolia	GU732739.1	Xie2006094	Xie *et al*. (2011)
Clematis apiifolia	AB116877.1		Miikeda *et al*. (2006)
Clematis armandii	GU732740.1	Xie2006050	Xie *et al*. (2011)
Clematis brachiata	GU732744.1	Wen9472	Xie *et al*. (2011)
Clematis brachiata	GU732743.1	Wen11077	Xie *et al*. (2011)
Clematis brachiata	GU732742.1	Wen11065	Xie *et al*. (2011)
Clematis brachiata	GU732741.1	Luke11695	Xie *et al*. (2011)
Clematis brachyura	AB116901.1		Miikeda *et al*. (2006)
Clematis brevicaudata	GU732745.1	Xie2006026	Xie *et al*. (2011)
Clematis cf. insidiosa	GU732806.1	Wen9513	Xie *et al*. (2011)
Clematis chinensis	GU732746.1	Xie2006055	Xie *et al*. (2011)
Clematis chrysocoma	GU732749.1	Xie2004DL32	Xie *et al*. (2011)
Clematis chrysocoma	GU732748.1	Tibet979	Xie *et al*. (2011)
Clematis chrysocoma	GU732747.1	Tibet1605	Xie *et al*. (2011)
Clematis confusa	GU732750.1	Tibet891	Xie *et al*. (2011)
Clematis crassifolia	AB116891.1		Miikeda *et al*. (2006)
Clematis crispa	GU732751.1	Wen10019	Xie *et al*. (2011)
Clematis delavayi	GU732752.1	Tibet1256	Xie *et al*. (2011)
Clematis delavayi	AB116899.1		Miikeda *et al*. (2006)
Clematis drummondii	GU732753.1	Wen9735	Xie *et al*. (2011)
Clematis eichleri	AB116906.1		Miikeda *et al*. (2006)
Clematis erectisepala	GU732754.1	Xie2003YJ01	Xie *et al*. (2011)

Clematis fasciculiflora	AB116900.1		Miikeda *et al.* (2006)
Clematis finetiana	GU732755.1	Xie2006052	Xie *et al.* (2011)
Clematis florida	AB116883.1		Miikeda *et al.* (2006)
Clematis fusca	GU732756.1	Xie2006004	Xie *et al.* (2011)
Clematis fusca	AB116876.1		Miikeda *et al.* (2006)
Clematis gentianoides	AB116907.1		Miikeda *et al.* (2006)
Clematis glycinoides	GU732757.1	Mcpherson622	Xie *et al.* (2011)
Clematis heracleifolia	GU732758.1	Xie & Shi 2003H03	Xie *et al.* (2011)
Clematis hexapetala	GU732759.1	Xie2003143	Xie *et al.* (2011)
Clematis integrifolia	GU732761.1	Wen10380	Xie *et al.* (2011)
Clematis integrifolia	GU732760.1	Horandl9512	Xie *et al.* (2011)
Clematis japonica	AB116884.1		Miikeda *et al.* (2006)
Clematis lasiandra	GU732762.1	Xie2004082	Xie *et al.* (2011)
Clematis lasiandra	AB116882.1		Miikeda *et al.* (2006)
Clematis lasiantha	GU732764.1	Janeway9369	Xie *et al.* (2011)
Clematis lasiantha	GU732763.1	Janeway9366	Xie *et al.* (2011)
Clematis lasiantha	AB116897.1		Miikeda *et al.* (2006)
Clematis leschenaultiana	GU732765.1	Xie2004001	Xie *et al.* (2011)
Clematis ligusticifolia	GU732767.1	Janeway9513	Xie *et al.* (2011)
Clematis ligusticifolia	GU732766.1	Castro	Xie *et al.* (2011)
Clematis ligusticifolia	AB116898.1		Miikeda *et al.* (2006)
Clematis linearifolia	GU732768.1	Li *et al.* 35	Xie *et al.* (2011)
Clematis loureiroana	GU732769.1	Tibet2157	Xie *et al.* (2011)
Clematis meyeniana	GU732771.1	Xie2003099	Xie *et al.* (2011)
Clematis montana	GU732773.1	Xie2004027	Xie *et al.* (2011)

Clematis montana	GU732772.1	Tibet1226	Xie *et al.* (2011)
Clematis nobilis	AB116903.1		Miikeda *et al.* (2006)
Clematis ochotensis	AB116879.1		Miikeda *et al.* (2006)
Clematis orientalis	GU732775.1	Li14	Xie *et al.* (2011)
Clematis orientalis	GU732774.1	Li13	Xie *et al.* (2011)
Clematis orientalis	AB116893.1		Miikeda *et al.* (2006)
Clematis patens	AB116881.1		Miikeda *et al.* (2006)
Clematis peterae	GU732777.1	Xie2004002	Xie *et al.* (2011)
Clematis peterae	GU732776.1	Li4918	Xie *et al.* (2011)
Clematis pierotii	AB116888.1		Miikeda *et al.* (2006)
Clematis pinnata	GU732778.1	Xie2004BT01	Xie *et al.* (2011)
Clematis pitcheri	GU732779.1	Wen9723	Xie *et al.* (2011)
Clematis pogonandra	GU732780.1	Xie2004024	Xie *et al.* (2011)
Clematis potaninii	GU732781.1	Xie2004023	Xie *et al.* (2011)
Clematis potaninii	AB116895.1		Miikeda *et al.* (2006)
Clematis pubescens	GU732782.1	Li *et al.* 200714	Xie *et al.* (2011)
Clematis ranunculoides	GU732783.1	Xie2004ZD25	Xie *et al.* (2011)
Clematis recta	GU732785.1	Wen10382	Xie *et al.* (2011)
Clematis recta	GU732784.1	Horandl9514	Xie *et al.* (2011)
Clematis rehderiana	GU732786.1	Xie2004DQ11	Xie *et al.* (2011)
Clematis reticulata	GU732787.1	Wen9780	Xie *et al.* (2011)
Clematis rutoides	GU732790.1	Wen9553	Xie *et al.* (2011)
Clematis rutoides	GU732789.1	Wen9548	Xie *et al.* (2011)
Clematis rutoides	GU732788.1	Wen9489	Xie *et al.* (2011)
Clematis serratifolia	AB116902.1		Miikeda *et al.* (2006)
Clematis siamensis	GU732791.1	Xie2004TM09	Xie *et al.* (2011)
Clematis sibirica	GU732792.1	Xie2004XJ029	Xie *et al.* (2011)

Clematis sp.	GU732807.1	Wen9476	Xie *et al*. (2011)
Clematis stans	AB116885.1		Miikeda *et al*. (2006)
Clematis strigillosa	GU732793.1	Wen9691	Xie *et al*. (2011)
Clematis taiwaniana	GU732794.1	Wen9385	Xie *et al*. (2011)
Clematis tangutica	GU732795.1	Tibet57	Xie *et al*. (2011)
Clematis tangutica	AB116892.1		Miikeda *et al*. (2006)
Clematis tashiroi	AB116889.1		Miikeda *et al*. (2006)
Clematis terniflora	AB116880.1		Miikeda *et al*. (2006)
Clematis terniflora var. *mandshurica*	GU732770.1	Wen10383	Xie *et al*. (2011)
Clematis texensis	GU732796.1	Wen9749	Xie *et al*. (2011)
Clematis texensis	AB116894.1		Miikeda *et al*. (2006)
Clematis tibetana	GU732798.1	Tibet917	Xie *et al*. (2011)
Clematis tibetana	GU732797.1	Tibet396	Xie *et al*. (2011)
Clematis uncinata	GU732799.1	Xie2004007	Xie *et al*. (2011)
Clematis uncinata var. *ovatifolia*	AB116886.1		Miikeda *et al*. (2006)
Clematis villosa	AB116908.1		Miikeda *et al*. (2006)
Clematis virginiana	GU732802.1	Wen10494	Xie *et al*. (2011)
Clematis virginiana	GU732801.1	Wen10387	Xie *et al*. (2011)
Clematis virginiana	GU732800.1	Wen10002	Xie *et al*. (2011)
Clematis vitalba	GU732805.1	Wen10373	Xie *et al*. (2011)
Clematis vitalba	GU732804.1	Wen10354	Xie *et al*. (2011)
Clematis vitalba	GU732803.1	Horandl9513	Xie *et al*. (2011)
Clematis vitalba	AB116904.1		Miikeda *et al*. (2006)
Clematis williamsii	AB116878.1		Miikeda *et al*. (2006)

Hepatica nobilis var. *japonica*	AB116911.1		Miikeda *et al.* (2006)
Knowltonia sp.	AB116912.1	OM073	Miikeda *et al.* (2006)
Naravelia laurifolia	GU732808.1	Wen10182	Xie *et al.* (2011)
Naravelia laurifolia	AB116905.1		Miikeda *et al.* (2006)
Pulsatilla chinensis	GU732812.1	Xie2006024	Xie *et al.* (2011)
Pulsatilla dahurica	GU732810.1	Xie2006025	Xie *et al.* (2011)
Pulsatilla sp.	GU732813.1	Xie 2007001	Xie *et al.* (2011)
Pulsatilla turczaninovii	GU732811.1	Xie2006027	Xie *et al.* (2011)

Table 4.3 JModeltest suggested models of evolutions used for the different regions in the MrBayes analyses.

	Nuclear (ITS)	**Chloroplast (*atp*B-*rbc*L)**	**Chloroplast (*rpo*B-*trn*C)**
Number of Character	644	927	1293
Model	GTR+I+G	GTR+I+G	GTR+G
Frequency of A	0.2245	0.3663	3549
Frequency of C	0.3426	0.1319	0.1349
Frequency of G	0.2648	0.1380	0.1554
Frequency of T	0.1681	0.3638	0.3548
p-inv	0.3250	0.6210	n/a
gamma	0.4630	0.7780	0.110

Divergence time estimates

Divergence times were estimated using a Bayesian approach implemented in BEAST v. 1.7.4 (Drummond & Rambaud, 2012) for the combined ITS, *atp*B-*rbc*L and *rpo*B-*trn*c dataset. The crown node for the group was constrained using the date from the results corresponding to the equivalent node in the Magallón *et al*. (2015) constrained and Anderson *et al*. (2005) constrained *Anemoneae* analyses in chapter 3.

For the BEAST analyses the Birth and Death incomplete sampling tree prior was selected because of incomplete coverage across the selected genera (Stadler, 2009). The analysis implemented a relaxed clock with uncorrelated log-normal model of rate variation (Drummond *et al*. 2006). The GTR+G+I model suggested by JModelTest was implemented. The input file for the analysis was generated using BEAUti v.1.7.4 (Drummond & Rambaut, 2007, Drummond *et al*. 2012).

Two independent runs of 100 million generations were carried out for the two different sets.

The two separate runs from each analysis were checked visually using Tracer v.1.6 (Rambaut *et al*. 2014) for convergence to stationarity to ensure that ESS values of greater than 200 were achieved. The two independent runs for each analysis were combined using LogCombiner v.1.7.4 (Drummond *et al*. 2012). A maximum clade credibility (MCC) tree was generated from the sampled trees using TreeAnnotator v.1.7.4 (Drummond *et al*. 2012), with mean ages, 95% highest posterior probability (HPD) intervals and posterior probabilities calculated.

Visualisation of Trees

Phlyogenetic and Divergence Time estimation trees were visualised using FigTree v.1.4 (Rambaut, 2012)

Phylogeographic Analysis - RASP

Historic geographical ranges were estimated using RASP (Reconstruct Ancestral State in Phylogenies) v.3.2 (Yu *et al*. 2012; Yu *et al*. 2015). The S-DEC (Statistical Dispersal, Extinction and Cladogenesis) analysis was used as it implements a Bayesian framework to generate a probability of an ancestral range occurring at a node. Akaike weights (AICw) for alternative models are calculated and interpreted as the relative probability of different ancestral ranges, displayed as pie charts on the nodes of a phylogenetic tree (Yu *et al*. 2015).

Distributions for each of the terminal taxa were coded following the geographical regions used in the Flora of Nepal (Watson *et al*, 2011) and added to a spreadsheet of terminal taxa. The geographical split of East and West Himalaya is at the Kali Gandaki, the Stainton line is taken to be 84°E in this study although Stainton (1972) takes 85°E as the absolute extent of West Himalayan elements in dry inner valleys behind the main Himalayan range. The longitude of 84°E in this study is also different to Stearn (1978) who stated Stainton took the divide to be 83°E. In the Dobremez (1976) vegetation classification the border of the western division was given as 83° 28'E (Hara *et al*. 1978). The Stainton Line and Dobremez vegetation divisions will be annotated on the visualisations of the species distribution models.

The Magallón constrained BEAST trees from the molecular dating analysis was resampled to generate a dataset of 1000 trees for the S-DEC analysis. The MCC tree used to annotate the results of the S-DEC analysis was generated from the resampled dataset of 1000 trees.

Table 4.4 Coded geographical entities used in the RASP analysis follows the delimitations of areas in the Flora of Nepal (Watson *et al*. 2013). S America excluded from analysis as there were no representative accessions.

Code	Geographical Area	Details
A	W Himalaya	From the Kali Gandaki westwards covering W Nepal, NW India, N Pakistan.
B	E Himalaya	From the Kali Gandaki eastwards covering E Nepal, Sikkim, Bhutan, Arunachal Pradesh.
C	Tibetan Plateau	Xizang and Qinghai.
D	Assam and Burma	Assam, Nagaland, Manipur, Myanmar
E	S Asia	E Pakistan, peninsular India, Sri Lanka, Bangladesh
F	E Asia	China (excluding Xizang, Xinjiang, and Qinghai), N & S Korea, Japan, Taiwan

G	SE Asia	Thailand, Laos, Cambodia, Vietnam, Malaysia, Indonesia, Philippines, New Guinea.
H	N Asia	Xinjiang, Russia, Mongolia.
I	C Asia	Kazakhstan, Uzbekistan, Turkmenistan, Tajikistan, Kyrgyzstan.
J	SW Asia	Afghanistan, W Pakistan, Iran, Middle East, Arabian Peninsula, Turkey, Azerbaijan, Armenia, Georgia
K	Europe	Including Ukraine, Belarus, Baltic states.
L	Africa	Including Madagascar
M	North America	Including C America south to Panama
N	Australasia	Australia, New Zealand and Pacific Islands.

Species Distribution Modelling (SDM)

Species distribution models were generated using MaxEnt version 3.3.3k for the West Himalayan elements, *Clematis graveolens*, *C. tibetana subsp. brevipes, C. tibetana subsp. vernayi, C. grata, C. phlebantha.* Examination of the BM, E and K specimens of the other widely distributed Himalayan species, *C. buchaniana*, *C. grewiiflora* and *C. connata* found that they are frequently misidentified. It was therefore decided that their records on GBIF are unreliable, and without these records there would be insufficient data to accurately model their distributions. *Clematis confusa* is a Himalayan endemic with too few geo-referenced records for the model to run.

MaxEnt analyses are generated from presence-only data, taken from known distributions. MaxEnt estimates distribution ranges by finding the closest to uniform set of constraints to the expected values of the environmental variables tested, a process called maximum entropy (Phillips *et al*. 2006). It performs well with low quality and quantity of records when estimating distributions (Person *et al*. 2007), and is useful for species with restricted or poorly known distributions.

The 19 standard Bioclimatic variables (table 4.7) and altitude were downloaded from (www.worldclim.org) at 5 km^2 resolution. Default MaxEnt setting were used including 25% test data. This uses the defined percentage of the presence data to test the rigour of the model based on 75% actual data (Phillips *et al*. 2006).

Table 4.5 Bioclimatic variables are derived from the monthly temperature and rainfall values in order to generate more biologically meaningful variables.

Name	Variable Description
BIO1	Annual Mean Temperature
BIO2	Mean Diurnal Range (Mean of monthly (max temp - min temp))
BIO3	Isothermality (BIO2/BIO7) (* 100)
BIO4	Temperature Seasonality (standard deviation *100)
BIO5	Max Temperature of Warmest Month
BIO6	Min Temperature of Coldest Month
BIO7	Temperature Annual Range (BIO5-BIO6)
BIO8	Mean Temperature of Wettest Quarter
BIO9	Mean Temperature of Driest Quarter
BIO10	Mean Temperature of Warmest Quarter
BIO11	Mean Temperature of Coldest Quarter
BIO12	Annual Precipitation
BIO13	Precipitation of Wettest Month
BIO14	Precipitation of Driest Month
BIO15	Precipitation Seasonality (Coefficient of Variation)
BIO16	Precipitation of Wettest Quarter
BIO17	Precipitation of Driest Quarter
BIO18	Precipitation of Warmest Quarter
BIO19	Precipitation of Coldest Quarter

Georeferenced data from the Flora of Nepal database, occurrence data from GBIF (www.gbif.org) and additional georeferenced specimens from E, were used as presence data for the analysis. *Clematis graveolens* had 60 presence points, *C. tibetana subsp. brevipes* 58 presence points, *C. tibetana subsp. vernayi* 16 presence points, *C. grata* 125 presence points and *C. phlebantha* 20 presence points.

Outputs were visualised in ArcMap 10.1. Each output has the Stainton Line at approximately 84°E added as the marker for the East / West Himalayan split in the vegetation (Stainton, 1972) and the Dobremez (1972) vegetation belts.

Results

Phylogenetics

The sectional classification used to present the results follows Johnson (2001) unless otherwise stated. Figure 4.1 is the strict consensus tree generated from the combined nDNA and cpDNA analysis, the nDNA (ITS) dataset is figure 4.2 and the combined cpDNA (*atp*B-*rbc*L & *rpo*B-*trn*C) tree is figure 4.3. It is well known that Bayesian posterior probabilities (pp.) values may overestimate support (Suzuki et al. 2002; Simmons et al. 2004), and therefore thresholds are modified to be more conservative the Swenson *et al*. (2008) greater than 0.95 pp. were considered equivalent to strong support, 0.90-0.95 pp. moderate support and less than 0.90 pp. poor support.

On the nDNA tree, figure 4.2, *Clematis* is sister to *Anemoclema* with strong support (0.98 pp). On the combined cpDNA tree, figure 4.3, *Anemoclema* is on the unresolved backbone with all *Clematis* accessions. On the combined nDNA and cpDNA tree, figure 4.1, the relationship is strongly supported (1.0 pp.).

Despite the generally strong support for these reported nodes, and accessions group together well there are exceptions and relationships between species in strongly supported clades can often be poorly supported or unclear as they form polytomies, especially in the cpDNA analysis.

Placement of new Nepalese accessions

On the nDNA tree, figure 4.1, the three *Clematis montana* accessions from Nepal form a strongly-supported polytomy (1.0 pp.) which is a poorly supported sister clade (0.79 pp.) to the two Tibetan *C. montana* and three Chinese *C. chrysocoma* accessions, which all belong to section *Cheiropsis* subsection *Montanae*. By contrast on the combined cpDNA tree, figure 4.2, the three Nepalese *C. montana* comprise the strongly supported sister clade (1.0 pp.) to the Tibetan *C. montana*. In the combined nDNA and cpDNA tree, figure 4.3, the three Nepalese *C. montana* are sister to the two Tibetan *C. montana* with strong support (1.0 pp.), and the *C. montana* clade is sister to the two Chinese *C. chrysocoma*, also with strong support (0.99 pp.). These accessions along with *C. fasciciflora* form a poorly supported sister clade (0.83 pp.) to the remaining accessions of *Clematis* in the nDNA, figure 4.1, and a poorly supported clade (0.81 pp.) on the backbone polytomy of the combined nDNA and cpDNA analysis, figure 4.3.

On the nDNA tree, figure 4.1, *Clematis alternata* (section *Archiclematis*) accessions form a clade with moderate support (0.92 pp.) on the unresolved polytomy of the remaining accessions of *Clematis*. On the combined cpDNA tree, figure 4.2, it forms part of a polytomy with *C. alpina* (section *Atragene*) and *C. ladakiana* and *C. tibetana* subsp. *brevipies* (both section Meclatis). On the combined nDNA and cpDNA tree, figure 4.3, they are part of the unresolved basal polytomy of members of section *Connatae* and section *Clematis* subsection *Africanae*.

On the nDNA tree, figure 4.1, the Nepalese accessions of section *Connatae* (*C. buchananiana, C. connata*, *C. grewiiflora* and *C. sp. nov*. BRD A119) are part of the unresolved polytomy that includes all the remaining accessions of *Clematis*. *Clematis sp. nov*. BRD A119 is the strongly supported sister (0.99 pp.) to *Clematis confusa*, but together they are in an unresolved position. In the combined cpDNA tree, figure 4.2, the Nepalese accessions of section *Connatae* are on the unresolved backbone polytomy, with the exception of *C. buchananiana* and *Clematis sp. nov*. BRD A119 with are part of a poorly supported clade (0.52 pp.) with one *C. phlebantha* accession (section *Fruticella* subsection *Phlebanthae*).

On the nDNA tree, figure 4.1, *Clematis ladakiana* (section *Meclatis*) from Pakistan is part of a clade containing *C. orientalis* (section *Meclatis*), *C. nobilis* (section *Atragene*) and C. *pogonandra* (section *Campanella*) with good support (0.93 pp.) and is the strongly supported sister lineage (1.0 pp.) to two accessions of *C. orientalis*.

On the nDNA tree, figure 4.1, *Clematis sp. nov.* GAK8802 (section *Meclatis*) from Oman is the poorly supported sister lineage (0.89 pp.) to the Nepalese *C. graveolens* clade (section *Meclatis*) which has moderate support (0.93 pp.). These three accessions are in a poorly supported clade with *C. orientalis* (section *Meclatis*) and *C. acerifolia* (section Cheiropsis). In the cpDNA tree, figre 4.2, *Clematis sp. nov.* GAK8802 forms part of a largely unresolved but well-supported clade of *C. akebiodes*, *C. tibetana*, *C. tangutica* and *C. tibetana* subsp. *brevipes* Manasalu08, all section *Meclatis*. On the cpDNA tree, figure 4.2, the two accessions of C. *graveolens* are on the unresolved backbone but strongly supported as sister (1.0 pp.). In the combined nDNA and cpDNA tree, figure 4.3, *C. graveolens* is the poorly supported sister lineage (0.51 pp.) to a strongly supported clade (0.99 pp.) corresponding to the same clade containing *Clematis sp. nov.* GAK8802 in the cpDNA tree.

On the nDNA tree, figure 4.1, both accessions of *Clematis tibetana* subsp. *brevipes* (section *Meclatis*) (BRD A219 and Manalsu08 20816085) from Nepal, but different localities ca. 200 km apart on different sides of the Kali Gandaki, are in a poorly supported clade (0.87 pp.) with *C. akebiodes C. tibetana*, *C. tangutica*, all section *Meclatis*. On the cpDNA tree, figure 4.2, the BRD accession of *C. tibetana* subsp. *brevipes* is in a poorly supported polytomy (0.52 pp.) with *C. alternata* (section *Archiclematis*), *C. alpina* (section *Atragene*) and *C. ladakiana* (section *Meclatis*) and the Manaslu08 accession is a strongly supported but unresolved clade with *C. akebiodes*, *C. tibetana*, *C. tangutica* and *C sp. nov.* GAK8802. In the combined analysis the BRD accession is the poorly supported sister lineage (0.54 pp.) to a *C. orientalis* on the unresolved backbone polytomy. The Manaslu08 accession is in a strongly supported clade (0.99 pp.) that contains the two accessions of C. *graveolens* are on the unresolved back bone of the cpDNA tree but strongly supported (1.0 pp.). In the combined analysis *C. graveolens* is the poorly supported sister (0.51 pp.) lineage to a strongly supported clade (0.99 pp.) containing *C. akebiodes C. tibetana*, *C. tangutica*, which is the poorly supported sister clade (0.51 pp.) to *C. graveolens*.

On the nDNA tree, figure 4.1, the two accessions of *Clematis phlebantha* (section *Flammula*) from Nepal form a strongly supported clade (1.0 pp.), in a moderately supported clade (0.9 pp.) containing accessions from Asian, European and North American (sections *Bebaeanthera*, *Clematis, Flammula*, *Lasithana* and *Tubulosae*). The *C. phlebantha* clade is the poorly supported sister clade (0.54 pp.) to the accessions from Asian, European and North American. However, in the cpDNA tree, figure 4.2, the position of *C. phlebantha* (BRD A262) is unresolved on the backbone polytomy and the other accession (BRD B151) is in a poorly supported clade (0.53 pp.) containing *C. buchananiana* and *C. sp. nov.* BRD

A11 (section *Connatae*). On the combined nDNA and cpDNA tree, figure 4.3, both *C. phlebantha* accessions form a strongly supported clade (1.0 pp.) in an unresolved position on the backbone polytomy.

On the nDNA tree, figure 4.1, the two accessions of *Clematis grata* (section *Clematis*) from Nepal form a poorly supported clade (0.59 pp.) and are the sister lineage, with moderate support (0.92 pp.), to Asian, European and North American species (sections *Bebaeanthera*, *Clematis*, *Lasithana* and *Tubulosae*). On the cpDNA tree, figure 4.2, they form a strongly supported clade (0.97 pp.) in an unresolved position on the backbone polytomy. In the combined nDNA and cpDNA tree, figure 4.3, they are a strongly supported clade (1.0 pp.) which is the poorly supported (0.75 pp.) sister lineage to species from sections *Bebaeanthera*, *Clematis*, *Lasithana* and *Tubulosae*.

The sectional classifications of Grey-Wilson 2000 and Johnson 2001 are annotated on figure 4.3 for a comparison between taxonomies and evolutionary relationships. The Grey-Wilson and Johnson taxonomies do not reflect the evolutionary relationship presented in the phylogeny.

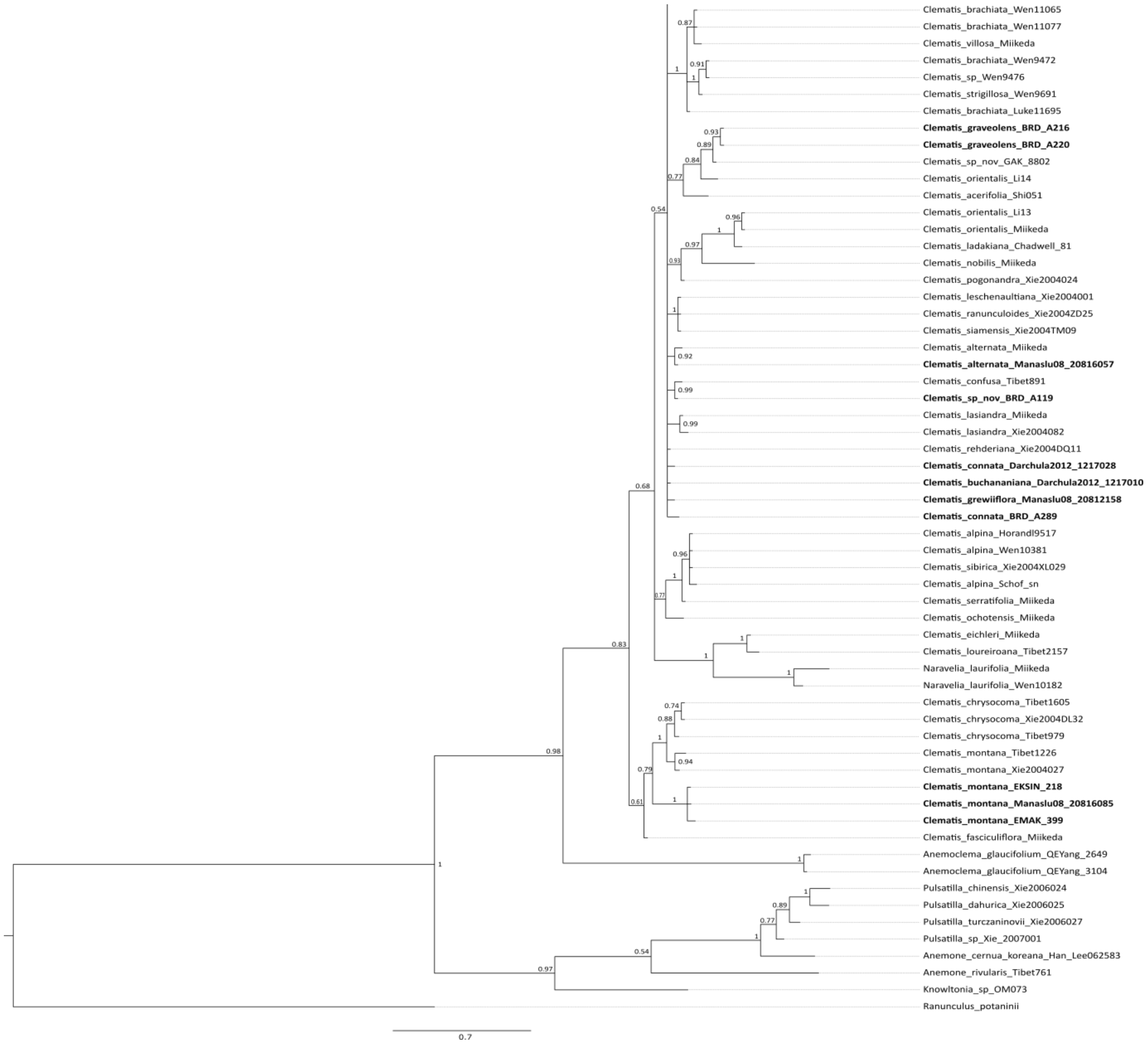
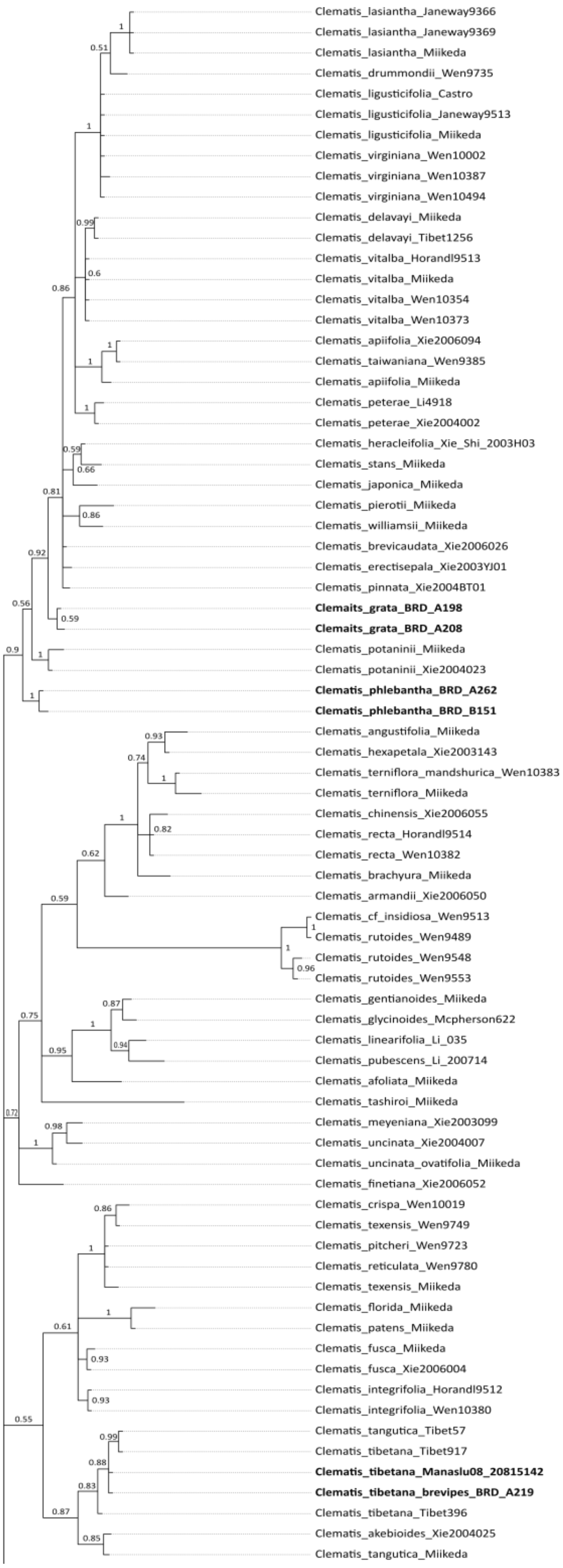


Figure 4.1 Strict Consensus tree generated from the nDNA (ITS) dataset Accessions in bold are new accessions from this project.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Clematis_ligusticifolia_Castro
Clematis_ligusticifolia_Janeway9513
1
Clematis_ligusticifolia_Miikeda
Clematis_virginiana_Wen10002
Clematis_virginiana_Wen10387
Clematis_virginiana_Wen10494
Clematis_delavayi_Miikeda
0.99
Clematis_delavayi_Tibet1256
Clematis_vitalba_Horandl9513
0.6
Clematis_vitalba_Miikeda
0.86
Clematis_vitalba_Wen10354
Clematis_vitalba_Wen10373
Clematis_apiifolia_Xie2006094
1
1
Clematis_taiwaniana_Wen9385
Clematis_apiifolia_Miikeda
1
Clematis_peterae_Li4918
Clematis_peterae_Xie2004002
Clematis_heracleifolia_Xie_Shi_2003H03
0.59
Clematis_stans_Miikeda
0.66
Clematis_japonica_Miikeda
0.81
Clematis_pierotii_Miikeda
0.86
Clematis_williamsii_Miikeda
Clematis_brevicaudata_Xie2006026
0.92
Clematis_erectisepala_Xie2003YJ01
Clematis_pinnata_Xie2004BT01
0.56
Clemaits_grata_BRD_A198
0.59
Clemaits_grata_BRD_A208
0.9
Clematis_potaninii_Miikeda
1
Clematis_potaninii_Xie2004023
1
Clematis_phlebantha_BRD_A262
Clematis_phlebantha_BRD_B151
Clematis_angustifolia_Miikeda
0.93
Clematis_hexapetala_Xie2003143
0.74
Clematis_terniflora_mandshurica_Wen10383
1
Clematis_terniflora_Miikeda
1
Clematis_chinensis_Xie2006055
0.82
Clematis_recta_Horandl9514
Clematis_recta_Wen10382
0.62
Clematis_brachyura_Miikeda
Clematis_armandii_Xie2006050
0.59
Clematis_cf_insidiosa_Wen9513
1
Clematis_rutoides_Wen9489
1
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0.96
Clematis_rutoides_Wen9553
Clematis_gentianoides_Miikeda
0.87
Clematis_glycinoides_Mcpherson622
0.75
1
Clematis_linearifolia_Li_035
0.94
0.95
Clematis_pubescens_Li_200714
Clematis_afoliata_Miikeda
Clematis_tashiroi_Miikeda
0.72
Clematis_meyeniana_Xie2003099
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Clematis_uncinata_Xie2004007
1
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Clematis_finetiana_Xie2006052
Clematis_crispa_Wen10019
0.86
Clematis_texensis_Wen9749
1
Clematis_pitcheri_Wen9723
Clematis_reticulata_Wen9780
Clematis_texensis_Miikeda
Clematis_florida_Miikeda
1
Clematis_patens_Miikeda
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Clematis_fusca_Miikeda
0.93
Clematis_fusca_Xie2006004
Clematis_integrifolia_Horandl9512
0.93
Clematis_integrifolia_Wen10380
0.55
Clematis_tangutica_Tibet57
0.99
Clematis_tibetana_Tibet917
0.88
Clematis_tibetana_Manaslu08_20815142
0.83
Clematis_tibetana_brevipes_BRD_A219
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Clematis_tibetana_Tibet396
0.85
Clematis_akebioides_Xie2004025
Clematis_tangutica_Miikeda

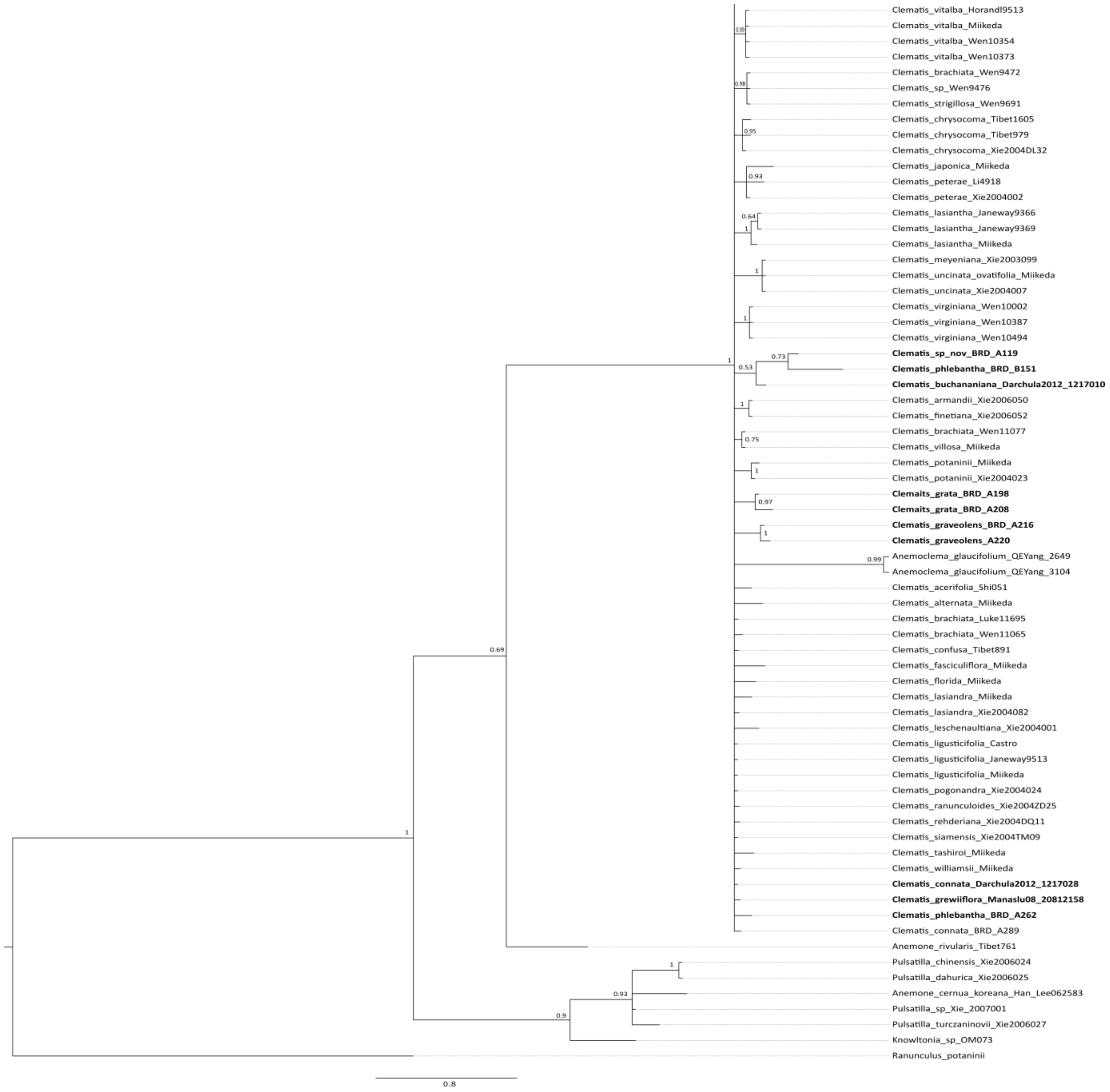
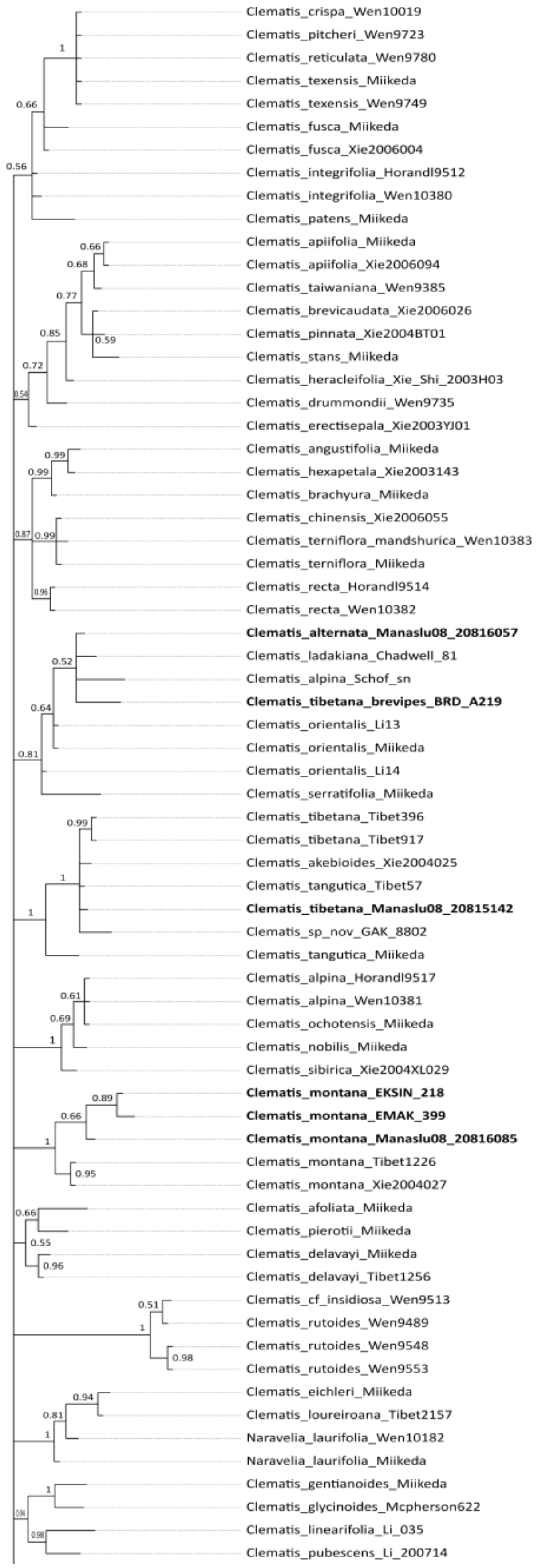


Figure 4.2 Strict consensus tree generated from Baysian analysis of combined cpDNA (*atp*b-*rbc*l & *rpo*B-*trn*C). Accessions in bold are the new accession from this project.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Clematis_pitcheri_Wen9723
Clematis_reticulata_Wen9780
Clematis_texensis_Miikeda
0.66
Clematis_texensis_Wen9749
Clematis_fusca_Miikeda
Clematis_fusca_Xie2006004
0.56
Clematis_integrifolia_Horandl9512
Clematis_integrifolia_Wen10380
Clematis_patens_Miikeda
0.66
Clematis_apiifolia_Miikeda
0.68
Clematis_apiifolia_Xie2006094
Clematis_taiwaniana_Wen9385
0.77
Clematis_brevicaudata_Xie2006026
0.85
Clematis_pinnata_Xie2004BT01
0.59
Clematis_stans_Miikeda
0.72
Clematis_heracleifolia_Xie_Shi_2003H03
0.54
Clematis_drummondii_Wen9735
Clematis_erectisepala_Xie2003YJ01
0.99
Clematis_angustifolia_Miikeda
0.99
Clematis_hexapetala_Xie2003143
Clematis_brachyura_Miikeda
Clematis_chinensis_Xie2006055
0.87
0.99
Clematis_terniflora_mandshurica_Wen10383
Clematis_terniflora_Miikeda
Clematis_recta_Horandl9514
0.96
Clematis_recta_Wen10382
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Clematis_alpina_Schof_sn
Clematis_tibetana_brevipes_BRD_A219
0.64
Clematis_orientalis_Li13
Clematis_orientalis_Miikeda
0.81
Clematis_orientalis_Li14
Clematis_serratifolia_Miikeda
0.99
Clematis_tibetana_Tibet396
Clematis_tibetana_Tibet917
Clematis_akebioides_Xie2004025
1
Clematis_tangutica_Tibet57
1
Clematis_tibetana_Manaslu08_20815142
Clematis_sp_nov_GAK_8802
Clematis_tangutica_Miikeda
Clematis_alpina_Horandl9517
0.61
Clematis_alpina_Wen10381
0.69
Clematis_ochotensis_Miikeda
1
Clematis_nobilis_Miikeda
Clematis_sibirica_Xie2004XL029
0.89
Clematis_montana_EKSIN_218
0.66
Clematis_montana_EMAK_399
1
Clematis_montana_Manaslu08_20816085
Clematis_montana_Tibet1226
0.95
Clematis_montana_Xie2004027
0.66
Clematis_afoliata_Miikeda
Clematis_pierotii_Miikeda
0.55
Clematis_delavayi_Miikeda
0.96
Clematis_delavayi_Tibet1256
0.51
Clematis_cf_insidiosa_Wen9513
1
Clematis_rutoides_Wen9489
Clematis_rutoides_Wen9548
0.98
Clematis_rutoides_Wen9553
0.94
Clematis_eichleri_Miikeda
0.81
Clematis_loureiroana_Tibet2157
1
Naravelia_laurifolia_Wen10182
Naravelia_laurifolia_Miikeda
1
Clematis_gentianoides_Miikeda
0.94
Clematis_glycinoides_Mcpherson622
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Clematis_pubescens_Li_200714

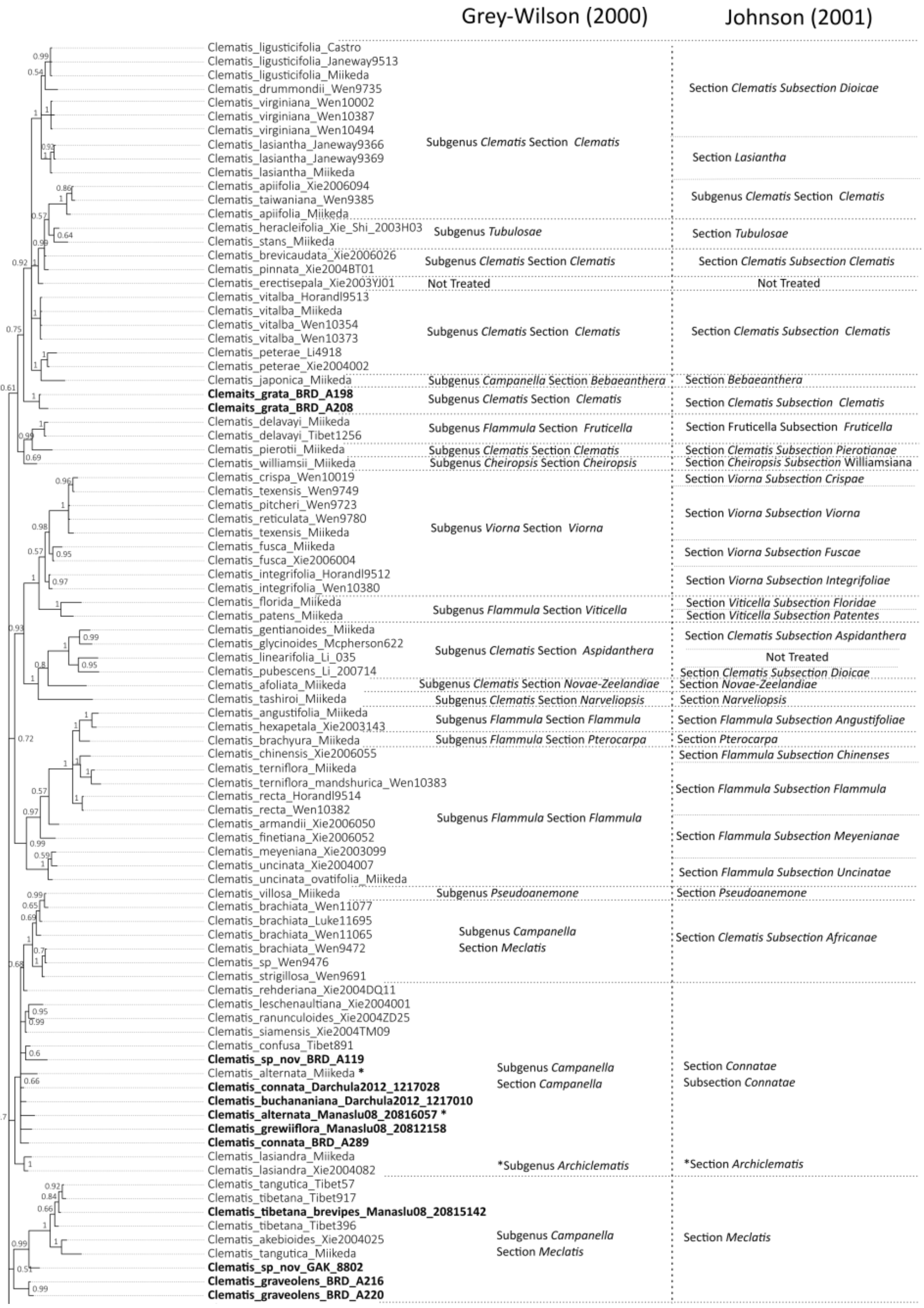
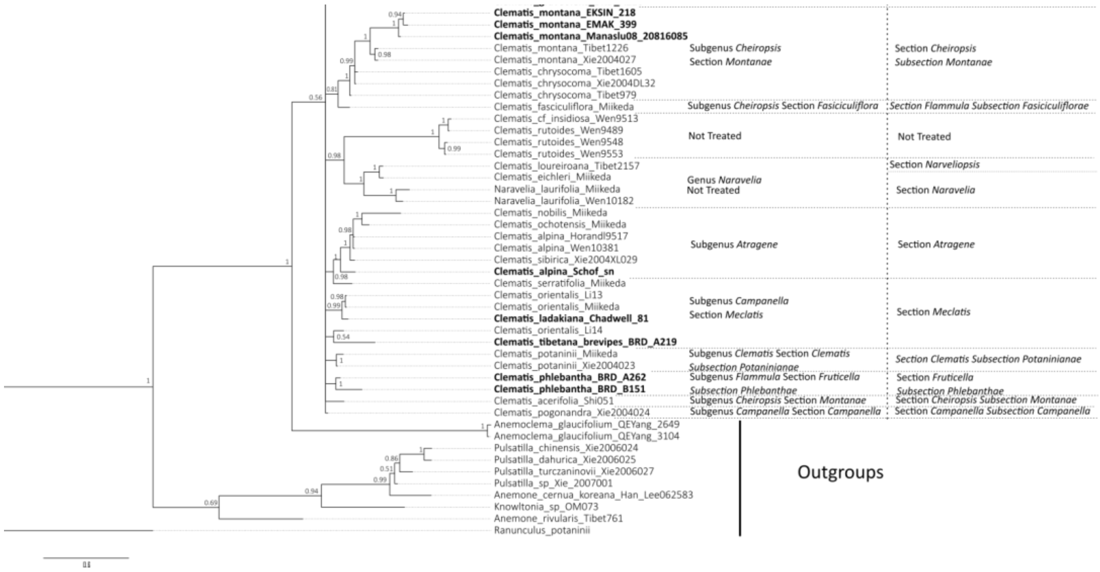


Figure 4.3 MrBayes strict consensus trees generated from combined ITS, *atp*B-*rbc*L & *rpo*B-*trn*C. Numbers at nodes are Bayesian posterior probability values. Infrageneric classifications Subgenus and section of Grey-Wilson (2000) and Johnson's (2001) section and subsection classifications are labelled for comparison. Species in bold are new data.



Divergence time estimate results

Table 4.5 shows the estimated ages of evolutionary events of equivalent nodes from the Anderson *et al*. (2005) and Magallón *et al*. (2015) constrained *Anemoneae* dataset, analyses from Chapter 3 and the equivalent nodes from the analyses of the *Clematis* dataset constrained with the results from the analyses of the *Anemoneae* dataset.

Figures 4.4 and 4.5 are the maximum clade credibility trees generated from the Anderson and Magallón constrained analyses of the *Clematis* dataset.

There were large differences in the estimated ages of evolutionary events at deeper nodes in the *Clematis* dataset when compared to the equivalent nodes in the *Anemoneae* dataset. The greatest differences were seen in the *Anemoninae* and *Clematinidae* split which was estimated at 55 Ma in the Anderson constrained *Anemoneae* dataset and 38 Ma in the Anderson constrained *Clematis* dataset; the age of this split was estimated at 49 Ma in the Magallón constrained *Anemoneae* dataset and only 35 Ma in the Magallón constrained *Clematis* dataset.

All analyses dated the *Clematis* crown to the middle of the Miocene, but the *Clematis* dataset analyses had much larger 95% confidence interval ranging from the Pleistocene to the Oligocene, compared with the *Anemoneae* dataset analyses which ranged from the Late Miocene to the Oligocene / Early Miocene boundary.

Equivalent derived nodes are closer in age estimates between the *Anemoneae* and *Clematis* analyses, table 4.5.

Table 4.6 The equivalent estimates ages of evolutionary events at nodes of interest from the Anderson and Magallón constrained *Anemoneae* and *Clematis* dataset analyses.

	Anemoninae - *Clematinidae* split	*Anemoclema* - *Clematis* Split	*Clematis* crown	section *Meclatis*	section *Cheiropsis* subsection *Montanae* crown	*C. chrysocoma* - *C. montana* split	Tibetan - Himalayan *C. montana* split
Anderson constrained *Anemoneae* dataset **(Figure 3.4)**	54.88 Ma (37.37 - 60.44 Ma) 0.99 pp.	30.18 Ma (16.35 - 47.83 Ma) 1.0 pp.	16.88 Ma (9.79 - 26.07 Ma) 1.0 pp.	6.42 Ma 0.57 pp.	5.76 Ma (2.17 - 11.76 Ma) 0.99 pp.	3.12 Ma (1.25 - 6.11 Ma) 0.99 pp.	2.08 Ma (0.84 - 4.24 Ma) 0.95 pp.
Anderson constrained *Clematis* dataset **(Figure 4.4)**	38.29 Ma 0.86 pp.	19. 97 Ma (3.22 - 43.89 Ma) 0.99 pp.	13.27 Ma (2.36 - 27.78 Ma) 1.0 pp.	6.42 Ma 0.22 pp.	4.52Ma (0.62 - 11.28 Ma) 0.95 pp.	2.91 Ma (0.37 - 6.9 Ma) 0.95 pp.	1.71 Ma (0.2 - 40.8 Ma) 0.95 pp.
Magallón constrained *Anemoneae* dataset **(Figure 3.5)**	49.33 Ma (95% HPD: 37.37-60.44 Ma) 0.99 pp.	26.69 (14.63 - 42.72 Ma) 0.99 pp.	15.01 (9.02 - 22.95 Ma) 0.99 pp.	6.42 Ma 0.55 pp.	5.18 Ma (2.04 - 10.11 Ma) 0.99 pp.	2.78 Ma (1.18 - 5.36 Ma) 0.99 pp.	1.87 Ma (0.68 - 3.66 Ma) 0.95 pp.

Magallón constrained *Clematis* dataset **(Figure 4.5)**	34.84 Ma 0.83 pp.	18.56 Ma (2.59 - 39.99 Ma) 0.99 pp.	12.28 Ma (1.83 - 26.05 Ma) 1.0 pp.	5.78 Ma 0.66 pp.	3.97 Ma (0.45 - 10.46 Ma) 1.0 pp.	2.5 Ma (0.28 - 6.45 Ma) 1.0 pp.	1.41 Ma (0.14 - 3.86 Ma) 1.0 pp.

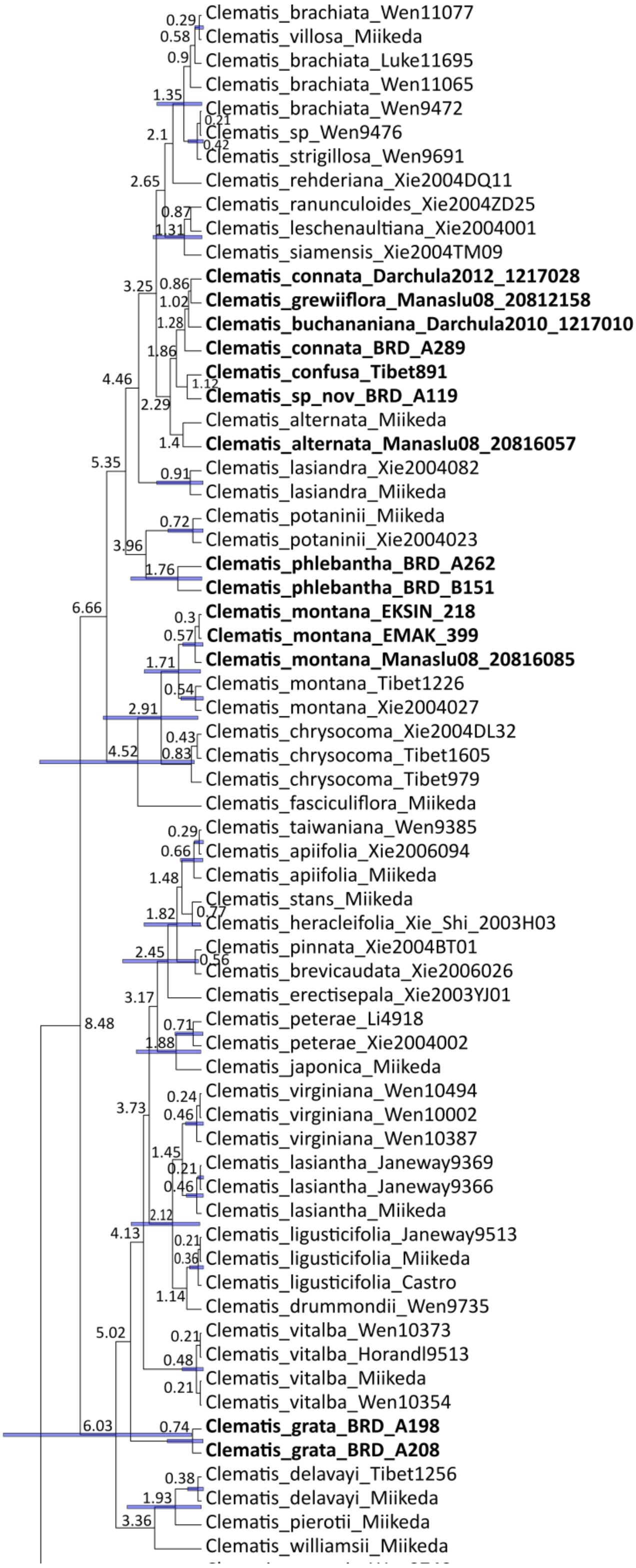
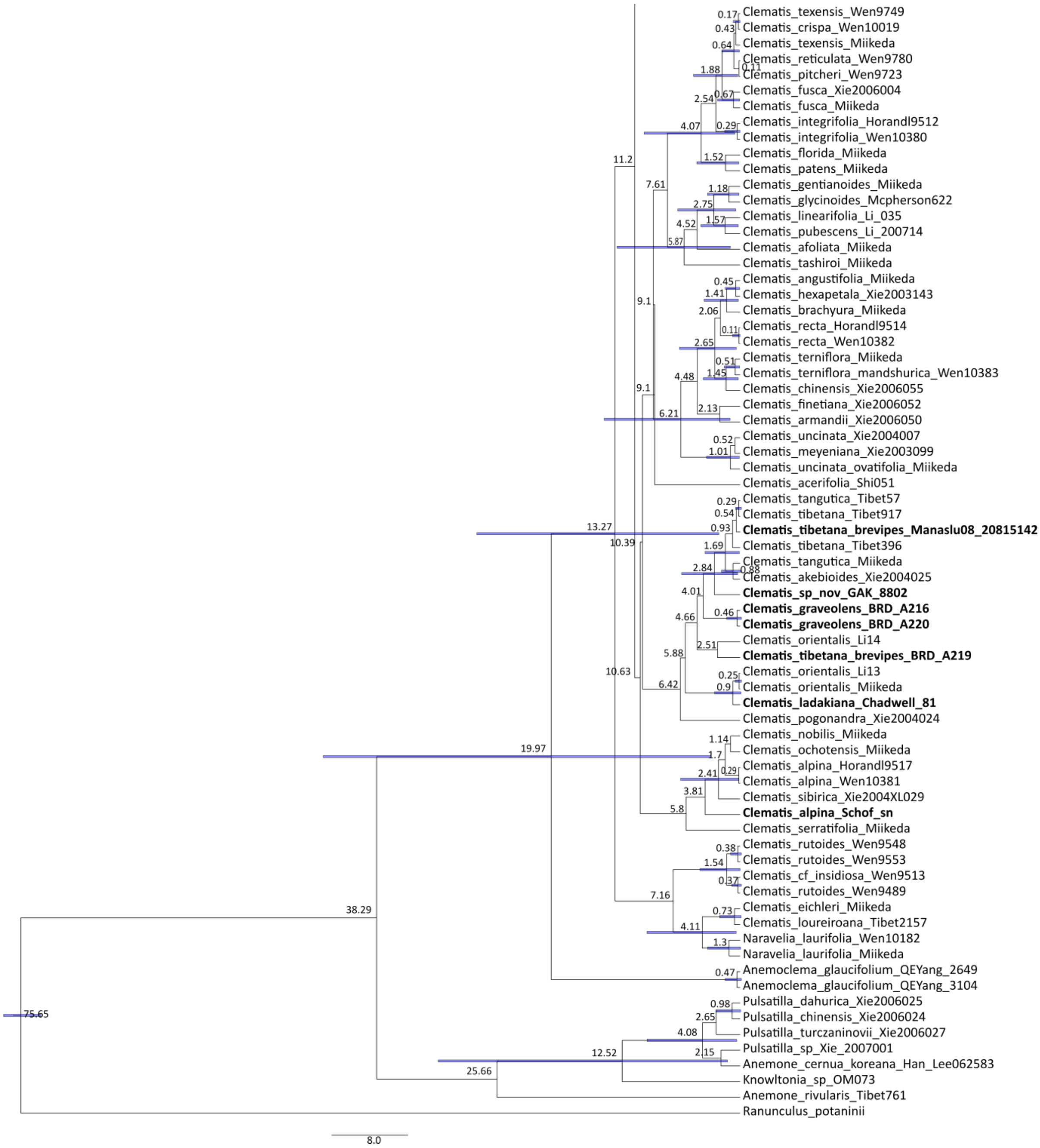


Figure 4.4 BEAST generated Maximum Clade Credibility tree of the Clematis dataset constrained with the crown node based on the dates derived from the Anderson constrained analysis of the *Anemoneae* dataset, chapter 3 figure 3.4. Numbers at nodes are divergence age estimates, bars at nodes are HPD 95% confidence intervals. Terminal taxa in bold indicate the new accessions in this study.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Clematis_crispa_Wen10019
Clematis_texensis_Miikeda
Clematis_reticulata_Wen9780
Clematis_pitcheri_Wen9723
Clematis_fusca_Xie2006004
Clematis_fusca_Miikeda
Clematis_integrifolia_Horandl9512
Clematis_integrifolia_Wen10380
Clematis_florida_Miikeda
Clematis_patens_Miikeda
Clematis_gentianoides_Miikeda
Clematis_glycinoides_Mcpherson622
Clematis_linearifolia_Li_035
Clematis_pubescens_Li_200714
Clematis_afoliata_Miikeda
Clematis_tashiroi_Miikeda
Clematis_angustifolia_Miikeda
Clematis_hexapetala_Xie2003143
Clematis_brachyura_Miikeda
Clematis_recta_Horandl9514
Clematis_recta_Wen10382
Clematis_terniflora_Miikeda
Clematis_terniflora_mandshurica_Wen10383
Clematis_chinensis_Xie2006055
Clematis_finetiana_Xie2006052
Clematis_armandii_Xie2006050
Clematis_uncinata_Xie2004007
Clematis_meyeniana_Xie2003099
Clematis_uncinata_ovatifolia_Miikeda
Clematis_acerifolia_Shi051
Clematis_tangutica_Tibet57
Clematis_tibetana_Tibet917
Clematis_tibetana_brevipes_Manaslu08_20815142
Clematis_tibetana_Tibet396
Clematis_tangutica_Miikeda
Clematis_akebioides_Xie2004025
Clematis_sp_nov_GAK_8802
Clematis_graveolens_BRD_A216
Clematis_graveolens_BRD_A220
Clematis_orientalis_Li14
Clematis_tibetana_brevipes_BRD_A219
Clematis_orientalis_Li13
Clematis_orientalis_Miikeda
Clematis_ladakiana_Chadwell_81
Clematis_pogonandra_Xie2004024
Clematis_nobilis_Miikeda
Clematis_ochotensis_Miikeda
Clematis_alpina_Horandl9517
Clematis_alpina_Wen10381
Clematis_sibirica_Xie2004XL029
Clematis_alpina_Schof_sn
Clematis_serratifolia_Miikeda
Clematis_rutoides_Wen9548
Clematis_rutoides_Wen9553
Clematis_cf_insidiosa_Wen9513
Clematis_rutoides_Wen9489
Clematis_eichleri_Miikeda
Clematis_loureiroana_Tibet2157
Naravelia_laurifolia_Wen10182
Naravelia_laurifolia_Miikeda
Anemoclema_glaucifolium_QEYang_2649
Anemoclema_glaucifolium_QEYang_3104
Pulsatilla_dahurica_Xie2006025
Pulsatilla_chinensis_Xie2006024
Pulsatilla_turczaninovii_Xie2006027
Pulsatilla_sp_Xie_2007001
Anemone_cernua_koreana_Han_Lee062583
Knowltonia_sp_OM073
Anemone_rivularis_Tibet761
Ranunculus_potaninii
0.17
0.43
0.64
0.11
1.88
0.67
2.54
0.29
4.07
1.52
11.2
7.61
1.18
2.75
1.57
4.52
5.87
0.45
1.41
9.1
2.06
0.11
2.65
0.51
1.45
4.48
9.1
2.13
6.21
0.52
1.01
0.29
0.54
0.93
13.27
10.39
1.69
0.88
2.84
4.01
0.46
4.66
2.51
5.88
0.25
10.63
0.9
6.42
1.14
19.97
1.7
0.29
2.41
3.81
5.8
0.38
1.54
0.37
7.16
0.73
4.11
1.3
38.29
0.47
75.65
0.98
2.65
4.08
12.52
2.15
25.66
8.0

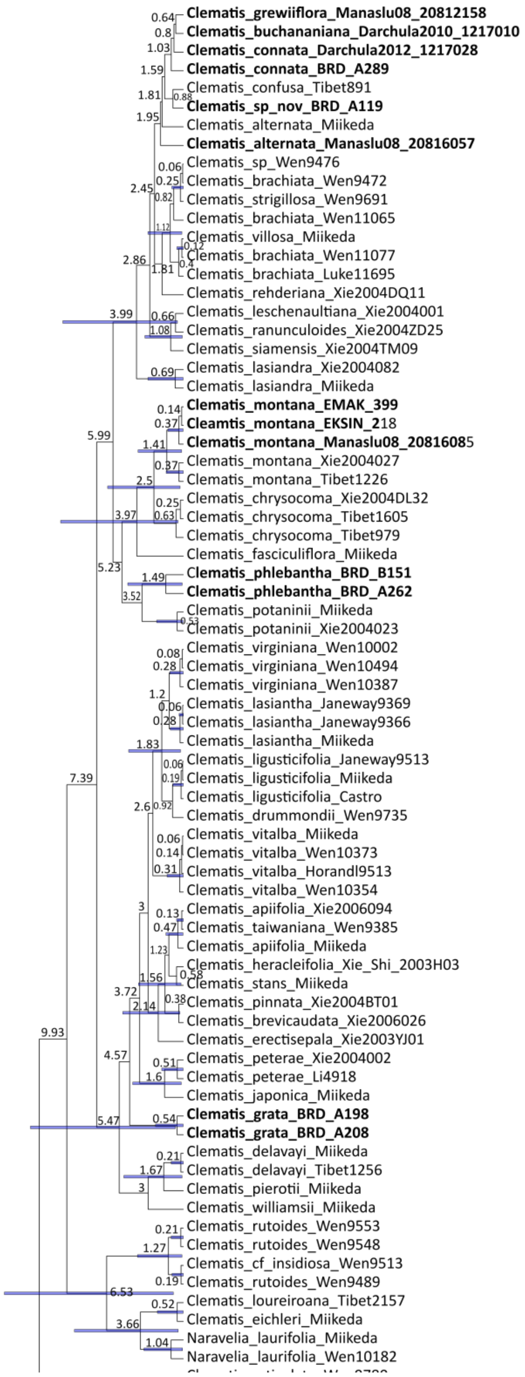
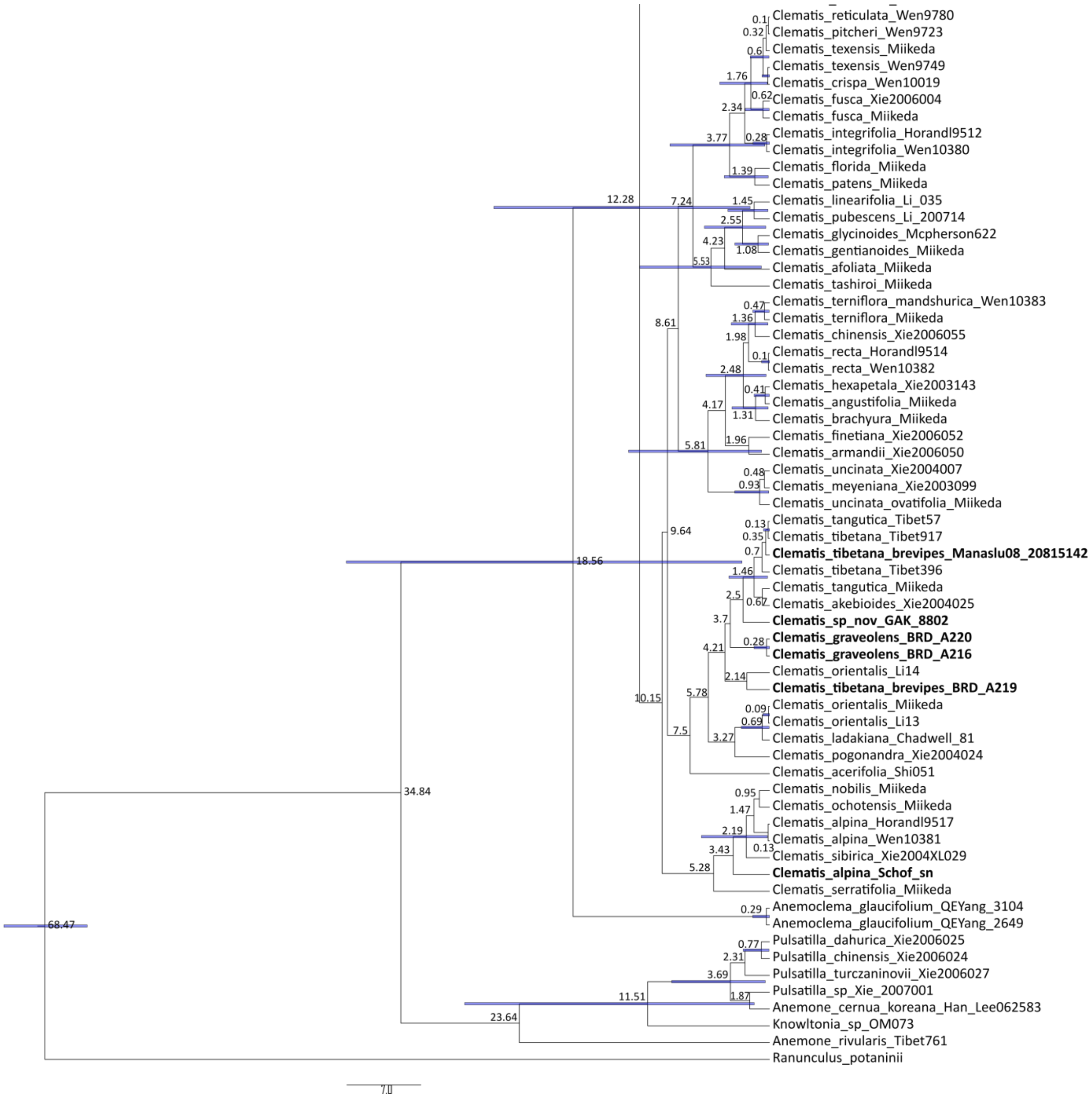


Figure 4.5 BEAST generated Maximum Clade Credibility tree of the Clematis dataset constrained with the crown node based on the results of the Magallón constrained analysis of the *Anemoneae* dataset, chapter 3 figure 3.5. Numbers at nodes are divergence age estimates (millions of years), bars at nodes are HPD 95% confidence intervals. Terminal taxa in bold indicate the new accessions in this study.


0.1
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0.32
Clematis_pitcheri_Wen9723
0.6
Clematis_texensis_Miikeda
Clematis_texensis_Wen9749
1.76
Clematis_crispa_Wen10019
0.62
Clematis_fusca_Xie2006004
2.34
Clematis_fusca_Miikeda
3.77
0.28
Clematis_integrifolia_Horandl9512
Clematis_integrifolia_Wen10380
1.39
Clematis_florida_Miikeda
Clematis_patens_Miikeda
12.28
7.24
1.45
Clematis_linearifolia_Li_035
Clematis_pubescens_Li_200714
2.55
Clematis_glycinoides_Mcpherson622
4.23
1.08
Clematis_gentianoides_Miikeda
5.53
Clematis_afoliata_Miikeda
Clematis_tashiroi_Miikeda
0.47
Clematis_terniflora_mandshurica_Wen10383
8.61
1.36
Clematis_terniflora_Miikeda
1.98
Clematis_chinensis_Xie2006055
0.1
Clematis_recta_Horandl9514
2.48
Clematis_recta_Wen10382
0.41
Clematis_hexapetala_Xie2003143
4.17
Clematis_angustifolia_Miikeda
1.31
Clematis_brachyura_Miikeda
1.96
Clematis_finetiana_Xie2006052
5.81
Clematis_armandii_Xie2006050
0.48
Clematis_uncinata_Xie2004007
0.93
Clematis_meyeniana_Xie2003099
Clematis_uncinata_ovatifolia_Miikeda
0.13
Clematis_tangutica_Tibet57
9.64
0.35
Clematis_tibetana_Tibet917
0.7
18.56
Clematis_tibetana_brevipes_Manaslu08_20815142
Clematis_tibetana_Tibet396
1.46
Clematis_tangutica_Miikeda
2.5
0.67
Clematis_akebioides_Xie2004025
3.7
Clematis_sp_nov_GAK_8802
0.28
Clematis_graveolens_BRD_A220
4.21
Clematis_graveolens_BRD_A216
Clematis_orientalis_Li14
2.14
Clematis_tibetana_brevipes_BRD_A219
10.15
5.78
0.09
Clematis_orientalis_Miikeda
0.69
Clematis_orientalis_Li13
7.5
3.27
Clematis_ladakiana_Chadwell_81
Clematis_pogonandra_Xie2004024
Clematis_acerifolia_Shi051
34.84
0.95
Clematis_nobilis_Miikeda
1.47
Clematis_ochotensis_Miikeda
Clematis_alpina_Horandl9517
2.19
Clematis_alpina_Wen10381
3.43
0.13
Clematis_sibirica_Xie2004XL029
5.28
Clematis_alpina_Schof_sn
Clematis_serratifolia_Miikeda
0.29
Anemoclema_glaucifolium_QEYang_3104
68.47
Anemoclema_glaucifolium_QEYang_2649
0.77
Pulsatilla_dahurica_Xie2006025
2.31
Pulsatilla_chinensis_Xie2006024
3.69
Pulsatilla_turczaninovii_Xie2006027
Pulsatilla_sp_Xie_2007001
11.51
1.87
Anemone_cernua_koreana_Han_Lee062583
23.64
Knowltonia_sp_OM073
Anemone_rivularis_Tibet761
Ranunculus_potaninii
7.0

Phylogeographic RASP results

The analysis showed it is a confused picture but a general trend indicating that the Himalayan species (table 4.6) and clades originated in E Asia before migrating to the Himalaya, but not a general stepwise progression from E Asia to the E Himalaya to the W Himalaya for example. The origin of section *Connatae* was suggested to be most likely the E Himalaya or E Asia before diversifying in the E Himalaya. The origin and suubseqent diversification of Section *Cheirposis* subsection *Montanae* is most likely to be E Asia. The *C. phlebantha* clade's origin is likely to be E Asia or the W Himalaya. *Clematis grata* is likely to be E Asia before evolving in the West Himalaya. Finally, section *Meclaits* is equivocal.

Figure 4.6 shows the full estimated geographical range for all sampled Clematis. The lack of support for relationships and the wide distributions of some species that occur in large clades make it difficult to assess evolutionary ancestral ranges.

Table 4.7 Results from the S-DEC analysis based on the Magallón constrained analysis MCC tree generated from BEAST. Most likely ancestral areas, likelihood support and number of vicariance and dispersal events in that clade.

Key Nodes	Most Likely Stem (likelihood pp.) + events		Geographical Area	Most Likely Crown (likelihood pp.)		Geographical Area
Clematis	F (0.79)		E Asia	F (0.95)		E Asia
section *Connatae*	BF (0.4)	Vicariance (1)	E Himalaya and E Asia	B (0.69)	Dispersal (1)	E Himalaya
section *Cheiropsis* subsection *Montanae*	F (1.0)	Dispersal (2)	E Asia	F (1.0)	Dispersal (1)	E Asia
C. phlebantha clade	AF (0.54)	Dispersal (1) Vicariance (1)	W Himalaya and E Asia	A (0.80)	n/a	W Himalaya

C. grata clade	F (0.60)	Dispersal (2) Vicariance (1)	E Asia	A (0.94)	n/a	W Himalaya
section *Meclatis*	Equivocal (1.0)	Dispersal (6) Vicariance (4)	?	Equivocal (1.0)	Dispersal (4) Vicariance (3)	?

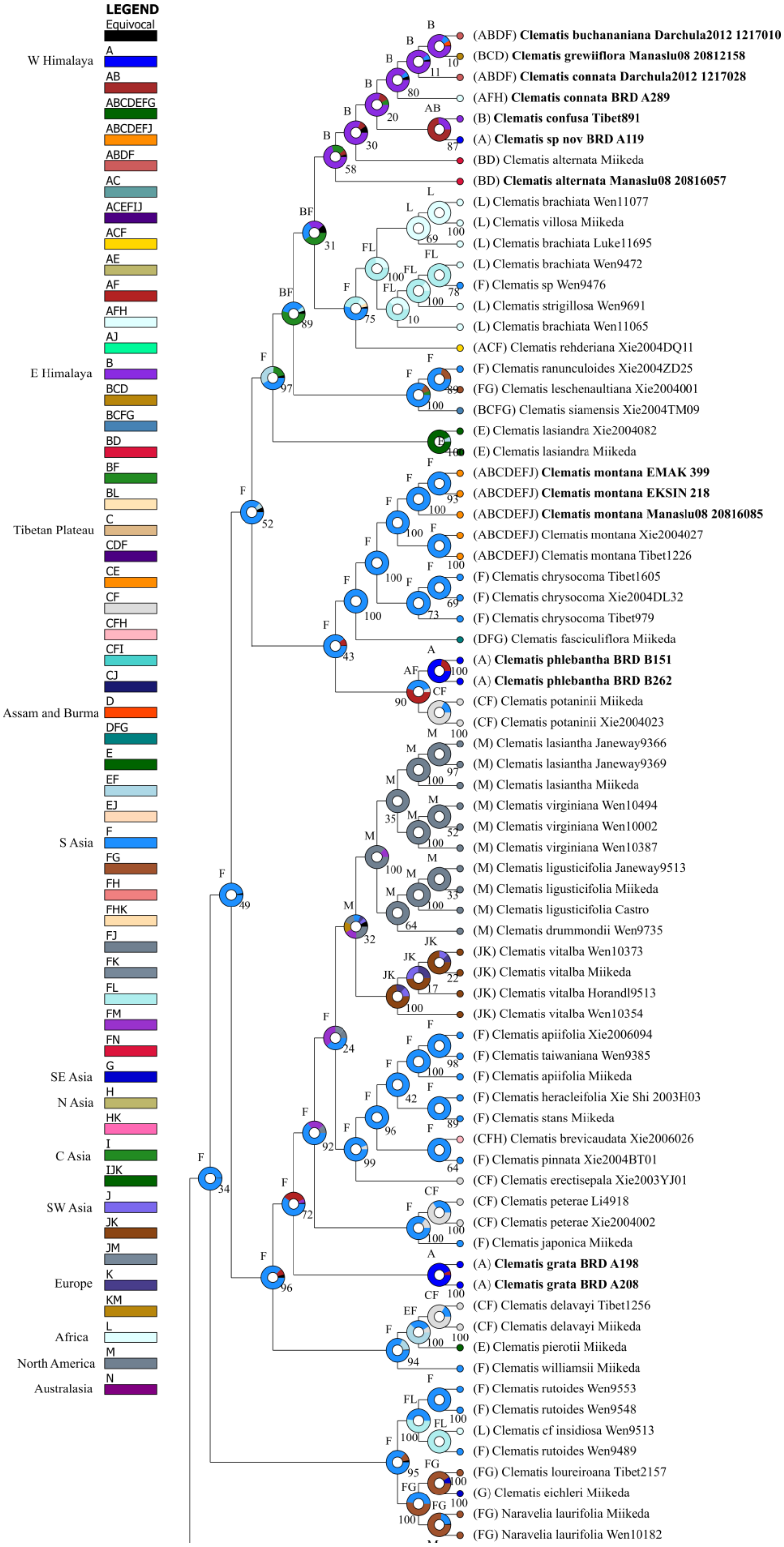
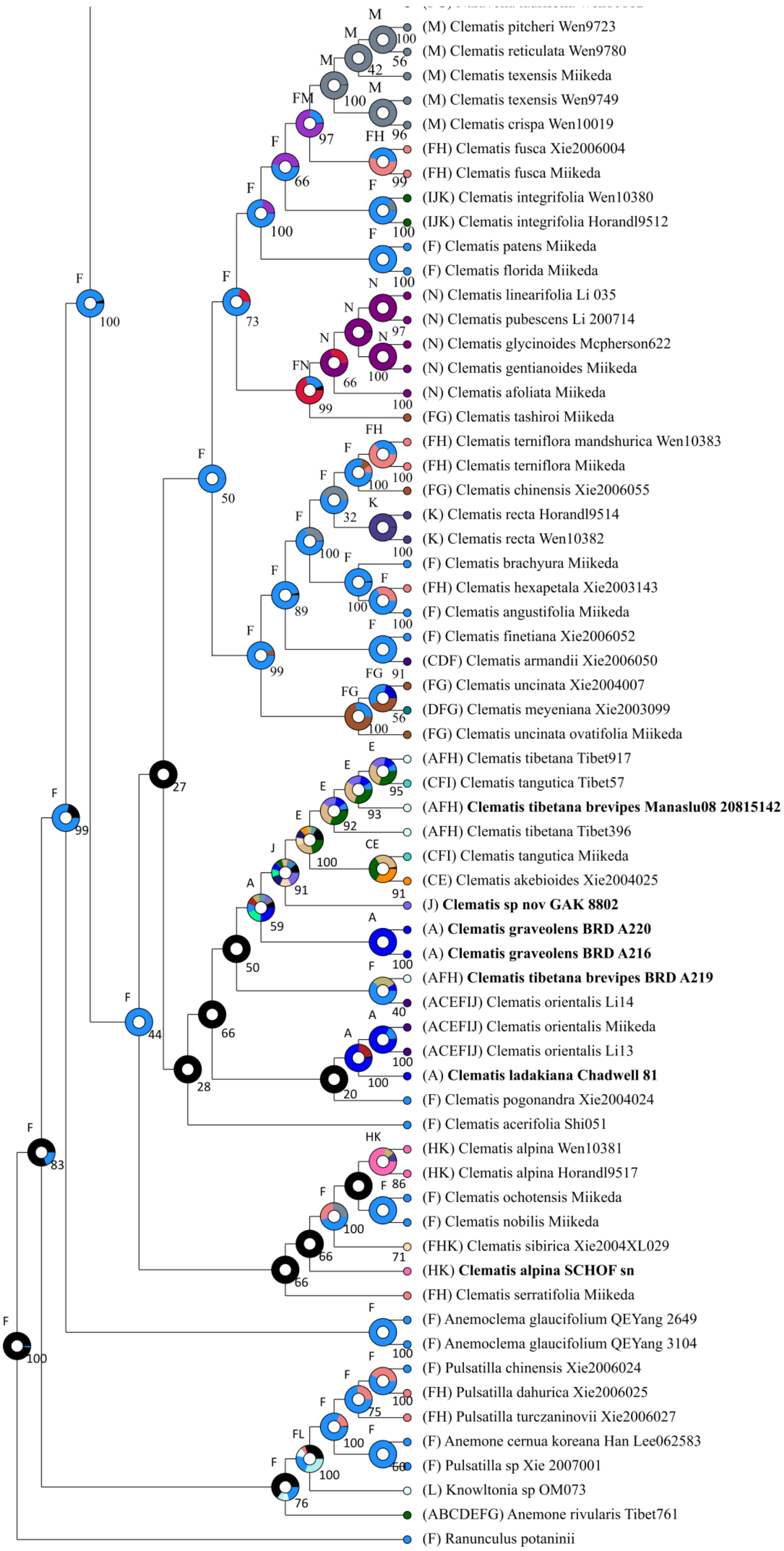


Figure 4.6 The most likely ancestral geographical ranges generated in S-DEC analysis. Results had the default restriction of reporting only composite results that contained the two most likely areas. Letters and node are most likely area, the legend reports all the possible states including those with more than two regions, pie charts show alternative states, numbers are bootstrap supports. Accessions in bold are the new data generated in this study. Table 4.4 has the full list of countries that are in each region.


(M) Clematis pitcheri Wen9723
(M) Clematis reticulata Wen9780
(M) Clematis texensis Miikeda
(M) Clematis texensis Wen9749
(M) Clematis crispa Wen10019
(FH) Clematis fusca Xie2006004
(FH) Clematis fusca Miikeda
(IJK) Clematis integrifolia Wen10380
(IJK) Clematis integrifolia Horandl9512
(F) Clematis patens Miikeda
(F) Clematis florida Miikeda
(N) Clematis linearifolia Li 035
(N) Clematis pubescens Li 200714
(N) Clematis glycinoides Mcpherson622
(N) Clematis gentianoides Miikeda
(N) Clematis afoliata Miikeda
(FG) Clematis tashiroi Miikeda
(FH) Clematis terniflora mandshurica Wen10383
(FH) Clematis terniflora Miikeda
(FG) Clematis chinensis Xie2006055
(K) Clematis recta Horandl9514
(K) Clematis recta Wen10382
(F) Clematis brachyura Miikeda
(FH) Clematis hexapetala Xie2003143
(F) Clematis angustifolia Miikeda
(F) Clematis finetiana Xie2006052
(CDF) Clematis armandii Xie2006050
(FG) Clematis uncinata Xie2004007
(DFG) Clematis meyeniana Xie2003099
(FG) Clematis uncinata ovatifolia Miikeda
(AFH) Clematis tibetana Tibet917
(CFI) Clematis tangutica Tibet57
(AFH) **Clematis tibetana brevipes Manaslu08 20815142**
(AFH) Clematis tibetana Tibet396
(CFI) Clematis tangutica Miikeda
(CE) Clematis akebioides Xie2004025
(J) **Clematis sp nov GAK 8802**
(A) **Clematis graveolens BRD A220**
(A) **Clematis graveolens BRD A216**
(AFH) **Clematis tibetana brevipes BRD A219**
(ACEFIJ) Clematis orientalis Li14
(ACEFIJ) Clematis orientalis Miikeda
(ACEFIJ) Clematis orientalis Li13
(A) **Clematis ladakiana Chadwell 81**
(F) Clematis pogonandra Xie2004024
(F) Clematis acerifolia Shi051
(HK) Clematis alpina Wen10381
(HK) Clematis alpina Horandl9517
(F) Clematis ochotensis Miikeda
(F) Clematis nobilis Miikeda
(FHK) Clematis sibirica Xie2004XL029
(HK) **Clematis alpina SCHOF sn**
(FH) Clematis serratifolia Miikeda
(F) Anemoclema glaucifolium QEYang 2649
(F) Anemoclema glaucifolium QEYang 3104
(F) Pulsatilla chinensis Xie2006024
(FH) Pulsatilla dahurica Xie2006025
(FH) Pulsatilla turczaninovii Xie2006027
(F) Anemone cernua koreana Han Lee062583
(F) Pulsatilla sp Xie 2007001
(L) Knowltonia sp OM073
(ABCDEFG) Anemone rivularis Tibet761
(F) Ranunculus potaninii

Species Distribution Model (SDM) results

All five SDMs performed well, with training and test data approaching an AUC of 1.0 in all cases (table 4.7). The Area Under Curve (AUC) results from the MaxEnt species distribution models is a measure of how well the models performed (Phillips et al. 2007). The AUC is generated by comparing Sensitivity, how well the model can correctly predict the presence of, in this case, a species, to Specificity, how well it can predict when there should not be a presence of, in this case, a species. So if the AUC results are close to of 0.5 then the models are no better than a random prediction. As AUC approaches 1.0 then they are considered to have performed well. The training data are the 75% of presence points that build the model and the test data are the 25% that are set aside to test the training data.

The distribution estimates are visualised and presented in figures 4.7 to 4.11

Table 4.7 The Area Under Curve (AUC) results generated by the SDM generated by MaxEnt.

Species Name	Training Data (AUC)	Test Data (AUC)	Figure
Clematis graveolens	0.99	0.981	4.7
C. tibetana subsp. *brevipes*	0.999	0.999	4.8
C. tibetana subsp. *vernayi*	0.998	0.998	4.9
C. grata	0.986	0.982	4.10
C. phlebantha	0.99	1.0	4.11

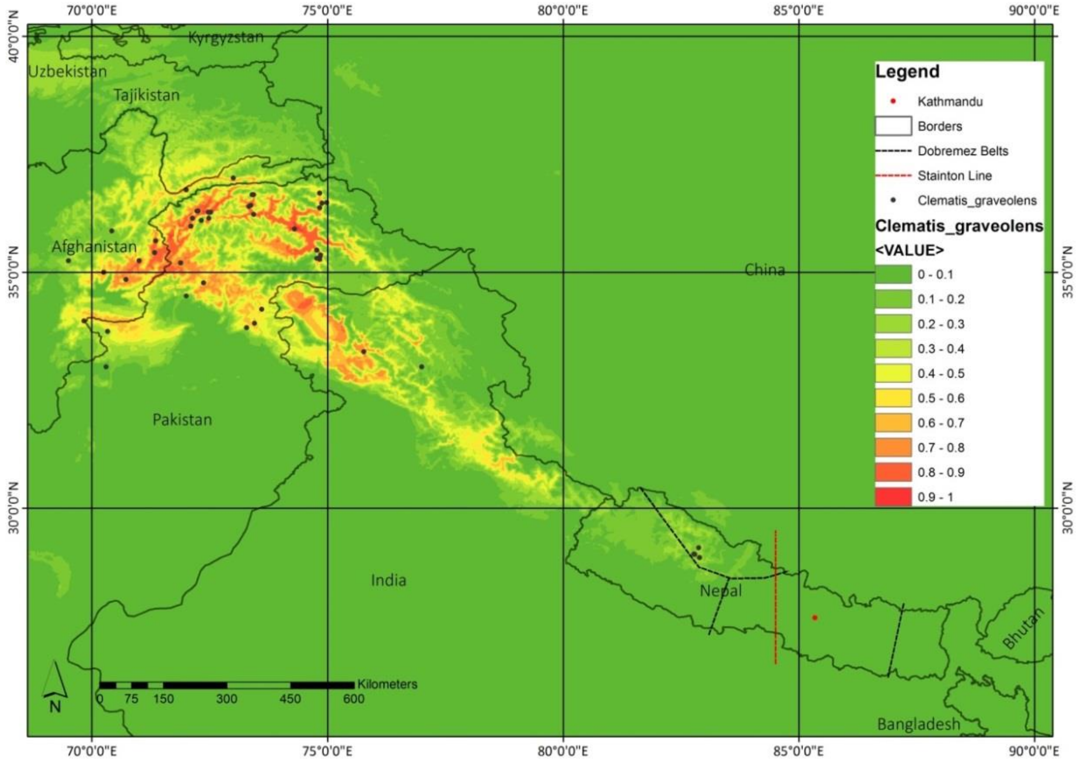


Figure 4.7 ***Clematis graveolens*** **species distribution model generated in MaxEnt using georeferenced herbarium specimens at E and GBIF data, black dots, using the standard 19 bioclimatic variables and altiude.**

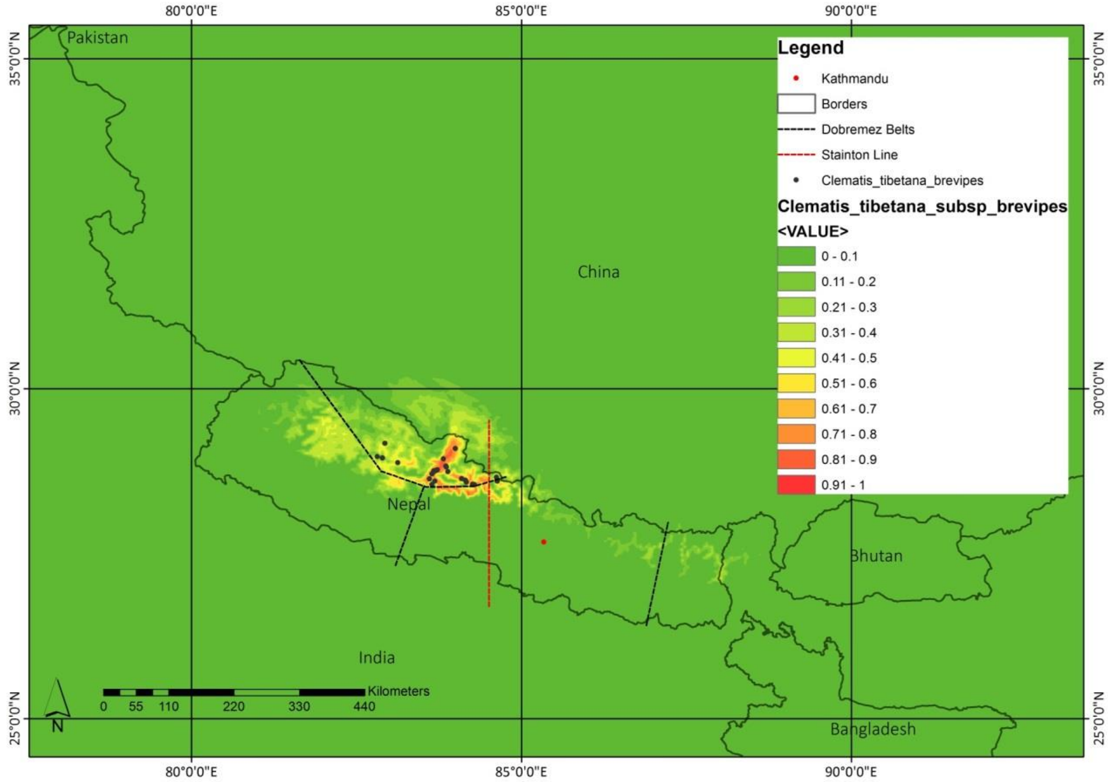


Figure 4.8 ***Clematis tibetana*** **subspecies** ***brevipes*** **species distribution model generates in MaxEnt using georeferenced herbarium specimens at E and GBIF data, black dots, using the standard 19 bioclimatic variables and altitude.**

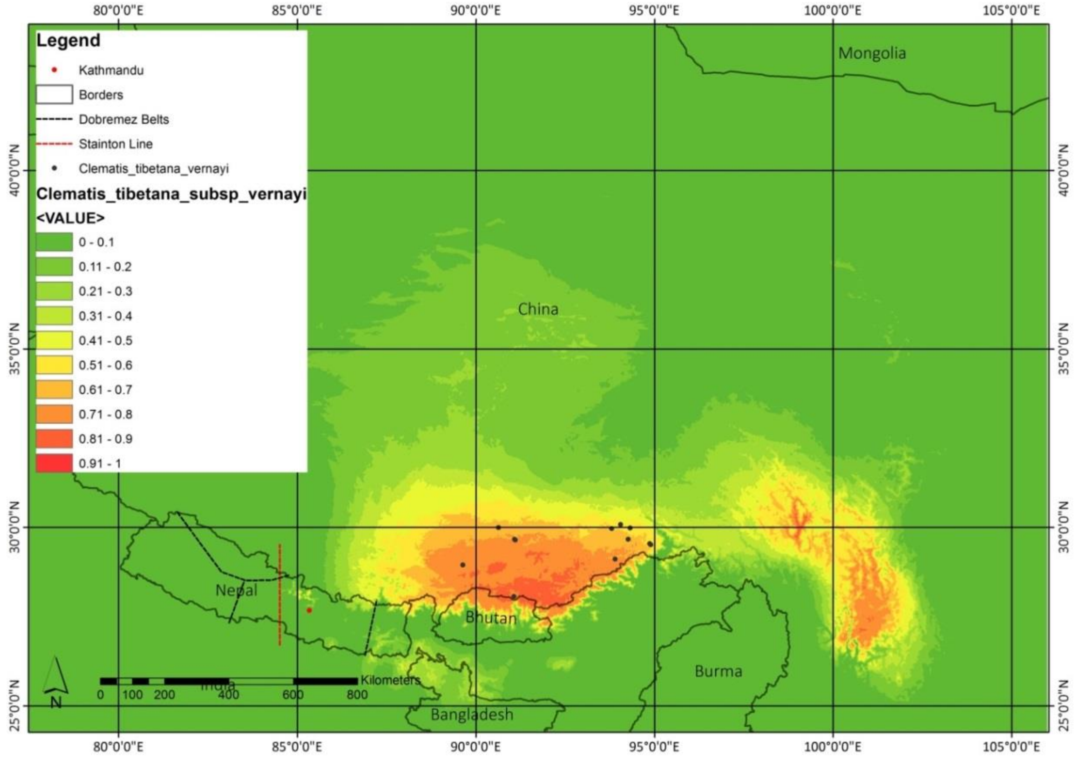


Figure 4.9 ***Clematis tibetana*** **subspecies** ***vernayi*** **species distribution model generated in MaxEnt using georeferenced herbarium specimens at E and GBIF data, black dots, using the standard 19 bioclimatic variables and altitude.**

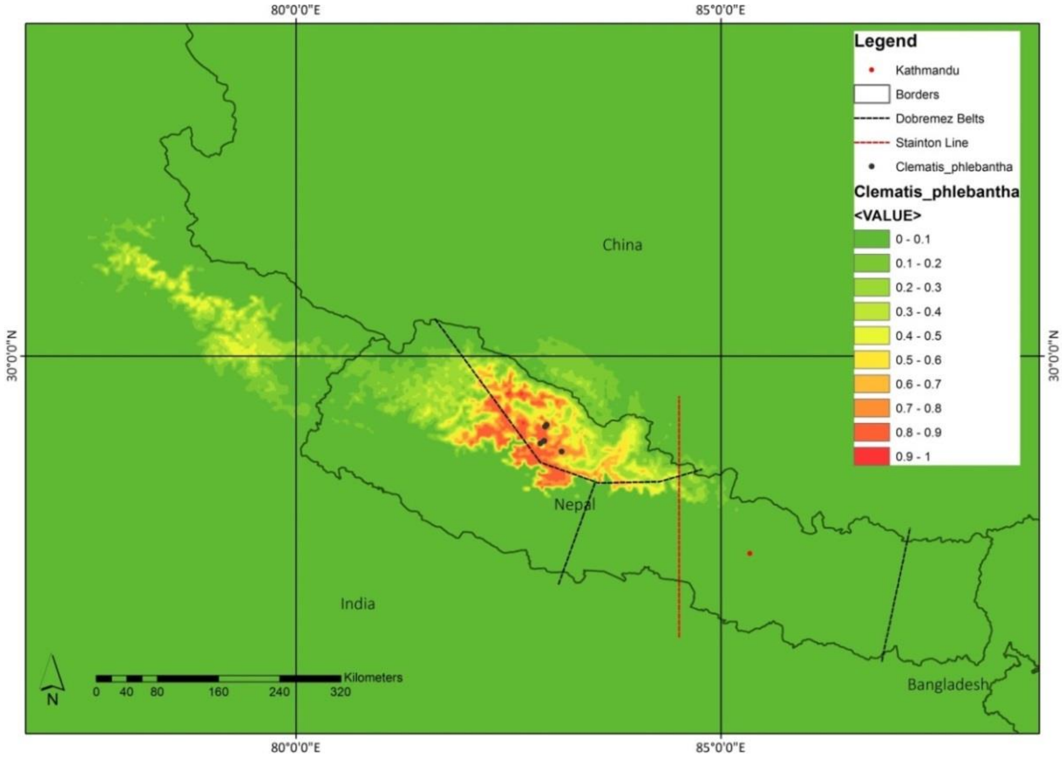


Figure 4.10 Clematis phlebantha species distribution model generated in MaxEnt using georeferenced herbarium specimens at E and GBIF data, black dots, using the standard 19 bioclimatic variables and altitude.

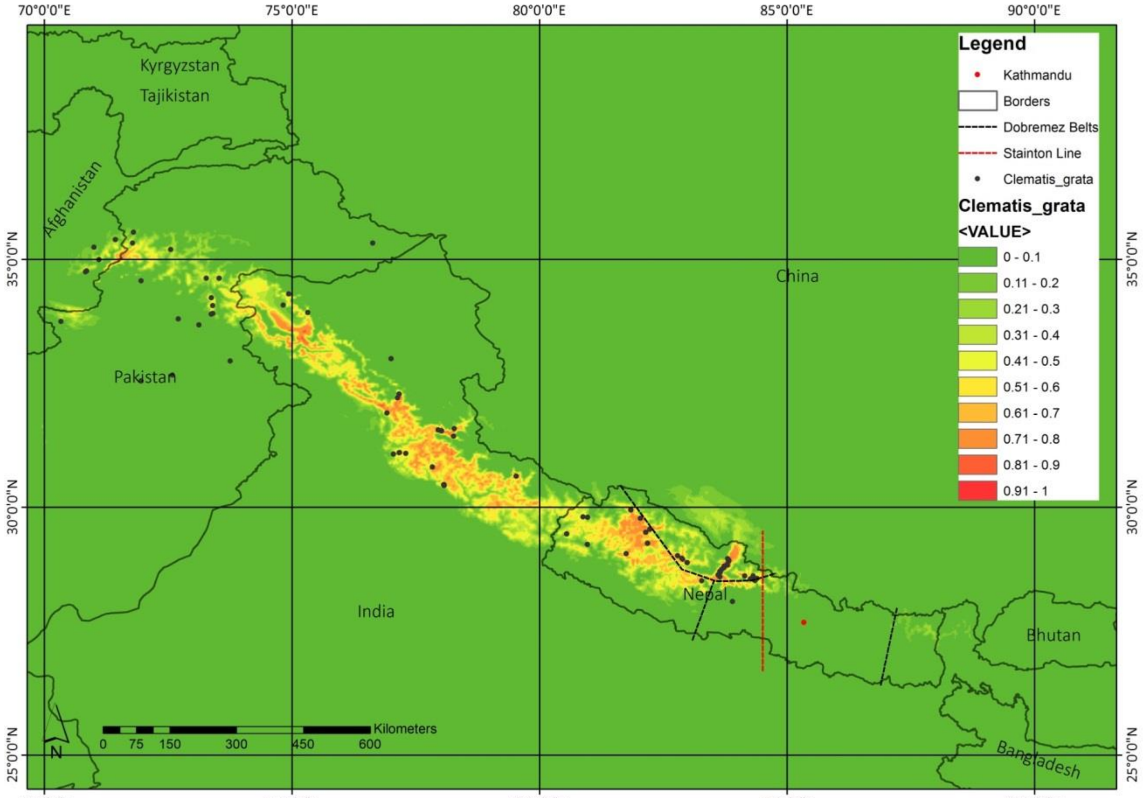


Figure 4.11 ***Clematis grata*** **species distribution model generated in MaxEnt using georeferenced herbarium specimens at E and GBIF data, black dots, using the standard 19 bioclimatic variables and altitude.**

Discussion

Although the terminal clades in the nDNA and cpDNA phylogenies are generally congruent there is very little support along the backbone for deeper relationships. As Xie *et al*. (2011) noted there are severe limitations in the infrageneric taxonomies of Tamura (1993) and Wang & Li (2005). This study also found that the taxonomies of Grey-Wilson (2000) and Johnson (2001) are also inconsistent with the phylogenetic relationships of the sampled species.

The relationships of the newly sampled Nepalese species ranged from well supported to poorly resolved or completely unresolved within the phylogeny. The results using the secondary calibration point based on the reassessed molecular dating analyses suggested the evolutionary events that have led to well-supported clades in *Clematis* are older than had been reported by Xie *et al*. (2011), but still originated in the Miocene. The Nepalese taxa do not form a single clade in any of the phylogenies which suggests that the Himalayan taxa had multiple distinct origins, and this is in part supported by the results of the ancestral range reconstruction.

The putative *Clematis* leaf fossil which was discussed in Chapter 3 was not used in any analyses because it was not examined in this study and furthermore Pigg & Devore (2005) did not believe it could definitively be assigned to an extant species group. However, its age is generally consistent with the dates that were generated independently through the molecular dating.

Congruence

The trees are largely congruent. Lack of resolution in the backbone of the cpDNA tree gives the impression of incongruence, but there is general congruence between terminal taxa. Incongruent accessions could have been excluded from the combined analysis but this would have reduced the dataset further. Increasing the dataset to include a second chloroplast molecular marker was to increase the resolution and statistical support for relationships in *Clematis* that were lacking in the *Anemoneae* dataset. This was the same approach as Xie *et al.* and it made the dataset as comparable as possible, especially as the *psb*A-*trn*H region used by Xie failed to work in this study. This lack of resolution and low statistical support echo the findings of both Miikeda *et al*. (2006) and Xie *et al*. (2011).

The incongruence seen between accessions of *Clematis tibetana* subsp. *brevipies* is a result of differing chloroplast sequence between the accessions. One appears to have sections of sequence more closely related to *C. orientalis* types and the other to *C. tibetana* - *C. tanguitica* cpDNA types, and this is likely to be an example of chloroplast capture as this is an interfertile closely related group of species with overlapping native ranges.

Some of the unexpected relationships in the results of the cpDNA analysis relate to missing data. The placement of *C. buchananiana* Darchula2012 1217010 and *C. phlebantha* BRD A151, both of which lack *atp*B-*rbc*L data, are placed in a small clade together with *Clematis* sp. nov. BRD A119. *Clematis alternata* Manaslu08 20816057 and *C. ladakiana* Chadwell 81 both lack *rpo*B-*trn*C sequence data and are placed on a polytomy with *C. alpina* and *C. tibetana* subsp. *brevipes* BRD A219.

The genus *Clematis*

There are clear differences between the evolutionary relationships found in this study and the classifications of Grey-Wilson (2000) and Johnson (2001). This echoes the findings of Xie *et al*. (2011) who analysed the taxonomies of Tamura (1993) and Wang & Li (2005) and also found little agreement between them and the phylogeny. However, given the poor resolution and low support in the backbones of the phylogenies there is little point re-aligning the taxonomy until a fuller and more robust picture emerges.

The divergence dates of deeper nodes in both the Anderson and Magallón *Clematis* dataset analyses are considerably more recent than they are in the *Anemoneae* datasets (table 4.6). In the strongly supported section *Cheiropsis* subsection *Montanae* clade the nodes closer to the terminal taxa have a mix of younger and older ages; however they are similar to the ages of equivalent nodes in the *Anemoneae* dataset. Section *Meclatis*, which is poorly supported, also has similar divergence ages at equivalent nodes.

Xie *et al*. (2011) suggested that extant *Clematis* began diversifying during the Neogene, in the middle Miocene to Pliocene. In this current study, despite the crown of *Clematis* being double the age of that reported by Xie *et al.* across all analyses, it still suggests that extant *Clematis* began diversifying in the Miocene. However, Xie *et al*. stated that all their well-supported clades began diversifying in the Quaternary (Pleistocene/Holocene). In this study, with increased sampling and the doubling of the crown age, it is evident that some of the clades began diversifying earlier, in the late Miocene continuing to the Holocene.

Xie *et al*. proposed that the most likely ancestral area for *Clematis* was China, and the S-DEC analysis also suggests that the stem and the crown nodes are most likely derived from E Asia. Although the stem node is poorly supported (0.79 pp.) the crown node is strongly supported (0.95 pp.) and the combination of the strong support for both of these nodes in the combined nDNA and cpDNA corroborate Xie *et al*.'s (2011) hypothesis.

With the backbone of the phylogeny so poorly resolved it is difficult to assess any but the most basic relationships within the genus. The placement of lineages on the MCC (maximum clade credibility) trees should be treated with caution as the backbone lacks statistical support. At this moment the molecular regions used in Sangar sequencing do not give enough variation to form well supported species relationships in the genus *Clematis*.

Section ***Cheiropsis*** subsection ***Montanae***

The sampling of this subsection was increased and this study included three accessions of *C. montana* from the Nepal Himalaya. The two of the three species from Johnson's section *Cheiropsis* subsection *Montanae*, *C. montana* and *C. chrysocoma*, have strongly supported relationships at all the nodes in the clade of the combined analysis, while *C. acerifolia* is in an unresolved position at the base of the tree. The poorly supported sister lineage to the subsection *Montanae* clade is *C. fasciculiflora*. This species was placed by Johnson in section *Flammula* but Grey-Wilson put it into subgenus *Cheiropsis* along with *C. montana* and *C. chrysocoma*, a clear example of conflict between these taxonomies and the phylogeny.

While all three species have flowers that emerge from fascicles on axillary shortshoots, the most obvious difference among them is the presence of persistent bud scales in *C. montana* and *C. chrysocoma*. With the poor support between the subsection *Montanae* clade and the *C. fasciculiflora* lineage it would be sensible to investigate these species further to rule out a misidentification of Miikeda's *C. fasicuflora* accession before altering the taxonomy.

The origin of the well-supported section *Chieropsis* subsection *Montanae* clade was in the late Miocene, with the date of divergence between *C. chrysocoma* and *C. montana* in the Pliocene. The subsequent split between the Tibetan and Himalayan *C. montana* dates to the Pleistocene. The topology of the clade suggests that *C. montana* is a derived lineage which has moved westwards along the Himalaya from China. It also suggests that *C. montana* has colonised the Himalaya rather than evolved there.

The most likely ancestral area of section *Cheiropsis* subsection *Montanae* is E Asia, and the topology of this clade is well supported in all analyses. While the S-DEC analysis suggests that the most likely area for all nodes in the clade is E Asia it does appear there has been a westward migration of lineages. The large geographical area where *C. montana* is found in comparison to the other lineages in the clade also suggests that C. *montana* has used the Himalaya as an effective means to disperse over a large area. *Clematis montana* also has the greatest altitudinal range of any *Clematis* species, suggesting that it has had the ability to take advantage of the vast range of habitats which have been produced during the ongoing Himalayan orogeny.

Section Meclatis

The expanded sampling of this clade to include Himalayan representation by both *Clematis graveolens* and *Clematis tibetana* subsp. *brevipes*, both of which are west Himalayan elements (Figures 4.7 and 4.8 respectively), both resolved several questions and raised others.

Wang (2004) argued that *C. graveolens* was misplaced in section *Meclatis*, noting the similarity of its floral morphology to that of *C. brachiata* in section *Brachiatae*. Wang argued that Grey-Wilson was wrong to place *C. brachiata* in section *Meclatis* and Johnson was wrong to place it in a section *Africanae*. Wang's reassessment that section *Brachiatae* is distinct from section *Meclatis* is supported by Xie *et al*.'s (2011) placement of the accessions of each section in different clades. The current study supports the phylogenetic distinction of section *Brachiatae* and section *Meclatis,* with *C. graveolens* included in section Meclatis. The two accessions of *C. graveolens*, the first of this species to be analysed phylogenetically, have at best ambiguous relationships. The Bayesian analysis of the *Clematis* dataset places them in a poorly supported clade with other *Meclatis* accessions, while the Bayesian analysis of the *Anemoneae* dataset places *C. graveolens* in a strongly supported *Meclatis* clade. Wang's repositioning in of C. *graveolens* in Section *Brachiatae* is therefore rejected.

The west Nepalese endemic *Clematis tibetana* subsp. *brevipes* is also in Section *Meclatis*. The two accessions of *C. tibetana* subsp. *brevipes* are not consistently resolved as sister lineages, as would be expected if these were indeed the same taxon. In the nDNA phylogeny the accessions are sister and are placed with accessions of *C. tangutica* and *C. tibetana*, in the combined and cpDNA phylogenies they are placed in the two separate *Meclatis* clades. This subspecies shows morphological variation and Grey-Wilson (1998) described several varieties in his revision of *C. orientalis* and its allies, under *C. tibetana* subspecies *vernayi* (Figure 4.9, SDM used as support for delimitation of subspecies in Chapter 2), to cover the diversity in Nepal. The placement of this subspecies across two clades, and the previous fractured taxonomic treatments by Grey-Wilson may reflect a hybrid origin for the species, as evidenced by possible chloroplast capture.

The *Meclatis* clade dates to the late Miocene. The poorly supported nodes that correspond to the crown of the *Meclatis* clade in the *Clematis* dataset date to the Late Miocene in both the Anderson and Magallón constrained datasets. In the *Anemoneae* dataset analyses the well-supported sub-clade, containing *C. graveolens*, one *C. orientalis* accession and the new species from Oman, dates to the Pleistocene / Pliocene boundary.

According to the S-DEC analysis, figure 4.6 the origin of section *Meclatis* is equivocal as a result of the levels of dispersal and vicariance events which are greater than in any of the other clades. Relationships in section *Meclatis sensu* Johnson (2001) and Grey-Wilson (2000) are generally poorly supported in all analyses. Coupled with the wide distributional range over much of Asia this has led to this equivocal result in the analysis, but suggests the group is and has been mobile, and potentially fast to diversify. Species from section *Meclatis* are found in semi-arid habitats and environments from Arabia to the Eastern Mediterranean and into the semi-arid central Asian interior. The late Miocene dates of diversification for these species suggest that they have evolved to take advantage of the increased aridification linked to the Tethys sea shrinkage seen in the northern Hemisphere at this time Zhang *et al*. (2014).

Clematis phlebantha

The phylogenetic placement of the Nepalese endemic species *Clematis phlebantha* was tested for the first time in this study. This species is restricted to a small area of Dolpa and Jumla districts in NW Nepal, in the dry trans-Himalaya region to the north of the main Himalayan ranges (figure 4.10). It is morphologically distinct from all other Nepalese *Clematis* with its sub-shrub habit. Its taxonomic position has varied across classifications. It was originally placed in section *Fruiticella* subsection *Rectae* by L.J.H. Williams in its protologue (Williams, 1956) and considered to be closely related to *Clematis delavayi* and *Clematis limprichtii*, which is currently considered to be a variety of *C. delavayi* (Grey-Wilson, 2000; Johnson, 2001; Wang & Bartholomew, 2001). It was placed in the monotypic subsection *Phlebanthae* of section *Fruiticella* by Grey-Wilson (2000) and Johnson (2001).

The position of *C. phlebantha* remains unresolved in the cpDNA and combined region Bayesian analyses. In the nDNA Bayesian analysis and the BEAST analyses it is a moderate to strongly supported basal lineage to a clade containing sections *Clematis, Fruticella, Bebaeanthera* and *Tubulosa*. In the BEAST trees *C. phlebantha* is sister to *C. potaninii* (section *Clematis* subsection *Potaninianae)* but moderately supported (Anderson 0.9 pp. & Magallón 0.91 pp.) and both are derived from the basal lineage of the clade containing sections *Clematis, Fruticella, Bebaeanthera* and *Tubulosa*. Grey-Wilson (2000) noted that the flowers of *C. phlebantha* ‘reminded’ him of *C. potaninii* but ultimately placed the two in different subgenera.

From the *Anemoneae* dataset, the strongly supported divergence date estimate for the *C. phlebantha* - C. *potaninii* split is in the Pliocene with the confidence interval stretching between the late Miocene and Pleistocene. These analyses only used one accession of *C. phlebantha* as the other lacked *atp*B-*rbc*L data. In the *Clematis* datasets with both accessions the divergence estimates are be broadly equivalent, though with lower support, and date to the Pliocene.

Clematis phlebantha is found only in the dry trans-Himalayan region of Nepal and is morphologically adapted to its arid habitat by being a compact subshrub which is covered in dense white hairs, a likely adaptation to reflect sunlight and reduce evapotranspiration. The trans-Himalayan aridification is linked to the uplift of the Himalaya and Tibetan plateau blocking the passage of the monsoon. Guo *et al*. (2004) suggest that aridification of the interior of Asia, with the trans-Himalaya on the southern edge, occurred during the late Miocene and Pliocene. As *C. phlebatha* is adapted to this semi-arid habitat, and the timing of diversification is appropriate, it would suggest that *C. phlebantha* represents a lineage that became adapted to the semi-arid interior during the Miocene-Pliocene.

Clematis grata

This species is also phylogenetically tested for the first time in this study and while not a Nepalese endemic, it is an example of a West Himalayan element mentioned by Stainton (1972), that has its most easterly occurrence at (84°E) at the Annapurna massif, just to the east of the Kali Gandaki (figure 4.11).

Clematis grata appears to be basal in a clade containing members of section *Clematis, Lasiantha*, *Fruticella*, *Tubulosae*, and this clade is one of the most geographically widespread of the genus. This clade’s relationship to the rest of the genus is unknown or is at best ambiguous because of the poorly supported backbone to the clade. This group has as a wide distribution across Eurasia, from the Mediterranean to central Asia, the Himalaya, S and SE Asia and north to Japan and NE Asia. There are also members of this clade in subsections that occur in N America.

The well-supported crown date for this clade at the Pliocene-Miocene boundary suggests that the group has taken advantage of the change in global wind patterns in the late Miocene

(Quan *et al*. 2014; Tang *et al*. 2015) to disperse and evolve over much of the Northern Hemisphere. *Clematis grata*'s position as a basal lineage suggests that this clade has taken advantage of *Clematis*' dispersal ability to successfully colonise a large part of the northern Hemisphere. The last common ancestor of the west Himalayan *C. grata* and the relatively large sister clade containing species found in N America, Europe and much of E Asia suggests that the *C. grata* lineage dispersed into the West Himalaya.

Himalayan *Connatae* grade

The expansion of the sampling in section *Connatae* includes greater representation of Himalayan species in addition to the species from E Asia presented in the Xie *et al*. (2011) study. As a result, some of the findings from that study have been reappraised.

The current study added five accessions of section *Connatae*, comprising four Himalayan species and one additional accession of *Clematis alternata*. While relationships among them are unresolved, they do all emerge on an unresolved polytomy at the base of a poorly supported clade in the Bayesian analyses and as a poorly supported clade within a larger well supported clade in the BEAST analysis. In the Xie *et al*. study *Clematis alternata* is reported to be a monotypic lineage in section *Archiclematis*, following Wang & Li (2005), on a poorly-supported polytomy. The general consensus from the recent morphological studies (Grey-Wilson, 2000; Johnson, 2001; Wang & Barthholemew, 2006) is that *C. alternata* is an ancestral lineage to *Clematis*. Xie *et al*. (2011) showed it to be derived and nested within *Clematis*. Previously it had been treated as a separate genus, and more recently as a monotypic section, *Archiclematis*, based on its alternate leaves which distinguish it from the remainder of *Clematis*. However Johnson (2001) noted that members of section *Connatae* exhibit alternate leaf morphology as seedlings and juvenile plants. In combination with the molecular data, this suggests that section *Archiclematis* is superfluous and the species would be better placed in section *Connatae*, following Johnson (2001).

There is insufficient sampling in this widespread group to draw any conclusions based on phylogeography. *Clematis connata*, *C. buchananiana* and *C. grewiiflora* are all widespread in the Himalaya and the relationships between these species are poorly supported. This is reflected in the morphology where there appears to be a good deal of overlapping characters and inconsistency in identification as a result. This is reflected in the taxonomic treatment in chapter 2 in which the concepts for these three species are broad and as a result incorporate taxa that have been considered distinct in other treatments.

The age from the *Anemoneae* dataset, which only included three additional accessions from section *Connatae* and was strongly supported at the crown, is Pliocene (4.7 and 4.2 Ma). The two further section *Connatae* and one additional section *Archiclematis* accessions which were used in the *Clematis* dataset all had one of the two cpDNA regions missing, resulting in less support for the relationship of the clade.

The topology in section *Connatae* differs between datasets because of the missing data and different number of taxa present and therefore is difficult to assess what has happened historically. The only conclusion that can be drawn is that this section had its origin in the Late Miocene.

Appraisal of *Clematis* fossils

The analyses of the *Clematis* dataset indicate that the strongly supported European *C. vitalba* clade dates from the Pleistocene. This is far younger than the fossilised achene from the Pliocene plant beds of the Netherlands ascribed to *C. grata* by Reid & Reid (1915). The last shared ancestor of *C. grata* and *C. vitalba* dates to the Pliocene (circa 3 - 5 Ma) across the analyses, on moderate to poorly supported nodes. This date is broadly in-line with the age of fossil plant-beds where the fossil achenes were recovered. Reid & Reid's (1915) identification of the achenes as *C. grata* should be reappraised in the light of the current taxonomy. At time of identification, *C. grata* was much more broadly circumscribed to contain what are now considered to be distinct species from China, and reappraisal of the achenes in light of current taxonomy might indicate that these fossils could be used to constrain a node that corresponds to *Clematis* section *Clematis*.

The possible *Clematis* leaf fossil from British Colombia which dates to the Eocene is substantially older than either of the late Miocene or Pliocene splits between the two extant North American clades from their Asian sister species. This fossil could represent an extinct early *Clematis* lineage or might be misidentified. Reappraisal of this fossil is required as its existence is only listed in Pigg & Devore (2005) as a personal communication from W.Wehr of the Burke Museum of Natural History and Culture.

Conclusion

The current study clearly supports the monophyly of *Clematis* and there is good evidence from this and previous studies (Miikeda *et al*. 2006; Xie *et al*. 2011) to support a broad circumscription of the genus to include *Clematopsis*, *Archiclematis* and *Naravelia*. This study corroborates the criticisms of Miikeda *et al*. and Xie *et al*. that the current infrageneric taxonomy does not reflect evolutionary relationships. Without fuller sampling of *Clematis* throughout its distribution and better resolution of relationships at the base of the *Clematis* clade it would be premature to realign that taxonomy.

The evolutionary age of *Clematis* estimated using only a secondary calibration point for the analysis is younger than the results of the larger *Anemoneae* dataset, but it is still older than that reported by Xie *et al*. (2011).

The diversity in Nepal appears to be the result of at least six immigration events as all dates of these nodes postdate the uplift of the Himalaya, but this may well be an underestimate as not all the species of *Clematis* from Nepal could be included in the analysis. The ancestral area analyses for these six events suggest that dispersal was the likely origin and this is substantiated in the high dispersal ability of the genus, with its wind-dispersed achenes.

Section *Meclatis* has an equivocal origin and nodes of the species included in the phylogeny had multiple suggested ancestral areas which are almost equally likely. There also appears to be evidence of chloroplast capture between the two lineages of *C. tibetana* subsp. *brevipes* which suggests a still more complicated picture in the evolution of section *Meclatis*.

The Nepalese endemics, *C. phlebantha* and *C. tibetana* subsp. *brevipes*, showed a preference for semi-arid habitats, as observed in the field, inferred from climatic niche modelling and indicated by their morphological adaptations. Their evolutionary dates of origin also correspond broadly to the Late Miocene dates of Asian interior aridification hypothesised by Sun *et al*. (2010; 2015). All of this suggests that the adaptation to aridification has driven diversification in some Nepalese *Clematis* species.

The phylogeographic analysis carried out in this study suggests that E Asia was the ancestral origin of *Clematis* and this broadly agrees with Xie *et al*.'s hypothesis of China being the likely ancestral origin of the genus. This analysis does not support Xie *et al*.'s other hypotheses relating to the geographical delimitations of the clades containing accessions of section *Meclatis* or section *Cheiropsis* subsection *Montanae* as strongly Chinese. Xie characterised both of these as being Chinese clades but that was a result of their sampling. Both these groups contain species with distribution ranges well beyond China therefore the hypothesis to characterise these clades as Chinese has been rejected.

It should be noted that, through the increased molecular sampling, this study has filled in geographical gaps from Asia in the phylogeny, there is still no representation of the ca. 9 *Clematis* species from S America.

Chapter 5: Phylogeography and the Dynamics of Speciation in the Himalaya

Himalayan Orogeny and Speciation

Introduction

Investigations into the plant phylogeography of Himalaya are in their infancy. This is in marked contrast to the Andes where meta-analyses of more than fifty dated phylogenies have given important insights into the assemblage of the biota of this other region of megadiversity (Pennington *et al*. 2010; Hoorn *et al*. 2010). Richardson *et al*. (2012) proposed that collaboration between systematists, palaeobiologists, palaeoclimatologists and geologists with their relatively independent datasets and methodologies would lead to a better understanding the abiotic and biotic history of SE Asia. This proposal holds true for any region including the Himalaya where these histories are not well known. Dated molecular phylogenies, calibrated with fossils and the collaboration between different disciplines give us the opportunity to put these divergence and speciation events into the context of geological time and support or refute the dated events of the Himalayan orogeny proposed by other scientific disciplines

Geoscientific Histories of the Himalaya

Tectonics

The geological history of the Himalaya begins with the complex collision event that occurred between the Indian and Eurasian plates (Powell & Conaghan, 1973). The supercontinent Gondwana began to break apart 140 Ma, the African plate moved north and the Indian plate moved north-eastwards and closing much of the Tethys Sea that lay between them and the Eurasian plate (Searle *et al*. 1987). Until recently the consensus was that the collision between the Indian and Eurasian plates was at 70 - 45 Ma, beginning the initial uplift events that would become the Himalaya. Under this scenario, by 40 Ma the Indian plate had begun the process of subduction below the Eurasian plate (Harrison *et al*. 1992; Tapponnier *et al*. 2001; Avouac, 2003; Royden *et al*. 2008). The Tethys Sea closed completely 20-15 Ma as the continental shelf of the Indian plate pushed against the Eurasian plate, causing the granite crust to fold and thrust upwards and created fold mountains. It is estimated that 2500 km of crust became folded and compressed and 900 km of this crust eventually became subducted (Patriat & Achache, 1984; Robinson *et al*. 2006; Royden *et al*. 2008). Molnar & Stock (2009) report the rapid slowdown in the convergence rate of the Indian plate with the Eurasian plate of 109 mm per year c. 65 Ma to 34 mm per year since 11 Ma in the west and

118 mm per year c. 65 Ma to 44 mm per year since 11 Ma. A recent estimate, using GPS data, has the Indian plate continuing to move northward and subducting beneath the Eurasian plate at a rate of ca.20 mm a year (Wang & Xu 2003). These rates vary across the Himalaya, with the convergence between the India plate and South Tibet estimated as 17.8 (±0.5) mm per year in central and eastern Nepal and 20.5 (±1) mm per year in western Nepal (Ader *et al*. 2012).

This model of Himalayan orogeny was generally accepted until the early 2000s, when alternative hypotheses began to appear. Ali & Atchinson (2004) concluded from paleomagnetic data that the 55-50 Ma position of the Eurasian plate differed from the location that is was assumed to be under the accepted model and therefore the timing of the Indian-Eurasian collision could not be correct. Furthermore they argued that as there was no major uplifting of the Himalaya until 20 Ma there has to be an alternative hypothesis to explain the event detected at 55-50 Ma. They proposed that the collision event detected at 55-50 Ma was between the Indian plate and an intraoceanic island arc, and that the true Indo-Eurasian collision occurred at 30-20 Ma (Ali & Aitchison, 2004; Aitchison *et al*. 2007).

A similar hypothesis was proposed by van Hinsbergen *et al*. (2012), who suggested that there had been less folding and subduction at the initial collision than expected by the traditional model and suggested a collision between Eurasia and a Himalayan-Tibetan microcontinent at 50 Ma, followed by the ‘hard India-Eurasia collision’ at 25-20 Ma. With the competing hypotheses surrounding the timings of the collision these differing ages will likely have a subsequent effect on the estimated timings of more recent periods of uplift and the paleoclimatic events such as the onset of monsoon.

At present the most widely-accepted theory to explain the immense height of the Himalaya and Tibetan Plateau is known as channel flow (Tapponnier *et al*. 2001). According to this model the creation of fold mountains in the Himalaya caused the repeatedly folded rocks to succumb to stresses and fracture and as the Indian plate began to become subducted, a belt of molten granite formed underneath the Tibetan Plateau. The relatively light granite magma floated upwards, pushed against the bottom of the Plateau plate where it began to cool and solidify lifting the Tibetan Plateau higher (Beaumont *et al*. 2001; Godin *et al*. 2006). The typical thickness of oceanic crust is 5-10 km and that of continental crust 20-70 km (Pidwirny, 2013), with the uplift processes being used in part to explain the crust thickness of up to 80 km in the Himalaya (Jackson, 2002; Ernst, 2004).

Exhumation, the uncovering of previous surfaces through erosion or uplift, is believed to have started in the Himalaya around 23 Ma. It may have been initiated before, but the record has yet to be uncovered by off-shore drilling (Clift & Plumb, 2011). The literature on exhumation was reviewed by Adlakha *et al.* (2013) and is summarised here. The initiation or rapid increase of exhumation of the NW Himalaya dates from 23-19 Ma and 18-13 Ma in the NE Himalaya. This was then followed by a decline from 19-4 Ma in the NW and 13-2 Ma in the NE, with an increasing rate since 4 and 2 Ma in the NW and NE respectively. Clift & Plumb (2011) state that all current models suggest that exhumation requires erosion through precipitation. They then suggest that a ‘positive feedback’ is in operation. The exhumation through precipitation caused erosion which ‘focusses rock uplift’ leading to a higher Himalaya and greater influence on the monsoon. Clift & Plumb are vague on the mechanism but it is assumed that as rock is eroded and transported away this removes material and therefore mass from over geological faults allowing tectonic stresses to be overcome and ‘focusses rock uplift’ at these points.

Valdyia (2002) suggested that the strong tectonic movement that uplifted the Great Himalayan mountains had begun by the Late Miocene (11.5 to 5.1 Ma) and exposed them to erosion. Estimates for paleoaltitudes for the Himalaya, important to the movement of the monsoon (see below), are not common but Garzione *et al*. (2000) estimated that the southern edge of the Tibetan plateau and the north central portion of Nepal had reached elevations of between 4500 m and 6000 m by 11 Ma. This altitude coupled with increasing sedimentation accumulations in the Arun and Tinau river systems dating to 10 to 9 Ma suggests that the Himalaya had become an effective barrier to the passage of the monsoon (Valdyia, 2002). This caused monsoon precipitation to fall at the southern edge of the Tibetan plateau rather than further into the Asian interior, the evidence coming from the increased sedimentation of these Himalayan river systems.

Paleoclimate

The formation of the Tibetan plateau and Himalaya is fundamental to the regional, and indeed global, climate. Wind direction is a major factor in the intensity of the monsoons and the temperature of the Himalaya. During the summer warm westerlies are blown across the Indian Ocean, arriving at India from the southwest. The wind is blocked by the Higher Himalaya, and forms a vortex to the south, where monsoons form. The winds blow across India and loop back close to Burma resulting in the initiation of the monsoon in the east of the Himalaya.

An initiation date of 8 Ma has been suggested for the initiation of the South Asian Monsoon which has been linked to an uplifted Tibetan Plateau (Prell and Kutzbach, 1992). The resulting aridification and shift from C_3 forests to C_4 grasslands in Pakistan ca.7.5 Ma and Tibet (Quade *et al*. 1989) were all linked by Molnar *et al*. (1993). Recording the abundance of certain types of plankton in upwelling of ocean sediments in the Arabian Sea was one of the first proxies to suggest a monsoon at 8 Ma (Prell and Curry, 1981). The growth of phytoplankton is stimulated by the increased nutrient availability in the Arabian Sea as sediment is washed out of the Indus delta by monsoonal floods.

The shift to C_4 grasslands was also recorded in North America (Cerling *et al*. 1993) and similar increases in plankton abundance dated to ca. 8 Ma in the Pacific and Atlantic (discussed in Clift & Plumb, 2011) casting doubt on the model of simple monsoon initiation at 8 Ma. It may be that the changes in plankton abundance were linked more to wider global climate change than a regional response to the elevation of Tibet (Clift & Plumb 2011).

Clift & Plumb (2011) also discussed the climate records of the west and east Himalaya. In the west the shift to C_4 grasslands in Pakistan ca.7.5 Ma (Quade *et al*. 1989) was linked to the monsoon by those authors as the current C_4 vegetation is supported by the intense seasonality of the monsoon. Quade *et al.* (1989) also measured oxygen isotope in carbonates found in fossils, the character of the isotopes being determined by the temperature at the time the precipitation fell. The oxygen isotopes recorded a shift in the dominant rainy season from winter to summer. Other studies using oxygen isotopes have suggested strong seasonality in precipitation as early as 10.7 Ma and a greater intensity by 7.5 Ma (Dettman *et al*. 2001). The climate of the east Himalaya in the late Miocene was also discussed by Clift & Plumb (2011). They described the analysis of clay minerals taken from deep water drilling in SE Indian Ocean that hold evidence of chemical weathering in the composition of the clays dating to after 8 Ma, and taken with lower rates of sediment accumulation in the Bay of Bengal after 7.5 Ma, they suggested less erosion then as a result of a weaker monsoon.

The uplift of the Himalaya coincided with a marked drop in global temperature. This drop has been linked to the uplift, and monsoon activity, as chemical weathering of the exposed rock surface may have absorbed CO_2, thus reducing the greenhouse effect (Molnar *et al*. 1993). This chemical weathering may have contributed to the global drop in temperature since the India-Eurasia collision (Raymo & Ruddiman 1992; Dalai *et al*. 2002), although the extent of which this occurred is uncertain.

Clift & Plumb (2011) suggest that the 23 Ma exhumation age of the Himalaya is linked to an earlier onset of the monsoon at 23 Ma, not the younger age of ca. 8 Ma. They conclude that there is positive feedback in operation with the monsoon driving erosion, essential for the exhumation of Himalaya, leading to uplift, a higher Himalaya and therefore greater impact on the monsoon. They argue the without the monsoon-driven erosion they find it difficult to see how the Himalaya could have formed.

This is disputed by Adlakha *et al*. (2013) who suggest that as there are different exhumation behaviours along the Himalaya despite changes in the climate happening at the same time and therefore are no correlations between climate and the exhumation of the Himalaya and that it is the tectonic forces are the main cause exhumation.

The current hypothesised timings of paleoclimate depend on a clear understanding of the early processes that have led to the Himalaya and Tibetan plateau reaching a height that significantly affected the regional and global climate. Exhumation rates of the Himalaya have been explained using a variety of proxies such as sediments related to upwelling of nutrient rich waters which are recorded in plankton fossil sediments from the Bay of Bengal and the Arabian Sea, oxygen isotope measurements from onshore fossils and the analysis of mineral composition in deep water sediments. Changes in rates are used to hypothesise uplift events and contribute to an understanding of the monsoon initiation and development. This cross-discipline working is the only way to approach explanations for these complex and interconnected systems.

Conflicts in the literature surrounding the Himalayan-Tibetan Orogeny

At the moment there are very little consensus between the fields of paleogeography and paleoclimatology with the proposed dates for the initiation of the uplift of the Himalaya. The timings of the formation of the high Himalaya and the onset and development of the Indian monsoon are all important as they will have played a role in the generations of biodiversity in the region. Schmidt & Opgenoorth (2015) summarised the current state of knowledge and uncertainties in geosciences that surround the uplift Himalaya and Tibet and the effect on paleoclimate in the region.

Schmidt & Opgenoorth (2015) argued that biogeographical studies of the evolution of plant groups within the Himalaya, Tibet and the mountains of S.W. China have not added much to the debate with authors simply cherry-picking historical scenarios to support their own narrative surrounding their data and failing to highlighting knowledge gaps in the data. In light of Schmidt & Opgenoorth (2015), I reassessed previous phylogeographic studies. It becomes clear that it would be impossible to study groups of interest in the Himalaya under their very strict criteria (table 5.1). The fossil record is too incomplete to have fossil calibration for every phylogeny, and plant groups are still relatively poorly known, to the extent that taxon sampling is far from complete. The authors also suggest that only groups which are at least as old as the Eocene (50 - 33Ma) should be used as study groups, so their evolutionary history spans the entire period of the Himalayan-Tibetan Orogeny. This is also very limiting as it excludes many widespread groups, like *Berberis* and *Clematis* that have migrated and diversified more recently, and limits their potential to help answer questions relating to more recent geological events within the Himalayan-Tibetan Orogeny.

While their criteria are suitable for a hypothesis-driven approach to the studying of the effect of the entire uplift on the evolution of a given taxon to test given geological processes, they are too restrictive for studying wider biogeographical theories, where a synthesis across many taxa may provide the correlations and conflicts in the data to explain what is undoubtedly an intricate picture.

There are currently no large-scale dated studies looking at wider biogeographical patterns in the Himalaya. This chapter synthesises existing knowledge by reviewing the literature, adding additional accessions and reanalysing *Meconopsis* and *Koenigia* datasets to supplement the analysis of *Clematis*. Its aim is to develop a broader picture of the processes generating the biodiversity of the dynamic and megadiverse Himalaya.

Table 5.1 Re-assessed literature and studies of Himalayan rich groups following the criteria set out by Schmidt & Opgenoorth (2015) to assess their usefulness in addressing hypotheses surrounding the development of the Himalaya.

Taxon	Author	Integration of diversity of geoscience scenarios	Sufficient knowledge regarding connections between cladogenesis and character evolution of the study group	Sufficient molecular (nDNA) data and molecular clock calibration	Sufficient evolutionary age [at least as old as Eocene]	Sufficient consideration of the species' biology and speciation process
Androsace	Roquet (2013)	No – Continental and region scale analysis not looking at geoscience literature in Himalaya	Yes - evolution of life strategy to range expansion and subsequent species radiation	No - nDNA + cpDNA secondary dated	Maybe - group on boundary of Eocene	Yes. Life strategy main consideration.
Begonia	Rajbhandary *et al*. (2011)	No - only fitting one geoscience scenario.	No - Plant habit linked to the seasonality of the monsoon. The adaptation to seasonality not necessarily a direct result of but a later adaptation.	No cpDNA and a secondary dated node	No genus and clades much younger.	Yes two clades have differing habit, tuberous & deciduous and evergreen & rhizomatous, occurring in different habitats.
Berberis	Adhikari (Unpublished PhD thesis 2010).	Yes. Adhikari (Unpublished PhD	Yes - chromosome numbers mapped and not linked to	No - Adhikari (Unpublished PhD thesis 2010). nDNA	Genus dates from the Eocene but derived	Yes adaptation to new environments

	Adhikari *et al*. (2014). Adhikari *et al*. (2015).	thesis 2010) reviewed geoscience literature.	evolutionary events in Nepal Himalaya	dated phylogeny but secondary dated.	Himalayan lineages much more recent.	and possible shift in niche ecology.
Cyananthus	Zhou *et al.* (2013)	No - a generalisation about Himalayan motion. Not related to any one scenario.	Yes - linked to dispersal ability and sexual system evolution	No - cpDNA, single fossil calibration point	No - younger than Eocene	No. Link to dispersal ability and sexual system evolution
Koenigia	Fan *et al.* (2013)	No alternatives scenarios given.	No - Lacking data	No - cpDNA, secondary dated	No – group younger than Eocence	No - Range expansion and evolution linked to glacial oscillations but no data to support.
Meconopsis	Kadereit *et al*. (2011)	No - Generalisation about continental climate change towards end of Oligocene. No alternatives given.	No - lacking data	Combine analysis of nDNA and cpDNA	No - younger than Eocene.	Yes speciation linked to evolutionary inheritance of niche preference to mesic habitats through ancestral state reconstruction.

Meconopsis	Xiao (unpublished PhD 2013)	None discussed	Yes. chromosome numbers mapped and linked to reticulate evolutionary events	No *rbc*L	Yes. *Meconopsis* are younger than Eocene the study derived dated from a large Ranunculales analysis.	Yes evolution linked to reticulate evolutionary events. Cautious about used of dated phylogeny which does not assess the role of hybridisation adequately.
Meconopsis	Xie *et al* (2014)	No – no alternatives given	Yes - chromosome numbers mapped and linked to evolutionary events	Maybe - nDNA used but no fossil calibration. Substitution rate used to estimate ages.	Yes - *Meconopsis* are younger than Eocene but the clade is nested in *Papaver* so is in the context of a full phylogeny.	No. The link between cold- and wet-tolerance by ploidy development is suggested but not tested.
Ranunculus	Emadzade *et al*. 2011, Hörandl & Emadzade 2011	No- Continental	No. Lacking data	Yes. Multiple calibration points.	No. Derived Himlayan lineages much more recent.	Linked to long range dispersal

Phylogeography and the Dynamics of Speciation in the Himalaya

Phylogeography can be used to answer some of the hypotheses, relating to timings of Himalayan uplift in relation the expansion and uplift of the Tibetan plateau, the initiation and development of the monsoon and the formations of high altitude habitats where many of the endemic Himalayan species are found, by identifying past population expansions and migrations revealed by the geographic patterns in the sampled taxa. This can be achieved by using the independent dated evidence from the geoscientific literature to put the estimated evolutionary ages of groups studied into context. Recent phylogeographic studies of *Begonia* (Begoniaceae) (Rajbhandary *et al.* 2010 & 2011) and *Berberis* (Berberidaceae) (Adhirkari 2010) have looked specifically at Nepalese rich groups within more widely distributed genera. Both these have attempted to shed light on their evolutionary histories in the Himalaya. Other recent studies with dated phylogenies have looked more broadly at the biogeographic histories of genera such as *Androsace* (Primulaceae) (Roquet (2013), *Cyananthus* (Campanulaceae) (Zhou *et al*. 2013), *Koenigia* (Polygonaceae) (Fan *et al*. 2013), *Meconopsis* (Papaveraceae)(Kadereit *et al.* 2011; Xiao 2013; Xiao & Simpson 2014 and Xie *et al*. 2014) *Ranunculus* (Ranunculaceae) (Emadzade *et al.* 2011, Hörandl & Emadzade 2011), also relate to events in the Himalaya (table 5.1).

Additional accessions from Nepal were added to the readily available *Meconopsis* and *Koenigia* datasets, and reanalysed. The results from these two genera along with those of *Clematis* from Chapter 4 were used with in conjunction with available phylogeographic studies provided an overview of the differing evolutionary histories and influences on the evolution of the Himalayan flora.

Reaassessment of existing datasets - *Meconopsis*

Materials and Methods

Seven accessions of *Meconospsis* were sampled from collections gathered on recent fieldwork for the Flora of Nepal and held in storage at Royal Botanic Garden Edinburgh (RBGE) (table 5.2). The seven accessions were successfully amplified for both one nDNA (ITS) and one cpDNA (*trn*L-*trn*F) region.

Additional taxon sampling

Total genomic DNA was extracted from silica gel dried leaf material using DNeasy Plant mini Kits (Qiagen Ltd, Crawley, West Sussex, UK) following the manufacturer's protocol. For PCR reactions universal nDNA and cpDNA primers were selected (Table 5.3).

Table 5.2 New accessions of Nepalese *Meconopsis*

Taxon	**Collection Number**	**EDNA Number**
Meconopsis manasaluensis	Manasalu08 20815156	EDNA14-0034767
Meconopsis simplicifolia	DNEP3 AY126	EDNA14-0034768
Meconopsis horridula	Manasalu08 20812249	EDNA14-0034769
Meconopsis paniculata	DNEP3 BY196	EDNA14-0034770
Meconopsis horridula	Manasalu08 20815157	EDNA14-0034771
Meconopsis simikotensis	JRSA 195	EDNA14-0034772
Meconopsis grandis subsp. *jumlaensis*	JRSA 21	EDNA14-0034773

Table 5.3 Primers selected for use in *Meconopsis* pcr reactions.

Primer	5' to 3'-end	Reference
ITS		
ITS5P	GGAAGGAGAAGTCGTAACAAG	White *et al.* 1990
ITS8P	CACGCTTCTCCAGACTACA	
Trnl-F		
Trnlc	CGAAATCGGTAGACGCTACG	Taberlet *et al.* 1991
Trnld	GGGGATAGAGGGACTTGAAC	

PCR was carried out on DNA Engine Tetrad 2 Peltier Thermal Cycler by Bio-Rad. in 25 µl reactions in the following PCR mix: 2.5 µl of 10 x NH4 reaction buffer, 2.5 µl of dNTPs (0.2mM), 1.25 µl of MgCl2 (50mM), 0.75 µl of forward primer (10µM), 0.75 µl of reverse primer (10µM), 0.2 µl of Taq (5U/µl), 10 µl of TBT-PAR, 6.05 µl of distilled H2o and 1 µl of template DNA. PCR protocol for the nDNA ITS region and cpDNA *trn*L-*trn*F region are displayed in tables 5.4 and 5.5.

Table 5.4 PCR protocol for nDNA (ITS)

Stage	Temperature	Time	Cycles
Initial Denature	94°C	3min	1
Denature	94°C	1min	30
Annealing	55°C	1min	
Extension	72°C	90sec	
Final Extension	72°C	5min	1
Termination	10°C	forever	1

Table 5.5 PCR protocol for cpDNA (*trn*L-*trn*F)

Stage	Temperature	Time	Cycles
Initial Denature	94°C	3min	1
Denature	94°C	1min	35
Annealing	49°C	1min	
Extension	72°C	5min	

Final Extension	72°C	10min	1
Termination	10°C	forever	1

The quality and quantity of PCR produce was assessed using 1% agarose gel. Samples of successfully amplified ITS with clearly visible bands were purified using ExoSAP-IT (USB Corporation) following the manufacturer's protocol. Initially the cpDNA bands were faint, so extension and annealing times were increased by 15 seconds each and an additional 5 cycles added to the PCR protocol, as suggested by troubleshooting protocols in the RBGE molecular laboratory handbook, before clearly visible bands were purified.

Sequencing reactions were 10 µl using DNA Engine Tetrad 2 Peltier Thermal Cycler by Bio-Rad using standard Bigdye reaction protocol: Bigdye 0.5 µl, 5 x Sequencing Buffer 2 µl, Primer 0.32 µl, Distilled H_2O 5.68 µl, Template DNA 1.5 µl. Product to be analysed were sent to the GenePool service at University of Edinburgh.

Analysis

All available *Meconopsis* and outgroups in Xie *et al*. (2014) that had ITS and *trn*L-*trn*F sequence data were downloaded from Genbank (table 5.6).

New forward and reverse sequences fragments were combined, aligned and edited in SequencherTM (V. 4.5, Genetic Codes Corporation, Ann Arbor, Michigan, US).

All downloaded and newly generated sequence data for *Meconopsis* accessions with ITS and *trn*L-*trn*F were aligned automatically using MAFFT version 7, Multiple alignment program for amino acid or nucleotide sequences, through the Computational Biology Research Centres' online portal (http://mafft.cbrc.jp/alignment/server/) then checked, edited manually and where possible minimising changes, using Mesquite (Maddison & Maddison 2011, 2015).

Table 5.6 Accessions from Genbank used in the *Meconopsis* reanalysis with ITS and trnL-trnF sequence data.

Name	Voucher	ITS	*trn*L-*trn*F
Argemone mexicana	Yuan 2000607	AY328303.1	AY328248.1
Chelidonium majus	Yuan 200052707	AY328308.1	AY328251.1
Corydalis sp.	Gong 20020431	AY328255.1	AY328203.1

Corydalis temulifolia	Shui 20020426	AY328256.1	AY328202.1
Dicranostigma franchetianum	Gong 20020516	AY328305.1	AY328250.1
Dicranostigma lactucoides	Yuan 2000704	AY328304.1	AY328249.1
Eomecon chionantha	Yuan 200052708	AY328306.1	AY328254.1
Lamprocapnos spectabilis	Gong 20010515	AY328310.1	AY328204.1
Macleaya cordata	Yuan 2000605	AY328307.1	AY328253.1
Meconopsis aculeata	NA 2091890	AY328263.1	AY328227.1
Meconopsis autumnalis	Egan 25	JX079011.1	JX087754.1
Meconopsis autumnalis	Egan 17	JX078977.1	JX087748.1
Meconopsis barbiseta	Qinghai-11	KM044430.1	KM044474.1
Meconopsis barbiseta	Qinghai-10	KM044429.1	KM044473.1
Meconopsis bella	E00045403	AY328279.1	AY328218.1
Meconopsis beticofolia	1998.0451	DQ250323.1	DQ251174.1
Meconopsis betonicifolia	Liao 20020608	AY328292.1	AY328236.1
Meconopsis betonicifolia	Laojunshan1	JQ798371.1	JQ798378.1
Meconopsis betonicifolia	Cuona3	JQ798369.1	JQ798377.1
Meconopsis cambrica	Yuan 200271106	AY328299.1	AY328243.1
Meconopsis chankheliensis	Bailey 1936June	JX078973.1	JX087753.1
Meconopsis chelidonifolia	MO 4008808	AY328300.1	AY328246.1
Meconopsis chelidonifolia	Yele	JQ798373.1	JQ798381.1
Meconopsis chelidonifolia	Leibo	JQ798372.1	JQ798380.1
Meconopsis concinna	Boufford 35133	JX079031.1	JX087759.1
Meconopsis delavayi	Yuan 2000633	AY328285.1	AY328211.1
Meconopsis delavayi	W.Xiao 90526	JX079017.1	JX087736.1
Meconopsis delavayi	Yunnan-22	KM044432.1	KM044476.1
Meconopsis delavayi	Yunnan-21	KM044431.1	KM044475.1
Meconopsis dhwojii	Sharma 1620937	AY328276.1	JX087745.1
Meconopsis dhwojii	W.Xiao RICB9	JX079001.1	JX087755.1
Meconopsis discigera	Long 394	AY328277.1	AY328221.1
Meconopsis discigera	Bowes Lyon15045	JX079038.1	JX087774.1
Meconopsis forrestii	Chamberlin 625	AY328287.1	AY328219.1
Meconopsis forrestii	Fang1154	JX079041.1	JX087734.1
Meconopsis ganeshensis	Miyamoto 9400059	JX079014.1	JX087772.1
Meconopsis georgei	Forrest 30595	JX078989.1	JX087768.1
Meconopsis grandis	Yuan 200271110	AY328290.1	AY328235.1
Meconopsis henrici	Yuan 2000680	AY328281.1	AY328209.1
Meconopsis henrici	Boufford 35710	JX079043.1	JX087739.1
Meconopsis henrici	W.Xiao 090722-1	JX078987.1	JX087762.1
Meconopsis horridula	Boufford 30011	AY328261.1	AY328208.1
Meconopsis horridula	Boufford 30022	AY328258.1	JX087764.1
Meconopsis horridula	ACE 1773	JX079044.1	JX087783.1
Meconopsis horridula	W.Xiao 080623-2	JX079039.1	JX087758.1

Meconopsis horridula	Boufford 32738	JX079005.1	JX087767.1
Meconopsis horridula	Boufford 38099	JX079000.1	JX087773.1
Meconopsis horridula	Boufford 35132	JX078992.1	JX087738.1
Meconopsis horridula	Miyamoto 9420086	JX078986.1	JX087769.1
Meconopsis horridula	W.Xiao 090707-1	JX078985.1	JX087784.1
Meconopsis horridula	Boufford 33530	JX078981.1	JX087761.1
Meconopsis horridula	Boufford 38460	JX078980.1	JX087771.1
Meconopsis horridula	Boufford 33724	JX078978.1	JX087770.1
Meconopsis horridula	Egan 15	JX078971.1	JX087742.1
Meconopsis horridula	Qinghai12	KM044439.1	KM044478.1
Meconopsis horridula	Tibet-58	KM044438.1	KM044477.1
Meconopsis impedita	Yuan 2000667	AY328284.1	AY328213.1
Meconopsis impedita	Sichuan-43	KM044440.1	KM044479.1
Meconopsis integrifolia	Yuan 2000684	AY328288.1	AY328229.1
Meconopsis integrifolia	ACE 705	JX078984.1	JX087756.1
Meconopsis integrifolia	isolate H25	JQ798323.1	JQ798344.1
Meconopsis integrifolia	isolate H24	JQ798322.1	JQ798343.1
Meconopsis integrifolia	isolate H23	JQ798321.1	JQ798342.1
Meconopsis integrifolia	isolate H22	JQ798320.1	JQ798341.1
Meconopsis integrifolia	isolate H21	JQ798319.1	JQ798340.1
Meconopsis integrifolia	isolate H20	JQ798318.1	JQ798339.1
Meconopsis integrifolia	isolate H19	JQ798317.1	JQ798338.1
Meconopsis integrifolia	isolate H18	JQ798316.1	JQ798337.1
Meconopsis integrifolia	isolate H17	JQ798315.1	JQ798336.1
Meconopsis integrifolia	isolate H16	JQ798314.1	JQ798335.1
Meconopsis integrifolia	isolate H15	JQ798313.1	JQ798334.1
Meconopsis integrifolia	isolate H12	JQ798310.1	JQ798333.1
Meconopsis integrifolia	isolate H9	JQ798307.1	JQ798332.1
Meconopsis integrifolia	isolate H8	JQ798306.1	JQ798331.1
Meconopsis integrifolia	isolate H7	JQ798305.1	JQ798330.1
Meconopsis integrifolia	isolate H6	JQ798304.1	JQ798329.1
Meconopsis integrifolia	isolate H5	JQ798303.1	JQ798328.1
Meconopsis integrifolia	isolate H4	JQ798302.1	JQ798327.1
Meconopsis integrifolia	isolate H3	JQ798301.1	JQ798326.1
Meconopsis integrifolia	isolate H2	JQ798300.1	JQ798325.1
Meconopsis integrifolia	isolate H1	JQ798299.1	JQ798324.1
Meconopsis lancifolia	Yuan 2000657	AY328282.1	AY328212.1
Meconopsis lancifolia	W.Xiao 090707-2	JX079033.1	JX087744.1
Meconopsis lancifolia	ACE 568	JX079021.1	JX087746.1
Meconopsis lancifolia	W.Xiao 080621-1	JX079008.1	JX087750.1
Meconopsis lancifolia	Boufford 33308	JX079002.1	JX087749.1
Meconopsis lancifolia	Boufford 34065	JX078994.1	JX087779.1

Meconopsis lancifolia	Yunnan-23	KM044443.1	KM044481.1
Meconopsis lancifolia	Sichuan-44	KM044441.1	KM044480.1
Meconopsis latifolia	Stewart 22563a	AY328264.1	AY328226.1
Meconopsis lyrata	E00107706	AY328267.1	AY328215.1
Meconopsis napaulensis	Gong 20020611	AY328269.1	AY328228.1
Meconopsis napaulensis	Egan 16	JX079024.1	JX087733.1
Meconopsis napaulensis	Egan 29	JX078979.1	JX087760.1
Meconopsis oliveriana	J.Z.Xiao 1	JX079016.1	JX087765.1
Meconopsis oliveriana	Shaanxi-1	KM044444.1	KM044482.1
Meconopsis paniculata	Yuan 200271108	AY328272.1	AY328223.1
Meconopsis paniculata	W.Xiao RICB5	JX079022.1	JX087743.1
Meconopsis paniculata	Egan 7	JX079004.1	JX087777.1
Meconopsis paniculata	Tibet-59	KM044449.1	KM044485.1
Meconopsis pinnatifolia	Tibet-62	KM044452.1	KM044486.1
Meconopsis primulina	E00060407	AY328266.1	AY328217.1
Meconopsis pseudohorridula	Tibet-65	KM044455.1	KM044488.1
Meconopsis pseudohorridula	Tibet-64	KM044454.1	KM044487.1
Meconopsis pseudovenusta	W.Xiao 090705-2	JX079009.1	JX087741.1
Meconopsis punicea	Yuan 2000707	AY328293.1	AY328238.1
Meconopsis punicea	Sichuan-48	KM044448.1	KM044484.1
Meconopsis punicea	Sichuan-46	KM044446.1	KM044483.1
Meconopsis quintuplinervia	Yuan 200271107	AY328295.1	AY328239.1
Meconopsis quintuplinervia	Qinghai-13	KM044456.1	KM044490.1
Meconopsis racemosa	Boufford 29486	AY328257.1	AY328206.1
Meconopsis regia	Stainton 4627	AY328273.1	AY328224.1
Meconopsis robusta	Bajhang 20913119	KF777122.1	KF777120.1
Meconopsis rudis	Yunnan-25	KM044459.1	KM044492.1
Meconopsis simplicifolia	Miyamota 9420218	AY328289.1	AY328230.1
Meconopsis simplicifolia	Egan 4	JX079040.1	JX087751.1
Meconopsis simplicifolia	Sejilashan1	JQ798368.1	JQ798375.1
Meconopsis simplicifolia	Cuona	JQ798366.1	JQ798374.1
Meconopsis sinomaculata	Sichuan-50	KM044460.1	KM044493.1
Meconopsis sinuata	E00148904	AY328268.1	AY328216.1
Meconopsis smithiana	GSE97 9592	AY328301.1	AY328247.1
Meconopsis sp.	Sichuan-51	KM044461.1	KM044494.1
Meconopsis sp.	Sichuan-52	KM044462.1	KM044495.1
Meconopsis sp.	Tibet-71	KM044463.1	KM044496.1
Meconopsis sp.	Xiao 090705-1	JX078998.1	JX087782.1
Meconopsis speciosa	Xie 199810	AY328286.1	AY328220.1
Meconopsis speciosa	W.Xiao 090703-2	JX078993.1	JX087781.1
Meconopsis speciosa	Tibet-66	KM044464.1	KM044497.1
Meconopsis superba	Yuan 200271111	AY328274.1	AY328225.1

Meconopsis superba	W.Xiao RICB7	JX079006.1	JX087735.1
Meconopsis torquata	MO 1659653	AY328278.1	AY328222.1
Meconopsis torquata	Ludlow 9904	JX078999.1	JX087737.1
Meconopsis villosa	Yuan 200271105	AY328302.1	AY328245.1
Meconopsis wallichii	W.Xiao 90522	JX079026.1	JX087780.1
Meconopsis wallichii	Miyamoto 9584100	JX079025.1	JX087747.1
Meconopsis wilsonii	Boufford 32733	JX078995.1	JX087740.1
Meconopsis wilsonii	WXiao_090522	KM044465.1	KF513555.1
Meconopsis wumungensis	Gong 20020610	AY328265.1	AY328214.1
Meconopsis x cookei	Yuan 200271112	AY328291.1	AY328234.1
Meconopsis x cookei	E00137066	AY328294.1	AY328231.1
Meconopsis zangnanensis	Chen 25-960	JX079018.1	KF513556.1
Papaver alpinum	W.Xiao 090527-2	JX079023.1	JX087766.1
Papaver lateritium	W.Xiao 090527-3	JX078983.1	JX087776.1
Papaver nudicaule	Beijing-23	KM044467.1	KF513557.1
Papaver pavoninum	Xingjiang-56	KM044468.1	KM044498.1
Papaver radicatum	Jilin-05	KM044470.1	KF513558.1
Papaver rhoeas	Gong 2003030701	AY328311.1	AY328240.1
Papaver somniferum	Yuan 200271102	AY328297.1	AY328242.1
Papaver sp.	Yuan 2000611	AY328298.1	AY328241.1
Papaver sp.	Xiao 090527-1	JX079012.1	JX087752.1
Papaver spicatum	Yuan 200271101	AY328296.1	AY328244.1
Roemeria refracta	Xingjiang-35	KM044472.1	KM044499.1
Stylophorum diphyllum	Yuan 200271103	AY328309.1	JX087757.1
Stylophorum diphyllum	W.Xiao 090527-4	JX079036.1	AY328252.1

Divergence time estimates

Divergence times were estimated using a Bayesian approach implemented in BEAST v. 1.7.4 (Drummond & Rambaud, 2007, Drummond *et al.* 2012) for the combined ITS & *trn*L-*trn*F dataset.

For the BEAST analyses the Birth and Death incomplete sampling tree prior was selected because of incomplete coverage across the genera selected (Stadler 2009). The analysis implemented a relaxed clock with uncorrelated log-normal model of rate variation (Drummond *et al.* 2006). The GTR+G model suggested by JModelTest was implemented. The input file for the analysis was generated using BEAUti ver.1.7.4 (Drummond & Rambaut, 2012). The crown node for the tree was set to 74.5 Ma which corresponds to age of the Late Cretaceous fossil taxon *Palaeoaster* assigned to the subfamily *Papaveroideae* by

Smith (2001) which was used in Kadereit *et al*. (2011) in an *rbc*L study to generate secondary dates to calibrate a smaller dataset of *Meconopsis*.

Two independent runs of 50 million generations were carried out for the two different sets.

The two separate runs from each analysis were checked visually using Tracer v. 1.6 (Rambaut *et al.* 2013) for convergence to stationarity and that ESS values of greater the 200 were achieved. The two independent runs for each analysis we combined using LogCombiner v. 1.7.4 (Drummond *et al.* 2012). A maximum clade credibility (MCC) tree was generated from the sampled trees using TreeAnnotator v. 1.7.4 (Drummond *et al.* 2012), with mean ages, 95% highest posterior probability (HPD) intervals and posterior probabilities calculated.

Visualisation of Trees

Phlyogenetic and Divergence Time estimation trees were visualised using FigTree v.1.4 (Rambaut, 2012)

Results

The evolutionary relationships in *Meconopsis* are congruent between this study and Xie *et al*. (2013). The evolutionary age estimates are also broadly similar to Xie *et al*. (2013), despite the different approaches in constraining the phylogenies. Table 5.7 are the evolutionary age estimates of nodes of interest from the reanalysed *Meconopsis* dataset and table 5.8 shows the equivalent crown node estimated age for *Meconopsis* from the other available studies. Figure 5.1 is the Maximum Clade Credibility tree generated in BEAST.

The crown of the strongly supported (0.98 pp.) *Robustae*/*Superbae*/*Discogyne* clade dates to the middle Miocene at 12.88 Ma (95% HPD: 9.37-16.45) and the crown of strongly supported (0.98 pp) subclade *Discogyne* dates to the late Miocene at 8.37 Ma (95% HPD: 5.81-11.19). The *Robustae*/*Superbae*/*Discogyne* suite are predominantly found in the Himalaya and especially in Nepal with 11 endemic species.

The strongly supported (1.0 pp.) clade containing the Nepalese accessions of the high altitude species *Meconopsis horriulda,* clade 2, dates to the Pleistocene at 1.61 Ma (95% HPD: 0.77-2.91 Ma).

The estimated evolutionary ages Asian *Meconopsis* range from the Oligocene / Miocene boundary at ca. 22 Ma to end of the early Miocene at 16.6 - 17.95 Ma. These ages were used come from studies using different methodologies to constrain the phylogenies.

Table 5.7 Evolutionary age estimates from nodes of interest from the reanalysed *Meconospis* dataset

Node	Evolutionary Age Estimate
Crown of *Meconopsis*	17.95 Ma (95% HPD: 13.56-23.03) 1.0 pp.
Crown of *Robustae*/*Superbae*/*Discogyne* Clade	12.88 Ma (95% HPD: 9.37-16.45) 0.98 pp.
Crown of *Discogyne*	8.37 Ma (95% HPD: 5.81-11.19) 0.98 pp.
Crown of *M. horridula* clades	4.68 (95% HPD: 2.67-7.39) 1.0 pp.
Crown of Nepalese accessions in M. *horridula* clade 2	1.61 Ma (95% HPD: 0.77-2.91 Ma) 1.0 pp.
Crown of *M. simplicifolia* clade	3.37 Ma (95% HPD: 1.8-5.49) 1.0 pp.
Crown of Nepalese accessions of *M.* s*implicifolia*	1.06 Ma (95% HPD: 0.42-1.99) 1.0 pp.

Table 5.8 Comparison of the crown ages of the Asian *Meconopsis* clade from the dated studies

Evolutionary Age Estimate	**Kadereit *et al*. (2011)**	**Xiao (unpublished PhD study 2013)**	**Xie *et al* (2014)**	**Current study**
Crown of Asian *Meconopsis* clade	Ca. 22.0 Ma (95% HPD: 14 - 32 Ma)	22 Ma (ca 95% HPD: 10-35 Ma)	Ca. 16.6 Ma (95% HPD: 7.5-30 Ma)	17.95 Ma (95% HPD: 13.56-23.03)

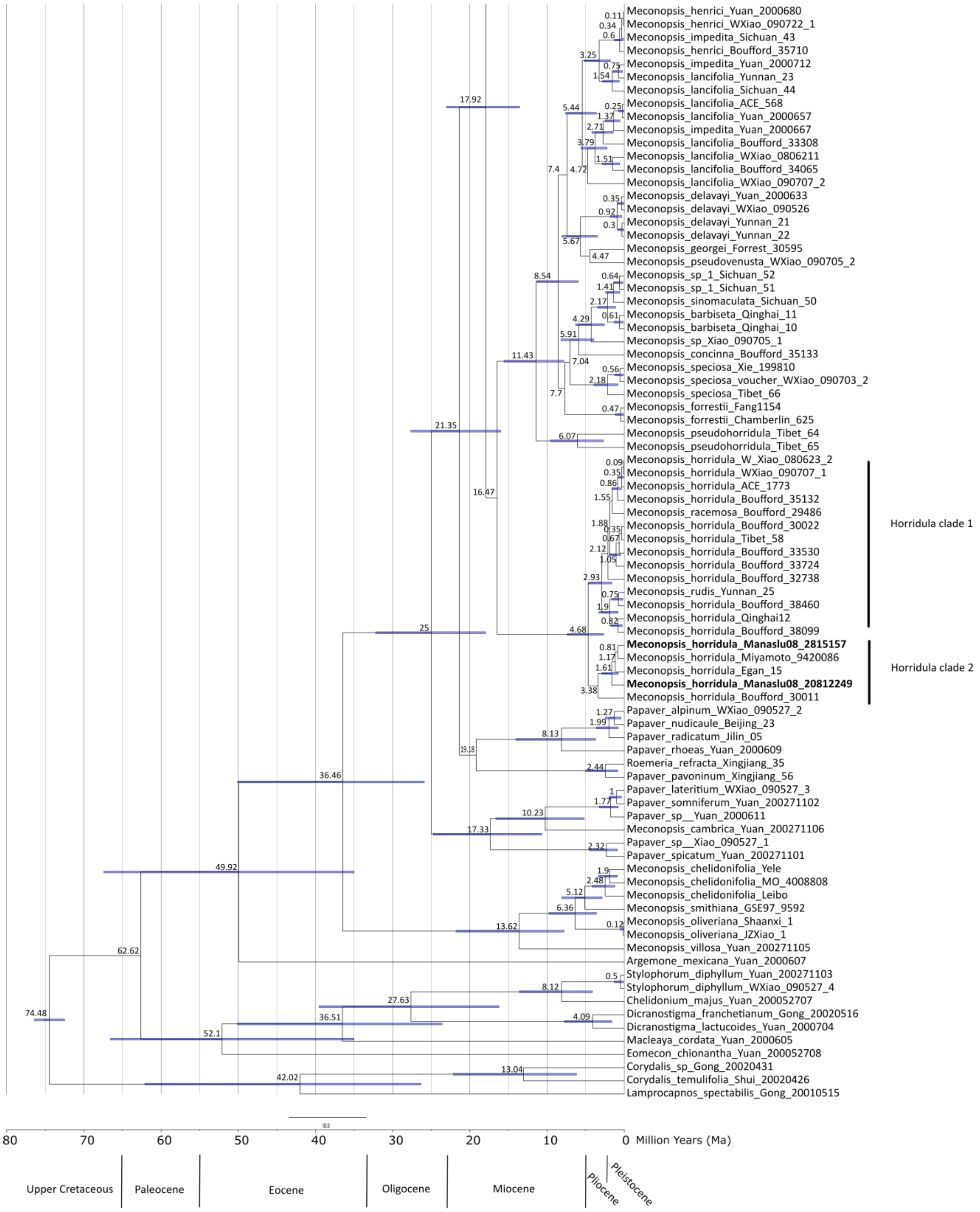
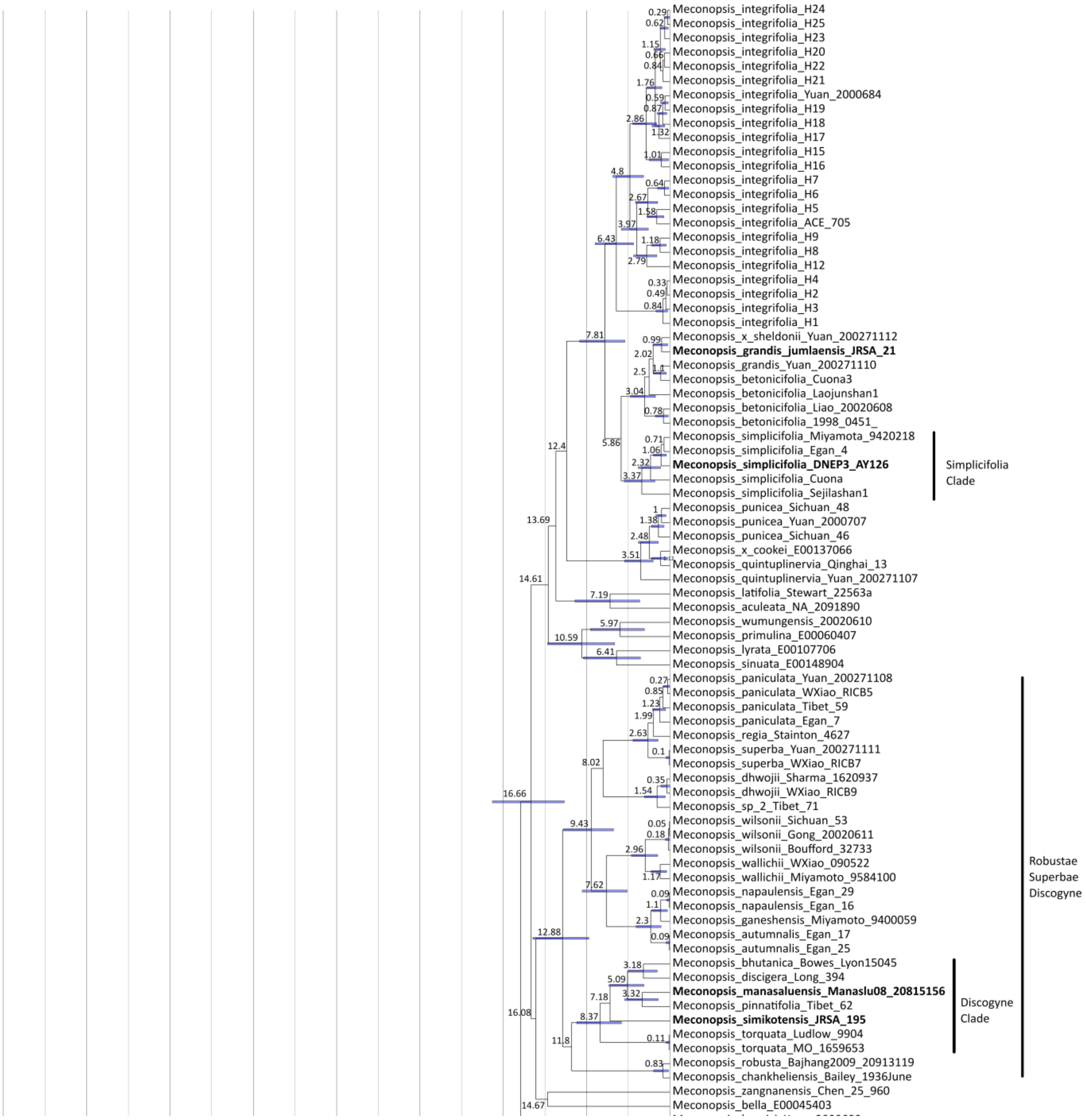


Figure 5.1 Maximum Clade Credibility tree generated from BEAST analysis of reanalysed *Meconsopsis* dataset. Numbers at nodes are divergence age estimates, bars at nodes are HPD 95% confidence intervals. Terminal taxa in bold indicate the new accessions in this study.



Reassessment of existing datasets - *Koenigia*

Materials and Methods

The dataset of existing *trn*L-*trn*F and *rbc*L sequences and Nepalese data generated by Scherrenberg (2014) as part of her MSc in Plant Diversity and Taxonomy at University of Edinburgh and Royal Botanic Garden Edinburgh were realigned using MAFFT version 7,Multiple alignment program for amino acid or nucleotide sequences, through the Computational Biology Research Center's online portal (http://mafft.cbrc.jp/alignment/server/) then checked and edited manually using Mesquite (Maddison & Maddison 2011, 2015).

Divergence time estimates

The divergence time of the Polygonaceae from the Plumbaginaceae was set to 67.9 Ma based on Magallón *et al*. (2015), which differed from Scherrenberg (2014) who used 42 Ma based on Wiikstrom *et al*. (2001 & 2003). As with the reassessment of the evolutionary dates of the Ranunculaceae tribe Anemoneae in Chapter 3 the most robust evolutionary dates used as a secondary calibration point.

For the BEAST analyses the Birth and Death incomplete sampling tree prior was selected, relaxed clock with uncorrelated lognormal model of rate variation and the GTR model, which was suggested by JModelTest, were all implemented.

Two independent runs of 50 Million generations were performed and each was checked visually using Tracer v. 1.6 for convergence to stationarity and to ensure that the ESS values were greater than 200 were achieved. The co-efficient of variance was the only variable to fail to achieve 200 and was reported as 196.

Visualisation of Trees

Phlyogenetic and Divergence Time estimation trees were visualised using FigTree v.1.4 (Rambaut, 2012)

Table 5.9 Genbank accessions of trnL-trnF and rbcl chloroplast regions used in Scherrenberg (2014) and used in the reanalysis of the *Koenigia* dataset.

Species name	**Voucher**	***trn*L-*trn*F**	***rbc*L**
Aconogonon campanulatum	FanDM-014	JN235034.1	JN234953.1
A. cathayanum	FanDM-022	JN235039.1	JN234958.1
A. hookeri	FanDM-019	JN235041.1	JN234960.1
A. lichiangense	HQ	HQ843169.1	-
A. molle	Kim Deng	EF653790.1	EF653764.1
A. tortuosum	FanDM-061	JN235038.1	JN234957.1
Bistorta amplexicaulis	FanDM-035	JN235033.1	JN234952.1
B. macrophylla	FanDM-031	JN235071.1	JN234988.1

B. paleacea	FanDM-063	JN235070.1	JN234987.1
B. sinomontana	FanDM-062	JN235032.1	-
B. vacciniifolia	FanDM-027	JN235037.1	JN234956.1
B. vivipara	FanDM-017	JN235072.1	JN234989.1
Koenigia delicatula	FanDM-024	JN235069.1	JN234986.1
*K. fertilis (*included as *K. islandica)*	FanDM-013	JN235036.1	JN234955.1
K. forrestii	FanDM-016	JN235043.1	JN234962.1
K. islandica	Arctic Wu & Yang	JN235046.1	JN234965.1
K. islandica	FanDM-012	JN235047.1	JN234966.1
K. islandica	Lutz USA	EF653789.1	EF653763.1
K. nepalensis	FanDM-023	JN235049.1	JN234968.1
K. nummularifolia	FanDM-010	JN235042.1	JN234961.1
K. pilosa	FanDM-021	JN235048.1	JN234967.1
Limoniastrum guyonianum		AJ391319.1	AJ286358.1
Limonium delicatulum		AJ391324.1	Y16903.1
Limonium dendroides		-	Z97644.1
Limonium rigualii		AJ391328.1	Z97645.1
Oxyria digyna	FanDM-058	JN235068.1	JN234991.1
Polygonum campanulatum var. *fulvidum (*included as *A. campanulatum* var. *fulvidum)*	HQ	HQ435347.1	HQ435358.1
*P. cyanandrum (*included as *K. pilosa)*	HQ	HQ435346.1	HQ435355.1
*P. delicatulum (*included as *K. delicatula)*	HQ	HQ435344.1	HQ435353.1
*P. forrestii (*included as *K. forrestii)*	DQ	FJ821779.1	-
*P. suffultum (*included as *B. suffulta)*	FanDM-065	JN235073.1	JN234990.1
P. suffultum	FanDM-065	JN235091.1	JN234950.1
Plumbago europaea		AJ391334.1	Y16906.1
Plumbago zeylanica		AJ312248.1	Y16905.1
Rheum nanum		AY566444.1	EU840313.1
Rheum officinale		AF303431.1	EU840310.1
Rheum sublanceolatum		AF303440.1	EU840306.1
*Rubrivena polystachya (*included as *A. polystachyum)*	FanDM-018	JN235040.1	JN234959.1

Table 5.10 Accessions of *Koenigia* with trnL-trnF and rbcL regions generated by Scherrenberg (2014) and used in the reanalysis of the *Koenigia* dataset.

EDNA-Number	Name	Collection	Country
EDNA14-0035445	*Koenigia nepalensis*	Manaslu08 20811256	Nepal
EDNA14-0035446	*Koenigia nepalensis*	Manaslu08 20816076	Nepal
EDNA14-0035448	*Koenigia nepalensis*	Bajhang09 20915018	Nepal
EDNA14-0035449	*Koenigia nepalensis*	Bajhang09 20915032	Nepal
EDNA14-0035450	*Koenigia nepalensis*	Bajhang09 20915088	Nepal
EDNA14-0035451	*Koenigia deliculata*	Bajhang09 20915108	Nepal
EDNA14-0035452	*Koenigia deliculata*	DNEP3 BX53	Nepal
EDNA14-0035453	*Koenigia deliculata*	DNEP3 AY149	Nepal
EDNA14-0035941	*Koenigia nepalensis*	DNEP3 BX143	Nepal
EDNA14-0035942	*Koenigia delicatula*	DNEP3 AX57	Nepal
EDNA14-0035943	*Koenigia nepalensis*	DNEP3 AY162	Nepal
EDNA14-0035944	*Koenigia nepalensis*	JRS A128	Nepal
EDNA14-0035946	*Koenigia nepalensis*	ENEP 292	Nepal
EDNA14-0035947	*Koenigia nepalensis*	ENEP 323	Nepal
EDNA14-0035949	*Koenigia nepalensis*	ESIK 574a	Nepal
EDNA14-0035950	*Koenigia nepalensis*	LKSRD 373	Nepal
EDNA14-0035951	*Koenigia nepalensis*	T. Wraber 382	Nepal
EDNA14-0035952	*Koenigia delicatula*	ENEP 230	Nepal
EDNA14-0035953	*Koenigia delicatula*	Sinclair I.W.J., Long D.G. 5042	Bhutan
EDNA14-0035954	*Koenigia delicatula*	EKSIN 151	Nepal
EDNA14-0035955	*Koenigia delicatula*	EMAK 358	Nepal
EDNA14-0035956	*Koenigia islandica*	McBeath, R.J.D. 2086	India
EDNA14-0035958	*Koenigia islandica*	ESIK 574b	Nepal
EDNA14-0035959	*Koenigia nummularifolia*	EMAK 357	Nepal
EDNA14-0035960	*Koenigia nummularifolia*	ESIK 621	Nepal

Results

There is congruence between the topology of the BEAST tree from thereanalysed *Koenigia* dataset and the equivalent from Scherrenberg (2013). The ages from the reanalysed *Koenigia* dataset are older than those reported by Fan *et al*. (2013) and Scherrenberg (unpublished MSc. thesis), the equivalent nodes are reported in table 5.11.

Table 5.11 Node age comparison between Scherrenberg (unpublished MSc. thesis 2014) and the reanalyised dataset using a crown age contraint based of 67.9 Ma from (Magallón *et al*. 2015).

Evolutionary Age Estimates	**Fan *et al*. (2013)**	**Scherrenberg (2014)**	**This study**
Koenigia/*Aconogonon*	Not reported	19.39 Ma (95% HPD: 9.95-31.01 Ma)	30.6 Ma (95% HPD:15.94-46.7 Ma)
Koenigia delicatula	-	ca. 6 Ma	13.57 Ma (95% HPD: 4.04-25.99 Ma)
‘core’ *Koenigia*	8.98 Ma (95% HDP: 4.82-13.58 Ma)	7.4 Ma (95% HPD: 3.12-12.24 Ma)	16.58 Ma (95% HPD:7.53-27.09 Ma)
Koenigia nepalensis	-	5.18 Ma (95% HPD: 1.82-8.08 Ma)	11.66 Ma (95% HPD:5.09-19.99 Ma)

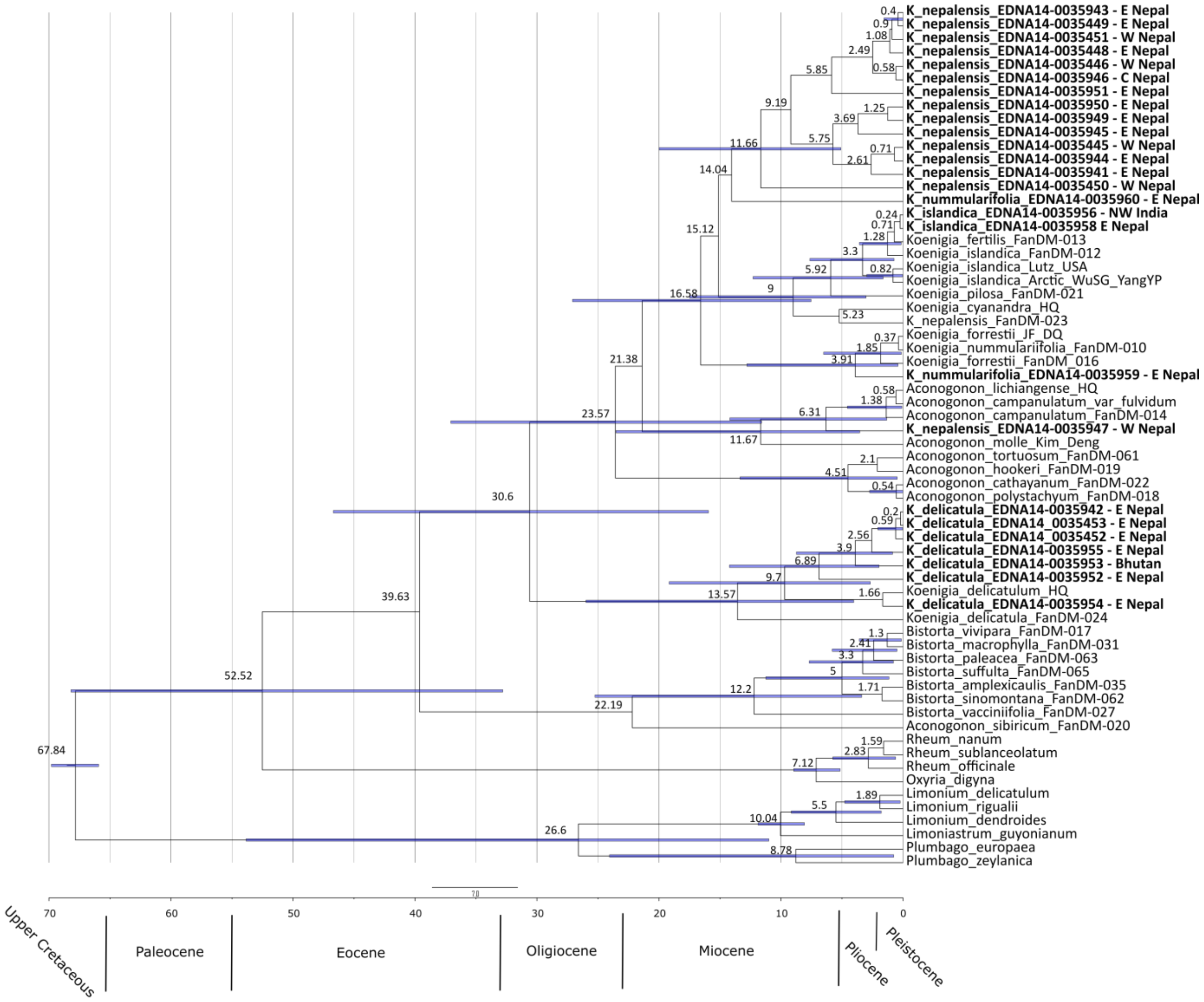


Figure 5.2 Maximum Clade Credibility tree generated from BEAST analysis of reanalysed *Koenigia* cpDNA (*trn*L-*trn*F) and *rbc*L dataset. Numbers at nodes are divergence age estimates, bars at nodes are HPD 95% confidence intervals. Terminal taxa in bold indicate the new accessions in this study.

Discussion

The reanalyses outlined above contribute to the overall synthesis that follows. Some studies suggest clades in genera that illustrate some of the drivers contributing to Himalayan floristic diversity, and are discussed under each of several broad themes relating to the uplift of the Himalaya, including the monsoon, and aridifcation. Other studies show several influences acting and are discussed under each area where appropriate. It is important to note that this is a complex picture, with mobile plant groups and a dynamic altitudinal and climatic series of systems, so no one climatic factor or tectonic event is likely to be responsible for the entire diversity in any given genus in the Himalaya.

Jabbour & Renner (2012) recorded a burst of diversification in a clade of tribe Delphineae (Ranunculaceae) containing species from the Himalayan and mountains of SW China. This was linked to a move to long life-cycles in cold, wet high-altitude habitats. A similar finding was recorded in Roquet (2013) which mapped the transition to long life-cycles in *Androsace* (Primulaceae) in different high altitude mountain environments including the Himalaya. Neither of these examples attempted to link the emergence of the high altitude habitats presumably due to the complicated, contradicting or scanty data surrounding the emergence of montane and alpine habitats in the Himalaya.

Uplift and Tectonics

Koenigia

The evolutionary ages calculated for *Koenigia* from the reanalysed dataset are older than those previously reported. Fan *et al*. (2013) suggested that ‘core’ *Koenigia* diverged from its close relatives, *Aconogonon* and the basal lineage of *K. delicatula* at 8.89 Ma (95% HPD: 4.82-13.58 Ma) and linked this to the uplifting of the eastern edge of the Tibetan plateau 15-10 Ma (Royden *et al*. 2008). The authors of the study suggest that China serves as the centre of diversity but do concede that *Koenigia* may have diversified in connection to Himalayan tectonic motions.

Nepalese *Koenigia* were further studied by Scherrenberg (unpublished MSc. thesis 2014) who confirmed that *Aconogonon* is nested in the *Koenigia* clade, through in the increased sampling of *Koenigia*. In Scherrenberg’s analysis the evolutionary date of the

Koenigia/*Aconogonon* clade dates to 15.04 Ma (95% HPD: 6.48-25.01 Ma) the evolutionary date of the 'core' *Koenigia* clade, excluding the basal lineage of *Koenigia* and the nested *Aconogonon*, is 7.4 Ma (95% HPD: 3.12-12.24) The date of the 'core' clade is consistent with Fan *et al*. (2013). Scherrenberg suggested that the last common ancestor of *Koenigia*/*Aconogonon* and *Bistorta* was probably widespread in the in the ancestral area of the Himalaya and evolved in conjunction with the uplift.

The use of the revised calibration point from the Magallón *et al.* (2015) study to constrain the reanalysed phylogeny had the effect of increasing the estimated ages of divergence events compared with the Fan *et al*. (2013) and Scherrenberg (2014) studies. The *Koenigia*/*Aconogonon* clade estimated to have diverged 30.6 Ma (95% HPD: 15.94-46.7 Ma compared with ca. 15.4 (95% HPD: 6.48-25.01 Ma). The revised analysis places the divergence of the *Koenigia*/*Aconogonon* clade in the Oligocene, coinciding with the closure of the Tethys Sea and the associated trend in global cooling (Allen & Armstrong 2008) and the collision of the India-Eurasian plate as hypothesised by Ali & Aitchison (2004, 2007) and by Hinsbergen *et al*. (2012).

Scherrenberg (2014) estimated the age of the extensively sampled *Koenigia nepalensis* clade at ca. 5.2 Ma, and its diversification was attributed by Scherrenberg to climatic fluctuations in the Pliocene and uplift (Zhiseng *et al*. 2001). The reanalysis estimates the age of this clade to be ca. 11.7 Ma, in the Miocene, so may be more likely linked to tectonic activity in the Himalaya (Valydia 2002).

Cyananthus

Not all previous studies of Himalayan-rich groups focus on the role that the uplift has played on its evolution. The topology of the *Cyananthus* phylogeny reported by Zhou *et al*. (2013) resolved to a clade of Himalayan species and a clade of species from SW China. Zhou *et al*. suggested that *Cyananthus* had a likely origin in the Himalayan at 15.12 Ma (95% HPD 12.12-22.94 Ma), before dispersing into the mountains of SW China and further diversifying. Apart from a brief mention of what Zhou *et al*. called 'the Himalayan motion', no other attempt was made to explain its origin and subsequent diversification in the light of the geoscientific literature.

Monsoon as a driver

Begonia

The large genus *Begonia* began to diversify in Africa during the Oligocene but the current hotspot of diversity for the genus is China and SE Asia, which must be the result of an eastward dispersal or migration across the Asian continent. Rajbhandary *et al.* (2011) investigated the role of the Himalaya as a mesic corridor that allowed *Begonia* to colonise the current hotspot of diversity.

Begonia arrived in the Himalaya ca. 15 Ma (Figure 5.3), and its strong preference for mesic habitats indicates that the monsoon had started by the early-mid Miocene, as suggested by Copeland (1997), allowing *Begonia* to establish in the Himalaya. Rajbhandary's study linked two groups of *Begonia* in the Himalaya with different life strategies to the seasonality of the monsoon, deciduous and tuberous vs. evergreen and rhizomatous.

The seasonally deciduous group has a likely, but equivocal, origin in the Himalaya. The radiation of these seasonally deciduous tuberous species, which grow in seasonally drier habitats with flowering times that are strongly linked to the onset of the monsoon, dated to between 6.4 (±4.8) and 7.4 (±4.6) Ma coinciding with the initiation, or strengthening, of the Asian monsoon 7.5-10 Ma (Quade *et al*. 1989, Prell and Kutzbach, 1922; Dettman et al. 2001). The second group of species is evergreen and rhizomatous and extends into aseasonal habitats. This group, which has extended flowering times and is less constrained by the monsoon, arrived in the Himalaya ca. 5.1 (±3.2) Ma, with an endemic radiation starting 3.0 (±2.1) Ma and continuing throughout the Pleistocene. This immigration coincides with a further intensification of the Asian monsoon around 5 to 3.6 Ma, by Zhou *et al*. and is possibly linked to a further surge in the uplift of the Himalaya and the Tibetan plateau.

Of the two groups of *Begonia* in the Himalaya, Rajbhandary *et al.* (2011) argue only the deciduous could possibly be relicts of an eastward migration. However, due to the unresolved relationships of clades within this group, their origins remain unknown. The evergreen species represent a re-entry to the Himalayan region, most likely from China, and their in-situ speciation in the Himalaya may well be due to the presence of suitable habitats with a constant supply of water as suggested by Rajbhandary *et al.* (2011), facilitated by changes in the monsoon intensity. If the Himalaya acted as a migration corridor in the early evolution of Asian *Begonia*, the expectation would be to see other Asian species nested within a Himalayan clade in the phylogeny. However, with the unsupported nodes at the base of the clade containing all the Himalayan species, evidence for this is equivocal. Some subclades in this group do show a likely Himalayan origin, but the supported nodes are dated in the late Miocene at ca. 7.5 Ma and so post-date the early divergence of the section.

While Schmidt & Opgenoorth (2015) suggested Rajbhandary *et al.* (2011) could not find additional support for the Himalaya acting as a mesic migration route, this was one of the first phylogeographic studies focused specifically on the Himalaya, and it does not mean that additional groups that support the hypothesis do not exist.

Meconopsis

Meconopsis (Papaveraceae) is restricted to the Himalaya, adjacent Tibet the mountains of SW China and north to Qinghai[1]. The *Meconopsis* dataset was reanalysed as part of this study because the evolution of this genus appears to be linked to the geological events in the Himalaya and the Tibetan plateau.

Previous phylogenetic reconstructions (Kadereit *et al*. 2011; Xiao 2013; Xiao & Simpson 2014; Xie *et al*. 2014,) showed that *Papaver* section *Meconella* and the main Asian clade of *Meconopsis*, which excludes *M. cambrica* and the *Meconopsis* species now in the genus *Cathcartia*, split from the remainder of *Papaver* ca. 28 Ma (Kadereit *et al*. 2011) or ca. 20 Ma (Xie e*t al*. 2014). Both *Meconopsis* and *Papaver* section *Meconella* groups share a mesic habitat preference, unlike the remainder of *Papaver sensu stricto* that has a semi-arid preference, as tested by Kadereit *et al.* (2011) through the phylogenetic reconstruction.

Meconopsis and *Papaver* section *Meconella* began to diverge from each other ca. 24 Ma (Kadereit *et al*. 2011) or 16.6 Ma (Xie *et al*. 2014) in the Miocene. *Papaver* section *Meconella*, has a much larger distribution than *Meconopsis*. It is a Holoarctic group, widely distributed across the Northern Hemisphere, with populations spanning central, inner and eastern Asia, Siberia, Scandinavia, Greenland, Canada, the Rocky Mountains, and regions of

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[1] The western European species *Meconopsis cambrica* has been clearly shown as early as the late 1990s (Kadereit *et al*. 1997) that it is nested in *Papaver sensu stricta* and not closely related to Asian *Meconopsis*. Grey-Wilson (2014) has recently transferred this species to a monotypic genus, *Parameconopsis*.

Europe. The Kadereit *et al.* date for the split of *Meconopsis* and *Papaver* section *Meconella* from *Papaver sensu stricto* is the end of the Oligocene, during a period of significant global cooling and increased aridity (Sun *et al*. 2010) and coincides with the revised date for the collision between the Indian Plate and the Lhasa Block proposed by Aitchison *et al.* (2007). Xie e*t al*.'s (2014) estimate of ca. 16.6 Ma postdates the 23 Ma date for monsoon initiation proposed by Clift *et al*. (2011) but predates Dettman *et al*.'s (2001) 10.7 - 7.5 Ma for initiation and intensification of the Monsoon. Therefore if the Xie *et al*. (2014) age is accurate then the reason for this split must be found elsewhere.

These discrepancies in evolutionary divergence date relate to the different methodologies in dating their phylogenies. Kadereit (*et al*. 2011) used a secondary calibration of the phylogeny after carrying out a higher level study that employed a single fossil and two secondary calibrated nodes, whereas Xie (*et al*. 2014) used the average rates of nucleotide substitution based in angiosperms to calculate the divergence dates. According to the criteria of Schmidt & Opgenoorth (2015) the use the average rates of nucleotide substitutions as an approach should be considered a provisional methodology.

Schmidt & Opgenoorth (2015) focused only on the Xie *et al*. (2014) study and noted that they failed to present any specific morphological adaptations to monsoonal climatic parameters. However Kadereit *et al*. (2011) suggested through ancestral state reconstruction that Asian *Meconopsis* and *Papaver* section *Meconella* inherited their preference for mesic habitats from their common ancestor shared with the *Meconopsis* species of SW China, now in the genus *Cathcartia*, while the remainder of *Papaver sensu lato* evolved to take advantage of semi-arid habitats as a result of the increased aridity during the Paleogene and Neogene. The implication is that the Himalayan orogeny and Tibetan Plateau uplift created a xeric geographic barrier limiting genetic exchange and causing *Meconopsis* and *Papaver* section *Meconella* to diverge allopatrically. The mesic conditions created by the monsoon and new habitats created by the orogeny allowed *Meconopsis* to diversify in the Himalaya and SW China while *Papaver* sect *Meconella* diversified across a much larger area at higher latitudes in the Northern Hemisphere. As additional samples of *Meconopsis* from Nepal were available for from previous *Flora of epal* fieldwork these were added to the existing dataset.

The three previous studies and the current reassessment used different techniques to estimate the evolutionary date of *Meconopsis* (table 5.8). Kadereit *et al*. used a Eudicot dataset of 87 accessions that was constrained by dates from Bell *et al*. (2010) and the root of subfamily

Papaveroideae constrained with the age of the fossil *Palaeoaster*. Wei (2013) used a dataset of over 532 accessions with rbc*L* data for the Ranunculales and Proteales, including 20 accessions of *Meconopsis,* with a Ceratophyllaceae outgroup. The age of the Ranunculales and accessions from the Menispermaceae were constrained using previously published ages. Xie *et al*. (2014) estimated the age of *Meconopsis* using a mean substitution rate for angiosperms. This study calibrated the crown of the phylogeny using the age of the *Palaeoaster* fossil as a minimum age for the group. Despite the four studies using different methods all the crown ages for Asian *Meconopsis* suggest a Late to Middle Miocene evolution for the genus.

The large and well represented Himalayan *Robustae*/*Superbae*/*Discogyne* clade, which contains species found in the Himalaya and adjacent Tibet and one species in SW China, shared a last common ancestor 12.88 Ma (95% HPD: 9.37-16.45), which coincides with the principal uplift of the Himalaya 11.5 -5.1 Ma (Valdyia, 2002). The core group of species date to 9.4 Ma, which includes a derived clade from the mountains of SW China. This suite of species is particularly diverse in Nepal with nine endemics.

The *Discogyne* subclade has an interesting pattern reflected in the topology. The basal lineage of *Meconopsis torquata* is restricted to very small area of c. 80 km^2 in the hills north of Lhasa with the remaining derived lineages scattered across the Himalaya. The last common ancestor of the derived Himalayan lineages and *M. torquata* dating from 8.37 Ma suggests that the Himalaya had reached a sufficient height to prevent the monsoon penetrating beyond and causing a rain shadow immediately to the north. The aridification of the ancestral area of the last common ancestor must have restricted the distributions, seen in the limited populations of M. *torquata* to the mesic areas in the hills north of Lhasa and south into the monsoonal areas of the Himalaya; ultimately over time causing the populations to speciate allopatrically. All species of *Meconopsis* are believed to have limited dispersal ability because of their seed being gravity dispersed, restricting the distributions of species.

The extra sampling from Nepal and dates from this study also supports the proposed hypothesis of Schmidt & Opgenoorth (2015) that *Meconopsis* share a similar evolutionary scenario with flightless beetles (Schmit *et al*. 2012). The scenario discussed by Schmit *et al*. (2012) proposed an ‘out of Tibet’ hypothesis to explain the diversity of the Beetle clades that became isolated in deep valleys of the Himalaya. Again this is supported by the limited seed dispersal ability of *Meconopsis* suggesting these populations are “trapped” in valley systems.

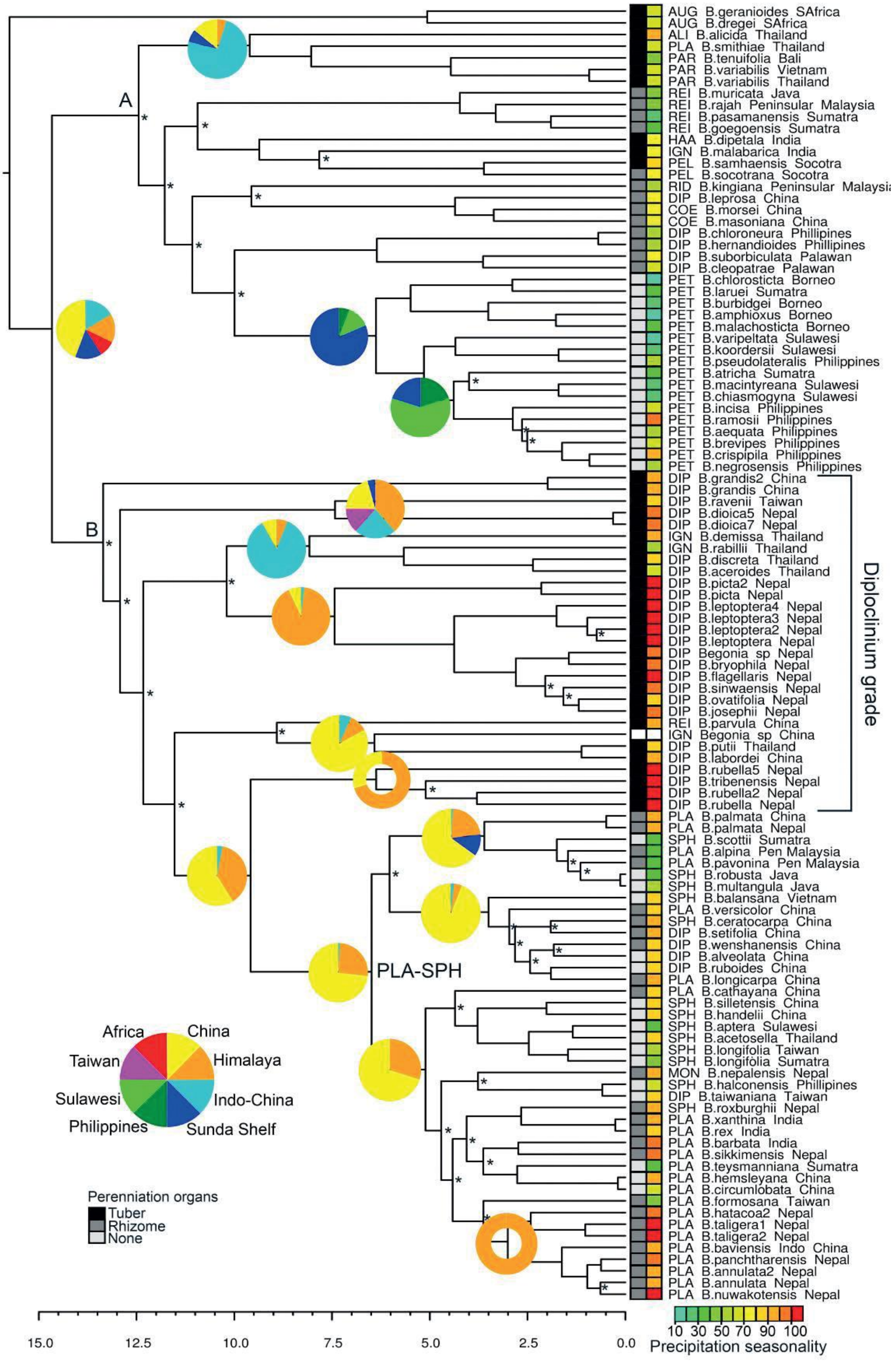
A
B
PLA-SPH
AUG B.geranioides SAfrica
AUG B.dregei SAfrica
ALI B.alicida Thailand
PLA B.smithiae Thailand
PAR B.tenuifolia Bali
PAR B.variabilis Vietnam
PAR B.variabilis Thailand
REI B.muricata Java
REI B.rajah Peninsular Malaysia
REI B.pasamanensis Sumatra
REI B.goegoensis Sumatra
HAA B.dipetala India
IGN B.malabarica India
PEL B.samhaensis Socotra
PEL B.socotrana Socotra
RID B.kingiana Peninsular Malaysi
DIP B.leprosa China
COE B.morsei China
COE B.masoniana China
DIP B.chloroneura Phillipines
DIP B.hernandioides Phillipines
DIP B.suborbiculata Palawan
DIP B.cleopatrae Palawan
PET B.chlorosticta Borneo
PET B.laruei Sumatra
PET B.burbidgei Borneo
PET B.amphioxus Borneo
PET B.malachosticta Borneo
PET B.varipeltata Sulawesi
PET B.koordersii Sulawesi
PET B.pseudolateralis Philippines
PET B.atricha Sumatra
PET B.macintyreana Sulawesi
PET B.chiasmogyna Sulawesi
PET B.incisa Philippines
PET B.ramosii Philippines
PET B.aequata Philippines
PET B.brevipes Philippines
PET B.crispipila Philippines
PET B.negrosensis Philippines
DIP B.grandis2 China
DIP B.grandis China
DIP B.ravenii Taiwan
DIP B.dioica5 Nepal
DIP B.dioica7 Nepal
IGN B.demissa Thailand
IGN B.rabillii Thailand
DIP B.discreta Thailand
DIP B.aceroides Thailand
DIP B.picta2 Nepal
DIP B.picta Nepal
DIP B.leptoptera4 Nepal
DIP B.leptoptera3 Nepal
DIP B.leptoptera2 Nepal
DIP B.leptoptera Nepal
DIP Begonia sp Nepal
DIP B.bryophila Nepal
DIP B.flagellaris Nepal
DIP B.sinwaensis Nepal
DIP B.ovatifolia Nepal
DIP B.josephii Nepal
REI B.parvula China
IGN Begonia sp China
DIP B.putii Thailand
DIP B.labordei China
DIP B.rubella5 Nepal
DIP B.tribenensis Nepal
DIP B.rubella2 Nepal
DIP B.rubella Nepal
PLA B.palmata China
PLA B.palmata Nepal
SPH B.scottii Sumatra
PLA B.alpina Pen Malaysia
PLA B.pavonina Pen Malaysia
SPH B.robusta Java
SPH B.multangula Java
SPH B.balansana Vietnam
PLA B.versicolor China
SPH B.ceratocarpa China
DIP B.setifolia China
DIP B.wenshanensis China
DIP B.alveolata China
DIP B.ruboides China
PLA B.longicarpa China
PLA B.cathayana China
SPH B.silletensis China
SPH B.handelii China
SPH B.aptera Sulawesi
SPH B.acetosella Thailand
SPH B.longifolia Taiwan
SPH B.longifolia Sumatra
MON B.nepalensis Nepal
SPH B.halconensis Phillipines
DIP B.taiwaniana Taiwan
SPH B.roxburghii Nepal
PLA B.xanthina India
PLA B.rex India
PLA B.barbata India
PLA B.sikkimensis Nepal
PLA B.teysmanniana Sumatra
PLA B.hemsleyana China
PLA B.circumlobata China
PLA B.formosana Taiwan
PLA B.hatacoa2 Nepal
PLA B.taligera1 Nepal
PLA B.taligera2 Nepal
PLA B.baviensis Indo China
PLA B.panchtharensis Nepal
PLA B.annulata2 Nepal
PLA B.annulata Nepal
PLA B.nuwakotensis Nepal
Diploclinium grade
Africa
China
Taiwan
Himalaya
Sulawesi
Indo-China
Philippines
Sunda Shelf
Perenniation organs
Tuber
Rhizome
None
15.0
12.5
10.0
7.5
5.0
2.5
0.0
10 30 50 70 90 100
Precipitation seasonality

Figure 5.3 Maximum clade credibility chronogram derived from an analysis of nuclear ribosomal ITS sequences using BEAST. Asterisks denote clades with a posterior probability of less than 0.8; pie charts show Bayesian ancestral area reconstructions on supported nodes; lower scale denotes time in millions of years; the monochrome and coloured boxes at the branch tips show the perennation organ type and precipitation seasonality respectively; missing data coded as white. Three-letter codes indicate the sections of Begonia (ALI, section Alicida; AUG, section Augustia; COE, section Coelocentrum; DIP, section Diploclinium; HAA, section Haagea; IGN, ignota (section unknown); MON, section Monopteron; PAR, section Parvibegonia; PET, section Petermannia; PLA, section Platycentrum; REI, section Reichenheimea; RID, section Ridleyella; SPH, section Sphenanthera). Adapted from (Rajbhandary *et al.* (2011) used with permission of authors.

Aridification as a Driver

Clematis

The tested diversity of *Clematis* in Nepal appears to be the result of at least six immigration events as all dates of these nodes postdate the uplift of the Himalaya, but this may well be an underestimate as not all the species of *Clematis* from Nepal could be included in the analysis. The ancestral area analyses for these six events suggest that dispersal was the likely origin and this is substantiated in the high dispersal ability of the genus, with its wind-dispersed achenes.

Aridification in the trans-Himalaya has also played a role in the evolution of some lineages of *Clematis*. The species best described as West Himalayan elements, those with their easternmost occurrences at 84° E (*C. graveolens* & *C. grata*), and the Nepalese endemics with restricted distributions in W Nepal, (*C. phlebantha* & C. *tibetana* subsp. *brevipes*), have all evolved as a consequence the two stage aridification of the Asian Interior in the late Miocene and then in the Pliocene (Sun *et al*. 2015) was hypothesised to begin and linked to region uplift of the Qinghai-Tibetan plateau and the Himalaya.

Berberis

Berberis is a large genus of 450-500 woody deciduous and evergreen shrubs with important centres of diversity in Eurasia, mostly in the Himalaya and China, with c. 300 species and South America with c. 200 species. The recent revision of Nepalese *Berberis* by Adhikari *et al.* (2012) recognised 21 species.

A continental-scale study of *Berberis* (Adhikari, 2010 and Adhikari *et al*. 2015) showed at least four different events leading to the extant diversity of *Berberis* in Nepal. A western Nepalese species, *B. petiolaris*, appear to be from a lineage that is Eurasian in origin, and in the east of Nepal there appears to be a mixture of immigration and emigration events between Nepal and southwest China and one in-situ rapid radiation of 14 species in Nepal, with 10 species being restricted to the arid west of the country.

A clade of evergreen species of *Berberis* from Nepal and China belonging to section *Wallichianae* probably evolved, or at least began to radiate, in the eastern Himalaya. The clade contains the Nepalese species *Berberis insignis* and *B. wallichiana* which grow on the forest floor of oak-laurel forests in eastern Nepal. These forests receive more than 2000 mm of monsoon precipitation annually. The mean age of this clade was 5 Ma (95% HPD: 1.3 - 9.8 Ma) suggesting that the Himalaya had achieved sufficient height and was receiving a high level of monsoonal rains by the Miocene-Pliocene boundary.

The large clade of deciduous Nepalese species appears to be due to a recent rapid species radiation. This group began to diversify in Nepal 8.8 Ma (95% HPD: 4.1 - 14.5 Ma), possibly driven by the creation of new habitats, environmental conditions and isolation associated with an intense period of uplift. Ten of the 14 *Berberis* taxa found in this clade show a preference for more xeric areas in western Nepal with annual monsoon precipitation of less than 800 mm, where the strength of the monsoon has faded and the trans-Himalaya, where the rain shadow effect is evident. These species have adapted to the seasonality of the monsoon by being deciduous and only come into leaf when the monsoon rains begin. In general *Berberis* species show a preference for mesic habitats, but the restriction of this clade to drier habitats indicates a shift in their ecology driven by the orogeny and consequent reduction of the impact of the monsoon. However without better resolution in the tree topology it cannot be definitively said if the remaining four widespread taxa in the clade represent the base of the clade or are evidence of a secondary shift from xeric back to a mesic habitat preference.

Adhikari (2010) also suggested an adaptation to calcareous soil type associated with the drying of the Tethys Sea in the trans-Himalaya may be the evolutionary driver behind the divergence of *Berberis mucrifolia*.

As evidenced from *Berberis* the ability to immigrate to new habitats and diversify in-situ relied the ability to disperse and there are examples of long distance dispersal being responsible for some of the species present in the Himalaya.

Long distance dispersal

Clematis

There is also evidence of long distance dispersal having a role in creating lineages that have established in the Himalaya. The wind-blown achenes of *Clematis* ensure that is high potential for long distance dispersal to play a role in diversification, and this supported by the results of the S-DEC analysis in Chapter 4. *Clematis phlebantha*, which is restricted to the NW of Nepal, and *C. potaninii*, from the eastern edge of the Qinghai-Tibetan plateau, are both found in semi-arid habitats, but some 1500-2000 km apart. This clade, although not strongly supported, dates to the mid-Pliocene and suggests a long distance dispersal event. The restricted distribution of *C. phlebantha* suggests that it has become ‘trapped’ in a small ecologically suitable pocket.

With the Himalayan *C. montana* and E Asian *C. chrysocoma* it was again suggested that their dispersal ability has led to the evolution of this clade as it migrated from E Asia at the Pliocene-Pleistocene boundary (ca. 2.7 Ma), with further diversification between Tibetan and Himalayan clades of *C. montana* in the Pleistocene. As discussed in the previous chapter, *C. montana* appears to have a wide ecological tolerance, and has an extensive distribution from the east to the west of the Himalaya and the greatest altitudinal range (1300-4900 m) of any of the Nepalese species of *Clematis*. This apparent ecological tolerance has allowed *C. montana* to effectively colonise a large area rather than become ‘trapped‘, as appears to have happened to *C. phlebantha*.

This pattern of long distance dispersal generating Himalayan lineages also appears in *Ranunculus*.

Ranunculus

Ranunculus is a cosmopolitan genus with a global distribution from the arctic to the tropics, and is especially diverse in temperate areas. In the Himalaya *Ranunculus* has had two independent colonisation events (Emadzade *et al.* 2011, Hörandl & Emadzade 2011). *Ranunculus makaluensis* originated from an ancestor in the eastern Irano-Turanian region about 3.9 Ma. The species is a geographically isolated local endemic of the Makalu glacier region in Nepal, growing at c. 4000–4500 m. Emadzade *et al.* (2011) hypothesised that a long distance dispersal event was the likely origin of this species as the clade contained no other Himalayan relatives.

The other Himalayan alpine species are nested in a clade, which is sister to Arctic, lowland European, and North American species. They arrived in the late Pliocene early Pleistocene (3.5 Ma) and began to radiate in the Himalaya. This suggests that Quaternary glaciations may have isolated the Himalayan group and they were able to diversify into the new habitats opening up in the Himalaya. It was also noted that the Himalayan alpine species are not related to the lowland Himalaya species which appear in a number of clades (Emadzade *et al.* 2011, Hörandl & Emadzade 2011).

Ranunculus appears to show good evidence for the Himalaya's connections with other mountain floras. Altai and Tian-Shan mountain species, like the Himalaya, appear to have had two independent immigration events (Emadzade *et al.* 2011, Hörandl & Emadzade 2011). The first clade is of Arctic origin and the second with species that extend into the Himalaya, which is noteworthy as the Altai and Tian-Shan and W Himalaya are classically thought to be strongly Irano-Turanian in floristic composition (Emadzade *et al.* 2011, Hörandl & Emadzade 2011). Two mountain species from Taiwan are nested within the Himalayan clade and suggest a long-distance dispersal event from the Himalaya to subtropical mountains, carried by the south-westerly winter winds or or dispersal through some form of zoochory.

More recent speciation events

Koenigia

Glacial cycles undoubtedly play a significant part in recent speciation in the region, with some studies showing evidence for this. Fan *et al.* (2013) reported a split between the Himalayan *Koenigia* and the single Arctic species *K. islandica* dating to 1.39 Ma (95% HPD: 0.4-2.62 Ma) triggered by Pleistocene glacial cycles. With the increased sampling by Scherrenberg (2014) of to include increased sampling of *K. islandica* the reassessment of the dataset puts the estimated date of the crown of the *K. islandica* clade to 3.3 Ma towards the end of the Pliocene, with subsequent diversification of linegaes in the Pleistocene. Fan *et al*. linked the wide range of *K. islandica* to its ability to effectively disperse and colonise new habitats through its short, fast lifecycle coupled with being a polypoid species which is capable of dealing with the harsh environmental conditions associated with glacial cycles.

Meconopsis

A more recent evolutionary event is recorded in the aggregate of species that form the high altitude species *Meconopsis horridula*. This species has been recently taxonomically split into numerous species by Grey-Wilson (2014). The large *M. horridula* clade 1 (figure 5.1) corresponding to accessions from the Tibetan Plateau and mountains of SW China and the smaller sister clade *M. horridula* clade 2 contains a basal lineage from the Tibetan plateau and the derived lineages from the Himalaya. The split of clades 1 and 2 dates from 4.68 Ma Pliocene and the diversification of the derived Himalayan lineages are at 1.6 Ma in the Pleistocene, suggest the Himalayan lineages are the result of Pleistocene glacial cycles.

Lathyrus

An example of a more recent speciation event can be found with the western Himalayan *Lathyrus emodi* and the central Asian *Lathyrus gmelinii* (Schaefer *et al.* 201). These species appear to have originated from an Irano-Turanian lineage since the late Plieocence- Early Pliestocene (c. 2.8 Ma). Six morphologically similar derivative species are found throughout western Eurasia as upland mesic forest marginals, with *L. emodii* and *L. gmelinii* lineages splitting in the last 0.1 - 1 Ma. The timing of this divergence again suggests Pleistocene glaciation and climatic shifts rather than Orogeny are the key factor, but this is a single example that could be further tested with more sampling.

Conclusion

Untangling the interconnected roles of uplift and paleoclimate and the effect they have on speciation in the Himalaya is in its early stages, but clearly there is no one driver of speciation in the Himalaya. The Himalaya are a complicated and dynamic mountain system, and as new environmental conditions and habitats have developed through uplift, increasing altitude, changes in the monsoon and recent periods of glaciation, their role in the evolution of plants will have been equally complicated. It is impossible to describe this complicated and incomplete picture, but this chapter has given an overview of the main themes driving biodiversity in the Himalaya. It is hoped that our understanding of this system will only increase as more data becomes available, and as molecular dating techniques become more sophisticated and the fossil record is reassessed, phylogeographic studies in the Himalaya will shed more light on their role in the evolution of the region's plant diversity.

As the geological processes shaped the Himalaya this will have shaped the evolution of groups with limited dispersal ability which were already in-situ, like *Meconopsis*. Patterns found in the *Discogyne* clade suggest that they become isolated as the valley systems developed around them, and ultimately speciated.

Groups like *Begonia* and *Meconopsis* with their preference for mesic habitats have evolved in the Himalaya in the presence of the monsoon.

Plants with effective means of dispersal, like *Berberis* and *Clematis,* will have been able to establish in the new habitats once they have reached the Himalaya. The species of *Clematis* restricted to the west all seem to show a preference of areas of reduced monsoon rainfall, with one species and one subspecies *Clematis tibetana* subsp. *brevipes* and C. *phlebantha* endemic to the semi-arid trans-Himalayan NW of Nepal. *Berberis* demonstrated a clear shift from a mesic preference to a preference for arid habits in a clade of endemic Nepalese species. There is also evidence of long distance dispersal in *Clematis phlebantha* and *Ranunculus makaluensis* with these endemic Himalayan species closely related species well outside the Himalaya.

This study has also demonstrated that evolutionary age estimates and the corresponding interpretations based on dated geoscientific literature must be treated with caution as demonstrated with *Clematis* and *Koenigia* and to an extent *Meconopsis.* The reassessment of these studies using Magallón *et al.*'s (2015) recent calibration of evolutionary events in the angiosperms roughly doubled the age of events deep in the *Clematis* and *Koenigia* phylogenies.

Chapter 6: Final conclusions and further work

Taxonomy of Nepalese *Clematis*

This study recognises 21 species of *Clematis* as occurring in Nepal, compared to 28 species in the *Annotated Checklist of Flowering Plants of Nepal* (Press *et al*. 2000). Of those 21 species three are endemic. Two species were not formally treated in the account, one non-native species that is cultivated in Nepal and one species only known from the type specimen in KYO herbarium, which was not seen.

Fieldwork in the Mid-West of Nepal 2014 a population *C.confusa* in Rukum District. Herbarium work for the revision also found a new species record for Nepal, *C. kilungensis,* previously placed into synonymy with *C. barbellata*, which has been collected on a number of occasions in Solu Khumbu District near Namche Bazar. These specimens had been previously identified as *C. barbellata*, but also misidentified as *C. montana* and *C. tibetana*.

Molecular evidence was used to inform some of the taxonomic decisions surrounding the broad taxonomic treatments of the *Clematis buchananiana*, *C. connata* and *C. grewiiflora,* which are all in section *Connatae*. The synonymies of these three species include many names because of a lack of good characters to differentiate previously accepted species. This was mirrored in the lack poor resolution to support this group on the phylogenetic trees, suggesting that this group is actively speciating.

The use of species distribution models (SDM) also supported the previous taxonomic decision to split the Nepalese endemic *Clematis tibetana* subsp. *brevipes* from the E Tibetan *C. tibetana* subsp. *vernayi* by Yonekura (2008). Although his decision was based on differences in anther length and the geographical distance between the Nepalese and Tibetan populations, the SDM clearly showed an ecological difference between the two subspecies in terms of the ecological niches they inhabit.

The molecular part of this study clearly supports the monophyly of *Clematis* and there is good evidence from this and previous studies (Miikeda *et al*. 2006, Xie *et al*. 2011) to support a broad circumscription of the genus to include *Clematopsis*, *Archiclematis* and *Naravelia*. This study corroborates the criticisms of Miikeda *et al*. and Xie *et al*. that the current infrageneric taxonomy does not reflect evolutionary relationships. As stated earlier, without fuller sampling of the throughout its distribution, the last major gap being S America, and better resolution of relationships at the base of the *Clematis* clade it would be premature to realign that taxonomy.

The evolution of tribe *Anemoneae*

The phylogenetic patterns of the *Anemoneae* resolve to two strongly supported clades supporting previous studies. The first clade consists of the subtribe *Anemoninae* which contains *Anemone* including the genera *Hepatica* and *Pulstatilla*, previously placed into synonymy. The second clade corresponds to subtribe *Clematidinae* which comprises a basal lineage of the monotypic genus *Anemonclema*, and sister to that the genus *Clematis*, including the genera *Clematopsis*, *Archiclematis* and *Naravelia*, all previously placed into synonymy.

This study has estimated the evolutionary age of *Anemone* for the first time to the mid-Eocene at 47.62 Ma (95% HPD: 34.63 - 63.42 Ma) from the Anderson constrained analysis or 43.88 Ma (95% HPD: 32.71-55.02 Ma) from the Magallón constrained analysis.

The evolutionary age of *Clematis* was estimated at the Middle Miocene in the analyses of the *Anemoneae* and *Clematis* datasets. The estimates of evolutionary age from this study are older than the age reported by Xie *et al*. (2011) which dated to the late Miocene, and this difference is attributed to the selection of different priors used to constrain phylogenies.

The ca. 300 species of *Clematis* appear to have had been generated as part of a rapid evolution in the last ca. 16 Ma, in comparison to ca. 150 species of *Anemone* in the last ca. 40 Ma. The most obvious differences in terms of morphology is the predominant life form in *Clematis* is secondary woodiness in the stems in most species compared to the herbaceous habit of *Anemone*, and the development of wind-blown achenes through an elongated, plumose and persistent style in *Clematis*. This achene characteristic appears to have arisen independently in a clade within *Anemone*, the species formerly in *Pulsatilla*. This apparent shift the rate in the generation of species could be investigated further.

Phylogeography of *Clematis* in Nepal

The diversity in Nepal appears to be the result of at least six immigration events as all dates of these nodes postdate the uplift of the Himalaya, but this may well be an underestimate as 11 species, 50%, of *Clematis* from Nepal could be included in the analysis. The ancestral area analyses for these six events suggest that dispersal was the likely origin, which fits well with the high dispersal ability of the genus, with its wind-dispersed achenes.

The species of *Clematis* best described as west Himalayan elements, *Clematis grata*, *C. graveolens* and the two which are also endemic to Nepal, *C. phlebantha* and *C. tibetana* subsp. *brevipes*, showed a preference for semi-arid habitats, as observed in the field, inferred from climatic niche modelling and in some cases indicated by their morphological adaptations. Their evolutionary dates of origin also correspond broadly to the Late Miocene dates of Asian interior aridification hypothesised by Sun *et al*. (2010; 2015). All of this suggests that the adaptation to aridification has driven diversification in some Nepalese *Clematis* species.

The general trend appears to be for a westward migration with Himalayan *Clematis* derived from E Asian species. This is illustrated well by *Clematis montana* in section *Cheiropsis* subsection *Montanae* which is widespread in the Himalaya and sister to Tibetan species *C. chyrsocoma*. The widespread species of section *Connatae* are poorly resolved but also indicate a westward migration from E Asia to the Himalaya, as can be seen with *C. grata* and *C. phlebantha*.

Although not fully tested because of how unresolved section *Meclatis* is in the phylogeny, *C. tibetana* subsp. *brevipes* from NW Nepal in the trans-Himalaya and *C. tibetana* subsp. *vernayi* from the east of the Tibetan plateau display an east west disjunct with differing ecological niches. The origin of *C. tibetana* subsp. *brevipes* suggests hybrid origin from different chloroplast sequences in the two accessions.

Phylogeography and Dynamics of Speciation in the Himalaya

Currently there is no clear picture in the geoscientific literature to explain the history of the Himalaya. There are competing hypotheses about the timings of the collision of India with Asia, the subsequent event surrounding the Tibetan uplift and mountain building that created the Himalaya. This is also the case in the paleoclimate literature for the dates of monsoon initiation and development, which are also disputed. All these competing hypotheses offer a range of possible dated events with which to link possible evolutionary scenarios and of course lead to a generally inconsistent picture.

The molecular phylogeny dating methods continue to develop and becoming more sophisticated, but this study demonstrates that the choice of priors with which to constrain analyses can have a large impact on the estimated ages of the evolutionary events. The revised ages from the latest work on the evolution of the angiosperms (Magallón *et al*. 2015) and expanded datasets had the effect of doubling the ages of crown nodes of *Clematis* and *Koenigia*.

At the moment the picture of what has driven the diversity in the Himalaya is far from clear, as there are too few well resolved and accurately dated phylogenies to work with. At present there is evidence of long distance dispersal into the Himalaya from both the east, e.g. *Clematis phlebantha* and the west, e.g. *Ranunculus makaluensis*. There is evidence of hybridisation from chloroplast capture in *Clematis tibetana* subsp. *brevipes*. The immigration of a lineage of *Berberis* to the Himalaya followed by a subsequent diversification appears to be in response to a shift in habitat preference from mesic to semi-arid. The species of *Begonia* and *Meconopsis* show a strong preference for mesic habitats and their diversifications in Himalayan clades appears to be related to the development of the monsoon. *Meconopsis* species have limited dispersal ability and so their evolution appears to have also been driven by vicariance and subsequent isolation in valley systems. There is still much to be resolved about the evolutionary history of the Himalaya and it is hoped that our understanding of how the region's biodiversity has been generated will develop as we gain new insights into its geological history, the development of its climate and the phylogenetic relationships of species that grow in this mega-diverse mountain range.

Publications from this PhD

A taxonomic account of *Clematis* in Nepal will be published in the *Flora of Nepal* volume 2 and will be available online through the www.floraofnepal.org. The new species from Oman with the provisional name *Clematis hajarensis* will be published in the *Edinburgh Journal of Botany*.

A paper on highlighting the issues and inconsitencisy with the competing infrageneric taxonomies for *Clematis* and the lack of phlyogentic support for any of them is another possible publication. This paper would propose a much simplified infrageneric taxonmy more in line with broad species groupings suggested by the phylogeny.

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