Systematics and Phylogeographic studies of *Berberis* L. (Berberidaceae) in the Nepal Himalaya

Bhaskar Adhikari

Doctor of Philosophy
The University of Edinburgh
Royal Botanic Garden Edinburgh
2010

Declaration

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

Bhaskar Adhikari

Abstract

Berberis L. contains more than 500 species, is the largest genus in the Berberidaceae, and is now recognised to comprise *Berberis s.s.* with simple leaves and compound-leaved species formally ascribed to *Mahonia*. Because of its sheer size, much basic taxonomy is required in *Berberis*, and this thesis provides a taxonomic revision for the species found in Nepal, which includes a key, full descriptions, illustrations, phenology, notes on ecology, distinguishing features and distribution maps. Twenty one species are recognised in Nepal, of which two, *B. karnaliensis* and *B. pendryi*, are newly described. Nine taxa are lectotypified during this study.

Berberis has a mainly Northern Hemisphere distribution, with its main centre of distribution in the Sino-Himalaya. *Berberis s.s.* extends into South America where it has a secondary centre of diversity. There have been few phylogenetic studies of *Berberis*, and previous studies were inadequate because they did not use outgroups to root their phylogenies. This thesis provides parsimony and Bayesian analyses of chloroplast *ndhF* data of 64 accessions of 59 *Berberis* taxa, rooted using multiple outgroups including *Ranzania*, the putative sister group of *Berberis*. The results support the monophyly of *Berberis s.l,* but compound-leaved *Berberis* are shown to be paraphyletic. *Berberis higginsae*, a member of section *Horridae*, is sister to all other taxa sampled. The *ndhF* phylogeny, dated using a fossil calibration, indicates the North American origin of compound-leaved *Berberis*, and the origin of the simple-leaved South American lineages in long distance dispersal events from Eurasia.

ITS sequences of 97 accessions of 79 species of *Berberis* are studied to investigate the origin and diversification of simple-leaved *Berberis* in Nepal. The Nepalese species are shown to have multiple origins from at least four different colonisations and have subsequently speciated in situ. The diversification of some groups appears to have been triggered by the active uplift phase of the Himalaya during the Miocene. The tendency of phylogenetically close Nepalese species to grow in similar ecological conditions indicates that phylogenetic niche conservatism is evident in Nepalese *Berberis* species.

Chromosome counts for nine taxa of Nepalese simple-leaved *Berberis* are obtained, of which five taxa are counted for the first time. All the taxa examined are diploids with 2n=28, suggesting that the polyploidy is not an important factor in the diversification of *Berberis* in Nepal.

Acknowledgements

It gives me great pleasure to express my deep sense of gratitude to my supervisors Dr. Toby Pennington, Dr. Colin Pendry and Dr. Richard Milne for their continuous help, encouragement and guidance during the research period.

My special thanks to Dr. Mark Watson for his suggestions, expert advice and encouragement. I would also like to thank to my previous supervisor Dr. Krishna K. Shrestha for his continuous encouragement and support since the beginning of my career in the field of plant taxonomy.

There are many people at the Royal Botanic Garden Edinburgh who helped me during this project. I am indebted to Dr. Michael Moller and Dr. Kwiton Jong for their invaluable advice and guidance on cytological work. I thank David Knott for his help with living collections of *Berberis* and Clare Morter who looked after the *Berberis* living collections in the glass house.

Thanks are also due to Dr. James Richardson, Tiina Sarkinen and Daniel Thomas for helping me with phylogenetic software. I would like to thank laboratory staff Dr. Michelle Hollingsworth and Alexandra Clark for their help on molecular work, and also Frieda Christie and Ruth McGregor for their assistance in the use of chemicals.

I am indebted to Dr. John David, Royal Horticultural Society (RHS), for his advice and help during this project.

I am heartily grateful to all my friends at Royal Botanic Garden Edinburgh, Kate Armstrong, Tobias Marczewski, Nicola Burton, Chantel Davies, Sumudu Rubasinghe, Rhiannon Crichton, Alex Twyford, Jane Droop, Carmen Puglisi, Emma Goodyer, Paulina Hechenleitner, Mobina Shaukat Ali and Saima Umbreen for their kind cooperation and assistance in various aspects during this project. I am especially grateful to Kate Armstrong for careful proofreading of this thesis and also for sharing her ideas on Nepalese plants.

I am indebted to Julian Harber for sharing his expertise on *Berberis* and also for providing some samples of *Berberis*.

Thanks are also due to Hari B. Chhetri for providing some useful literatures and silica gel dried DNA samples and also Kailash Nepal and Ram Chandra Paudel for their companionship, help and support. I am also grateful to expedition group members for their kind cooperation and help during field trips to different parts of Nepal.

I would also like to thank to the curators and staff of the herbaria of BM, K, KATH, TI and TUCH for granting permission to study the herbarium specimens and for their help during my visit.

My special thanks to my parents, my brother and sister for their support and encouragement.

Last but not least thanks to my beloved wife Anshu for her continuous support and care. Thank you so much!

Financial support for this project has been kindly provided by The University of Edinburgh (Torrance Bequest), Royal Horticultural Society (RHS) and the Royal Botanic Garden Edinburgh (RBGE).

Contents

CHAPTER 2: A REVISION OF *BERBERIS* S.S. (BERBERIDACEAE) IN NEPAL

CHAPTER 3: SYSTEMATIC AND BIOGEOGRAPHIC PATTERNS OF BERBERIS S.L. INFERRED FROM CHLOROPLAST NDHF GENE SEQUENCES

CHAPTER 4: ORIGIN AND DIVERSIFICATION OF *BERBERIS* IN THE NEPAL HIMALAYA: INSIGHT FROM NUCLEAR RIBOSOMAL ITS SEQUENCES

CHAPTER 5: CHROMOSOME COUNTS FOR NEPALESE SPECIES OF SIMPLE-LEAVED *BERBERIS*

CHAPTER 6: FINAL CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE WORK

APPENDIX 1

APPENDIX 2

APPENDIX 3

List of figures

CHAPTER 2

CHAPTER 3

CHAPTER 4

CHAPTER 5

List of tables

CHAPTER 2

CHAPTER 3

CHAPTER 4

CHAPTER 5

CHAPTER 1: INTRODUCTION

Large genera of plants, usually defined as those containing 500 or more species, offer an excellent opportunity to study the generation and maintenance of biodiversity (Mabberley, 1997, 2008). However, genera of this size often represent taxonomic black holes (Landrum, 1999), since to understand them takes a single researcher many years of work (Frodin, 2004) or a large team which needs considerable funding, for example, US National Science Foundation (NSF) funding for teams to monograph *Solanum* and *Euphorbia.* An alternative approach to the taxonomic study of mega-diverse genera is to focus on limited areas of their distribution (Landrum, 1999). This thesis takes the geographic approach in *Berberis* L. (Berberidaceae), which has *ca*. 600 species (including compound-leaved *Mahonia*) (Ahrendt, 1961), by focussing on the species that grow in Nepal.

Berberis is a prominent genus growing in most vegetation types throughout Nepal from 1000-4600 m. The taxonomy of Nepalese *Berberis* is poorly understood, with considerable disagreement on the number of species growing in Nepal. Tebbs (1979, p 29) enumerated 30 species and 12 varieties of *Berberis* from Nepal but indicated that "A critical revision of this genus is urgently needed based on the abundant material recently collected in Nepal and adjacent regions". This thesis provides a thorough revision of Nepalese species of *Berberis s.s.* (simple-leaved) based upon both field and herbarium study with keys, type information, distribution maps, detailed descriptions and illustrations.

Berberis s.l. comprises two groups: simple-leaved species of *Berberis* (*Berberis s.s.*) and compound-leaved species of *Berberis* (commonly known as *Mahonia*). *Berberis s.s.* has two important centres of diversity, Asia and South America, and compound-leaved species mainly grow in Asia and North America. The origins of disjunction patterns within these groups are not well understood. This thesis presents a phylogenetic investigation of *Berberis s.l.* based on chloroplast *ndhF* sequences to examine the relationships between the simple-leaved and compound-leaved species of *Berberis*, and the disjunction patterns in *Berberis s.l.*, especially the origin of its intriguing antitropical distribution.

The effect of mountain building events in the evolution and diversification of plant groups has not been studied so far in the Nepal Himalaya. *Berberis* is an ideal group to study the effect of the Himalayan orogeny on plant diversification as it grows in wide range of habitats including forest floor, forest margin, open pastures at higher altitudes, semi-desert vegetation, and the margins of cultivated land. Some species of *Berberis* in Nepal are widespread whilst others are much more restricted both geographically and altitudinally (Mouri *et al.* 2004). Moreover, they grow in wetter parts of eastern Nepal to drier regions of trans-Himalayan regions, which makes *Berberis* an ideal case study to investigate the role of switching between biomes in diversification. The phylogenetic analysis using nuclear ribosomal ITS sequences in this thesis provides an important framework to understand the role of Himalayan orogeny in diversification of *Berberis* in the Nepal Himalaya, the number of independent lineages within the genus which are found in Nepal, and the influence of ecology on *Berberis* diversification.

The final area of research in this thesis is chromosome counts for some species of Nepalese *Berberis* to investigate the possible role of polyploidy in the diversification of *Berberis* species in the Nepal Himalaya. This study was motivated by the suggestion that during the Quaternary, the Himalayas were one of the most active regions in the world for the speciation of flowering plants (Yoshida 2006). Plants capable of surviving glacial cycles must be able to adapt to their changing environment, and one potential means of adaptation is through polyploidy, which is one way of increasing diversity in plant forms. Understanding the ploidy level in *Berberis* species is also important for interpreting molecular phylogenies of *Berberis* because polyploidy can complicate phylogenetic inferences.

In summary, the principal aims of this thesis are:

- To provide a taxonomic revision of *Berberis s.s.* in Nepal including identification keys, full descriptions, illustrations and distribution maps.
- To use chloroplast *ndhF* sequence data to examine the relationship between simple and compound-leaved species of *Berberis*, and the global disjunction patterns in *Berberis s.l.*
- To use nuclear ribosomal ITS sequence to investigate the origin and diversification of simple-leaved species in the Nepal Himalaya.

- To count the chromosome numbers of Nepalese *Berberis* to investigate the possible role of polyploidy in the diversification of *Berberis* in the Nepal Himalaya.

CHAPTER 2: A REVISION OF *BERBERIS* S.S. (BERBERIDACEAE) IN NEPAL

2.1 INTRODUCTION

The family Berberidaceae consists of the species-rich woody genera *Berberis* with a few species-poor herbaceous genera. The Berberidaceae is a morphologically diverse family which led some authors to segregate the genera into small families. For example, several authors (Hutchinson, 1959; Airy Shaw, 1965; Takhtajan, 1969) recognized small families such as Nandinaceae (*Nandina*), Podophyllaceae (*Podophyllum*) and Berberidaceae (*Berberis*, *Mahonia*, *Epimedium*). APG II (2003) and APG III (2009) placed all these small families into the Berberidaceae. The family is represented by 14 genera and about 715 species (Mabberley, 2008). In Nepal, the Berberidaceae is represented by *Berberis* (including *Mahonia*) and *Podophyllum*.

The name *Berberis* derives from '*Berberys*', the Arabian name for the fruit (Quattrocchi, 1947). *Berberis* is the largest genus in Berberidaceae with *ca*. 600 species (including *Mahonia*) (Ahrendt, 1961). There are conflicting views on generic delimitation in *Berberis* and *Mahonia*. Ahrendt (1961) postulated that simple-leaved *Berberis* are derived from the compound-leaved *Mahonia* and described *Mahonia* as a distinct genus. Studies examining chromosome number (Derman, 1931), floral anatomy (Terabayashi, 1978) and seedling morphology (Terabayashi, 1987) found that there were no significant differences between *Berberis* and *Mahonia*. Kim and Janson (1994) reported the sharing of a 11.5 kb expansion of the inverted repeat (IR) region of the chloroplast genome between *Berberis* and *Mahonia* which suggests a close phylogenetic relationship. Laferriere (1997) transferred all the species of *Mahonia* to *Berberis*. As *Mahonia* is not included in this revision the term *Berberis sensu stricto* is used to refer simple-leaved *Berberis*.

Berberis s.s has two important centres of diversity, Eurasia with *ca.* 300 species mainly in the Himalayas and in China, and South America with *ca.* 200 (Ahrendt, 1961). The actual number may be smaller as Landrum (1999) recognized only 20 out of the 60 species reported by Ahrendt (1961) from Chile. Landrum (1999) reduced the number of *Berberis* species in Chile through synonymy. A notable example is the merging of three species (*B. microphylla*, *B. buxifolia* and *B. heterophylla*) from three different sections under *B. microphylla*.

There are conflicting views regarding the number of species growing in Nepal because of the lack of critical revisionary work and taxonomic problems within the genus. Different authors reported different figures, with Tebbs (1979) listing 30 species and 12 varieties; Bista *et al.* (2001) reporting 36 species and 20 varieties; and Press *et al*. (2000) recording 30 species and 13 varieties. This chapter aims to provide a thorough revision of *Berberis* for Nepal in order to resolve this situation of taxonomic uncertainty.

2.2 TAXONOMIC HISTORY

2.2.1 Taxonomic history of Nepalese Berberis s.s.

The genus *Berberis* was first described by Linnaeus in *Species Plantarum* in 1753. The first species of *Berberis* collected from Nepal was *B. aristata*, by Nepal's earliest plant collector Dr Francis Buchanan (later Hamilton) in 1802. *Berberis aristata* was published in De Candolle's *Regni Vegetabilis Systema Naturale* in 1821 along with another Himalayan species, *B. asiatica.* At that time, De Candolle included a total of 29 species of *Berberis* from all over the world in his *Regni Vegetabilis*. In 1824, he listed 32 species of *Berberis* in his *Prodromus* which included the addition of another Nepalese species, *B. wallichiana*, based on Dr Nathaniel Wallich's specimens.

Using the Buchanan and Wallich specimens, D. Don produced the first account of the plants of Nepal, *Prodromus Florae Nepalensis* in 1825, in which he mentioned only the three species of *Berberis* which had already been published by De Candolle in 1824. Altogether 43 species of *Berberis* from all over the world were included in G. Don's *A General History of the Dichlamydeous Plants* in 1831 which listed seven species from Nepal. Almost four decades later, J.D. Hooker and T. Thomson enumerated 12 species of *Berberis* from the Himalayan region in *The Flora of British India* in 1872, among them eight species were reported from Nepal.

The first major account of the genus was published by C.K. Schneider in 1904-1905. In 1941-1942, the number of *Berberis* species increased significantly when L.W.A Ahrendt described new species of *Berberis* from Bhutan, Assam, Southern Tibet, Upper Burma, and N.W. Yunnan and went on to publish his worldwide monograph in 1961. Chamberlain and Hu (1985) provided a synospsis of section *Wallichianae* and recognized 75 species with two new taxa. Among them, most of the species included were from China, and four species were from Nepal.

A revision of the genus from India was first published by Chatterjee in 1953 in which he included 68 species. Later, Rao *et al*. (1998 a,b) revised Indian *Berberis* and recognised 55 species.

2.2.2 History of infrageneric classification of Berberis s.s.

C.K. Schneider (1904-1905) classified the genus into 21 sections, 32 subsections and included 155 species. In 1908, he updated his monograph and also divided the genus into two subgeneric groups, the *Septentrionales* of Eurasia and South American *Australes*. In 1942, he again revised the section *Wallichianae* and recognized 71 species.

In 1941-1942, L.W.A Ahrendt revised the *Berberis* from Bhutan, Assam, Southern Tibet, Upper Burma, and N.W. Yunnan, and published a worldwide monograph in 1961. Ahrendt (1961) classified the genus into 33 sections and 50 sub-sections and listed 496 species (including hybrid species) which included 114 new species described by him.

The validity of Ahrendt’s (1961) infra-generic classification was questioned by Landrum’s (1999) revision of *Berberis* from continental Chile, Juan Fernandez Islands and adjacent southern Argentina in which he merged three species from different sections recognised by Ahrendt under a single name. Landrum recognized only 20 out of the 60 species reported by Ahrendt (1961).

Table 2.1. Nepalese species of *Berberis* in major floras and monographic works.

De Candolle (1824)	**D. Don (1825)**	**G. Don (1831)**	**J.D. Hooker & T. Thomson (1872)**	**Schneider (1904-1905)**	**Ahrendt (1961)**
B. aristata	*B. aristata*	*B. aristata*	*B. angulosa*	*B. angulosa*	*B. angulosa*
B. asiatica	*B. asiatica*	*B. asiatica*	*B. aristata*	*B. aristata*	*B. aristata*
B. wallichiana	*B. wallichiana*	*B. ceratophylla*	*B. asiatica*	*B. asiatica*	*B. asiatica*
		B. floribunda	*B. concinna*	*B. chitria*	*B. ceratophylla*
		B. petiolaris	*B. insignis*	*B. concinna*	*B. chitria*
		B. umbellata	*B. umbellata*	*B. hookeri*	*B. concinna*
		B. wallichiana	*B. vulgaris*	*B. insignis*	*B. duthieana*
			B. wallichiana	*B. jaeschkeana*	*B. everestiana*
				B. koehneana	*B. floribunda*
				B. kumaonensis	*B. glaucocarpa*
				B. orthobotrys	*B. hamiltoniana*
				B. thomsoniana	*B. hookeri*
				B. umbellata	*B. insignis*
				B. virescens	*B. jaeschkeana*
				B. wallichiana	*B. koehneana*
					B. kumaonensis
					B. mucrifolia
					B. orthobotrys
					B. parisepala
					B. petiolaris
					B. poluninii
					B. sikkimensis
					B. tsarica
					B. umbellata
					B. virescens
					B. wallichiana
					B. walterana

Ahrendt's species concept was unrealistically narrow and delimitation of his species was based on dubious characters such as the colour of stems and margin of leaves. The true number of *Berberis* species worldwide is likely to be significantly lower than the 496 he reported.

2.3. MATERIALS AND METHODS

This revision is based on a study of *ca.* 700 herbarium specimens, and field observations of wild populations of *Berberis* in Nepal. Field work was conducted in Western, Central and Eastern parts of Nepal (Appendix 1). Among 21 species recognized in this study, 19 species were collected and studied in the wild. All herbarium specimens collected during the field visits were deposited in the herbarium of the Royal Botanic Garden Edinburgh (E) and the National Herbarium in Kathmandu (KATH), Nepal.

Study visits were conducted to herbaria with major holdings of Himalayan specimens (BM, K and KATH). Loans were received from BM and herbarium specimens were gifted from Tokyo University (TI). Some European herbaria (G, WU) were also consulted for the images of type specimens but most of the important collections from Himalayan regions were found to be deposited in BM, K and E. Specimens from other neighbouring areas (India, Bhutan and China) were also examined for specific taxa.

Data from herbarium labels of all *Berberis* specimens and associated information were entered into the PADME database developed at the Royal Botanic Garden Edinburgh (RBGE). PADME is a Microsoft access database designed to manage information required to carry out floristic and monographic studies. It allows the user to enter all specimen data along with nomenclature, literature, geography, associated images and type information. The stored data can be easily extracted to generate reports and distribution maps and is also used for the publication of floras and monographs.

All specimens were gathered for each species and a set of the best herbarium specimens were chosen for dissections. Vegetative and reproductive characters were then recorded in an excel data sheet. Morphological characters were examined with a stereo microscope. To examine floral characters, flowers were rehydrated by boiling in water for 5-10 minutes before dissection. Photographs were taken of all dissected parts, and dissections were then pasted on paper and placed in the capsules of the respective herbarium sheets. All flower measurements were made on mature flowers. Recorded characters were then compared and a set of characters which were unique and best described the particular species were chosen for the descriptions, and also to produce dichotomous keys. The final descriptions were then checked and updated with all the available specimens.

Illustrations were made for all species based on herbarium specimens and photographs taken in the field. Distribution maps were produced using Arcview GIS 3.2a based on specimen label geographic data. As the distribution maps are based on the collection localities of available herbarium specimens, they can be improved in the future for under collected species.

2.4 SPECIES CONCEPT

The species is the fundamental component of any taxonomic revision. The process of assigning any individual to a given species largely depends on the criteria by which the species are delimited and defined, which in turn is based on the author's concept of a species. There is a vast and growing literature on species concepts (Eldredge & Cracraft, 1980; Donoghue, 1985; Nixon & Wheeler, 1990; Mayr, 1992; Baum & Donoghue, 1995; Mayden, 2002; De Queiroz, 2007). All these concepts are mainly based on either 'process based' or 'pattern based' approaches.

The process based approach such as the 'biological species concept' mainly depends on the biological processes which shape species. The biological species concept of Mayr (1942) defines a species as: "groups of interbreeding natural populations that are reproductively isolated from other such groups." Although the biological species concept is widely accepted, one of the major problems for the taxonomist is the practical impossibility of studying the reproductive isolation mechanisms and gene flow between the large numbers of populations in the wild.

An alternate approach, the 'pattern based' concept, mainly depends on character patterns as the main source of species delimitation. The most common method used by the classical taxonomist to assign individuals as members of a particular species is based on shared morphological characters unique to the particular species. Numerical taxonomy is also based on the morphological species concept but uses a large number of phenotypic characters instead of only diagnostic ones, and defines species as a cluster derived from a measure of overall similarity (Sneath & Sokal, 1973).

The phylogenetic species concept also depends on the pattern based concept which may be history based (De Queiroz & Donoghue, 1988) or character based (Eldredge & Cracraft, 1980; Nixon & Wheeler, 1990). Character based concepts define species as ‘the smallest aggregation of populations or lineages diagnosable by a unique combination of character states in comparable individuals (Nixon & Wheeler, 1990).

In this revision, I mainly followed the character based morphological criteria to define *Berberis* species. During this study, I had an opportunity to observe populations of most Nepalese *Berberis* species in the wild. To determine the diagnostic characters of a particular species, the morphology of all herbarium specimens was studied along with field observation of *Berberis* species in the wild. The phylogenetic analysis of both nuclear and chloroplast sequence data also helped to some extent in species delimitation. Therefore, the species concept used in this study is close to the phylogenetic species concept of Nixon and Wheeler (1990).

Infraspecific classification in this revision mainly follows Du Rietz (1930) (in Stace 1980) which defines subspecies as “a population of several biotypes forming a more or less distinct regional facies of a species” and variety as “a population of one or several biotypes, forming more or less distinct local facies of a species”. Recommendations made by Stuessy (1990, 2009) were also taken into consideration while defining infraspecific categaories.

2.5 MORPHOLOGY

Habit: All species of Nepalese *Berberis* are woody and range from small shrubs to large tree-like shrubs up to 6 m tall. On the basis of height, Nepalese *Berberis* can be divided into three catogories.

1. Small shrubs rarely exceeding 1 m tall: *B. mucrifolia, B. tsarica, B. pendryi, B. everestiana* var. *ventosa* and *B. kumaonensis*.

2. Shrubs usually more than 1 m and up to 2 m tall: *B. angulosa*, *B. concinna*, *B. jaeschkeana* var. *usteriana*, *B. karnaliensis*, *B. hookeri* and *B. wallichiana*.

3. Large shrubs or small tree-like shrubs usually more than 2 m and up to 6 m tall: *B. aristata*, *B. asiatica*, *B. glaucocarpa*, *B. hamiltoniana*, *B. insignis*, *B. virescens*, *B. petiolaris*, *B. orthobotrys* var. *rubicunda*, *B. koehneana* and *B. thomsoniana*.

Most of the species are erect and profusely branched from the base. *Berberis kumaonensis* is the only exception and is a semi-erect or prostrate shrub.

Stem: Stems are terete, angled or sulcate. They are sometimes classified in two catagories: primary stem (long shoots) and short axillary stems (short shoots). Short shoots are distinct and elongated in some species like *B. petiolaris* and *B. thomsoniana* while very short in other Nepalese species. Mature long shoots are usually reddish brown, ash grey or sometimes yellowish grey, while young twigs are usually green. Twigs which receive more exposure to sun turn reddish brown. Therefore twig colour is variable on a single plant and cannot be taken as a diagnostic character for species delimitation. Young twigs are puberulous in some species, but they are soon glabrescent.

Leaves and spines: Sometimes spines have been interpreted as the reduced leaves of long shoots, and normal leaves as the leaves of short shoots (Landrum, 1999). The shape of spines in *Berberis* varies from leaf like in *B. actinacantha* (South-American *Berberis*) to strong 1-5-fid true spines in many Asian species, while intermediate forms can be found in *B. validiviana* (South-American *Berberis*) and in *B. koreana* (Fig. 2.1). As the spines of all Nepalese species are distinct and strong, and not foliaceous, the terms spines and leaves are used consistently in this revision. Spines are usually present in all Nepalese species. The least spiny individuals with solitary and spineless stems are found in *B. insignis* and *B. petiolaris*. Usually the spines are 3—5-fid and solitary towards the apex of the twigs. Spines in all the Nepalese species are 3-fid only with the exception of *B. tsarica* which has 5-fid spines. They are usually terete, angled or slightly sulcate below. Foliage leaves borne only on short shoots (Fig. 2.2), and are simple and pinnate-veined in all species of *Berberis* s.s. They are arranged in whorls or in fascicles. Petioles are usually very small or indistinct in the Nepalese taxa except in *B. petiolaris* (Fig. 2.3, k), which has a petiole up to 3.5 cm. Leaves are obovate, elliptic, lanceolate, obovate to elliptic or obovate to oblanceolate in shape and their sizes range from 0.5 cm in *B. tsarica* to 17 cm in *B. insignis* (Fig. 2.3).

FIG. 2.1. Foliaceous spine in *B. koreana*.

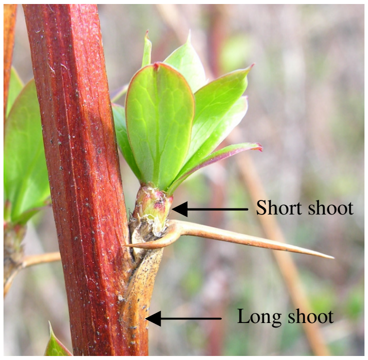


FIG. 2.2 Spine, short shoot and long shoot in *B. thomsoniana*.

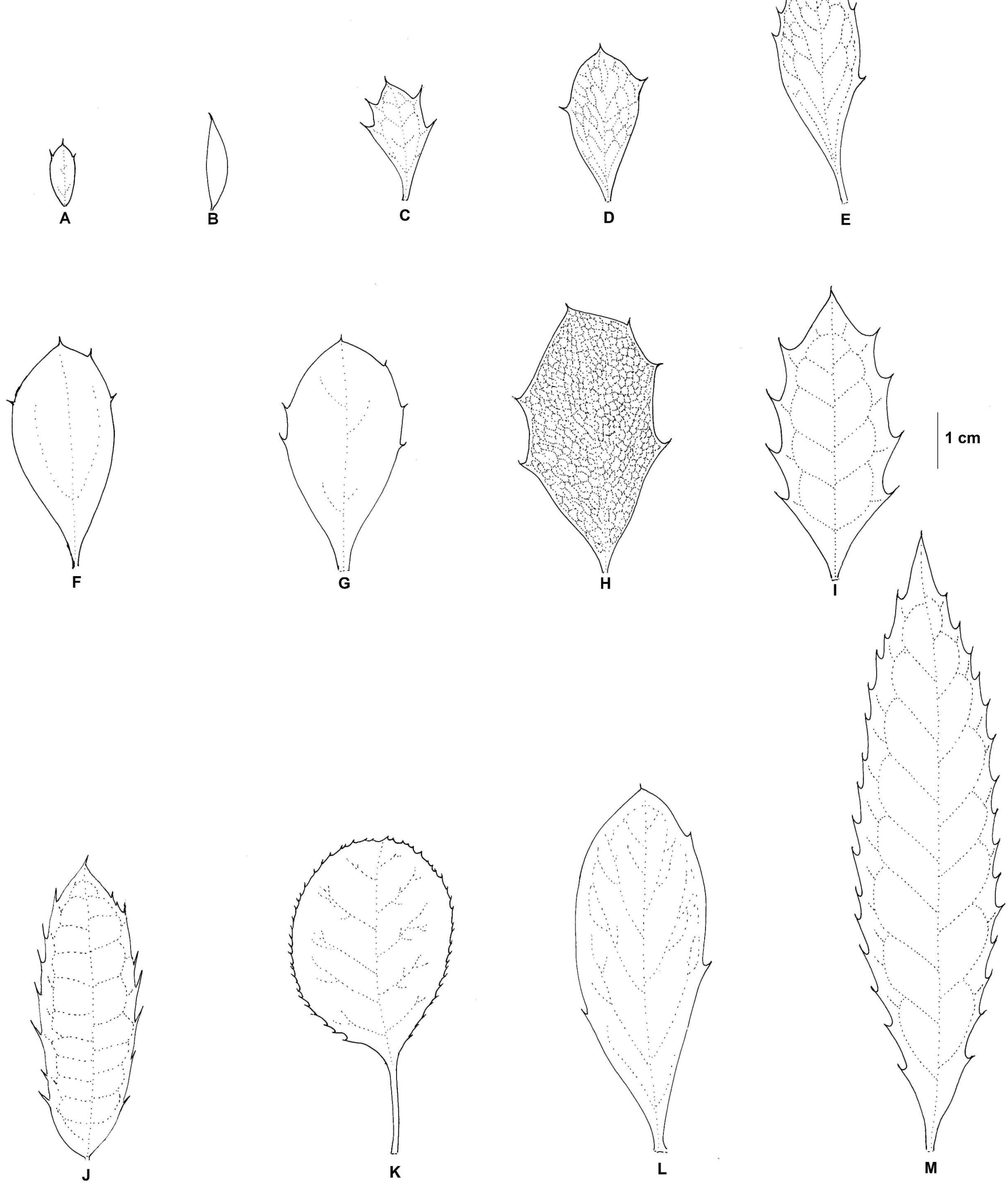


FIG. 2.3. Leaves of various species of *Berberis*. All leaves are drawn at the same scale. A. *B. mucrifolia* (SSW 8111). B. *B. tsarica* (DNEP3 BY132). C. *B. kumaonensis* (JRSB 163). D. *B. concinna* (Manaslu 08 20812277). E. *B. thomsoniana* (LKSRB 181). F. *B. angulosa* (Adhikari BL2 38). G. *B. koehneana* (Adhikari BL2 51). H. *B. asiatica* (Adhikari B105). I. *B. hookeri* (D. Proud 1). J. *B. wallichiana* (Adhikari BL 123). K. *B. petiolaris* (JRSA 122). L. *B. glaucocarpa* (JRSA 134). M. *B. insignis* (EMAK 875).

The apices of the leaves are obtuse to acute and mucronate, and the bases are cuneate or attenuate to the small petiole. The margins are entire or spinose-spinulose toothed and variable within the species. Four types of venation were recognized in Nepalese taxa: few veins which are slightly prominent below and less distinct above (*B. angulosa*, *B. hamiltoniana* and *B. tsarica*); venation prominent on both sides with distinct primary and few secondary veins (*B. aristata*, *B. concinna*, *B. kumaonica*, *B. petiolaris* and *B. thomsoniana*); venation prominent on both sides with reticulate veins (*B. asiatica*) and venation prominent on both sides with distinctly looping closed primary veins (*B. wallichiana*, *B. insignis* and *B. hookeri*). The veins are completely invisible in *B. mucrifolia*. Leaf texture varies from papery in *B. petiolaris* to leathery coriaceous in *B. asiatica.* Leaves are usually glabrous above and papillose below. Some leaves are distinctly glaucous on their lower surfaces of leaves (*B. asiatica* and *B. concinna*).

Inflorescence: The common types of inflorescence are fasciculate (*B. insignis*, *B. wallichiana* and *B. hookeri*), pedunculate racemes (*B. petiolaris*, *B. thomsoniana* and *B. aristata*) and shortly pedunculate or epedunculate racemes (*B. asiatica*). Some intermediate types such as sub-umbellate types of inflorescence are also present in some taxa. Some species (*B. pendryi*, *B. tsarica* and *B. kumaonensis*) have solitary flowers. Bracts are visible and distinct in racemes and panicles but not in fasciculate inflorescences or with solitary flowers.

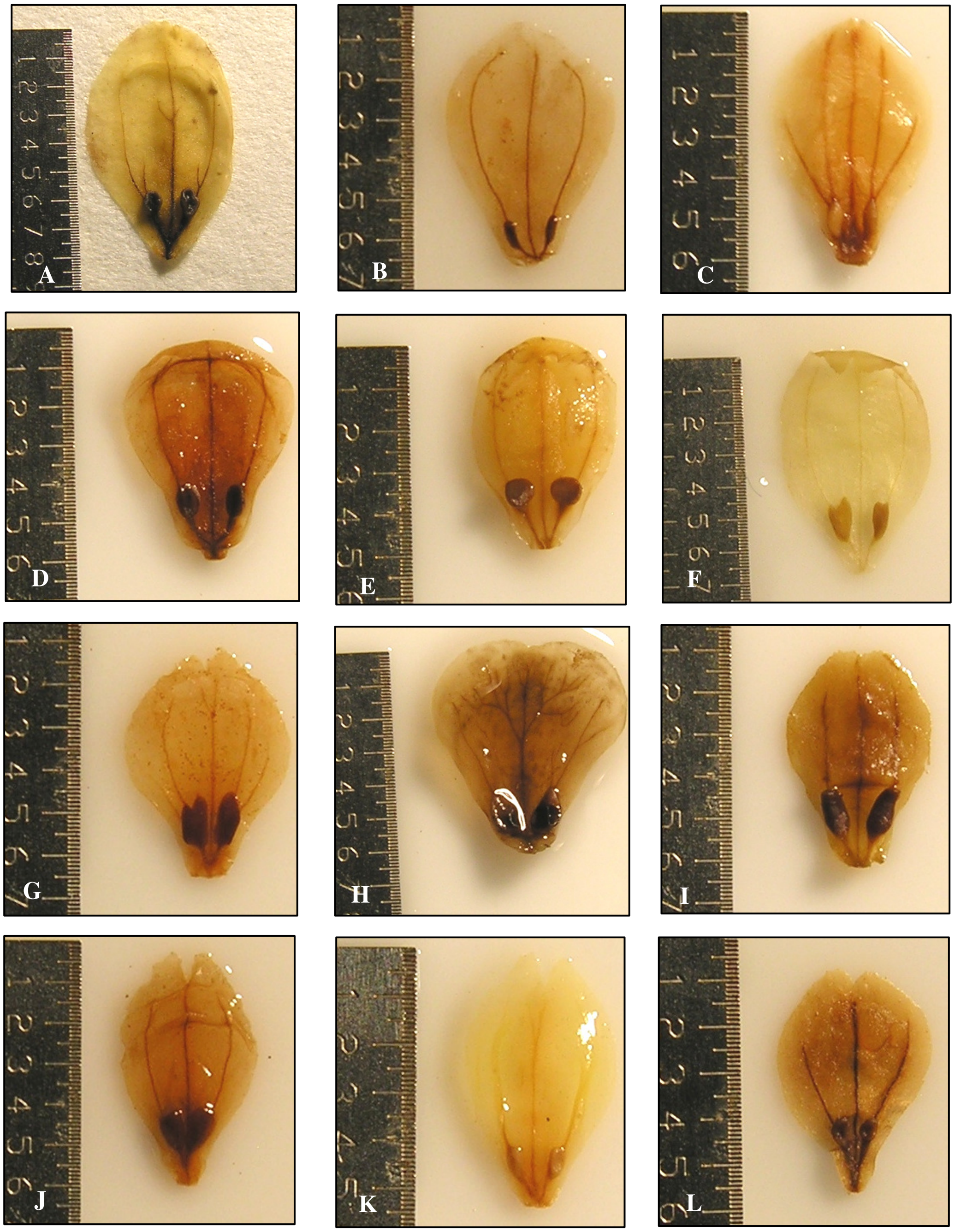


FIG. 2.4. Petals of various species of *Berberis*. A. *B. angulosa* var. *angulosa* (LKSR 45). B. *B. aristata* (D. Proud 5). C. *B. asiatica* (Adhikari, B 101). D. *B. concinna* var. *concinna* (SSW 5595). E. *B. concinna* var. *extensiflora* (Manaslu 08 20812277). F. *B. glaucocarpa* (JRSA 80). G. *B. hamiltoniana* (PSW 4579). H. *B. hookeri* (LKSRB 22). I. *B. insignis* (Suzuki *et al*. 9263024). J *B. jaeschkeana* var. *usteriana* (SSW 1225). K. *B. karnaliensis* (JRSA 59). L. *B. koehneana* (Polunin 506). Scale in millimetres.

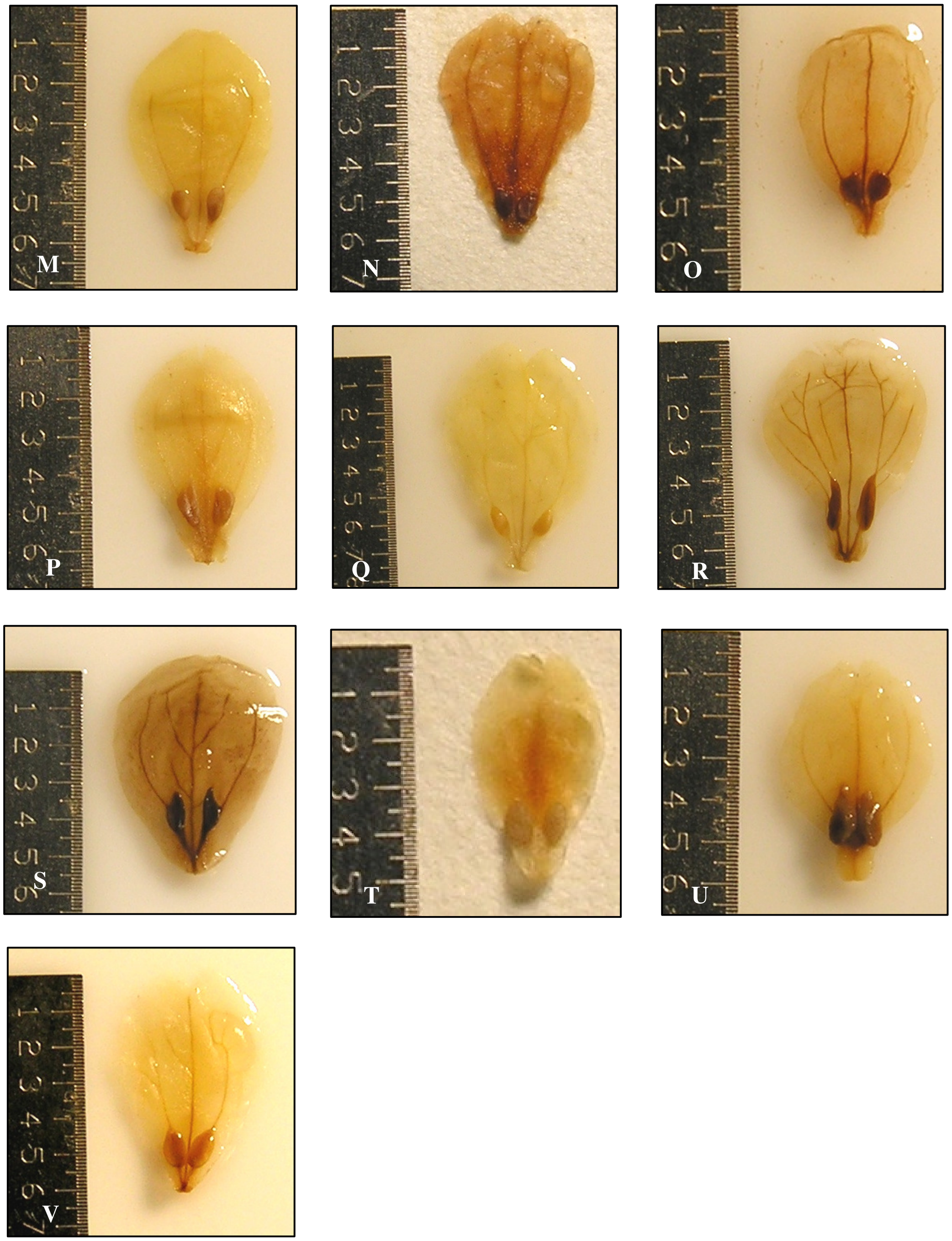


FIG. 2.4. (Continued). M. *B. kumaonensis* (JRSB 163). N. *B. mucrifolia* (Stainton 4847). O. *B. orthobotrys* var. *rubicunda* (Polunin 514). P. *B. pendryi* (EA 34). Q. *B. petiolaris* var. *petiolaris* (JRSB 39). R. *B. petiolaris* var. *garhwalana* (Dobremez 1967). S. *B. thomsoniana* (LKSRB 15). T. *B. tsarica* (DNEP1 153). U. *B. virescens* (DNEP1 238). V. *B. wallichiana* (G 18). Scale in millimetres

Flowers: Flowers are 0.8—2.5 cm in diameter, bright yellow or sometimes greenish yellow in some species (*B. hookeri* and *B. wallichiana*). The flowers have 2—4 whorls of sepals with 3 sepals in each whorl. The sepals of the outermost whorl are small and have been called prophylls in many publications (Ahrendt, 1961; Rao *et al.*, 1998). As there is no clear distinction between the sepals and the prophylls, the term 'outermost sepal' is used throughout the descriptions where 4 whorls of sepals are present. The shapes of the outer sepals and inner sepals are usually ovate and obovate respectively. The petals are in two whorls with 3 in each whorl and usually obovate. They are usually smaller than the inner sepals and are distinguished from them by the two nectariferous glands on the base of the inner surface (Fig 2.4). The shape of glands varies within Nepalese species and cannot be taken as a diagnostic character for species delimitation. The apices of the petals range from entire or emarginate to distinctly notched. Petal venation is usually 3-5 and varies within species. Stamens are attached at the base of each petal. The connective of the stamens is sometimes distinctly protruded beyond the anther locules and is an important character in distinguishing several species. The apices of connectives are apiculate, bifurcated, retuse or conical in shape (Fig. 2.5). The pistil is simple and the ovary contains 1-13 ovules. The stigma is sessile or sub-sessile and the style is usually indistinct in flowers.

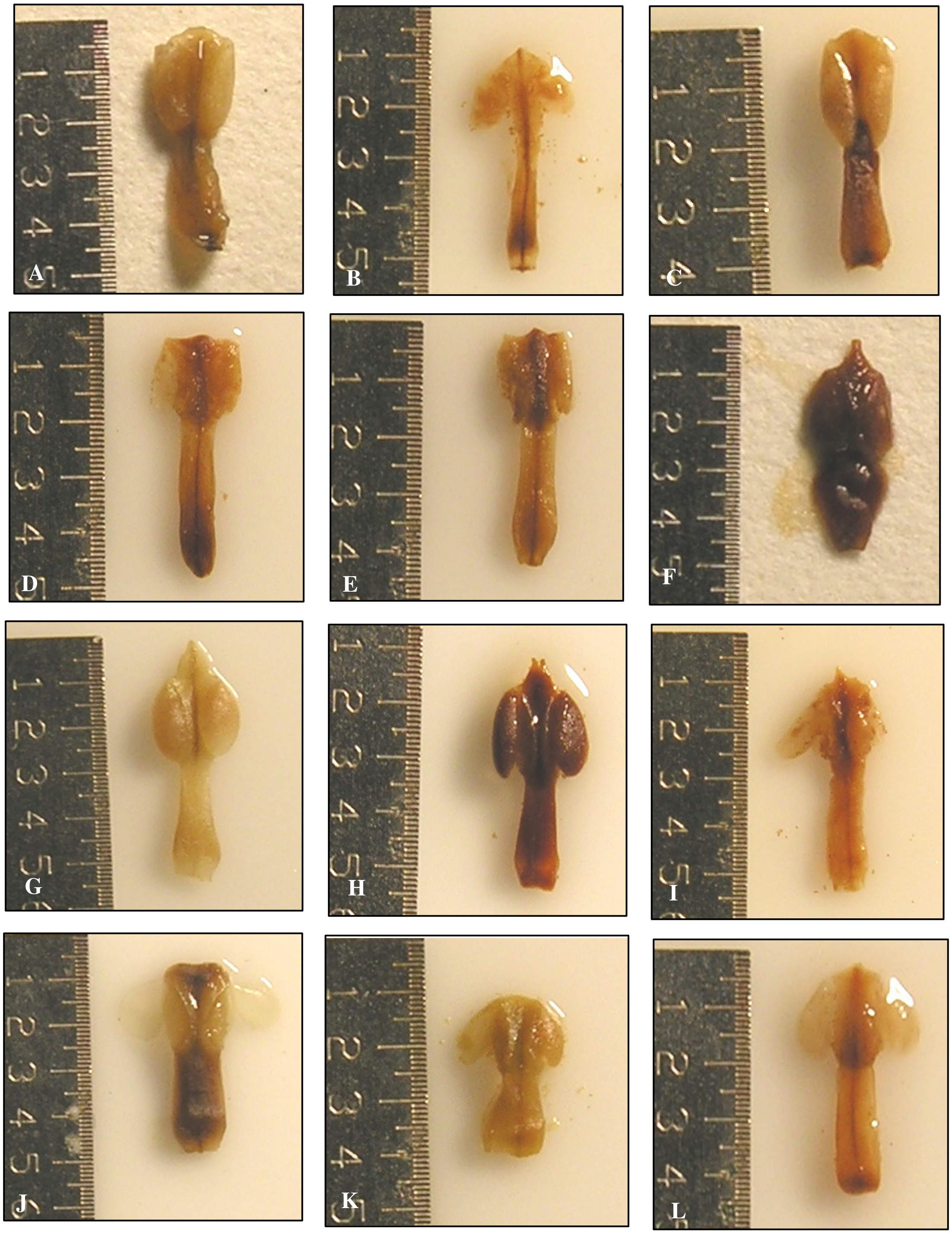


FIG. 2.5. Stamens of various species of *Berberis*. A. *B. angulosa* var. *angulosa* (LKSR 45). B. *B. aristata* (D. Proud 5). C. *B. asiatica* (Adhikari, B 101). D. *B. concinna* var. *concinna* (SSW 5595). E. *B. concinna* var. *extensiflora* (Manaslu 08 20812277). F. *B. everestiana* var. *ventosa* (Stainton 4289). G. B. *glaucocarpa* (JRSA 80). H. *B. hamiltoniana* (PSW 4579). I. *B. hamiltoniana* (Lowndes 941). J. *B. hookeri* (LKSRB 22). K. *B. insignis* (Suzuki *et al*. 9263024). L. *B. jaeschkeana* var. *usteriana* (SSW 1225). Scale in millimetres.

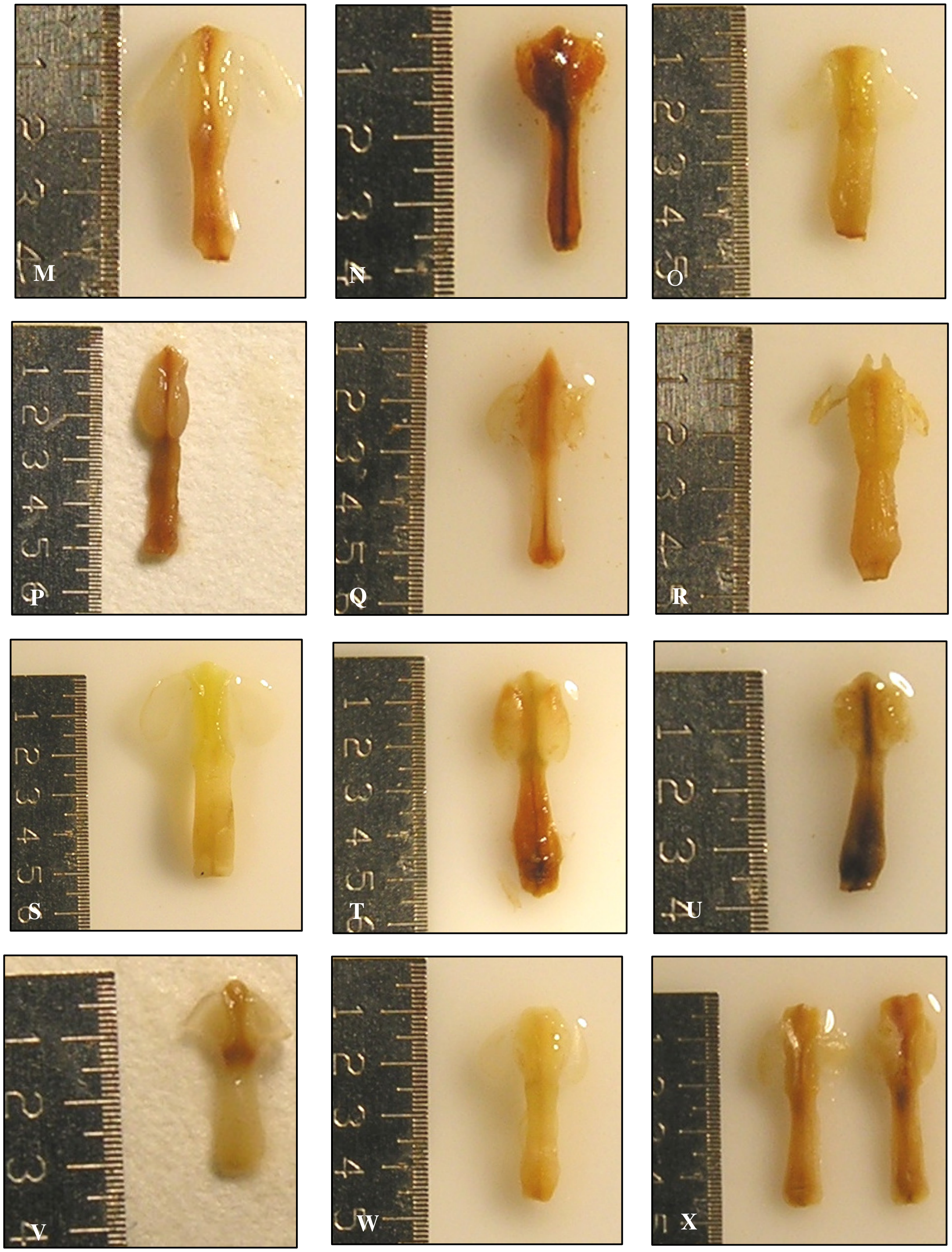


FIG. 2.5. (Continued). M. *B. karnaliensis* (JRSA 59). N. *B. koehneana* (Polunin 506). O. *B. kumaonensis* (JRSB 163). P. *B. mucrifolia* (Stainton 4847). Q. *B. orthobotrys* var. *rubicunda* (Polunin 514). R. *B. pendryi* (EA 34). S. *B. petiolaris* var. *petiolaris* (JRSB 39). T. *B. petiolaris* var. *garhwalana* (Dobremez 1967). U. *B. thomsoniana* (LKSRB 15). V. *B. tsarica* (DNEP1 153). W. *B. virescens* (DNEP1 238). X. *B. wallichiana* (G 18). Scale in millimetres.

Pollen: The structure of *Berberis* pollen ranges from inaperturate, 3-4 poly/syn-colpate, semi-spiraperturate to spiraturate, and exine ornamentation ranges from foveolate to foveolate-fossulate (Rao *et al.* 1999). Nepalese species of *Berberis* have pollen with 3-polycolpate apertures and spiraperturate pollen (Rao *et al.* 1999). Although pollen morphology of Indian species has been shown to be variable, the pollen morphology of closely related Nepalese taxa is not variable enough to distinguish species.

Fruits: Fruits are berries and are globose, ellipsoid, ovoid or obovoid in shape. As the fruits develop, the style becomes more distinct in some species, and berries are distinctly stylose. The colour of berries ranges from bright red to dark purplish black. Some of the species (*B. glaucocarpa* and *B. asiatica*) have glaucous berries. Seeds are usually ellipsoid in shape and ranges from 3-7 mm long.

2.6 ANATOMICAL STRUCTURES

On the basis of leaf anatomy, *Berberis* can be distinguished from other closely related genera within Berberidaceae by the presence of polygonal cells of the upper epidermis, oval unlignified stomata, and the absence of hairs on leaves (Metcalfe & Chalk, 1950).

Singh & Hussain (2007) studied the leaf epidermis in Indian species of *Berberis* and found that stomata are situated exclusively on the lower surface of the leaf with guard cells slightly raised above the subsidiary cells. They also found that the cuticular ornamentations are of taxonomic significance at species level in some taxa.

Berberis wood is usually yellow due to the presence of berberin. Mean vessel diameter in Berberidaceae is reported to be significantly lower than other dicotyledons (Metcalfe & Chalk, 1950; Carlquist, 1995). Vessels of *Berberis* wood are larger in earlywood than latewood (Carlquist, 1995). Rays in *Berberis* are usually multiseriate, and prismatic calcium oxalate crystals are found in ray cells of some species (Carlquist, 1995). Perforation in ray cells of *Berberis* is usually bordered or sometimes simple, not abundant, and found in the body of multiseriate rays among procumbent cells (Carlquist, 1995; Merev *et. al.* 2005).

2.7 ECOLOGY AND DISTRIBUTION

The Nepalese species of *Berberis* usually grows in disturbed habitats of forest margin, open pastures at higher altitudes, semi-desert vegetation, and the margins of cultivated land. A few exceptions include *B. insignis* and *B. wallichiana* which usually grow on the forest floor of Oak-Laurel forest in east Nepal. Similarly, *B. petiolaris* is a component of temperate moist *Abies*-*Betula*-*Acer* forest in west Nepal. Nepalese *Berberis* grow in wide range of rainfalls from drier regions of western Nepal to wetter parts of eastern Nepal. They grow from as low as 1000 m altitude (*B. asiatica*) up to 4700 m (*B. tsarica*). The distribution of *Berberis* collections in Nepal is shown in Fig. 2.6.

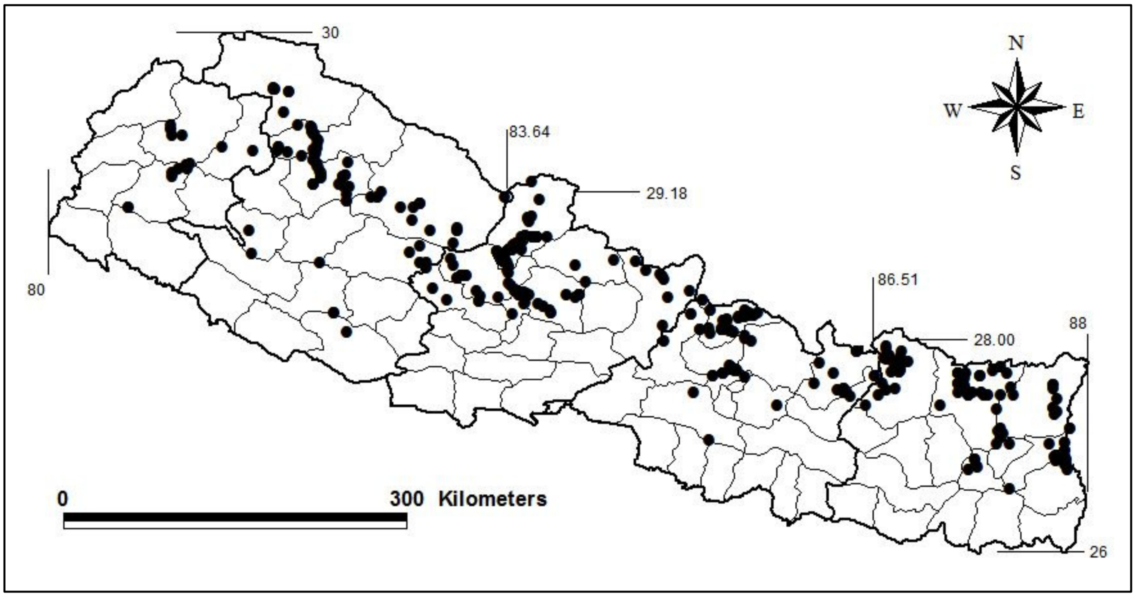


FIG. 2.6. Distribution of *Berberis* collections in Nepal.

2.8 POLLINATION

Berberis are usually pollinated by bees and wasps. Most of *Berberis* species have sensitive stamens. When insects visit the flower and touch the filament, it bends towards the pistil. The sensitive stamens in *Berberis* might be a mechanism to support self fertilization in the genus but more detailed study is required to understand their role. Honey bees and wasps were observed visiting several species of *Berberis* (Fig. 2.7). In some high altitude species, flies were seen visiting flowers (Fig. 2.8).

FIG. 2.7. Honey bee visiting *B. hamiltoniana* (JRSB 162). Humla, 3650 m (2008).

FIG. 2.8. Fly visiting *B. angulosa* (LKSRB 34). Panchthar, 3350 m (2007).

2.9 USES

Most of the Nepalese species of *Berberis* have medicinal uses because of the presence of the alkaloid berberine. Extracts from the stem and root of *Berberis* are used in ophthalmic medicine, and to treat jaundice, malarial fever, diarrhoea and peptic ulcers (Manandhar, 2002). Fruits are eaten fresh and sometimes used for making local alcoholic drinks. In Nepali, all the species are commonly known as 'Chutro'.

2.10 TAXONOMIC TREATMENT

BERBERIS L., Sp. Pl.: 330. 1753

Shrubs or small tree-like shrubs, evergreen or deciduous, usually with yellow wood. Stem or branches terete, sulcate or angled, reddish brown or yellowish brown becoming ash grey when older. Spines 3—5-fid, terete, sulcate or angled, strong or weak. Leaves simple, petiolate or sessile, in fascicles or in whorls, margin entire to spinescently toothed, venation pinnate, prominent or obscure. Flowers solitary, fascicled, or in pedunculate or epedunculate racemes, umbels or in panicles. Bracts often present. Flowers greenish yellow to yellow, 3-merous. Perianth segments in 4 to 6 whorls, outer 2—4 whorls of sepals, inner 2 whorls of petals with nectariferous glands at the base. Outermost sepals small, bract-like, median sepals usually smaller than the inner sepals, innermost sepals larger than the petals. Stamens 6; filament thick, sometimes with connective protuded beyond the apex of anther-locules; anthers-locules opening by valves. Pistil usually barrel shaped. Ovary 1-locular, ovules solitary to 13, stigma peltate. Berries ellipsoid, sub-globose, ovoid, ovoid to oblong, obovoid to oblong, usually red or purplish black, with or without style, sometimes covered with white or bluish white bloom (glaucous).

About 500 species: wide spread in N Hemisphere (especially in Himalayas and E Asia) and extending into Africa (2 species), and Central and South America (secondary centre of diversity); 21 species in Nepal.

KEY TO THE SPECIES

1a. Flower solitary on in fascicles.. 2

1b. Flowers in racemes, panicles or in umbels 11

2a. Evergreen shrubs. Berries black... 3

2b. Deciduous shrubs. Berries red 5

3a. Spines usually absent. Leaves large up to 17 cm long. Anther connectives slightly protuded or not ....**9. B. insignis**

3b. Spines present. Leaves small up to 8 cm (rarely up to 11 cm in *B. wallichiana*). Anther connectives distinctly protruded 4

4a. Berries with distinct style. Flowers 10—25(—30). Ovule solitary **21. B. wallichiana**

4b. Berries without style. Flowers 3—10. Ovules 3—6 ....**8. B. hookeri**

5a. Semi-prostrate shrubs less than 20 cm tall .... **13. B. kumaonensis**

5b. Erect shrubs more than 50 cm tall.. 6

6a. Berries without style... 7

6b. Berries with style…….. 9

7a. Spines 5-fid, usually equally or longer than leaves................................ **19. B. tsarica**

7b. Spines 3-fid, usually shorter than leaves.. 8

8a. Leaves glaucous below, venation distinct both sides, glands cup shaped.................... .... **4. B. concinna**

8b. Leaves not glaucous, venation slightly prominent below, glands obovoid................. .... **1. B. angulosa**

9a. Leaves rigidly coriaceous, anther connectives slightly protruded. ..**14. B. mucrifolia**
9b. Leaves thin or slightly coriaceous, anther connectives distinctly protruded 10

10a. Sepals in 4 whorls. Anther connectives apiculate ..... **5. B. everestiana** var**. ventosa**
10b. Sepals in 3 whorls. Anther connectives protruded into 2 or 3 teeth like appendages
.... **16. B. pendryi**

11a. Secondary and tertiary leaf venation usually reticulate. Berries thickly glaucous. 12
11b. Secondary leaf venation with closed or open loops, tertiary venation obscure. Berries non-glaucous.. 13

12a. Flowers in stiff pedunculate racemes ....**6. B. glaucocarpa**
12b. Flowers in flexible epedunculate racemes or in fascicles ....**3. B. asiatica**

13a. Leaves with distinct petiole up to 3.5 cm long, lamina elliptic-rounded or elliptic to broadly obovate...**17. B. petiolaris**
13b. Leaves without petiole, lamina obovate.. 14

14a. Inflorescence up to 16 cm long panicle, with up to 70 flowers .... **12. B. koehneana**
14b. Inflorescence up to 6 cm long raceme, umbellate or sub-umbellate raceme, with 2—25 flowers.. 15

15a. Berries dark purple or black, slightly glaucous.....................................**2. B aristata**
15b. Berries red, berries non-glaucous.. 16

16a. Sepals in 4 whorls, outermost sepal nearly equal to outer sepal.
....**18. B. thomsoniana**
16b. Sepals in 3 whorls, outermost sepal absent.. 17

17a. Shrubs usually less than 2 m tall. Berries with distinct style 18
17b. Shrubs usually more than 2 m tall. Berries without style (sometimes very small style in *B. virescens*) 19

18a. Young branches glabrous. Peduncle usually less than 1 cm long.
....**10. B. jaeschkeana** var. **usteriana**
18b. Young branches densely puberulous. Peduncle usually more than 1 cm long
....**11. B. karnaliensis**

19a. Outer sepal less than 3.5 mm long. Apex of connectives obtuse or retuse.
....**20. B. virescens**
19b. Outer sepal more than 3.5 mm long. Apex of connectives pointed or bifurcated.. 20

20a. Berries ovate-ellipsoid. Petals notched. ....**7. B. hamiltoniana**
20b. Berries ellipsoid to oblong-obovoid. Petals obtuse, rarely emarginate or crenate.....
....**15. B. orthobotrys** var. **rubicunda**

1. Berberis angulosa Wall. ex Hook.f. & Thoms., Fl. Ind. 1: 227. 1855.

Hooker f. & Thomson in Hooker.f., Fl. Brit. Ind. 1(1): 111 (1872); Hooker f., Bot. Mag. 115: t. 7071 (1889); Schneider, Bull. Herb. Boiss. 2.5: 398 (1905); Chatterjee, Rec. Bot. Surv. India 16(2): 24 (1953); Ahrendt, J. Linn. Soc. Bot. 57: 113 (1961); Tebbs in Hara & Williams, Enum. Fl. Pl. Nepal 2: 29 (1979); Grierson & Long, Fl. Bhutan 1(2): 323 (1984); Rao *et al.*, Rheedea 8(2): 110 (1998); Press, Shrestha & Sutton, Annot. Checkl. Fl. Pl. Nepal: 25 (2000).

Shrub to 2 m. Stems and branches terete to sulcate, glabrous, reddish brown when young becoming greyish and verruculose when older. Internodes 1—2.5 cm. Spines 3(—5)-fid, strong, usually terete, central spine 1—3 cm, lateral spines 0.5—1.5 cm. Leaves deciduous, slightly coriaceous. Petiole indistinct or short, 2—5 mm. Lamina obovate to oblanceolate, 1.5—4.5 × 0.5—1.5 cm, base cuneate to shortly attenuate, apex obtuse, mucronate, margin usually entire, sometimes with 1—3 spinulose teeth on each side, dark green above, paler beneath, venation slightly prominent below. Flowers solitary or in fascicles of 2—6 flowers. Bracts indistinct. Flowers yellow, 1.5—2 cm in diameter. Pedicel 0.5—2 cm, glabrous to puberulous. Sepals in 2 whorls, outer sepals ovate or spathulate, 6—10 × 3.5—4.5 mm; inner sepals obovate, 7—10 × 5—7 mm. Petals obovate, 5.5—8.5 × 3.5—5.5 mm, base cuneate, apex undulate or rounded, margin entire, venation distinct with one central and 2 or 3 pairs of lateral veins; glands obovoid, *ca.* 1 mm long. Stamens 4—5 mm long, connectives slightly produced, tip conical. Pistil 3—4 mm long; ovules 4—6. Berries bright red, sub-globose, 8—10 mm long; style absent.

Distinguishing features: This species is easily identified by its smaller habit of less than 2 m, solitary bright red and estylose fruits (2—6 in var. *fasciculata*).

Key to the varieties of *Berberis angulosa.*

1a. Flowers solitary .... a. var. **angulosa**

1b. Flowers in fascicles of 2—6 flowers .... b. var. **fasciculata**

1a. Berberis angulosa Wall. ex Hook.f. & Thoms. var. **angulosa**

Ahrendt, J. Linn. Soc. Bot. 57: 113 (1961). (Fig. 2.9; 2.51 A, B).

TYPE: NEPAL: Wallich 1475.1 [Lectotype, designated by Harber (unpublished) K-W!; isolectotype: K! with barcode K000077366 labelled as ‘a’].

Berberis parisepala Ahrendt, Gard. Chron. (3): 109 (1941).

TYPE: Cultivated from KW 8350. (Holotype: BM!).

Flowers always solitary. Pedicels glabrous to puberulous.

Flowering time: May—July.

Fruiting time: Jun—Oct.

Ecology: In dry open rocky places.

Altitudinal range: 3000—4500 m.

Distribution: Central, Eastern [E Himalaya, Tibetan Plateau].

Selected specimens: CENTRAL: Manang, Bimatang, 3730 m, 13.08.2008, 20815195 Manaslu08 (E); Lamjung, Rambrong, Lamjung Himal, 12000 ft, 29.06.1954, Stainton, Sykes & Williams 6047 (BM); Baglung, East of Dhorpatan, 11500 ft, 21.10.1954, Stainton, Sykes & Williams 9109 (BM); Kaski, Annapurna Himal, Setikhola, 12500 ft, 3.8.1954, Stainton, Sykes & Williams 6656 (BM); Myagdi, South of Gurjakhani, 12500 ft, 08.06.1954, Stainton, Sykes & Williams 3062 (E,BM); Rasuwa, Langtang khola, 11100 ft, 24.05.1962, S. Bowes-Lyon 147(E,BM); Rasuwa, Gosaikund-Cholangpati, 4030 m, 9.10.2001, ENEP 280 (E). EASTERN: Panchthar, Prangbung 6, Pasi Bhanjyang, 3236 m, 11.06.2007, LKSR 45 (E, TUCH); Taplejung, Topkegola, 12000 ft, 19.10.1971, Beer, L.W., Lancaster C.R. & Morris, D. 88 (BM); Sankhuwasabha, N bank of Barun Khola below Repu Kharka, 3560 m, 8.10.1991, EMAK 585 (E); Sankhuwasabha, Numbuk-Yangri Kharka, 3470 m, 18.07.1988, , Suzuki *et al.* 8840287 (E). Number of specimens examined: 76

1b. Berberis angulosa var. **fasciculata** Ahrendt, J. Bot. Lon (Suppl): 42. 1941.

Ahrendt, J. Linn. Soc. Bot. 57: 113 (1961). (Fig. 2.51 C).

TYPE: INDIA: Sikkim: 10,700 ft, Hooker & Thomson (Holotype: K!)

Flowers in fascicles of 2—6 flowers. Pedicels usually puberulous.

Flowering time: May—July.

Fruiting time: Jun—Oct.

Ecology: In dry open rocky places.

Altitudinal range: 3000 m.

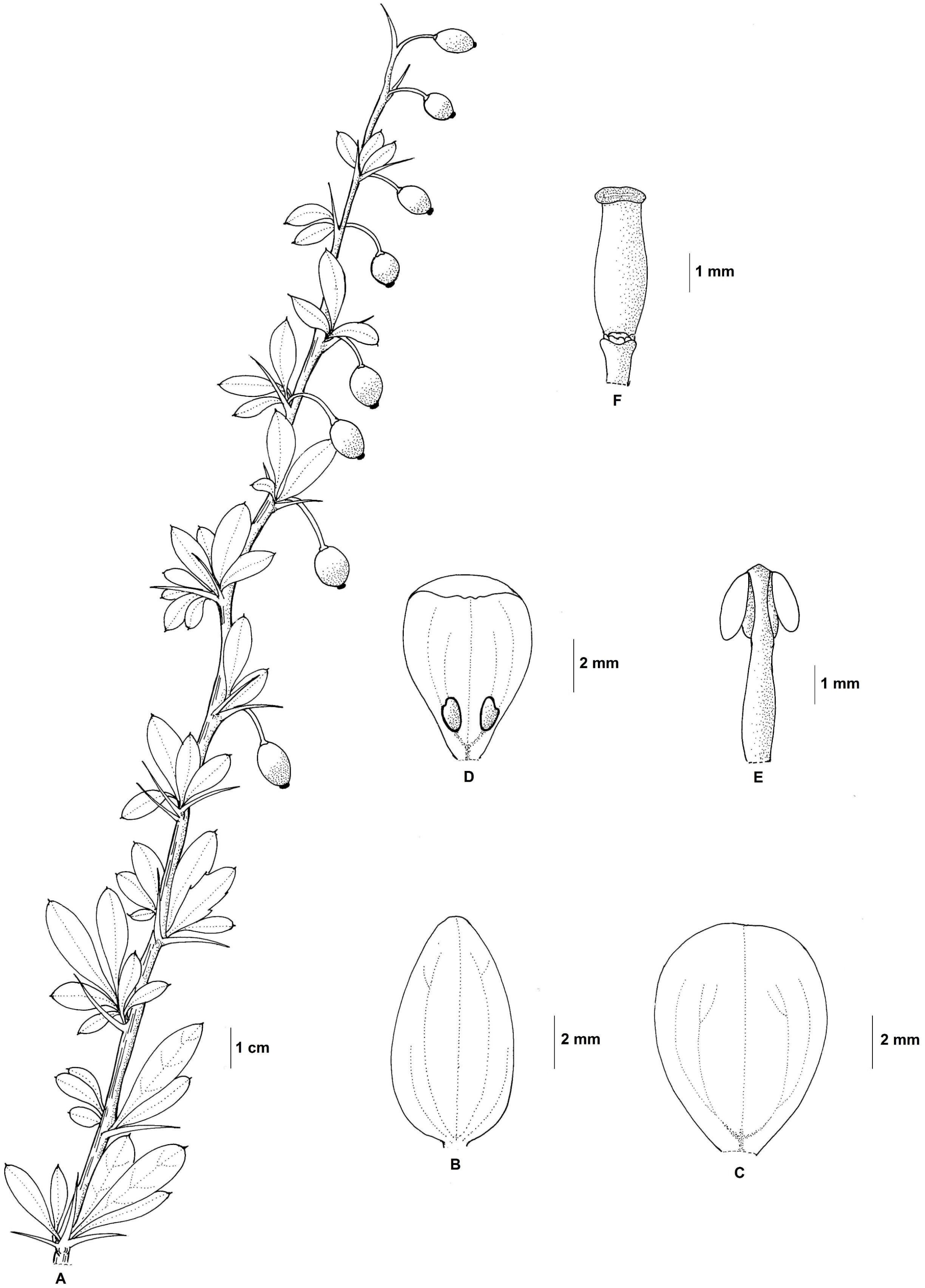


FIG. 2.9. ***Berberis angulosa*** var. ***angulosa***. A. Fruiting branch. B. Outer sepal. C. Inner sepal. D. Petal. E. Stamen. F. Pistil. (Based on: A, EMAK 585; B-F, LKSR 45).

Distribution: Central [E Himalaya].

New record for Flora of Nepal.

Specimens examined: CENTRAL: Rasuwa, Cholangpati, 3623 m, 20.10.2006, Adhikari, B. EL 127 (E). Number of specimens examined: 2

Notes: Hook.f. & Thomson (1855) in *Flora Indica* mentioned the variety *fasciculata* as β and later Ahrendt (1941) described it as a new variety *fasciculata*. Rao *et al*. (1998) did not recognise var. *fasciculata*, stating that the isotype of *B. angulosa* from BM (Wallich 1475) has fasciculate to sub-umbellate inflorescences. Wallich 1475 actually comprises two gatherings; 1475.1 which is *B. angulosa* from Nepal and 1475.2 which is *B. umbellata* from Kumaon. Wallich 1475 in BM is more likely to be 1475.2 or from different gatherings. Wallich 1475.1 in K-W! has all solitary flowers. Both varieties were closely observed in the field and are considered to be distinct varieties. Variety *angulosa* is common in central and eastern Nepal but only a few individuals of var. *fasciculata* were observed at a single location in central Nepal.

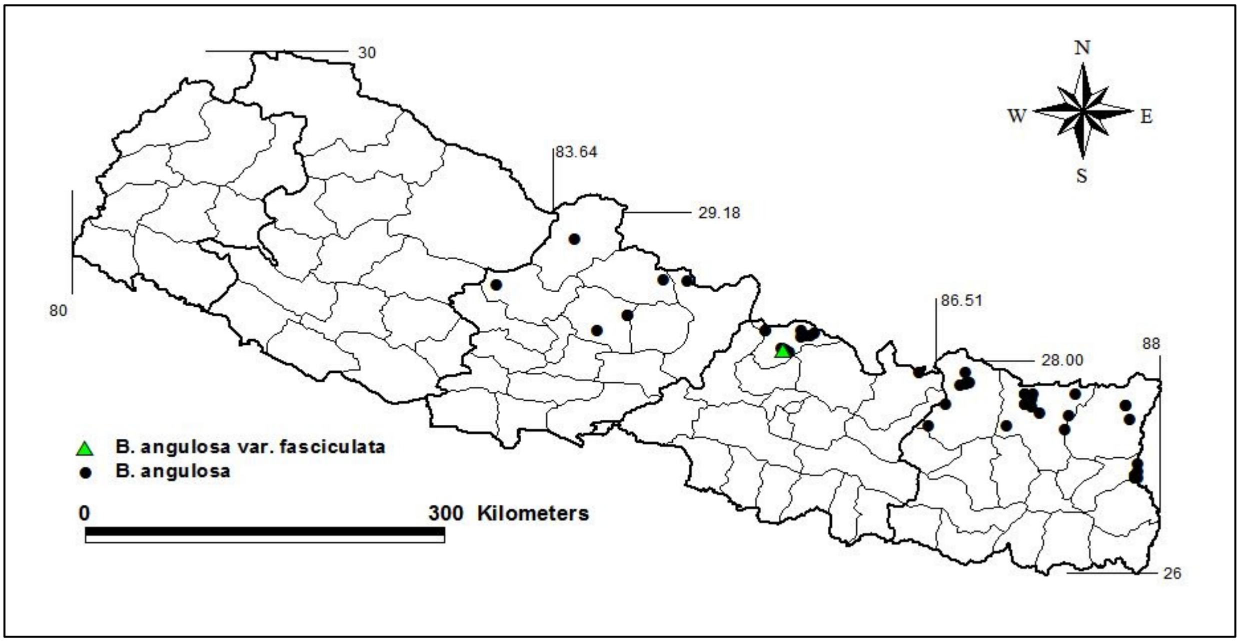


FIG. 2.10. Distribution of *Berberis angulosa.*

2. Berberis aristata DC., Syst. Nat. 2: 8. 1821.

Hooker f. & Thomson, Fl. Ind: 222 (1855); Hooker f. & Thomson in Hooker f., Fl. Brit. Ind. 1(1): 110 (1872); Schneider, Bull. Herb. Boiss. 2.5: 451 (1905); Chatterjee, Rec. Bot. Surv. India 16(2): 20 (1953); Ahrendt, J. Linn. Soc. Bot. 57: 101(1961); Tebbs in Hara & Williams, Enum. Fl. Pl. Nepal 2: 29 (1979); Grierson & Long, Fl. Bhutan 1(2): 326 (1984); Rao *et al.*, Rheedea 8(1): 24 (1998); Press, Shrestha & Sutton, Annot. Checkl. Fl. Pl. Nepal: 25 (2000). (Fig. 2.11; 2.51 D, E).

TYPE: NEPAL: Buch-Ham s.n. (Lectotype, here designated, sheet 622.7 LINN-SM!).

B. ceratophylla G.Don, Sys. Gard. 1: 115 (1831).

TYPE: Wallich *s.n.* (Lectotype, designated as type by Ahrendt, 1961, OXF).

B. chitria Ker, Bot. Reg. 9: t. 729 (1823).

TYPE: Buch-Ham s.n. (Lectotype, here designated, sheet 622.7 LINN-SM!).

B. sikkimensis (C.K.Schneid.) Ahrendt, J. Bot. Lon. (Suppl): 85 (1942).

TYPE: Hooker & Thomson, s.n. (Lectotype, here designated, Sikkim Himal, 9000ft K!).

Shrub to 5 m. Stems and branches sulcate or angled, glabrous or puberulous, reddish brown. Internodes 1—5 cm. Spines 3-fid, solitary towards twig apex, strong, usually terete, central spine 1—2 cm, the lateral spines equal or slightly shorter than the central. Leaves deciduous, slightly coriaceous. Petiole indistinct. Lamina obovate to elliptic, 2—6 × 0.5—1.5 cm, base cuneate, apex acute or obtuse, mucronate, margin entire or with 2—10 spinulose teeth on each side, dark green above, paler beneath, venation distinct and slightly raised both sides. Inflorescence 4—6 cm long, a pedunculate or sub-paniculate raceme of 10—20 flowers. Bracts ovate, reddish brown, 2—2.5 mm long. Flowers yellow, *ca.* 1 cm in diameter. Peduncle 0.5—2 cm, glabrous. Pedicel 0.5—1 cm, slightly glaucous. Sepals in 3 whorls, outer sepals ovate, 2—3 × 1—2 mm; median sepals elliptic or elliptic obovate, 3—5 × 1.5—3 mm; inner sepals obovate, 6—8.5 × 3—5 mm. Petals obovate, 5—8 × 3—5 mm, base cuneate, apex obtuse, rarely notched, 0.2—0.3 mm deep, margin entire, venation distinct with one central and one pair of lateral veins; glands elliptic or obovoid, 0.7—1 mm long. Stamens 4—5.5 mm long, connectives scarcely produced, tip conical. Pistil 5—6 mm long; ovules 3—4. Berries greenish purple becoming dark purple to black on ripening, oblong-ovoid, sometimes asymmetric, 8—10 mm long, slightly glaucous; style thick, 1—2.5 mm.

Flowering time: Apr—July.

Fruiting time: May—Oct.

Ecology: Common in forest clearings and disturbed vegetation along forest edges and roadsides.

Altitudinal range: 1300—3400 m.

Distribution: Western, Central, Eastern, [E Himalaya, W Himalaya].

Distinguishing features: Identifiable by the combination of pedunculate or sub-paniclulate raceme, dark purple or black oblong-ovoid berries, and short and thick style.

Selected specimens: WESTERN: Dailekh, 2100 m, 31.07.1991, Suzuki *et al.* 9194038 (BM). CENTRAL: Sheopuri, North of Kathmandu, 8000 ft, 6.05.1957, Proud, D. 5 (BM, E); Rasuwa, Route to Langtang Khola, 9000 ft, 6.06.1949, Polunin, O. 161 (E, BM); Rasuwa, Near Dhunche towards Deurali 1990 m, 19 Oct 2006, Adhikari B EL 122 (E). EASTERN: Sagarmatha, Jorsale, 2842 m, 28.09.2005, DNEP3 BY207 (E); Dhankuta, Hile-Shidua, 2100 m, 12.07.1991, Ohba *et al.* 9154008 (E). Number of specimens examined: 109

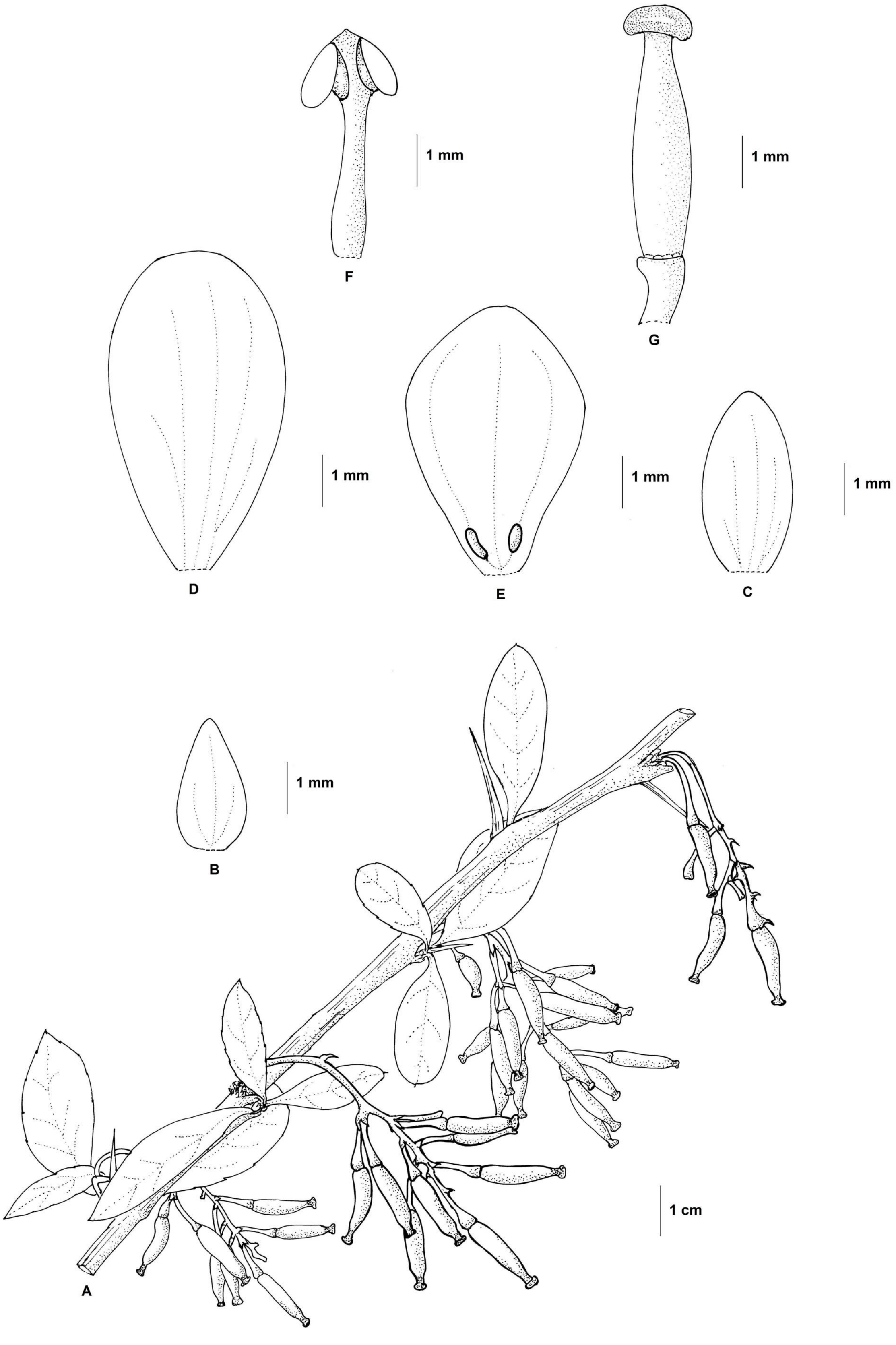


FIG. 2.11. ***Berberis aristata***. A. Fruiting branch. B. Outer sepal. C. Median sepal. D. Inner sepal E. Petal. F. Stamen. G. Pistil. (Based on: A, Adhikari, SB 15; B-G, D. Proud 5).

Note: *B. aristata* is one of the commonest species of *Berberis* growing in the Himalayas. It shows a great range of variation in shape and size of leaf and inflorescence. Several attempts have been made (Schneider, 1905; Ahrendt, 1961) to describe new species and varieties based on small variable characters of this species which makes the identity of the species unclear.

The species was first collected by Francis Buchanan-Hamilton from 'Chitlong' (today's Chitlang), Central Nepal, on 10 April 1802 (Press & Shrestha, 2000). The first name Buchanan intended to give was *B. chitria* which is perhaps from its local name as 'chutro'. Buchanan gave his original set of Nepalese collections along with large collections of drawings and manuscripts of Indian plants to his friend J.E. Smith, with another set being given to Aylmer Bourke Lambert (popularly known as Lambert).

Based on Buchanan collections in Lambert's herbarium, De Candolle (1821) described the species *B. aristata* instead of using the name *B. chitria*, first given by Buchanan. Later Ker[1] in 1823 (Bot. Reg. 9: t 729) rejected the first published name *B. aristata* in preference to the *B. chitria*, the original name given by Buchanan. Since then, both names have been used in parallel as the accepted name. Based on Lambert's materials together with those of Wallich, D. Don (1825) produced *Prodromus Florae Nepalensis* in which he accepted the Buchanan name *B. chitria*. Later, Hooker and Thomson (1855, 1872) accepted *B. aristata* as the first validly published name. Schnieder (1905) treated both *B. aristata* and *B. chitria* as separate species saying, ''Buch-Ham seems to have

---

[1] Lindley has been cited as a author of Botanical Register volume 9 in most of the published literature but according to TL2, Ker Gawler should be a author of all descriptions for volume 1-14 (1815-1829) and Lindley for volume 16-23 (1829-1847).

distributed other forms under the same name and Lindley described some of this in Bot. Reg. t 729''. Ahrendt (1941-1942, 1961) also accepted both names and treated them as separate species.

Buchanan collected *Berberis* specimens from Chitlang and from the surroundings of Kathmandu valley, since he was not permitted to visit other parts of the country during his year-long stay in Nepal. The populations of *B. aristata* from Central Nepal and the hills surrounding the Kathmandu valley have been observed during this study and it is concluded that there are no other species of *Berberis* similar to *B. aristata* which grow in that region. Therefore, in this account, *B. chitria* is considered to be a synonym.

After the death of Lambert, the herbarium was split up and sold at auction. Buchanan specimens collected from Nepal along with Mysore and Malabar were purchased by Robert Brown for the British Museum and incorporated there. However, some of the specimens did not find their way into the collections (Press & Shrestha, 2000) and Buchanan's *B. aristata* specimens have not been found in BM. The only surviving Buchanan collection of *B. aristata* was found at LINN-SM (herbarium sheet no 622.7), and it has therefore been chosen for the lectotype.

Specimens with longer peduncles and sub-paniculate inflorescences are usually identified as *B. chitria* corresponding to Ker's figure (Bot. Reg. t 729.1823). Ker's drawing was from a cultivated plant with no information on origin. There are some collections of *Berberis* with longer peduncles growing in the western Himalayas (Kumaon, India) which could be a different species or varieties and need a different name. No specimens of this kind were observed in the field and no such herbarium specimens from Nepal were located. Therefore, this matter is beyond the scope of this work.

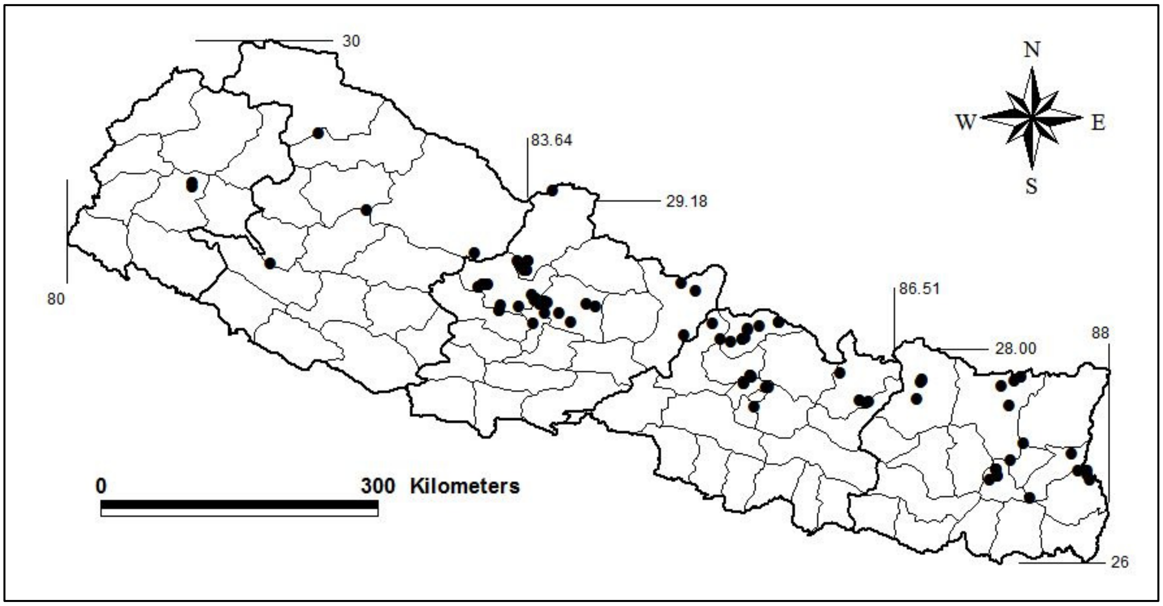


FIG. 2.12. Distribution of *Berberis aristata.*

3. Berberis asiatica Roxb. ex DC., Syst. Nat. 2: 13.1821.

Don, Prodr. Fl. Nepal: 204 (1825); Roxburgh, Fl. Ind. 2: 182 (1832); Hooker f. & Thomson in Hooker f., Fl. Brit. Ind. 1(1): 110 (1872); Schneider, Bull. Herb. Boiss. 2.5: 456 (1905); Chatterjee, Rec. Bot. Surv. India 16(2): 13 (1953); Ahrendt, J. Linn. Soc. Bot. 57: 86 (1961); Tebbs in Hara & Williams, Enum. Fl. Pl. Nepal 2: 29 (1979); Grierson & Long, Fl. Bhutan 1(2): 326 (1984); Rao *et al.*, Rheedea 8(1): 52 (1998); Press, Shrestha & Sutton, Annot. Checkl. Fl. Pl. Nepal: 25 (2000). (Fig. 2.13; 2.51 F).

TYPE: NEPAL: Wallich 1477 (1821). (Lectotype here designated, G-DC!; isolectotype: K!, E!).

Shrub to 3 m. Stems and branches terete or angled, glabrous, yellowish grey, sparsely verruculose, young shoots slightly sulcate. Internodes 1—6 cm. Spines usually 3, solitary towards twig apex, strong, terete or angular, central spine 1—2 cm, the lateral spines equal or slightly smaller than the central. Leaves deciduous, leathery. Petiole indistinct or up to 0.8 cm. Lamina obovate, obovate-elliptic or oblanceolate, 2—6(—9) × 1—3(—5) cm, base cuneate or attenuate to the small petiole, apex obtuse, usually mucronate, margin with 1—4 spinose teeth on each side, rarely entire, light green above, glaucous and papillose beneath, venation reticulate, prominent both sides. Inflorescence 1.5—6 cm long, a fascicle or a condensed epedunculate raceme of 8—20(—35) flowers with a few flowers arising from the base of the rachis. Bracts ovate-triangular, 1—2 mm. Flowers yellow, 1—1.5 cm in diameter. Pedicel 0.5—2 cm, glabrous. Sepals in 3 whorls, outer sepals ovate-triangular, 1.5—2.5 × 1—1.5 mm; median sepals ovate, ovate-triangular or elliptic, 2.5—3.5 × 1.5—2.5 mm; inner sepals obovate, oblong-obovate or elliptic, 4.5—6 × 2—4 mm. Petals obovate, 4.5—6.5 × 3.5—4.5 mm, base cuneate or slightly clawed, apex slightly emarginate, sometimes obtuse, margin entire, venation distinct with one central and 2 pairs of lateral veins; glands oblong-obovate, 0.8—1.2 mm long. Stamens 3.5—5 mm long, connective slightly produced or not, tip obtuse or conical. Pistil 4.5—6 mm long; ovules 3—6. Berries dark purple, ovoid or globose, 7—10 mm long, glaucous; style 0.5—1.5 mm.

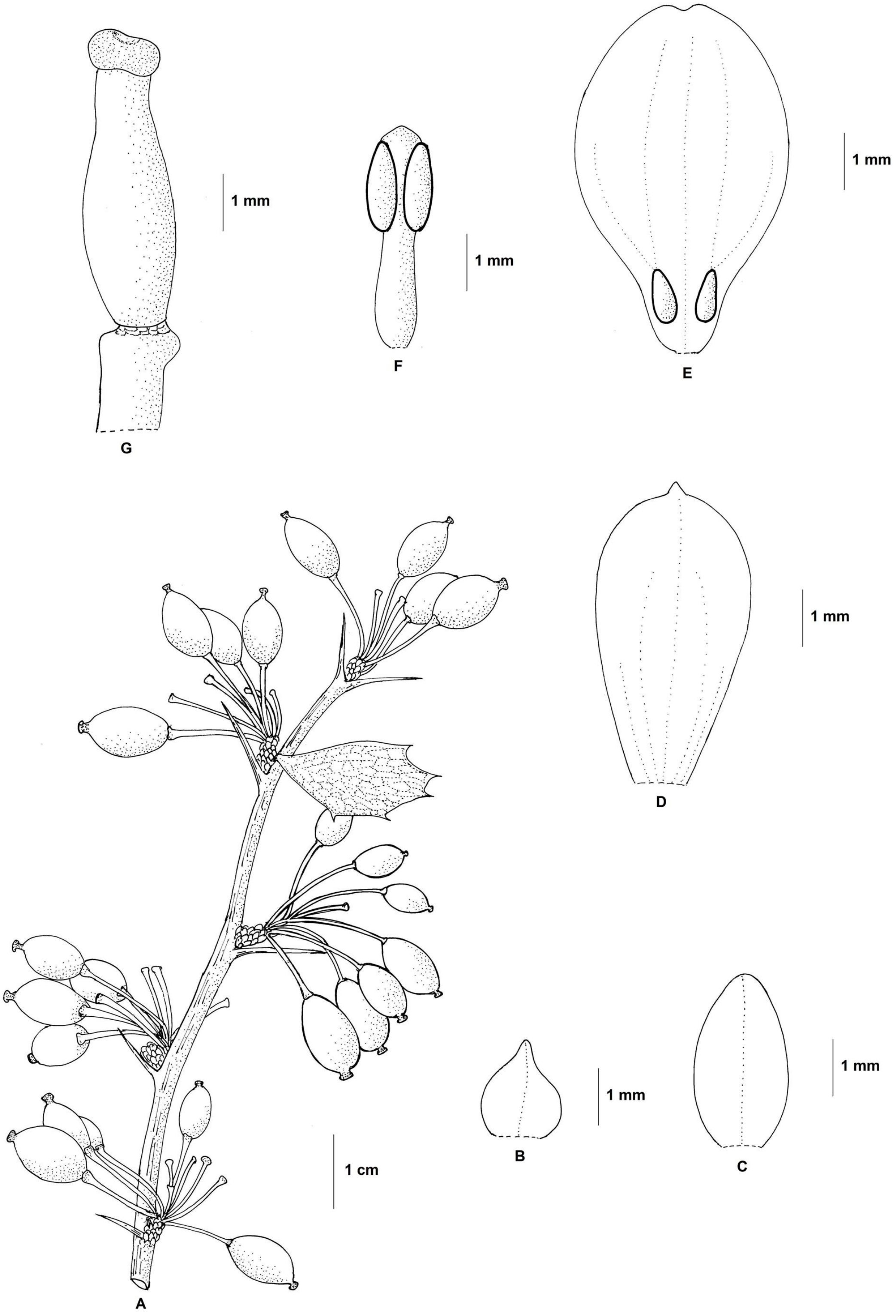


FIG. 2.13. ***Berberis asiatica***. A. Fruiting branch. B. Outer sepal. C. Median sepal. D. Inner sepal. E. Petal. F. Stamen. G. Pistil. (Based on: A, Adhikari, G2; B-G, Adhikari, B 101).

Flowering time: Feb—May.

Fruiting time: Mar—Jul.

Ecology: Disturbed vegetation beside roads and trails, and forest clearings.

Altitudinal range: 1000—2700 m.

Distribution: Western, Central [W Himalaya, Tibetan Plateau].

Distinguishing features: This species is easily identified by its leathery leaves with distinct reticulate venation and glaucous berries.

Selected specimens: WESTERN: Mugu, Karkhan: Khater Dara, 2120 m, 24.04.1952, Polunin, Sykes and Williams 836 (E, BM); Mugu, Above Luma, 2180 m. 11.06.2008, JRSA 108 (E). CENTRAL: Palpa, Bhairabsthan, 1300 m, 05.05.2006. Adhikari, B. B 101 (E); Myagdi, Lumsum, 1820 m, 22.03.1974, Vickery, A.R. 407 (BM); Kaski, Lumle, 5600 ft, 15.03.1970, Flatt, H 163 (BM); Kaski: Ghabung Khola, 5000 ft, 19.05.1954, Stainton, Sykes and Williams 5316 (E); Kathmandu, Thankot, 5000 ft, 28.03.1975, Stainton 7327 (E); Dolkha, Khimti Khola, 8000 ft, 13.04.1964, Stainton 4471 (KATH, BM). Number of specimens examined: 120

Note: De Candolle (1821) cited Wallich's specimens as "in Napaulia (*Wallich*)" but without mentioning any numbers. A single sheet of *B. asiatica* in the De Candolle herbarium (G-DC) has three gatherings from Bengal, Nepal (Napaul) and a leaf specimen from Lambert. Ahrendt (1961) cited the type specimen as, "Nepal: 1819, Wallich 1477" but no specimens were found with the date 1819. The specimen "Wallich 1477.1" in the Wallich herbarium (K-W) is dated as 1821 while the specimen in Vienna (W) is dated 1820. The specimen most likely to have been seen by De Candolle was the specimen in G-DC, and, therefore, this has been chosen as the lectotype. The date on the sheet might refer to the publication date or may have been copied later from the Wallich Catalogue. This specimen was most likely to have been collected by Edward Gardner and his team in (1817-1819) (see note in *B. wallichiana*).

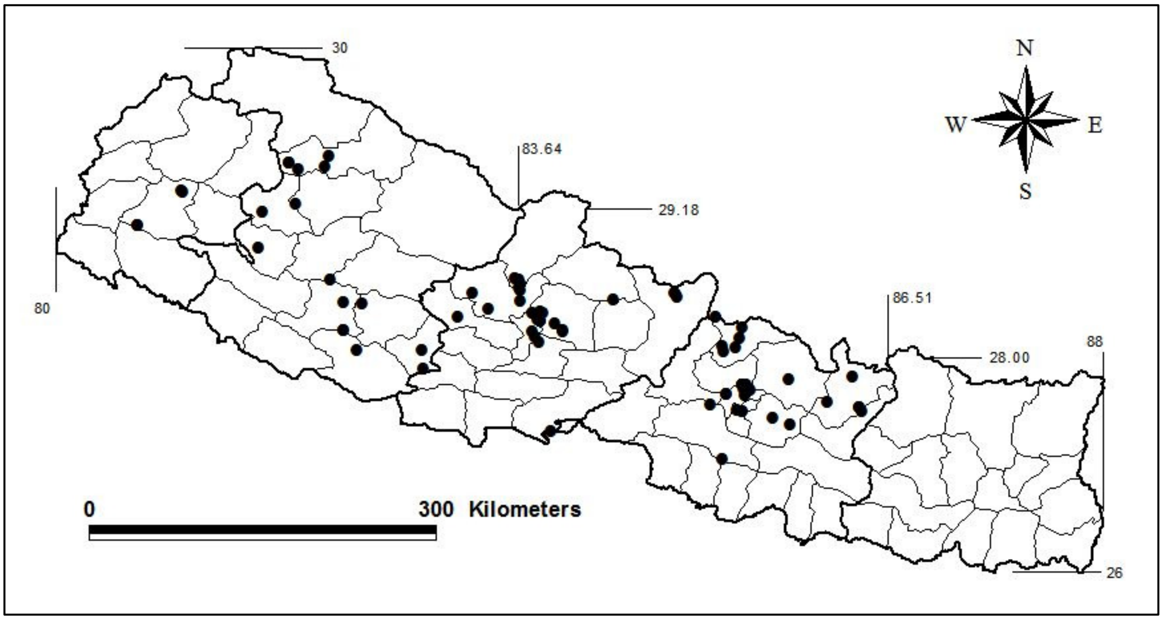


FIG. 2.14. Distribution of *Berberis asiatica.*

4. Berberis concinna Hook.f., Bot. Mag. 79: t. 4744. 1853.

Hooker f. & Thomson, Fl. Ind: 228 (1855); Hooker f. & Thomson in Hooker f., Fl. Brit. Ind. 1(1): 111 (1872); Schneider, Bull. Herb. Boiss. 2.5: 397 (1905); Chatterjee, Rec. Bot. Surv. India 16(2): 26 (1953); Ahrendt, J. Linn. Soc. Bot. 57: 118 (1961); Tebbs in Hara & Williams, Enum. Fl. Pl. Nepal 2: 30 (1979); Grierson & Long, Fl. Bhutan 1(2): 324 (1984); Rao *et al*., Rheedea 8(2): 111 (1998); Press, Shrestha & Sutton, Annot. Checkl. Fl. Pl. Nepal: 25 (2000).

Shrub to 2 m. Stems and branches usually sulcate, glabrous, yellowish grey, sparsely verruculose. Internodes 1—2.5 cm. Spines usually 3, terete, angled or slightly sulcate below, strong, central spine 0.8—1.5 cm, lateral spines 0.5—1.2 cm. Leaves deciduous, slightly coriaceous. Petiole absent. Lamina obovate, 1—3 × 0.5—1.5 cm, base cuneate, apex obtuse, sometimes acute, usually mucronate, margin with 1—3 spinose teeth on each side, dark green above, glaucous and papillose beneath, venation reticulate, prominent both sides. Inflorescence 1—5 cm long, flowers solitary or an umbel of 2 or 3 flowers. Bracts ovate-triangular, 1.5—3 × 1—1.5 mm. Flowers yellow, 1.5—2.5 cm in diameter. Peduncle 1.5—3 cm in umbellate flowers. Pedicel 0.5—2 cm, glabrous, red, slightly swollen at the base of fruit. Sepals in 4 whorls, outermost sepals ovate, 2—4 × 1.5—2.5 mm; outer sepals ovate, elliptic, obovate or narrowly obovate, 5—8.5 × 2.5—4 mm; median sepals elliptic, ovate, broadly obovate-elliptic, 5—8.5 × 3—5.5 mm; inner sepals broadly ovate to obovate-elliptic, 5.5—9 × (2.5—)4—6.0 mm. Petals obovate, 4.5—6.5 × 2.5—4.5 mm, base cuneate, apex obtuse, slightly emarginate or crenate,

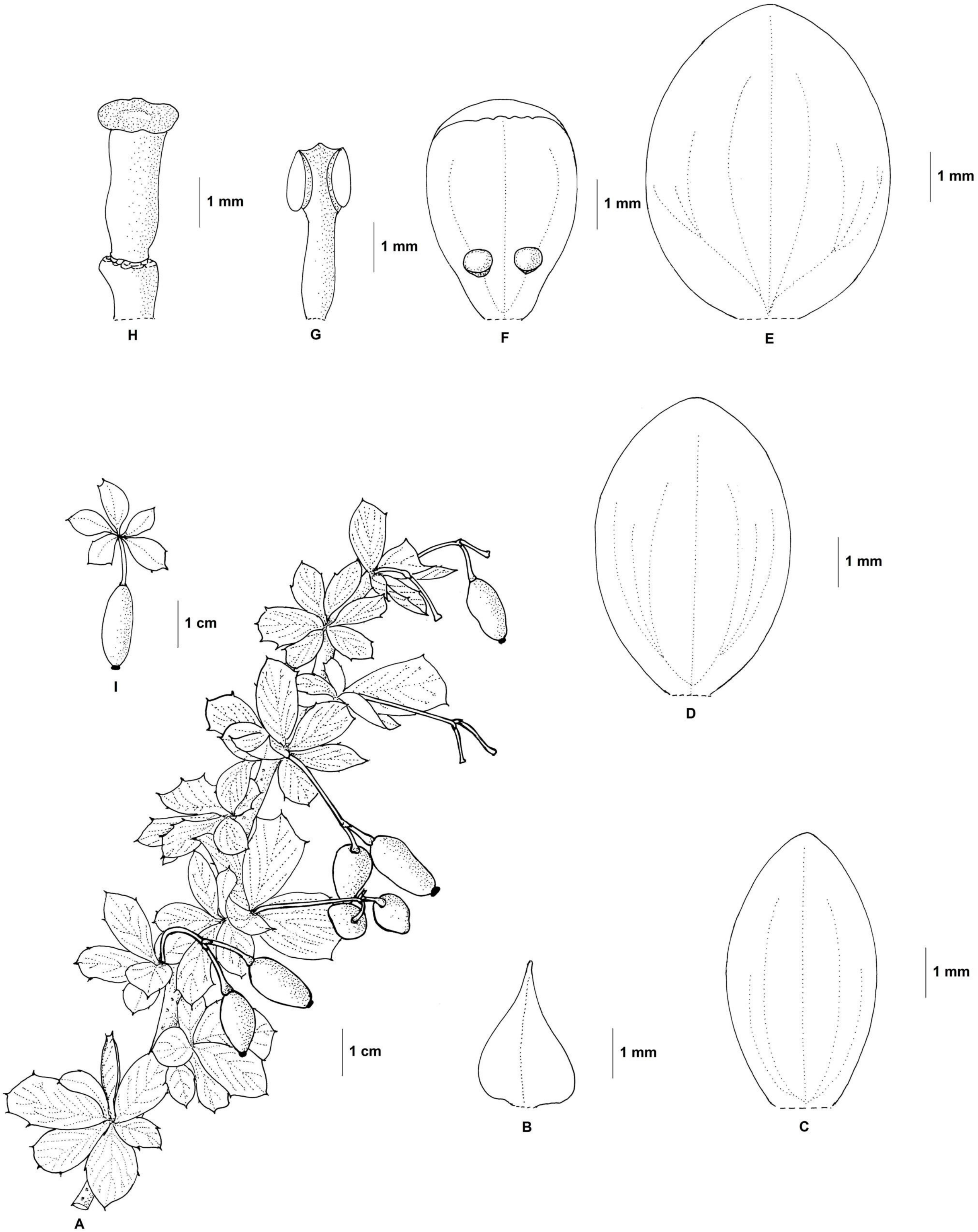


FIG. 2.15. (A-H). ***Berberis concinna*** var. ***extensiflora***. A. Fruiting branch. B. Outermost sepal. C. Outer sepal. D. Median sepal. E. Inner sepal. F. Petal. G. Stamen. H. Pistil. (Based on: A, EA 105; B-H, Manaslu 08, 20812277). I. ***Berberis concinna*** var. ***concinna***. Fruiting branch. (Based on Adhikari, EL 125).

margin entire, venation distinct with one central and one pair of lateral veins; glands cup shaped, 0.3—0.5 mm in diameter. Stamens 3.5—4.5 mm long, connective scarcely produced. Pistil 3—4 mm long; ovules 7—13. Berries red, oblong-ovoid, 1—1.5 cm long; style absent.

Distinguishing features: Identifiable by the combination of glaucous leaves with reticulate venation, solitary or 2—3 flowers in an umbels; large up to 1.5 cm long estylose berries.

Key to the varieties of *Berberis concinna.*

1a. Inflorescence 0.8—1.5 cm long, flowers solitary without bracts on the middle of pedicel. .... a. var. **concinna**

1b. Inflorescence 2—5 cm long, flowers 2—3, usually in umbels, if solitary subtended by bracts on the middle of pedicel. .... b. var. **extensiflora**

4a. Berberis concinna Hook.f. var. **concinna**

Ahrendt, J. Linn. Soc. Bot. 57: 118 (1961). (Fig. 2.15, I; 2.51, G).

TYPE: INDIA: Sikkim: Lachen valley, Hooker, s.n. (Lectotype, here designated, K! with barcode K000077361).

Berberis concinna var. *brevior* Ahrendt

TYPE: NEPAL: Namlang, Sharma E269 (Holotype: BM! Barcode: BM000573943; isotype: E!).

Shrub up to 1 m. Flowers solitary. Ovules usually 7—10.

Flowering time: Jun—Aug.

Fruiting time: Aug—Oct.

Ecology: Open areas in sunny dry slopes.

Altitudinal range: 2600—4550 m.

Distribution: Western, Central, Eastern [E Himalaya].

Notes: Ahrendt (1961) described two varieties of *B. concinna* from Nepal. He separated variety *brevior* from variety *concinna* on the basis of length of the pedicels and size of the fruits, but these characters are not consistent within these two varieties, so variety *brevoir* is considered as a synonym of variety *concinna*.

Selected specimens: WESTERN: Rukum, above Ranmagaon, 12000 ft, 02.07.1954, Stainton, Sykes and Williams 3352 (E). CENTRAL: Mustang, Kaligandaki, Lete, 11500 ft, 04.06.1954, Stainton, Sykes and Williams 5595 (BM, E). EASTERN: Sankhuwasabha, Milkedada, 3480 m, 1971, Beer, Lancaster and Morris 41 (BM). Sankhuwasabha, Ridge near Kauma, S of Shipton La, 3530 m, 26.09.1991, EMAK 315 (E). Number of specimens examined: 31

4b. Berberis concinna Hook.f. var. **extensiflora** Ahrendt, J. Linn. Soc. Bot. 57: 118 (1961). (Fig. 2.15, A-H; 2.51, H).

TYPE: NEPAL: Larjung, Kaligandaki valley, Stainton, Sykes & Williams, 8175 (Holotype: BM! Barcode: BM000551279; isotype: E!).

Shrub up to 2 m. Flowers usually 2—3 in umbels. Ovules usually 10—13.

Flowering time: Jun—Aug.

Fruiting time: Aug—Oct.

Ecology: Open areas in sunny dry slopes.

Altitudinal range: 3000—3400 m.

Distribution: Central (Nepal).

Selected specimens: CENTRAL: Myagdi, Ghorepani, Poon hill, 3120 m, 4.10.2006, EA 105 (E); Manang: Bimtang-Gho, 3280 m, 14.08.2008, 20812277 Manaslu 08 (E); Lamjung, Rambrong, Lamjung Himal, 11000 ft, 27.10.1954, Stainton, Sykes and Williams 9352 (BM, E). Number of specimens examined: 13

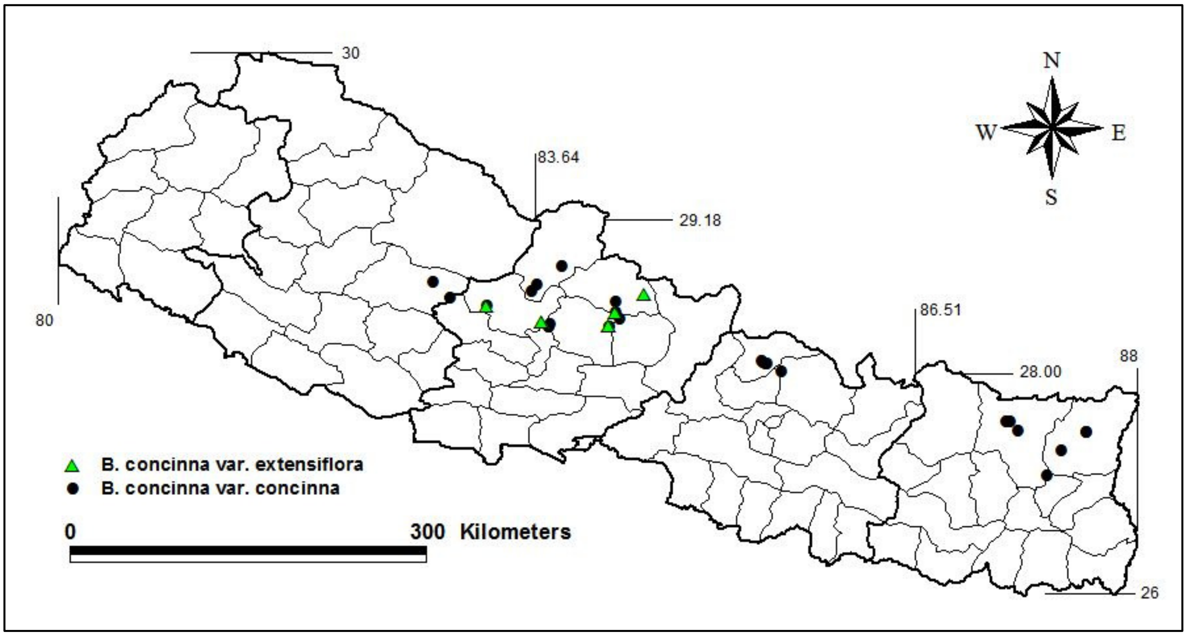


FIG 2.16. Distribution of *Berberis concinna.*

5. Berberis everestiana Ahrendt var. **ventosa** Ahrendt, J. Linn. Soc. Bot. 57: 117. 1961.

Tebbs in Hara & Williams, Enum. Fl. Pl. Nepal 2: 30 (1979); Rao *et al.*, Rheedea 8(2): 116 (1998); Press, Shrestha & Sutton, Annot. Checkl. Fl. Pl. Nepal: 26 (2000). (Fig. 2.17).

TYPE: NEPAL: Dolpa, between Pudamigaon and Ringmigaon, Polunin, Sykes and Williams 3554 (Holotype: BM!).

Shrub to 1 m. Stems and branches terete to slightly sulcate, glabrous, yellowish brown when young becoming greyish and verruculose when older. Internodes 0.5—2 cm. Spines 3(—5)-fid, strong, usually terete, central spine 1—1.5 cm, lateral spines 0.5—1.2 cm. Leaves deciduous, thin or slightly coriaceous. Petiole absent. Lamina usually obovate, 0.5—2.5 × 0.5—1 cm, base cuneate, apex obtuse, mucronate, margin entire or sometimes with 1—3 spinulose teeth on each side, dark green above, paler beneath, venation prominent below. Flowers solitary, rarely 2, yellow, *ca.* 1 cm in diameter. Bracts indistinct. Pedicel 0.3—1 cm. Sepals in 4 whorls, outermost sepals ovate with acute apex, 2 × 1.5 mm; outer sepals ovate or elliptic ovate, 6—7 × 3—4 mm; median sepals ovate-elliptic, 7—7.5 × 5—5.5 mm; inner sepals broadly obovate, 6.5—7.5 × 5—6 mm. Petals obovate, 5—6.5 × 3.5—4.5 mm, base cuneate, apex obtuse, undulate or slightly emarginate, margin entire, venation distinct with 1 central and 1 or 2 pairs of lateral veins; glands obovoid, 1—1.5 mm long. Stamens 3.5—5 mm long, connectives distinctly produced, tip apiculate. Pistil 3—4 mm long; ovules 5—7. Berries red, sub-globose or oblong-ovoid, *ca.* 1 cm long; style small *ca.* 1 mm.

Flowering time: Jun—Sep.

Fruiting time: Aug—Oct.

Ecology: Usually grows on exposed dry slopes.

Altitudinal range: 2700—4550 m.

Distribution: Western, Central, Eastern [E Himalaya (Sikkim)].

Distinguishing features: Small shrub less than 1.5 m; flowers usually solitary; connective distinctly produced; berries red with short style. This species is most likely to be confused with *B. angulosa* but is easily distinguished by its smaller flowers and leaves, and distinctly produced anther connective.

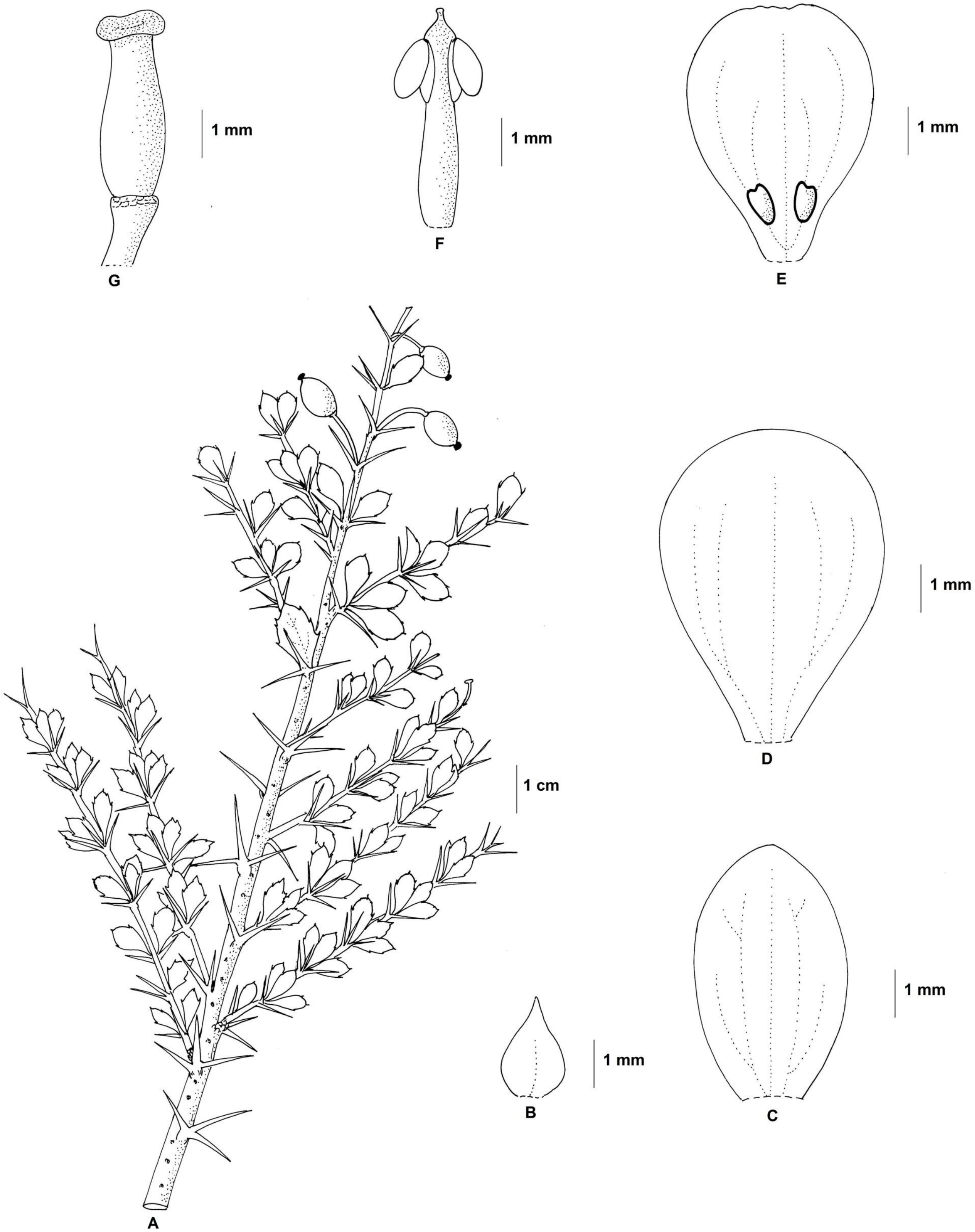


FIG. 2.17. ***Berberis everestiana*** var. ***ventosa***. A. Fruiting branch. B. Outer sepal. C. Median sepal. D. Inner sepal. E. Petal. F. Stamen. G. Pistil. (Based on: A, PSW 3554; B-G, Stainton 4289).

Selected specimens: WESTERN: Dolpa, Phoksundo Tal, 12500 ft, 11.10.1952, Polunin, Sykes & Williams 2202 (E, BM); Dolpa, Barbung Khola, Pemrigaon, 15000 ft, 09.06.1952, Polunin, Sykes & Williams 1104 (E). CENTRAL: Mustang, Kaligandaki Valley, 27.09.2006, EA 46(E). EASTERN: Solukhumbu, Bhotekoshi Valley, 3917 m, 23.09.2005, DNEP3 BY156 (E). Number of specimens examined: 30

Note: Ahrendt (1961) described two varieties of *B. everestiana*. The only specimen of variety *everestiana* cited by him from Nepal is Bailey 46 from Cholong Pati. Bailey 46 has been identified in this study as *B. angulosa* and no other specimens were recorded from Nepal which match the description of variety *everestiana*.

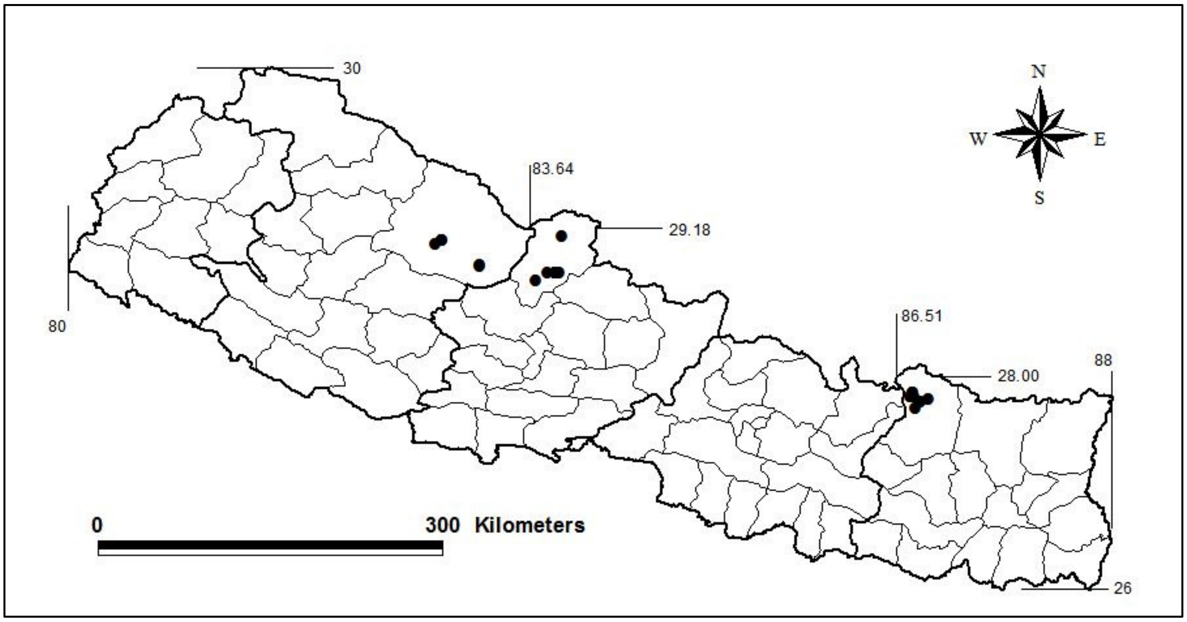


FIG. 2.18. Distribution of *B. everestiana* var. *ventosa.*

6. Berberis glaucocarpa Stapf, B. Mag. 151: sub t. 9102. 1926.

Ahrendt, J. Bot. Lond. (Suppl.): 101 (1942); Chatterjee, Rec. Bot. Surv. India 16(2): 14 (1953); Ahrendt, J. Linn. Soc. Bot. 57: 90 (1961); Tebbs in Hara & Williams, Enum. Fl.

Pl. Nepal 2: 30 (1979); Rao *et al*., Rheedea 8(1): 57 (1998); Press, Shrestha & Sutton, Annot. Checkl. Fl. Pl. Nepal: 26 (2000). (Fig 2.19; 2.51, I).

TYPE: INDIA: Jaunsar: Brandis 746 (Lectotype here designated, K! with barcode K000644848).

Shrub to 4 m. Stems and branches terete, glabrous, dark grey to greyish yellow, verruculose. Internodes 2—4 cm. Spines usually 3, solitary towards the apex of twig, strong, terete or angular, central spine 0.8—2 cm, lateral spines 0.5—1.5 cm. Leaves deciduous, thinly coriaceous to coriaceous. Petiole absent. Lamina obovate-oblanceolate, 2—5.5(—7) × 1—3 cm, base cuneate, apex obtuse, usually mucronate, margin usually entire, sometimes with 2—6 spinose teeth on each side, lustrous green above, paler beneath, venation reticulate, prominent both sides. Inflorescence 3—6 cm long, a stiff pedunculate raceme of 10—20 flowers, sometimes with a few flowers arising from the base of the rachis. Bracts ovate, 1.5—2 mm long. Flowers yellow, *ca.* 1.5 cm in diameter. Peduncle 0.2—1.5 cm. Pedicel 0.5—1 cm. Sepals in 3 whorls, outer sepals ovate, 4.5—6 × 2—2.5 mm; median sepals ovate-elliptic, 5.5—8 × 3.5—4.5 mm; inner sepals elliptic-orbiculate, 7.5—8.5 × 5—7 mm. Petals obovate, 5—7.5 × 4—4.5 mm, base cuneate, margin entire, apex notched, 0.2—0.5 mm deep, venation distinct with one central and one pair of lateral veins; glands obovoid, 1—1.5 mm long. Stamens 4.5—5.5 mm long, connective distinctly produced, tip obtusely pointed. Pistil 3.5—4.5 mm long; ovules 3—4. Berries dark purple, oblong ovoid or ovoid globose, 0.8—1.2 cm long including style, glaucous; style 0.5—1.5 mm long.

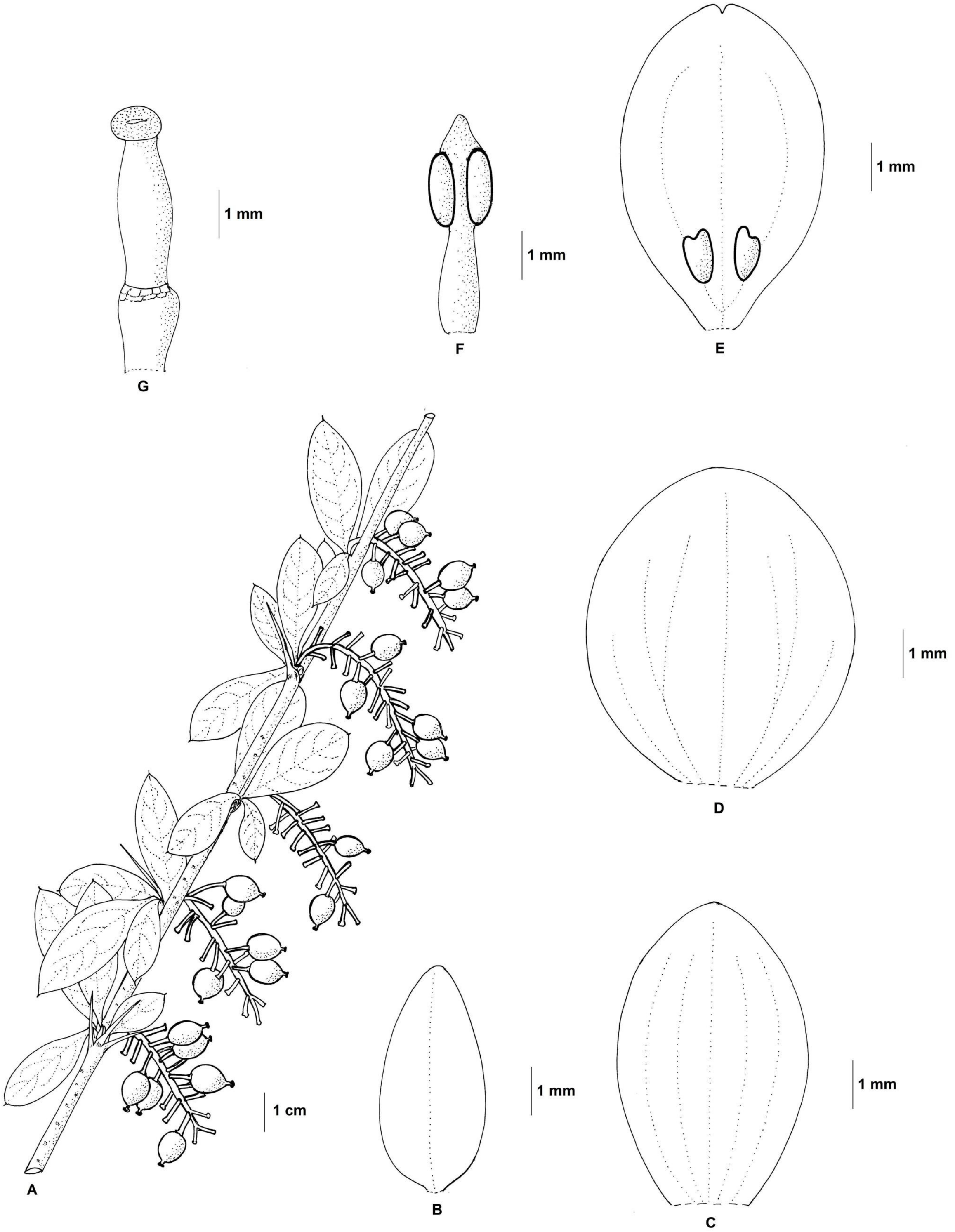


FIG. 2.19. ***Berberis glaucocarpa***. A. Fruiting branch. B. Outer sepal. C. Median sepal. D. Inner sepal. E. Petal. F. Stamen. G. Pistil. (Based on: A, PSW 5072; B-G, JRSA 80).

Flowering time: May—Jul.

Fruiting time: Jun—Oct.

Ecology: Along forest edges.

Altitudinal range: 2300—3450 m.

Distribution: Western, Central [W Himalaya (Kumaon)].

Distinguishing features: This species is easily identified by its glaucous berries in stiff racemes and distinctly produced anther connectives.

Selected specimens: WESTERN: Mugu, Below Ghuruchi Lagna, 3400 m, 07.06.2008, JRSA 80 (E); Humla, Below Saatthapla towards Rimi, 2640 m, 13.06.2008, JRSA 134 (E); Jumla, Chanki, NW of Jumla, 2580 m, 09.08.1952, Polunin, Sykes and Williams 5072 (BM); Dolpa, Near Hurikot, 3030 m, 25.09.1952, Polunin, Sykes and Williams 5407 (BM); Jumla, 2420 m, 10.05.1952, Polunin, Sykes and Williams 930 (BM). CENTRAL: Myagdi, Above Sauwala Khola, 10500 ft, 13.09.1954, Stainton, Sykes and Williams 4376 (E); Baglung, Near Sirtibang Lekh, 11000 ft, 14.10.1984, Stainton, Sykes and Williams 9024 (E). Number of specimens examined: 16

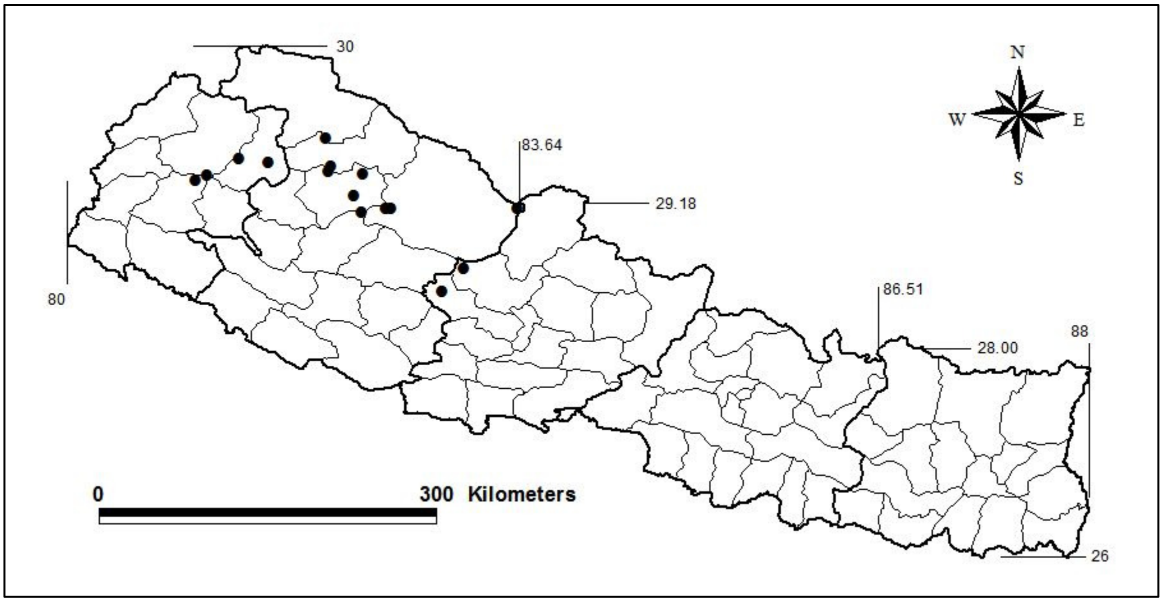


FIG. 2.20. Distribution of *B. glaucocarpa.*

7. Berberis hamiltoniana Ahrendt, Gard. Ill. 64: 426. 1944.

Chatterjee, Rec. Bot. Surv. India 16(2): 22 (1953); Ahrendt, J. Linn. Soc. Bot. 57: 137 (1961); Tebbs in Hara & Williams, Enum. Fl. Pl. Nepal 2: 30 (1979); Rao *et al.*, Rheedea 8(2): 111 (1998); Press, Shrestha & Sutton, Annot. Checkl. Fl. Pl. Nepal: 25 (2000). (Fig. 2.21; 2.51, J).

TYPE: NEPAL: Cultivated (probably at Watlington in Oxfordshire), L.A. 347 (from seed from Jey Jey, Kew 1056/34), fl. 3 June 1942, 10 June 1943; fr. 15 Oct. 1943 (Lectotype, here designated: BM! specimen no 534 with barcode: 000554686).

Shrub to 4 m. Stems and branches terete, glabrous, reddish brown when young becoming grey when older, sparsely verruculose. Internodes 1—2(—4) cm. Spines usually 3, solitary towards twig apex, strong, terete or slightly angled, central spine 0.8—1.8 cm, lateral spines 0.5—1.5 cm. Leaves deciduous, thinly coriaceous. Petiole absent. Lamina obovate or obovate-elliptic, 2—3.5 × 0.7—1.5 cm, base cuneate, apex obtuse, mucronate, margin usually entire, rarely with 2 or 3 spinulose teeth on each side, dark green above, paler and papillose beneath, venation sub-conspicuous above, prominent and slightly raised below. Inflorescence 1.5—4 cm long, an umbellate or sub-umbellate raceme of 3—8 flowers with a few flowers arising from the base of rachis. Bracts ovate-triangular, 1—2.5 mm long. Flowers yellow, 1—1.5 cm in diameter. Peduncle 0.5—2 cm. Pedicel 0.5—2.0 cm. Sepals in 3 whorls, outer sepals ovate or ovate-elliptic, 3.5—5.5 × 2—3 mm; median sepals elliptic or elliptic-obovate, 4.5—7 × 2.5—5 mm; inner sepals broadly obovate, 7—9 × 5—6.5 mm.

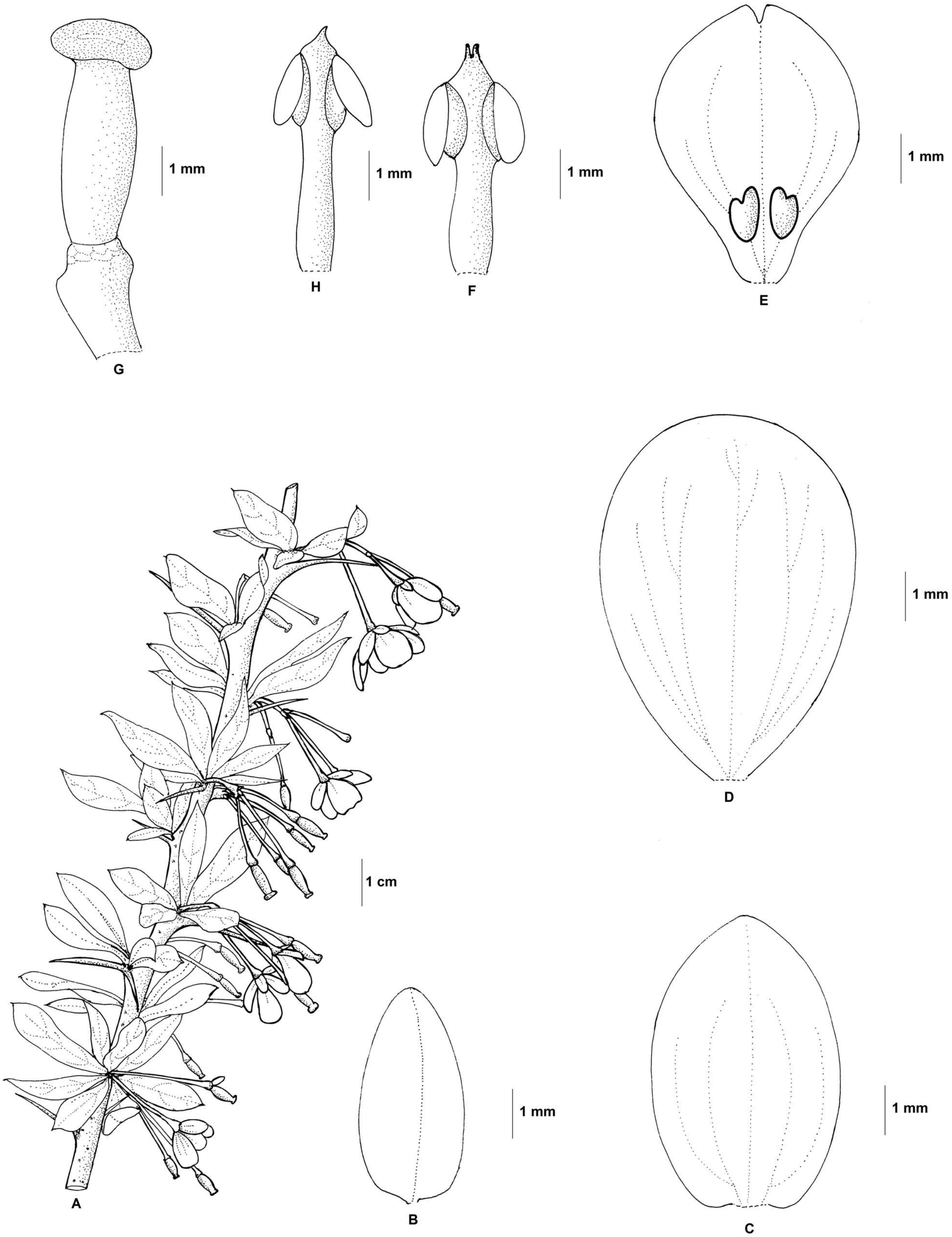


FIG. 2.21. ***Berberis hamiltoniana***. A. Flowering branch. B. Outer sepal. C. Median sepal. D. Inner sepal. E. Petal. F, H. Stamen. G. Pistil. (Based on: A, JRSB 162; B-G, PSW 4579; H, Lowndes, 941).

Petals obovate, 5—7 × 3—5 mm, base cuneate, apex notched, 0.2—0.4 mm deep, margin entire, venation distinct with 1 central and 1 or 2 pairs of lateral veins; glands obovoid-ellipsoid, 0.8—1.5 mm long. Stamens 4—5 mm long, connective distinctly produced, tip pointed, sometimes bifurcated. Pistil 3.5—4.5 mm long; ovules 3—5. Berries red, ovate-ellipsoid, 0.6—1 cm long; style absent.

Flowering time: May—Jul.

Fruiting time: Jun—Oct.

Ecology: Open hillside.

Altitudinal range: 2900—4250 m.

Distribution: Western, Central [W Himalaya (Himachal Pradesh)].

Distinguishing features: Identifiable by its usually entire leaves, umbellate or sub-umbellate raceme, notched petals and distinctly produced anther connectives.

Selected specimens: WESTERN: Mugu, Below Ghuruchi Lagna, 3400 m, 07.06.2008, JRSA 80 (E); Humla, Below Saathapla towards Rimi, 2640 m, 13.06.2008, JRSA 134 (E); Jumla, Chanki, NW of Jumla, 2580 m, 09.08.1952, Polunin, Sykes and Williams 5072 (BM); Dolpa, Near Hurikot, 3030 m, 25.09.1952, Polunin, Sykes and Williams 5407 (BM); Jumla, 2420 m, 10.05.1952, Polunin, Sykes and Williams 930 (BM). Number of specimens examined: 16

Note: Ahrendt (1944) stated the type to be in Herb. Oxon whereas in 1961 he cited type as ‘Cultivated: L.A. 347 (from seed from Jey Jey, Kew 1056/34), fl. 3 June 1942, 10 June 1943; fr. 15 Oct. 1943 (Type, BM)’. Two herbarium sheets were found in BM with the same annotations but different numbers, 534 and 535. The specimen numbered 534 is in better condition and has been chosen as the lectotype.

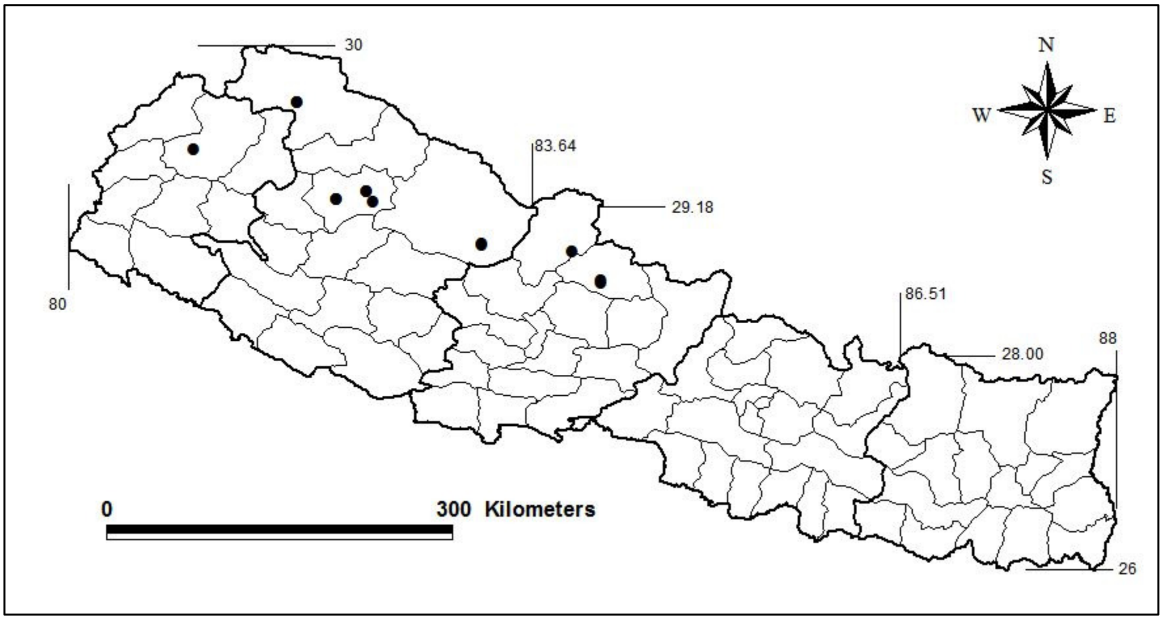


FIG. 2.22. Distribution of *Berberis hamiltoniana.*

8. Berberis hookeri Lem., Ill. Hort. 6: 207. 1859.

Schneider, Bull. Herb. Boiss. 2.4: 401 (1905); Chatterjee, Rec. Bot. Surv. India 16(2): 8 (1953); Ahrendt, J. Linn. Soc. Bot. 57: 39 (1961); Tebbs in Hara & Williams, Enum. Fl. Pl. Nepal 2: 30 (1979); Grierson & Long, Fl. Bhutan 1(2): 326 (1984); Chamberlain & Hu, Notes RBG Edinb. 42(3): 533 (1985); Rao *et al.*, Rheedea 8(1): 40 (1998); Press, Shrestha & Sutton, Annot. Checkl. Fl. Pl. Nepal: 26 (2000). (Fig. 2.23; 2.51, K).

TYPE: Cultivated plant from a garden in Ghent Belgium. [Holotype: Illustration: Illustration Horticole, Planche 207 (1859); Epitype, here designated: Sikkim: Hooker 255, K!].

Shrub to 1.5 m. Stems and branches glabrous, terete, dark grey to greyish yellow, verruculose, young shoots slightly sulcate. Internodes 2—5(—8) cm. Spines usually 3,

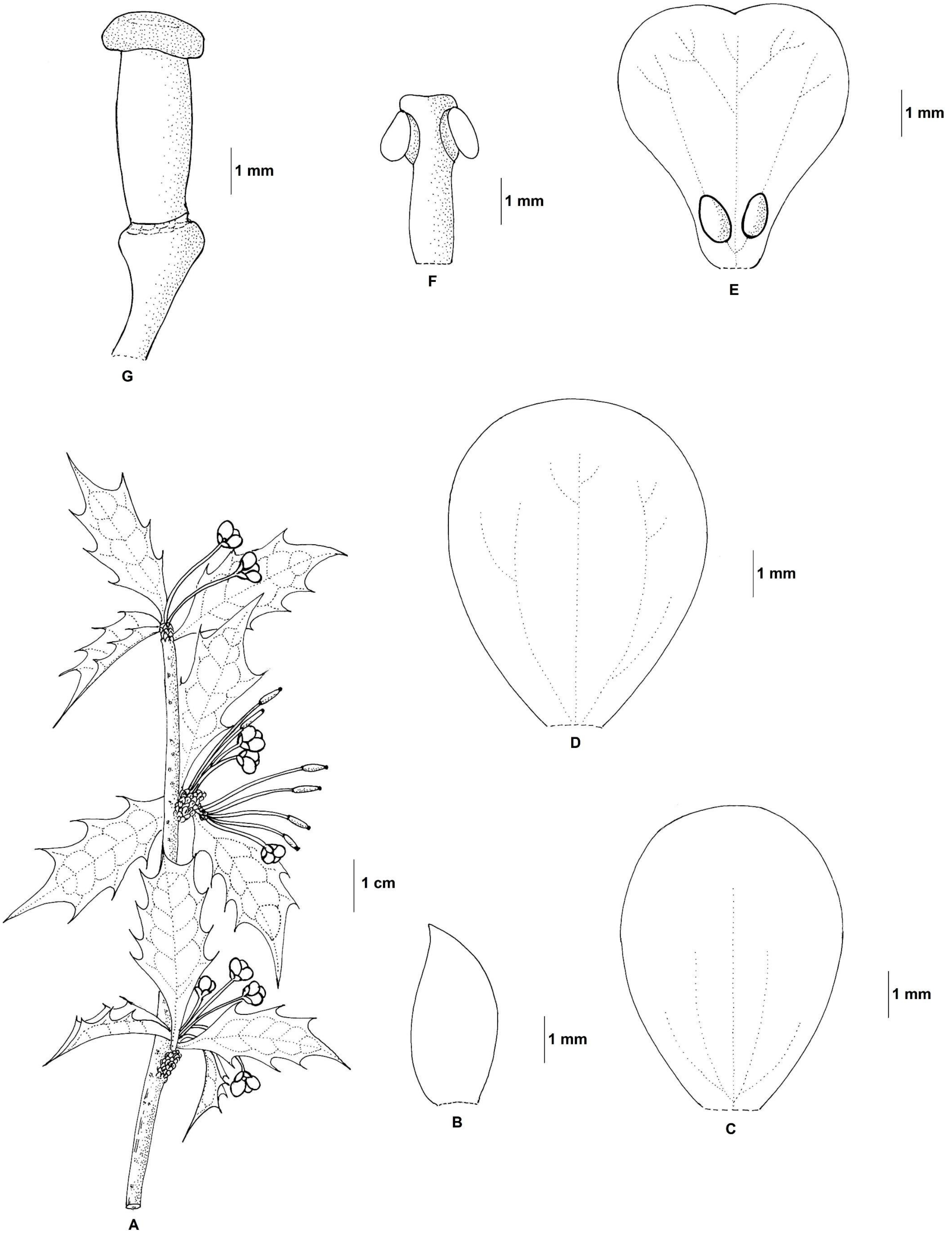


FIG. 2.23. ***Berberis hookeri***. A. Flowering branch. B. Outer sepal. C. Median sepal. D. Inner sepal. E. Petal. F. Stamen. G. Pistil. (Based on: A, D. Proud, 1; B-G, LKSRB 22).

strong, terete, central spine 1—2.2 cm, the lateral spines equal or slightly shorter than the central. Leaves evergreen, coriaceous. Petiole absent or sometimes up to 5 mm. Lamina lanceolate-elliptic, rarely obovate, 3—7 × 1—3 cm, base cuneate or attenuate to a small petiole, apex acute, usually mucronate, margin with 2—7(—14) spinose teeth on each side, lustrous green above, paler and sometimes glaucous beneath, venation prominent both sides. Inflorescence a fascicle of 3—11 flowers. Flowers yellowish green, *ca.* 1.5 cm in diameter. Pedicel (0.5—)1—2.5 cm, glabrous. Sepals in 4 whorls, outermost sepals ovate-triangular with acute or acuminate apex, 3—4 × 1—1.5 mm; outer sepals ovate or oblong ovate, 3.5—4.5 × 1.5—2.5 mm; median sepal elliptic-obovate or broadly obovate, 4.5—8 × 3—5.5 mm; inner sepals broadly obovate, 6—9 × 3.5—7.5 mm. Petals obovate, 4.5—6.5 × 3.5—6 mm, base cuneate, apex obtuse or emarginate, margin entire, venation distinct with one central and one pair of lateral veins; glands ovate, 0.8—1.5 mm long. Stamens 3—4.5 mm long, connective produced, truncate or slightly retuse. Pistil 3—4.5 mm long; ovules 3—6. Berries black, oblong-ovoid, 1—1.5 cm long, glaucous; style absent.

Flowering time: May—July.

Fruiting time: July—Oct.

Ecology: Open hillsides, edges of evergreen forest.

Altitudinal range: 2000—3400 m.

Distribution: Central, Eastern [E Himalaya, Tibetan Plateau, Assam-Burma].

Distinguishing features: Identifiable by its evergreen habit, greenish yellow flowers in fascicles, and glaucous black estylose berries.

Selected specimens: CENTRAL: Rasuwa, Gosaikund Ridge, 3180 m, 14.05.1960, Proud,D. 1 (BM), Myagdi, Near Kuinekhani, 2730 m, 25.05.1954, Stainton, Sykes and Williams 2846 (E). EASTERN: Taplejung, Between Koping and Noondaki, 3000m, 24.10.1991, EMAK 1021 (E); Panchthar, Sidin-1, Lamapokheri, 3007 m, 08.06.2007, LKSRB 22 (TUCH, E); Taplejung, Mewakhola, ca 3300 m, 25.10.1971, Beer, Lancaster and Morris, 157 (BM). Number of specimens examined: 18

Note: Lemaire's (1859) description is based on a cultivated plant and he did not cite any specimen in his original description. Therefore, Lemaire's illustration has been identified as the holotype and Hooker's specimen has been designated as an epitype.

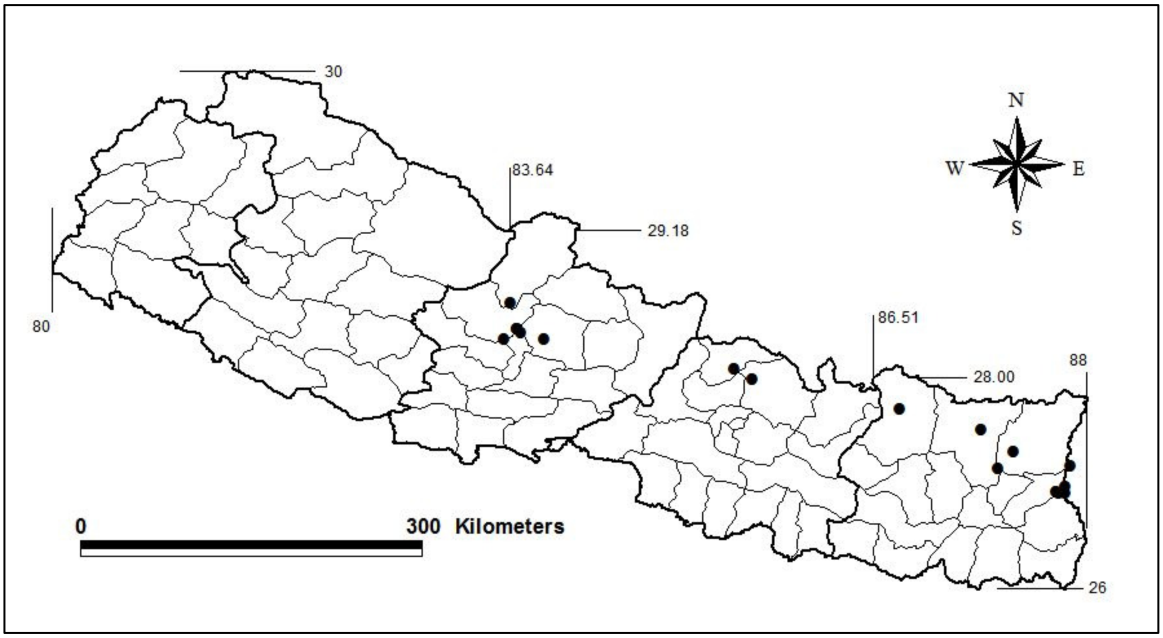


FIG. 2.24. Distribution of *Berberis hookeri.*

9. Berberis insignis Hook.f. & Thoms., Fl. Ind. 1: 226.1855.

Hooker f. & Thomson in Hooker f., Fl. Brit. Ind. 1(1): 111 (1872); Schneider, Bull. Herb. Boiss. 2.4: 401 (1905); Chatterjee, Rec. Bot. Surv. India 16(2): 7 (1953); Ahrendt, J. Linn. Soc. Bot. 57: 36 (1961); Tebbs in Hara & Williams, Enum. Fl. Pl. Nepal 2: 30 (1979); Grierson & Long, Fl. Bhutan 1(2): 324 (1984); Chamberlain & Hu, Notes RBG

Edinb. 42(3): 536 (1985); Rao *et al*., Rheedea 8(1): 32 (1998); Press, Shrestha & Sutton, Annot. Checkl. Fl. Pl. Nepal: 25 (2000). (Fig. 2.25).

TYPE: INDIA: Sikkim: Hooker s.n., May 23, 1849 (Lectotype here designated, K ! with Barcode: K000077368).

Shrub to 4 m. Stems and branches terete, glabrous, grey, young stems reddish brown. Internodes 2—7 cm. Spines absent or 3, rarely 5, central spine 2—2.5 cm, lateral spines 1—1.5. Leaves evergreen, slightly coriaceous. Petiole absent or up to 8 mm. Lamina oblong or elliptic-lanceolate, 8—17 × 2—4 cm, base cuneate or attenuate to the small petiole, apex acuminate, sometimes acute, margin with 10—26 spinose teeth on each side, green above, paler beneath, venation sub conspicuous above, prominent below. Inflorescence a fascicle of 5—25 flowers. Flowers yellow, *ca.* 1.5—2 cm in diameter. Pedicel 0.5—1.5 cm, glabrous. Sepals in 4 whorls, outermost sepals broadly ovate, 1.5—3.5 × 1.5—3 mm; outer sepals broadly ovate-rounded, 3—6 × 2.5—4 mm; median sepal broadly ovate or elliptic-ovate, 4—7 × 3.5—4.5 mm; inner sepals broadly obovate or elliptic-rounded, 4—7 × 4—6 mm. Petals obovate or narrowly obovate, 4.5—6.0 × 2.5—4.0 mm, base cuneate, apex emarginate, margin entire, venation distinct with one central and one pair of lateral veins; glands ellipsoid or oblong obovoid, 1—1.5 mm long. Stamens 3—4.5 mm long, connective slightly produced or not, tip obtuse. Pistil 3—4 mm long; ovules 2—4. Berries black, oblong-obovoid or ellipsoid, *ca.* 1 cm long; style absent.

Flowering time: Apr—Jun.

Fruiting time: Jun—Nov.

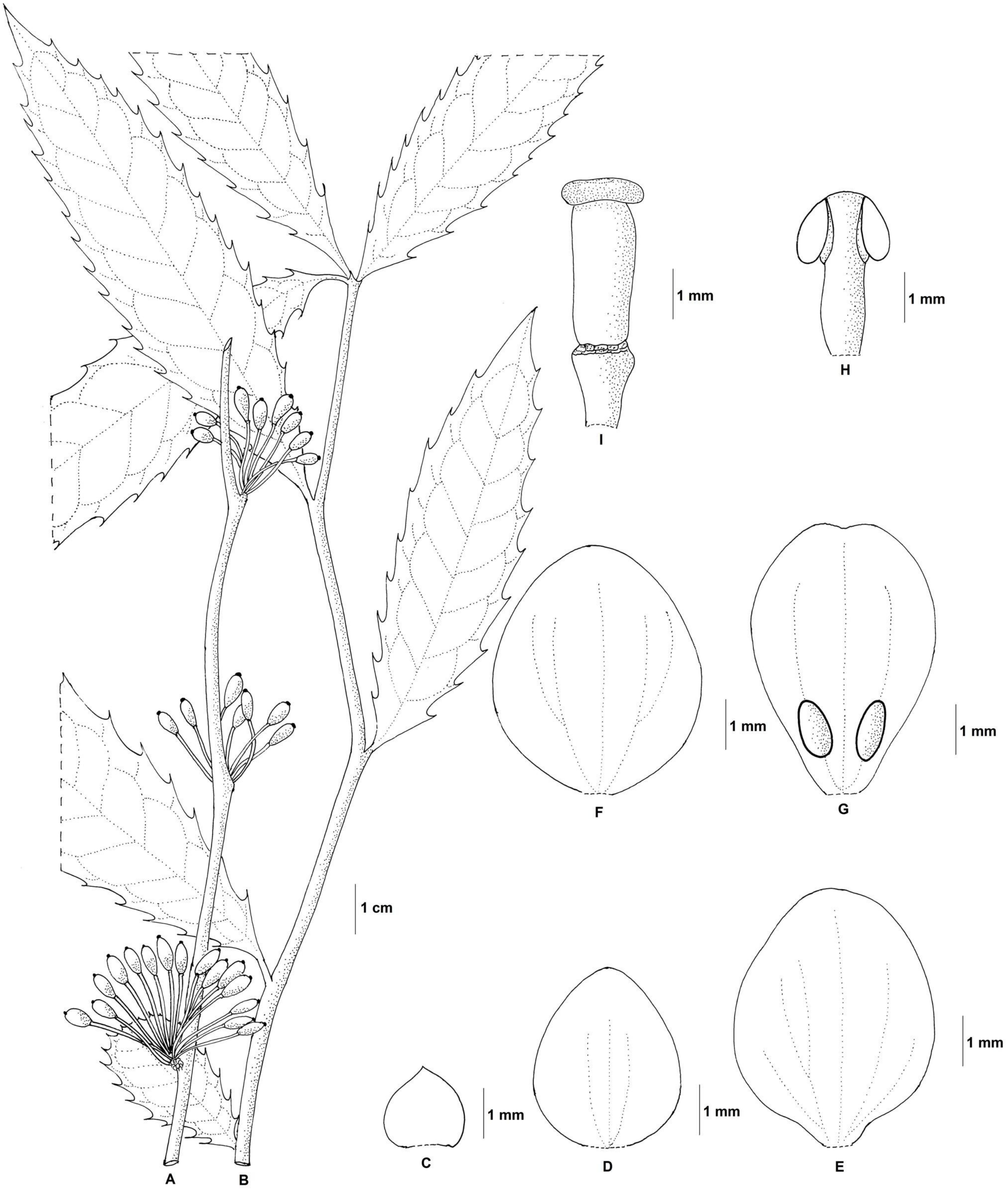


FIG. 2.25. ***Berberis insignis***. A. Fruiting branch. B. Vegetative branch. C. Outermost sepal. D. Outer sepal. E. Median sepal. F. Inner sepal. G. Petal. H. Stamen. I. Pistil. (Based on: A & B, EMAK 875; C-I, Suzuki *et al*. 9263024).

Ecology: Moist forest floor of evergreen forests.

Altitudinal range: 2000—3400 m.

Distribution: Eastern [E Himalaya, Assam-Burma].

Distinguishing features: This species is easily identified by its large leaves up to 17 cm, flowers in fascicles and black berries.

Selected specimens: EASTERN: Sankhuwasabha, West side of Panch Pokhari Khola, 2460 m, 17.10.1991, EMAK 875 (E); Sankhuwasabha, Arun Valley, Maghang Khola, 3330 m, 01.05.1956, Stainton 182 (E); Taplejung, Sewaden-Topkegola, 2520 m, 15.05.1992, Suzuki et al. 9263064 (E). Sankhuwasabha, Milke Bhanjyang, 2420 m, 03.07.1969, Williams L.H.J 1104 (BM); Taplejung, Mewa Khola, 3030 m, 25.10.1971, BLM 145 (BM). Number of specimens examined: 21

Note: Hooker and Thomson (1855) mentioned three gatherings, that of Griffith from Bhutan and Hooker's own collections from Sikkim and Nepal. Later Ahrendt (1961) cited one of Hooker's specimens as a type. For lectotypification, Hooker's specimen at Kew, which was cited by Ahrendt (1961), has been chosen because the specimen is from Hooker's herbarium and was collected by him in 1849.

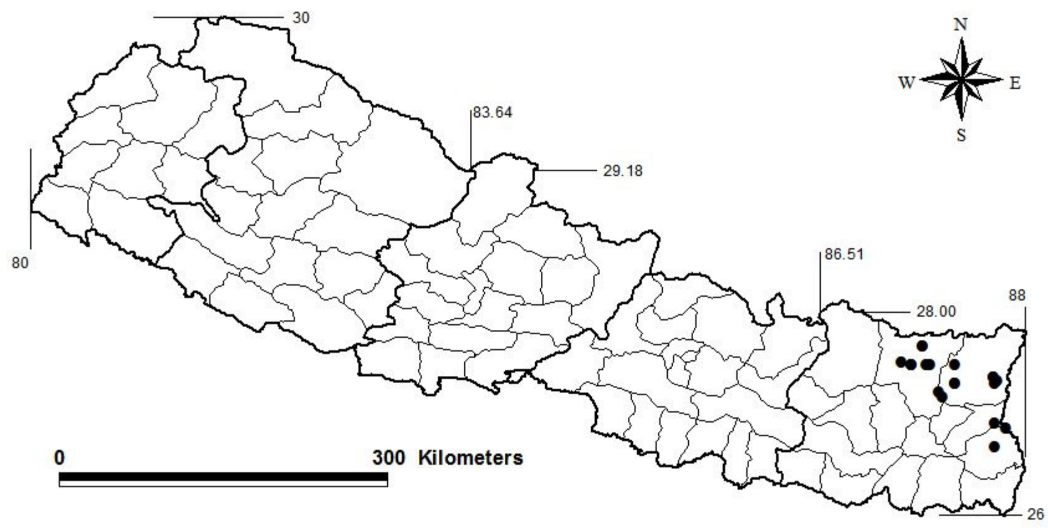


FIG. 2.26. Distribution of *Berberis insignis.*

10. Berberis jaeschkeana C.K.Schneid. var. **usteriana** C.K.Schneid., Bull. Herb. Boiss. 2.4: 399. 1905.

Ahrendt, J. Linn. Soc. Bot. 57: 139 (1961); Tebbs in Hara & Williams, Enum. Fl. Pl. Nepal 2: 30 (1979); Rao *et al*., Rheedea 8(2): 121 (1998); Press, Shrestha & Sutton, Annot. Checkl. Fl. Pl. Nepal: 26 (2000). (Fig. 2.27; 2.51, L).

TYPE: INDIA: Kumaon, Byans, Duthie 5307 (Lectotype, here designated, WU!; isolectotype: K!).

Shrub to 2 m. Stems and branches terete or angled, glabrous, greyish brown. Internodes 1—2 cm. Spines usually 3, strong, sulcate, central spine 1—2 cm, the lateral spines equal or slightly shorter than the central. Leaves deciduous, coriaceous. Petiole absent. Lamina obovate or narrowly obovate-oblanceolate, 1.5—4 × 0.7—1 cm, base cuneate, apex acute or obtuse, tapering to a spine like mucro, margin entire or with 1—3 spinulose teeth on each side, green above, papillose beneath, venation obscure above, sub-conspicuous below. Inflorescence 2—4 cm long, a simple pedunculate raceme or sub-umbellate raceme of 5—15 flowers. Bracts ovate-triangular, 1—2 mm long. Flowers yellow, *ca.* 1 cm in diameter. Peduncle 0.3—1.2 cm, sometimes puberulous. Pedicel 0.5—1 cm. Sepals in 3 whorls, outer sepals ovate or oblong ovate, 3—5.5 × 1—3 mm; median sepals obovate-elliptic, 4—7.5 × 2—4 mm; inner sepals broadly obovate, 6—8.5 × 4—6 mm. Petals obovate, 5—6 × 3—3.5 mm, base cuneate, apex notched, 0.5—1 mm deep, margin entire, venation distinct with one central and one pair of lateral veins;

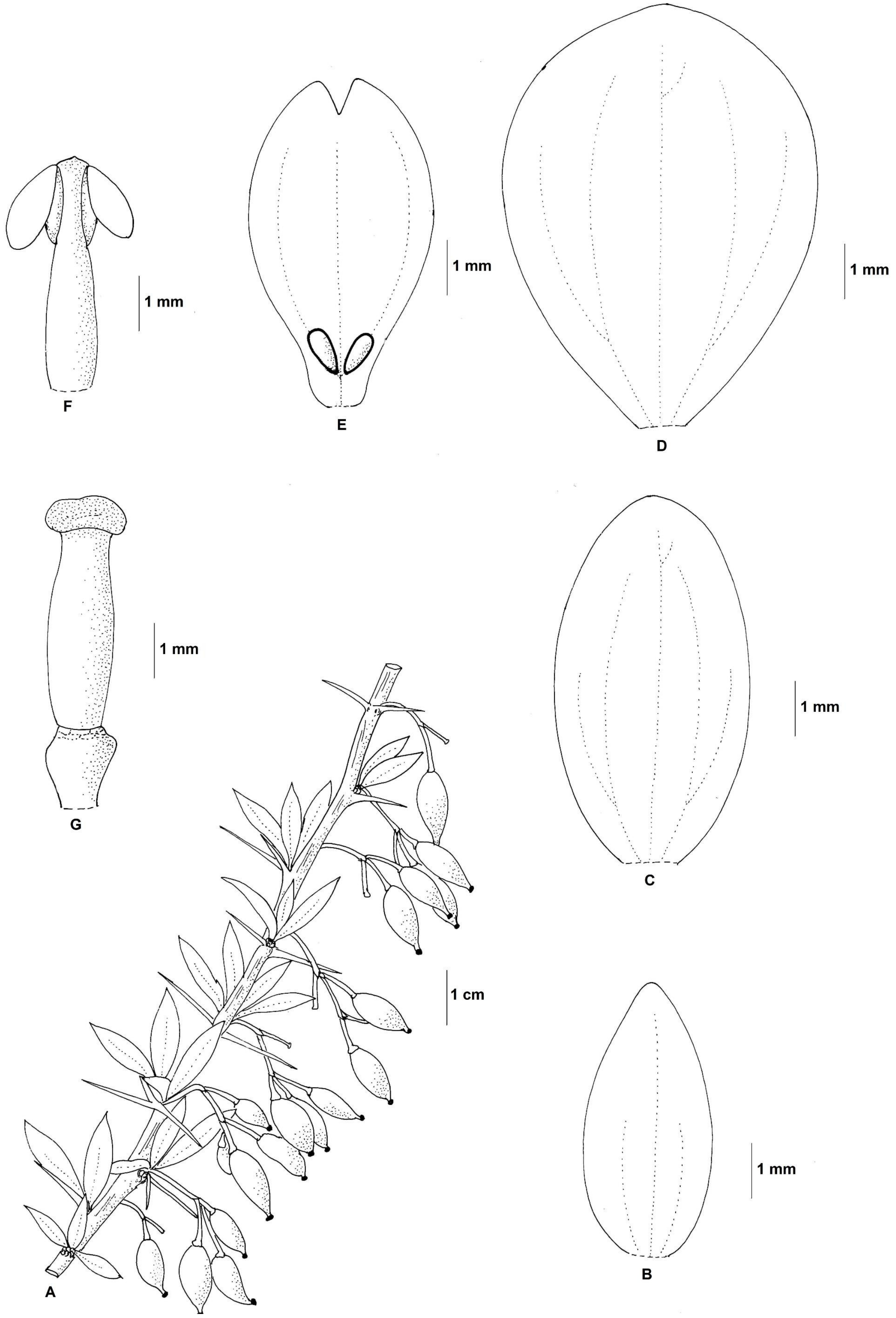


FIG. 2.27. ***Berberis jaeschkeana*** var. ***usteriana***. A. Fruiting branch. B. Outer sepal. C. Median sepal. D. Inner sepal. E. Petal. F. Stamen. G. Pistil. (Based on: A, EA2 33; B-G, SSW 1225).

glands ellipsoid or obovate-ellipsoid, 0.8—1 mm long. Stamens 3.5—4 mm long, connective scarcely produced or not. Pistil 3—4 mm long; ovules 2—4. Berries red, 1—1.2 cm long, oblong-ovoid tapering to a small style; style 1—2 mm long, sometimes indistinct.

Flowering time: May—Aug.

Fruiting time: Jul—Oct.

Ecology: In open dry exposed areas.

Altitudinal range: 2600—3800 m.

Distribution: Western, Central [W Himalaya (Kumaon)].

Distinguishing features: Identifiable by the combination of coriaceous leaves, pedunculate sub-umbellate racemes and red oblong ovoid berries.

Selected specimens: WESTERN: Dolpa, Bheri river, below Tarakot, 9500 ft, 02.06.1952, Polunin, Sykes and Williams 1014 (E). CENTRAL: Mustang, Muktinath, 12500 ft, 09.06.1954, Stainton, Sykes and Williams 5694 (BM); Mustang, Thinigaon, Muktinath Himal, 13000 ft, 20.06.1954, Stainton, Sykes and Williams 1225 (BM). Number of specimens examined: 14

Note: Schneider (1905) cited two specimens collected by Duthie (no 5306 & 5307) from the same locality, Byans, Kumaon. Duthie no 5307 deposited in WU has been chosen as lectotype because the specimen is in better condition than 5306 and also has a duplicate in Kew. The Nepalese specimens differ from the Indian type by their slightly coriaceous leaves and 2-4 ovules.

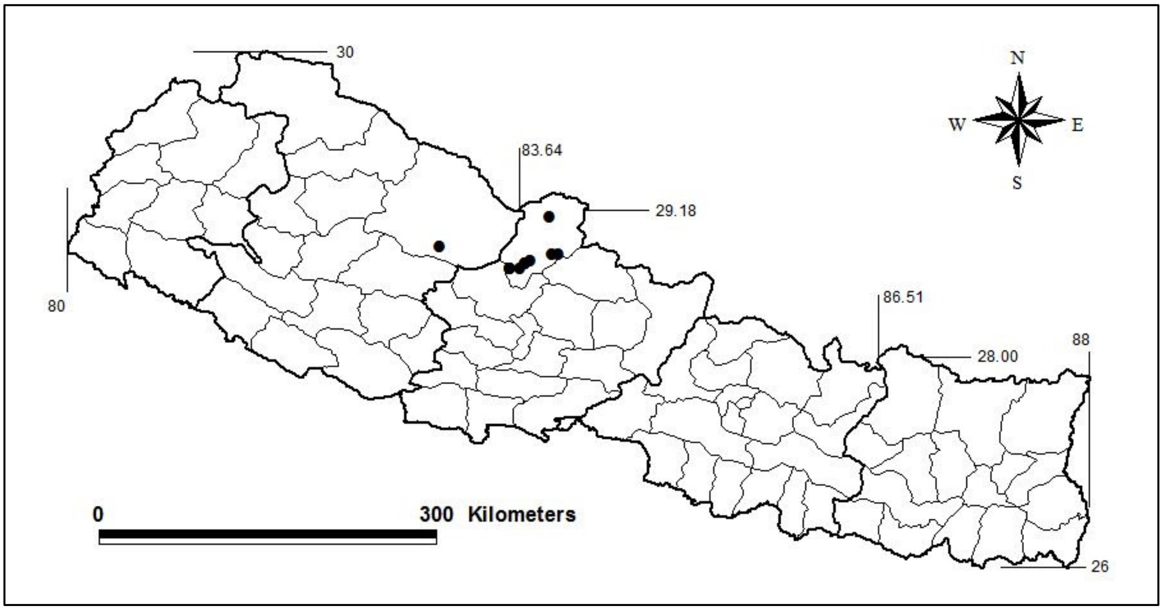


FIG. 2.28. Distribution of *Berberis jaeschkeana* var. *usteriana.*

11. Berberis karnaliensis Adhikari *sp. nov.* (Fig 2.29; 2.51 M).

Species *B. jaeschkeana* C.K.Schneid. affinis; spinae 3-fidae, ramuli puberuli, inflorescentiae umbellatae, petala apice incisura, stamina apice leviter producta, ovulis 2—3.

TYPE: NEPAL: Jumla, Bumra, JRSA 59 (Holotype: E!; isotype KATH!).

Shrub to 2 m. Stems and branches terete or slightly angled, densely puberulous when young becoming less puberulous when older, yellow or yellowish brown, slightly verruculose. Internodes 0.5—2.5 cm. Spines usually 3, solitary towards twig apex, strong, terete or angular, central spine 1—1.5 cm, the lateral spines equal or slightly shorter than the central. Leaves deciduous, thinly coriaceous. Lamina elliptic or narrowly obovate, 1—3 × 0.3—1 cm, base cuneate, apex acute, rarely obtuse,

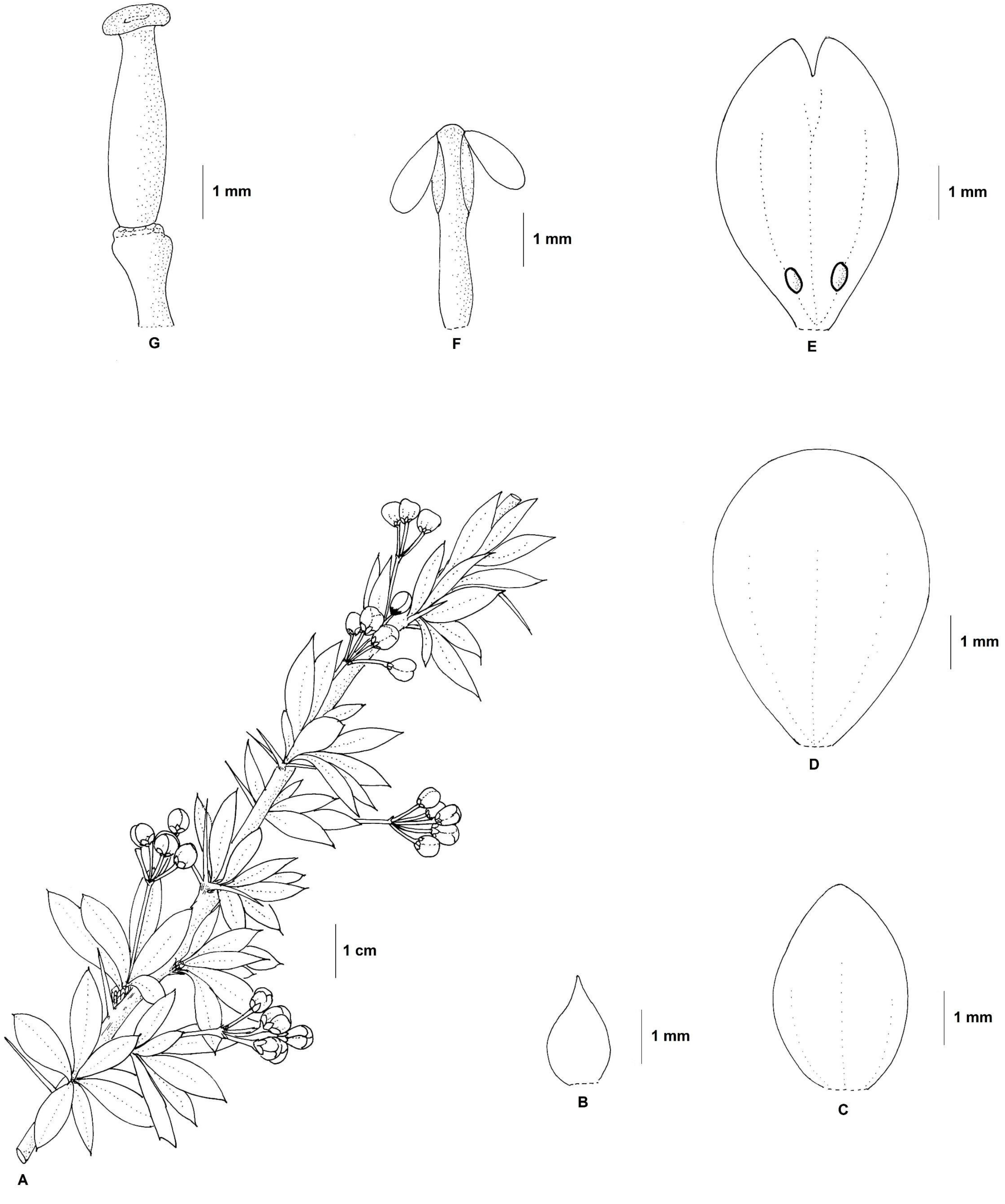


FIG. 2.29. ***Berberis karnaliensis***. A. Flowering branch. B. Outer sepal. C. Median sepal. D. Inner sepal. E. Petal. F. Stamen. G. Pistil. (Based on: JRSA 59).

mucronate, margin usually entire, sometimes with 3—5 spinulose teeth on each side, lustrous green above, paler and slightly papillose beneath, venation sub-conspicuous above, slightly raised and prominent below. Inflorescence 2—5 cm long, an umbellate or sub-umbellate raceme of 2—15 flowers. Bracts ovate with acuminate apex, 1—1.5 mm. Flowers yellow, *ca.* 1 cm in diameter. Peduncle 1—2.5 cm, glabrous, red. Pedicel 0.5—1.5 cm, glabrous. Sepals in 3 whorls, outer sepals ovate, 1.5—2 × 1—1.5 mm; median sepal elliptic or elliptic-ovate, 3—5 × 2—3 mm; inner sepals obovate to broadly obovate, 5—7 × 3—6 mm. Petals narrowly obovate to elliptic, 5—7 × 2—4 mm, base cuneate, margin entire, apex notched, 0.8—1 mm deep, venation distinct with one central and one pair of lateral veins; glands ellipsoid, 0.5—0.7 mm long. Stamens 3—5 mm long, connective slightly produced, tip obtuse or conical. Pistil 3—5 mm long; ovules 2—3. Berries dark red, oblong-obovoid, 0.8—1 cm long; style 1 mm long.

Flowering time: Apr—Jun.

Fruiting time: Jun—Oct.

Ecology: Along roadsides and forest margins of *Pinus*-*Rhododendron* forest.

Altitudinal range: 1800—3400 m.

Distribution: Western Nepal.

Distinguishing features: Identifiable by its pubescent branches, umbellate or sub-umbellate inflorescences, notched petals and stylose red berries.

Selected specimens: WESTERN: Humla, Unapani-Limne, 1820 m, 15.06.2008, JRSA 146 (E); Jumla, Gothichaur-Chutra, 2760 m, 27. 09. 1991, Minaki *et al.* 9104265 (E); Mugu, Khater Dara, 1850 m, 21.04.1952, Polunin, Sykes and Williams 834 (E); Dolpa, Dunai, 2100 m, 26.04.1974, Dobremez 2781

(E); Dolpa, Kolagaun, 11000 ft, 3.06.1952, Polunin, Sykes and Williams 1365 (BM); Rukum, 2700 m, 20.05.1969, Dobremez 142 (BM). Number of specimens examined: 11

Notes: This species is recorded from the Karnali and Rapti Zones of Western Nepal. It is most closely related to *B. jaeschkeana* C.K. Schneid. but differs in its densely pubescent branches, long peduncle, umbellate inflorescence and smaller fruits.

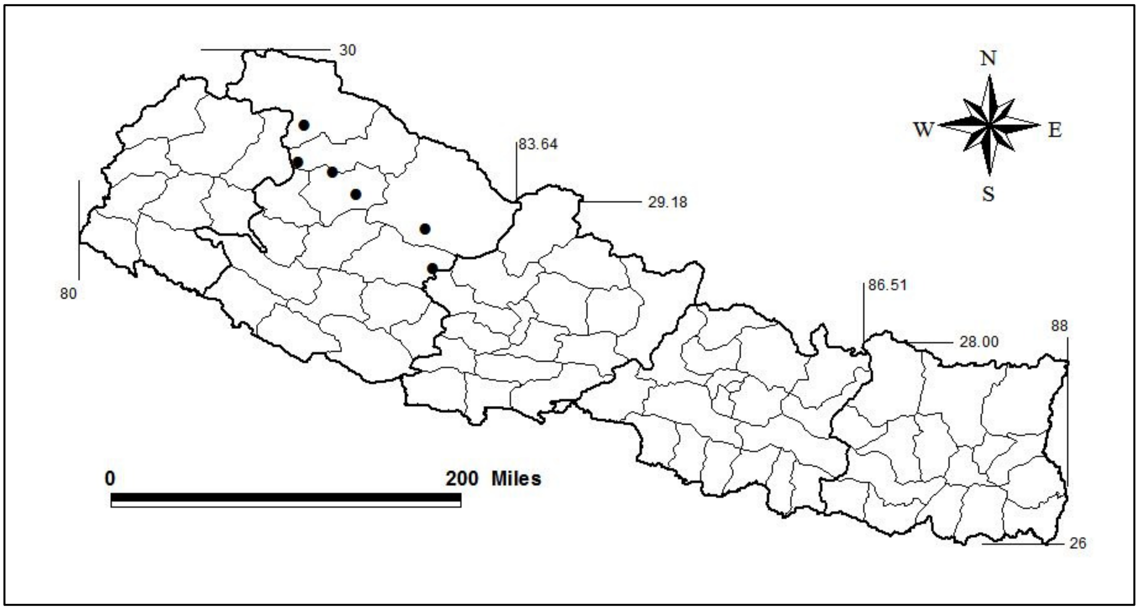


FIG. 2.30. Distribution of *Berberis karnaliensis.*

12. Berberis koehneana C.K.Schneid., Bull. Herb. Boiss. 2. 5: 814.1905.

Chatterjee, Rec. Bot. Surv. India 16(2): 32 (1953); Ahrendt, J. Linn. Soc. Bot. 57: 210 (1961); Tebbs in Hara & Williams, Enum. Fl. Pl. Nepal 2: 30; (1979); Rao *et al*., Rheedea 8(2): 129 (1998); Press, Shrestha & Sutton, Annot. Checkl. Fl. Pl. Nepal: 26 (2000). (Fig. 2.31; 2.51, N).

TYPE: INDIA: Kumaon, Near Budhi, Byans, Duthie 5309 (Holotype: WU!; isotype: K!, DD!).

Berberis koehneana var. *auramea* Ahrendt.

TYPE: NEPAL: Langtang, Polunin 506 (Holotype: BM!; isotype E!, A!).

Shrub to 3 m. Stems and branches terete or slightly angled, glabrous, yellow or yellowish brown, verruculose. Internodes 1—4 cm. Spines usually 3-fid, solitary towards twig apex, strong, terete or angular, central spine 0.5—1.5 cm, the lateral spines equal or slightly shorter than the central. Leaves deciduous, thin or slightly coriaceous. Petiole absent or 2—5 mm. Lamina obovate, 2—5 × 0.7—1.5 cm, base cuneate, tapering to the small petiole, apex obtuse, rarely acute, usually mucronate, margin usually entire, sometimes with 1—4 spinulose teeth on each side, lustrous green above, paler and papillose beneath, venation prominent both sides. Inflorescence 3—16 cm long, a panicle of 15—70 flowers. Bracts ovate-triangular, 1.5—3 mm. Flowers yellow, *ca.* 0.5—1 cm in diameter, yellow. Peduncle 0.5—4 cm, glabrous, reddish brown. Pedicel 0.5—1 cm. Sepals in 3 whorls, outer sepals ovate-triangular, 1.5—2.5 × 1.0—1.5 mm; median sepals elliptic or elliptic-ovate, 2.5—5 × 2—3.5 mm; inner sepals obovate to broadly obovate, 3.5—6.5 × 3.5—4.5 mm. Petals obovate, 3.5—6 × 2.5—4.5 mm, base cuneate or slightly clawed, margin entire, apex notched, 0.3—0.5 mm deep, venation distinct with one central and one pair of lateral veins; glands elliptic or elliptic-ovate, 0.5—0.7 mm long. Stamens 2—3.5 mm long, connective scarcely produced, tip conical. Pistil 2—3.5 mm long; ovules 2—3. Berries bright red, oblong ovoid or ellipsoid, 0.5—1.0 cm long; style absent.

Flowering time: Apr—Aug.
Fruiting time: Aug—Oct.

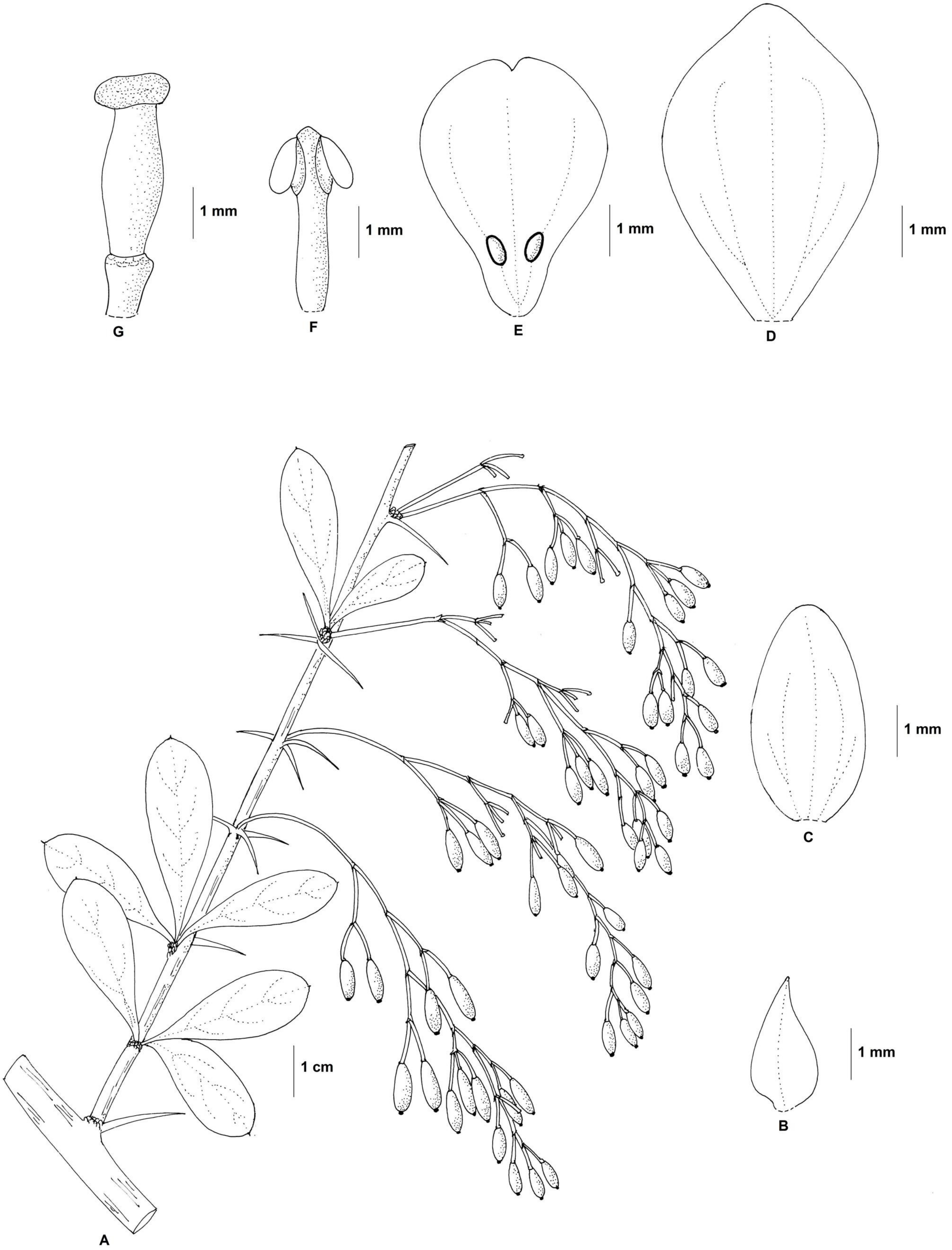


FIG. 2.31. ***Berberis koehneana***. A. Fruiting branch. B. Outer sepal. C. Median sepal. D. Inner sepal. E. Petal. F. Stamen. G. Pistil. (Based on: A, SSW 8136; B-G, Polunin 506).

Ecology: Dry, open, degraded areas along forest margins, settlements and agricultural lands.
Altitudinal range: 2400—4000 m.

Distribution: Western, Central [W Himalaya (Kumaon)].

Distinguishing features: This species is easily identified by its long paniculate inflorescence with up to 70 flowers and red estylose berries.

Selected specimens: WESTERN: Humla, Dozam, near Simikot, 8500 ft, 04.06.1952, Polunin, Sykes and Williams 4272 (E); Jumla, Bhurchula lekh, 9000 ft, 10.07.1952, Pulunin, Sykes and Williams 4514 (E); Rukum, Above Ghustung Khola, 10500 ft, 11.10.1954, Stainton, Sykes and Williams 4786 (BM, E). CENTRAL: Rasuwa, Ghodatabala, 3018 m, 4 Aug 2007, Adhikari, B, BL2 55 (E); Mustang, Below Muktinath, 3604 m, 16 Aug 2007, Adhikari, B., EA2 14 (E); Mustang, Kaligandaki Valley, Thinigaon, 3480 m, 26.09.1954, Stainton, Sykes and Williams 8022 (BM, E). Number of specimens examined: 55

Note: Ahrendt (1961) described the variety *auramea* from Central Nepal on the basis of colour of its yellow stem. Colour of stem greatly varies within the species and not consistently within any subgroup, so is not considered a reliable character to separate taxa. Therefore, the variety *auramea* is reduced to synonymy.

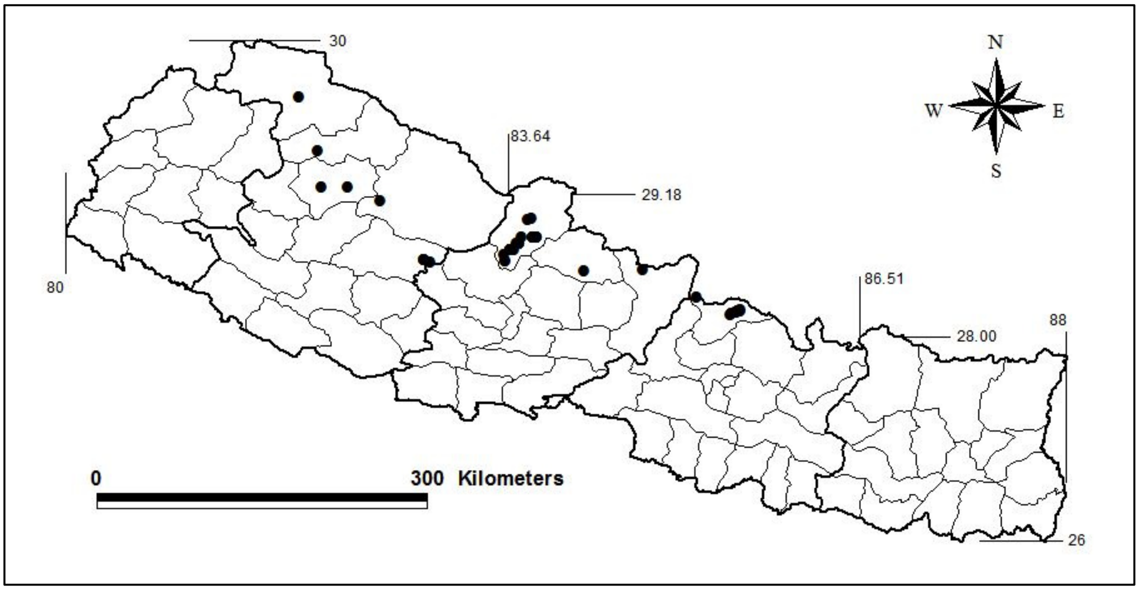


FIG. 2.32. Distribution of *Berberis koehneana.*

13. Berberis kumaonensis C.K.Schneid., Bull. Herb. Boiss. 2.5: 397. 1905.

Chatterjee, Rec. Bot. Surv. India 16(2): 25 (1953); Ahrendt, J. Linn. Soc. Bot. 57: 120 (1961); Tebbs in Hara & Williams, Enum. Fl. Pl. Nepal 2: 30 (1979); Rao *et al.*, Rheedea 8(2): 112 (1998); Press, Shrestha & Sutton, Annot. Checkl. Fl. Pl. Nepal: 26 (2000). (Fig. 2.33; 2.51, O).

TYPE: INDIA: Kumaon, rocks near Garbyang, Kalivalley, 13000 ft, 15 Sep 1894, Duthie 2697 (Holotype: G!; isotype: K!).

Semi-prostrate shrub to 20 cm. Stems and branches sulcate, glabrous, brownish-grey. Internodes 0.5—2 cm. Spines usually 3-fid, sulcate, central spine 1—3 cm, the lateral spines equal or slightly shorter than the central. Leaves deciduous, slightly coriaceous. Petiole absent. Lamina obovate, 1—2 × 0.3—1 cm, base cuneate, apex acute or obtuse, tapering to a spine like mucro, margin with 1—2(—3) spinose teeth on each side, green above, papillose and slightly glaucous beneath, venation prominent both sides. Flowers solitary, yellow, 1.5—2.0 cm in diameter. Pedicel 0.5—1.2 cm, reddish yellow. Sepals in 4 whorls, outermost sepals linear or oblong ovate, 4.5—6 × 1—2 mm; outer sepals ovate-elliptic, 4—6 × 2—4 mm; median sepals broadly obovate to elliptic, 6—8 × 3.5—5 mm; inner sepals broadly obovate to elliptic, 5.5—8.5 × 3.5—5 mm. Petals obovate, 4.5—6.0 × 3.0—4.0 mm, base cuneate, apex obtuse or slightly notched to 0.2—0.4 mm, margin entire, venation distinct with one central and one pair of lateral veins; glands ellipsoid, 0.5—1 mm long. Stamens 3.5—4 mm long, connective scarcely produced or not. Pistil 3—3.5 mm long; ovules 7—12. Berries not seen.

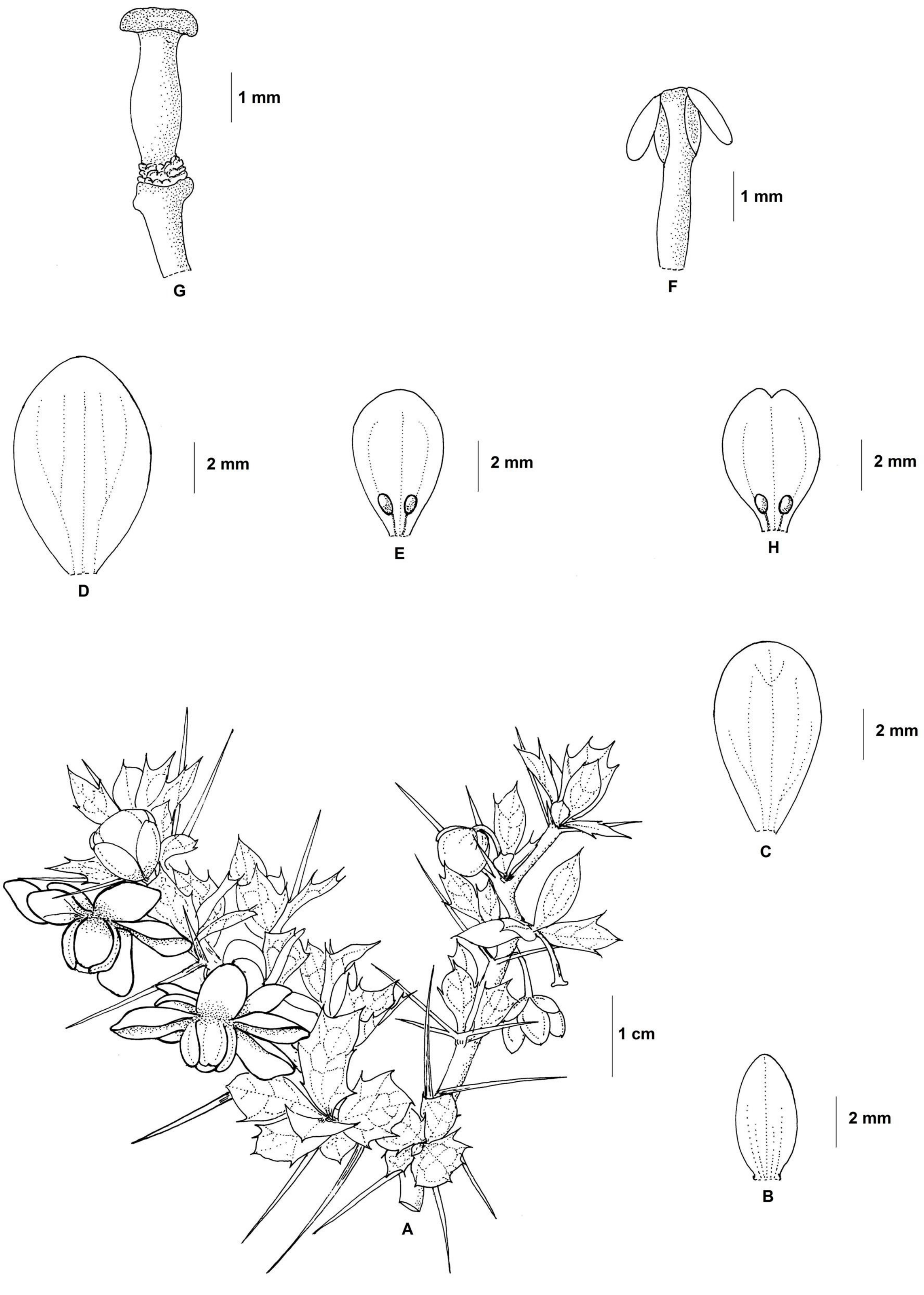


FIG. 2.33. ***Berberis kumaonensis***. A. Flowering branch. B. Outer sepal. C. Median sepal. D. Inner sepal. E,H. Petal. F. Stamen. G. Pistil. (Based on: A-G, JRSB 163; H, JRSB 172).

Flowering time: May—Jul.

Fruiting time: Jun—Sep.

Ecology: In dry, rocky, open and exposed areas.

Altitudinal range: 3000—4000 m.

Distribution: Western [W Himalaya (Kumaon)].

Distinguishing features: Small spiny semi-prostrate shrub; flowers large up to 2 cm in diameter, ovules 7—12.

Selected specimens: WESTERN: Humla, Norkeni, above Simikot, 3650 m, 21.06.2008, JRSB 163 (E); Doti, Khaptad National Park, 2967 m, 02.07.2009, Bajhang 09 20915029 (E); Mugu, Ghurchi lekh, between Lumsa and Murma, 3330 m, 11.08.1952, Polunin, Sykes and Williams 5124 (BM). Number of specimens examined: 6

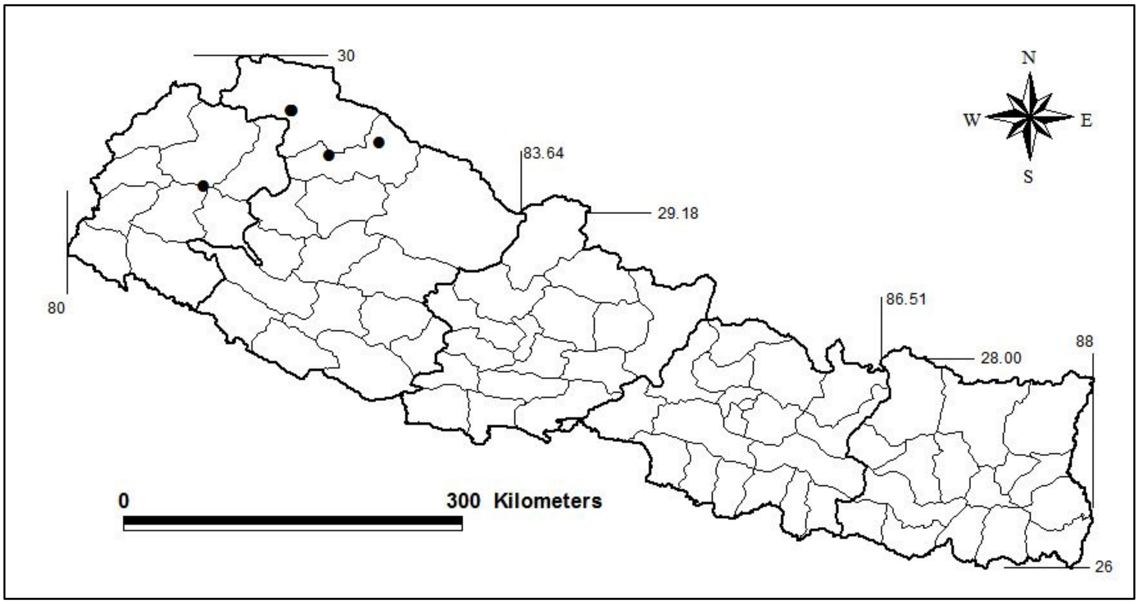


FIG. 2.34. Distribution of *Berberis kumaonensis.*

14. Berberis mucrifolia Ahrendt, J. Roy. Hort. Soc. 81: 135.1956.

Ahrendt, J. Linn. Soc. Bot. 57: 135 (1961); Tebbs in Hara & Williams, Enum. Fl. Pl. Nepal 2: 31 (1979); Press, Shrestha & Sutton, Annot. Checkl. Fl. Pl. Nepal: 26 (2000). (Fig. 2.35; 2.51, P).

TYPE: NEPAL: Tegar, N of Mustang, Stainton, Sykes and Williams 8111 (Holotype: BM!; isotype: E!).

Dwarf shrub, sometimes semi-prostrate, up to 70 cm. Stems and branches terete, puberulous to glabrous, dark grey to greyish yellow, verruculose, young shoots reddish green. Internodes 0.3—1.2 cm. Spines 3-fid, strong, terete or slightly angular, central spine 1—2 cm, lateral spines 0.5—1.5 cm. Leaves deciduous, coriaceous. Petiole absent. Lamina narrowly elliptic to narrowly obovate, 0.8—2.5 × 0.2—0.5 cm, base cuneate, apex acute to acuminate, tapering to a spine like mucro of 0.5—1.5 mm, margin usually entire, rarely one spine-like tooth on either side, lustrous green above, paler whitish green beneath, venation indistinct. Flowers solitary or in a fascicles of 2—3 flowers. Bracts ovate-triangular, 0.5—1.0 mm. Flowers yellow, *ca.* 0.8 cm in diameter. Pedicel 0.5—1.0 mm. Sepals in 3 whorls, outer sepals oblong ovate, 2.5—3 × 1.5—2 mm; median sepals ovate-elliptic, 4.0—4.5 × 1.5—2.8 mm; inner sepals broadly obovate, 5.5—7 × 3.5—4.5 mm. Petals broadly obovate, 5—6 × 3—5 mm, base cuneate, apex slightly notched, 0.2—0.5 mm deep, margin entire, venation distinct with one central and one pair of lateral veins; glands oblong or oblong ovate, 0.8—1.3 mm long.

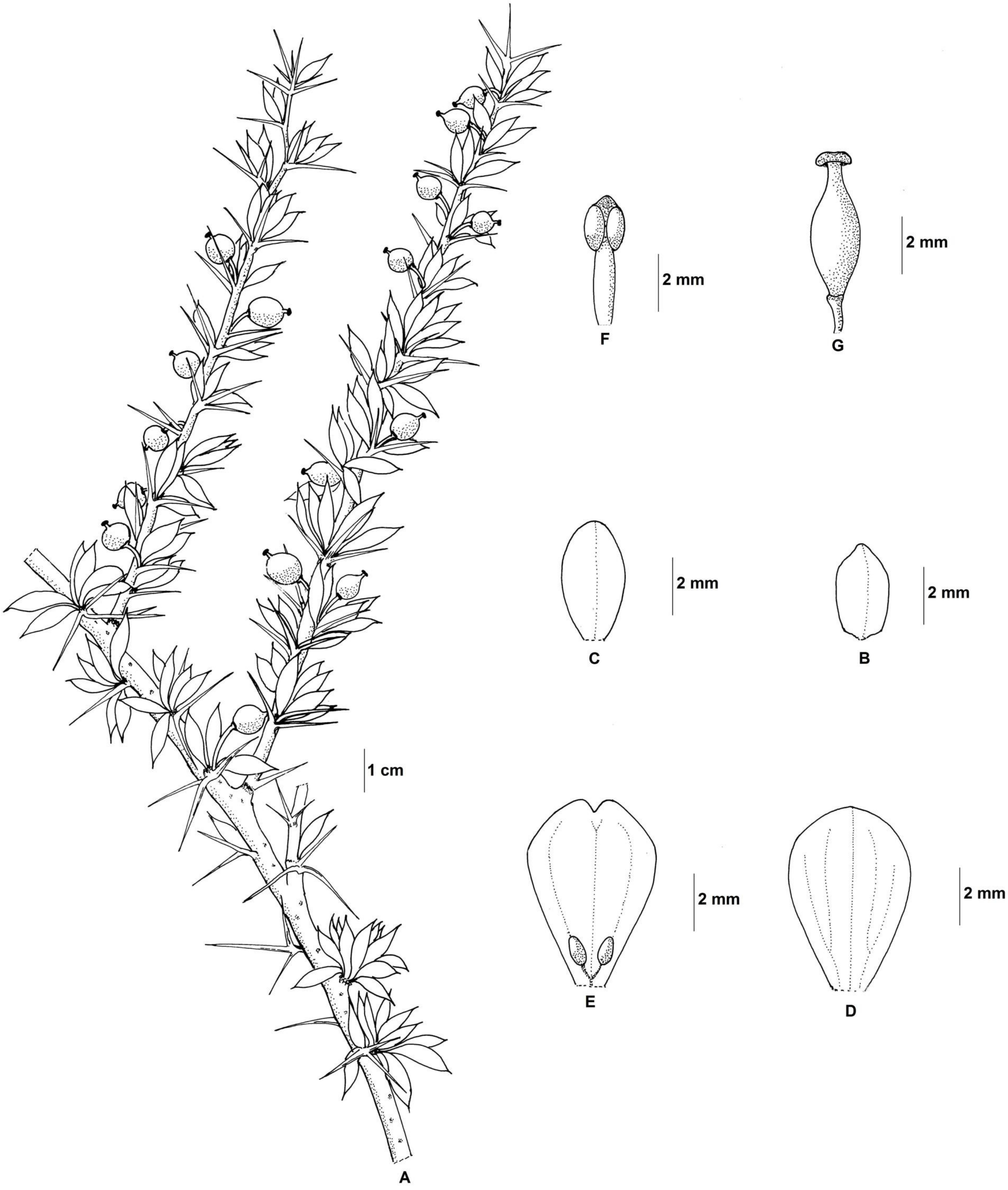


FIG. 2.35. ***Berberis mucrifolia***. A. Fruiting branch. B. Outer sepal. C. Median sepal. D. Inner sepal. E. Petal. F. Stamen. G. Pistil. (Based on: A, JRSA 1 ; B-G, Stainton 4847).

Stamens 3.5—5 mm long, connective slightly produced, tip conical. Pistil 4—5 mm long; ovules 3—6(—9). Berries red, globose or obovoid-globose, 5—7 mm long; style 1—1.5 mm.

Flowering time: Apr—Jun.

Fruiting time: Jun—Oct.

Ecology: In dry, rocky, open areas.

Altitudinal range: 2000—4400 m.

Distribution: Western, Central (Endemic to Nepal).

Distinguishing features: Identifiable by its small habit up to 50 cm, coriaceous leaves and red stylose berries.

Selected specimens: WESTERN: Jumla, Munigaon, 9500 ft, 28.09.1952, Polunin, Sykes and Williams 5444 (BM). CENTRAL: Baglung, Okhaldhungagaon, South of Dhorpatan, 11000 ft, 01.05.1954, 367 Stainton, Sykes and Williams 367 (E, BM); Mustang, Tetang, 3600 m, 18.05.1974, Dobremez & Manandhar 3029 (74-658) (E); Myagdi, Above Gurjakhani, 9500 ft, 21.07.1954, Stainton, Sykes and Williams 3567 (E, BM); Mustang: Kaligandaki, 500 m north of Jomsom, 2750 m, 26 Sep 2006, EA 1(E); Mustang, South of Eklobhatti, 28 Sep 2006, EA 40 (E). Number of specimens examined: 34

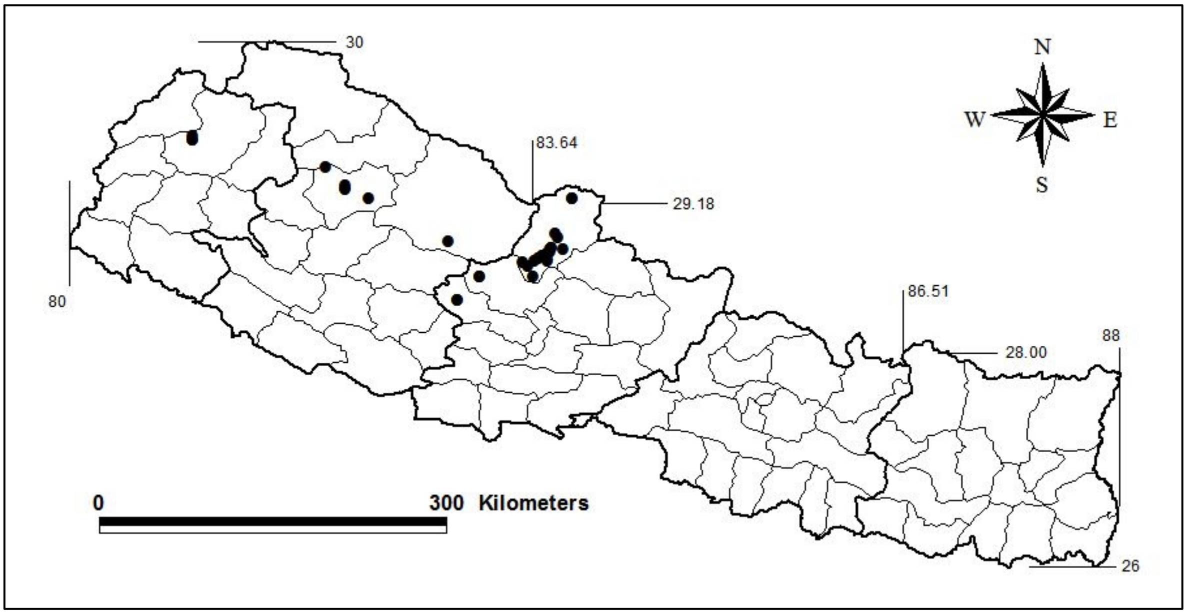


FIG. 2.36. Distribution of *Berberis mucrifolia.*

15. Berberis orthobotrys Bienert ex Aitch. var. **rubicunda** Ahrendt, J. Linn. Soc. Bot. 57: 133. 1961.

Rao *et al*., Rheedea 8(2): 126 (1998). (Fig. 2.37; 2.51 Q).

TYPE: NEPAL: Langtang, Polunin 514 (Holotype: BM!; isotype: E!).

Shrub to 3 m. Stems and branches terete, glabrous, yellowish grey to black, verruculose, young shoots dark brown, slightly sulcate or angular. Internodes 0.7—1.5 cm. Spines 3-fid, terete or angled, central spine 0.8—1.5 cm, the lateral spines equal or slightly shorter than the central. Leaves deciduous, slightly coriaceous. Petiole absent. Lamina obovate-elliptic, 1—2.5 × 0.4—1.5 cm, base cuneate, apex acute or obtuse, mucronate, margin usually entire, sometimes with 1—3 spinulose teeth on each side, green above, slightly papillose beneath, venation sub-conspicuous above, distinct and slightly raised below. Inflorescence 1—3 cm long, a shortly pedunculate or sub-umbellate raceme of 2—12 flowers, sometimes with few flowers arising from the base of the rachis. Bracts ovate or oblong-ovate, 1—2 mm. Flowers yellow, *ca.* 1 cm in diameter. Peduncle 0.2—1.2 cm. Pedicel 0.3—1.3 cm. Sepals in 3 whorls, outer sepals ovate or oblong ovate, 4—5 × 2—3 mm; median sepals ovate or obovate, 5—7 × 3—5 mm; inner sepals elliptic, broadly obovate to rounded, 6—8 × 4—6 mm. Petals obovate, 5.5—6.5 × 3—4.5 mm, base cuneate, apex obtuse or crenate, rarely slightly emarginate, margin entire, venation distinct with one central and one pair of lateral veins; glands elliptic, rounded or obovate, 0.5—1 mm long. Stamens 4—5 mm long, connective distinctly produced, tip pointed, sometimes with 2—3 tips. Pistil 4—5 mm long; ovules (2—)3—4. Berries red, ellipsoid to oblong-obovoid, 0.8—1 cm long; style absent.

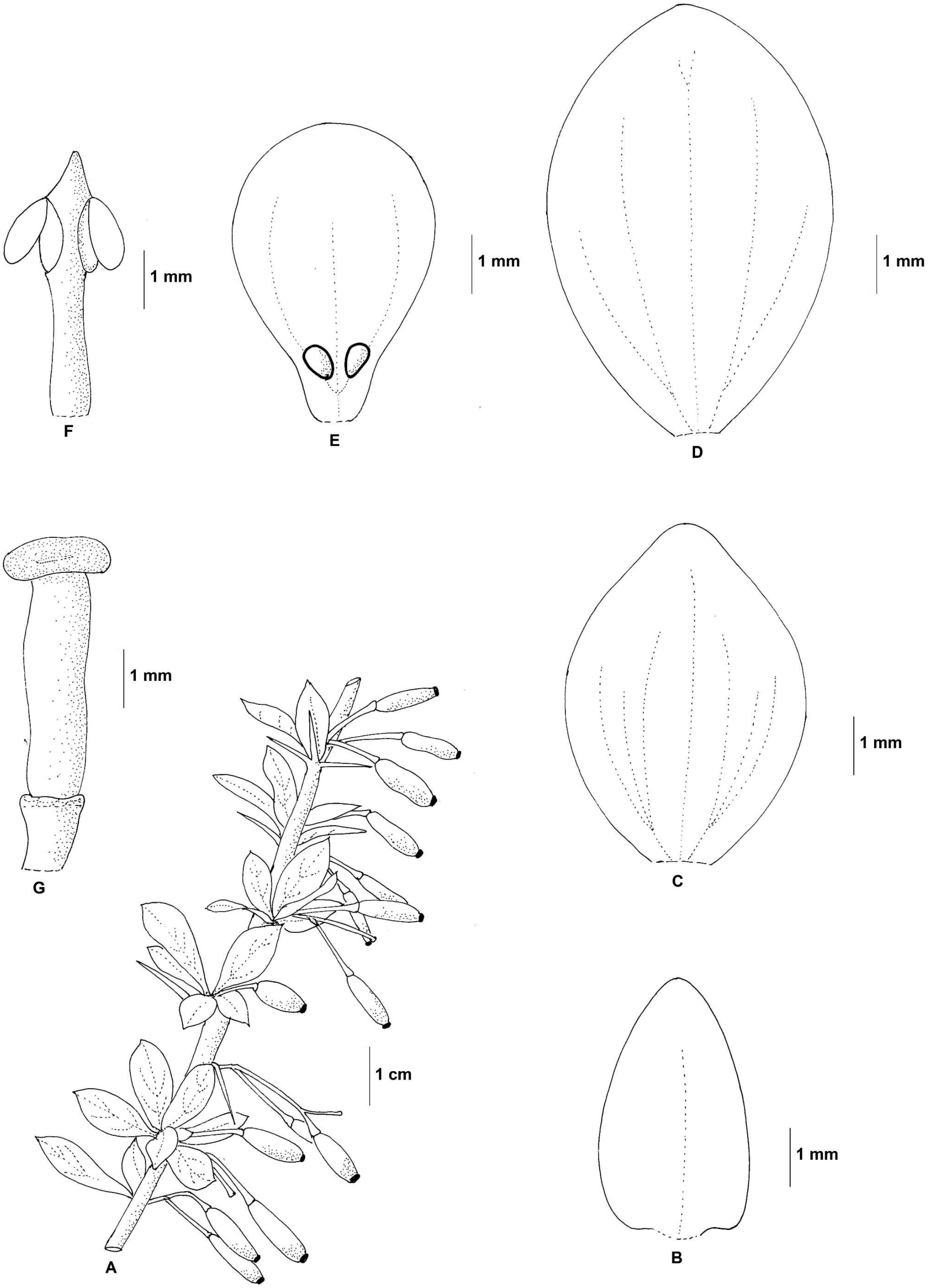


FIG. 2.37. ***Berberis orthobotrys*** *var*. ***rubicunda***. A. Fruiting branch. B. Outer sepal. C. Median sepal. D. Inner sepal. E. Petal. F. Stamen. G. Pistil. (Based on: A, Adhikari, G2; B-G, Polunin 514).

Flowering time: May—Jul.

Fruiting time: Aug—Oct.

Ecology: Open, exposed areas with *Rhododendron* and *Rosa* species.

Altitudinal range: 2800—4200 m.

Distribution: Central, Eastern (Nepal).

Distinguishing features: Leaves usually entire; connectives distinctly produced to long pointed tips; berries red ellipsoid to oblong-obovoid; style absent.

Selected specimens: CENTRAL: Rasuwa, Langtang, 11500 ft, 22.06.1949, O. Polunin 514 (BM, E). Rasuwa, above Langtang, 11500-12500 ft, 21.06.1949, O. Polunin 483 (BM). Rasuwa, Langtang-Kyanjin, 3579 m, 3.08.2007, Adhikari, B. BL2 39 (E). Number of specimens examined: 8

Note: *Berberis orthobotrys* var. *rubicunda* replaces *B. koehneana* above around 3200 m altitude in Central Nepal from where this plant was first described. *Berberis orthobotrys* is a variable species and has been reported from Afganistan, Pakistan, Kashmir, Nepal and Tibet. The variety *rubicunda* which has been reported only from Nepal might be a separate species. A detailed comparison across the entire range of the species would be necessary to confirm its status, and this is beyond the scope of this work.

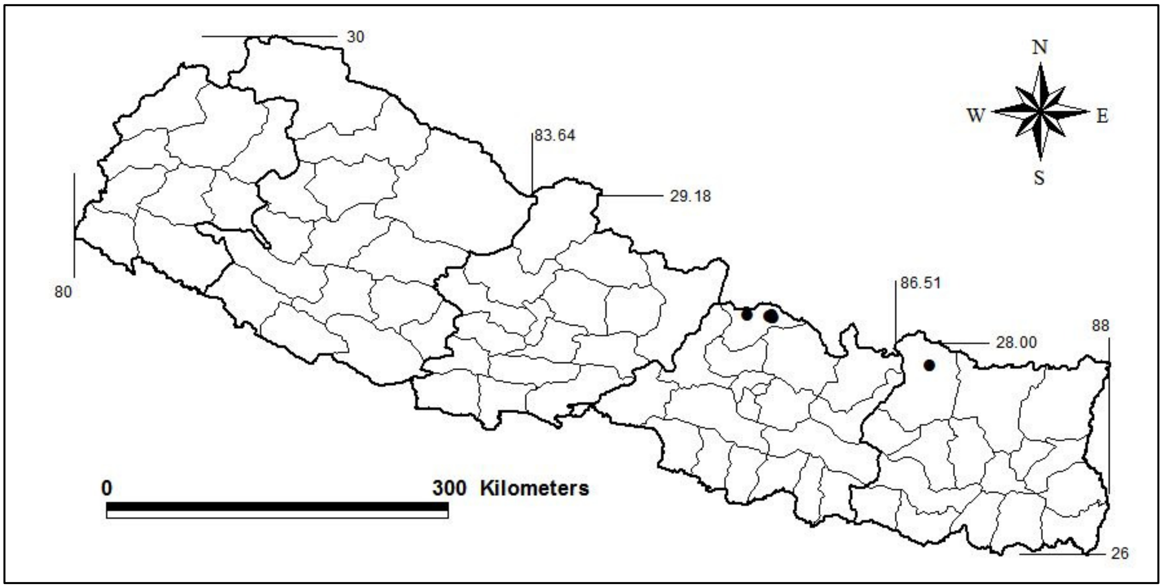


FIG 2.38. Distribution of *Berberis orthobotrys* var. *rubicunda.*

16. Berberis pendryi Adhikari *sp. nov.* (Fig. 2.39; 2.51 R).

Species *B. tsarica* Ahrendt affinis; spinae 3-fidae, flores solitarii, petala apice incisura, stamina apice producta bicornis, ovulis 3-5.

TYPE: NEPAL: Mustang, Below Muktinath, Pendry, Milne and Adhikari EA 34 (Holotype E!; isotype KATH!).

Low growing shrub to 70 cm. Stems and branches terete, young shoots slightly sulcate, reddish brown, puberulous, becoming greyish and sparsely verruculose when older. Internodes 0.2—1 cm. Spines usually 3-fid, rarely 5-fid with smallest outermost pair, strong, terete or angular, central spine 0.5—1.2 cm, lateral spines 0.5—1 cm, much smaller in older stem *ca.* 0.3—0.5 cm. Leaves deciduous, thin. Petiole absent. Lamina obovate, 0.5—2 × 0.4—0.8 cm, base cuneate, apex obtuse, apiculate or mucronate, margin usually entire, sometimes with 1—3(—5) spinulose teeth on each side, dark green above, paler beneath, venation slightly prominent below. Flowers solitary, yellow, *ca.* 1 cm in diameter. Pedicel 0.3—1 cm. Sepals in 3 whorls, outer sepals ovate, 3.5—6 × 2.5—4.5 mm; median sepals obovate to broadly obovate, 5—7.5 × 3—5 mm, inner sepals obovate, 6—8.5 × 4—6.5 mm. Petals obovate, 5—6.5 × 3—4.5 mm, base cuneate, margin entire, apex notched, 0.5—0.8 mm deep, venation distinct with one central and 1 or 2 pair of lateral veins; glands obovoid, 0.8—1.5 mm long. Stamens 4—5 mm long, connective produced into 2 or sometimes 3 tooth-like appendages, rarely truncate. Pistil 3—5 mm long; ovules 3—5.

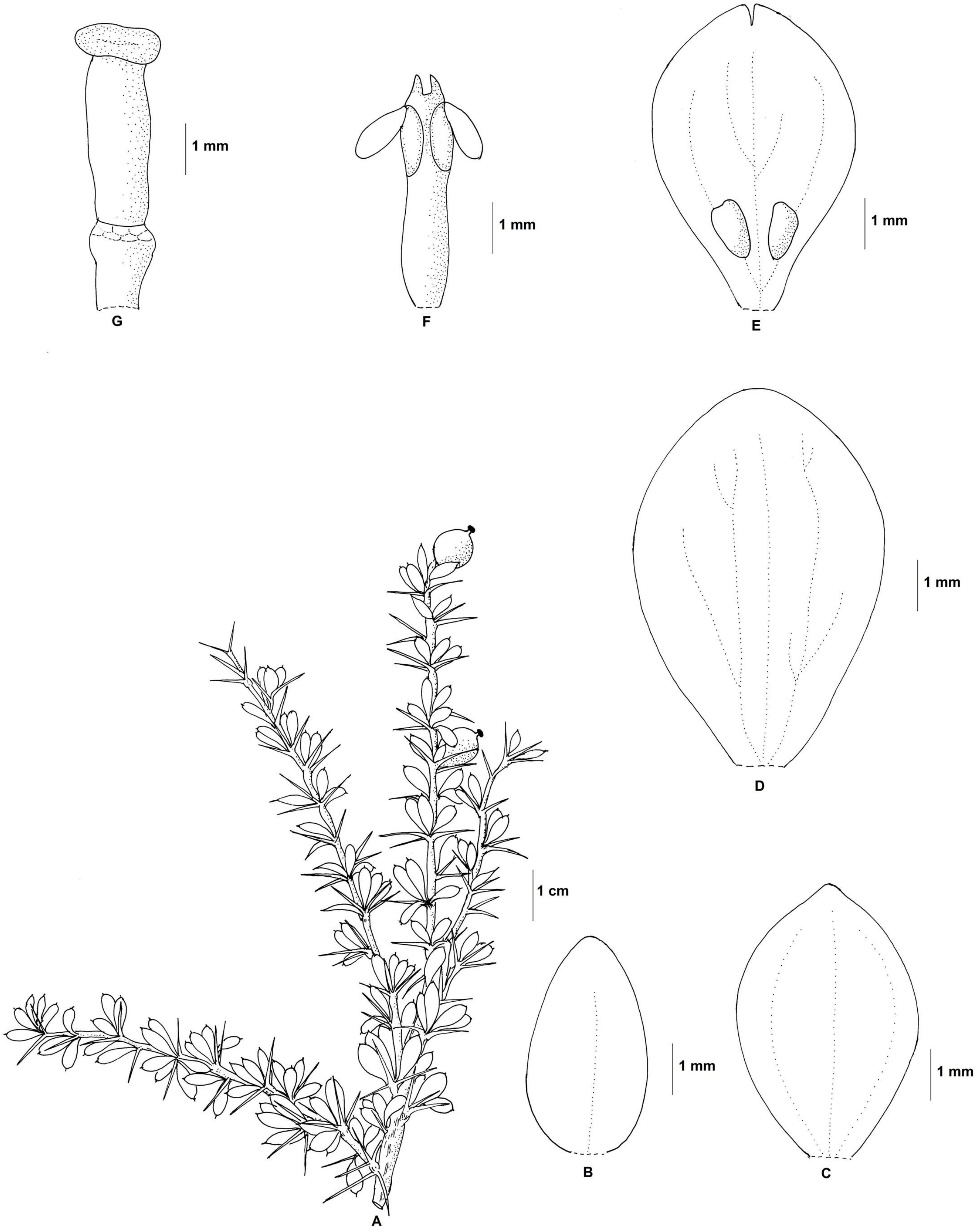


FIG. 2.39. ***Berberis pendryi***. A. Fruiting branch. B. Outer sepal. C. Median sepal. D. Inner sepal. E. Petal. F. Stamen. G. Pistil. (Based on: A, EA 28; B-G, EA 34).

Berries red, sub-globose to ellipsoid, 0.5—1 cm long; style 1 mm long.

Flowering time: Jun—Aug.

Fruiting time: July—Nov.

Ecology: In open dry rocky areas with thin soil.

Altitudinal range: 3000—3700 m.

Distribution: Central.

Selected specimens: CENTRAL: Mustang, Near muktinath temple, 28.09.2006, 3600 m, Pendry, Milne & Adhikari EA 32 (E, KATH); Mustang, outside of main gate of Muktinath temple, 28.09.2006, 3600 m, Pendry, Milne & Adhikari, EA 31 (E, KATH). Number of specimens examined: 8

Distinguishing features: It is most closely related to *B. tsarica* Ahrendt, from which it differs by it 3-fid spines, and distinctly produced bifurcated horn like connective.

Note: This species at present is known only from the type locality. It replaces *B. mucrifolia* in the Annapurna region of Central Nepal above 3000 m. This species is dedicated to Dr Colin Pendry who coordinated my PhD project and who led the expedition to the Annapurna region of Central Nepal during which this species was discovered.

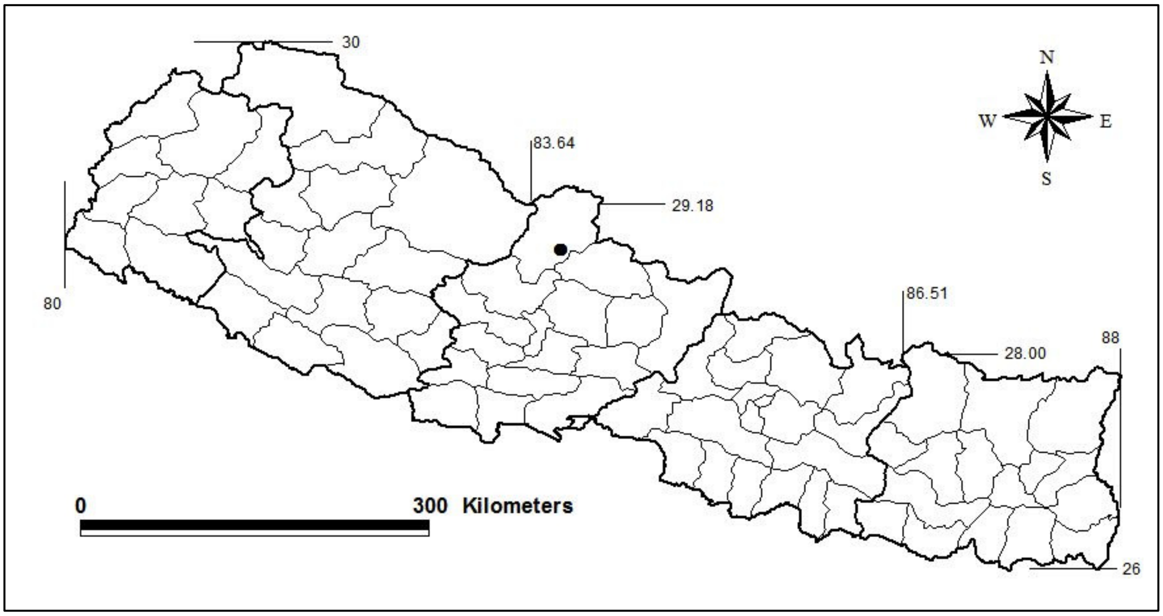


FIG. 2.40. Distribution of *Berberis pendryi.*

17. Berberis petiolaris Wall. ex G.Don, Gen. Syst. 1: 116. 1831.

Chatterjee, Rec. Bot. Surv. India 16(2): 16 (1953); Ahrendt, J. Linn. Soc. Bot. 57: 93 (1961); Tebbs in Hara & Williams, Enum. Fl. Pl. Nepal 2: 31 (1979); Rao *et al.*, Rheedea 8(1): 10 (1998); Press, Shrestha & Sutton, Annot. Checkl. Fl. Pl. Nepal: 26 (2000).

Shrub or small tree to 6 m. Stems and branches terete, purplish brown becoming greyish black when older. Internodes 2—6 cm. Spines absent or solitary, rarely 3-fid, terete. Leaves deciduous, papery. Petiole 1—3.5 cm. Lamina elliptic-rounded or elliptic-obovate, 3—6 × 1—4.5 cm, base cuneate, apex obtuse, margin finely serrate with 10—36 spinules on each side, rarely entire, dull green above, paler beneath, venation prominent both sides. Inflorescence 3—5 cm long, a simple pedunculate raceme of 3—25 flowers, sometimes fasciculate or with a few flowers arising from the base of the rachis. Bracts ovate-triangular, 1—2 mm long. Flowers yellow, *ca.* 0.5—1 cm in diameter. Peduncle 0.5—1.7 cm. Pedicel 1—1.5 cm, swollen at the base of the flower. Sepals in 3 whorls, outer sepals ovate-triangular, 2—3 × 1—2 mm; median sepals ovate-elliptic, 2.5—4.5 × 1.5—3.5 mm; inner sepals broadly obovate, 4—7 × 2.5—6 mm. Petals broadly obovate, 5—8.5 × 4—5.5 mm, base cuneate or slightly clawed, apex obtuse, emarginate or slightly notched, margin entire, venation distinct with one central and one pair of lateral veins; glands ovate-elliptic or lanceolate, 1—2 mm long. Stamens 4—6 mm long, connective scarcely produced or not. Pistil 5—7 mm long; ovules 2—4. Berries red, oblong ovoid; style very short or absent.

Distinguishing features: Small tree-like shrubs with few spines; leaves with distinct long petiole.

Key to the varieties of *Berberis petiolaris.*

1a. Inflorescence a 8—25-flowered raceme. Glands ovate-elliptic. .... a. var. **petiolaris**

1b. Inflorescence a 3—9-flowered sub-umbellate raceme. Glands lanceolate ....b. var. **garhwalana**

17a. Berberis petiolaris Wall. ex G.Don var. **petiolaris** (Fig 2.41, A-G; 2.51, S).

TYPE: INDIA: Kumaon: Wallich 1474.2 (B) (Lectotype here designated, K-W!; isolectotype: K!).

Berberis petiolaris Wall. ex G.Don var. *extensa* Ahrendt ex R.R. Rao, T.Husain & Datt.

TYPE: NEPAL: Polunin, Sykes and Williams 2066 (Holotype: BM!).

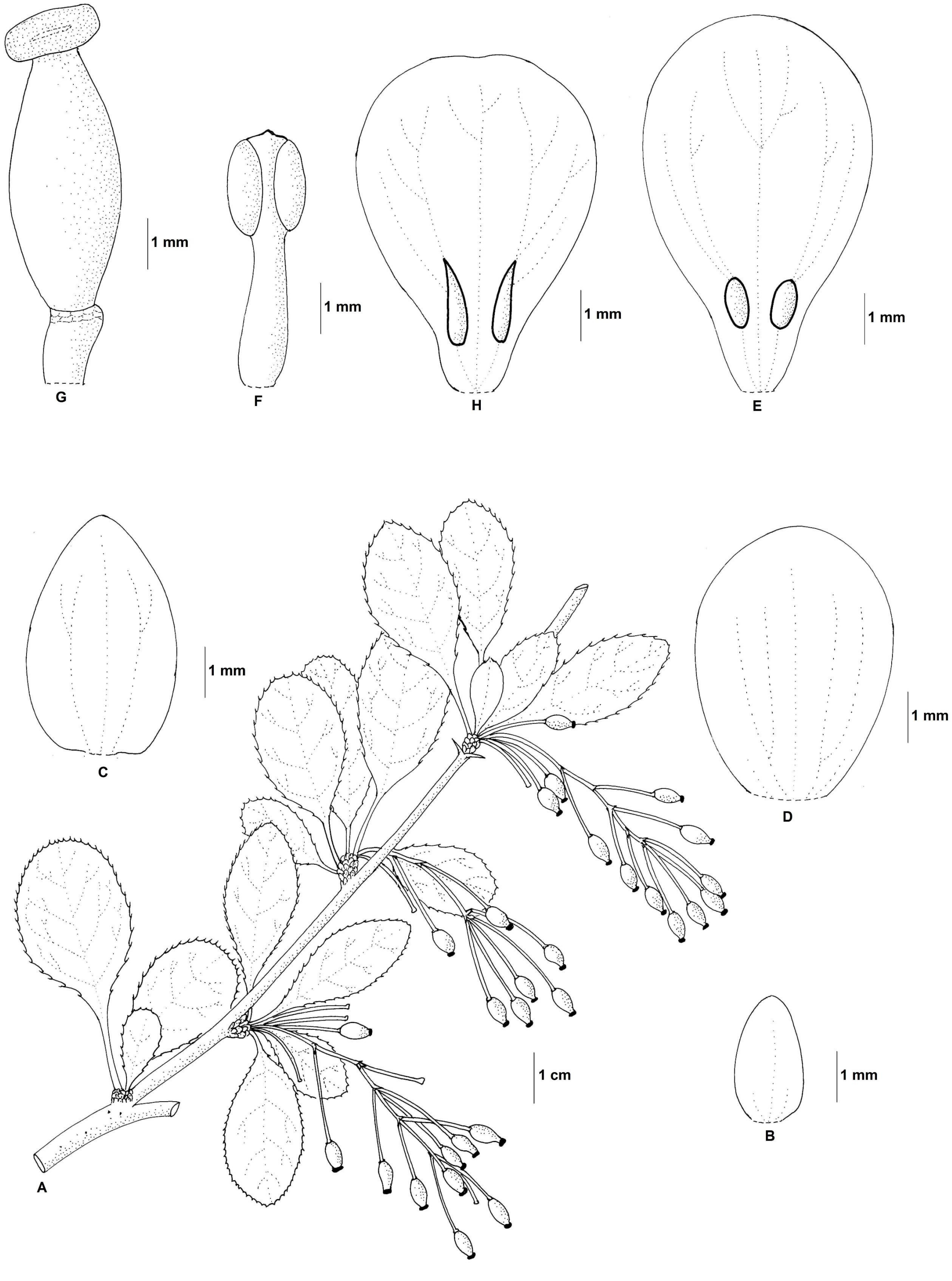


FIG. 2.41. ***Berberis petiolaris*** var. ***petiolaris***. (A-G) A. Fruiting branch. B. Outer sepal. C. Median sepal. D. Inner sepal. E. Petal. F. Stamen. G. Pistil. (Based on: A, JRSA 122; B-G, PSW 2066). ***Berberis petiolaris*** var. ***garhwalana***. H. Petal. (Based on: Dobremez 1967).

Inflorescence 4—8 cm long, a raceme of 8—25 flowers. Petals with ovate-elliptic glands.

Flowering time: Apr—Jun.

Fruiting time: Jun—Aug.

Ecology: In moist temperate forests.

Altitudinal range: 2700—3500 m.

Distribution: Western [W Himalaya (Kumaon)].

Notes: Rao *et al*. (1994) described the variety *extensa* based on the specimen of Polunin, Sykes and Williams 2066 from Nepal. While examining the specimens of *B. petiolaris* collected in Kumaon along with the type of variety *extensa* and other specimens from Western Nepal, I did not find enough significant differences between them to maintain them as different varieties. The characters given by Rao *et al*. (1994) to separate var. *extensa* from var. *petiolaris* are entire leaves and uni-or biserrate bracts. The type of variety *petiolaris* itself has serrate leaves and the serration of bracts alone is not sufficient to separate this variety.

Selected specimens: WESTERN: Jumla, Below Khalichaur, 2890 m, 5.06.2008, JRSB 39 (E); Mugu, Chankheli Lagna, 3330 m, 19.05.1952, Polunin, Sykes and Williams 4123 (E); Mugu, N of Chankheli Lagna, 3250 m, 12.06.2008, JRSA 122 (E); Jumla, Sialgarhi, Chudhabise Khola, 3030 m, 19.05.1952, Polunin, Sykes and Williams 2066 (E, BM). Number of specimens examined: 4

17b. **Berberis petiolaris** Wall. ex G.Don var. **garhwalana** Ahrendt, J. Bot. Lond. (Suppl.): 82 (1942). (Fig. 2.41, H).

Ahrendt, J. Linn. Soc. Bot. 57: 93 (1961). Rao *et al*., Rheedea 8(1): 11 (1998).

TYPE: INDIA: Strachey & Winterbottom 5. (Holotype: BM!).

Inflorescence 3—4 cm long, sub-umbellate raceme of 3—9-flowered. Petals with lanceolate glands.

Flowering time: April—Jun.

Fruiting time: Jun—Aug.

Ecology: In moist temperate forests.

Altitudinal range: *ca.* 2700 m.

Distribution: Western [W Himalaya].

Specimen examined: WESTERN: Doti, Khaptad, 2700 m, 24.04.1973, J.F. Dobremez 1967 (BM). Number of specimens examined: 2

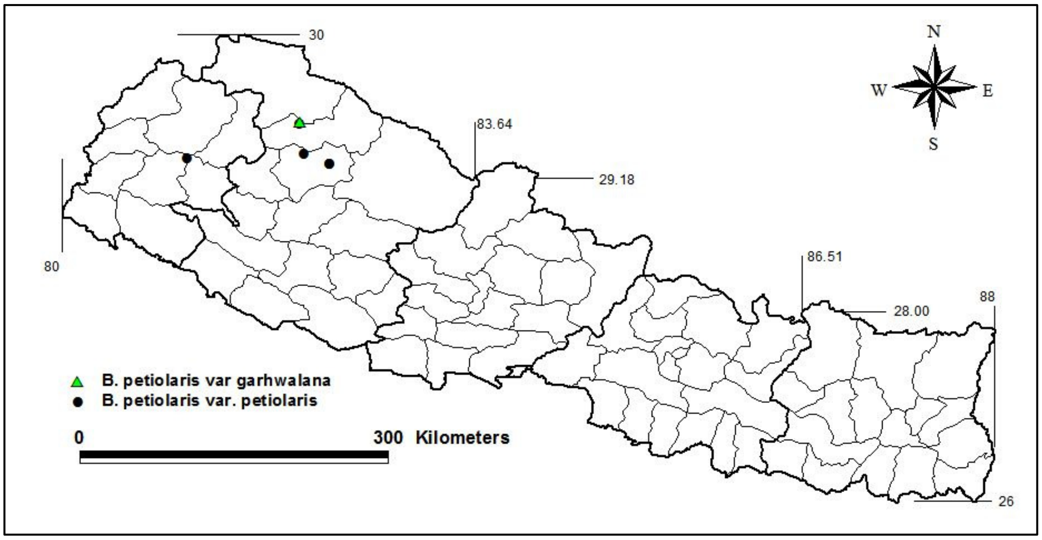


FIG. 2.42. Distribution of *Berberis petiolaris*.

18. Berberis thomsoniana C.K.Schneid., Bull. Herb. Boiss. 2.5: 454. 1905.

Ahrendt, J. Linn. Soc. Bot. 57: 106 (1961); Grierson & Long, Fl. Bhutan 1(2): 327 (1984); Rao *et al*., Rheedea 8(1): 27 (1998). (Fig. 2.43; 2.51, T).

TYPE: INDIA: Sikkim: Hooker s.n. [Holotype: W! (W 0024722); isotype: K!].

Shrub to 3 m. Stems and branches sulcate, glabrous, dark grey to greyish yellow, verruculose, young shoots reddish brown. Internodes 1.5—4 cm. Spines usually 3-fid, smaller and absent towards the apex of twig, terete or angular, central spine 1—2 cm, lateral spines 0.5—1.2 cm. Leaves deciduous, thin or slightly coriaceous. Petiole absent or 0.2—1 cm. Lamina obovate, 2—4.5 × 1—2 cm, base cuneate or attenuate to a small petiole, apex obtuse, usually mucronate, margin usually entire, sometimes with 2—6 spinulose teeth on each side, green above, paler beneath, venation prominent both sides. Inflorescence 2—4.5 cm long, a pedunculate raceme or sub-umbellate raceme of 4—10 flowers, sometimes with few flowers arising from the base of the rachis. Bracts narrowly ovate to triangular, 1.5—2.5 mm. Flowers yellow, *ca.* 1.0—1.5 cm in diameter. Peduncle 0.2—1 cm, glabrous to puberulous, red. Pedicel 1—2 cm, glabrous to puberulous. Sepals in 4 whorls, outermost sepals boat-shaped, linear or oblong ovate, 4—6 × 1—2 mm; outer sepals narrowly ovate, 4.5—7 × 2—4 mm; median sepals broadly obovate-elliptic, 6—8 × 4—6 mm; inner sepals broadly obovate-elliptic, 5—8 × 3—5 mm. Petals obovate, 4.5—6.5 × 3.5—5 mm, base cuneate, apex obtuse or emarginate, margin entire, venation distinct with one central and one pair of lateral veins; glands obovoid, 0.8—1.3 mm long. Stamens 3—4 mm long, connective

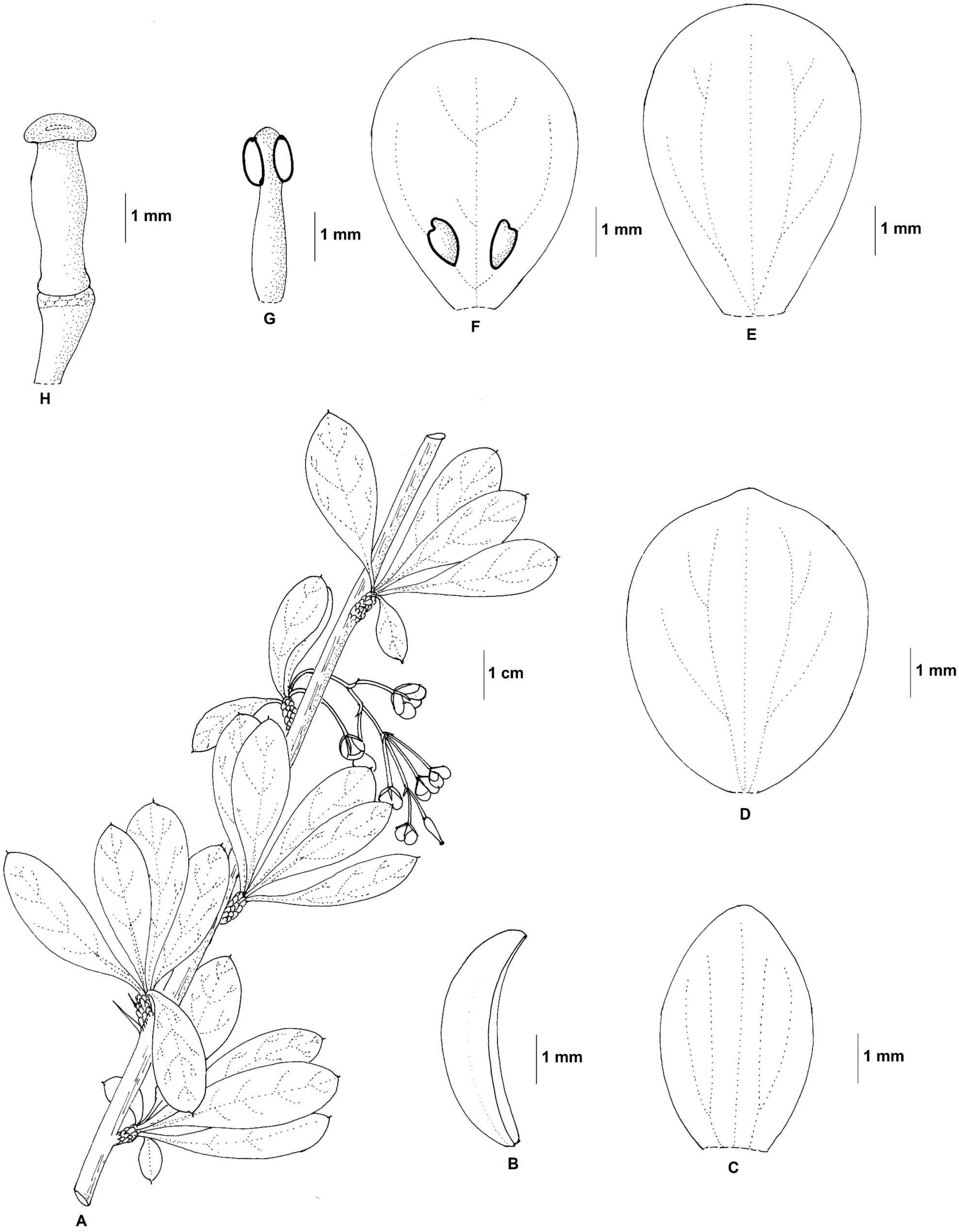


FIG. 2.43. ***Berberis thomsoniana***. A. Flowering branch. B. Outermost sepal. C. Outer sepal. D. Median sepal. E. Inner sepal. F. Petal. G. Stamen. H. Pistil. (Based on: A, LKSRB 46; B-H, LKSRB 17).

scarcely produced or not. Pistil 3.5—4 mm long; ovules 2—5. Berries red, oblong-obovoid, *ca.* 1 cm long; style absent.

Flowering time: May—Aug.

Fruiting time: Aug—Oct.

Ecology: Open hill sides and the edges of *Oak-Rhododendron* forest.

Altitudinal range: 3000—3650 m.

Distribution: Western, Central, Eastern [E Himalaya].

Distinguishing features: Identifiable by the combination of pedunculate or sub-umbellate raceme, 4 whorls of sepals with the outermost sepals nearly equal to the outer sepals.

Selected specimens: WESTERN: Rukum, Near Seng Khola, 11000 ft, 06.10.1954, Stainton, Sykes and Williams 4706 (BM). CENTRAL: Rasuwa, Near Chandanbari, 3166 m, 19.10.2006, Adhikari, B. EL 124 (E); Rasuwa, Langtang Khola, 3180 m, 24.05.1962, Bowes-Lyon 148 (E,BM). EASTERN: Panchthar, Maimajuwa-8, Bharlang, 2904 m, 8.06.2007, LKSRB 17 (E); Illam, Jaubari-Jogmai: 2621 m, 18.06.2007, LKSRB 181 (E); Sankhuwasabha, N bank of Barun Khola, below Nehe Kharka, 3560 m, 8 Oct 1991, EMAK 583 (E). Number of specimens examined: 42

Note: Schneider (1905) cited the type as ‘‘Sikkim: c 3000 m (Hooker), *typus* in Herb. Hofm. Wien’’. Later in 1908, he cited Hooker specimen no 39 and Clarke specimen no 27575 a, without distinguishing a type. Ahrendt (1961) identified the type as Hooker 39 (K). Hooker's specimen in Hofmuseum Wien (W) which has no number is more likely to be the specimen which Schneider saw when describing the species and therefore has been identified as the holotype.

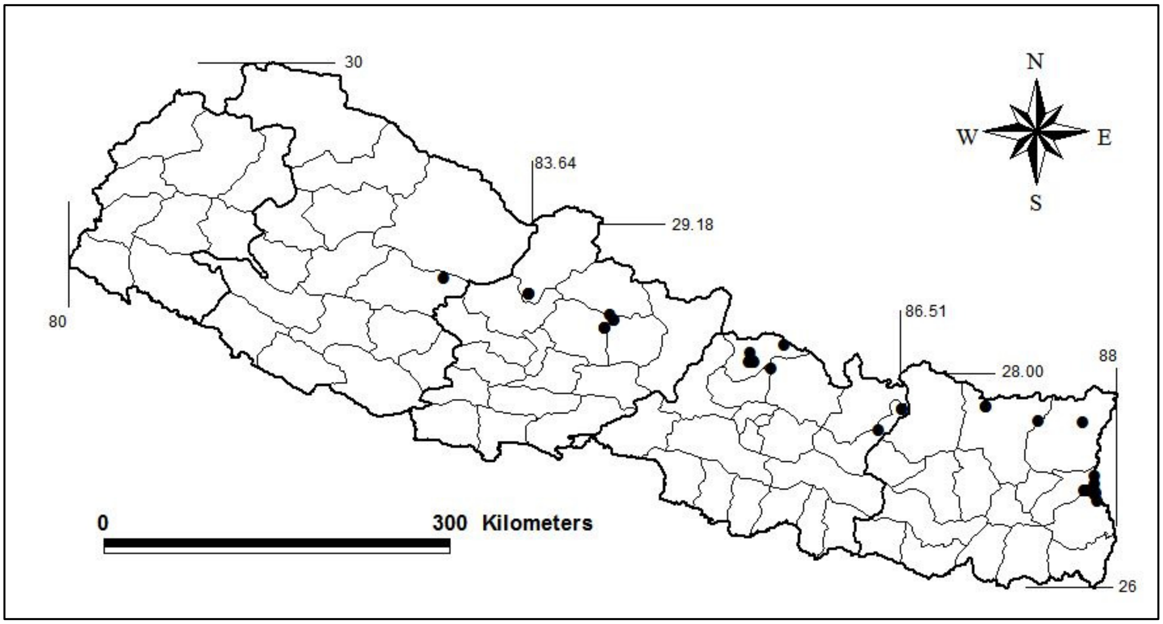


FIG. 2.44. Distribution of *Berberis thomsoniana.*

19. Berberis tsarica Ahrendt, J. Bot. Lon. (Suppl.): 48.1941.

Ahrendt, J. Linn. Soc. Bot. 57: 133 (1961); Tebbs in Hara & Williams, Enum. Fl. Pl. Nepal 2: 31 (1979); Grierson & Long, Fl. Bhutan 1(2): 324 (1984); Press, Shrestha & Sutton, Annot. Checkl. Fl. Pl. Nepal: 26 (2000). (Fig 2.45).

TYPE: S.E.Tibet: Tsari district, near Langong, Chianang, Ludlow, Sherriff & Taylor 3961 (Holotype: BM!).

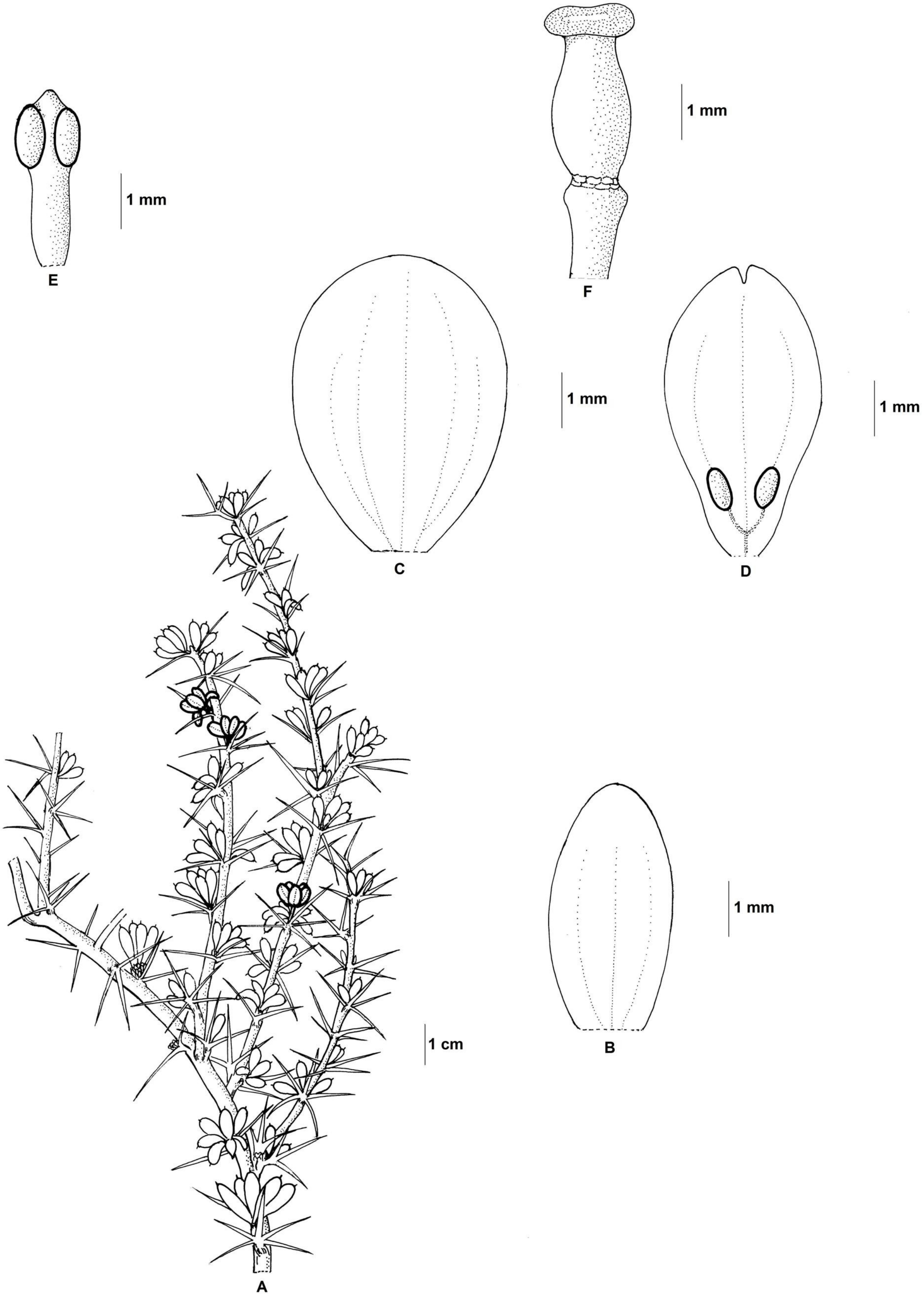


FIG. 2.45. ***Berberis tsarica***. A. Flowering branch. B. Outer sepal C. Inner sepal. D. Petal E. Stamen. F. Pistil. (Based on DNEP 1 153).

Selected specimens*:* CENTRAL: Dolkha, Rolwaling, 15000 ft, 30.06.1964, Stainton 4718 (BM).EASTERN: Solukhumbu, Dole-Luza, 4300 m, 15.05.2004, DNEP1 153 (E); Solukhumbu, Bhote Koshi, 4700 m, 21.09.2005, DNEP3 BY134 (E). Solukhumbu, Langmuche Valley, 4400 m, 24.09.2005, DNEP3 BY188 (E). Solukhumbu, Khumbu, Tsolu Khola, 4550 m, 25.06.1964, S-Bowes Lyon, SA 2103 (BM). Number of specimens examined: 7

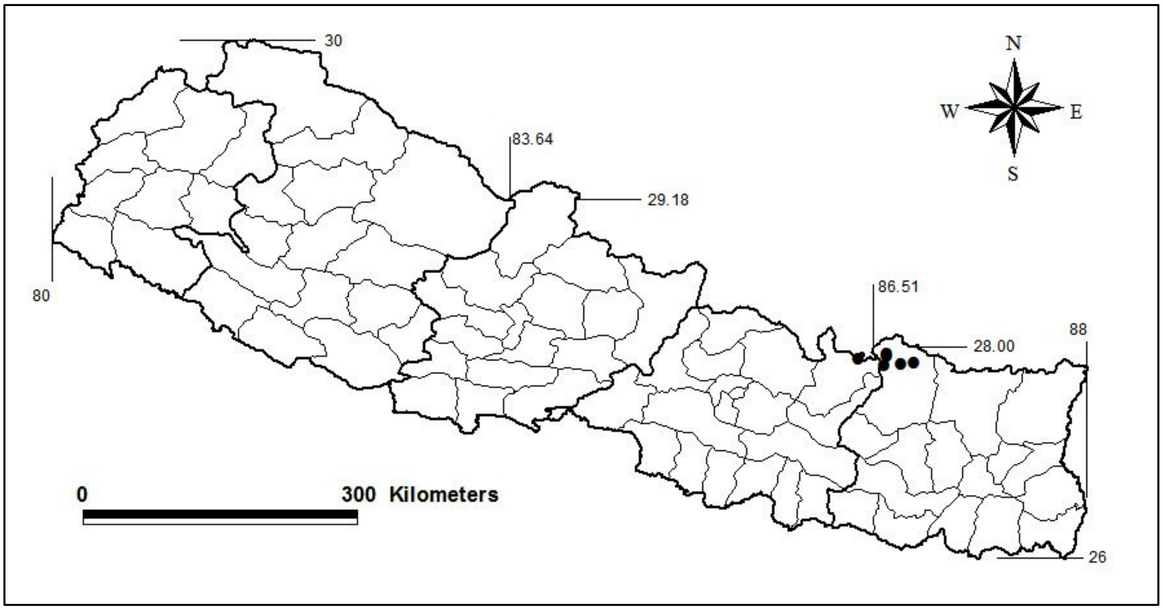


FIG. 2.46. Distribution of *Berberis tsarica.*

20. Berberis virescens Hook.f., Bot. Mag. 116: t. 7116. 1890.

Chatterjee, Rec. Bot. Surv. India 16(2): 27 (1953); Ahrendt, J. Linn. Soc. Bot. 57: 125 (1961); Tebbs in Hara & Williams, Enum. Fl. Pl. Nepal 2: 31 (1979); Grierson & Long, Fl. Bhutan 1(2): 326 (1984); Rao *et al*., Rheedea 8(2): 109 (1998); Press, Shrestha & Sutton, Annot. Checkl. Fl. Pl. Nepal: 27 (2000). (Fig 2.47).

TYPE: INDIA: Sikkim: Lachen Valley, 9000 ft, May 28, 1849 (Holotype: K! Barcode: K000340167).

Shrub to 3 m. Stems and branches terete or slightly angular, reddish brown when young becoming greyish brown and verruculose on older. Internodes 1—2 cm. Spines 3-fid, strong, terete or angular, central spine 0.8—1 cm, the lateral spines equal or slightly smaller than the central. Leaves deciduous, thin to slightly coriaceous. Petiole absent. Lamina obovate or obovate-elliptic, 0.8—2 × 0.3—1 cm, base cuneate, apex obtuse, mucronate, margin entire or 3—5 spinulose toothed, dark green above, slightly glaucous beneath, venation sub-conspicuous, above prominent below. Inflorescence 1—3 cm long, a short condensed raceme or sub-umbellate raceme of 2—8 flowers, rarely fasciculate. Flowers yellow, *ca.* 0.8 cm in diameter. Peduncle 0.2—0.5 cm (if present). Pedicel 0.3—1 cm. Bracts ovate-triangular, 2—2.5 × 1—1.5 cm. Sepals in 3 whorls, outer sepals ovate, 2.5—3.5 × 1—2 mm; median sepals ovate-elliptic, 4—5 × 2—3 cm; inner sepals broadly obovate, 7—8 × 5—7 mm. Petals obovate-elliptic, 5—6 × 3—4 mm, base clawed, apex notched, 0.5 mm deep, margin entire, venation distinct with one central and one pair of lateral veins; glands ovoid, *ca.* 1 mm long. Stamens 3—4 mm long, connectives produced, tip obtuse or slightly retuse. Pistil 3—4 mm long; ovules 3—4. Berries, oblong-ellipsoid, 8—12 mm long; style very small or absent.

Flowering time: April—June.

Fruiting time: May—Oct.

Ecology: Open degraded woodland.

Altitudinal range: 3500—4000 m.

Distribution: Eastern [E Himalaya (Sikkim, Bhutan)].

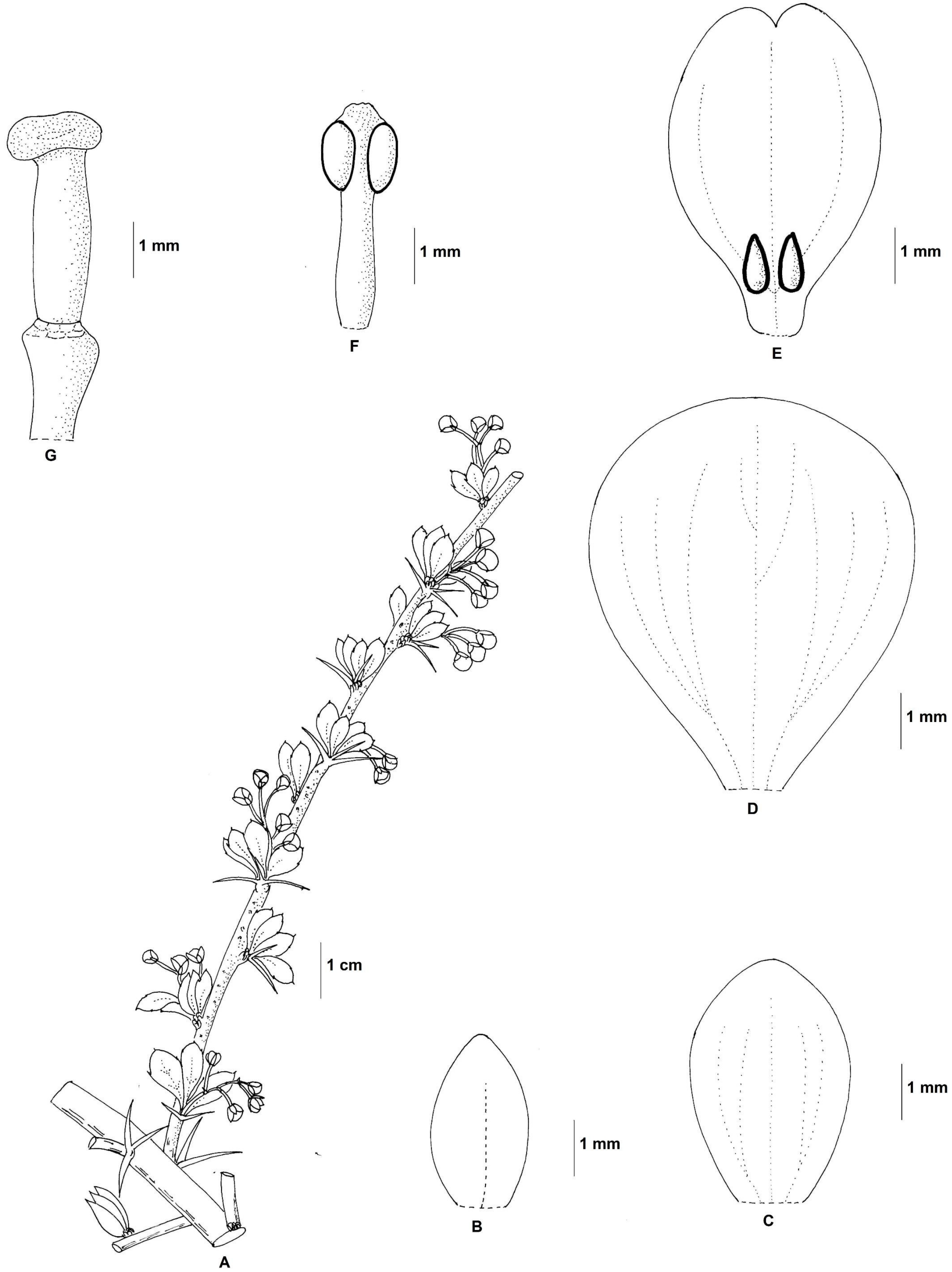


FIG. 2.47. ***Berberis virescens***. A. Flowering branch. B. Outer sepal. C. Median sepal. D. Inner sepal. E. Petal. F. Stamen. G. Pistil. (Based on: DNEP1 238).

Distinguishing features: Inflorescence a short, condensed or sub-umbellate raceme; petals distinctly notched; connective produced, tip obtuse or retuse.

Selected specimens: EASTERN: Sagarmatha, Tengboche-Phunki Tenga, 3670 m, 23 May 2004, DNEP1 238 (E); Sagarmatha, Solukhumbu, 3700 m, 12 May 2004, DNEP1 93(E). Number of specimens examined: 3

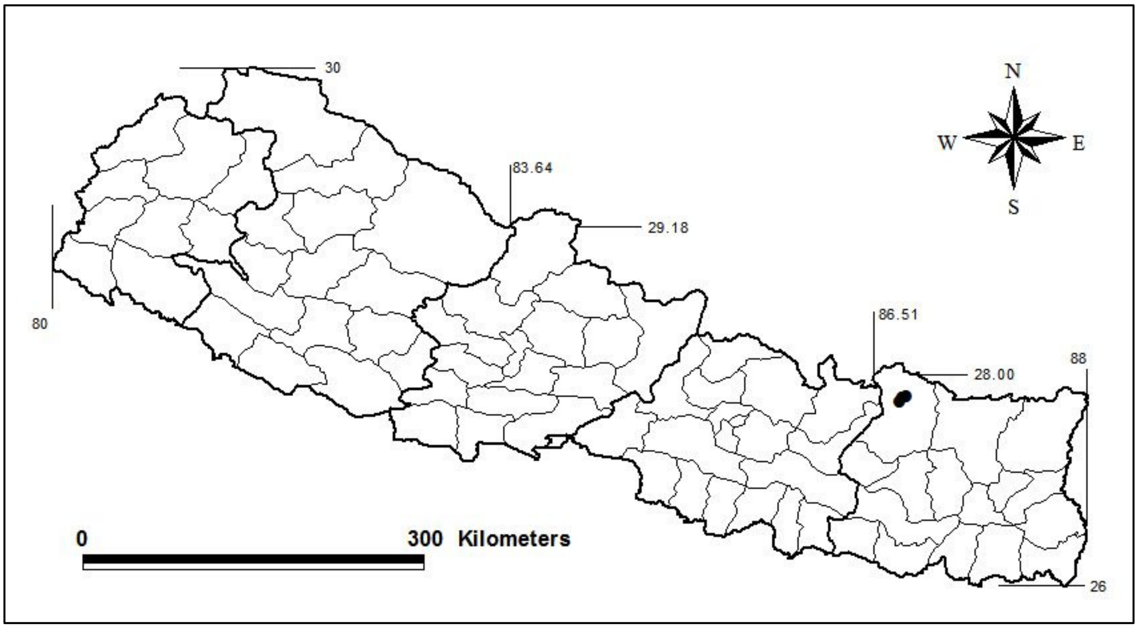


FIG. 2.48. Distribution of *Berberis virescens.*

21. Berberis wallichiana DC., Prodr. 1: 107. 1824.

Don, Prodr. Fl. Nepal: 204 (1825); Wallich, Pl. Asiat. Rar. 3: 23. t. 243 (1832); Schneider, Bull. Herb. Boiss. 2.4. 403 (1905); Chatterjee, Rec. Bot. Surv. India 16(2): 10 (1953); Ahrendt, J. Linn. Soc. Bot. 57: 71 (1961); Tebbs in Hara & Williams, Enum. Fl. Pl. Nepal 2: 31 (1979); Rao *et al.*, Rheedea 8(1): 42 (1998); Press, Shrestha & Sutton, Annot. Checkl. Fl. Pl. Nepal: 27 (2000). (Fig. 2.49; 2.51, U,V).

TYPE: NEPAL: Gardner in Wallich (1819). (Lectotype here designated, G-DC! with barcode G00201780; isolectotype: K!).

Berberis poluninii Ahrendt, J. Linn. Soc. Bot. 57: 73 (1961).

TYPE: NEPAL: Polunin 746 (Holotype: BM!).

Shrub to 2 m. Stems and branches glabrous, terete, dark grey, verruculose, young stems yellowish grey, sulcate or angled. Internodes 2—5 cm. Spines usually 3-fid, sometimes absent on old stems, terete or sulcate, central spine 1—3 cm, lateral spines 1.5—2 cm. Leaves evergreen, coriaceous. Petiole absent or 2—5 mm. Lamina oblong lanceolate, 3—11 × 1—2 cm, base cuneate, sometimes attenuate to a small petiole, apex acute to accuminate, mucronate, margin 6—13(—25) spinose toothed on each side, lustrous green above, paler beneath, venation prominent to sub-conspicuous above, prominent below. Inflorescence a fascicle of 10—25(—30) flowers. Flowers yellow, 1—2 cm in diameter. Pedicel 0.5—1.5(—4) cm, glabrous or puberulous, dark red. Sepals in 3 whorls, outer sepals ovate or ovate-triangular, 1—2.5 × 0.8—2 mm; median sepals ovate or ovate-elliptic, 3.5—4.5 × 1.5—2.5 mm; inner sepals obovate, 6—7 × 3—5 mm. Petals obovate, 5—6.5 × 3.5—4.5 mm, base cuneate, apex undulate or notched, 0.5 mm deep, margin entire, venation distinct with one central and one pair of lateral veins; glands ovate, 0.8—1.2 mm long. Stamens 3.5—4.5 mm long, connective produced, truncate or sometimes slightly bilobed. Pistil 4—4.5 mm long; ovule solitary. Berries black, ellipsoid, 0.5—1 cm long; style 0.5—1 mm.

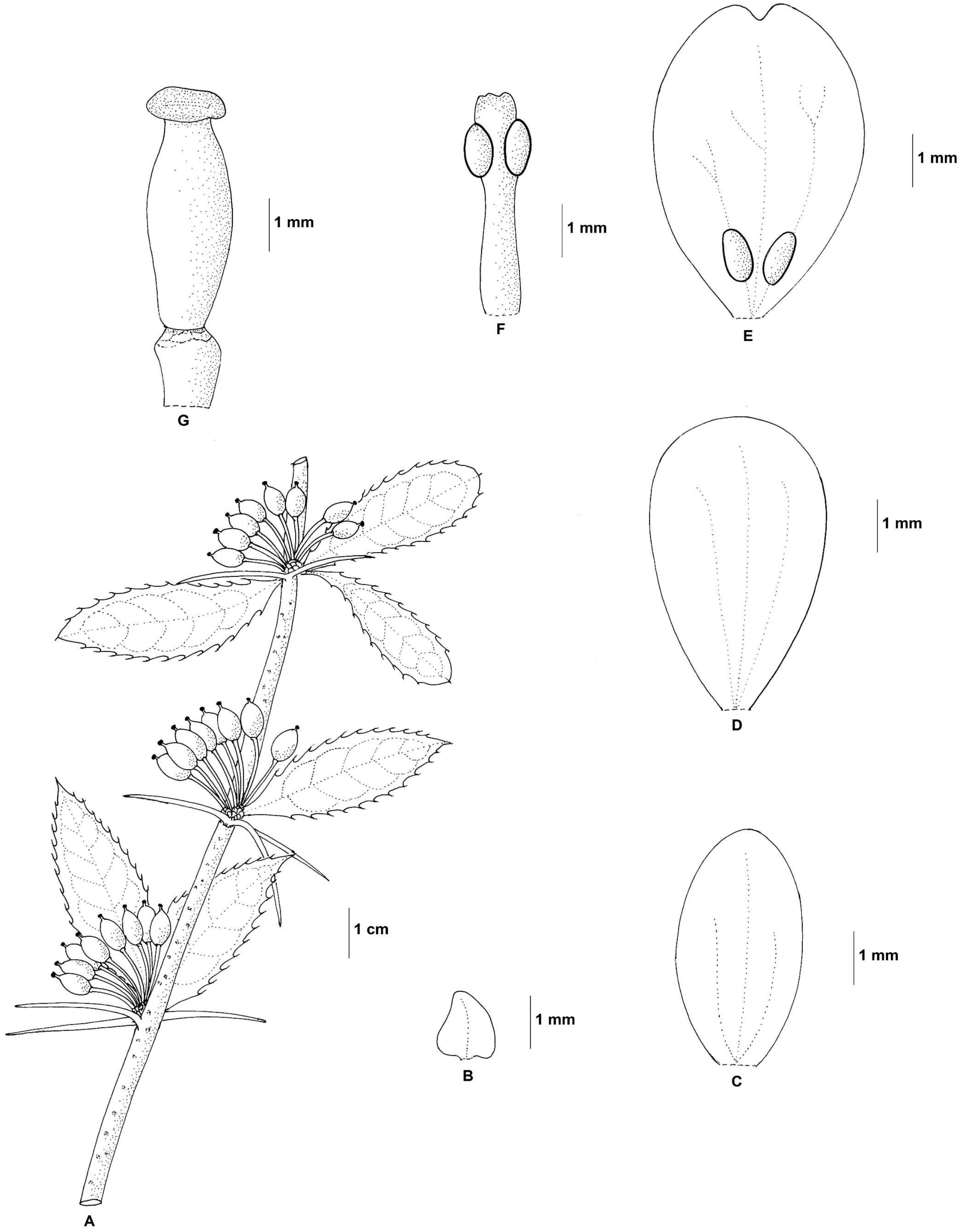


FIG. 2.49. ***Berberis wallichiana***. A. Fruiting branch. B. Outer sepal. C. Median sepal. D. Inner sepal. E. Petal. F. Stamen. G. Pistil. (Based on: A, Proud 2; B-G, Adhikari G 18).

Flowering time: Mar—Jun.

Fruiting time: May—Nov.

Ecology: Edges of *Rhododendron-Oak* forests.

Altitudinal range: 2300—3300 m.

Distribution: Central, Eastern [Eastern Himalaya, Assam-Burma].

Distinguishing features: Identifiable by its evergreen habit, flowers in fascicles, black stylose berries and solitary ovules.

Selected specimens: CENTRAL: Kathmandu, Nangi Dada, 2730 m, 16.04.1957, D. Proud 3b (E) ; Rasuwa, Khanjing-Sherpagaon, 2295 m, 2.08.2007, Adhikari, B BL2 24 (E); Rasuwa, Chandanbari, 3300 m, 21.05.2008, Adhikari, B G18 (E). EASTERN: Solukhumbu, Junbesi, 2420 m, 24.04.1974, Stainton 6977 (E); Terathum, Near Chauki, 2620 m, 26.10.1991, EMAK 1066 (E); Dhankuta: Above Hille, 2580 m, 13.11.1975, L.W. Beer 25741 (BM). Number of specimens examined: 24

Note: Specimens from two different gatherings, Wallich 1478 and Wallich s.n.(1819) have been cited as the type by different authors (Ahrendt, 1941, 1961; Chamberlain & Hu, 1985; Rao *et al*. 1998). Wallich 1478 is the Wallich catalogue number and specimens were collected by him in 1821. Early Wallich collections (1817-1819) were actually collected by Edward Gardner and his team (Fraser-Jenkins, 2005) but distributed in different herbaria under the Wallich name. The specimen in the De Candolle herbarium is from Gardner's 1819 collection and has been chosen as lectotype. As suggested by Fraser-Jenkins (2005), the specimen is cited as Gardner in Wallich (1819).

Ahrendt (1961) described the species *B. poluninii* based on the specimen collected by Polunin, no 746 near Timure, Central Nepal. Chamberlain and Hu (1985) treated *B. poluninii* as a synonym of *B. praecipua*. The specimens from the same locality have been collated for this study and all have been identified as *B. wallichiana*. The type specimen of *B. poluninii* differs from *B. wallichiana* only by its slightly smaller leaves. Therefore, *B. poluninii* has been treated as synonym of *B. wallichiana*.

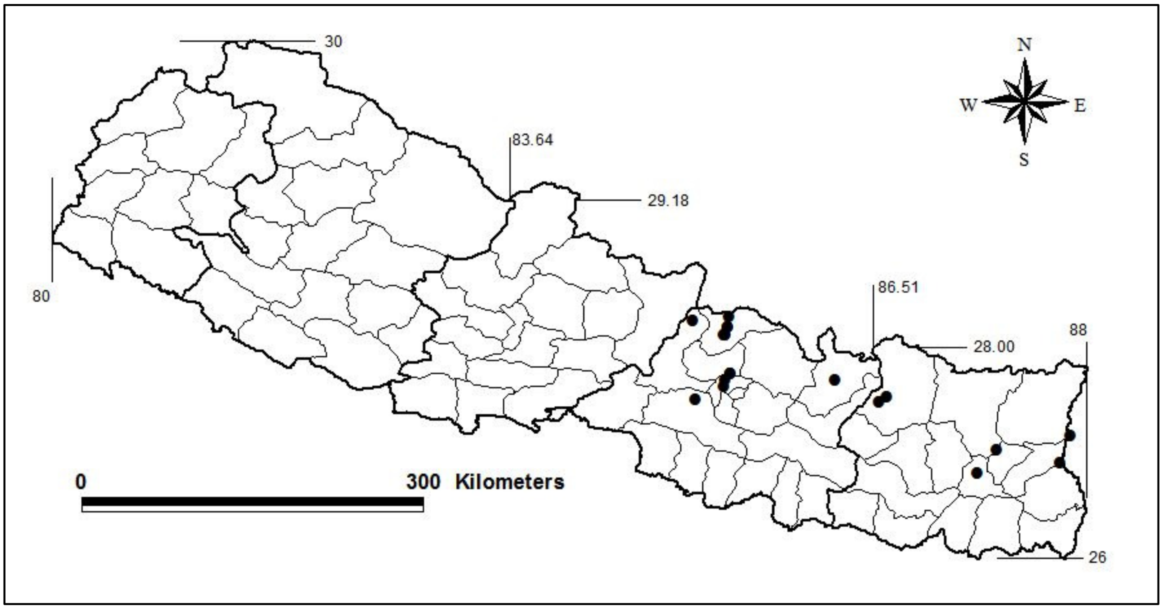


FIG. 2.50. Distribution of *Berberis wallichiana.*

FIG. 2.51. Various species of *Berberis*. A, B. *B. angulosa* var. *angulosa*. A. Fruiting branch. (EL 126: Rasuwa, 3525 m). B. Flowering branch. (LKSRB 38: Panchthar, 3410 m). C. *B. angulosa* var. *fasciculata*. Fruiting branch. (EL 127: Rasuwa, 3620 m). D, E. *B. aristata*. D. Fruiting branch. (SB 15: Kathmandu: 2380 m). E. Flowering branch. (Adhikari G3: Rasuwa, 2670 m). F. *B. asiatica*. Fruiting branch. (JRSA 106: Mugu, 2180 m).

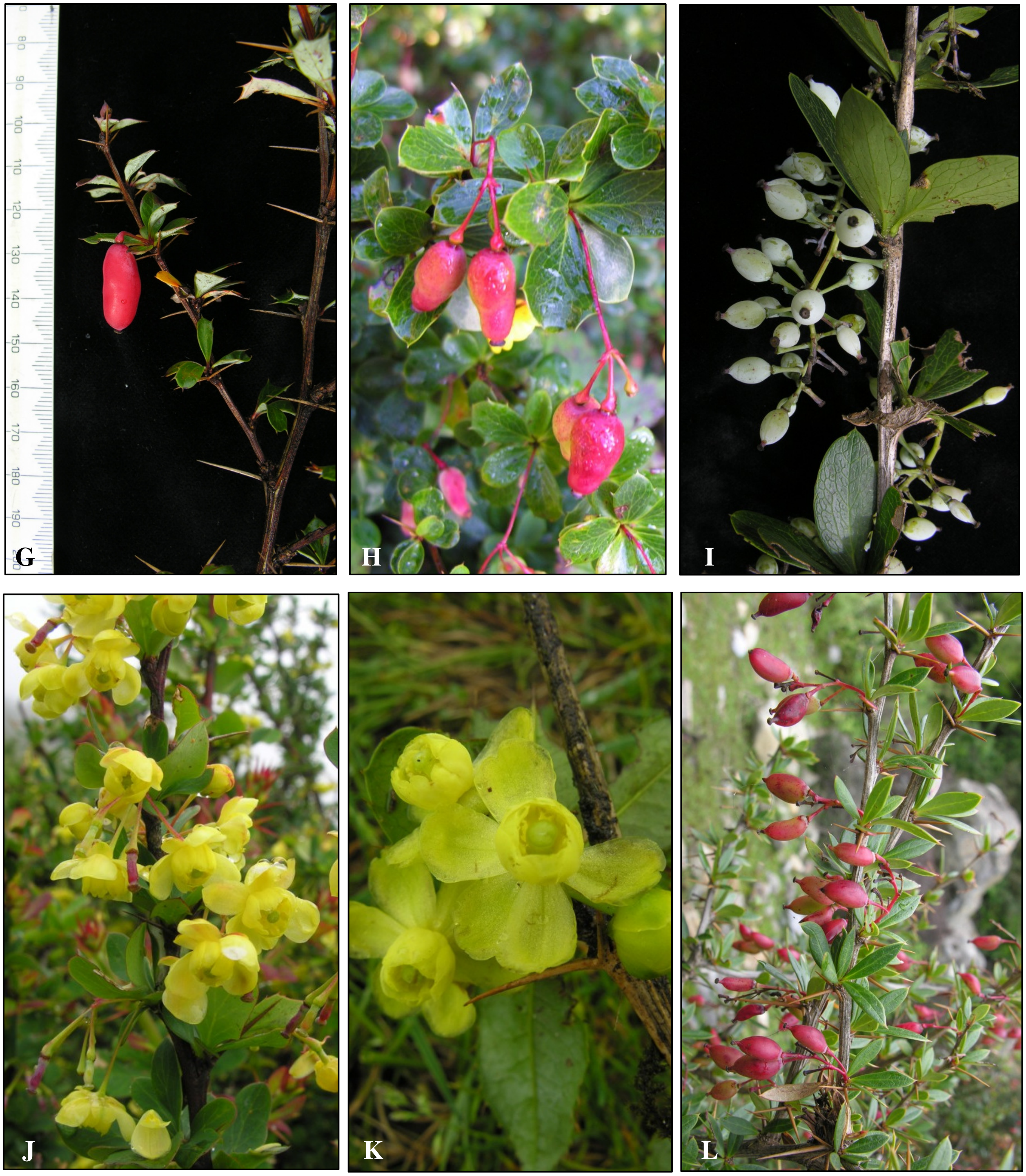


FIG. 2.51. (Continued). G. *B. concinna* var. *concinna*. Fruiting branch. (EL 125: Rasuwa, 3410 m). H. *B. concinna* var. *extensiflora*. Fruiting branch. (EA 104: Myagdi, 3150 m). I. *B. glaucocarpa*. (JRSA 134: Humla, 2640 m). J. *B. hamiltoniana*. (JRSB 162: Humla, 3640 m). K. *B. hookeri*. (LKSRB 22: Panchthar, 3000 m). L. *B. jaeschkeana* var. *usteriana*. (EA2 33: Mustang, 2600 m).

FIG. 2.51. (Continued). M. *B. karnaliensis*. (JRSA 59: Jumla, 2950 m). N. *B. koehneana*. (BL2 26: Rasuwa, 3010 m). O. *B. kumaonensis*. (JRSB 163: Humla, 3650 m). P. *B. mucrifolia*. (EA2 44: Mustang, 2600 m). Q. *B. orthobotrys* var. *rubicunda*. (BL2 51: Rasuwa, 3640 m). R. *B. pendryi*. (EA 32: Mustang, 3600 m).

FIG. 2.51. (Continued). S. *B. petiolaris* var. *petiolaris*. (JRSB 39: Jumla, 2890 m). T. *B. thomsoniana*. (LKSRB 112: Illam, 3050 m). U,V. *B. wallichiana*. U. Fruiting branch. (EL 123: Rasuwa, 2710 m). V. Flowering branch. (G18: Rasuwa, 3300 m).

CHAPTER 3: SYSTEMATIC AND BIOGEOGRAPHIC PATTERNS OF *BERBERIS S.L.* INFERRED FROM CHLOROPLAST *NDH*F GENE SEQUENCES

3.1 INTRODUCTION

3.1.1 Taxonomic and phylogenetic studies in Berberis

Berberis sensu lato (including *Mahonia*) is the largest genus in the family Berberidaceae. Although *Berberis s.s.* (simple-leaved *Berberis*) and *Mahonia* are morphologically quite distinct, several studies (Derman, 1931; Terabayashi, 1978; Kim & Jansen, 1998) have suggested their close phylogenetic relationship, and Laferriere (1997) transferred all the species of *Mahonia* to *Berberis.*

The most recent worldwide revision of *Berberis* (including *Mahonia*) was made by Ahrendt (1961) in which he recognised *ca.* 600 species. However, the true number may be much lower than this as Landrum (1999) recognized only 20 species of *Berberis* from Chile and adjacent southern Argentina out of the 60 reported by Ahrendt (1961). Similarly the revision of Nepalese species for this study (Chapter 2) recognizes only 21 species out of the 30 reported from Nepal (Ahrendt, 1961). Ahrendt (1961) mainly followed the classification scheme of Fedde (1902) and Schneider (1904-1905, 1908) with small modifications of a few sections and several subsections. Both Schneider

(1908) and Ahrendt (1961) divided the simple-leaved *Berberis* into *Septentrionales* and *Australes* and the compound-leaved *Berberis* into *Occidentales* and *Orientales*. Ahrendt (1961) recognized 33 sections and 50 sub-sections of simple-leaved species. The validity of these sections was called into question by Landrum's merging of three species from three different sections under a single species (Landrum, 1999).

Phylogenetic relationships among the genera within the Berberidaceae have been fairly well studied (Kim & Jansen, 1996; Kim & Jansen, 1998; Kim *et al.*, 2004a). For example, Kim and Jansen (1996) performed a phylogenetic analysis using *rbcL* sequence data and concluded that the Berberidaceae *s.l.* is monophyletic and that *Berberis s.l.* and *Ranzania* are sister taxa. Kim and Jansen (1998) used chloroplast restriction site data to study further relationships among the genera within the family, and the results were mostly congruent with their previous findings. Kim *et al.* (2004a) performed a phylogenetic analysis of 23 species of Berberidaceae based on *ndhF* to study intergeneric relationships. Their *ndhF* results were also largely congruent with *rbcL* and chloroplast DNA restriction site analysis, corroborating the monophyly of the family and the sister group relationships of *Berberis* and *Ranzania*.

Berberis itself has been the focus of rather few molecular studies. The species level ITS phylogeny of *Berberis s.l.* by Kim *et al.* (2004b) to date is the only published phylogeny. Although several studies (Meacham, 1980; Terabayashi, 1985; Kim & Jansen, 1996; Kim & Jansen, 1998; Wang *et al.*, 2007) suggested that *Ranzania* is the sister genus to *Berberis*, Kim *et al.* (2004b) used the midpoint rooting method to root their ITS phylogeny of *Berberis* because the ITS sequence of *Ranzania* was too divergent for proper alignment with *Berberis* species. The mid point rooting method (MPR) places the root of the tree at the midpoint between the two most divergent operational taxonomic units (Nei & Kumar, 2000) and is usually recommended when outgroup rooting is not possible (Sanderson & Shaffer, 2002). MPR does not necessarily find the correct location for the root of a phylogenetic tree. Todd (2006) produced an ITS phylogeny of *Berberis* for his MSc dissertation using Kim's published sequences with the addition of 22 sequences from the species growing in the Royal Botanic Garden Edinburgh, but was also unable to root his phylogeny with well known sister genus *Ranzania* because of sequence divergence. Both of these studies therefore may have failed to identify the basally divergent taxa in *Berberis s.l.* because the phylogenies had not been rooted with an outgroup.

An alternative means of rooting a phylogenetic tree of *Berberis* is to use a more slowly evolving genome region to reconstruct the phylogeny which permits an alignment with related genera that can be used as outgroups. *NdhF* is a protein coding gene located in the small single-copy region of the chloroplast genome close to the inverted repeats (Soltis & Soltis, 1998) (Fig. 3.1). It encodes a subunit of the nicotinamide dehydrogenase complex and is reported to be more informative than *rbcL* (Olmstead & Sweere, 1994; Olmstead & Reeves, 1995). The *ndhF* gene has been widely used to reconstruct phylogenies both within and among different plant families and also to infer the biogeographic histories of many groups (Clark *et al.*, 1995; Kim & Jansen, 1995; Neyland & Urbatsch, 1996; Ferguson, 1998; Pyck *et al.*, 1999; Davis *et al.*, 2002; Schnabel *et al.*, 2003; Kim *et al.*, 2004a; Wen *et al.*, 2008).

Therefore, the gene *ndh*F was chosen in this investigation so that the widely accepted outgroup rooting method can be used to root the *Berberis* phylogeny for the first time. Another reason for chosing *ndh*F was the availability of sequences of *Berberis* and closely related outgroups in GenBank. This chapter presents a phylogenetic analysis for *Berberis s.l.* derived from *ndh*F sequences from 59 *Berberis* species and eight outgroups.

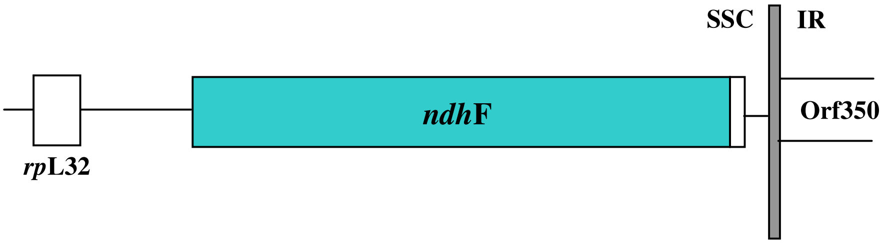


FIG. 3.1. Map of *ndh*F and adjoining regions of cpDNA in tobacco. Boxes indicate reading frame, line connecting boxes indicate noncoding DNA. Vertical bar at the left end of Orf350 indicates junction between inverted repeats (IR) and small single-copy region (SSC). Modified from Olmstead and Reeves (1995).

3.1.2 Biogeography of Berberis

A second goal of this chapter is to use the *ndhF* phylogenetic hypothesis to investigate the origin of the distribution patterns in *Berberis s.l.*, which hence provides new information about how the intercontinental distribution of *Berberis s.l.* developed. This chapter presents the first dated phylogeny of *Berberis s.l.* to illustrate the intercontinental relationships of *Berberis s.l.* All the genera of the Berberidaceae have a Northern Hemisphere distribution, with only *Berberis* extending to South America (Heywood, 2007). *Berberis s.s.* (simple-leaved) has two important centres of diversity, corresponding to the *Septentrionales* of Eurasia and the *Australes* of South America. Most of the species of *Septentrionales* (*ca.* 320 species) grow in Eurasia, except for two in North America and four in North Africa, while *Australes* (*ca.* 175 species) are exclusively South American (Ahrendt, 1961). Of the two compound-leaved groups, *Occidentales* (*ca.* 43 species) grow in North and Central America while *Orientales* (*ca.* 60 species) are Asian with one species (*B. nervosa*) in North America (Ahrendt, 1961).

3.1.2.1 Compound-leaved Berberis: Northern hemisphere disjunction

The extant members of compound-leaved *Berberis* are disjunctly distributed mainly in Western North America and Asia. Northern Hemisphere intercontinental plant disjunctions have received considerable attention from botanists and biogeographers (Wolfe, 1975; Wen, 1999; Donoghue *et al.*, 2001; Milne & Abbott, 2002). Eastern Asia (EA), Eastern North America (ENA), Western North America (WNA), and Europe are the main areas recognized by many authors which are involved with Northern Hemisphere plant disjunctions (Milne & Abbott, 2002; Donoghue & Smith, 2004). Among these four areas, disjunctions between ENA and EA are especially common among various plant groups, and have been studied more intensively than other disjunctions (Xiang *et al.*, 2000; Donoghue *et al.*, 2001), with the WNA-EA disjunction comparatively less well understood (Schultheis & Donoghue, 2004).

The movement of different groups of plants between Asia and North America through Beringia and the North Atlantic Land Bridge (NALB) at different times is well documented (Tiffney, 1985; Donoghue *et al.*, 2001; Milne & Abbott, 2002). The NALB consisted of two distinct connections: a northerly link between Greenland and Fennoscandia, and a more southerly link between Greenland and Scotland (Tiffney, 1985). The northern route was available until around 40 million years ago, but the lifespan of the southern route is under active debate, in part because the Iceland hotspot has been continuously producing land between Greenland and Scotland since these landmasses began moving apart (Tiffney, 1985; Tiffney & Manchester, 2001; Milne and Abbott, 2002). Therefore, both connections are thought to have been available during the early Eocene, but the Greenland–Scotland route might have persisted until the late Oligocene or even the early Miocene (*ca*. 20-7 mya) (Tiffney, 1985; Tiffney & Manchester, 2001; Milne & Abbott, 2002).

The Bering land bridge is thought to have been available for interchange of taxa far later than the NALB, until approximately 5.5 million years ago (Gladenkov *et al.*, 2002). However, climatic cooling in the Beringia region between 15 and 5 million years ago might have made the bridge too cold for many taxa at an earlier time than this (Tiffney & Manchester, 2001; Milne, 2006), although as many *Berberis* species are fairly cold-tolerant, they might have been less affected by this.

Wang *et al.* (2007) studied the Northern Hemisphere disjunction pattern in six disjunct groups of Berberidaceae: *Achlys* (WNA-EA), *Epimedium* and *Vancouveria* (WNA-Eurasia), *Diphylleia* (EA-ENA), *Caulophyllum* (EA-ENA), *Sinopodophyllum* and *Podophyllum* (EA-ENA) and *Plagiorhegma* and *Jeffersonia* (EA-ENA). His study was mainly in the context of the phylogeny of Berberidaceae; he included only one species of simple-leaved and two species of compound-leaved *Berberis*, which was not enough to examine the disjunction pattern within *Berberis*, especially because no South American species were included.

3.1.2.2 Simple-leaved Berberis: Anti-tropical disjunction

The simple-leaved *Berberis* is the only group within the family Berberidaceae which extends its distribution to the Southern Hemisphere. The term 'amphitropical' is usually used to describe those taxa which are disjunctly distributed between the Northern and Southern Hemispheres. However, several authors remarked that the most appropriate term would be antitropical (Cox, 1990; Kenicer, 2007) because the term amphitropical means both tropics. Moreira-Muñoz and Muñoz-Schick (2007) used the term 'wide antitropical' to define the disjunction patterns of 10 genera of Asteraceae distributed in cool regions of both hemispheres.

The antitropical pattern of distribution is shown by many temperate taxa. Raven (1963) found approximately 160 examples of this type of disjunction between closely related or identical species. The antitropical disjunction pattern between North America and South America is well documented in many plant groups (Chambers, 1963; Vargas *et al*. 1998; Ickert-Bond *et al*., 2009). The review of Wen & Ickert-Bond (2009) showed 34 cases of this type of disjunction in plants, and most of them resulted from long distance dispersal.

Disjunctions between temperate South America and Asia are rare among plant groups (Thorne, 1972). However, some molecular studies suggest that the origin of South American-Eurasian disjunctions have occured through dispersal from Eurasia to South America (Kenicer *et al*., 2005; Schnabel *et al*., 2003; Soltis *et al*., 2001).

Kim *et al.* (2004b) hypothesized vicariance events during the Cretaceous to explain the origin of South American simple-leaved *Berberis*, arguing that by the time South

America split from Africa, many major lineages of angiosperm had already diversified and migrated resulting in broad distributions in Asia, Africa and South America. This hypothesis therefore predicts a Cretaceous origin for *Berberis*, a prediction that can be tested by molecular dating.

This chapter uses a dated *ndh*F phylogeny of *Berberis* to investigate the competing hypotheses of long distance dispersal and vicariance to explain the origin of South American simple-leaved *Berberis*.

3.2 AIMS AND OBJECTIVES

The main aims of this chapter are to use an *ndh*F phylogeny to:

1. Determine the relationship between the simple-leaved and compound-leaved *Berberis* species.

2. Determine when the major geographic disjunctions arose within simple-leaved and compound-leaved *Berberis*, and whether they are best explained by overland migration or long distance dispersal.

3.3 MATERIALS AND METHODS

3.3.1 Taxon sampling and outgroup selection

Sixty-four accessions of *Berberis s.l.* were examined, representing 48 species from 21 of 33 sections of simple-leaved and 11 species from all four sections of compound-leaved *Berberis*. These accessions cover the geographic range of *Berberis*. One accession each of *Ranzania*, *Nandina* and *Caulophyllum* were included in the analysis to serve as outgroups. The use of multiple outgroups permits an evaluation of the monophyly of *Berberis* (Nixon & Carpenter, 1993). For the biogeographic analysis, five additional accessions from the families Ranunculaceae, Menispermaceae and Lardizabalaceae were also included so that additional fossil calibration points could be employed, increasing the total number of accessions to 69. Table 3.1 shows the samples used in this study with voucher information, origin of sample, distribution range (Ahrendt, 1961) and section and sub-sectional classification following Ahrendt (1961).

3.3.2 DNA extraction, PCR and sequencing

Total genomic DNA was extracted from silica gel-dried leaves using a CTAB method (Doyle & Doyle, 1987) and the DNeasy Plant Mini Kit (Qiagen Ltd, Crawley, West Sussex, UK) following the manufacturer’s protocol. The extracted DNA was checked in 1% agarose gel electrophoresis. The extracted DNA was purified with the Wizard DNA clean up system (Promega Corporation, 2800 Woods Hollow Road, Madison, USA) following the manufacturer’s protocol.

Table 3.1. Samples used in this study with voucher information, origin of sample, distribution range (Ahrendt, 1961), and sectional and sub-sectional classification following Ahrendt (1961). BL2, EL, EA, EA2, JRSA, G and LKSRB are expedition abbreviations (see appendix 1). RBGE: Royal Botanic Garden Edinburgh.

Taxa	Section/subsection	Voucher information	Origin	Distribution (Ahrendt, 1961)
Simple-leaved *Berberis* (Septentrionales)				
Berberis angulosa Wall. ex Hook.f. & Thoms.	Angulosae/Euangulosae	BL2 38	Nepal: Rasuwa	Nepal, Sikkim, SE Tibet
Berberis angulosa Wall. ex Hook.f. & Thoms.	Angulosae/Euangulosae	EL 126	Nepal: Rasuwa	Nepal, Sikkim, SE Tibet
Berberis angulosa Wall. ex Hook.f. & Thoms. var *fasciculata* Ahrendt	Angulosae/Euangulosae	EL 127	Nepal: Rasuwa	Nepal, Sikkim
Berberis sibirica Pallas	Angulosae/Sibiricae	Harber: MF0505	China: N of Beijing	Siberia, N Mongolia
Berberis mucrifolia Ahrendt	Angulosae/Jaeschkeanae	EA2 29	Nepal: Mustang	Nepal
Berberis mucrifolia Ahrendt	Angulosae/Jaeschkeanae	EA 20	Nepal: Mustang	Nepal
Berberis jaeschkeana C.K.Schneid.	Angulosae/Jaeschkeanae	EA2 40	Nepal: Mustang	Kashmir, Punjab Himalaya, Nepal
Berberis pendryi Adhikari	Angulosae/Jaeschkeanae	EA2 5	Nepal: Mustang	Nepal
Berberis karnaliensis Adhikari	Angulosae/Jaeschkeanae	JRSA 59	Nepal: Jumla	Nepal
Berberis orthobotrys Bienert ex Aitch.	Angulosae/Subangulosae	RBGE-19810556	Iran	Kashmir, Nepal, SE Tibet
Berberis asiatica Roxb. ex DC.	Asiaticae	G 2	Nepal: Rasuwa	Nepal, Kumaon, Garhwal, Assam
Berberis asiatica Roxb. ex DC.	Asiaticae	JRSA 88	Nepal: Mugu	Nepal, Kumaon, Garhwal, Assam
Berberis mitifolia Stapf	Brachypodae	Harber	China: Hubei, W	China: W Hubei
Berberis aetnensis C. Presl	Crataeginae	Harber	Italy: Mt Etna Sicily	Sicily, South Italy, East Spain
Berberis garciae Pau.	Crataeginae	Harber	Spain: Guadalajara province	East Spain
Berberis minutiflora C.K.Schneid.	Franchetianae/Minutiflorae	Harber ACE 1848	China: Yunnan, NW	Yunnan, SW Szechuan, SE Tibet
Berberis stearnii Ahrendt	Franchetianae/Eufranchetiana	RBGE-19310185	China: Yunnan	Yunnan
Berberis tsarongensis Stapf	Franchetianae/Eufranchetiana	RBGE-19381165	China: Tibet	SE Tibet, NW Yunnan
Berberis papillifera (Franch.) Koehne	Franchetianae/Yunnanenses	Harber BSWJ 7738	China: Yunnan, NW	Yunnan
Berberis microtricha C.K.Schneid.	Franchetianae/Yunnanenses	RBGE-19910513	China: Yunnan, NW	S Szechuan, Yunnan

Table 3.1. (Continued).

Taxa	Section/subsection	Voucher information	Origin	Distribution (Ahrendt, 1961)
Simple-leaved Berberis (Septentrionales)				
Berberis cretica L.	Heteropodae/Creticae	RBGE-19687158 A	Unknown (cultivated)	Crete, Cyprus
Berberis heteropoda Schrenk	Heteropodae/Pseudumbellatae	Harber	Kyrgyzstan: Tien-Shan	Sinkiang (China)
Berberis iliensis Popov	Integerrimae	Rae, Gardner & Ogar, 11	Kazakhstan: Ili Intermountain Valley	NW Sinkiang
Berberis prattii C.K.Schneid.	Polyanthae/Eupolyanthae	RBGE-19687172	China: Sichuan, W	W Szechuan
Berberis koehneana C.K.Schneid.	Polyanthae/Subpolyanthae	BL2 26	Nepal: Rasuwa	Nepal, Kumaon
Berberis koehneana C.K.Schneid.	Polyanthae/Subpolyanthae	BL2 55	Nepal: Rasuwa	Nepal, Kumaon
Berberis gyalaica Ahrendt	Sherriffianae	Harber-AC 3647	China: Tibet	SE Tibet
Berberis sherriffii Ahrendt	Sherriffianae	RBGE-19744027	China: Tibet	SE Tibet
Berberis forrestii Ahrendt	Sinenses	Harber-AC 1548	China: Yunnan, NW	Yunnan
Berberis petiolaris Wall.ex G.Don	Tinctoriae/Eutinctoriae	JRSA 122	Nepal: Mugu	Nepal, India
Berberis aristata DC.	Tinctoriae/Chitriae	G 19	Nepal: Rasuwa	Nepal
Berberis thomsoniana C.K.Schneid.	Tinctoriae/Umbellatae	G 4	Nepal: Rasuwa	Sikkim, E Nepal
Berberis tschonoskyana Regel	Tschonoskyanae	RBGE-19940193B	Japan: Shikoku	Japan
Berberis koreana Palib	Vulgares	RBGE-19731131A	Korea: Kyonggi-do	Korea
Berberis regeliana Koehne ex C.K.Schneid.	Vulgares	RBGE-19920784A	Japan: Hokkaido	Japan
Berberis vulgaris L.	Vulgares	Harber	Spain: N Pyrenees	Europe: Switzerland, Albania
Berberis candidula C.K.Schneid.	Wallichianae/Verruculosae	RBGE-19902425A	Unknown (cultivated)	W. Hupeh
Berbeis coxii C.K.Schneid.	Wallichianae/Manipuranae	RBGE-19251027	China: Tibet	Upper Burma
Berberis sanguinea Franch.	Wallichianae/Sanguineae	RBGE-19390166B	Unknown (cultivated)	W Szechuan
Berberis kawakamii Hayata	Wallichianae/Barandanae	RBGE-19933979B	Taiwan: Miaoli	Taiwan (Formosa)
Berberis bergmanniae C.K.Schneid.	Wallichianae/Euwallichianae	RBGE-19754095A	Unknown (cultivated)	W Szechuan
Berberis wallichiana DC.	Wallichianae/Euwallichianae	BL2 20	Nepal: Rasuwa	Nepal
Berberis sargentiana C.K.Schneid.	Wallichianae/Euwallichianae	RBGE-19784169	China: Hubei, W	W. Hubei

Table 3.1. (Continued).

Taxa	Section/subsection	Voucher information	Origin	Distribution (Ahrendt, 1961)
Simple-leaved *Berberis* (Australes)				
Berberis congestiflora C. Gay	Actinacanthae/Congestiflorae	RBGE-19892971B	Chile: Araucania	Chile
Berberis empetrifolia Lam.	Buxifoliae/Empetrifoliae	RBGE-19761088A	Argentina : Tierr. Nac. Tierra del Fuego	Chile
Berberis hieronymi C.K.Schneid.	Buxifoliae/Eubuxifoliae	Harber	Argentina, NW: Sierras Grandes de Córdoba	Argentine
Berberis chilensis Gillies ex Hook.	Illicifoliae/Chilenses	RBGE-19900509	Chile: Maule	Chile
Berberis ilicifolia Forst.	Illicifoliae/Euilicifoliae	RBGE-19880919A	Chile: Los Lagos	Argentine, Chile
Berberis negeriana Tischler	Illicifoliae/Euilicifoliae	RBGE-20040497A	Chile: Biobio	Chile
Berberis valdiviana Phil.	Laurinae/Flexuosae	RBGE-19900563C	Chile: Biobio	Chile
Berberis microphylla Forst.	Microphyllae	RBGE-19670638B	Chile	Chile
Berberis montana Gay	Montanae	RBGE-19921060A	Chile: Los Lagos	Chile
Berberis trigona Kunze ex Poepp. & Endl.	Trigonae	Harber-HCM 98123	Chile: Araucanía	Chile
Compound-leaved *Berberis* (*Mahonia*) (Orientales)				
Berberis polyodonta (Fedde) Laferr.	Longibracteatae/Dolichostyles	RBGE-19911138B	China: Yunnan, W.	Szechuan
Berberis duclouxiana (Gagnep.) Laferr.	Longibracteatae/Dolichostyles	RBGE-19923156A	China: Yunnan, NW	Yunnan, W. Yunnan
Berberis napaulensis (DC.) Laferr.	Longibracteatae/Napaulenses	LKSRB 1	Nepal: Panchthar	Nepal
Berberis gracilipes Oliv.	Longibracteatae/Dolichopodae	RBGE-19891713B	China: Sichuan, W	Szechuan
Berberis nervosa Pursh	Longibracteatae/Nervosae	RBGE-19782559 B	Canada: British Columbia	British Columbia, Idaho, California, Washington

Table 3.1. (Continued).

Taxa	Section/subsection	Voucher information	Origin	Distribution (Ahrendt, 1961)
Compound-leaved *Berberis* (*Mahonia*)				
(Occidentales)				
Berberis repens Lindl.	Aquifoliatae/Euaquifoliatae	RBGE-19800553A	Canada: British Columbia	Colorado
Berberis aquifolium Pursh	Aquifoliatae/Euaquifoliatae	RBGE-19912984A	Canada: British Columbia	California
Berberis pallida Hartw. ex Benth.	Paniculatae/Eupaniculatae	RBGE-19930103C	Mexico: Hidalgo	Mexico, Oaxaca
Berberis fremontii Torr.	Horridae	RBGE-19716619A	Unknown (cultivated)	Colorado, Arizona, Mexico
Berberis nevinii A. Gray.	Horridae	Rancho Santa Ana Botanical Garden, s.n.	Unknown (cultivated)	S. California
Berberis higginsae Munz	Horridae	Gen Bank Acc: AY145164	Y-D Kim *et al.* (2004) (YDK 90-1 TEX)	California
Outgroup	**Family**	**GenBank Accession No.**		
Nandina domestica Thunb.	Berberidaceae	AY145148	Y-D Kim *et al.* (2004) (YDK 94-1 TEX)	Japan, China, India
Caulophyllum robustum Maxim.	Berberidaceae	AY145149	Y-D Kim *et al.* (2004) (YDK 92-5 TEX)	Japan, E. Asia
Ranzania japonica T.Ito	Berberidaceae	AY145150	Y-D Kim *et al.* (2004) (Edinburgh, 841879)	Japan
Additional outgroups for BEAST analysis				
Hydrastis canadensis L.	Ranunculaceae	AY145146	Y-D Kim *et al.* (2004) (YDK 90-1 TEX)	N. America
Clematis drummondii Torr. & A.Gray	Ranunculaceae	AY145147	Y-D Kim *et al.* (2004)	N. America
Glaucidium palmatum Siebold & Zucc.	Ranunculaceae	AY145145	Y-D Kim *et al.* (2004) (Arnold Ar. sn)	Japan
Cocculus carolinus DC.	Menispermaceae	AY145144	Y-D Kim *et al.* (2004) (YDK 93-2 TEX)	N. America
Akebia quinata Decne.	Lardizabalaceae	AY145143	Y-D Kim *et al.* (2004) (Johansson 166 (CONN)	Japan, Asia

The *ndh*F gene was amplified using the newly designed primer *ndh*F 20F (5′-TGC ATG AT CAT ACC TTG G-3′) and *ndh*F 2155R (5′-TCC GCG CCC TAT ATA TTT T -3′), both manufactured by Invitrogen. Primers were designed with the help of published sequences of *B. higginsae* and *B. koreana* (Kim *et al.*, 2004a) and using Primer3 software (Rozen & Skaletsky, 2000). The PCR reaction was carried out in the DNA Engine Tetrad 2 Peltier Thermal Cycler by Bio-RAD in 25 µl reactions containing 1-2 µl of template DNA, 2.5 µl of 10 x NH_4 reaction buffer, 2.5 µl of dNTPs (0.2 mM), 1.25 µl of $MgCl_2$ (50 mM), 0.3 µl of Taq Polymerase (5U/µl), 0.8 µl of Bovine Serum Albumin (BSA, 1ug/ml), 14.65-15.65 µl of distilled water and 1 µl of each primer (10 µM). The thermal cycler was programmed as: initial denaturation at 95 ^{0} C for 3 minutes; 1 minute annealing at 50^0 C , 1 minute extension at 72 ^{0} C followed by 30 cycles of 1 minute denaturation at 95 ^{0} C; 1 minute annealing at 50^0 C and 1 minute extension at 72 ^{0} C . The cycle was terminated with an extension at 72 ^{0} C for 10 minutes followed by 10^0 C for forever. Quality and quantity of PCR product was checked in a 1% agarose gel and purified with ExoSAP-IT (USB Corporation) following the manufacturer's protocol. The forward and reverse primer noted above, and the internal primer 727F [5′- CAA TTC CCC CTT CAT GTA TGG TTA CC-3′; (Kim *et al.*, 2004a)] were used in sequencing reactions, each carried out in 10 µl reaction containing 1-2 µl of template DNA, 0.32 µl of primer (10 µM), 2 ul of 5 x sequencing buffer, 1 µl of Bigdye mix and 4.68-5.68 µl of distilled water. Sequencing amplification was done in the DNA Engine Tetrad 2 Peltier Thermal Cycler by Bio-RAD using the following conditions: 25 cycles of 95^0 C for 30 seconds, 50^0 C for 20 seconds and 60^0 C for 4 minutes. The product was analysed at the GenePool sequencing facility at The University of Edinburgh, UK. Forward, reverse and internal sequence fragments were aligned, visualized and edited using Sequencher^{TM} (Version 4.5, Genetic Codes Corporation, Ann Arbor, Michigan, US). Sequences were aligned initially in BioEdit (Version 7.0.9) and later at amino acid level by eye in MacClade 4.06 for OS X (Maddison & Maddison, 2003).

3.3.3 Phylogenetic analysis

The phylogenetic analyses were conducted using maximum parsimony and Bayesian methods. Maximum parsimony searches employed the heuristic search method using Tree-Bisection-Reconnection (TBR) branch swapping in PAUP* 4.0 β10 (Swofford, 2002). Heuristic search was conducted with 10000 random addition sequences which saved 10 trees per replicate (with MulTrees on and steepest descent off). For character optimisation the option ACCTRAN was used and starting trees were obtained by stepwise addition. There were only a few gaps and they were treated as missing characters. To calculate the support for each clade of the tree, bootstrap analysis (Felsenstein, 1985) was conducted using 10000 replicates with full heuristic search and the TBR branch-swapping algorithm, which saved 10 trees per replicate (MulTrees option off).

Bayesian analysis was carried out using Markov Chain Monte Carlo (MCMC) methods implemented in MrBayes ver. 3.1.2. (Huelsenbeck & Ronquist, 2001). Nucleotide substitution models for Bayesian analyses were evaluated using the Akaike information criteria (AIC) and the hierarchical likelihood ratio test (LRT) in MrModeltest ver. 2.3 (Nylander, 2004). The model GTR+G was suggested by both AIC and LRT, and was therefore implemented in the analysis. Two parallel independent runs, each consisting of one cold and three heated chains were run for 5 million generations. The Markov chains were sampled every 100th generation, resulting in 50000 sampled trees from each chain. The trace files were analysed in Tracer version 1.5 (Rambaut & Drummond, 2007) and it was determined that stationarity was reached after 5000 trees were sampled (500000 generations); therefore the first 5000 trees (10%) were discarded as 'burn-in'.

3.3.4 Divergence time estimation

Divergence times were estimated using a Bayesian approach implemented in BEAST ver. 1.5.3 (Drummond & Rambaut, 2007). Fossils of compound-leaved *Berberis* date back to the middle Eocene (Manchester, 1999; Erwin & Schorn, 2000) and were used to constrain the age of the stem group of *Berberis s.l.* to a miminum of 45 million years ago (mya). The crown divergence of the Menispermaceae-(Berberidaceae-Ranunculaceae) clade was estimated at 91 mya, based on the fossil genus *Prototinomiscium* recovered from the Turonian of central Europe (Knobloch & Mai, 1986; Anderson *et al.*, 2005), so this was used as a second calibration point in this analysis. Wang *et al.* (2007) also used these two fossils to calibrate their chronogram of Berberidaceae. For the BEAST analysis, the Yule speciation prior was implemented, which has been recommended for species level phylogenies (Drummond *et al.*, 2007). The analysis was implemented under a relaxed clock with an uncorrelated log-normal model of rate variation

(Drummond *et al.*, 2006). The SRD06 model of sequence evolution was used (Shapiro *et al.*, 2006). Though this model has fewer parameters than GTR +G+I, it has been found to be superior for protein coding nucleotide data (Drummond *et al.*, 2007). The xml input file for this analysis was generated using BEAUti ver.1.5.3 (Drummond & Rambaut, 2007).

Two independent runs were performed, each of 100 million generations, with sampling every 10000 generations. Two separate runs were combined using LogCombiner ver 1.5.3 (Drummond & Rambaut, 2007). Convergence of the chain to a stationary distribution was confirmed by visualizing trace files in Tracer ver. 1.5 (Rambaut & Drummond, 2007). Tracer ver. 1.5 was also used to measure effective sample size (ESS) of each parameter. A maximum clade credibility tree was generated from the sampled trees using TreeAnnotator ver.1.5.3 (Drummond & Rambaut, 2007), and mean ages, 95% highest posterior probability (HPD) intervals and posterior probablities were also calculated. The resulting tree was visualized using FigTree ver.1.3.1 (Rambaut, 2009).

3.3.5 Optimisation of geographic areas and morphological characters

Optimisation of distribution areas and morphological characters of the species of *Berberis s.l.* was performed in Mesquite ver. 2.72 (Maddison and Maddison, 2009). The distribution areas and morphological characters were coded as multistate unordered characters and mapped on to the most parsimonious trees generated in PAUP* 4.0 β10 (Swofford, 2002).

3.4 RESULTS

3.4.1 Sequence characteristics

The region of the *ndh*F gene sequenced in this analysis begins at position 76 and ends at 2094 of the *ndh*F gene of Berberidaceae published by Kim *et al.* (2004a). The length of unaligned sequences varied from 2010 base pairs in *Ranzania japonica* to 2082 base pairs in *B. koreana*. The aligned *ndh*F data set including gaps consisted of 2088 characters. The longest indel in the data set was 18 codons long in *B. koreana*. The sequence divergence between ingroup and outgroup taxa was manageable and alignment was entirely unambiguous. The sequence was rich in A+T with only 33.6% C+G content. In the aligned matrix, the number of parsimony informative characters was 182, and 1724 characters were constant.

3.4.2 Parsimony analysis

Parsimony analysis of *ndh*F data generated three most parsimonious trees with 449 steps, with consistency index (CI) of 0.90 and retention index (RI) of 0.91. For the first time, the outgroup rooting method was applied to root a *Berberis* phylogeny, and the North American compound-leaved *Berberis higginsae* was found to be a sister to the rest of the *Berberis* species (Fig. 3.2).

3.4.3 Bayesian analysis

The topologies of both parsimony and Bayesian trees were congruent. Although some clades have relatively higher posterior probability than bootstrap support, these two values represent different statistical support measures and are not directly comparable to each other (Douady *et al.*, 2003). The *ndh*F data supported the monophyly of the simple-leaved *Berberis*, but the compound-leaved *Berberis* were paraphyletic. Both maximum parsimony and Bayesian analyses placed *B. higginsiae*, the North American compound-leaved *Berberis* of section *Horridae*, as sister to all other *Berberis* species. Among the remaining species of compound-leaved *Berberis*, there were two clades (Clade 1 & 2; Fig. 3.2) corresponding to North American *Occidentales* and Asian *Orientales*. However, the position of two North American species, *B. nevinii* and *B. nervosa* were not resolved. Simple-leaved *Berberis* formed six major clades. Of these, clade 3 was exclusively South American, clade 4 contained species from Eurasia (Japan, Korea, Spain, Italy, China, Iran and Kazakstan); clade 5 contained Nepalese and Chinese species; clades 6 and 7 were exclusively Nepalese, and clade 8 comprised only Chinese species (Fig. 3.2).

The South American clade (Clade 3; Fig. 3.2) contained all species examined from sections *Actinacanthae* (*B. congestiflora*), *Buxifoliae* (*B. empetrifolia* and *B. hieronymi*), *Illicifoliae* (*B. chilensis*, *B. illicifolia* and *B. negeriana*), *Laurinae* (*B. valdiviana*), *Microphyllae* (*B. microphylla*), *Montanae* (*B. montana*) and *Trogonae* (*B. trigona*).

Clade 4 comprised species from sections *Integerrimae* (*B. iliensis*), *Tschonoskyanae* (*B. tschonoskyana*), *Angulosae* (*B. sibirica* and *B. orthobotrys*), *Heteropodae* (*B. cretica* and *B. heteropoda*), *Crateginae* (*B. aetnensis* and *B. garciae*) and *Vulgares* (*B. koreana*, *B. regeliana* and *B. vulgaris*).

Clade 5 contained only species from section *Wallichianae* (*B. wallichiana*, *B. sanguinea*, *B. kawakamii*, *B. bergmanniae*, *B. candidula* and *B. sargentiana*) with relatively good support [Posterior probability (pp): 1; Bootstrap (bs): 80). These are all but one of the species from section *Wallichianae* examined; the position of *B. coxii* remained unresolved.

Clade 6 comprised the species from section *Angulosae* (*B. mucrifolia*, *B. jaeschkeana* and *B. karnaliensis*) and *Asiaticae* (*B. asiatica*), and clade 7 contains the species from three different sections: *Polyanthae* (*B. koehneana*), *Tinctoriae* (*B. thomsoniana*) and *Angulosae* (*B. angulosa*).

Within clade 8, section *Sherriffianae* (*B. gyalaica* and *B. sherriffii*) formed a small monophyletic subclade, while other species in clade belonged to sections *Franchetianae* (*B. papillifera*, *B. stearnii*, *B. minutiflora*, *B. tsarongensis* and *B. microtricha*), *Sinenses* (*B. forrestii*) and *Polyanthae* (*B. pratii*).

Notable polyphyly was shown by sections *Angulosae* and *Polyanthae*. The seven members of section *Angulosae* examined (*B. angulosa*, *B. sibirica*, *B. mucrifolia*, *B. jaeschkeana*, *B. pendryi*, *B. karnaliensis* and *B. orthobotrys*) were scattered across three different clades (4, 6 and 7). Within section *Polyanthae*, the two species examined, *B. koehneana* and *B. pratii*, were placed in clades 7 and 8 respectively (Fig. 3.2).

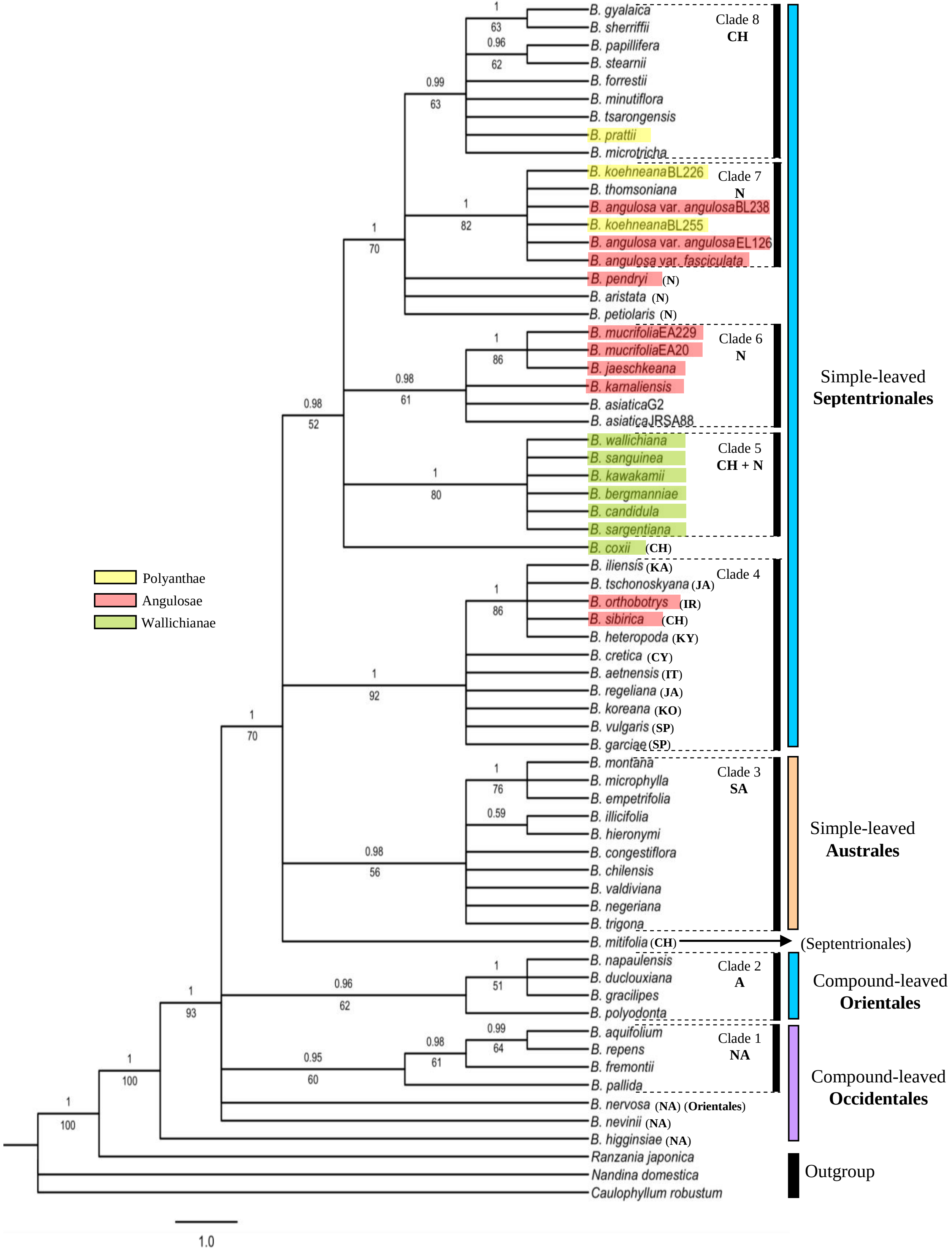


FIG. 3.2. Bayesian majority rule consensus tree obtained from *ndh*F sequences of 64 accessions representing 22 sections of simple-leaved and 4 sections of compound-leaved *Berberis*. Numbers above branches are posterior probabilities and below are bootstrap support. Both strict consensus and bootstrap consensus trees from Parsimony analysis are identical to the Bayesian majority rule consensus tree. NA: North America; A: Asia, SA: South America; SP: Spain; KO: Korea; JA: Japan; IT: Italy; CY: Cyprus; KY: Kyrgyzstan; IR: Iran; KA: Kazakhstan; CH: China; N: Nepal.

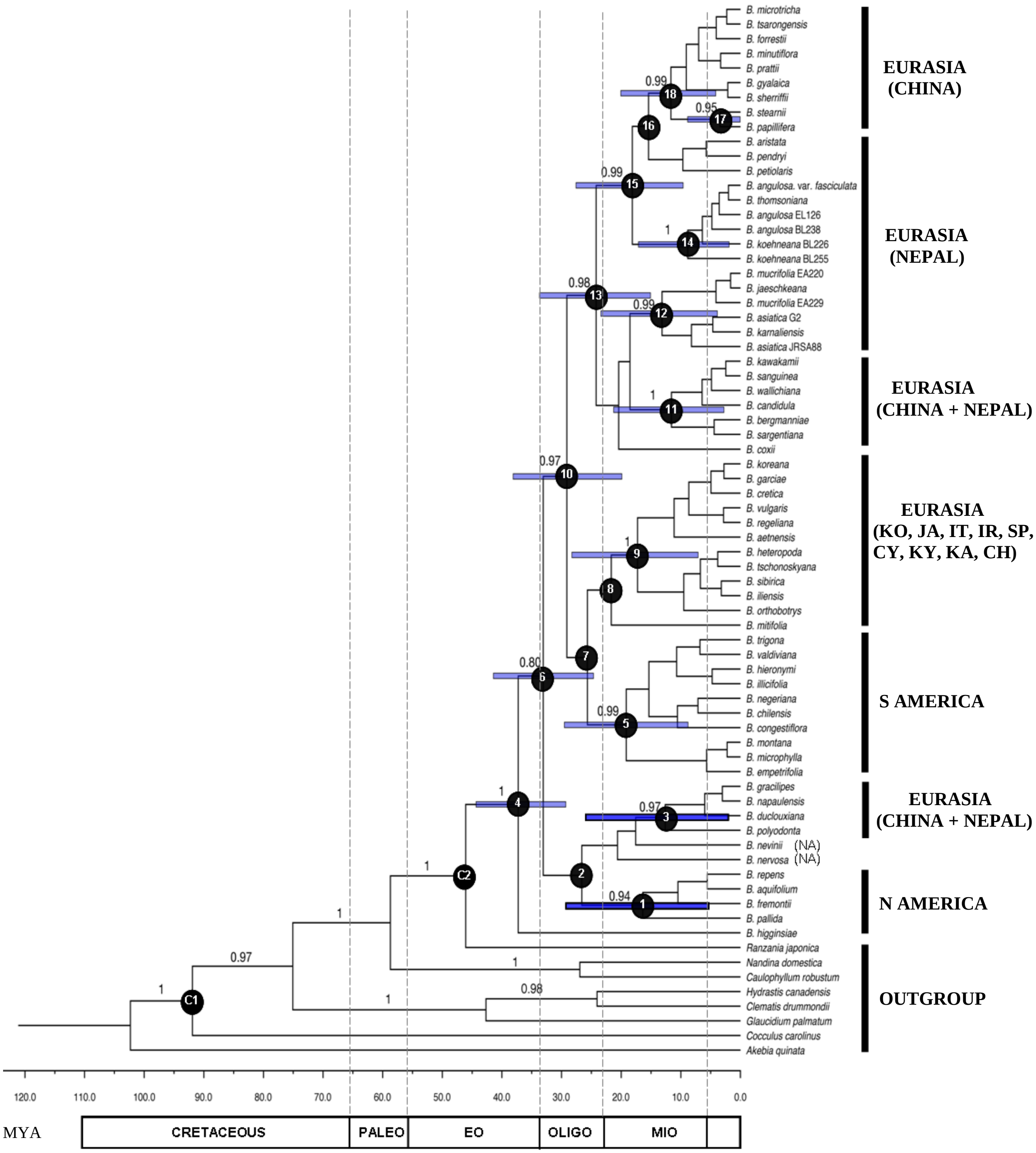


FIG. 3.3. Chronogram of *Berberis s.l.*: maximum clade credibility tree obtained from BEAST analysis. Nodes are posterior mean ages (mya), with blue bars indicating the 95% HPD intervals (see Table 3.2 for more details). C1: constrained minimum age = 91 mya, C2: constrained minimum age = 45 mya. Bayesian posterior probablities support values more than 0.5 are indicated above the branches. Mio: Miocene; Oligo: Oligocene; Eo: Eocene; Paleo: Paleocene. KO: Korea; JA: Japan; IT: Italy; IR: Iran; SP: Spain; CY: Cyprus; KY: Kyrgyzstan; KA: Kazakhstan; CH: China.

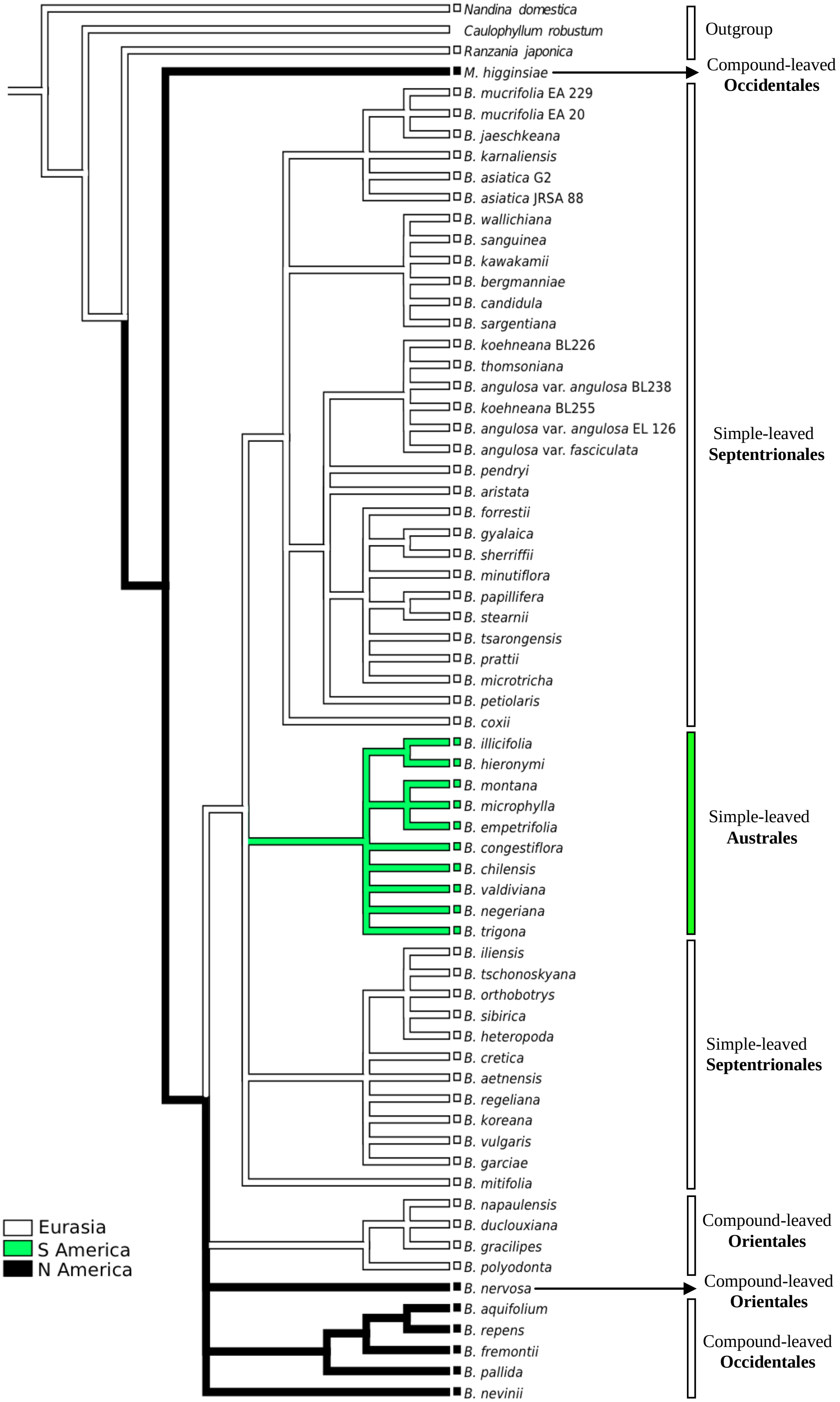


FIG. 3.4. Optimization of geographic areas on one of the three most parsimonious trees obtained from maximum parsimony analysis of *ndh*F sequences of 64 accessions of *Berberis s.l.*

3.4.4 Divergence time estimation

The chronogram derived from the BEAST analysis of *ndh*F sequences is largely congruent with the phylogeny resulting from maximum parsimony and Bayesian analysis.

Table 3.2. Mean ages, 95% HPD intervals of divergence time and Bayesian posterior probabilities of major nodes in *Berberis s.l.* based on Beast analysis of *ndh*F sequences.

Node number	**Mean age (Mya)**	**95% HPD (Mya)**	**Bayesian posterior probabilities**
C1 (Calibration node)	91.0		1.00
C2 (Calibration node)	45.0		1.00
1	16.4	5.12-29.19	0.94
2	26.6		0.49
3	12.6	2.19-25.69	0.97
4	37.3	29.29-44.30	1.00
5	19.1	8.79-29.50	0.99
6	33.0	24.65-41.42	0.80
7	25.7		0.37
8	21.7		0.33
9	17.3	7.10-28.28	1.00
10	29.1	19.89-38.13	0.97
11	11.6	2.76-21.23	1.00
12	13.1	3.86-23.4	0.99
13	24.2	15.08-33.69	0.98
14	8.8	1.89-17.10	1.00
15	18.1	9.61-27.58	0.99
16	15.4		0.12
17	3.4	0.05-8.86	0.95
18	11.6	4.13-20.07	0.99

The North American compound-leaved species *B. nevinii* and *B. nervosa*, whose positions were unresolved in Bayesian and Parsimony analysis, are grouped with section *Orientales* in the Asian clade. However, the support for the clade is low (Pp< 0.5).

The simple-leaved and compound-leaved *Berberis* clade diverged from one another *ca.* 33 mya during the late Eocene or the Oligocene (Fig. 3.3, node 6; Table 3.2). The South American crown clade dates from *ca*. 20 mya (Fig. 3.3, node 5; Table 3.2) which is well before the closure of Isthmus of Panama, indicating that the *Australes* must have reached South America by long distance dispersal.

3.4.5 Optimisation of geographic areas and morphological characters

One of the most parsimonious trees with geographic areas and morphological characters optimized using parsimony is shown in Fig. 3.4 and Fig 3.5-3.7 respectively. The mapping of areas and characters on all three most parsimonious trees gave similar results, and suggests that the Asian compound-leaved species evolved from North American lineages, and South American simple-leaved species from Eurasian lineages.

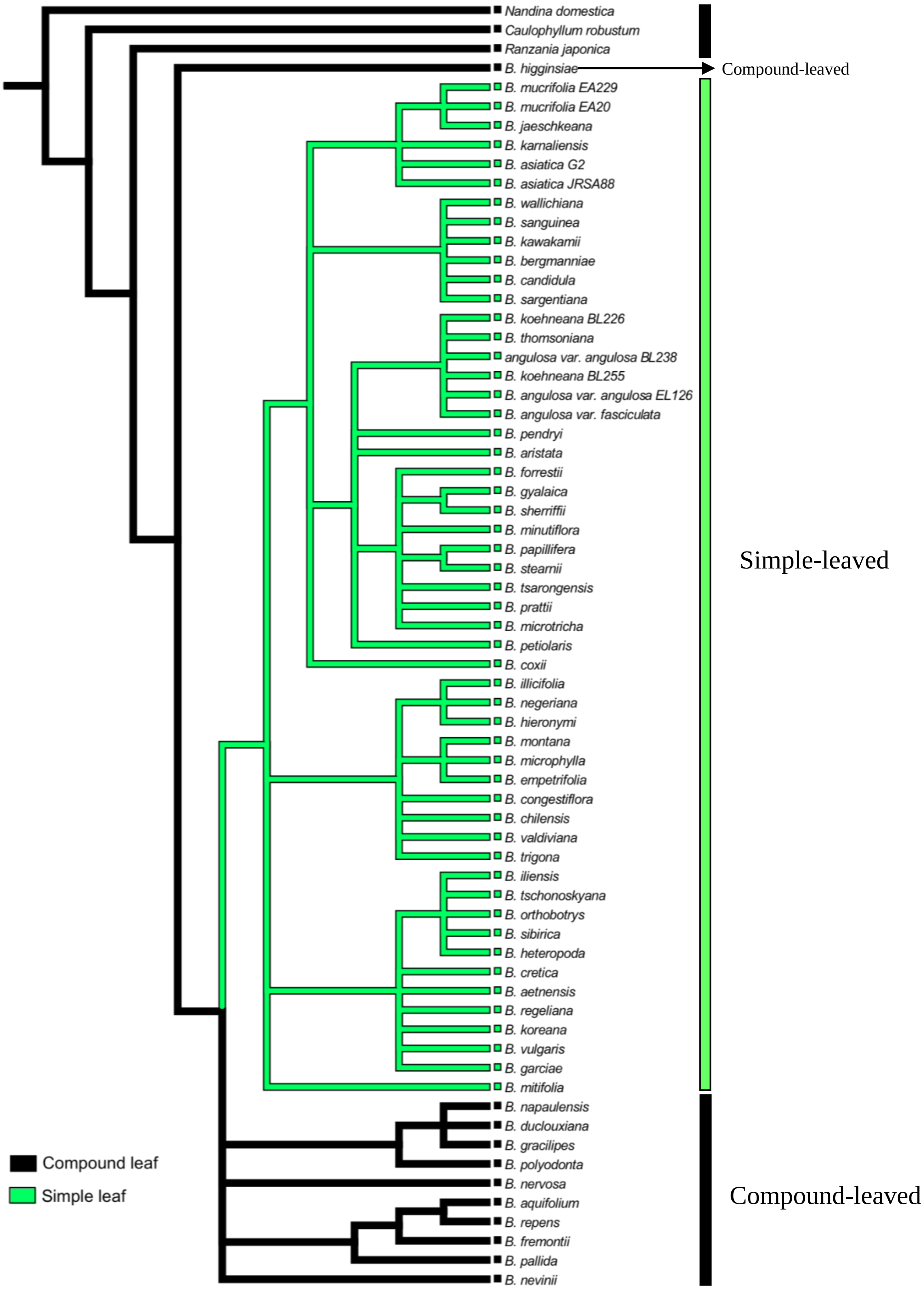


FIG. 3.5. Optimization of leaf characters on one of the three most parsimonious trees obtained from maximum parsimony analysis of *ndh*F sequences of 64 accessions of *Berberis s.l.*

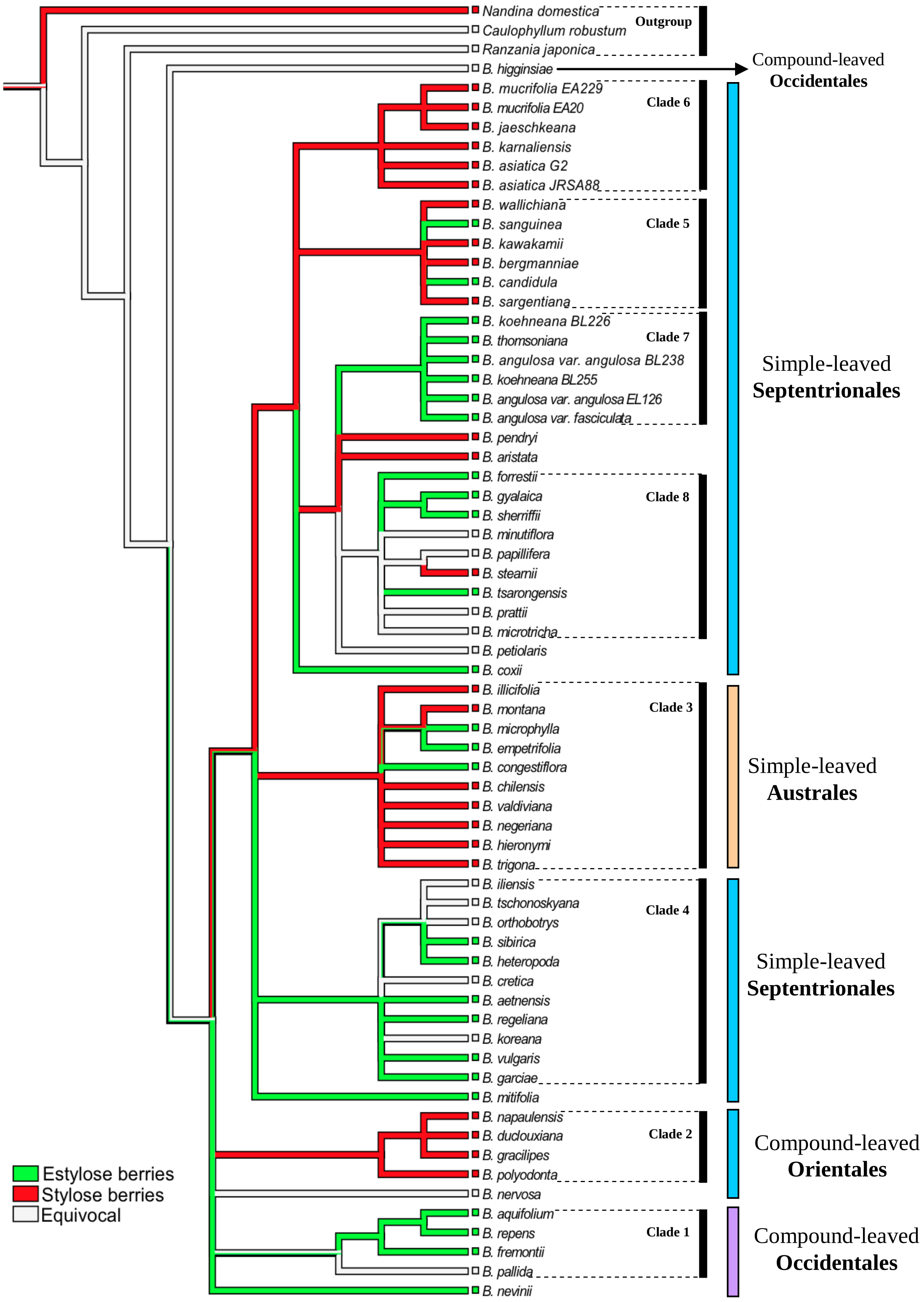


FIG. 3.6. Optimization of berries characters on one of the three most parsimonious trees obtained from maximum parsimony analysis of *ndh*F sequences of 64 accessions of *Berberis s.l.*

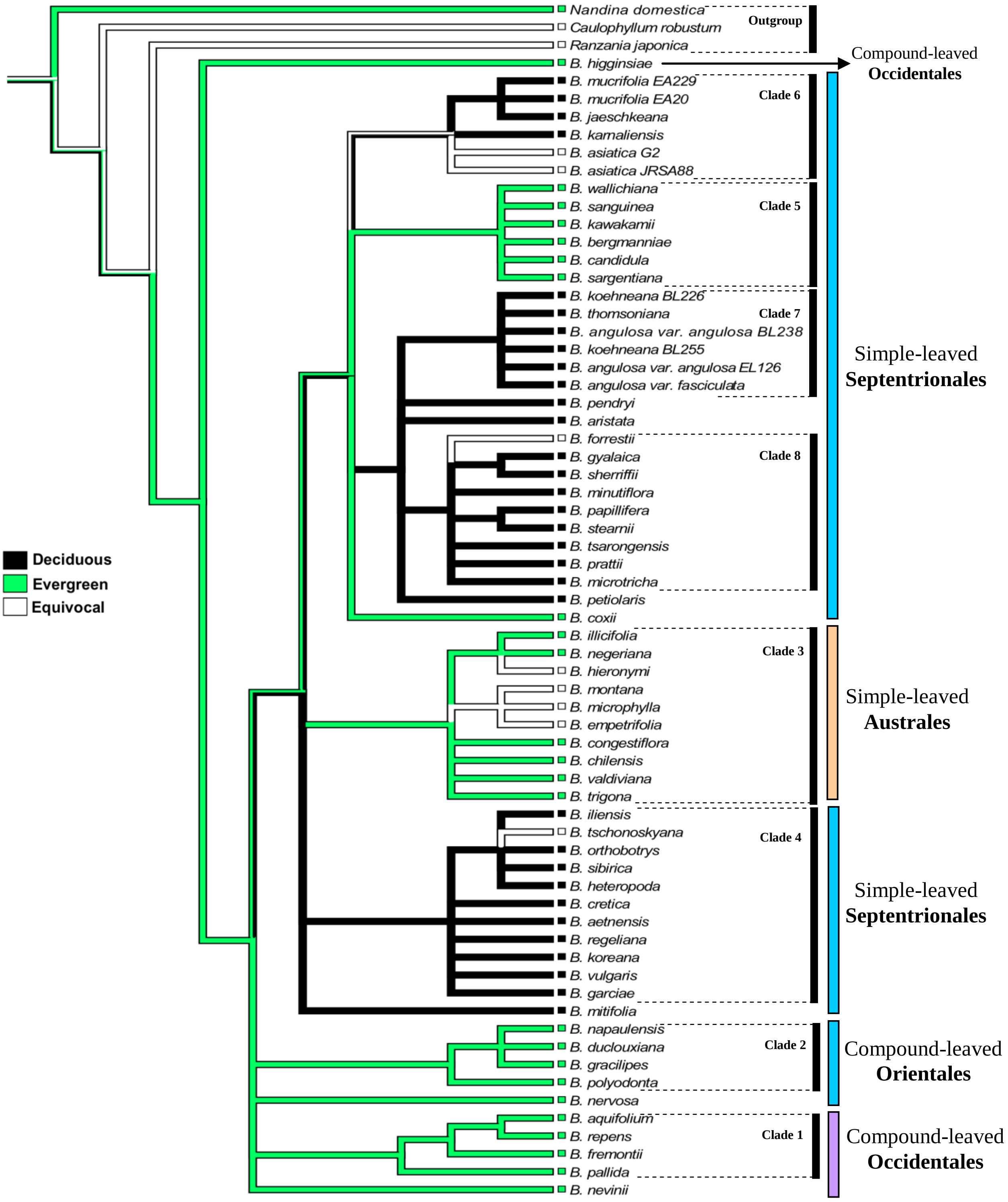


FIG. 3.7. Optimization of deciduous and evergreen habits on one of the three most parsimonious trees obtained from maximum parsimony analysis of *ndh*F sequences of 64 accessions of *Berberis s.l.*

3.5 DISCUSSION

3.5.1 Early branching lineages of Berberis s.l.

The use of the outgroup rooting method for the first time in a phylogenetic analysis of *Berberis s.l.* provides new insight into the phylogenetic structure of the genus, especially the branching order of its early diverging lineages. Kim *et al*. (2004b) used the mid point rooting method to root the ITS phylogeny of *Berberis s.l.* because the ITS sequence of *Ranzania* was too difficult to align with the ingroup *Berberis* taxa. The tree topology generated by the present analysis slightly differs from the Kim *et. al*. (2004b) topology, and this is probably because of the difference in rooting methods used. Both the maximum parsimony and Bayesian analysis in this study placed *B. higginsae*, a member of section *Horridae* of compound-leaved *Berberis*, as a sister to all other *Berberis* species with good branch support (Fig. 3.2), whereas in Kim's topology *B. higginsae* was placed as sister to *Berberis s.s.*.

3.5.2 Phylogeny and taxonomic implications

The analysis agrees with Ahrendt's (1961) view that simple-leaved *Berberis* are derived from compound-leaved *Mahonia*-like lineages (Fig. 3.5). Higher level taxonomic divisions within both simple and compound-leaved *Berberis* (Schneider, 1908; Ahrendt, 1961) were congruent to some extent with the present phylogeny. For example, the simple-leaved South American *Australes* and compound-leaved Asian *Orientales* each formed monophyletic clades (Fig. 3.2; Clades 3 and 2 respectively). However, simple-leaved Eurasian *Septentrionales* and the compound-leaved North American *Occidentales* were paraphyletic.

The origin of the South American *Australes* clade (Fig. 3.2; Clade 3) within the Eurasian *Septentrionales* makes the *Septentrionales* paraphyletic. The compound-leaved *Occidentales* also appeared as paraphyletic because *B. higginsae*, a member of section *Horridae* within *Occidentales*, was found to be sister to all other *Berberis* species. Some species of compound-leaved section *Horridae* exhibit some morphological characters such as smoother dark red stems and fascicle or umbel type inflorescences which are otherwise found only in simple-leaved *Berberis* and absent in all other compound-leaved species. This suggests possible affinities of these species of section *Horridae* with simple-leaved *Berberis*. The paraphyly of compound-leaved species was also reported by previous authors (Kim & Jansen, 1998; Kim *et al.*, 2004a).

The ITS phylogenies of Kim *et al*. (2004b) and Todd (2006) were not congruent with the sections and subsections of Schneider (1904-1905, 1908) and Ahrendt (1961). The validity of these sections had already been questioned by Landrum (1999) who merged three species from different sections under a single species. Although the present *ndh*F phylogeny is not well resolved at species level and has limited sampling (*ca*. 14% of total *Berberis* spp.), it does indicate that the general scheme of sectional and sub-sectional classification is not fully consistent with phylogenetic relationships. For example, two species from section *Angulosae* (*B. sibirica* and *B. orthobotrys*) are grouped in clade 4 while others are in clades 6 and 7 (Fig. 3.2). Otherwise the species in clade 4 mainly belong to sections *Crateginae*, *Vulgares, Integerrimae*, *Heteropodae* and *Tschonoskyanae*, giving the clade a distribution stretching across Eurasia from Japan in the East to Spain in the west.

The members of section *Wallichianae* (*B. wallichiana*, *B. sanguinea*, *B. kawakamii*, *B. bergmanniae*, *B. candidula*, *B. sargentiana*) form a clade (clade 5) with good support (Bs: 80; Pp: 1). However, the position of *B. coxii* remained unresolved. This group was poorly supported in Todd's (2006) phylogeny (bootstrap <50 %). The section *Wallichianae* is a well defined natural group of species (Chamberlain & Hu, 1985) and is supported by morphological characters including its evergreen habit (Fig. 3.7). It contains *ca*. 75 species (Chamberlain & Hu, 1985) and to confirm its monophyly increased sampling is required, especially from China.

Clade 6 is exclusively Nepalese and comprises the species from sections *Angulosae* (*B. mucrifolia*, *B. jaeschkeana* and *B. karnaliensis*) and *Asiaticae* (*B. asiatica*). All the species in this clade are characterized by their stylose berries (Fig. 3.6) and coriaceous leaves, and are adapted to drier habitats. Clade 7 is also exclusively Nepalese and the species in it fall in three different sections; *Angulosae* (*B. angulosa*), *Polyanthae* (*B. koehneana*) and *Tinctoriae* (*B. thomsoniana*). All the species in this clade are morphologically linked by their estylose berries (Fig. 3.6).

Clade 8 consists of Chinese species of *Berberis* belonging to four different sections. The majority of the species are from sections *Sherriffianae* (*B. gyalaica* and *B. sherriffii*) and *Franchetianae* (*B. papillifera*, *B. stearnii*, *B. minutiflora*, *B. tsarongensis* and *B.*

microtricha). The remaining two species belong to section *Sinenses (B. forrestii*) and *Polyanthae* (*B. pratii*). The subclade within clade 8 (Fig. 3.2) which contains the species *B. gyalaica* and *B. sherriffii* supports the sectional circumscription of section *Sherriffinae*.

The polyphyly of several sections such as *Angulosae* and *Polyanthae* clearly indicates the current need for re-classification of the genus to reflect phylogenetic relationships. Congruence of new molecular phylogenetic groups with morphology suggests a route to a new infrageneric classification.

3.5.3 Biogeography of the genus Berberis s.l.

3.5.3.1 Compound-leaved Berberis: Northern Hemisphere disjunction

The extant members of compound-leaved *Berberis* are mainly distributed in WNA and Asia (China and Himalayas) with a few species extending their range to Southeast Asia. The position of North American *B. higginsae*, a member of section *Horridae*, as a sister to all other *Berberis* species suggests a possible North American origin of *Berberis s.l.*

According to fossil evidence, compound-leaved *Berberis* species are well represented in the WNA Tertiary flora (Wolfe, 1975; Manchester, 1999). Western North America is thought to be one of the major hot spots of evolution and diversification of many plants during the early Tertiary (Xiang & Soltis, 2001; Milne & Abbott, 2002). The fossils of compound-leaved *Berberis* are dated back to 45 mya (Manchester, 1999; Erwin & Schorn, 2000). They are well represented in the Eocene flora of Thunder mountain of

Central Idaho (Axelrod, 1998a; Erwin & Schorn, 2000), the late Oligocene flora of Oregon (Axelrod, 1987) and the Oligocene of lower Hyanes Creek (Axelrod, 1998b), all in North America. The dated phylogeny (Fig 3.3) with Eurasian species sister to N American species, and the results of the area optimization (Fig. 3.4) suggest a probable North American origin of compound-leaved *Berberis*. This is supported by the oldest fossils for *Berberis*, which are North American. The divergence time between the North American and Eurasian clades was estimated as *ca*. 26 mya (Fig. 3.3, node 2; Table 3.2) which indicates that the southern route of the NALB is the most probable for the dispersal of compound-leaved *Berberis* towards Eurasia. That this disjunction arose via Europe and the NALB is further supported by fossil evidence for the existence of compound-leaved *Berberis* in France during the Oligocene (Saporta, 1865), whereas such fossils are not found in eastern Asia until the early Miocene (Tanai, 1972). The phylogenies (Fig. 3.2 & 3.3) and the results from the area optimization (Fig 3.4) support the hypothesis of migration from North America to Asia. Although the common pattern shown by many disjunct taxa is migration from Asia to North America (Donoghue *et al.*, 2001; Xiang & Soltis, 2001), compound-leaved *Berberis* appears to have moved in opposite direction, as was the case for *Ribes* (Schultheis & Donoghue, 2004).

3.5.3.2 Simple-leaved Berberis: Antitropical disjunction

Berberis fendleri and *B. canadensis* are the only extant simple-leaved *Berberis* species in North America (Ahrendt, 1961) and Ahrendt (1961) grouped them with Eurasian *Septentrionales* as they are morphologically similar to Eurasian species. However, the fossil record indicates a much higher diversity in North America during the Tertiary (Axelrod, 1987; Axelrod, 1998b; Ramirez & Cevallos-Ferriz, 2000). Most of the fossil leaves of simple-leaved *Berberis* from North America are comparable with today's extant *Septentrionales*, particularly resembling some Chinese species (Axelrod, 1987; Axelrod, 1998b). The oldest fossils for simple-leaved *Berberis* are from Oligocene (23-35 mya) sediments from Upper Hyanes Creek (Idaho) (Axelrod, 1998b) and from Puebla, Mexico (Ramirez & Cevallos-Ferriz, 2000). Late Oligocene and Miocene (5-23 mya) *Berberis* leaf fossils have been documented in Europe (Kvacek & Erdei, 2001; Kovar-Eder *et al.*, 2004). The fossil leaves from the European Tertiary are similar to the present day extant *Berberis* growing in the Himalayas and eastern Asia (Kvacek & Erdei, 2001; Kovar-Eder *et al.*, 2004).

The presence of fossil records from North America and Europe from the Oligocene and Miocene indicates that interchanges of the lineages of simple-leaved species of *Berberis* between these two continents had already taken place at least in the Oligocene. The age of *Berberis* leaf fossils from Argentina is uncertain following the Archangelsky's disagreement with the exact dating of fossil *B. corymbosa* in Osri (1976).

In Asia, fossil leaves of simple-leaved *Berberis* have been reported from Miocene sediments of Hokkaido, Japan (Tanai, 1961) and Pleistocene sediments of Kashmir India (Puri, 1947). Li *et al.* (2010) described the Palaeocene fossil species *B. wuyunensis* from NE China. The exact dating and identification of this fossil species needs to be reinvestigated because compound-leaved *Berberis,* the early diverging lineages of

Berberis, first appeared in the fossil record during the middle Eocene. Therefore, the presence of fossils of simple-leaved *Berberis* from the Palaeocene seems unlikely based on these phylogenetic data.

The ancestors of South American simple-leaved *Berberis* species may have reached South America by dispersal from Eurasia during the late Oligocene *ca*. 26 mya (Fig 3.3, table 3.2). The single origin of South American *Australes* in the phylogeny (Fig. 3.2) and the area optimization (Fig 3.4) indicate an ancient long distance dispersal event from Eurasia to South America. Long distance dispersal has been identified as one of the major forces in determining plant distributions (Pennington *et al.*, 2004). A similar pattern of dispersal from Eurasia to South America has been reported in *Chrysosplenium* (Soltis *et al.*, 2001), *Gleditsia* (Schnabel *et al.*, 2003) and in *Lathyrus* (Kenicer *et al.*, 2005). The fruits of *Berberis* are red and adapted to dispersal by birds. However, the gut passage rates for *Berberis* seeds and their potential for long distance dispersal is untested.

An alternative hypothesis would be dispersal from North America to Eurasia and South America followed by the extinction of the North American lineages. Though this is supported by the slightly younger fossils towards the east, more precise dating of *Berberis* fossils and robust sampling is necessary to further test this hypothesis. The North American species of simple-leaved *Berberis* were not included in the *ndh*F phylogeny because the samples available were discovered to be wrongly identified. However, in the ITS phylogeny (Chapter 4) the North American species (sequences derived from the GenBank) are nested within a Eurasian species group suggesting that their ancestors dispersed from Eurasia to North America. Furthermore, they are not sister to the South American Australes, which gives no support to the hypothesis of dispersal from North America to South America.

The chronogram (Fig 3.3 & Table 3.2) indicates that the ancestors of South American species may have reached South America *ca*. 26 million years ago. However, the extant species diversity has resulted from comparatively recent diversification within the last 15 million years. Data from the present study support Landrum's (1999) view that *Berberis* was already diverse in South America long before land connections formed between North and South America.

Kim *et al.* (2004b) favoured a vicariance event during the Cretaceous to explain the origin of South American *Australes* but the dated phylogeny along with fossil evidence suggest that was highly unlikely. Compound-leaved *Berberis* first appeared in fossil records in North America during the middle Eocene, fully 65 million years after the separation of South America from Africa during the Cretaceous. The dated phylogeny (Fig. 3.3) also clearly shows the ability of *Berberis* species to disperse over long distances through geological time, so invoking vicariance to explain its global distribution is not necessary.

3.6 CONCLUSIONS

Ahrendt's (1961) classification is incompatible with all recent phylogenetic investigations of *Berberis*, including the present study using *ndh*F sequence data. Monophyletic clades supported with some morphological characters indicate that a new classification scheme for the genus can be developed based upon monophyly and morphological characters. However, as *ndh*F resolution is not strong enough, a multi-gene approach including nuclear loci is recommended to reclassify the genus.

The compound-leaved species of *Berberis* are most likely to have originated in North America during the Eocene, from ancestors resembling the compound-leaved *B. higginsiae*. The lineages of compound-leaved *Berberis* most probably reached Eurasia through the NALB with subsequent migration eastwards to China and the Himalayas.

The simple-leaved species of *Berberis* might have originated in Eurasia or in North America, and more precise dating of fossils is necessary to confirm their place of origin The South American simple-leaved *Berberis* diversification took place before the union of North and South America. The present study suggests that the South American species may have been dispersed from Eurasia. However, the possibility of dispersal from North America to South America, with subsequent extinction in North America cannot be completely ruled out.

CHAPTER 4: ORIGIN AND DIVERSIFICATION OF *BERBERIS* IN THE NEPAL HIMALAYA: INSIGHT FROM NUCLEAR RIBOSOMAL ITS SEQUENCES

4.1 INTRODUCTION

The Himalayan region is a major hotspot of biodiversity. Despite the long interest in the flora of the Nepal Himalaya, no attempt has yet been made to elucidate the origin and diversification of Nepalese taxa using dated phylogenies. This contrasts strongly with, for example, the Andes where about 22 such published studies have yielded many insights into the origin and diversification of the flora of that region (Pennington *et al.*, submitted). The present study is therefore the first to attempt this for the Nepal Himalaya, and includes wide sampling of Nepalese *Berberis*, with 35 accessions of 20 species out of the 24 reported from Nepal, along with worldwide sampling (62 accessions of 59 species) to understand their relationships with other *Berberis* growing elsewhere.

The early diversification of simple-leaved and compound-leaved *Berberis* has already been discussed in chapter 3, based on an *ndhF* phylogeny, dealing with global trends and the origin of major clades. In this chapter, the origin and diversification of *Berberis* species in the Nepal Himalaya will be discussed.

4.1.1 Origin of the Himalayan mountain range

The Himalaya is a 2400 km long and 250-350 km wide mountain range stretching from northern Myanmar in the east to Pamir Knot on the Afghan border in the west; at its highest point (Mount Everest), it reaches up to 8848 m (Valdiya, 1998). It lies between the Indo-Gangetic plain in the south and the Tibetan Plateau in the north. The Himalayan range includes about 30 peaks exceeding 7600 m altitude, and resulted from rapid mountain-building tectonic forces and vigorous erosion processes (Gurung, 1999), which have led to a complex topography of high mountains and narrow ridges traversed by extremely deep river gorges. The Nepal Himalaya lies along the central part of the Himalayan range.

The uplifting of the Himalaya started when the Indian subcontinent collided with the Eurasian plate. Initial contact probably occurred in the Paleocene or early Eocene *ca*. 65-50 mya (Windley, 1977; Beck *et al.*, 1995; Patzelt *et al.*, 1996; Valdiya, 1998; Valdiya, 2002). The junction of these two collided land masses is known as the Indus-Tsangpo Suture (I-TS) (Gansser, 1980), and that area is now occupied by the river Indus in the West and Tsangpo (Brahmaputra) in the East. It is believed that the northern edge of the Indian plate slid down *ca*. 220-400 km under the Asian plate (Patriat & Achache, 1984; Harrison *et al.*, 1992; Zhao *et al.*, 1993; Valdiya, 2002) which subsequently increased the thickness of the Tibetan crust by nearly two-fold. The great elevation of the Tibetan plateau to above 5000 m is thought to be the result of the subduction and underthrusting of the Indian plate under the Asian plate (Valdiya, 2002). When the buoyant front part of the colliding Indian continent buckled up with Asia, it gave rise to a series of small uplifts all along the collision zones. With continuing tectonic movement, the Northern edge of the Indian crust broke up throughout its length, producing what is known as the Main Central Thrust (MCT) in the south and Trans Himadri Fault (THF) in the north (Fig 4.1; Valdiya, 1998). The basement complex between the MCT and THF was thrust up and later gave rise to the Great Himalaya (Valdiya, 2002). After the formation of the MCT and the THF in the northern part, the crust in the southern Himalaya also broke along a series of faults known as the Main Boundary Thrust (MBT). The Lesser Himalaya formed along these faults and the area south of these uplifted mountains sagged and became an elongated foreland basin, known as the Siwalik basin. The vast amount of sediments brought by rivers resulting from the erosion of rising mountains gave rise to the Siwalik range, which is mainly composed of fluvial sandstones and siltstones resulted from the rising Himalaya that were laid down from the early Miocene to the Pleistocene (Johnson *et al.*, 1985; Harrison *et al.*, 1992). The Siwalik is separated from the Indo-Gangetic Plain in the extreme south by Himalayan Frontal Fault (HFF).

Based on these east-west trending tectonic boundaries, the Himalaya can be divided into five major zones:
Tethys Himalaya
Great Himalaya
Lesser Himalaya
Siwaliks
Indo-Gangetic Plain

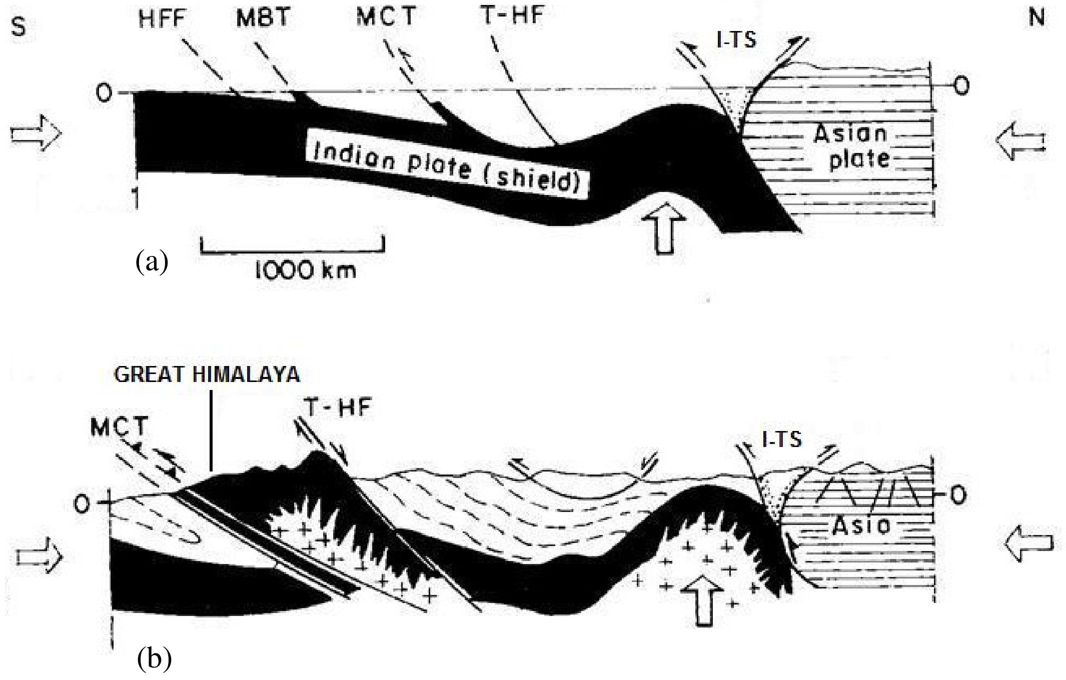


FIG. 4.1. Positions of different thrusts and faults. (a) The frontal part of the Indian crust buckled up and broke up along a number of faults in the Middle Miocene (35-45 mya). (b) The basement complex between the Trans-Himadri Fault (T-HF) and the Main Central Thrust (MCT) which later gave rise to Great Himalaya. I-TS: Indus-Tsangpo Suture, MBT: Main Boundary Thrust, HFF: Himalayan Frontal Fault. From Validya (2002).

The Himalaya is the result of a series of orogenic movements and its topography is the result of the interaction between uplift and erosion. There is some disagreement on the timing of the major phases of the Himalayan uplift. Based on the early Miocene to Quaternary sedimentary deposits in the Bengal fan in the Central Indian Ocean, Amano and Taira (1992) proposed a two-phase active uplift of the Great Himalaya with the first phase between 10.9 and 7.5 mya and the second phase 0.9 mya later. Copeland *et al.* (1993) argued that the growth of the Himalaya is not the result of major impulses but a series of many episodes of uplift each of which affected a relatively small area. Some studies suggested that the uplift of the Tibetan and Himalayan mountains began gradually at 40 mya and became intense at 25 to 20 mya in the Himalaya and Southern Tibetan plateau (Harrison *et al.*, 1992; Guo *et al.*, 2002). The muscovite Rb-Sr (rubidium-strontium) dated age of 34±3 mya from Langtang (Inger & Harris, 1992) and the monazite U-Pb (Uranium-lead) dated age of 34-36 mya from Annapurna (Hodges *et al.*, 1996) Nepal shows the initial formation of small uplifts during the early Oligocene in the central Himalaya. The Miocene epoch probably represents the time of maximum uplift of the Himalaya and southern Tibet during which the large volume of granites were eroded from the Great Himalaya (Mani, 1978; Copeland *et al.*, 1987; Copeland & Harrison, 1990). In the beginning of the late Miocene, about 11.5-5.1 mya, the Great Himalayan mountains had been thrust up and exposed to erosion because of very strong tectonic movement (Valdiya, 2002). The Pb/TH monazite ages also suggested the reactivation of the MCT zone in Central Nepal at *ca*. 6-8 mya (Harrison *et al.*, 1997).

Several studies suggest that the Himalaya is still rising (Jackson & Bilham, 1994; Valdiya, 2002). Based on spirit levelling data from the Nepal Himalaya between 1977 and 1990, Jackson and Bilham (1994) reported localized uplift at the rate of 2-3 mm/yr in the Lesser Himalaya and 4-6 mm/yr in the Great Himalaya.

Based on GPS (Global Positioning System) studies, the movement of Indian plate has been recorded at the rate 37mm/yr in south India (Catherine, 2004). Similarly, the northward movement of the Indian plate at the rate of 35.7±5.8 mm/yr has been recorded by GPS in Nepal (Freymueller *et al.*, 1996).

The major river systems in the Himalayan range, the Brahmaputra, Sutlej, Indus and Jahnavai rise to the north of Great Himalaya, and are older than the mountains (Mani, 1974a). They therefore have considerable biogeographical importance. The major river systems in the Nepal Himalaya are the Koshi, Gandaki, Karnali and Mahakali. These rivers added their tributaries during and after the uplift of the Himalayas.

The effect of Himalayan orogeny on plant diversification in the Himalayas has not been well studied. Time-calibrated phylogenies have been proven to be useful tool to assess possible links between the past geological history and the diversification of several plant groups (Richardson *et al.*, 2001; Wang *et al.*, 2009). The effect of the Himalayan orogeny on diversification of *Berberis* species in the Nepal Himalaya is tested using the dated phylogeny in this chapter.

4.1.2 Climate and vegetation

The presence of the Himalayan mountain ranges blocks the northward movement of moisture-laden winds rising from the Indian Ocean during the summer months resulting in intense precipitation on their southern flanks as moist air rises and cools. The monsoon climate is thought to have been initiated in the mid or late Miocene when the Himalaya and south Tibet attained a sizeable height (Molnar *et al.*, 1993; Guo *et al.*, 2002; Valdiya, 2002). Subsequent further uplift of the Himalaya strengthened the effect of the summer monsoon, creating the very wet climate south of the Himalaya and also greatly reduced rainfall in the parts of Asia that are in the rain shadow of the mountains.

These dramatic changes to the regional climate have had considerable impacts on plant and animal life.

FIG. 4.2. Trans-Himalayan region of north-western Nepal. Mustang, Kaligandaki valley *ca*. 3000 m (2006)

FIG. 4.3. Oak-Laurel forest in eastern Nepal. Illam, *ca*. 3000 m (2007)

The vegetation patterns in the Nepal Himalaya are highly influenced by the pattern of rainfall during the summer monsoon. In a typical year, monsoon rainfall starts from Myanmar at the beginning of June and rapidly advances west during June and is in full swing over the southern mountain range of the Himalaya by the middle of July (Ramdas, 1974). The western Himalaya receives significantly less monsoon rainfall than the eastern Himalaya (Appendix 2). A large part of western Nepal has a drier climate and the most of the area is sub-arid and sub-humid. The northern part of western Nepal which lies within the rain shadow of the Annapurna and Dhaulagiri mountain ranges, is termed as the trans-Himalayan region, and has a dry climate characteristic of the Tibetan plateau (Fig. 4.2).

Most parts of eastern Nepal receive the full force of the summer monsoon, and the area is characterised by a humid and semi-humid climate. The dramatic effect of rainfall on vegetation can be seen by comparing the vegetation at similar altitude in Illam and Mustang districts. At 3000 m, the southern slopes of the Himalaya in Illam support evergreen Oak and Laurel forests (Fig. 4.3) with *B. insignis*, *B. wallichiana* and *B. hookeri* while in the trans-Himalayan Mustang, the vegetation is mainly composed of thorny cushion plants and includes *Berberis* species such as *B. mucrifolia* and *B. jaeschkeana* var. *usteriana*.

4.1.3 Plant migrations in the Himalaya

The mountains of the Himalaya have experienced rapid erosion due to their high altitude and heavy monsoon rainfall, leading to steep slopes and deep valleys. This, along with the wide range of altitudinal and latitudinal variations has provided an abundant range of habitats for plants to colonize and adapt to, promoting speciation.

By the time the Himalaya started to uplift during the Eocene, the angiosperms had already diversified across the globe (Wikström *et al.*, 2001). The joining of India and mainland Asia opened a migration route from Eurasia to the rising Himalaya. Based on the early appearance of some mammals in India such as Rhinocaratoids and Brontotheras, which have a close affinity with Eurasian species, it is clear that the migration path had already been established across the rising Himalaya during the early Eocene (Valdiya, 2002).

The most important pathway for the migration and interchanges of flora during the Eocene was the establishment of Assam-Asia contact, also known as the Assam-Gateway (Mani, 1974b). This pathway provided the route for the extensive interchanges of the autochthonous flora of the Indian Peninsula and the Asiatic Tertiary Mountain flora (Mani, 1974b).

The mountain chain of the Himalaya is a major migration corridor between the eastern and western parts of Asia, permitting east-west movement of temperate and boreal plants. Some boreal plants appear to have entered into alpine zone of the Himalaya from the north via the mountains surrounding the Tibetan plateau (Ohba, 1988). For example, several boreal plant species such as *Potentilla fruticosa* L., *Epilobium angustifolium* L., *Viola biflora* L., *Bistorta vivipara* (L.) Gray have their most southerly distributions across the Himalayan range (Yoshida, 2006).

The Himalayan chain provides the pathway linking the temperate floras of the Central Asian Mountains to the northwest and the Sino-Japanese element to the northeast. Plants adapted to wet summer conditions tend to occupy the southern side of the range, whereas those from arid western Asia are found on the northern side (Hooker, 1906; Yoshida, 2006). Many species of Central Asiatic affinity are found in the arid region of the western Himalaya. For example, species of *Caragana* and Chenopodiaceae which are characteristic of cold desert are found in the western Himalaya (Gupta, 1994).

Several tropical Indian groups of Gondwanan origin found their way through the deep valleys at the foot of mountains towards the north. For example, species of *Incarvillea*, members of the largely tropical family Bignoniaceae, are found in the Himalaya and adjacent mountains of south-western China (Yoshida, 2006). Thus, the deep river valleys appear to have been an important corridor for north-south migration of plants in the Himalayan mountain chain.

The Pleistocene glaciations had enormous effects on plant distributions and have contributed greatly to the evolution of the Himalayan flora. During glaciations the temperate and boreal elements of the Himalaya spread southwards towards Peninsular India, whereas during the interglacial periods, the tropical elements advanced northwards through the Himalaya. Because the Himalaya is a series of more or less parallel or nearly converging mountain chains, migrations would not have been in a straight line from north to south but would have followed the river systems (Yoshida, 2006). Therefore, the migrations were affected by the intervening mountains and species ranges are likely to have been subject to considerable fragmentation with the consequent opportunity for speciation during these climatic oscillations.

All biogeographic studies in the Himalayan region to date are mainly based on contemporary plant distribution patterns, and no phylogenetic biogeographic study has been published so far for this region.

4.1.4 Biogeographic and ecological divisions of Nepal

Nepal lies between the latitudes of $26^{o}\ 22^{'}$ N and $30^{o}\ 27^{'}$ N and longitudes of $80^{o}\ 04^{'}$ E and $88^{o}\ 12^{'}$ E, and occupies a central position in the Himalaya acting as the boundary between two major phytogeographical Kingdoms; the Holarctic and the Paleotropical. Phytogeographically, Nepal lies on the crossroad of five floristic regions defined by Takhtajan (1986): (i) Eastern Asiatic (ii) Irano-Turanian (iii) Sudano-Zambezian (iv) Indian (v) Indochinese (Fig. 4.4).

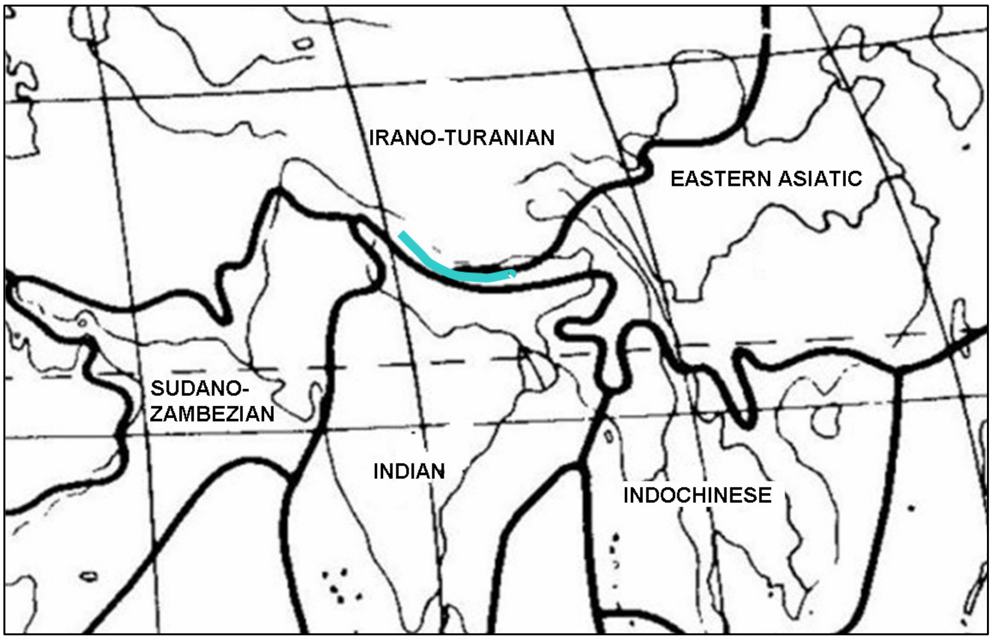


FIG. 4.4. Floristic regions of Asia surrounding the Himalaya (Takhtajan, 1986). Nepal Himalaya is highlighted with blue.

Nepal's extreme altitudinal gradients allows for the occurrence of a wide range of climatic conditions from tropical in the lowland Terai to the nival in the Great Himalaya, within a distance of less than 150 km. The vegetation map of Nepal prepared by

Nepalese and French scientists recognized 189 different ecosystems in Nepal which fall under five categories (Shrestha, 2002):

1. Alpine: 4000-5000 m, alpine grassland with *Juniper* spp, *Rhododendron* bushes and cushion forming plants.
2. Sub-alpine: 3000-4000 m, dominated by *Abies* and *Betula* forest.
3. Temperate: 2000-3000 m, dominated by Oak-Laurel and *Rhododendron* forest.
4. Sub-tropical: 1000-2000 m, dominated by *Pinus* forest in the west and *Schima-Castanopsis* forest in central and western Nepal.
5. Tropical: below 1000 m, dominated by *Shorea robusta* and *Terminalia* spp.

Stearn (1960) divided the Himalaya into Eastern and Western botanical provinces with the border lying along 83^o E (Fig. 4.5), with one third of Nepal falling within the Eastern botanical provinces which experience much higher rainfall (Hara *et al.*, 1978). He divided Nepal into three major regions with the central region between 83^o E and $86^o30^'$ E (Fig. 4.5). The longitudinal line $86^o30^'$ E runs through the the Giant Massif of Yoshida (2006) in Eastern Nepal which includes high peaks namely Makalu, Lohtse, Everest, Cho Oyu and Gaurishankar. Stainton (1972) recognized five main geographical divisions (Fig. 4.5): (i) terai and outer foothills (with Bhabar and Dun valleys); (ii) the midland areas (east, central and west); (iii) arid zone; (iv) inner valleys; (v) Humla-Jumla region. Based on more extensive climatological, floristic and ecological data, Dobremez (1972) divided Nepal into four phytogeographical divisions: (i) Domaine ouest-nepalais (from the Kumaon frontier to Dhaulagiri); (ii) Domaine nord-ouest-nepalais (arid north-west region north of Dhaulagiri and Annapurna); (iii) Domaine centre-nepalais (from Dhaulagiri to the Arun valley); (iv) Domaine est-nepalais (from the Arun valley to the Sikkim frontier).

In the absence of phylogenetic studies of plant groups in the Nepal Himalaya, it has so far not been possible to study the evolutionary interchanges of species between these biomes. The Himalayan orogeny along with the onset of the monsoon played an important role in the evolution of different ecological niches. *Berberis* species are distributed across these regions and provide an opportunity to investigate the relative frequency of speciation between ecologically distinct biomes. Recent investigations of 'phylogenetic niche conservatism' the concept that lineages tend to maintain their ancestral ecology have indicated that evolutionary switches between major biomes are infrequent (Crisp *et al*. 2009). In this chapter, the extent of phylogenetic niche conservatism in Nepalese *Berberis* will be evaluated, by examining whether closely related species tend to occur within the same biome.

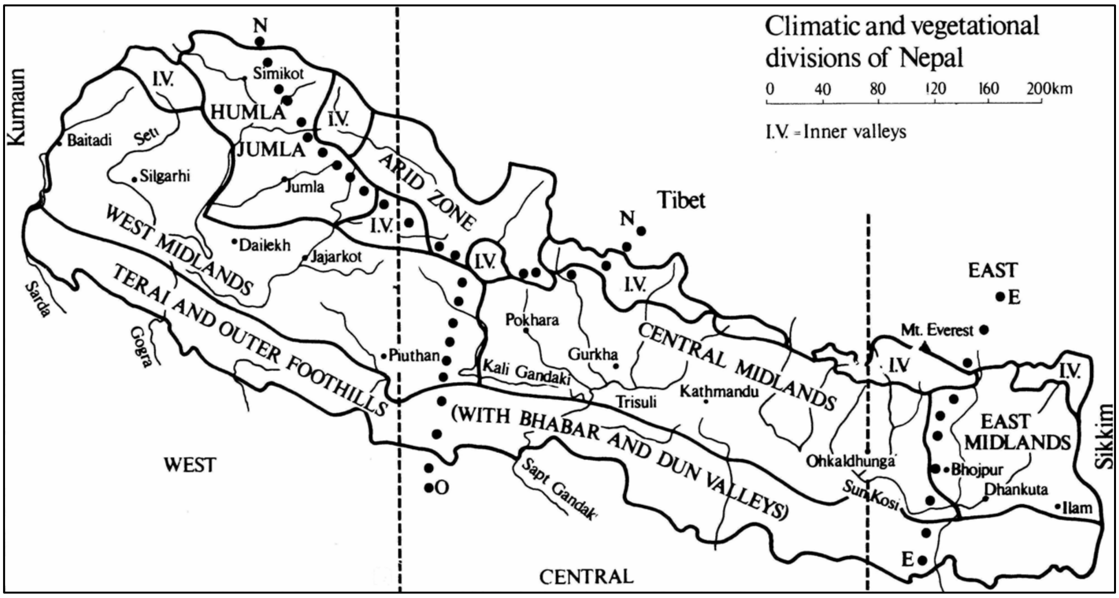


FIG. 4.5. Map of Nepal with climatic and vegetational divisions of Nepal proposed by Stainton (1972). Vertical lines showing three major divisions (west, central, east) proposed by Stearn (1960). Dotted lines showing four domains proposed by Dobremez (1972). From Hara *et al.* (1978).

4.1.5 Distribution patterns of Berberis in the Nepal Himalaya

The species of *Berberis* are distributed all over Nepal from 1000 m (*B. asiatica*) to 4600 m (*B. tsarica*). The altitudinal range and distribution is shown in Table 4.1.

Table 4.1. Distribution of *Berberis* in Nepal.

Taxa	**Altitudinal range (m)**	**Distribution**
B. asiatica Roxb. ex DC.	1000-2700 (1700)	WC Nepal [W Himalaya, Tibetan Plateau]
B. aristata DC.	1300-3400 (2100)	WCE Nepal [E Himalaya, W Himalaya]
B. borealis (Takeda) Laferr.	1600-2700 (1100)	CE Nepal [E Himalaya (Sikkim)]
B. karnaliensis Adhikari	1800-3400 (1600)	W Nepal
B. napaulensis (DC.) Laferr.	2000-2900 (900)	WCE Nepal [E Himalaya (Bhutan)]
B. hookeri Lem.	2000-3400 (1400)	CE Nepal [E Himalaya, Tibetan Plateau, Assam-Burma]
B. insignis Hook.f. & Thoms.	2000-3400 (1400)	E Nepal [E Himalaya, Assam-Burma]
B. mucrifolia Ahrendt	2000-4400 (2400)	WC Nepal (Endemic to Nepal)
B. wallichiana DC.	2300-3300 (1000)	CE Nepal [Eastern Himalaya, Assam-Burma]
B. glaucocarpa Stapf	2300-3450 (1150)	WC Nepal [W Himalaya (Kumaon)]
B. acanthifolia Wall. ex Walp.	2400-2900 (500)	WC Nepal [E Himalaya]
B. koehneana C.K.Schneid	2400-4000 (1600)	WC Nepal [W Himalaya (Kumaon)]
B. jaeschkeana C.K.Schneid var. *usteriana* C.K.Schneid.	2600-3800 (1200)	WC Nepal [W Himalaya (Kumaon)]
B. concinna Hook.f.	2600-4550 (1950)	WCE Nepal [E Himalaya]
B. petiolaris Wall. ex G.Don	2700-3500 (800)	W Nepal [W Himalaya (Kumaon)]
B. everestiana Ahrendt var. *ventosa* Ahrendt	2700-4550 (1800)	WCE Nepal [E Himalaya (Sikkim)]
B. orthobotrys Bienert ex Aitch. var. *rubicunda* Ahrendt	2800-4200 (1400)	CE Nepal
B. hamiltoniana Ahrendt	2900-4250 (1350)	WC Nepal [W Himalaya (Himachal Pradesh)]
B. thomsoniana C.K.Schneid.	3000-3650 (650)	WCE Nepal [E Himalaya]
B. pendryi Adhikari	3000-3700 (700)	C Nepal
B. kumaonensis C.K.Schneid	3000-4000 (1000)	W Nepal [W Himalaya (Kumaon)]
B. angulosa Wall. ex Hook.f. & Thoms.	3000-4500 (1500)	CE Nepal [E Himalaya, Tibetan Plateau]
B. virescens Hook.f.	3500-4000 (500)	E Nepal [E Himalaya (Sikkim, Bhutan)]
B. tsarica Ahrendt	4000-4700 (700)	CE Nepal [E Himalaya, Tibetan Plateau]

The distribution pattern of Nepalese species of *Berberis* shows that only *B. aristata* has wide range of distribution along the whole Himalayan range while all others have restricted distributions. A few species like *B. petiolaris* and *B. glaucocarpa* grow in the western Himalaya and have distributions extending only as far east as central Nepal, whereas species like *B. insignis* and *B. hookeri* grow in Eastern Himalaya and did not find their way west to central Nepal. A few species like *B. tsarica*, *B. pendryi* and *B. angulosa* grow in the Alpine region of the Nepal Himalaya above 3000 m while others like *B. asiatica* are mainly distributed in the subtropical and temperate regions around 2000 m. The range of altitude and habitats they occupy make *Berberis* species an ideal case study for diversification in the Nepal Himalaya.

4.1.6 The Internal Transcribed Spacers (ITS)

The ITS region is a part of the transcriptional unit of 18S-26S nuclear ribosomal DNA, but these spacers are absent within mature ribosomes (Baldwin *et al.*, 1995). The spacers (ITS1 and ITS2) appear to play a role in the maturation of nuclear RNAs (Baldwin *et al.*, 1995; Soltis & Soltis, 1998). The order of the ITS region is shown in Fig 4.6 with the primers used in this study.

ITS sequences are widely used for phylogenetic reconstruction in angiosperms (Baldwin *et al.*, 1995) and have proven extremely useful for inferring relationships at lower taxonomic levels (Stuessy, 2009). For this reason, ITS was chosen here as it would be likely to provide more phylogenetic resolution than *ndh*F, and this chapter present a phylogenetic analysis of *Berberis* from Nepal Himalaya that samples the genus more thoroughly than any prior molecular investigation of the genus.

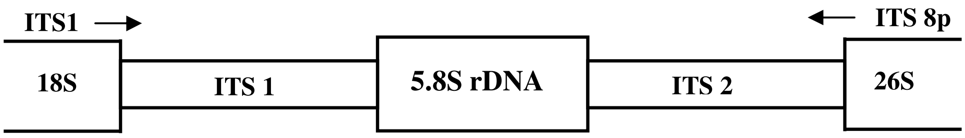


FIG. 4.6. Schematic diagram of the repeat unit of 18S and 26S nuclear ribosomal DNA showing the ITS 1 and ITS 2 regions and the primers used in this study.

4.2 AIMS AND OBJECTIVES

The main aims of this chapter are to use phylogenetic trees derived from ITS sequences to answer the following questions:

1. Has the uplift of the Himalaya played a major role in the diversification of *Berberis* species within Nepal?
2. How many distinct colonisation events from outside Nepal need to be inferred to explain the diversity of species now present there?
3. Is there evidence of phylogenetic niche conservatism within the radiation of Nepalese species?
4. Are ITS sequences helpful for resolving species-level relationships in *Berberis*?

4.3 MATERIALS AND METHODS

4.3.1 Taxon sampling

Ninety-seven accessions were sampled in this phylogenetic analysis, representing 79 species from 25 sections of simple-leaved and five sections of compound-leaved species. Among the simple-leaved species, 68 species were examined, representing 52 species from Eurasian *Septentrionales* and 16 species from South American *Australes*. Eleven species of compound-leaved *Berberis* were included, representing four species from Eurasian *Orientales* and seven from North American *Occidentales*. This study includes 35 accessions of Nepalese species of *Berberis s.l.* representing 19 simple-leaved and one compound-leaved species. The only Nepalese species not sampled were *B. tsarica*, *B. virescens*, *B. acanthifolia* and *B. borealis*. All the samples of Nepalese species of *Berberis* were collected during a programme of field work to cover the full range of *Berberis* habitats (Appendix 1). *Berberis higginsae*, which was found to be a sister to all other *Berberis s.l.* in a phylogeny based on *ndh*F (Chapter 3), was used to root the tree. Table 4.1 shows the samples used in this study with voucher information, origin of sample, distribution range (Ahrendt, 1961) and section and sub-sectional classification following Ahrendt (1961).

4.3.2 DNA extraction, PCR and sequencing

Total genomic DNA was extracted from silica gel-dried leaves using a CTAB method (Doyle & Doyle, 1987) and the DNeasy Plant Mini Kit (Qiagen Ltd, Crawley, West Sussex, UK) following the manufacturer's protocol. The extracted DNA was checked and quantified by 1% agarose gel electrophoresis. The extracted DNA was purified with the Wizard DNA clean up system (Promega Corporation, 2800 Woods Hollow Road, Madison, USA) following the manufacturer's protocol.

The ITS region was amplified using the ITS1 primer (5´-GGA AGG AGA AGT CGT AAC AAG G-3´) designed by Kim *et al.* (2004b), and ITS8P (5´-CAC GCT TCT CCA GAC TAC A-3´), which is identical to that used in Moller & Cronk (1997).

The PCR reaction was carried out in the DNA Engine Tetrad 2 Peltier Thermal Cycler by Bio-RAD in 25 µl reactions containing 1-2 µl of template DNA, 2.5 µl of 10 x NH_4 reaction buffer, 2.5 µl of dNTPs (0.2 mM), 1.25 µl of $MgCl_2$ (50mM), 0.125 µl of Taq Polymerase (5U/µl), 15.125-16.125 µl of distilled water and 0.75 µl of each primer (10 µM).

The thermal cycler for ITS amplification was programmed as: initial denaturation at 95 ^{0} C for 3 minutes; 1 minute annealing at 52^0 C , 1 minute extension at 72 ^{0} C followed by 30 cycle of 1 minute denaturation at 95 ^{0} C; 1 minute annealing at 52^0 C and 1 minute extension at 72 ^{0} C . The cycle was terminated with an extension at 72 ^{0} C for 10 minutes followed by 10^0 C for forever. Quality and quantity of PCR product was checked in 1% of agarose gel prior to sequencing.

Table 4.2. Samples used in this study with voucher information, origin of sample, distribution range (Ahrendt, 1961) and sectional and sub-sectional classification following Ahrendt (1961). BL2, EL, EA, EA2, JRS, G and LKSR are expedition abbreviations (see appendix 1). RBGE: Royal Botanic Garden Edinburgh.

Taxa	Section/subsection	Voucher information	Origin	Distribution
Simple-leaved *Berberis* (Septentrionales)				
Berberis angulosa Wall. ex Hook.f. & Thoms. var. *angulosa*	Angulosae/Euangulosae	EL 126	Nepal: Rasuwa	Nepal, Sikkim, SE Tibet
Berberis angulosa Wall. ex Hook.f. & Thoms. var. *fasciculata* Ahrendt	Angulosae/Euangulosae	EL 127	Nepal: Rasuwa	Nepal, Sikkim, SE Tibet
Berberis angulosa Wall. ex Hook.f. & Thoms. var. *angulosa*	Angulosae/Euangulosae	BL2 44	Nepal: Rasuwa	Nepal, Sikkim, SE Tibet
Berberis kumaonensis C.K.Schneid.	Angulosae/Euangulosae	JRSA 176	Nepal: Humla	Kumaon, Nepal, Garhwal
Berberis kumaonensis C.K.Schneid.	Angulosae/Euangulosae	JRSB 163	Nepal: Humla	Kumaon, Nepal, Garhwal
Berberis concinna Hook.f. var. *concinna*	Angulosae/Euangulosae	EL 125	Nepal: Rasuwa	Nepal, Sikkim, SE Tibet
Berberis everestiana var. *ventosa* Ahrendt	Angulosae/Euangulosae	EA2 16	Nepal: Mustang	SE Tibet, Nepal
Berberis concinna Hook.f. var. *extensiflora* Ahrendt	Angulosae/Euangulosae	EA 104	Nepal: Myagdi	Nepal, Sikkim, SE Tibet
Berberis mucrifolia Ahrendt	Angulosae/Jaeschkeanae	EA2 29	Nepal: Mustang	Nepal
Berberis mucrifolia Ahrendt	Angulosae/Jaeschkeanae	EA2 20	Nepal: Mustang	Nepal
Berberis jaeschkeana var. *usteriana* C.K.Schneid.	Angulosae/Jaeschkeanae	EA2 40	Nepal: Mustang	Kashmir, Punjab Himalaya, Nepal
Berberis jaeschkeana var. *usteriana* C.K.Schneid.	Angulosae/Jaeschkeanae	EA2 33	Nepal: Mustang	Kashmir, Punjab Himalaya, Nepal
Berberis jaeschkeana C.K.Schneid. var. *bimbilaica* Ahrendt	Angulosae/Jaeschkeanae	RBGE-19381159	China: Tibet	SE Tibet
Berberis pendryi Adhikari	Angulosae/Jaeschkeanae	EA2 5	Nepal: Mustang	Nepal
Berberis pendryi Adhikari	Angulosae/Jaeschkeanae	EA 32	Nepal: Mustang	Nepal
Berberis pendryi Adhikari	Angulosae/Jaeschkeanae	EA2 8	Nepal: Mustang	Nepal
Berberis karnaliensis Adhikari	Angulosae/Jaeschkeanae	JRSA 59	Nepal: Jumla	Nepal
Berberis karnaliensis Adhikari	Angulosae/Jaeschkeanae	JRSA 5	Nepal: Jumla	Nepal
Berberis hamiltoniana Ahrendt	Angulosae/Jaeschkeanae	JRSB 165	Nepal: Humla	Nepal
Berberis sibirica Pallas	Angulosae/Sibiricae	Harber: MF0505	China: Beijing	Siberia, N Mongolia

Table 4.2. (Continued)

Taxa				
Simple-leaved *Berberis* (Septentrionales)	**Section/subsection**	**Voucher information**	**Origin**	**Distribution**
Berberis orthobotrys Bienert ex Aitch. var. *rubicunda* Ahrendt	Angulosae/Subangulosae	BL2 39	Nepal: Rasuwa	Nepal
Berberis orthobotrys Bienert ex Aitch.	Angulosae/Subangulosae	RBGE-19810556	Iran	Kashmir, Nepal, SE Tibet
Berberis cooperi Ahrendt	Angulosae/Subangulosae	RBGE-19891045	Bhutan: Bumthang	Bhutan
Berberis asiatica Roxb. ex DC.	Asiaticae	G2	Nepal: Rasuwa	Nepal, Kumaon, Garhwal, Assam
Berberis asiatica Roxb. ex DC.	Asiaticae	BL2 1	Nepal: Rasuwa	Nepal, Kumaon, Garhwal, Assam
Berberis glaucocarpa Stapf	Asiaticae	JRSA 65	Nepal: Jumla	Punjab Himalaya, Kashmir, Nepal
Berberis mitifolia Stapf	Brachypodae	Harber: Wilson 4416	China: Hubei, W	W hupeh
Berberis canadensis Mill.	Canadenses	GenBank: AH013472.1		USA
Beberis fendleri A. Gray	Canadenses	GenBank: AH013471.1		USA
Berberis aetnensis C. Presl	Crataeginae	Harber	Italy: Mt Etna Sicily	Sicily, South Italy, East Spain
Berberis garciae Pau.	Crataeginae	Harber: Abel Moreno	Spain: Guadalajara	East Spain
Berberis maderensis Lowe	Crataeginae	Harber	Unknown (Cultivated)	Madeira
Berberis stearnii Ahrendt	Franchetianae/Eufranchetiana	RBGE-19310185	China: Yunnan	Yunnan
Berberis minutiflora C.K.Schneid.	Franchetianae/Minutiflorae	Harber-ACE 1848	China: Yunnan, NW	Yunnan, SW Szechuan, SE Tibet
Berberis papillifera (Franch.) Koehne	Franchetianae/Yunnanenses	Harber-BSWJ 7738	China: Yunnan, NW	Yunnan
Berberis cretica L.	Heteropodae/Creticae	RBGE-19687158 A	Unknown (Cultivated)	Crete, Cyprus
Berberis heteropoda Schrenk	Heteropodae/Pseudumbellatae	Harber-Durbye 1706	Kyrgyzstan: Tien-Shan	Sinkiang (China)
Berberis integerrima Bunge	Integerrimae	RBGE-19912970	Turkey: Kastamonu	S Turkestan
Berberis iliensis Popov	Integerrimae	Rae, Gardner & Ogar, 11	Kazakhstan	NW Sinkiang
Berberis jamesiana Forrest & W.W. Sm.	Integerrimae	RBGE-19181021	China : Yunnan, NW	Yunnan, W Szechuan
Berberis prattii C.K.Schneid.	Polyanthae/Eupolyanthae	RBGE-19687172	China: Sichuan, W	W Szechuan
Berberis koehneana C.K.Schneid.	Polyanthae/Subpolyanthae	BL2 55	Nepal: Rasuwa	
Berberis koehneana C.K.Schneid.	Polyanthae/Subpolyanthae	BL2 54	Nepal: Rasuwa	Nepal, Kumaon
Berberis gyalaica Ahrendt	Sherriffianae	Harber-AC 3647	China: Tibet	SE Tibet

Table 4.2. (Continued)

Taxa				
Simple-leaved *Berberis* (Septentrionales)	**Section/subsection**	**Voucher information**	**Origin**	**Distribution**
Berberis sherriffii Ahrendt	Sherriffianae	RBGE-19744027	China: Tibet	SE Tibet
Berberis forrestii Ahrendt	Sinenses	Harber-AC 1548	China: Yunnan, NW	Yunnan
Berberis aristata DC.	Tinctoriae/Chitriae	G 19	Nepal: Rasuwa	Nepal
Berberis aristata DC.	Tinctoriae/Chitriae	EA2 47	Nepal: Mustang	Nepal
Berberis petiolaris Wall. ex G.Don	Tinctoriae/Eutinctoriae	JRSA 122	Nepal: Mugu	Nepal, India
Berberis thomsoniana C.K.Schneid.	Tinctoriae/Umbellatae	G 4	Nepal: Rasuwa	Sikkim, E Nepal
Berberis thomsoniana C.K.Schneid.	Tinctoriae/Umbellatae	LKSRB 96	Nepal: Illam	Sikkim, E Nepal
Berberis tschonoskyana Regel	Tschonoskyanae	RBGE-19940193B	Japan: Shikoku	Japan
Berberis thunbergii DC	Tschonoskyanae	RBGE-19931362	Japan: Honshu	Japan
Berberis koreana Palib	Vulgares	RBGE-19731131A	Korea: Kyonggi-do	Korea
Berberis regeliana Koehne ex C.K.Schneid.	Vulgares	RBGE-19920784A	Japan: Hokkaido	Japan
Berberis vulgaris L.	Vulgares	Harber-Fillan	Spain: N Pyrenees	Europe: Switzerland, Albania
Berberis kawakamii Hayata	Wallichianae/Barandanae	RBGE-19933979B	Taiwan: Miaoli	Taiwan (Formosa)
Berberis bergmanniae C.K.Schneid.	Wallichianae/Euwallichianae	RBGE-19754095A	Unknown (Cultivated)	Szechuan
Berberis wallichiana DC.	Wallichianae/Euwallichianae	BL2 20	Nepal: Rasuwa	Nepal, India, China
Berberis wallichiana DC.	Wallichianae/Euwallichianae	G 18	Nepal: Rasuwa	Nepal, India, China
Berberis sargentiana C.K.Schneid.	Wallichianae/Euwallichianae	RBGE-19784169	China: Hubei, W	W. Hupeh
Berberis dumicola C.K.Schneid.	Wallichianae/Euwallichianae	RBGE-19220032	China : Yunnan, W	NW Yunnan
Berberis hookeri Lem.	Wallichianae/Hookerianae	LKSRB 22	Nepal: Panchthar	Bhutan, Assam, Sikkim, Nepal
Berberis calliantha Mulligan	Wallichianae/Hookerianae	RBGE-19381019	China : Xizang (Tibet)	SE Tibet
Berberis insignis Hook.f. & Thoms.	Wallichianae/Insignes	LKSRB 145	Nepal: Illam	Assam Himalaya
Berbeis coxii C.K.Schneid.	Wallichianae/Manipuranae	RBGE-19251027	China: Tibet	Upper Burma
Berberis sanguinea Franch.	Wallichianae/Sanguineae	RBGE-19390166B	Unknown (Cultivated)	W Szechuan
Berberis candidula C.K.Schneid.	Wallichianae/Verruculose	RBGE-19902425A	Unknown (Cultivated)	W. Hupeh

Table 4.2. (Continued)

Taxa	Section/subsection	Voucher information	Origin	Distribution
Simple-leaved *Berberis* (Australes)				
Berberis congestiflora C. Gay	Actinacanthae/Congestiflorae	RBGE-19892971B	Chile: Araucania	Chile
Berberis horrida C. Gay	Actinacanthae/Congestiflorae	GenBank: AH013506.1	Unknown (Cultivated)	Chile
Berberis actinacantha Mart.	Actinacanthae/Euactinacanthae	Gardner & Knees 8421	Chile	Chile
Berberis grevilleana Gillies ex Hook. & Arn.	Actinacanthae/Euactinacanthae	GenBank: AH013507.1	Unknown (Cultivated)	Chile, Argentina
Berberis empetrifolia Lam.	Buxifolia/Empetrifoliae	RBGE-19761088A	Argentina	Chile, Argentina
Berberis hieronymi C.K.Schneid.	Buxifolia/Eubuxifoliae	Harber	Argentina NW	Argentine
Berberis rotundifolia Poepp. & Endl.	Corymbosae	RBGE-20080789 C	Chile: Biobío	Chile
Berberis chilensis Gillies ex Hook.	Illicifoliae/Chilenses	RBGE-19900509	Chile: Maule	Chile
Berberis ilicifolia Forst.	Illicifoliae/Euilicifoliae	RBGE-19880919A	Chile: Los Lagos	Argentine, Chile
Berberis negeriana Tischler	Illicifoliae/Euilicifoliae	RBGE-20040497A	Chile: Biobio	Chile
Berberis valdiviana Phil.	Laurinae/Flexuosae	RBGE-19900563C	Chile: Biobío	Chile
Berberis microphylla Forst.	Microphyllae	RBGE-19670638B	Chile	Chile, Argentina
Berberis montana Gay	Montanae	RBGE-19921060A	Chile: Los Lagos	Chile
Berberis jamesonii Lindl.	Paniculatae	GenBank: AH013505.1		Ecuador
Berberis trigona Kunze ex Poepp. & Endl.	Trigonae	Harber-HCM 98123	Chile: Araucanía	Chile
Berberis trigona Kunze ex Poepp. & Endl.	Trigonae	GenBank: AH013514.1		Chile
Berberis lutea Ruíz & Pav.	Virgatae	GenBank: AH013504.1		Ecuador

Table 4.2. (Continued)

Taxa **Compound-leaved *Berberis* (*Orientales*)**	**Section/subsection**	**Sample information**	**Origin**	**Distribution**
Berberis napaulensis (DC.) Laferr.	Longibracteatae/Napaulenses	EA2 53	Nepal: Mustang	Nepal
Berberis *napaulensis* (DC.) Laferr.	Longibracteatae/Napaulenses	LKSRB 1	Nepal: Panchthar	Nepal
Berberis polyodonta (Fedde) Laferr.	Longibracteatae/Dolichostyles	RBGE-19911138B	China: Yunnan, W	Szechuan
Berberis duclouxiana (Gagnep.) Laferr.	Longibracteatae/Dolichostyles	RBGE-19923156A	China: Yunnan, NW	Yunnan, W. Yunnan
Berberis gracilipes Oliv.	Longibracteatae/Dolichopodae	RBGE-19891713B	China: Sichuan, W	Szechuan
Berberis nervosa Pursh	Longibracteatae/Nervosae	RBGE-19782559 B	Canada: British Columbia	British Columbia, Idaho, California, Washington
Compound-leaved *Berberis* (*Occidentales*)				
Berberis repens Lindl.	Aquifoliatae/Euaquifoliatae	RBGE-19800553A	Canada: British Columbia	Colorado
Berberis aquifolium Pursh	Aquifoliatae/Euaquifoliatae	RBGE-19912984A	Canada: British Columbia	California
Berberis fremontii Torr.	Horridae	RBGE-19716619A	NA	Colorado, Arizona, Mexico
Berberis nevinii A. Gray	Horridae	Rancho Santa Ana Botanic Garden, sn	NA	S. California
Berberis higginsae Munz	Horridae	GenBank: AH013515.1		
Berberis pallida Hartw. ex Benth.	Paniculatae/Eupaniculatae	RBGE-19930103C	Mexico: Hidalgo	Mexico, Oaxaca

The PCR product was purified with the GFX PCR purification kit (Amersham Bioscience) following the manufacturer's protocol. The product was then sequenced in 10 µl reactions containing 4 µl of DTCS Quickstart mix, 1 µl of each primer (10 µM), 2 µl of PCR product and 3 µl of distilled water. The thermal cycle was: 35 cycles of 96^0 C for 20 seconds, 50^0 C for 5 seconds and 60^0 C for 4 minutes. The sequencing products were cleaned with ethanol and analysed on CEQ 8000 Genetic Analysis System.

A few PCR products were purified with ExoSAP-IT (USB Corporation) following the manufacturer's protocol and for these, sequencing reactions were carried out with the same protocol used for the *ndh*F gene (chapter 3) with the same primers as those for ITS PCR. These samples were analysed in the GenePool sequencing facility at the University of Edinburgh, UK.

Raw sequences (i.e. forward and reverse) were analysed individually by the CEQ^{TM} 8000 Genetic Analysis System (Beckmann Coulter Ltd. US) and both reverse and forward sequences were assembled using Sequencher^{TM} (Version 4.5, Genetic Codes Corporation, Ann Arbor, Michigan, US). The sequences analyzed in the GenePool sequencing facility were assembled directly in Sequencher^{TM}.

Sequences were aligned initially in BioEdit (Version 7.0.9) using ClustalW and later by eye in MacClade 4.06 for OS X (Maddison & Maddison, 2003). Boundaries of ITS1 and ITS2 were identified with the help of published ITS sequences (Kim *et al.*, 2004b).

4.3.3 Phylogenetic analysis

Phylogenetic analyses were conducted using maximum parsimony and Bayesian methods. Maximum parsimony searches comprised two heuristic searches using Tree-Bisection-Reconnection (TBR) in PAUP* 4.0 β10 (Swofford, 2002). An initial search of 10000 random addition sequence replicates saved 10 trees per replicate (with Multrees on and steepest descent off) creating a range of possible starting trees for a second search. The second search was performed with the same settings using all saved trees from the first search, and saved a maximum of 10000 shortest trees which, according to Sanderson and Doyle (1993), is sufficient to capture all topological variation. For character optimisation the option ACCTRAN was used and starting trees were obtained by stepwise addition. Parsimony informative gaps were coded as binary characters. To calculate the support for each clade of the tree, bootstrap analysis (Felsenstein, 1985) was conducted using 10000 replicates with full heuristic search and the TBR branch-swapping algorithm, which saved 10 trees per replicate (Multrees option off).

Bayesian analysis was carried out using Markov Chain Monte Carlo (MCMC) methods implemented in MrBayes ver. 3.1.2. (Huelsenbeck & Ronquist, 2001). Nucleotide substitution models for Bayesian analyses were evaluated using Akaike information criteria (AIC) and a hierarchical likelihood ratio test (LRT) in MrModeltest version 2.3 (Nylander, 2004). The model GTR+G was suggested by both AIC and LRT, and was therefore implemented in the analysis. Two parallel independent runs, each consisting of one cold and three heated chains were run for 5 million generations. The Markov chains were sampled every 100th generation, resulting in 50000 sampled trees from each chain. The trace files were analysed in Tracer version 1.5 (Rambaut & Drummond, 2007) and it was determined that stationarity was reached after 5000 trees (500000 generations); therefore the first 5000 (10%) trees were discarded as ‘burn-in’.

4.3.4 Divergence time estimation

Divergence times were estimated using a Bayesian approach implemented in BEAST ver. 1.5.3 (Drummond & Rambaut, 2007). The time to the most recent common ancestor (tMRCA) of *B. higginsae* and the rest of the *Berberis* species inferred from the BEAST analysis of *ndhF* sequences (Chapter 3; Fig. 3.3; Node 4) was used as prior information to calibrate the ITS phylogeny. A normal prior distribution was employed which is thought to reflect the uncertainty associated with secondary calibration (Couvreur *et al.*, 2008). Therefore, the tMRCA of *B. higginsae* and the remaining *Berberis* species (Fig 4.10; Node C) was calibrated with a mean age of 37.27 and standard deviation of 2.5 to accommodate the 95% HPD intervals inferred from the *ndhF* analysis.

For the BEAST analysis, the Yule speciation prior was implemented, which has been recommended for species level phylogenies (Drummond *et al.*, 2007). The analysis was implemented under a relaxed clock with an uncorrelated log-normal model of rate variation (Drummond *et al.*, 2006). The GTR+G model of sequence evolution was used as suggested by MrModeltest version 2.3 (Nylander, 2004). The xml input file for this analysis was generated using BEAUti ver.1.5.3 (Drummond & Rambaut, 2007).

Two independent runs were performed, each of 100 million generations, with sampling every 10000 generations. Two separate runs were combined using LogCombiner ver. 1.5.3 (Drummond & Rambaut, 2007). Convergence of the chain to a stationary distribution was confirmed by visualizing trace files in Tracer ver. 1.5 (Rambaut & Drummond, 2007). Tracer ver. 1.5 was also used to measure effective sample size (ESS) of each parameter. A maximum clade credibility tree was generated from the sampled trees using TreeAnnotator ver.1.5.3 (Drummond & Rambaut, 2007), and mean ages, 95% highest posterior probability (HPD) intervals and posterior probablities were also calculated. The resulting tree was visualized using FigTree ver.1.3.1 (Rambaut, 2009).

4.4 RESULTS

4.4.1 Sequence characteristics

The full aligned sequences of all taxa used in this study can be found in the accompanying CD-ROM. Sequence characteristics for the ITS region are summarized in table 4.2.

Table 4.3. Sequence characteristics of the 97 *Berberis* accessions included in this study.

Sequence characteristics	**ITS**
Aligned length (including 5.8S region)	640
Length range (without gaps)	612-616
G and C content (%)	51
Length of ITS1 (in aligned sequences)	262
Length of ITS2 (in aligned sequences)	238
Length of 5.8S	140
Constant sites	524
Variable sites	56
Parsimony informative characters	68
Parsimony informative gaps	7

4.4.2 Phylogenetic analysis

The parsimony analyses produced 1799 trees of 176 steps with a consistency index of 0.756 and retention index of 0.906, and rescaled consistency index of 0.685. The strict consensus tree with bootstrap support is shown in Fig. 4.8.

The topologies of the parsimony and Bayesian tree were congruent except for slightly better resolution in Bayesian tree. The position of a few Chinese species of *Berberis* was unresolved in both trees. The ITS and *ndhF* phylogenies are largely congruent. Both confirm the monophyly of simple-leaved *Berberis* and paraphyly of compound-leaved *Berberis*, and were therefore congruent with major taxonomic divisions of both simple-leaved and compound-leaved *Berberis*. One potential incongruence is the position of the Nepalese species *B. petiolaris* in the widespread Eurasian clade (Clade F; Fig 4.7 & 4.8) in the ITS phylogeny. This species was grouped with Chinese and Nepalese species in *ndh*F phylogeny (Chapter 3; Fig 3.2) though with weak branch support. Another potential incongruence is a group of Chinese species (*B. gyalaica*, *B. sherriffii*, *B. papillifera*, *B. stearnii*, *B. forrestii*, *B. minutiflora*, and *B. pratii*) which formed a monophyletic clade in the *ndh*F phylogeny (Chapter 3; Clade 8; Fig 3.2) but remain unresolved in the ITS phylogeny (Fig 4.7 & 4.8). Phylogenies derived from more plastid and nuclear loci are needed to determine if these potential incongruences are real or merely reflect lack of resolution in the ITS tree.

Seven clades (Clades A-G; Fig 4.7 & 4.8) were common to both the Bayesian and parsimony ITS trees, but other than a moderately well supported sister relationship between clades A and B, relationships between these clades were unresolved. Clades A and B correspond to the compound-leaved North American *Occidentales* and Eurasian *Orientales* respectively. Clade C comprised the Nepalese species *B. asiatica* and *B. glaucocarpa*. In the Bayesian tree (Fig 4.7), *B. concinna* was grouped in clade C with low support [Posterior probability (Pp): 0.54] while in the parsimony tree the position of *B. concinna* remained unresolved.

Clade D contained only evergreen species from Nepal and China and is largely equivalent to section *Wallichianae*. However, the position of a few evergreen species (*B. candidula*, *B. hookeri* and *B. coxii*) from section *Wallichianae* remains unresolved. *Berberis insignis* was sister to the *Wallichianae* group (clade D) in the Bayesian tree (Fig 4.7) with low support (Pp: 0.53) but remained unresolved in the parsimony tree. The Chinese evergreen species *B. sanguinea* was sister to clade G in the parsimony tree (Fig 4.8) but with low support [Bootstrap (Bs): 54] and its position was unresolved in the Bayesian tree.

The South American *Australes* formed a monophyletic clade (clade E) in both trees with good support (Pp: 1; Bs: 76). Clade F contains widespread Eurasian species from Japan, Korea, Spain, Portugal, Turkey, Iran, Italy, Cyprus, Kyrgyzstan, Kazakhstan and northern China. Two North American species *B. canadensis* and *B. fendleri*, and one Nepalese species *B. petiolaris*, were also grouped in this clade.

The majority of the Nepalese species formed a reasonably well supported monophyletic clade (Clade G) (Pp: 0.99; Bs: 69) in both the Bayesian and parsimony trees (Fig 4.7 & 4.8).

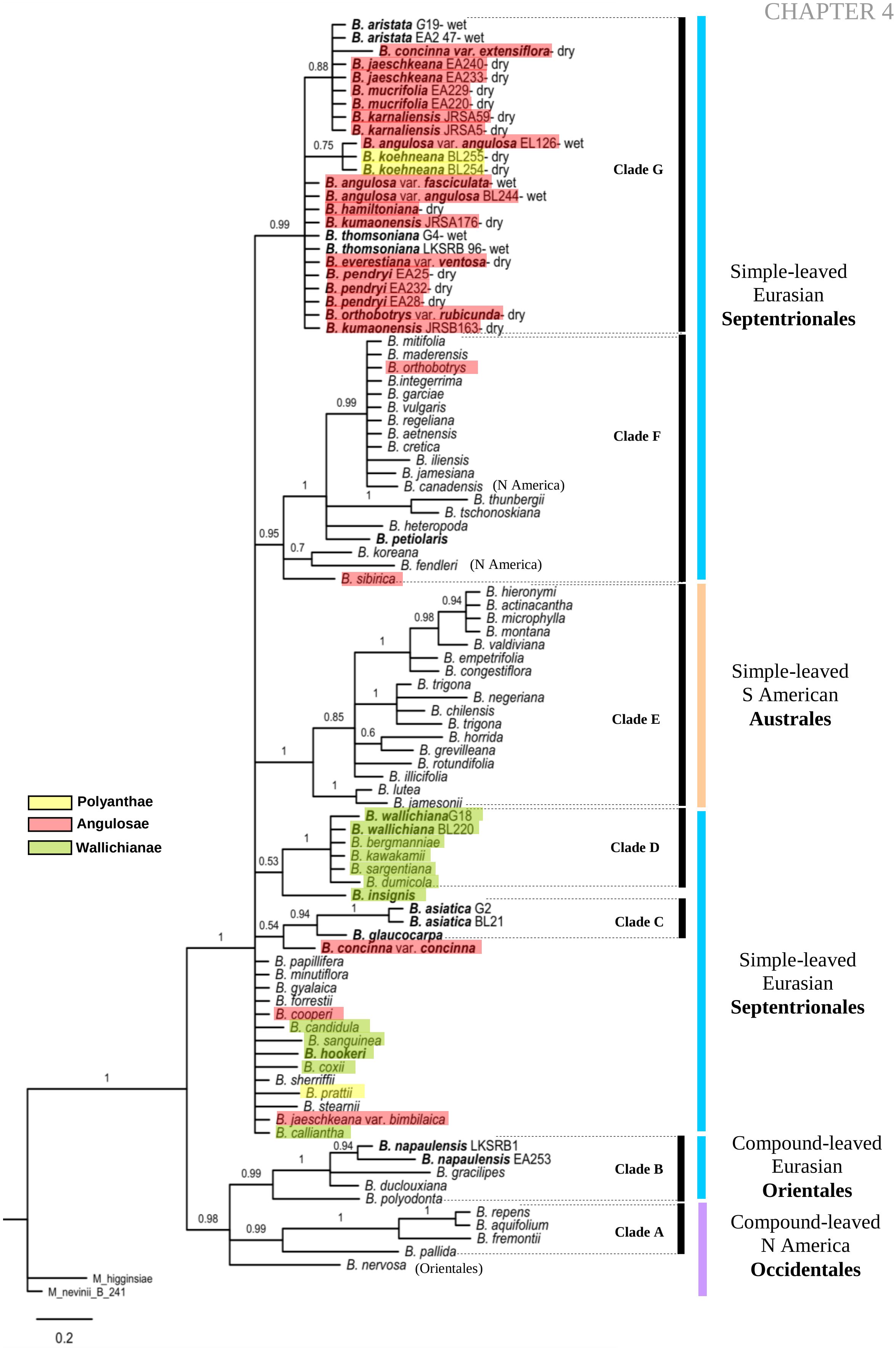


FIG. 4.7. Bayesian majority rule consensus tree obtained from ITS sequences of 97 accessions representing 25 sections of simple-leaved and 5 sections of compound-leaved *Berberis*. Numbers above branches are posterior probabilities. Nepalese species are in bold; wet-wet adapted species, dry-dry adapted species.

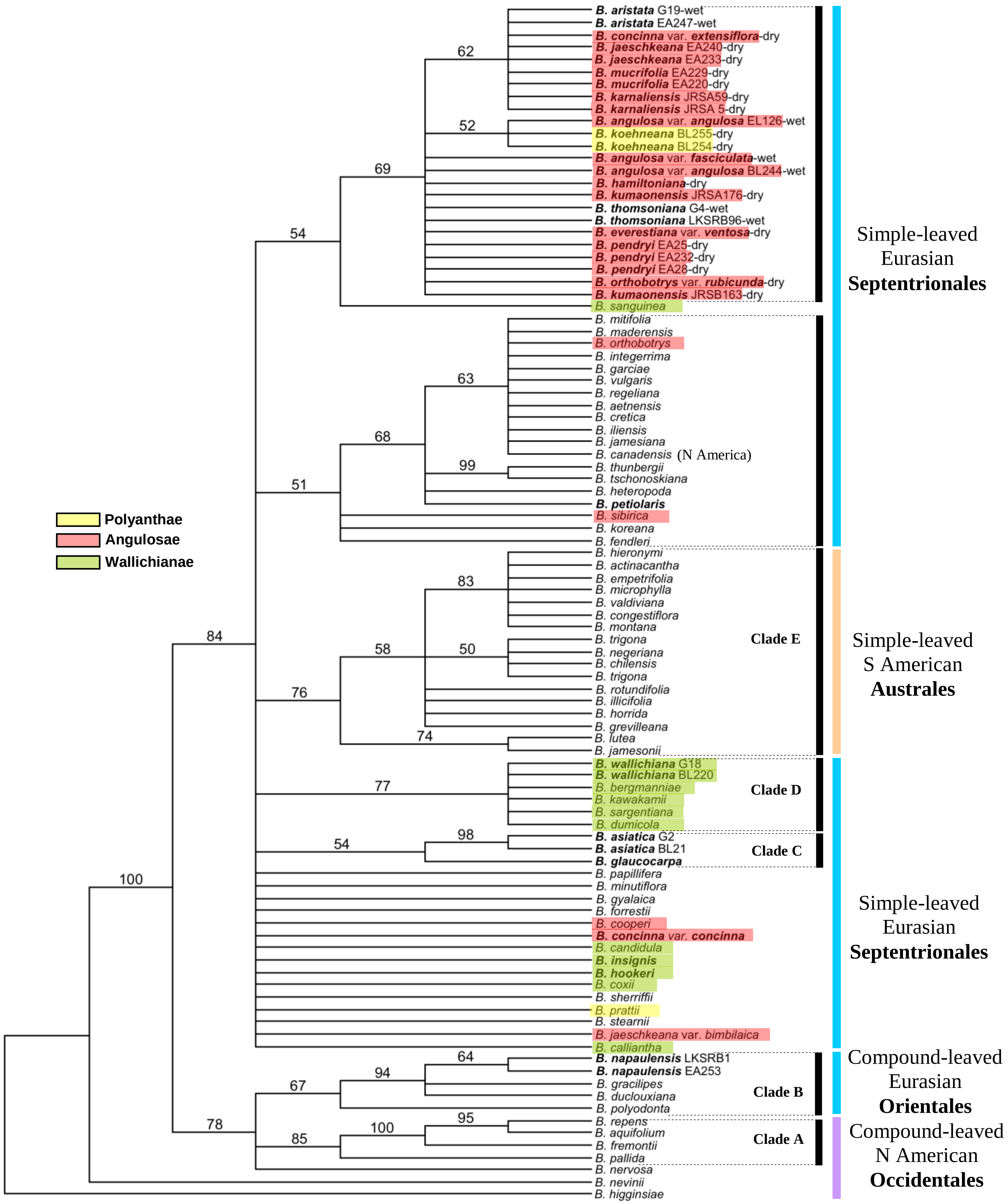


FIG. 4.8. Strict consensus tree of 1799 equally parsimonious trees obtained from ITS sequences of 97 accessions representing 25 sections of simple-leaved and 5 sections of compound-leaved *Berberis*. Numbers above branches are bootstrap support (>50). Nepalese species are in bold; wet-wet adapted species, dry-dry adapted species.

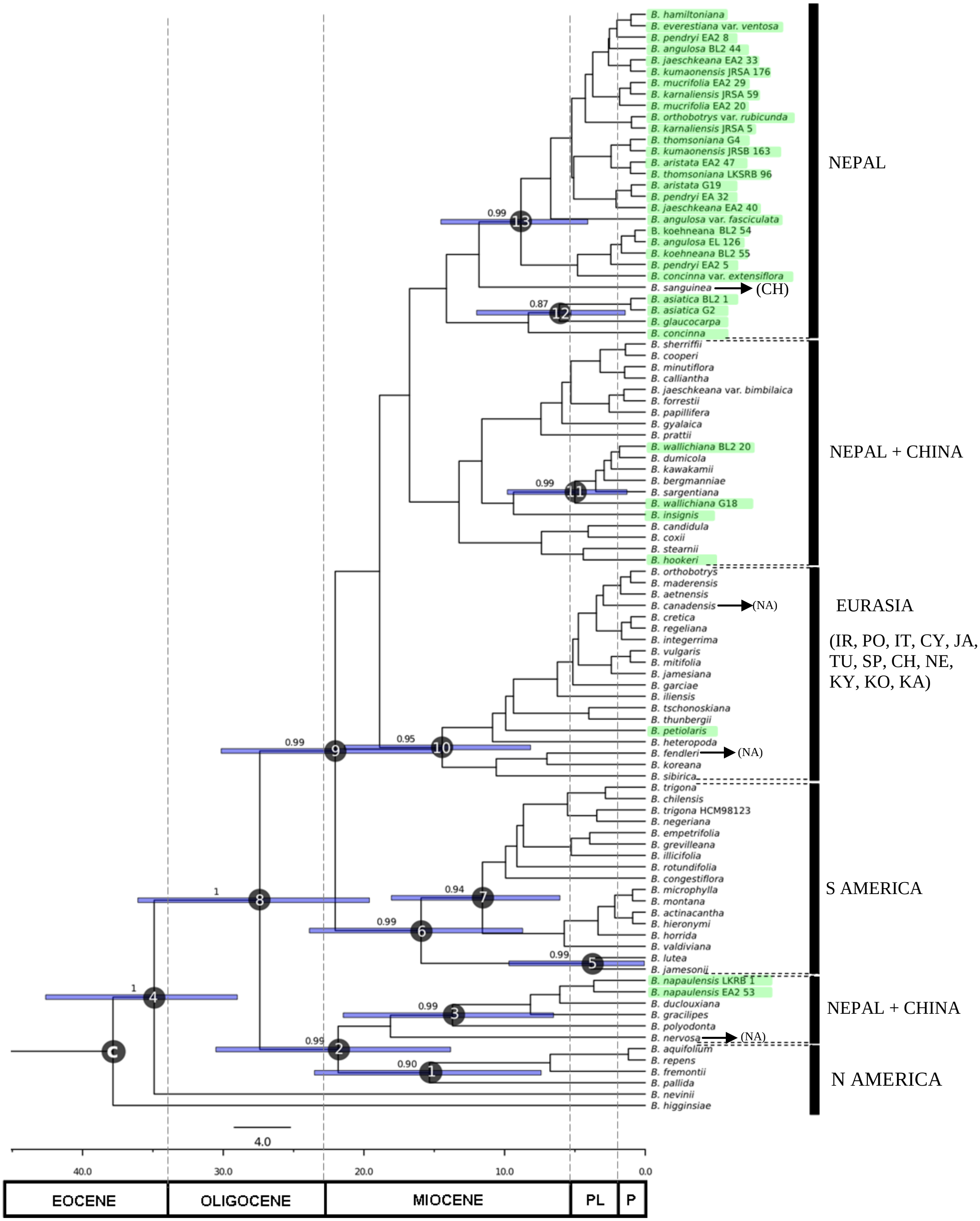


FIG. 4.9. ITS chronogram of *Berberis s.l.*: maximum clade credibility tree obtained from BEAST analysis. Nodes are posterior mean ages (mya), with blue bars indicating the 95% HPD intervals (see Table 4.4 for more details). C: constrained age = mean age 37.27 mya. Bayesian posterior probabilities support values are indicated above the branches. PL: Pliocene. P: Pleistocene. Nepalese species are highlighted with green. IR: Iran; PO: Portugal; IT: Italy; CY: Cyprus; JA: Japan; TU: Turkey; SP: Spain; CH: China; KA: Kazakhstan; NE: Nepal; KY: Kyrgyzstan; KO: Korea.

4.4.3 Divergence time estimation

The chronogram obtained from the BEAST analysis of ITS sequences is shown in Fig 4.9. The age of the largest clade of Nepalese species (Fig 4.9; Node 13; Table 4.4) is estimated as 8.8 mya with 95% HPD intervals of 4.1-14.5 mya.

Table 4.4. Mean ages, 95% HPD intervals of divergence time and Bayesian posterior probabilities of major nodes in *Berberis s.l.* based on BEAST analysis of ITS sequences.

Node number (see fig. 4.10)	**Mean age (mya)**	**95% HPD (mya)**	**Bayesian posterior probabilities**
C (Calibration node)			1.00
1	15.3	7.4-23.5	0.90
2	21.8	13.8-30.5	0.99
3	13.7	6.5-21.4	0.99
4	34.9	29.0-42.6	1.00
5	3.8	0.07-9.7	0.99
6	15.9	8.7-23.8	0.99
7	11.6	6.0-18.0	0.94
8	27.4	19.5-36.0	1.00
9	22.0	14.4-30.1	0.90
10	14.4	8.1-21.4	0.95
11	5.0	1.3-9.8	0.99
12	6.1	1.43-11.9	0.87
13	8.8	4.1-14.5	0.99

4.5 DISCUSSION

4.5.1 Effect of Himalayan orogeny on recent radiation of Nepalese Berberis

The majority of Nepalese taxa (14 taxa) form a clade (Clade G; Fig. 4.7 & 4.8) with good support (Pp: 0.99; Bs: 69), and the low sequence divergence detected within this clade is consistent with rapid radiation of these *Berberis* species in the Nepal Himalaya. The estimated mean age of this clade, *ca.* 8.8 mya with 95% HPD interval 4.08-14.5 mya (Fig. 4.9; Node 13; Table 4.4), coincides with the active uplift phase of the Himalaya during the late Miocene. Several studies suggest that the maximum uplift of the Great Himalaya took place during the Miocene (Mani, 1978; Copeland *et al.*, 1987; Copeland & Harrison, 1990). This, therefore, is consistent with a hypothesis that the speciation process in this clade was triggered by the uplift of the mountains and associated changes in the climate during the Miocene. This type of rapid radiation has been reported also in the genus *Saussurea* in Tibet (Wang *et al.*, 2009).

4.5.2 Colonisations of Berberis lineages in the Nepal Himalaya

The ITS phylogeny of *Berberis* shows that the Nepalese species have multiple origins, deriving from at least four different colonizations. The largest exclusively Nepalese Clade G (Fig 4.7 & 4.8) suggests the origin of this clade in the Nepal Himalaya.

Berberis petiolaris falls into the widespread Eurasian clade (clade F, Fig 4.7 & 4.8) with species from Japan, Korea, Spain, Iran, Italy, Cyprus, Kyrgyzstan, Kazakhstan and northern China. This is a western Himalayan species and the ITS tree suggests that it originated from Eurasian lineages. However, *B. petiolaris* was nested with Nepalese and Chinese species in the *ndhF* phylogeny (Chapter 2; Fig 3.2), though with weak branch support. More data are needed to determine if incongruence between the *ndhF* and ITS trees could be the result of hybridisation or incomplete lineage sorting, or merely lack of phylogenetic resolution.

Clade D comprises the evergreen species of *Berberis* from Nepal and China belonging to section *Wallichianae*. The positions of the other evergreen species (*B. candidula*, *B. hookeri* and *B. coxii*) remained unresolved in the ITS phylogeny. *Berberis insignis*, a Nepalese evergreen species, was grouped with other evergreen species in the Bayesian and BEAST trees but remained unresolved in the parsimony tree. China is the centre of diversity for section *Wallichianae* with *ca.* 73 species (Chamberlain & Hu, 1985), among which 60 species are endemic. The term 'Sino-Himalayan' is generally used to define the floristic region covering the Himalaya and south-western China. In his discussion of the land forms, climatic zones and biogeographic links between the China and the Himalaya, Yoshida (2006) proposed that a smaller area comprising the wetter part of the Himalaya, and the mountainous part of south-western China, or the Hengduan Mountains and adjacent northern highlands should be considered as the Sino-Himalayan region. The Sino-Himalayan region is believed to be a centre for the speciation of eastern Himalayan plants and it is likely that the section *Wallichianae* evolved, or at least began to radiate, in this region. The evergreen simple-leaved Nepalese *Berberis* grow in eastern Nepal in temperate forests which receive more monsoon rain fall than the western Himalaya

(Fig 4.10). Their close relationship with other Chinese evergreen species mainly from Sichuan and Yunnan suggests the likelihood of their having come to eastern Nepal from China. The mean age of diversification of extant members of *Wallichianae* in Clade D was estimated as 5 mya (Fig 4.9; Node 11; Table 4.4). Several studies suggest that the Himalaya and south Tibet attained a sizeable height at least by the late Miocene to create a monsoon climate (Molnar *et al.*, 1993; Guo *et al.*, 2002; Valdiya, 2002). Therefore, the suitable habitats for species like *B. wallichiana* and *B. insignis* were available by that time to allow them colonize eastern Nepal.

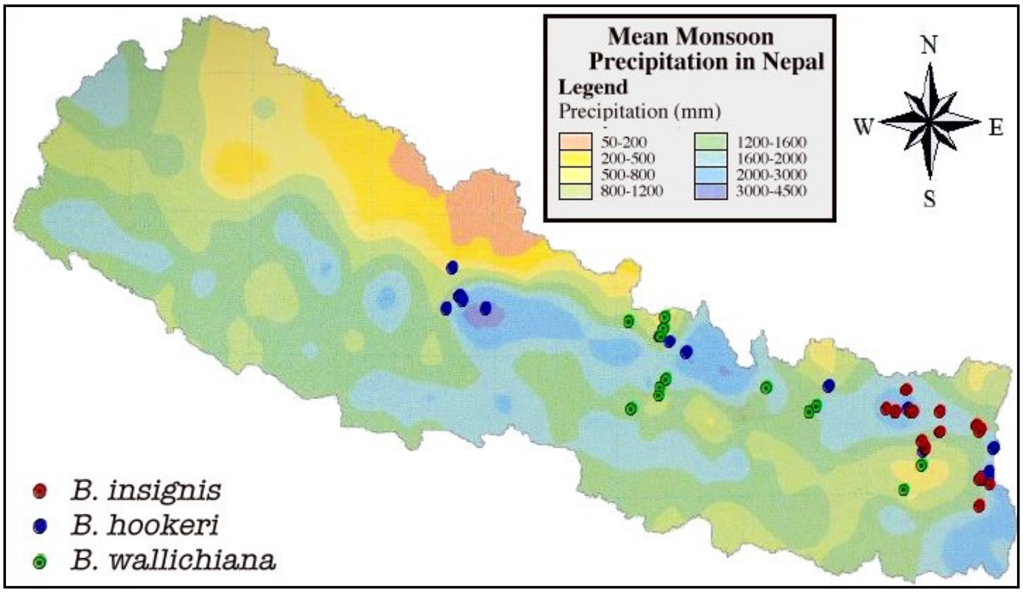


FIG. 4.10. Distribution of Nepalese species of section *Wallichianae* plotted with mean monsoon precipitation (modified from UNEP, 2001).

Berberis asiatica is sister to *B. glaucocarpa*, the western Himalayan species (Clade C; Fig 4.7 & 4.8), and grows at comparatively lower altitude in Nepal (Fig 4.11). This clearly suggests a western Himalayan origin of *B. asiatica*.

The placement of the compound-leaved species *B. napaulensis* within a clade of Chinese compound-leaved species indicates the dispersal of this species from the Eastern Himalaya.

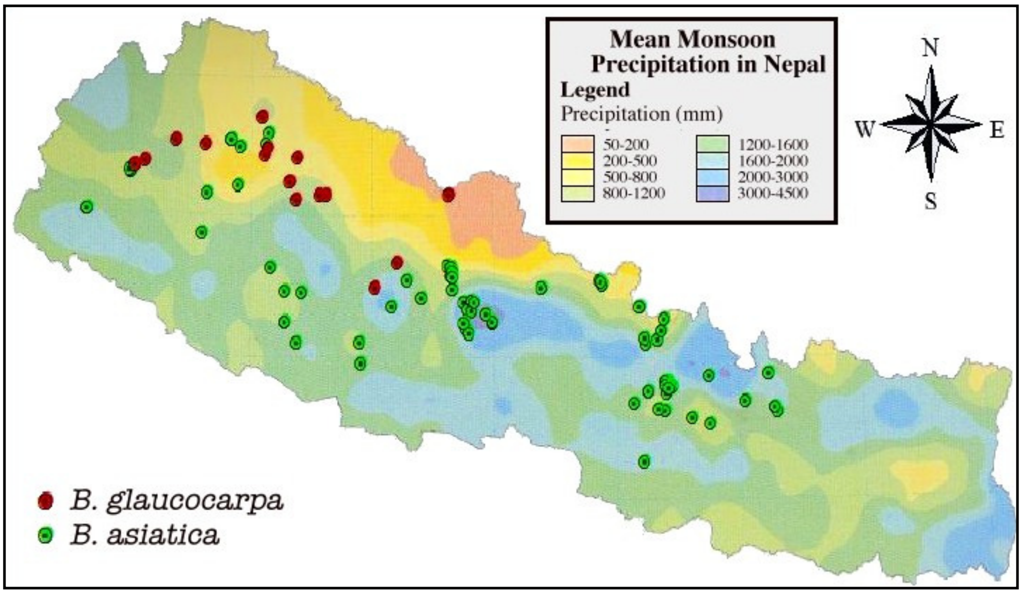


FIG. 4.11. Distribution of *B. glaucocarpa* and *B. asiatica* plotted with mean monsoon precipitation (modified from UNEP, 2001).

4.5.3 Phylogenetic niche conservatism among the Nepalese species of Berberis

Most of the taxa (10 out of 14) of the exclusively Nepalese clade G are found in the drier regions of western and the trans-Himalayan region of Nepal where the mean annual monsoon precipitation has been reported to be less than 800 mm (Fig 4.12). These regions fall under the arid and Humla-Jumla geographical divisions of Stainton (1972), and Domaine ouest-nepalais and Domaine nord-ouest nepalais of Dobremez (1972) (Fig 4.6).

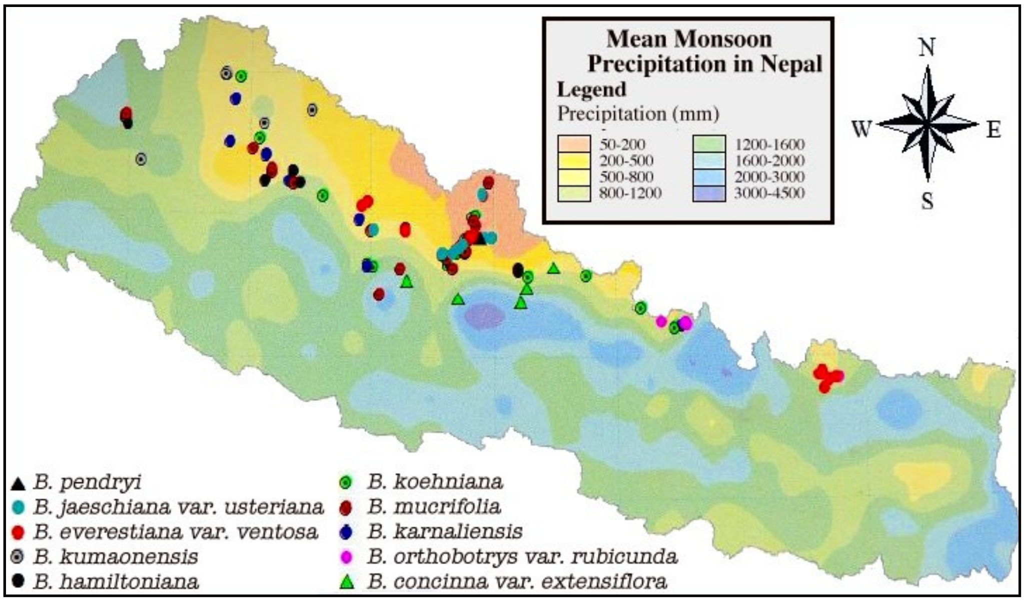


FIG. 4.12. Distribution of 10 out of 14 taxa of Nepalese Clade G plotted with mean monsoon precipitation (modified from UNEP, 2001).

The remaining four taxa (*B. angulosa* var. *angulosa*, *B. angulosa* var. *fasciculata*, *B. thomsoniana* and *B. aristata*) are distributed in wide range of habitats (Fig. 4.13).

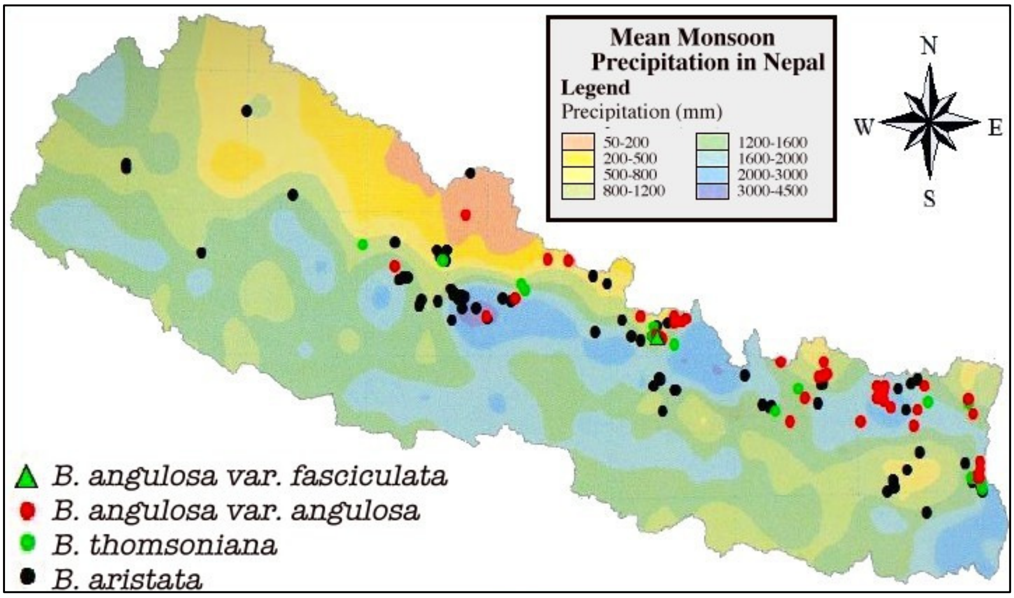


FIG 4.13 Distribution of four taxa of Nepalese Clade G plotted with mean monsoon precipitation (modified from UNEP, 2001).

The clear preference of the most of the species of Clade G for the drier areas of western and trans-Himalayan region of Nepal indicates that the past geological history of the region, i.e. like uplift of the mountains and the formation of the drier climate in the western and trans-Himalayan region during the Miocene might have played an important role for the evolution and diversification of Clade G. However, wider sampling of species from other dry areas of Western Himalaya (Kumaon, Himachal Pradesh etc) and Tibetan Plateau is necessary to ensure that all the members of this clade are included in the analysis to evaluate the role of niche differentiation and diversification of *Berberis* species in the Himalaya.

Four taxa of the Clade G (*B. angulosa* var. *angulosa*, *B. angulosa* var. *fasciculata*, *B. thomsoniana* and *B. aristata*) are more widely distributed in a wide range of habitats throughout the Nepal Himalaya (Fig 4.13), in contrast to the majority of the species in this clade, which are confined to the drier regions of western Nepal. The habitat preferences of the species of Clade G suggest that niche conservatism may have played a role. In general *Berberis* species prefer wet habitats, and restriction of these species to drier climates suggests this transition from wet to dry ecology may have only occurred once. Only four taxa are found in a wide range of habitats, but with the poor resolution of the ITS phylogeny it is not clear whether they are basal in the clade or secondary transitions were made from dry to wet ecology.

Similarly, wet loving Nepalese evergreen species of section *Wallichianae* (*B. wallichiana* and *B. insignis*) formed a clade with other Sino-Himalayan species in the BEAST tree (Fig 4.9; Nepal + China clade; node 11) which grow in the wetter part of the eastern Himalaya.

Berberis mucrifolia, an endemic species to Nepal, which is one of the members of the clade G, might have evolved in response to the formation of drier calcareous habitats associated with the drying of Tethys sea. *Berberis mucrifolia* is restricted to drier climates and calcareous soils in the trans-Himalayan region and some parts of western Nepal. Other examples of calciphiles with similar types of restricted distribution include *Ajuga lupilina*, *Androsace yunanensis* and *Draba involucrata* Yoshida (2006).

4.6 CONCLUSIONS

The ITS phylogeny suggests that the Nepalese species of *Berberis* have originated at least from four colonisation events from outside Nepal. Species from wetter habitats of Nepal are closely related to species from China and the Eastern Himalaya, and have migrated westwards along the Himalayas. However, the present distribution of *Berberis* in the Nepal Himalaya is not only the result of introduction from neighbouring areas but also the rapid radiation within one clade, in with response to the mountain building events during the Miocene. Rapid adaptive radiation was a force for speciation of some western Himalayan species.

Phylogenetic niche conservatism is evident in Nepalese *Berberis* as most of the species of the exclusively Nepalese clade grow in the drier regions of western Nepal.

CHAPTER 5: CHROMOSOME COUNTS FOR NEPALESE SPECIES OF SIMPLE-LEAVED *BERBERIS*

5.1 INTRODUCTION

The importance of cytological data for the study of evolution and diversification of plant species has long been recognized (Stebbins, 1971; Stace, 2000). Polyploidy is considered to be one of the major forces behind evolution and speciation in higher plants (Stebbins, 1971; Soltis & Soltis, 1995). It has been estimated that 50-70% of species of angiosperms are polyploids (Stebbins, 1971; Grant, 1981; Masterson, 1994). Polyploids are thought to have a higher tolerance of a wide range of climatic conditions than diploids, and to be better adapted to more diverse and severe environmental conditions (Hara, 1969). Many authors have suggested that polyploidy is one way by which plants can adapt to harsh climates at high altutudes (Janaki Ammal, 1950; Yoshida, 2006). Polyploids are, therefore, expected to be more frequent at high altitudes than low altitudes. The Nepal Himalaya exhibits extreme elevation ranges within even short distances and high proportions of polyploids could be expected at higher altitudes. Indeed, polyploidy has been shown to play an important role in the speciation and diversification of some taxa from the Himalaya (Janaki Ammal, 1950; Wakabayashi & Ohba, 1999), Tibet (Meng *et al.*, 2010) and the Hengduan Mountains (Nie *et al.*, 2005).

The Berberidaceae include about 12 herbaceous and two woody genera (*Berberis s.l.* and *Nandina*) (Mabberley, 2008). Using character compatibility analysis, Meacham

(1980) recognized four major groups within the family and these were supported by chromosome numbers and fruit characters. An *ndhF*, *rbcL* and chloroplast DNA restriction site analysis also recognized four chromosomal clades (x=10, 8, 7 and 6) in Berberidaceae (Kim & Jansen, 1996; Kim & Jansen, 1998; Kim *et al.*, 2004). The highest base number x=10 has been reported for *Nandina* while the majority of herbaceous genera have a base number of 6 (*Achlys*, *Bongardia, Diphylleia, Dysosma*, *Epimedium*, *Jeffersonia*, *Plagiorhegma, Podophyllum*, *Sinopodophyllum*, *Vancouveria*). The x=7 group consisted of only *Berberis s.l.* and *Ranzania*. *Caulophyllum*, *Leontice* and *Gynnospermium* form the x=8 group (Kim & Jansen, 1998; Kim *et al.*, 2004).

The Argentinean species of simple-leaved *Berberis* have been relatively well studied cytologically (Bottini *et al.*, 1997; Bottini *et al.*, 1999; Bottini *et al.*, 2000), and most have 2n=28 chromosomes (Bottini *et al.*, 1999). Higher ploidy levels were reported only for *B. buxifolia* and *B. heterophylla*, both with 2n=56 (Bottini *et al.*, 1997).

The chromosome numbers of Nepalese species of simple-leaved *Berberis* species are almost completely unknown. Out of the 21 simple-leaved species in Nepal, chromosome counts for only one species (*B. asiatica*) have been published so far from a plant of Nepalese origin. For other Nepalese species such as *B. aristata*, *B. angulosa*, *B. concinna* and *B. wallichiana*, the published counts were based on either plants of Indian origin or a cultivated specimen of unknown origin. Regarding compound leaved-species, one species, *B. napaulensis* has been counted so far. The published chromosome numbers for Nepalese species of *Berberis* and some closely related species from the Himalaya is shown in Table 5.1.

The main objectives of this study are to determine the chromosome numbers of Nepalese species of *Berberis* and to investigate the possible role of polyploidy in the evolution and diversification of simple-leaved species of *Berberis* in this group. Furthermore, the presence of hybrid allopolyploids complicates the phylogenetic analysis of species relationships. Therefore, investigating ploidy level in Nepalese *Berberis* is important to evaluate the accuracy of the phylogenetic inferences made in Chapter 3 and 4.

Table 5.1. List of published chromosome numbers for Nepalese *Berberis* and closely related species from the Himalaya with information on origin and references. IPCNR: Index to Plant Chromosome Number Report. TROPICOS: www.tropicos.org. NA: Not available. PMC: Pollen mother cell

Taxa	**Chromosome no.**		**Ploidy level**	**Origin**	**Primary References**	**Secondary References, database**	**Methods**
	n	**2n**					
B. angulosa Wall. ex Hook.f. & Thoms.		28	Diploid	Not known	Giffen, H (1936)	Bolkhovskikh *et al.* (1969)	Root tip
B. angulosa Wall. ex Hook.f. & Thoms.	14			Not known	Sharma, A.K (1970) Original not seen	IPCNR (1975-1978), TROPICOS	NA
B. aristata DC.		28	Diploid	Not known	Giffen, H (1936)	Bolkhovskikh *et al.* (1969)	Root tip
B. aristata DC.	14		Diploid	Not known	Sandhu, P.S. & S.K. Mann (1988)	IPCNR (1988-1989), TROPICOS	NA
B. aristata DC.	14		Diploid	Not known	Gill *et al.* (1984)	IPCNR (1984-1985)	NA
B. aristata DC.	14		Diploid	Not known	Mehra, P.N. (1976)	IPCNR (1975-1978), TROPICOS	NA
B. aristata DC.	14		Diploid	India: West Himalayas	Mehra, P.N. & Sareen, T.S. (1969)	IPCNR (1967-1971)	NA
B. aristata DC.	14		Diploid	Not known	Mehra, P.N. & Sareen, T.S. (1973)	IPCNR (1973/1974)	NA
B. asiatica Roxb. ex DC.	14		Diploid	India: Central	Singhal & Gill (1984)	IPCNR (1986-1987), TROPICOS	NA
B. asiatica Roxb. ex DC.	14		Diploid	Not known	Gill *et al.* (1984)	IPCNR (1984-1985)	NA
B. asiatica Roxb. ex DC.	14		Diploid	Nepal: Godawari	Malla *et al.* (1975)	IPCNR (1975-1978), TROPICOS	NA
B. chitria Lindl.	14		Diploid	Not known	Gill *et al.* (1984)	IPCNR (1984-1985)	NA
B. chitria Lindl. var. *chitria*	14		Diploid	India: Uttar Pradesh	Singhal *et al.* (1980)	IPCNR (1979-1981), Khatoon, S & Ali, S.I. (1993), TROPICOS	NA
B. chitria var. *occidentalis* Ahrendt	14		Diploid	India: Uttar Pradesh	Singhal *et al.* (1980)	IPCNR (1979-1981), TROPICOS	NA
B. chitria var. *occidentalis* Ahrendt	14		Diploid	Not known	Gill *et al.* (1984)	IPCNR (1984-1985)	NA
B. concinna Hook.f.		28	Diploid	Not known	Giffen, H (1936)	Bolkhovskikh *et al.* (1969)	Root tip
B. coriaria Royle	14		Diploid	Not known	Sandhu, P.S. & S.K. Mann (1989)	IPCNR (1988-1989), TROPICOS	NA
B. lycium Royle	14		Diploid	Not known	Giffen, H (1936)	Bolkhovskikh *et al.* (1969)	PMC
B. lycium Royle	14		Diploid	India: Kashmir Himalaya	Jee *et al.* (1989)	IPCNR (1990-1991), TROPICOS	PMC

Table 5.1. Continued

Taxa	**Chromosome no.**		**Ploidy level**	**Origin**	**Primary References**	**Secondary References, database**	**Methods**
	n	**2n**					
B. lycium Royle	14		Diploid	Not known	Sandhu, P.S. & S.K. Mann (1988)	IPCNR (1988-1989), TROPICOS	NA
B. lycium Royle var. *lycium*	14		Diploid	India: Uttar Pradesh	Singhal *et al.* (1980)	IPCNR (1979-1981), Khatoon, S & Ali, S.I. (1993), TROPICOS	NA
B. lycium Royle var. *lycium*	14		Diploid	Not known	Gill *et al.* (1984)	IPCNR (1984-1985)	NA
B. lycium Royle var. *subfascicularis* Ahrendt	14		Diploid	India: Uttar Pradesh	Singhal *et al.* (1980)	IPCNR (1979-1981), TROPICOS	NA
B. lycium Royle var. *subfascicularis* Ahrendt	14		Diploid	Not known	Gill *et al.* (1984)	IPCNR (1984-1985)	NA
B. napaulensis (DC.) Laferr.		28	Diploid	Not known	Sharma, A.K (1970). Original not seen.	IPCNR (1975-1978)	NA
B. napaulensis (DC.) Laferr.		28	Diploid	Nepal: Godawari	Malla *et al.* (1974)	IPCNR (1973-1974)	NA
B. orthobotrys Bienert ex Aitch. subsp *orthobotrys*	24		Hypotetraploid	Pakisthan, Thalle	Khatoon, S (1991) (PhD Thesis). Original not seen.	Khatoon, S & Ali, S.I. (1993), TROPICOS	NA
B. ulicina Hook.f. & Thoms.	14		Diploid	India (Kashmir Himalaya)	Jee *et al.* (1989)	IPCNR (1990-1991), TROPICOS	PMC
B. umbellata Wall. ex G. Don	14		Diploid	India, Uttar Pradesh	Singhal *et al.* (1980)	IPCNR (1979-1981), Khatoon, S & Ali, S.I. (1993), TROPICOS	NA
B. umbellata Wall. ex G. Don	14		Diploid	India: West Himalayas	Bir & Thakur (1984)	IPCNR (1986-1987), TROPICOS	NA
B. umbellata Wall. ex G. Don	14		Diploid	Not known	Gill *et al.* (1984)	IPCNR (1984-1985)	NA
B. wallichiana DC.	14		Diploid	Not known	Sharma, A.K (1970). Original not seen.	IPCNR (1975-1978), TROPICOS	NA

5.2 MATERIALS AND METHODS

Chromosomes were counted in roots harvested from plants growing in the living collections at the Royal Botanic Garden Edinburgh (RBGE). All collections were grown from seeds of wild origin collected in Nepal. Prior to harvesting roots, plants were re-potted with new compost and transferred to an environmentally controlled glasshouse (average day temp 18°C and night temp 16°C) to stimulate root growth.

Two pretreatments were applied to prevent the formation of mitotic spindles (Jong, 1997): α-bromo-naphthaline for 3hrs at room temperature and 0.002M 8-hydroxyquinolene for 5 hrs at 4^0 C. 8-hydroxyquinolene gave better chromosome spread than α-bromo-naphthalene. As root growth is sensitive to light, pretreatments were carried out in the dark to prevent any deleterious effect of light. Root tips were then fixed with freshly prepared Farmer's Fluid (3:1, absolute ethanol: glacial acetic acid) and kept at 4°C overnight.

Root tips were rinsed in distilled water and transferred to 5M HCl for hydrolysis. They were then washed for 1 minute in distilled water to remove acid and transferred to Feulgen reagent prepared according to Fox (1969; see Jong, 1997) for 2-3 hours in the dark. The root tips were then washed in tap water twice and softening of the root was carried out in a 1:1 enzyme mixture of 4% cellulose and 4% pectinase for 30 min at 35° C.

Each root tip meristem was excised onto a slide with a few drops of acetocarmine (0.04% counter stain). A coverslip was then placed carefully over the root tip which was first dispersed with a chopstick, and then the slide was placed within a folded filter paper and squashed using the thumb. The coverslip was then sealed with rubber solution to prevent drying out. This gave a semi-permanent preparation that allowed observation and some manipulation of the slides. Chromosomes were observed with a Zeiss Axioskop microscope and images were taken at different magnifications using AxioVision Rel. 4.7 (Carl Zeiss MicroImaging GmbH, Germany). Chromosomes were counted in at least 5 different cells to confirm the number.

The most informative slides were made permanent using the alcohol exchange method without the removal of coverslips as described in Jong (1997).

5.3 RESULTS

Chromosome counts from the present study are listed in Table 5.2. All chromosome numbers found in this study were n=14.

1. ***Berberis angulosa*** Wall. ex Hook.f. & Thoms.

Berberis angulosa is distributed in central and Eastern Nepal. It grows above 3000 m in dry open places in Nepal, and also in the Eastern Himalayas and the Tibetan Plateau. Root tips were collected from plants grow from seeds collected in the Rasuwa district of Central Nepal at 3525 m. It has a somatic chromosome number of 2n=28 (Fig. 5.1 A). Large satellites were prominent in prometaphasic stages of mitotic divisions. This report of a chromosome number of 2n=28 for *B. angulosa* from Nepal is consistent with previous reports by Giffen (1936) and Sharma (1970), though this is the first report of chromosome numbers for this species from Nepal.

Table 5.2. Chromosomes counts of *Berberis* taxa obtained in this study.

Taxon	Accession	Origin	2n	Fig.
B. angulosa Wall. ex Hook.f. & Thoms.	20071214A	Nepal: Rasuwa, near Cholangpati	28	5.1 A
B. aristata DC.	20071210A	Nepal: Rasuwa, Dovan	28	5.1 B
B. aristata DC.	20071210B	Nepal: Rasuwa, Dovan	28	5.1 C
B. concinna Hook.f.	20071213	Nepal: Rasuwa, near Cholangpati	28	5.1 D
B. concinna Hook.f. var. *extensiflora* Ahrendt	20071208A	Nepal: Myagdi, Ghorepani	28	5.2 A
B. jaeschkeana C.K.Schneid. var. *usteriana* C.K.Schneid.	20071203B	Nepal: Mustang, Kaligandaki	28	5.2 B
B. jaeschkeana C.K.Schneid var. *usteriana* C.K.Schneid.	20071203A	Nepal: Mustang, Kaligandaki	28	5.2 C
B. mucrifolia Ahrendt	20071201A	Nepal: Mustang, Kaligandaki	28	5.2 D
B. pendryi Adhikari	20071200C	Nepal: Mustang, Muktinath	28	5.3 A
B. thomsoniana C.K. Schneid.	20071212A	Nepal: Rasuwa, near Chandanbari	28	5.3 B
B. thomsoniana C.K. Schneid.	20071212B	Nepal: Rasuwa, near Chandanbari	28	5.3 C
B. wallichiana DC.	20071211A	Nepal: Rasuwa, above Deurali	28	5.3 D
B. wallichiana DC.	20071211B	Nepal: Rasuwa, above Deurali	28	5.4

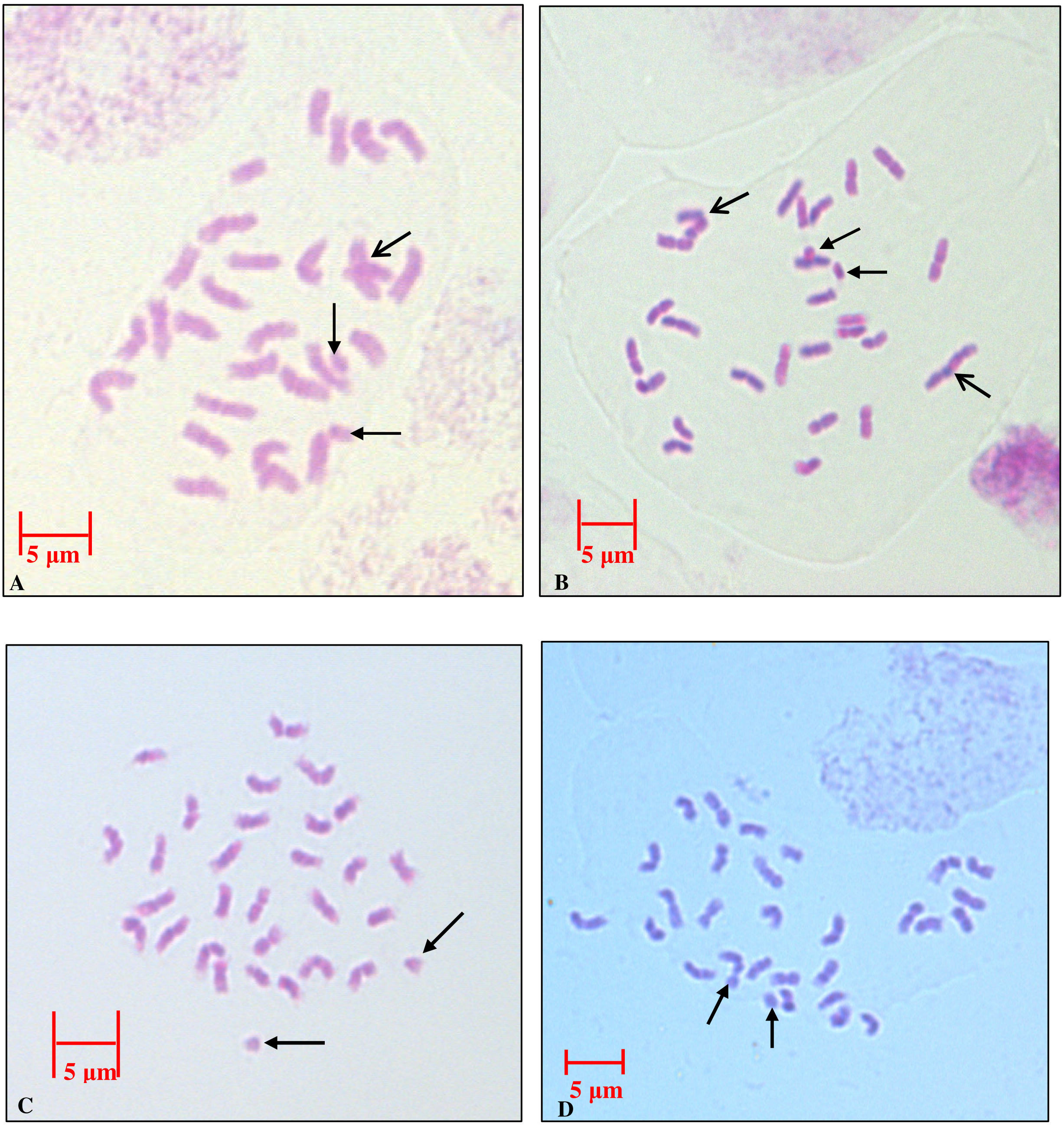


Fig 5.1 Mitotic chromosomes of *Berberis*. A. *B. angulosa* (20071214 A), prometaphase, 2n=28. B. *B. aristata* (20071210 A) metaphase, 2n=28. C. *B. aristata* (20071210 B), prometaphase, 2n=28. D. *B. concinna* (20071213) prometaphase, 2n=28. ⟶ satellites; ⟶ overlapping chromosomes.

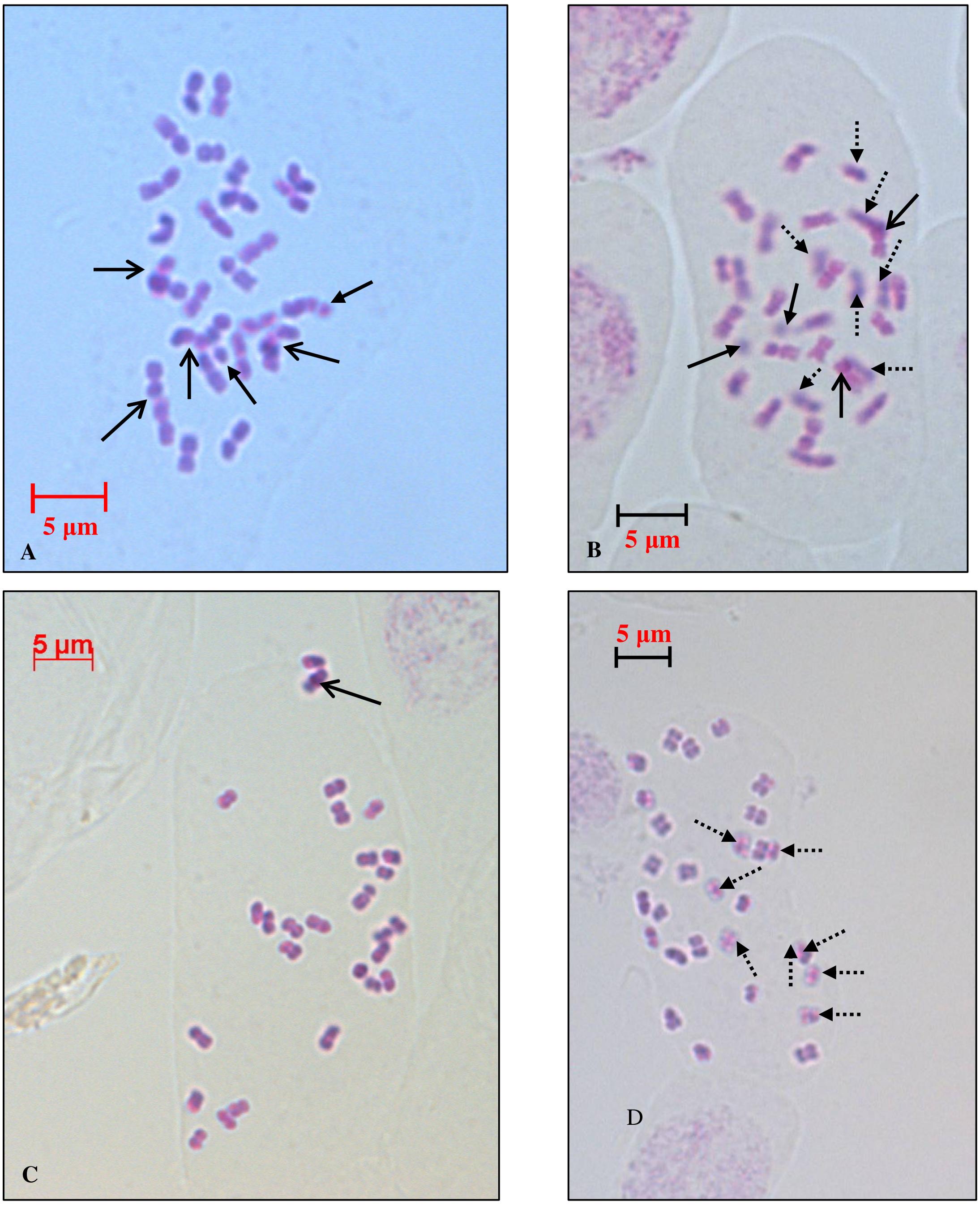


Fig 5.2 Mitotic chromosomes of *Berberis*. A. *B. concinna* var. *extensiflora* (20071208 A), 2n=28. B. *B. jaeschkeana* var. *usteriana* (20071203 B), 2n=28. C. *B. jaeschkeana* var. *usteriana* (20071203 A), 2n=28. D. *B. mucrifolia* (20071201 A) 2n=28. ⟶ satellites, → overlapping chromosomes, ⋯▸ out of focus chromosomes.

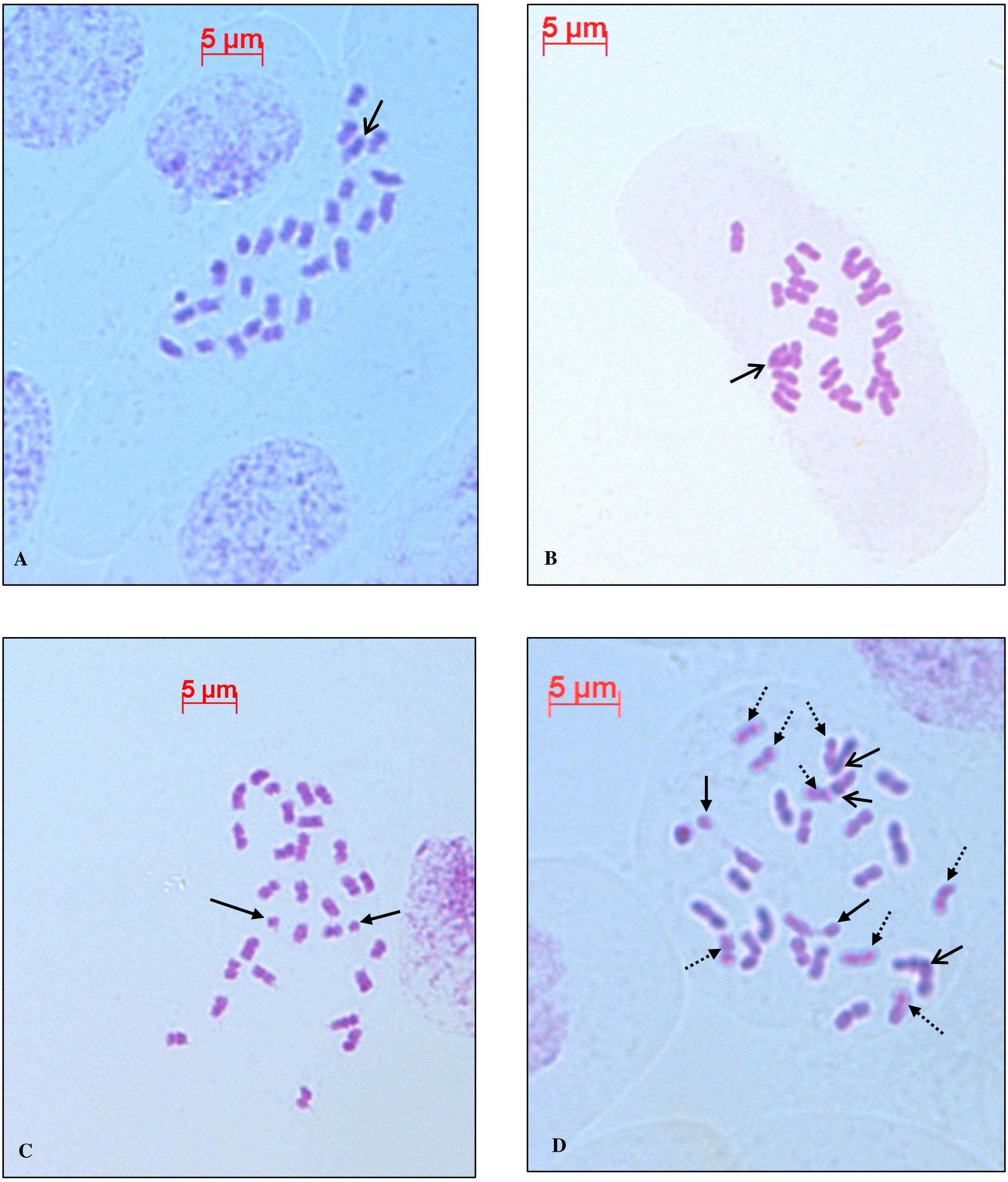


Fig 5.3 Mitotic chromosomes of *Berberis*. A. *B. pendryi* (20071200C), 2n=28. B. *B. thomsoniana* (20071212A), 2n=28. C. *B. thomsoniana* (20071212 B), 2n=28. D. *B. wallichiana* DC. (20071211A) 2n=28. ⟶ satellite, → overlap, ······► out of focus.

Fig 5.4. Mitotic chromosomes of *Berberis wallichiana* DC. (20071211B) 2n=28.
⟶ satellites

2. ***Berberis aristata*** DC.

This species is widely distributed across the whole range of the Himalayas, from 1300 m up to 3400 m. Seeds of this species were collected from Rasuwa, Dovan of Central Nepal at 2000 m. Root tips from two individuals of the same accession were examined and both had chromosome numbers of 2n=28 (Fig 5.1 B, C). Satellites were prominent in all spreads. In Fig 5.1 C, the chromosomes are in prometaphasic stages and the satellites are detached. The present count of 2n=28 agrees with previous reports by Giffen (1936), Sandhu and Mann (1988), Gill *et al.* (1984), Mehra (1976), and Mehra & Sareen (1969, 1973). This is the first report of chromosome number for this species from Nepal.

3. ***Berberis concinna*** Hook.f.

This species grows on dry, sunny slopes between 2500-4500 m in Nepal and in the Eastern Himalayas. Seeds of this species were collected from Rasuwa district of Central Nepal at 3400 m. The present count confirms the previous count of 2n=28 by Giffen (1936). Satellites are prominent in Fig. 5.1 D. This is the first chromosome count for this species from Nepal.

4. ***Berberis concinna*** Hook.f. var. ***extensiflora*** Ahrendt

This variety of *B. concinna* grows in Central Nepal and also in the Eastern Himalayas above 3000 m. Seeds were collected from the Myagdi district of Central Nepal at 3100 m. A somatic chromosome number of 2n=28 was counted for this species (Fig 5.2A). Satellites are prominent in Fig 5.2 A. This is the first report of the chromosome number for *B. concinna* var. *extensiflora.*

5. ***Berberis jaeschkeana*** C.K.Schneid. var. ***usteriana*** C.K.Schneid.

This variety of *B. jaeschkeana* is distributed in the drier regions of Central and Western Nepal and in the Western Himalayas above 2500 m. Seeds were collected from the Kaligandaki Valley of Central Nepal at 2850 m. Root tips from two different individuals of the same accession were examined and both had a somatic chromosome number of 2n=28. Satellites were prominent in prometaphase (Fig 5.2 B) squashes but not so clear in metaphase stages (Fig 5.2 A). This is the first report of the chromosome number for *B. jaeschkeana*.

6. ***Berberis mucrifolia*** Ahrendt

This species is known only from the Nepal Himalaya and grows mainly above 2500 m. Root tips were collected from a plant that was grown from seeds collected in the Kaligandaki valley of Central Nepal at 2800 m. A somatic chromosome number of 2n=28 was counted for this species (Fig 5.2 D). This is the first report of the chromosome number for this species.

7. ***Berberis pendryi*** Adhikari

Berberis pendryi is known only from Muktinath area of Central Nepal growing at 3500 m, from where seeds were collected. A somatic chromosome number documented was 2n=28 (Fig 5.3 A), and this is the first report of the chromosome number for this newly described species.

8. ***Berberis thomsoniana*** C.K.Schneid.

Berberis thomsoniana is found throughout Nepal and also in the Eastern Himalayas and usually grows above 3000 m. Seeds of this species were collected from the

Langtang region in Central Nepal at 3170 m. Root tips of two individuals from the same accession were examined and both had the diploid chromosome number 2n=28. Satellites were prominent in prometaphase chromosomes (Fig. 5.3 C) but not in metaphase chromosomes (Fig 5.3 B). This is the first report of the chromosome number for *B. thomsoniana*.

9. ***Berberis wallichiana*** DC.

Berberis wallichiana, an evergreen species of *Berberis,* is distributed in Central and Eastern Nepal and in the Eastern Himalayas between 2000-3500 m. Seeds of this species were collected from the Langtang region of Central Nepal at 2700 m. Root tips of two individuals from the same accession were examined and both had a somatic chromosome number 2n=28. Satellites were prominent in both prometaphasic and metaphasic chromosomes (Fig 5.3 D & Fig 5.4). The satellite stalk was clearly visible in the metaphasic chromosomes (Fig. 5.3 D). This count agrees with previous count by Shrama (1970), and is the first count for this species from Nepal.

5.4 DISCUSSION AND CONCLUSIONS

This is the first report of the chromosome numbers of nine taxa of *Berberis* from the Nepal Himalaya. Among them, five taxa (*B. concinna* var. *extensiflora*, *B. jaeschkeana* var. *usteriana*, *B. mucrifolia*, *B. pendryi* and *B. thomsoniana*) were counted for the first time.

All the taxa examined in this study were found to be 2n=28. Though polyploids seem to play an important role in speciation and diversification of some high alititude taxa (Janaki Ammal, 1950; Wakabayashi & Ohba, 1999; Nie *et al.*, 2005; Meng *et al.*, 2010), the genus *Berberis* does not seem to follow this trend as not a single polyploid was observed among the species examined. This agrees with the findings of Wakabyashi and Ohba (1988) in *Saxifraga*, in which a low number of polyploids suggested that polyploidy is not an important factor in species diversification in the alpine flora of Nepal Himalaya (Ohba, 1988; Wakabayashi & Ohba, 1988). However, further studies in other taxa are needed to investigate the role of polyploidy in the speciation and diversification of Himalayan plants.

Most of the *Berberis* species examined during this study grow between 2500 and 4500 m in different types of environmental conditions. Examining only eight species out of 21 cannot rule out the presence of polyploids among Nepalese *Berberis*, but it suggest that polyploidy does not seem to play an important role in the diversification of Nepalese *Berberis*.

The congruence of nuclear and plastid phylogenies in Chpater 3 and 4 is further supported by the absence of polyploids in Nepalese *Berberis*. However, as the ITS and *ndhF* phylogenies are not well resolved at species level and this investigation of polyploidy only covers a limited number of taxa, the possibility of reticulate evolution in *Berberis* cannot be completely ruled out.

CHAPTER 6: FINAL CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE WORK

6.1 TAXONOMY OF NEPALESE *BERBERIS*

This study recognises 21 species of *Berberis s.s.* in Nepal compared with over 30 species reported in recent enumerations (Tebbs, 1979; Press *et al*. 2000; Bista *et al.* 2001). Two species, *Berberis pendryi* and *Berberis karnaliensis*, are newly described, but seven taxa are reduced to synonymy. Nine taxa are lectotypified. For species delimitation, floral characters provide the most important diagnostic characters, in particular inflorescence type, petal shape, connective shape, colour of berries, presence or absence of a style on the berries, and the number of ovules. Although the shape and size of leaves can greatly vary within the species, they are useful in distinguishing some species such as *B. asiatica* and *B. insignis*.

This study is mainly based on Nepalese species of *Berberis*. To resolve the taxonomic problems of some species like *B. orthobotrys* and *B. aristata*, a more extensive study is required which could cover the whole geographical range of these widespread species.

Hybridization is thought to be common in *Berberis*. However, during this study only a few individuals with morphologically intermediate forms were observed in the wild populations which might be hybrids. These forms were usually found in sympatric populations of different species of wide distribution, for example, the populations of *B. aristata* and *B. thomsoniana* in central Nepal. To detect hybridization in wild populations of *Berberis*, population level studies on selected groups are necessary based on molecular markers such as amplified fragment length polymorphisms (AFLP).

6.2 ORIGIN OF DISJUNCTION PATTERNS IN *BERBERIS S.L.*

Both nuclear and plastid data suggest the North American origin of *Berberis s.l.* which is also supported by the presence of the oldest fossils of *Berberis* which are found in North America. The dated phylogeny indicates that the disjunction pattern in compound-leaved species of *Berberis* is more likely to have arisen via the North Atlantic Land Bridge, and the origin of simple-leaved South American species was from a dispersal event from Eurasia. These data do not support Kim's (2004b) hypothesis of an ancient vicariance origin for the simple-leaved South American *Berberis*.

Any further biogeographical study should increase taxon sampling with multiple accessions of taxa which could provide a better understanding of the origin of simple-leaved species of *Berberis*. A thorough study of *Berberis* fossils is important in historical biogeographic studies to understand the timing of diversification and past extinction events.

6.3 SECTIONAL CIRCUMSCRIPTIONS IN *BERBERIS*

The sectional circumscription of *Berberis* in the most recent monograph of the genus (Ahrendt, 1961) is incompatible with the present phylogenetic analysis. The incongruence of these sections with molecular data has also been reported by previous authors (Kim *et al.*, 2004 b; Todd, 2006). Some monophyletic clades supported by morphological characters in this study may indicate new routes for the classification of *Berberis s.l.*

Species-level relationships are only partially resolved in the ITS and *ndhF* phylogenies. Different sampling strategies were employed for these two regions utilizing different numbers of accessions. Sixty-seven *ndh*F sequences were analysed to study the worldwide historical biogeography of *Berberis*, whereas ninety-four ITS sequences were analysed to specifically study the diversification of *Berberis* in Nepal. Due to this discrepancy in sampling strategy it was not deemed appropriate to combine datsets in this thesis. However, combined analysis will be performed with North American species of *Berberis* in future publications. As the *ndh*F and ITS phylogeneis are largely congruent, inferences drawn from a combined analysis will most likely be the same as the results from this study. Any further molecular studies should use a multiple gene approach with both nuclear and chloroplast genes. Low copy nuclear genes like *LEAFY*, *WAXY, PHY* along with other chloroplast markers may provide better understanding of species level relationships, as they have done in studies of *Nolana*, *Amorphophallus* and *Verbena* (Grob *et al.* 2004; Tu *et al.*, 2008, Yuan & Olmstead, 2008).

6.4 ORIGIN OF NEPALESE SPECIES OF *BERBERIS S.S*

The nuclear ribosomal ITS sequence data indicates that Nepalese *Berberis* are derived from at least four different colonization events. The dated phylogeny indicates that the speciation of some species of Nepalese *Berberis* coincided with uplift of the mountains during the Miocene. The tendency of phylogentically closely related Nepalese species to grow in similar ecological conditions is evidence for phylogenetic niche conservatism. Most New World *Berberis* species grow in the wetter part of southwestern South America (Chile, Argentina) with only few species growing in drier regions in northern Chile. Further studies on South American *Berberis* would help to identify whether phylogenetic niche conservatism is evident in New World *Berberis*.

This is the first dated phylogenetic study carried out in the Nepal Himalaya to investigate the possible link between past geological events and the diversification of Nepalese plant species. The uplift of the mountains in the Miocene probably played an important role in the diversification of many taxa in the Nepal Himalaya, but more dated phylogenies from other groups are needed to clarify the effect of these events. Multiple studies would permit a meta-analysis approach to seek common patterns in phylogenetically unrelated groups, which would be indicative of common underlying drivers of diversification.

Dated phylogenies of large shrubby genera like *Rhododendron* (Ericaceae) and *Astragalus* (Leguminosae) with extensive sampling from the Himalaya would be helpful in understanding the relative contributions of Pleistocene glaciation and Himalayan uplift in the diversification of Himalayan plants. In the Nepal Himalaya only, about 30 species of *Rhododendron* and *Astralgalus* grow from 1500 m up to 5600 m altitude. Moreover, *Astragalus* is also present in the Andes and previously published data on New World species (Scherson *et al*., 2008) will aid comparative studies in the diversification of plants in these two young mountains. Though the geological histories of these mountains are different, comparative studies in different groups will give further insights into the effect of past geological events and associated climate change in the diversification of species in these geologically active regions.

The biogeographical divisions of Nepal proposed by different authors (Stearn, 1960; Dobremez, 1972; Stainton, 1972), and their evolutionary significance have not been tested yet using molecular phylogenies. Several plant groups which grow throughout the Nepal Himalaya from the wetter part of Eastern Nepal to the arid regions of the Trans-Himalayan regions can be used to study the evolutionary interchanges of species between these biomes. The genus *Artemisia* (Asteraceae) could be an ideal taxon to study the niche conservatism and ecological switching between biomes because *ca*. 20 species of Nepalese *Artemisia* are distributed across the Nepal Himalaya from as low as 300 m (*Artemisia indica* Willd) up to 5000 m (*Artemisia stricta* Edgew.).

Any further molecular studies in the Nepal Himalaya should include samples from across the Himalayan range rather than being constrained by political boundaries to better understand the species relationships and effect of Himalayan orogeny in plant diversification.

6.5 CHROMOSOME NUMBERS IN NEPALESE SPECIES OF BERBERIS

Chromosomes are counted for nine taxa of simple-leaved species of *Berberis* from Nepal. All are first reports of chromosome numbers of these taxa from Nepal and five taxa are counted for the first time. The absence of polyploids in Nepalese species of *Berberis* suggests that the polyploidy is not a major factor in the diversification of Nepalese *Berberis*. Moreover, the absence of polyploids and congruence of nuclear and plastid phylogenies tend support to the notion that the inferences drawn from the phylogenies in this study are robust and reflect underlying evolutionary history.

Any future chromosome study in *Berberis* should include multiple accessions from different populations to study genome evolutions. Techniques like genomic insitu hybridisation (GISH) and fluorescence in situ hybridization (FISH) may be useful to study the hybridization in *Berberis*.

BIBLIOGRAPHY

AHRENDT, L. W. A. (1941-1942). A survey of the genus *Berberis* L. in Asia. *Journal of Botany* (Supplement): 1-116.

AHRENDT, L. W. A. (1944). Newcomers to the Barberries-II, old and new species from casual collections. *Gardening Illustrated* 64: 425-426.

AHRENDT, L. W. A. (1956). A new dwarf Barberry from Nepal. *Journal of the Royal Horticultural Society* 81: 135.

AHRENDT, L. W. A. (1961). *Berberis* and *Mahonia*: A taxonomic revision. *Journal of the Linnean Society Botany* 57: 1-410.

AIRY SHAW, H. K. (1965). Diagnoses of new families, new names, etc., for the seventh edition of Willis's Dictionary. *Kew Bulletin* 18: 249-273.

AMANO, K. & TAIRA, A. (1992). Two-phase uplift of Higher Himalayas since 17 Ma. *Geology* 20(5): 391-394.

ANDERSON, C. L., BREMER, K. & FRIIS, E. M. (2005). Dating phylogenetically basal eudicots using *rbcL* sequences and multiple fossil reference points. *American Journal of Botany* 92(10): 1737-1748.

ANGIOSPERM PHYLOGENY GROUP (2003). An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG II. *Botanical Journal of the Linnean Society* 141: 399-436.

ANGIOSPERM PHYLOGENY GROUP (2009). An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. *Botanical Journal of the Linnean Society* 161(2): 105-121.

AXELROD, D. I. (1987). The late Oligocene Creede flora. *Colorado University California Publication Geological Sciences* 130: 1-235.

AXELROD, D. I. (1998a). The Eocene Thunder Mountain flora of Central Idaho. *University of California Publications Geological Sciences* 142: 1-61.

AXELROD, D. I. (1998b). The Oligocene Haynes Creek flora of Eastern Idaho. *University of California Publications Geological Sciences* 153: 1-235.

BALDWIN, B. G., SANDERSON, M. J., PORTER, J. M., WOJCIECHOWSKI, M. F., CAMPBELL, C. S. & DONOGHUE, M. J. (1995). The ITS region of nuclear ribosomal DNA: A valuable source of evidence on angiosperm phylogeny. *Annals of the Missouri Botanical Garden* 82(2): 247-277.

BAUM, D. A. & DONOGHUE, M. J. (1995). Choosing among alternative "Phylogenetic" species concepts. *Systematic Botany* 20(4): 560-573.

BECK, R. A., BURBANK, D. W., SERCOMBE, W. J., RILEY, G. W., BARNDT, J. K., BERRY, J. R., AFZAL, J., KHAN, A. M., JURGEN, H., METJE, J., CHEEMA, A., SHAFIQUE, N. A., LAWRENCE, R. D. & KHAN, M. A. (1995). Stratigraphic evidence for an early collision between northwest India and Asia. *Nature* 373(6509): 55-58.

BIR, S. S. & THAKUR, H. (1984). SOCGII plant chromosomes number report-II. *Journal of Cytology and Genetics* 19: 114-115.

BISTA, M. S., ADHIKARI, M.K., & RAJBHANDARI, K.R, Ed. (2001). Flowering Plants of Nepal (Phanerogams). *Bulletin of Department of Plant Resources* No.18. Department of Plant Resources, National Herbarium and Plant Laboratories, Kathmandu, Nepal.

BOLKHOVSKIKH, Z., GRIF, V., MATVEJEVA, T. & ZAKHARYEVA, O. (1969). *Khromosomnye chisla tsvetkovykh rastenii.* [*Chromosome numbers of flowering plants*]. Izdatel'stvo 'Nauka' Leningradskoe Otdelenyi, Leningrad.

BOTTINI, M. C. J., GREIZERSTEIN, E. J. & POGGIO, L. (1997). Numeros chromosomicos y contenido de adn de cuatro especies patagonicas del genero *Berberis (Berberidaceae)*. *Boletin de la Sociedad Argentina de Botanica* 32(3-4): 235-239.

BOTTINI, M. C. J., GREIZERSTEIN, E. J. & POGGIO, L. (1999). Ploidy levels and their relationships with the rainfall in several populations of Patagonian species of *Berberis* L. *Caryologia* 52: 75-80.

BOTTINI, M. C. J., GREIZERSTEIN, E. J., AULICINO, M. B. & POGGIO, L. (2000). Relationships among genome size, environmental conditions and geographical distribution in natural populations of NW Patagonian species of *Berberis* L. (Berberidaceae). *Annals of Botany* 86(3): 565-573.

CANDOLLE, A. P. DE (1821). *Regni Vegetabilis Systema Naturale.* Vol.2. Parisiis Sumptibus Sociorum Treuttel et Würtz.

CANDOLLE, A. P. DE (1824). *Prodromus Systematis Naturalis Regni Vegetabilis*. Vol.1. Parisiis Sumptibus Sociorum Treuttel et Würtz.

CARLQUIST, S. (1995). Wood anatomy of Berberidaceae: Ecological and phylogenetic considerations. *Aliso* 14(2): 85-103.

CATHERINE, J. K. (2004). A preliminary assessment of internal deformation in the Indian plate from GPS measurements. *Journal of Asian Earth Sciences* 23(4): 461-465.

CHAMBERLAIN, D. F. & HU, C. M. (1985). A synopsis of *Berberis* section *Wallichianae*. *Notes from the Royal Botanic Garden Edinburgh* 42(3): 529-557.

CHAMBERS, K. L. (1963). Amphitropical species pairs in *Microseris* and *Agoseris* (Compositae: Cichorieae). *The Quarterly Review of Biology* 38(2): 124-140.

CHATTERJEE, R. (1953). Studies in Indian Berberidaceae from botanical, chemical and pharmaceutical aspects. *Records of the Botanical Survey of India* 16(2): 1-42.

CLARK, L. G., ZHANG, W. & WENDEL, J. F. (1995). A Phylogeny of the grass family (Poaceae) based on *ndh*F sequence data. *Systematic Botany* 20(4): 436-460.

COPELAND, P. & HARRISON, T. M. (1990). Episodic rapid uplift in the Himalaya revealed by 40Ar/39Ar analysis of detrital K-feldspar and muscovite, Bengal fan. *Geology* 18(4): 354-357.

COPELAND, P., AMANO, K. & TAIRA, A. (1993). Two-phase uplift of Higher Himalayas since 17 Ma: Comment and reply. *Geology* 21(2): 188-190.

COPELAND, P., MARK HARRISON, T., KIDD, W. S. F., XU, R. & ZHANG, Y. (1987). Rapid early Miocene acceleration of uplift in the Gangdese belt, Xizang (southern Tibet), and its bearing on accommodation mechanisms of the India-Asia collision. *Earth and Planetary Science Letters* 86(2-4): 240-252.

COUVREUR, T., CHATROU, L., SOSEF, M. & RICHARDSON, J. (2008). Molecular phylogenetics reveal multiple tertiary vicariance origins of the African rain forest trees. *BMC Biology* 6(1): 54.

COX, C. B. (1990). Guest essay: New geological theories and old biogeographical problems. *Journal of Biogeography* 17(2): 117-130.

CRISP, M. D., ARROYO, M. T. K., COOK, L. G., GANDOLFO, M. A., JORDAN, G. J., MCGLONE, M. S., WESTON, P. H., WESTOBY, M., WILF, P. & LINDER, H. P. (2009). Phylogenetic biome conservatism on a global scale. *Nature* 458(7239): 754-756.

DAVIS, C. C., BELL, C. D., MATHEWS, S. & DONOGHUE, M. J. (2002). Laurasian migration explains Gondwanan disjunctions: Evidence from Malpighiaceae. *Proceedings of the National Academy of Sciences of the United States of America* 99(10): 6833-6837.

DE QUEIROZ, K. (2007). Species concepts and species delimitation. *Systematic Biology* 56(6): 879-886.

DE QUEIROZ, K. & DONOGHUE, M. J. (1988). Phylogenetic systematics and the species problem. *Cladistics* 4(4): 317-338.

DERMAN, H. (1931). A study of chromosome number in two genera of *Berberidaceae*: *Mahonia* and *Berberis*. *Journal of the Arnold Arboretum* 12: 281-287.

DON, D. (1825). *Prodromus Florae Nepalensis*. Londini.

DON, G. (1831). *A general history of the dichlamydeous plants.* Vol. 1. London: J.G. and F. Rivington.

DONOGHUE, M. J. & SMITH, S. A. (2004). Patterns in the assembly of temperate forests around the Northern Hemisphere. *Philosophical Transactions: Biological Sciences* 359(1450): 1633-1644.

DONOGHUE, M. J. (1985). A critique of the biological species concept and recommendations for a phylogenetic alternative. *The Bryologist* 88: 172-181.

DONOGHUE, M. J., BELL, C. D. & LI, J. (2001). Phylogenetics patterns in Northern Hemisphere plant geography. *International Journal of Plant Science* 162: S41-S52.

DOUADY, C. J., DELSUC, F., BOUCHER, Y., DOOLITTLE, W. F. & DOUZERY, E. J. P. (2003). Comparison of Bayesian and maximum likelihood bootstrap measures of phylogenetic reliability. *Molecular Biology and Evolution* 20(2): 248-254.

DOYLE, J. J. & DOYLE, J. L. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* 19: 11-15.

DRUMMOND, A. & RAMBAUT, A. (2007). BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology* 7(1): 214.

DRUMMOND, A. J., HO, S. Y. W., PHILLIPS, M. J. & RAMBAUT, A. (2006). Relaxed phylogenetics and dating with confidence. *PLoS Biol* 4(5): e88.

DRUMMOND, A. J., HO, S. Y. W., RAWLENCE, N. & RAMBAUT, A. (2007). *Beast Manual: A rough guide to BEAST 1.4*. Available from http://beast.bio.ed.ac.uk.

DU RIETZ, G. E. (1930). The fundamental units of biological taxonomy. *Svensk Botanisk Tidskrift* 24: 333-428.

ELDREDGE, N. & CRACRAFT, J. (1980). *Phylogenetic patterns and the evolutionary process: Methods and theory in comparative biology*. Columbia University Press, New York.

ERWIN, D. M. & SCHORN, H. E. (2000). Floristic revision of the Eocene Thunder Mountain megaflora of Idaho. *Botany 2000 (Abstract).*

FEDDE, F. (1902). Versuch einer Monographie der Gattung *Mahonia*. *Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie. Leipzig* 31: 30-131.

FELSENSTEIN, J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* 39(4): 783-791.

FERGUSON, D. M. (1998). Phylogenetic analysis and relationships in Hydrophyllaceae based on *ndhF* sequence data. *Systematic Botany* 23(3): 253-268.

FOX, D. P. (1969). Some characteristics of the cold hydrolysis technique for staining plant tissue by the Feulgen reaction. *Journal of Histochemistry and Cytochemistry* 17(4): 266-272.

FRASER-JENKINS, C. R. (2005). *The first botanical collectors in Nepal-The Fern collections of Hamilton, Gardner and Wallich-lost herbaria, lost letters and lost* *books somewhat rediscovered*. Bishen Singh Mahendra Pal Singh, Dehra Dun, India.

FREYMUELLER, J., BILHAM, R., BÜRGMANN, R., LARSON, K. M., PAUL, J., JADE, S. & GAUR, V. (1996). Global positioning system measurements of Indian plate motion and convergence across the Lesser Himalaya. *Geophysical Research Letters* 23(22): 3107-3110.

FRODIN, G. D. (2004). History and concepts of big plant genera. *Taxon*. 53(3): 753-776.

GANSSER, A. (1980). The significance of the Himalayan suture zone. *Tectonophysics* 62(1-2): 37-40, 43-52.

GIFFEN, M. H. (1936). The chromosomes numbers of *Berberis*. Transactions of Royal Society of South Africa 24: 203-206.

GILL, B. S., BIR, S. S. & SINGHAL, V. K. (1984). *Cytological studies in some western Himalayan wood species II. Polypetalae*. Puja Publishers, Delhi.

GLADENKOV, A. Y., OLEINIK, A. E., MARINCOVICH, L. & BARINOV, K. B. (2002). A refined age for the earliest opening of Bering Strait. *Palaeogeography, Palaeoclimatology, Palaeoecology* 183(3-4): 321-328.

GRANT, V. (1981). *Plant Speciation*. Columbia University Press.

GRIERSON, A. J. C. (1984). Berberidaceae. In: A. J. C. Grierson & D. G. Long. *Flora of Bhutan* 1(2): 322-328. Royal Botanic Garden Edinburgh.

GROB, G. B. J., GRAVENDEEL, B. & EURLINGS, M. C. M. (2004). Potential phylogenetic utility of the nuclear FLORICAULA/LEAFY second intron: comparison with three chloroplast DNA regions in Amorphophallus (Araceae). *Molecular Phylogenetics and Evolution* 30(1): 13-23.

GUO, Z. T., RUDDIMAN, W. F., HAO, Q. Z., WU, H. B., QIAO, Y. S., ZHU, R. X., PENG, S. Z., WEI, J. J., YUAN, B. Y. & LIU, T. S. (2002). Onset of Asian desertification by 22 Myr ago inferred from loess deposits in China. *Nature* 416(6877): 159-163.

GUPTA, R. K. (1994). Arcto-alpine and boreal elements in the high altitude flora of north west Himalaya. In: Y. P. S. Pangtey & R. S. Rawal (eds.). *High Altitudes of the Himalaya*. pp. 11-32. Gyanodaya Prakashan, Nainital.

GURUNG, H. (1999). *Mountain of Asia: A regional inventory*. International Centre for Integrated Mountain Development (ICIMOD), Kathmandu, Nepal.

HARA, H. (1969). Remarkable examples of speciation in Asiatic plants. *American Journal of Botany* 56(7): 732-737.

HARA, H., STEARN, W. T. & WILLIAMS, L. H. J. (1978). *An Enumeration of the Flowering Plants of Nepal.* Vol 1. British Museum (Natural History).

HARRISON, T. M., COPELAND, P., HALL, S. A., QUADE, J., BURNER, S., OJHA, T. P. & KIDD, W. S. F. (1993). Isotopic preservation of Himalayan/Tibetan Uplift, denudation, and climatic histories of two molasse deposits. *The Journal of Geology* 101(2): 157-175.

HARRISON, T. M., COPELAND, P., KIDD, W. S. F. & YIN, A. (1992). Raising Tibet. *Science* 255(5052): 1663-1670.

HARRISON, T. M., RYERSON, F. J., LE FORT, P., YIN, A., LOVERA, O. M. & CATLOS, E. J. (1997). A Late Miocene-Pliocene origin for the Central Himalayan inverted metamorphism. *Earth and Planetary Science Letters* 146(1-2): E1-E7.

HEYWOOD, V. H., BRUMMITT, R.K., CULHAM, A., AND SERBERG, O. (2007). *Flowering plant families of the World*. Royal Botanical Garden, Kew.

HODGES, K. V., PARRISH, R. R. & SEARLE, M. P. (1996). Tectonic evolution of the central Annapurna Range, Nepalese Himalayas. *Tectonics* 15(6): 1264-1291.

HOOKER, J. D. & THOMSON, T. (1855). *Flora indica.* Vol.1. London: W. Pamplin.

HOOKER, J. D & THOMSON, T. (1872). Berberis. In: J. D. Hooker (eds.). *The Flora of British India*. 1(1): 108-112. London: L. Reeve & Co.

HOOKER, J. D. (1889). *Berberis angulosa*. Native of the Himalaya. *Curtis's Botanical Magazine* 115: Tab 7071.

HOOKER, J. D. (1890). *Berberis virescens*. Native of the Sikkim Himalaya. *Curtis's Botanical Magazine* 116: Tab 7116.

HOOKER, J. D. (1906). *A Sketch of the Flora of British India*. Oxford Clarendon Press.

HUELSENBECK, J. P. & RONQUIST, F. (2001). MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17(8): 754-755.

HUTCHINSON, J. (1959). *The Families of Flowering Plants*. Oxford at the Clarendon Press.

ICKERT-BOND, S. M., RYDIN, C. & RENNER, S. S. (2009). A fossil-calibrated relaxed clock for *Ephedra* indicates an Oligocene age for the divergence of Asian and New World clades and Miocene dispersal into South America. *Journal of Systematics and Evolution* 47(5): 444-456.

INGER, S. & HARRIS, N. B. W. (1992). Tectonothermal evolution of the High Himalayan crystalline sequence, Langtang valley, northern Nepal. *Journal of Metamorphic Geology* 10(3): 439-452.

JACKSON, M. & BILHAM, R. (1994). Constraints on Himalayan deformation inferred from vertical velocity fields in Nepal and Tibet. *Journal of Geophysical Research* 99(B7): 13897-13912.

JANAKI AMMAL, E. K. (1950). Polypoloidy in the genus *Rhododendron*. *The Rhododendron Year Book* 5: 92-98.

JEE, V., DHAR, U. & KACHROO, P. (1989). Cytogeography of some endemic taxa of Kashmir Himalaya. *Proceedings of the Indian National Science Academy* B 55: 177-184.

JOHNSON, N. M., STIX, J., TAUXE, L., CERVENY, P. F. & TAHIRKHELI, R. A. K. (1985). Paleomagnetic chronology, fluvial processes, and tectonic implications of the siwalik deposits near Chinji village, Pakistan. *The Journal of Geology* 93(1): 27-40.

JONG, K. (1997). *Laboratory manual of plant cytological techniques*. Royal Botanic Garden Edinburgh.

KENICER, G. J., KAJITA, T., PENNINGTON, R. T. & MURATA, J. (2005). Systematics and biogeography of *Lathyrus* (Leguminosae) based on internal transcribed spacer and cpDNA sequence data. *American Journal of Botany* 92(7): 1199-1209.

KENICER, G.J. (2007). *Systematics and biogeography of Lathyrus L. (Leguminosae, Papilionoideae)*. PhD thesis. The University of Edinburgh.

KER GAWLER, J. B. (1823). *Berberis chitria*. Nepal Barberry. *Botanical Register* 9: Tab 729.

KHATOON, S. & ALI, S. I. (1993). *Chromosome atlas of the angiosperms of Pakistan*. Department of Botany, University of Karachi, Karachi, Pakistan.

KIM, Y.-D. & JANSEN, R. K. (1994). Characterization and phylogenetic distribution of chloroplast DNA rearrangement in the Berberidaceae. *Plant Systematics and Evolution* 193: 107-114.

KIM, K. J. & JANSEN, R. K. (1995). *ndh*F sequence evolution and the major clades in the sunflower family. *Proceedings of the National Academy of Sciences of the United States of America* 92(22): 10379-10383.

KIM, Y.-D. & JANSEN, R. K. (1996). Phylogenetic implications of *rbcL* and ITS sequence variation in the Berberidaceae. *Systematic Botany* 21(3): 381-396.

KIM, Y.-D. & JANSEN, R. K. (1998). Chloroplast DNA restriction site variation and phylogeny of the Berberidaceae. *American Journal of Botany* 85(12): 1766-1778.

KIM, Y.-D., KIM, S.-H., KIM, C. H. & JANSEN, R. K. (2004a). Phylogeny of Berberidaceae based on sequences of the chloroplast gene *ndhF*. *Biochemical Systematics and Ecology* 32(3): 291-301.

KIM, Y.-D., KIM, S.-H. & LANDRUM, L. R. (2004b). Taxonomic and phytogeographic implications from ITS phylogeny in *Berberis* (Berberidaceae). *Journal of Plant Research* 117: 175-182.

KNOBLOCH, E. & MAI, D. H. (1986). Monographie der Früchte und Samen in der Kreide von Mitteleuropa. *Rospravy Ustredniho Ustavu Geologickeho Svazek* 47: 1-219.

KOVAR-EDER, J., KVACEK, Z. & STROBITZER-HERMANN, M. (2004). The Miocene flora of Parschlug (Styria, Austria)-revision and synthesis. *Annalen Des Naturhistorischen Museum in Wien* 105A: 45-159.

KVACEK, Z. & ERDEI, B. (2001). Putative proteaceous elements of the *Lomatites*-type reinterpreted as new *Berberis* of the European Tertiary. *Plant Systematics and Evolution* 226: 1-12.

LAFERRIERE, J. E. (1997). Transfer of specific and infraspecific taxa from *Mahonia* to *Berberis*. *Acta Botanica Indica* 25: 243-246.

LANDRUM, L. R. (1999). Revision of *Berberis* (Berberidaceae) in Chile and Adjacent Southern Argentina. *Annals of the Missouri Botanical Garden* 86(4): 793-834.

LEMAIRE, C. A. (1859). *Berberis hookerii*. *L'Illustration horticole* 6: Planche 207.

LI, Y.-L., KVACEK, Z., FERGUSON, D. K., WANG, Y.-F., LI, C.-S., YANG, J., YING, T.-S., ABLAEV, A. G. & LIU, H.-M. (2010). The fossil record of *Berberis* (Berberidaceae) from the Palaeocene of NE China and interpretations of the evolution and phytogeography of the genus. *Review of Palaeobotany and Palynology* 160(1-2): 10-31.

LINNAEUS, C. (1753). *Species Plantarum* 1. Stockholm.

MABBERLEY, D. J. (1997). *The Plant-book*. Cambridge University Press, Cambridge.

MABBERLEY, D. J. (2008). *The Plant-Book*. Cambridge University Press, Cambridge.

MADDISON, D. R. & MADDISON, W. P. (2003). *MacClade ver. 4.06*. Sinauer Associates, Sunderland.

MADDISON, W. P. & MADDISON, D. R. (2009). Mesquite: a modular system for evolutionary analysis. Version 2.72. Available from http://mesquiteproject.org.

MALLA, S. B., BHATTARAI, S., GORKHALI, M. & SINGH, M. P. (1975). In IOPB Chromosomes number reports XLIX. *Taxon* 24: 501-516.

MALLA, S. B., BHATTARAI, S., SHRESTHA, M. & SINGH, M. P. (1974). In IOPB chromosomes number reports-XLVI. *Taxon* 23: 801-812.

MANANDHAR, N. P. (2002). *Plants and People of Nepal*. Timber Press, Portland, Oregon.

MANCHESTER, S. R. (1999). Biogeographical relationships of North American Tertiary floras. *Annals of the Missouri Botanical Garden* 86(2): 472-522.

MANI, M. S. (1974a). Physical features. In: M. S. Mani (eds.) *Ecology and Biogeography in India*. pp.11-59. Dr. W.Junk Publishers.

MANI, M. S. (1974b). Biogeographical evolution in India. In M. S. Mani (eds.) *Ecology and Biogeography in India*. pp. 698-724. Dr. W.Junk Publishers

MANI, M. S. (1978). *Ecology and phytogeography of high-altitude plants of the Northwest Himalaya*. Chapman and Hall, London.

MASTERSON, J. (1994). Stomatal size in fossil plants: evidence for polyploidy in majority of angiosperms. *Science* 264(5157): 421-424.

MAYDEN, R. L. (2002). On biological species, species concepts and individuation in the natural world. *Fish and Fisheries* 3(3): 171-196.

MAYR, E. (1942). *Systematics and the origin of species*. Columbia University Press.

MEACHAM, C. A. (1980). Phylogeny of the Berberidaceae with an evaluation of classification. *Systematic Botany* 5(2): 149-172.

MEHRA, P. N. & SAREEN, T. S. (1969). In IOPB chromosome number reports XXII. *Taxon*18: 433-442.

MEHRA, P. N. & SAREEN, T. S. (1973). Cytology of some Himalayan trees. *Silvae Genetica* 22: 66-70.

MEHRA, P. N. (1976). *Cytology of Himalayan hardwoods*. Sree Saraswaty Press, Calcutta.

MENG, Y., SUN, H., YANG, Y.-P. & NIE, Z.-L. (2010). Polyploidy and new chromosome counts in *Anaphalis* (Asteraceae: Gnaphalieae) from the Qinghai-Tibet Plateau of China. *Journal of Systematics and Evolution* 48(1): 58-64.

MEREV, N., GERCEK, Z., SERDAR, B., ERSENBAK, F. & BIRTÜRK, T. (2005). Wood anatomy of some Turkish plants with special reference to perforated ray cells. *Turkish Journal of Botany* 29: 269-281.

METCALFE, C. R. & CHALK, L. (1950). *Anatomy of the Dicotyledons*. Vol.1. Oxford Clarendon Press.

MILNE, R. I. & ABBOTT, R. I. (2002). The origin and evolution of Tertiary relict floras. *Advances in Botanical Research* 38: 281-314.

MILNE, R. I. (2006). Northern Hemisphere plant disjunctions: A window on Tertiary land bridges and climate change? *Annals of Botany* 98(3): 465-472.

MOLLER, M. & CRONK, Q. C. B. (1997). Origin and relationships of *Saintpaulia* (Gesneriaceae) based on ribosomal DNA internal transcribed spacer (ITS) sequences. *American Journal of Botany* 84(7): 956-965.

MOLNAR, P., ENGLAND, P. & MARTINOD, J. (1993). Mantle dynamics, uplift of the Tibetan Plateau, and the Indian monsoon. *Reviews of Geophysics* 31(4): 357-396.

MOREIRA-MUÑOZ, A. & MUÑOZ-SCHICK, M. (2007). Classification, diversity, and distribution of Chilean Asteraceae: implications for biogeography and conservation. *Diversity and Distributions* 13: 818-828.

MOURI, C., MIKAGE, M. & NOSHIRO, S. 2004 Distribution maps and a tentative key of *Berberis* (Berberidaceae) in Nepal. *Newsletter of Himalayan Botany* 33: 26-32.

NEI, M. & KUMAR, S. (2000). *Molecular evolution and phylogenetics*. New York: Oxford University Press.

NEYLAND, R. & URBATSCH, L. E. (1996). Phylogeny of subfamily Epidendroideae (Orchidaceae) inferred from *ndhF* chloroplast gene sequences. *American Journal of Botany* 83(9): 1195-1206.

NIE, Z.-L., WEN, J., GU, Z.-J., BOUFFORD, D. E. & SUN, H. (2005). Polyploidy in the flora of the Hengduan Mountains hotspot, Southwestern China. *Annals of the Missouri Botanical Garden* 92(2): 275-306.

NIXON, K. C. & CARPENTER, J. M. (1993). On outgroups. *Cladistics* 9(4): 413-426.

NIXON, K. C. & WHEELER, Q. D. (1990). An amplification of the phylogenetic species concept. *Cladistics* 6(3): 211-223.

NYLANDER, J. A. A. (2004). *MrModeltest 2.3*. Evolutionary Biology Centre, Uppsala University.

OHBA, H. (1988). The alpine flora of the Nepal Himalayas: An introductory note. In: H. Ohba & S. B. Malla (eds.) *The Himalayan Plants*. pp. 19-46.

OLMSTEAD, R. G. & REEVES, P. A. (1995). Evidence for the polyphyly of the Scrophulariaceae based on chloroplast *rbcL* and *ndhF* sequences. *Annals of the Missouri Botanical Garden* 82(2): 176-193.

OLMSTEAD, R. G. & SWEERE, J. A. (1994). Combining data in phylogenetic systematics: An empirical approach using three molecular data sets in the Solanaceae. *Systematic Biology* 43(4): 467-481.

OSRI, M. C. (1976). Sinopsis de las especies Argentinas del genero *Berberis* (Berberidaceae). *Boletin de la Sociedad Argentina de Botanica* 17: 127-149.

PATRIAT, P. & ACHACHE, J. (1984). India-Eurasia collision chronology has implications for crustal shortening and driving mechanism of plates. *Nature* 311(5987): 615-621.

PATZELT, A., LI, H., WANG, J. & APPEL, E. (1996). Palaeomagnetism of Cretaceous to Tertiary sediments from southern Tibet: evidence for the extent of the northern margin of India prior to the collision with Eurasia. *Tectonophysics* 259(4): 259-284.

PENNINGTON, R. T., CRONK, Q. C. B. & RICHARDSON, J. A. (2004). Introduction and synthesis: plant phylogeny and the origin of major biomes. *Philosophical Transactions of the Royal Society of London series B: Biological Sciences* 359: 1455-1464.

PENNINGTON, R.T., LAVIN, M, SARKINEN, T., LEWIS, G.P., KLITGAARD, B.B., HUGHES, C.E. (submitted). *Proceedings of the National Academy of Sciences of the United States of America.*

PRESS, J. R. & SHRESTHA, K. K. (2000). Collections of flowering plants by Francis Buchanan-Hamilton from Nepal, 1802-1803. *Bulletin of the British Museum- Natural History (Botany)*30(2): 101-130.

PRESS, J. R., SHRESTHA, K. K. & SUTTON, D.A. (2000). *Annotated Checklist of the Flowering Plants of Nepal*. The Natural History Museum, London.

PURI, G. S. (1947). Some fossils leaves of *Berberis* from the Karewas of Kashmir. *The Journal of the Indian Botanical Society* 26: 177-183.

PYCK, N., ROELS, P. & SMETS, E. (1999). Tribal relationships in Caprifoliaceae: Evidence from a cladistic analysis using *ndh*F sequences. *Systematics and Geography of Plants* 69(2): 145-159.

QUATTROCCHI, U. (1947). *CRC World Dictionary of Plant Names*. CRC Press LLC Florida.

RAMBAUT, A. & DRUMMOND, A. J. (2007). *Tracer ver. 1.5*. Available from http://beast.bio.ed.ac.uk/Tracer.

RAMBAUT, A. 2009. *FigTree ver.1.3.1*. Available from http://tree.bio.ed.ac.uk/ software/figtree.

RAMDAS, L. A. (1974). Weather and climatic patterns. In: M. S. Mani (eds.) *Ecology and Biogeography in India*. pp 99-134. Dr. W.Junk Publishers

RAMIREZ, J. L. & CEVALLOS-FERRIZ, S. R. S. (2000). Leaves of Berberidaceae (*Berberis* and *Mahonia*) from Oligocene sediments, near Tepexi de Rodriguez, Puebla. *Review of Palaeobotany and Palynology* 110: 247-257.

RAO, R. R., HUSAIN, T. & DUTT, B. (1994). An undescribed variety of *Berberis petiolaris* Wall. ex G.Don (Berberidaceae) from the Himalaya. *Botanical Bulletin of Academia Sinicia* 35: 229-232.

RAO, R. R., HUSAIN, T., DUTT, B. & GARG, A. (1998a). Revision of the family Berberidaceae of India-I. *Rheedea* 8(1): 1-66.

RAO, R. R., HUSAIN, T., DUTT, B. & GARG, A. (1998b). Revision of the family Berberidaceae of the Indian Region-II. *Rheedea* 8(2): 109-143.

RAO, R. R., HUSAIN, T., DUTT, B. & GARG, A. (1999). Palynology of *Berberis*. *Rheedea* 9(2): 115-146.

RAVEN, P. H. (1963). Amphitropical relationships in the floras of North and South America. *The Quarterly Review of Biology* 38(2): 151-177.

RICHARDSON, J. E., PENNINGTON, R. T., PENNINGTON, T. D. & HOLLINGSWORTH, P. M. (2001). Rapid diversification of a species-rich genus of neotropical rain forest trees. *Science* 293(5538): 2242-2245.

ROXBURGH, W. (1832). *Flora Indica* 2: 181-184. Serampore: Printed for W. Thacker.

ROZEN, S & SKALETSKY, H.J. (2000). Primer3 on the WWW for general users and for biologist programmers. In: S. Krawetz & S. Misener (eds.)

Bioinformatics Methods and Protocols: Methods in Molecular Biology. pp. 365-386 Humana Press, Totowa, NJ.

SANDERSON, M. J. & DOYLE, J. J. (1993). Phylogenetic relationships in North American *Astragalus* (Fabaceae) based on chloroplast DNA restriction site variation. *Systematic Botany* 18(3): 395-408.

SANDERSON, M. J. & SHAFFER, H. B. (2002). Troubleshooting molecular phylogenetic analyses. *Annual Review of Ecology and Systematics* 33: 49-72.

SANDHU, P. S. & MANN, S. K. (1988). SOCGI plant chromosome number reports-VII. *Journal of Cytology and Genetics* 23: 219-228.

SANDHU, P. S. & MANN, S. K. (1989). SOCGI plant chromosomes number reports-VIII. *Journal of Cytology and Genetics* 24: 179-183.

SAPORTA, G. (1865). Etudes sur la vegetation du Sud-Est de la France a l'epoque tertiaire. *Annales des Sciences Naturelles, Botanique* 4: 5-264.

SCHERSON, R. A., VIDAL, R. & SANDERSON, M. J. (2008). Phylogeny, biogeography, and rates of diversification of New World Astragalus (Leguminosae) with an emphasis on South American radiations. *American Journal of Botany* 95(8): 1030-1039.

SCHNABEL, A., MCDONEL, P. E. & WENDEL, J. F. (2003). Phylogenetic relationships in *Gleditsia* (Leguminosae) based on ITS Sequences. *American Journal of Botany* 90(2): 310-320.

SCHNEIDER, C. K. (1904-1905). Die Gattung *Berberis* (Euberberis). Vorarbeiten fur eine Monographie. *Bulletin De L'Herbier Boissier* 2: 33-48, 133-148, 391-403, 449-464, 655-670, 800-831.

SCHNEIDER, C. K. (1908). Weitere beitrage zur kenntnis der gattung *Berberis* (Euberberis). *Bulletin De L'Herbier Boissier* 8(2): 192-204, 258-266.

SCHNEIDER, C. K. (1942). Die Berberis der section *Wallichianae*. *Mitteilungen der Deutschen Dendrologischen Gesellschaft* 55: 1-60.

SCHULTHEIS, L. M. & DONOGHUE, M. J. (2004). Molecular phylogeny and biogeography of *Ribes* (Grossulariaceae), with an emphasis on gooseberries (Subg. Grossularia). *Systematic Botany* 29(1): 77-96.

SHAPIRO, B., RAMBAUT, A. & DRUMMOND, A. J. (2006). Choosing appropriate substitution models for the phylogenetic analysis of protein-coding sequences. *Molecular Biology and Evolution* 23(1): 7-9.

SHRESTHA, T. B. (2002). *Forest and vegetation types of Nepal*. Tree Improvement and Silviculture Component (TISC).

SINGH, S. M. & HUSAIN, T. (2007). SEM study of the leaf epidermis in Indian species of the family Berberidaceae. *Journal of Economic and Taxonomic Botany* 31(4): 818-838.

SINGHAL, V. K. & GILL, B. S. (1984). SOCGI plant chromosomes number reports-II. *Journal of Cytology and Genetics* 19: 115-117.

SINGHAL, V. K., GILL, B. S. & BIR, S. S. (1980). In plant chromosomes number reports- LXVII. *Taxon* 29: 355-357.

SNEATH, P. H. A. & SOKAL, R. R. (1973). *Numerical Taxonomy*. W. H. Freeman and Company, San Francisco.

SOLTIS, D. E. & SOLTIS, P. S. (1995). The dynamic nature of polyploid genomes. *Proceedings of the National Academy of Sciences of the United States of America* 92(18): 8089-8091.

SOLTIS, D. E. & SOLTIS, P. S. (1998). Choosing an approach and an appropriate gene for phylogenetic analysis. In: D. E. Soltis, P. S. Soltis & J. J. Doyle (eds.) *Molecular Systematics of Plants II DNA Sequencing*. pp. 1-42. Kluwer Academic Publishers.

SOLTIS, D. E., TAGO-NAKAZAWA, M., XIANG, Q.-Y., KAWANO, S., MURATA, J., WAKABAYASHI, M. & HIBSCH-JETTER, C. (2001). Phylogenetic relationships and evolution in *Chrysosplenium* (Saxifragaceae) based on matK sequence data. *American Journal of Botany* 88(5): 883-893.

STACE, C. A. (1980). *Plant taxonomy and biosystematics*. Edward Arnold (Publishers) Limited.

STACE, C. A. (2000). Cytology and cytogenetics as a fundamental taxonomic resource for the 20th and 21st Centuries. *Taxon* 49(3): 451-477.

STAINTON, J. D. A. (1972). *Forests of Nepal*. London (John Murray).

STEARN, W. T. (1960). *Allium* and *Milula* in the Central Nepal. *Bulletin of the British Museum- Natural History (Botony)* 2: 159-191.

STEBBINS, G. L. (1971). *Chromosomal evolution in higher plants*. Edward Arnold (Publishers) Ltd., London.

STUESSY, T. F. (1990). *Plant Taxonomy: The Systematic Evaluation of Comparative Data*. Columbia University Press.

STUESSY, T. F. (2009). *Plant Taxonomy: The Systematic Evaluation of Comparative Data*. Columbia University Press.

SWOFFORD, D. L. (2002). PAUP*. *Phylogenetic analysis using parsimony* (*and Other Methods). Version 4. Sinauer Associates, Sunderland, Massachusetts.

TAKHTAJAN, A. (1969). *Flowering Plants: Origin and Dispersal*. Smithsonian Institute Press, Washington.

TAKHTAJAN, A. (1986). *Floristic Regions of the World*. University of California Press, Berkeley.

TANAI, T. (1961). Neogene floral change in Japan. *Journal of the Faculty of Science, Hokkaido University. Series 4, Geology and mineralogy* 11(2): 119-398.

TANAI, T. (1972). Tertiary history of vegetation in Japan In: A. Graham (eds.) *Floristic and Paleofloristic of Asia and Eastern North America.* pp. 235-255. Elsevier Publ. Co.

TEBBS, M.C. (1979). Berberidaceae. In: HARA, H. & WILLIAMS, L.H.J. *An Enumeration of the Flowering Plants of Nepal.* 2: 29-31. British Museum (Natural History).

TERABAYASHI, S. (1978). Studies in morphology and systematics of Berberidaceae, II: Floral anatomy of *Mahonia japonica* (Thunb.) DC. and *Berberis thunbergii* DC. *Acta Phytotaxonomica et Geobotanica* 29: 106-118.

TERABAYASHI, S. (1985). The comparative floral anatomy and systematics of Berberidaceae. I. Morphology. *Memoirs of the Faculty of Science, Kyoto University, Series B(Biology)* 10: 73-90.

TERABAYASHI, S. (1987). Seedling morphology of the Berberidaceae. *Acta Phytotaxonomica et Geobotanica 38*: 63-74.

THORNE, R. F. (1972). Major disjunctions in the geographic ranges of seed plants. *The Quarterly Review of Biology* 47(4): 365-411.

TIFFNEY, B. H. & MANCHESTER, S. R. (2001). The use of geological and paleontological evidence in evaluating plant phylogeographic hypotheses in the Northern Hemisphere Tertiary. *International Journal of Plant Sciences* 162(6): S3-S17.

TIFFNEY, B. H. (1985). Perspectives on the origin of the floristic similarity between eastern Asia and eastern North America. *Journal of the Arnold Arboretum* 66: 73-94.

TODD, M. (2006). Phytogeographic and taxonomic relationship of *Berberis* (Berberidaceae) inferred from an Internal Transcribed Spacer (ITS) Phylogeny. MSc Thesis (unpublished). The University of Edinburgh.

TU, T., DILLON, M. O., SUN, H. & WEN, J. (2008). Phylogeny of Nolana (Solanaceae) of the Atacama and Peruvian deserts inferred from sequences of four plastid markers and the nuclear LEAFY second intron. *Molecular Phylogenetics and Evolution* 49(2): 561-573.

UNEP, 2001. *Nepal: State of the Environment 2001.*

VALDIYA, K. S. (1998). Dynamic Himalaya. Universities Press (India) Limited.

VALDIYA, K. S. (2002). Emergence and evolution of Himalaya: reconstructing history in the light of recent studies. *Progress in Physical Geography* 26(3): 360-399.

VARGAS, P., BALDWIN, B. G. & CONSTANCE, L. (1998). Nuclear ribosomal DNA evidence for a western North American origin of Hawaiian and South American species of *Sanicula* (Apiaceae). *Proceedings of the National Academy of Sciences of the United States of America* 95(1): 235-240.

WAKABAYASHI, M. & OHBA, H. (1988). Cytotaxonomic study of the Himalayan *Saxifraga*. In: H. Ohba & S. B. Malla (eds.) *The Himalayan Plants I.* pp. 71-89.

WAKABAYASHI, M. & OHBA, H. (1999). Chromosome numbers of seven species of *Sedum* and *Sinocrassula indica* (Crassulaceae) in East Himalaya. *Journal of Japanese Botany* 74: 228-235.

WALLICH, N. (1832). *Plantae Asiaticae Rariores.* Vol. 3. London: Treuttel and Würtz.

WANG, W., CHEN, Z.-D., LIU, Y., LI, R.-Q. & LI, J.-H. (2007). Phylogenetic and biogeographic diversification of Berberidaceae in the Northern Hemisphere. *Systematic Botany* 32(4): 731-742.

WANG, Y.-J., SUSANNA, A., RAAB-STRAUBE, E. V., MILNE, R. & LIU, J.-Q. (2009). Island-like radiation of *Saussurea* (Asteraceae: Cardueae) triggered by uplifts of the Qinghai-Tibetan Plateau. *Biological Journal of the Linnean Society* 97(4): 893-903.

WEN, J. & ICKERT-BOND, S. M. (2009). Evolution of the Madrean-Tethyan disjunctions and the North and South American amphitropical disjunctions in plants. *Journal of Systematics and Evolution* 47(5): 331-348.

WEN, J. (1999). Evolution of eastern Asian and eastern North American disjunct distributions in flowering plants. *Annual Review of Ecology and Systematics* 30: 421-455.

WEN, J., BERGGREN, S. T., LEE, C.-H., ICKERT-BOND, S., YI, T.-S., YOO, K.-O., XIE, L., SHAW, J. & POTTER, D. (2008). Phylogenetic inferences in *Prunus* (Rosaceae) using chloroplast *ndh*F and nuclear ribosomal ITS sequences. *Journal of Systematics and Evolution* 46(3): 322-332.

WIKSTRÖM, N., SAVOLAINEN, V. & CHASE, M. W. (2001). Evolution of the angiosperms: calibrating the family tree. *Proeedings of the Royal Society London B* 268: 2211-2220.

WINDLEY, B. F. (1977). *The Evolving Continents*. John Wiley and Sons.

WOLFE, J. A. (1975). Some aspects of plant geography of the Northern Hemisphere during the late Cretaceous and Tertiary. *Annals of the Missouri Botanical Garden* 62(2): 264-279.

XIANG, Q.-Y. & SOLTIS, D. E. (2001). Dispersal-vicariance analyses of intercontinental disjuncts: Historical biogeographical implications for angiosperms in the Northern Hemisphere. *International Journal of Plant Sciences* 162(6): S29-S39.

XIANG, Q.-Y., SOLTIS, D. E., SOLTIS, P. S., MANCHESTER, S. R. & CRAWFORD, D. J. (2000). Timing the Eastern Asian-Eastern North American floristic disjunction: Molecular clock corroborates paleontological estimates. *Molecular Phylogenetics and Evolution* 15(3): 462-472.

YOSHIDA, T. (2006). Geobotany of the Himalaya I. *Newsletter of Himalayan Botany* 37: 1-24.

YUAN, Y.-W. & OLMSTEAD, R. G. (2008). A species-level phylogenetic study of the Verbena complex (Verbenaceae) indicates two independent intergeneric chloroplast transfers. *Molecular Phylogenetics and Evolution* 48(1): 23-33.

ZHAO, W., NELSON, K. D., CHE, J., QUO, J., LU, D., WU, C. & LIU, X. (1993). Deep seismic reflection evidence for continental underthrusting beneath southern Tibet. *Nature* 366(6455): 557-559.

APPENDIX 1

ITINERARIES OF FIELD TRIPS CARRIED OUT DURING THIS PROJECT

EDINBURGH ANNAPURNA EXPEDITION (EA) 2006

(Dr C.Pendry, Dr. R. Milne & B. Adhikari)

25 September: Kathmandu - Pokhara

26 September: Pokhara-Jomsom (2750 m) - Kagbeni (2820 m)

27 September: Kagbeni (2820 m) - Khinga (3400 m) - Muktinath (3600 m)

28 September: Muktinath (3600 m) - Jomsom (2750 m)

29 September: Jomsom (2750 m) -Marpha (2700 m) - Larjung (2550 m)

30 September: Larjung (2550 m) - Lete (2450 m) - Ghasa (1850 m)

1 October: Ghasa (1850 m) - Ghara (1630 m)

2 October: Ghara (1630 m) - Sikha (2000 m) - Chitre (2570 m)

3 October: Chitre (2570 m) - Ghorepani (2900 m)

4 October: Ghorepani (2900 m) - Poon Hill Ghorepani (3150 m) - Tadapani (2700 m)

5 October: Tadapani (2700 m) - Ghandruk (2000 m)

6 October: Ghandruk (2000 m) - Birethati (1350 m) - Pokhara

7 October: Pokhara - Kathmandu

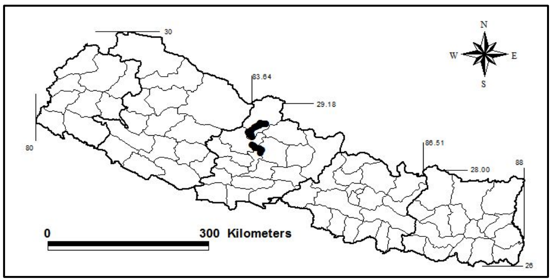


FIG 1. A

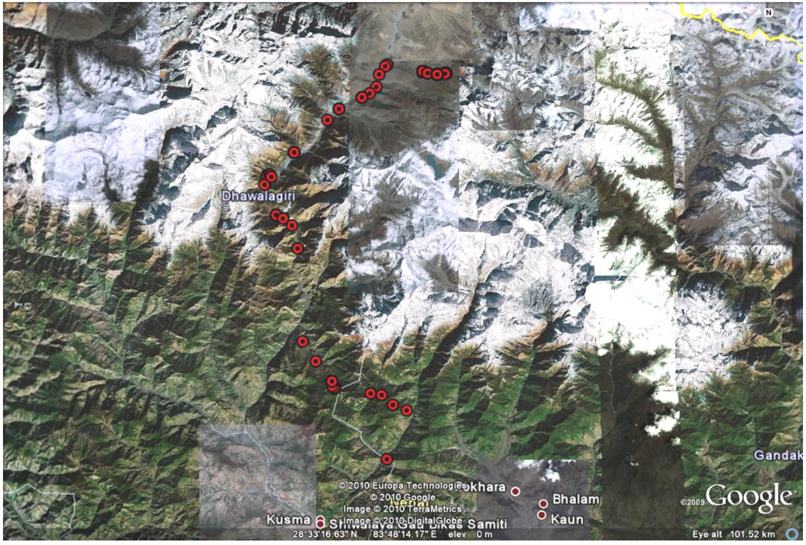


FIG 1. B

FIG 1. A, B. *Berberis* collection localities of EA expedition

EDINBURGH LANTANG (GOSAIKUND) TRIP (EL) 2006

(B. Adhikari & B. Nepal)

18 October: Kathmandu - Dhunche

19 October: Dhunche (2000 m) - Deurali (2710 m) - Chandanbari (3400 m)

20 October: Chandanbari (3400 m) - Gosaikund (4100 m)

21 October: Gosaikund (4100 m) - Dhunche (2000 m)

22 October: Dhunche - Kathmandu

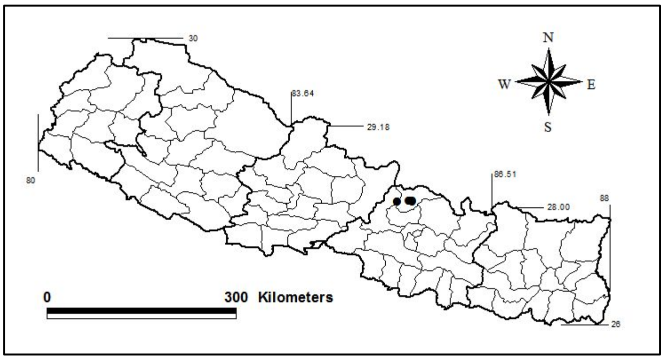


FIG 1. C

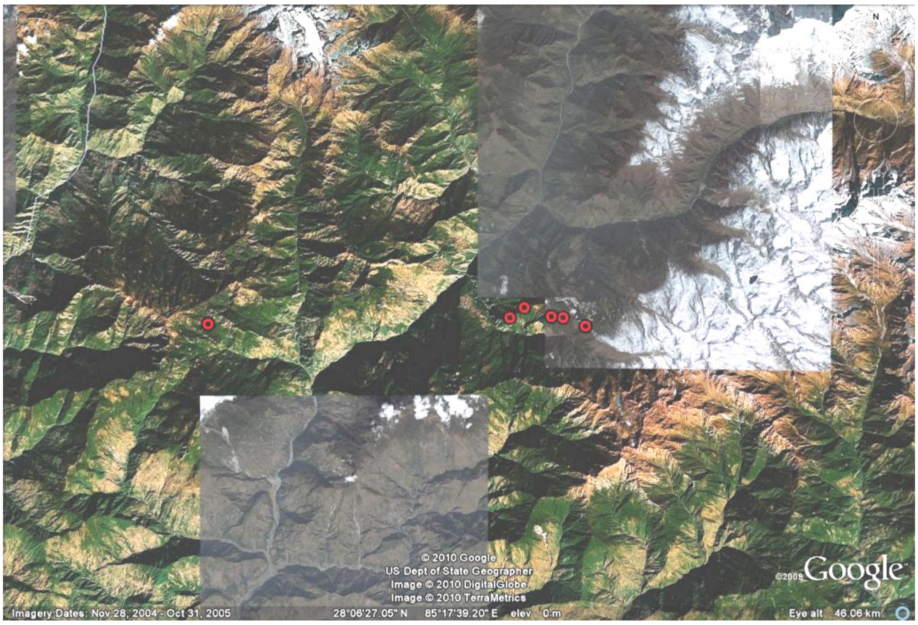


FIG 1. D

FIG 1. C, D. *Berberis* collection localities of EL expedition.

LOWER KANCHENJUNGA SINGALILA RIDGE EXPEDITION (LKSR) 2007

(Dr. K.K. Shrestha, B. Adhikari, S. Rajbhandary, R. Kunwar, R.C. Poudel, K. Humagain, J. Pandey & N.B. Chhetri)

4 June 2007: Kathmandu - Bhadrapur - Illam Bazzar

5 June 2007: Illam Bazzar

6 June 2007: Illam Bazzar (1213 m) - Maimajuwa, Hattiya (1800 m)

7 June 2007: Hattiya (1800 m) - Banduke (2802 m)

8 June 2007: Banduke (2802 m) - Dhupi Chaur (3357 m)

9 June 2007: Dhupi Chaur (3357 m) - Chandu (3529 m)

10 June 2007: Chandu (3529 m) - Pasibhanjyang (3236 m)

11 June 2007: Pasibhanjyang (3236 m) - Gorkhe Pani (3300 m)

12 June 2007: Gorkhe Pani (3300 m)

13 June 2007: Gorkhe Pani (3300 m) - Aahal (3566 m)

14 June 2007: Aahal (3566 m) - Kalapokhari (3032 m)

15 June 2007: Kalapokhari (3032 m)

16 June 2007: Kalapokhari (3032 m) - Hangetham (2238 m)

17 June 2007: Hangetham (2238 m) - Jaubari (2621 m)

18 June 2007: Jaubari (2621 m) - Jogmai (2278 m)

19 June 2007: Jogmai (2278 m) - Nayabazzar - Illam Bazzar (1213 m)

20 June 2007: Illam Bazzar - Birtamod

21 June 2007: Birtamod - Kathmandu

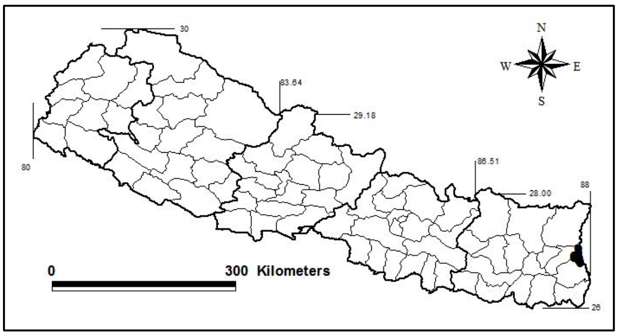


FIG 1. E

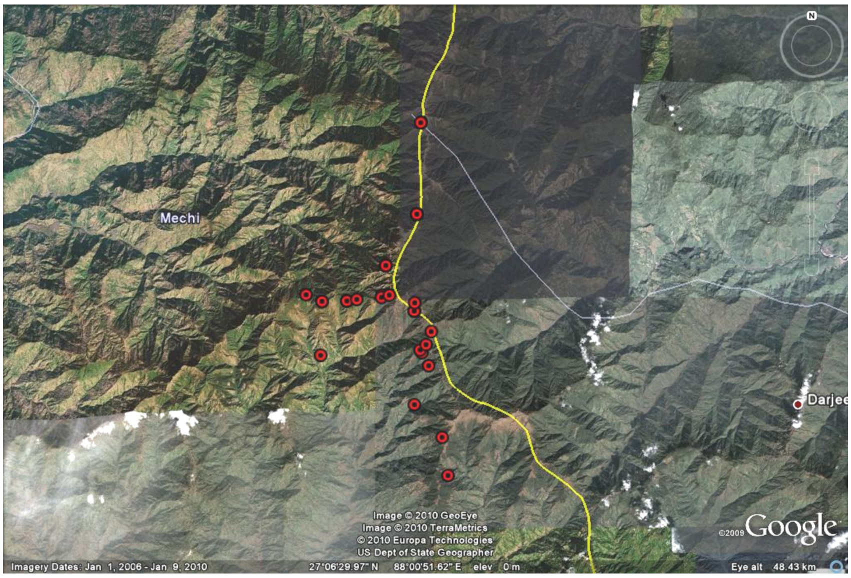


FIG 1. F

FIG 1. E, F. *Berberis* collection localities of LKSR expedition.

EDINBURGH ANNAPURNA EXPEDITION 2 (EA2) 2007

(B. Adhikari)

12 Aug 2007: Kathmandu - Pokhara

13 Aug 2007: Pokhara - Beni - Tipling

14 Aug 2007: Tipling - Ghasa (1850)

15 Aug 2007: Ghasa (1850 m) - Muktinath (3650 m)

16 Aug 2007: Muktinath (3650 m) - Jomsoom (2750 m) - Marpha (2700 m)

17 Aug 2007: Marpha (2700 m) - Lete (2450)

18 Aug 2007: Lete (2450) - Tatopani (1400 m)

19 Aug 2007: Tatopani (1400 m) - Beni

20 Aug 2007: Beni - Pokhara - Kathmandu

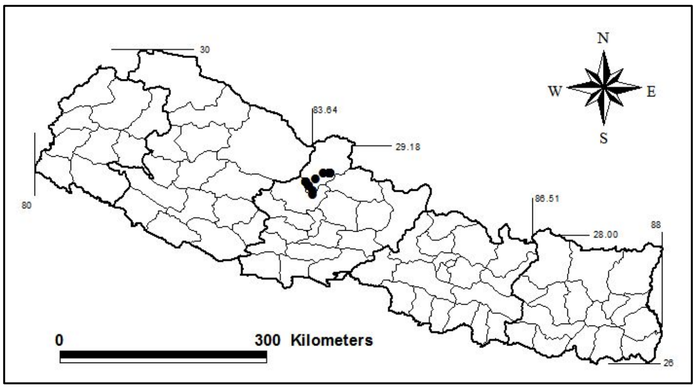


FIG 1. G

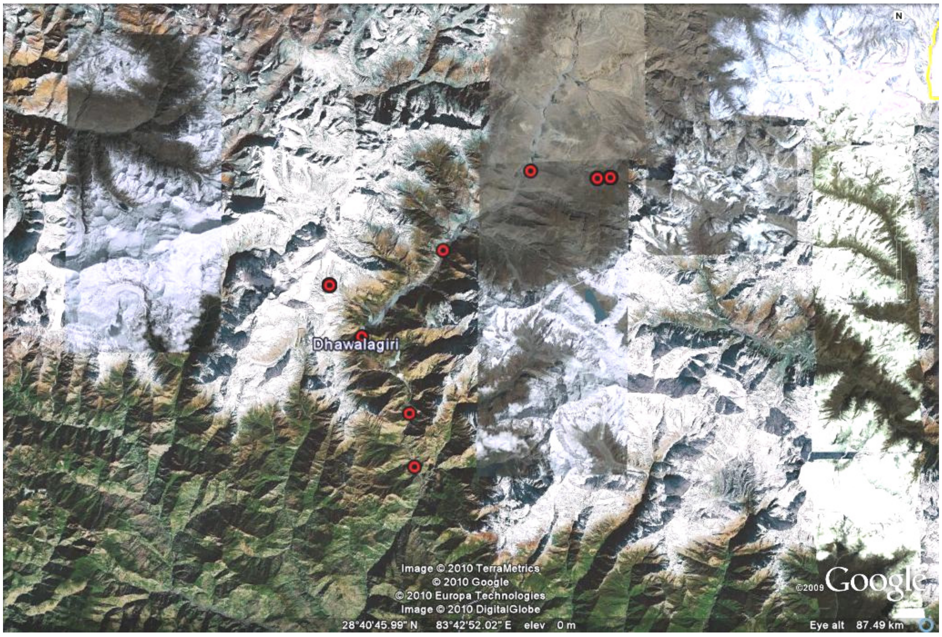


FIG1. H

FIG1. G, H. *Berberis* collection localities of EA2 expedition.

EDINBURGH LANTANG (KYANJIN) TRIP (BL2)

(B. Adhikari & B. Raskoti)

30 July 2007: Kathmandu - Dhunche

31 July 2007: Collecting around Dhunche

1 Aug 2007: Dhunche (1850 m) - Syabrubensi (1500 m) - Khangjing (2280 m)

2 Aug 2007: Khangjing (2280 m) - Lama Hotel (2450 m) - Ghodatabala (3013 m)

3 Aug 2007: Ghodatabala (3013 m) - Langtang (3363 m) - Kyanjin (3826 m)

4 Aug 2007: Kyanjin (3826 m) - Lamahotel (2450 m)

5 Aug 2007: Lamahotel (2450 m) - Syabrubensi (1500 m)

6 Aug 2007: Syabrubensi (1500 m) - Dhunche (1864 m)

7 Aug 2007: Dhunche - Kathmandu

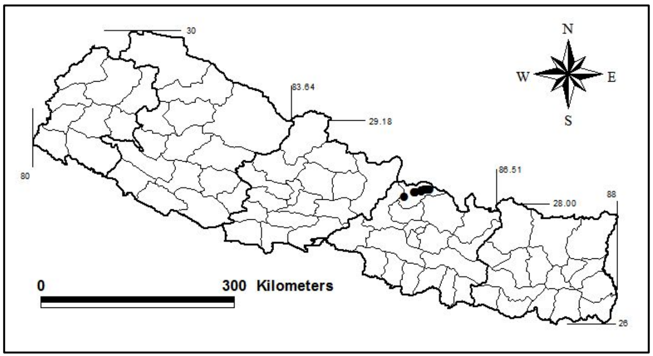


FIG. 1 I

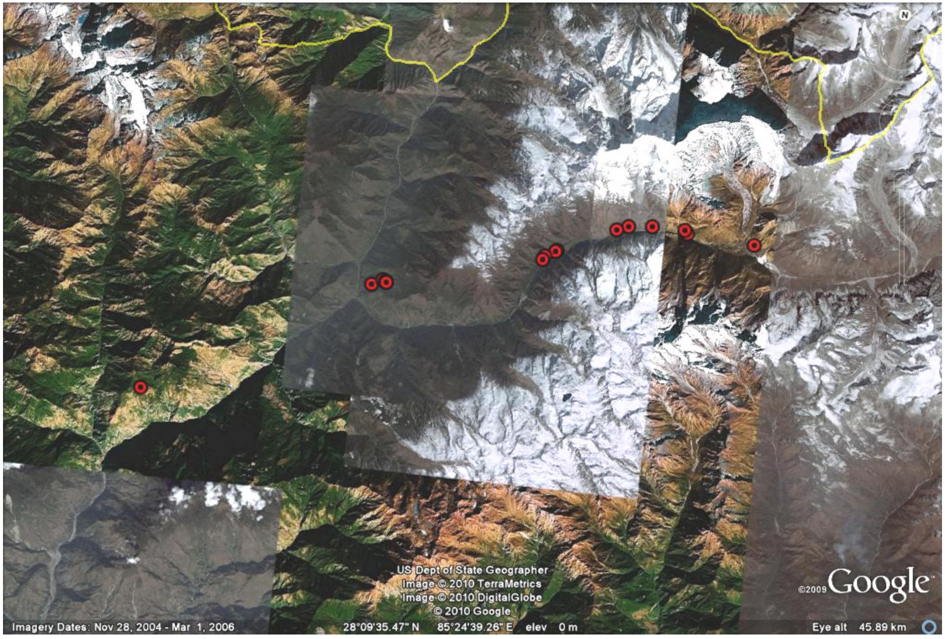


FIG. 1 J

FIG 1. I, J. *Berberis* collection localities of BL2 expedition.

JUMLA-RARA-SIMIKOT EXPEDITION (JRS) 2008

(C. Pendry, S. Noshiro, S. Baral, P. Kurmi, S. Rajbhandary, B. Dell, B. Adhikari)

1 June: Kathmandu - Nepalgunj - Surkhet
2 June: Surkhet - Jumla (2340 m)
3 June: Jumla, Khalanga (2340 m) - Khalichaur (3480 m)
4 June: Collecting around Khalichaur (3480 m)
5 June: Khalichaur (3480 m) - Kabra (2800 m)
6 June: Kabra (2800 m) - Bulbule (3000 m)
7 June: Bulbule (3000 m) - Jhyari (2500 m)
8 June: Jhyari (2500 m)- Rara (2900 m)
9 June: Rara (2900 m) - Gamgadi (2083 m)
10 June: Gamgadi (2083) - Luma (2070 m)
11 June: Luma (2070 m) - Darkhya (2770 m)
12 June: Darkhya (2770 m) - Sat Thapla (3120 m)
13 June: Sat Thapla (3120 m) - Darma (2300 m)
14 June: Darma (2300 m) - Nyashi Khola (1550 m)
15 June: Nyashi Khola (1550 m) - Limne (1800 m)
16 June: Limne (1800 m) - Lali (2300 m)
17 June: Lali (2300 m) -Yangchu (2120 m)
18 June: Yangchu (2120 m) - Simikot (3100 m)
19 June: Simikot (3100 m) - Norkeni (Above Simikot, 3640 m)
20 June: Collecting around Norkeni
21 June: Noekeni - Simikot
22 June: Simikot - Surkhet - Nepalgunj
23 June: Nepalgunj - Kathmandu

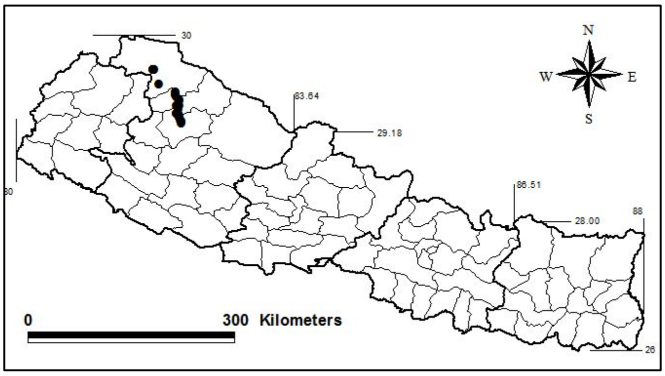


FIG 1. K

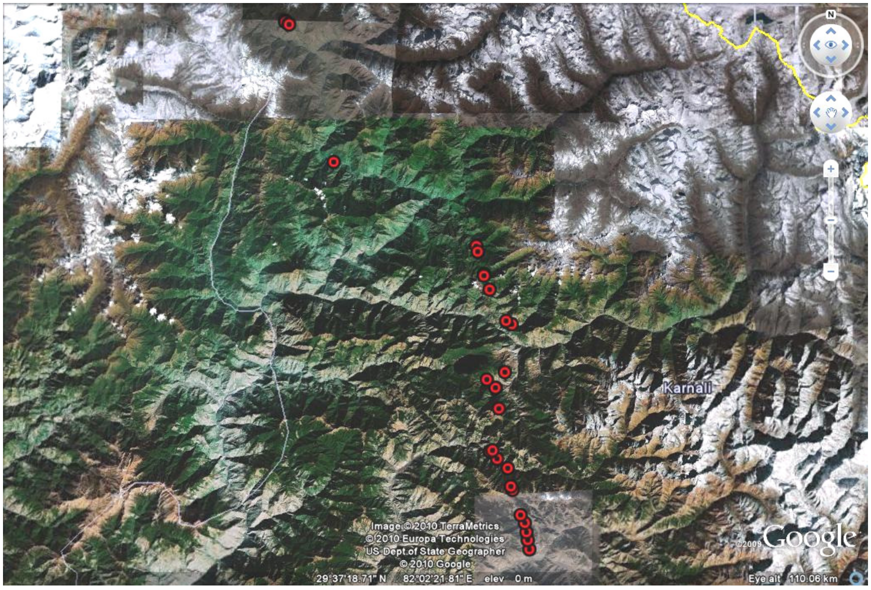


FIG 1. L

FIG1. K, L. *Berberis* collection localities of JRS expedition.

GOSIAKUND TRIP (G) 2008

(B. Adhikari)

18 May: Kathmandu - Dhunche

19 May: Dhunche (1850 m) - Chandanbari (3400 m)

20 May: Chandanbari (3400 m) - Lauribinayak (4000 m)

21 May: Lauribinayak (4000 m) - Gosaikund (4450) - Suryakund (4600 m) - Lauribinayak (4000 m)

22 May: Lauribinayak (4000 m) - Dhunche (1850 m)

23 May: Dhunche - Kathmandu

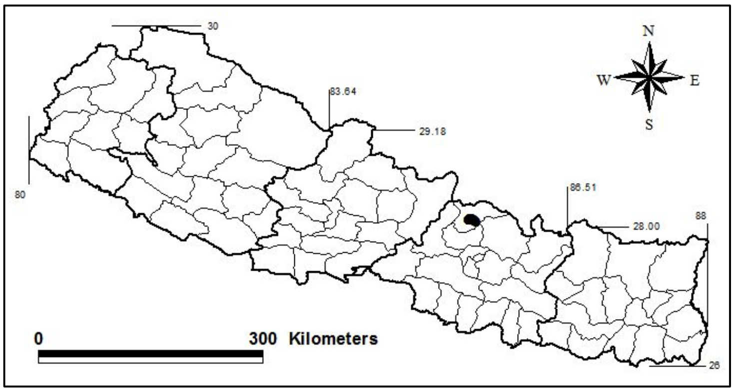


FIG 1. M

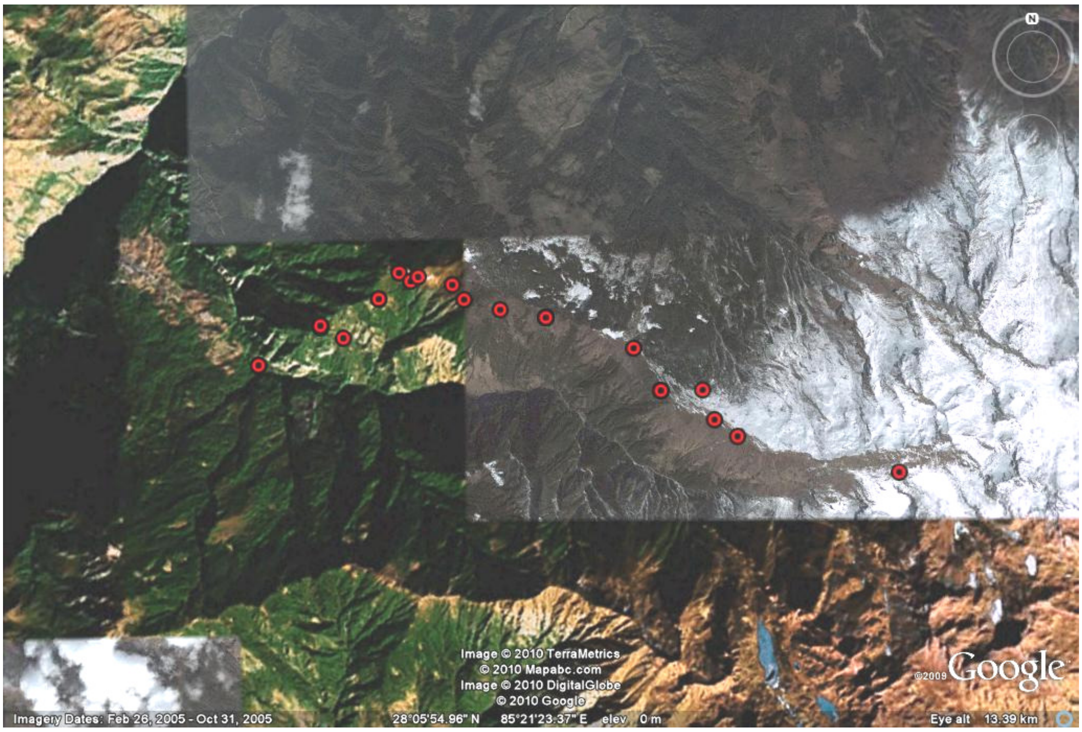
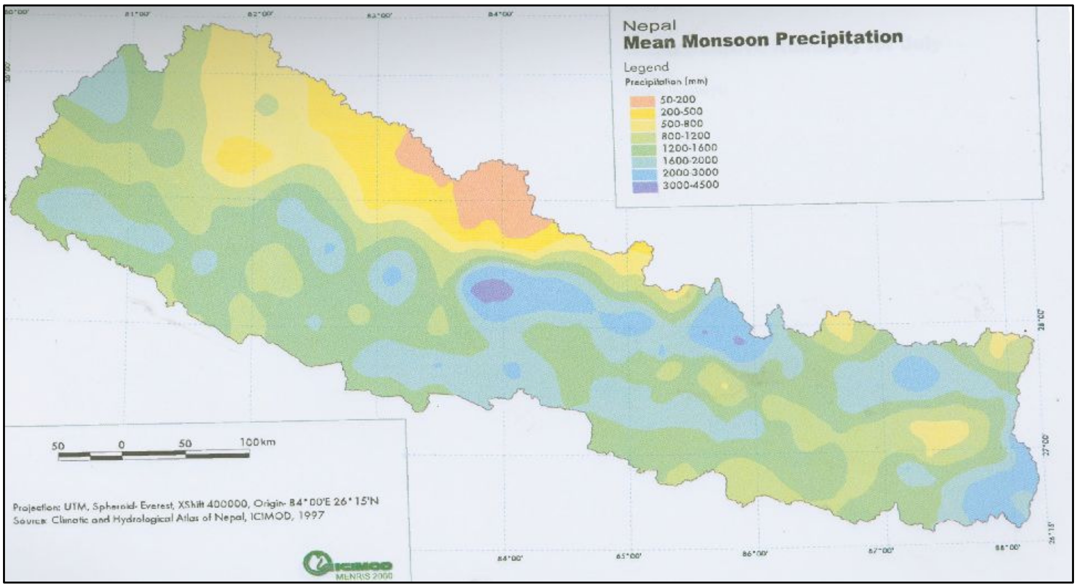


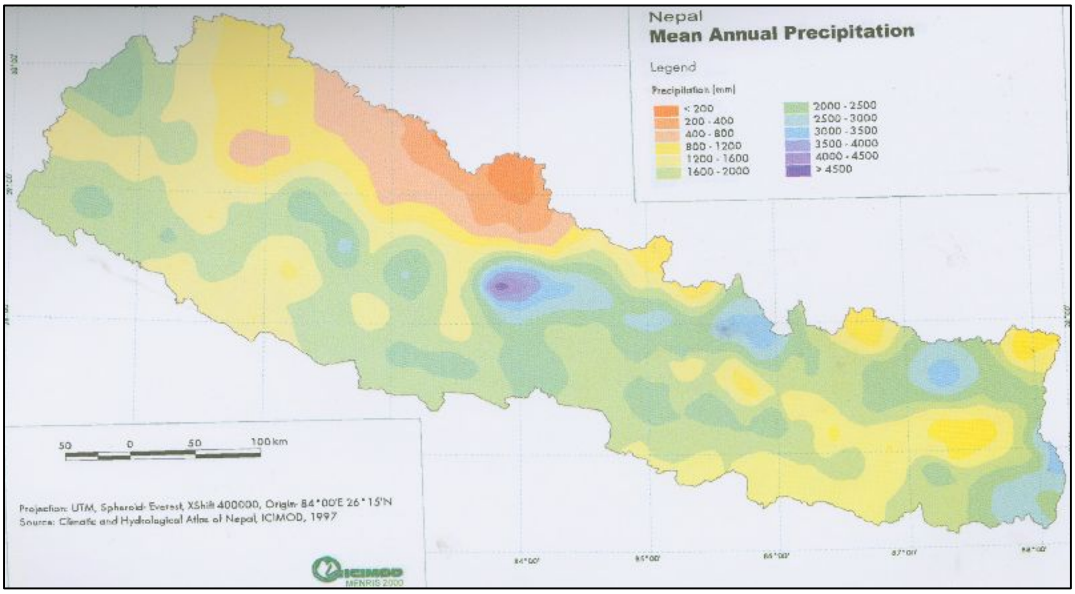
FIG 1. N

FIG 1. M, N. *Berberis* collection localities of Gosaikund trip.

APPENDIX 2

MEAN MONSOON PRECIPITATION AND MEAN ANNUAL PRECIPITATION MAP OF NEPAL (Taken from UNEP 2001, Nepal: State of the Environment).





APPENDIX 3

CD-ROM CONTENTS LIST

The CD-ROM that accompanies this thesis contains the following files:

List of specimens examined

Sequence data

*ndh*F aligned matrix (Chapter 3).

ITS aligned matrix (Chapter 4).

Images

Selected images of *Berberis* species taken during the field trips.

Thesis PDF version