Asian *Begonia*: out of Africa via the Himalayas?

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ABSTRACT. The large genus *Begonia* began to diverge in Africa during the Oligocene. The current hotspot of diversity for the genus in China and Southeast Asia must therefore be the result of an eastward dispersal or migration across the Asian continent. To investigate the role of the Himalayas as a mesic corridor facilitating this migration, we constructed a time-calibrated molecular phylogeny using ITS sequence data. Himalayan species of *Begonia* were found to fall into two groups. The first is an unresolved grade of tuberous, deciduous species of unknown geographic origin, with evidence of endemic radiations in the Himalayan region beginning c. 7.4 Ma, coinciding with the onset of the Asian monsoon. The second is a group of evergreen rhizomatous species with a probable origin in China, which immigrated to the Himalayan region c. 5.1 Ma, coinciding with an intensification of the monsoon. The hypothesis of the Himalayas being a mesic migration route during the colonisation of Asia is not refuted, but further data is needed.

Keywords. Begonia, biogeography, China, Himalayas, molecular phylogeny, southeast Asia

Introduction

The large genus *Begonia* has around 750 species in Asia, with the bulk occurring in Southeast Asia, and the Malesian region having c. 440 of these (Hughes 2008). Reconstructions of the phylogenetic history of *Begonia* show an early divergent African clade, with Asian and American species nested within an African grade (Plana et al. 2004, Forrest et al. 2005, Goodall-Copestake et al. 2010) and Asian (including Socotran) species supported as monophyletic (Goodall-Copestake et al. 2010, Thomas 2010). Given the initial diversification of *Begonia* in Africa in the Oligocene or late Eocene (Goodall-Copestake et al. 2009) and the monophyly of Asian species, there must have been a migration or dispersal of an ancestor eastwards from Africa which has speciated and led to the various diversity hotspots across Asia.

Molecular divergence age estimates indicate that the origins of Asian (including Socotran) *Begonia* date to 18–15 Ma (Thomas 2010) coinciding with the mid-Miocene climatic optimum, a warm phase which led to the expansion of tropical vegetation in Asia as far north as southern Japan and as far east as the northwest of the Indian subcontinent (Zachos et al. 2001, Morley 2007). However it would seem that a straightforward migration of *Begonia* across the Arabian peninsula to Asia is unlikely, given the dry conditions thought to have existed across much of southwest Asia and the Arabian peninsula at the time (Morley 2007). Hence a long distance dispersal event does not seem an unlikely hypothesis for the entry of an African ancestor into Asia, although this was possibly facilitated by the greater expanse of tropical climate during the mid-Miocene. *Begonia* from Sri Lanka, the Western Ghats and Socotra are at the western limit of the Asian *Begonia* clade (Fig. 1), and have been found to be early divergent lineages within a phylogeny of Asian species (Thomas 2010).

The Himalayas began to form during the initial collision of the Asian and Indian plates during the early Eocene c. 35 Ma (Ali & Aitchison 2008) and by the time of the arrival of *Begonia* in Asia c. 15 Ma, the High Himalayas were present (Amano & Taira 1992). Hence, depending on climate, there was potentially a significant area of montane habitat at the north of the Indian continent which could have provided a mesic link as a migration route eastwards towards the current *Begonia* diversity hotspots of Indo-China and Malesia (Fig. 1).

To investigate the role of the Himalayas as an easterly migration route during the colonisation of Asia, Himalayan species of *Begonia* were placed in a phylogeny to allow their interpretation in a geographical and temporal context. This paper builds on previous studies by considerably increasing the sampling of Himalayan species and coding the region as a distinct geographic unit in a biogeographic analysis. In particular, this paper addresses the following questions:

—Is there evidence for the Himalayas behaving as a link in an eastward migration of *Begonia*?

—How has paleo-climatic change and the Himalayan orogeny influenced the evolution of Nepalese *Begonia*?

Materials and methods

The molecular phylogenetic analysis was based on nuclear ribosomal internal transcribed spacer (ITS) sequences, obtained from Genbank and also *de novo* for this study (Appendix A) following the methods in Thomas (2010). Phylogenetic reconstruction, divergence times and ancestral area reconstruction were performed simultaneously using Bayesian inference as implemented in BEAST v1.6.1 (Drummond & Rambaut 2007), using 4×10^7 generations and sampling every 1000th generation. The sequence data was divided into three partitions, namely the two internal transcribed spacers (ITS1 and ITS2) and the 5.8s ribosomal gene. Models of sequence evolution for each nucleotide sequence partition were determined using jModelTest (Posada 2008). The divergence time for the split between African and Asian species was set as 17.8 Ma
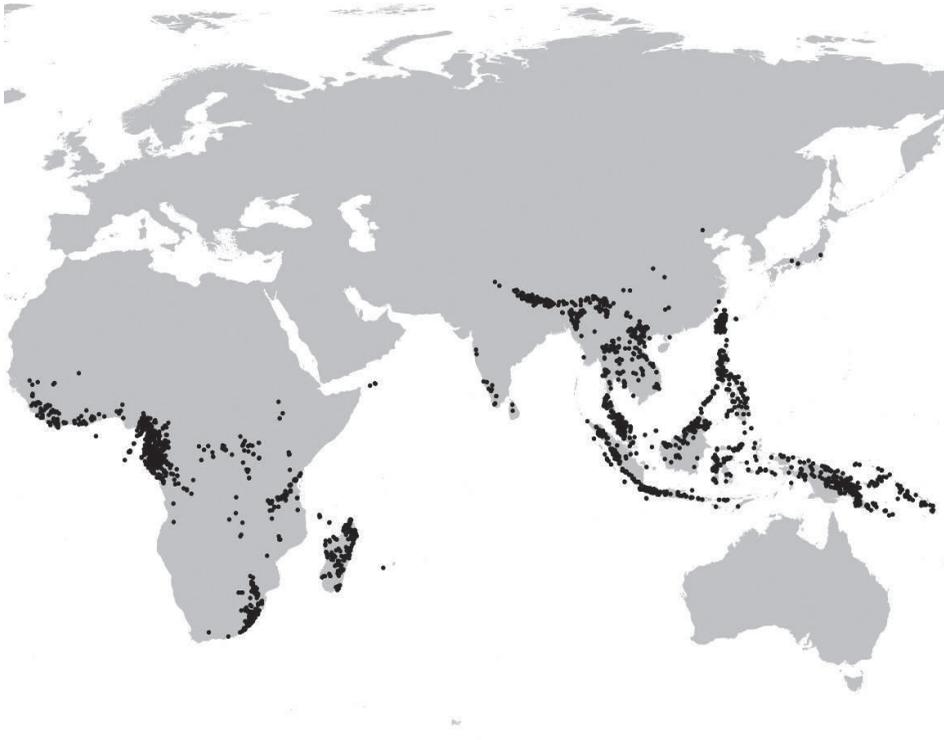
Fig. 1. The distribution of *Begonia* in the Old World, highlighting the disjunction between the African and Asian floras, and the westward linear distribution along the Himalayas. Data from the GBIF data portal and Hughes & Pullan (2007).

(Thomas 2010), implemented as a normally distributed prior with a standard deviation of 3.4. Bayesian ancestral area reconstructions were performed using the continuous-time Markov chain (CTMC) model for discretized diffusion specified by Lemey et al. (2009), considering diffusion amongst 10 geographic areas defined as Africa, China, Himalaya, India, Indo-China, Philippines, Socotra, Sulawesi, Sunda Shelf and Taiwan. Maximum clade credibility consensus trees were made using TreeAnnotator v1.6.1 (Drummond & Rambaut 2007) with a burn-in of 25%, and visualised using FigTree v1.3.1 (Rambaut 2007).

The Bioclim parameter precipitation seasonality (Busby 1991) was extracted from the WorldClim database (Hijmans et al. 2005a) using DIVA-GIS (Hijmans et al. 2005b) and georeferenced herbarium specimen data from Hughes & Pullan (2007). It is calculated as the standard deviation of weekly precipitation estimates expressed as a percentage of the annual mean of those estimates. Scores for species with multiple localities were averaged.

Perennation organ type was scored from literature records and herbarium specimens available from Hughes & Pullan (2007).

Results

Sequence characterisation
The aligned sequences gave a dataset of 873 characters in length (ITS1 330 bases, 5.8s 148 bases, ITS2 395 bases). The bases at positions 492–499 and 505–525 were excluded from the analysis due to difficulty in reliably assigning positional homology. The remaining 845 bases were included in the analysis, of which 561 were variable and 433 (51%) were potentially informative (ITS1, 237 variable and 190 (58%) potentially informative; 5.8s, 31 variable and 11 (7%) potentially informative; ITS2, 294 variable and 232 (63%) potentially informative). All three regions were analysed under a GTR+G+I model (general time reversible model, gamma distributed rate variation, plus a proportion of invariable sites).

Phylogenetic analysis—geography and dating
The Asian + Socotra ingroup was supported as monophyletic with a posterior probability of 1 (Fig. 2). Relationships at the base of the tree were generally poorly supported, and hence inferences regarding timing and geography of cladogenesis on this part of the tree are not possible with confidence.

Clade A contains species from China, Indo China and Malesia, with several well supported sub-clades. One of these contains the Thai species *B. alicida* and *B. smithiae*, together with three species of *Begonia* sect. *Parvibegonia*. Two other supported subclades are highlighted (Fig. 2) concerning the large *Begonia* sect. *Petermannia* with a probable origin on the Sunda Shelf in the late Miocene followed by later diversification in Wallacea.

Himalayan species are present only in Clade B (Fig. 2), in both the 'Diploclinium grade' and the clade consisting of members of *Begonia* sect. *Platycentrum* and *Sphenanthera*, marked PLA-SPH in Fig. 2. The 'Diploclinium grade' has a largely unsupported topology at the base, but began diversifying sometime between 14.7 (±7.6) and 12.3 (±6.7) Ma in the mid-Miocene. Species from the Himalayan region in this grade are intermixed with species from China, Indo-China and Taiwan. Due to the unresolved backbone, the area of origin for this tuberous, seasonally adapted grade cannot be reconstructed. Supported subclades within the grade have areas of origin reconstructed as the Himalaya, Indo-China or China. One consists of the Taiwan endemic *B. ravenii* sister to two accessions of the Himalayan *B. dioica*; the geographic origin of this clade is equivocal, with the Himalayas having the highest posterior probability, though only of 0.37; it dates from 7.4 (±5.7) Ma. Another clade consists of four species from Thailand, probably representing speciation originating in that country (PP 0.84), dating from 8.1 (±5.2) Ma. A clade of species from Nepal, with *B. picta* appearing as sister to the remaining species, likely has a Himalayan origin (PP 0.90) dating 7.4 (±4.6) Ma. A clade with *Begonia puttii* (Thailand) and *B. labordei* (China) along with an unidentified species from China diverged 6.4 (±4.8) Ma in China (PP 0.82). The most highly nested subclade in the Diploclinium grade, sister to the PLA-SPH clade, contains three accessions of *Begonia* rubella from Nepal
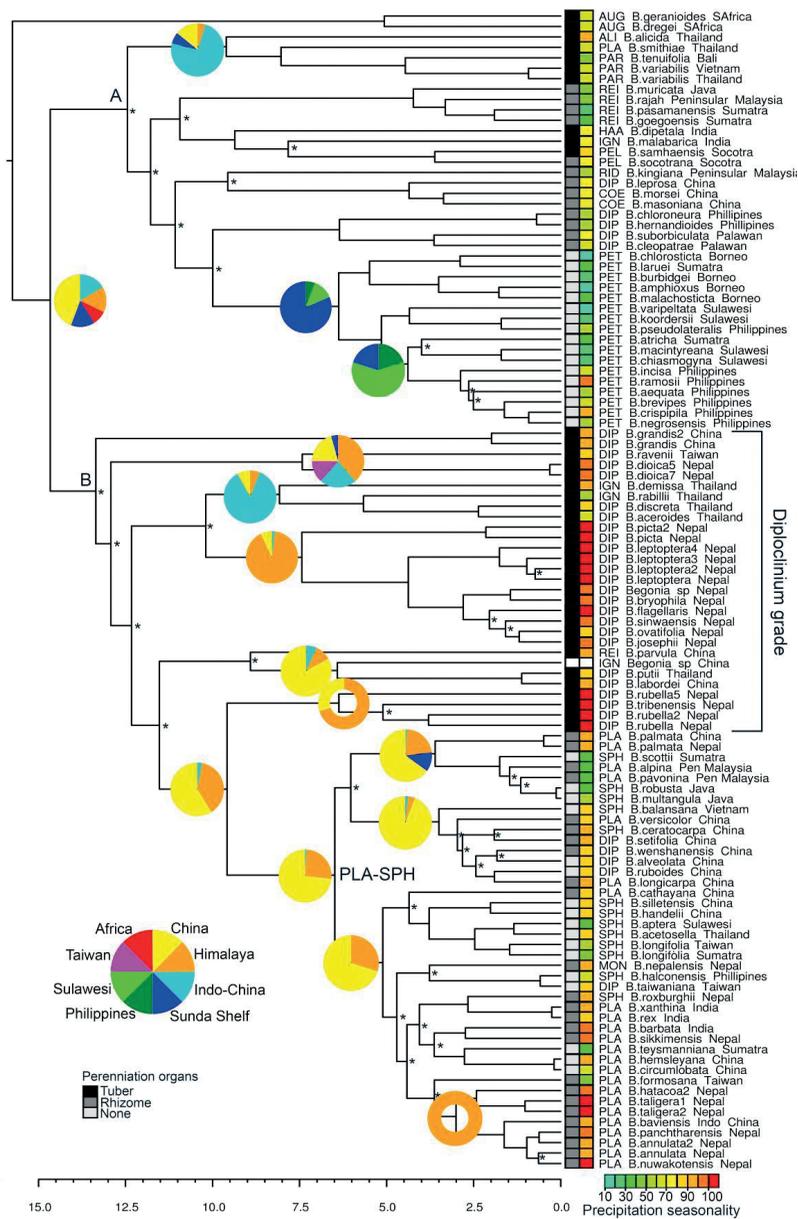
Fig. 2. Maximum clade credibility chronogram derived from an analysis of nuclear ribosomal ITS sequences using BEAST v1.6.1 (Drummond & Rambaut 2007). Asterisks denote clades with a posterior probability of less than 0.8; pie charts show Bayesian ancestral area reconstructions on supported nodes; lower scale denotes time in millions of years; the monochrome and coloured boxes at the branch tips show the perenniation organ type and precipitation seasonality respectively; missing data coded as white. Three-letter codes indicate the sections of *Begonia* (ALI, sect. *Alicida*; AUG, sect. *Augustia*; COE, sect. *Coelocentrum*; DIP, sect. *Diploclinium*; HAA, sect. *Haagea*; IGN, *ignota* (section unknown); MON, sect. *Monopteron*; PAR, sect. *Parvibegonia*; PET, sect. *Petermannia*; PLA, sect. *Platycentrum*; REI, sect. *Reichenheimea*; RID, sect. *Ridleyella*; SPH, sect. *Sphenanthera*).

and one of *B. tribenensis*, also from Nepal. This subclade probably has an origin in the Himalayas (PP 0.95) dated 6.4 (±4.3) Ma.

The origin of the PLA-SPH clade dates from 6.5 (±4) Ma and hence is considerably younger than the 'Diploclinium grade'. Himalayan species in the PLA-SPH clade are intermixed with species from China, Indo-China, the Sunda shelf, Taiwan, the Philippines and Sulawesi. The geographic area of origin for this clade is most probably China (PP 0.73). Three supported subclades within the PLA-SPH clade also have an area of origin optimised as China, with posterior probabilities of 0.65, 0.70 and 0.94. Only one subclade has a probable area of origin in the Himalaya (PP 0.99), dating from 3.0 (±2.3) Ma and with one dispersal into Indo-China. The overall picture for the PLA-SPH clade is of a fairly rapid diversification with its origins in China following loss of the tuberous habit, with dispersal to the Himalayan region and throughout Southeast Asia followed by localised speciation.

Phylogenetic analysis—perennation organs, precipitation and seasonality
All of the species in the 'Diploclinium grade' are tuberous and the vast majority are found in climates with a marked seasonality in rainfall (Fig. 2). They tend to die back and lose their leaves completely in the dry season. Species within the PLA-SPH clade either have a rhizome or no specialised perennation organs. The rainfall seasonality of the distributions of most of the species is much less marked than for those in the 'Diploclinium grade', and they do not lose their leaves during the dry season. Only one clade lacks perennation organs entirely, consisting of *Begonia* sect. *Petermannia* species distributed in the largely everwet Malesian region.

Discussion

Although support for the backbone of the tree was weak, the taxon composition of the two major clades, A and B, matches that of strongly supported clades found using chloroplast sequence data by Thomas (2010), with much greater sampling of Himalayan species in this study. In addition, the overall topology of clade B matches the results of Thomas (2010) with respect to a clade of *Begonia* sects. *Platycentrum* and *Sphenanthera* (PLA-SPH in Fig. 2) being nested within a grade consisting mainly of *Begonia* sect. *Diploclinium* species from the continent (labelled 'Diploclinium grade' in Fig. 2); also congruent is the Chinese species *B. grandis* being sister to the remaining taxa. The basal relationships in Clade A are unsupported, but the supported subclades within Clade A are similar to those found by Thomas (2010), and generally correspond to species of a single section or geographic region. This congruence gives some confidence in the tree topology, despite the disappointing levels of support.

The Himalayas as a link in an eastward Begonia migration
If the Himalayas acted as a migration corridor in the early evolution of Asian *Begonia*, we would expect to see other species in Asia nested within a Himalayan grade in the phylogeny. Due to the unsupported nodes at the base of the clade containing all the

Himalayan species, evidence for this is equivocal. Of the two major sections in the Himalayan *Begonia* flora (*Begonia* sects. *Diploclinium* and *Platycentrum*), *Begonia* sect. *Diploclinium* has the oldest lineages which date to between 14.7 (±7.6) and 12.3 (±6.7) Ma and accounts for nearly 60% of the species in Nepal. Some subclades in this group do show a likely Himalayan origin, but the supported nodes are dated in the late Miocene at c. 7.5 Ma and so post-date the early divergence of the section. Further work on other gene regions is needed to resolve the relationships in the 'Diploclinium grade'. The hypothesis of basally branching Himalayan lineages in *Begonia* sect. *Diploclinium* is not refuted by our results. However, the fact that the Chinese species *B. grandis* is possibly sister to the rest of the 'Diploclinium grade' is tantalising and raises the possibility of China being an ancestral area for this group.

The other major section, *Begonia* sect. *Platycentrum*, accounts for 35% of Nepalese species. The species are all included in the PLA-SPH clade with an origin in the late Miocene–early Pliocene. There is no evidence for the Himalayan species in *Begonia* sect. *Platycentrum* being relicts from an eastward migration early in the evolution of Asian *Begonia*, in fact the emerging picture is the exact opposite. This clade has a probable Chinese origin, and represents an entry of the genus into the Himalayan region separate from those in the 'Diploclinium grade'. The Himalayan species are largely highly nested within this clade and there is evidence for more than one migration into the Himalayan region, including a very recent one in the late Pliocene–early Pleistocene (bottom of Fig 2; clade from *B. hatacoa* to *B. nuwakotensis*).

Other major clades containing the bulk of species diversity in Southeast Asia (*Begonia* sect. *Petermannia* from Malesia; *Begonia* sect. *Diploclinium* from the Philippines; *Begonia* sect. *Reichenheimia* from the Sunda shelf) remain unsupported in Clade A. Our results do not show any affinity of these clades with Himalayan taxa, and the origin of these large radiations remains enigmatic.

Paleo-climatic change, Himalayan orogeny and evolution of Nepalese Begonia
It is possible to highlight two main events in the evolution of the Himalayan *Begonia* flora—the mid- to late Miocene diversification of the tuberous, seasonally adapted 'Diploclinium grade' and the late Miocene–Pliocene immigration and diversification of the rhizomatous and evergreen PLA-SPH clade.

The early diversification of tuberous clades with a likely origin in the Himalayas can be dated to between 6.4 (±4.8) and 7.4 (±4.6) Ma. This coincides with the development of the Asian monsoon 7.4 Ma (Copeland 1997), due to the Tibetan plateau having reached sufficient altitudes to affect a major re-organisation of atmospheric circulation over the Asian continent (Zheng et al. 2004). The tuberous species would be well adapted to this seasonal monsoon climate, with concentrated periods of intense rainfall interspersed with significant dry seasons. These species are deciduous, and their flowering periods are strongly constrained to the three months after the onset of the monsoon (Rajbhandary et al. 2010). The subsequently formed dry dehiscent capsular fruits are then able to disperse their seeds either passively or through wind assistance during the following dry season.

The immigration of members of the PLA-SPH clade into the Himalayan region began 5.1 (±3.2) Ma, with an endemic radiation starting 3.0 (±2.1) Ma and continuing throughout the Pleistocene. The immigration coincides with a further intensification of the Asian monsoon around 5 to 3.6 Ma, possibly linked to a further surge in the uplift of the Himalayas and the Tibetan plateau (Zheng et al. 2004, Zhisheng et al. 2001) and changes in ocean currents in the Indo-Pacific region (Srinivasan & Sinha 2000). The evolution of an evergreen, rhizomatous habit in this clade suggests adaptations to wetter conditions, but the details of how the Pliocene monsoon intensification affected total and seasonal rainfall in the Chinese (presumably SW China) region where the PLA-SPH clade originated are unknown. The Himalayan species in this clade currently occur in areas with similar rainfall seasonality to the tuberous species, but occupy different micro habitats where water is more constantly available; their flowering is not strongly constrained by the monsoon (Rajbhandary et al. 2010). They have fruits which are adapted to rain-splash seed dispersal, and hence depend on either rainfall or drips and splashes from waterfalls to disperse. Our results are congruent with those of Tebbitt et al. (2006), who suggested that members of *Begonia* sect. *Platycentrum*, which have fruit morphologies indicative of rain dispersal, evolved from wind-dispersed Asian taxa following the colonisation of wetter habitats.

Of the two groups of *Begonia* in the Himalayas, seasonally deciduous and evergreen, only the former could possibly be relicts of an eastward migration. However, due to the unresolved relationships of clades within this group, their origins remain unknown. The evergreen species represent a re-entry to the Himalayan region, most likely from China, and have speciated in response to further tectonic uplift and Pliocene–Pleistocene climatic cycles and changes in the monsoon intensity. These factors are also likely to have strongly influenced the recent diversification of other elements of the Himalayan flora. Further phylogenetic studies of Himalayan plants will reveal the relative contribution of relict clades and recent speciation to the considerable plant diversity in the region.

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Appendix A. List of the Genbank accession numbers of the DNA sequences used in the phylogenetic analysis.

B. aceroides, HQ729023; *B. acetosella,* AY048976; *B. aequata,* AF48514; *B. alicida,* HQ729022; *B. alpina,* AY753717; *B. alveolata,* AY048977; *B. amphioxus,* AF485150; *B. annulata,* HQ729060; *B. annulata2,* HQ729059; *B. aptera,* AF48510; *B. atricha,* HQ729047; *B. balansana,* AF485091; *B. barbata,* AF485095; *B. baviensis,* AF485087; *B. brevipes,* HQ729048; *B. bryophila,* HQ729030; *B. burbidgei,* HQ729049; *B. cathayana,* AF280106; *B. ceratocarpa,* AY048978; *B. chiasmogyna,* HQ729050; *B. chloroneura,* AF485134 ; *B. chlorostricta,* AF485153; *B. circumlobata,* AY753721; *B. cleopatrae,* AF485133; *B. crispipila,* HQ729051; *B. demissa,* HQ729026; *B. dioica1,* HQ729039; *B. dioica2,* HQ729038; *B. dipetala,* AF485142; *B. discreta,* HQ729024; *B. dregei,* AY429336; *B. flagellaris,* HQ729031; *B.formosana,* AF485119; *B. geranioides,* AF469120; *B. goegoensis,* AF485138; *B. grandis,* AF485089; *B. grandis2,* AF485088; *B. halconensis,* AF485106; *B. handelii,* AY048982; *B. hatacoa2,* AF485111; *B. hemsleyana,* AF485099; *B.hernandioides,* AF485135; *B. hyatae,* AJ287262; *B. incisa,* AF485148; *B. josephii2,* HQ729037; *B. kingiana,* AF485139; *B. koordersii,* HQ729052; *B. labordei,* AF485122; *B. laruei,* HQ729058; *B. leprosa,* AY753722; *B. leptoptera,* HQ729036; *B. leptoptera2,* HQ729033; *B. leptoptera3,* HQ729034; *B. leptoptera4,* HQ729035; *B. longicarpa,* AF485109; *B. longifolia,* AF485105; *B. macintyreana,* HQ729054; *B. malabarica,* AF468141; *B. malachosticta,* AF485156; *B. masoniana,* AF485123; *B. morsei,* AF485130; *B. multangula,* AY753724; *B. muricata,* AY753725; *B. negrosensis,* HQ729055; *B. nepalensis,* AY753726; *B. nuwakotensis,* HQ729061; *B. ovatifolia,* HQ729032; *B. palmata,* AF485113.1; *B. palmata,* AF485115.1; *B. panchtharensis,* HQ729062; *B. parvula,* GU176066; *B. pasamanensis,* HQ729070; *B. pavonina,* AY753727; *B. picta,* HQ729042; *B. picta2,* HQ729041; *B. pseudolateralis,* HQ729053; *B. putii,* HQ729025; *B. rabillii,* HQ729027; *B. rajah,* AF485136; *B. ramosii,* HQ729057; *B. ravenii,* HQ729040; *B. rex,* AY753728; *B. robusta,* AY753729; *B. roxburghii,* AF485092; *B. rubella,* AF485112; *B. rubella2,* HQ729043; *B. rubella5,* HQ729044; *B. ruboides,* AY048987; *B. samhaensis,* AF469122; *B. scottii,* HQ729063; *B. setifolia,* AY048990; *B. sikkimensis,* HQ729064; *B. silletensis,* AF485094; *B. sinwaensis,* HQ729029; *B. smithiae,* HQ729065; *B. socotrana,* AF469121; *B. suborbiculata,* HQ729069; *B. taiwaniana,* AY753731; *B. taligera1,* HQ729066; *B. taligera2,* HQ729067; *B. tenuifolia,* HQ873478; *B. teysmanniana,* HQ729068; *B. tribenensis,* HQ729045; *B. variabilis,* AY753732; *B. variabilis,* HQ729046; *B. varipeltata,* HQ729056; *B. versicolor,* AF485090; *B. wenshanensis,* AY048974; *B. xanthina,* AY753733; *Begonia* sp. (China)*,* GU176063; *Begonia* sp. (Nepal)*,* HQ729028.