Diversity of Genus *Begonia* L. (Begoniaceae) of the Sikkim Himalayas

A Thesis Submitted

To

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In Partial Fulfilment of the Requirement for the

Degree of Doctor of Philosophy

By

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This is to certify that the Ph. D thesis entitled "Diversity of Genus *Begonia* L. (Begoniaceae) of the Sikkim Himalayas" submitted to Sikkim University in partial fulfilment for the requirements of the degree of Doctor of Philosophy in Botany embodies the research work carried out by **Mr. Aditya Pradhan** at the Department of Botany, School of Life Sciences, Sikkim University. It is a record of bonafide investigation carried out and completed by him under my supervision. He has followed the rules and regulations prescribed by the University. The results are original and have not been submitted anywhere else for any other degree or diploma.

It is recommended that this Ph. D thesis be placed before the examiners for the evaluation.

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DECLARATION

I declare that the present Ph. D thesis entitled "Diversity of Genus *Begonia* L. (Begoniaceae) of the Sikkim Himalayas" submitted by me for the award of the degree of **Doctor of Philosophy in Botany** is a bonafide research work carried out by me at the Department of Botany, Sikkim University under the supervision of **Dr. Arun Chettri**.

The thesis contains no materials which has been accepted for a degree or diploma of any other University or Institution, except by way of background information and duly acknowledged in the thesis, and to the best of my knowledge and belief no materials previously published or written by another person except where due acknowledgement is made in the text of the thesis, nor does the thesis contain any materials that infringes copyright.

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"Diversity of Genus *Begonia* L. (Begoniaceae) of the Sikkim Himalayas"

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1.1. Taxonomy and Distribution of genus Begonia L.

Begonia L. is a mega-diverse genus belonging to the family *Begoniaceae*. It is the 5th largest genus of flowering plants with 1825 species distributed worldwide (Hoover et al. 2004; Hughes et al. 2015). The greatest diversity of *Begonia* is in Asia and America (>600 species each), with the bulk of species occurring in Southeast Asia having *ca*. 440 species (Hughes 2008) while Africa is relatively poor (*ca*. 160 species) in species diversity. The genus *Begonia* is believed to have originated from Africa, while the species found in South America and Southeast Asia are the results of parallel radiations over the last 20-50 million years (Plana et al. 2004; Gooddall Copestake et al. 2010; Thomas et al. 2011). The genus is absent in Australia as long-distance dispersal is very rare and uncommon in *Begonia* unable to cross the Torres strait from Papua New Guinea to Australia (Goodall-Copestake et al. 2010). The genus is represented by 59 species in India (Uddin 2007), with four new records and six new species discovered from Northeast India (Hughes and Camfield 2018; Pradhan et al. 2018) bringing a total to 69 species. The family consists of two genera *Begonia* Linnaeus and *Hillebrandia* Oliver. The genus *Hillebrandia* differs from *Begonia* in having a semi-inferior ovary. The genus *Hillebrandia* with its single species i.e. *H. sandwicensis* Oliver is endemic to Sandwich Island of the Hawaiian Islets, Northern America. The third genus, *Symbegonia*, which was previously included within the *Begoniaceae*, was later segregated based on floral characters and further based on molecular data the genus was incorporated within the *Begonia* section *Petermannia* (Forest and Hollingsworth 2003).

The family *Begoniaceae* is easily recognizable in having asymmetrical leaves, unisexual monoecious flowers, twisted-papillose stigmas and three winged capsules (Doorenbos 1998). However there is a large range of morphological diversity within the genus, mainly in vegetative form, and this morphological diversity is linked to adaptation to different ranges of ecological conditions. Characters such as perenating rhizome, leaf micro-morphology, and stomatal cluster underlie their ability to thrive in diverse niches. The distribution of species is uneven across the globe, with more species diversity found in the tropics than any other ecosystem in the world (Dewitte 2011). Even though plant diversity is contained in the tropics and shows the more morphological variation in the tropics, most of the study on evolution and development have used temperate or near temperate species as model species (Clausen et al. 1948; Bradshaw and Schemske 2003; Reiseberg et al. 2003). *Begonia* being one of the most highly diverse species-rich genera provides a model for investigating the process responsible for the generation of biodiversity and morphological innovation in large genera (Forrest et al. 2005; Neale et al. 2006). Morphological characters have been used to address the evolution of *Begonia* and as such often support well-resolved molecular clades (Forest et al. 2005) and therefore give insights into the evolution of morphology within the group. *Begonia* suffers from a considerable amount of taxonomic confusion. Many species are still unassigned to any section and this is due to lack of knowledge about the species, especially the Asian one (Rajbhandary et al. 2011). Medullary and cortical vascular bundles in the petiole and stem anatomical pattern resemble more like monocotyledons than dicotyledons. Correlations between leaf shape and the numbers and size of trichomes were examined by Mclellan (2005) and thus concluded the limitation of the existence of independent evolution of leaf shapes and trichomes in some *Begoniaceae*.

Begonia having a wide/broad distribution however shows differentiation at local and regional levels (Hughes 2008). In this regard, numerous studies have conducted to prove the differentiation at local and regional scales. The allozymes variation in 12 population of *B. dregei* and seven populations of *B. homonyma* from South Africa resulted in little/low gene flow amongst populations or even between populations isolated a few kilometres apart (Matolweni et al. 2000; Hughes et al. 2003; Hughes and Hollingsworth 2008). The low level of gene flow is been reported due to poor seed dispersal and limited pollen flow (Dewitte et al. 2011).

The molecular divergence age estimates indicate the origins of Asian *Begonia* date back to 18-15 Ma (Thomas 2010) by the time the Himalayas had already formed due to the collision of the Asian and Indian Plates by early Eocene c. 35 Ma (Ali and Aitchison 2008). The Himalaya is an important area in terms of its position as it is the centre of several different floristic regions (Bhuju et al. 2007) and hence *Begonia* being a narrow endemic species and poorly dispersed often makes it ideal for biogeographic analysis (Hughes and Hollingsworth 2008). One of the fundamental questions in biogeography is how species accumulate in different ecological regions? The ecological explanation for speciation patterns often includes environmental tolerance and niche partitioning (Nyari and Reddy 2013).

1.2. Ecological Niche

Sir Joseph's Grinnell (1917) first coined the term 'niche' in his classical paper "*The niche relationships of the California Thrasher*". According to him, the niche of the species is the total of all the ecological condition that allows the species to thrive and produce an offspring. Later Charles S. Elton (1927) defined 'niche' as the functional role played by the species in an ecosystem. G. E. Hutchinson (1957) later theorized the ecological niche as an n-dimensional hyper volume of all the environmental conditions (Fig.1).

The fundamental niche is the entire set of conditions under which a population or species can survive and reproduce it.

Realized niche is a set of environmental conditions used by a species or population, after interactions with other associated species. Sometimes Fundamental Niche and Realized niche are referred to as **precompetitive** and **post competitive** niches, reflecting a traditional focus on inter-specific competition's effect on niches.

The base of ENM is entirely dependent on environmental factors and is completely based on Grinnell's ecological niche. Therefore ecological niche based on ENM can be defined as '......*the set of ecological conditions within which the species can maintain its population without immigration*'.

1.3. Ecological Niche Modelling

Ecological niche modeling (ENM) or species distribution modeling (SDM) is a computer-based model which integrates the study of climate tolerances through combining the species occurrence data (i.e. geographic coordinates) with a set of environmental variable (bioclimatic, topographic, NDVI, etc.) to create a predicted distribution maps (Franklin 2009). One of the most popular methods to investigate species response to climate is through the construction of bioclimatic models (Nix 1986). The bioclimatic model is statistically more similar among sister groups (species) and that are conserved across evolutionally time (Peterson et al. 1999). Apart from predicting the suitable habitat of a species ENM can also be used to examine niche similarity and divergence between taxonomically uncertain species (Martı´nez-Gordillo et al. 2009; Adhikari et al. 2012). ENM has now become a powerful tool for biogeographers, conservation biologists, evolutionary biologists and ecologists in recent years (Marina and Peterson 2009; Pradhan and Chettri 2017; Adhikari et al. 2018). Predicting and mapping the suitable habitat for threatened and endangered species is critical for monitoring and restoration of their declining native populations (Kumar and Stohlgren 2009). In this regard species reintroduction is one such ecological engineering technique for restoration of the depleted species populations, degraded habitats and ecosystems (Adhikari et al. 2012). To reintroduce and rehabilitate the threatened species in terrestrial ecosystems, detailed knowledge on the distribution of their potential habitats is essential. Species distribution modeling or ENM, therefore, helps to identify the suitable habitat for reintroduction, and in developing effective species-specific conservation measures. It has been successfully used in restoring critical habitats and predicting the impact of environmental and climate change on species and ecosystems (Brooks et al. 2004; Samway 2005; Giriraj et al. 2008; Franklin 2009; Gogol-Prokurat 2011; Khanum et al. 2013). ENM also permits exploration of geographic and ecological phenomena based on known occurrence records of species (Peterson et al. 2011). ENM is a tool that can be used to explore niche divergence or conservatism among organisms (Arribas et al. 2012), so niche related characters may offer other lines of evidence by which to assess lineage separation.

A variety of SDM methods are available to predict potentially suitable habitat for a species (Guisan and Thuiller 2005; Elith et al. 2006; Guisan et al. 2007a; Guisan and Zimmermann 2007b; Wisz et al. 2008). However, comparatively, there are fewer examples of studies using small sample sizes. One such method is based on the principle of maximum entropy (MaxEnt) which has high success rates with sample sizes as low as five (Thuiller et al. 2005; Pearson et al. 2007; Pradhan et al.

2020) and has the best predictive power across all the sample sizes (Papes and Gaubert 2007; Wisz et al. 2008).

1.4. Correlative vis-a-vis Mechanistic approaches for ENM

The correlative approach for ENM relates point distributional data or present data in the form of latitude and longitude (i.e. geographic coordinate) of the species with the set of environmental data (i.e. raster data) to make a full distribution map of a species. On the other hand in mechanistic modelling, the physiological tolerance of the species is quantified to create a distribution map of a species. The correlative model is quite preferred due to the sufficient availability of species presence data and environmental raster data freely available online.

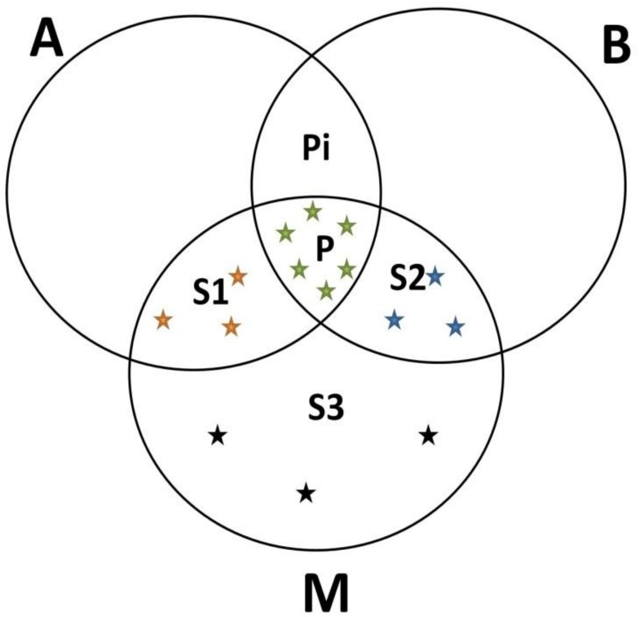

Fig 1: The three factors that determine the geographical distribution of a given species: **A** is the geographical projection of the fundamental niche. **B** is the geographical area where the biotic interactions would foster the survival of the species. **M** is the area of the world that is accessible to the species within a time period. P is the occupied area of distribution. A∩B = P+Pi is the geographical projection of the realized niche. Pi is the area that is suitable because both biotic and abiotic conditions co-occur, but is not accessible to the species (e.g., due to geographical barriers). S1 is the area where sink populations due to competitive exclusion occur. S2 is the areas where sink populations due to negative intrinsic grow rates occur. S3 is where sink populations due the combinations of the previous two reasons occur (modified from Soberón 2007).

1.5. ENM Packages/Algorithms

Many ecological niche modelling packages/algorithms are available in the web and most of them can be freely downloaded. Some of the commonly used programs are as follows:

i. MAXENT
ii. GARP
iii. DIVA
iv. BIOMAPPER
v. BIOCLIM
vi. DOMAIN
vii. MODECO
viii. FLORAMAP
ix. Support Vector Machine
x. Artificial Neural Networks
xi. Generalized Linear Models
xii. Generalized Additive Models
xiii. Maximum Likelihood
xiv. Classification & Regression Trees
xv. Boosted Regression Trees
xvi. Random Forests
xvii. Bayesian Methods
xviii. Relative Environmental Suitability

1.7. Plant secondary metabolite

Plant secondary metabolites (PSM) are a broad group of chemical compounds generally produced by the majority of the plant to cope abiotic and biotic stresses. These metabolites are not necessary for growth and development and hence term as 'secondary metabolites' (Hartmann 2007).

PSM can be roughly classified into three classes:

(1) Alkaloids

(2) Phenolic compounds and

(3) Terpenes.

1.7.1. Alkaloids

As many as 10,000 alkaloids compounds have been isolated for the past 200 years. These compounds are readily found in plants, animals, bacteria, and fungi (Cushnie et al. 2014), usually having toxic or analgesic properties on animals (Hopkins 2003). These properties of alkaloids make the compound used in both modern and traditional medicine. The toxic alkaloids compound has multiple roles in plants which includes protection against infection (Faeth and Saari 2012). However not all plant produces alkaloids, several plant families like Poaceae do not produce alkaloids. These plants synthesize alkaloids by fungal endophyte, which controls the production and accumulation level of alkaloids (Schardl et al. 2013).

1.7.2. Phenolic compounds

There are around 10,000 phenolic compounds isolated are highly diverse, ranging from simple phenol to more complex ones, like tannins and lignins (Hopkins 2003). These compounds produced by plants, fungi, and bacteria. Polyphenol is compounds containing several hydroxyl groups and is often used as a reference to the whole family of tannins (Chomel et al. 2016).

Based on their structure phenolic compounds can be classified into simple phenol (6 carbon skeleton), phenolic acid (C6-C3), flavonoids (C6-C3-C6), Tannins ([(C6-C3-C6)]n). Sometimes based on the degree of polymerization, phenolic compounds can be further classified as (a) phenolic compound with low molecular weight (b) phenolic compound with a high molecular weight

1.7.3. Terpenes

Terpenes are the most diverse group of plant secondary metabolite (Hartmann 2007). These groups of PSM is usually produced by plants ranging in concentration from 1-2 % in dry matter, greater concentration is found in certain plant structures like trichomes (Langenheim 1994). Some terpenes are volatile and are sometimes referred to as volatile organic compounds. Latex, carotenoids, hormone, carotenoids pigments and essential oils are all included in terpenes family (Hopkins 2003).

1.8. Biochemical variation along the elevation gradient

Biosynthesis of the secondary metabolites in plants is not only controlled genetically but is also strongly affected by different biotic and abiotic stresses (Naghdi Badi et al. 2004). In different ecological niches, plants behave differently in terms of biochemical aspects to better adapt to their environment. This broad range of environmental factors across altitude thus affects the chemical composition (Aslam et al. 2015). Plants at various altitudes can adapt by avoiding and overcoming the stress conditions utilizing various physiological and biochemical mechanisms or can have a different response related to their resistance to these stress conditions which depends mainly on the morphology, anatomy and life cycle (Kuss 2005). It has been reported that environmental factors are known to influence the quality and quantum of secondary metabolites (Gautier et al. 2008). For example, an increase in altitude showed a decreasing trend in the berberine content of *Berberis asiatica* (Andola et al.

2010). In contrast, phenolic content increased with increasing altitude in *Hedychium spicatum*, etc (Rawat et al. 2011). These studies are indicative of the fact that responses of a species for secondary metabolites do affect by environmental factors. Studies on these aspects are not systematically investigated in *V. jatamansi*. Earlier studies on the variation in morphological and genetic diversity of *V. jatamansi* suggest that environmental and genetic factors influence the diversity of the plant and its chemical constituents (Sundaresan et al. 2012). However, a systematic investigation on the response of the species for its secondary metabolites activity across altitudinal range and habitat conditions is poorly known. This is important because the species grow in different habitat conditions at the wide altitudinal range, where microclimatic conditions like temperature, moisture regime, and soil nutrients varied considerably (Rawal et al. 2003). All these factors are reported to have direct bearing with secondary metabolites of a plant species (Andola and Rawal 2011). Thus attempts were made to investigate the level of variation in morphological and chemical constituents (*viz*. total phenolic, flavonoid, and tannin contents) in *B. satrapis* along the elevation gradient.

Objectives

1. To study the taxonomy and distribution pattern of *Begonia* in Sikkim Himalaya, in relation to bioclimatic variables.
2. To model the distribution of two rare species of *Begonia* using Ecological niche modelling technique.
3. Study of Biochemical and Phytochemical variation in different population of *Begonia satrapis* in Sikkim Himalayas

2.1. Taxonomic history of genus Begonia L.

The genus *Begonia* L. was for the first time discovered from the West Indies by Charles Plumier in the year 1690. This new genus proposed by Plumier (1703) was named in the honor of French superintendent of Santo Domingo (1638-1710) Michel Begon. The first nine species of *Begonia* which was discovered before 1753 from Neotropical and Pantropical regions were given different names and were later reclassified and renamed as *Begonia*.

Linnaeus (1753) in "*Genera Plantarum"* placed the genus under section *'Fragmenta Tournefortiana'*. The genus was first considered it as "genus *incertaesedis*" placed underclass Polygamiadioecia'. However, the clear description of the genus *Begonia* was provided by Dryander (1791) using the foliage leaf character.

The credit for the establishment of family *Begoniaceae* was given to various authors like Klotzsch (1854), Lindley (1836), Endlicher (1836-1840) and Bentham and Hooker (1865-1867). The revisionary work published by Klotzsch (1855) identified and described 194 species grouped into 41 genera. The family was divided into two groups *Stephanocarpeae* with persistent stylodia in fruit and *Gymnocarpeae* with deciduous stylodia. His classification based on a difference in the shape of the styles and complicated stigmas prime significance (Clarke 1879).
Later Candolle (1864) proposed three genera namely Casparya, Begonia and Mezierea reducig the genera from earlier 41 by Klotzsch. The proposed genera by Candolle were differentiated based on fruit structure and the mode of dehiscence.

Bentham and Hooker (1867) recognized 330 species within the family *Begoniaceae*, amongst which 329 were placed within a single genus *Begonia*. The monotypic genus *Hillebrandia*, described by Oliver (1866) was maintained by them. Based on a combination of characters of the androecium, the styles and the placentas, the genus *Begonia* was arranged into 5 series. These 5 series were subdivided into species-specific groups using morphological and geographical criteria. Clarke (1879) further developed a classification on genus *Begonia* wherein 6 sections were proposed based on the number of cells in the fruit and the mode of the fruit dehiscence. Warburg's (1895) revisionary work on *Begoniaceae* incorporated new sections and species whereby increasing the number of sections to 62 with *ca* 470 species. Four genera were recognised *viz*. *Hillebrandia*, *Begonia*, *SymBegonia* and *Beganiella*. Irmscher's (1925) classification on genus *Begonia* further added two sections viz. *Apteron* A. DC and *Warburgina* O. Kuntze and recognised five genera *viz*. *Hillebrandia*, *Begonia*, *SymBegonia*, *Semibegoniella* and *Begoniella*. Doorenbos et al. (1998) recognised 1403 species of *Begonia* under 63 sections.

2.2. Diversity of genus Begonia L.

The mega diverse pantropical genus *Begonia* were initially diversified in Africa but at present the region is relatively species-poor (*ca*. 160 species) compared to the Neotropics (*ca*. 690 species) and Asia (*ca*. 650 species) (Goodall-Copestake et al. 2010). The diversification rates of African, Asian and two Neotropical *Begonia* clades were estimated in Bayes rate 1.7.5 (Silvestro et al. 2011). The diversity of Asians *Begonia* is significantly faster than African *Begonia*. The diversification rate of Africa was estimated at 0.23 Myr _1 (SD 0.06) whereas those of Asian *Begonia* at 0.61 Myr _1 (SD 0.14) (Valente et al. 2010). The faster diversification rate of *Begonia* in Southeast Asia is striking and deserves closer investigation (Moonlight 2015).

2.3. Classification of Begonia

On the basis of molecular and morphological data the family *Begoniaceae* is now firmly placed within the order Cucurbitales. The order also includes the economically and species rich family *Cucurbitaceae* (950-980 species) and species poor family *Tetramelaceae* (2 species) (De Witte et al. 2011). The family *Begoniaceae* is strongly related to family *Datiscaceae* and *Tetramelaceae* based on 14 nuclear, mitochondrial and plastid marker (Schaefer and Renner 2011). Two genera are presently recognised within the family *Begoniaceae* (Forest and Hollingsworth 2003) *viz*. *Begonia* (a diverse genus which now includes the American genera *Semibegoniella* and *Begoniella* as well as genus *SymBegonia*) (Wilde 2011) and *Hillebrandia* (Taxonomically and geographically isolated Pacific genus). The genus *Begonia* is currently divided into 66 sections comprising 1920 species (Hughes et al. 2015). The continental African *Begonia* with 115 species distributed over 12 sections is currently well studied (Wilde 2011). The African continent is comparatively poor in species diversity but shows extreme morphological variations. Keraudren-Aymonin (1983) recognized 50 species of *Begonia* from Madagascar and the Comores.

In India, 64 species of *Begonia* were reported, under 6 sections (*viz. Casparya, Alaecida, Knesebeckia, Plactycentrum, Papayraceae, and Uniplacentales*) along with 9 infraspecific taxa and one doubtful species *B. khasiana* C. B. Clarke (Clarke 1879), which is still categorized as a doubtful species (Camfield and Hughes 2018). Kumar and Bhattacharya (1992) listed 23 species endemic to India. Rao et al. (2003) reported 55 species of which 18 are listed under the red category. Uddin (2007) reported 56 species for India of which 10 being endemic to Northeast India, 8 to Western Ghats and 1 to Andaman and Nicobar Island. Nayar et al. 2006 reported 17 wild species of *Begonia* from the Western Ghats. *B. aliciae* C.E.C. Fisch. and *B. crenata* Dryand, rediscovered by Kumar et al. (2002) showed overlapping characters and was later considered as conspecific by Aitawade and Yadav (2012). Camfiled (2009) revisionary work on *Begonia* of Northeast India, confirmed 41 species under 5 sections of which 13 being endemic to the region. However, Camfiled and Hughes (2018) later revised the genus *Begonia* from the region and confirmed 38 species of which 10 being endemic with one new species (*viz. Begonia koelzii* R.Camfield) discovered from Nagaland bringing a total of 39 species from the Northeast region with one new record (Pradhan et al. 2019) and two endemic to Sikkim Himalaya bringing a total to 42 species from Northeast India (Pradhan et al. 2019). *Begonia naga* described from Mokokchung, Nagaland, India by Krishna et al. (2018) were later treated as synonyms of *Begonia manhaoensis* (Aung et al. 2020). *Begonia keralensis* Pradeep, Sinj. Thomas and Britto described by Krishna et al. (2019) is also treated as synonyms of *Begonia bachulkarii* Aitawade, Kattuk and S.R.Yadav both described from Nelliyampathy in Kerala, India (Aitawade et al. 2019).

2.4. Morph taxonomy

The morphological study in *Begonia* species is fairly negligible. The morph taxonomy of genus *Begonia* from the Western Ghats is studied by Aitawade (2013). The male flower and androecium morphology of Nepalese *Begonia* were studied by Rajbhandary et al. (2011). Moreover, using scanning electron microscopy (SEM) the ornamentation of pollen was analysed on 28 Nepalese *Begonia* based on size, shape in polar and equatorial views, P/E ratio, aperture, and exine (Rajbhandary et al. 2012).

2.5. Ethno-botanical importance

The malasar tribe of Kerala and Tamil Nadu traditionally uses the stem extract of *B. malabarica* to treat diabetes, arthritis and common joint pain (Pandi Kumar and Ignacimuthu 2007; Pandikumar et al. 2009). The leaf extracts are also used as the substitute of *Tamarindus indica* L. (Tamarind) by Paliyan tribes of Tamil Nadu against respiratory problems, stomach ulcers and stomach-ache (Ramesh et al. 2002). The dried aerial part is made a paste with coconut oil and is taken against rheumatic pain (Bose et al. 2014). The leaf juice mixed with ginger is used in treating anemia (Suresh and Nagarajan 2009). Kanikkar tribes of the Agasthyamalai Biosphere Reserve, Tamil Nadu, used the fresh leaf juice of *B. malabarica* with salt to treat dizziness (Mohan and Rajesh 2008). Since ancient times *B. fimbristipula* is used as food and also as herbal medicine, as it contains a low percentage of lipids and a high proportion of dietary fiber, polyphenolic substances and fructan-oligosaccharides (Ke and Pan 2007).

Tribal people of Kanikkars believe that this plant has rejuvenation capacity and its juice is consumed to treat fever and in blood purification (Ariharan et al. 2012). Fresh leaves of *B. floccifera* are consumed to treat stomach pain (Kalpanadevi and Mohan 2012). *B. malabarica* leaves are used variously to treat various diseases such as fresh ones to treat respiratory infections, diarrhea, skin diseases and blood cancer (Kirtikar and Basu 1975).

2.6. Molecular investigation

The genus *Begonia* L. is believed to have originated from Africa and based on combined internal transcribed spacer (ITS) and trnL intron sequences the historical biogeographic analysis of African *Begonia* were investigated. The study concluded that a large number of *Begonia* diversity in Africa were of pre-Pleistocene origin. The Pleistocene origin species were mostly concentrated within sections *Tetraphila*, *Loasibegonia* and *Scutobegonia* having their centre of diversity in Western Central Africa (Plana et al. 2004).

The phylogeny of Asian *Begonia* was constructed using non-coding plastid DNA sequence data for 84 major Asian species. The fruit and ovary characters were mapped on the phylogeny to assess their utility in infra-generic classifications, which is very crucial in the present sectional circumscriptions (Thomas et al. 2011).

Begonia having a wider distribution shows differentiation at a very local and regional scale (Hughes 2008). Several studies were made on local patterns of differentiation based on molecular markers. The allozyme variation was investigated in the populations of *B. dregei* and closely related species *B. homonyma* (Matolweni et al. 2000). The results of which very little or no gene flow among populations or even within populations located only a few kilometres apart.

2.7. Discovery of new species since 2015

In recent years, a significant number of *Begonia* species were discovered. In 2007, Flora of China was published recording 173 species of *Begonia* (Gu et al. 2007). Recently published articles on diversity and conservation of wild Chinese *Begonia* predicted 250-300 species from China alone (Tian et al. 2018). An updated checklist on *Begonia* of Myanmar recognized 73 species (Hughes et al. 2019). 18 species of *Begonia* have been recognised from New Guinea (Gagul et al. 2018). 14 species recognised from Sumatra, Indonesia (Girmansyah et al. 2019). So far 19 species have been described from Laos (Hong Bo et al. 2020). The flora of Bangladesh has recognized 19 species of *Begonia* (Deb and Rahman 2018). In Nepal 29 species of *Begonia* have been reported (Rajbhandary et al. 2011). In addition the list of newly discovered species in last five years is given in table 1.

Table 1: List of newly discovered species of *Begonia* since 2015

Species	Country	Citation
Begonia abbreviata C.-I Peng	Vietnam	Peng et al. 2015
Begonia acidulenta Sang and Kiew	Borneo	Sang and Kiew 2016
Begonia adamsensis Magtoto and R. Rubite	Philippines	Magtoto et al. 2018
Begonia addrinii S. Julia and Kiew	Borneo	Sang and Kiew 2016b
Begonia aiensis C.W. Lin and C.-I Peng	Sarawak, Boreo	Lin et al. 2017
Begonia alabensis Kiew	Malaysia	Repin et al. 2015
Begonia albopunctata Y.M. Shui, W.H. Chen and H.Q. Nguyen	Northern Vietnam and Southern China	Chen et al. 2018
Begonia amidalae C. W. Lin and C.-I Peng	Borneo	Lin et al. 2014b
Begonia anserina C. W. Lin and C.-I Peng	Borneo	Lin et al. 2014b
Begonia apiensis Kiew and S.Julia		Sang et al. 2013
Begonia armykapil S. Julia and C.Y. Ling	Malaysia	Sang et al. 2016
Begonia austrovietnamica C.-I Peng, C.W.Lin, D.D. Nguyen and N.D. Truong	Vietnam	Peng et al. 2018
Begonia baik C. W. Lin and C.-I Peng	Malaysia	Lin et al. 2015
Begonia baikoides S. Julia and C.Y. Ling	Malaysia	Sang et al. 2016
Begonia balgooyi D.C.Thomas and Ardi	Indonesia	Thomas et al. 2018
Begonia bambusetorum H.Q. Nguyen, Y.M. Shui and W.H. Chen	Northern Vietnam and Southern China	Chen et al. 2018
Begonia bayae Sang and Kiew	Borneo	Sang and Kiew 2016
Begonia benaratensis S.Julia		Sang et al. 2013
Begonia bintang Rimi	Malaysia	Repin et al. 2015
Begonia bosuangiana S.Julia	Malaysia	Repin et al. 2015
Begonia calciphila C.-I Peng	Vietnam	Peng et al. 2015
Begonia caobangensis C.-I Peng and C. W. Lin	Vietnam	Peng et al. 2015
Begonia celata S. Julia and Kiew	Borneo	Sang and Kiew 2016b
Begonia circularis C.-I Peng and C. W. Lin	Vietnam	Peng et al. 2015
Begonia compacta Sang and Kiew	Borneo	Sang and Kiew 2016
Begonia conipila Irmsch. ex Kiew		Sang et al. 2013
Begonia conniegeriae S.Julia and Kiew		Sang et al. 2013
Begonia crassa S. Julia and Kiew	Borneo	Sang and Kiew 2016b
Begonia cremnophila Tebbitt		Tebbit 2013
Begonia crockerensis Rimi	Malaysia	Repin et al. 2015
Begonia culasiensis C.W. Lin and C.-I Peng	Philippines	Peng et al. 2017
Begonia curvifolia Ardi	Indonesia	Ardi and Hughes 2018
Begonia darthvaderiana C. W. Lin and C.-I Peng	Borneo	Lin et al. 2014b
Begonia delicata Gregorio and J.A.S. Costa	Brazil	Gregorio et al. 2015
Begonia devexa S. Julia and Kiew	Borneo	Sang and Kiew 2016b
Begonia dinosauria C.W. Lin and C.-I Peng	Sarawak,Boreo	Lin et al. 2017
Begonia divergens Kiew and S.Julia		Sang et al. 2013

Begonia dolichobracteata Girm	Borneo	Girmansyad and Susanti 2015
Begonia doloisii Rimi	Malaysia	Repin et al. 2015
Begonia droseroides C.I. Peng, Rubite and C.W. Lin	Philippines	Rubite et al. 2018
Begonia duhungensis Girm	Indonesia	Girmansyah 2017
Begonia edgariana Sang and Kiew	Borneo	Sang and Kiew 2016
Begonia elachista Moonlight and Tebbitt	Peru	Moonlight et al. 2017
Begonia elianeae Gregorio and J.A.S. Costa	Brazil	Gregorio et al. 2015
Begonia erectocarpa H.Q. Nguyen, Y.M. Shui and W.H. Chen	Northern Vietnam and Southern China	Chen et al. 2018
Begonia erythrothrix Tebbitt and Moonlight	Peru	Moonlight and Tebbitt 2017
Begonia exposita Phutthai and M.Hughes,	Thailand	Phutthai and Hughes 2017
Begonia felis C. W. Lin and C.-I Peng	Malaysia	Lin and Peng 2017
Begonia flammea Rimi	Malaysia	Repin et al. 2015
Begonia fractiflexa S. Julia and Kiew	Borneo	Sang and Kiew 2016b
Begonia fulgurata C.-I Peng, C.W. Lin and Phutthai	Thailand	Peng et al. 2017
Begonia gabaldonensis Rubite, C.I. Peng and C.W. Lin	Philippines	Rubite et al. 2018
Begonia guixiensis Yan Liu, S.M. Ku and C.-I Peng	Vietnam	Peng et al. 2014
Begonia gulongshanensis Y.M. Shui and W.H. Chen	Northern Vietnam and Southern China	Chen et al. 2018
Begonia henrilaportei Scherber. and J. Duruisseau	Madagascar	Scherberich and Duruisseau 2016
Begonia hinnamnoensis Souvann. and Lanors.	Central Laos	Souvannakhoummane et al. 2018
Begonia hirsuticarpa C.W. Lin and C.-I Peng	Sarawak, Boreo	Lin et al. 2017
Begonia hititepala S. Julia and Kiew	Borneo	Sang and Kiew 2016b
Begonia hosensis C.W. Lin and C.-I Peng.	Malaysia	Lin et al. 2014a
Begonia hughesii R. Rubite and C.-I Peng	Philippines	Rubite et al. 2015
Begonia inobongensis Kiew	Malaysia	Repin et al. 2015
Begonia intermedia D.K. Tian and Y.H. Yan	China	Tian et al. 2014
Begonia iridifolia C.W. Lin and C.-I Peng	Sarawak, Boreo	Lin et al. 2017
Begonia jamilahana Y.W.Low, Joffre and Ariffin	Brunei Darussalam	Low et al. 2015
Begonia jamilahanuiana S. Julia and Kiew	Borneo	Sang and Kiew 2016b
Begonia jenginensis Sang and Kiew	Borneo	Sang and Kiew 2016
Begonia jinyunensis C.-I Peng, B. Ding and Q. Wang		Ding et al. 2014
Begonia kanaensis Kiew and C.Y. Ling	Borneo	Kiew et al. 2016
Begonia kanburiensis Phutthai	Thailand	Phutthai et al. 2014
Begonia khammouanensis Souvann. and Lamxay	Central Laos	Souvannakhoummane et al. 2018
Begonia kinahimiae Rimi	Malaysia	Repin et al. 2015
Begonia kipandiensis S.Julia	Malaysia	Repin et al. 2015
Begonia kuchingensis C. W. Lin and C.-I Peng	Malaysia	Lin and Peng 2017
Begonia langsonensis C.-I Peng and C. W. Lin	Vietnam	Peng et al. 2015

Begonia lawii C.W. Lin and C.-I Peng	Sarawak, Boreo	Lin et al. 2017
Begonia lecongkietii N.S.Lý and M.Hughes	Vietnam	LY et al. 2018
Begonia leipingensis D. K. Tian, L. H. Yang and C. Li	China	Li et al. 2016
Begonia lichenora C.W. Lin and C.-I Peng	Sarawak, Boreo	Lin et al. 2017
Begonia lingiae S. Julia	Borneo	Kiew et al. 2016
Begonia locii C.-I Peng, C. W. Lin and H. Q. Nguyen	Vietnam	Peng et al. 2015
Begonia longa C.-I Peng and W.C. Leong	Vietnam	Peng et al. 2014
Begonia ltahensis Girm.	Indonesia	Girmansyah 2017
Begonia lucychongiana S.Julia and Kiew		Sang et al. 2013
Begonia lugrae Ardhaka and Undaharta	Bali	Ardi et al. 2013
Begonia marinae Tebbitt,	Bolivia	Tebbit 2015
Begonia madulidii Rubite, C.I. Peng and C.W. Lin	Philippines	Rubite et al. 2018
Begonia magentifolia Kiew and S.Julia		Sang et al. 2013
Begonia magnicarpa C.W. Lin and C.-I Peng	Sarawak, Boreo	Lin et al. 2017
Begonia mariachristinae Wahlsteen	Myanmar	Wahlsteen 2018
Begonia matarombeoensis D.C.Thomas and Ardi	Indonesia	Thomas et al. 2018
Begonia medogensis Jian W. Li, Y.H.Tan and X.H.Jin	Western China and Northern Myanmar	Li et al. 2018
Begonia melanobullata C.-I Peng and C. W. Lin	Vietnam	Peng et al. 2015
Begonia melinauensis S.Julia and Kiew		Sang et al. 2013
Begonia mentewangensis Girm	Borneo	Girmansyad and Susanti 2015
Begonia merrilliana C.W. Lin and C.-I Peng	Philippines	Peng et al. 2017
Begonia metallicolor C.W. Lin and C.-I Peng	Sarawak, Boreo	Lin et al. 2017
Begonia minissima H.Q. Nguyen, Y.M. Shui and W.H. Chen	Northern Vietnam and Southern China	Chen et al. 2018
Begonia mollissima Y.M. Shui, H.Q. Nguyen and W.H. Chen	Northern Vietnam and Southern China	Chen et al. 2018
Begonia moneta C.-I Peng, Rima and C.W. Lin	Malaysia	Peng et al. 2015
Begonia montaniformis C.-I Peng, C. W. Lin and H. Q. Nguyen	Vietnam	Peng et al. 2015
Begonia myanmarica C.-I Peng and Y. D. Kim	Myanmar	Tseng et al. 2017
Begonia namkadingensis C.-J. Yang, Souladeth and Tagane	Laos	Yang et al. 2018
Begonia natunaensis C.W. Lin and C.-I Peng.	Indonesia	Lin and Peng 2014
Begonia nephrophylla Undaharta and Ardi	Indonesia	Undaharta and Ardi 2016
Begonia nix C.W. Lin and C.-I Peng	Sarawak, Boreo	Lin et al. 2017
Begonia octopetala L'He´r., Stirp.		Tebbit 2015
Begonia ocellata Ardi	Indonesia	Ardi and Hughes 2018
Begonia padawanensis baik C. W. Lin and C.-I Peng	Malaysia	Lin et al. 2015
Begonia paganuccii Gregorio and J.A.S. Costa	Brazil	Gregorio et al. 2015
Begonia papulifolia S. Julia and C.Y. Ling	Malaysia	Sang et al. 2016
Begonia pengchingii Phutthai and M.Hughes	Thailand	Phutthai and Hughes

		2017
Begonia peridoticola Rimi, C.-I Peng and C.W. Lin	Malaysia	Peng et al. 2015
Begonia pleiopetala A. DC.		Tebbit 2015
Begonia phutthaii M.Hughes	Thailand	Phutthai and Hughes 2017
Begonia pseudosubperfoliata Phutthai and M.Hughes)	Thailand	Phutthai and Hughes 2017
Begonia pseudopleiopetala Tebbitt		Tebbit 2015
Begonia rambutan Rimi	Malaysia	Repin et al. 2015
Begonia ramosissima Kiew and S.Julia		Sang et al. 2013
Begonia ranaiensis Girm.	Indonesia	Girmansyah 2013
Begonia rhodoneura S.Julia		Sang et al. 2013
Begonia rhytidophylla Y.M. Shui and W.H. Chen	Northern Vietnam and Southern China	Chen et al. 2018
Begonia rubrobracteolata S. Julia and C.Y. Ling	Malaysia	Sang et al. 2016
Begonia rubrotepala S. Julia	Borneo	Kiew et al. 2016
Begonia sadirensis Kiew and S. Julia	Borneo	Kiew et al. 2016
Begonia semongkatensis Girm.	Indonesia	Girmansyad 2016
Begonia sendangensis Ardi	Lombok	Ardi et al. 2013
Begonia serianensis C. W. Lin and C.-I Peng	Malaysia	Lin and Peng 2017
Begonia solaniflora Jara	Colombia	Jara-Muñoz et al. 2017
Begonia speculum Moonlight and Tebbitt	Peru	Moonlight and Tebbitt 2017
Begonia sphenantheroides C.-I Peng	Vietnam	Peng et al. 2015
Begonia supercillaris C.W. Lin and C.-I Peng	Sarawak, Boreo	Lin et al. 2017
Begonia sykakiengii C.W. Lin and C.-I Peng	Philippines	Peng et al. 2017
Begonia tagbanua M. Hughes, C.-I Peng and R. Rubite	Philippines	Rubite et al. 2015
Begonia tamdaoensis C.-I Peng	Vietnam	Peng et al. 2015
Begonia taraw C.-I Peng, R. Rubite and M. Hughes	Philippines	Rubite et al. 2015
Begonia tebiang Sang and Kiew	Borneo	Sang and Kiew 2016
Begonia tenasserimensis Phutthai and M. Hughes		Phutthai and Hughes 2017
Begonia tinjanii S. Julia and C.Y. Ling	Malaysia	Sang et al. 2016
Begonia tomaniensis Rimi	Malaysia	Repin et al. 2015
Begonia triangularis S. Julia and C.Y. Ling	Malaysia	Sang et al. 2016
Begonia ubahribuensis S. Julia and Kiew	Borneo	Sang and Kiew 2016b
Begonia umbratica S.Julia		Sang et al. 2013
Begonia vulgare S.Julia and Kiew		Sang et al. 2013
Begonia wallacei C.W. Lin and C.-I Peng	Sarawak, 147Boreo	Lin et al. 2017
Begonia wui-senioris C.-I Peng	Myanmar	Peng et al. 2014
Begonia wuzhishanensis C.-I Peng, X.H. Jin and S.M. Ku	China	Peng et al. 2014
Begonia xiphophylloides Kiew		Sang et al. 2013
Begonia yapenensis M.Hughes	Indonesia	Hughes et al. 2015

Begonia yiii Kiew and S.Julia		Sang et al. 2013
Begonia elnidoensis C.I Peng, Rubite and C.W.Lin	Palawan	Hughes et al. 2018
Begonia gironellae C.I Peng, Rubite and C.W.Lin	Palawan	Hughes et al. 2018
Begonia quinquealata C.I Peng, Rubite and C.W.Lin	Palawan	Hughes et al. 2018
Begonia tabonensis C.I Peng, Rubite and C.W.Lin	Palawan	Hughes et al. 2018
Begonia tenuibracteata C.I Peng, Rubite and C.W.Lin	Palawan	Hughes et al. 2018
Begonia germaineana	Bolivia	Tebbitt 2015
Begonia chrysantha	Bolivia	Tebbitt 2015
Begonia suaviola Jara	Colombia	Munoz-Jara 2016
Begonia silverstonni Jara	Colombia	Munoz-Jara 2016
Begonia manuselaensis Ardhaka and Ardi	Seram	Ardhaka et al. 2016
Begonia lamxayiana Souvann	Lao	Souvannakhoummane et al. 2016
Begonia heliantha Tebbitt	Peru	Tebbitt 2016
Begonia urubambensis Tebbitt	Peru	Tebbitt 2016
Begonia nosymangabensis Scherber. and Duruiss	Madagascar	Scherberich and Duruisseau 2017
Begonia pteridoides Scherber. and Duruiss	Madagascar	Scherberich and Duruisseau 2017
Begonia qingchengshanensis H.Z. Li, C.-I Peng and C.W. Lin	China	Li et al. 2018
Begonia dasycaulis Kiew and C.Y.Ling	Borneo	Ying et al. 2018
Begonia minutitepala S. Julia and Kiew	Borneo	Ying et al. 2018
Begonia incudiformicarpa Ardi and D.C.Thomas	Sulawesi	Ardi et al. 2018
Begonia iskandariana Ardi and D.C. Thomas	Sulawesi	Ardi et al. 2018
Begonia johntania Ardi and D.C.Thomas	Sulawesi	Ardi et al. 2018
Begonia tumburanoensis D.C.Thomas and Ardi	Sulawesi	Ardi et al. 2018
Begonia trevisoensis J.C. Jaramillo	Brazil	Jaramillo et al. 2018
Begonia argentii Kiew and S.Julia	Borneo	Sang et al. 2018
Begonia lansatensis Kiew and S.Julia	Borneo	Sang et al. 2018
Begonia muluensis C.Y.Ling and Kiew	Borneo	Sang et al. 2018
Begonia vulgarioides S.Julia and Kiew	Borneo	Sang et al. 2018
Begonia jokwaniana C.Y.Ling and S.Julia	Borneo	Sang et al. 2018
Begonia langenbergiana L. Kollmann	Brazil	Kollmann 2018
Begonia itingae E.L. Jacques	Brazil	Jacques 2018
Begonia mamedeana E.L. Jacques and Gomes da Silva	Brazil	Jacques 2018
Begonia rivularis E.L. Jacques	Brazil	Jacques 2018
Begonia tripicoensis E.L. Jacques	Brazil	Jacques 2018
Begonia ehuangzhangensis Q.L. Ding, W.Y. Zhao and W.B. Liao	China	Ding et al. 2018
Begonia amoeboides Moonlight	Peru	Moonlight and Reynel 2018
Begonia lamolina Moonlight	Peru	Moonlight and Reynel 2018
Begonia zhongyangiana	China	Wang et al. 2019

Begonia yenyeniae J.P.C. Tan	Malaysia	Tan et al. 2018
Begonia medeiroii	Brazil	Funez et al. 2019
Begonia puerensis	China	Wang et al. 2020
Begonia cunhambebii	Brazil	Jacques 2020
Begonia lorenzii	Brazil	Jacques 2020
Begonia puerensis	China	Wang et al. 2020
Begonia phouchomvoyensis Lanors., Lamxay and Souvann	Laos	Lanorsavanh et al. 2020
Begonia maguniana H.P.Wilson	New Guinea	Wilson et al. 2019

2.8. **Table 2:** *IUCN threatened species of Begonia*

Species	Threat Status	Citation
Begonia salaziensis Warb.	Critically Endangered D ver. 2.3	Rutty 2000
Begonia acerifolia Kunth	Near Threatened ver 3.1	Quintana and Pitman 2003
Begonia acerifolia Kunth	Near Threatened ver 3.1	Quintana and Pitman 2003
Begonia aeranthos L.B. Sm. and B. Schub	Endangered B1ab(iii) ver 3.1	Quintana and Pitman 2003
Begonia asympeltata L.B. Sm. and Wassh.	Critically Endangered A2c; B1ab(iii) ver 3.1	Quintana and Pitman 2003
Begonia holmnielseniana L. B. Sm. and Wassh.	Vulnerable D2 ver 3.1	Quintana and Pitman 2003
Begonia serotina A.DC.	Endangered A4c ver 3.1	Quintana and Pitman 2003
Begonia sparreana L.B. Sm. and Wassh.	Vulnerable D2 ver 3.1	Quintana and Pitman 2003
Begonia valvata L.B. Sm. and B..SchuB	Endangered B1ab(iii) ver 3.1	Quintana and Pitman 2003
Begonia samhaensis M.Hughes and A.G.Mill.	Endangered B2ab(iii) ver 3.1	Miller 2004
Begonia socotrana Hook.f.	Least Concern ver 3.1	Miller 2004
Begonia seychellensis Hemsl.	Vulnerable B1ab(iii)+2ab(iii) ver 3.1	Gerlach 2011
Begonia oxyanthera WarBegonia	Vulnerable A4bc ver 3.1	Cheek 2015
Begonia pelargoniiflora J.J.de Wilde and J.C.Arends	Endangered B1ab(iii)+2ab(iii) ver 3.1	Cheek 2015
Begonia pseudoviola Gilg	Endangered A2c; B2ab(ii,iii,iv,v) ver 3.1	Cheek 2015

2.9. **Table 3:** *Discovery of new species, new distributional records and threatened species of Begonia from India*

Species	Citation
Begonia koelzii R. Camfield	Camfield and Hughes 2018
Begonia integrifolia Dalzell	Aitawade and Yadav 2014
Begonia dampae Odyuo, Sinha, Murugesan and Uddin	Odyou et al. 2018
Begonia bachulkarii	Aitawade et al. 2019
Begonia handibadaganathensis	Aitawade et al. 2019
Begonia bracteolata N.Krishna, Pradeep and B.Mani	Krishna et al. 2020
New distributional record to India since 2014-2019	
Begonia panchtharensis S. Rajh.	Pradhan et al. 2018
Begonia difformis (Irmsch.) W.C.Leong, C.I Peng and K.F.Chung,	Camfield and Hughes 2018

Begonia labordei H.Lév.		Camfield and Hughes 2018
Begonia handelii Irmsch.		Camfield and Hughes 2018
Threatened species of *Begonia* in India		
Begonia aborensis Dunn	Rare	Rao et al. 2003
Begonia aliciae C.E.C. Fisch.	Endangered	Rao et al. 2003
Begonia anamalayana Bedd.	Endangered	Rao et al. 2003
Begonia brevicaulis DC.	Endangered/Extinct	Rao et al. 2003
Begonia burkillii Dunn	Rare	Rao et al. 2003
Begonia canarana Miq.	Endangered	Rao et al. 2003
Begonia cordifolia (Wight) Thwaites	Rare	Rao et al. 2003
Begonia lushaiensis C.E.C. Fisch.	Rare	Rao et al. 2003
Begonia phrixophylla Blatt. and McCann	Rare	Rao et al. 2003
Begonia rubro-venia Hook. var. *meisneri* C. B. Clarke	Rare	Rao et al. 2003
Begonia satrapis C. B. Clarke	Rare; Critically endangered	Nayar and Sastry 1990; Rao et al. 2003; Adhikari et al. 2018
Begonia scintillans Dunn	Inderminate	Rao et al. 2003
Begonia scutata Wall. ex DC.	Rare	Nayar and Sastry 1990; Rao et al. 2003
Begonia subpeltata Wight	Rare	Rao et al. 2003
Begonia tessaricarpa C. B. Clarke	Inderminate	Rao et al. 2003
Begonia trichocarpa Dalzell	Vulnerable	Rao et al. 2003
Begonia watti C. B. Clarke	Endangered	Rao et al. 2003
Begonia wengeri C.E.C. Fisch.	Inderminate	Rao et al. 2003

2.10. Ecological Niche Modeling

A sufficient number of occurrence records, i.e. locality data or geographic coordinate, are needed to establish a model for distribution of species. The adaptive radiation hypothesis was validated using *Begonia* presence data and thus three markers for 574 species and 809 accessions were used to reflect *Begonia* phylogeny. Species were compared through the comparison of their distributions in environmental space calculated from species distribution models. The ecological diversification patterns observed in the two *Begonia* clades vary widely from those predicted in the adaptive model and thus indicate that *Begonia* is not an adaptative climatic radiation (Moonlight 2017).

2.11. Application of Ecological Niche Modelling

2.11.1. Conservation of rare, endemic and threatened species

Mapping the distribution of rare, endemic and threatened species is an important step for the survival and regeneration of threatened species (Babar et al. 2012; Adhikari et al. 2012). These species are typically intended for protection initiative and one such measure is by re-introduction in the area defined by ENM (Gil-Sanchez et al. 2011). The usages of ENM on recovery of threatened species may even reverse the threat category of such species (Martinez-Meyer et al. 2006; Wilson et al. 2011; Adhikari et al. 2011). However, such sites may not completely satisfy the conservation of biodiversity. Thus it is also important to prioritize future protected areas by ENM (Fajardo et al. 2014). Furthermore, the global protected area network, which serves as a refuge for rare, endemic and threatened species, has expanded exponentially over the last 25 years (Naughton-Treves 2005). These sites aids for in-situ conservation of threatened plant species through identification of protected area (Pereira et al. 2014; Pradhan and Chettri 2017).

2.11.2. Climate change impact

Climate is typically complex in nature, and these fluctuations in temperature also contribute to shifts in the species' climatic niche. Plants have been recorded to typically migrate > 1 km each year into a suitable environment in response to climate change (Corlett and Westcott 2013). ENM has been used to predict the effect of climate change on the distribution of several species and plan for conservation and ecological restoration (Tanaka et al. 2012; Gelviz-Gelvez et al. 2014; Hu et al. 2015). Nevertheless, obtaining a reliable model of species based on climate change is often challenging and crucial and may likely mislead the policymaker and conservation enthusiast (Morin and Thuiller 2009). The potential invasion of alien species may also be projected using future climate change data (Peterson et al. 2003; Adhikari et al. 2012). The knowledge of the ecological requirement of invasive species both on native and introduced habitat informs environmental management as well as an ecologist (Albright et al. 2009). The effect of phenotypic plasticity and intra-specific variation in thermal niche affects the distribution of species in a warming climate (Valladares et al. 2014).

2.11.3. Discovery of new population locality and sister species

The discovery of new populations of rare, endemic and endangered species is crucial for conservation and assessment of threat (Adhikari et al. 2018). ENM has enabled the researcher to locate/identify a new population of such rare and threatened species (Marinez et al. 2006; Marinez et al. 2007; Menon et al. 2010).

2.11.4. Mapping the prehistoric distribution of species

Mapping and predicting the climatic niche of the past is a prerequisite in solving taxonomic disagreement and miss identification of certain taxa. Using an archaeological collection of species record, model can be generated for current and past climate to determine the prehistoric niche (Pearman et al. 2008; Pinto et al. 2015). Population genetics and ecological niche model have also been used to address the impact of climate change on the geographic distribution of certain plant species (Bystriakova et al. 2013; Beatty and Provan 2014).

2.11.5. Mapping the suitable habitat for infectious disease

ENM has also been used to predict the potential risk of several emerging infectious diseases worldwide (Adhikari et al. 2009; Slater and Michael 2012; Du et al. 2013; Ordoñez-Sierra 2019).

2.11.6. Threat assessment

EOO is one of the IUCN's most important criteria for determining the threat of the species. ENM has recently been used to assess the threat of such species, both plants and animals (Thorn et al. 2009; Joppa et al. 2015; Adhikari et al. 2018). The IUCN has recommended that extent of occurrence (EOO) be calculated by drawing MCP (i.e. minimum convex polygon) around the presence locality of species. For the species with a small sample size, the ENM derived EOO appears to be much larger than the EOO calculated by point presence locality (Syfert et al. 2014).

2.12. Biochemical variation

2.12.1. Phytochemistry

From the rhizome of *B. heracleifolia* six known cucurbitacins have been isolated and, due to different structural features, cucurbitacin B, cucurbitacin D, and 23, 24-dihydro cucurbitacin D have demonstrated clear antiproliferative activity against tumors and immune cells (Frei et al. 1998). The differential cytotoxicity profile was detected in extracts of *B. plebeja* towards the renal tumor, brain tumor and melanoma cell lines (Fuller et al. 1994). *B. malabarica* and *B. rex-cultorum* (Baby rainbow) exhibited the highest antioxidant activities and the anthocyanin concentration positively correlates with the antioxidant potentialities among the cultivars (Aswathy 2016). The bioactive compound was also determined for the same species using Gas Chromatography-Mass Spectrometry (Aswathy 2015). The total phenolic, flavonoid contents and in vitro antioxidant of methanol extracts of *B. malabarica* Lam. and *B. floccifera* Bedd. the whole plant was performed by Kalpanadevi and Mohan (2012).

2.12.2. Terpenoids isolated from Begonia

Monoterpenoid such as Piperonylic acid, diterpenoids such as 2,4-diphenylbut-1-ene and 2,4,6-diphenylbut-1-ene, Triterpenoids such as Glyceryl-1-tetracosanoate, Transdocosanylferulate, Stigmasterol, Stigmast-4-en-3-one, Oleanoic acid, 3β,22a -dihydroxy olean-12-en-29-oic acid,β- -sitosterol, β-sitosterol-3- -D-glucopyranoside, 6β-hydroxysitost-4-en-3-one, 6β-hydroxystigmasta-4,22dien-3-one, Cucurbitacin B, Dihydrocucurbitacin B, 23,24-dihydrocucurbitacin B, Cucurbitacin E, Dihydrocucurbitacin E, Cucurbitacin F, Cucurbitacin I, Dihydrocucurbitacin I, Tetraterpenoid such as Lutein and Aminophosphosphingolipid such as 2-(2-hydroxy tricosanoyl amino)-1,3,4-hexadecane were isolated from the rhizome of *B. nantoensis* (Wu et al. 2004). Triterpenoids such as Stigmasterol, β- amyrin, β-sitosterol, β- -sitosterol, β-sitosterol-3- -D-glucopyranoside were also isolated from *B. evansiana (*Zhang et al. 1997). Ramesh et al. (2002) isolated Triterpenoids such as Friedelin and β-sitosterol-3- -D-glucopyranoside from *B. malabarica*.

2.12.3. Phenolic compounds

Phenolic compounds show antioxidant (Orcis et al. 2011) and are used to treat cardiovascular diseases, atherosclerosis, cancer, and disbetes (Miniati 2007). Compounds such as Vanillin, p-hydroxybenzaldehyde, 5,7 dihydroxychromone, Vanillic acid, Eudesmic acid, Methylparaben, Methyl vanillate, Protocatechuic acid methyl ester, Protocatechuic acid, Caffeic acid were isolated from B. nantoensis (Wu et al (2004) and compounds such as anthraquinone from B. fimbristipula (Wang et al. 2013).

2.12.4. Flavonoids

Compounds such as Catechin and Quercetin are flavonols isolated from *B. nantoensis* and *B. metallica* (Harborne and Hall 1964; Wu et al. 2004). Compounds such as Quercetin 3-xyloside, Quercetin 3-glucoside, Quercetin 3-O-rhamnoside, Quercetin 3-O-rutinoside, Quercetin 3-O-rutinoside and Kaempferol 3-glucoside are flavonol glycosides isolated from *Begonia* spp. (Harborne and Hall 1964;

Vereskovskii et al. 1987a). Compounds such as 3-methylquercetin and 3-methylkaempferol are methylated flavonols islolated *B. manicata*, *B. haageana var. drostii*, *B. glaucophylla*, *B. pearceio* (Harborne and Hall 1964). Compounds sun as 3-O-methylkaempferol 7-O-glucoside, Glucosides of flavonol 3-methyl esters, 8-methoxy-3,3',7-tri-Omethylquecetin (ternation), 3,3',7-tri-O-methylquercetin7-O'Glucoside are methylated flavonol glycosides isolated from *Begonia spp.* (Harborne and Hall 1964; Vereskovskii et al. 1987a). A compound like Luteolin is isolated from *B. erythrophylla* and *B.malabarica* (Vereskovskii et al. 1987a; Ramesh et al. 2002). Compounds such as Luteolin-7-glucoside, Orientin, Isoorientin, Vitexin**,** and Isovitexin are glycoflavones isolated from *B. erythrophylla* and *B. nantoensis* (Vereskovskii et al. 1987b; Wu et al. 2004). A compound like 4',5',7-trihydroxy-flavone-6-glucopyranoside is a methylated flavone isolated from *B. evansiana* (Zhang et al. 1997). Compound **88** is an isoflavone (Wu et al. 2004). Compounds like Daidzein is isoflavone isolated from *B. nantoensis* (Wu et al. 2004). Compounds like 1-caffeoyl glucose and 1-feruloylglucose are hydroxycinnamic acids isolated from *B. manicata* (Harborne and Hall 1964). Methyl (S)-glycerate is a methyl ester isolated from *B. nantoensis* (Wu et al. 2004).

2.12.5. Organoleptic Characters

Sweet plants are usually preferred for the treatment of respiratory ailments, bitter ones for the treatment of dysentery, pain, and fever and as an antidote to snake venom. Diarrhea is usually treated with astringent and bitter plants. Astringent polyphenols-containing plants are preferred for gastrointestinal and dermatological treatment. Tannins were used for the treatment of gastrointestinal illnesses (Heinrich 2003). Since the varieties of *Begonia* species have sour tastes, they are used in treatment of several diseases in the Indigenous Systems of Medicine. Scientific evidence generated from the biological screening studies postulates promise in the field of drug discovery for the treatment various diseases. Sweet *Begonia* species containing fructose are called flavouring ingredients (Castillo and Smith 1997). Sour plants like *B. floccifera* and *B. malabarica* produce a significant amount of vitamin C and are used to treat respiratory disorders, stomach aches and stomach ulcers (Kirtikar and Basu 1975; Ramesh et al. 2002; Ariharan et al. 2012). Astringent plants like as *B. fimbristipula* possess polyphenols used to combat dizziness and foot sores (Heinrich 2003; Kalpanadevi and Mohan 2012). *B. gracilis,* a bitter cucurbitacins-containing herb, has antitumor property (Fuller et al. 1994; Castillo and Smith 1997).

3.1. Sikkim Himalayas

The district of Darjeeling is an important geological component of Sikkim and, on the basis of physiography and geology, these two areas are inextricable (Basu 2013). The physical features of Darjeeling and Sikkim are very similar, separated by river Teesta and Rungit which acts as a natural boundary dividing the two geographically consonant regions (Fig. 2). Therefore the state of Sikkim along with Darjeeling together constitutes the Sikkim Himalayas. The two regions from herein will be referred to as Sikkim Himalayas (Rai et al. 2000). The region lies amid the Eastern Himalayan regions, roofed by a snow clad-mountain in the North and planes in the South. It is bordered by countries such as Nepal in the East, China in the North, and Bhutan in the West, and is tectonically one of the most active areas of the Himalayas. It consists of three tectonic units: (1) The Higher Himalayas (2) The Lower Himalayas and (3) The Siwaliks, separated by thrusts, but merged by great river Teesta (Starkel and Sarkar 2014).

3.2. Protected areas

India has established 769 protected area (PA), covering an area of 162072.49 km^2 which is just 4.93 % coverage of the country's geographic area (i.e. 32, 87, 590 km^2). The state of Sikkim has nine protected areas including 1 National park (i.e. Khangchendzonga National Park), 7 wildlife sanctuary (*viz*. Barsey Rhododendron Sanctuary, Fambonglho Wildlife Sanctuary, Kyongnosla Alpine Sanctuary, Maenam Wildlife Sanctuary, Pangolakha Wildlife Sanctuary, Shingba Rhododendron Sanctuary, Kitam Bird Sanctuary) and one conservation reserve (i.e. Sling Dong

Fairrieanum Orchid Conservation Reserve) covering a total area of 2931.12 km^2 which is 41.3 % of the total area of Sikkim (7096 km^2), a much higher than the national average of 4 % (ENVIS Centre: Sikkim).

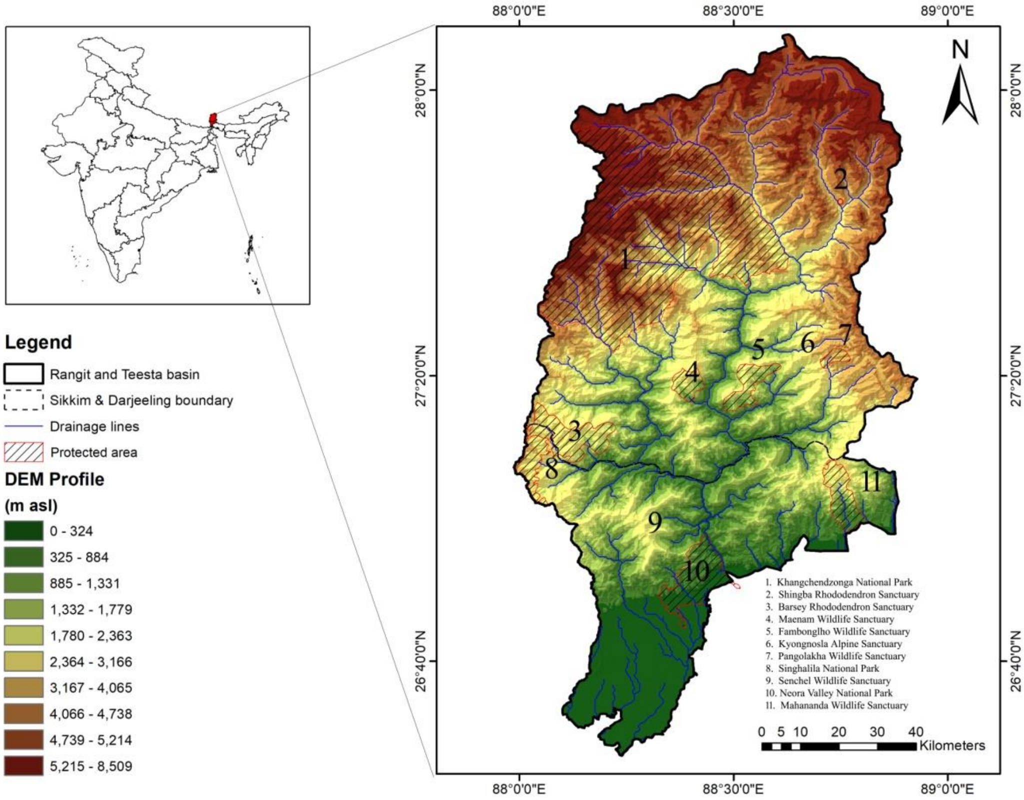

Fig. 2: Protected areas in Sikkim and Darjeeling of northeast India. (Protected area data source: http://protectedplanets.net; Digital elevation model (DEM) data source: https://search.earthdata.nasa.gov/search/)

Likewise, there are five protected areas in the Darjeeling district *viz.* Singhalila National Park, Senchel Wildlife Sanctuary, Mahananda Wildlife Sanctuary, Neora Valley National Park, Jorepokhari Salamander Sanctuary covering a total of 333.04 km^2 which is 10.23 % of the total area of Darjeeling (3,255 km^2) (Das et al.). In total Sikkim and Darjeeling (i.e. Sikkim Himalaya) comprises 14 protected area covering an area of 3264.16 km^2, which is 9.92 % of the overall area of India (Fig. 2).

3.3. Landuse Landcover

The present land use and landcover pattern of Sikkim Himalaya is largely dominated by the evergreen broadleaf forest (27.50 %), followed by snow and ice cover (19.52 %), Deciduous broadleaf forest (14.61 %), cropland (12.74 %), barren land (7.89 %), grassland (6.53 %), planatations (5.04 %), mixed forest (3.26 %), water bodies (1.68 %), buit-up-land (1.04 %), shrubland (0.09 %), fallow land (0.01 %) and permanent wetland (0.01 %) (Fig. 3, 4) (Roy et al. 2016).

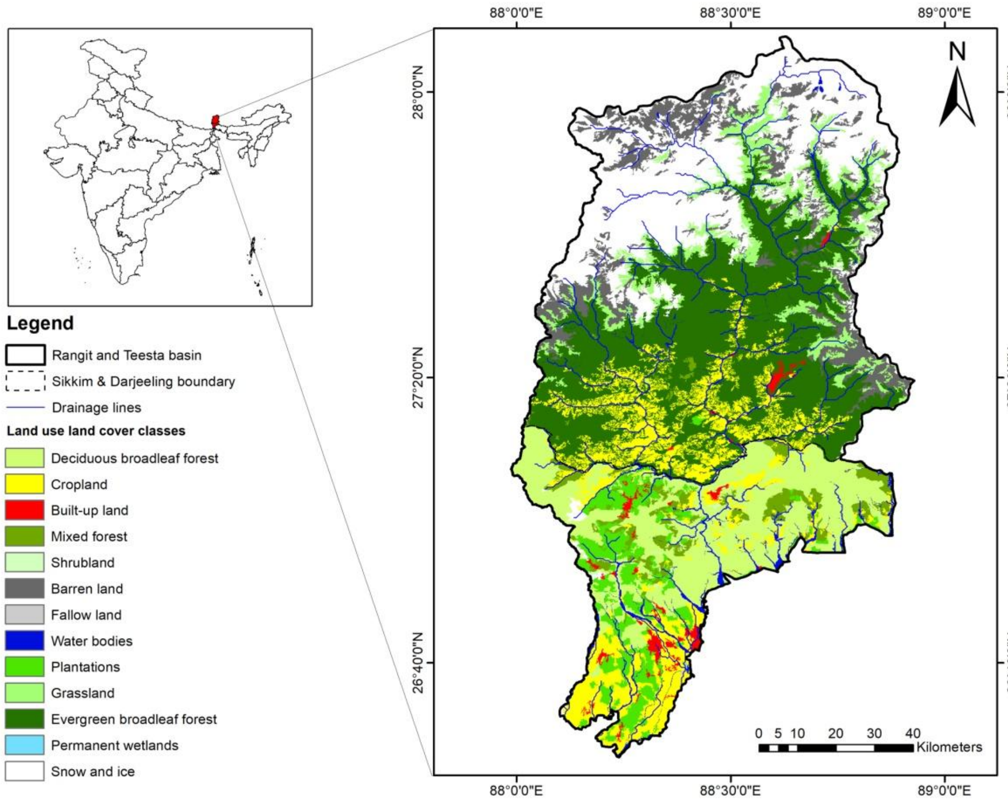

Fig. **3**: Land use land cover in Sikkim and Darjeeling of northeast India. Data source for land use land cover: Roy et al. (2016), https://daac.ornl.gov/VEGETATION/guides/Decadal_LULC_India.html).

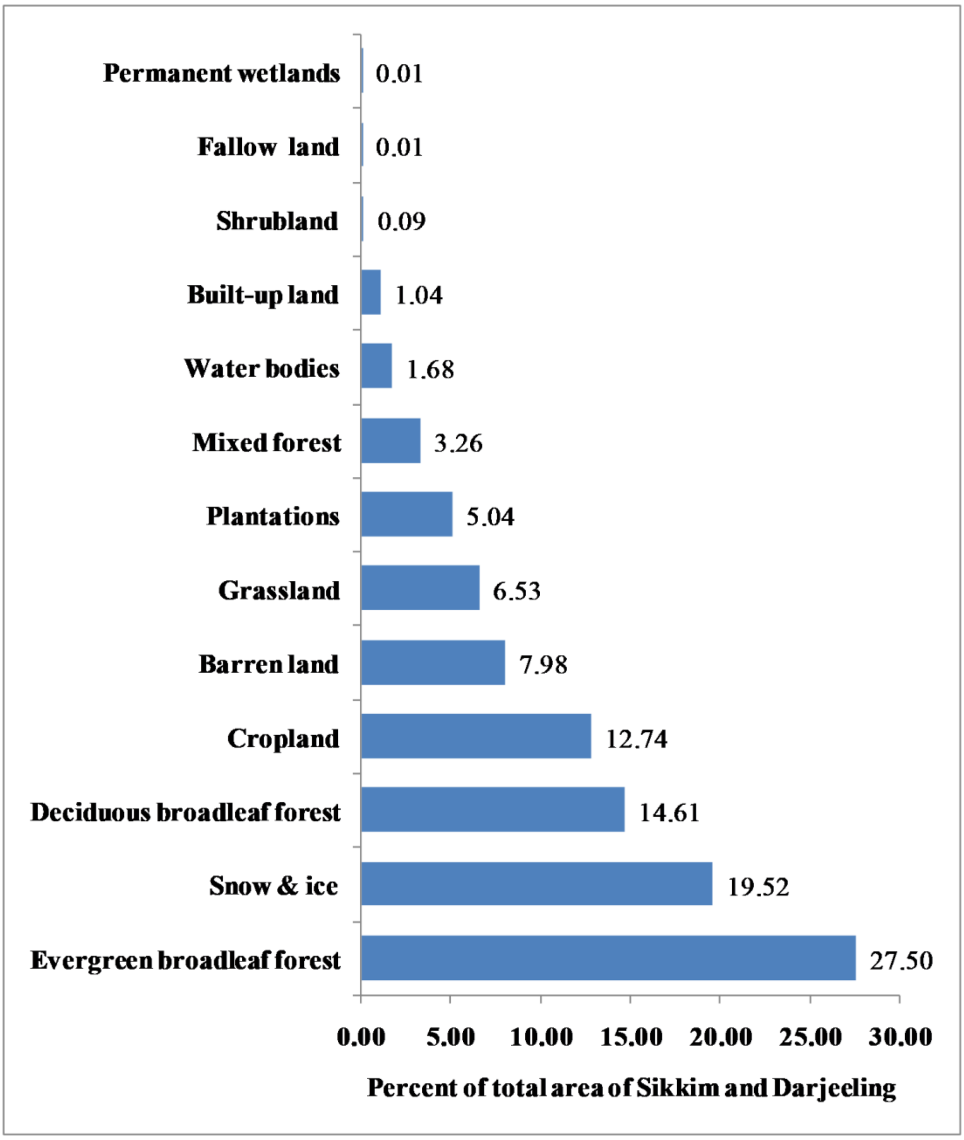

Fig. 4: Proportion of area under different land use land cover classes in Sikkim and Darjeeling. (Data source: Roy et al. 2016)

3.4. Floral Wealth

The Sikkim Himalayan region was first explored by J.D. Hooker (1871-1897) accompanied by a comprehensive work by Biswas (1956), is home to some of the rarest, threatened and endemic floras, and is often regarded as a botanical paradise for Botanist. The subtropical type of vegetation extending up to an altitude of 1500 m includes some rare and threatened species such as *Calamus inermis*, *Angiopteris* *evecta*, *Lagerstroemia minuticarpa*, *Pandanus ungifer*, *Begonia satrapis*, *Phoenix rupicola*, *Cymbidium whiteae*, etc. The temperate forest which ranges from 1500 m to 4000 m includes some rare and threatened species such as *Acer hookeri*, *Paris polyphylla*, *Codonopsis affinis*, *Swertia chirita*, etc. The alpine vegetation (above 4000 m asl) is distinguished by flowering plants such as *Rhododendron spp*., *Hippophae spp*., *Primula spp*., *Aconitum ferox*., *Gentiana urnula, Saussurea spp., Potentilla spp. Picrorhiza kurrooa, Meconopsis spp. Podophyllum spp*., etc.

3.5. Faunal Wealth

Sikkim Himalayas is also host to some of the most threatened species of fauna and offers a tremendous opportunity for research. The Tso Lhamo Plateau in North Sikkim harbours the only population of Southern kiang (*Equus kiang polydon*). Many mammalian fauna, such as Tibetan argali, Tibetan gazelle, Blue sheep, Tibetan wolf, Tibetan sand fox, Pallas's cat, Snow leopard, Eurasian lynx, Himalayan marmot, Tibetan woolly hare, Plateau pika, Siberian weasel of Tso Lhamo Plateau have been categorized to different threat category by IUCN (Rawat and Tambe 2011).

3.6. Climate and Soil

The varying climatic conditions of the Sikkim Himalayas are largely attributed to altitude variation. The temperature ranges from the low valley tropical heat to alpine cold of higher reaches. The moist tropical climate is experienced at an average altitude of 600 m and above this a subtropical climate prevails at an elevation of about 1500 m. The cold temperate climatic environments are the located at an altitude of around 3000 m and progressively cooler further up. The proximity of the Bay of Bengal to the South and exposure to summer monsoon resulted in one of the most humid regions in the Himalayas. Most of the annual rainfall is received during May through October and depending on elevation, the annual rainfall varies from 500 to 5000 mm.

The soil of Sikkim Himalaya is largely comprised of Leptosols (35.89 %), Cambisols (27.41 %), Acrisols (13.36 %), Glaciers (11.50 %), Fluvisols (7.33 %) and Arenosols (4.51 %) (Fig. 5, 6) (FAO 2009).

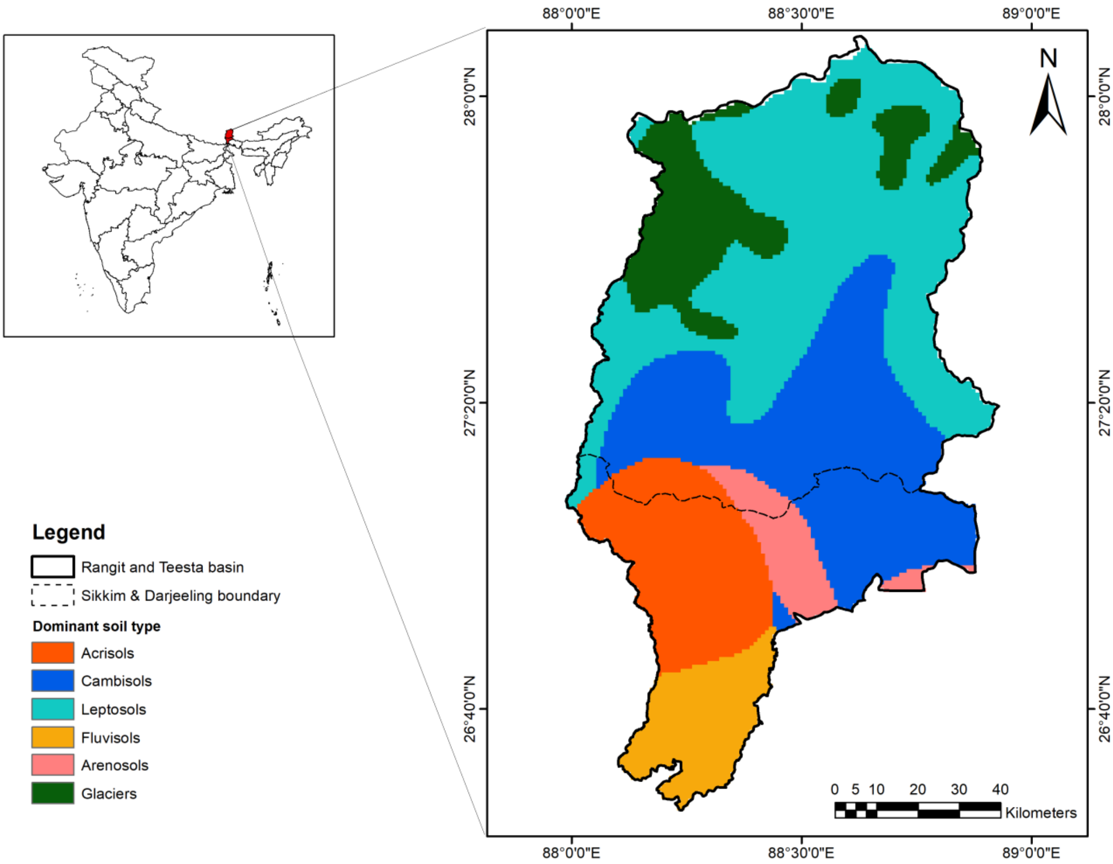

Fig. 5: Map showing dominant soil types in the Teesta and Rangit basin. (Data source: FAO, 2009; http://www.fao.org/soils-portal/soil-survey/soil-maps-and-databases/harmonized-world-soil-database-v12/en/)

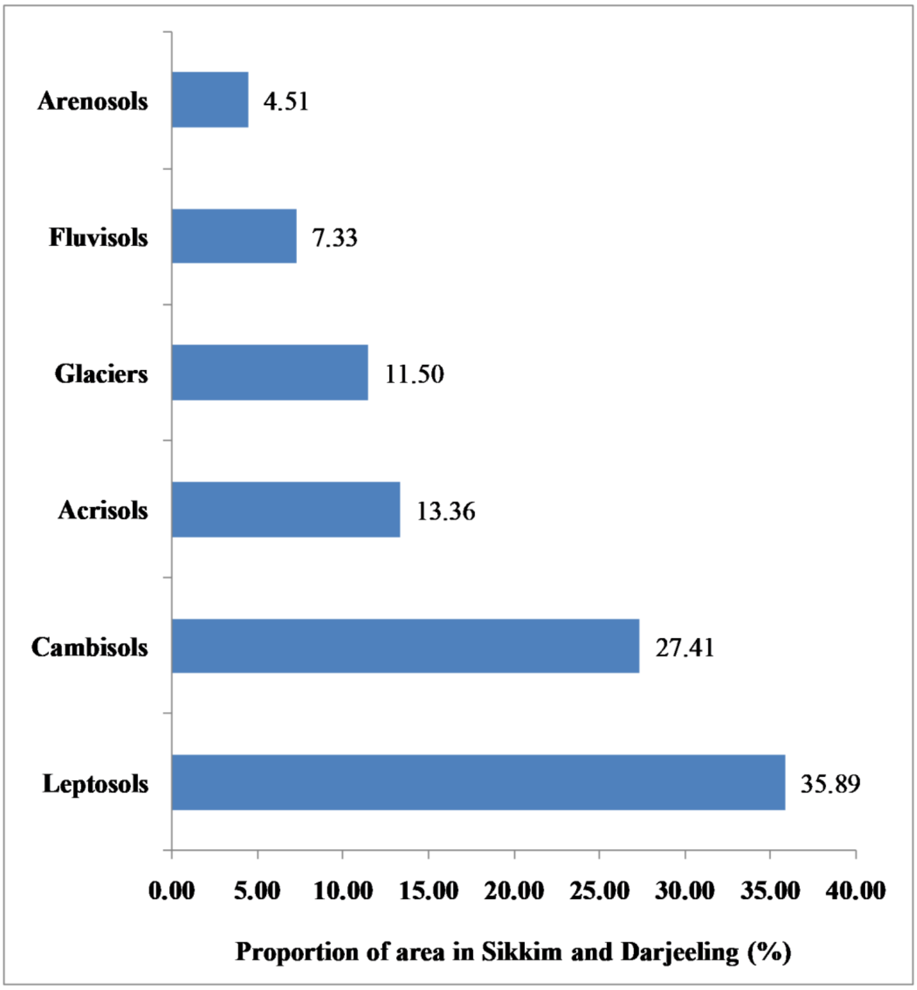

Fig. 6: Proportion of area in Sikkim and Darjeeling with different soil types (Source: FAO, 2009; http://www.fao.org/soils-portal/soil-survey/soil-maps-and-databases/harmonized-world-soil-database-v12/en/).

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4.1. Introduction

Begonia L. belonging to the family *Begoniaceae* is the 5th largest genus of flowering plants with 1825 species distributed worldwide (Hoover et al. 2004; Hughes et al. 2015). The greatest diversity of *Begonia* is in Asia and America (>600 species each), with the bulk of species occurring in Southeast Asia having *ca*. 440 species (Hughes 2008) while Africa is relatively poor (*ca*. 160 species) in species diversity. The genus *Begonia* is believed to have originated from Africa, while the species found in South America and Southeast Asia are the results of parallel radiations over the last 20-50 million years (Plana et al. 2004; Gooddall Copestake et al. 2010; Thomas et al. 2010). The genus is absent in Australia as long-distance dispersal is very rare and uncommon in *Begonia* and unable to cross the Torres strait from Papua New Guinea to Australia (Goodall-Copestake et al. 2010). The genus is represented by 59 species in India (Uddin 2007), with four new records and six new species discovered from Northeast India (Camfield and Hughes 2018; Pradhan et al. 2018) bringing a total to 69 species. The family consists of two genera *Begonia* Linnaeus and *Hillebrandia* Oliver. The genus *Hillebrandia* differs from *Begonia* in having a semi-inferior ovary. The genus *Hillebrandia* with a single species i.e. *H. sandwicensis* Oliver is endemic to Sandwich Island of the Hawaiian Islets, Northern America. The third genus, *Symbegonia*, which was previously included within the *Begoniaceae*, was later segregated based on floral characters and molecular data, the genus was incorporated within the *Begonia* section *Petermannia* (Forest and Hollingsworth 2003).

4.2. Materials and Methods

4.2.1. Literature survey

A preliminary literature survey was carried through Flora, Books, and manuals (Clarke 1879; Grierson 1991; Hara 1971; Uddin 2007). The major herbaria (*viz*. BSHC, CAL) and Botanical Garden (Lloyd Botanic Garden) were also visited to collect the distributional data and exact geographical locality of *Begonia* in Sikkim Himalaya, India.

4.2.2. Field visits

Based on the above literature survey and herbarium data extensive surveys were carried out throughout 2013-2019 in all the four districts of Sikkim (*viz.* North, South, East, and West) and Darjeeling District of West Bengal. To collect the phenological data regular visits were made during the month of May-October. Data on flowering, fruiting, habitat, habit, and geographic coordinates were recorded in the field itself. Each species was photographed in its natural habitat using the Nikon D5200 camera. Two specimens of each species were collected for processing herbarium specimens.

4.2.3. Identification

Specimens of *Begonia* were brought in a laboratory and detailed morphological characters were recorded and described. The collected specimens were identified using various Floras, Books, monographs, revisionary work (Clarke 1879; Grierson 1991; Uddin 2007; Rajbhandary et al. 2010; Camfield and Hughes 2018). The identification of each specimen was confirmed comparing it with the type specimens available online.

4.2.4. Herbarium preparation

The herbarium was processed following a standard herbarium technique of Jain and Rao (1977). The specimens were pressed dried and processed in a standard herbarium sheet (29 × 42 cm). The processed voucher specimens were deposited in the herbarium of the Department of Botany, Sikkim University and at the herbarium of Botanical Survey of India (BSI), Sikkim Himalayan Circle (BSHC) for future references.

4.2.5. Systematic treatment

Bracketed key has been prepared based on the diagnostic characters of each species for easy identification.

4.2.6. Nomenclature and citation of taxa

The citation of each taxon was obtained from the International Plant Names Index (IPNI) database (http://www.ipni.org), 'w^3 Tropicos' (http://www.tropicos.org) and The Plant List, (http://www.theplantlist.org). The citation of each species is followed by additional specimens examined, description, habitat, phenology, potential values, conservation status, specimen examined, and a note.

4.2.7. Preliminary threat assessment

Species were assigned to a Red List Category following the IUCN criteria (IUCN 2012). The area of occupancy (AOO) and extent of occurrence (EOO) was calculated using ArcView GIS 3.x version 1.2. (Moat 2007) and geographic coordinates collected from the field.

4.3. Results

4.3.1. Habitat Diversity and Leaf variegation

The habitat of Sikkim Himalayan *Begonia* is varied and consists of nearly all growth forms, from tuberous to rhizomatous. Natural reproduction in *Begonia* is *via* seeds however some species produce tubers and stolen like structure for a vegetative mode of reproduction. Moreover, one such species (*B. gemmipara*) produces gemma cup (specialized vegetative organs) for vegetative mode of reproduction (Thomas 2010).

Sikkim Himalayan *Begonia* also has diverse leaf types and colours. Leaves are usually peltate and non-peltate from entire to deeply lobed. Only one species (*B. josephii*) has a peltate leaf and two species had compound leaves *viz*. *B. sikkimensis* and *B. panchtharensis*, rest were all non-peltate. Sub-peltate leaf was observed only in *B. gemmipara*. Leaf margin was entire to deeply lobed. Most of the species showed green colour, however diverse colouration and leaf variegation were commonly observed in *B. xanthina*, *B. picta*, *B. palmata*, and *B. flaviflora* (Plate 1).

4.3.2. Diversity in tepals number and colours

The most commonly occurring flower colours in Sikkim Himalayan *Begonia* are white, pink, red, and yellow. The number of tepals in male flowers ranged from 2 in the case of *Begonia nepalensis* and 4 in the rest of the species (Plate 2). The number of tepals in female flowers ranged from 4 in *B. roxburghii* and 5 in remaining of the species (Plate 3).

4.3.3. Diversity in fruits and wings

The morphological characteristics of fruits and wings are diverse in Sikkim Himalayan *Begonia*. The length and shape of the wings vary between species. The fruits are usually 3 lobed and winged expect in *B. roxburghii* 4 lobed wingless fruits is present (Plate 4).

4.3.4. Distribution of Begonia along an elevation gradient

The vertical distribution of *Begonia* species in Sikkim Himalaya ranges from 200-3000 m asl (Fig. 7). Most of the species are found in the range between 1000-1500 m (8 species) and least number of species (2 species each) found below 500 and above 2500 m elevation respectively (Fig. 8).

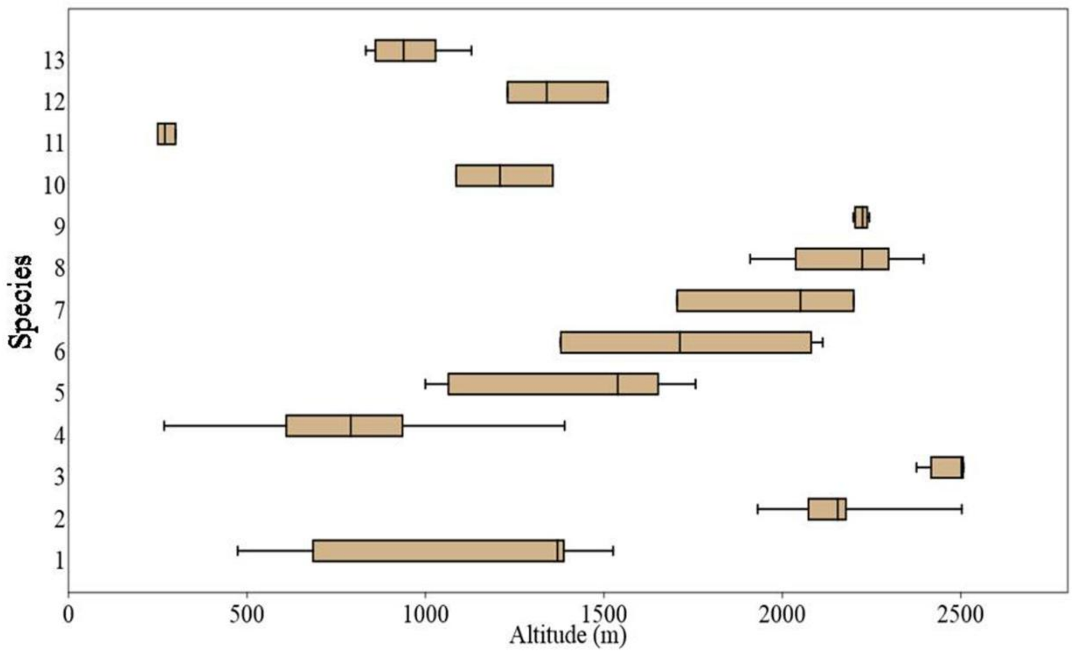

Fig. 7: Species range along an elevation gradient (1) *B. picta* (2) *B. josephii* (3) *B. gemmipara* (4) *B. satrapis* (5) *B. sikkimensis* (6) *B. palmata* (7) *B. panchtharensis* (8) *B. catcarthii* (9) *B. flaviflora* (10) *B. megaptera* (11) *B. nepalensis* (12) *B. xanthina* (13) *B. roxburghii*

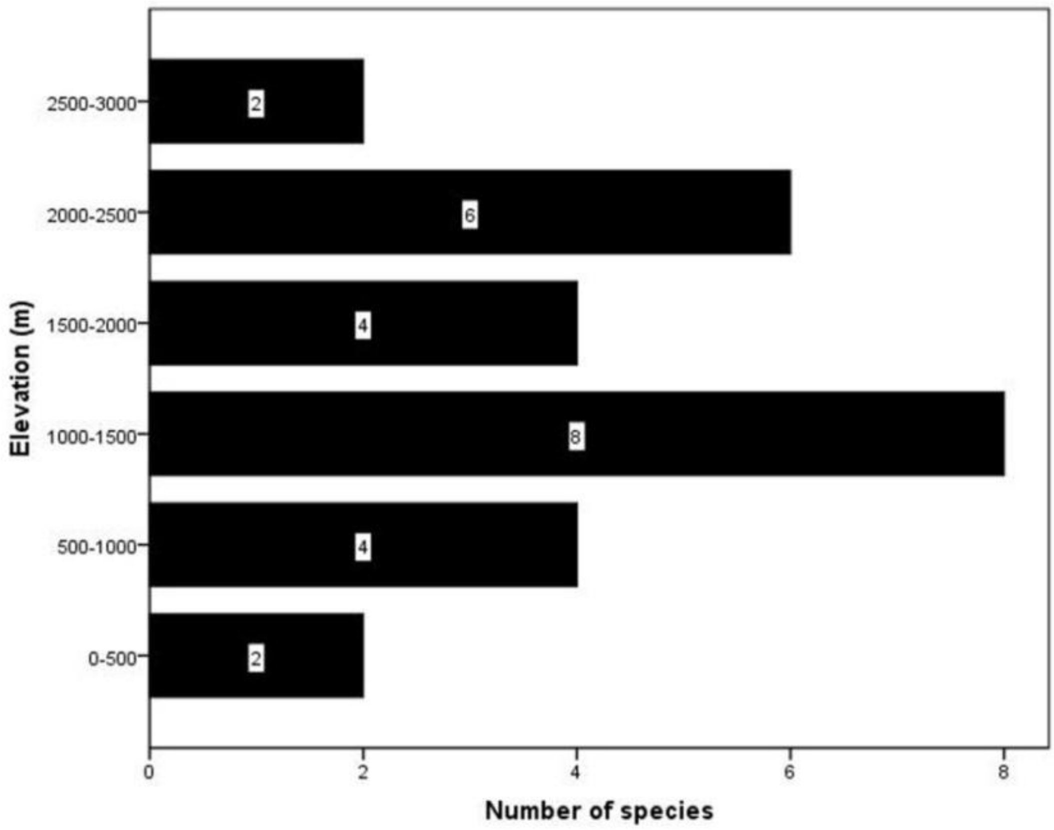

Fig. 8: Number of *Begonia* species per 500 m elevation gradient

4.3.3. Taxonomic treatment

Class Equisetopsida C.Agardh (Agardh et al. 1825)

Subclass Magnoliidae Novák ex Takht. (Takhtajan 1967)

Superorder Rosanne Takht. (Takhtajan 1967)

Order Cucurbitales Juss. ex Bercht. & J.Presl (Von Berchtold & Presl 1820)

Family Begoniaceae C.Agardh (Agardh 1824)

Genus *Begonia* L. (Linnaeus 1753)

4.3.4. Taxonomic description of the genus Begonia

Begonia Linnaeus, Sp. Pl. 2: 1056. 1753.

Perennial, succulent herbs, sometimes sub-shrubs. Stems: erect, occasionally acaulescent. Rootstock: rhizomatous, tuberous, sometimes climbing with adventitious roots or stoloniferous. Leaves: simple, palmately compound, alternate or all basal; blade oblique and asymmetric, rarely symmetric, margin often irregularly serrate, occasionally entire, venation palmate; petiole long, weak; stipules membranous, usually deciduous. Flowers unisexual, plants monoecious, rarely dioecious, (1 or) 2–4 to several, rarely numerous, in dichotomous cymes, sometimes in panicle, with pedicels and bracts. Male flower: tepals 2 or 4 and decussate, usually outer ones larger, inner ones smaller; stamens usually numerous; filaments free or connate at base; anthers 2–celled, apical or lateral; connectives extended at the apex, sometimes apiculate. Female flower: tepals 2–5(–10); ovary inferior, 1–3(–7)-loculed; placenta axile or parietal; styles 2 or 3(or more), free or fused at base, forked once or more; stigma turgid, spirally twisted-tortuous or U-shaped, capitate or reniform, setose-papillose. Fruits: Capsule dry, sometimes berrylike, unequally or sub equally 3-winged, rarely wingless and 3–4 horned; seeds very numerous, pale brown, oblong, minute, testa reticulate.

Begonia sect. Diploclinium

This section occurs from Sri Lanka and the Western Ghats in India, across the Himalayas to China and into continental Southeast Asia. A small number of species occur in the Malesian region. Species in this section are monoecious (rarely dioecious), mostly tuberous, sometimes creeping, with or without an erect stem. Inflorescences: axillary to terminal, cymose, flowers white to pink. Male flowers: 2–5 tepals and oblong-ellipsoid to obovate anthers, opening *via* pore-like slits and connectives not extended, rarely extended. Female flowers: 3–5 tepals, 3 styles, and 3 locular ovaries with 3 equal to subequal wings. Fruits: pendulous and dehiscent.

The number of species [03]: *B. josephii* A.DC. (de Candolle 1859); *B. satrapis* Clarke (C. B. Clarke 1857); *B. picta* Sm. (Smith 1805).

Begonia sect. Platycentrum

This section is the largest in the region in terms of species number and is distributed from Nepal eastwards into China and southwards to Sumatra and Java. Species in this section are monoecious with rhizomatous and/or upright stems. Inflorescences: axillary, cymose, flowers white, pink, or yellow. Male flowers: 4 tepals and obovate anthers opening via long side slits and with extended connectives. Female flowers: 3–8 tepals, 2–3 styles, and 2 locular ovaries with 3 unequal wings. Fruits: nodding and dehiscent.

The number of species [07]: *B. cathcartii* Hook. f. and Thomson.; (Hooker 1855); *B. megaptera* A. DC. (de Candolle 1859); *B. palmata* D. Don (Don 1825); *B. sikkimensis* A. DC. (de Candolle 1859); *B. xanthina* Hook. (Hooker 1852); *B. panchtharensis* S. Rajh. (Rajbhandari et al. 2010); *B. flaviflora* H. Hara (Hara 1970).

Begonia sect. Monopteron

This section comprises just one species and is restricted to the Himalayas. They are monoecious, rhizomatous plants with erect stems. Inflorescences: axillary, cymose, flowers white to pink. Male flowers: 2–4 tepals, globose anthers, opening *via* pore like slits, connective not extended. Female flowers: 4–6 tepals, 2 styles, and 2 locular ovaries with 1 large wing and 2 undeveloped ridge-like wings. Fruits: pendulous and dehiscent.

The number of Species [01]: *B. nepalensis* (A.DC.) Warb. (Warburg 1894; basionym: de Candolle 1859).

Begonia sect. Sphenanthera

This section has a similar distribution to the allied sect. Platycentrum, but is also found further east in the Malesian region. The species are either monoecious or dioecious and are rhizomatous with or without upright stems. Inflorescences: axillary, cymose, flowers white to pink. Male flowers: 4 tepals and oblong anthers, opening via long side slits and with extended connectives. Female flowers: 4–6 tepals, 3–4 styles, and 3–4 locular ovaries which are wingless or horned. Fruits: fleshy on a stiff pedicel.

The number of Species [01]: *B. roxburghii* (Miq.) A.DC. (de Candolle 1864; basionym: Miquel 1856).

Begonia sect. Putzeysia

This section occurs in Asia. The species are terrestrials, perennial with rhizomes; tubers present; stem herbaceous; tubercles in leaf axil present; stipules persistent, entire; junction petiole and leaf blade without a tuff of hairs. Leaves alternate, more then 2, straight, asymmetric, not peltate, simple or palmately lobed; venation palmate. Inflorescence are axillary, dichasial, bisexual or occasionally dioecoius; bracts persistent during flowering. Flower with 2 bracteoles. Male flower: tepals 4;

zygomorphic androecium, filaments fused below, anthers are obovate, longer than the filaments. Female flower: 5 free tepals; fruits 3 winged, equal, not hooked, locules 3, placentatation axillary, styles 3, free, 2 lobed, stigma is kidney shaped. Fruits: berry, dehiscent near the wings, with or without beaks.

The number of Species [01]: *B. gemmipara* Hook. f. and Thomson

Key to the Section

1.a. The plant is tuberous.. sect. *Diploclinium*

1.b. The plant is rhizomatous or trailing...sect. *Platycentrum*

Key to the species of sect. Diploclinium

1.a. Leaves peltate, solitary.. *B. josephii*

1.b. Leaves more than one and not peltate..2

2.a. Leaves reniform, apex obtuse or rounded, capsule unequal.................. *B. satrapis*

2.b. Leaves ovate-cordate, apex acuminate, capsule is elongated, lateral wings equal...*B. picta*

Key to the species of sect. Platycentrum

1.a. Leaves variegated and white flower...*B. palmata*

1.b. Leaves variegated with yellow flower...2

2.a. Light green patches between veins, leaf margin entire..........................*B. xanthina*

2.b. Leaf with a dark center, green middle, and dark edges.........................*B. flaviflora*

3.a. Leaves lobed to deeply lobed..4

3.b. Leaves margin entire..5

4.a. Flowers white, single leaf arising directly from the base............*B. panchtharensis*

4.b. Flower red, multiple leaves arising from the base............................*B. sikkimensis*

5.a. Flower white to light pink in colour, stigma yellow, leaves smooth...*B. megaptera*

5.b. Flower white, leaves pubescent, stigma green....................................*B. cathcartii*

The section, *Monopteron*, *Sphenanthera,* and *Putzeysia* each consists of one species each *viz*. *B. nepalensis*, *B. roxburghii,* and *B. gemmipara.*

B. nepalensis is characterized by having 2 tepals male and female flower; *B. roxburghii* is characterized by having a sessile flower with 4 locular ovaries and *B. gemmipara* is characterized by having gemma cup-like structure for the vegetative mode of reproduction (Thomas 2010).

4.3.5. **Begonia josephii** A. DC., Ann. Sci. Nat. ser. 4, 11:126.1859; Hara in Flora of Eastern Himalaya 2: 84.1971; C.B. Clarke in Hook. f. FI. Brit. India 2:648.1879; Grierson in Grierson and Long, Flora of Bhutan 2(1):237–246.1991; Uddin et al. in Journal of Economic and Taxonomic Botany 31(3):591–597.2007.

Additional specimen examined: INDIA. Sikkim: Kyanghnosla, on the way to Kupuk, 4.08.1988, *D.C.S. Raju & S. Sing* 3662, on the way to Yangang, 22.05.1985, *A.K. Verma* 3742, Bakhim, 24.07.1999, *D. Maity* 21659, Bakhim to Yuksam, 2500 m, 31.07.99, *D. Maity* 21229, Rabongla Reserve Forest, 23.08.1986, *A.K. Verma* 6557, Tendong Reserve Forest, 17.08.1985, *A.K. Verma* 4209, Lingthem, 13.09.1986, *D.C.S. Raju* 7306, Mensithang, 17.09.1994, *S. Pradhan* 17122, Pentong, 16.09.1986, *B. Mitra* 6928, on the way to Raj Bhawan, beyond CPWD Rest House, 08.08.1983, *B. Krishna* 2763, Phadamchem to Zuluk, 25.08.1986, *B. Krishna & B. Mitra* 6679, Okhrey to Sombaria, 2300 m, 20.08.1994, *P. Singh* 13907, Hilley Reserve Forest, 2400-2700 m, 21.08.1994, *P. Singh* 13969, Gangtok, on the way to Raj Bhawan, 17.08.1982, *B. Krishna* 2272, Near Rajbhawan, Gangtok, 18.08.1980, *P.K. Hajra* 561; Enchey Monastery Surrounding, Gangtok, 19.08.1980, *P.K. Hajra* 581, Near Raj Bhawan, Gangtok, 18.08.1980, *P.K. Hajra* 561; West Bengal: Maney Bhanjan, 03.09.1985, *B. Krishna* 5675 (BSHC).

Description: Tuberous, succulent, monoecious herb, 15–20 cm tall. Stipules: lanceolate, 2–4 mm long, glabrous, deciduous. Leaves: petiole slender, 10–15 cm long; lamina ovate, peltate at the base, 10.2–16 × 8.5–13.2 cm, ovate-broadly ovate, symmetric, green and pubescence on abaxial surface, red and sparsely hirsute on adaxial surface, margin denticulate, with sparse hair, acuminate apex. Inflorescence: axillary, cymose, bracts lanceolate, peduncle glabrous, 3 mm long. Male flowers: pedicel 10–20 mm long, glabrous, tepals 4, outer large and broadly ovate, white, 3–9 × 2.5–4 mm, margin entire, dorsal surface glabrous; inner tepals spathulate, white, 2.5–4 × 1.5–2.5 mm, stamens 10–18, shortly monadelphous; filaments 0.6–0.8 mm, anthers 1–1.2 mm, oblong–obovoid, apex emarginate. Female flowers: pedicel 7–15 mm long, glabrous, tepals 4–5 unequal, ovate, outer tepals 4–9 × 3–5 mm, entire margin, 3 locules ovary, bifid placentae, 3 winged, styles 3, persistent, stigmas tortuous or lunate, U-shaped. Fruit: Capsule 3 winged wings unequal, falcate, triangular, seeds short, ellipsoid (Plate 5).

Habitat: Found growing as understory in the moist temperate deciduous forest.

Distribution: Nepal, China, Bhutan, Myanmar; India: Arunachal Pradesh, Assam, Meghalaya, Nagaland, Sikkim (Pangthang, Sadam); West Bengal (6th Mile, Senchal Wildlife Sanctuary).

Phenology: *B. josephii* flowers during August and fruits thereafter (Table 4).

Potential value: Tender Shoots/Petiole fermented as a pickle (Shrestha and Dhillion 2003)

Conservation status: The calculated range of *B. josephii* is small (EOO is 497.27 km^2 and AOO is 148.19 km^2), which would suggest a status of endangered. However this likely reflects the lack of collections throughout their distribution range, as the species is distributed throughout the entire northeast India and with the inclusion of population data may further lead to a change in threat status.

Specimen examined: **INDIA**. Sikkim: East district, Pangthang, 2000m alt. (Aditya Pradhan, No. 00112, 3rd October 2016, BSHC, Plate 18).

Note: *B. josephii* grows naturally in the temperate forest along the roadside. This is the only species of *Begonia* reported in Sikkim Himalaya having a peltate leaf base.

Table 4: Phenology of *B. josephii* from Sikkim Himalaya (e=early; m=mid; l=late)

		Jan			**Feb**			**Mar**			**Apr**			**May**			**Jun**			**Jul**			**Aug**			**Sept**			**Oct**			**Nov**			**Dec**		
ANNUAL CYCLE		e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l
	A																			■	■	■															
	B																					■	■	■													
	C																								■	■	■	■	■	■	■						
	D																													■	■	■	■	■			
	E																																■	■	■		

(A= Emergence of seedlings; B= Establishment of seedlings; C= Flowering; D= Fruiting; E= Seed set)

4.3.6. **Begonia satrapis** C.B. Clarke; Hook. f. Fl. Brit. India 2:638.1879; K.D. Kumar and U.C. Bhattach. in M. P. Nair and A. R. K. Sastry (ed) Red Data Book 3:85.1990; Grierson in Grierson and Long, Flora of Bhutan 2(1):237–246.1991; Uddin et al. in Journal of Economic and Taxonomic Botany 31(3):591–597.2007; Nautiyal et al. in Journal of the Botanical Research Institute of Texas 3(2):823–830.2009.

Additional specimen examined: **INDIA**. Sikkim: Rungeet, 2000 ft, 04.08.1910, *Ribu & Rhomo* (Lloyd Botanical Garden, Darjeeling).

Description: Tuberous, succulent, monoecious herb, 30–45 cm tall. Stem: erect, 20–35 cm long, elongated above the leaves, pubescent. Leaves: lamina basal, 5–

7.5 × 7.5–10 cm, cordate at the base, serrate margin, acute apex, pubescent, multicostate; petiole red, 1.2–1.8 cm long; stipules lanceolate, densely pubescent. Inflorescence: biparous cymes, peduncle elongate, 2.4–3 cm long, densely pubescent. Male flowers: pedicel 16–25 mm long, tepals 2, outer large, pubescent, ovate, 12–15 × 8–12 mm, dark pink, inner tepals, lanceolate, light pink, 8–10 × 5–7 mm, stamens 20–41, shortly monadelphous, column 5–6 mm long, anthers obovoid, connective not produced. Female flowers: pedicel 20–30 mm long, tepals 5, unequal, pink, outer tepals large, 10–12 × 4–8, inner tepals small, 7–8 × 3–5 mm, pubescent; styles 3, ovary 3 locular, bifid placentae. Fruits: capsule pubescent, 3 winged, unequal, seeds ellipsoid, dehiscent by longitudinal slits (Plate 6).

Habitat: Grows in tropical and subtropical mixed deciduous forest dominated by tree species like *Schima wallichii*, *Pinus* spp. etc

Distribution: India: Sumbuk, Kitam, Sadam (Sikkim); West Bengal (Rambi, Badamtam).

Phenology: *B. satrapis* flowering during July and fruiting starts from September (Table 5).

Potential Value: Ornamental

Conservation status: Critically endangered (Nayar and Sastry 1990; Adhikari et al. 2018)

Specimens examined: **INDIA**. Sikkim: South District, Sumbuk, 900 m alt. (Aditya Pradhan, No. 00127, 15th August 2016, BSHC, Plate 19).

Note: This species is strictly endemic to Sikkim Himalaya and has an ornamental value. It grows naturally at a lower elevation (300-900 m asl) in association with tree species like *Shorea robusta* Roth and *Schima wallichii* (DC.) Korth. etc.

Table 5: Phenology of *B. satrapis* from Sikkim Himalaya (e=early; m=mid; l=late)

		Jan			Feb			Mar			Apr			May			Jun			Jul			Aug			Sept			Oct			Nov			Dec		
ANNUAL CYCLE		e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l
	A													■	■	■																					
	B																■	■	■																		
	C																			■	■	■	■	■	■	■	■										
	D																											■	■	■	■						
	E																															■	■	■			

(A= Emergence of seedlings; B= Establishment of seedlings; C= Flowering; D= Fruiting; E= Seed set)

4.3.7. **Begonia picta** Smith, Exot. Bot. 2: 81.1805; C.B. Clarke in Hook. f. Fl. Brit. India 2: 638.1879; Hara in Flora of Eastern Himalaya 2.84.1971; Polunin and Stainton in Concise Flowers of the Himalaya 57.1990; Grierson in Grierson and Long, Flora of Bhutan 2(1): 237–246.1991; Uddin et al. in Journal of Economic and Taxonomic Botany 31(3):591–597.2007.

Additional specimen examined: **INDIA**. Sikkim: Nam Nam Road Gangtok, 16.08.1983, *S. Singh* 2768, Dikchu Khola, 07.09.1997, *B.K. Shukla* 19591 (BSHC); West Bengal: Darjeeling, 07.09.1809, s.n. 9110 (CNH), 10 Km to Mussoorie Uttar Pradesh, 23.08.1985, *P.K. Hajra, R.N. Banerjee & Syamali Das Gupta* 07 (CNH), Chumba State, 6000 ft, 16.07.1896, 1762 (CNH), On the way to Algarh Kalimpong district, 1520 m, 08.09.1989, *Dilip Kumar* 19648, Pachalama, 11.07.1862, 312 (CNH), Mingling-Takepokong, Nepal, 1160 m, 18.11.1958, *Rollahagiri Ram* 17795 (CNH).

Description: Tuberous, succulent herb, 8–12 cm tall. Leaves: petiole 5–6 cm long, pubescent, lamina 8–13 × 7–12 cm, ovate, basifixed, lower surface green with red ting on the veins, margin double serrate and acute, stipules lanceolate. Inflorescence: cymose and terminal, peduncle pilose, bracts lanceolate, 2.5–3 × 1–1.3

mm, dense hair on margin, entire. Male flower: pedicel 10–12 mm long, tepals 4, villose, 5–9 × 4–8 mm, pink to white, entire, glabrous, androecium with 30–40 stamens, filaments 2 mm long, symmetric, equal, fused at base, anther oblong, 1–1.5 mm long, dehiscent through slit at the tip. Female flower: pedicel 7-10 mm long, pilose, bracteoles not present, tepals 5 (rarely 6), outer tepals 3–5 × 2.3–4 mm, white to pink, glabrous, inner tepals smaller, 1.2–1.4 × 0.6–0.8 mm, ovary 3 locular, styles 3, nearly separated, bifid placentae. Fruits: capsule 3 winged, oblong-ellipsoid, wings unequal, extended along the pedicel, pubescent, seeds short, ellipsoids and light brown (Plate 7).

Habitat: It is a very common species found growing luxuriantly along road side in the subtropical forest.

Distribution: China, Tibet, Nepal, Bhutan, Myanmar; India: Arunachal Pradesh, Meghalaya, Mizoram, Manipur, Nagaland, Sikkim (Assam Lingay, Sadam), Kurseong, Darjeeling (West Bengal).

Phenology: *B. picta* flowers during July and fruiting starts from September (Table 6).

Potential value: Ornamental/Fermented as a pickle (Shrestha 1987; Bhattarai 1992; Sigdel 2004; Pathak and Pandey 2005; Gurung 2006; Shrestha and Dhillion 2006)

Conservation status: The calculated range of *B. picta* is small (EOO is 351.45 km^2 and AOO is 206.16 km^2), which would suggest a status of endangered. However this likely reflects the lack of collections throughout their distribution range, as the species is distributed throughout the entire northeast India and with inclusion of population data may further lead to a change in threat status.

Specimen examined: **INDIA**. Sikkim: East District, Assam Linzey, 1500 m alt. (Aditya Pradhan, No. 00126, 15th September 2016, BSHC, Plate 20).

Note: *B. picta* is similar to *B. satrapis* however differs in having multiple leaves arising from the tuber. The species grows naturally in a subtropical forest.

Table 6: Phenology of *B. picta* from Sikkim Himalaya (e=early; m=mid; l=late)

		Jan			Feb			Mar			Apr			May			Jun			Jul			Aug			Sept			Oct			Nov			Dec		
ANNUAL CYCLE		e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l
	A																■	■	■																		
	B																			■	■	■															
	C																					■	■	■	■	■	■										
	D																										■	■	■	■	■						
	E																													■	■	■					

(A= Emergence of seedlings; B= Establishment of seedlings; C= Flowering; D= Fruiting; E= Seed set)

4.3.8. **Begonia palmata** D. Don.; Prodr. Fl. Nepal. 223.1825; *B. laciniata* var. *tuberculosa* C.B. Clarke in Hook. f., Fl. Brit. India 2:645.1879; Hara in Flora of Eastern Himalaya 2.84.1971; Grierson in Grierson and Long, Flora of Bhutan 2(1):237–246.1991; Uddin et al. in Journal of Economic and Taxonomic Botany 31(3):591–597.2007.

Additional specimen examined: INDIA. West Bengal: Mungpo, 08.05.1987, *D.C.S. Raju* 7186; Sikkim: Phumsebong, 09.06.1988, *P. Sanu & S. Pradhan* 8359, Near Selep tank, 02.06.1981, *B. Krishna* 1604, Dhatray Reserve Forest (Rabong), 16.05.1985, *A.K. Verma* 3534 (BSHC); Assam, *S.N. Bal* 184, 609 (CNH), Fubza Manipur, 02.1906 (CNH), *A. Mekbold* 5514 (CNH), Nepal, 26.03.1959, *G. Panigrahi* 6052 (CNH), Hanuman top bypass road, Gangtok, 27.08.1989, *K.D. Kumar* 19623 (CNH), Chumang, 26.04.1965, *H. Santapau & S.K. Mukerjee* 115 (CNH), Dikchu, 27.05.1959, *S.K. Mukerjee* 4959 (CNH), Gangtok, 1520 m, 06.09.1989, *Dilip Kumar* 19650 (CNH), Khasi Hill, 09.1945, *G. Mani*, Notrong Forest Assam, 10.11.1988, *K. Biswas* 3858 (CNH), Nongpoo, 10.11.1938, *K. Biswas* 3857 (CNH).

Description: Rhizomatous, herbs or subshrubs, 25–70 cm tall, caulescent, rhizomes elongate, 1.9–2.1 cm in diameter. Stem erect, brown villous, floccose-pubescent, or tomentose. Stipules: lanceolate, 9–25 × 2–8 mm, glabrous, persistent. Leaves: petiole 2.5–20 cm long, densely pubescent, lamona ovate, basifixed, cordate at base, asymmetric, upper surface green, with pale ring marking, underneath reddish green, pubescent, venation palmate, acute-acuminate apex. Inflorescence: axillary to terminal, biparous cymes, peduncle 3.3–8.9 cm, tomentose. Male flowers: pedicel 10–30 mm long, glabrous or sparsely tomentose, tepals 4, outer tepals ovate, 7–20 × 4–15 mm, white, tomemntose on reverse, base dense, entire margin, inner tepals obovate to oblong, 5–10 × 4–5 mm, white, glabrous, entire, 50–80 stamens, symmetric, nearly free or slightly monadelphous; filaments 0.5–2.5 mm, anthers 1.9–2.3 mm, obovoid; connective produced little above the anther lobes. Female flower: pedicels 10–20 mm long, glabrous or sparsely tomentose, bracteoles not present, tepals 5, ovate, unequal, outer tepals 2 obovate, 8–23 × 8–22 mm; inner tepals 2–3, lanceolate, 15–19 × 5–9 mm; ovary brown tomentose or villous, 2 locular; placentation axile, bifid; styles 2, divided from near the base into 2 much-contorted branches. Fruits: Capsule 3 winged; lateral wings 5–8 mm long; distil wing 25–32 mm, seeds ellipsoid, obovoid (Plate 8).

Habitat: Commonly grows in the moist subtropical forest as an understory.

Distribution: China, Nepal, Bhutan, Bangladesh, Myanmar, Thailand, Laos, Vietnam; India: Northeast India, Sikkim (Assam linzay, Dzongu), Lebong road, Ghoom (West Bengal).

Phenology: *B. palmata* flowers during May and fruiting starts from August (Table 7).

Potential value: Ornamental, food and beverage (Malla and Kurmi 2004; Pathak and Pandey 2005; Shrestha 2008; Deb and Rahman 2018).

Conservation status: The calculated range of *B. palmata* is small (EOO is 642.87 km^2 and AOO is 170.60 km^2), which would suggest a status of endangered. However this likely reflects the lack of collections throughout their distribution range, as the species is distributed throughout the entire northeast India and with inclusion of population data may further lead to a change in threat status.

Specimen examined: INDIA. Sikkim: East Sikkim, Assam Lingay, 1300 m alt. (Aditya Pradhan, No. 00131, 15th September 2016, BSHC, Plate 21).

Note: *B. palmata* is a subtropical species having a variegated leaf and white-coloured flowers. Leaf variegation is prominent during flowering stage and gradually disappears after fruiting.

Table 7: Phenology of *B. palmata* from Sikkim Himalaya (e=early; m=mid; l=late)

		Jan			Feb			Mar			Apr			May			Jun			Jul			Aug			Sept			Oct			Nov			Dec		
ANNUAL CYCLE		e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l
	A										■	■	■																								
	B												■	■	■																						
	C														■	■	■	■	■	■	■																
	D																					■	■	■	■	■											
	E																									■	■	■									

(A= Emergence of seedlings; B= Establishment of seedlings; C= Flowering; D= Fruiting; E= Seed set)

4.3.9. **Begonia xanthina** Hook.; Bot. Mag. 78. 78: t. 4683.1852; C.B. Clarke in Hook. f., Fl. Brit. India 2:644.1879; Grierson in Grierson and Long, Flora of Bhutan 2(1):237–246. 1991; Peng and Leong in Botanical Studies 47:89–96.2006; Uddin et al. in Journal of Economic and Taxonomic Botany 31(3):591–597.2007.

Additional specimen examined: INDIA. Sikkim: Sakyong towards Pentong, 16.09.1986, *A.K. Krishna* 7019 (BSHC).

Description: Rhizomatous herb, 42–50 cm tall. Rhizome: 12–15 mm wide, pilose. Stipule: lanceolate, 14–22 × 7–12 mm, caduceus. Leaves: petiole 10-20 cm long, lamina ovate, pilose to villose, asymmetric, basifixed, 23–35 × 13.3–12.8 cm, upper surface green with white spots, glabrous, underneath red, entire margin, acute apex. Inflorescence: terminal, cymose, peduncle glabrous, bracts ovate, 18–20 × 16–18 mm, glabrous. Male flower: pedicel yellowish-green, 26–41 cm long, tepals 4, outer tepals 10–13 × 4 cm, yellow, ovate, pubescent on reverse; inner tepals ovate, 6–10 × 3–4 mm, yellow, glabrous, stamens 100–133, symmetric, anther oblong, yellow , filaments 2.4–3.8 mm long. Female flowers: pedicel green, 13–25 mm long, glabrous, tepals 5, equal, outer tepals 15–18 × 6–10 mm, yellow, pubescent, inner similar yet smaller, ovary 2 locular, bifid placentae, ellipsoid, styles 2, deciduous. Fruits: capsule ovate, 8–14 × 6–10 mm, glabrous, unequal (Plate 9).

Habitat: Grows at subtropical to temperate forest in the human settled area.

Distribution: China, Bhutan; India: Arunachal Pradesh, Meghalaya, Sikkim (Gangtok).

Phenology: *B. xanthina* flowers during September and fruits during October (Table 8).

Potential value: None

Conservation status: The calculated range of *B. xanthina* is small (EOO is 74.21 km^2 and AOO is 38.91 km^2), which would suggest a status of critically endangered. However this likely reflects the lack of collections throughout their distribution range, as the species is distributed throughout the entire northeast India and with inclusion of population data may further lead to a change in threat status.

Specimen examined: **INDIA**. Sikkim: East District, Gangtok, 2200m alt. (Aditya Pradhan, No. 0088, 3rd October 2016, BSHC, Plate 22).

Note: *B. xanthina* is the only species of *Begonia* having patches of variegated leaves and yellow flowers.

Table 8: Phenology of *B. xanthina* from Sikkim Himalaya (e=early; m=mid; l=late)

		Jan			**Feb**			**Mar**			**Apr**			**May**			**Jun**			**Jul**			**Aug**			**Sept**			**Oct**			**Nov**			**Dec**		
ANNUAL CYCLE		e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l
	A																	■	■																		
	B																			■	■	■															
	C																						■	■	■	■	■	■									
	D																											■	■	■	■						
	E																														■	■	■				

(A= Emergence of seedlings; B= Establishment of seedlings; C= Flowering; D= Fruiting; E= Seed set

4.3.10. **Begonia flaviflora** (C.B. Clarke) H. Hara in Journal of Japanese Botany 14.91.1970; Hara in Flora of Eastern Himalaya 2.84.1971; *Begonia laciniata* var. flava C.B. Clarke in Hook. f., Fl. Brit. India 2:647.1879; Grierson in Grierson and Long, Flora of Bhutan 2(1): 237–246.1991; Uddin et al. in Journal of Economic and Taxonomic Botany 31(3):591–597. 2007.

Additional specimen examined: INDIA. Sikkim: Damthang to Temi, 1950 m, 08.1991, *R. Gopalan* 97629, 02.08.1985, *D.C.S. Raju* 4000, Bakhim to Yuksum, 2500 m, 31.07.1999, *D. Maity* 21228 (BSHC).

Description: Rhizomatous herb, erect, 30–50 cm in height. Stem: stout, brown tomentose when young, 4 mm wide, 5–15 cm long internodes, pubescent. Stipules: lanceolate, 9–14 × 2–4 mm, persistent. Leaves: petiole 18–20 cm long, ovate, cordate at base, 10–23 × 7–18 cm, ovate, asymmetric, green upper surface with darker centre, glabrous, red purple underneath, acute margin, deeply lobed, acute apex. Inflorescence: axillary, cymose, pilose peduncle, 9.5–11 cm long, bracts not present. Male flowers: pedicel 15–25 mm, pubescent, tepals 4, yellow, outer tepals ovate, 15–20 × 6–9 mm, entire margin, inner tepals obovate, 10–15 × 8 mm, glabrous, stamens 50-70, symmetric, filaments 1–1.3 mm long, anther elliptic, dehiscent through slits, connective extended. Female flowers: pedicel 7–12 mm long, pubescent, bracteoles not present, tepals 5, yellow, ovate to oblong, equal, outer tepals 11–14 × 6–12 mm, entire margin, inner tepals smaller, glabrous, 2 locular ovary, bifid placentae, capsule ellipsoid, styles 3. Fruits: capsule, 5–8 × 4–8 mm, wings unequal, oblong (Plate 10).

Habitat: Grows in the dense subtropical-temperate forest.

Distribution: Nepal, Myanmar, China, South Tibet; India: Arunachal Pradesh, Sikkim (Phademchen, Yuksam), West Bengal (3rd Mile, 6th Mile Road, Lebong).

Phenology: *B. flaviflora* flowers during July and fruits during September (Table 9).

Potential value: None

Conservation status: The calculated range of *B. flaviflora* is small (EOO is 1128.32 km^2 and AOO is 127.89 km^2), which would suggest a status of endangered. However this likely reflects the lack of collections throughout their distribution range, as the species is distributed throughout the entire northeast India and with inclusion of population data may further lead to a change in threat status.

Specimen examined: INDIA. West Bengal: Darjeeling District, Senchal Wildlife Sanctuary, 2000 m alt. (Aditya Pradhan, No. 00130, 3rd July 2005, BSHC, Plate 23).

Note: *B. flaviflora* is succulent *Begonia* species found naturally growing in the temperate forest along the roadside. Like *B. palmata* this species also have variegated leaves which disappears after fruiting phenology. However this species differ in having bright yellow flowers.

Table 9: Phenology of *B. flaviflora* from Sikkim Himalaya (e=early; m=mid; l=late)

		Jan			**Feb**			**Mar**			**Apr**			**May**			**Jun**			**Jul**			**Aug**			**Sept**			**Oct**			**Nov**			**Dec**		
ANNUAL CYCLE		e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l
	A														■	■	■																				
	B																■	■	■	■																	
	C																			■	■	■	■	■													
	D																							■	■	■	■										
	E																										■	■	■	■	■						

(A= Emergence of seedlings; B= Establishment of seedlings; C= Flowering; D= Fruiting; E= Seed set)

4.3.11. **Begonia panchtharensis** S. Rajbhandari, in Garden's Bulletin Singapore 62(1):151–162.2010; Pradhan et al. in Journal of Japanese Botany 94(1):56–57.2019.

Additional specimen examined: NEPAL. Eastern Nepal: Panchthar: Tinibote, Sisire, Prangbung, 2240-2300 m, 10.10.2007, *Rajbhandari E00428086*.

Description: Rhizomatous, monoecious herb, 30–50 cm tall. Rhizome 1.6–12.3 cm long, 20.1–28.9 mm in diameter, covered with long roots. Stipules: caducous 15.4–22.5 × 7.4–10.3 mm, cymbiform, broadly ovate, pink with a green tinge, red spots on the adaxial surface, margin entire, apex acuminate. Leaves arising directly from the rhizome, petiole yellowish-green with red linear dots, 31.3–43.2 cm long, smooth, succulent, grooved; lamina slightly asymmetric, deeply lobed, 23–39 × 35–46 cm, margin serrulate or deeply dentate, lobes 7–9, apex acuminate, adaxial surface sparsely hirsute, abaxial surface glabrous, palmately veined. Inflorescence: axillary, cymose, protandrous; peduncle 32–45 cm long, glabrous, grooved with red linear dots; bracts membranous, cymbiform, elliptic to broadly elliptic, 23–28 × 12–18 mm. Male Flower: pedicel 1.1–2.3 cm long, glabrous; tepals 4, white to pink, outer 2 tepals broadly ovate, margin entire, apex acute, inner 2 tepals oval to narrowly oval, glabrous, base cuneate, retuse apex; stamens 128–149, anthers 1.7–2 mm long. Female flower: pedicel 1.2–1.8 cm, glabrous; tepals 6–8, tepals pink, acute apex, styles 2, persistent, ovary oblong, 3 unequal wings, placenta axile, capsule oblong, dehiscing along the sutures between the two small wings (Plate 11).

Habitat: Grows in a temperate forest in association with *Begonia flaviflora, Galium* spp. *Thysaenolaena maxima, Urtica dioca,* etc.

Distribution: Nepal, India: Sikkim (Yuksam, Thangsing).

Phenology: *B. panchtharensis* flowers during September and fruits during October (Table 10).

Potential value: Medicinal (Rajbhandari 2013)

Conservation status: The calculated range of *B. panchtharensis* is small (EOO is 61.15 km^2 and AOO is 44.37 km^2), which would suggest a status of critically endangered. However this likely reflects the lack of collections throughout their distribution range, as the species is reported from Nepal as well and with inclusion of population data may further lead to a change in threat status.

Specimen examined: **INDIA**. Sikkim: East District, Thangsing, 2200 m alt. (Aditya Pradhan, No. 00129, 26th October 2016, BSHC, Plate 24).

Note: *B. panchtharensis* is similar to *B. sikkimensis* however differs in having grooved petiole and deeply lobed single leaf arising directly from the rhizome and six tepals in the female flower. A deeply lobed leaf of *B. panchtharensis* is similar to newly discovered species *B. koelzii* R. Camfield from Northeast India.

Table 10: Phenology of *B. panchtharensis* from Sikkim Himalaya (e=early; m=mid; l=late)

		Jan			Feb			Mar			Apr			May			Jun			Jul			Aug			Sept			Oct			Nov			Dec		
ANNUAL CYCLE		e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l
	A																			■	■	■															
	B																						■	■	■												
	C																								■	■	■	■									
	D																											■	■	■							
	E																													■	■	■					

(A= Emergence of seedlings; B= Establishment of seedlings; C= Flowering; D= Fruiting; E= Seed set)

4.3.12. **Begonia sikkimensis** A. DC.; Ann. Sci. Nat. ser. 4(11):134.1859; C.B. Clarke in Hook. f., Fl. Brit. India 2:646.1879; Grierson and Long in Flora of Bhutan 2(1):241.1991; Uddin et al. in Journal of Economic and Taxonomic Botany 31(3):591–597.2007.

Additional specimen examined: **INDIA**. Sikkim: Tendong Reserve Forest, 6500 ft., 19.06.2003, *A.K. Sahoo* 26407, Enchey Monastery surrounding, Gangtok, 19.08.1980, P.K. Hajra 577, Near Raj Bhawan Gangtok, 18.08.1980, *P.K. Hajra* 560 (BSHC); Darjeeling, 4500 ft, 8721 (CNH), In between Chungthang and Lachung, 1672 m, 30.08.1989, *Dilip Kumar* 19606 (CNH).

Description: Rhizomatous, erect, succulent herbs, 59–100 cm tall. Rhizome: 10–12 mm wide, glabrous. Stem: slightly woody, 2 cm wide, glabrous. Stipules: lanceolate, 15–25 × 2–5 mm, glabrous. Leaves: petiole 5–12 cm long, glabrous, lamina broadly ovate, basifixed, 8–19 × 10–20 cm, asymmetric, peltate and deeply lobed, shallowly cordate, denticulate, apex acuminate. Inflorescence: terminal biparous cymes; peduncle glabrous 8–15 cm long, bracts ovate, 2–23 × 2–24 mm, ovate-elliptic, acuminate and bright pink. Male flower: pedicel 12–15 mm long, glabrous, tepals 4, outer ovate, 12–17 × 6–12 mm, pink to red, glabrous, entire, inner tepals oblong, 10–15 × 4–10, pink, glabrous, stamens 100–136, monadelphous, symmetric, filaments unequal, anther elliptic, 1–1.6 mm long. Female flower: pedicel 10–20 mm long, glabrous, bracteoles not presnt, tepals 5, equal, outer tepals, 4–10 × 3–7 mm, ovate, bright pink to red, entire margin, ovary 2 locular, bifid placentation, styles 2 and fused at the base; stigma 2 cleft. Fruits: capsule 3 winged, 10–18 × 15–38 mm, oblong-ellipsoid, 18–23 × 7–9 mm, unequal winged; the distal wing is oblong-triangular (Plate 12).

Habitat: Grows in the temperate forest as an understory.

Distribution: China, Tibet, Nepal, Myanmar; India: Arunachal Pradesh, Mizoram, Nagaland, Sikkim (Pangthang), West Bengal (Mungpo).

Phenology: *B. sikkimensis* flowers during August and fruiting starts from September (Table 11).

Potential value: None

Conservation status: The calculated range of *B. sikkimensis* is small (EOO is 1103.20 km^2 and AOO is 221.66 km^2), which would suggest a status of endangered. However this likely reflects the lack of collections throughout their distribution range, as the species is distributed throughout the entire northeast India and with inclusion of population data may further lead to a change in threat status.

Specimen examined: **INDIA**. Sikkim: East Sikkim, Pangthang, 1757 m alt. (Aditya Pradhan, No.0128, 3rd October 2016, BSHC, Plate 25).

Note: *B. sikkimensis* is a temperate species found growing along roadside and river cutting. Two varieties of *B. sikkimensis* have been recognised *viz*. *B. sikkimensis* var. *sikkimensis* and *B. sikkimensis* var. *kamengensis*. The later differ in having bright red coloured flower, tall and slightly woody stem.

Table 11: Phenology of *B. sikkimensis* from Sikkim Himalaya (e=early; m=mid; l=late)

		Jan			Feb			Mar			Apr			May			Jun			Jul			Aug			Sept			Oct			Nov			Dec		
ANNUAL CYCLE		e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l
	A																■	■																			
	B																		■	■	■																
	C																					■	■	■	■	■											
	D																									■	■	■									
	E																											■	■	■	■						

(A= Emergence of seedlings; B= Establishment of seedlings; C= Flowering; D= Fruiting; E= Seed set)

4.3.13. **Begonia megaptera** A. DC.; Ann. Sci. Nat. Bot. 4(11):134.1859; C.B. Clarke in Hook. f., Fl. Brit. India 2:646.1879; Hara in Flora of Eastern Himalaya 2.84.1971; Grierson in Grierson and Long, Flora of Bhutan 2(1):237–246.1991; Uddin et al. in Journal of Economic and Taxonomic Botany 31(3):591–597.2007.

Additional specimen examined: INDIA. Sikkim: Chalisay on the way to Rongli, 4000 ft, 07.12.1980, *P. Chakraborty* 1012, Damthang to Namchi forest, 6000 ft, 22.06.2003, *A.K. Sahoo* 26519; West Bengal: on the way to Lopchu, 26.10.1985, *D.C.S. Raju* 4473 (BSHC); Sitong, 4000 ft, 14.01.1977, 2395 (CNH), Kalimpong, 606-712 m, 05.09.1989, *Dilip Kumar* 19626 (CNH).

Description: Rhizomatous, caulescent, 18–60 cm tall. Stem: stout, erect, branched, glabrous, pale green, internodes 10–13 cm long. Stipules: lanceolate, 10–14 × 5–8 mm, persistant, glabrous. Leaves: petiole 4–11 cm long, glabrous; lamina ovate, basifixed, 11–16 × 7–12 cm, oblique, cordate base, apex acuminate, shallowly lobed or undulate, glabrous on both the surfaces. Inflorescence: axillary, two times branched, few-flowered cymes; peduncle 11–18 long, glabrous; bracts large, oblong, deciduous. Male flowers: pedicel 18 mm long, glabrous, tepals 4; outer broadly ovate, 6–13 × 3–7 mm, white to light pink, glabrous, entire margin; inner tepals, ovate, 3–8 × 2–5 mm, white to light pink, glabrous, stamens 60–80, monadelphous; anthers oblong, 2 mm long. Female flowers: pedicel 10–15 mm long, glabrous, bracteoles not present tepals 5, outer 10–14 × 5–10 mm, white to light pink, glabrous, styles 2, fused at base; stigmas spiralled and contorted; ovary 2 locular. Fruits: capsule recurved, oblong, 3 winged, 2.9 × 1.2 cm including the wings; lateral wings *ca*. 3 mm long; distal wing 1.2–1.5 cm long, upper edge concave (Plate 13).

Habitat: Found growing on walls and rocky crevices at an elevation ranging from 500-1500 m asl.

Distribution: Nepal, Bhutan, Bangladesh, Myanmar; India: Arunachal Pradesh, Meghalaya, Mizoram, Nagaland, Sikkim (Ahoo Busty, 6th mile, Ranipool).

Phenology: *B. megaptera* starts flowering during October and fruiting starts from November (Table 12).

Potential value: None

Conservation status: The calculated range of *B. megaptera* is small (EOO is 222.66 km^2 and AOO is 102.28 km^2), which would suggest a status of endangered. However this likely reflects the lack of collections throughout their distribution range, as the species is distributed throughout the entire northeast India and with inclusion of population data may further lead to a change in threat status.

Specimen examined: INDIA. Sikkim: East District, Ahoo Yangtang, 892 m alt. (Aditya Pradhan, No.00121, 3rd October 2016, BSHC, Plate 26).

Note: *B. megaptera* is a species of *Begonia* having large winged fruit, grows naturally on the roadside forest at lower elevation in association with *B. roxburghii*.

Table 12: Phenology of *B. megaptera* from Sikkim Himalaya (e=early; m=mid; l=late)

		Jan			Feb			Mar			Apr			May			Jun			Jul			Aug			Sept			Oct			Nov			Dec		
ANNUAL CYCLE		e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l
	A																					■	■	■													
	B																								■	■	■										
	C																											■	■	■	■	■					
	D																														■	■	■				
	E																																■	■			

(A= Emergence of seedlings; B= Establishment of seedlings; C= Flowering; D= Fruiting; E= Seed set)

4.3.14. **Begonia cathcartii** Hook. f.; lll. Him. Pl. t. 13.1855; C.B. Clarke in Hook. f. Fl. Brit. India.2:646.1879. Hara in Flora of Eastern Himalaya 2.84.1971; Grierson in Grierson and Long, Flora of Bhutan 2(1):237–246.1991; Uddin et al. in Journal of Economic and Taxonomic Botany 31(3):591–597.2007.

Additional specimen examined: INDIA. Sikkim: Fambonglho Wildlife Sanctuary, 15.07.1998, *S.K. Rai* 20824, Yuksam to Tsoka trek, 11.05.1994, *G.P. Sinha* 16022, Towards Sosing, 10.06.1987, *B. Krishna* 7506, Tendong Reserve Forest, 07.06.1986, *A.K. Verma* 5434, Yuksom to Tsoka trek, 2700 m, 11.05.1994, *G.P.Sinha* 16022, Barmeli to Maenam Ghumba trail, 3100 m, 19.06.2004, *T.M. Hynniewta* 28676, Kabi Sacred Grooves, 27.08.1998, *S.S. Das* 20712 (BSHC).

Description: Rhizomatous, monoecious herb, 25–30 cm tall. Rhizome: 8 mm wide. Leaves: petiole 5–15 cm long, red strigose, lamina ovate, cordate at base, 12–13 × 4–6 cm, asymmetric, red strigose all over, serrate margin, apex acuminate. Inflorescence: terminal, biparous cymes; peduncle 1.5 cm long, sparsely red strigose.

Male flowers: pedicel 15–20 mm long, pilose, tepals 4, outer tepals 8–13 × 7–10 mm, obovate-cordate, white, red pilose near the base, inner tepel white, lanceolate, 6–10 × 5–9 mm, glabrous, stamens 70–90 in number, filament 1mm, anther 2 mm long, monadelphous. Female flower: pedicel 15–18 mm long, bracteoles is not present, pilose, tepals 5, equal, 18–23 × 12–18 mm, white, red pilose near base, 2 locular ovary, bifid placentae, capsule densely strigose, with one long and two shorter wings, styles 3. Fruits: 32–40 × 10–18 mm, capsule ellipsoid, sparsely red strigose, 3 wing, 2 locular (Plate 14).

Habitat: Grows naturally in the temperate moist deciduous forest.

Distribution: Nepal, Bhutan, Myanmar, Thailand, India: Arunachal Pradesh, Sikkim (Pangthang), Ghoom, 6th Mile (Darjeeling).

Phenology: *B. cathcartii* starts flowering during May and fruiting during July (Table 13).

Potential value: None

Conservation status: The calculated range of *B. cathcartii* is small (EOO is 1053.37 km^2 and AOO is 192.27 km^2), which would suggest a status of endangered. However this likely reflects the lack of collections throughout their distribution range, as the species is distributed throughout the entire northeast India and with inclusion of population data may further lead to a change in threat status.

Specimen examined: **INDIA**. Sikkim: East District, Pangthang, 1911 m alt. (Aditya Pradhan, No. 00132, 3rd October 2016, BSHC, Plate 27).

Note: *B. cathcartii* is the species of *Begonia* having the green-coloured stigma and grows naturally in a deciduous temperate forest in association with *B. flaviflora* and *B. josephii.* The species is distinct in having red pubescent hair in leaves and stem and is commonly known as red haired *Begonia*.

Table 13: Phenology of *B. cathcartii* from Sikkim Himalaya (e=early; m=mid; l=late)

		Jan			Feb			Mar			Apr			May			Jun			Jul			Aug			Sept			Oct			Nov			Dec		
ANNUAL CYCLE		e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l
	A									■	■	■																									
	B											■	■																								
	C													■	■	■	■																				
	D																	■	■	■																	
	E																				■	■	■														

(A= Emergence of seedlings; B= Establishment of seedlings; C= Flowering; D= Fruiting; E= Seed set)

4.3.15. **Begonia nepalensis** (A. DC.) Warb., in Engl. Pflfam. Lll 6a:142.1894. *Begonia gigantea* Wall. ex C.B. Clarke in Hook. f., Fl. Brit. India 2:643.1879; Grierson in Grierson and Long, Flora of Bhutan 2(1):237–246.1991; Uddin et al. in Journal of Economic and Taxonomic Botany 31(3):591–597.2007.

Additional specimen examined: **INDIA**. West Bengal: Hallapara, Jaldapara National Park, 27.11.2013, *K. Karthigeyan* 61234 (CNH), 02.1836, *Courtallum* 838 (CNH)

Description: Rhizomatous, caulescent, monoecious herbs, 44–80 cm tall. Stem: 7–12 mm wide, glabrous. Stipules lanceolate. Leaves: petiole: 2–4 cm long, lamina ovate-lanceolate, cordate at base, basifixed, cauline, 12–20 × 2.5–9.5 cm, asymmetric, glabrous upper surface, lower surface pubscent only on veins, dentate margin, caudate-acuminate apex. Inflorescence: axillary or terminal biparous cymes; bracts ovate, acute, deciduous. Male flowers: pedicel 1–3 cm long, glabrous, tepals 2, ovate, 6–9 × 6–7 mm, white, glabrous, inner tepals not present, stamens 40–48, shortly monadelphous; anthers obovoid, dehiscent through slits at the tip, connective absent. Female flower: pedicel 2–4 cm long, glabrous, bracteoles absent, tepals 4; equal, outer tepals 2, ovate, 9–15 × 6–9 mm, white, glabrous, entire margin, inner tepals small, 2 locular ovary, bifid placentae, capsule ellipsoid, 1 winged, styles 2. Fruits: capsule ellipsoid, distal wing 18–22 mm, lateral wings 2–4 mm (Plate 15).

Habitat: Grows in tropical forest dominated by tree species like *Tectona grandis*, *Schima wallichii*, *Shorea robusta* etc.

Distribution: Nepal, Bhutan, Myanmar; India: Arunachal Pradesh, Assam, Meghalaya, Mizoram, Sikkim, West Bengal (Rambi).

Phenology: *B. nepalensis* flowers during late winter during November and fruits during January (Table 14).

Potential value: None

Conservation status: The calculated range of *B. nepalensis* is very small (EOO is 8.97 km^2 and AOO is 6.86 km^2), which would suggest a status of critically endangered. However this likely reflects the lack of collections throughout their distribution range, as the species is distributed throughout the entire northeast India and with inclusion of population data may further lead to a change in threat status.

Specimen examined: **INDIA**. West Bengal: Darjeeling District, Kalijhora, 245 m alt. (Aditya Pradhan, No. 0133, 5th February 2017, BSHC, Plate 28).

Note: This species grows naturally along the roadside in a tropical forest. *B. nepalensis* is the only species of *Begonia* having 2 tepals male and female flowers. The species is also quite unique in having late flowering and fruiting phenology, late during winter.

Table 14: Phenology of *B. nepalensis* from Sikkim Himalaya (e=early; m=mid; l=late)

		Jan			Feb			Mar			Apr			May			Jun			Jul			Aug			Sept			Oct			Nov			Dec		
ANNUAL CYCLE		e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l
	A																								■	■											
	B																										■	■	■								
	C																													■	■	■	■	■			
	D	■	■																															■	■	■	■
	E		■	■	■																																

(A= Emergence of seedlings; B= Establishment of seedlings; C= Flowering; D= Fruiting; E= Seed set)

4.3.16. **Begonia gemmipara** Hook. f., lll. Him. Pl. t. 14. 1855; C.B. Clarke in Hook. f. Fl. Brit. India 2:641.1879; Hara in Flora of Eastern Himalaya 2:84.1971; Hara in Hara and Williams, Enum. Fl. Pl. Nepal 2:181.1979; Grierson in Grierson and Long, Flora of Bhutan 2(1):237–246.1991; Uddin et al. in Journal of Economic and Taxonomic Botany 31 (3):591–597.2007.

Additional specimen examined: **INDIA**. Sikkim: Chitrey, 09.09.2008, *S.K. Rai* 33982, Hilley Reserve Forest, 2800 m, 26.08.1994, *P.Singh* 16308, Chitrey to Taal, 24.06.2003, *S.K. Rai & S. Pradhan* 26156, Barsey Rhododendron Sanctuary to Bhaluchowk, 18.09.2002, *S.K. Rai & S. Pradhan* 25312, Tiger Hill areas, 01.09.1985, *B. Krishna* 4619 (BSHC).

Description: Tuberous, succulent, monoecious herb, 13–25 cm tall. Stipules ovate, caduceus. Leaves: petioles 2.5–5 cm long, peltate at the base, ovate to cordate, abaxial surface glabrous, adaxial surface pilose, serrate or doubly serrate margin, apex acuminate. Inflorescence: axillary, peduncle absent, bracts 2, large, persistent. Male flower: tepals 4, equal, white with pink stripes, stamens numerous, slightly monadelphous, anthers obovoid, connective absent. Female flower: tepals 5, equal, persistent on the mature fruit, styles 3, distinct, stigmas lunate. Fruits: capsule 3 winged, 5–12 mm, seeds ellipsoid (Plate 16).

Habitat: Grows as an epiphyte on trees like *Cryptomeria japonica* at elevation 2500-3000 m asl.

Distribution: Nepal, India: Sikkim (Barsey Rhododendron Sanctuary) West Bengal (Senchal Wildlife Sanctuary).

Phenology: Flowering and fruiting from August to November in the natural habitat (Table 15).

Potential value: None

Conservation status: The calculated range of *B. gemmipara* is small (EOO is 866.70 km^2 and AOO is 98.83 km^2), which would suggest a status of critically endangered. However this likely reflects the lack of collections throughout their distribution range, as the species is reported from Nepal as well and with inclusion of population data may further lead to a change in threat status.

Specimen examined: **INDIA**. West Bengal: Darjeeling District, Senchal Wildlife Sanctuary, 2500m alt. (Aditya Pradhan, No. 00123, 5th October 2016, BSHC, Plate 29).

Notes: *B. gemmipara* is the only epiphytic species of *Begonia* reported in India. The species is usually found growing as epiphytes on trees like *Cryptomeria japonica*. It produces gemma-cup like structure for the asexual mode of reproduction.

Table 15: Phenology of *B. gemmipara* from Sikkim Himalaya (e=early; m=mid; l=late)

		Jan			Feb			Mar			Apr			May			Jun			Jul			Aug			Sept			Oct			Nov			Dec		
ANNUAL CYCLE		e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l
	A																			■	■																
	B																					■	■	■													
	C																								■	■	■	■									
	D																											■	■	■							
	E																													■	■	■	■				

(A= Emergence of seedlings; B= Establishment of seedlings; C= Flowering; D= Fruiting; E= Seed set

Sect. Sphenanthera

4.3.17. **Begonia roxburghii** (Miq.) A. DC., Prodr. 15(1):398.1864; C. B. Clarke in Hook. f., Fl. Brit. India 2:635.1879; Uddin et al. in Journal of Economic and Taxonomic Botany 31 (3):591–597.2007; Grierson in Grierson and Long, Flora of Bhutan 2(1):237–246.1991.

Additional specimen examined: INDIA. West Bengal: Chuk Chuki, Gorumara National Park, 19.09.2008, *V. Ranjan & Anant Kumar* 44449 (CNH).

Description: Rhizomatous, caulescent, erect, herb, 30–120 cm tall. Stem: slightly woody, 7–15 mm wide, glabrous, 6–12 cm long internode. Stipules: lanceolate, 12–15 × 2–4 mm, glabrous, deciduous. Leaves: petiole 4–12 cm long, glabrous, ovate, basifixed, cordate at base, 15–23 × 12–20 cm, entire margin, acute-acuminate apex. Inflorescence: cymose, axillary, peduncle pubescent, bracts 10–12 × 3–5 mm, glabrous, margin entire. Male flowers: pedicels 5–13 mm long, tepals 4, glabrous, outer tepals ovate-obovate, 4–9.7 × 2.4–9.2 mm, white or sometimes pale pink, inner tepals elliptic, 35–84 × 18–77 mm, rounded, white, glabrous, margin entire. Male flower: pedicel 10–25 mm long, glabrous, tepals 4, outer ovate, 5–10 ×

2–4 mm, white, glabrous, stamens 66–83, symmetric, filament base fused, anther oblong, 1.1–2.6 mm long. Female flower: pedicel 8–20 mm long, glabrous, bracteoles not presnt, tepals 4, equal, obovate, 5–12 × 3–7 mm, white, glabrous, entire margin, inner tepals smaller, ovary 4 locular, capsule ovoid, 3–4 × 4–8 mm, without wings, styles 4, persistent. Fruits: capsule pyramidal-ovoid, green or reddish-green, glabrous, globose, ellipsoid (Plate 17).

Habitat*:* Found growing in roadside forest and riverside at an elevation ranging from 600-1300 m asl.

Distribution: Nepal, Bangladesh, Myanmar, India: Northeast India, Sikkim (Ahoo Busty, 9th mile, Ranipool), West Bengal.

Phenology: *B. roxburghii* flowers during June and fruits during September (Table 16).

Potential value: Ornamental, food**,** anti-nociceptive, anti-arthritic, clot lysis and cytotoxic (Uddin 2010; Mobarak et al. 2018, Deb and Rahman 2018)

Conservation status: The calculated range of *B. roxburghii* is very small (EOO is 94.58 km^2 and AOO is 100.14 km^2), which would suggest a status of critically endangered. However this likely reflects the lack of collections throughout their distribution range, as the species is distributed throughout the entire northeast India and with inclusion of population data may further lead to a change in threat status.

Specimen examined: **INDIA**. Sikkim: East District, Ahoo busty, 892 m alt. (Aditya Pradhan, No. 0090, 15^{th} September 2016, BSHC, Plate 30).

Note: *B. roxburghii* is the only species of *Begonia* in Sikkim Himalaya with 4 locular ovaries, slightly woody and wingless fruits.

Table 16: Phenology of *B. roxburghii* from Sikkim Himalaya (e=early; m=mid; l=late)

		Jan			**Feb**			**Mar**			**Apr**			**May**			**Jun**			**Jul**			**Aug**			**Sept**			**Oct**			**Nov**			**Dec**		
ANNUAL CYCLE		e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l
	A												■	■	■																						
	B															■	■																				
	C																■	■	■	■	■																
	D																				■	■	■														
	E																						■	■	■												

(A= Emergence of seedlings; B= Establishment of seedlings; C= Flowering; D= Fruiting; E= Seed set

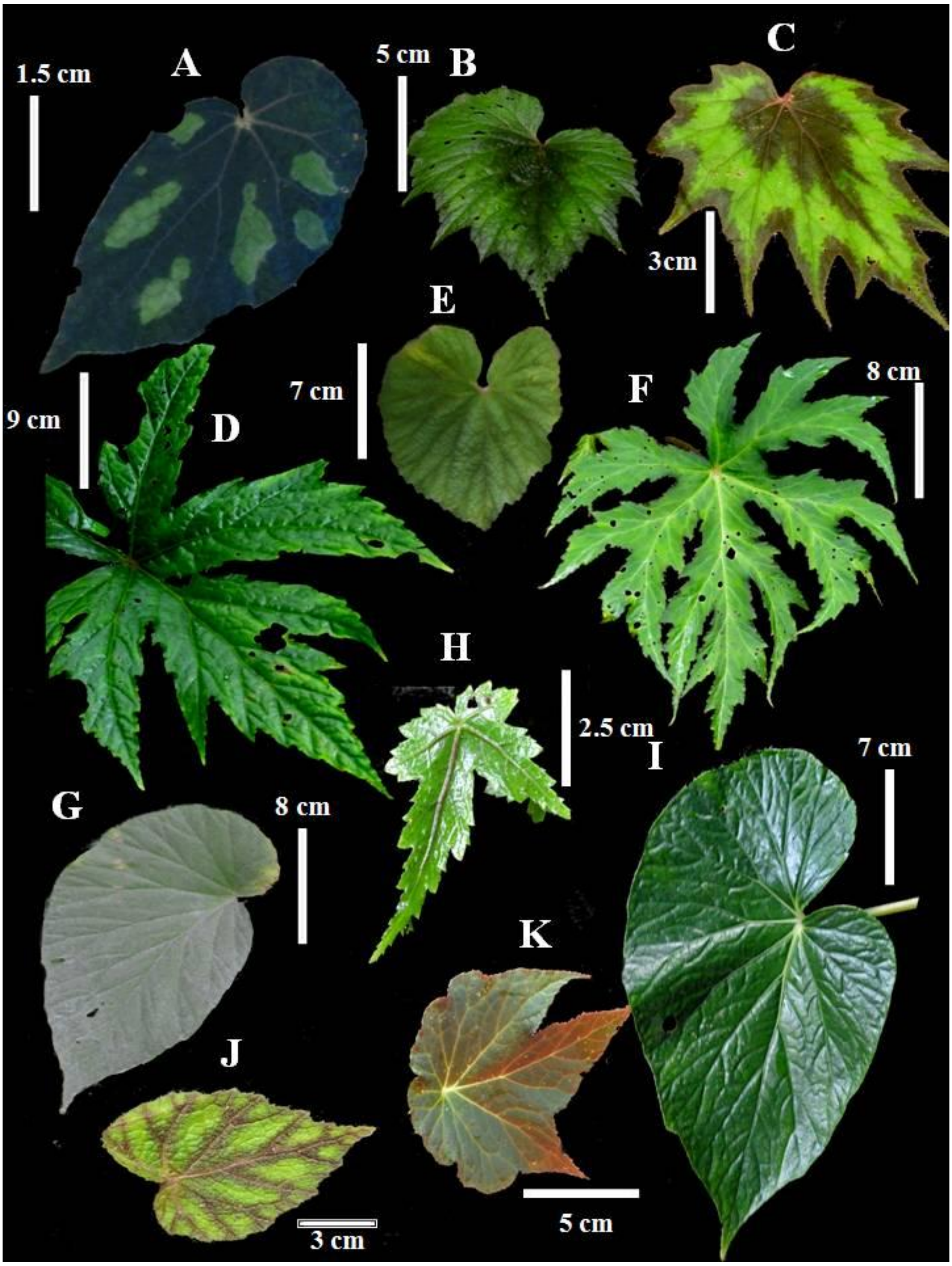

Plate 1 Diversity in leaf morphology, colour and variegation pattern in Sikkim Himalayan Begonias**:** A**: B. xanthina**; B: **Begonia flaviflora;** C: **B. palmata;** D**: B. sikkimensis;** E: **B. satrapis;** F**: B. panchtharensis**; G: **B. nepalensis**; H**: B. gemmipara**; I: **B. roxburghii**; J: **B. picta**; K: **B. josephii**

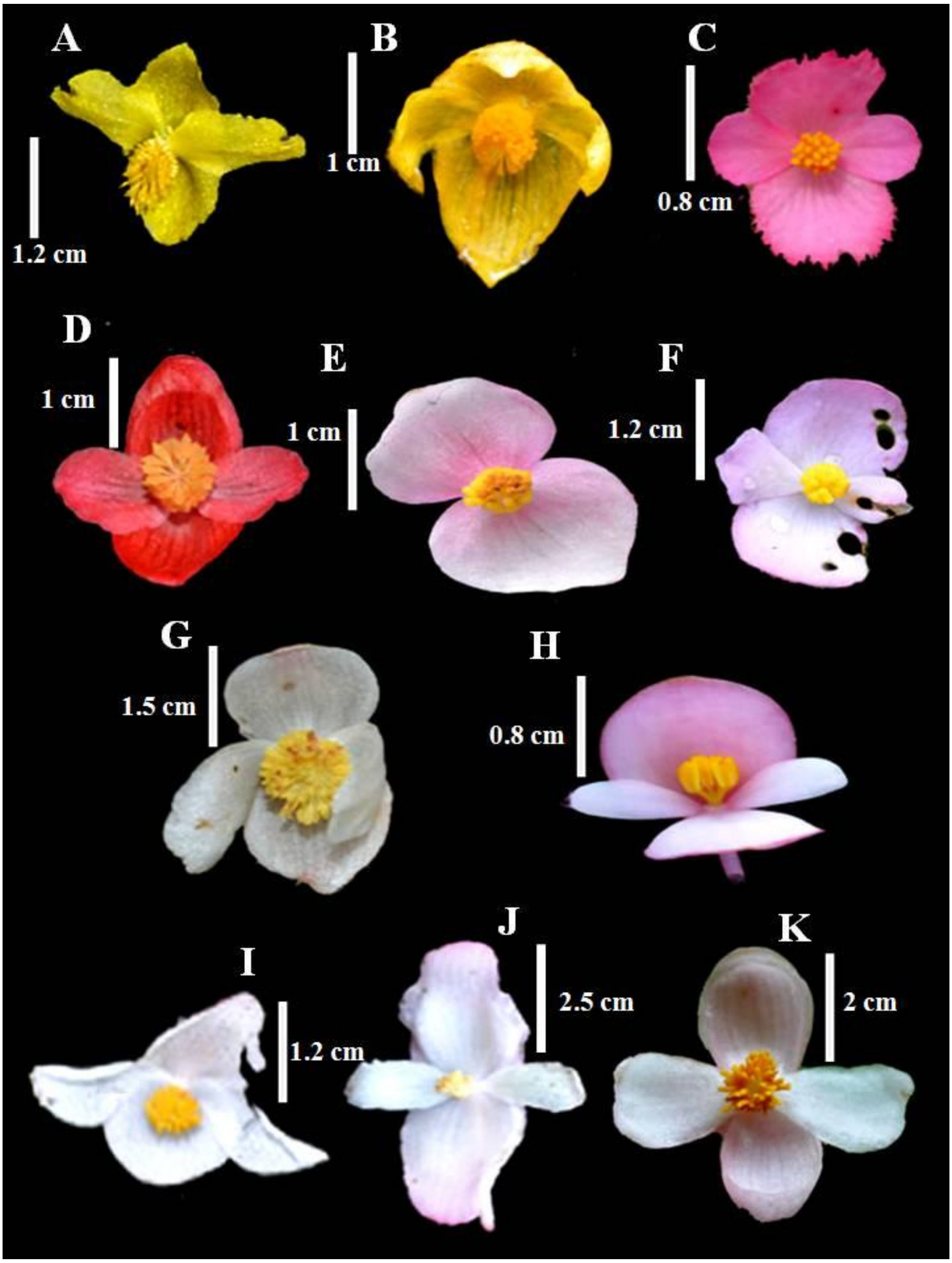

Plate 2 Diversity in male flower tepals number and colour in Sikkim Himalayan Begonias, A: **B. xanthina**; B: **B. flaviflora**; C: **B. satrapis**; D: **B. sikkimensis**; E: **B. nepalensis**; F: **B. picta**; G: **B. panchtharensis**; H: **B. josephii**; I: **B. palmata**; J: **B. megaptera**; K: **B. roxburghii.**

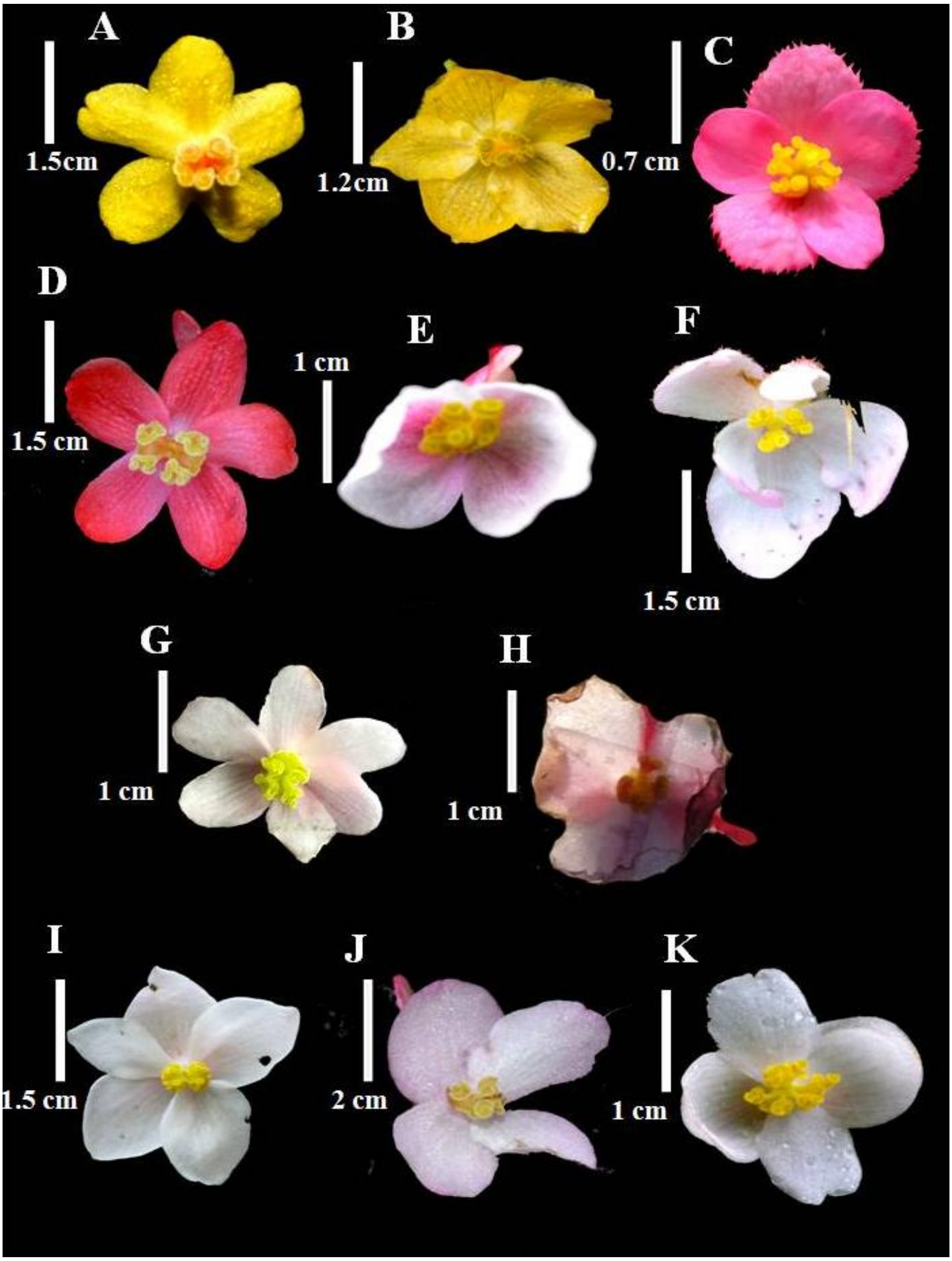

Plate 3 Diversity in female flower tepals number and colour in Sikkim Himalayan Begonia, A: **B. xanthina**; B: **B. flaviflora**; C: **B. satrapis**; D: **B. sikkimensis**; E: **B. nepalensis**; F: **B. picta**; G: **B. panchtharensis**; H: **B. josephii**; I: **B. palmata**; J: **B. megaptera**; I: **B. roxburghii**

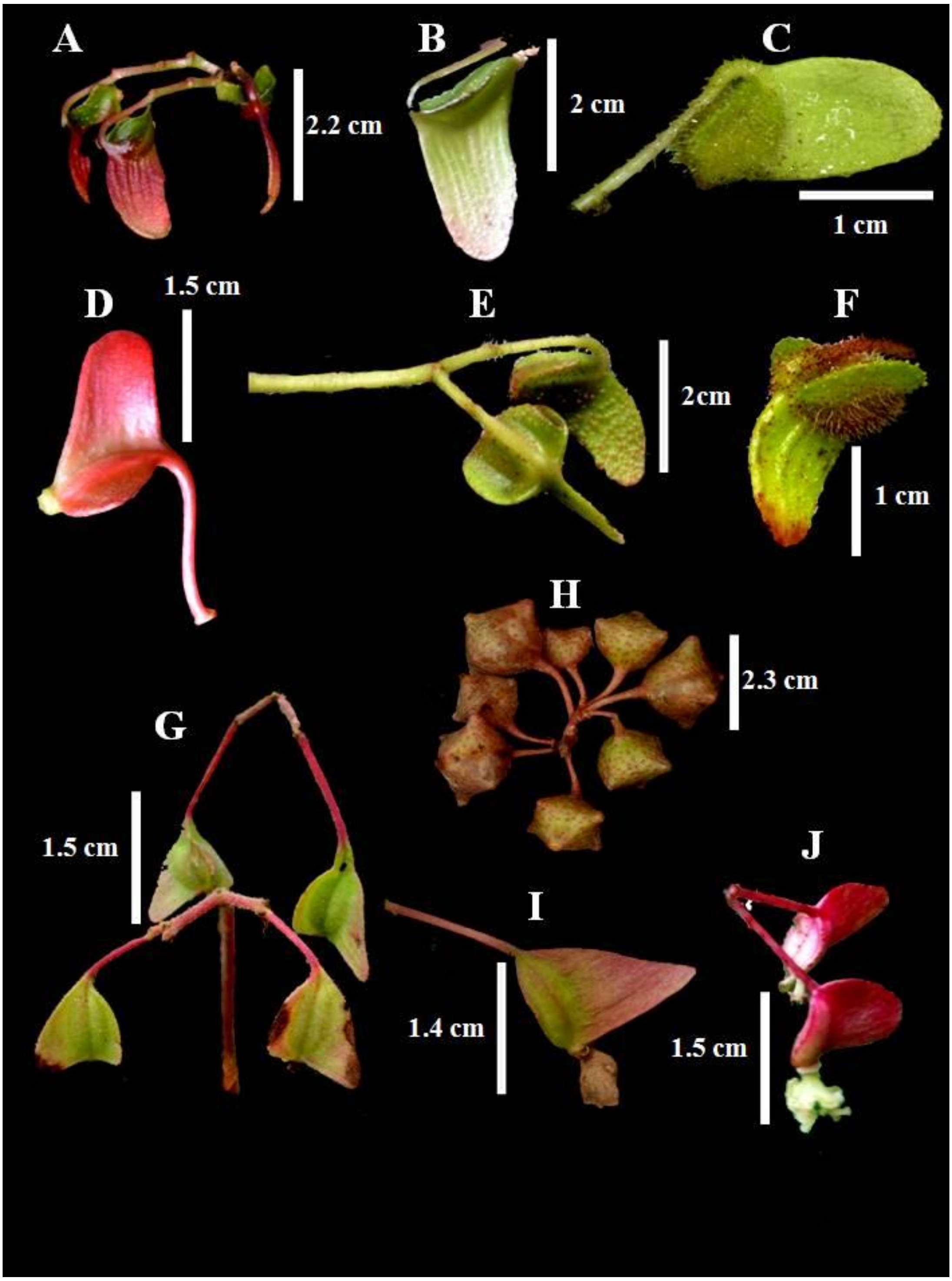

Plate 4 Diversity in fruits; A: **B. xanthina**; B: **B. megaptera**; C: **B. flaviflora**; D: **B. sikkimensis**; E: **B. palmata**; F: **B. cathcartii**; G: **B. satrapis**; H: **B. roxburghii**; I: **B. nepalensis**; J: **B. panchtharensis**

Plate 5 Diversity in tuber and rhizome; A: **B. josephii**; B: **B. satrapis**; C: **B. panchtharensis**; D: **B. nepalensis**; E: **B. palmata**; F: **B. picta**; G: **B. gemmipara**

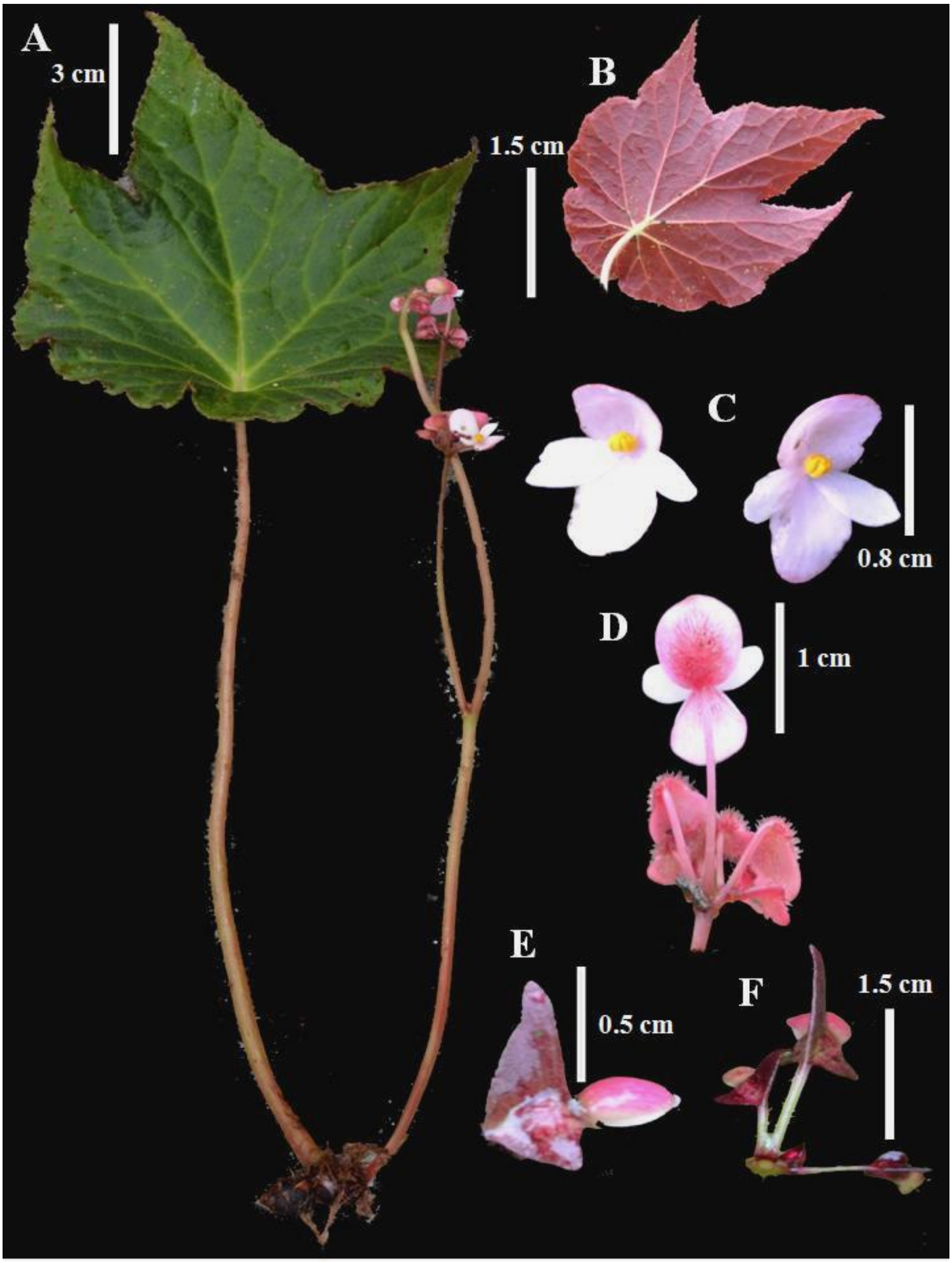

Plate 6 B. josephii A: Habit; B: Dorsal view of leaf; C: Male Flower; D: Dorsal view male flower; E: Female flower bud; F: Dorsal view of female flower bud

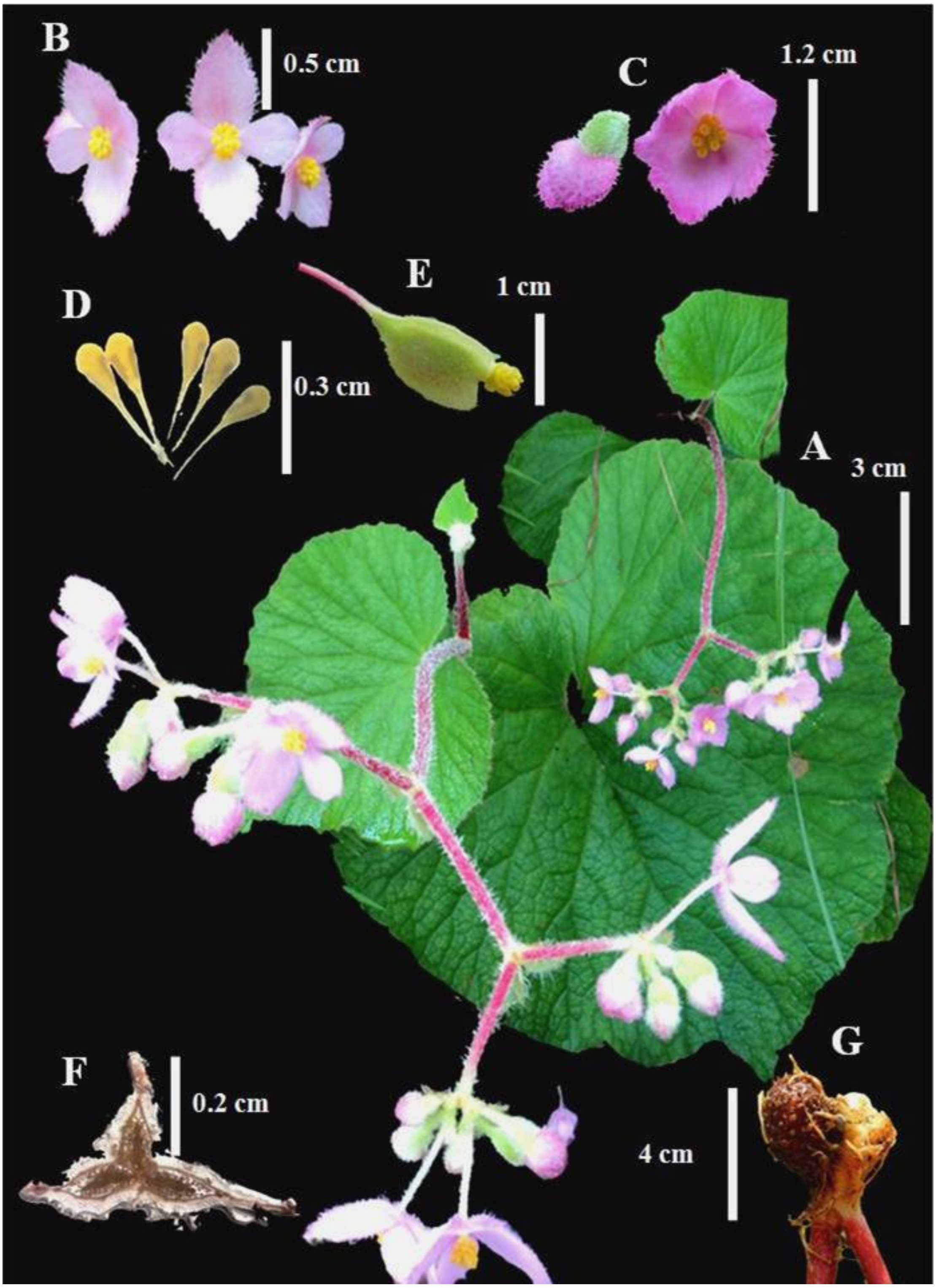

Plate 6 B. satrapis A: Habit; B: Dorsal view of leaf; C: Male Flower; D: Dorsal view male flower; E: Female flower bud; F: Dorsal view of female flower bud

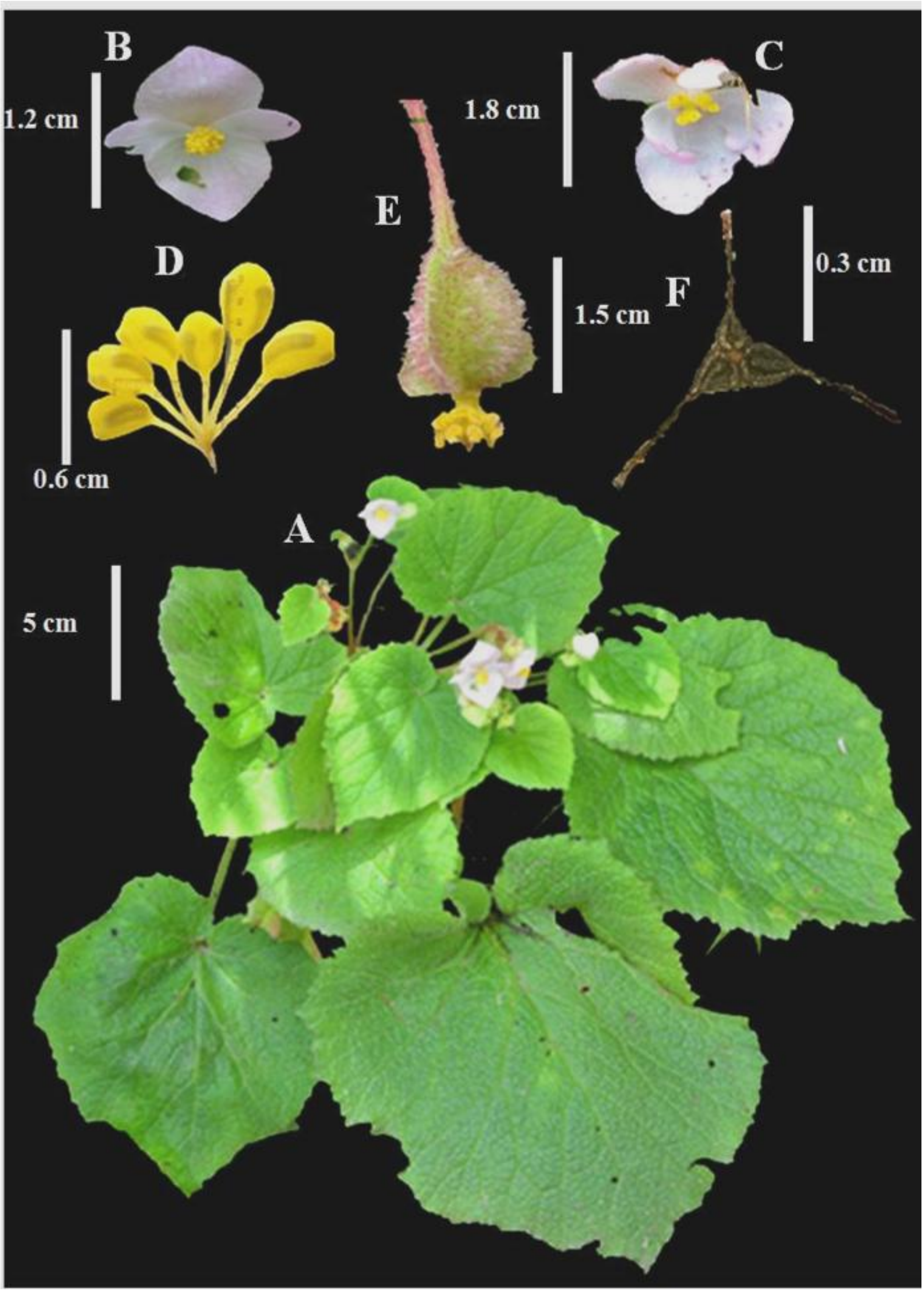

Plate 7 B. picta A: Habit; B: Male Flower; C: Female Flower; D: Stamens; E: Carpel; F: Transverse section of ovary

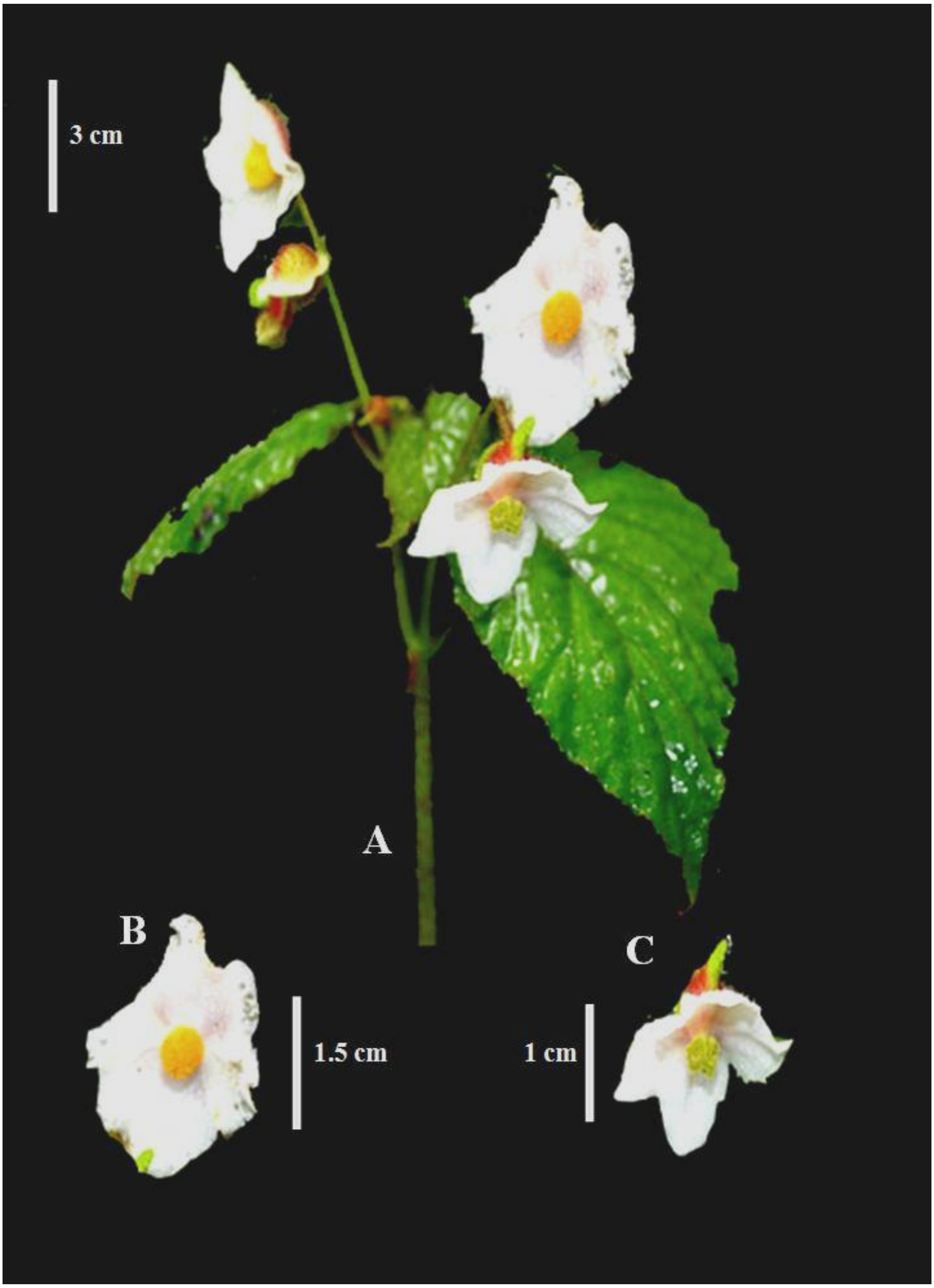

Plate 8 B. catcarthii A: Habit; B: Male Flower; C: Female Flower

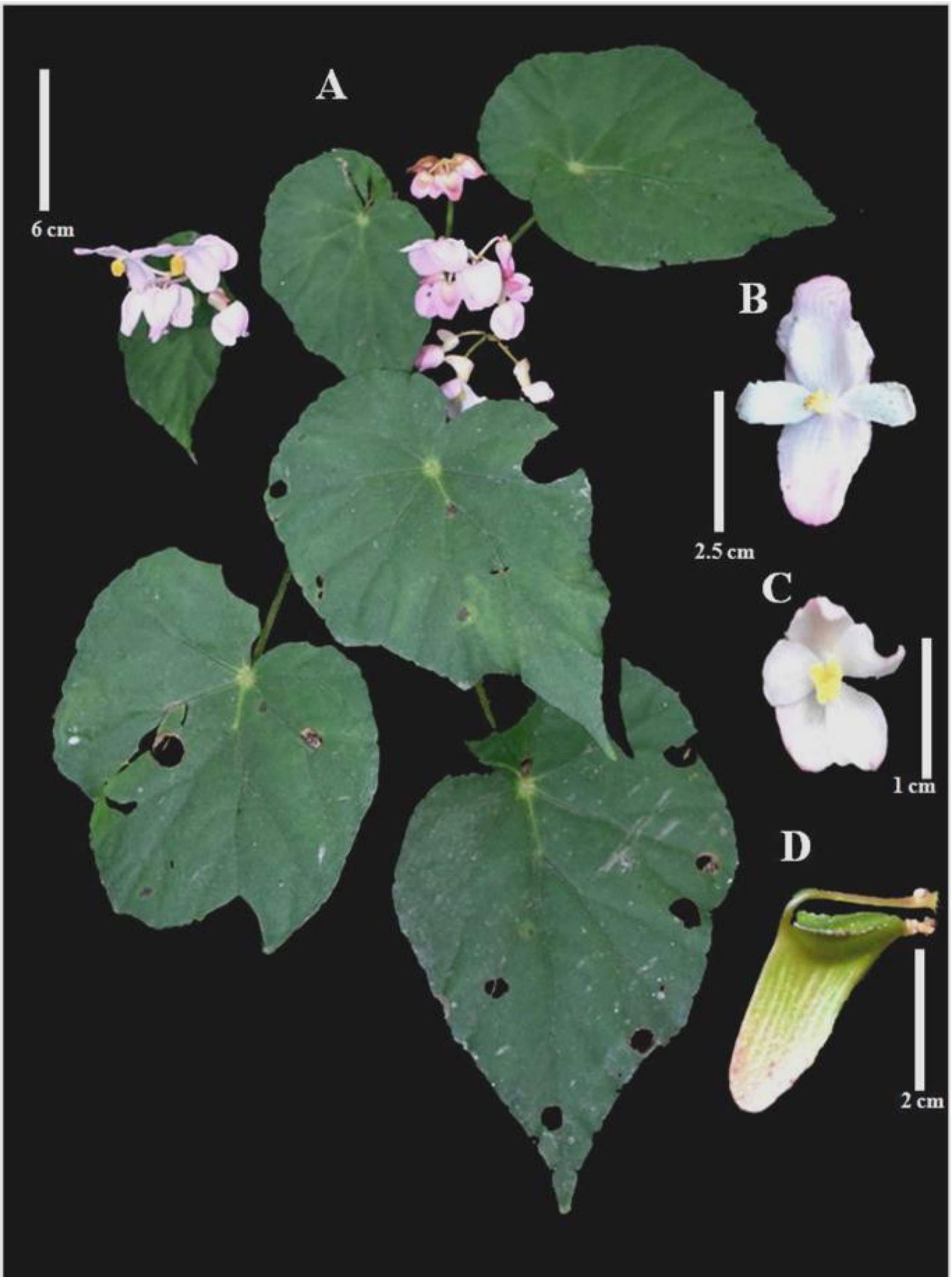

Plate 9 B. megaptera A: Habit; B: Male Flower; C: Female Flower; D: Fruit

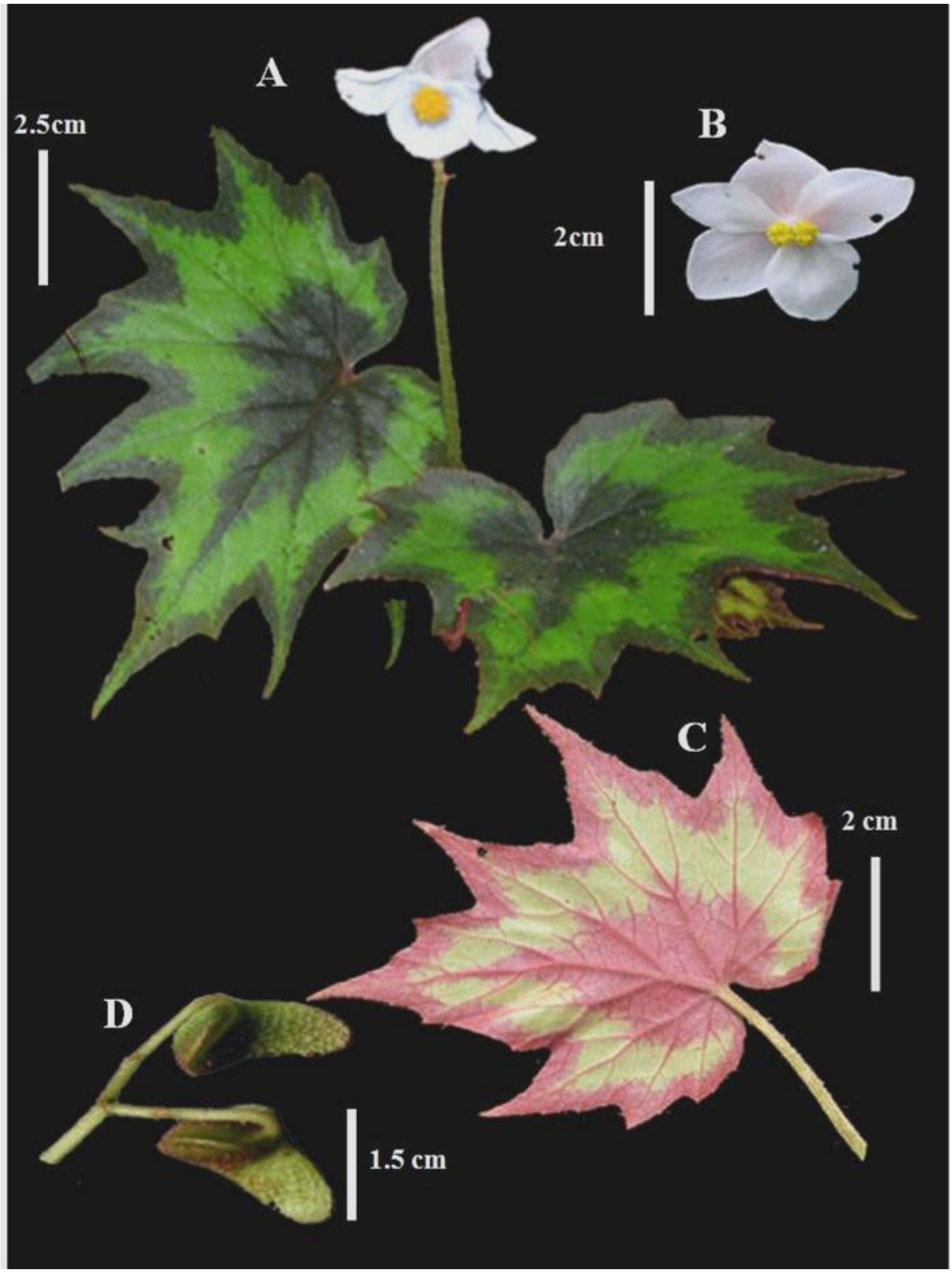

Plate 10 B. palmata A: Habit with Male Flower; B: Female Flower; C: Leaf (Dorsal view); D: Fruit

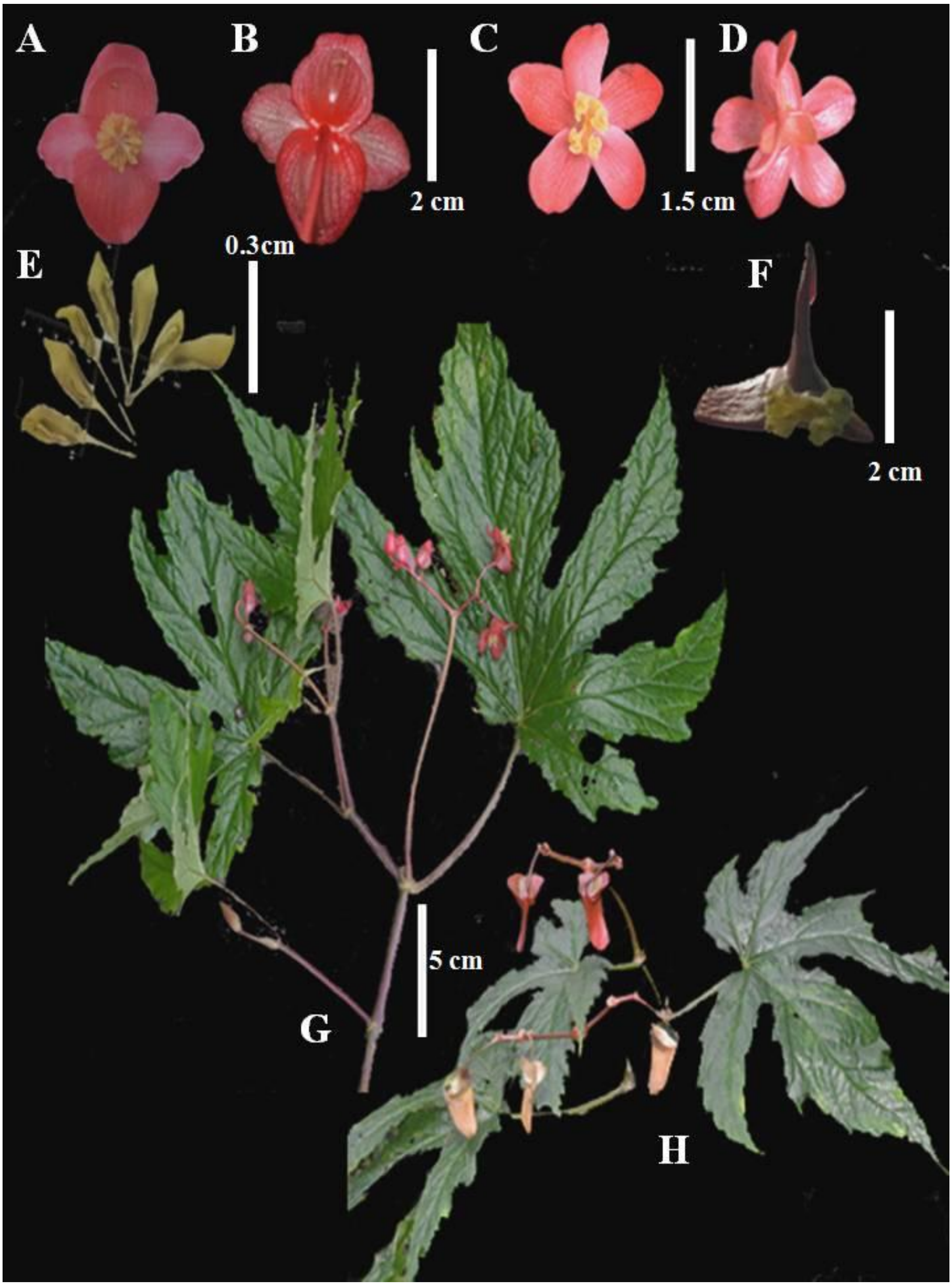

Plate 11 B. sikkimensis A-B: Male Flower (top view-bottom view); C-D: Female Flower (top view-bottom view); E: Stamens; F: Carpel; G: Habit; H: Mature fruit

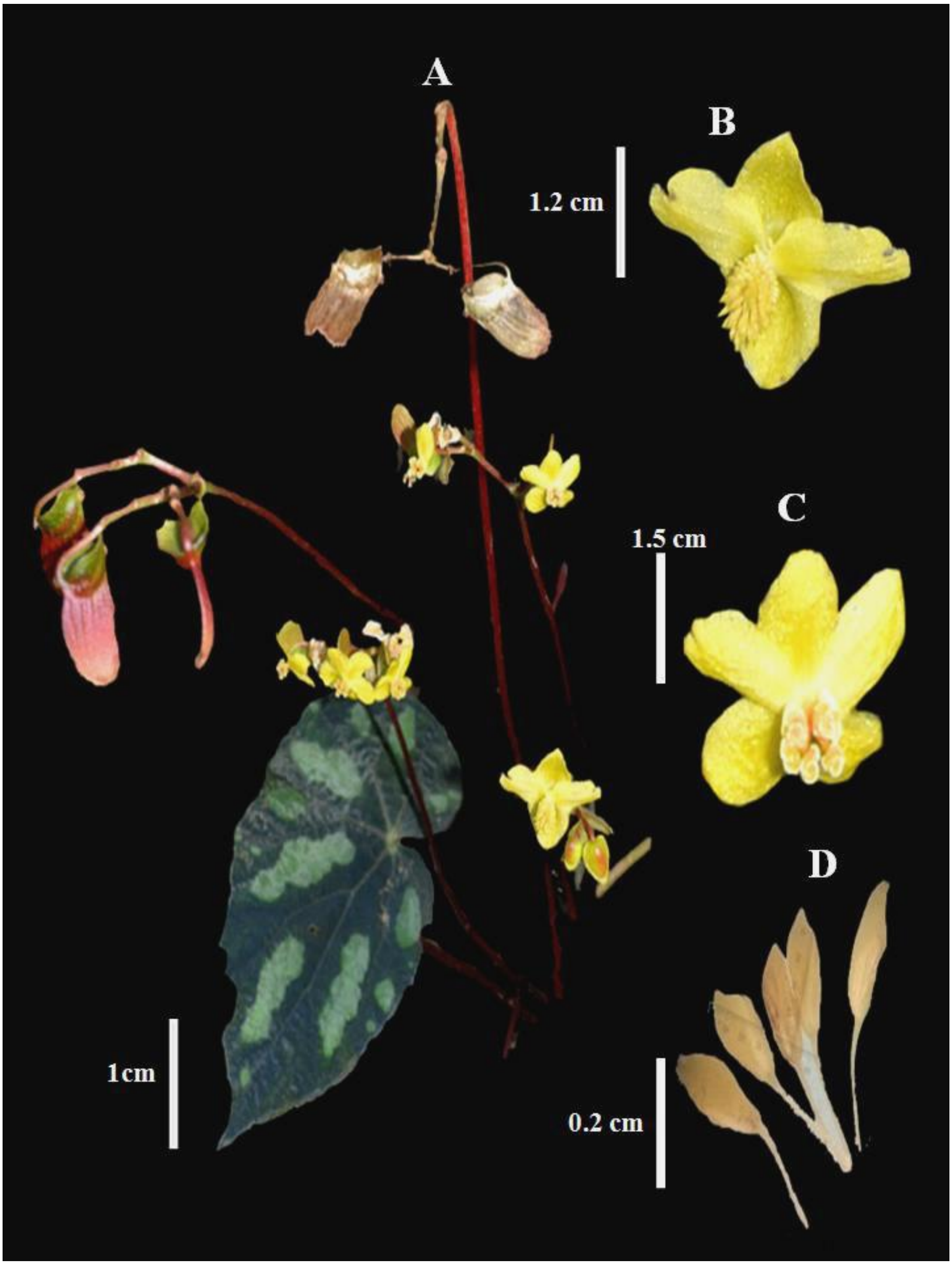

Plate 12 B. xanthina A: Habit; B: Male Flower; C: Female Flower; D: Stamens

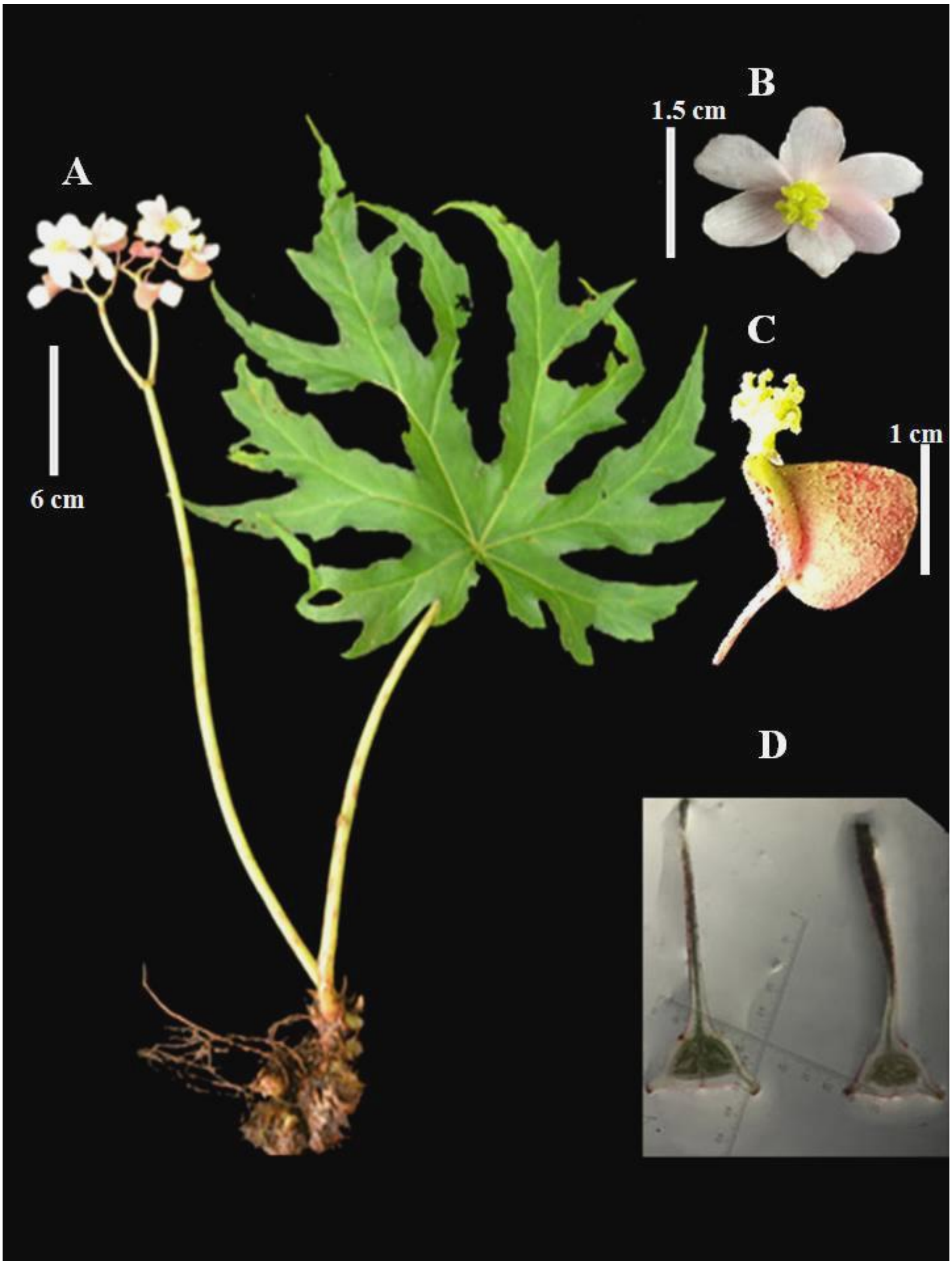

Plate 13 B. panchtharensis A: Habit; B: Female Flower; C: Fruit; D: Transverse section of ovary

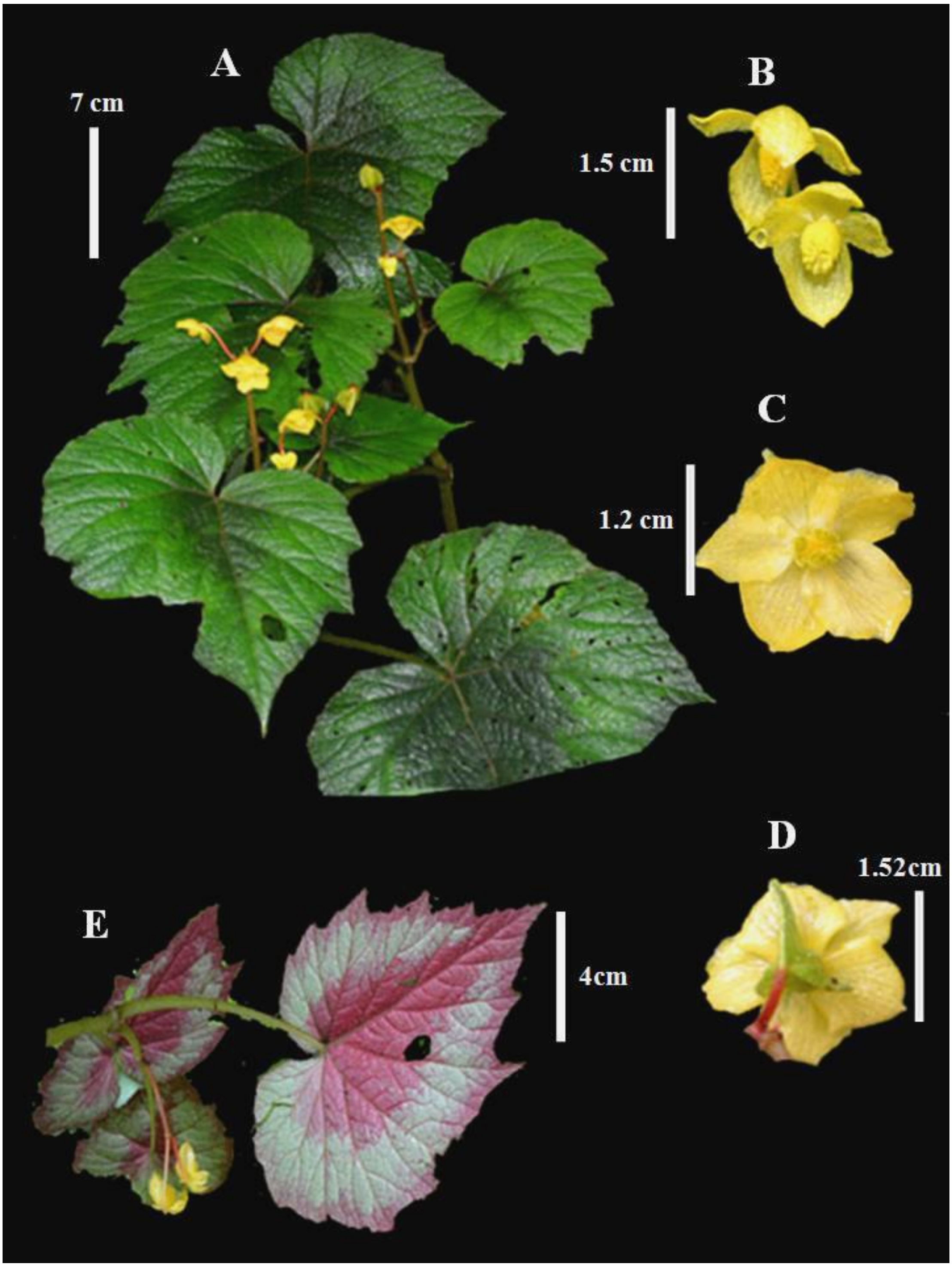

Plate 14 B. flaviflora A: Habit; B: Male Flower; C-D: Female Flower (front view and bottom view); E: Leaves (dorsal view)

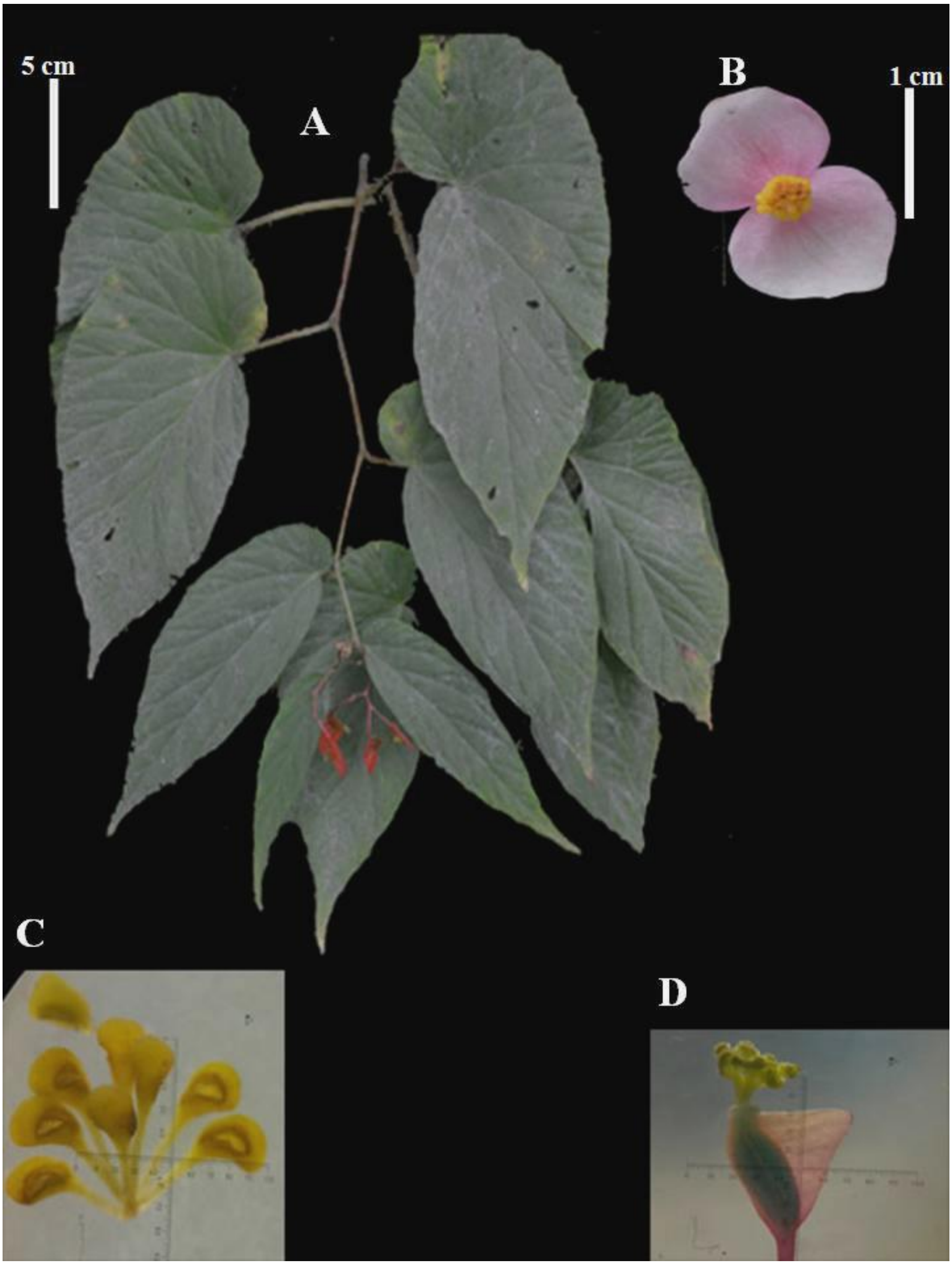

Plate 15 B. nepalensis A: Habit; B: Male Flower; C: Stamens; D: Carpel

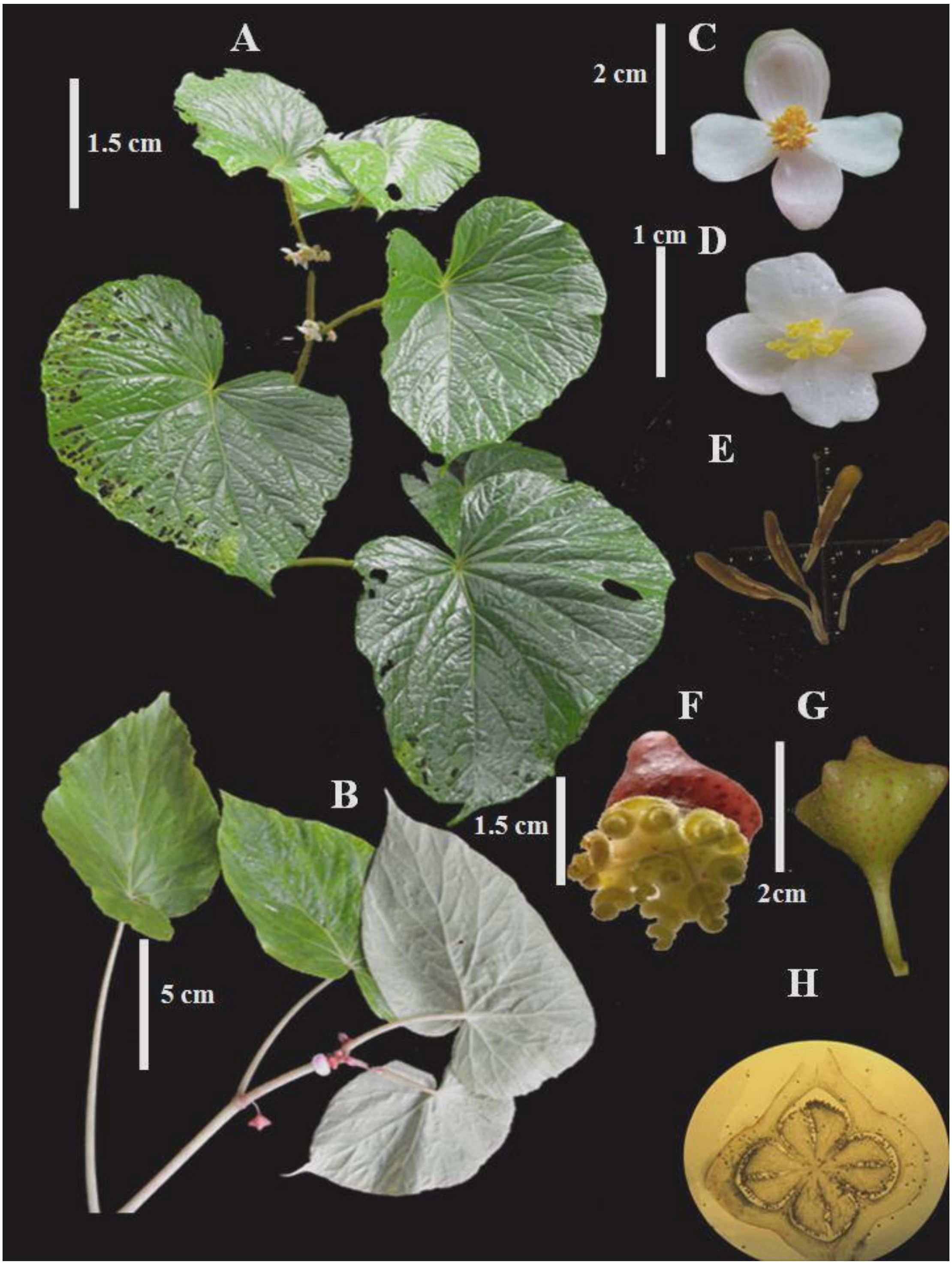

Plate 16 B. roxburghii A: Habit; B: Leaves (dorsal and ventral view); C: Male Flower; D: Female Flower; E: Stamens; F: Carpel; G: Fruit; H: Transverse section of ovary

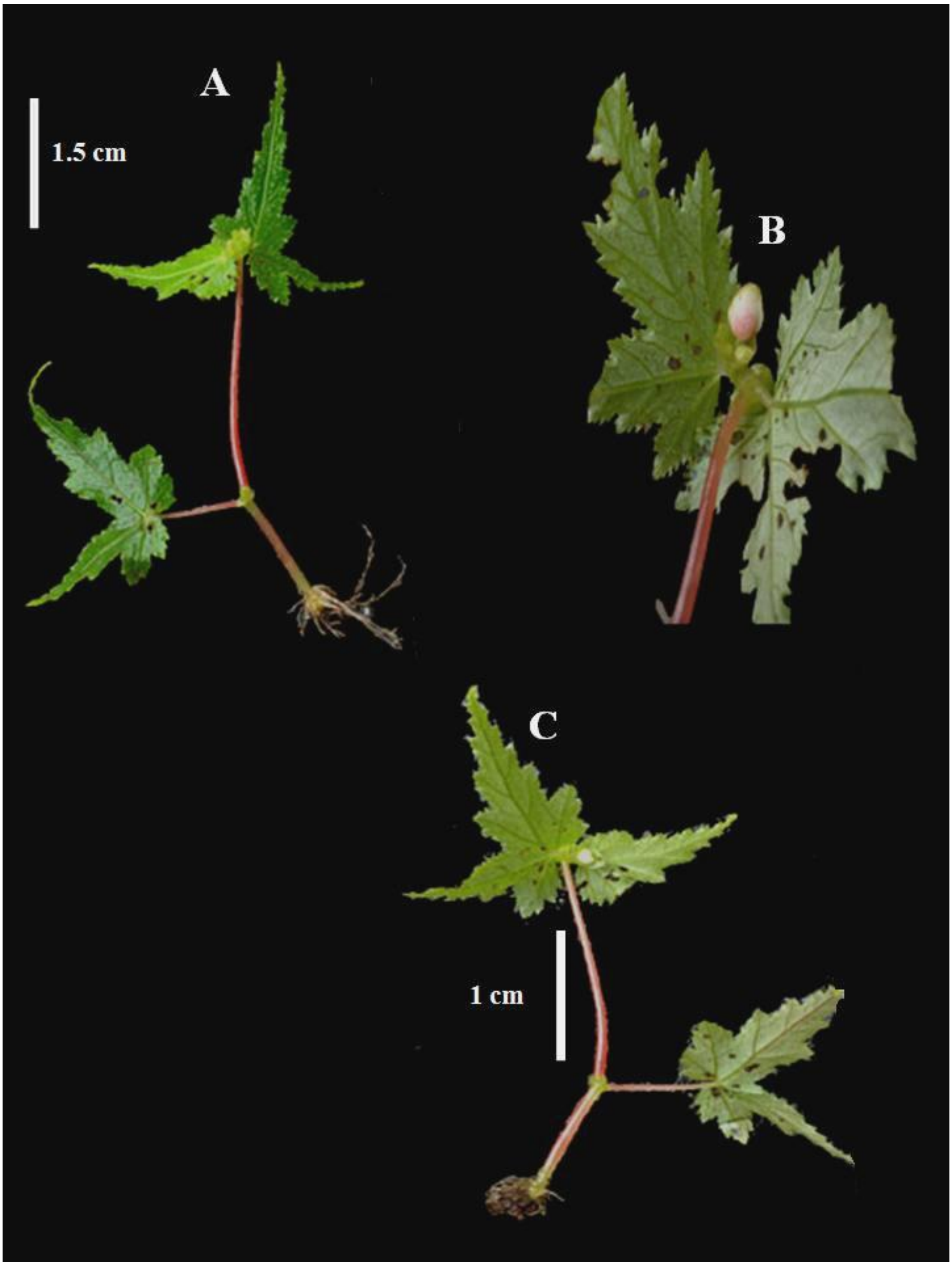

Plate 17 B. gemmipara A: Habit; B: Flower bud; C: Leaf (ventral view)

4.4. Discussion

4.4.1. Taxonomy and Distribution

The Sikkim Himalayan *Begonia* largely comprises two sections *viz*. *Begonia* sect. *Diploclinium* (03 species) and *Begonia* sect. *Platycentrum* (07 species) and one species, each in the *Begonia* sect. *Monopteron*, *Begonia* sect. *Putseyzia* and *Begonia* sect. *Sphenanthera*. Amongst the 13 species of *Begonia* recorded, most of the species are also reported from Northeast India and a few from neighbouring countries such as Nepal, Bhutan, and Bangladesh, with the exception of *B. satrapis* Clarke, which has a narrow distribution range and is strictly endemic to the Sikkim Himalayas. Species such as *B. panchtharensis* and *B. gemmipara* which are endemic to Sikkim Himalaya have also been reported from Nepal. Considering regional distribution, only two species (*B. nepalensis* and *B. satrapis*) are found in the rather drier part of the region. The rest of the species is known to grow abundantly in the temperate forest with abundant moisture content and closed canopy cover. Recent revision on Begonias of Northeast India (excluding Sikkim Himalaya) by Camfield and Hughes (2018) reported 38 species of *Begonia*. Amongst them, 10 species (*viz*. *B. josephii*, *B. picta*, *B. sikkimensis*, *B. megaptera*, *B. nepalensis*, *B. roxburghii*, *B. palmata*, *B. cathcartii*, *B. xanthina* and *B. flaviflora*) are found in the Sikkim Himalaya region and three species endemic to the region *viz*. *B. satrapis*, *B. panchtharensis*, and *B. gemmipara*. Rajbhandari et al. 2010 reported 29 species of *Begonia* from Nepal, out of which 11 species (*viz*. *B. josephii*, *B. picta*, *B. sikkimensis*, *B. panchtharensis*, *B. megaptera*, *B. nepalensis*, *B. roxburghii*, *B. palmata*, *B. gemmipara*, *B. cathcartii*, and *B. flaviflora*) are present in Sikkim Himalaya. Grierson (1991) described 20 species of *Begonia* from Bhutan, out of which 12 species are present in Sikkim Himalaya. Thus in terms of percentage, the Sikkim Himalayan *Begonia* displayed 77 % similarity with the

North-eastern *Begonia*, 85 % similarity with that of Nepalese *Begonia*, and 60 % with the of Bhutanese *Begonia*. The higher similarity in number of species to that of Northeast India, Nepal, and Bhutan may have resulted as Sikkim Himalayas lies in the midst of the Eastern Himalayas and the result is inevitable with the consonant climatic condition, topography, and complex bioclimatic zones from near tropical to alpine meadows. Bangladesh is home to 19 species (Deb and Rahman, 2018) of which four (*viz*. *B. cathcartii*, *B. megaptera*, *B. roxburghii*, *B. palmata*) are found in Sikkim Himalaya with 30 % numerical similarities.

The Sikkim Himalayan *Begonia* is also diverse in terms of both growth form and distribution. Diversity exists not only at the species level but also within populations. The genus is mostly succulent, shade-loving perennial herbs (sometimes soft-wooded shrub), adapted to different microhabitat and is often found associated in rocky slopes along streamlines and waterfalls (Goodall-Copestake 2005; Kiew 2005; Phutthai et al. 2009). Population studies based on morphology and genetic approaches are extremely necessary, particularly for highly diverse species with a wide distribution range and large variation in morphology, including *B. palmata*, *B. satrapis* and *B. picta.*

The vertical distribution of *Begonia* species in Sikkim Himalaya ranges from 400 m to 3000 m asl from tropical to the temperate forest. The bulk of species are found in the sub-tropical belt between1000 m to 1500 m asl (8 species). The *Begonia* sect. *Platycentrum* with a greater number of species has narrow altitudinal range. Likewise, the *Begonia* sect. *Monopteron*, *Begonia* sect. *Putzeyzia* and *Begonia* sect. *Sphenanthera* also has a narrow range of distribution. Nevertheless, the *Begonia* sect. *Diploclinium* which has a smaller number of species has a broader altitudinal distribution showing wider altitudinal tolerance. Thus it can be concluded that the

Begonia sect. *Diploclinium* has a wider altitudinal tolerance compared to the *Begonia* sect. *Platycentrum, Begonia* sect. *Monopteron*, *Begonia* sect. *Putzeyzia* and *Begonia* sect. *Sphenanthera*.

4.4.2. Phenology

The phenology of *Begonia* species belonging to section *Diploclinium* (*viz*. *B. josephii* A.DC., *B. satrapis* Clarke, *B. picta* Smith) shows marginal flowering before monsoon onset (i.e. July) and usually, flowers after 1-2 months of heavy rainfall have accumulated in the soil leading to early spout of a sapling from the tuber and lasted for only 2-3 months, after which the plants die. The species of section *Platycentrum* (*viz*. *B. cathcartii* Hook. f., *B. megaptera* A.DC., *B. palmata* D. Don, *B. sikkimensis* A.DC., *B. xanthina* Hook., *B. panchtharensis* S. Rajh., *B. flaviflora* Hara) had a longer flowering period (Rajbhandary et al. 2010) compared to sect. *Diploclinium*. The *Begonia* sect. *Monopteron* [*B. nepalensis* (A. DC.) Warb.] flowers late in the winter during the drier season. The *Begonia* sect. *Sphenanthera* [*B. roxburghii* (Miq.) A. DC.] and *Begonia* sect. *Putzetsia* (*B. gemmipara*) also flowers late after the monsoon.

4.4.3. Threat

The IUCN (International Union for Conservation of Nature) and CDC (Conservation Data Centre) have devised various methodologies to categorize species under different threat categories. It involves population inventories through field survey and EOO (Extent of occurrence) and AOO (Actual area of occupancy) determination. Based on population data and EOO/AOO, *B. satrapis* is categorized as critically endangered (Adhikari et al. 2018). Species like *B. palmata*, *B. cathcartii*, and *B. megaptera* which is common in occurrence in Sikkim Himalaya and Nepal Himalaya is considered possible extinct/endangered in Bangladesh (Afroz 2008; Deb and

Rahman 2018). However, these species are quite common in Northeast India and Sikkim Himalaya (Camfield and Hughes 2018).

5.1. Introduction

For centuries, ecologist and biologists have facinated on why species vary greatly in the extent of its distribution (Gaston 1960). Some species have a narrow distribution range whilst some closely related species have a broader distribution, ranging from continental to global scale (Wallace 1876; Willis 1922). It is widely believed that narrowly distributed species have specialized environmental requirements while the widly distributed species have broader environmental tolerance. Therefore a positive correlation between environmental niche breadth and range size is widely accepted in macroecological studies (Gaston 2000; Gaston and Spicer 2001; Slatyer et al. 2013). However, it is difficult to conclude the above hypothesis because the environmental niche of a species is usually defined by the set of occurrence records. Hence a larger number of presence locality data are likely to have a wider distribution range, unlike species having a lesser number of occurrence records (Burgman 1989; Gregory and Gaston 2000; Gaston and Blackburn 2000; Gaston and Spicer 2001). Therefore, the species-rich genus *Begonia* in Sikkim Himalaya was chosen as the model plant to anser rthis question.

Begonia L. being the sixth largest genus of flowering plants often provides an opportunity to study the processes underlying the theory of rapid radiation. To develop a robust distribution model and to test the above method theory requires a sufficient amount of occurrence data (Moonlight 2017). However, the unavailability of geo-referenced occurrence data in herbaria and other online sources such as GBIF (Global Biodiversity Information Facility) (www.gbif.org) limits the use of such techniques. At present, there is a growing need to estimate the species distribution range for theoretical as well as applied reasons e.g. understanding species geography to its conservation. However, limited species occurrence data poses enormous challenges to the researchers. Moreover, quantifying the environmental factors which contribute the most in the distribution of species becomes even more complicated and challenging (Guisan and Thuiller 2005; Colwell and Rangel 2009). The factors that govern the distribution of species are biotic factors, abiotic factors (soil and topography), species interaction, competition, predators, and parasites (Gaston 2003). In practice, the species distribution model is developed using only the occurence data and abiotic variables. Recently several studies have indicated the importance of biotic interaction in shaping the spatial distribution of species (Gotelli et al. 2010; Sunday et al. 2011). The factors such as biotic interaction and dispersal are usually ignored and their effect is usually considered negligible at broader geographical scale or spatial scales (Soberon 2007; Colwell and Rangel 2009; Gotzenberger et al. 2012; Araújo et al. 2014). Thus, abiotic factors, such as bioclimatic variables, NDVI, slope, aspect, etc are often used in predicting and identifying the suitable habitat of species (Pradhan and Chettri 2017; Chettri et al. 2018; Pradhan et al. 2020). The selection of predictor variables is fundamental before modeling, yet the choice of input variables is still debatable (Synes and Osborne 2011). The ecologically relevant variables are capable of generating robust model and vice versa. For example, the soil type variables might be good predictor variables for plants whilst temperature and forest fragmentation related variables might be a good choice for animals (Bradley et al. 2012). The use of NDVI contributes to the modeling process by providing information about the phenological status, canopy cover, and the water content variation (Amaral et al. 2007). Thus this study aimed to (1) predict and indentify the suitable habitat of B. satrapis for insitu conservation (2) define the ecological niche of *Begonia* species and quantify the similarities between them using ENM techniques. The ENMs constructed were compared to assess the similarities of the ecological niche of the *Begonia* species and to know if they share the same ecological niche or not.

5.2. Materials and Methods

5.2.1. Collection of occurrence record

The primary occurrence data or the presence data (i.e. geographic coordinate/Latitude and Longitude) for 13 species of *Begonia* (*viz*. *Begonia satrapis, Begonia gemmipara*, *Begonia josephii*, *Begonia picta*, *Begonia xanthina*, *Begonia cathcartii, Begonia flaviflora, Begonia megaptera, Begonia nepalensis, Begonia palmata*, *Begonia sikkimensis, Begonia panchtharensis*, *Begonia roxburghii*) were collected from the hills of Darjeeling and Sikkim Himalaya using Garmin GPS (Global Positioning System). The occurrence data were collected with an accuracy of 3-10 m.

The geographic coordinate was collected in the form of Degree Minute Second (DMS) which was later converted to decimal degrees (DD) using the formula:

$$\mathbf{DD = D + M/60 + S/3600}$$

Example: DD = 88º, M = 36', S = 14.6"

$$DD = 88 + 36/60 + 14.6/3600$$

$$= 88.60406$$

The converted presence data was later rearranged in Microsoft excels in the following order i.e. species name, longitude, latitude and then saved in CSV (comma delimited) format and was later used for modeling.

3.2.2. Predictor Variables

Two sets of the model were developed for each species of *Begonia*. The first model was developed using bioclimatic variables obtained from the worldclim database (https://www.worldclim.org/) at 30 arc-second resolution grid (~ km^2 resolution), which is the highest resolution available. The desired environmental layer was first clipped from the parent layer using ArcView ver. 3.x. software.

The 19 bioclimatic variables were then subjected to a correlation test (r>0.9) using ENM Tools 1.3 software (Warren et al. 2010) (Table 18). Therefore out of 19 bioclimatic variables, three temperature-related variables (i.e. BIO, BIO2, and BIO3) and two precipitation related variables (i.e. BIO15 and BIO19) (in bold) were used for modeling the distribution of 13 species of *Begonia* in Sikkim Himalaya (Table 17).

The second model was developed using NDVI (Normalized Difference Vegetative Index) raster data for January to December obtained from GLCF (Global Land Cover Facility) (University of Maryland, USA).

The Normalized Difference Vegetation Index (NDVI) is a numerical indicator that quantifies vegetation by measuring the difference between near-infrared (which vegetation strongly reflects) and red light (which vegetation absorbs) and is given by the formula:

NDVI= (NIR-RED) / (NIR+RED)

Where; NIR= near-infrared and

RED=Red light

The 12 NDVI variables were first subjected to correlated tests (r>0.9) using ENM Tools 1.3 software (Warren et al. 2010) (Table 19). Thus out of 12 NDVI variables, 10 were used to model the distribution of *Begonia* in Sikkim Himalaya along with altitude.

Table 17: List of 19 bioclimatic variables used for modeling the distribution of *Begonia* in Sikkim Himalaya

Sl. no.		**Bioclimatic variables**	**Units**
1	**BIO1**	**Annual Mean Temperature**	**˚C**
2	**BIO2**	**Mean Diurnal Range [Mean of monthly (max temp - min temp)]**	**˚C**
3	**BIO3**	**Isothermality (Bio2/Bio7) (* 100)**	**˚C**
4	BIO4	Temperature Seasonality (standard deviation *100)	˚C
5	BIO5	Max Temperature of Warmest Month	˚C
6	BIO6	Min Temperature of Coldest Month	˚C
7	BIO7	Temperature Annual Range (Bio5-Bio6)	˚C
8	BIO8	Mean Temperature of Wettest Quarter	˚C
9	BIO9	Mean Temperature of Driest Quarter	˚C
10	BIO10	Mean Temperature of Warmest Quarter	˚C
11	BIO11	Mean Temperature of Coldest Quarter	˚C
12	BIO12	Annual Precipitation	mm
13	BIO13	Precipitation of Wettest Month	mm
14	BIO14	Precipitation of Driest Month	mm
15	**BIO15**	**Precipitation Seasonality (Coefficient of Variation)**	**mm**
16	BIO16	Precipitation of Wettest Quarter	mm
17	BIO17	Precipitation of Driest Quarter	mm
18	BIO18	Precipitation of Warmest Quarter	mm
19	**BIO19**	**Precipitation of Coldest Quarter**	**mm**

Note: Bold variables are used for modeling

3.2.3. Ecological Niche Modelling

MaxEnt v.3.3. 3k Software (Phillips and Dudik 2008) was used to model the distribution of *Begonia* species in Sikkim Himalaya. MaxEnt modeling was used because it has a high accuracy rate and performs better with small size (Elith et al. 2006; Pearson et al. 2007; Kumar and Stohlgren 2009). The 10 percentile training presence logistic threshold was used, with 20 replicate run and maximum of 5000 iterations for each species. All other settings were kept default as it has been calibrated with a wide range of species (Fig. 9) (Phillips and Dudik 2008). From 20 replicated runs for each species, the average, and maximum, minimum, median, and standard deviation was obtained. Each *Begonia* species were modelled individually using a set of both bioclimatic and NDVI variables. Further, for *B. satrapis* the potential area of distribution and/or reintroduction was categorized into five classes i.e. very-high (0.77-1), high (0.57-0.77), medium (0.38-0.57), low (0.19-0.38) and very low (0-0.19). The complete process from obtaining data and modeling is given in the flow diagram (Fig. 11)

Using Niche Toolbox (http://shiny.conabio.gob.mx:3838/nichetoolb2/) binary maps were obtained and suitable areas for each species of *Begonia* were calculated using 10 percentile training presence logistic threshold cut off values.

3.2.4. Assessment of habitat status

The assessment of the actual habitat status of species in the area identified through ENM was carried out by superimposing the predicted potential areas on Google Earth Ver. 6 (www.google.com/earth) imageries for habitat quality assessment. The final predicted MaxEnt suitability maps were exported using DIVA-GIS ver. 7.5.0 software in KMZ format. KMZ (Keyhole Markup Language) is an extension file used by Google earth for the placemark file. The exported KMZ files were overlaid on Google Earth to determine the actual habitat type prevalent in the areas of occurrence (Fig. 10) (Adhikari et al. 2011).

3.2.5. Population status via model threshold

To examine the robustness of the final predicted model, the population status of *B. satrapis* was determined by taking into account all the individuals of species in a 250 × 250 m grid at different probability threshold values. The population data were then correlated with each model threshold values to find out whether the area covering the very high and high suitability thresholds of the model maintain higher populations or not and vice-versa.

3.2.6. Threat assessment using IUCN categories and criteria version 3.1

B. satrapis Clarke was assigned to a Red List Category following the IUCN criteria (IUCN 2012). The area of occupancy (AOO) and extent of occurrence (EOO) was calculated using ArcView GIS 3.x, version 1.2. (Moat 2007) and geographic coordinates collected from the field.

3.2.7. Niche Overlap

ENM Tools software was used to examine the degree of niche overlap between *Begonia* species. Schoener's D (Schoener 1968) and Hellinger's I metrics were used to estimate the niche overlap between the species.

Schoener's D is given by the formula:

$$D(p_X, p_Y) = 1\frac{1}{2}\sum_i \left|p_{X,i} - p_{Y,i}\right|,$$

Where $p_{X,i}$ and $p_{Y,i}$ are the normalized suitability scores for species X and Y in grid cell *i*, similarly Hellinger's I is given by the formula:

$$I\left(p_X, p_Y\right) = 1 - \frac{1}{2}\sqrt{\sum_i \left(\sqrt{p_{X,i}} - \sqrt{p_{Y,i}}\right)}$$

The niche similarity measures are obtained after comparing the predicted suitable habitat calculated for each grid cell from a model developed through MaxEnt. The niche overlap values ranges vary from 0-1. The value 0 indicates no overlap and the value of 1 indicates a complete overlap of niches. If only two ENM outputs of two species are loaded in ENM Tools, single values of *D* and *I* will be produced and if more than two populations of different species are loaded pair wise *D* and *I* values will be produced in simple Microsoft excel file (Warren et al. 2008; 2010).

3.2.8. Niche Breadth

Niche breadth was also assessed using the same set of output predicted distribution models for each species (Phillips et al. 2006; Warren et al. 2010). Niche breadth is calculated by estimating the inverse concentration metric developed by Levins (1968).

3.2.9. Model evaluation and Performance

The model developed for each species was classified and evaluated based on "area under the curve" or AUC values. The model was further graded as: poor (AUC < 0.8), fair (0.8 < AUC < 0.9), good (0.9 < AUC < 0.95) and very good (0.95 < AUC < 1.0) following Thuiller et al. 2005. The model was also evaluated based on the partial AUC metric using Niche Toolbox (http://shiny.conabio.gob.mx:3838/nichetoolb2/). The evaluation was based on 500 bootstrap iterations with 5 % omission which gives a curve for AUC_{random} and AUC_{actual} (Lobo et al. 2008; Peterson et al. 2008).

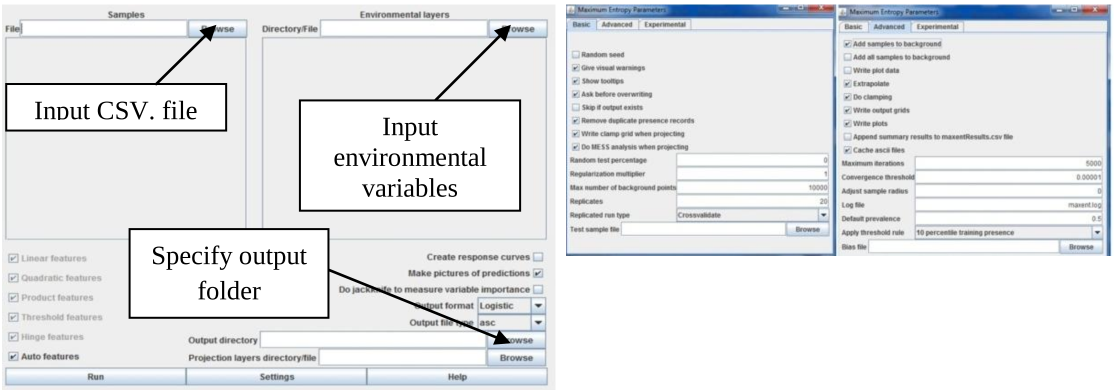

Fig. 9: Modeling process using MaxEnt

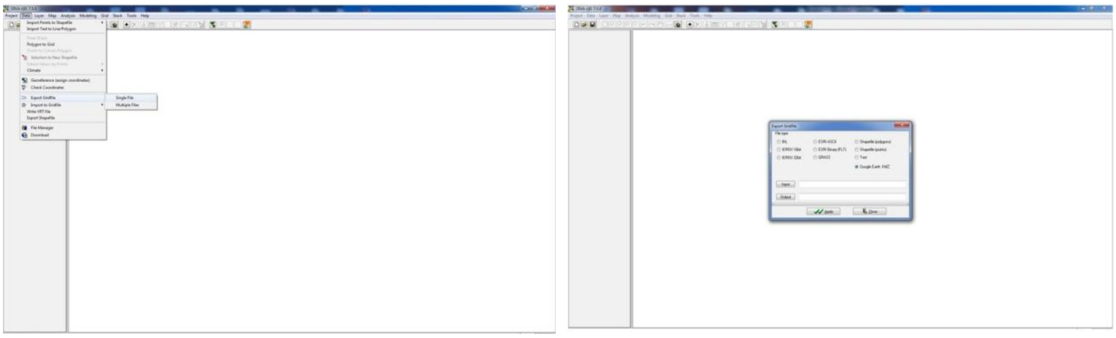

Fig. 10: Process of exporting ENM output file using DIVA-GIS

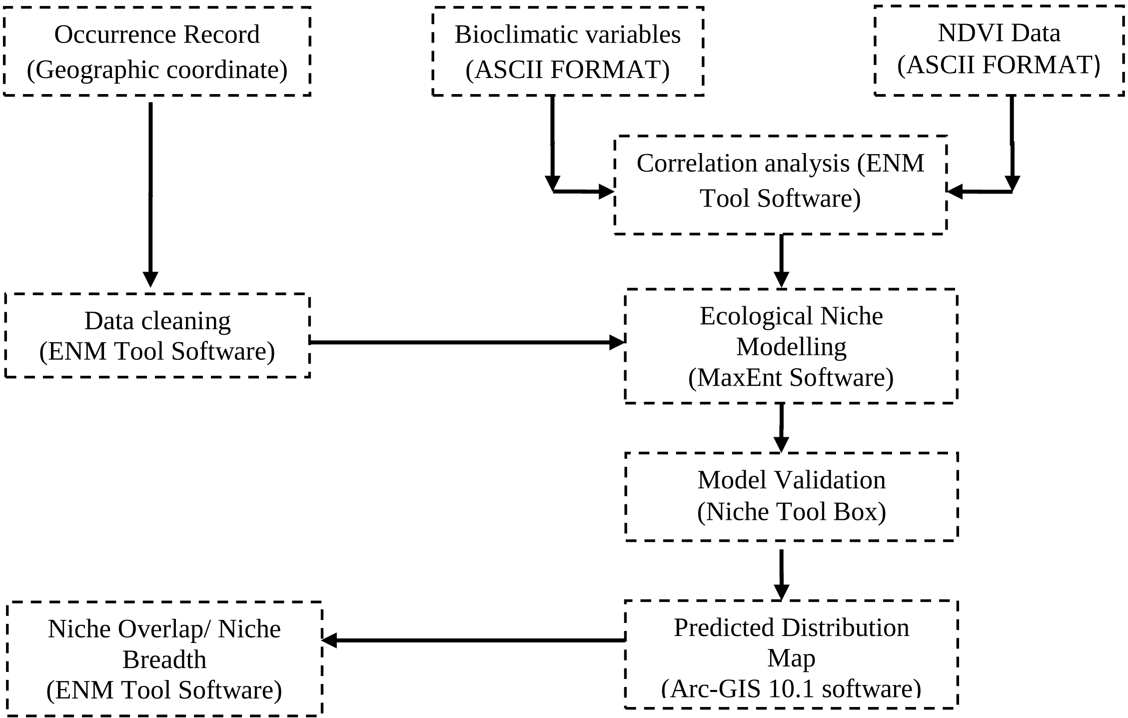

Fig. 11: Diagrammatic/Flow Chart representation of Ecological Niche Modeling

Table 18: Correlation analysis for the 19 bioclimatic variables to check multicollinearity using ENM Tools 1.3 (Warren et al. 2010)

	bio1	bio2	bio3	bio4	bio5	bio6	bio7	bio8	bio9	bio10	bio11	bio12	bio13	bio14	bio15	bio16	bio17	bio18
bio19	0.47	-0.74	-0.07	-0.68	0.40	0.56	-0.75	0.46	0.50	0.45	0.50	0.48	0.44	**0.92**	-0.66	0.44	**0.97**	0.45
bio1		-0.74	0.26	**-0.92**	**0.99**	**0.99**	-0.81	**1.00**	**1.00**	**1.00**	**1.00**	**0.96**	**0.95**	0.27	-0.07	**0.95**	0.58	**0.95**
bio2			0.27	**0.93**	-0.65	-0.83	0.99	-0.72	-0.77	-0.71	-0.77	-0.82	-0.81	-0.61	0.15	-0.81	-0.85	-0.82
bio3				-0.05	0.34	0.18	0.14	0.28	0.24	0.29	0.25	0.15	0.14	-0.17	-0.09	0.15	-0.09	0.14
bio4					-0.86	-0.96	0.97	-0.90	-0.93	-0.90	-0.93	-0.94	-0.93	-0.50	0.14	-0.93	-0.79	-0.94
bio5						**0.97**	-0.72	**1.00**	**0.99**	**1.00**	**0.99**	**0.92**	**0.91**	0.19	-0.05	**0.91**	0.50	0.91
bio6							-0.88	**0.98**	**0.99**	**0.98**	**0.99**	**0.96**	**0.95**	0.36	-0.10	**0.95**	0.67	0.96
bio7								-0.78	-0.83	-0.78	-0.83	-0.86	-0.85	-0.60	0.18	-0.85	-0.86	-0.86
bio8									**1.00**	**1.00**	**1.00**	**0.95**	**0.94**	0.24	-0.07	**0.94**	0.56	**0.94**
bio9										**1.00**	**1.00**	**0.96**	**0.95**	0.29	-0.07	**0.95**	0.61	**0.95**
bio10											**1.00**	**0.95**	**0.94**	0.24	-0.06	**0.94**	0.55	**0.94**
bio11												**0.96**	**0.95**	0.29	-0.07	**0.95**	0.61	**0.95**
bio12													**1.00**	0.27	0.03	**1.00**	0.61	**1.00**
bio13														0.23	0.10	**1.00**	0.58	**1.00**
bio14															-0.60	0.23	0.88	0.24
bio15																0.08	-0.50	0.07
bio16																	0.58	**1.00**
bio17																		0.60

Table 19: Correlation analysis for the 12 NDVI layers to check multicollinearity using ENM Tools 1.3 (Warren et al. 2010)

Predictor variables	**eu1 (Jan)**	**eu2 (Feb)**	**eu3 (Mar)**	**eu4 (Apr)**	**eu5 (May)**	**eu6 (Jun)**	**eu7 (Jul)**	**eu8 (Aug)**	**eu9 (Sep)**	**eu10 (Oct)**	**eu11 (Nov)**	**eu12 (Dec)**
Alt	-0.56	-0.77	-0.24	-0.53	-0.57	-0.16	-0.76	-0.75	-0.61	-0.10	-0.53	-0.51
eu1 (Jan)		0.57	0.15	0.45	0.35	0.07	0.58	0.61	0.60	0.13	0.46	0.45
eu2 (Feb)			0.12	0.54	0.60	0.22	0.62	0.58	0.46	0.23	0.54	0.54
eu3 (Mar)				0.03	-0.01	-0.16	0.18	0.22	0.22	-0.15	0.09	0.04
eu4 (Apr)					0.67	0.56	0.69	0.67	0.60	0.59	0.69	0.75
eu5 (May)						0.47	0.58	0.54	0.43	0.45	0.63	0.65
eu6 (Jun)							0.39	0.35	0.29	0.73	0.51	0.59
eu7 (Jul)								**0.96**	0.88	0.37	0.75	0.71
eu8 (Aug)									**0.92**	0.35	0.74	0.69
eu9 (Sep)										0.33	0.71	0.63
eu10 (Oct)											0.56	0.72
eu11 (Nov)												0.75

Note: eu1-eu12 are the NDVI variables for the month of January to December

5.3. Results

5.3.1. Studied species and occurrence records

B. satrapis is a rare plant species endemic to Sikkim and Darjeeling district of West Bengal, India. The species with broad basal leaf and the pink colour flower is of horticultural importance. To model the distribution of *B. satrapis* a total of 12 occurrence records were collected from the field.

5.3.2. Ecological Niche Modeling

Potential habitat with very high suitability thresholds was distributed in the lower elevations of Darjeeling district and South district of Sikkim along river Rungit up to the confluence of Rungit and Teesta River (Fig. 16). The primary field survey revealed the predicted potential habitat was mostly located in the open, degraded tropical moist deciduous forest dominated by tree species like *Shorea robusta* and *Schima wallichii* with sparse tree cover, human settlement, cultivated land and roads along the bank of river Teesta. The high suitability area predicted towards Darjeeling district is mostly under tea plantations (*viz*. Badamtam Tea Garden, Kambal Tea Garden, Singla Tea Garden, and Peshok Tea Garden) which do not serve as a site for species reintroduction. Areas with medium habitat suitability are those with dense forest cover with less human settlement. A total potential area of 754 km^2 was predicted to be suitable for *B. satrapis* in Sikkim Himalaya. Most of the area falls under a low suitability class and covers an area of about 432 km^2. The area with very high suitability was very much restricted to about 69 km^2. The area with high and medium suitability was restricted to 93 km^2 and 160 km^2 respectively (Fig 15).

5.3.3. Predictor variables and model evaluation

The model calibration test for *B. satrapis* yielded satisfactory results (AUC_{train} = 0.9929 ± 0.0029 and AUC_{test} = 0.9619 ± 0.084) (Fig. 12). Amongst the input environmental variables, NDVI for May was the most influential and contributed 40.4% to the MaxEnt model. The rest of the remaining layers collectively contributed 59.6% to the habitat model of the species (Fig. 13 and 14 and Table 20). Considering the permutation importance, NDVI for May also had the maximum influence on the habitat model and contributed to 18.7%, while rest together contributed to 81.3% (Table 20).

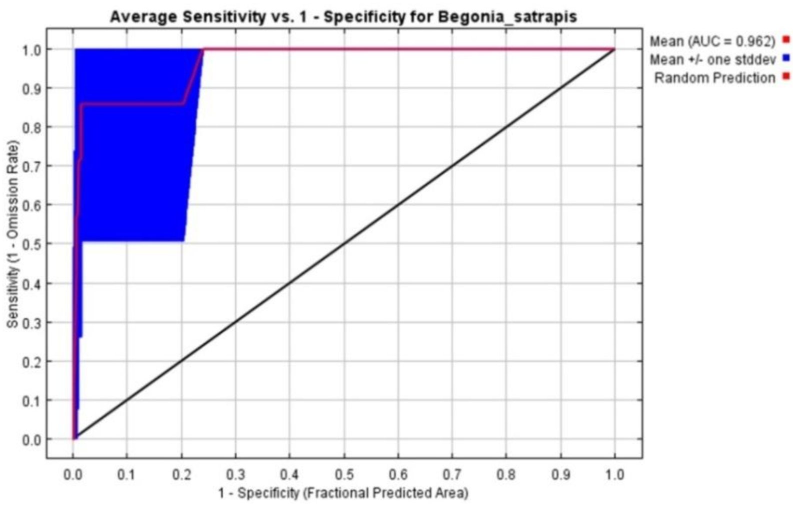

Fig. 12: AUC curve for model accuracy

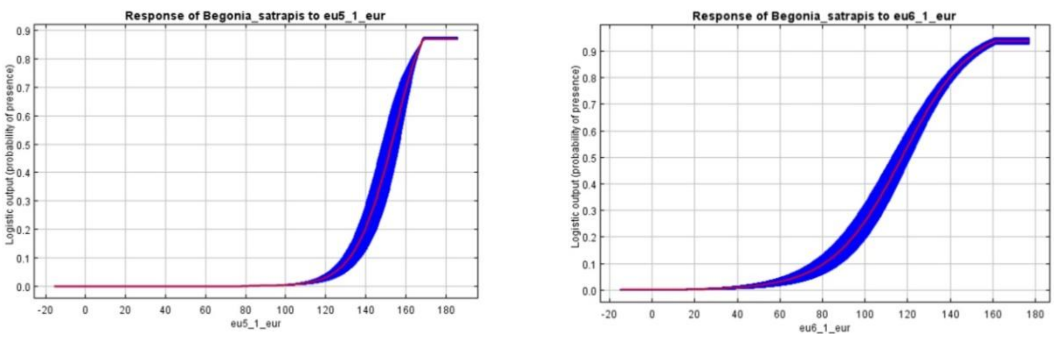

Fig. 13: Response curves revealing the influence of individual variables on the predicted probability of presence. The curves show the mean response of the 10 replicate MaxEnt runs (red) and the mean ± 1 standard deviation.

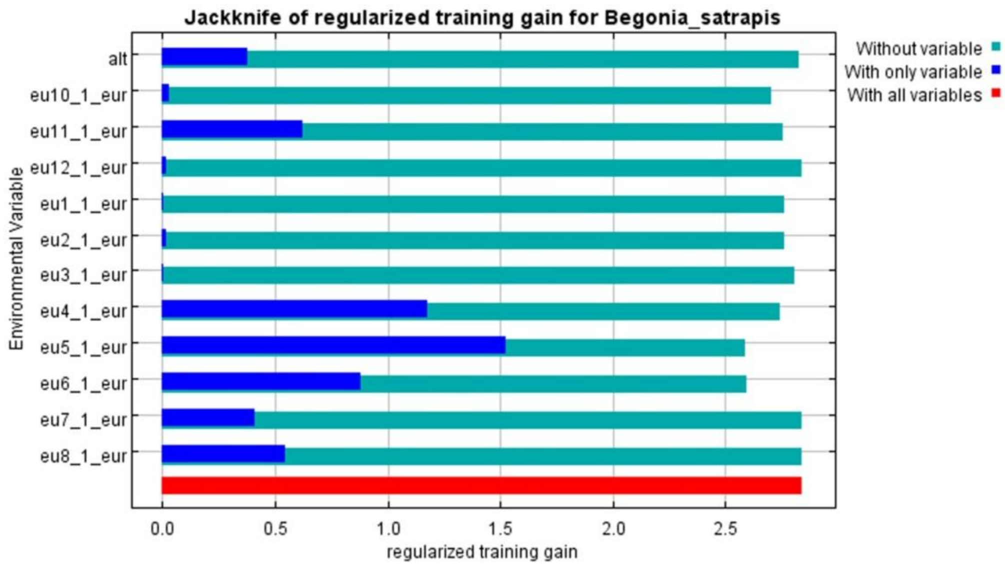

Fig. 14: Results of jackknife test for *B. satrapis*

Table 20: Estimates of relative contributions and permutation importance of the predictor environmental variables to the MaxEnt model

Variable	Percent contribution	Permutation importance
eu12	0	0.3
eu8	0	0
eu7	0	0
eu3	0.5	3
eu11	3.3	13.5
eu2	4.3	4.1
eu1	6.4	15.7
Alt	7.2	4.5
eu10	10.1	15.3
eu4	12.1	15.2
eu6	15.7	9.7
eu5	40.4	18.7

Note: eu1_1_eur – eu12_1_eur are NDVI from January – December

5.3.4. Population assessment

A total of 1948 individuals were inventoried within the area of occurrence spread over seven 1km × 1km grids. Of these 505 were mature flowering individuals, 600 sapling, and 890 seedlings. The analysis of population structure at each locality revealed that the highest number of individuals were in Sumbuk (974 and 423 individuals), followed by Kitam (208 individuals), Badamtam (147 and 11 individuals), Rambi (84 individuals) and Sadam (208 individuals). The total number of individuals, including flowering, sapling, and seedlings, was larger in the localities under very high suitability class and vice-versa (Table 21). However, when the model threshold values and population size were regressed, a weak positive correlation was observed indicating that the high suitability class does not necessarily have a higher individual ($r = 0.454$; $p = 0.304$).

Table 21: Population structure and current habitat status of *B. satrapis* with model threshold

Occurrence localities	**Elevation (m asl)**	**Habitat suitability threshold (values:0-1)**	**Current habitat status**	**Number of individuals**			
				FL	SP	SD	Total
Sumbuk 1	829	Very high (0.87)	Tropical Sal forest, Human settlement	190	274	510	974
Sumbuk 2	936	Very high (0.90)	Tropical Sal forest, Human settlement	111	125	187	423
Badamtam 1	622	Very High (0.81)	Tropical forest, human settlement, Tea garden	28	87	32	147
Badamtam 2	610	Very High (0.81)	Tropical forest, human settlement, Tea garden	3	0	8	11
Kitam	791	High (0.61)	Tropical forest, human settlement	47	21	33	101
Sadam	1390	High (0.63)	Human settlement, road cutting	72	47	89	208
Rambi	268	Low (0.20)	Tropical forest, Disturbed habitat, landslide prone area, road cutting	23	46	15	84
Total							**1948**

Note: FL=Flowering, SP=Sapling, SD=Seedling

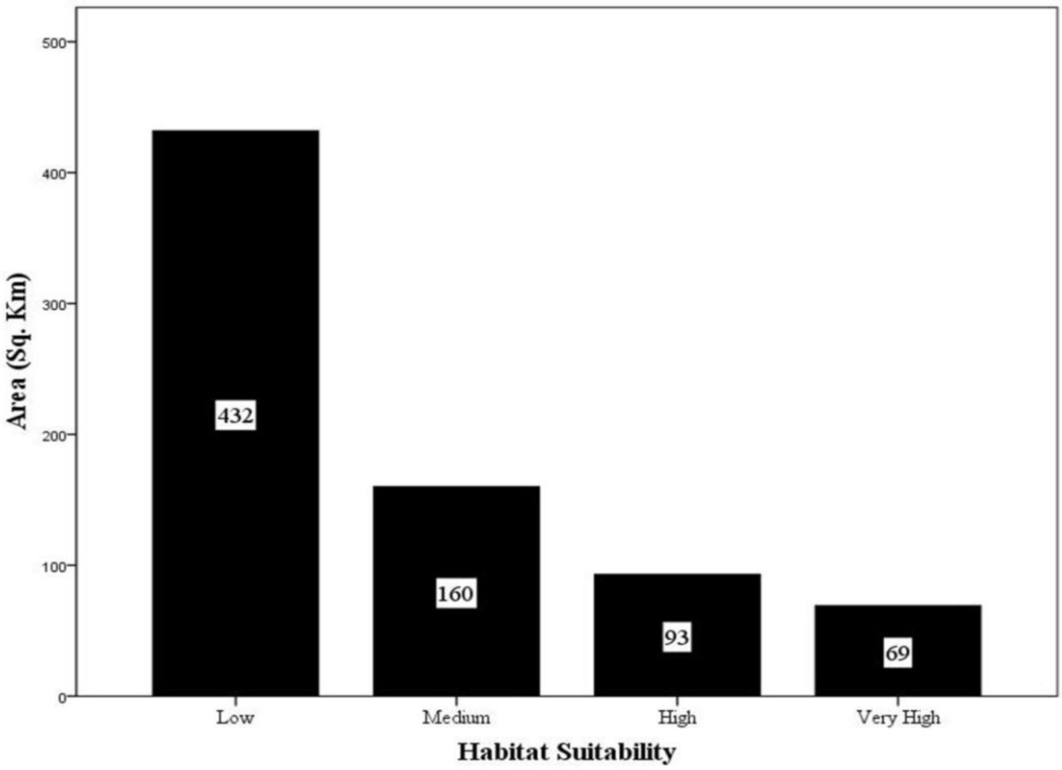

Fig. 15: Area under different suitability class (values on the bar represents the area in km^2)

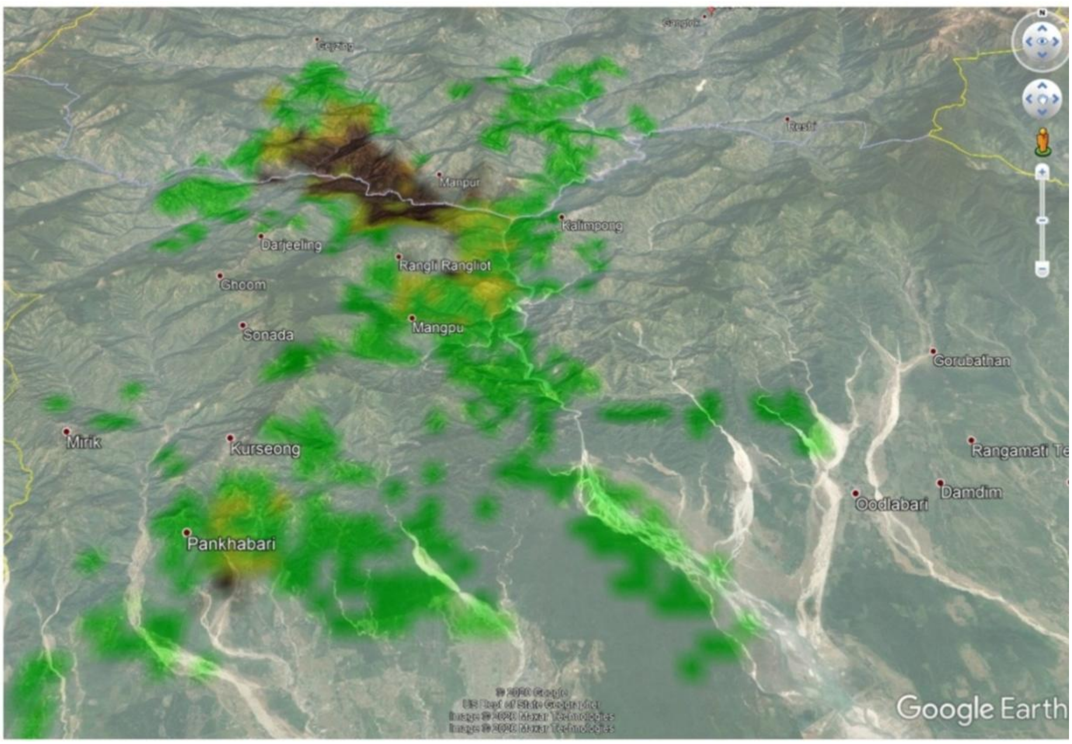

Fig. 16: Superimposed predicted distribution map onto Google earth

5.3.5. Superimposed predicted distribution map

The predicted potential area superimposed on Google Earth imageries depicted different forest area viz. Teesta valley forest, Sumbuk forest Block, Kitam forest Block, Majitar forest block. Areas like *Chisopani, Sangbung, Shyampani, Tinik*, *Naya Bazar, Harabotey*, in the South district of Sikkim. These areas predicted to be suitable can serve for the in-situ conservation of species through reintroduction in its natural habitat (Fig. 16). Localities such as *Sumbuk 1*, *Sumbuk 2,* and *Kitam* maintained higher population individuals owing to nearby Kitam Bird Sanctuary. The population encountered towards Darjeeling district was low because the predicted area falls under different tea estates (*viz*. Badamtam Tea Garden, Kambal Tea Garden, Singla Tea Garden, and Peshok Tea Garden) thereby restricting the distribution of species in the region. Similarly, the number of the individuals at Rambi was low too because of a constant landslide in its natural habitat. Interestingly, both the population encountered at *Badamtam* and *Rambi* had an open canopy, unlike populations at *Sumbuk* and *Kitam*, where the habitat was dominated by tree species like *Shorea robusta*, *Schima wallichii,* etc. Considering the number of the seedling, sapling, and flowering individuals at different localities, the populations at *Sumbuk 1*, *Sumbuk 2,* and *Kitam* may be considered healthy and thriving properly while populations at Badamtam and Rambi were declining.

Habitat status assessment through primary field survey and secondary through the superimposition of the predicted map on Google Earth satellite imageries revealed the predicted potential areas of the species under different threshold levels incorporating different vegetation types like forest patches, human settlement, cultivation areas, etc. Some areas under tropical deciduous *sal* forest in the South district of Sikkim offer potential habitats for species reintroduction showing higher levels of habitat suitability.

5.3.6. Threat assessment

The extent of occurrence (EOO) calculated for *B. satrapis* was 81 km^2 which is only 0.78 % of the total area of Sikkim Himalaya (10,345 km^2). The EOO thus calculated to be less than 100 km^2 and using IUCN criteria ver. 3.1 the assessment of threat was made using criteria B1 i.e. Extent of occurrence (EOO) estimated to be less than 100 km^2, and estimates indicating at least two of a-c, we used criteria 'b' (i.e. continuing decline, observed, inferred or projected, (iv) (i.e. a number of locations or subpopulations), 'c' extreme fluctuations in any of the following:

(i) extent of occurrence (EOO)

(ii) area of occupancy (AOO)

(iii) number of locations or subpopulations.

(iv) number of mature individuals

Based on the above criteria *B. satrapis* was categorized as **critically endangered.**

The extent of occurrence (EOO) (Km Sq.)	**Constraints**	**Current IUCN status**	**Assessment based on the present study using IUCN Criteria ver. 3.1**	**Status based on the present study**
81	Agriculture expansion, road widening, grazing, fodder collection and landslide	Not assessed	B1b(iv)c(i,ii,iii,iv); D	Critically endangered (CR)

5.3.7. Niche characterization in Begonia species

5.3.7.1. Collection Locality

A total of 108 occurrence records or geographic coordinates (*B. gemmipara*=8, *B. josephii=12*, *B. satrapis=10*, *B. picta*=12, *B. nepalensis*=4, *B. palmata*=14, *B. panchtharensis*=5, *B. sikkimensis*=8, *B. cathcartii*=7, *B. megaptera*=5, *B. xanthina*=5, *B. flaviflora*=5, *B. roxburghii*=13) were collected from all the four districts of Sikkim (North, South, East, and West) and Darjeeling district of West Bengal, India. The individual occurrence data were then correlated with the set of NDVI and bioclimatic variables. The presence and absence of *Begonia* species in each district are given in table 22.

Table 22: District wise distribution of *Begonia* species in Darjeeling and Sikkim

Species	**Sikkim**				**West Bengal**
	North	**South**	**East**	**West**	**Darjeeling**
B. gemmipara	-	-	-	+	+
B. josephii	-	-		+	+
B. satrapis	-	+	-	-	+
B. picta	-	+	+	+	+
B. nepalensis	-	-		-	+
B. palmata	+	-	+	-	+
B. panchtharensis	-	+	+	+	-
B. sikkimensis	-	-	+	-	+
B. cathcartii	-	-	+	-	+
B. megaptera	-	-	+	-	-
B. xanthina	-	-	+	-	-
B. flaviflora	-	-	+	+	+
B. roxburghii	-	-	+	-	+

Note: (+) denotes present; (-) denotes absent

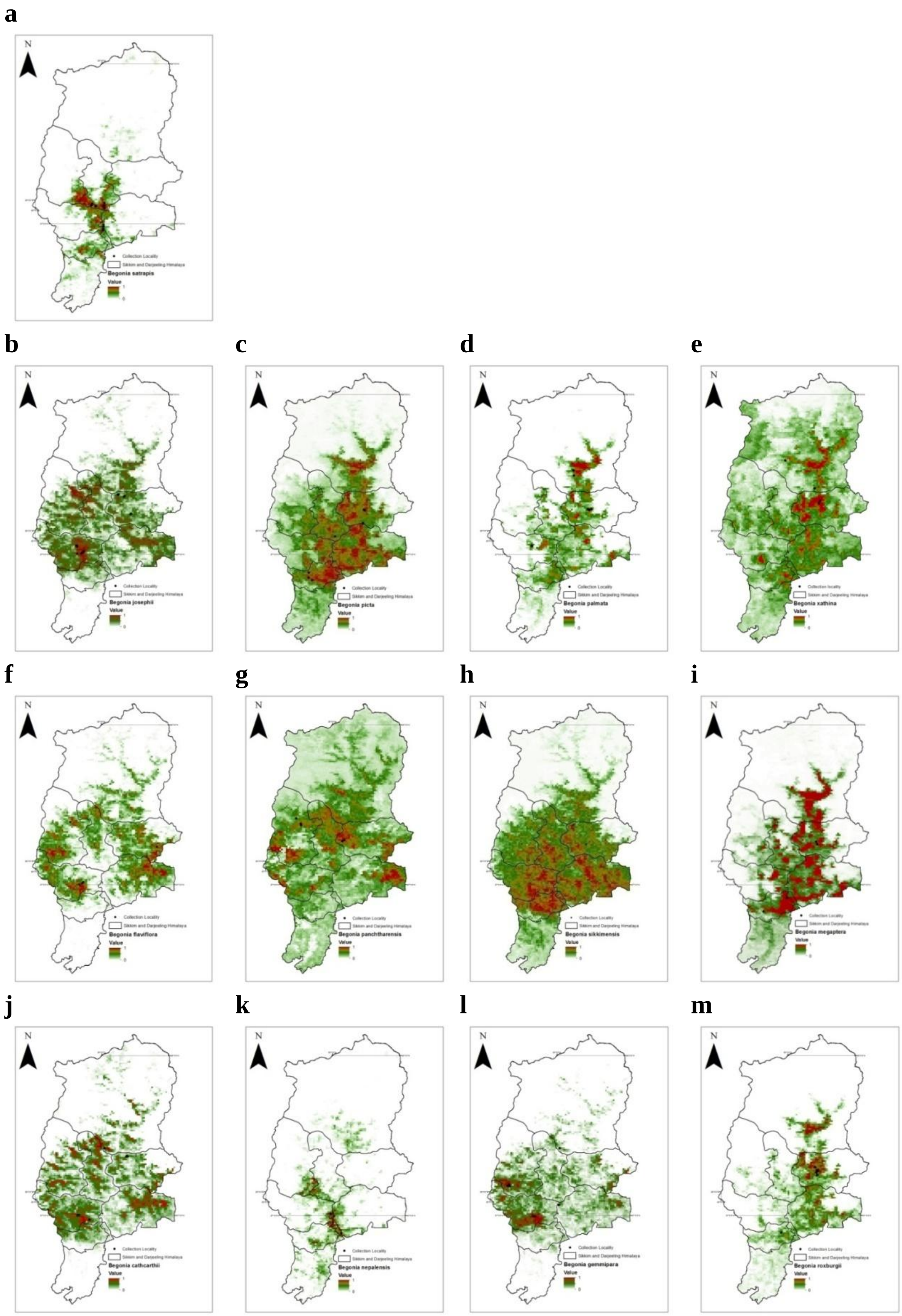

Fig. 17: Predicted distribution map based on NDVI variables: (a) *B. satrapis* (b) *B. josephii* (c) *B. picta* (d) *B. palmata* (e) *B. xanthina* (f) *B. flaviflora* (g) *B. panchtharensis* (h) *B. sikkimensis* (i) *B. megaptera* (j) *B. cathcartii* (k) *B. nepalensis* (l) *B. gemmipara* (m) *B. roxburghii*

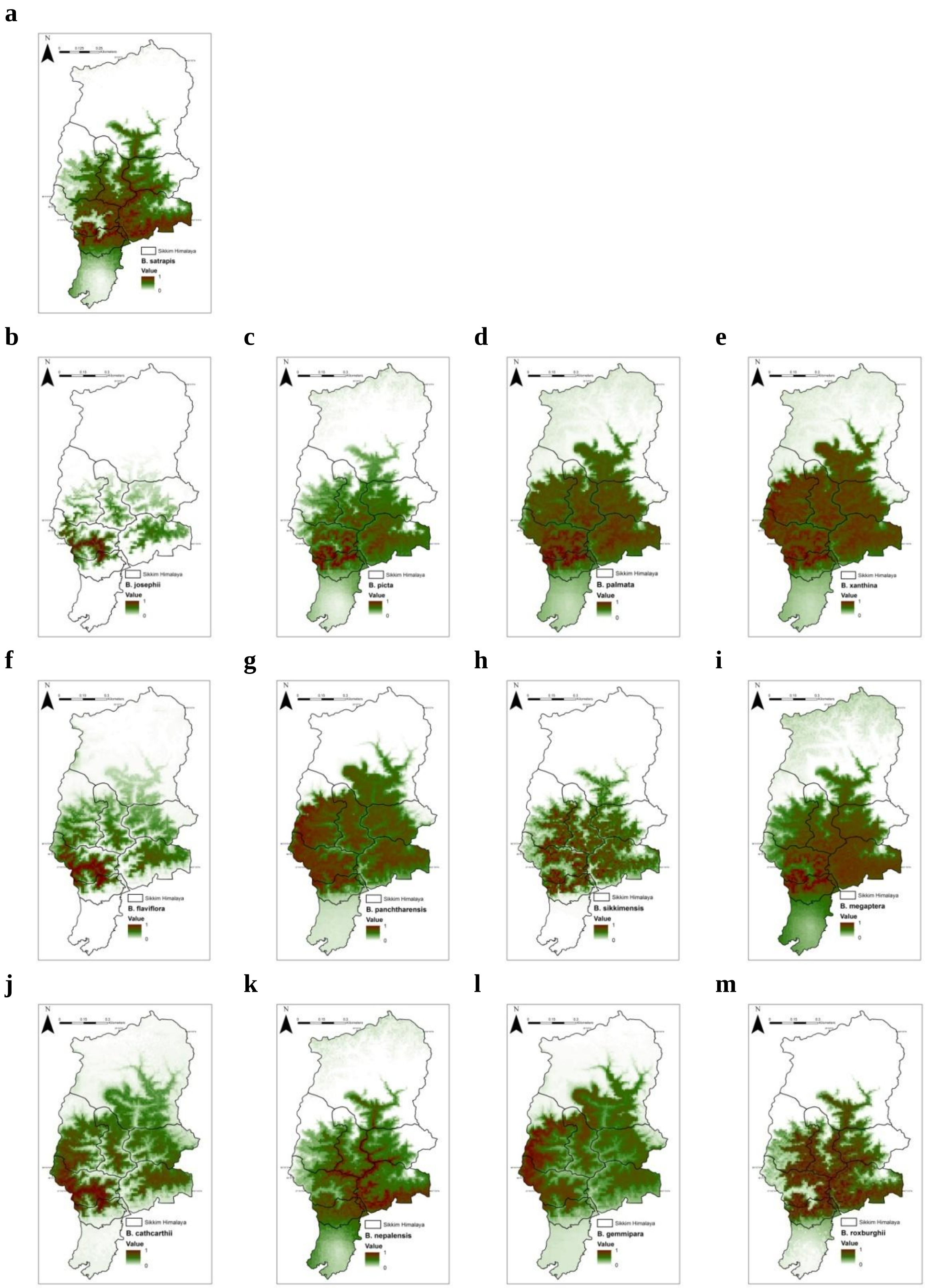

Fig. 18: Predicted distribution map based on bioclimatic variables: (a) *B. satrapis* (b) *B. josephii* (c) *B. picta* (d) *B. palmata* (e) *B. xanthina* (f) *B. flaviflora* (g) *B. panchtharensis* (h) *B. sikkimensis* (i) *B. megaptera* (j) *B. cathcartii* (k) *B. nepalensis* (l) *B. gemmipara* (m) *B. roxburghii*

5.3.7.2. Predicted habitat distribution

ENM was computed individually for each *Begonia* species. The model developed for 13 species of *Begonia* using both NDVI and bioclimatic variables is presented in Fig. 17 and 18. The 10 percentile training presence logistic threshold values for each species of *Begonia* are also provided in table 26. Using the threshold values of individuals *Begonia* species suitable habitat was calculated. Therefore based on NDVI dataset, *B. panchtharensis* had the maximum area predicted to be suitable (~4306.88 km^2), followed by *B. sikkimensis* (~3804.62 km^2), *B. picta* (~3785.4 km^2), *B. cathcartii* (~2480.01 km^2), *B. xanthina* (~1905.8 km^2), *B. josephii* (~1833.28 km^2), *B. flaviflora* (~1634.74 km^2), *B. megaptera* (~1412.58 km^2), *B. satrapis* (~1274.37 km^2), *B. gemmipara* (~1131.25 km^2), *B. palmata* (~783.446 km^2), *B. roxburghii* (~766.19 km^2), *B. nepalensis* (~60.36 km^2) (Fig. 19).

The predicted suitable areas were not consistent when two different sets of variables were used independently. The area predicted to be suitable based on bioclimatic variables were highest in *B. gemmipara* (~5712 km^2) followed by *B. palmata* (~ 4194.74 km^2), *B. panchtharensis* (~ 3040.09 km^2), *B. megaptera* (~ 3023.2 km^2), *B. xanthina* (~ 2603.65 km^2), *B. cathcartii* (~ 2593.21 km^2), *B. sikkimensis* (~ 2386.15 km^2), *B. josephii* (~ 2380.78 km^2), *B. flaviflora* (~ 2366.38 km^2), *B. picta* (~ 2296.18 km^2), *B. roxburghii* (~ 2206.23 km^2), *B. satrapis* (~ 1941.15 km^2), *B. nepalensis* (~ 367.45 km^2) (Fig. 19).

In both the models, *B. nepalensis* had the lowest area suitable. However, when bioclimatic variables were used as input variables, the area predicted was much higher compared to NDVI.

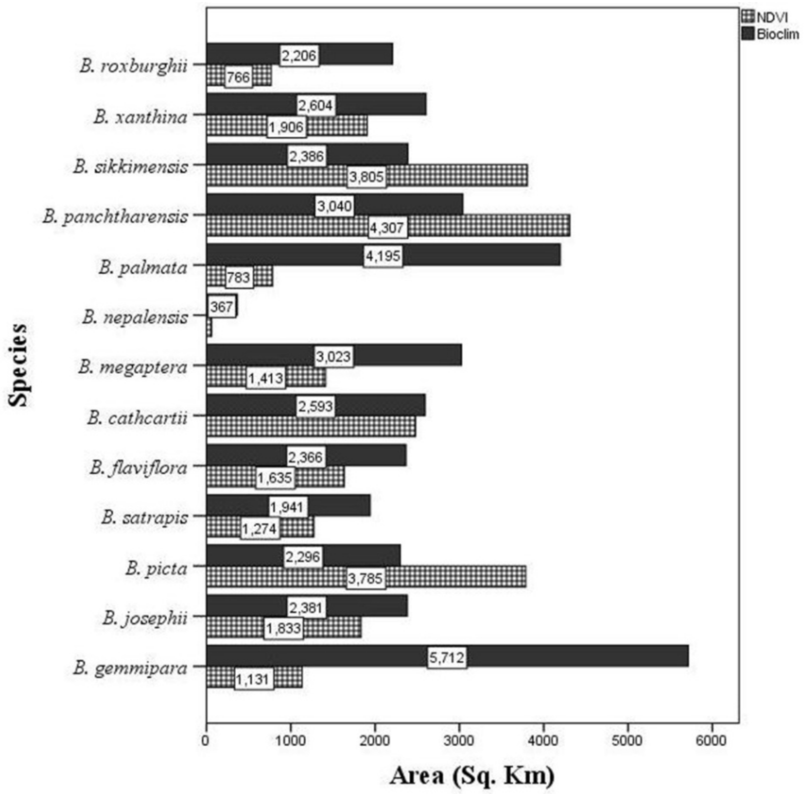

Fig. 19: Predicted suitable habitat of 13 species of *Begonia* in Sikkim Himalaya (*10 percentile training presence logistic threshold*)

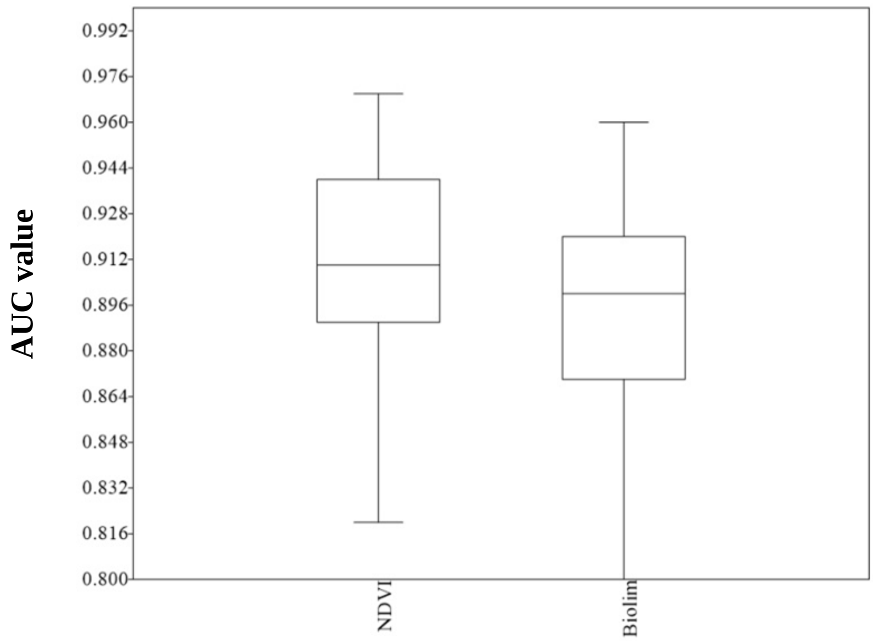

Fig. 20: Boxplots of the distribution of AUC values to compare the model fitness pertaining to the two predictor variables for the distribution of 13 *Begonia* species in Sikkim Himalaya.

Table 23: List of *Begonia* species, number of records and AUC score for model evaluation

Species	No. of records	Full AUC (mean±SD)		AUC ratio (mean±SD)		AUC ratio (mean ± SD	
		NDVI	Bioclim	NDVI	Bioclim	NDVI	Bioclim
B. gemmipara	8	0.93	0.84	0.96 ±0.02	**0.93 ±0.03**	1.92 ±0.05	1.86 ±0.07
B. josephii	12	0.94	**0.96**	0.96 ±0.009	0.95 ±0.01	1.93 ±0.01	1.91 ±0.03
B. satrapis	10	**0.97**	0.92	**0.98 ±0.007**	0.96 ±0.02	1.97 ±0.01	1.93 ±0.04
B. picta	12	0.89	0.91	0.94 ±0.02	0.94 ±0.03	1.89 ±0.04	1.88 ±0.06
B. nepalensis	4	0.89	0.87	0.99 ±0.002	0.97 ±0.01	1.99 ±0.004	1.94 ±0.03
B. palmata	14	0.95	**0.80**	0.98 ±0.004	0.97 ±0.02	1.97 ±0.008	1.94 ±0.04
B. panchtharensis	5	**0.82**	0.88	0.93 ±0.09	0.96 ±0.04	1.86 ±0.19	1.93 ±0.08
B. sikkimensis	8	**0.82**	0.93	**0.92 ±0.03**	0.94 ±0.02	1.84 ±0.07	1.88 ±0.04
B. cathcartii	7	0.96	0.90	0.96 ±0.01	0.944 ±0.02	1.93 ±0.03	1.88 ±0.04
B. megaptera	5	0.91	0.85	0.97 ±0.02	0.94 ±0.04	1.94 ±0.04	1.89 ±0.09
B. xanthina	5	0.84	0.87	0.95 ±0.04	**0.99± 9.33521 E-15**	1.90 ±0.09	1.99± 2.04486E -14
B. flaviflora	5	0.91	0.94	0.97 ±0.02	0.9 ±0.01	1.94 ±0.05	1.95 ±0.03
B. roxburghii	13	0.94	0.92	0.98 ±0.005	0.96 ±0.02	1.97 ±0.01	1.93 ±0.05

Note: The bold values are highest and lowest

5.3.7.3. Model evaluation and validation

Our model performance showed high accuracy and demonstrated high predictive ability based on AUC scores. When NDVI variables were used, the ROC_{full} i.e. mean AUC; ranged from 0.82 in *B. panchtharensis* and *B. sikkimensis* to 0.97 in *B. satrapis* and $ROC_{partial}$ ranging from 0.92 in *B. sikkimensis* to 0.98 in *B. satrapis*, *B. palmata* and *B. roxburghii*. Moreover, when bioclimatic variables were used, the mean AUC values ranged from 0.80 in *B. palmata* to 0.96 in *B. josephii* (Fig. 20). $ROC_{partial}$

ranged from 0.93 in *B. gemmipara* to 0.99 in *B. xanthina.* The AUC ratio values thus calculated as $AUC_{partial}/AUC_{random}$ for all the 13 species of *Begonia* were higher than random indicating good model consistency (Table 23).

5.3.7.4. Contributing variables and Environmental constraints for Begonia species

The different NDVI and bioclimatic variables used to model the distribution of *Begonia* species in Sikkim Himalaya showed a varying degree of contribution to each individual's model developed. The NDVI for July contributed the most in *B. cathcartii* (68.7 %) followed by *B. sikkimensis* (62.6 %), *B. josephii* (50.5 %), *B. flaviflora* (48.5 %) and *B. gemmipara* (47.6 %). The NDVI for November contributed the most in the case of *B. picta* (32.3 %), *B. palmate* (37.3 %), *B. megaptera* (56.8 %), and *B. roxburghii* (29.6 %). The NDVI for May, January, and March each contributed the most in *B. satrapis* (63.6 %), *B. nepalensis* (24.5 %), and *B. xanthina* (53.1 %) respectively to the final predictive model (Fig. 21; Table 24). Considering the permutation importance, the NDVI for July contributed the most in *B. gemmipara* (71.9 %), *B. josephii* (39.5 %), *B. catcarthii* (60.6 %) and *B. flaviflora* (67.7 %). The NDVI for November contributed the most in *B. picta* (72.7 %), *B. palmata* (40.3 %), and *B. megaptera* (53.3 %), *B. xanthina* (33.6 %). The NDVI for January, May, October and December contributed the most in *B. nepalensis* (30.3 %), *B. satrapis* (72.1 %), *B. sikkimensis* (43.0 %) and *B. panchtharensis* (53.3 %) and altitude contributed the most in *B. roxburghii* (35.0 %) (Table 24).

Table 24: Average contribution of input NDVI variables to model output for each species of *Begonia* distributed in Sikkim Himalaya

Taxon / Variables	*B. gemmipara*	*B. josephii*	*B. satrapis*	*B. picta*	*B. nepalensis*	*B. palmata*	*B. panchtharensis*	*B. sikkimensis*	*B. cathcartii*	*B. megaptera*	*B. xanthina*	*B. flaviflora*	*B. roxburghii*
Percentage contribution													
Eu1 (Jan)	0	0.9	12.5	0.3	**24.5**	0	1.3	0.8	0	0.3	0.1	0	0.1
Eu2 (Feb)	14.1	3.8	1.2	0	0.9	30.9	6.0	0	0.3	21.2	0	4.2	5.3
Eu3 (Mar)	19.2	0	1.0	5.0	2.0	4.0	1.2	0	0	0	**53.1**	4.3	18.2
Eu4 (Apr)	11.3	4.7	3.2	0	10.8	0.2	0.1	0.8	0.5	0.3	1.3	2.0	2.6
Eu5 (May)	0	4.6	**63.6**	0	18.2	1.8	0	0	0.2	0	0.5	31.6	12.3
Eu6 (Jun)	0.1	0	1.1	0	3.5	1.2	0.7	0	0.2	1.1	4.1	0.1	0.6
Eu7 (Jul)	**47.6**	**50.5**	0	12.5	0	0.6	37.2	**62.6**	**68.7**	0.4	0.1	**48.5**	0
Eu10 (Oct)	1.6	23.2	0.7	28.2	9.6	24.1	2.4	26.9	9.3	12.1	9.6	4.4	12.0
Eu11 (Nov)	2.2	0	6.2	**32.3**	10.2	**37.3**	0	8.2	0	**56.8**	22.6	1.5	**29.6**
Eu12 (Dec)	0	0	7.0	0	2.5	0	**45.9**	0	0	0.2	1.1	0	0.1
Altitude	0	0	3.5	21.5	17.9	0.1	5.1	0.6	20.8	7.5	7.5	3.3	19.2
Permutation importance													
Eu1 (Jan)	0	0.9	4.5	0.2	**30.3**	0	5.7	0	0	1.3	0	0.2	0
Eu2 (Feb)	7.2	8.4	0.3	0.3	0.6	23.7	7.9	0	0	0	0	2.7	0
Eu3 (Mar)	6.2	0	0.1	2.2	0.3	1.5	0	0	0	0	17.6	0.6	7.5
Eu4 (Apr)	2.4	1.8	1.6	0	2.3	0	0.3	4.7	0.2	0.4	2.9	5.1	5.1
Eu5 (May)	0	1.4	**72.1**	0	17.0	0.1	0	0	0	0	2	7.3	19.5
Eu6 (Jun)	0.7	0	0.2	0	2.9	3.4	2.7	0.1	0.7	3.4	12.3	1	1.7
Eu7 (Jul)	**71.9**	**39.5**	0	6.2	0	3.1	25.8	41.8	**60.6**	1.9	0	**67.7**	0
Eu10 (Oct)	1.5	27.7	0.6	9.4	6.4	27.4	0.1	**43.0**	9.9	15.9	5.9	10.4	5.5
Eu11 (Nov)	0.8	0.1	14.8	**72.7**	18.5	**40.3**	0	9.7	0	**67.3**	**33.6**	0	25.6
Eu12 (Dec)	0	0	5.8	0	0.4	0	**53.3**	0	0	2.2	11.7	0	0.1
Altitude	9.2	20.2	0	8.9	21.3	0.6	4.0	0.6	28.5	7.5	14	4.9	**35.0**

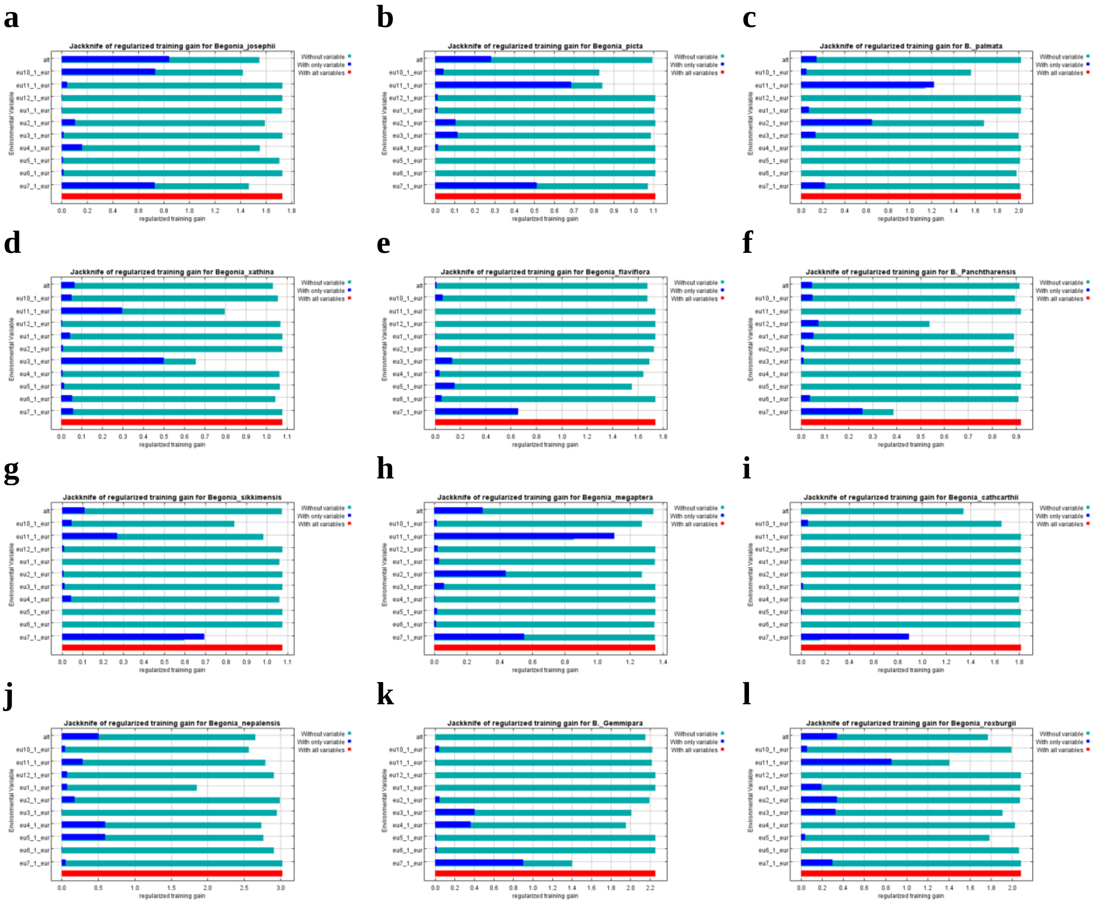

Fig. 21: Results of jackknife test for variables importance in *Begonia* species using NDVI variable: (a) *B. josephii* (b) *B. picta* (c) *B. palmata* (d) *B. xanthina* (e) *B. flaviflora* (f) *B. panchtharensis* (g) *B. sikkimensis* (h) *B. megaptera* (i) *B. cathcartii* (j) *B. nepalensis* (k) *B. gemmipara* (l) *B. roxburghii*

Based on bioclimatic variables the distribution of *Begonia* species was mostly governed by a combination of two major environmental factors *viz*. Mean Diurnal Range [Mean of monthly (max temp - min temp)] (Bio2) and Precipitation of Coldest Quarter (Bio19), which contributed the most to the final predicted distribution model of *Begonia* (Table 25). The jackknife plot for each species is given in fig. 22. The list of different bioclimatic variables used to model the distribution of each species of *Begonia* is given in table 18. The percentage contribution of mean diurnal range [(mean of monthly (max temp - min temp)] (Bio2) was highest in *B palmata* (87.3 %) followed by *B. sikkimensis* (82.4 %), *B. panchtharensis* (75.1 %), *B. xanthina* (71.6

%), *B. roxburghii* (67.6 %), *B. picta* (67.1 %), *B. flaviflora* (65 %), *B. josephii* (54.9 %), *B. cathcartii* (48.4 %), *B. megaptera* (45.5 %). The precipitation of coldest quarter (Bio19) contributed the most in *B. gemmipara* (64.1 %) and *B. satrapis* (42 %) and Bio1 i.e. annual mean temperature contributed the most in *B. nepalensis* (40.5 %). Considering the permutation importance, BIO2 contributed the most in *B josephii* (60 %), *B. panchtharensis* (42.6 %), *B. sikkimensis* (73.8 %), *B. flaviflora* (67.5 %) and *B. roxburghii* (47.6 %). BIO19 contributed the most in *B. gemmipara* (47.7 %), *B. nepalensis* (35 %), *B. palmata* (46.9 %) and *B. catcarthii* (39.5 %). BIO15 contributed the most in *B picta* (46 %), *B. megaptera* (60.7 %) and *B. xanthina* (36.9 %). BIO1 contributed the most in *B. satrapis* (44.1 %).

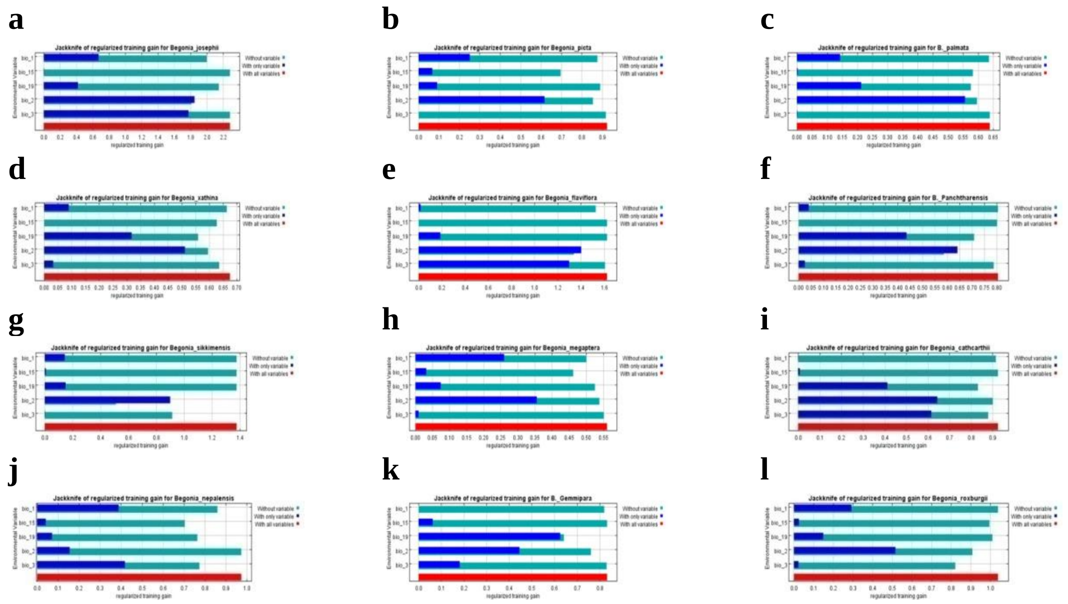

Fig. 22: Results of jackknife test of variables importance for *Begonia* species using bioclimatic variable: (a) *B. josephii* (b) *B. picta* (c) *B. palmata* (d) *B. xanthina* (e) *B. flaviflora* (f) *B. panchtharensis* (g) *B. sikkimensis* (h) *B. megaptera* (i) *B. cathcartii* (j) *B. nepalensis* (k) *B. gemmipara* (l) *B. roxburghii*

Table 25: Average contribution of input bioclimatic variables to model output for species of *Begonia* distributed in Sikkim Himalaya

Taxon / Variables	*B. gemmipara*	*B. josephii*	*B. satrapis*	*B. picta*	*B. nepalensis*	*B. palmata*	*B. panchtharensis*	*B. sikkimensis*	*B. cathcartii*	*B. megaptera*	*B. xanthina*	*B. flaviflora*	*B. roxburghii*
Percentage contribution													
BIO1	1	5.2	15.7	6.4	**40.5**	0.9	0	0.1	1.8	33.0	0.6	3.9	7.1
BIO2	31.0	**54.9**	0	**67.1**	6.5	**87.3**	**75.1**	**82.4**	**48.4**	**45.5**	**71.6**	**65.0**	**67.6**
BIO3	3.9	54.7	3.9	0.5	17.4	0.8	1.3	17.5	28.7	1.4	2.2	31.1	20.4
BIO15	0	0	38.4	24.4	22.6	4.8	0.2	0	0	16.6	3.7	0	1.5
BIO19	**64.1**	4.2	**42.0**	1.7	13.0	6.3	23.3	0	21.1	3.5	21.9	0	1.5
Permutation importance													
BIO1	7.4	32.1	**44.1**	3.0	19.9	0	0.3	1.3	7.4	21.9	5.1	13.1	0
BIO2	44.1	**60.0**	1.7	27.2	0	24.4	**42.6**	**73.8**	17.4	5.0	22.0	**67.5**	**47.6**
BIO3	0.8	0	2.2	0.7	20.3	0.4	11.6	24.9	35.6	1.2	0.9	19.3	10.9
BIO15	0	0.1	30.1	**46.0**	24.8	28.3	4.2	0	0	**60.7**	**36.9**	0	24.9
BIO19	**47.7**	7.8	21.9	23.1	**35.0**	**46.9**	41.3	0	**39.5**	11.2	35.1	0	16.5

5.3.7.5. Niche overlap

5.3.7.5.1. NDVI variables

The niche overlap test resulted in significantly different levels of overlaps in *Begonia* species. Based on NDVI variables used, the Hellinger's I niche overlap values were highest between *B. picta* and *B. sikkimensis* and *B. megaptera* (overlap value=0.96) indicating the high level to niche overlap whereas the lowest level of niche overlap was estimated between *B. satrapis* and *B. flaviflora* (overlap value=0.35) (Table 26).

Similarly, the Schoener's D niche overlap values were highest between *B. picta* and *B. sikkimensis* and *B. megaptera* (overlap value=0.81) indicating the high level to niche overlap whereas a low level of niche overlap was estimated between *B. satrapis* and *B. flaviflora* (0.12) (Table 26).

5.3.7.5.2. Bioclimatic variables

Based on bioclimatic variables used, the Hellinger's I niche overlap values were highest between *B. megaptera* and *B. nepalensis* (overlap value=0.99) and *B. xanthina and B. palmata* (overlap value=0.99) indicating the high level to niche overlap whereas the lowest level of niche overlap was estimated between *B. satrapis* and *B. josephii* (overlap value=0.59) (Table 27).

Similarly, the Schoener's D niche overlap values were highest between *B. xanthina and B. palmata* (overlap value=0.93) indicating the high level to niche overlap whereas a low level of niche overlap was estimated between *B. satrapis* and *B. josephii* (0.32) (Table 27).

5.3.7.6. Niche Breadth

5.3.7.6.1. NDVI variables

The niche breadth analysis based on NDVI variables resulted in narrower niches in some *Begonia* species. *B. panchtharensis* had the highest niche breadth value (NBV) of 0.642, indicating broader niches compared to other related species of *Begonia*, which also presented the broadest distribution of suitable habitat. Similarly, the niche breadth for *B. sikkimensis* (NBV = 0.412) and *B. picta* (NBV = 0.384) were also high with broader distribution of suitable habitat compared to other species of *Begonia*. The lowest niche breadth value was estimated in *B. satrapis* (NBV = 0.102) indicating a very narrow niche. Species like *B. nepalensis* (NBV = 0.110) and *B. palmata* (NBV = 0.180) also showed low niche breadth with a narrow distribution of suitable habitat (Table 28).

5.3.7.6.2. Bioclimatic variables

The niche breadth analysis, based on bioclimatic variables resulted in narrower niches in *B. josephii* (NBV = 0.136) compared to other species of *Begonia*, whilst species like *B. megaptera* (NBV = 0.633), *B. palmata* (NBV = 0.581), *B. panchtharensis* (NBV = 0.481), *B. megaptera* (NBV = 0.508) showed broader niche breadth, with broader distribution of suitable habitat ranging from 3000-6000 km^2 area (Table 28). Species like *B. flaviflora* and *B. sikkimensis* exhibited both narrow distribution range and low niche breadth value. Moreover one such species *i.e*. *B. josephii* showed low niche breadth value (0.136) exhibiting broader suitable habitat, unlike *B. nepalensis* where the niche breadth value was higher with a narrow distribution of suitable habitat.

5.3.7.7. Ecological differentiation between Begonia species

The detailed analysis of NDVI and bioclimatic variables revealed important ecological properties of the niche. The PCA plot showed some differentiation in the NDVI and bioclimatic profile of 13 species of *Begonia*. In both bioclimatic and NDVI plots, *B. satrapis* were slightly separated from the rest of the species along the first axis. In the case of PCA drawn from the NDVI dataset, 46.72 % was explained by the first axis, 22.59 % by the second, and 11.37 % by the third. Whilst in the case of PCA drawn from bioclimatic variables, 59.25 % explained by the first axis, 21.76 % by the second, and 15.80 % by the third. The UPGMA cluster also confirmed the separation similar to PCA drawn from both the types of variables used.

5.3.7.8. Relationship between predicted suitable habitat and niche breadth

The correlation between predicted suitable area and niche breadth of *Begonia* species was not significant (r = 0.217, p = 0.475) when bioclimatic data were used as an input variable (Fig. 24). This indicates that narrowly distributed species do not necessarily have narrow niche breadth. However, a positive correlation (r = 0.747, p = 0.003) was observed when the NDVI dataset was used as input variables (Fig. 23).

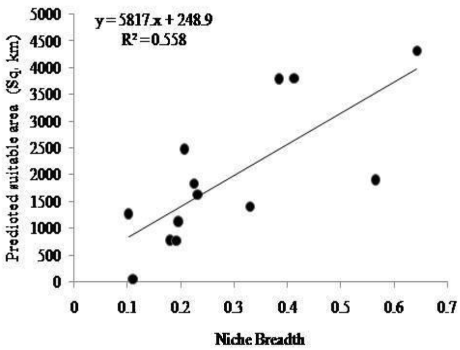

Fig. 23: Correlation between predicted suitable area and niche breadth of *Begonia* species using NDVI variables

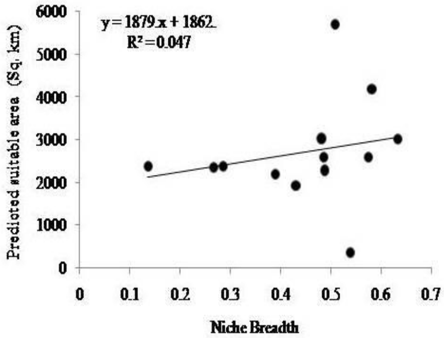

Fig. 24: Correlation between predicted suitable area and niche breadth of *Begonia* species using bioclimatic variables

Table 26: Niche breadth values and predicted suitable area (10 percentile training presence logistic threshold value) using NDVI and bioclimatic variables

Sl. No.	Species	Niche Breadth	Threshold value/Area (km^2)	Niche Breadth	Threshold value/Area (km^2)
		NDVI		**Bioclim**	
1	*B. gemmipara*	0.195	0.304/1131.25	**0.508**	**0.298/5712.11**
2	*B. josephii*	0.225	0.384/1833.27	**0.136**	**0.115/2380.78**
3	*B. picta*	**0.384**	**0.396/3785.40**	0.488	0.476/2296.18
4	*B. satrapis*	**0.102**	0.133/1274.37	0.430	0.523/1941.15
5	*B. flaviflora*	0.231	0.416/1634.74	**0.266**	**0.295/2366.38**
6	*B. cathcartii*	0.206	0.283/2480.00	0.486	0.470/2593.22
7	*B. megaptera*	0.329	0.521/1412.57	**0.633**	0.535/3023.19
8	*B. nepalensis*	**0.110**	**0.605/60.36**	**0.539**	**0.628/367.45**
9	*B. palmata*	**0.180**	**0.469/783.44**	**0.581**	**0.504/4194.74**
10	*B. panchtharensis*	**0.642**	**0.333/4306.88**	**0.481**	**0.548/3040.09**
11	*B. sikkimensis*	**0.412**	**0.445/3804.61**	0.285	0.412/2386.14
12	*B. xanthina*	0.565	0.449/1905.79	0.575	0.554/2603.65
13	*B. roxburghii*	0.191	0.448/766.19	0.389	0.532/2206.23

Note: The highest and the lowest niche breadth are highlighted in bold. Values range from 0-1: 0 is equal to one grid cell being suitable (specialized niche); whereas 1 is where all grid cells are suitable (broad niche).

Table 27: Summary of niche overlap values based on NDVI dataset [Schoener's D (above diagonal) and Hellinger's I (below diagonal)]

Schoener's D / **Hellinger's I**	*B. gemmipara*	*B. josephii*	*B. satrapis*	*B. picta*	*B. nepalensis*	*B. palmata*	*B. panchtharensis*	*B. sikkimensis*	*B. cathcartii*	*B. megaptera*	*B. xanthina*	*B. flaviflora*	*B. roxburghii*
B. gemmipara		0.65	0.25	0.50	0.24	0.33	0.49	0.63	0.69	0.38	0.45	0.68	0.32
B. josephii	0.89		**0.22**	0.55	0.27	0.46	0.49	0.68	0.76	0.46	0.43	0.68	0.37
B. satrapis	0.53	0.49		0.43	0.55	0.38	0.32	0.37	0.20	0.43	0.34	**0.12**	0.28
B. picta	0.81	0.81	0.73		0.44	0.67	0.57	**0.81**	0.49	**0.81**	0.70	0.48	0.66
B. nepalensis	0.50	0.52	0.81	0.73		0.39	0.31	0.39	0.21	0.44	0.34	0.18	0.33
B. palmata	0.61	0.71	0.68	0.91	0.68		0.37	0.56	0.35	0.75	0.52	0.32	0.67
B. panchtharensis	0.79	0.75	0.63	0.85	0.59	0.67		0.64	0.49	0.48	0.65	0.53	0.40
B. sikkimensis	0.89	0.89	0.68	**0.96**	0.68	0.82	0.88		0.64	0.67	0.63	0.60	0.54
B. cathcartii	0.93	0.95	0.46	0.77	0.45	0.62	0.76	0.88		0.39	0.40	0.75	0.31
B. megaptera	0.67	0.69	0.74	**0.96**	0.73	0.95	0.79	0.89	0.65		0.65	0.37	0.70
B. xanthina	0.76	0.71	0.66	0.91	0.88	0.88	0.88	0.87	0.68	0.88		0.43	0.55
B. flaviflora	0.90	0.90	**0.35**	0.77	0.41	0.60	0.82	0.87	0.93	0.65	0.75		0.34
B. roxburghii	0.60	0.62	0.57	0.90	0.61	0.91	0.67	0.81	0.57	0.92	0.80	0.63	

Note: Species are grouped by sections. Highest and lowest overlap values are in bold.

Table 28: Summary of niche overlap values based on bioclimatic variables [Schoener's D (above diagonal) and Hellinger's I (below diagonal)

Schoener's D / **Hellinger's I**	*B. gemmipara*	*B. josephii*	*B. satrapis*	*B. picta*	*B. nepalensis*	*B. palmata*	*B. panchtharensis*	*B. sikkimensis*	*B. cathcartii*	*B. megaptera*	*B. xanthina*	*B. flaviflora*	*B. roxburghii*
B. gemmipara		0.46	0.55	0.62	0.59	0.78	0.87	0.58	0.74	0.65	0.83	0.68	0.59
B. josephii	0.75		**0.32**	0.42	**0.32**	0.43	0.47	0.46	0.53	0.36	0.43	0.74	0.48
B. satrapis	0.82	**0.59**		0.85	0.88	0.74	0.64	0.65	0.48	0.79	0.69	0.44	0.85
B. picta	0.85	0.70	0.98		0.86	0.82	0.71	0.69	0.57	0.87	0.77	0.53	0.80
B. nepalensis	0.85	0.61	0.98	0.98		0.79	0.65	0.61	0.53	0.90	0.74	0.44	0.79
B. palmata	0.95	0.73	0.93	0.96	0.97		0.85	0.66	0.72	0.85	**0.93**	0.63	0.74
B. panchtharensis	0.98	0.75	0.87	0.90	0.89	0.97		0.68	0.80	0.70	0.89	0.68	0.70
B. sikkimensis	0.89	0.89	0.89	0.96	0.86	0.89	0.91		0.53	0.61	0.65	0.58	0.77
B. cathcartii	0.98	0.81	0.77	0.83	0.82	0.93	0.95	0.80		0.59	0.77	0.74	0.51
B. megaptera	0.89	0.66	0.96	0.98	0.99	0.98	0.91	0.85	0.87		0.80	0.50	0.74
B. xanthina	0.97	0.73	0.91	0.91	0.94	**0.99**	0.98	0.88	0.68	0.95		0.65	0.71
B. flaviflora	0.92	0.93	0.74	0.81	0.77	0.89	0.92	0.85	0.95	0.81	0.90		0.48
B. roxburghii	0.86	0.63	0.97	0.95	0.95	0.94	0.92	0.95	0.80	0.93	0.92	0.79	

Note: Species are grouped by sections. Highest and lowest overlap values are in bold

5.4. Discussion

5.4.1. Identification of suitable habitat for B. satrapis

The use of a vegetation index contributes to the species distribution modeling process by providing information about the canopy closure, the phenological status, and the water content variation. The species distribution models that use vegetation index (NDVI) as an additional environmental variable would improve the representation of a species spatial distribution (Amaral et al. 2007). The average model output and field survey revealed that suitable habitat of *B. satrapis* occurred in the tropical deciduous *sal* forest at an elevation ranging from 400-1200 m asl of Sikkim Himalaya. The restricted distribution of suitable habitats of *B. satrapis* at lower elevations indicates its narrow endemism. The past field survey, herbarium records, and published literature also reported that species do not occur beyond Sikkim Himalaya (Nautiyal et al. 2009; Nayar and Sastry 1990). Narrowly endemic species have specific requirements that often make them vulnerable due to narrow ecological tolerances. Environmental factors such as climate, geology, and soil affect vegetation indices in time and space (Soleimani et al. 2008). Since vegetation is a reflex of the total of all such environmental factors at a site; it is also amenable to quantification by using NDVI. This makes NDVI an ideal factor representing the complex environment that determines the potential habitats of *B. satrapis.* In the present study, NDVI for May played a key role in determining the distribution of potential habitat of *B. satrapis* in its native range. Interestingly, the NDVI for May which contributed most to the final habitat model corresponds to the period of seed germination and regeneration, indicating the significance of seed germination in determining the distribution of the species and survival.

5.4.2. Population assessment

The higher number of individuals in the area of high probability threshold such as Sumbuk and Kitam in South Sikkim indicates optimal habitat conditions for the survival of the species. However irrespective of higher threshold values lower number of individuals were encountered at Badamtam and Rambi. Extensive anthropogenic disturbance such as fodder collection, habitat fragmentation, construction of roads, and regular landslides at NH 31A may have resulted in lower numbers of individuals at such sites. Nevertheless, the area with a higher number of individuals maintained greater threshold values and vice versa. The higher number of seedling and sapling at Sumbuk and Kitam is considerably high compared to Badamtam and Rambi and therefore such populations can be considered as healthy or thriving populations and later as declining or dying populations.

5.4.3. Habitat assessment

The results of the superimposed predicted distribution map on Google Earth were similar to the field observation. The habitat assessment through Google earth image and primarily through field survey revealed that the distribution of *B. satrapis* is primarily confined to lower elevation along riverside and road cutting mostly dominated by human settlement leading to anthropogenic disturbance such as road widening and cultivation. The area predicted to be suitable towards Darjeeling were mostly tea gardens owned by the private company leading to a reduction in the suitable habitat of the species, decreasing niche breadth.

5.4.4. Niche characterization in Begonia species

5.4.4.1. NDVI variables

The distribution of *Begonia* species is correlated with NDVI based on niche modeling. The importance of NDVI variables contributing to the final predictive model varied across species. The model developed for *B. gemmipara*, *B. josephii*, *B. sikkimensis*, *B. cathcartii,* and *B. flaviflora* showed the highest contribution by NDVI for July. Species like *B. picta, B. palmata, B. megaptera,* and *B. roxburghii*, usually flowers late after the monsoon and thereby NDVI for November might have been the most important predictor variables affecting the distribution of species. Interestingly NDVI for November contributed the most in predicted the distribution of *B. nepalensis*. The month of November might have contributed the most in *B. nepalensis* as the species flowers late during dry season i.e. December-January. Amongst all the 13 species of *Begonia*, *B. satrapis* is considered critically endangered and is endemic to Sikkim and Darjeeling district of West Bengal (Adhikari et al. 2018). Due to narrow geographic range having restricted distribution such taxa are more sensitive to habitat disturbance leading to extinction (Peterson and Watson 1998). The distribution of *B. satrapis* is strictly affected by NDVI for May which is the month when regeneration of early seedling from the tuber emerges.

5.4.4.2. Bioclimatic variables

The distribution of *Begonia* species is determined by response to different bioclimatic variables and each taxon presents a different level of response to environmental variables. In most of the species, temperature-related variables contributed the most in the distribution of species. The distribution of species like *B. josephii, B. picta, B. palmata, B. panchtharensis, B. sikkimensis, B. cathcartii, B. megaptera, B. xanthina, B. B. flaviflora* and *B. roxburghii* were mostly governed by BIO 2 i.e. Mean Diurnal

Range [Mean of monthly (max temp - min temp)] and BIO1 contributed the most in case of *B. gemmipara*. These are the species which flowers late after enough moisture has settled after the monsoon and therefore temperature-related variables might have contributed the most in these *Begonia* species. However, species such as *B. satrapis* start to regenerate during the late summer and early monsoon and thus BIO19 (precipitation of the coldest quarter) might have contributed the most to the final model.

5.4.5. Niche overlap and Niche Breadth

5.4.5.1. NDVI variables

It is often assumed that closely related species are morphologically and physiologically alike and have similar environmental requirements i.e. niche retention (Futuyma and Mitter 1996; Webb 2000; Violle et al. 2011). However the niche overlap test for *Begonia* species resulted in great variability in niche overlap values between morphologically similar species. The low niche overlap values between species of section *Diploclinium viz. B. satrapis* and *B. josephii* might have resulted due to competitive interaction leading to niche partitioning (Hardin 1960). Moreover, the highest niche overlap values between species of section *Diploclinium viz. B. picta* and *Platycentrum viz. B. sikkimensis* and *B. megaptera* supports the 'limiting similarity hypothesis' which suggests that "*competition exclusion of closely related species would result in frequent coexistence of distantly related species in ecological communities*".

The results of niche breadth analysis support the idea that better-performing models are associated with more specialized and narrow niche breadth and vice-versa (Fuchs et al. 2018). The model developed for *Begonia* species *viz. B. picta*, *B. panchtharensis*, *B. sikkimensis,* and *B. xanthina* were considered fair (0.8 < AUC <

0.9), with higher niche breadth indicating more ecological flexibility compared to other species of *Begonia*. These species in addition to having broader niche breadth have larger distribution areas, ranging from Western Himalaya to entire Eastern Himalaya, covering countries like Nepal, Bhutan and China (Rajbhandari et al. 2010; Rana 2016; Camfield and Hughes 2018; Hughes et al. 2019; Pradhan et al. 2019). Thus these species have wider climatic tolerance with larger variation within and amongst the population and sometimes even recognized at a variety level (Camfield and Hughes 2018). In addition to having a wider niche breadth, these species also have a wider predicted distribution area compared to other species. A case apart in *B. xanthina* with broader niche breadth and smaller area (~1905 km^2) predicted to be suitable. However, the model developed for *B. satrapis*, *B. gemmipara*, and *B. nepalensis* were considered a very good performing model with the lowest niche breadth (ranging from 0.102-0.195) indicating lesser ecological flexibility. Such species with smaller niche breadth have lesser tolerance to climatic variation preferring homogenous environmental conditions (Kassen 2002; Dennis et al. 2011). The study thus displays a positive correlation between species' niche breadth and suitable predicted area, except in the case of *B. xanthina* the results were otherwise. Niche breadth of most species was consistent with their geographic distributions, as narrowly distributed species have smaller niche breadth and broader distributed species have wider niche breadth (Gaston 1993; Kunin and Gatson 1997). The narrow distribution range of *B. satrapis*, *B. gemmipara*, and *B. nepalensis* might be primarily due to narrow niche breadth. The study is in line with the study on the Mexican genus of globular cacti and numerous other similar studies (Zhu et al. 2016; Mosco 2017). Therefore such rare species with narrow niche breadth have a higher probability of extinction (Futuyma and Moreno 1988; McKinney 1997) and thus require immediate conservation initiatives to conserve the existing extant population.

5.4.5.2. Bioclimatic variables

Similarly the niche overlap test for *Begonia* species exhibited different levels of niche overlaps when bioclimatic data were used as input variables. Unlike NDVI here the morphologically similar species *viz. B. palmata and B. xanthina* (belonging to the section *Platycentrum*) exhibited higher niche overlap and thus the assumption that closely related species have similar environmental requirements is maintained.

The niche breadth study also supported the idea that better-performing models are associated with more specialized and narrow niche breadth and vice-versa (Fuchs et al. 2018). The model developed for *B. josephii, B. flaviflora* is considered a very good performing model with AUC score of 0.94 and 0.91 with the lowest niche breadth i.e. 0.0.136 and 0.266, and except in case of *B. sikkimensis,* it showed a lower niche breadth value and fair performing model. Interestingly *B. satrapis* an endemic species displayed a wider niche breadth compared to other widely distributed species. Therefore the niche breadth hypothesis that a positive correlation between niche breadth and geographical range size (Boulangeat et al. 2012; Botts et al. 2013) is not justified. Thus the species might be having a wider climatic tolerance irrespective of having a very narrow distribution range. Similar was the case in *B. nepalensis* with higher niche breadth (0.539) and narrow area predicted to be suitable (~376 km^2). Thus, the niche breadth hypothesis, which has recently gained more support, suggesting a positive correlation between niche breadth and geographical range size is not justified and thus rejected. Brown (1984) indicated that species that can utilize a greater array of resources and that can maintain viable populations under a wider variety of conditions should become more widespread. Based on this, the niche breadth hypothesis states that species with a broad niche can persist in a wide range of different habitat types, while species with a narrow niche will be restricted to those places where their specific niche requirements are met.

Chapter 6

Morphological and Phytochemical diversity in different population of *B. satrapis* Clarke

6.1. Introduction

The variation in climate along the elevation gradients provides an excellent experimental set-up for investigating the impact of changing climate on ecosystems and terrestrial organisms (Körner 2007; De Frenne et al. 2013). In addition to several studies on species distribution and species interaction between host and herbivores (Hodkinson 2005; Halbritter et al. 2013; Read et al. 2014), attention are given to studies related to morphological and biochemical variation along the gradient (Sakata et al. 2006, Kumar et al. 2008, Guerin et al. 2012). With the increase in elevation, plants are subjected to gradually decreasing mean temperature. Therefore elevation gradient is ideal for studying the variation in species trait (Landolt 1967; Körner 2003). The plant adaptive traits evolved in response to varying environmental conditions exhibits considerable variation along the elevation gradient (Westoby and Wright 2006). The plant adapted to adverse environmental conditions at higher elevation shows reduction in plant size compared to low elevation population (Jenny-Lips 1948; Körner 2003). Apart from trait variation, species growing along elevation gradients exhibit variation in life history.

Similarly, environmental factors and habitat conditions have also been reported to cause considerable variation in both qualitative and quantitative plant secondary metabolite (PSM) among species and also across populations (Lavola et al. 1988, 1994; Julkunen-Tiitto et al. 1996). The variation in environmental factors such as season, altitude, soil etc significantly modifies the quantity and quality of PSM (Chang et al. 2006; McIntyre et al. 2009). The environmental factors such as precipitation, mean temperature, soil and atmospheric pressure etc. changes with altitude (Zidorn 2010). Studies related to variations in biochemical and morphological traits provide opportunities to examine variation in plant populations (Kim and Donohue 2011). Therefore the study on biochemical and phytochemical variation in different populations of *B. satrapis* was undertaken.

6.2. Materials and Methods

6.2.1. Field survey and phenotypic data collection

The field visit was conducted coinciding with the early flowering stage of plants at each site, *viz.* Sadam (first week of August), Kitam (second week of August), Sumbuk (Second week of August), and Rambi (third week of August). Details of geographical locations of the populations are given in Table 29. The habitat of the Sadam populations was found in fertile hill slopes where the Kitam population was found on the roadside. The population at Sumbuk was found in the terrace field and the population of Rambi was on the roadside near the river. Each site was then divided into four patches for the ease of sampling. From each patch 10 individuals were selected randomly and data on morphology were recorded (Singh et al. 2015). A total of 17 phenotypic traits (both qualitative and quantitative) were recorded in the field itself.

6.2.2. Plant sample preparation

Fresh plant sample of *B. satrapis* was thoroughly washed, sliced, and oven-dried at 60˚ C until completely dried and acquire constant weight. The dried samples were grinded into a fine powder using a grinder. The powder sample was placed in clean labeled bottles and stored at room temperature in desiccators until prominent and analysed.

Table 29: Field data collection locality of *B. satrapis* in Sikkim Himalaya

Sl. No.	District/State	Place	Geographic coordinates	Elevation (m)
1	South district/Sikkim	Sadam	27°06.766' N 88° 25.948' E	1350
2	South district/Sikkim	Sumbuk	27° 06.749' N 88° 22.771' E	800
3	South district/Sikkim	Kitam	27° 06.986'N 88° 21.145' E	600
4	Darjeeling/West Bengal	Rambi	26°59.787' N 88° 25.616' E	400

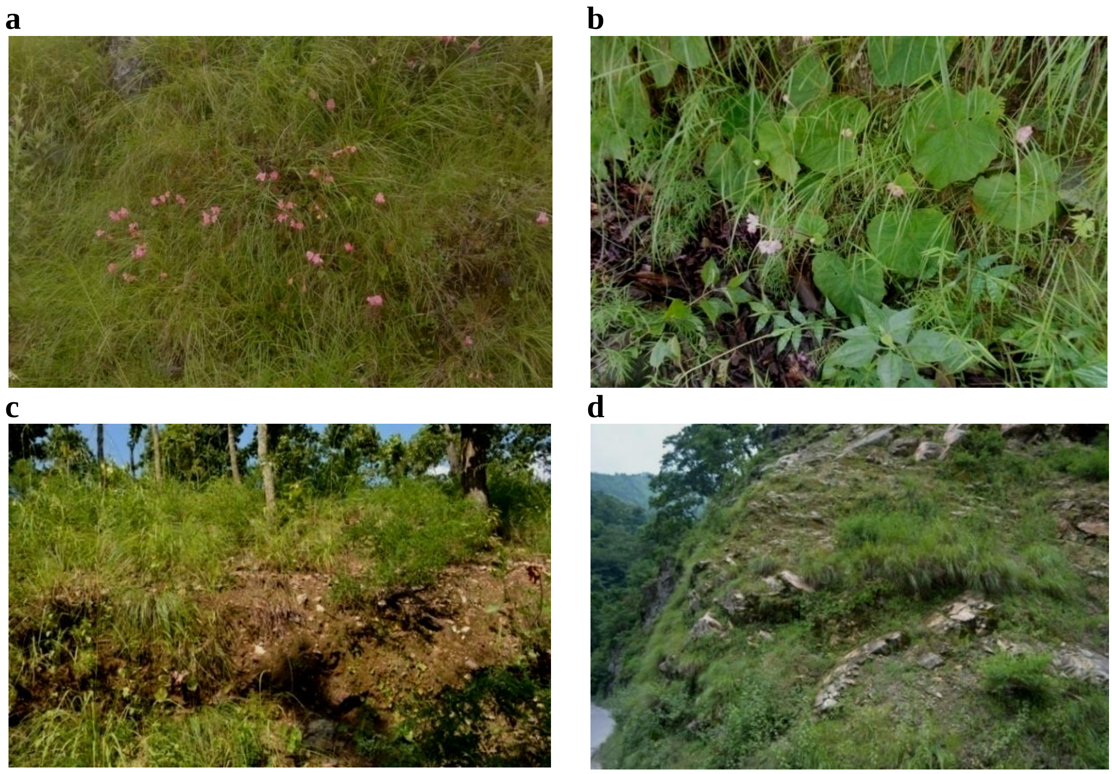

Fig. 25: Natural habitat of *B. satrapis* (a) Sadam (b) Sumbuk (c) Kitam (d) Rambi

6.2.3. Preparation of plant extracts

20 g of dried plant sample material was used for extract preparation. Samples were extracted separately in 250 ml of 80% aqueous methanol in a soxhlet apparatus at 40º C for 8 hours. The plant extracts were then filtered through a muslin cloth and then through Whatman filter paper. The process was repeated twice i.e., to the residue remaining after filtration, 250 ml of respective solvents were added and extracted. The three extracts were then concentrated under rotavac and combined. The filtrate concentrated under rotavac at 50^{0}C, brought to room temp, and incubated at -80^{0}C overnight and lyophilized.

Stock solutions (mg/ml) were prepared by dissolving the powdered extracts in respective solvents. Working solution 200µg/ml was prepared from the stock to be used for different analyses unless otherwise mentioned.

6.2.4. Qualitative Phytochemical analysis

To determine the presence or absence of different phytoconstituents (*viz*. alkaloids, flavonoids, tannins, phlobatanins, triterpenes, steroids, saponins, glycosides, cardiac glycosides, anthraquinones, resins), preliminary phytochemical tests were performed following the standard protocols (Harborne 1973; Trease and Evans 1989; Sofowora 1993).

6.2.5. Quantitative Phytochemical analysis

6.2.5.1. Crude Alkaloids Determination

The alkaloids content was determined using the method of Harborne (1973). 2.5 g sample was weighed and to which was added 50 ml of 5% acetic acid in ethanol and was allowed to stand for 4h. The filtered was extracted and concentrated on a water bath to $1/4^{th}$ of the original volume. To which was added a concentrated ammonium hydroxide until the precipitation was complete. The solution was settled, precipitated, collected and washed with dilute ammonium hydroxide and filtered. The residue is the alkaloid, which was dried and weighed.

6.2.5.2. Determination of total Phenol

The total phenolic contents in each extract were estimated following the method by Lin et al. 2011. Aliquot of 1.0 ml of extracts prepared in each solvent was mixed with 5 ml of 10 fold diluted Folin ciocalteu reagent and 4 ml of 7.5% sodium carbonate. The mixture was allowed to stand for 90 minutes at room temperature and absorbance was measured at 760 nm in a UV vis spectrophotometer. The amount of phenol content was determined using Gallic acid as standard.

6.2.5.3. Determination of total Flavonoid

The total flavonoid content was measured using the Aluminium chloride colorimetric method (Lin et al. 2011).

6.2.5.4. Determination of total Flavonol

The total flavonols content was measured by the standard methods (Kumaran and Karunakaran 2006).

6.2.5.5. Determination of total Tannins

The total tannins content was determined by the Folin – Ciocalteu method (Tambe and Bhamber 2014).

6.2.6. Climatic data

The climatic data were extracted using the climatic data interpolation methods as described by Hijmans et al. 2005. These data having a high spatial resolution (1 km^2) consider being a more accurate global elevation dataset and can be used for studies in mountain environments (Hijmans et al. 2005). The climatic data were obtained from the Worldclim database (https://www.worldclim.org/) and the data were extracted using DIVA-GIS software version 7.5. (http://www.diva-gis.org/). The details of the climatic data for the four study sites are given in table 31. The 19 bioclimatic and altitudinal data were further regressed with quantitative phytochemical and morphological traits.

6.2.7. Statistical Analysis

Analysis of variance (ANOVA) was performed to access the significance of the variation observed in the both quantitative phytochemical and phenotypic traits between populations. The cluster analysis was performed via unweighted pair group method to develop a dendogram. A two dimensional principal component analysis (PCA) was constructed to provide another means of testing the relationship among *Begonia* species. All the statistics was computed using PAST software available online.

6.3. Results and Discussion

6.3.1. Habitat type

The natural habitat of *B. satrapis* in four different study sites along the elevation gradient varied significantly. The habitat at Rambi and Sadam had open canopy cover, less human settlement with south-facing slope. Kitam and Sumbuk had close canopy cover and were densely human-populated (Fig. 25; Table 30).

Table 30: Study sites along with elevation and geographic coordinates

State	District	Locality	Altitude (m)	Slope	Geographic coordinate	Habitat type
Sikkim	South	Sadam	1300	70-80	27° 06.766' N 88° 25.948' E	Canopy open, less human settlement
Sikkim	South	Sumbuk	800	40-50	27° 06.749' N 88° 22.771' E	Human settlement, closed canopy cover
Sikkim	South	Kitam	600	50-60	27° 06.986' N 88° 21.145' E	Human settlement, closed canopy cover
West Bengal	Darjeeling	Rambi	400	70-80	26° 59.787' N 88° 25.616' E	Canopy open, Roadside close to river Teesta

6.3.2. Climatic conditions along the altitudinal gradient

There was a significant variation in the overall climatic conditions in the selected sites (Table 31). The maximum and minimum temperatures during the peak flowering period (i.e. August) ranged from 29.4-22.6˚ C at Sumbuk, 27.8-21.5˚ C at Kitam, 27.5-21.3˚ C at Sadam and 29.3-23.3˚ C at Rambi. The rainfall varied from 566, 575, 576 and 622 mm respectively. The minimum and maximum annual temperature along all the four study site decreases with increase in altitude (for tmax $R^2 = 0.93$, $p = 0.004$; tmin $R^2 = 0.92$, $p = 0.0009$) along with marginal decrease in annual rainfall ($R^2 = 0.69$, $p = 0.142$).

Table 31: List of 19 bioclimatic variables extracted using DIVA-GIS software (www.worldclim.org)

Sl. No.	Bioclimatic variables	Sadam	Sumbuk	Kitam	Rambi
1	Annual mean temperature (BIO1)	20.8	22.1	20.8	22.7
2	Mean monthly temperature range (BIO2)	8.7	9.6	8.8	9.8
3	Isothermality (BIO3)	46.5	48.5	46.8	47.8
4	Temperature seasonality (BIO4)	397	405.4	398.7	409.4
5	Maximum temperature of the warmest month (BIO5)	27.6	29.5	27.9	30.3
6	Minimum temperature of the coldest month (BIO6)	8.9	9.7	9	9.8
7	Temperature annual range (BIO7)	18.7	19.8	18.9	20.5
8	Mean temperature of wettest quarter (BIO8)	24.3	25.9	24.6	26.5
9	Mean temperature of driest quarter (BIO9)	15.8	17.1	16	17.7
10	Mean temperature of warmest quarter (BIO10)	24.3	25.9	24.6	26.5
11	Mean temperature of coldest quarter (BIO11)	14.9	16.2	15	16.8
12	Annual precipitation (BIO12)	2729	2766	2730	3047
13	Precipitation of wettest month (BIO13)	762	768	760	847
14	Precipitation of driest month (BIO14)	3	4	3	3
15	Precipitation seasonality (CV) (BIO15)	117.2	116	116.9	117
16	Precipitation of wettest quarter (BIO16)	1874	1887	1871	2088
17	Precipitation of driest quarter (BIO17)	33	34	33	32
18	Precipitation of warmest quarter (BIO18)	1874	1887	1871	2088
19	Precipitation of coldest quarter (BIO18)	33	35	35	33

6.3.2. Phenotypic variation between different populations

The phenotypic variation in *Begonia* species is common; mostly in leaf colouration resulting in higher chlorophyll content (Wang et al. 2016). The correlation matrix constructed for *B. satrapis* using 17 quantitative characters showed a significant variation in most of the traits. At the population level, the Rambi population was characterized by a taller plant sample with a larger leaf size compared to the other three populations. The corresponding variation in plant trait (i.e. increase in size with decrease in elevation) was also reported in Mediterranean mountain grasslands (Pescador et al. 2015). Moreover, the population at Rambi also had more female flower dominated plant specimens (Table 32). The result supports the idea that, sex ratio (male: female) increases significantly with elevation (i.e. male biased at higher elevation) (Ortiz et al. 2002). The higher number of female flower at lower elevation may have resulted due to inflorescence architecture leading to alteration in floral sex ratio; such was the case in *B. conchifolia* and *B. plebeja* (Twyford et al. 2014). However, statistically there was no significant difference between the number of the female flower and altitude ($P = 0.16$) (Table 32).

The mean of all the quantitative characters along with the standard error of all the four populations is given in table 32. ANOVA analysis showed 15 out of 17 traits differed significantly amongst populations (Table 33). There was a significant difference in most of the traits in four populations; however there was no significant variation encountered in traits like number of stamens ($p<0.05$) and stamen's length ($p<0.05$). The results of the pair-wise comparison of morphological traits in four populations of *B. satrapis* are given in table 34. Most of the traits were significantly different between Rambi and Sadam populations. The altitudinal difference between these two populations might have resulted in variation in most of the traits.

Table 32: Mean of 17 quantitative trait and regression analysis with altitude

Sl. No.	Trait	Sadam (±SE)	Sumbuk (±SE)	Kitam (±SE)	Rambi (±SE)	F-value	p-value
	Quantitative traits						
1	**Plant height (cm)**	23.10 ±0.42	37.80 ±0.94	56.84 ±1.54	75.14 ±1.34	20.60	**0.04***
2	**Leaf length (cm)**	6.01 ±0.22	12.89 ±0.41	15 ±0.54	23.22 ±0.47	21.29	**0.04***
3	**Leaf breadth (cm)**	8.33 ±0.31	15.93 ±0.45	18.72 ±0.64	22.71 ±0.48	557.78	**0.001****
4	**Petiole length (cm)**	2.47 ±0.17	11.75 ±0.78	14.66 ±1.08	22.88 ±0.46	46.46	**0.02***
5	No. of male flowers	5.52 ±0.49	7.37 ±0.75	8.07 ±1.07	11.07 ±0.98	12.39	0.07 ns
6	Pedicel length (cm)	0.46 ±0.05	0.94 ±0.08	0.57 ±0.08	2.02 ±0.17	2.12	0.28 ns
7	Peduncle length (cm)	0.45 ±0.05	1.86 ±0.20	0.71 ±0.11	2.16 ±0.18	1.61	0.33 ns
8	No. of Stamens	37.07 ±2.40	30.1 ±2.15	27.05 ±2.88	29.175 ±2.41	8.84	0.09 ns
9	Stamens length (cm)	0.30 ±0.02	0.3 ±0.02	0.27 ±0.03	0.35 ±0.03	0.28	0.64 ns
10	Connectivity length(cm)	0.20 ±0.04	0.14 ±0.01	0.13 ±0.01	0.295 ±0.03	0.15	0.72 ns
11	No. of female flowers	0.42 ±0.18	2.62 ±0.49	3.55 ±0.55	11.22 ±0.95	4.58	0.16 ns
12	Stipule breadth (cm)	0.16 ±0.02	0.24 ±0.01	0.24 ±0.01	0.37 ±0.03	8.61	0.09 ns
13	Stipule length (cm)	0.19 ±0.02	0.26 ±0.02	0.26 ±0.02	0.402 ±0.03	6.38	0.12 ns
14	Bracts length (cm)	0.40 ±0.02	0.46 ±0.02	0.52 ±0.03	0.81 ±0.06	4.19	0.17 ns
15	Bracts breadth (cm)	0.35 ±0.01	0.33 ±0.02	0.42 ±0.01	0.875 ±0.08	1.96	0.29 ns
16	**Length of style (cm)**	0.02 ±0.01	0.13 ±0.02	0.15 ±0.24	0.282 ±0.02	18.99	**0.04***
17	Fruit size (cm)	0.09 ±0.05	0.67 ±0.12	0.74 ±0.12	0.625 ±0.05	5.92	0.13ns

Note: p<0.05 is highlighted in bold; ns = Non-significant

Table 33: Result of ANOVA testing in morphological traits amongst populations of *B. satrapis* from different altitudes

Sl. No.	Traits	DF	F-value	P-value	Significance
1	Plant height	3	492.471	**7.99E-13**	***
2	Leaf length	3	114.646	**4.24353E-09**	***
3	Leaf breadth	3	135.455	**1.61E-09**	***
4	Petiole length	3	42.908	**1.09E-06**	***
5	No. of male flowers	3	4.499	**0.024577798**	*
6	Pedicel length	3	67.150	**9.03E-08**	***
7	Peduncle length	3	18.096	**9.48E-05**	***
8	No. of stamens	3	2.770	0.087362	ns
9	Stamens length	3	1.352	0.303978	ns
10	Connectivity length	3	9.762	**0.001532**	**
11	No. of female flowers	3	43.343	**1.03E-06**	***
12	Stipule breadth	3	8.367	**0.002853**	**
13	Stipule length	3	6.858	**0.006059**	**
14	Bracts length	3	14.330	**0.000285**	***
15	Bracts breadth	3	10.697	**0.001043**	**
16	Length of style	3	12.077	**0.000616944**	***
17	Fruit size	3	4.808	**0.02008**	*

Note: The P-value of significant trait are in bold; (ns, Non-significant, $p>0.05$; $^{*}p<0.05$; $^{**}p<0.001$; $^{***}p<0.0001$)

6.3.3. Phenotypic trait vs altitude vs climate

The quantitative phenotypic trait in four populations of *B. satrapis* was significantly associated with altitude as indicated by regression analysis of 17 morphological traits combined with altitude. Out of the 17 quantitative traits, five (*viz*. plant height, F = 20.6, P = 0.04; leaf length, F = 21.29, P = 0.04; leaf breadth, F = 557.78, P = 0.001; petiole length, F = 0.46.46, P = 0.02 and length of style, F = 18.99, P = 0.04) were significantly associated with altitude. The rest of the traits were not significant with altitude (Table 32). The 19 bioclimatic variables, treated as independent variables, were also not significant with phenotypic traits.

Table 34: Results of pair wise comparison of morphological traits (ns, Non-significant, p>0.05; *p<0.05; **p<0.001; ***p<0.0001)

Sl. No.	Traits	Sadam vs Sumbuk	Sadam vs Kitam	Sadam vs Rambi	Sumbuk vs Kitam	Kitam vs Rambi	Sumbuk vs Rambi
1	Plant height	***	***	***	***	***	***
2	Leaf length	***	***	***	ns	***	**
3	Leaf breadth	***	***	**	*	**	***
4	Petiole length	***	**	***	ns	*	***
5	No. of male flowers	Ns	ns	**	ns	*	Ns
6	Pedicel length	**	ns	***	*	***	***
7	Peduncle length	**	ns	***	*	**	Ns
8	No. of stamens	Ns	ns	*	ns	Ns	Ns
9	Stamens length	Ns	ns	ns	ns	Ns	Ns
10	Connectivity length	Ns	ns	ns	ns	**	**
11	No. of female flowers	*	*	***	ns	***	***
12	Stipule breadth	Ns	ns	**	ns	**	*
13	Stipule length	Ns	**	**	ns	*	Ns
14	Bracts length	Ns	ns	**	ns	*	**
15	Bracts breadth	Ns	ns	*	ns	*	*
16	Length of style	*	*	***	ns	*	*
17	Fruit size	*	*	**	ns	Ns	Ns

6.4. Comparative estimation of phytochemical between four populations of B. satrapis

6.4.1. Qualitative phytochemical analysis

Phytochemicals such as flavonoid, alkaloid, tannin, phlobatannin, and resin were present in all the four populations (*viz*. Sumbuk, Kitam, Sadam, and Rambi). However, their concentration varied amongst the population. The three phytochemicals such as alkaloids, tannins and phlobatannin were present in moderate concentration and phytochemicals such as flavonoids and resins in low concentration. The phytochemicals such as triterpene, steroids, saponins, glycoside, cardiac glycosides and anthraquinones were not present in all the four sampled populations (Table 35).

Table 35: Preliminary qualitative phytochemical analysis

Sl. No.	Phytochemical analysis	Sadam	Sumbuk	Kitam	Rambi
1	Alkaloids	++	++	++	++
2	Flavonoids	+	+	+	+
3	Tannins	++	++	++	++
4	Phlobatannins	++	++	++	++
5	Triterpene	-	-	-	-
6	Steroids	-	-	-	-
7	Saponins	-	-	-	-
8	Glycoside	-	-	-	-
9	Cardiac glycosides	-	-	-	-
10	Anthraquinones	-	-	-	-
11	Resins	+	+	+	+

Note: Sign (+) denotes presence of low concentration, (++) denotes presence of moderate concentration, (-) denotes absence.

6.4.2. Quantitative phytochemical analysis

Phytochemicals such as phenols, alkaloids, flavonoids, flavonols and tannins were present in all the four sampled population, but their quantity varied across populations. The quantity of phytoconstituents increased with increase in altitude (Aslam et al. 2015). The highest amount of phenol and flavonoids were detected at Sadam (1300 m asl) and lowest at Rambi population (400 m asl). Similarly, alkaloid was highest at Rambi and lowest at Sumbuk. The flavonol concentration was highest at Kitam and lowest at Sadam. The concentration of tannins was highest at Sadam and lowest at Sumbuk (Table 35).

6.4.2. Phytochemical constituent vs altitude

A strong positive correlation was observed between phytoconstituent and altitude. Moreover there was a significant variation in total phenol content (R^2 = 0.92, P = 0.04) and total flavonoids content (R^2 = 0.90, P = 0.04) along the elevation gradient. However the amount of phytoconstituents *viz*. alkaloids (R^2 = 0.56, P = 0.24), flavonol (R^2 = 0.75, P = 0.13) and tannins (R^2 = 0.79, P = 0.11) were not significant and did not differ among the four populations along the elevation gradient (Table 36; Fig. 26).

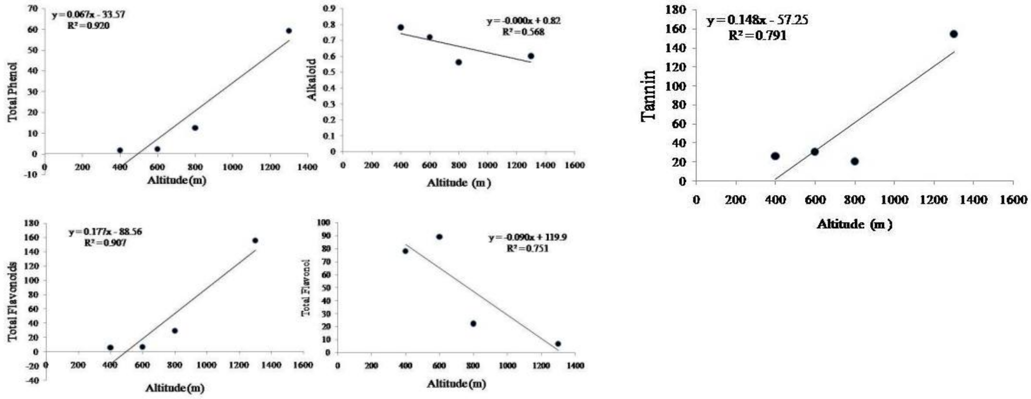

Fig. 26: Fitted regression lines and scatter plots for five phytoconstituent as dependent variables and altitude as independent variables.

Table 36: Quantitative phytochemical analysis between the different populations of *B. satrapis*

Phytoconstituent	**Populations/altitude**				**F value**	**P value**	**R**
	Sadam (1300 m)	Sumbuk (800 m)	Kitam (600 m)	Rambi (400 m)			
Total Phenol (µg/GAE/DW)	59.21 ±0.02	12.47 ±0.009	2.37 ±0.004	1.75 ±0.003	23.02	**0.04**	0.95
Alkaloids (µg/Colchicine E/DW)	0.60	0.56	0.72	0.78	2.63	0.24	0.75
Total Flavonoids (µg/QE/DW)	155.37 ±0.01	28.8 ±0.008	6.02 ±0.095	5.09 ±0.10	19.58	**0.04**	0.95
Total Flavonol (µg/Rutin E/DW)	7.29 ±0.008	22.89 ±0.01	89.35 ±0.01	78.50 ±0.01	6.03	0.13	0.86
Tannin (µg/Catechin E/DW)	153.98 ±0.01	20.3 ±0.003	30.47 ±0.002	25.80 ±0.001	7.58	0.11	0.88

(Note: p<0.05 is highlighted in bold)

6.4.3. PCA and Cluster analysis

The two dimensional plot generated from PCA using both morphological and biochemical attributes showed clustering patterns of the UPGMA dendrogram. In the 2D plot, Sadam population was found farthest and distinct as in UPGMA dendrogram. The population of Rambi and Kitam were closely related and formed a group similar to the UPGMA dendrogram (Fig. 28, 29). The analysis gave four principal components (PCs), out of which the first three principal components contributed 99.64 % of the total variability. The first two principal components accounted for 95.79 % of the total variability, in which the highest variation was contributed by the first components (69.1 %), followed by second (26.69 %) and third components (3.84 %) (Fig. 27). The first PC was influenced by the biochemical attributes like total flavonoid, flavonol, tannin content and morphological parameters like plant height, leaf length, leaf breadth, petiole length and number of stamens. The second PC was mostly influenced by the biochemical attributes like total phenol, flavonoid and tannin content and one morphological character i.e. number of stamens. The third PC was influenced by alkaloids, flavonol and tannin content and morphological attributes like pedicel and peduncle length, stamens and connectivity length, stipules length and breadth, bracts length and breadth, style length, fruit size and number of female flowers (Table 37).

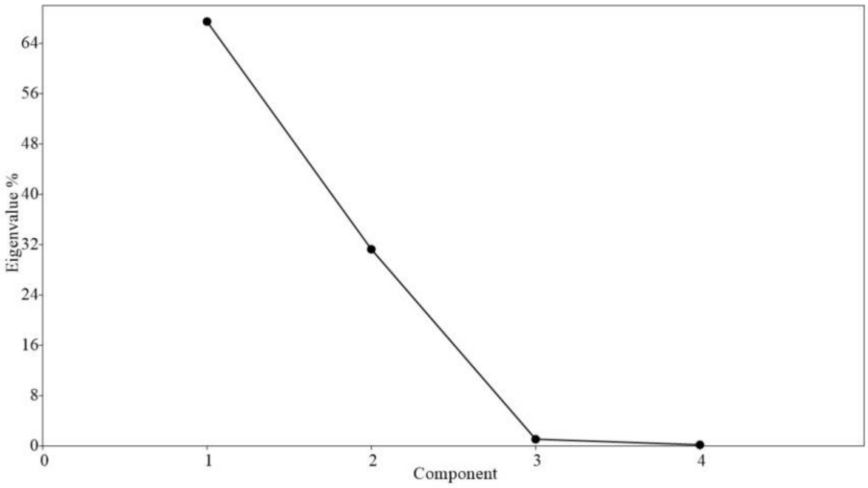

Fig. 27: Percentage variance of four principal components

Table 37: Details of principal components based on phytochemical and morphological attributes

Sl No.	Axis	PC1	PC2	PC3	PC4
	Phytoconstituents				
1	Total Phenol	-0.21459	**1.1063**	-0.11359	-0.08191
2	Alkaloid	-1.1731	-0.20546	**0.087255**	-0.02122
3	Total Flavonoid	**1.2738**	**3.1542**	-0.21154	-0.07714
4	Total Flavonol	**4.0858**	-1.9745	**0.93337**	-0.2043
5	Total Tannin	**1.9962**	**2.3163**	**0.9321**	0.15794
	Morphological traits				
1	Plant Height	**3.9767**	-0.88295	-0.64268	0.26421
2	Leaf Length	**0.35788**	-0.37776	-0.27693	0.10551
3	Leaf Breadth	**0.59539**	-0.31448	-0.36618	-0.06732
4	Petiole Length	**0.26707**	-0.45817	-0.24398	0.11527
5	No. of Male Flowers	-0.38255	-0.22567	-0.11357	-0.00067
6	Pedicel Length	-1.1288	-0.21674	**0.060789**	0.015399
7	Peduncle Length	-1.0785	-0.20209	**0.00521**	-0.0038
8	No. of Stamens	**1.797**	0.30861	-0.81212	-0.27634
9	Stamens Length	-1.2091	-0.20396	**0.089918**	-0.01922
10	Connectivity Length	-1.2222	-0.20634	**0.09592**	-0.01441
11	No. of Female Flowers	-0.74556	-0.36018	**0.04128**	0.20855
12	Stipule Breadth	-1.213	-0.20772	**0.091866**	-0.01695
13	Stipule Length	-1.2106	-0.20746	**0.091377**	-0.0168
14	Bracts Length	-1.1832	-0.20902	**0.087316**	-0.01351
15	Bracts Breadth	-1.1907	-0.21216	**0.093022**	-0.00621
16	Length of Style	-1.2235	-0.21005	**0.095397**	-0.0157
17	Fruit Size	-1.1743	-0.21071	**0.075768**	-0.03136

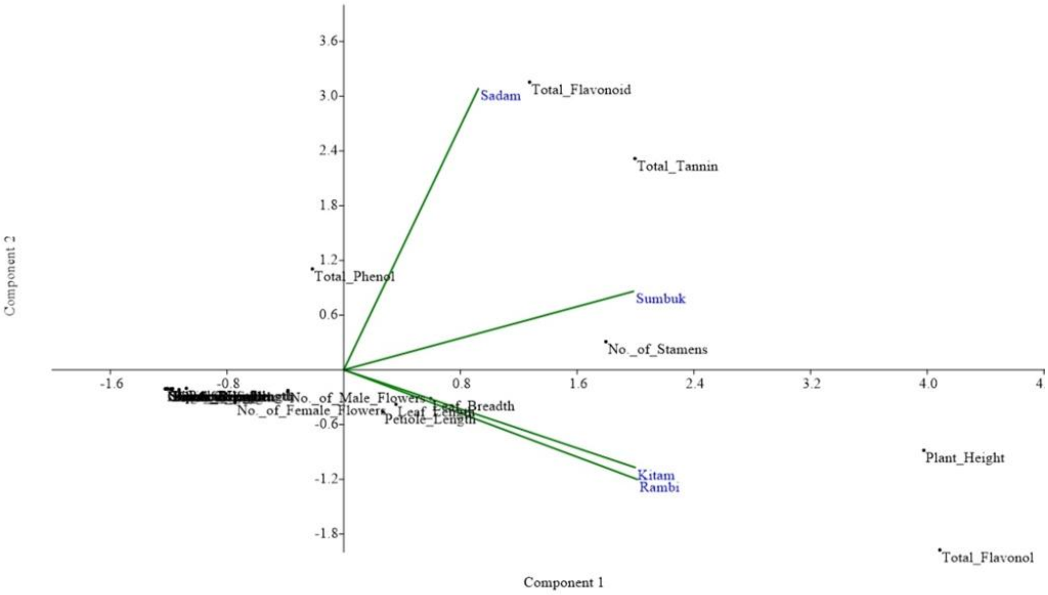

Fig. 28: 2D plot generated for four population of *B. satrapis* using UPGMA analysis based on phytochemical and morphological attributes

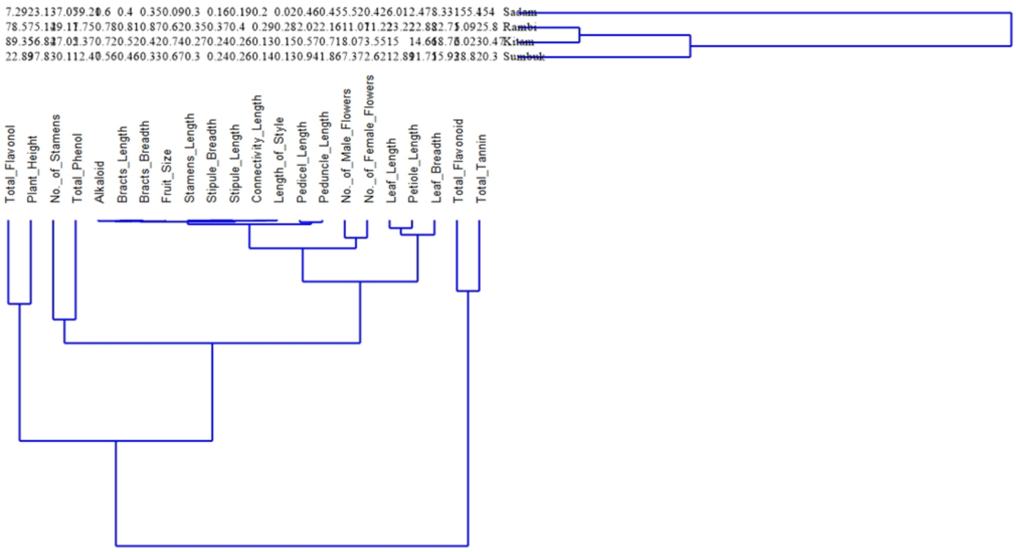

Fig. 29: Two way clusters generated for four population of *B. satrapis* using UPGMA analysis based on phytochemical and morphological attributes

6.5. Comparative phytochemical analysis between six species of Begonia

6.5.1. Total Phenols Content

The data presented in table 38 indicated a significant difference in total phenol content in *Begonia* species. The concentration of phenols varied from 31.9 µg/GAE in *B. sikkimensis* -103.9 µg/GAE in *B. picta*. The highest concentrations of phenols were present on *B. picta* followed by *B. megaptera*, *B. panchtharensis*, *B. josephii*, *B. roxburghii* and *B. sikkimensis*.

6.5.2. Total Flavonoid and Flavonol Content

Similarly the data presented in table 38 indicated a significant difference in total flavonoid and flavonol content in *Begonia* species. The highest concentration of flavonoid was present in *B. megaptera* (73.6 µg/QE) followed by *B. josephii* (57.4 µg/QE), *B. roxburghii* (52.01 µg/QE), *B. sikkimensis* (29.2 µg/QE), *B. picta* (15.7 µg/QE) and *B. panchtharensis* (14.09 µg/QE).

The concentration of flavonol was also significantly different in *Begonia* species. The highest amount of was present in *B. picta* (129.2 µg/Rutin E) followed by *B. roxburghii* (81.8 µg/Rutin E), *B. megaptera* (60.3 µg/Rutin E), *B. josephii* (47.4 µg/ml), *B. sikkimensis* (41.4 µg/Rutin E) and *B. panchtharensis* (17.3 µg/Rutin E).

6.5.3. Total Tannin

The total tannin present in *Begonia* species was comparable low with respect to other three metabolites. The highest concentration of tannins was present in *B. picta* (54.9) followed by *B. josephii* (41.8 µg/Catechin E), *B. megaptera* (39.6 µg/Catechin E), *B. panchtharensis* (34.05 µg/Catechin E), *B. sikkimensis* (33.1 µg/Catechin E) and *B. roxburghii* (3.9 µg/Catechin E).

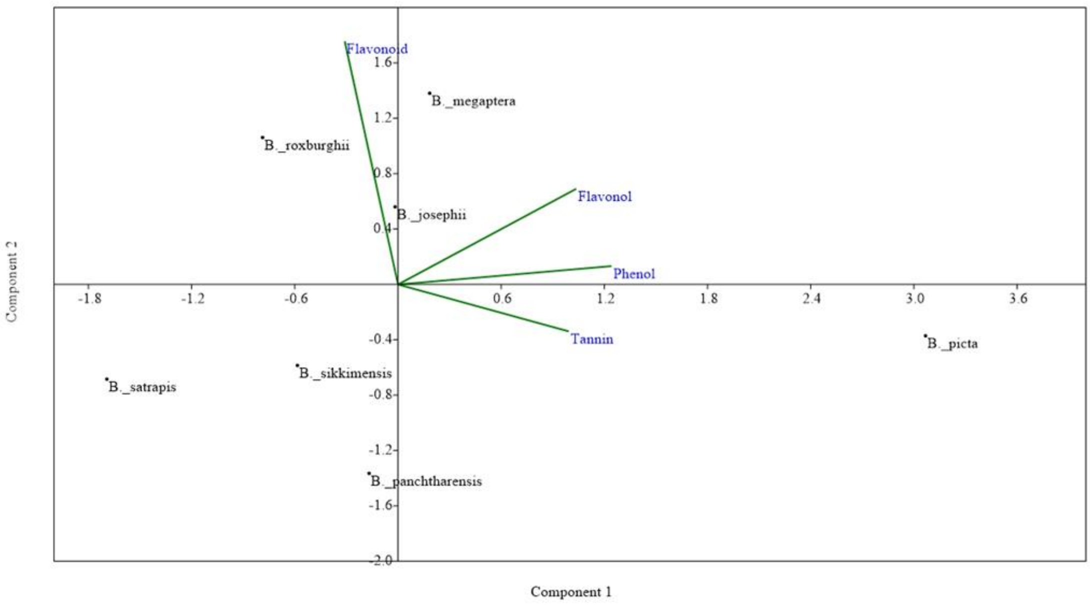

Fig. 30: 2D plot generated for seven species of *Begonia* using UPGMA analysis based on biochemical attributes

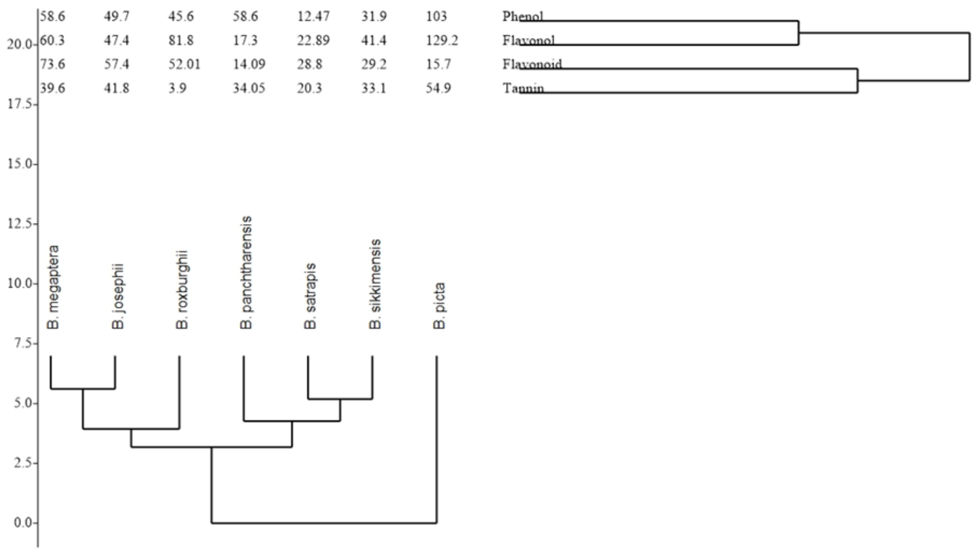

Fig. 31: Two way clusters generated for seven species of *Begonia* using UPGMA analysis based on biochemical attributes

Table 38: Comparative quantitative phytochemical screening amongst the few selected species of *Begonia*

Sl. No.	Species	Phenol (µg/GAE/DW) ±SD	Flavonoids (µg/QE/DW) ±SD	Flavonol (µg/Rutin E/DW) ±SD	Tannin (µg/Catechin E/DW) ±SD
1	*B. sikkimensis*	31.9±0.02	29.2±0.02	41.4±0.01	33.1±0.005
2	*B. picta*	103.9±0.4	15.7±0.01	129.2±0.05	54.9±0.009
3	*B. megaptera*	58.6±0.02	73.6±0.004	60.3±0.01	39.6±0.008
4	*B. roxburghii*	45.6±0.007	52.01±0.06	81.8±0.13	3.9±0.003
5	*B. panchtharensis*	58.6±0.02	14.09±0.004	17.3±0.001	34.05±0.003
6	*B. josephii*	49.7±0.009	57.4±0.02	47.4±0.03	41.8±0.005

Table 39: Details of principal components based on biochemical attributes

Sl. No.	Axis	PC1	PC2	PC3	PC4
	Phytoconstituent				
1	Phenol	0.9097	-0.223	0.2878	-0.1997
2	Flavonoid	-0.08065	0.9281	0.3627	-0.02256
3	Flavonol	0.9627	0.1758	-0.196	0.06261
4	Tannin	0.5086	-0.3981	0.6623	0.3797

6.5.4. PCA and Cluster analysis

The two dimensional plot generated from PCA showed three groups that were found similar to the clustering pattern of the UPGMA dendrogram (Fig. 30, 31). In the 2D plot, *B. picta* was found farthest and distinct as in UPGMA dendrogram. The second group was formed by *B. satrapis*, *B. sikkimensis* and *B. panchtharensis* and the third group by *B. megaptera*, *B. josephii* and *B. roxburghii* (Fig. 29). The analysis gave four principal components (PCs), out of which the first three principal components contributed 96.999 % of the total variability. The first two principal components accounted for 81.075 % of the total variability, in which the highest variation was contributed by the first components (55.808 %), followed by second (25.667%) and third components (15.924 %). The first PC was influenced by total phenol content, total flavonoid content and total tannin content. The second PC was influenced by the total flavonoid and flavonol content. The third PC was influenced by total phenol, flavonoid and tannin content (Table 39).

Cluster analysis of biochemical constituents' revealed a clear separation of *Begonia* species into three groups indicating chemical differentiation between species. The cluster formed by *B. sikkimensis* and *B. panchtharensis* is also supported by their distinctive morphological character (Rajbhandari et al. 2010). Furthermore, the above result is limited in support of biochemical classification of *Begonia* species and hence incorporation of other biochemical parameters may lead to further differentiation.

7.1. Taxonomy

A total of 13 species of *Begonia* have been inventoried from Sikkim Himalayas. These 13 species of *Begonia* belonged to the four section *viz*. *Diploclinium* (3 species), *Platycentrum* (7 species), *Monopteron* (1 species), *Sphenanthera* (1 species), and *Putzeysia* (1 species). Of the 13 species, 10 are recorded from Northeast India and some from neighbouring countries such as Nepal, Bhutan, and Bangladesh. The remaining three are either strictly endemic (*viz*. *B. satrapis*) or only reported from the Sikkim Himalayan region (*viz*. *B. panchtharensis* and *B. gemmipara*) and Nepal. *B. panchtharensis*, previously believed to be native to Nepal, was discovered from the Sikkim Himalayas, adding one more species of *Begonia* to the floral riches of India. The variety *B. sikkimensis* var. *kamengensis* earlier reported from the Indian state of Arunachal Pradesh state was reported from Sikkim.

The Sikkim Himalayan *Begonia* is diverse in number and colour of tepals. The number of tepals varies from two to four in male flowers and four to five in female flowers. The colour of the flower varies from white, pink, red and yellow. Usually the fruits are winged except for *B. roxburghii* where fruits are wingless and four lobed.

7.2. Preliminary threat assessment

Preliminary threat assessment using IUCN criteria B1 i.e. Extent of occurrence (EOO) and Area of Occupancy (AOO) categorized the *Begonia* species into different threat categories. Thus on the basis of assessment, seven species *viz*. *B. josephii*, *B. picta*, *B. palmata*, *B. flaviflora*, *B. sikkimensis*, *B. megaptera* and *B. catcarthii* have been categorized as **endangered** and five species *viz*. *B. gemmipara*, *B. nepalensis*, *B.*

xanthina, *B. panchtharensis* and *B. roxburghii* were categorized as **critically Endangered**. In addition, the IUCN ver. 1.3 classifications scheme was followed for assigning the threat status of *B. satrapis* and based on criteria B, C and D, the species was assigned to **critically endangered**.

7.3. Ecological Niche Modeling

B. satrapis which was categorized as **critically endangered** were inventoried within the area of occurrence spread over seven 1km × 1km grids. Of the 1948 individuals, 505 were mature flowering individuals, 600 sapling and 890 seedlings. The total number of individuals, including flowering, sapling, and seedlings, was higher in the localities under very high suitability class and vice-versa. However, when the model threshold values and population size were regressed, a weak positive correlation was observed indicating that the high suitability class does not necessarily have a higher individual ($r = 0.454$; $p = 0.304$).

The present study thus demonstrated the importance of ecological niche modeling (ENM) in predicting the potential habitats of endangered species for in-situ conservation through reintroduction. The potential habitat distribution map for *B. satrapis* can aid in the planning of land use management for existing populations, define high-priority survey sites, or set priorities for preserving its natural habitat for more effective conservation. The approach discussed here could be used to quantify habitat management trends for many threatened and vulnerable plant and animal species in other areas and could support field surveys and allocation of conservation and restoration efforts. The areas identified for the reintroduction of *B. satrapis* will not only aid in the restoration of degraded habitats where the species had existed previously; it will also serve to rehabilitate and improve its conservation status.

Consequently, the findings will be quite useful for natural resource managers to manage the species and conserving overall biological diversity in the region.

The comparative study of the ecological niche on the basis of NDVI and the bioclimatic variables revealed an accurate and effective model. However, when NDVI was used as input variables (AUC ranging from 0.82-0.97), the model showed greater accuracy compared to bioclimatic variables (AUC ranged from 0.80-0.96). In addition, the analysis of niche breadth and niche overlap revealed a positive correlation ($r = 0.747$, $p = 0.003$) between niche breadth and predicted suitable area when NDVI was used as input variables. The predicted suitable area and niche breadth of the *Begonia* species was not significant ($r = 0.217$, $p = 0.475$) when bioclimatic data were used as an input variable.

7.4. Phenotypic and phytochemical variation

The phenotypic and phytochemical variation in B. satrapis along the altitudinal gradient was studied. The correlation matrix constructed for *B. satrapis* using 17 quantitative characters displayed a significant variation in most of the traits. The population of Rambi was characterized by taller plant samples with a larger leaf size compared to the other three populations. In addition the population of Rambi also had more female flower dominated plant specimens. The result supports the idea that, sex ratio (male: female) increases significantly with elevation. The higher number of female flowers at lower elevation could have arisen from inflorescence architecture due to modification of the floral sex ratio. Statistically, though there was no significant difference between the number of the female flowers and altitude.

A strong positive correlation was observed between phytoconstituent and altitude. In addition, there was a significant variation in total phenol content (R^2 =

0.92, P = 0.04) and total flavonoids content (R^2 = 0.90, P = 0.04) along the elevation gradient. However the amount of phytoconstituents *viz*. alkaloids (R^2 = 0.56, P = 0.24), flavonol (R^2 = 0.75, P = 0.13) and tannins (R^2 = 0.79, P = 0.11) were not significant and did not differ between the four populations along the elevation gradient. Even though the technique is relatively simple the results provide evidence of how elevation shapes the morphology and phytochemical constituent in *Begonia*.

7.5. Phytochemical variation in Begonia species

Cluster analysis of seven *Begonia* species based on four phytoconstituent *viz*. total phenol, total flavonoid, total flavonol and total tannins showed a clear separation of *Begonia* species into three groups indicating chemical differentiation between species. In addition, the above finding is restricted in support of biochemical classification of *Begonia* species and therefore the inclusion of other biochemical parameters may contribute to further differentiation. A further molecular level study may provide a more in-depth analysis of the relationship between *Begonia* species.

The family Begoniaceae is the major angiospermic group. The genus *Begonia* under Begoniaceae is one of the species rich genus and is often used as a model plant to study the bio-geographic pattern of species. The list of future prospects and opportunities with regard to study of *Begonia* are as follows:

1. **Phylogeny and ecological niche modeling**

   Comparative phyloclimatic analysis and ecological niches evolution, based on bioclimatic variables and molecular phylogeny, will provide an opportunity to address questions related to speciation and evolution.

2. **Anthocyanins**

   Anthocyanins, which are thought to provide photo-protection under stressful conditions, provide opportunity to examine the role of *Begonia* species in photo-protection. In addition, the intra and inter-specific variation in the colouration of lower leaves (abaxial) serves as a model to test physiology related hypotheses.

3. **Morpho-anatomy**

   Morpho-anatomical analysis along the elevation gradient may provide information about habitat-driven traits and functional adaptations and therefore such hypotheses can be tested using *Begonia*.

4. **Threat assessment of Indian Begonia**

   Threat assessment using the IUCN criteria will provide insights into the current status of *Begonia* in India.

5. **Development of new varieties of Begonia**

Interspecific hybridization of *Begonia* may lead to new varieties with increased phenotypic diversity compared to parent plants. Such plants can be developed for commercial purposes.

Annexure

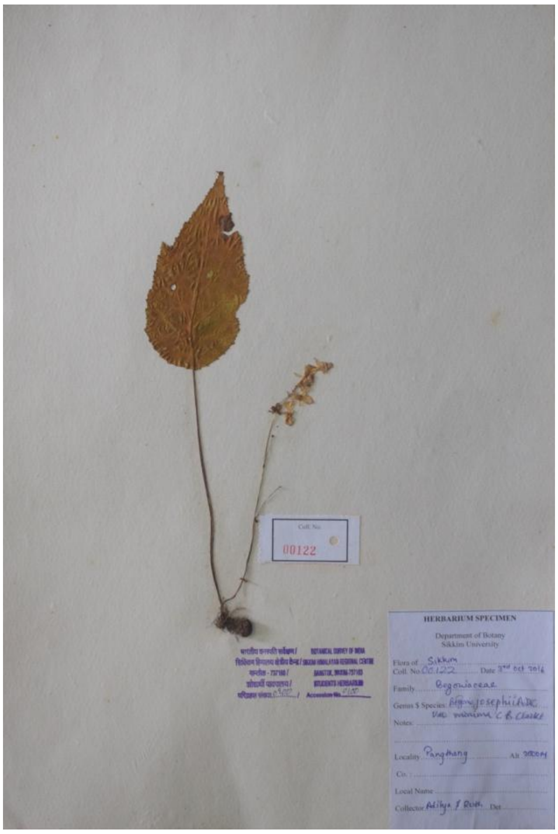

Plate 18: Begonia josephii

Plate 19: Begonia satrapis

Plate 20: Begonia picta

Plate 21: Begonia cathcartii

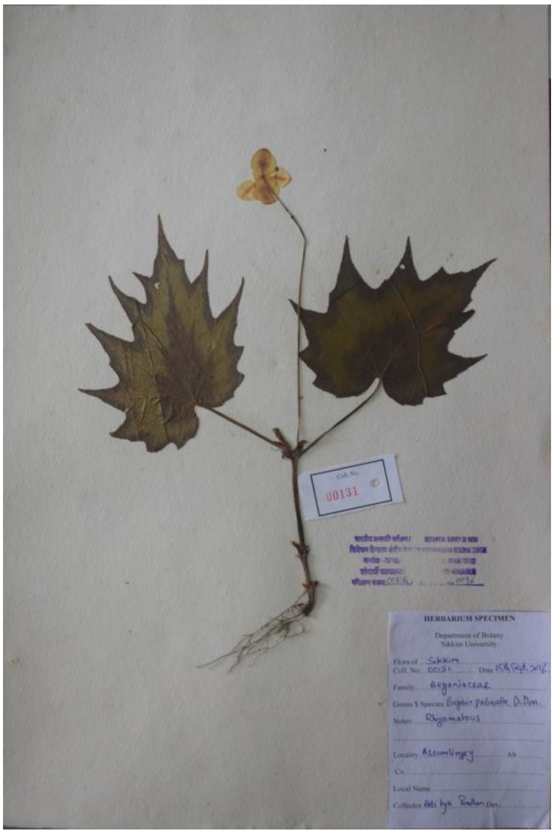

Plate 22: Begonia megaptera

Plate 23: Begonia palmata

Plate 24: Begonia sikkimensis

Plate 25: Begonia xanthina

Plate 26: Begonia panchtharensis

Plate 27: Begonia flaviflora

Plate 28: Begonia nepalensis

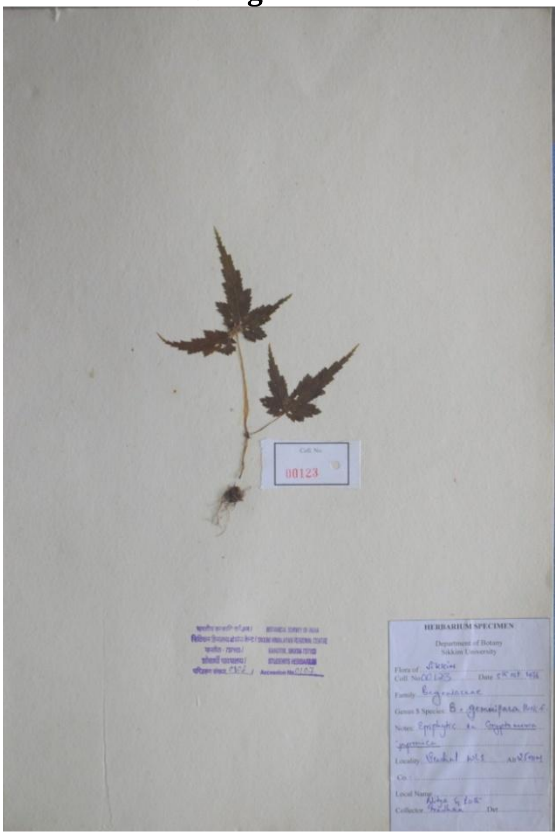

Plate 29: Begonia gemmipara

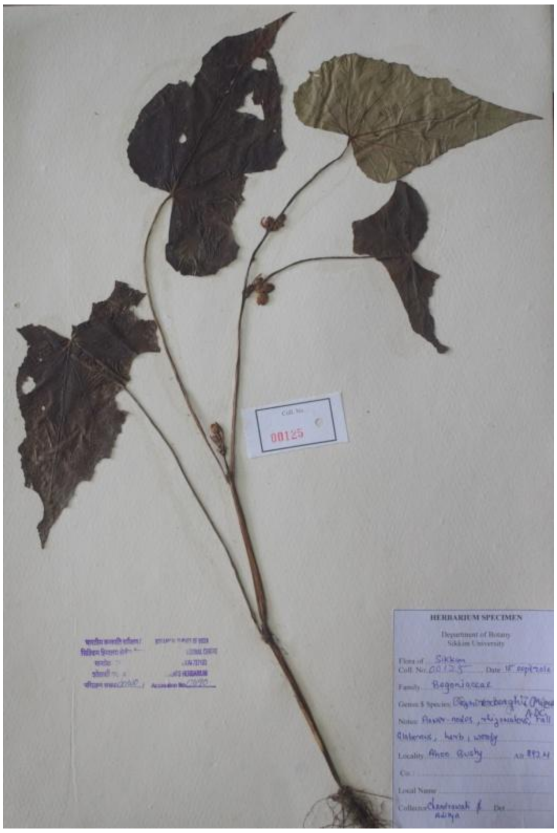

Plate 30: Begonia roxburghii

Bibilography

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Publications in peer-reviewed Journals

1. **Aditya Pradhan**, Dibyendu Adhikari and Arun Chettri. 2020. Predicting the distribution of suitable habitats for *Pandanus unguifer* Hook. f. - a dwarf endemic species from Sikkim Himalayas, through Ecological Niche Modeling. **International Journal of Conservation Science 11** (1) 145–152
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3. Arun Chettri, **Aditya Pradhan** and Dhani Raj Chhetri. 2019. Impact of Climate Change on Distribution of Caterpillar Fungus, Ophiocordyceps sinensis in Sikkim Himalaya, India. **Asian Journal of Conservation Biology 8** (2) 110–114
4. Subhankar Gurung, **Aditya Pradhan** and Arun Chettri. 2019. Pollination in an endemic and threatened monoecious herb *Begonia satrapis* C.B. Clarke (Begoniaceae) in the eastern Himalaya, India. **Journal of Threatened Taxa 11** (10) 14328–14333 https://doi.org/10.11609/jott.4256.11.10.14328-14333
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8. Chettri A, **Pradhan Aditya**, Sharma G, Pradhan B K and Chhetri D R. 2018. Habitat Distribution Modelling of Seabuckthorn (*Hippophae salicifolia* D. Don.) in Sikkim, Eastern Himalaya, India **Indian Journal of Ecology 45** (2) 266–269
9. **Aditya Pradhan** and Arun Chettri. 2017. Identifying Protected Areas Suitable for Conservation of Cycas pectinata Buch. Ham. in Southeast Asia Under Climate Change Scenario. **International Journal of Ecology and Environmental Sciences 43** (2) 129–135
10. Ashis Rai, Arun Chettri, **Aditya Pradhan**, Santosh Kumar Rai, Arun Kumar Rai and Nima Tshering Lepcha (2016). Diversity of climbing plants in 'Gadi' sacred grove of Central Pendam in East Sikkim, India. **Pleione 10** (1) 97–107

11. **Aditya Pradhan**, Arun Chettri and Subhankar Gurung (2015). Diversity and Distribution of Genus Dioscorea in Sikkim Himalaya, India: an application of DIVA-GIS. **NeBIO 6** (3) 29–31

(**Publications in * symbol are included in thesis**)

Seminar/Symposium/Conferences

1. Presented a paper on a topic entitled "**Population assessment and identification of suitable niche of Rhus chinensis Mill. And Hippophae salicofolia D. Don., two important medicinal plants from Sikkim Himalaya, India**" in the oral session, organized by Department of Botany, University of North Bengal on 20–21th February 2020
2. Presented a paper on topic entitled "**A comparative account on ecological niches of few selected species of *Begonia* L. (*Begoniaceae*) from Sikkim Himalaya**" in the oral session, organized by Department of Zoology, Sikkim University on 9–10th August 2019
3. Participated in training workshop on "**Application of open source geospatial tools in ecological niche modeling and widening the utilization on niche models for conservation of threatened plants**" organized by Centre for Advanced Studies in Botany, North-Eastern Hill University, Shillong , Meghalaya, from 21st–23rd September 2016
4. Participated in Northeast Winter School on "**Human Genetics: Techniques and Statistical Analysis**" organized by Indian Statistical Institute (ISI), Kolkata and Sikkim University from 16–20th March 2015
5. Presented a paper on a topic entitled "**Predicting the distribution range of *Begonia satrapis* Clarke, a rare and endemic plant species from Sikkim Himalaya in the present Climatic scenario**" in the oral session, organised by Department of Botany, St. Joseph's College, Darjeeling, from 26–28th June 2015
6. Participated in **Himalayan Young Researcher's Meet-II** organised by G. B. Pant Insititute of Himalayan Envirinment and Development, Indian Himalayas Climate Adaptation Programme (IHCAP) and Swiss Agency for Developent & Cooperation (SDC) from 15-17th September 2015, Kosi-Katarmal, Almora, Uttarakhand, India
7. Presented a poster on a topics entitled "**Status of *Begonia satrapis* Clarke (Begoniaceae): a rare plant species from Sikkim Himalaya**" organised by UBKV (Hill Campus), Kalimpong, Darjeeling, West Bengal, India on 22–24th May 2014
8. Participated in a workshop on "**Climate change and Mountain Areas: Awareness, Adaptation and Sustainabilty**" organized by FOSEP, Darjeeling and supported by FRIEDRICH EBERT STIFTUNG on 27–28th September 2014
9. Participated in a workshop on "**Conservation Assessment and Management Prioritizations on wild medicinal plants of Sikkim**" organized by State Medicinal Plant Board, Forest Environment and Wildlife Management Department, Government of Sikkim from 17–19th November 2014

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Aditya PRADHAN[a], Deliza RAI[a], Saroj K. BARIK[b] and Arun CHETTRI[a,*]: *Begonia panchtharensis* (*Begoniaceae*), a New Record to India from Sikkim, Eastern Himalaya

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Summary: We present a new record of *Begonia panchtharensis* S. Rajbh. (*Begoniaceae*) from the Eastern Himalaya in India. This record extends the known geographical distribution of this species from Nepal to India.

Begonia L. (*Begoniaceae*) is the sixth largest genus of the flowering plants (Frodin 2004) consisting of 1920 species (Hughes et al. 2015). The genus is represented by 56 species in India (Uddin 2007). During our recent floristic exploration we collected a specimen of *Begonia* from Thangsing (East Sikkim), India. On critical examination of morphological characters and perusal of literature, we identified this plant as *Begonia panchtharensis* S. Rajbh., characterized by having grooved petiole with red linear dots, six tepals in a female flower, and several large basal leaves arising directly from the rhizome (Rajbhandary et al. 2010a) (Fig. 1). *Begonia panchtharensis* was described from eastern Nepal, and has been considered to be narrow endemic to Nepal (Rajbhandary et al. 2010b). Therefore, this is a new record for flora of India, extending its geographical distribution eastward to India.

Fig 1. *Begonia panchtharensis* collected in Sikkim, India for the first time (A. Pradhan & D. Rai 0108, BSHC).

Begonia panchtharensis S. Rajbh. in Gard. Bull. Singapore **62**(1): 147 (2010).

Type: E NEPAL. Panchthar, Tinubote, Sisire, Prangbung VDC (Village Development Committee) , 2240–2300 m alt., cultivated at Eastern Nepal, 2 Oct. 2007, U. Thamsuhang s.n. (S. Rajbhandary S74) (E–holotype; KATH–isotype).

Distribution: E Nepal and India (Sikkim).

Flowering: September–October.

Specimen examined: **INDIA**. Sikkim, East district, Thangsing, 2200 m alt., 26 Oct. 2016, A. Pradhan & D. Rai

Inventory and characterization of new populations through ecological niche modelling improve threat assessment

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Categorization of species under different threat classes is a pre-requisite for planning, management and monitoring of any species conservation programme. However, data availability, particularly at the population level, has been a major bottleneck in the correct categorization of threatened species. Till date, threat assessments have been mostly based on expert opinion and/or herbarium records. The availability of primary data on distribution of species and their population attributes is limited in India because of inadequate field survey, which has been ascribed to resource constraints and inaccessibility. In this study, we demonstrate that ecological niche modelling (ENM) can be an economical and effective tool to guide surveys overcoming the above two constraints leading to the discovery of new populations of threatened species. Such data lead to improved threat assessment and more accurate categorization. We selected 14 threatened plants comprising 5 trees (*Acer hookeri* Miq., *Bhesa robusta* (Roxb.) Ding Hou, *Gynocardia odorata* Roxb., *Ilex venulosa* Hook. f. and *Lagerstroemia minuticarpa* Debb. ex P.C. Kanjilal), 8 herbs (*Angelica glauca* Edgew., *Aquilegia nivalis* Falc. ex Jackson, *Artemisia amygdalina* DC., *Begonia satrapis* C.B. Clarke, *Corydalis cashmeriana* Royle, *Dactylorhiza hatagirea* (D. Don) Soo, *Podophyllum hexandrum* Royle, and *Rheum australe* D. Don), and 1 pteridophyte (*Angiopteris evecta* (Forst.) Hoffm.) having distribution range in North East India, Eastern and Western Himalaya, and Jammu and Kashmir. The study was carried out between 2012 and 2016. ENM-based survey led to the discovery and characterization of 348 new populations. The data so obtained helped in assigning conservation status to 10 species, which earlier were never classified due to data deficiency. Using the new population and distribution data of the remaining four species, only one was confirmed regarding its existing status and two species were classified as 'Critically endangered' instead of the present classification as 'Endangered'. The fourth species was classified as 'Critically endangered' against the earlier category of 'Least concerned'.

Keywords: Niche modelling, population characterization, threatened plants, threat assessment.

Introduction

PRIORITIZATION of species through threat assessment is a pre-requisite for conservation planning, action, monitoring and evaluation purposes[1–3]. Lack of data pertaining to occurrence and population status of threatened species is a major bottleneck in the proper threat assessment and categorization process[4]. The data deficiency has been attributed to: (i) limited knowledge about the potential distributional range, (ii) inadequate survey, and (iii) poor knowledge about the population status in nature and other population attributes[5]. The International Union for Conservation of Nature (IUCN) and the Conservation Data Center (CDC) have devised various approaches/methodologies to categorize species under various threat classes. These include population inventory and characterization (following field surveys), herbarium records, expert opinions, qualitative scoring techniques, and determining area of occupancy (AOO) and extent of occurrence (EOO). Conservation assessment following field survey is an exhaustive process requiring substantial

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PREDICTING THE DISTRIBUTION OF SUITABLE HABITATS FOR *PANDANUS UNGUIFER* HOOK.F. - A DWARF ENDEMIC SPECIES FROM SIKKIM HIMALAYAS, THROUGH ECOLOGICAL NICHE MODELING

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Abstract

Pandanus unguifer is a threatened species endemic to Sikkim and Darjeeling district of West Bengal. The species bears mildly fragrant creamy white colored flower and is the only Pandanus species that produces flowers in potted condition and hence, is of ornamental value. We used field collected-coordinates of five presence occurrence localities of the species for habitat distribution modeling. Additionally, downloaded environmental data from worldclim database were used for correlation. Amongst the input environmental variables, Mean diurnal range in temperature (Bio2) and Precipitation seasonality (CV) (Bio15) were influential factors and contributed 48.7% and 40.8% respectively to the MaxEnt model. The Rest of the remaining layers collectively contributed 10.5% to the final habitat model. Of the total 5963 sq.km suitable area only 153 sq. km has a very high probability of having P. unguifer which is only 2.57% of the total area. The study revealed the highest potential distribution of the species in Darjeeling district of West Bengal compared to Sikkim. However, resource extraction due to the felling of trees for fodder and small timber, road-widening and agricultural expansion are the causative factors threatening the existence of the species in its natural habitat. Thus urgent conservation initiatives are required to protect the existing endangered endemic species richness of these regions.

***Keywords**: Darjeelin; Endangered; Endemic; Pandanus; Sano-Tarika; Sikkim*

Introduction

Ecological Niche Modeling (ENM) is a computer-based tools used to predict the distribution of species in correlation with the set of species occurrence locality and the environmental variables [1]. The availability of high-resolution bioclimatic data has lead to the wide use in ecological research and restoration ecology [2-4]. These tools find use in predicting the distribution of species across landscapes and have been used for prioritizing field surveys [5,6]. There are various algorithm used to predict the distribution of species [7-12]. However, comparatively, there are fewer examples of studies using small sample sizes [13]. We, therefore, used a method based on the principle of maximum entropy (MaxEnt) which has high success rates with sample sizes as low as five [14, 15].

P. unguifer belonging to the family *Pandanaceae* is a threatened and endangered species, endemic to Sikkim and Darjeeling district of West Bengal, India. The species has been assigned to Endangered category (EN B1ab [i,ii,iii] + B2ab [i,ii,iii]) as per IUCN Red List Criteria [16]. The species was first described by *J.D. Hooker* in *Flora of British India* in 1878 from Mungpo localities below Surail, West Bengal, India. The species have high ornamental

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POLLINATION IN AN ENDEMIC AND THREATENED MONOECIOUS HERB *BEGONIA SATRAPIS* C.B. CLARKE (BEGONIACEAE) IN THE EASTERN HIMALAYA, INDIA

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Abstract: *Begonia satrapis* was studied for its pollination aspects at Sumbuk, Sikkim, India. The floral details and the foraging behaviour of insects visiting the flowers were examined to define the pollination syndrome and its functionality for the success of sexual reproduction in this species. The flowers do not produce nectar and offer only pollen as floral reward to foraging insects. Therefore, male flowers were foraged more for its pollen than the female flowers. There was a significant difference in the visit to male and female flowers by both *Apis florea* and *Bombus breviceps*, respectively. The bees spent more time on male flowers than on female flowers. The bees appear to rely on visual stimuli to visit male and female flowers. The plant produces abundant fruit and seed set in both hand and open-pollinations indicating that it is facultatively xenogamous. The female flowers lacking any reward resemble male flowers and in effect are pollinated by deceit.

Keywords: *Apis florea*, *Bombus breviceps*, deceit, northeastern India, Sumbuk.

***Nepali* सार:** भारतको उत्तर-पूर्वीय राज्य सिक्किमको सुम्बुकमा पाइने मंगरकाँजे (*Begonia satrapis*)को परागण प्रक्रिया माथीको विशेष अध्ययन हो। यहाँ पाइने यस प्रजातिको प्रजनन प्रक्रिया तथा स्त्री फूल अनि नर फूलको पराग मिलन प्रक्रियालाई बुझ्न, फूलको मुख्य भागको जाँच साथै फूलको पराग खान आउने किटपतङ्गको क्रिया कलापमाथी विशेष जाँच गरिएको छ।फूलमा रस नहुनाले गर्दा किटपतङ्गले परागको धुलोलाई नै टिप्ने गर्दछ। विशेष गरि नर फूलमा परागको धुलो पाइनाले गर्दा मौरी (*Apis florea*) अनि भमरा (*Bombus breviceps*)ले नर फूलमा मात्र अधिक समय बिताएको पायौं।आफ्नै हातले गरिएको परागण प्रक्रिया साथै प्राकृतिक स्वभावले हुने परागण (Open pollination)द्वारा अधिक मात्रामा फल र बिज लागेको हुनाले मँगरकाँजेको यस प्रजातिलाई *फ्याकलटेटिभ जिनोग्यामस्* भन्न सकिन्छ। मौरी अनि भमराले आफ्नो नजरमाथी भर परेर नै नर फूल र स्त्री फूलमाथी बसेको अवलोकन गर्यौं। स्त्री फूलमा किटपतङ्गको निम्ति कुनै रस अथवा आहार नभए पनि नर फूल झैं दुरुस्त देखिने हुनाले किटपतङ्ग झुक्किएर स्त्री फूलमा बस्दा नर फूलबाट टिपेको परागको धुलो झरिन्छ, यसो हुँदा फूलमा परागण प्रक्रिया सम्भव भएको पाइन्छ।

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Competing interests: The authors declare no competing interests.

Author details: SUBHANKAR GURUNG is currently doing his PhD in the Department of Botany, Sikkim University, India. His interest lies in studying the reproductive biology of plants with special emphasis on understanding the role of pollinators. ADITYA PRADHAN, a PhD scholar and a Junior Project Fellow (JPF), is studying the diversity and distribution of the genus *Begonia* in Sikkim Himalayas. DR. ARUN CHETTRI is a plant taxonomist/ecologist who has worked extensively on plant diversity, taxonomy, ecology and conservation of threatened plants in different forests types in northeastern India, in particular in Sikkim.

Author contribution: Study designed by SG; Data collected by SG, AP; analysis of data done by AC, and SG wrote the manuscript.

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Impact of Climate Change on Distribution of Caterpillar Fungus, *Ophiocordyceps sinensis* in Sikkim Himalaya, India

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ABSTRACT

MaxEnt modelling has been used to predict the present and future distributions of caterpillar fungus, *Ophiocordyceps sinensis* in Sikkim Himalaya in four future climate change trajectories (viz. RCP 2.6, RCP 4.5, RCP 6.0 and RCP_8.5) for the year 2050. The result predicted an area of 311 km^2 (4.77 % of total area) to be suitable under current climatic condition. Under future climate change scenario the suitable habitat of caterpillar fungus would get drastically reduced (RCP_2.6, RCP_4.5 and RCP_8.5) with a minor expansion (i.e. ~71 km^2) in addition to current potential suitable habitat under scenario RCP_6.0. Niche overlap analysis resulted in more than 90 % niche overlap among current and future distribution of species. The existing protected areas (PAs) accounts for only 0.54 % (~35 km^2) of the total area of state of Sikkim and 1.64 % of the total area of PAs and shows variability in suitable habitat under climate change scenario. Therefore the establishment of new PAs especially towards Far-East and North-East region of Sikkim could be an alternative measure for the conservation of suitable habitat of caterpillar fungus. Alternatively, trans-boundary conservation programs connecting country like Bhutan in the West, Nepal in the East and Tibet in the North of Sikkim could be a feasible long term alternative plan for conservation of species.

Key words: Niche Modelling, *Ophiocordyceps sinensis*, Protected Area, Sikkim, *Yartsha-gumbu*

INTRODUCTION

Ecological Niche Modelling (ENM) has a variety of applications in conservation biology. One such application is an estimation of present and future suitable habitat of threatened species through identification of protected area for conservation of such species (Pradhan and Chettri, 2017; Chettri *et al*., 2018). The future distribution of species due to climate change depends on a variety of biotic and abiotic factors leading to change in distribution patterns (Hellmann *et al*., 2008). Even though the interactions between species and climate have been widely considered in conservation of species, the effect of climate change can produce shifts in species distribution (Mawdsley *et al*., 2009). In the last 25 years (1982-2006) the Himalaya has warmed up by about 1.5° C, which is three times more than that of the global average (Shrestha *et al*., 2012). Like other Himalayan states of India, climate in Sikkim is changing rapidly and that more changes are at the forefront leading to loss in Biodiversity, human health, local livelihoods, agriculture and water availability (Bawa and Ingty, 2012).

Ophiocordyceps sinensis locally known as *Yartsha -gumbu* in Sikkim, is a fungal parasite of larvae (caterpillars), hence also known as Caterpillar Fungus (CF). The CF has patchy distribution and known to occur in the Tibetan plateau, Bhutan, China, Nepal and India (Namgyel & Tshitila, 2003; Pegler *et al*., 1994; Winkler, 2005; Sharma, 2004; Devkota, 2006).

In the present study, we used presence record of CF in Sikkim Himalaya (Figure 1), with the following two objectives: (1) to predict and identify protected areas suitable for CF in Sikkim Himalaya (2) to compare the future (2050) predicted distribution of CF with present climate scenario.

MATERIALS AND METHODS

Occurrence data, Niche Modelling and Climate data

Due to a narrow geographic range of CF in Sikkim Himalaya, we collected five occurrence data, i.e. geographic coordinates from Northern and Eastern District of Sikkim (Supplementary material 1). We used MaxEnt (maximum entropy) model to predict the current and future habitat distribution of CF in Sikkim Himalaya. MaxEnt model is one of the most widely used software package for environmental niche modelling and can achieve high predictive accuracies even with the low presence only data (Phillips and Dudík, 2008). MaxEnt have been previously used to predict the impact of climate change on the distribution of CF in Nepal Himalaya (Shrestha and Bawa 2014). The climate data for current and future climate change scenario (2050) were obtained from the Worldclim data base (www.worldclim.org) at the resolution of 30 arc Sec (~1 km^2). The 19 bioclimatic variables were subjected to a correlation test using ENM Tools 3.3. (Warren *et al*., 2010) and therefore out of 19 variables 13 variables were found to be highly correlated (r^2>0.90) leaving six variable for modelling (Table 1).

Model evaluation and validation

The performance of the model was evaluated based on area under the curve (AUC) metric. The AUC value of

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Population assessment and species distribution modeling of *Paris polyphylla* in Sikkim Himalaya, India

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Abstract. *Lepcha DL, Pradhan A, Chhetri DJ. 2019. Population assessment and species distribution modeling of* Paris polyphylla *in Sikkim Himalaya, India. Biodiversitas 20: 1299-1305.* *Paris polyphylla* Smith is an important therapeutic plant that grows in the Indian Himalayan region. In Sikkim, the survival of the species is threatened by illegal extraction of its rhizome. Therefore, immediate conservation initiative is required to conserve the existing species population in the wild. Population assessment through field survey followed by ENM revealed a positive correlation between predicted suitable habitats with the actual sites of its occurrence, except in disturbed habitats. Precipitation of driest month (Bio14) and slope were the most influential factors that contributed 41.9 % and 30 % respectively to the MaxEnt model. Field survey revealed that the density of the plants varied between 0.45 (pl/m^2) and 3.89 (pl/m^2) and the frequency varied from 36% to 76%. The IVI for *P. polyphylla* ranged between 2.68 to 8.66 based on locations. On the other hand, the IVI of associated species varied from 3.57 to 18.14 based on species. *P. polyphylla* is a vulnerable plant in Sikkim Himalaya and it is facing an imminent threat of extinction. Under this situation, it is imperative that works on predictive modeling will help conserve the species. This study identified the potential habitats for *P. polyphylla* in the higher elevations of Sikkim Himalaya where it could be reintroduced.

Keywords: Ecological niche modeling, habitat, Himalaya, *Paris polyphylla,* Sikkim

INTRODUCTION

Paris polyphylla Smith commonly known as Love apple belongs to the Family Melanthiaceae (earlier Trilliaceae or Liliaceae) (Figure 1), and is mostly found in India, China, Bhutan, Laos, Myanmar, Nepal, Sikkim, Thailand and Vietnam (Sharma et al. 2014). In India the species have been recorded from the Himalayan states like Arunachal Pradesh, Himachal Pradesh, Jammu and Kashmir, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim, and Uttarakhand (Paul et al. 2015).

In traditional medicine, the roots are used as analgesic, antiphlogistic, antipyretic, antispasmodic, antitussive, depurative, febrifuge and narcotic (Duke and Ayensu 1984) Furthermore, it has been used to treat liver cancer in China for several decades (Lee et al. 2005; Shoemaker et al. 2005). In Nepal, the rhizome is indigenously used against snake bites, insect bites, alleviate narcotic effects, internal wounds, external wounds, fever, food poisoning and are fed to cattle during diarrhea and dysentery (Dutta 2007; Baral and Kurmi 2006). It is also used to treat headache, vomiting, and worms (Uprety et al. 2010). In the Indian Himalayan Region, *P. polyphylla*, used against burn, cut or injury, diarrhea, dysentery, fever, gastritis, skin diseases, stomach pain and wounds (Farooquee et al. 2004; Maity et al. 2004; Tiwari et al. 2010; Jamir et al. 2012; Lalsangluaii et al. 2013; Pfoze et al. 2013; Sharma and Samant 2014).

A B C D

Figure 1. A. Pictorial representation *P. polyphylla* from Sikkim, Himalaya, India. A. Whole plant; B. Plant with flower; C. Mature fruits with exploded pod; D. Sprouting rhizome

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Habitat Distribution Modelling of Seabuckthorn (*Hippophae salicifolia* D. Don.) in Sikkim, Eastern Himalaya, India

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Abstract: Seabuckthorn (*Hippophae salicifolia*) is one of the underutilized plant species having enormous potential for socio-economic upliftment and sustainable livelihood of rural communities. In Sikkim it grows naturally in Lachen and Lachung valleys of North district and it has not been explored for economic benefits. Due to its restricted distribution in Sikkim, ecological niche modelling (ENM) was performed to identify suitable habitat for in-situ conservation through identification of suitable areas and for further reintroduction in its natural habitat. The model was developed using Maximum Entropy (MaxEnt) distribution modelling algorithm and predicted that the suitable habitats of Seabuckthornare restricted to an area of *ca.* 286 km^2 in Sikkim. The distribution of the potential habitat was strongly influenced by NDVI layer for October and January, which corresponds to the period of fruiting phenology of the species, thus indicating the importance of fruiting phenology in determining the distribution of the species. Population status was positively correlated with very high model thresholds at three locations, confirming the usefulness of the habitat model in population monitoring. Therefore the approach presented here emerge to be quite promising in predicting the suitable habitat for species with restricted distribution and moreover it can be an effective tool for conservation planning, monitoring and management of the species.

Keywords: Seabuckthorn, ENM, Conservation, Macro propagation, Protected area

Seabuckthorn (*Hippophae salicifolia* D. Don) is a thorny, deciduous shrub (Singh 1998, Nepal *et al* 2001) belonging to the family Elaeagnaceae. The species is restricted to the Himalayan region, between 1500-3500 m asl (Gaur 1999, Hooker1894). In India *H. salicifolia* grows naturally in high altitude areas of Himachal Pradesh, Jammu and Kashmir, Uttar Pradesh (Singh et al1995, Singh 1998) and Sikkim (Basistha 2001). In Sikkim, the species is found naturally growing in Lachen and Lachung Valleys of North district between the altitude of 2700 and 3700 m. Ecological niche modelling (ENM) also known as *species distribution modelling* is a recent tool which uses computer algorithms to generate predictive maps of species distribution in a geographic space by correlating the point distributional data with a set of environmental raster data. Predicting and mapping the suitable habitat for threatened and endangered species is critical for monitoring and restoration of their declining native populations (Kumar and Stohlgren2009). Species reintroduction is one such ecological strategy for restoration of depleted species population reclamation of degraded habitats and ecosystems (Adhikari et al 2012). To reintroduce and rehabilitate a threatened species a detailed knowledge of its potential habitats is essential. Species distribution modelling or ENM therefore helps to identify the suitable habitats for reintroduction, and in developing effective species specific conservation measures. It has been successfully used in restoring critical habitats and predicting the impact of environmental and climate change on species and ecosystems (Brooks et al 2004, Franklin 2009, Giriraj et al 2008, Gogol-Prokurat 2011, Khanum et al 2013).

Identification of suitable habitat is prerequisite for species restoration programme (Adhikari et al 2012, Pradhan and Chettri 2017).To achieve such restoration of threatened species require GIS based techniques like ENM which reconstructs the niche of a species in an ecological space by correlating the occurrence points (geographic coordinate) with a set of environmental variables. The present work was therefore undertaken with the aim of identifying suitable habitat for conservation through large scale propagation of species. Such predicted habitat identified through ENM could be used for reintroduction of species in near future.

MATERIAL AND METHODS

Species occurrence records: Primary occurrence data

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Identifying Protected Areas Suitable for Conservation of *Cycas pectinata* Buch. Ham. in Southeast Asia Under Climate Change Scenario

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ABSTRACT

Cycads are considered one of the most threatened groups of plants, with 62 % of all species listed on the IUCN Red List. Therefore in order to implement sustainable conservation strategy, it is imperative to understand the impacts of changing climate on both ecosystem and at species level. We therefore presented an assessment on current and future habitat suitability distribution of *Cycas pectinata* Buch.-Ham., using a maximum entropy (MaxEnt) species distribution model. The projected future distribution map was then overlaid onto protected areas of Southeast Asia for further analysis. Out of 1938 protected areas identified in the 10 southeast countries, only 190 protected areas would remain suitable by the end of 2050 under climate change scenario (RCP 2.6, RCP 4.5, RCP 6.0 and RCP 8.5). The MaxEnt model performed better than random with an average test AUC value of 0.975 (±0.001). The AUC values thus confirm the accuracy of model prediction based on 73 occurrence points. A significant reduction in the suitable habitat was also observed under future climate change scenario, as a result of which the species may not be able to adapt to modification caused by climate change further leading to reduction in population size.

Key Words: Climate change, Cycads, ENM, MaxEnt, Protected area, Vulnerable

INTRODUCTION

According to IPCC Fourth Assessment Report, South East Asia is expected to be adversely affected by changing climate. Thus climate change is projected to have pressures on natural resources and the environment associated with rapid urbanization, industrialization and economic development in the region (IPCC 2007). Numerous studies have shown rapid increase in global climatic conditions in the last decade and thus have estimated similar trends for the coming years (Cordellier and Pfenninger 2009). The changes on the distribution patterns of the species and biodiversity as a whole are expected and are considered one of the major consequences of climate change (Botkin et al. 2007, Svenning et al. 2009). The Intergovernmental Panel on Climate Change therefore underpins the importance of conserving biodiversity in the face of changing climate conditions. Some of the major consequences of climate change include changes in distribution pattern of the species, increased rate of extinction, changes in phenology (Iverson and Prasad 2001, Thompson et al. 2009). In this regard, Species distribution modeling (SDMs) or Ecological niche modeling (ENM) has become an imperative tool for predicting the probable impact of climate change on floral distribution (Bakkenes et al. 2002, Chris et al. 2004, Thuiller et al. 2005). The model is developed by correlating species existence and its biophysical environment to predict the current as well as the future distribution of the species (Elith et al. 2006, Peterson 2006, Kumar and Stohlgren 2009). There are many modeling technique (MaxEnt GARP, and BIOCLIM) available for predicting the suitable habitat distribution of a species, but the input parameters and criteria differ. Most of the SDMs use both presence as well as absence data to model the