# Evolution and biogeography of Balsaminaceae: insights from molecular phylogeny


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# Evolution and biogeography of Balsaminaceae: insights from molecular phylogeny

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The lists of species and origins of materials used in this thesis

**THE PUBLISHED PAPERS AND MANUSCRIPTS**

I. Chromosomal evolution in Balsaminaceae, with cytological observations on 45 species from Southeast Asia.

II. Seedcoat Micromorphology of *Impatiens* (Balsaminaceae) from China.

III. Phylogeny and biogeography of Balsaminaeae inferred from ITS sequences.

IV. Phylogenetics of *Impatiens* and *Hydrocera* (Balsaminaceae) using chloroplast atpB-rbcL spacer sequence.

V. Phylogenetic analysis of Balsaminaceae based on one nuclear and two chloroplast markers

# Summary

Balsaminaceae are a family of predominantly annual or perennial herbs, with strikingly beautiful flowers. The family consists of two genera: the monotypic *Hydrocera* and the large genus *Impatiens*, with over 1000 species. This family is distributed all over the world except in the South America and Australia. While, *Hydrocera* is found in the Indo-Malaysia areas, *Impatiens* is highly clustered in tropical Africa, Madagascar, southern India, the eastern Himalayas, Southeast Asia. Only a few *Impatiens* species are found in the temperate areas of the northern hemisphere. The tremendous diversity of the flowers is embodied in the diversity of the shape and size of the spur and the two united lateral petals, which are hypothesized to be mediated by pollinators.

In order to have a better understanding of the morphological and karyological evolution, a further morphological investigation with special emphasis on seedcoat micromorphology of 38 species of *Impatiens* mostly from southwestern China and a further karyological investigation with a special emphasis on 45 species of *Impatiens* from southwest China and the adjacent areas was carried out. A high diversity of seedcoat sculpture patterns were found in seedcoat micromorphology of *Impatiens*. Four morphological types: laevigate, granulate, reticulate and protrusive type was distinguished. Taxonomic and phylogenetic implications of the seedcoat micromorphology are also discussed. Karyological investigations revealed the somatic chromosome numbers vary greatly from 2n=6 to 2n=66 in *Impatiens*. Considering all the available chromosomal data, x=7, 8, 9, 10 are the most frequent basic numbers of the family. Based on the present chromosome data, it is supposed that x=8, x=9, or x=10 are all possible of the ancestral basic numbers in *Impatiens*. Geographic distributions of the most frequent basic numbers show interesting patterns: x=7, 8 in Africa, x=7, 8, 10 in southern India and Sri Lanka, x=7, 9, 10 in the Himalayas, x=7, 8, 9, 10 in Southeast Asia and x=10 in Northern Asia, Europe and North America.

In order to avoid being trapped by the obvious morphological and karyological homoplasy, the DNA molecular phylogenetics of this family were explored. Using phylogenetic reconstruction, I try to elucidate relationships in this family, particularly the relationships within *Impatiens*. DNA markers of both biparental origin internal transcribed spacers (ITS) and maternal inheritance (trnL intron and trnL-F spacer, atpB-rbcL spacer) have been chosen. 182 species of this family coming from different distribution centers and three species of its closest relatives Marcgraviaceae and two species of Tetrameristaceae were amplified by PCR, sequenced and phylogenetically analyzed applying distance estimates and Bayesian method. Finally, five phylogenies were received: ITS NJ tree, trnLF NJ tree, atpB-rbcL NJ tree, combined NJ tree and combined Bayesian tree. They are congruent with each other, but not identical. Only combined Bayesian tree is well supported in most of the lineages. However, both of the analyses results based on separate and combined DNA data confirmed the monophyly of Balsaminaceae and *Impatiens*. From the combined Bayesian tree, it is seen more clear: the phylogenetic relationships in *Impatiens* are associated with geographic distribution, less defined by gross morphology.

Balsaminaceae show interesting biogeographic patterns in the paleotropics. Before the present study, it was not clear whether the great majority of species that occur disjunctively in the paleotropical regions is the result of the fragmentation of Gondwana or more recent dispersal. The present molecular phylogeny study does reveal that extant *Impatiens* species are of mainland Southeast Asian origin, from where dispersal to boreal Eurasia and North America,

to central Asia and Eastern Europe via the Himalayas, and to India and Africa have occurred. The Madagascan *Impatiens* show an African origin.

In order to get a clearer idea about the floral evolution and chromosome evolution of this family, the basic chromosome number and five morphological characters: the occurrence of flower spur, the number of lateral sepals the shape of fruit, the type of pollen, and the type of seedcoat micromorphology were mapped onto the combined Bayesian tree. The parsimonious optimization suggests that spurred flower, narrowly-fusiform capsule, 4 lateral sepals, 3-colpate pollen and reticulate seedcoat represent the plesiomorphic states within *Impatiens*. Both x=8 or 10 might be the ancestral basic chromosome number in Balsaminaceae.

The size and form of spur, lateral united petals and dorsal petals were integrated with molecular phylogeny as well. It shows that the diverse and complicate specialization of floral structures of Balsaminaceae is pollinator-mediated, and therefore highly homoplasious.

**Key words:** Balsaminaceae, *Hydrocera*, *Impatiens*, nrDNA, internal transcribed spacers (ITS), trnL intron and trnL-F spacer, atpB-rbcL, PCR, sequencing, molecular phylogeny, karyology, chromosome number, basic number, chromosomal evolution, bimodal karyotypes, systematics, seedcoat morphology.

# Chapter 1 Introduction

## 1.1 Balsaminaceae brief overview

Balsaminaceae are a medium-sized family comprised of two genera, the monotypic *Hydrocera* and the prolific *Impatiens*. Plants are more or less succulent, annual, or perennial, with a basal aggregation of leaves, or with neither basal nor terminal aggregations of leaves, helophytic or mesophytic, with solitary flowers, or aggregated in cymose‘inflorescences’. The two genera are easily distinguished from each other: *Hydrocera* has free petals and an indehiscent ‘berry-like’ fruit, whereas *Impatiens* has the petals variously united, and a characteristic, rather explosive, dehiscent fruit capsule. Other generic names such as: *Petalonema* Peter, *Semeiocardium* Zoll and *Impatientella* H. Perrier, are synonyms of *Impatiens* (Grey-Wilson, 1980a; Rao et al., 1986). *Hydrocera* is restricted to the Indo-Malesian countries, while *Impatiens* occurs in Asia, Africa, Madagascar, India, Europe and North America, and is absent from Australia and South America. But its ‘hotspots’ of diversification are in tropical and subtropical montane regions of the Old World. *Impatiens* has high diversity in floral morphology (Fig. 1.1). This made the Balsaminaceae an idea model to study floral evolution.




**Fig. 1.1 Examples of varied flower morphology of *Impatiens***

### 1.1.1 Position of Balsaminaceae

Most classic classifications based on morphology place Balsaminaceae in Geraniales and suggest that it is closely related to Tropaeolaceae and Geraniaceae (Cronquist, 1981; Thorne, 2000), while some important taxonomists treated Balsaminaceae as an order: the Balsaminales (Dahlgren, 1989; Takhtajan, 1997). However, recent molecular analyses indicate that Balsaminaceae belong to the order Ericales (Morton et al., 1996, 1997; Soltis et al., 2000; Albach et al., 2001; Anderberg et al., 2002; Bremer et al., 2002; Geuten et al., 2004). Both plastid (rbcL) and nuclear (18S rDNA) or combined sequence data suggest a close relationship between Balsaminaceae and Tetrameristaceae, Marcgraviaceae, and Fouquieriaceae (Fig. 1.2). This knowledge is helpful to choose suitable outgroups for the phylogenetic analyses of Balsaminaceae.

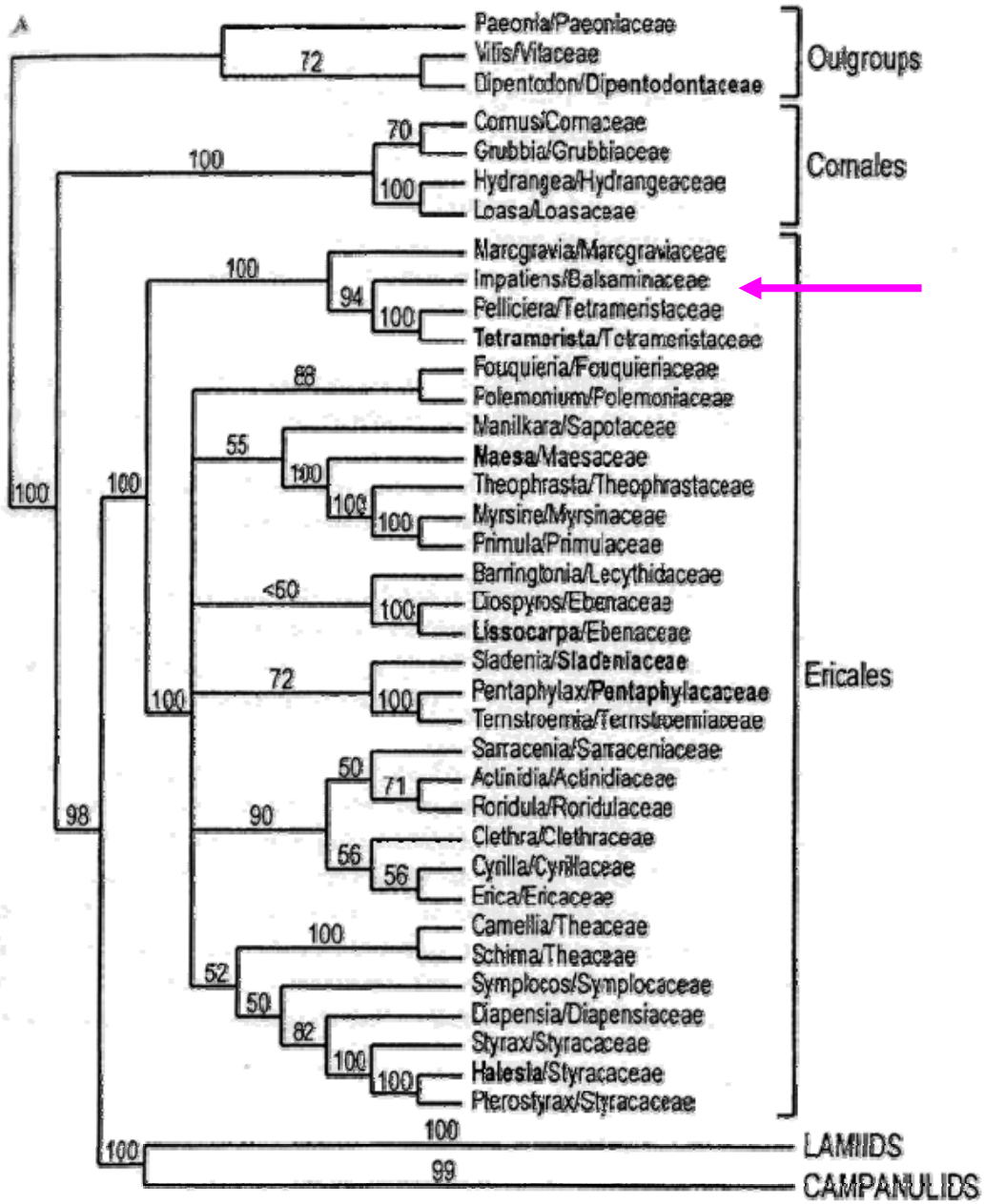



**Fig. 1.2 A phylogenetic tree shows the position of Balsaminaceae and its relationships with the other families (Bremer et al., 2002)**

### 1.1.2 The genus *Hydrocera*

#### 1.1.2.1 Habitat and distribution

*Hydrocera* is a semi-aquatic plant that grows in stagnant water in pools, lakes and ditches, with the lower part of the stem submerged up to 70 cm of water. It is monotypic with *Hydrocera triflora* Wight & Arn., and occurs in southern India, Ceylon, Thailand, Vietnam, Laos, Cambodia, Malaysia, Indonesia and south China.

#### 1.1.2.2 Morphology

**Plant:** The portion of the stem above water is leafy, unbranched and erect, while the submerged portion is thickened and spongy in texture, leafless, but rooting at the lower nodes.
**Flower:** The flower is resupinate through 180 degrees. The basic structure consists of 4 whorls: 5 sepals, 5 petals, 5 stamens and 5-carpellary ovary. The sepals and petals are free from each other. All sepals are conspicuous and colored like the petals. The outer pair of lateral sepals lie below the upper lateral sepals. They are large and project downwards and backwards so as to enfold the lower sepal and spur completely. The lower sepal has a broad mouth, and in lateral view is more or less navicular, being constricted into a short, stout, incurved spur. It serves to carry nectar as well as a support for the lateral petals. The 5 petals are large and conspicuous. The partial hood-like dorsal petal lies outside the other petals, protecting the androecium. The lateral petals have two pairs, an upper pair and lower pair. The upper seems to serve no particular function other than to attract pollinators. The lower provides adequate support for visiting insects. They are a little bit overlap, but still remain free to each other.
The five stamens are united together by the upper part of the filaments and the anthers and completely encircle the gynoecium. The anthers project downwards so that they act like a ‘brush’ against the body of the pollinator. The ovary consists of 5 fused carpels, each carpel containing a single ovule (Fig. 1.3).
**Fruits:** Fleshy indehiscent pseudoberry, globose, 5-angled, 5-loculed, with one seed per locule, with a short beak which represents the remains of the stigma.

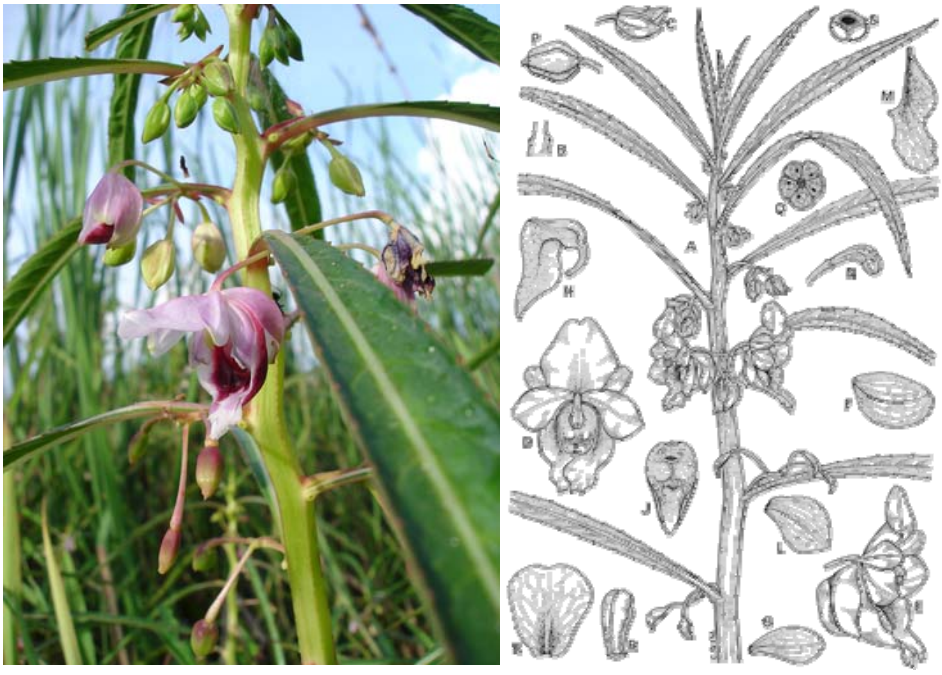


**Fig. 1.3 Picture of *Hydrocera triflora***

### 1.1.3 The genus *Impatiens*

#### 1.1.3.1 Habitat and distribution

*Impatiens* is nearly always associated with wet and humid places, frequently growing along the sides of rivers and streams, in grassy marshlands, or in the spray zones of waterfalls and cascades. It is rare to find *Impatiens* growing by stagnant waters. Most species are terrestrial and a few are epiphytic. It is a large genus with over 1000 species scattered throughout in the highlands and mountains of the tropical and subtropical regions of the Old World. It has five conspicuous diversity hotspots: tropical Afica (ca. 120 spp. Grey-Wilson, 1980a); Madagascar (ca. 200 spp. Fischer & Rahelivololona, 2002, 2003, 2004); southern India and Sri Lanka (ca. 220 spp. Bhaskar & Razi, 1981); the eastern Himalayas (ca. 120 spp.), and Southeast Asia in its broad sense (including Burma, Thailand, southwest China, the Indochina peninsula, and the Malesian archipelagos, ca. 250 spp.). There are 7 native species in North America and 2 native species in Europe and 2 native species in Boreal Eurasia. There are no native species in South America or Australia.

#### 1.1.3.2 Morphology

**Plant:** *Impatiens* are primarily annual or perennial herbs. The stems are typically rather thick and fleshy and often semi-translucent, only rarely becoming thickened and woody. The leaves are generally becoming membranous, often almost transparent when dried. Some species are rhizomatous or tuberous.

**Flower:** *Impatiens* flowers show a fascinating diversity in shapes and colors. They are zygomorphic and usually resupinate through twisting of the pedicel. In most species each flower has three sepals, two of which are usually small-sized lateral sepals and one petaloid lower sepal that is modified into a nectary-tipped spur. Some species have five sepals, two pairs of small-sized lateral sepal plus the peraloid lower sepal. All species of *Impatiens* have five petals. One upper dorsal petal is usually hood-like. The other four lower petals are connate into two lateral pairs. The tremendous floral diversity is mainly embodied in the diversity of the shape and size of the spur and the two united lateral petals. The floral morphology is so important for classification in this family, thus, I will describe the sepals and petals in detail with examples respectively. The terminology of floral structure used in the research is as follows and refers entirely to the resupinate flower (Fig. 1.4).

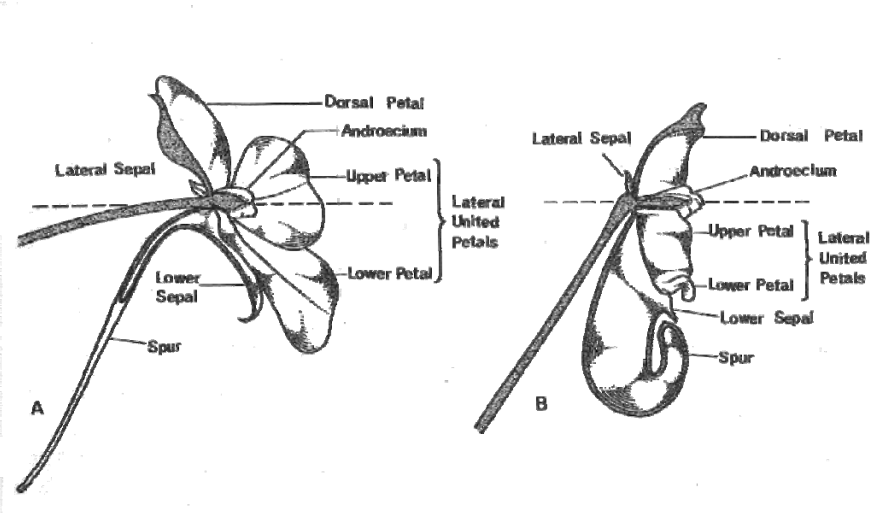



**Fig. 1.4 Longitudinal half flowers of A*: Impatiens walleriana*; B: *I. niamniamensis*. (Grey-Wilson, 1980b)**

**Lateral sepals:** In most *Impatiens*, there are only 2 lateral sepals (one pair) which have the form of narrow -lanceolate as in *I. rubrostriata* Hook.f. (Fig. 1.5) or broadly ovate as in *I. purpurea* Hand.-Mazz. Some species have 4 lateral sepals (2 pair), usually the inner pair is linear, smaller and narrower than the out pair which sometimes is a little bit ovate, as in *I. arguta* Hook.f. & Thomas. (Fig. 1.5). Sometimes the two pair of lateral sepals are slightly over-lapped, but together lie to the outside the lower sepal. This 4 lateral sepals type is not common in *Impatiens*, but exists in different regions: 7 African *Impatiens* among 109 (Grey-Wilson, 1980b), e.g. *I. tinctoria* Grey-Wilson; 35 Chinese *Impatiens* among 240 (Chen, 2001; Huang et al., 2003), e.g. *I. barbata* Comber; 7 species in Vietnam, e.g. *I. clavigera* Hook.f.; 2 species in Madagascar (Fischer & Rahellivololona, 2003), e.g. *I. kuepferi* Eb.Fisch. & Raheliv. Species with 4 lateral sepals are represented in India as well, but only in north India (Grey-Wilson 1980b, Bhaskar & Razi, 1981). The number and the form of lateral sepals is a key character for the classification in *Impatiens*.



**1** **2**

**Fig. 1.5 1. *I. rubrostriata* with 2 lateral sepals 2. *I. arguta* with 4 lateral sepals**



**1** **2** **3**

**4** **5** **6**

**Fig. 1.6 Different types of spur in *Impatiens*: 1. *I. balfourii*, shallowly navicular spur; 2. *I. fenghuaiana*, long filiform spur; 3. *I. poculifer*, saccate spur; 4. *I. radiata*, straight spur; 5. *I. souliana*, spit spur; 6. *I. YMG7* (from Madagascar), no spur**

**Lower sepal (developing into nectary-tipped spur):** The lower sepals of *Impatiens* exhibits an extraordinary wide range of variation in form and size: shallowly navicular, e.g. *I. balfourii* Hook.f., bucciniform, e.g. *I. tuberosa* H.Perrier., deeply saccate, e.g. *I. poculifer* Hook.f., short, e.g. *I. parviflora* DC., long filiform, e.g. *I. fenghwaiana* Y.L.Chen, straight, e.g. *I. radiata* Hook.f., twisted, e.g. *I. ruiliensis* S.Akiyama & H.Ohba, curved, e.g. *I. forrestii* Hook. f. ex W.W.Smith, divided, e.g. *I. soulieana* Hook.f. etc. (Fig. 1.6). Usually the spur has nectar inside. Obviously, these variations are related with different pollinator. Some Madagascan endemic species have no spur at all, e.g. *I. malcomberi* Eb.Fisch. & Raheliv.

**Lateral petals:** The 4 lateral petals of *Impatiens* are fused together in 2 pairs, some are joined only near the base as in *I. walleriana* Hook.f. and *I. aureliana* Hook.f. (Fig. 1.7), but most of *Impatiens* are more firmly joined as in *I. chungtienensis* Y. L.Chen and *I. mengtzeana* Hook.f. Most of *Impatiens* I studied, , the upper part is reduced while the lower of each pair is very much elaborated. For example, in *I. andringtrensis* H. Perrier, *I. tinctoria* and *I. rectangula* Hand.-Mazz., the lower part is much larger than upper part (lower/uppper $\geqslant$ 2); in *I. nolitangere* L. and *I. uligenosa* Franch., the lower part is larger than upper part but the proportion is between 1-2 times. Some species such as in *I. lyalii* Baker and *I. javensis* Steud., the lateral united pairs are almost equal in size (Fig. 1.7). The variable united lateral petals are adapted to different pollination syndromes as they provide a suitable landing platform and entrance guide for pollinators to the spur and the nectar.




**Fig. 1.7 Different types of lateral petals in *Impatiens*: 1. *I. aureliana*; 2. *I. tinctoria*; 3. *I. mengtzeana*; 4. *I. nolitangere.***

Grey-Wilson (1980b) proposed based on African species that, the shape of the petals and their size is related to the type of lower sepals and spur. In general, though not in all instances, the petals are large in the filiform-spurred species and small in the bucciniform or saccate-spurred species.

**Dorsal petal:** The dorsal petals of *Impatiens* can be divided into two types: (1). the dorsal petal is a basically flat, rounded structure with a crest behind, somewhat concave. It was mentioned that this kind of flat dorsal petal is companied with filiform spur and equally large lateral petals in African species (Grey-Wilson, 1980b), as in *I. walleriana* and *I. sodenii* Engl. & Warb. ex Engl., but I observed flat dorsal petals companied with other navicular spur or saccate spur in Asian species, as in *I. lateristachys* Y.L. Chen & Y.Q. Lu and *I. lingzhiensis* Y. L. Chen. (2). The dorsal petal may be distinctly cucullate, forming a characteristic hood over the top of the androecium and projecting obliquely forward. Grey-Wilson (1980b) mentioned that this kind of dorsal petal is usually associated with a deeply navicular, bussiniform or saccate spur type, with the result that there is little room for the upper petal of each lateral united pair, as in *I. auricoma* Baill. and *I. keilii* Gilg. This type exists in other places, as in *I. repens* Moon. (southern India), *I.corchorifolia* Franch. (China). The shape and position of dorsal petals are related to the type of pollinator and the flower color.

The gynoecium and androecium are basically very similar to *Hydrocera*. The five stamens have short and flat filaments. The upper parts of the filaments and the anthers are more or less united forming a cap over the superior gymoecium which has 5 fused carpels and contains many ovules.

**Fruits:** Elongated linear type or broadly fusiform explosive capsule, often containing many seeds.

### 1.1.4 A historical survey of Balsaminaceae

#### 1.1.4.1 Classification

This family is a well-known example of a taxonomically difficult group as ‘terror for botanists’, because of the delicate yet fragile nature and hypervariable structure of its flowers. It is extremely difficult to examine in dried specimens if prepared conventionally (Gray-Wilson, 1980b, c). So far, there is no comprehensive infrageneric classification available for *Impatiens*. The only global attempt was the early work of Warburg and Reiche (1895), in which 14 sections were recognized. He first divided this genus into two subgenera according to the leaves appearing the basic or top position of the stem, then divided into 14 groups based on the arrangement of the leaves, the number of the flowers on the inflorescences and the proportion of the length of the flower and spur. Grey-Wilson (1980b) thought this classification was neither natural nor practical, and was thus not followed by the later authors.

Table1.1 lists the main researchers who have contributed to the classification of Balsaminaceae from different regions. The three most important reports with large number of *Impatiens* is: 1. Within African *Impatiens*, the present of peduncle and shape of lower sepal is important for the classification. Grey-Wilson (1980b,e) proposed important revision which recognized six informal infragenetic groups based on these characters, though he agreed that the *Impatiens* species were difficult to divide into sections mainly because apparently important taxonomic characters seem to be rather randomly distributed through the species; 2. Within Chineses *Impatiens*, Chen (2001) gave the identification key which mainly depends on the shape of capsule, inflorescences, number of lateral sepals and shape of perianth. But no clear group were distinguished. Floral morphology like shape of lateral sepals, the shape of

**Table 1.1 Main research of the classification of Balsaminaceae from different region**

| Region | Authors | Name of paper | Species number |
|---|---|---|---|
| Africa | Wilczek & Schulze (1960) | Balsaminaceae. Flore du Congo et du Ruana-Burundi | 35 |
| | Grey-Wilson (1980b) | *Impatiens* of Africa | 109 |
| Madagascar | Perrier de la Bathie (1934) | Les *Impatiens* de Madagascar | 83 |
| | Fischer & Rahelivololona (2002, 2003, 2004) | New taxa of *Impatiens* (Balsaminaceae) from Madagascar. I, II, III | 30 |
| China | Akiyama et al. (1995) | Notes of *Impatiens* (Balsaminaceae) from Southwestern Yunnan, China | 5 |
| | Akiyama et al. (1996b) | Further notes of *Impatiens* (Balsaminaceae) from Yunnan, China | 5 |
| | Chen (1978) | Notulae de genere *Impatiens* L. florae sinicae | 65 |
| | Chen (1989) | Four new species of *Impatiens* (Balsaminaceae) from China | 4 |
| | Chen (1990) | Six new species of *Impatiens* from SiChuan | 6 |
| | Chen (1990) | New plants from the HengDuan mountains | 2 |
| | Chen (1999) | Six new species of *Impatiens* L. from China | 6 |
| | Chen (2000) | Three new species of *Impatiens* L. from China | 3 |
| | Chen (2001) | Flora of China | 220 |
| | Hooker (1908-1911) | Les espèces du genre «*Impatiens*» | 80 |
| India | Baskhar (1975) | Studies in Balsaminaceae | 38 |
| Sri Lanka | Grey-Wilson (1981) | Balsaminaceae: The handbook of the flora of Ceylon | 26 |
| Myanmar | Toppin (1920) | Notes on the Balsams of Chitral and the Kachin hills | 25 |
| Nepal | Hara (1979) | An enumeration of the flowering plants of Nepal (Balsaminaceae) | 39 |
| Thailand | Shimizu (1979) | The lectures on the flora of thailand | 40 |
| Himalaya | Akiyama et al. (1991) | Taxonomic notes of the East Himalayan species of *Impatiens*. Studies of Himalayan *Impatiens* (Balsaminaceae) (1). | 18 |
| Nieuw-guinea | Grey-Wilson (1980e) | *Impatiens* in Papuasia. Studies in Balsaminaceae | 12 |
| Sumatra | Grey-Wilson (1989) | A revision of Sumatran *Impatiens*. Studies in Balsaminaceae | 12 |
| Pakistan | Yasin J. Nasir (1980) | Flora of Pakistan | 12 |

spur, flower color, size and hairy etc. helped a lot for distinguishing the close species, these characters are useful for identifying the speices: *I. omeiana* Hook.f. is a yellow flower with narrowly infundibuliform lower sepal, with involute stout spur and *I. wilsonii* Hook.f. Eb. Fisch. is a white flower with saccate lower sepal, with very short curved spur; *I. siculifer* Hook.f. has navicular lateral sepals and *I. stenantha* Hook.f. has ovate-oblong lateral sepals; *I. imbecilla* Hook.f. is a small pink flower with puberulous the pedicels and bracts and *I. faberi*

Hook.f. is a big violet flower with glabrous pedicels and bracts. 3. Within Madagascan *Impatiens*, Perrier de la Bathie (1934) divides *Impatiens* first according the presence of spur, then using the type of spur and the way of anthers dehiscent. He proposed three sections based on floral morphology: 1) sect. *PreImpatiens* (=sect. *Impatiens*) (flowers with conspicuous spur), 2) sect. *Trimorphopetalum* (flowers without spur, anthers dehiscent apically), and 3) the monotypic sect. *Impatientella* (flowers without spur, anthers dehiscent laterally), the latter two being endemic to Madagascar. Fischer and Rahelivololona (2002) recently elevated sect. *Trimorphopetalum* to subgeneric level. The monotypic sect. *Impatientella* contains only the spurless and entirely cleistogamous species, *Impatiens inaperta* H. Perrier.

Most studies on the classification of *Impatiens* are restricted to distinct distribution regions. Therefore, a reliable ingragenetic classification is still missing. Although aggregates of closely allied species can be steadily recognized, it is rather hard to unravel the phylogenetic relationships among the species aggregates on the basis of gross morphology. These difficulties have necessarily limited most studies on *Impatiens* so far to regional and purely descriptive taxonomic treatments (e.g. Perrier de la Bathie, 1949; Humbert, 1956; Chen, 1978; Grey-Wilson, 1980a,b, 1989; Akiyama et al., 1992a,b, 1995; Akiyama & Ohba, 2000; Fischer & Rahelivololona, 2002).

New species are still being described from different regions (e.g. Bos, 1990; Fischer, 1997; Cheek & Fischer,1999; Shimizu, 2000; Cheek & Csiba, 2002; Huang et al., 2003; Raymond et al., 2005; Akiyama et al., 2005). More work needs to be done to give more investigation and confirmation about the classification of this family.

#### 1.1.4.2 General morphology

There are quite a lot of morphological studies on this family. Nearly all the previous classifications have been based on the gross morphology. The general gross morphology, taxonomic characters of Balsaminaceae include fruit shape, phyllotaxis, leaf shape, and inflorescence etc. (e.g. Perrier de la Bathie, 1934; Baskhar, 1975; Shimizu, 1979; Grey-Wilson, 1980b; Akiyama et al., 1991; Akiyama & Ohba**,** 2000; Chen, 2001). Floral morphology is the most important character, and seedcoat morphology and palynological studies have been done in this family and will be done more in the present study, thus, I just chose these three points to discuss here.

##### 1.1.4.2.1 Floral morphology and evolution

The fancinating diversity in floral morphology makes it a good candidate to study floral morphology and evolution. Grey-Wilson (1980b) has formulated a set of hypothesis on floral morphological evolution based on the study of African *Impatiens*: The ancestral type of *Impatiens* is with 5-merous structure with separate and equal sepals and petals (Fig. 1.8). The developing from lower sepals (adaxial) into elaborated nectariferous spur and the uniting of the lateral petals can preserve the symmetry of the flower. Resupination, by simple twisting of the pedicel through 180 degrees would bring the sepal into a ventral position and bring the anthers into the upper part of the blossom.

Resupination is good for the flower as it allows it to support a wide range of bulky structures, and the remaining four sepals would be displaced upwards (Pijl & Dodson, 1966). The upper pair of lateral sepals have disappeared in many instances, resulting in the evolution of flowers

with five sepals to flowers with three sepals (i.e. calyx merosity 5→3). Dorsal petal, lying on the outside of the other petals, could protect them in bud. The remaining petals have become united into two lateral pairs and together they attract pollinators, act as a landing stage for the pollinator (except in the case of hawkmoths) and provide an access point to the lower sepals and hence the nectary, situated in the tip of spur. Change to zygomorphic flower and resupination appear to be closely associated with pollinator, and all these evolution is to get efficient cross-pollination.

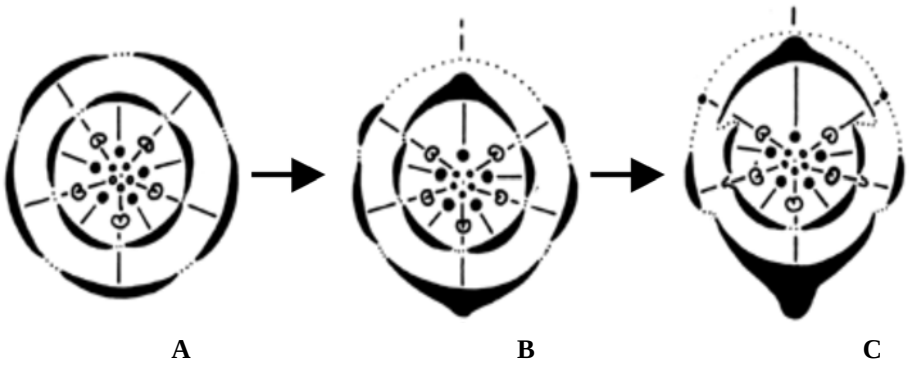



**Fig. 1.8 Floral diagrams showing the hypothetical development of an *Impatiens* flower from a regular structure. A, with free sepals and petals to an increasingly zygomorphic structure. B, with an increase in the size of the lower sepal and the upper petal, causing a subsequent decrease in size and displacement of the lateral sepals. C, with a much greater enlargement of the lower sepal and the upper petal, the disappearance of the upper pair of lateral sepals and the subsequent displacement and uniting of the lateral petals into pairs. All shown in the resupinate position (Grey-Wilson, 1980b)**

A great majority of species of the family have not yet been explicitly studied for pollination biology. Grey-Wilson (1980b) reviewed this in African species: Out of the 109 morphologically diverse *Impatiens* species, 58 are pollinated by butterflies, 3 by moths, 27 by birds, and 21 by bees. He analyzed the relation of the recombination of different floral traits, flower color and the adaptation to different pollinators, and proposed: erect or hood like dorsal petal, shallow lower petal, filiform spur, pink, mauve or purple flower for butterflies; hood like dorsal petal, shallow lower petal, filiform spur, white flower for moths; hood like dorsal petal, deeply navicular or bucciniform lower sepal, incurved spur, and white, yellow or pink flower for bees; hood like dorsal petal, broadly saccate lower sepal, incurved spur, and pink or mauve flower for large solitary bees; hood like dorsal petal, shallow or bucciniform lower petal, filiform or incurved spur, and red flower for birds, etc.

An intimate relationship between pollinators, floral morphology and angiosperm diversification has been suggested (Grant, 1949, 1971; Stebbins, 1970; Macior, 1971; Kiester et al., 1984). In *Impatiens*, if the multiple and independent gains of floral traits is prevalent, the diverse and complicated specialization of floral structures such as the shapes and sizes of the spurs and the morphology of the lateral united petals are probably pollinator-mediated, and therefore highly homoplasious. Phylogenetic assessments on the relationships among the complicate floral types and related pollination syndromes are still missing for Balsaminaceae.

Most species concepts incorporate the necessity of reproductive isolation (Dobzhanky, 1937; Mayr, 1942; Grant, 1963). Therefore, characters that can promote reproductive isolation may increase speciation and thus diversification rates. Because the length, shape, color, and orientation of nectar spurs can influence the types of pollinators that visit flowers, they are

closely linked with reproduction and reproductive isolation. Thus, if species differ in their floral morphology and consequently differ in the types of visitors that pollinate their flower, then even if the species come into secondary contact, selection for divergent flower morphologies via pollinator preferences may provide a prezygotic reproductive isolating mechanism and prevent the taxa from merging (Grant, 1952; Hodges & Arnold, 1994a, 1995).

#### 1.1.4.2.2 Seedcoat

Seedcoat ornamentation of *Impatiens* has been given much attention since Hooker and Thomson (1860), but without any comprehensive surveys. Before the present study, seed morphology of Balsaminaceae had been observed by Grey-wilson (1980b) in some African species, by Shimizu (1979), and Lu and Chen (1991) in some Chinese and Thailand species.

In Lu and Chen (1991)'s observation on 12 *Impatiens* species from Mt. Omei (southwest China) under scanning electron microscopy (SEM) , two types of seed surface morphology were identified: (1) Seeds without conspicuous differentiation of surface, represented by *I. wilsoni*; and (2) Seeds with the large cell elevation at different levels, surface scabrous, represented by the other 11 species. The species with the first type of seeds is indicated with 3-colpate pollen grains and the species with the second type is indicated with 4-colpate pollen type. Their results verify that the seed morphology of the genus can supply characters significant to taxonomy of the genus at species level. Shimizu (1979) examined the seedcoat of 20 Thailand species in his paper of discussing the classification and ecology of the Thailand *Impatines* species. But no detailed information about seedcoat was given except few describe of three species. *I. chinensis* L. has global smooth seeds, *I. platypetala* Lindl. has flat seeds covered with long hairs and *I. violaeflora* Hook.f. has two kinds of projection, big and minute ones. Grey-Wilson (1980b) examined seeds of 55 African species and five main seed types were distinguished: smooth seeds; warted seeds; shorted-haired seeds; long-haired seeds; seeds with club-shaped appendages. It is found that closely related species seem to have similar seed structures so that these characters would probably be useful in distinguishing groups or aggregates rather than individual species. Utami and Shimizu (2005) have done the seedcoat observation in 65 Southeast Asian species almost the same time with the present study. Some pictures were showed but the comparison and discussion with the classification of Warburg and Reiche (1895) made the seedcoat information disordered. No clear relationship between gross morphology and chromosome number was found. Seedcoat morphology alone does not provide universally applicable key characters for identification, but can be as helpful as many other characters used in taxonomy. Detailed observations on more species and a systematic overall evaluation of seedcoat micromorphology in *Impatiens* are necessary.

#### 1.1.4.2.3 Palynology

Some pollen morphological studies have been made on this family by some authors. Huynh (1968) has studied pollen morphology of nearly 350 *Impatiens* species from different areas and *Hydrocera*. 4-colpate with rectangular, 4-colpate with square, 3-colpate and 5-colpate were reported in *Impatiens*, and 3-colpate was found in *Hydrocera*. But, no discussion about pollen morphology and gross morphology were provided in his study. Grey-Wilson (1980e) reported some pollen morphology observations on some African hybrid species, and two main group were divided: (1) 3-colpate grains with a triangular equator or 4-colpate with a square or squarish equator, and (2) 4-colpate grains with a rectangular or oblong equator. Some mixture types were found from the same individual flower and hybridization within *Impatiens*

probably gave rise to many convergent pollen characters. The presence of 5-aperturate pollen in *Impatiens*, which is typical for hybrids was proposed by him. Lu (1991) studied 13 *Impatiens* species from Mt. Omei (southwest China) and both of 3-colpate and 4-colpate were found. She supposed that *Impatiens* with 3-colpate pollen type is a natural group and may have close relationship with *Hydrocera*. 4-colpate type pollen in *Impatiens* was suggested to be the advanced type in this genus. She proposed that the pollen morphology is of important significance in classification of the taxa and evaluation of systematic position of this family. Recently, Janssens et al. (2005) did more palynological study on African and Asian *Impatiens* species. Both 3-colpate and 4-colpate pollen type were reported and it is found that close related species have similar pollen morphology.

The pollen morphology of Balsaminaceae was also examined by Bhaskar and Raiz (1973, 1979), Tara and Namboodri (1974), Bhaskar et al. (1975), Durdana and Nair (1988), Perveen and Qaiser (2001) etc., but more extensive pollen study should be carried out within this family to find palynological support for classification and future molecular phylogeny.

The genus *Impatiens* can be roughly divided into two major groups based on the pollen data, like based on the shape of the fruit (Grey-Wilson, 1980). An examination of the pollen types of the northerly distributed group (those with linear fruit) shows that the 4-colpate rectangular type predominates. In Africa, southern India, Sri Lanka, Indochina and Southeast Asia, the 4-colpate rectangular type is about equal in number to the 4-colpate square and 3-colpate triangular grain types. In the transition zone, Burma-Indochina, there are about equal quantities of the three types, as far as it is known. However, the divergence of the northerly group seems to be supported again by the fact that the major pollen type in Europe, Central Asia, Japan and North America is the 4-colpate rectangular one.

#### 1.1.4.3 Cytotaxonomy

A great deal of karyological studies have been done on Balsaminaceae. Knowledge of chromosomal evolution of Balsaminaceae stands as an important aspect in understanding the extraordinary diversity, endemism, and biogeography of the family. *Hydrocera triflora* were found only with 2n=16 (Govindaran & Subramanian 1986; Rao et al. 1986). About 157 species of *Impatiens* from most of the distribution areas have been karyologically studied prior to our present study. Some important observations were made by Smith (1934), Khoshoo (1955a,b, 1956, 1957, 1966), Jones and Smith (1966), Bhaskar (1976, 1980), Larsen (1981), Zinov'eva-Stahevitch and Grant (1982, 1984, 1985), Rao et al. (1986), Govindarajan and Subramanian (1986), Akiyama et al. (1992b, 1993, 1996a), Sugawara et al. (1994, 1997), etc. The following somatic chromosome numbers have been reported: 2n=6, 8, 10, 12, 14, 15, 16, 17, 18, 19, 20, 24, 26, 28, 30, 32, 34, 36, 40, 44, 48, 50, 56, 66. The lowest chromosome number 2n=6 was found in *I. latifolia* L. and *I. leschenaultii* Wall. from southern India (Govindarajan & Subramanian, 1986), and the highest number 2n=66 was found in *I. mooreana* Schltr. from New Guinea (Jones & Smith, 1966). The most frequent chromosome number encountered so far are 2n=16, 2n=18, 2n=20, 2n=14, 2n=12 and 2n=32. As to the evolution of the basic chromosome numbers, Khoshoo (1957) proposed that 7 and/or 10 were the ancestral basic numbers of *Impatiens*, then he suggested (1966) that x=10 was perhaps more typical of the Himalayan species and this supposition supported the fact that x=10 was spread to temperate Asia, North America and Europe. Jones and Smith (1966) and Akiyama et al. (1992b) suggested x=7 to be the ancestral type and from which the other numbers were derived by mainly ascending dysploidy, whereas Rao et al. (1986) suggested that x=8 might

be the original basic number from which the other numbers, lower or higher, were derived by both descending and ascending dysploidy.

### 1.1.4.4 Biogeography

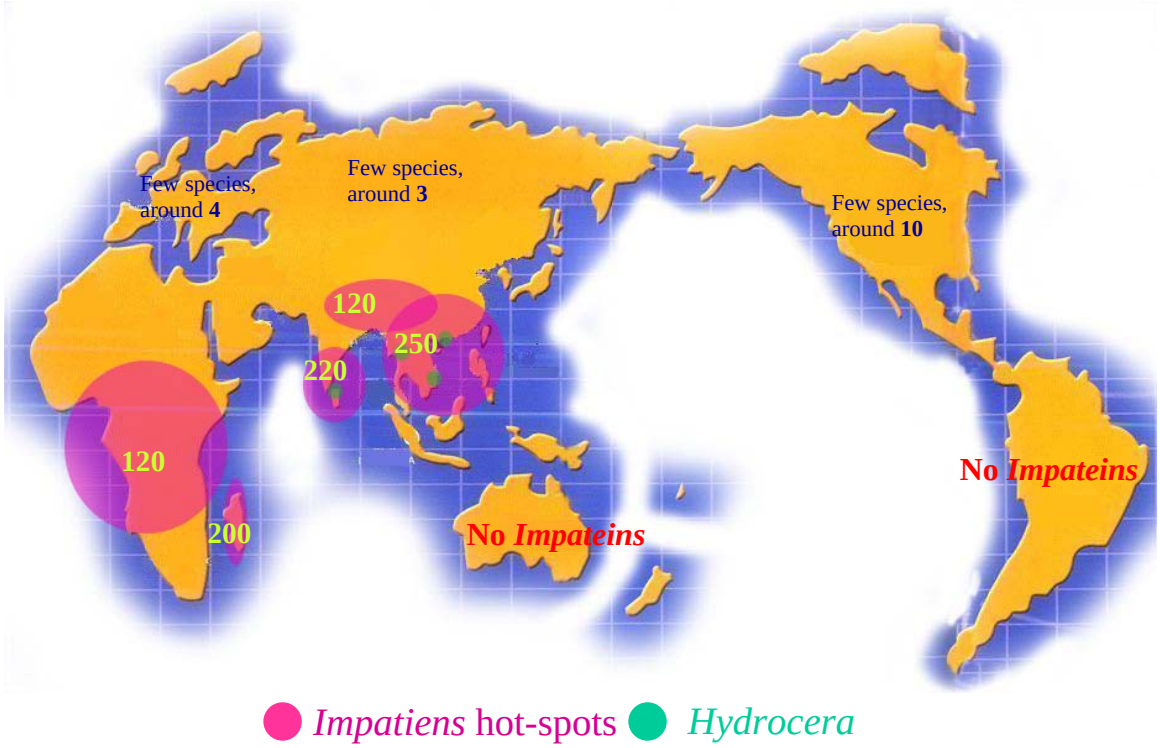



**Fig. 1.9 Distribution map of Balsaminaceae**

Balsaminaceae also show interesting biogeographic patterns in the paleotropics. *Hydrocera* is an Indo-Malesian genus, and *Impatiens* occurs in Asia, Africa, Madagascar, India, Europe and North America, and is absent from Australia and South America (Fig. 1.9). *Impatiens* has five conspicuous diversification centers in the paleotropical regions and the high degree of endemism were found in these centers. In tropical Africa, there are around 120 species (Grey-Wilson, 1980b; Bos, 19990; Fischer, 1997; Cheek & Fischer, 1999; Cheek & Csiba, 2002). Almost two-thirds of the African species of *Impatiens* confined to only six mountain regions: Uluguru/Ukaguru Mts (E. Tanzania); Ruwenzori and neighbouring Mts (E. Zaire, W. Uganda, Rwanda & Burundi); Mts of Nigeria, Cameroon and Gabon; Mt. of S. Tanzania & N. Malawi; Mt. of S. Malawi & E. Rhodesia/W. Mozambique; Usambara Mts N. Tanzania (Mt Meru & Kilimanjaro) (Grey-Wilson, 1980b). As of 1980, 44 species of *Impatiens* occur in East Africa, 23 occur in West Africa and 16 species occur in central Africa. Only two species occur in all three regions, four species shared in common between the east and central regions and five between the central and west. This showed the 'local endemic' distributions of *Impatiens* on the African continent, and no African species occur outside the continent (Grey-Wilson, 1980b). In Madagascar, there are around 200 species (Perrier de la Bathie, 1934, 1949; Fischer & Rahelivololona, 2002, 2003, 2004a, 20004b; Goodman & Benstead, 2003) and almost all the native species of Madagascar are endemic. Most of Madagascan *Impatiens* are found in eastern humid or rainy high-elevation regions and the mountains of the northern highlands. In southern India and Sri Lanka, there are around 220 species (Bhaskar & Razi, 1981), 91% of the southern Indian species are endemic (Chatterjee, 1940; Rao et al. 1986) and most of Indian *Impatiens* are concentrated in Western Ghats. There is no common species

shared between northern and southern India, and nearly 7 species both in Ceylon and south India (Bhaskar, 1981). In the eastern Himalayas, there are around 120 species, and there are few species that occur in both the west and the east. In the Southeast Asia area (including Burma, Thailand, Vietnam, southwest China) and the neighboring islands (including Indochina peninsula, Malesia archipelagos), there are around 250 species (Chen, 2001, Huang et al., 2003). It is indicated a high degree of endemism in this region and it was reported that the Southeastern Asian islands, the Philippines, Borneo, Java and New Guinea in particular, probably have an endemic rate per island or island group exceeding 90%. The work for flora of Thailand by Shimizu (1969) indicates 19 of 40 *Impatiens* species are endemic. The degree of endemism suggests active recent evolution and many taxa may be regarded as neoendemics.

On the contrary to the paleotropical areas, only a few species of *Impatiens* are represented in the temperate areas and tend to be very much more widespread. In northern and central Asia, only *I. brachycentra* Kar. & Kir. and *I. parviflora* and *I. nolitangere* occur. In Europe, *I. nolitangere*, *I. parviflora* occur and *I. balfourii* and *I. glandulifera* Arn. were introduced. In North America, there are 7 native species: *I. aurella* Rydb., *I. ecalcarata* Collett & Hemsl., *I. capensis* Meerb., *I. pallida* Nutt., *I. turrialbana* Donn. Sm., *I. nolitangere,* and *I. mexicana* Rydberg. Several species like *I. balsamina*, *I. walleriana*, *I. glandulifera* have been introduced and cultivated all of these places. There are no native species in South America and Australia. The absence of species from other regions such as North and Southwest Africa, southwest U. S. A., and central and east India can be largely accounted for by a climate too dry for *Impatiens*.

The area of endemism is defined as a geographic region comprising the distributions of two or more monophyletic taxa that exhibit a phylogenetic and distributional congruence and having their respective relatives occurring in other such defined regions (Harold & Mooi, 1994; Morrone**,** 1994; Szumik et al., 2002). Plant genera with large numbers of closely related, narrowly endemic species are thought to be undergoing rapid speciation (Cowling & holmes, 1992; Linder & Hardy, 2004). The large distribution area, the high diversity associated with localized endemism in *Impatiens* suggests that active and recent diversification via explosive speciation and rapid radiation have been prevalent in the evolution of this genus. This rapid radiation, subsequent parallel evolution and hybridization (Arisumi, 1977; Grey-Wilson, 1980b; Merlin & Grant, 1986) can further obscure the phylogenetic relationships among the species.

The historical biogeographic connection across the paleotropical regions stands out as a noticeable pattern and a key topic in the biogeography of plants and animals (Raven & Axelrod, 1974; Schatz, 1996; Raxworthy et al., 2002). For Balsaminaceae, it is still a debate whether the disjunctive distribution of the main diversity centers across the paleotropical regions of Balsaminaceae is the result of the fragmentation of the Gondwana or more recent dispersals. Jones and Smith (1966) believed that Himalayas represented the centre of origin from which species radiated both to the north and to the south. Khoshoo (1966) suggested the species spread from the Himalayas to temperate Asia, North America and Europe. But these hypothesis were mainly based on species diversity and karyological data. Bhaskar (1981) thought that southern India contains most of the primitive or phylogenetically old species and Western Ghats is the place of origin of *Impatiens* considering the concentration of diploids, primitive radial pollen grains, and shrubby habit. Grey-Wilson (1980a) believed that the Balsaminaceae originated in the western Gondwana in the Paleogene ca. 50 million years ago (Ma), and subsequent spread to Southeast Asia through Madagascar and India at the time of or after the Indian plate collided with the Laurasia (ca. 45 Ma). His conclusions were based

on pronounced similarity of species among Africa, Madagascar, and southern India, as well as on the assumed sister relationship between Balsaminaceae and Tropaeolaceae. He further suggested that the spreading drought during the Neogene (ca. 27 Ma) was the main cause of the isolation of species among the paleotropical diversity centres, i.e. tropical Africa, Madagascar, southern Indian and Sri Lanka, and Southeast Asia. Subsequently from Southeast Asia and the adjacent Sino-Himalayan area, *Impatiens* diversified secondarily into two lineages: one lineage radiated to the temperate Eurasian areas and North America, and another radiated to the tropical and subtropical areas in Southeast Asian islands. Meanwhile, he rejected the possibility of an overland migration between Africa and India.

## 1.2 DNA as systematic markers

### 1.2.1 Plant molecular systematics

Phylogenetic systematics is a discipline with three major objectives: 1) reconstructing the phylogenetic (evolutionary) relationships among organisms; 2) studying character evolution in reference to such reconstructions; and 3) producing classifications that reflect such relationships (Wiley, 1980). Molecular systematics have been widely studied in the last two decades applying of nucleic acid data to resolve plant relationships. Since DNA molecules have been proved not only the key to heredity, but also documents of evolutionary history. Therefore, DNA molecules inevitably become choices for modern systematics. Two primary classes of DNA data have been used to assess phylogenetic reconstruction in plants: the nuclear ribosomal DNA (nrDNA) and the chloroplast DNA (cpDNA). Usually, slowly evolving regions are used to resolve large-scale relationships; faster evolving regions are used to resolve relationships among closely related species (Mast & Givnish, 2002).

### 1.2.2 Chloroplast DNA (cpDNA)

Chloroplast DNA sequences are a primary source of data for plant molecular systematic studies. Early in the plant molecular systematics era, chloroplast DNA was surveyed through restriction site polymorphism studies (Olmstead & Palmer, 1994). As DNA sequencing technology became more available, comparative studies of cpDNA gene sequences began to accumulate sparked by the observations of Ritland and Clegg (1987) and Zurawski and Clegg (1987). The properties of cpDNA which made it suitable for evolutionary and phylogenetic studies include: quantitatively abundant and easy to amplify; small sized molecule and conservative in structure and gene organization, few arrangement in the evolutionary process (Palmer, 1995) (Fig. 1.10); relatively conservative sequence evolution of coding genes but relatively higher evolutionary rates in noncoding regions (introns and intergenic spacers). Noncoding regions have been presumed to be more useful at lower taxonomic ranks because they are less functionally constrained and are therefore freer to vary, thereby potentially providing more phylogenetically informative characters per unit of sequencing effort (Clegg et al., 1994).

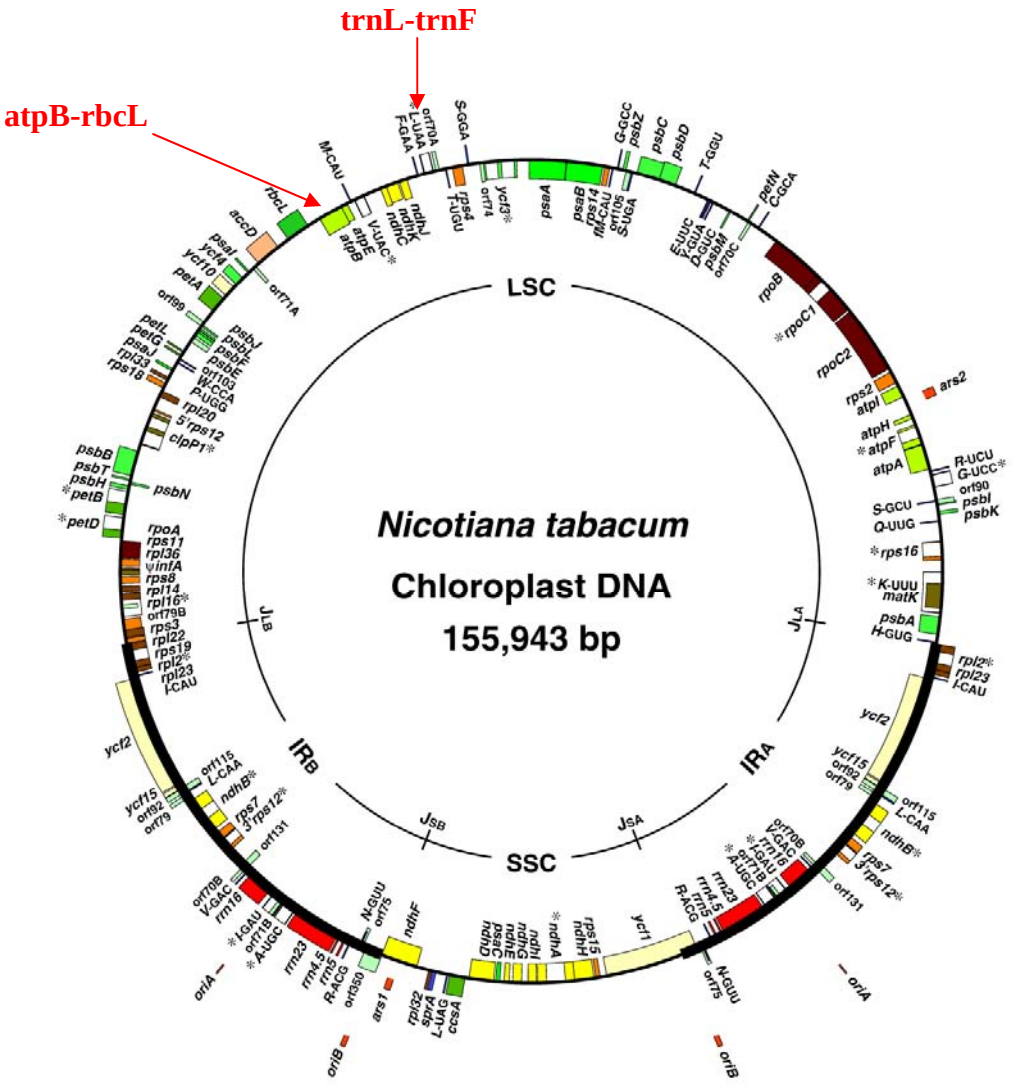



**Fig. 1.10** **Gene map of the chloroplast genome from tobacco (*N. tabacum*). Genes shown on the inside of the circle are transcribed clockwise, and those on the outside are transcribed counterclockwise. Genes for transfer RNAs are represented by 1-letter code of amino acids with anticodons. Asterisks denote split genes. Open-reading frames are shown by orf plus codon number (≥ 70 codons). When two genes overlap, the one that is located downstream or inside the other gene is displayed with a lower-height box (Yukawa et al., 2005)**

Many surveys have been published within the last decade (e.g. Downie & Palmer, 1987; Clegg et al., 1994; Graham et al., 2000). A landmark publication, the angiosperm rbcL study of Chase et al. (1993), set the stage for the increased use of cpDNA sequences for phylogenetic studies. Most early publications employed sequences of rbcL and were focused on supergeneric taxonomic questions (e.g. Chase et al., 1993). Subsequent workers began to explore additional gene sequences such as ndhF (Olmstead & Palmer, 1994; Olmstead & Reeves, 1995; Clark et al., 1995; Backlund et al., 2000; Beilstein et al., 2006); atpB (Hoot et al., 1995; Wolf, 1997; Soltis et al., 2000; Savolainen et al., 2000; Kathriarachchi et al., 2005); matK (Johnson & Soltis, 1994; Steele & Vilgalys, 1994; Nishfikaw et al., 2002; Quint & Classen-Bockhoff, 2006), these genes are usually used for higher classification level for phylogeny studies (Soltis et al., 1999; Qiu et al., 1999). Noncoding regions of the chloroplast were being explored for lower level taxonomic studies, such as trnT-trnL-trnL-trnF region (Taberlet et al., 1991), the atpB-rbcL intergenic spacer (Golenberg et al., 1993; Hodge & Arnold, 1994; Manen et al., 1994), and the noncoding intron portions of the trnK/matK region

(Johnson & Soltis, 1994; Steele & Vilgalys, 1994) are first regions to be exploited (Shaw et al., 2005). Some others noncoding regions, such as the rpS16, rpL16, trnH-psbA, trnS-trnG, rpl20-rps12, trnM-rbcL, trnS-trnT are now routinely employed for studies of phylogeny at intergeneric and interspecific levels (Renner, 1999; Hamilton, 1999; Clausing & Renner, 2001; Hamilton et al., 2003).

Choosing an appropriate cpDNA region for phylogenetic investigation is often difficult because of the paucity of information about the relative tempo of evolution among different noncoding cpDNA regions. Shaw et al. (2005) gave an excellent paper about the utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. They sampled across all of the major lineages of phanerogames sensu APG II (2003) and comparing the phylogenetic utility of each. They provide a discussion of the often used noncoding cpDNA regions and present a general protocol for selecting potential noncoding cpDNA regions useful to systematic investigations. However, it is inferred that even noncoding cpDNA regions often fail to provide significant phylogenetic information at low taxonomic levels (Small et al., 1998, 2004).

### 1.2.3 Nuclear ribosomal DNA (nrDNA)

The nuclear genes that code for the rRNA are repeated thousand of times in plant genomes and may comprise as much as 10 % of the total plant DNA. Ribosomal DNA arranged in tandem repeats in one or a few chromosomal loci. 18s-26s nr DNA gene family is important for the reconstruction of plant phylogeny. Nowadays, the internal transcribed spacer (ITS) region of nuclear ribosomal DNA has become popular in phylogenetic studies in many angiosperm families at lower taxonomic levels, especially at intergeneric and intrageneric levels (Baldwin et al., 1995). The ITS region (including 5.8s, ITS1 and ITS2) has a short sequence (600-700 base pair), with two highly conserved flanking regions, multi copies (Rogers & Bendich, 1987; Hamby & Zimmer, 1992), relative stable structure, concerted evolution (Arnheim et al., 1980; Hills et al., 1991; Baldwin et al., 1995), evolving fast and a high rate of nucleotide subsititutions. ITS characters have improved our understanding of angiosperm phylogeny in several groups by: 1) corroborating earlier unexpected findings; 2) resolving conflicts between other data sets; 3) improving resolution of species relationships; or 4) providing direct evidence of reticulate evolution (Baldwin et al., 1995). In addition, ITS data can be highly informative and accurate for detecting hybridization (Sang et al., 1995).

However, ITS data were not useful at both high taxonomic level and at low taxonomic level. At high taxonomic level such as family level or above, the high sequence divergence and length mutation of ITS makes sequence alignment very problematical (Wilson, 2003). It is due to orthology/paralogy conflation and problems in alignment due to indel accumulation (Alvarez & Wendel, 2003), or lack of resolving power (Whitcher & Wen, 2001).

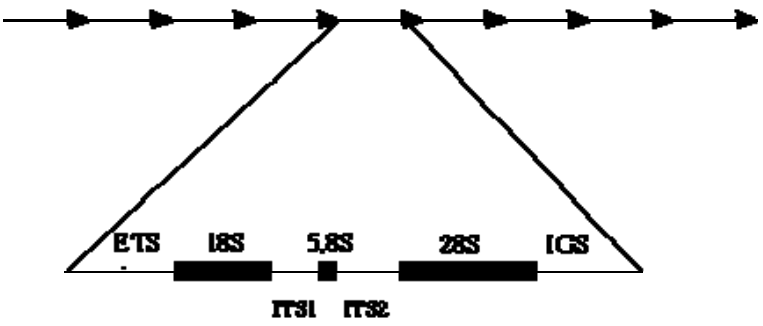



**Fig. 1.11 nr DNA and ITS region**

### 1.2.4 Low-copy nuclear DNA

Growing examples in the recent literature have demonstrated that low-copy compensate cpDNA and nrDNA for improvement of resolution and robustness of plant phylogenetic reconstruction (Soltis & Soltis, 1998; Doyle & Doyle, 1998; Sang, 2002; Grob et al. 2004). Low-copy nuclear genes in plants are a rich source of phylogenetic information because of its fast evolving rates. They hold a great potential to improve the robustness of phylogenetic reconstruction at all taxonomic levels, especially where universal markers such as cpDNA and nrDNA are unable to generate strong phylogenetic hypothesis. It has recently become clear that low-copy nuclear genes are particularly helpful in resolving close interspecific relationships and in reconstructing allopolyploidization in plants. The lack of the universal markers or universal PCR primers of low-copy nuclear genes has also hampered their phylogenetic utility. Although utilizing low-copy nuclear genes usually requires extra lab work such as designing PCR primers, PCR-cloning, and/or Southern blotting, rapid accumulation of gene sequences in the databases and advances in cloning techniques have continued to make such studies more feasible.

## 1.3 Phylogenetic inference methods

### 1.3.1 Parsimony methods

Maximum parsimony (MP) is the most popular method for reconstructing ancestral relationships (Stewart, 1993). In the parsimony approach, the goal is to identify the phylogeny that requires the fewest necessary changes to explain the differences among the observed sequences. The process is to identify all informative sites in the multiple alignments, then for each possible tree, to calculate the number of changes at each informative site, to sum the number of changed for each possible tree, and to select the tree with the smallest number of changes as the most likely tree. But, it is not practical to evaluate every possible tree with large data sets. The score for each tree is based on the minimum number of changes in character states that are required to explain the data. PAUP (Phylogenetic Analysis Using Parsimony) is the most generally useful computer package implementing parsimony (Swofford, 2000). It offers a set of flexible choices of character assumptions and tree searching regimes so that it can be used to analyze type of character data to find the most parsimonious trees.

### 1.3.2 Distance methods

A variety of distance algorithms are available to calculate pairwise distance. Distance analysis compares two aligned sequences at a time, and builds a matrix of all possible sequence pairs. During each comparison, the number of changes (base substitutions and insertion/deletion events) are counted and presented as a proportion of the overall sequence length. These final estimates of the difference between all possible pairs of sequences are known as pairwise distances. Different corrections of the transformation exist based on different evolutionary models, for examples, the Jukes-Cantor one-parameter model which treats any change at a nucleotide position as equally likely, and Kimura two-parameter model which takes into account the difference between transition and transversion frequency. Kimura's two-parameter model is probably the most widely used correction. The commonly used methods of this kind are Unweighted Pair Group Method Using Arithmetic Averages (UPGMA) (Sokal & Sneath, 1963) and Neighbour-Joining (NJ) (Saitou & Nei, 1987). UPGMA need strong assumptions that the distances between pairs of oererational taxonomic unit (OUT) reflect the sum of all mutations that occurred in the OTUs since their separation from a common ancestor, and that mutations accumulate at the same rate in all lineages. In other

words, the distance must be additive and generated in a clock-like fashion. NJ method is preferred for distance analyses over UPGMA method, because unlike UPGMA it does not assume that all the sequences evolve at the same rate The NJ method represents a more sophisticated distance matrix algorithm that attempts to correct for differences in evolutionary rate. This method is also computationally simple and can be easily applied to large data sets. It is found evolutionary context and tree imbalance to be the most important factors affecting the accuracy to the NJ method. NJ was more accurate than UPGMA or MP in terms of the average strict consensus index over all treatments. However, no one method was more accurate than the other two for all combinations of treatments (Kim, Rohlf & Sokal, 1993).

### 1.3.3 Maximun Likelihood (ML)

This method is a family of statistical approaches commonly used throughout the biological sciences. Its application in phylogenetics involves estimating the likelihood of observing a particular set of aligned sequences given a model of nucleotide substitution and a tree topology. As with distance methods, choice of the substitution model and its particular parameters provides the means of allowing for multiple hits. However, maximum likelihood is more like parsimony in that each character (aligned nucleotide position) contributes directly and individually to the overall optimality score, in this case the probability of data given the model and tree. Given a tree topology, branch lengths are estimated according to the model and parameters chosen, and the probability of obtaining the particular character states is estimated for each nucleotide position. These probabilities are multiplied together to obtain the overall likelihood for the topology in question, which is usually presented as its negative logarithm. Like distances, these values can be expressed to several decimal places, so there is little chance of ties among nearly equally optimal trees. One advantage of likelihood methods is that a statistical test called the likelihood ratio can be used to evaluate many properties of trees (Kishino & Hasegawa, 1989; Huelsenbeck & Crandall, 1997), However, likelihood methods are computationally very intensive. Likelihood searches are usually practical with only relatively small numbers of sequences even using heuristic search strategies. When nucleotide compositions of the sequences under study show big difference, the results generated by ML should be considered cautiously since ML algorithm assumes that base composition remains constant across the phylogeny (Doyle & Gaut**,** 2000; Holder & Lewis, 2003).

### 1.3.4 Bayesian methods

Bayesian inference of phylogeny, a method recently developed and widely used, shows the superiority for the phylogeny study (Huelsenbeck et al., 2000, 2001; Alfaro et al., 2003, Ronquist & Huelsenbeck, 2003). It is based on maximum likelihood function, but unlike maximum likelihood, Bayesian inference of phylogeny can incorporate a systematist's prior information about phylogeny through the specification of a prior probability distribution trees (Huelsenbeck, 2002). Bayesian methods allow complex models of sequence evolution to be implemented. For example, estimating divergence times, finding the residues that are important to natural selection and detecting recombination points, Bayesian approaches to achieve aims that might be difficult or intractable for ML. It uses the complex evolutionary models and represents a powerful tool for addressing a number of long-standing, complex questions in evolutionary biology (Huelsenbeck et al., 2001). The advantages of this method are: 1) it can incorporate prior information; 2) it is much faster; and 3) it accurately reflects the probability that a branch is correct.

When interpreting trees for biogeography using a taxon-area phylogeny, Bayesian inference has the advantage over both parsimony and ML methods of trait reconstruction of taking phylogenetic uncertainty, such as topology, parameter values, and branch length estimations, into account (Huelsenbeck & Bollback**,** 2001; Huelsenbeck et al., 2003; )

**Table 1.2 Comparison of MP, NJ, ML and Bayesian methods (Holder & Lewis, 2003.)**

| Method | Advantages | Disadvantages | Sofrware |
|---|---|---|---|
| Neighbour joining | Fast | Information is lost in compressing sequences into distances; reliable estimates of pairwise distances can be hard to obtain for divergent sequences | PAUP*<br>MEGA<br>PHILIP |
| Parsimony | Fast enough for the analysis of hundreds of sequences; robust if branches are short (closely related sequences or dense sampling) | Can perform poorly if there is substantial variation in branch lengths | PAUP*<br>NANA<br>MEGA<br>PHILIP |
| Maxinum likelihood | The likelihood fully captures what the data tell us about the phylogeny under a given model | Can be prohibitively slow (depending on the thoroughness of the search and access to computational resources) | PAUP*<br>PAML<br>PHILIP |
| Bayesian | Has a strong connection to the maximum likelihood methods; might be a faster way to assess support for treesthan maximum likelihood bootstrapping | The prior distributions for parameters must be specified; it can be difficult to determine whether the Markov chain Monte Carlo (MCMC) approximation has run for long enough | MrBayes<br>BAMBE |

### 1.3.5 Tree search methods

For a given phylogenetic method, many inferred trees may be produced. Several kind of searches may be used in order to find the best tree for the optimally criterion chosen. To find all the optimal trees, the guaranteed methods are the exact searches including exhaustive search and Branch-and-Bound method. **The exhaustive search** is to evaluate every possible tree. However, this method is feasible only for small data sets (practical for a few sequences (<20)). For large data sets such a search needs a prohibitive amount of computing time. That is because as the number of terminal taxa (OTU) increase, the number of possible trees increases dramatically. **Branch and bound search** is useful for data sets containing from 12-25 or so taxa, depending on the 'messiness' of the data. This technique can eliminate some bad trees from consideration and still guarantee that they will return the best tree. Although this approach requires checking fewer trees than the exhaustive one, it is still time consuming. **Heuristic search** is used for large data sets. Which give a starting tree containing all sequences of interest, performs branch swapping to generate alternative trees in an attempt to find a better tree under a given optimum is attained. Typically uses a stepwise addition or neighbour-joining tree as the starting tree.

### 1.3.6 Phylogenetic confidence and accuracy

The most commonly used confidence method in phylogenetics has been nonparametric bootstrapping, and first applied to the phylogeny problem by Felsenstein (1985). Bayesian Markov chain Monte Carlo sampling has become increasingly popular in phylogenetics as a method for both estimating the maximum likelihood topology and for assessing nodal confidence. Nonparametric bootstrapping is appropriate if one is interested in the sensitivity of observed results to the sampling error associated with collecting characters from a hypothesized underlying character distribution. If one is willing to specify a fully probable

model of character evolution and wishes to place confidences limits on the results of analysis conditioned on the observed data and that model, Bayesian posterior probabilities are the appropriate confidence measure to use. In cases where one decides to bootstrap, it is useful to note that it may require on short internodes compared with BMCMC-PP (Bayesian Markov chain Monte Carlo-posterior probabilities). When assessing posterior probabilities, it is important to remember that confidence values estimated on extremely short intermodes may sometimes be sensitive to the underlying stochastic process. It is suggested that BMCMC-PP is useful method to use when systematists wish to show how well the data support the results of model-based phylogenetic analysis (Alfaro et al., 2003). BMCMC-PP also appears to have increased sensitivity to phylogenetic signal, which may allow workers to achieve high confidence in a correct result with fewer characters. However, BMCMC-PP also appears to be more susceptible than likelihood bootstrapping to assigning high confidence to incorrect short internodes.

The standard approach in phylogenetics has been to reconstruct phylogenetic hypotheses by maximizing the number of taxa, utilizing one or a few genes (Baldauf et al., 2000; Peterson & Eernisse, 2001; Moncalvo et al., 2002). But the study of Rokas and Carroll (2005) shows that increasing taxon number correlates with a slight decrease in phylogenetic accuracy. In contrast, increasing gene number has a significant positive effect on phylogenetic accuracy. Thus, for typical data sets, the number of genes utilized may be a more important determinant of phylogenetic accuracy than taxon number.

## 1.4 The aims and context of the present study

### 1.4.1 Plant collection

In order to have a general concept of the relationships and phylogeny of this family Balsaminaceae, we have made considerable efforts to collect both fresh material and herbarium samples from all main distribution centers and places all over the world.

Collection of materials has been the greatest challenge in the investigation. Five dedicated expeditions have been made to Yunnan province, Sichun province, Tibet, some other mountains in China and Madagascar mainly by Dr. Yong-ming Yuan. The expedition to Vietnam was mainly for *Hydrocera triflora* since this monotypic genus has an important position of this family. More samples from Madagascar and Africa were received by international collaborations. Samples from southern India, North America, boreal Eurasia and Central Asia have been kindly supplied by friends. We paid more attention to get the species with different floral type and from different places, so that it can give more useful information to understand the floral evolution and biogeography of this family with my study. All vouchers specimens are deposited at the herbarium of University of Neuchâtel.

### 1.4.2 Taxonomy and floral evolution based on molecular phylogeny

I want to verify intragenetic divisions to give more insight to the classification of this family through this study. Floral morphology and evolution is a hot-topic in this family, though there are some hypothesis about it, but still need to be confirmed. In the present study, I aim to build the molecular phylogeny of this family using the suitable gene markers to see the phylogenetic relationship between the two genera and intrageneric relationship within *Impatiens*, then to combine with some floral morphological data, such as number of lateral sepals, forms of lateral petals and dorsal petals, shape and size of spur, together with the shape of fruits, pollen type, chromosome numbers etc., to see the evolutionary trend. Seed coat is

regarded as one of the important character in *Impatiens* also, and is expected to give some insight for the taxonomy. Thus, some collected seed samples from southwest China will be observed in the present study.

### 1.4.3 Karyology

Previously karyological studies on *Impatiens* were mostly carried out on Indian and African species. Although the mainland Southeast Asia and the adjacent Sino-Himalayan area represent the biggest diversification centre of the family, only a few studies were done on the species growing in these regions. In order to get a complementary karyological information for this family, I chose more species from this area to observe their chromosome number and karyotype structure and cytogeography implication.

### 1.4.4 Biogeography

Lastly, a global investigation of this family was performed to understand the relationships among the main diversification centers. Where is the original place and how the species colonies to the other regions?

The main targets of the thesis are:

**1)** To investigate the phylogenetic relationship between the two genera *Hydrocera* and *Impatiens* and the intrageneric relationship within the genus *Impatiens*, using the non-coding sequences of the nuclear ribosomal internal transcribed spacers (ITS), and of the chloroplast trnL intron, trnL-trnF intergenic spacer, and atpB-rbcL spacer.

**2)** To gain insights into the origin and biogeographic history of the family from a molecular phylogenetic perspective and to determine the relationships among the species of the main diversification hotspots.

**3)** To evaluate the evolution of floral morphology and vegetative morphology by tracing several chosen traits on the molecular phylogeny tree.

**4)** To investigate the chromosomal variation and evolution of Balsaminaceae

**5)** To observe the evolution of the diagnostic characters of seedcoat micromorphology using scanning electron microscopy.

# Chapter 2 Seedcoat micromorphology (paper I)

## 2.1 Introduction

The significance of seedcoat morphology in solving problems of classification and phylogeny has long been recognized (Hooker & Thomson, 1860; Wunderlich, 1967; Corner, 1976; Kumar & Singh, 1990). Recent examples of successful applications of seed morphology include studies on *Veronica* (Martinez-Ortega & Rico, 2001), *Massonieae* (Pfosser et al., 2003), Caryophyllaceae (Yildiz, 2002) and Brassicaceae (Abdel Khalik & Van der Maesen, 2002). However, seed morphology of Balsaminaceae has been observed for few species (Shimizu, 1979; Grey-Wilson, 1980b; Lu & Chen, 1991). These studies revealed that seed morphology of the genus provide useful taxonomic characters at species level, but it was not possible to elucidate the overall variation in seedcoat micromorphology and its implementation for taxonomy, due to insufficient sampling. Detailed observations on more species and a systematic overall evaluation of seedcoat micromorphology in *Impatiens* are necessary. southwest China is one of the most important diversification centers of *Impatiens*. Scanning electron microscopy (SEM) observations on seeds of *Impatiens* from this region, can give insight to elucidate the systematic value of seedcoat micromorphological characters and detect evolutionary trends among seedcoat microcharacters and their correlations with other traits.
The remaining of this chapter is a summary of the article of Song et al. (2005) (see Paper I), but with minor update considering the recent article about seed morphology of *Impatiens* (Utami & Shimizu, 2005) and more detail information of seeds of African species by Grey-Wilson (1980b).

## 2.2 Materials and methods

35 species of *Impatiens* from southwest China, 2 species with Himalayan origin and 1 species with African origin were examined using scanning electron microscopy. Seed sizes were measured from five seeds randomly chosen. The shape, colour and structure of the seedcoat were observed.

## 2.3 Results

### 2.3.1 Observation of seedcoat micromorphology

These observations indicated that a high diversity of seedcoat micromorphology exist among the species of the *Impatiens*. Four morphological types and more subtypes were divided based on the structure and ornamentation of epidermal cells of the seedcoat, particularly the pattern of the arrangement of the cells and degree of elevation of the anticlinal cell walls. The laevigate type and granulate type are unique, occurring in *I. chinensis* and *I. balsamina* L. respectively. The reticulate type and protrusive type are common in present observation and have been further divided into four subdivisions respectively. For reticulate type: fine reticulate subtype observed in 8 species, colliculate subtype observed in 10 species, carinate subtype observed in 2 species, striate subtype observed in 3 species; for protrusive type: digitiform subtype observed in 5 species, clustered subtype observed in 1 species, squamalate subtype observed in 5 species, cristate subtype observed in 2 species (Table 2.1 ). Among the considerable diversity of seedcoat micromorphological observation, the granulate type in *I.*

*balsamina*, carinate subtype in *I. delavayi* Franch. and *I. poculifer*, striate subtype in *I. arguta*, *I. balfourii* and *I. parviflora*, digitiform subtype in *I. davidi* Franch., *I. bahanensis* Hand.-Mazz., *I. drepanophora* Hook. f., *I. cyathiflora* Hook. f. and *I. siculifer*, and clustered subtype in *I. ruiliensis* were observed for the first time.

### 2.3.2 Seed size, outline, color

The size of seedcoat range are from 1.3-1.5 x 0.6-0.9 mm (length x width) (*I. xanthina* Comber) to 4.1-4.5 x 2.0-2.1 mm (*I. parviflora*). Outline of seed varies from globose, ovate, ellipsoid, subellipsoid or long ellipsoid. Seeds colour varied from yellow, beige, grey to various shades of brown, to black (Table 1 in paper I).

## 2.4 Discussion

### 2.4.1 Comparison with previous observations

Comparing the present observation with previous studies on seedcoat, it is found that *I. wilsonii* (From Mt. Omei, southwest China), which Lu and Chen (1991) described as having the laevigate seedcoat, belongs to reticulate seedcoat type defined in the present study. The other 11 *Impatiens* species they studied from the same region correspond to the present reticulate and colliculate subtypes according to their micrographs, except *I. monticola* Hook.f. corresponding to squamalate subtype of the protrusive category. Shimizu (1979) has studied the seed of *I. violaeflora*, the micrographs of its seedcoat reveals that it is very similar to the cristate subtype observed in *I. walleriana* (Africa origin, cultivated in Yunnan, China) in the present study. While in gross morphology, *I. violaeflora* is very similar to *I. aureliana* (distributed mainly in southwest China and Burma), which has a squamalate subtype of seedcoat. This suggests that the cristate and squamalate subtype sculpture may have a similar origin. Utami and Shimizu (2005) reported seedcoat morphology of 65 species mostly from Southeast Asia. Their observations on some species is congruent with the present study: as in *I. chinensis* with laevigate seedcoat, *I. balsamina* with granulate seedcoat, *I. nolitangere* with fine reticulate seedcoat, *I. walleriana* with cristate seedcoat. Their observations on other species from Indochina region reveals some similarity with present study: two Thailand species, *I. psittacina* Hook.f. and *I. kerriae* Craib with finely granulate seedcoat are similar with granulate type in the present study, but a little bit bigger; one Indian species *I. cordata* Wright) is similar with striate subtype (reticulate type) seedcoat in the present study; another Indian species *I. henslowiana* Arn. and one Japanese species *I. textori* Miq. is similar with fine reticulate subtype (reticulate type) in the present study. But many other Indian, Thailand and Indonesian species have the seedcoat similar to protrusive type in the the present study. Grey-Wilson (1980b) reported seed type of 55 African species and identified five type of seedcoat. The smooth seed like *I. tinctoria* is similar to reticulate type in the present study, the warty seed like *I. usambarensis* Grey-Wilson and short-haired seed like *I. kilimanjari* Oliver are similar to cristate subtype (protrusive type) in the present study. The seedcoat with long hair as in *I. gardneriana* Wight (Indian species) (Utami & Shimizu, 2005), *I. platypetala* (Indonesia species) (Shimizu, 1979) and *I. keilii* (African species) (Grey-Wilson, 1980) were never observed in the present study.

From the main previous references of seedcoat morphology of Balsaminaceae (Shimizu, 1979; Grey-Wilson, 1980; Lu & Chen, 1991; Utami & Shimizu, 2005), taxa only used in the present study (including molecular study) were chosen to compare the seedcoat micromorphological differences and give phylogenetical implications. The seedcoat micromorphology of these taxa were identified and matched according to the categories defined in the present study and

the taxa names are listed in Table 2.1. From all available data about seedcoat micromorphology, it is noticed that reticulate type is common in East Asia (China, Japan) while protrusive type is common in Africa and South Asia (India, Thailand and Indonesia et al.). But more species need to be observed from different region.

**Table 2.1 List of *Impatiens* species have been observed with micromorphology in the present study and previous studies (samples chosen only used in the present study).**

| | | | Song (present study) | Lu & Chen (1991) | Utami & Shimizu (2005) | Grey-Wilson (1980) | Shimizu (1979) |
|---|---|---|---|---|---|---|---|
| **Laevigate** | **1** | | *I. chinensis* L. | | *I. chinensis* L. | | *I. chinensis* L. |
| **Granulate** | **2** | | *I. balsamina* L. | | *I. balsamina* L. | | |
| | | | | | *I: kerriae* Craib | | |
| | | | | | *I. psittacina* Hook.f. | | |
| **Reticulate** | **3A** | **fine reticulate** | *I. dolichoceras* Pritz. ex Diels | *I. oxyanthera* Hook.f. | *I. henslowiana* Arn. | *I. tinctoria* Grey-Wilson | |
| | | | *I. gongshanensis* Y.L.Chen | *I. conchibracteata* Y.L.Chen et Y.Q.Lu | *I. nolitangere* L. | *I. rothii* Hook.f. | |
| | | | *I. holocentra* Hand.-Myzz. | *I. undulata* Y. L. Chen et Y. Q. Lu | *I. textori* Miq. | | |
| | | | *I. forrestii* Hook. f. ex W.W.Smith | *I. faberi* Hook.f. | | | |
| | | | *I. infirma* Hook.f. | *I. imbecilla* Hook.f. | | | |
| | | | | *I. lateristachys* Y.L.Chen et Y.Q.Lu | | | |
| | | | *I. nolitangere* L. | | | | |
| | | | *I. rostellata* Franch. | *I. platychlaena* Hook.f. | | | |
| | | | | *I. alpicola* Y.L.Chen et Y.Q.Lu | | | |
| | | | *I. scabrida* DC. | | | | |
| | **3B** | **colliculate** | *I. aquatilis* Hook.f. | | | | |
| | | | *I. chungtienensis* Y.L.Chen | | | | |
| | | | *I. cyanantha* Hook.f. | | | | |
| | | | *I. fenghwaiana* Y.L.Chen | | | | |
| | | | *I. pseudokingii* Hand.-Mazz | | | | |
| | | | *I. purpurea* Hand.-Mazz. | | | | |
| | | | *I. radiate* Hook.f. | | | | |
| | | | *I. rectangular* Hand.-Mazz. | | | | |
| | | | *I. apsotis* Hook.f. | | | | |
| | | | *I. soulieana* Hook.f. | | | | |
| | **3C** | **carinate** | *I. delavayi* Franch. | | | | |
| | | | *I. poculifer* Hook.f. | | | | |
| | | | *I. arguta* Hook.f. et Thomas. | | *I. cordata* Wight | | |
| | **3D** | **striate** | *I. balfourii* Hook.f. | | | | |
| | | | *I. parviflora* DC. | | | | |
| **Protrusive** | **4A** | **digitiform** | *I. davidi* Franch. | | | | |
| | | | *I. bahanensis* Hand.-Mazz. | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | *I. drepanophora* Hook. f.<br>*I. cyathiflora* Hook. f. | | | | |
| **4B** | **clustered** | *I. siculifer* Hook.f.<br>*I. ruiliensis* S.Akiyama et H.Ohba<br>*I. begoniifolia* S.Akiyama et H.Ohba | | | | |
| **4C** | **squamalate** | *I. yingjiangensis* S.Akiyama et H.Ohba<br>*I. mengtszeana* Hook.f.<br>*I. trichosepala* Y.L.Chen<br>*I. aureliana* Hook.f. | *I. monticola* Hook.f. | | | |
| **4D** | **cristate** | *I. walleriana* Hook.f.<br>*I. xanthina* Comber | | *I. walleriana* Hook.f.<br>*I. platypetala* Lindl. | *I. assurgens* Baker<br>*I. hians* Hook. f.<br>*I. hoehnelii* T.C.E.Fries<br>*I. keilii* Gilg.<br>*I. kilimanjari* Oliver<br>*I. mackeyana* Hook.f.<br>*I. mannii* Hook.f.<br>*I. meruensis* Gilg.<br>*I. niamniamensis* Gilg.<br>*I. sodenii* Engl. et Warb. ex Engl.<br>*I. usambarensis* Grey-Wilson<br>*I. walleriana* Hook.f. | *I. platypetala* Lindl. |

### 2.4.2 Seed size

As to the size of the seed, it is found that seeds of reticulate type are comparatively bigger as in *I. parviflora* (4.1-4.5 x 2.0-2.1 mm), *I. infirma* (3.1-3.8 x 1.9-2.1 mm), *I. chungtienensis* (3.1-3.8 x 1.6-1.8 mm), *I. cyanantha* (2.9-3.1 x 1.8-2.3 mm) and *I. arguta* (2.5-3.0 x 1.9-2.2 mm), while the seeds of protrusive type are comparatively small as in *I. trichosepala* (1.3-1.6 x 0.9-1.2 mm) and *I. xanthina* (1.3-1.5 x 0.6-0.9 mm) in the present study. In Grey-Wilson's report, smooth seeds as in *I. tinctoria* (4.1-4.8 x 2.9-3 mm) and *I. rothii* (5.2-3.8 mm) are relatively larger than the other seeds with embellishments on the seedcoat surface as in *I. usambarensis* (1.1-1.4 x 0.7-0.8 mm) and *I. hians* (1.7-2.2 x 1.1-1.9 mm). But it is not always this rule that seeds with reticulate type are bigger and seeds with protrusive type are smaller, e.g. *I. holocentra* has fine reticulate subtype (reticulate type) seedcoat, but quite small seed (1.5-1.8 x 1.0-1.1 mm); *I. bahanensis* has digitiform subtype (protrusive type) seedcoat, but quite big seed (2.2-3.1 x 1.4-1.7 mm); *I. meruensis* has cristate subtype (protrusive type) seedcoat, but very big seed (3.9-4.5 x 2.5-2.7 mm). Thus, there is not always a correlation between seed size and seedcoat micromorphology.

### 2.4.3 Phylogenetic and taxonomic implications of seedcoat micromorphology

The present observations confirmed the possibility that types or subtypes of seedcoat can be diagnostic or indicative of phylogenetic relationships. Some important correlations among the seedcoat micromorphological characters and gross morphological characters and karyological character have been identified. For example, the species with a squamalate subtype and the cristate subtype seedcoat (in present observation), all are limited to a lineage of species that have globose or broadly fusiform (length/diameter $\leq$ 4) capsules (Grey-Wilson, 1980b); all are very similar to each other in aspects of morphology; all were found to have lower basic chromosome numbers (x=8, 7, 6). Thus, the squamalate and cristate sculpture of seedcoats seems to be associated with the fruit shape. But *I. balsamina* and *I. chinensis* are exception because though they are having globose or broadly fusiform capsules, they are having a granulate and laevigate seedcoat respectively. Some closely related species seem to have similar seed structures, e.g. *I. tinctoria* and its allies all have rather large smooth seeds (Grey-Wilson, 1980b), *I. delavayi* and *I. poculifer* are very similar to each other in gross morphology and have the unique carinate seedcoat.

The sculpture on seedcoats thus offers a set of characters useful for the taxonomy of the genus. However, more species need to be examined and analysed before taxonomic importance of the seed character can be properly and adequately assessed. In some way, seedcoat type reflects habitat type, for example, species with cristate subtype seedcoat (as in *I. filicornu* Hook.f.) usually grow along the river, while the species with smooth seedcoat (as in *I. tinctoria*) are often from forest margins (Grey-Wilson, 1980b). Thus, seedcoat morphology alone could not provide universally applicable key characters for identification.

# Chapter 3 Karyology (paper II)

## 3.1 Introduction

Chromosomal variation contributes to the processes of species evolution (Hong, 1990; Stebbins, 1971). Knowledge of chromosomal evolution of Balsaminaceae stands as an important aspect in understanding the extraordinary diversity, endemism, and biogeography of the family. About 157 species of *Impatiens* from most of the distribution areas have been karyologically studied prior to the present study (e.g. Smith, 1934; Khoshoo, 1955a,b, 1956, 1957, 1966; Jones & Smith, 1966; Bhaskar, 1976, 1980; Larsen, 1981; Zinov'eva-Stahevitch & Grant, 1982, 1984, 1985; Rao et al.,1986; Govindarajan & Subramanian, 1986; Akiyama et al. 1992b, 1993, 1996a; Sugawara et al., 1994, 1997), but these species are mostly Indian and African species. Although the mainland Southeast Asia and the adjacent Sino-Himalayan area represent the biggest diversification centre of the family, only a few studies were done on the species growing in these regions. In order to get complementary karyological information for this family, more species from this area were chosen to observe their chromosome number and karyotype structure. By incorporating the present results into a thorough survey of previous studies, a review of the chromosome numbers and the possible evolutionary relationships among different basic numbers are also presented.

## 3.2 Materials and methods

The karyological investigations have been made for 52 samples of 45 species of *Impatiens* collected from southwest China, Taiwan and Sumatra. Chromosome number determinations were made from flower buds fixed in Carnoy fluid. The staining method is with alcohol-HCL –carmine (Snow, 1963). After staining the buds for 48 hrs and heated in 45% acetic acid for 2-3 minutes, anthers or young ovaries were squashed in the standard way and observed using a light microscope.

## 3.3 Results

### 3.3.1 Chromosome number

The details of the results are showed in Table 3.1. The gametic numbers n=6, 7, 8, 9, 10, 20, 27 or the corresponding somatic numbers were found. The number n=27 found in *I. pseudokingii* Hand.-Mazz was new for the family. The number n=9 (or 2n=18) appeared most frequently in 26 species observed, followed by the number n=10 (or 2n=20) in 8 species, n=7 (or 2n=14) and n=8 (or 2n=16) in 4 species respectively and n=6 and n=27 in 1 species respectively. The chromosome numbers for 32 species are reported for the first time (see the species marked with an asterisk in Table 3.1). The chromosome numbers were confirmed for 11 species. 7 of them, the chromosomal number are congruent with the previous reports (*I. aureliana* n=6, *I. chinensis* n=8, *I. mengtszeana* n=8, *I. siculifer* n=9, *I. stenantha* n=9, *I. uliginosa* Franch. n=9 and *I. yingjiangensis* n=8); 4 of them, the present results confirmed some previous reports but were different from some other reports: *I. arguta* (present result n=10, previous reports n=6, 9 or 10), *I. bicornuta* Wall. (present 2n=18, previous n=8 or 2n=18), *I. radiata* (present 2n=18, previous n=10 or 2n=18), and *I. rubrostriata* (present 2n=20, previous 2n=20 or 40). Different numbers from previous reports were found for two

**Table 3.1 The chromosome numbers observed, and the origins of materials and voucher specimens. The chromosome numbers reported for the first time are marked with "*", the number different from previous reports is marked with "!", and the numbers confirmed some of the previous reports but different from other reports are marked with "◊"**

| Taxon | Coll. No. | Origin and altitude | Chromosome Number |
|---|---|---|---|
| *I. acehensis* C. Grey-Wilson | AF1 | Gunung Sinabung, Indonesia | 2n = 14* |
| *I. aquatilis* Hook. f. | cn2k1-75 | Kunming, Yunnan, China, 2100m | n = 9* |
| *I. apsotis* Hook. f. | cn2k2-159 | Kangding, Sichuan, China, 2915m | n = 9* |
| *I. arguta* Hook. f. et Thomas. | cn2k-52 | Gongshan, Yunnan, China, 2600 m | n = 10◊ |
| *I. aureliana* Hook. f. | cn2k1-63 | Longchuan, Yunnan, China, 1700m | n = 6 |
| *I. bahanensis* Hand.-Mazz. | cn2k-30 | Gongshan jidu, Yunnan, China, 2550m | n = 9* |
| *I. bicornuta* Wall. | cn2k-55 | Gongshan, Yunnan, China, 2600m | 2n = 18◊ |
| *I. chimiliensis* Comber | cn2k-51 | Gongshan, Yunnan, China, 3500m | 2n = 18* |
| *I. chinensis* L. | cn2k1-49 | Tengchuan, Yunnan, China, 1630m | n = 8 |
| *I. chungtienensis* Y. L. Chen | cn2k2-179 | Zhongdian, Yunnan, China, 2700m | n = 9* |
| *I. crassicaudex* Hook. f. | cn2k2-104 | Batang, Sichuan, China, 3800m | n = 9* |
| *I. cyathiflora* Hook. f. | cn2k1-74 | Kunming, Yunnan, China, 2100m | 2n = 18* |
| *I. delavayi* Franch. | cn2k-76 | Dali, Yunnan, China, 2650m | 2n = 20* |
| | cn2k2-14 | Zhongdian, Yunnan, China, 3160m | n = 10* |
| *I. desmantha* Hook. f. | cn2k2-30 | Zhongdian, Yunnan, China, 3410m | n = 9* |
| *I. dolichoceras* Pritz. ex Diels | cn2k1-80 | Nanning, Guangxi, China, 1519m | 2n = 18* |
| *I. drepanophora* Hook. f. | cn2k1-39 | Longling, Yunnan, China, 1580m | n = 9! |
| | cn2k-16 | Fugong, Yunnan, China, 1335m | n = 9! |
| | cn2k-20 | Gongshan, Yunnan, China, 1433m | n = 9! |
| *I. eubotrya* Miq. | AF2 | Gunung Merapi, Indonesia, 2502m | 2n = 18! |
| *I. fenghwaiana* Y. L. Chen | cn2k1-78 | Nanning, Guangxi, China, 1519m | 2n = 18* |
| *I. gongshanensis* Y. L. Chen | cn2k-26 | Gongshan, Yunnan, China, 2040m | n = 10* |
| *I. holocentra* Hand.-Mazz. | cn2k-54 | Gongshan, Yunnan, China, 2700m | n = 9* |
| *I. imbecilla* Hook. f. | Hao 426 | Omei Mt., Sichuan, China | 2n = 20* |
| *I. infirma* Hook. f. | cn2k2-60 | Xiangcheng, Sichuan, China, 2950m | n = 9* |
| *I. lecomtei* Hook. f. | cn2k2-202 | Lijiang, Yunnan, China, 2900m | 2n = 18* |
| *I. mengtszeana* Hook. f | cn2k1-60 | Longchuan. Yunnan, China, 1450m | n = 8, 9 |
| | cn2k2-214 | Jiangcheng, Yunnan, China, 1100m | n = 8 |
| *I. microcentra* Hand.-Mazz. | cn2k-50 | Gongshan, Yunnan, China, 2800m | n = 9* |
| *I. napoensis* Y. L. Chen | cn2k1-61 | Longchuan, Yunnan, China, 1450m | n = 8* |
| *I. oxyanthera* Hook. f. | 00-s008 | Omei Mt., Sichuan, China | n = 7* |
| *I. poculifer* Hook. f. | cn2k2-209 | Weixi, Yunnan, China, 3000m | 2n = 20* |
| *I. principis* Hook. f. | 01-yjp006 | Lushui, Yunnan, China | n = 9* |
| *I. pseudokingii* Hand.-Mazz. | cn2k-29 | Gongshan, Yunnan, China, 2550m | n = 27* |
| *I. radiata* Hook. f. | cn2k1-43 | Tengchun, Yunnan, China, 2097m | 2n = 18◊ |
| *I. rectangula* Hand.-Mazz. | cn2k-19 | Gongshan, Yunnan, China, 1433m | n = 9* |
| | cn2k-28 | Gongshan, Yunnan, China, 2050m | n = 9* |
| | cn2k1-17 | Gongshan, Yunnan, China, 1580m | 2n = 18* |
| *I. rostellata* Franch. | cn2k2-167 | Shimian, Sichuan, China, 2245m | n = 10* |
| *I. rubrostriata* Hook. f. | cn2k1-44 | Tengchun, Yunnan, China, 2097m | 2n = 20◊ |
| *I. scutisepala* Hook. f. | cn2k-56 | Gongshan, Yunnan, China, 2500m | n = 9* |
| *I. siculifer* Hook. f. | cn2k2-215 | Jiangcheng, Yunnan, China, 1200m | n = 9 |
| *I. stenantha* Hook. f. | 01-yjp008 | Lushui, Yunnan, China | n = 9 |
| *I. taronensis* Hand.-Mazz. | cn2k-57 | Gongshan, Yunnan, China, 2600m | 2n = 18* |
| *I. tenuibracteata* Y. L. Chen | 01-yjp003 | Lushui, Yunnan, China | n = 9* |
| *I. tortisepala* Hook. f. | cn2k2-166 | Shimian, Sichuan, China, 2245m | n = 10* |
| *I. trichocepala* Y. L. Chen | cn2k1-68 | Longling, Yunnan, China, 1260m | n = 7* |
| *I. uliginosa* Franch. | 99-174 | Binchuan, Yunnan, China, 2200m | n = 9 |
| | cn2k2-173 | Huaping, Yunnan, China, 2150m | n = 9 |
| *I. uniflora* Hayata | AF3 | Taiwan, China, 2565m | n = 20* |
| *I. xanthina* Comber | cn2k1-35 | Gongshan, Yunnan, China, 2070m | n = 7* |
| *I. yingjiangensis* S. Akiyama et H. Ohba | cn2k1-55 | Yingjiang, Yunnan, China, 1435m | n = 8 |

species: 2n=18 for *I. eubotrya* Miq. from Sumatra for which 2n=28 was reported previously; n=9 for *I. drepanophora* for which n=10 was reported previously.

### 3.3.2 Karyomorphology

Most of the observations were made on meiosis of pollen mother cells, thus, few karyomorphological data were obtained. Nevertheless, noticeably almost all the species observed with n=9 or 2n=18 showed a bimodal karyotype structure: one pair of chromosomes (or one in gamete) is distinctly longer than other chromosomes, for examples, *I. drepanophora*, *I. holocentra*, *I. radiata*, *I. rectangula*, etc. Only one species *I. pseudokingii* observed with n=27, new for the family, showed three distinctively longer chromosomes than the other chromosomes in its gametes suggesting the species as a hexaploid. Such bimodal karyotype structure seems to be specific to the species with the basic number x=9. All the species observed with basic number x=9 show such karyotype, except *I. apsotis*, which has 9 chromosomes with similar size in its gametes. All the species observed with other chromosome numbers do not show such bimodal structure, where all the chromosomes are more or less the same size, for examples, *I. delavayi*.

## 3.4 Discussion

### 3.4.1 Variation and evolution of chromosome numbers in *Impatiens*

The present study contributed new documentation of chromosome numbers for 32 species from one of the most important, yet poorly studied, diversification centers of *Impatiens*. The present observations confirmed previous reports for seven species, some differences from previous reports were also revealed: in four species, the present results confirmed some previous reports but were different from some other reports; in two species, different numbers from previous were observed. These different numbers found for a species could be due to true intraspecific variation (as in *I. mengtszeana*), but it might be also the results of misidentifications of different authors, since the determination of species is a well-known difficulty in *Impatiens*.

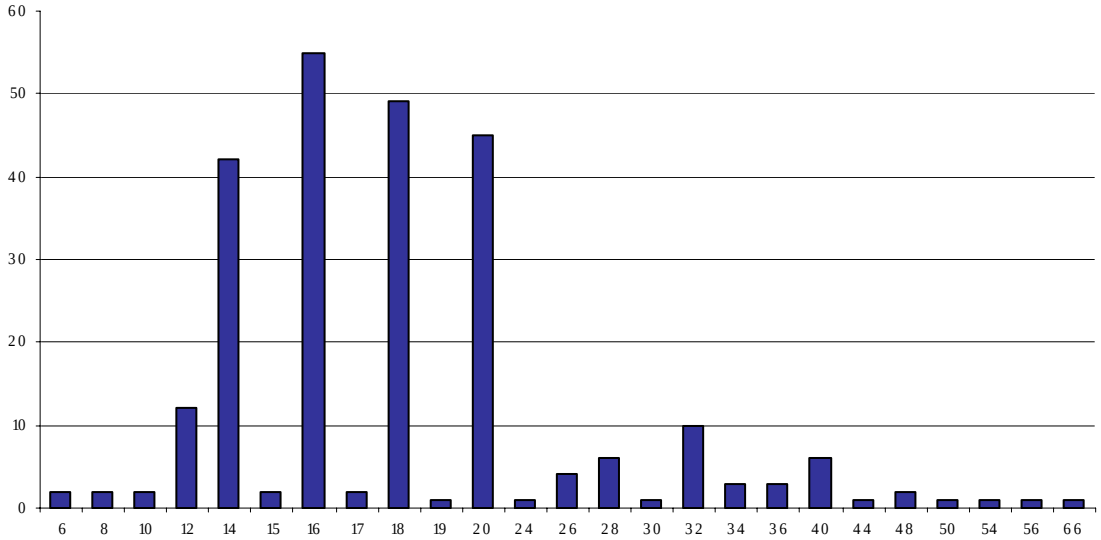



**Fig. 3.1 The somatic chromosome numbers in *Impatiens* varies from 2n=6 to 2n=66. 2n=14, 16, 18, 20 are the four most frequent numbers in this family, and 2n=16 found in 55 taxa is the commonest one.**

In Balsaminaceae, the present counting for the 45 species, together with the previous counting for 157 species, established a wide range of chromosome number variation: 2*n* = 6, 8, 10, 12, 14, 15, 16, 17, 18, 19, 20, 24, 26, 28, 30, 32, 34, 36, 40, 44, 48, 50, 54, and 66, which suggested a complicated chromosomal variation. Among these numbers, 2*n* = 14, 16, 18 and 20 are the most frequent numbers and they take the major part (78%) of the species observed (Fig. 3.1).

The chromosome numbers of *Impatiens* shows a typical dysploid variation, except for a few aneuploid cases (2n=15, 17 and 19). Most of the species can be considered as diploids with the basic number of x=3, 4, 5, 6, 7, 8, 9, 10, 12, 13, etc., and some higher numbers such as 2n=32, 36, 40 etc. can be considered as polyploidy of these lower basic numbers. Higher numbers such as 2n=34, 44, 54, and 66 can be explained either as diploid with higher secondary basic numbers, or dysploid at polyploidy levels. Polyploidy holds certain importance in the chromosomal evolution in *Impatiens* as well, particularly in the natural hybridisations. Different hypotheses have been proposed regarding the evolution of the basic numbers through dysploidy in conjunction with polyploidy in *Impatiens*. Jones and Smith (1966) and Akiyama et al. (1992b) suggested x = 7 to be the ancestral type, from which the other numbers were derived mainly by ascending dysploidy, whereas Rao et al. (1986) suggested evolution of x = 7, 9 and 10 from the basic number x = 8 through both descending and ascending dysploidy (Fig. 3.2). *Hydrocera triflora* was consistently reported 2n=16, considering *Hydrocera* is the sister group of *Impatiens*, Rao et al. (1986)'s suggestion about x=8 is the ancestral number is reasonable. However, the possibility of the basic numbers x = 9 and 10 being ancestral in *Impatiens* cannot be ruled out yet. Phylogenetic study might give some insight on this question.

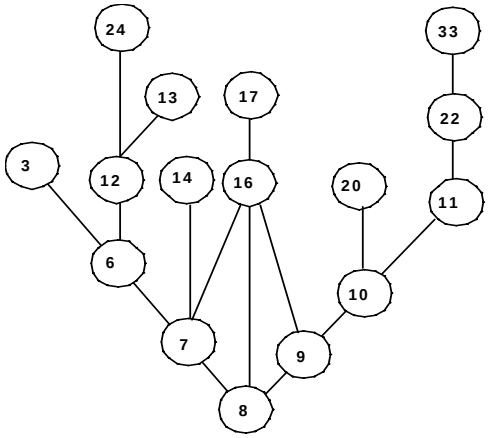



**Fig. 3.2 Evolutionary scheme of basic chromosome numbers (Rao et al. 1986)**

### 3.4.2 Karyotype structure

Most *Impatiens* species have symmetrical karyotypes, i.e. all the chromosomes of a species are similar to each other in size and are mostly metacentric or submetacentric (Levan et al. 1964). In the present observations, almost all the species with n=9 or 2n=18 showed a special bimodal karyotype: one pair (one in gamete) of chromosomes is distinctly longer than other chromosomes, except one species, *I. apsotis*, which has 9 chromosome of similar size in its gametes. *Impatiens pseudokingii* with n=27 showed three chromosomes conspicuously longer than others were thought to belong to the same type as well since it is suggested as a hexaploid. Such bimodal karyotype was noticed before in *I. glandulifera* from the Himalayas (Smith, 1934) and some species in central and eastern Nepal (Akiyama et al., 1992b). It has been not observed that species with any other number showing the bimodal karyotype in the

present study. However, Sugawara et al. (1994) reported such bimodal karyotypes for species with 2n=12, 20 from China.

The origin of the asymmetrical bimodal karyotype might result from symmetrical karyotypes via unequal translocations (Stebbins, 1971). Khoshoo (1957) suggested that the bimodal karyotype with x=9 was derived from a symmetrical karyotype with x=10 by translocation of the essential material of one chromosome onto one of the arms of another chromosome followed by the loss of one centromere. The hypothesis for explaining the origin is related with the assumption of ancestral basic number for *Impatiens*. If accept the assumption of x=8 as the ancestral basic number for *Impatiens* as suggested by Rao et al. (1986), an alternative hypothesis has to be proposed to explain the origin of the bimodal karyotypes via ascending dysploidy which is not available yet.

### 3.4.3 Cytogeography of *Impatiens*

Chromosome number variations in *Impatiens* show interesting geographical patterns, that strongly related to the centers of species diversity and endemism. Of the five hotspots of species diversity, karyological data are almost blank for Madagascar. The brief distribution of the available chromosome numbers in other hotsports of species diversity is as following: in Africa, most species have the chromosome number of 2n=16 (shown by 52% of species observed) or 2n=14 (21%), other numbers such as 2n=10, 20, 32 occurred less frequently, and the number 2n=18 has never been found; in south India and Sri Lanka, most species have chromosome number of 2n=16 (38%), followed by 2n=20 (20%), 2n=14 (17%) and 2n=18 (6%). The lowest chromosome number in *Impatiens*, 2n=6, occurred in *I. latifolia* and *I. leschenaultia* only in this region; in Himalayas, species have similar frequencies for three main basic numbers: 2n=14 (30%), 2n=18 (26%), and 2n=20 (24%); in broad Southeast Asia including mainland Southeast Asia, southwest China, Indochina peninsula, and Malesia harbours, species mostly with 2n=18 (40%), 2n=14 (13%), 2n=20 (12%), and 2n=16 (8%). All native species in Northern Asia, Europe and North America have the number of 2n=2x=20 only (Fig. 3.3).

Grey-Wilson (1980b) suggested that west Gondwana is the origin place for *Impatiens* and spread to Southeast Asia through the Indian subcontinent based on the similarity of the species occurring in Africa, Madagascar, and southern India. The subsequently migration is from Southeast Asia and the adjacent Himalayan area, one lineages radiating to the temperate Eurasian areas and North America, another lineages radiating in the tropical and subtropical areas. Mapping the most frequent basic chromosome numbers of the diversity centers on Grey-Wilson's migration map, it is seen that the basic number x=7, 8 are predominantly shared by species from Africa, southern India, Sri Lanka and Southeast Asia; x=10 from the northern hemisphere and x=9 from Himalayas and Southeast Asia. However, this pattern does not necessarily support Grey-Wilson's (1980) hypothesis about the origin and migration of *Impatiens*, because assuming a Southeast Asian origin of *Impatiens* and subsequent radiations to all other areas including southern India, Sri Lanka, Africa and Madagascar can equally result the same pattern. But karyological studies alone is not enough to test the hypothesis, molecular phylogenetic studies will hopefully give more insights.

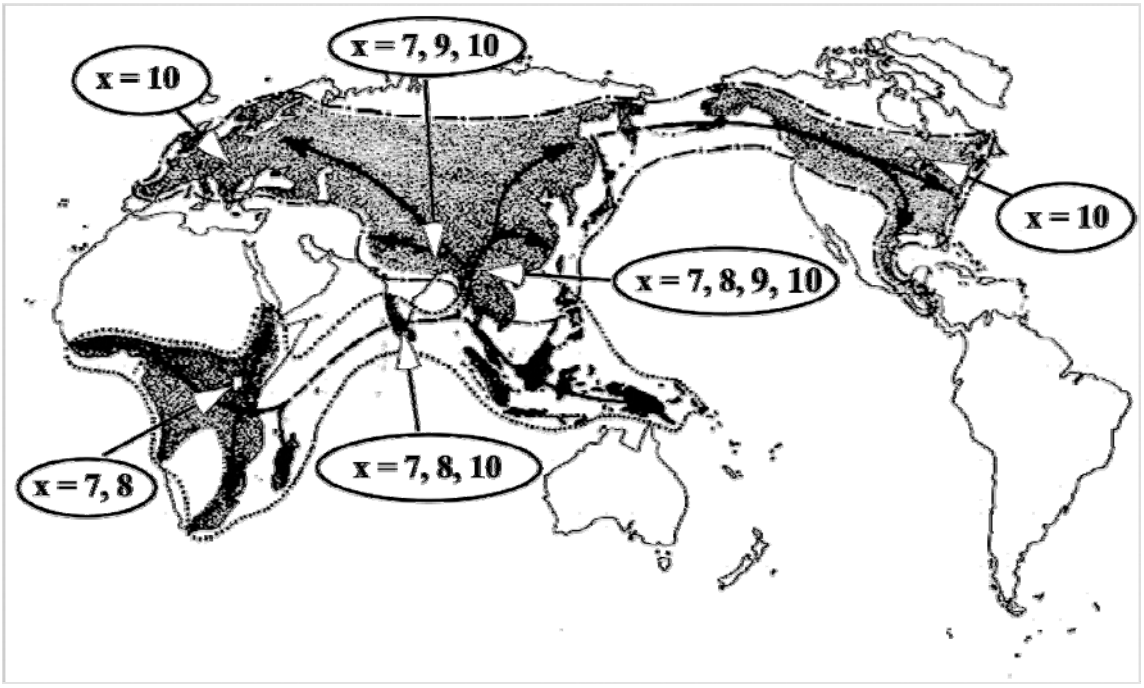



**Fig. 3.3 Distribution of the most frequent basic chromosome numbers and the migration routes proposed by Grey-Wilson (1980b)**

# Chapter 4 Molecular phylogeny (Papers III, IV, V)

## 4.1 Introduction

The understanding of phylogenetic relationships among angiosperms has greatly increased in the last few years, in particular as a result of analyses of molecular data from multiple DNA fragments. Molecular methods have been used to reconstruct the phylogeny of plant families, for biogeographical studies, character evolution tracing etc. (e.g. Clausing & Renner, 2001; Crawford & Mort, 2005; Guo & Ge, 2005; Fior et al., 2006).

In Balsaminaceae, previous work has focused on morphology (e.g. Perrier de la Bathie, 1934; Grey-Wilson, 1980b; Chen, 2001 etc.), and karyology (e.g. Jones & Smith, 1966; Bhaskar, 1976, 1980; Rao et al., 1986; Akiyama et al., 1992a, 1993, 1996b etc.) and few molecular phylogenetic studies have been made. Several studies aimed at addressing phylogenetic relationships at the family level or above have included a few representative species (no more than five) of Balsaminaceae (Morton et al., 1996, 1997; APG, 1998; Soltis et al., 2000; Albach et al., 2001; Anderberg et al., 2002; Bremer et al., 2002; Geuten et al., 2004). These studies have confirmed Balsaminaceae as a member of the order of Ericales of the basal asterids (mentioned before). Within the family, the only molecular phylogenetic study has been done with 25 species from the eastern Himalayan area using chloroplast rbcL and trnL-F sequences (Fujihashi et al., 2002). Besides its inappropriate analyses (too distant outgroups from rosids were used), this study was too sketchy to resolve questions about phylogeny and biogeography of the family. Global phylogenetic relationships within the family remain poorly known, and the more general problems such as diversification patterns of floral and vegetative morphology and historical biogeography of the family need to be addressed through more comprehensive global studies.

To conduct a comprehensive molecular phylogenetic study of Balsaminaceae, the present study began by using nucleotide sequence data of the internal transcribed spacer region (ITS) of nuclear ribosomal DNA for 112 species of Balsaminaceae and five species of its closest relatives in Marcgraviaceae and Tetrameristaceae. This ITS phylogenetic analysis applying parsimony and distance estimates confirmed the monophyly of Balsaminaceae and suggested the monophyly of *Impatiens*. Within *Impatiens*, a few clades received strong support. ITS phylogeny reveals that extant *Impatiens* species are of Southeast Asian origin, and has dispersed to other center of distribution. It suggested the ancestral basic chromosome number and two morphological character evolution trends (details see Paper III). But, ITS data alone could not resolve relationships among most of the lineages with confident support values. Further phylogenetic studies with additional molecular data from cpDNA were launched to improve the resolution and clade supports of the molecular phylogenies.

Then, chloroplast atpB-rbcL was sequenced from 86 species of Balsaminaceae and five outgroups. Phylogenetic reconstruction using parsimony and Bayesian approaches provide a well-resolved phylogeny that confirmed the monophyly of Balsaminaceae and the sister group relationship between *Impatiens* and *Hydrocera*. It suggested similar biogeographical distribution patterns as the ITS phylogeny. The intrageneric taxonomic implications and several character evolutions were discussed according to this atpB-rbcL phylogeny (detail see Paper IV). But, this study has less samples, and more extensive taxa and additional data from other DNA sequences was necessary to obtain a better molecular phylogeny.

Therefore, one more chloroplast region trnL-trnF was chosen and more taxa of *Impatiens* were added. The phylogeny of the family was reconstructed using all three DNA sequences: ITS, atpB-rbcL and trnL-F. Interesting biogeographic and morphological evolutionary patterns were revealed by the molecular phylogenies. The following description is according to the final study based on three DNA fragments of 187 species.

## 4.2 Materials and methods

### 4.2.1 Materials

The species chosen for molecular study include 182 species representing two genera of Balsaminaceae. *Hydrocera* was collected in Vietnam and *Impatiens* in east and Southeast Asia, Madagascar, Africa, Himalayas, southern India and Sri Lanka, North America, central Asia and Europe (Table 4.1). The species chosen represent the greatest amount of morphological variation in the genus. Three species of Marcgraviaceae and two species of Tetrameristaceae were sampled as outgroups since it was revealed these two familes are closely related to Balsaminaceae by recently molecular phylogenetic studies (Morton et al., 1996, 1997; APG, 1998; Soltis et al., 2000; Albach et al., 2001; Anderberg et al., 2002; Bremer et al., 2002; Geuten et al., 2004).

**Table 4.1 samples chosen from different distribution center for molecular phylogeny study of Balsaminaceae**

| | **E & SE Asia** | **Madagascar** | **Africa** | **Himalaya** | **S. India & Sri Lanka** | **N. America & Boreal Eurasia** | **Central Asia & Europe** |
|---|---|---|---|---|---|---|---|
| Species number | 93 | 31 | 25 | 15 | 10 | 6 | 2 |

### 4.2.2 Molecular markers

Three molecular markers were chosen that would provide the most amount of information at the species level, i.e. internal transcribed spacer of nrDNA (ITS1, 5.8S and ITS2), and noncoding regions of chloroplast DNA (trnL intron, trnL-F spacer and atpB-rbcL spacer) (Table 4.2).

**Table 4.2 the names and sequences of the primers used.**

| **Primer** | **Primer sequence** | **Reference** |
|---|---|---|
| **ITS** | | White et al., 1990 |
| ITS 5 | 5’-GGAAGTAGAAGTCGTAACAAGG-3’ | |
| ITS 4 | 5’-TCCTCCGCTTATTGATATGC-3’ | |
| **trnLF** | | Taberl et al., 1991 |
| trnLF C | 5’-CGAAATCGGTAGACGCTACG-3’ | |
| trnLF D | 5’-GGGGATAGAGGGGACTTGA -3’ | |
| trnLF E | 5’-GGTTCAAGTCCCTCTATCCCC -3’ | |
| trnLF F | 5’-ATTTGAACTGGTGACACGAG-3’ | |
| **atpB-rbcL** | | Hoot et al, 1995 |
| atpB-rbcL F | 5’-GAATCCAACACTTGCTTTAGTCTCT-3’ | |
| atpB-rbcL R | 5’ AGA AGT AGT AGG ATT GAT TCT CAT A -3’ | |

### 4.2.3 Molecular methods

Total DNA was extracted from fresh silica gel dried leaf tissue (Chase & Hills, 1991) or herbarium leaves using either the CTAB procedure of Doyle and Doyle (1987) or the DNeasy Plant Mini Kit (QIAGEN AG, Basel). DNA was then directly amplified by PCR of all markers, after purification of PCR products, cycle sequencing reactions were performed with one of the same primers used for PCR. The products of cycle sequencing were cleaned then run on ABI310 automated sequencer (Applied Biosystems). All samples were successfully sequenced with the three markers, except one species of Marcgraviaceae and two species of Tetrameristaceae that were unable to be sequenced with trnLF. The sequences were aligned with Clustal X (Thompson et al., 1997), then adjusted by manual alignment.

### 4.2.4 Phylogenetic analysis

Neighbor-joining analyses (Saitou & Nei, 1987) were conducted on individual ITS, trnLF and atpB-rbcL data sets and on a combination of the three gene using PAUP v4.0b10 (Swofford, 2000). The number of nucleotide substitutions per site was estimated by Kimura's (1980) two-parameter method and used to estimate the genetic distance. The reliability of the clades on the shortest tree(s) was assessed using bootstrapping with PAUP* (Felsenstein, 1985) performed with 1000 replicates. Bootstrap support (BS) was categorized according to Kress et al.'s (2002) criteria, i.e. strong (>85%), moderate (70-85%), weak (50-70%), or poor (<50%) support. The consistency index (CI) (Kluge & Farris, 1969) and the retention index (RI) (Farris, 1989) were calculated.

A Bayesian analysis (Rannala & Yang, 1996; Mau et al., 1999) was performed using Mr. Bayes v. 3.1 (Ronquist & Huelsenbeck, 2003) and involved the same taxon sampling as used for the Neighbor-Joining analysis of the combined dataset. Data were partitioned into functional categories (ITS, trnLF and atpB-rbcL). The most appropriate molecular model of sequence evolution for each data set was selected using Modeltest 2.2 (Nylander, 2004). A combined dataset of all partitions, including gaps, was analyzed applying separate models to each data partition and with parameters estimated separately for each individual partition. Two million generations were run with parameters sampled every 100 generations, based on the inspection of the likelihood scores for each generation. Runs were repeated twice to confirm results. After discarding the trees saved prior to this point as burn-in (Huelsenbeck & Bollback**,** 2001), the remaining trees were imported into PAUP* and a 50% majority-rule consensus tree was produced to obtain posterior probabilities of the clades. A tree with a posterior probability of 0.90 has a 90% chance of being true given that the model and the priors are correct.

To assess the level of congruence among the ITS, trnLF and atpB-rbcL data sets, each data set was analyzed independently to see if they produced a similar topology. Following the conditional data combination approach (Huelsenbeck et al., 1996), the incongruence length difference (ILD) test of Farris et al. (1995) of each two data sets were implemented in PAUP* 4.0b10 (Swofford, 2000) with 100 replicates, TBR branch swapping and simple sequence addition (other default settings). Following Cunningham (1997), P value of 0.01 is taken as a significance criterion for this test.

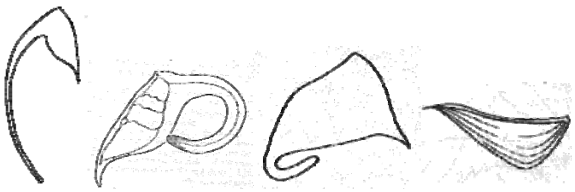
### 4.2.5 Character state optimization and biogeographic analyses

A total of 182 ingroup and two outgroup species were used in all analyses. Evolution of structural characters was examined in two ways using MacClade version 3.08 (Maddison & Maddison, 1992): (1) molecular data were used to create a topology onto which morphological characters were mapped. Bayesian consensus tree based on combined datasets

was chosen in the present study; (2) five morphological characters (presence of flower spur, shape of fruit, number of lateral sepals, type of pollen and type of seedcoat micromorphology), and basic chromosome numbers, have been considered as unordered binary or multi-state characters and were optimized onto the molecular phylogeny to examine their evolution. The geographic distribution was also examined in the same way to trace the historical biogeography of Balsaminaceae. Seven areas of endemism were designated on the basis of species diversity: 1) Madagascar, 2) Africa, 3) South India and Sri lanka, 4) east and Southeast Asia (comprising Japan, Taiwan and the other Southeastern Asian islands, the Southeast Asian countries, and the area of the southwest China), 5) North America and boreal Eurasia, 6) the Himalayas and Tibet, 7) Central Asia and Europe. Wide distributions over more than two areas of endemism were counted as polymorphisms. The other floral morphological characters (shape and form of the spur, lateral petals and dorsal petals) were integrated with the molecular phylogeny as well. (Table 4.3)

**Table 4.3 Morphological, palynological, karyological and biogeographical characters states used in this study**

| No. | Character and state descriptions |
|---|---|
| 1. | Presence of spur: N: no spur; Y: with spur |
| 2. | Shape of fruits: 1: capsule broadly fusiform; 2: capsule narrowly fusiform; 3: berry like |
| 3. | Number of lateral sepals: 2: two lateral sepals; 4: four lateral sepals |
| 4. | Pollen morphology: 3: 3-colpate pollen type; 4: 4-colpate pollen type |
| 5. | Basic chromosome number: 1: x=7; 2: x=8; 3: x=9; 4: x=10; 5: x=5; 6: x=6; 7: x=17; 8: x=13; 9: x=3 |
| 6. | Species distribution area: 1: Madagascar; 2: Africa; 3: South India & Sri Lanka; 4: East & Southeast Asia (comprising Japan, Taiwan & the other Southeast Asian islands, the Southeast Asian countries & the area of southwestern China); 5: North America & Boreal Eurasia; 6: the Himalayas & Tibet; 7: Central Asia & Europe |
| 7. | Shape of the spur: 1: filiform; 2: navicular; 3: saccate; 4: almost no spur |
| 8. | Shape of the lateral petals: Lateral petals united: 1-3: 1: lower/upper > 2times, 1A : lower part narrow; 1B: lower part broad; 1C : lower part 2 split; 2: lower/upper 1~2times; 3: lower<upper; 4: lateral petals almost seperated |
| 9. | Shape of the dorsal petals: 1: flat or slightly concave; 2: hooklike |
| 10. | Seedcoat micromorphology: 1: laevigate; 2: granulate; 3: reticultate: 3A: fine reticulate; 3B: colliculate; 3C: carinate; 3D: striate; 4: protrusive: 4A: digitiform; 4B: clustered; 4C: squamalate; 4D: cristate |



**1 2 3 4**

**Fig. 4.1 shape of the spur (lower sepals) in Balsaminaceae**




1A 1B 1C 2 3 3 4

**Fig. 4.2 shape of the lateral petals in Balsaminaceae**



1 2

**Fig. 4.3 shape of dorsal petals in Balsaminaceae**

## 4.3 Results

### 4.3.1 Sequence characteristics

The length of the complete unaligned fragments of the ingroup taxa ranged from 579 to 655 base pairs for ITS, from 866 to 998 base pairs for trnLF, and from 657 to 762 base pairs for atpB-rbcL. The full ITS dataset including 187 sequences of ingroup and outgroup taxa have 809 aligned characters, of which 92 (11.4%) involved alignment ambiguity and were excluded from phylogenetic analyses, 183 (22.6%) are constant, 112 (13.8%) are autaponorphic, 422 (52.2%) are informative when considered with the criteria of parsimony. The full trnLF dataset including 184 sequences of ingroup and outgroup taxa have 1243 aligned characters, of which 652 (52.4 %) are constant, 236 (19.0%) are autaponorphic, 355 (28.6 %) are informative, in some species, there are large deletions among sequences, e.g. *I. cyathiflora, I. delavayi* and *I. barbata*. The full atpB-rbcL dataset including 187 sequences of ingroup and outgroup taxa have 961 aligned characters, of which 120 (12.2%) involved alignment ambiguity and were excluded from phylogenetic analyses, 457 (47.6 %) are constant, 154 (16.0%) are autaponorphic, 230 (23.9%) are informative. The sequence showed higher divergence in ITS (from 0% - 38.7%) than in trnLF (from 0% - 10.5%) and in atpB-rbcL (from 0% - 13.6%).

### 4.3.2 ITS phylogeny

NJ analysis of the ITS dataset including all outgroup and ingroup taxa using Kimura-2-parameter resulted in a NJ tree (Fig. 4.4). The bootstrap clade support (when greater than 50%) are shown in the Fig. 4.4 and it is seen that this tree is moderately resolved. Despite the relative high sequence divergence, the resolution among the main lineages is poor or receives less significant support, while some lineages of closely related taxa are better resolved with strong support. The internal branches connecting the main lineages are conspicuously shorter in comparison to the long terminal branches, suggesting a high proportion of autapomorphic variations of the sequences. The monophyly of Balsaminaceae is highly supported (BS: 100%). The monotypic genus *Hydrocera* was resolved as sister to a monophyletic *Impatiens* with 100% bootstrap support.

Based on this tree, numbers (A through V) were assigned to the resolved lineages of *Impatiens*. In addition, a group of closely related non-monophyletic taxa were also referred as a clade, e.g. “clade F”, for convenience of illustration and discussion. These numbers were used on the other NJ tree as well. Within *Impatiens*, there are four clades that have independent and different positions if considering all topologies: clade A including *I. clavigera* (Vietnam species), *I. omeiana* (southwest Chinese species) and *I. sp200416* ( a new species from Mt. QingCheng in southwest China); clade B including *I. kerriae, I. psittacina* (Thailand species), *I. stenosepala* Pritz. ex Diels and *I. gongshanensis* (southwest Chinese species) (but on ITS NJ tree, *I. stenosepala* and *I. gongshanensis* was clusted closed to clade C); clade D including *I. arguta* (southwest Chinese species), *I. lingzhiensis* and *I. namchabawensis* Morgan R., Yuan Y-M & Ge X-J (Himalayan species); clade E including

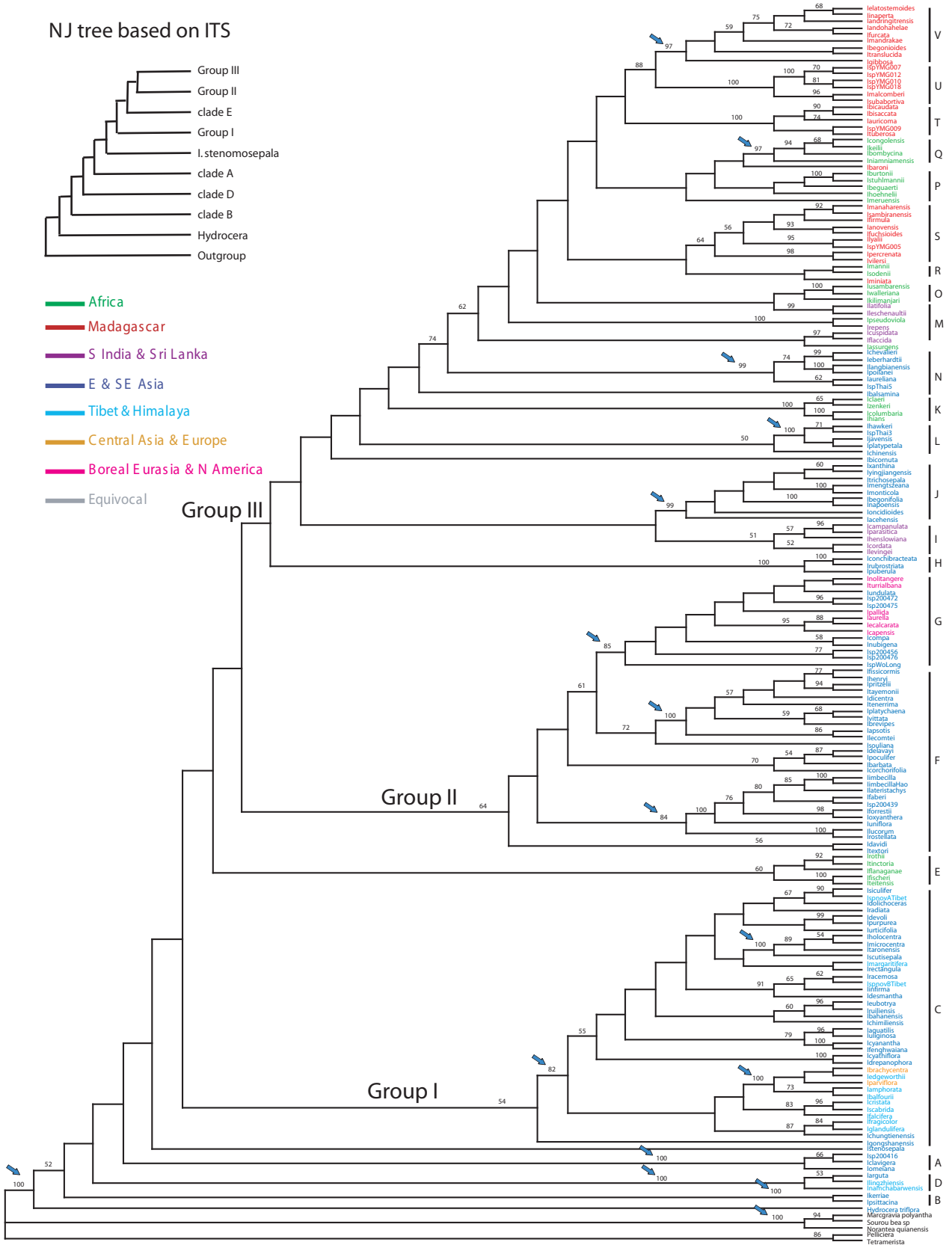



Fig. 4.4 The phylogram of the Neighbor-joining tree based on Kimura-2-parameter of the complete ITS dataset (including 182 ingroup and 5 outgroup). Numbers above the branches are bootstrap values supporting the corresponding branch when greater than 50%. Species name with different color indicate different sampling places: Red-Madagascar; Green-Africa; Violet-South India & Sri Lanka; Blue-East & Southeast Asia; Light blue-Tibet & Himalaya; Yellow-Central Asia & Europe; Pink-Boreal Eurasia & N America; Grey-Equivocal (same color indicate for Fig. 4.5-4.9).

five East African species: *I. rothii*, *I. tinctoria*, *I. flanaganae* Hemsl., *I. fischeri* Warb., *I. teitensis* Grey-Wilson. The remaining representatives of *Impatiens* are divided into three large groups. On ITS NJ tree, clades A, B, D were split from the others and stayed the most basal position. Clade A and D were strongly supported (100%), and clade B split into two parts, but these splits are not well supported.

Group I (clade C) staying in the basal position of the tree and is well supported (82%). It comprises all the Himalayan and Tibet species (except *I. puberula* DC.), some southwest Chinese species, one European species *I. parviflora*, and one central Asian species *I. brachycentra*. Inside clade C, the Himalayan species were well mixed with southwest Chinese species. Some small lineages with closely related morphological species were highly supported (84% - 100%). The eastern European species, *I. parviflora* and central Asian species *I. brachycentra* were grouped with three Himalayan species (100%), these species showed affinity to the other Himalayan and Southwest Asian species in Clade C. *Impatiens gongshanensis* was separated from clade B and sister to Goup I in ITS NJ tree.

Group II (clade F, G) staying in the middle position of the tree, comprises five North American species, one boreal species (*I. nolitangere*) and some southwest Chinese species. The North American species together with the boreal species *I. nolitangere* and other eight central Chinese species formed clade G with high support (85%), then it showed the affinity to the other southwest Chinese species. It is worth noting that clade E including five East African species is sister to Goup II and Goup III, and stayed in the basal position of Group II, but no significant bootstrap supports its position.

Group III (clade H-V) staying in the terminal position of the tree, is a heterogeneous collection of Madagascan, African, south Indian and Southeast Asian species. Almost every well-supported lineage in this group is strongly correlated with a specific area of distribution. Madagascan species formed four clades (S, T, U, V). The spurless Madagascan endemic taxa including two strongly supported clades (clade U, 100% & clade V, 97%) are resolved as being monophyletic with a high support (88%). Another Madagascan clade T was strongly supported (100%) and closed to the monophyletic spurless clades. Madagascan clade S was grouped with some African species without significant support and didn't show the direct connection with other Madagascan species. Madagascan species *I. baroni* Baker and *I. miniata* Grey-Wilson were grouped with African species directly. African species were divided into five clades (K, O, P, Q, R) except clade E (five East African species) was nested out of them but closed to Southeast Asian clades F of Group II. Four West African (Gabon, Cameroon) species formed a highly supported clade K (100%) and showed close connection with Southeast Asian species clade L and N, while the other African species (clade O, P, Q, R) were grouped with Madagascan, Indian species, but didn't receive good support. South Indian species occurred in two lineages (clade I, M) which show no immediate affinity to each other, but clade I showed close connections with Southeast Asian species and clade M including two African species showed Southeast Asian and African connections. Seven southwest Chinese species were well grouped with one Indonesian species and one Malaysian species (clade J, 100%). Clade H containing *I. conchibracteata* Y.L.Chen et Y.Q.Lu, *I. rubrostriata* (southwest Chinese species) and *I. puberula* (Himalayan species) was highly supported (100%) and stayed the basal position of Group III. Southwest Chinese species *I. bicornuta* stayed among Southeast Asian species felled within this group, which is different from the other topologies (see later).

### 4.3.3 trnLF phylogeny

NJ analysis of the trnLF dataset including all ingroup taxa and two outgroup taxa (two species of Margraviaceae) using Kimura-2-parameter resulted in a NJ tree (Fig. 4.5). The resolution among the main lineages is poor, while some small lineages of closely related taxa are better resolved with strong support. Balsaminaceae and *Impatiens* were resolved as monophyletic with 100% bootstrap support. Clade A including *I. clavigera*, *I. omeiana* and *I. sp200416* splits from other *Impatiens* with a moderate bootstrap support (78%) and stayed in the basalmost position in NJ trnLF tree. clade D including *I. arguta*, *I. lingzhiensis* and *I. namchabarwensis* splits from other *Impatiens* and stays in the basal position as well, but without significant support. The remaining representatives of *Impatiens* clustered into three groups and every group contains almost the same species as in ITS NJ tree.

Group I (clade C) staying in the middle position of the tree, was highly supported (99%). The Eastern European species, *I. parviflora* and central Asian species *I. brachycentra* grouped with three Himalayan species with a high bootstrap support (97%), and these species showed affinity to the other Southeast Asian and Himalayan species in Clade C. Clade B including *I. kerriae*, *I. psittacina I. stenosepala* and *I. gongshanensis* is sister to Group I, but marginally supported only 63%.

Group II (clade F, G) staying in the basal position of the tree, was highly supported (91%). Inside this group, five North American species were together with the boreal species *I. nolitangere*. Then these species and other seven central Chinese species formed clade G with strong support (89%). They showed the affinity to the other East Asian species.

Group III (clade H-V) staying in the terminal position of the tree. Clade H containing *I. conchibracteara*, *I. rubrostriata* and *I. puberula* was highly supported (98%) and stays in the basal position of the Group III, the other clades (I-V) containing Madagascan, African, southern Indian and Southeast Asian species formed highly supported clade I-V (90%). Most of the Madagascan species were together, except clade S was separated and grouped with African species. The Madagascan spurless clades (U, V) were separated and mixed with other Madagascan spurred species (clade STUV), but these resolutions did not receive significant bootstrap support in either clades. African species including six clades (E, K, O, P, Q, R) showed different position on the trnLF NJ tree compared with ITS NJ tree. Clade E (five East African species) was nested out of them but was sister to Group III. Clade K (four West African species) was highly supported (100%) and sister to clade M-V including Madagascan species, other African species, five Indian species and few Southeast Asian species with moderate support 80%. Clade P and R was mixed a little bit each other then grouped with a few Madagascan species, together with clade Q, O showing on the terminal position of the tree, except *I. assurgens* stayed in the basal position of Madagascan species. South Indian species include two clades: clade I and clade M. Clade I shows close connection with clade J (Southeast Asian species), except *I. henslowiana* was out and closer to clade L (Southeast Asian species). Species of Clade M and clade N (Southeast Asian species) were separated and mixed together, but neither of them received good support.

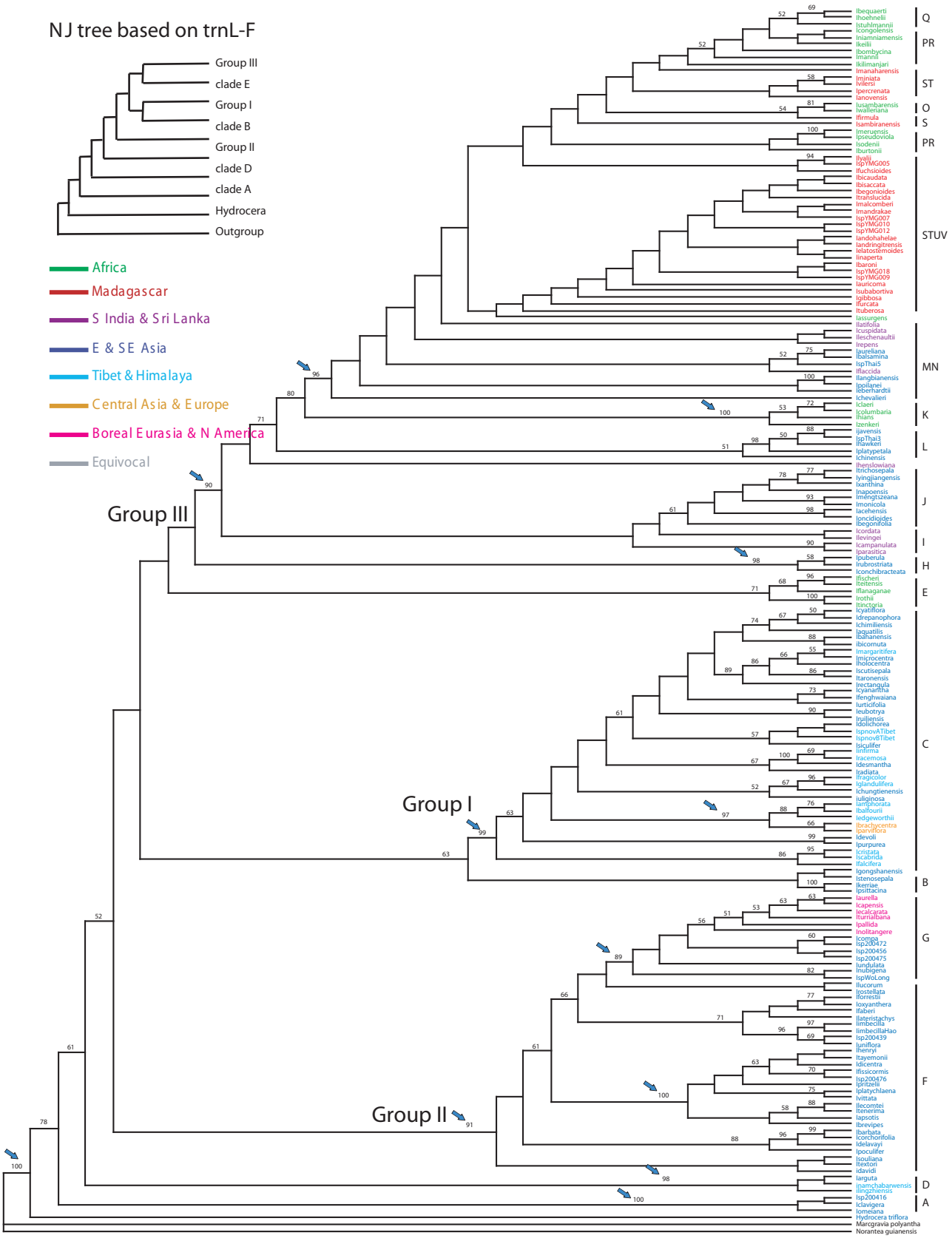



Fig. 4.5 The phylogram of the Neighbor-joining tree based on Kimura-2-parameter of the complete trnLF dataset (including 182 ingroup and 2 outgroup). Numbers above the branches are bootstrap values supporting the corresponding branch when greater than 50%.

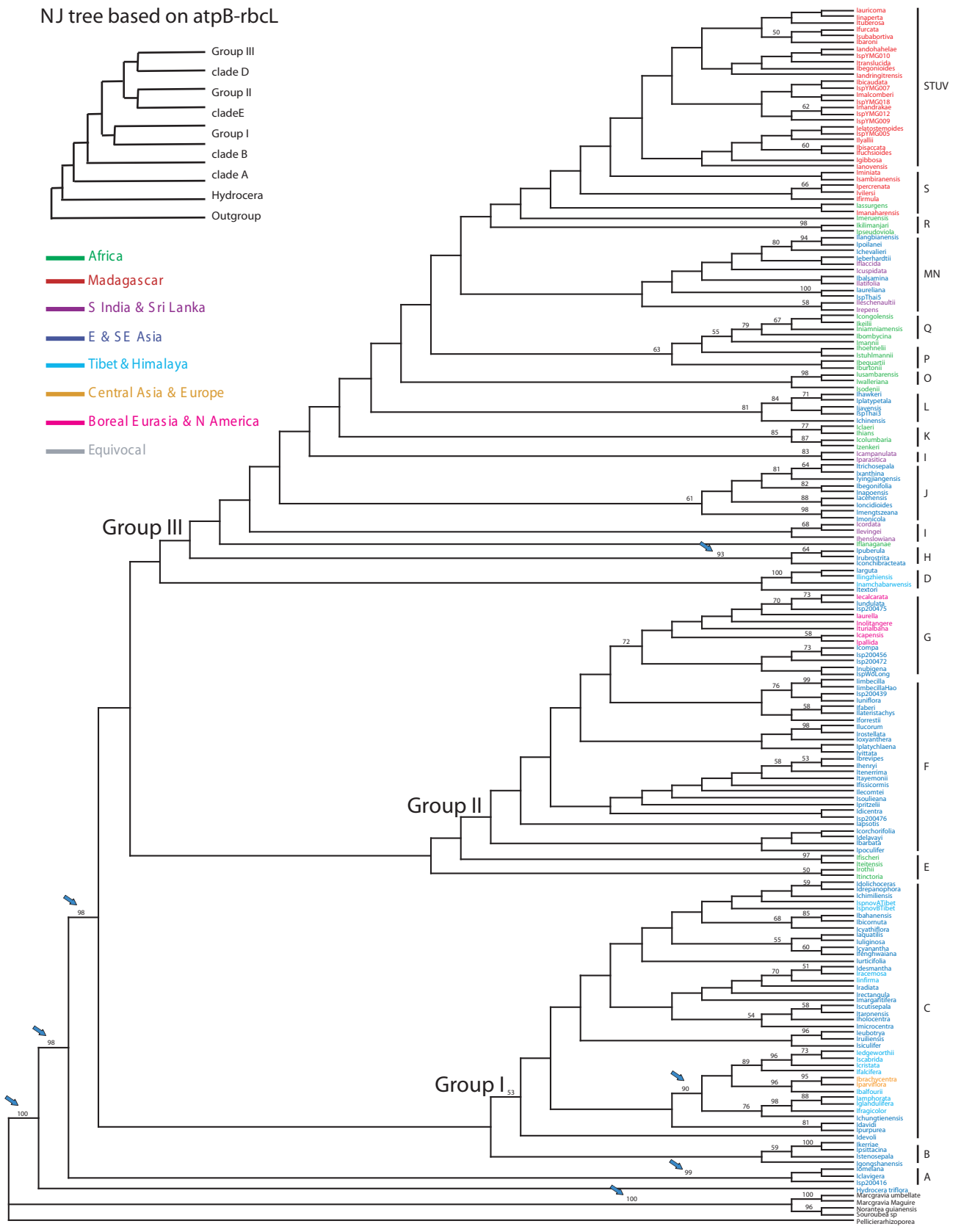



Fig. 4.6 The phylogram of the Neighbor-joining tree based on Kimura-2-parameter of the complete atpB-rbcL dataset (including 182 ingroup and 5 outgroup). Numbers above the branches are bootstrap values supporting the corresponding branch when greater than 50%.

### 4.3.4 atpB-rbcL phylogeny

NJ analysis of the atpB-rbcL dataset including all outgroup and ingroup taxa using Kimura-2-parameter resulted in a NJ tree (Fig. 4.6). The resolution is generally poor on this tree except three basal split and some small lineage got good supports. Balsaminaceae and *Impatiens* were resolved as monophyletic with 100% strong bootstrap support. Clade A including *I. clavigera*, *I. omeiana* and *I. sp200416* is sister to all the other *Impatiens* species with strong support (98%). The remaining representatives of *Impatiens* are divided into three groups and every group contains almost the same species as the ITS NJ tree and trnLF NJ tree.

Group I (clade C) staying in the basal position of the tree. The eastern European species, *I. parviflora* and central Asian species *I. brachycentra* grouped with eight Himalayan species and one southwest Chinese species with a high support (90%), and these species showed affinity to the other Southeast Asian and Himalayan species in Clade C. Clade B including *I. kerriae*, *I. psittacina I. stenosepala* and *I. gongshanensis* is sister to this group, but without significant support.

Group II (clade F, G) staying in the middle position of the tree. One North American species *I. ecalcarata* was grouped with two southwest Chinese species with moderate support (70%), then with another four North American species and the boreal species *I. nolitangere* and five central Chinese species formed clade G with moderate support (72%). They showed the affinity to the other East Asian species. Clade E (five East African species) is sister to this group, but without significant support.

Group III (clade H-V) staying in the terminal position of the tree. Madagascan species were together appearing the most advanced position of the tree, but the spurless species were mixed with spurred species and formed the clade STUV. Several African species (clade R) were closed to them. The other African species in this group showed three lineages: clade P,Q were grouped together, close with clade O showed the connection with Southeast Asian and south Indian species; clade K (four West African species) was well supported (85%) and separated from clade O, P, Q by Southeast Asian clade L; *I. flanaganae* was nested out of the other African species but stayed alone between Southwest Asian species (clade H) and south Indian species. The two clades of south Indian species showed some different distribution: clade J (Southeast Asian species) was nested inside clade I; clade M was separated and mixed with clade N (Southeast Asian species) like in trnLF NJ tree, but Indian species showed the basal position. Clade D including *I. arguta*, *I. lingzhiensis* and *I. namchabarwensis* with Japanese species *I. textori* together were sister to Group III. It is worth noting that within this group none of the relationship among the caldes were well supported.

### 4.3.5 Data sets conflict test and combined nuclear and chloroplast DNA phylogeny

The partition homogenetity test (PHT) between each two data sets received statistic support with P=0.01, it means that there are conflict among the different sequences. The ILD test has been criticized by several authors (e.g. Dolphin et al., 2000; Yoder et al. 2001; Darlu & Lecoinre, 2002). It has been suggested that PHT p values greater than p=0.01 reflect congruent data sets that, if combined, will either improve or will not negatively affect phylogenetic accuracy (Cunningham et al., 1998). Even in some cases, tests of detecting highly significant incongruence (P< 0.001) through combining the incongruent partitions actually increased phylogenetic accuracy (Templeton, 1993; Rodrigo, 1993). Nevertheless, in the present study, a comparison of the cladograms obtained for each separate partition

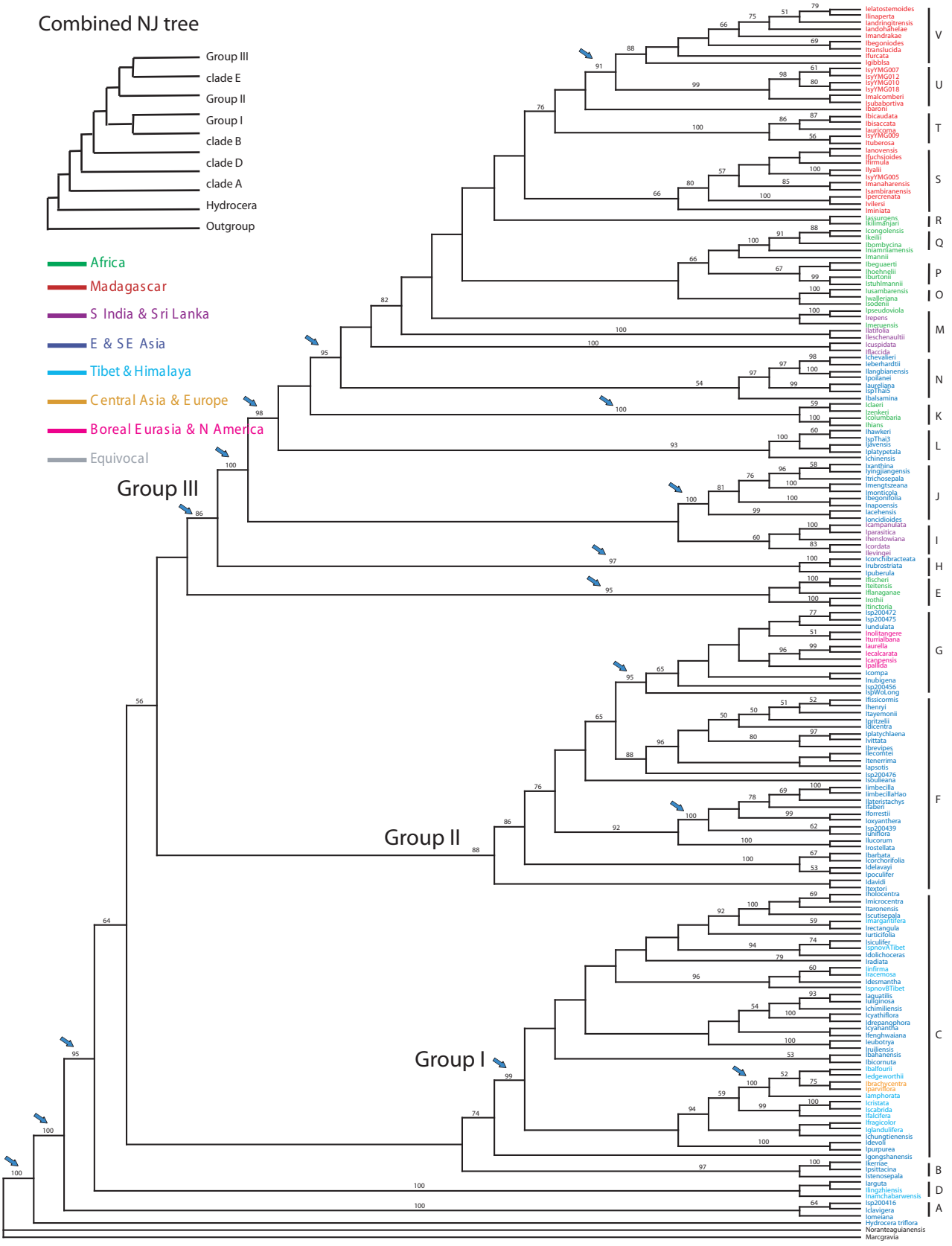
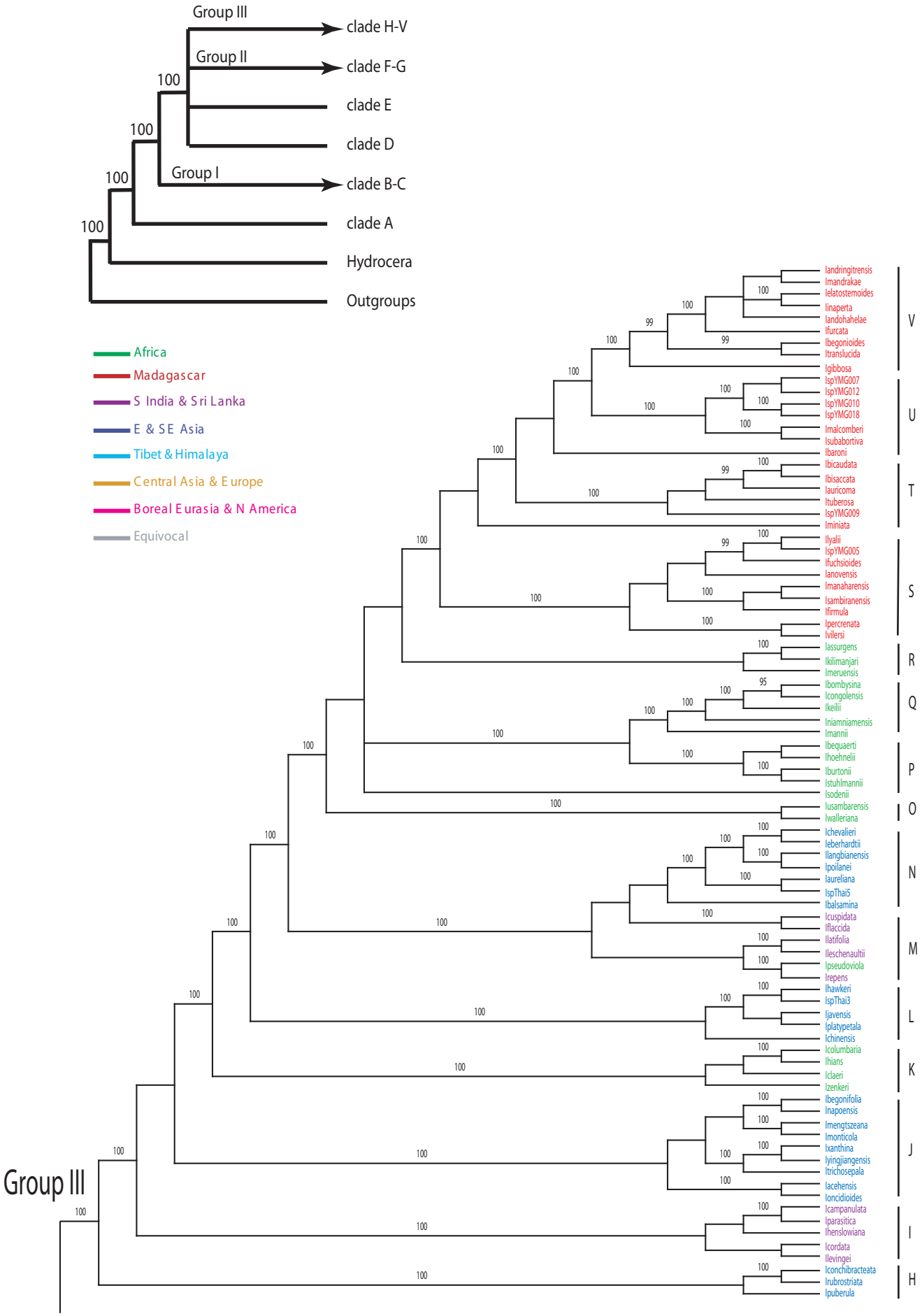



Fig. 4.7 The phylogram of the Neighbor-joining tree based on Kimura-2-parameter of the combined ITS, trnLF, and atpB-rbcL (including 182 ingroup and 2 outgroup). Numbers above the branches are bootstrap values supporting the corresponding branch when greater than 50%.


Group III
clade H-V
Group II
clade F-G
clade E
clade D
Group I
clade B-C
clade A
Hydrocera
Outgroups
Africa
Madagascar
S India & Sri Lanka
E & SE Asia
Tibet & Himalaya
Central Asia & Europe
Boreal Eurasia & N America
Equivocal
landringitrensis
lmandrakae
lelatostemoides
linaperta
landohahelae
lfurcata
lbegonioides
ltranslucida
lgibbosa
lspYMG007
lspYMG012
lspYMG010
lspYMG018
lmalcomberi
lsubabortiva
lbaroni
lbicaudata
lbisaccata
lauricoma
ltuberosa
lspYMG009
lminiata
llyalii
lspYMG005
lfuchsioides
lanovensis
lmanaharensis
lsambiranensis
lfirmula
lpercrenata
lvilersi
lassurgens
lkilimanjari
lmeruensis
lbombysina
lcongolensis
lkeilii
lniamniamensis
lmannii
lbequaerti
lhoehnelii
lburtonii
lstuhlmannii
lsodenii
lusambarensis
lwalleriana
lchevalieri
leberhardtii
llangbianensis
lpoilanei
laureliana
lspThai5
lbalsamina
lcuspidata
lflaccida
llatifolia
lleschenaultii
lpseudoviola
lrepens
lhawkeri
lspThai3
ljavensis
lplatypetala
lchinensis
lcolumbaria
lhians
lclaeri
lzenkeri
lbegonifolia
lnapoensis
lmengtszeana
lmonticola
lxanthina
lyingjiangensis
ltrichosepala
lacehensis
loncidioides
lcampanulata
lparasitica
lhenslowiana
lcordata
llevingei
lconchibracteata
lrubrostriata
lpuberula
V
U
T
S
R
Q
P
O
N
M
L
K
J
I
H
Group III

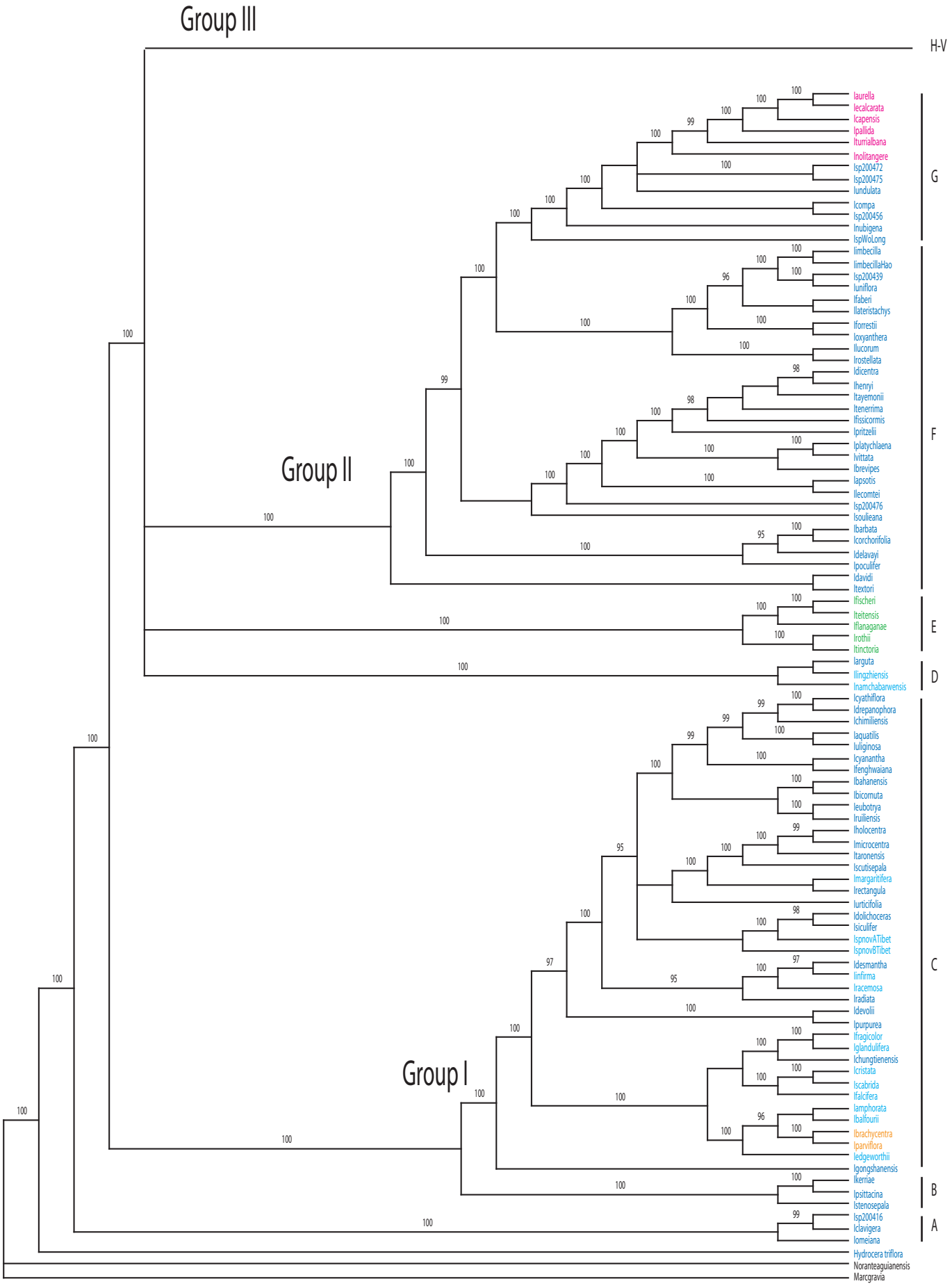



Fig. 4.8 Bayesian consensus cladogram of the combined ITS, trnLF, and atpB-rbcL (including 182 ingroup and 2 outgroup). Numbers above the branches indicate posterior probabilities higher than 95%.

suggests a general congruence in topologies. If congruence occurs, the differently resolved linages are often poorly supported. Therefore, all data sets were combined and were subjected to a combined analysis.

Such combined data matrix covered 184 taxa (including the 2 outgroup species) and 3019 characters, of which 212 (7%) were excluded from analyses, 1309 (43.4%) were constant, 1000 (33.1 %) were informative. The NJ tree using Kimura-2-parameter produced by combining the data sets is mainly congruent with the other NJ tree based on individual data sets (Fig. 4.7). For the Bayesian analysis, the model GTR + I +G was selected for partitions ITS, trnLF and atpB-rbcL respectively by Modeltest 2.2. Bayesian analysis resulted in an almost identified topology with combined NJ tree except the position of clade D and E, although posterior probability (BPP) values for clades were higher than neighbor joining bootstrap values (Fig. 4.8). The consensus tree resulting from Bayesian analysis of the combined dataset is considered as the preferred topology and used for discussions unless mentioned otherwise.

Bayesian analysis based on combined datasets support the monophyly of the family and the sister relationship between *Hydrocera* and *Impatiens* with very high support value (BPP: 1.0). Within *Impatiens*, the most basal lineage clade A, including *I. omeiana*, *I. clavigera*, and *I. sp200416*, splits from the others with a strong bootstrap support (BPP: 1.0). The remaining representatives of *Impatiens* are divided into three large groups the same as in separate ITS, trnLF and atpB-rbcL NJ tree.

Group I (clade C) staying in the basal position of the tree, was well supported (BPP:1.0). The eastern European species, *I. parviflora* and the central Asian species *I. brachycentra* are closely grouped together (BPP:1.0), then grouped with three Himalayan species (BPP:1.0), these species showed affinity to the other Himalayan and Southeast Asian species in clade C. *Impatiens gongshanensis* spliting from clade B (*I. kerriae*, *I. psittacina* and *I. stenosepala* ) stayed at the basal position of Group I. Clade B is sister to Group I.

Group II (clade F, G) staying in the middle position of the tree, was well supported (BPP:1.0). Five North American species were grouped with the boreal species *I. nolitangere* (BPP:1.0), then grouped with another seven central Chinese species to form clade G (BPP:1.0) like in every individual NJ tree They showed the affinity to the other East Asian species.

Group III (clade H-V) staying in the terminal position of the tree, was well supported (BPP:100). Madagascan species were all grouped together (clade S, T, U, V, BPP:1.0), appearing the most terminal position of the whole tree. The spurless Madagascan endemic taxa (clade U, V) were resolved as being monophyletic with a high support (BPP:1.0), this group is the most derived within *Impatiens*. African species are not monophyletic. But African species in this group were clustered together and close to Madagascan species except that clade K (four West African species) was nested out and grouped with Southeast Asian species. One African species *I. pseudoviola* was grouped with Indian species in clade M. South Indian species occurred in two lineages (clade I, M) which show no immediate affinity to each other, but showed close connections with Southeast Asian species, e.g. clade M was grouped with clade N (Southeast Asian species) with high support (BPP:1.0). Other Southeast Asian speceis in clade H, J, L were well supported respectively (BPP:1.0) and fell within this group.

Clade D including *I. arguta*, *I. lingzhiensis* and *I. namchabarwensis* ; clade E including five East African species were paraphyletic to Group II and Group III.

### 4.3.6 Character-state mapping and biogeographic analysis

Chromosome numbers and five important morphological characters, occurrence of flower spur, shape of fruits, number of lateral sepals, type of pollen, and type of seedcoat micromorphology were optimized onto the consensus tree generated from Bayesian analyses on the combined dataset. Parsimonious optimization suggests that spurred flower, linear-fusiform capsules, 4 lateral sepals, 3-colpate pollen type, and reticulate seedcoat type represent plesiomorphic states within *Impatiens*. The basic chromosome number x=8 or 10 are possible ancestral numbers. Optimizing the shape and form of the spur, lateral petals and dorsal petals with molecular phylogeny, no clear trends can be seen in respect to the shape and form of the spur, lateral petals and dorsal petals through out all the Balsaminaceae species. Multiple gains and loss of the floral character have occurred, which confirmed that floral characters are highly mediated by pollinators.

Fitch parsimony optimization of centers of endemism as a multi-state character infers mainland Southeast Asia as the ancestral area for extant Balsaminaceae. *Impatiens* in other areas are inferred to have dispersed from mainland Southeast Asia. Two to three dispersals from mainland Southeast Asia to Africa are suggested: one dispersal resulted in clade E consisting five East African species, and another one or two dispersals involved divergence of other African species (clades K, O-R). The Madagascan *Impatiens* are inferred as having dispersed from Africa, whereas the *Impatiens* species from southern India and Sri Lanka seem to have two origins: some (clade I) were dispersed from mainland Southeast Asia, and others (clade M) from Africa. The central Asian and European *Impatiens* were dispersed from mainland Southeast Asia via the Himalayas, whereas the boreal Eurasian and North American *Impatiens* are resolved as being dispersed from mainland Southeast Asia (Fig. 4.9).

## 4.4 Discussion

### 4.4.1 Similarity and incongruence of different phylogeny analyses

Results from the distance based analyses were congruent for both the individual and combined datasets and were congruent to the Bayesian inference for combined datasets. They are similar in topology: all phylogenies have the same three large groups I, II, III from basal position up except Group I appearing between Group II and III in trnLF NJ tree, but not well supported. Every group contains almost the same species, but only the combined Bayesian tree is well supported in most of lineages. In fact, even in the same well supported lineage, e.g. in combined NJ tree and combined Bayesian tree, bootstrap values did not always correlate well with the posterior probabilities, and were often considerable lower. This irregularity was noted by Huelsenbeck et al. (2002) and has been identified in a number of other studies utilising these two analytical methods (e.g. Soltis et al., 2002; Valiejo-Roman et al., 2002; Simmons et al., 2003; Erixon et al., 2003).

The incongruence mainly embodied in the placement of four clades (A, B, D, E): Clade A including *I. clavigera, I. omeiana* and *I. sp200416* is always in the basalmost position within *Impatiens* in all the phylogeny except ITS NJ tree; Clade B is always close to Group I but two Thailand species (*I. kerria* and *I. psittacina*) split out and appeared in the most basal position within *Impatiens* in ITS NJ tree; Clade D including *I. arguta*, *I. lingzhiensis* and *I.*

*namchabarwensis* appeared in the basal position of the whole topology in ITS NJ tree, trnLF NJ tree and combined NJ tree, but appeared in the middle position in atpB-rbcL NJ tree and Bayesian tree; Clade E including five West African species is close to Group III in trnLF NJ tree and combined NJ tree but close to Group II in the other tree. In addition, the spurless taxa endemic to Madagascar are resolved as being monoplyletic with good support on the ITS NJ tree and combined NJ tree and Bayesian tree, they were separated and mixed with spurred flower in trnLF and atpB-rbcL NJ trees.

The minor dissimilarities among the five trees can be explained in two ways:
1). nrDNA and cpDNA provide different information. The non-coding fast-evolving ITS sequences are more variable and homoplasious than the chloroplast (Dolphin et al., 2000; Barker & Lutzoni, 2002; Dowton & Austin, 2002), and contain phylogenetic information adequate for addressing relationships within more terminal parts of the trees, while plastid data brought resolutions for deeper level branches. Some molecular data of ITS was affected by long-branch attraction and showed the wrong combining of the species from different ancestry (Hendy & Penny, 1989; Bergsten, 2005). Usually in this case, these species divergence a lot with their closely related species, thus, they formed a long branch on phylogeny; or these species showed incredible rapid mutation rate. For example, *I. bicornuta* (Himalayan species sampled in southwest China) was nested out of Group I and showed in Group III and together with some Southeast Asian species in ITS NJ trees. The hybridization and the following evolution to the different direction might be another reason for the difference of the topology of ITS and cpDNA (Rieseberg & Carney, 1998).

Multiple independent DNA fragments can yield the gene trees that represent the true phylogeny relationship (Miyamoto & Fitch, 1995; Sang, 2002), and combining the DNA fragments of different origins can improve the phylogeny information and avoid noise, to improve the accurate of phylogeny reconstruction (Donoghue & Sanderson, 1992; Olmstead & Sweere, 1994; Miyamoto & Fitch, 1995).

2). A great deal of work has been put into studies on the accuracy of tree reconstruction methods based on DNA or amino acid sequences, where accuracy is understood as the ability of a reconstruction method to recover the true branching pattern topology of the underlying tree from a given data set (Strimmer & Haeseler, 1996).

**Neighbor-Joining method** has been widely used and is still used in phylogenetic studies (Wu et al., 2006), but it has been proven that the accuracy of neighbor joining is influenced by the number of taxa, sample size, sequence differences etc.

**Bayesian method** is a practical alternative to the other methods, because it has advantages over the other methods in terms of ability to use complex models of evolution, different models for separate partition, ease of interpretation of the results, and computational efficiency (Huelsenbeck, 2002; Nylander et al., 2004). Bayesian inference of phylogeny, a methods recently quickly developed and widely used, showed superiority for phylogenetic studies (Huelsenbeck et al. 2000, 2001; Alfaro et al., 2003).

In the present study, among all the trees, the combined Bayesian tree is well supported in most lineages. Comparing with this tree, the different position of the groups and clades on the other tree is never well supported. Bayesian tree is chosen for further discussion.

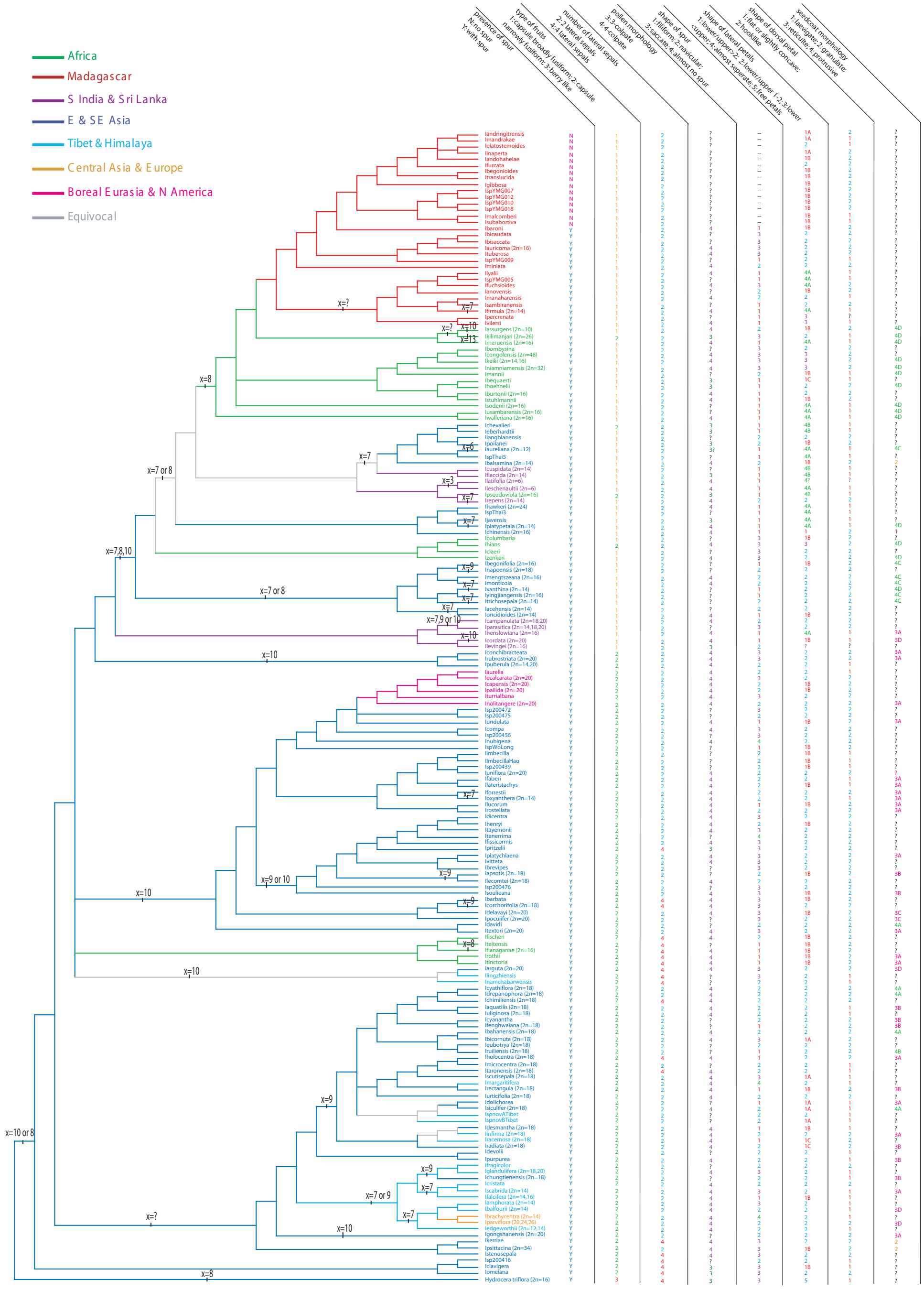



Fig. 4.9 Parsimonious optimization of the distribution centers of endemism, basic chromosomal numbers, and selected morphological characters onto the Bayesian combined tree. Vertical bars indicate positions of character state changes. Chromosome numbers when available are shown after the species names.

### 4.4.2 Phylogenetic relationships within Balsaminaceae

**Molecular phylogeny confirmed the monophyly of Balsaminaceae.** Some recent molecular analyses indicated that Balsaminaceae belong to the order Ericales and are part of the basal clade (Morton et al., 1996; Savolainen et al., 2000; Soltis et al., 2000; Alhach et al., 2001; Anderberg et al., 2002; Bremer et al., 2002; APG, 2003; Geuten et al., 2004). The present molecular phylogeny also confirmed that Balsaminaceae are monophyletic with strong support (BPP:1.0 in combined Bayesian tree/ BS: 100 in all NJ tree).

**Molecular phylogeny confirmed the monophyly of *Impatiens***. The monotypic *Hydrocera* is regarded as a distinct genus in Balsaminaceae because of its unique floral structure and indehiscent fruit (Grey-Wilson, 1980d, 1989; Chen, 2001). It was considered as a species of *Impatiens* in some classic works such as Linnaeus's Species Plantatum. Since the floral morphology of *Impatiens* is highly diversified, the unique floral morphology of *H. triflora* does not necessarily ensure it as a distinct genus rather than a specialized ingroup of *Impatiens*. The present molecular phylogeny, however, recognized *Hydrocera* as a distinct genus sister to *Impatiens* and confirmed the monophyly of *Impatiens* with strong support (BPP: 100 in combined Bayesian tree/BS: 100 in combined NJ tree).

### 4.4.3 Phylogenetic relationships within *Impatiens*

**The phylogenetic relationships within *Impatiens* are associated with geographic distribution, less defined by gross morphology.** The phylogenetic relationships with the different lineages of *Impatiens* have never been explicitly studied before. The present molecular phylogeny showed that many of the clades are associated with a specific area of distribution but it is very difficult to find morphological synapomorphies for the different clades revealed by molecular phylogeny. For example, four species from Vietnam *I. chevalieri* Tardieu and *I. eberhardtii* Tardieu have almost separate lateral petals and a long filiform spur, while *I. langbianensis* Tardieu has a naviculate spur, united lateral petals (lower/upper part:1-2 time) and *I. poilanei* Tardieu has a small distinctly cucullate dorsal petal and large lower part of the lateral petals. But these four species are always clustered together in different molecular trees. Five North American species don't have very similar floral morphology, *I. aurella* Rydb. has quite flat dorsal petal and naviculate spur, *I. ecalcarata* Collett & Hemsl. and *I. turrialbana* Donn. Sm. has saccate spur, *I. capensis* Meerb. and I. pallida Nutt. have larger lower part of the lateral petals etc., but they are always clustered together (clade G) except including three southwest Chinese species in ITS NJ tree and two southwest Chinese species in atpB-rbcL NJ tree. Two Thailand species, *I. kerriae* Craib and *I. psittacina* Hook.f. were always clusted in the same clade B, but they don't have similar floral morphology: the former one is a middle yellow flower with naviculate to saccate spur, but the latter one is big violet flower with a deep saccate spur.

### 4.4.4 Biogeographic implications of the molecular phylogeny

**Mainland Southeast Asia is suggested to be the original region**, **from where dispersals to other diversification centers occurred.**

The geographic distribution of species and the relationship to abundance have long been of interest to biologists (Darwin, 1859; Brown, 1995; Gaston, 2003). Balsaminaceae show an interesting mainly paleotropical distribution pattern, with several diversity hotsports: tropical Africa, Madagascar, southern India and Sri Lanka, the eastern Himalayas, and Southeast Asia.

Jones and Smith (1966) and Khoshoo (1966) suggested that *Impatiens* originated in the Himalayan region. Their hypothesis was mainly based on species diversity and karyological data. Bhaskar (1981) thought that south India contains most of the primitive or phylogenetically old species and Western Ghats is the places of origin of *Impatiens* considering the concentration of diploids, primitive radial pollen grains and shrubby habit. Grey-Wilson (1980b) formulated a hypothesis suggesting that Balsaminaceae originated in western Gondwana and spread to Southeast Asia through Madagascar and India. From Southeast Asia and the adjacent Sino-Himalayan area, *Impatiens* diversified secondarily into two lineages: one lineage radiating to the temperate Eurasian areas and North America, and another radiating to the tropical and subtropical areas in Southeast Asian islands. Meanwhile, he rejected the possibility of an overland migration between Africa and India.

Biogeographical optimization onto the combined Bayesian phylogeny illustrates the region of origin and dispersal route in this family. It revealed that mainland Southeast Asia is the ancestral area of extant *Impatiens*. The boreal species *I. nolitangere* and North American species (clade G) are suggested as having dispersed from mainland Southeast Asia. The central Asian species *I. brachycentra* and European species *I. parviflora* may have been dispersed from mainland Southeast Asia through the Himalayas. Africa seems to have been colonized at least twice by ancestors from mainland Southeast Asia: once led to the clade E (East African species, possessing long-linear fruits, flower with five sepals, large seeds with reticulate seedcoat) through a dispersal directly from mainland Southeast Asia, and at another one by a colonizer with broad fusiform fruits that gave rise to the others (Clade K, O-R) through a dispersal from mainland Southeast Asia probably via India subcontinent. However, it is to be further confirmed if the colonization of Africa might have been achieved via India as stepping-stones, as our present sampling of India species is still insufficient. Nevertheless, as far as the species sampled from India and Sri Lanka are concerned, some species (clade I) showed Southeast Asian connection and some (clade M) showed African connections. Thus, it is suggested that south India was colonized at least twice, once by a mainland Southeast Asia ancestor (clade I), and another time most likely by an ancestor with African affinities (clade M). Madagascan *Impatiens* shows clearly an African origin and the colonization event might be only once.

The historical biogeography of *Impatiens* suggested by molecular phylogenies is, in fact, in concordance with the conspicuous species similarity among Africa, Madagascar, southern India, and Southeast Asia noticed by Grey-Wilson (1980b). However, the present results suggest a mainland Southeast Asian origin of *Impatiens* and dispersals to Africa and Madagascar, whereas on the contrary, Grey-Wilson (1980) suggested an opposite direction of dispersal from an assumed west Gondwanan (African) origin and spread to Southeast Asia and the adjacent Sino-Himalayan area.

Bayesian inference was well applied to the biogeography and the identification of past dispersal patterns (Nepokroeff et al., 2003; Sanmartin & Ronquist, 2004; Waters & Roy, 2004). Extensive dispersal patterns across the paleotropical regions were also well documented in other families such as Melastomataceae. The study on the historical biogeography of this family revealed that it initially diversified in the tropical forest north of the Tethys sea, from where it spread throughout Eurasia and to North America. They also entered South America, and from where one clade (*Melastomeae*) reached Africa by long distance dispersal. From Africa, this clade further spread to Madagascar, India, and Indochina (Renner et al., 2001). *Exacum* (Gentianaceae) represents another example (Yuan et al., 2005). The ancestral area reconstruction suggests that *Exacum* originated in Madagascar. One clade

colonized Sri Lanka and southern India via long-distance dispersals, from where it dispersed extensively to Southeast Asia. These examples showed that similar geographical distribution patterns in the paleotropical regions may have very different historical origins. The present molecular study revealed Balsaminaceae are of mainland Southeast Asia origin, from where extensive overland dispersals to boreal Eurasia and North America, to central Asia and eastern Europe via the Himalayas, and to India and Africa via the Himalayas have happened. The historical biogeography of Balsaminaceae shows additional patterns and thus can contribute to a better comprehension of the historical biogeography across the paleotropical regions.

### 4.4.5 Chromosomal number evolution implicated by molecular phylogeny

It is proved that Bayesian statistical methods enable the study of character evolution (Ronquist, 2004). The present study on karyology together with previous reports on karyology in this family revealed that chromosomal number in Balsaminaceae have a high diversity: from 2n=6 to 2n=66, x=7, 8, 9, 10 are the most frequent basic numbers of the family. It was proposed that x=8, 9 or 10 are all possible to be the ancestral basic chromosomal number only based karyological study. Tracing all the available chromosomal number onto the molecular phylogeny, it suggested both x=8 or 10 are the most likely ancestral base number in *Impatiens* (Fig. 4.9). The evolution of chromosome basic number in *Impatiens* might be: from x=10 derived to the other numbers mainly by descending dysploidy or from x=8 derived to the other numbers by both descending and ascending dysploidy.

Since some important chromosomal information of the species on the molecular tree is still missing, e.g. the most basal clade A (*I. omeiana*, *I. clavigera* and *I. sp200416*), Madagascan species, and the chromosome number of many other species is still not available , it is still impossible to say which number is the base ancestral number in *Impatiens* for sure.

### 4.4.6 Morphology character evolution implicated by molecular phylogeny

The use of molecular data in the investigation of relationships within morphologically complex families has been a powerful approach for achieving well-delimited taxa (e.g. Asteraceae: Kim & Jansen, 1995; Ericaceae: Kron et al., 2002; Myrtales, Schönenberger & Conti, 2003). Combining data sets for phylogenetic analysis assumes (1) that the same history is true for each character set and (2) that the chosen methods of tree reconstruction are equipped to handle the differences in their evolutionary rules (Miyamoto & Fitch, 1995).

In the present study, the chosen morphological features were optimized on molecular tree, more insights were given about their evolution trend.

#### 4.4.6.1 Fruit shape

***Hydrocera* has unique berry-like fruits, in *Impatiens*, the evolution of fruits is from narrowly fusiform to broadly fusiform.** Fruit shape is one of the most important taxonomic characters in Balsaminaceae. *Hydrocera* has unique indehiscent berry-like fruits, while *Impatiens* has two types of dehiscent capsules: narrowly fusiform (length/diameter >5) and broadly fusiform (length/diameter $\leq$ 4). Tracing this character on the molecular phylogeny (Fig. 4.10), it is showed that the elongated linear type of fruit occurred in Group I, II and one clade of Group III (clade H) which include most of Southeast Asian species, Himalayan

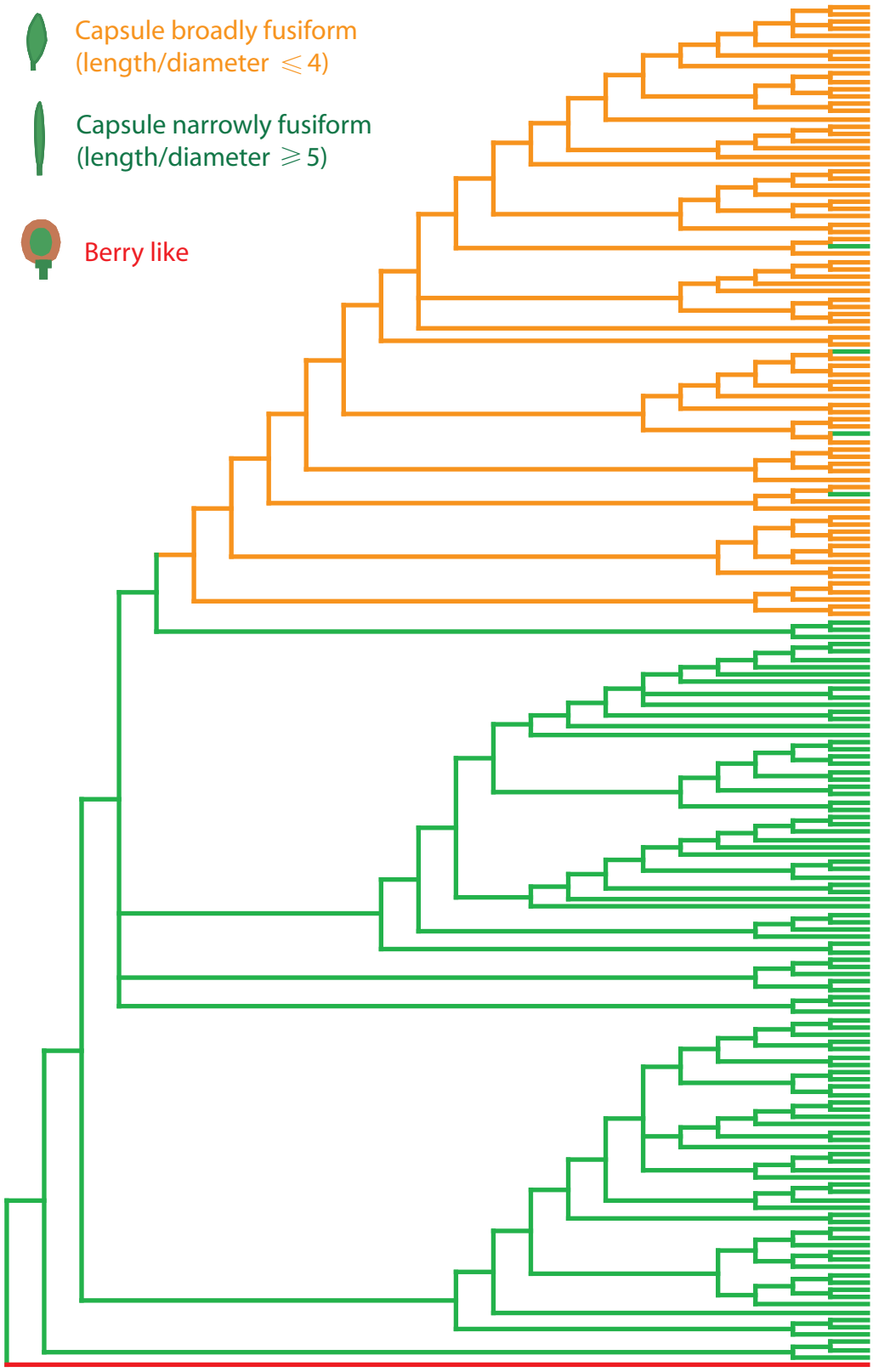



Fig. 4.10 Type of fruits in Balsaminaceae: Red-berry like; Green-capsule narrowly fusiform; Orange-capsule broadly fusiform

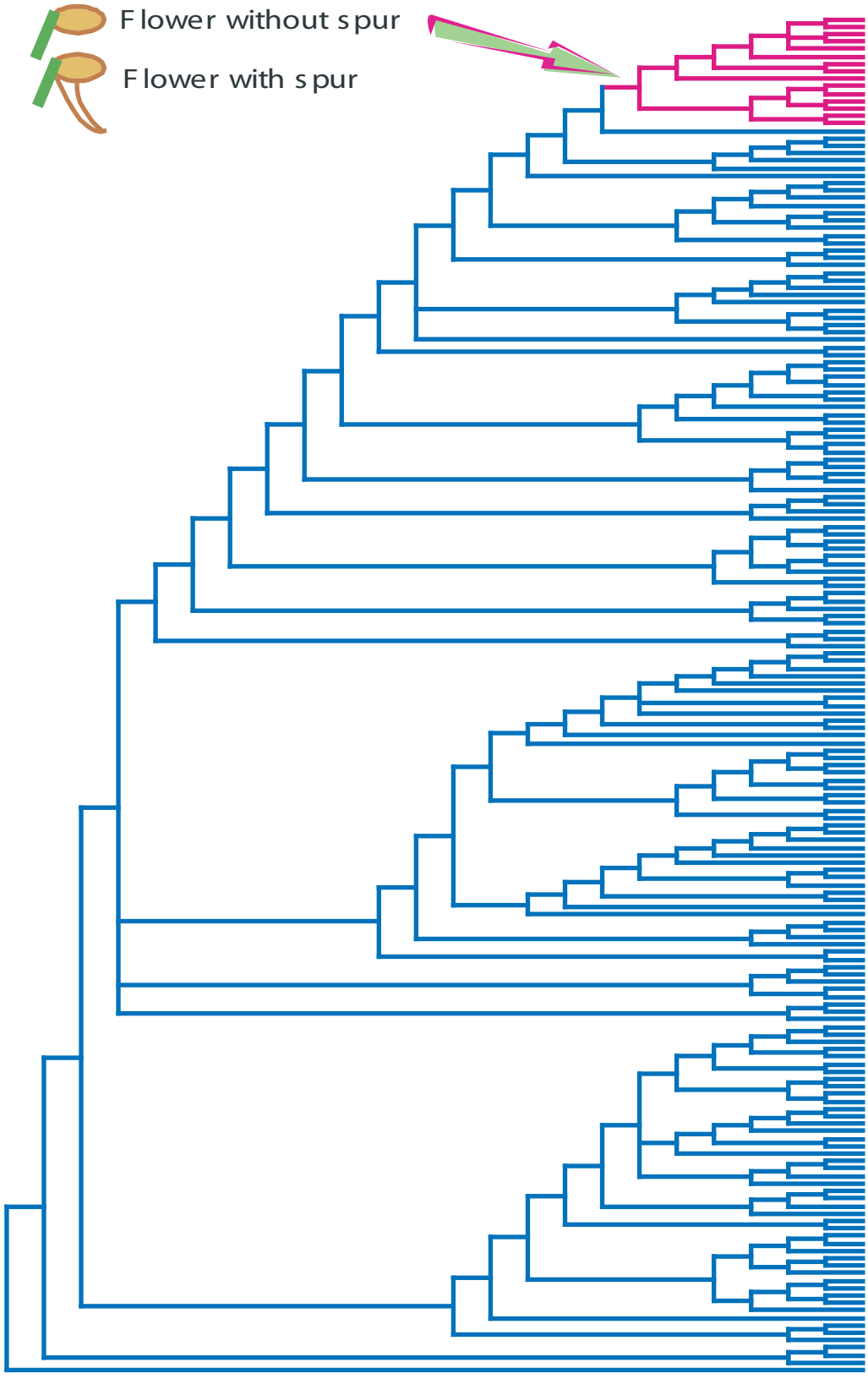



Fig. 4.11 The presence of spur in Balsaminaceae: Blue-flower with spur; Pink-flower without spur (Trimorphopetalum group)

species, East African species and species from North temperate regions and appear in the basal position of phylogeny. Wherears the broadly fusiform type of fruit occurred only in Group III which includes Madagascan species, African species, southern Indian and Sri Lanka species and some Southeast Asian species that appear in the advanced position of phylogeny. There are several reversals from narrowly fusiform to broadly fusiform but the present results still suggested that narrowly fusiform fruit is plesiomorphic and the broadly fusiform fruit are derived from the linear type. Considering the distribution area related with the fruits shape, the present results roughly support Grey-Wilson's (1980b) division about North group with long-linear fruits and South group with broadly fusiform fruits. He mentioned that there are overlaps between these two groups, in the molecular tree, it is seen that southwest Chinese *Impatiens* has both of the two type fruits.

#### 4.4.6.2 The presence of spur

**In Balsaminaceae, the floral evolution is from spurred flower to spurless flower.** *Hydrocera* and most of *Impatiens* have one lower sepal which developed into a nectary-tipped spur. But there are some species endemic to Madagascar that have no spur at all and they were called '*Trimorphopetalum*' group (Perrier de la Bathie, 1934). This group takes almost half of the species of the Madagascar *Impatiens* and showed a big diversification. On the present molecular phylogeny, 15 spurless species were grouped together (clade U, V) with strong support (BPP: 1.0), and stay in the most advanced position of phylogeny. It suggested this is the derived character in *Impatiens* flower. The evolutionary trend is from spurred flower to spurless flower (Fig. 4.11).

In *Aquilegia* and its relatives, the comparison of spurred flower and nonspurred flower was done and it revealed at what developmental stage the morphology of spurred versus nonspurred taxa diverge (Hoddges, 1997). Such studies are important to identify the developmental stage where gene action may promote spur production and how the timing of spur development may constrain or promote its evolution in Angiosperms. The future study about floral ontogeny of *Impatiens* could ascertain the origin and development of spurless *Impatiens.*

#### 4.4.6.3 Number of lateral sepals

**In Balsaminaceae, 4 lateral sepals is a plesiomorphic state, and 2 lateral sepals is derived by reduction.** *Hydrocera* has 1 lower sepal and 4 free lateral sepals. Most *Impatiens* have 1 lower sepal and 2 lateral sepals, but some *Impatiens* have 1 lower sepal and 4 lateral sepals which form two lateral pairs in the flowers that exists in different diversity centers. Grey-Wilson (1980b) suggested an important trend of floral evolution in *Impatiens* is the loss of upper pair of lateral sepals resulting the evolution of a flower type from 5 sepals to 3 sepals. Though species possessing 4 lateral sepals have a very small number, but still present a plesiomorphic state in *Impatiens.* Among 182 Balsaminaceae species used for the present molecular study, 19 species have 4 lateral sepals in mature flowers, 12 of them are South Asian species (clade A ,B, C, D, F), 2 of them are Himalayan species (clade D) and 5 of them are East African species (clade E). After tracing this character of 4 lateral sepals onto the phylogeny, it is confirmed that five sepals is plesiomorphic in *Impatiens*, the evolutionary trend is from 4 lateral sepals flower to 2 lateral sepals flower. This important floral morphology character is parallel evolution from different distribution centre (Fig. 4.12).

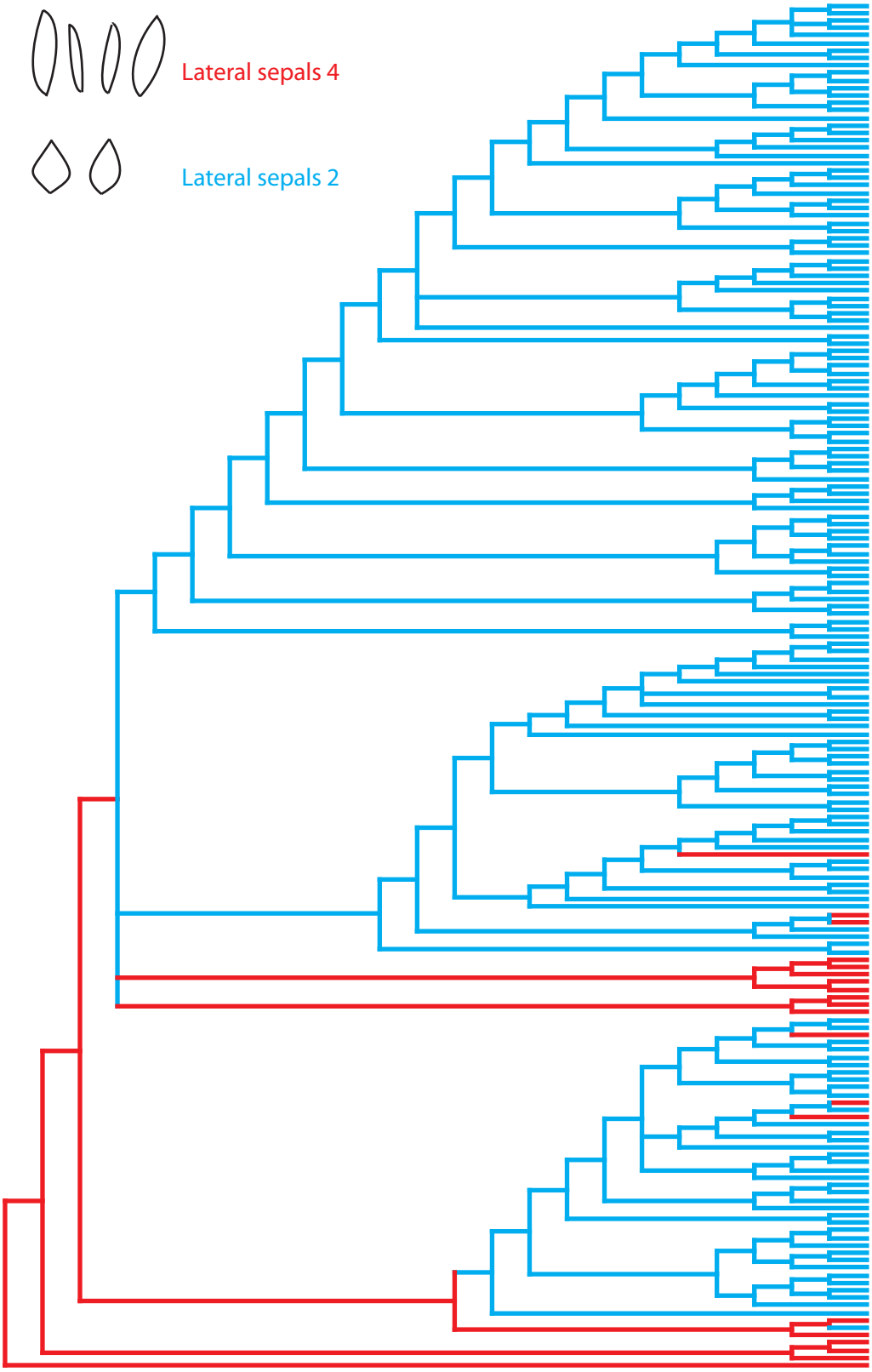



Fig. 4.12 The numbers of lateral sepals in Balsaminaceae: Red-species with 4 lateral sepals: Blue-species with 2 lateral sepals

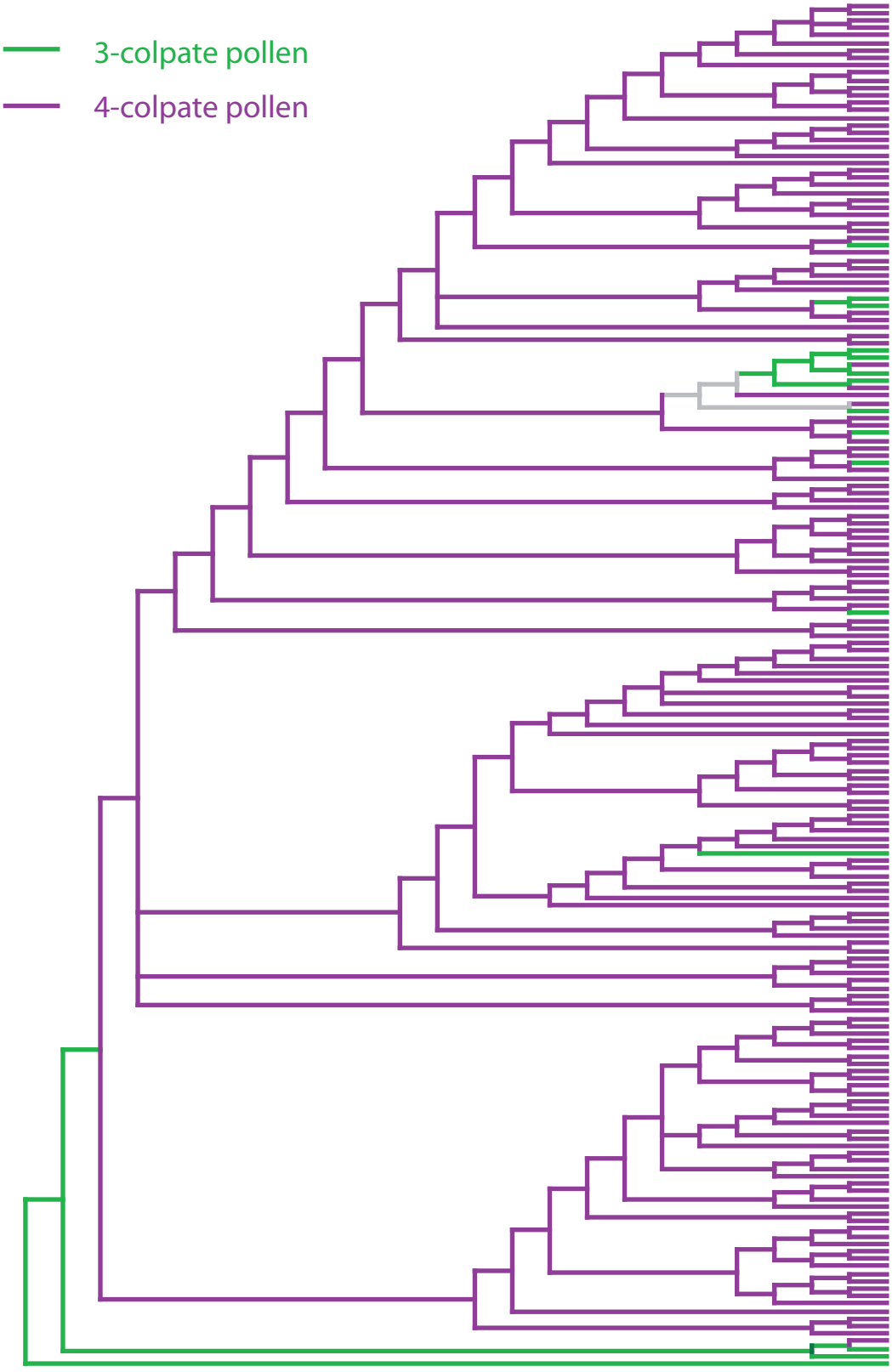



Fig. 4.13 The type of pollen in Balsaminaceae: Green-species with 3-colpate pollen; Violet-species with 4-colpate pollen

#### 4.4.6.4 Pollen type

**In Balsaminaceae, 3-colpate pollen is the ancestral type, 4-colpate pollen is derived.** *Hydrocera triflora* was found to have tricolpate pollen grains with a triangular, oblate shape and elongated colpi and was thought to be plesiomorphic pollen type in Balsaminaceae (Lu 1991; Janssens et al., 2005). Lu (1991) reported *I. omeiana* and *I. wilsoni* from Mount Omei in southwest China and confirmed *I. clavigera* (southeast Chinese species) have the same pollen type like *Hydrocera triflora*. She suggested that these Chinese species with 3-colpate pollen are very close to *Hydrocera triflora*. The present molecular phylogeny showed that *I. omeiana* and *I. clavigera* were grouped together and resolved as sister to the remaining species of *Impatiens*, they exist just between *Hydrocera* and other *Impatiens.* This confirmed the hypothesis of Lu (1991).

The most common pollen type of *Impatiens* is 4-colpate type, but few 3-colpate type were reported in African, Indian, Southeast Asian species as well (Huynh, 1968; Lu 1991; Perveen et al., 2001; Jassens et al., 2005). From all the available pollen data of Balsaminaceae, there is information about 114 species (among 184 species used in present study) on pollen colpate type. Tracing this character on the molecular phylogeny (Fig. 4.13), it is concluded that the evolution trend is from triangular 3-colpate pollen to rectangular 4-colpate pollen in the lineage of *Impatiens*, but there are reverses in several lineages. This confirmed that 3-colpate pollen grains with a triangular, oblate shape and elongated colpi are the ancestral pollen type in Balsaminaceae.

#### 4.4.6.5 Seedcoat micromorphology

**In *Impatiens*, reticulate seedcoat is an ancestral primitive type, squamalate and cristate seedcoat is advanced type.** Among the present seedcoat micromorphology observations from 38 *Impatiens* species, together with previous reports on seedcoat micromorphology of *Impatiens* (Shimizu, 1979; Lu & Chen, 1991; Utami & Shimizu, 2005), the seedcoat information of the species used in the present study was chosen and to be matched into the following four types distinguished here: laevitgate, granulate, reticulate, protrusive. Tracing these characters with the molecular phylogeny (Fig. 4.14), it showed that species with reticulate type are more common in *Impatiens* and they occurred relatively basal position on the molecular phylogenetic tree. This suggested that the reticulate type is comparatively ancestral primitive type. The species with complex sculptures, such as squamalate and cristate, occurred in the more apical branches of the phylogenetic tree and they might represent more advanced type. There are reversals: *I. davidi, I. cyathiflora, I. drepanophora, I. bahanensis, I. ruiliensis, I. siculifer* which have protrusive seedcoat appearing the comparatively basal position on the phylogenetic tree. Two Thailand species *I. kerriae* and *I. psittacina* having granulate seedcoat (Utami & Shimizu, 2005), appeared the very basal position of the phylogeny tree (clade B). From the molecular phylogeny, it is indicated that the evolutionary trend is from reticulate type to protrusive type. But the information about the seedcoat micromorphology of this family is still insufficient (only 67 of 182 species known on the present molecular tree), more species need to be studied to confirm this conclusion.

#### 4.4.6.6 Floral morphology evolution

**In *Impatiens*, the evolution of floral morphology is highly parallel and convergent mediated by the pollinators.** The extremely high floral diversity of *Impatiens* makes it a good candidate to study the floral evolution. Its diversity is mainly embodied in the diversity

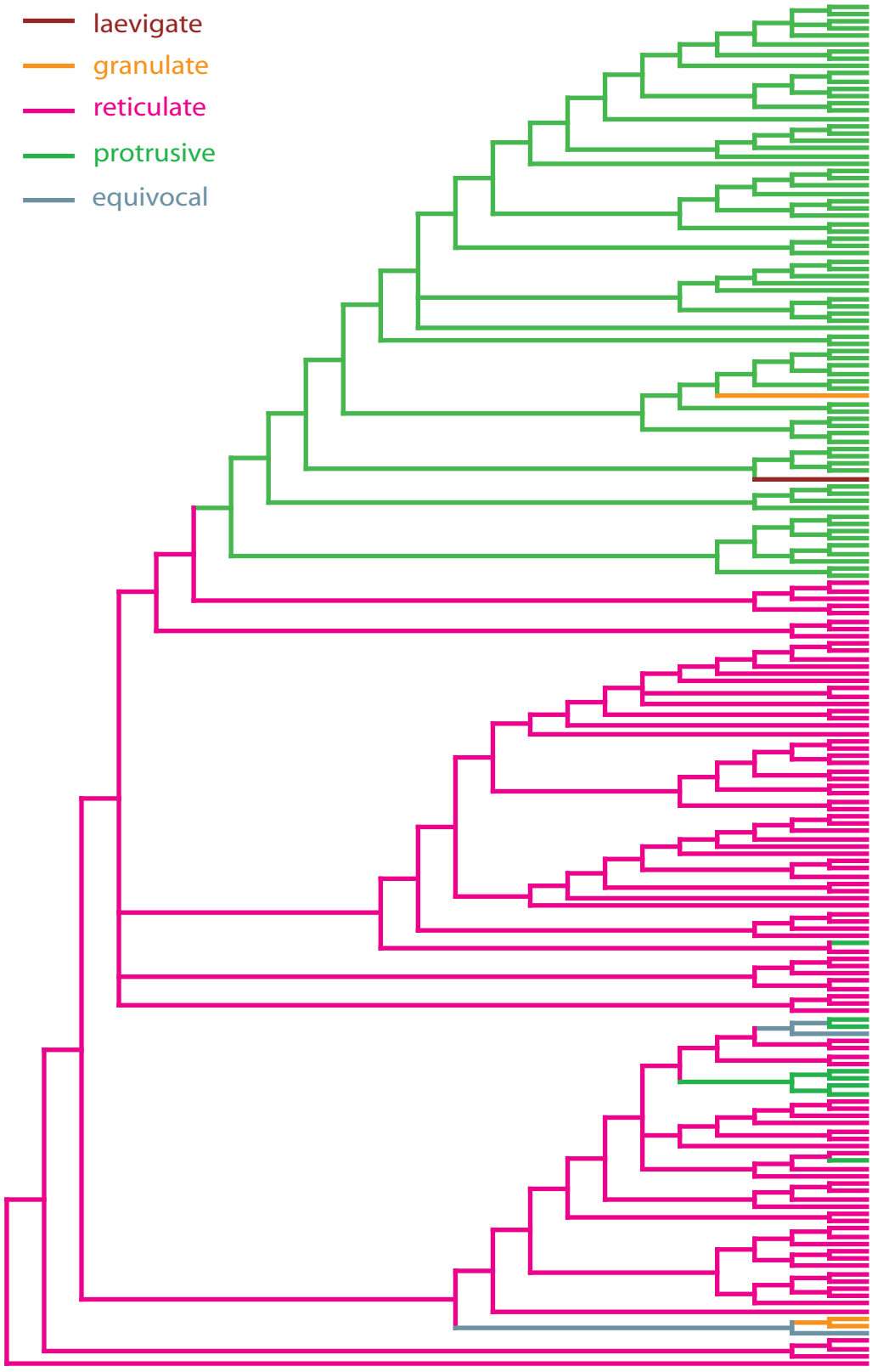



Fig. 4.14 Type of seedcoat micromorphology in Balsaminaceae: Brown-Laevigate type; Orange-Granulate type; Pink-Reticulate type; Green-Protrusive type.

of the shape and size of the spur and the two united lateral petals, but the correlations among the different floral parts have remained unclear. Lower sepals, lateral petals, dorsal petals were divided into different types (Table 4.3) and these characters were integrated with the present molecular phylogeny (Fig. 4.9). It is found that there is no regular correlation between floral characters and the phylogeny of the species in the general sense. For example, *I. lyallii* Baker (Madagarcar speices, in clade S), *I. sodenii* (African species, in clade P), *I. aureliana* (southwest Chinese species, in clade N), *I. javensis* (Indonesian species, in clade L), and *I. flacida* Arn. (south Indian species, in clade M), all have similar flower morphology with long filiform spur, almost separate lateral petals and flat dorsal petal, but they come from different regions and belong to different clades in the molecular tree (Fig. 4.8). Similarly, *I. auricoma* (Madagascan species, in clade T), *I. niamniamensis* Gilg (African species, in calde Q), *I. parasitica* Bedd. (south Indian species, in clade I), *I. conchibracteata* Y.L. Chen & Y.Q. Lu (southwest Chinese species, in clade H), *I. ecalcarata* Collett & Hemsl. (North American species, in clade G), all have similar flower morphology with a saccate spur, but they come from different region and nested in different clades in the molecular tree (Fig. 4.8).

However, in some small aggregates of species, different floral characters are associated with localized endemism. In Madagascan group (clade S-V) and African group (clade O-R), it showed that the trend is from filiform spur to saccate spur or no spur. This confirms the hypothesis of Grey-Wilson (1980b) about the evolutionary processes of the lower sepals within African species.

The rich variation in the floral structure is linked to the pollinators of the species. Grey-Wilson (1980b) thought, among African species, the ‘flat type’ flower (a shallow lower sepal with a long filiform spur and flat dorsal petal) usually have pollinator like butterflies or moths, and the ‘funnel type’ flower (a deeply navicular or saccate lower sepal and a hooklike dorsal petal) usually have pollinators like bees or bumble-bees. In the observations of Yuan and Boehler (personal communication) in *Impatiens*, bees, butterflies, hawkmoth and moth were all seen as pollinators. Though, a few pollinators were observed, it is still suggested, in Balsaminaceae, floral parts may well function in an integrated manner and pollinators mediated the evolution of the flower. Therefore, there must be conspicuous parallel and convergent evolution in floral morphology, i.e. floral morphology is not always a reliable indication of phylogenetic relationships.

Innovation in floral form has been proposed as one of the primary mechanisms of diversification in angiosperms (Barrett et al., 1996). This phenomenon has been most intensively studied in group such as the Orchidaceae (Johnson et al., 1998; Aceto et al., 1999) and the Polemoniaceae (Grant & Grant, 1965). In the study of phylogeny and evolution of *Orchis* and allied genera based on ITS DNA variation (Aceto et al., 1999), the results indicate that floral morphology is highly flexible and current generic and infrageneric limits are artificial. Even if some floral characters closely correspond to the molecular data, most are highly homoplastic and thus unsuitable for phylogenetic reconstruction. Similar with *Impatiens*, various traits pertaining to floral morphology may be interpreted as a result of ecological convergence related to pollinator-mediated selection; such characters can undergo drastic modifications without correspondingly dramatic genetic changes. The study on floral characters, phylogeny and pollinator shifts of the twin spurred orchid genus *Satyrium*, represents another example, which showed the floral characters reflected pollinator class more than the phylogeny of the orchid species (Van der Niet, 2006).

# Chapter 5 General conclusion

In the present study, three DNA markers ITS, trnLF, atpB-rbcL spacer were chosen to reconstruct the the phylogeny of the family using these sequences. The phylogenetic relationship between the two general *Hydrocera* and *Impatiens* and the infragenetic relationship within the genus *Impatiens* were revealed. The origin place of the family, the dispersal route was discussed based on the molecular phylogeny. Floral morphology and chosen vegetative morphology were traced on the molecular phylogeny tree to evaluate their evolutionary trends. Chromosomal variation and evolution were investigated and the chromosomal number was traced on the molecular phylogeny tree to discuss the possible basal chromosomal number. Seedcoat micromorphology was studied to reveal their high diversity and phylogenetic and taxonomic implications.

1) The molecular phylogeny study using ITS, trnLF, atpB-rbcL spacer confirmed the monophyly of Balsaminaceae and *Impatiens*. *Impatiens* is sister to *Hydrocera*. The phylogenetic relationship in *Impatiens* is associated with geographic distribution, and less defined by gross morphology.

2) Biogeographical optimization onto the molecular phylogeny reveals that extant *Impatiens* species originated in the nainland Southeast Asia, from where dispersals to boreal Eurasia and North America, to central Asia and Europe via Himalayas, to Southeast Asian islands, and to India and Africa have occurred. Madagascan species show a clear African origin.

3) By tracing several chosen morphological characters onto the molecular phylogeny, it is concluded that, the evolution trend in *Impatiens* is from narrowly fusiform fruits to broadly fruits, from spurred flower to spurless flower, from 4 lateral sepals to 2 lateral sepals, from 3-colpate pollen to 4-colpate pollen. Integrating the character of lower sepals, lateral petals and dorsal petals with the molecular phylogeny, it is found that there is no regular relation between floral character and their phylogeny in the general sense. In *Impatiens*, floral evolution is parallel and convergence, mediated with their pollinator.

4) The chromosome counting of 45 *Impatiens* species, together with the previous counting for 157 species, showed that there is a wide range of chromosome number: 2n=6-66 and 2n= 14, 16, 18, 20 are the most frequent numbers. The bimodal karyotype was typically observed in the species with n=9 (2n=18) or n=27. Geographic distributions of the most frequent basic numbers show interesting patterns: x=7 and 8 in Africa, x=7, 8, 10 in southern India and Sri Lanka, x=7, 9, 10 in the Himalayas, x=7, 8, 9, 10 in Southeast Asia, and x=10 in Northern Asia, Europe and North America. Tracing this character with molecular phylogeny, it is concluded that x=8 or 10 are the most likely ancestral base numbers in *Impatiens*. More species are expected to be studied.

5) The observations of Seedcoat micromorpholgy in *Impatiens* revealed its high diversity. Considering all the seedcoat micromorphology information in *Impatiens*, it is concluded that the type and subtype of seedcoat can be diagnostic and indicative of phylogenetic relationships, but seedcoat micromorphology is not always concordant with morphology. Tracing this character with molecular phylogeny, it is concluded that reticulate seedcoat is ancestral primitive type and squamalate and cristate seedcoat is advanced type. More species need to be studied to confirm this conclusion.

# Chapter 6 Perspectives

Floral ontogeny studies of Balsaminaceae are very attractive for future studies because this will contribute insights into a better understanding of the relationships among the different flower types from a developmental point of view. It will not only enrich the existing morphological data but also further reinforce the phylogenetic inferences on the floral evolution. Floral ontogeny has been scarcely investigated in this family. Only Caris et al. (2006) have recently studied floral development in two African *Impatiens* species: *I. niamniamensis* and *I. columbaria* J.J.Bos and one New Guinea *Impatiens* species: *I. hawkeri* W.Bull, ex Gard. They proposed the hypotheses based on their observations and the literature (Grey-Wilson, 1980b; Ramadevi & Narayana, 1989): in the course of evolution, the anterolateral sepals become reduced and that a morphocline can be recognized from species with five sepals, over species with rudimentary sepals that fuse postgenitally with the anterior petal, to species where congenital fusion between these sepals and the anterior petal has taken place.

Their study shed important light on understanding of floral evlution, however, *Impatiens columbaria* is not the species that has a true pentamerous calyx character. True free petals species *Hydrocera triflora* should be studied to see the difference of the development of the lateral petals compare with the other united lateral petals in *Impatiens*. The three species they observed are all quite advanced floral type (proved by the present study), more species with diverse floral types are expected to be studied. Thus, more species of Balsaminaceae, especially using the species representing typical different floral structures and species from the basal branch of the phylogeny should be studied further.

*Hydrocera triflora*, possessing 5 free petals and 5 sepals, will be chosen to see the position and the developmental sequence of the different perianth members. The differences between these two genera from the developmental point of view will be revealed as well. *Impatiens nanchabawensis* (new species from Tibet, Raymond et al., 2005) and *I. arguta* (southwest Chinese species) will be chosen because they have the typical two pairs of lateral sepals which proved to be a plesiomorphic character in *Impatiens* (from the present study). The observation of these two species can understand the development link between species with 5 and 3 sepals. *Impatiens elatostemmoides* H.Perrier and *I. mandrakae* Eb.Fisch. & Raheliv. will be chosen as the representative of the spurless species from Madagascar, to ascertain the origin and development of spurless *Impatiens*. In addition, more *Impatiens* species which from the basic branch of phylogeny: *I. siculifer* (southwest Chinese species), yellow flower with a narrowly recurved long spur and *I. radiata* (southwest Chinese species), small white flower with navicular, narrowed straight spur will be chosen for a further study on the development of different type of spurs in *Impatiens*.

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# References

**Abdel K. K. and Van der Maesen L. J. G**. 2002. Seed morphology of some tribes of Brassicaceae (implications for taxonomy and species identification for the flora of Egypt). *Blumea* 47: 363–383.

**Aceto S., Caputo P., Cozzolino S., Gaudio L. and Moretti A.** 1999. Phylogeny and evolution of *Orchis* and allied genera based on ITS DNA variation: morphological gaps and molecular continuity. *Mol. Phylogenet Evol.* 13: 67-76.

**Akaike H.** 1974. A new look at the statistical model identification. *IEEE Trans Autom. Contr*. 19: 716–723.

**Akiyama S. and Ohba H.** 2000. Inflorescences of the Himalayan species of *Impatiens* (Balsaminaceae). *J. Jap. Bot.* 75: 226-240.

**Akiyama S., Ohba H. and Wu S.-K.** 1996a. Cytotaxonomic comparison of *Impatiens* distributed in Nepal Himalaya and Yunnan. In: "*International Symposium on Floristic Characteristics and Diversity of East Asian Plants*", Abstracts p138. *Botanical Society of China*, Kunming.

**Akiyama S., Ohba H. and Wu S.-K.** 1996b. Further notes of *Impatiens* (Balsaminaceae) from Yunnan, China. *Bull. Nation. Sci. Mus*. B (Tokyo) 22: 135-144.

**Akiyama S., Ohba H. and Wakabayashi M.** 1993. Correlation between flower form and chromosome number in the Himalayan *Impatiens*. p. 254, in «XV International Botanical Congress», Abstracts P254. Tokyo.

**Akiyama S., Ohba H., Sugawara T., Yang Y.-P. and Murata J.** 1995. Notes on *Impatiens* (Balsaminaceae) from southwestern Yunnan, China. *J. Jap. Bot.* 70: 95-106.

**Akiyama S., Ohba H. and Suzuki M.** 1992a. Notes on east Himalayan species of *Impatiens*: 1. *Jap. Bot.* 67: 187-193.

**Akiyama S., Wakabayashi M. and Ohba H.** 1992b. Chromosome evolution in Himalayan *Impatiens (*Balsaminaceae*). Bot. J. Linn. Soc.*, 109: 247-257.

**Akiyama S., Ohba H. and Wakabayashi M.** 1991. Taxonomic notes of the East Himalayan species of *Impatiens*. Studies of Himalayan *Impatiens* (Balsaminaceae) (1). In Ohba H. and Malla S.B. (eds), *The Himalayan Plants*, vol. **2**: 67-94. University of Tokyo Press, Tokyo, Japan.

**Akiyama S.**, **Ohba H., Ikeda H., Tsukaya H., and Mohamed M.** 2005. A new species of *Impatiens* (Balsaminaceae) from Mt. Kinabalu, Borneo. *J. Jap. Bot.* 80: 266-270.

**Albach D. C., Soltis P. S., Soltis D. E. and Olmstead R. G.** 2001. Phylogenetic analysis of asterids based on sequences of four genes. *Ann. Missouri Bot. Gard.* 88: 163–212.

**Alfaro M. E., Zoller S. and Lutzoni F.** 2003. Bayes or bootstrap? A simulation study comparing the performance of Bayesian Markov Chain Monte Carlo sampling and bootstrapping in assessing phylogenetic confidence. *Mol. Biol. Evol.* 20: 255-266.

**Alvarez I. and Wendel J. F.** 2003. Ribosomal ITS sequences and plant phylogenetic inference. *Mol. Phylogenet. Evol.* 29: 417-434.

**Anderberg A. A., Rydin C. and Källersjö M.** 2002. Phylogenetic relationships in the order Ericales s.l.: analyses of molecular data from five genes from the plastid and mitochondrial genomes. *Am. J. Bot.* 89: 677–687.

**APG** 1998. An ordinal classification for the families of flowering plants. *Ann. Missouri Bot. Gard.* 85: 531–553.

**APG II.** 2003. An update of the angiosperm phylogeny group classification for the orders and families of flowering plants: APG II. *Bot. J. Linn. Soc.* 141: 399–436.

**Arisumi T.** 1977. Interspecific hybridization among certain Indonesian and Indian *Impatiens* spp. *Hortscience* 12: 410-410.

**Arnheim N., Krystal M., Schmickel R., Wilson G., Ryder O. and Zimmer E.** 1980. Molecular evidence for genetic exchanges among ribosomal genes on nonhomologous chromosomes in man and apes. *Proc. Natl. Acad. Sci. U.S.A.* 77: 7323-7327.

**Backlund M., Oxelman B. and Bremer B.** 2000. Phylogenetic relationships within the Gentianales based on ndhF and rbcL sequences, with particular reference to the Loganiaceae. *Am. J. Bot.* 87: 1029-1043.

**Baldauf S. L., Roger A. J., Wenk-Siefert I. and Doolittle W. F**. 2000. A kingdom-level phylogeny of eukaryotes based on combined protein data. *Science* 290: 972-977.

**Baldwin B. G.** 1992. Molecular phylogenetics of *Calycademia* (Compositae) based on ITS sequences of nuclear ribosomal DNA: chromosomal and morphological evolution reexamined. *Am. J. Bot.* 80: 222-238.

**Barker F. K. and Lutzoni F. M.** 2002. The utility of the incongruence length difference test. *Sys. Bio.* 51: 625-637.

**Barrett S. C., Harder L. D. and Worley A. C.** 1996. The comparative biology of pollination and mating in flowering plants. *Philosophical Transactions of the Royal Society of London,* B 351: 1271-1280.

**Beilstein M. A., Al-Shehbaz I. A. and Kellogg E. A.** 2006. Brassicaceae phylogeny and trichome evolution. *Am. J. Bot.* 93: 607-619.

**Bergsten J.** 2005. A review of long-branch attraction. *Cladistics* 21: 163-193.

**Bhaskar V.** 1976. In IOPB chromosome number reports LI. *Taxon* 25: 155-164.

**Bhaskar V.** 1975. Studies in Balsaminaceae. Vol. i. Phd-thesis. University of Mysore.

**Bhaskar V.** 1980. Chromosome drift in *Impatiens* L. (Balsaminaceae). *Cytologia* 45: 233-239.

**Bhaskar V.** 1981. The genus *Impatiens* L. in south India: endemism and affinities. *Indian Forester* 107: 368-376.

**Bhaskar V. and Razi B. A.** 1973**.** A new kind of exine sculpturing in *Impatiens* L. (Balsaminaceae) from south India. *Curr. Sci.* 42: 510-512.

**Bhaskar V. and Razi B. A.** 1979. Pollen morphology of South Indian *Impatiens* L. *Journ. Palyn. Lucknow*. 15: 12-23.

**Bhaskar V. and Razi B. A.** 1981. Peninsular Indian *Impatiens* L. (Balsaminaceae). *Bull. Bot. Surv. India*. 23: 191-196.

**Bhaskar V., Razi B. A. and Yoganarasimban S. N.** 1975. A ¨ Pollen variety¨ of *Impatiens* acaulis Arn. (Balsaminaceae). *Curr. Sci.* 44: 622-623.

**Bos J. J.** 19990. Novitates gabonenses 4. Another new species of *Impatiens* (Balsaminaceae) from Gabon. *Bull. Mus. Nation. Hist. Nat., B, Adansonia, Ser.* 4, 12(3–4): 239-242.

**Bremer B., Bremer K., Heidari N., Erixon P., Olmstead R. G., Anderberg A. A., Källersjö M. and Barkhordarian E.** 2002. Phylogenetics of asterids based on 3 coding and 3 non-coding chloroplast DNA markers and the utility of non-coding DNA at higher taxonomic levels. *Mol. Phylogenet. Evol.* 24: 274-301.

**Buckley T. R., Arensburger P., Simon C. and Chambers G. K.** 2002. Combined data, Bayesian phylogenetics, and the origin of the New Zealand cicada genera. *Syst. Biol.* 51: 4-18.

**Caris P. L., Geuten K. P., Janssens S. B. and Smets E. F.** 2006. Floral development in three species of *Impatiens* (Balsaminaceae). *Am. J. Bot.* 93: 1-14.

**Chase M. W. and Hills H. H.** 1991. Silica-gel: An ideal material for field preservation of leaf samples for DNA studies. *Taxon* 40: 215-220.

**Chatterjee D.** 1940. Studies on the endemic flora of India and Burma. *Jour. Roy. As. Sci.* Bengal, 5: 19-67.

**Cheek M. and Csiba L.** 2002. A new epiphytic species of *Impatiens* (Balsaminaceae) from western Cameroon. *Kew Bull.* 57: 669-674.

**Cheek M. and Fischer E**. 1999. A tuberous and epiphytic new species of *Impatiens* (Balsaminaceae) from Southwest Cameroon. *Kew Bull.* 54: 471-475.

**Chen Y.-L.** 1978. Notulae de genere *Impatiens* L. florae sinicae. *Act. Phytotax. Sin.* 16: 36-55.

**Chen Y.-L.** 1989. Four new species of *Impatiens* (Balsaminaceae) from China. *Acta Phytotax. Sin.* 27: 395-404.

**Chen Y.-L.** 1990. Six new species of *Impatiens* from SiChuan. *Acta Botanica Yunnanica* 12: 22-30.

**Chen Y.-L.** 1990. New plants from the HengDuan mountains. *Acta Phytotax. Sin.* 28: 483-490.

**Chen Y.-L.** 1999. Six new species of *Impatiens* L. from China. *Acta Phytotax. Sin.* 37: 88-99.

**Chen Y.-L.** 2000. Three new species of *Impatiens* L. from China. *Acta Phytotax. Sin.* 38: 557-562.

**Chen Y.-L.** 2001. Balsaminaceae, Flora Reipublicae Popularis Sinicae, *Tomus* 47 (2). Science Press, Beijing.

**Clark L. G., Zhang W. and Wendel J. F.** 1995. A phylogeny of the grass family (Poaceae) based on ndhF sequence data. *Syst. Bot.* 20: 436-460.

**Clausing G. and Renner S. S.** 2001. Molecular phylogenetics of Melastomataceae and Memecylaceae: implications for character evolution. *Am. J. Bot.* 88: 486-498.

**Clegg M. T., Gaut B. S., Learn G. H. and Morton B. R.** 1994. Rates and pattern of chloroplast DNA evolution. *Proc. Natl. Acad. Sci. U.S.A.* 91: 6795-6801.

**Corner E. J. H.** 1976. The seeds of Dicotyledons I. Cambridge University Press. London.

**Cowling R. M. and Holmes P. M.** 1992. Endemism and speciation in a lowland flora from the Cape floristic region. *Bot. J. Linn. Soc.* 47: 367-383.

**Crawford D. J. and Mort M. E.** 2005. Phylogeny of Eastern North American *Coreopsis* (Asteraceae-Coreopsideae): insights from nuclear and plastid sequences, and comments on character evolution**.** *Am. J. Bot.* 92: 330-336.

**Cronquist A.** 1981. An integrated system of classification of flowering plants. Columbia University Press, New York.

**Cunningham C. W.** 1997. Can three incongruence tests predict when data should be combined? *Mol. Biol. Evol.* 14, 733-740.

**Cunningham C. W., Omland K. E. and Oakley T. H.** 1998. Reconstructing ancestral character states: a critical reappraisal. *Trends Ecol. Evol.* 13: 361-366.

**Dahlgren G**. 1989. An updated angiosperm classification. *Bot. J. Linn. Soc*. 100: 197-203.

**Darlu P. and Lecointre G.** 2002. When does the incongruence length difference test fail? *Mol. Biol. Evol*.19:432-437.

**Dolphin K., Belshaw R., Orme C. D. L. and Quicke D. L. J.** 2000. Noise and incongruence: interpreting results of the incongruence length difference test. *Mol. Phylogenet. Evol.* 17, 401–406.

**Donoghue M. J. and Sanderson M. J.** 1992. The suitability of molecular and morphological evidence in reconstructing plant phylogeny. In: Soltis P S, Soltis D E, Doyle J J. (eds.). Molecular Systematics of Plants. London: Chapman and Hall. pp: 340-368.

**Downie S. R. and Palmer J. D.** 1992. Use of chloroplast DNA rearrangments in reconstructing plant phylogeny. In Molecular Systematics of Plants: 14-35. Soltis D. E. and Doyle J. J. Editors. Chapman and hall. New York.

**Dowton M. and Austin A. D.** 2002. Increased congruence does not necessarily indicate increased phylogenetic accuracy--the behavior of the incongruence length difference test in mixed-model analyses. *Syst. Biol.* 51: 19-31.

**Doyle J. J. and Doyle J. L.** 1987. A rapid DNA isolation method for small quantities of fresh tissues. *Phytochem. Bull.* 19: 11–15.

**Doyle J. J.** 1992. Gene trees and species trees – molecular systematics as one-character taxonomy. *Syst. Bot* 17: 144-163.

**Doyle J. J.** 1997. Trees within trees: genes and species, molecules and morphology. *Syst. Biol.* 46: 537-553.

**Doyle J. J. and Gaut B. S.** 2000. Evolution of genes and taxa: a primer. *Plant Mol. Biol.* 42: 1-23.

**Durdana Y. and Nair P. K. K.** 1988. Pollen morphology of Indian Geraniales. Vol. XI-XVI. New delhi.

**Erixon P., Svennblad B., Britton T. and Oxelman B.** 2003. Reliability of Bayesian posterior probabilities and bootstrap frequencies in phylogenetics. *Syst. Biol.* 52: 665-673.

**Farris J. S.** 1989. The retention index and the rescaled consistency index. *Cladistics* 5: 417-419.

**Farris J. S., Källersjö M., Kluge A. G. and Bult C.** 1995. Testing significance of incongruence. *Cladistics* 10: 315-319.

**Felsenstein J.** 1985**.** Confidence limits on phylogenied: an approach using the bootstrap. *Evolution* 39: 783-791.

**Fior S., Karis P. O., Casazza G., Minuto L. and Sala F.** 2006. Molecular phylogeny of the Caryophyllaceae (Caryophyllales) inferred from chloroplast *matK* and nuclear rDNA ITS sequences. *Am. J. Bot.* 93: 399-411.

**Fischer E. and Rahelivololona M. E.** 2004a. New taxa of *Impatiens* (Balsaminaceae) from Madagascar. III. *Adansonia* 26:37–52.

**Fischer E. and Rahelivololona M. E.** 2004b. A new epiphytic species of *Impatiens* (balsaminaceae) from the Comoro Islands. *Adansonia* 26: 93-95.

**Fischer E. and Rahelivololona M. E.** 2002. New taxa of *Impatiens* (Balsaminaceae) from Madagascar. I. *Adansonia* 24: 271–294.

**Fischer E.** 1997. Contributions to the flora of Central Africa V: Two new species of *Impatiens* (Balsaminaceae) from Eastern Zaire. *Bull. Jard. Bot. Nat. Belg.* 66**:** 63-71.

**Fischer E. Wohlhauser S. and Rahelivololona M. E.** 2003. New taxa of *Impatiens* (Balsaminaceae) from Madagascar. II. A collection from Masoala Peninsula. *Adansonia* 25: 17–31.

**Fujihashi H., Akiyama S. and Ohba H.** 2002. Origin and relationships of the Sino-Himalayan *Impatiens* (Balsaminaceae) based on molecular phylogenetic analysis, chromosome numbers and gross morphology. *J. Jap. Bot.* 77: 284-295.

**Geuten K., Smets E., Schols P., Yuan Y.-M., Janssens S., Küpfer P. and Pyck, N.** 2004. Conflicting phylogenies of balsaminoid families and the polytomy in Ericales: combining data in a Bayesian framework. *Mol. Phylogenet. Evol.* 31: 711–729.

**Golenberg E. M., Clegg M. T., Durbin M. L., Doebley J. and Ma D. P.** 1993. Evolution of a noncoding region of the chloroplast genome. *Mol. Phylogenet. Evol.* 2: 52-64.

**Goodman S. M. and Benstead J. P.** 2003. The Natural History of Madagascar. 402-409.The University of Chicago Press Chicago and London.

**Graham S. W. and Olmstead R. G.** 2000. Utility of 17 chloroplast genes for inferring the phylogeny of the basal angiosperms. *Am. J. Bot.* 87: 1712-1730.

**Grant V.** 1949. Pollination systems as isolating mechanisms in angiosperms. *Evolution* 3: 82-97.

**Grant V.** 1971. Plant speciation. New York: Columbia University Press.

**Grant V. and Grant K. A.** 1965. Flower pollination in the phlox family. Columbia University Press, New York.

**Grey-Wilson C.** 1980a. *Impatiens* in Papuasia, Studies in Balsaminaceae: I. *Kew Bull.* 34: 661–688.

**Grey-Wilson C.** 1980b. *Impatiens* of Africa. A. A. Balkema, Rotterdam.

**Grey-Wilson C**. 1980c. *Hydrocera triflora*, its floral morphology and relationships with *Impatiens*. Studies in Balsaminaceae: V. *Kew Bull.* 35: 213–219.

**Grey-Wilson C.** 1980d. Notes on collecting *Impatiens* (Balsaminaceae). *Fl. Males. Bull.* 33: 3435–3436.

**Grey-Wilson C.** 1980e. Hybridisation in African *Impatiens*. Studies in Balsaminaceae. *Kew Bull.* 34: 689-722.

**Grey-Wilson C.** 1981. Balsaminaceae: The handbook of the flora of Ceylon. I: 77-120.

**Grey-Wilson C.** 1989. *Semeiocardium* Zoll. Is it a good genus? Studies in Balsaminaceae: IX. *Kew Bull.* 44: 107-113.

**Grob G. B. J., Gravendeel B. and Eurlings M. C. M.** 2004. Potential phylogenetic utility of the nuclear FLORICAULA/LEAFY second intron: comparison with three chloroplast DNA regions in *Amorphophyllus* (Araceae). *Mol. Phylogenet. Evol.* 30: 12-23.

**Guo Y.-L. and Ge S**. 2005. Molecular phylogeny of Oryzeae (Poaceae) based on DNA sequences from chloroplast, mitochondrial, and nuclear genomes**.** *Am. J. Bot.* 92: 1548-1558.

**Hamby R. K and Zimmer E. A.** 1992. Ribosomal RNA as a phylogenetic tool in plant systematics. In：Soltis P. S, Soltis D E. and Doyle J. J. (eds.). Molecular Systematics of Plants. Chapman and Hall, New York, pp: 50-91.

**Hamilton M. B., Braverman J. M. and Soria-Hernanz D. F**. 2003. Patterns and Relative Rates of Nucleotide and Insertion/Deletion Evolution at Six Chloroplast Intergenic Regions in New World Species of the Lecythidaceae. *Mol. Biol. Evol.* 20: 1710-1721.

**Hamilton M. B.** 1999. Four primer pairs for the amplification of chloroplast intergenic regions with intraspecific variation. *Mol. Ecol.* 8: 521-523.

**Hara H. and Williams L. H. J.** 1979. An enumeration of the flowering plants of Nepal (Balsaminaceae). 2: 78-80.

**Harold A. S. and Mooi R. D.** 1994 Areas of endemism: definition and recognition criteria**.** *Syst. Biol.* 43: 261-266.

**Heinrich B.** 1979. Resource heterogeneity and patterns of movements in foraging bumblebees. *Oecologia* 40: 235-245.

**Hendy M. D. and Penny D.** 1989. A framework for the quantitative study of evolutionary trees. *Syst. Zool.* 38: 297-309.

**Hillis D. M., Moritz C., Porter A. and Baker R. J**. 1991. Evidence for biased gene conversion in concerted evolution of ribosomal DNA. *Science* 251: 308-310.

**Hoddge S. A. and Arnold M. L.** 1994. Columbines: a geographically widespread species flock. *Proc. Natl. Acad. Sci. U.S.A.* 91: 5129-5132.

**Hoddges S. A.** 1997. Floral nectar spurs and diversification. *Int. J. Plant Sci*. 158: 581-588.

**Holder M. and Lewis P. O.** 2003. Phylogeny estimation: traditional and Bayesian approaches. *Nat. Rev. genet.* 4: 275-284.

**Hong D. Y.** 1990. Plant Cytotaxonomy. p. 120, Science Press, Beijing.

**Hooker J. D.** 1875. *Impatiens* Linn. Flora of British India I: 440-483.

**Hooker J. D.** 1905. An epitome of the British Indian species of *Impatiens*. *Rec. Bot. Surv. India* 4:1-58.

**Hooker J. D.** 1908-1911. Les espèces du genre «*Impatiens*». Dans l'herbier du muséum de Paris.

**Hooker J. D. and Thomson T.** 1860. Praecursores and floram Indicam. Balsaminaceae. *J. Linn. Soc.* 4: 106-157.

**Hoot S. B., Culham A. and Crane P. R**. 1995. The utility of atpB gene sequences in resolving phylogenetic relationships: comparison with rbcL and 18S ribosomal DNA sequences in the Lardizabalaceae. *Ann. Missouri Bot. Gard.* 82: 194-207.

**Huang S.-H., Shui Y.-M. and Chen W.-H.** 2003. New taxa of *Impatiens* from Yunnan. *Acta Bot. Yunnan.* 25: 261–280.

**Huelsenbeck J. P.** 2002. Potential applications and pitfalls of Bayesian inference of phylogeny. *Syst. Bot.* 51: 673-688.

**Huelsenbeck J. P. and Bollback J. P.** 2001. Emperical and hierarchical Bayesian estimation of ancestral states. *Syst. Bot.* 50: 351-366.

**Huelsenbeck J. P. and Crandall K. A.** 1997. Phylogeny estimation and hypothesis testing using maximum likelihood. *Annu. Rev. Ecol.* 28: 437-466.

**Huelsenbeck J. P. and Hillis D. M.** 1993. Success of phylogenetic methods in the four-taxon case. *Syst. Biol.* 42: 247-264.

**Huelsenbeck J. P., Bull J. J. and Cunningham C. W.** 1996. Combining data in Phylogenetic analysis. *Tree* 11: 152-157.

**Huelsenbeck J. P., Larget B., Miller R. E. and Ronquist F.** 2002. Potential Applications and Pitfalls of Bayesian Inference of Phylogeny. *Syst. Biol.* 51: 673-688.

**Huelsenbeck J. P., Nielsen R. and Bollback J. P.** 2003. Stochastic mapping of morphological characters. *Syst. Biol.* 52: 131-158.

**Huelsenbeck J. P., Rannala B. and Masly J. P.** 2000. Accommodating phylogenetic uncertainty in evolutionary studies. *Science* 288: 2349-2350.

**Huelsenbeck J. P., Ronquist F., Nielsen R. and Bollback J. P.** 2001. Bayesian inference of phylogeny and its impact on evolutionary biology. *Science* 294: 2310-2314.

**Huynh K.-L.** 1968. Morphologie du pollen des Tropaeolacées et des Balsaminacées I and II. *Grana Palynologica* 8: 88-184 and 277-516.

**Janssens S., Geuten K., Yuan Y.-M., Song Y., Küpfer P. and Smets E.** 2006. Phylogenetics of *Impatiens* and *Hydrocera* (Balsaminaceae) using chloroplast atpB-rbcL spacer sequences. *Syst. Bot.* 31: 171-180.

**Janssens S., Lens F., Dressler S., Geuten K., Smets E. and Vinckier S.** 2005. Phynological variation in balsaminoid Ericales. II. Balsaminaceae, Tetrameristaceae, Pellicieraceae and general conclusions. *Ann. Bot.* 96: 1061-1073.

**Johnson L. A. and Soltis D. E.** 1994. matK DNA sequence and phylogenetic reconstruction in Saxifragaceae s.str. *Syst. Bot.* 19: 143-156.

**Johnson S. D. and Steiner K. E.** 1997. Long-tongued fly pollination and evolution of floral spur length in the *Disa draconis* complex (Orchidaceae). *Evolution* 51: 45-53.

**Johnson S. D., Linder P. and Steiner K. E.** 1998. Phylogeny and radiation of pollination systems in *Disa* (Orchidaceae). *Am. J. Bot.* 85: 402-411.

**Jones K. and Smith J. B.** 1966. The cytogeography of *Impatiens* L. (Balsaminaceae). *Kew Bull*. 20: 63-72.

**Kathriarachchi H., Hoffmann P., Samuel R. Wurdack K. J. and Chase M. W.** 2005. Molecular phylogenetics of Phyllanthaceae inferred from five genes (plastid atpB, matK, 3'ndhF, rbcL, and nuclear PHYC). *Mol. Phylogenet. Evol.* 36: 112-134.

**Kato M., Itino T., Hotta M., Abbas I. and Okada H.** 1989. Flower visitors of 32 plant species in West Sumatra. Occasional Papers of the Kagoshima University Research Centre for the South Pacific No. 16: 15-31.

**Kato M, Itino T, Hotta M,Inoue T.** 1991. Pollination of four Sumatran *Impatiens* species by hawkmoths and bees. *Tropics* 1: 59-73.

**Khoshoo T. N.** 1955a. Cytology of *Impatiens*. *Curr. Sci.*, 24: 423-424.

**Khoshoo T. N.** 1955b. Biosystematies of the *Sisymbrium irio* complex. *Nature (London)* 176: 608.

**Khoshoo T. N.** 1956. Chromosomes from herbarium sheets of *Impatiens*. *Stain Technol* 31: 31-33.

**Khoshoo T. N.** 1957. Cytology of some *Impatiens* species. *Caryologia* 10: 55-74.

**Khoshoo T. N.** 1966. Cytology of pollen with particular reference to *Impatiens* and Allieae. *Proc. Indian Acad. Sci., Seat.* B, 63: 35-45.

**Kiester A. R., Lande R., and Schemske D. W.** 1984. Models of coevolution and speciation in plants and their pollinators. *Am. Nat.* 124: 220-243.

**Kim J., Rohlf J. and Sokal R.** 1993. The accuracy of phylogenetic estimation using the Neighbour-Joining method. *Evolution* 47: 471-486.

**Kim K.-J. and Jansen R. K. 1995.** ndhF Sequence Evolution and the Major Clades in the Sunflower Family. *Proc. Natl. Acad. Sci. U.S.A.* 92: 10379-10383.

**Kimura M.** 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.* 16: 111-120.

**Kishino H. and Hasegawa M.** 1989. Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order in the Hominoidea. *J. Mol Evol* 29: 170-179.

**Kluge A. G. and Farris J. S**. 1969. Quantitative phyletics and the evolution of anurans. *Syst. Zool.*18: 1-32.

**Kress W. J., Prince L. M. and Williansm K. J.** 2002. The phylogeny and a new classification of the gingers (Zingiberaceae): evidence from molecular data. *Am. J. Bot.* 89: 1682-1696.

**Kron K. A., Judd W. S., Stevens P. F., Crayn D. M., Anderberg A. A., Gadek P. A., Quinn C. J. and Luteyn J. L.** Phylogenetic classification of Ericaceae: Molecular and morphological evidence. *Bot. Rev.* 68: 335-423.

**Kumar P. and Singh D.** 1990. Develoment and structure of seed coat in *Hibiscus*. *Phytomorph*. 40: 179-188.

**Larsen K.** 1981. Chromosome numbers in *Impatiens* from Thailand. *Nordic J. Bot.* 1: 43-44.

**Levan A., Fredga K. and Sandberg A.A.** 1964. Nomenclature for centrimeric position on chromosomes. *Hereditas* 52: 201-220.

**Linder H. P. and Hardy C. R.** 2004. Evolution of the species-rich Cape flora. *Philosophical Transactions of the Royal Society of London, B: Biol. Sci.* 359: 1623-1632.

**Lu Y.-Q.** 1991. Pollen morphology of *Impatiens L.* (Balsaminaceae) an dits taxonomic implications. *Acta Phytotaxonomica Sinica* 29: 352-357.

**Lu Y.-Q. and Chen Y-L.** 1991. Seed morphology of *Impatiens* L. (Balsaminaceae) and its taxonomic significance. *Acta Phytotaxonomica Sinica* **29:** 252–257.

**Macior L. W.** 1971. Co-evolution of plants and animals – systematic insights from plantinsect interactions. *Taxon* 20: 17-28.

**Maddison D. R. and Maddison W. P.** 2000. MacClade 4: analysis of phylogeny and character evolution, version 4.0. Sinauer Associates, Sunderland, Massachusetts, USA.

**Manen J. F., Natali A. and Ehrendorfer F.** 1994. Phylogeny of Rubiaceae-Rubieae inferred from the sequence of a cpDNA intergene region. *Plant Syst. Evol.* 190: 195-211.

**Martinez-Ortega M. M. and Rico E.** 2001. Seed morphology and its systematic significance in some *Veronica* species (Scrophulariaceae) mainly from the Western Mediterranean. *Plant Syst. Evol.* 228: 15–32.

**Mast A. R. and Givnish T.** 2002. Historical biogeography and the origin of stomatal distributions in *Banksia* and *Dryandra* (Proteaceae) based on their cpDNA phylogeny. *Am. J. Bot.* 89:1311-1323.

**Mau B., Newton M. A. and Larget B.** 1999. Bayesian phylogenetic inference via Markov chain Monte Carlo methods. *Biometric* 55: 1-12.

**Merlin C. M. and Grant W. F.** 1986. Hybridization studies in the genus *Impatiens*. *Canad. J. Bot*. 64: 1069-1074.

**Miyamoto M. M. and Fitch M. W.** 1995. Testing species phylogenies and phlogenetic methods with congruence. *Syst. Bot.* 44: 64 -76.

**Moncalvo J. M., Vilgalys R. Redhead S. A. et al. (14 co-authors).** 2002. One hundred and seventeen clades of euagarics. *Mol. Phylogenet. Evol.* 23: 357-400.

**Morrone J. J.** 1994. On the identification of areas of endemism. *Syst. Biol.* 43: 438-441.

**Morton C. M., Chase M.W., Kron K.A. and Swensen S. M.** 1996. A molecular evaluation of the monophyly of the order Ebenales based upon rbcL sequence data. *Syst. Bot*. 21: 567-586.

**Munoz-Centeno L. M., Albach D. C., Sanchez-Agudo J. A. and Martinez-ortege M. M.** 2006. Systematic significance of seed morphology in *Veronica* (Plantaginaceae): a phylogenetic perspective. *Ann. Bot.* 98: 335-350.

**Nelson G. and Platnick N. I.** 1981. Systematics and biogeography: cladistics and vicariance: Columbia University Press. New York

**Nepokroeff M., Sytsma K. J., Wagner W. L. and Zimmer E. A.** 2003. Reconstructing ancestral patterns of colonization and dispersal in the Hawaiian understory tree genus *Psychotria* (Rubiaceae): a comparison of parsimony and likelihood approaches. *Syst. Bot.* 52: 820-838.

**Nilsson L. A.** 1988. The evolution of flowers with deep corolla tubes. *Nature* 334: 147-149.

**Nishfikaw T., Salomon B., Komatsuda T., Bothmer von R. and Kadowaki K**. 2002. Molecular phylogeny of the genus *Hordeum* using three chloroplast DNAS sequences. *Genome* 45: 1157-1166.

**Nylander J. A. A.** 2004. MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.

**Nylander J., Ronquist F., Huelsenbeck J. and Nieves-aldrey J.** 2004. Bayesian Phylogenetic Analysis of combined data. *Syst. Biol.* 53: 47-67.

**Olmstead R. G. and Palmer J. D.** 1994. Chloroplast DNA systematics: a review of methods and data analysis. *Am. J. Bot.* 81: 1205-1224.

**Olmstead R. G. and Reeves P. A.** 1995. Evidence for the polyphyly of the Scrophulariaceae based on chloroplast rbcL and ndhF sequences. *Ann. Missouri Bot. Gard.* 82: 176-193.

**Olmstead R. G. and Sweere J. A.** 1994. Combining data in phylogenetic systematics: an empirical approach using three molecular data sets in the Solanaceae. *Syst. Biol.* 43: 467-481.

**Palmer J. D.** 1995**.** Rubisco rules fall; gene transfer triumphs. *Bioessays* 17: 1005-1008.

**Perrier de la Bathie H.** 1934. Les *Impatiens* de Madagascar. *Arch. Bot. Mem.* 7: 1-124.

**Perrier de la Bathie H.** 1949. Révision des *Impatiens* de Madagascar et des Comores. *Mem. Acad. Sci.* 67: 1—16.

**Perveen A. and Qaiser M.** 2001. Pollen flora of Pakistan-XXVI. Balsamincaceae. *Turk J. Bot*. 25: 35-38.

**Peterson K. J. and Eernisse D. J.** 2001. Animal phylogeny and the ancestry of bilaterians: inferences from morphology and 18S rDNA gene sequences. *Evol. Dev.* 3: 170-205.

**Pfosser M., Wetschnig W., Ungar S. and Prenner G.** 2003. Phylogenetic relationships among genera of *Massonieae* (Hyacinthaceae) inferred from plastid DNA and seed morphology. *J. Plant Res.* 116: 115–132.

**Pijl L. van der and Dodson C. H.** 1966. Orchid flowers: their pollination and evolution. Coral Gables, Fairchild Tropical Garden and Miami U. P.

**Posada D. and Crandall K. A.** 1988. Modeltest: testing the model of DNA substitution. *Bioinformatics* 14: 817-818.

**Qiu Y. L., Lee J. H., Bernasconi-Quadroni F., Soltis D. E., Soltis P. S., Zanis M., Zimmer E. A., Chen Z. D., Savolainen V. and Chase M. W.** 1999. The eairlest angiosperms: endivence from mitochondrial, plastid and nuclear genomes. *Nature* 402: 404-407.

**Quint M. and Classen-Bockhoff R.** 2006. Phylogeny of Bruniaceae Based on *mat*K and ITS Sequence Data. *Int. J. Plant Sci*. 167 : 135-146.

**Rao R. V. S., Ayyangar K. R. and Sampathkumar R.** 1986. On the karyological characteristics of some members of Balsaminaceae. *Cytologia* 51: 251--260.

**Rannala B. and Yang Z.-H.** 1996. Probability distribution of molecular evolutionary trees: A new method of phylogenetic inference. *J. Mol. Evol.* 43: 304-311.

**Raven P. H. and Axelrod D. I.** 1974. Angiosperm biogeography and past continental movements. *Ann. Missouri Bot. Gard.* 61: 539-673.

**Raxworthy C. R., Forstner M. R. J. and Nussbaum R. A.** 2002. Chameleon radiation by oceanic dispersal. *Nature* 415:784–787.

**Raymond J. M., Yuan Y.-M., Ge X.-J.** 2005. *Impatiens namchabarwensis* Balsaminaceae. *Curtis's Botanical Magazine* 22 : 205-208.

**Renner S. S.** 1999. Circumscription and phylogeny of the *Laurales*: Evidence from molecular and morphological data. *Am. J. Bot.* 86: 1301-1315.

**Renner S. S., Clausing G. and Meyer K.** 2001. Historical biogeography of Melastomataceae: the roles of Tertiary migration and long-distance dispersal. *Am. J. Bot.* 88: 1290-1300.

**Rieseberg L. H.** 1997. Hybrid origins of plant species. *Ann. Rev. Ecol. Syst.* 28: 359-389.

**Rieseber G. L. H. and Carney S. E.** 1998. Tansley review no. 102: plant hybridization. *New Phytologist* 140: 599-624.

**Ritland K. and Clegg M. T.** 1987. Evolutionary analysis of plant DNA sequenes. *American Naturalist* 130: S74-S100.

**Rodrigo A. G., Kelly-borges M., Bergoquist P. R. and Bergquist P. L.** 1993. A randomisation test of the null hypothesis that two cladograms are sample estimates of a parametric phylogenetic tree. *New Zealand J. Bot.* 31:257-268.

**Rogers S. O. and Bendich A. J.** 1987. Ribosomal RNA genes in plants: variability in copy number and in the intergenic spacer. *Plant Mol. Biol.* 9: 509-520.

**Rokas A. and Carroll S. B.** 2005. More genes or more taxa? the relative contribution of gene number and taxon number to phylogenetic accuracy. *Mol. Biol. Evol.* 22: 1337-1344.

**Ronquist F.** 2004. Bayesian inference of character evolution. *Trends Ecol. Evol.* 19: 475-481.

**Ronquist F. and Huelsenbeck J. P.** 2003. Mr. Bayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572-1574.

**Rust R. W.** 1977. Pollination in *Impatiens capensis* and *Impatiens pallida* (Balsaminaceae). *Bull. Torrey Bot. Club* 104: 361 - 367.

**Rust R. W.** 1979. Pollination of *Impatiens capensis*: pollinators and nectar robbers. *J. Kansas Entomol. Soc.* 52: 297 - 308.

**Saitou N. and Nei M.** 1987. The neighbor-joining methods: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* 4: 406–425.

**Sang T.** 2002. Utility of low-copy nuclear gene sequences in plant phylogenetics. *Critical Rev. in Biochem. Mol. Biol.* 37: 121-147.

**Sang T. and Zhong Y.** 2000. Testing hybridization hypothesis based on incongruent gene trees. *Syst. Bot.* 49: 422-434.

**Sang T. Crawford D. J. and Stuessy T. F.** 1995. Documentation of reticulate evolution in peonies (*Paeonia*) using internal transcribed spacer sequenced of nuclear ribosomal DNA:

Implications for biogeography and concerted evolution. *Proc. Natl. Acad. Sci U.S.A.*. 92: 6813-6817.

**Sanmartin I. and Ronquist F.** 2004. Southern hemisphere biogeography Inferred by event-based models: plant versus animal patterns. *Syst. Bot.* 53: 216-243.

**Savolainen V., Chase M. W., Hoot S. B., Morton C. M., Soltis D. E., Bayer C., Fay M. F., Brujun A. Y., Sullivan S. and Qui Y. L.** 2000. Phylogenetics of flowering plants based on combined analysis of plastid atpB and rbcL gene sequences. *Syst. Bot.* 49: 306-362.

**Schatz G. E.** 1996. Malagasy/Indo-Australo-Malesian phytogeographic connections. Pages 73–83 *in* Biogeography of Madagascar (W. R. Lourenco, ed.). ORSTOM editions, Paris.Steenis, C. G. G. J. van. 1962. The land-bridge theory in botany. *Blumea* 11:235–372.

**Schönenberger J. and Conti E.** 2003. Molecular phylogeny and floral evolution of Penaeaceae, Oliniaceae, Rhynchocalycaceae, and Alzateaceae (Myrtales). *Am. J. Bot.* 90: 293-309.

**Shaw J., Lickey E. B., Beck J. T. l., Farmer S. B., Liu W.-S., Miller J., Siripum K. C., Winder C. T., Schiling E. E. and Small R. L.** 2005. The tortoise and the hare II: relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. *Am. J. Bot.* 92: 142-166.

**Shimizu T.** 1979. The lectures on the flora of Thailand. *Acta Phytotax. Geobot*. 30: 180-187.

**Simmons M. P., Pickett K. M. and Miya M.** 2003. How meaningful are Bayessian support values? *Mol. Biol. Evol.* 21: 188-199.

**Small R. L., Cronn R. C. and Wendel J. F.** 2004. L. A. S. JOHNSON REVIEW No. 2. Use of nuclear genes for phylogeny reconstruction in plants. *Australian Syst. Bot.* 17: 145 – 170.

**Small R. L., Ryburn J. A., Cronn R. C., Seelanan T. and Wendel J. F.** 1998. The tortoise and the hare: choosing between noncoding plastome and nuclear Adh sequences for phylogeny reconstruction in a recently diverged plant group. *Am. J. Bot.* 85: 1301-1315.

**Smith F. H.** 1934. Prochromosomes and chromosome structure in *Impatiens*. *Proc. Amer. Phil. Soc.* 74: 193-215.

**Sokal R. R. and Smeath P. H.** 1963. Principles of numerical taxonomy. Freeman and Cmpany, San Fransisco.

**Soltis D. E., Soltis P. S. and Chase M. W.** 1999. Angiosperm phylogeny inferred from multiple genes as a tool for comparative biology. *Nature* 402: 402-404.

**Soltis D. E. and Soltis P. S.** 1998. Choosing an approach and an appropriate gene for phylogenetic analysis. In Soltis D. E., Soltis P. S. and J.doyle [eds.], Molecular systematics of plants II: DNA sequencing. Kluwer, Dordecht, Netherlands.

**Soltis D. E., Soltis P. S., Chase M. W., Mort M. E., Albach D. C., Zanis M., Savolainen V., Hahn W. H., Hoot S. B., Fay M. F., Axtell M., Swensen S. M., Prince L. M., Kress**

**W. J., Nixon K. C. and Farris J. S.** 2000. Angiosperm phylogeny inferred from 18S rDNA, rbcL, and atpB sequences. *Bot. J. Linn. Soc.* 133: 381-461.

**Soltis D. E., Soltis P. S. and Zanis M. J.** 2002. Phylogeny of seed plants based on evidence from eight genes. *Am. J. Bot.* 89: 1670-1681.

**Song Y., Yuan Y.-M. and Küpfer P.** 2003. Chromosomal evolution in Balsaminaceae with cytological observations on 45 species from Southeast Asia. *Caryologia* 56: 463-481.

**Song Y., Yuan Y.-M. and Küpfer P.** 2005. Seedcoat micromorphology of *Impatiens* (Balsaminaceae) from China. *Bot. J. Linn. Soc*. 149: 195-208.

**Stebbins G. L.** 1970. Adaptive radiation of reproductive characteristics in angiosperms I: pollination mechanisms. *Ann. Rev. Ecol. Syst.* 1: 307-326.

**Stebbins G. L.** 1971. Chromosomal Evolution in Higher plants. Edward Arnold Ltd., London.

**Steele K. P. and Vilgalys R.** 1994. Phylogenetic analyses of Polemoniaceae using nucleotide sequences of the plastid gene matK. *Syst. Bot.* 19: 126-142.

**Stewart C. B.** 1993. The powers and pitfalls of parsimony. *Nature* 361: 603-607.

**Strimmer K. and Haeseler A.** 1996. Accuracy of Neighbor Joining for n-Taxon Trees. *Syst. Bot.* 45: 516-523.

**Sugawara T., Akeyama S., Murata J. and Yang Y.-P.** 1994. . Karyology of ten species of *Impatiens* (Balsaminaceae) from SW.Yunnan, China. *Acta Phytotax. Geobot.*, 45: 119-125.

**Sugawara T., Akeyama S., Yang Y.-P., and Murata J.** 1997. Karyological characteristics of *Impatiens* (Balsaminaceae) in Yunnan, China. *Acta Phytotaxonomica Geobot.* 48: 7-14.

**Swofford D. L.** 2000. PAUP* Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4. Sinauer Associates, Sunderland, Massachusetts.

**Szumik C. A., Cuezzo F., Goloboff P. A. and Chalup A. E.** 2002. An Optimality Criterion to Determine Areas of Endemism. *Syst. Biol.* 51: 806-816.

**Taberlet P., Gielly L., Pantou G. and Bouver J.** 1991. Universal primers for amplification of three non-coding regions of plastid DNA. *Plant Mol. Biol.* 17: 1105-1105.

**Takhtajan A.** 1997. Diversity and classification of flowering plants. New York: Cambridge University Press.

**Tara C. P. and Namboodri A. N.** 1974. Aberrant microsporogenesis and sterility in *Impatiens sultani* (Balsaminaceae). *Am. J. Bot.* 61: 585-591.

**Templeton A. R.** 1983. Phylogenetic inference from restriction endonuclease cleavage site maps with particular reference to the humans and apes. *Evolution* 37:221-244.

**Thompson J. D., Gibson T. J., Plewniak F., Jeanmougin F. and Higgins D. G.** 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.* 24: 4876–4882.

**Thorne R. F.** 2000. The classification and geography of the flowering plants: dicotyledons of the class angiospermae: (subclasses Magnoliidae, Ranunculidae, Caryophyllidae, Dilleniidae, Rosidae, Asteridae, and Lamiidae). *Bot. Rev.* 66: 441-647.

**Tian J.-P., Liu K.-M. and Hu G.-W.** 2004. Pollination Ecology and pollination system of *Impatiens reptans* (Balsaminaceae) endemic to China. *Ann. Bot.* 93: 167-175.

**Toppin S. M.** 1920. Notes on the Balsams of Chitral and the Kachin hills. *Bull. Misc. Inf. Kew* 345-366.

**Utami N. and Shimizu T.** 2005. Seed morphology and classification of *Impatiens* (Balsaminaceae). *Blumea* 50: 447-456.

**Valiejo-Roman C., Terentieva E. I., Samigullin T. H. and Pimenov M. G.** 2002. nrDNA ITS sequences and affinities of Sino-Himalayan Apioideae. *Taxon* 51: 685-701.

**Van der Niet T.** 2006. Systematics and radiation patterns of *Satyrium* Sw. (Orchidaceae). Phd-thesis: 80. University of Zurich.

**Warburg O. and Reiche K.** 1895. Balsaminaceae. In: Die natürlichen Planzenfamilien. Engler and Prantl (eds.) 3, 5: 583-592. Dunckel and Humblott, Berlijn.

**Waters J. M. and Roy M. S.** 2004. Out of Africa: The train to Australasia. *Syst. Biol.* 53: 18-24.

**Wen J.** 1999. Evolution of eastern Asian and eastern North American disjunct distributions in flowering plants. *Ann. Rev. Ecol. Syst.* 30: 421-455.

**Whitcher I. N. and Wen J.** 2001. Phylogeny and biogeography of *Corylus* (Betulaceae): inferences from ITS sequences. *Syst. Bot.* 26: 283-298.

**Wilczek R. and Schulze G. M.** 1960. Balsaminaceae. Flore du Congo et du Ruana-Burundi 9 : 396-428.

**Wiley E. O.** 1980. Phylogenetic systematics and vicariance biogeography. Syst. Biol 5: 194-220.

**Wilson C. A.** 2003. Phylogenetic relationships in *Iris* series *Californicae* based on ITS sequences of nuclear ribosomal DNA. *Syst. Bot.* 28: 39-46.

**Wolf P. G.** 1997. Evaluation of atpB nucleotide sequences for phylogenetic studies of ferns and other pteridophytes. *Am. J. Bot.* 84: 1429-144.

**Wu G.-S., Fang J.-F. and Zhang Y.-P.** 2006. Molecular Phylogeny and Phylogeography of Suidae. *Zool.l Res.* 27: 197-201.

**Wunderlich R.** 1967. Some remarks on the taxonomic significance of the seed coat. *Phytomorpology* 17: 301-311.

**Yildiz K.** 2002. Seed morphology of Caryophyllaceae species from Turkey (North Anatolia). *Pakistan J. Bot.* 34: 161–171.

**Yoder A. D., Irwin, J. A. and Payseur B.** 2001. Failure of the ILD to determine data combinability for slow loris phylogeny. *Syst. Biol.* 50: 408-424.

**Yuan Y.-M., Song Y., Geuten K., Rahelivololona E., Wohlhauser S., Fischer E, Smets E. and Küpfer P.** 2004. Phylogeny and biogeography of Balsaminaceae inferred from ITS sequence data. *Taxon* 53: 391–403.

**Yuan Y.-M., Wohlhauser S., Möller M., Klackenberg J., Callmander M.W. and Küpfer P.** 2005. Phylogeny and Biogeography of *Exacum* (Gentianaceae): A disjunctive distribution in the Indian ocean basin resulted from long distance dispersal and extensive radiation. *Sys. Bio.* 54: 21-34.

**Yukawa M., Tsudzuki T. and Sugiura M.** 2005. The 2005 Version of the Chloroplast DNA sequence from Tobacco (*Nicotiana tabacum*). *Plant Mol. Bio. Rep.* 23: 359-365.

**Zinov'eva-Stahevitch A. E. and Grant W. F.** 1982. In IOPB chromosome number reports LXXIV. *Taxon*, 31: 365-366.

**Zinov'eva-Stahevitch A. E. and Grant W. F.** 1984. Chromosome numbers in *Impatiens* (Balsaminaceae). *Canad. J. Bot.*, 62: 2630-2635.

**Zinov'eva-Stahevitch A. E. and Grant W. F.** 1985. Aberrant microsporogensis *Impatiens* L. (Balsaminaceae) and its bearing on the taxonomy of the genus. *Cytologia*, 50: 825-836.

**Zurawske G. and Clegg M. T.** 1987. Evolution of higher plant chloroplast DNA-coded genes: implications for structure-function and phylogenetic studies. *Ann. Rev. Plant Physiol.* 38: 391-418.

# Appendix

The lists of species and origins of materials used in this thesis

| Taxon | Collector | Coll. N° | Date | Origin | Alt.[ m] |
|---|---|---|---|---|---|
| *I. acehensis* C.Grey-Wilson | A. Favre | AF1 | 29 Aug. 2002 | Sumatra, Indonesia | |
| *I. amoena* H. Perrier | S. Wohlhauser, M. Callmander & Rakotomamonjy | SW 61422 (PBZT) | | Antsatrotro, Madagascar | |
| *I. amphorata* Edgew. | B. de Retz (K. Geuten s. n. ) | B. de Retz 5566 (BR) | | Asia-West Himalaya | |
| *I. andohahelae* Eb. Fisch. & Rahelivololona | M. E. Rahelivololona, P. Druard, Jerome & I. Fiadana | And6 (PBZT) | 16 Nov. 2000 | Andohahela, Madagascar | 450 |
| *I. andringitrensis* H. Perrier | M. E. Rahelivololona | Ambatofitorahana 2 | 01 Feb.2002 | Ambatofitorahana, Madagascar | |
| *I. anovensis* H. Perrier | M. E. Rahelivololona, Saola & Scenario | Ambanizana 11 (PBZT) | 19 Feb. 2002 | Ambanizana, Madagascar | 900 |
| *I. aposotis* Hook. f. | Y.-M. Yuan | CN2k2-159 | 14 Aug. 2002 | Mt. Zheduoshan, Kangding, Sichuan, China | 2915 |
| *I. aquatilis* Hook. f. | Y. Song | CNY017 | 31 Aug. 2001 | Lijiang, Heilongtan park, Yunnan, China | 2100 |
| *I. arguta* Hook. f. & Thoms. | Y.-M. Yuan | CN2k-74 | 9 Aug. 2000 | Wuqu, Gongshan,Yunnan, China | 1550 |
| *I. assurgens* Baker | S. Dessein | 707 | 2004 | Zambia, Afica | |
| *I. aureliana* Hook. f. | Y.-M. Yuan | CN2k1-56 | 27 Aug. 2001 | nearby Husa river, Yingjiang, Yunnan, China | 1435 |
| *I. aurella* Rydb. | Zika (J. Grant x. n.) | Zika 20125 | 2004 | Washington, USA | |
| *I. auricoma* Baill. | E. Fischer | EF1 | | Comores origin, cult. Bot. Gard. Koblenz Univ. | |
| *I. bahanensis* Hand.-Mazz. | Y.-M. Yuan | CN2k-30 | 2 Aug. 2000 | Above Qiqi, Gongshan, Yunnan, China | 2550 |
| *I. balfourii* Hook.f. | Ray Morgan s. n. | NE1 | 5 May. 2003 | West Himalaya origin, , cult. by Ray Morgan, UK | |
| *I. balsamina* L. | Y.-M. Yuan | CN2k1-06 | 14 Ari. 2001 | cult. South China Institute of Botany, Guanzhou, China | 50 |
| *I. barbata* Comber | Y.-M. Yuan | CN2k2-178 | 19 Aug. 2002 | Yunnan, China | 2000 |
| *I. baroni* Baker | M. E. Rahelivololona, Jacky & Remi | RNM-4 (PBZT) | 11 Feb. 2002 | Ranomafana, Madagascar | |
| *I. baroni* Baker | Y.-M. Yuan & M. Ravokatra | YMG39 | 22 Mar. 2005 | Mandraka, Madagascar | 1206 |
| *I. begoniifolia* S. Akiyama & H. Ohba | Y.-M. Yuan | CN2k1-51 | 26 Aug. 2001 | Yingjiang,Yunnan, China | 1300 |

Appendix

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| *I. begonioides* Eb.Fisch. & Raheliv. | Y.-M. Yuan & M. Ravokatra | YMG56 | 27 Mar. 2005 | Vatoharanana Camp, Madagascar | 1000 |
| *I. bequaerti* De Wild. | E. Fischer | EF2 | | Congo origin, cult. Bot. Gard. Koblenz Univ. | |
| *I. bicaudata* H.Perrier | Y.-M. Yuan & M. Ravokatra | YMG4 | 8 Mar. 2005 | Cascade sacrie, Madagascar | 1046 |
| *I. bicornuta* Wall. | Y.-M. Yuan | CN2k-55 | 3 Aug. 2000 | Under the Xishaofang, Gongshan, Yunnan, China | 2800 |
| *I. bisaccata* Warb | Y.-M. Yuan & M. Ravokatra | YMG3 | 8 Mar. 2005 | Joffreville, Madagascar | 742 |
| *I. bombycina* W. Lobin & E. Fischer | E. Fischer | EF3 | | African origin, cult. Bot. Gard. Koblenz Univ. | |
| *I. brachycentra* Kar. & Kir. | Burtt | Burtt 3834 (E) | | Central Asia | |
| *I. brevipes* Hook.f. | Y.-M. Yuan | 2004-37 | 8 Aug. 2005 | Tianquan, Sichun, China | 1280 |
| *I. burtonii* Hook. f. | E. Fischer | EF4 | | Kenya origin, cult. Bot. Gard. Koblenz Univ. | |
| *I. campanulata* Wight | Ray Morgan s. n. | | 12 Nov. 2002 | South India origin, cult. by Ray Morgan, UK | |
| *I. capensis* Meerb. | P. Küpfer | | 8 Nov. 2001 | Montreal, Quebec, Canada | |
| *I. chevalieri* Tardieu | Y. Song & N. L. Phuong | 2004-07 | 18 Aug. 2004 | Dalat, Vietnam | |
| *I. chimiliensis* Comber | Y.-M. Yuan | CN2k-51 | 3 Aug. 2000 | Gaoligongshan Pass, Gongshan, Yunnan, China | 3500 |
| *I. chinensis* L. | Y.-M. Yuan | CN2k1-49 | 25 Aug. 2001 | Tengchun, Yunnan, China | 1630 |
| *I. chungtienensis* Y. L. Chen | Y.-M. Yuan | CN2k2-204 | 23 Aug. 2002 | Lidiping, Weixi, Yunnan, China | 3100 |
| *I. claeri* N. Halle | E. Fischer | EF5 | | Gabon origin, cult. Bot. Gard. Koblenz Univ | |
| I. *clavigera* Hook. f. | A. Favre | AF23 | 15 Sep. 2003 | Fannipan, Sapa, Vietnam | 1500 |
| *I. columbaria* J. J. Bos | E. Fischer | EF6 | | Gabon origin, cult. Bot. Gard. Koblenz Univ | |
| *I. compa* Hook. f. | Y.-M. Yuan | 2004-58 | 17 Aug. 2004 | Mt. ShenNongJia, Hubei, China | 2340 |
| *I. conchibracteara* Y. L. Chen & Y. Q. Lu | G. Hao | Hao 427 | 18 Aug. 2002 | Yixiantian to Hongchunping, Mt. Emei, Sichuan, China | 1700 |
| *I. congolensis* G. M. Schulze & R. Wilczek | E. Fischer | EF7 | | Congo orin, Africa, cult. Bot. Gard. Koblenz Univ. | |
| *I. corchorifolia* Franch. | P. Chassot & Y.-M. Yuan | 99-173 | | Jizushan, Binchuan, Yunnan, China | 2200 |
| *I. cordata* Wight | Ray Morgan s. n. | | 12 Nov. 2002 | Kerala, South India origin, cult. by Ray Morgan, UK | |
| *I. cristata* Wall. | Y.-M. Yuan & X.-J. Ge | CN2k3-15 | 21 Sep. 2003 | Baiba, Lingzhi, Tibet, China | 3150 |
| *I. cuspidata* Wight & Arn. | Ray Morgan s. n. | | 12 Nov. 2002 | South India origin, cult. by Ray Morgan, UK | |
| *I. cyanantha* Hook. f. | Y.-M. Yuan | CN2k1-84 | 14 Jul. 2001 | Shizong town, Yunnan, China | 1850 |
| *I. cyathiflora* Hook. f. | P. Chassot & Y.-M. Yuan | 99-29 | 5 Oct. 1999 | Qiongzhusi, Kunming, Yunnan, China | 2200 |
| *I. davidi* Franch. | Y.-M. Yuan | CN2k-09 | 20 Jul. 2000 | Yixiantian, Wuyishan, Fujian, China | 300 |
| *I. delavayi* Franch. | P. Chassot & Y.-M. Yuan | 99-154 | 15 Oct. 1999 | Tuguancun, Zhongdian, Yunnan, China | 2900 |
| *I. desmantha* Hook. f. | Y.-M. Yuan | CN2k2-30 | 5 Aug. 2002 | Grand Snow Mt. Wenshui, Zhongdian, Yunnan, China | 3410 |
| *I. devolii* T. C. Huang | Z.-Y. Jiang | Taiwan3 | 1973 | Nan Tou Guan Wu, Taiwan, China | |
| *I. dicentra* Franch.ex | Y.-M. Yuan | 2004-62 | 17 Aug. 2004 | Mt. ShennongJia, Hubei, China | 2310 |

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| Hook.f. | | | | | |
| *I. dolichoceras* Pritz. ex Diels | Y.-M. Yuan | CN2k1-80 | 9 Jul. 2001 | Damingshan, Nanning, Guangxi, China | 1519 |
| *I. drepanophora* Hook. f. | Y.-M. Yuan | CN2k1-41 | 24 Aug. 2001 | Longling, Yunnan, China | 2100 |
| *I. eberhardtii* Tardieu | Y. Song & N. L. Phuong | 2004-10 | 19 Aug. 2004 | Pongour waterfall, Dalta, Vietnam | |
| *I. ecalcarata* Collett & Hemsl. | Zika (J. Grant x. n.) | Zika 20068 | 2004 | Tillamook Co., Oregon, USA | |
| *I. edgeworthii* Hook.f. | Ray Morgan s. n. | NE2 | 5 May 2003 | Nepal origin, , cult. by Ray Morgan, UK | |
| *I. elatostemmoides* H.Perrier | Y.-M. Yuan & M. Ravokatra | YMG30 | 20 Mar. 2005 | Ambatovy, Madagascar | 1190 |
| *I. eubotrya* Miq. | A. Favre | AF2 | 29 Aug. 2002 | Gunung Merapi, Sumatra, Indonesia | 2502 |
| *I. faberi* Hook. f. | Y. Song | CN S007 | 3 Sep. 2000 | Hongchunping, Mt.Emei, Sichuan, China | |
| *I. falcifera* Hook.f. | Ray Morgan s. n. | NE3 | 5 May 2003 | West Himalaya origin, , cult. by Ray Morgan, UK | |
| *I. fenghwaiana* Y. L. Chen | Y.-M. Yuan | CN2k1-78 | 9 Jul. 2001 | Damingshan, Nanning, Guangxi, China | 1519 |
| *I. firmula* Baker | M. E. Rahelivololona, Jacky & Remi | RNM 4 (PBZT) | 1 Mar. 2001 | Ranomafana, Madagascar | 1300 |
| *I. fischeri* Warb. | O. Phaehler & M. Schnell | I-01 | 18 Jul. 2001 | Kenya, Africa | 2479 |
| *I. fissicornis* Maxim. | M.-L. Wang | CNsh003 | 10 Aug. 2000 | Shaanxi, China | 1900 |
| *I. flacida* Arn. | | Meise,FB/S3925 (BR) | | South India origin, Cult. United Kingdom, Richmond, Royal H.B. Kew | |
| *I. flanaganae* Hemsl. | RBG Edinburgh | 19860179 (E) | | Africa origin | |
| *I. forrestii* Hook. f. ex W. W. Smith | Y.-M. Yuan | CN2k-79 | 11 Aug. 2000 | Dali, cangshan, Yunnan, China | 2650 |
| *I. fragicolor* Marq. et Airy-Shaw | Y.-M. Yuan & X.-J. Ge | CN2k3-14 | 21 Sep. 2003 | Taizhao, Gongbujiangda, Tibet, China | 3580 |
| *I. fuchsioides* H. Perrier | R. Ranaivojaona | ROR 395 | 12 Feb. 2002 | Tsaratanana, Madagascar | |
| *I. furcata* H.Perrier | E. Fischer | EF8 | | African origin, cult. Bot. Gard. Koblenz Univ | |
| *I. gibbosa* H. Perrier (*I.lemuriana* Eb.Fisch. & Raheliv) | M. E. Rahelivololona, S.Wohlhauser & M. Callmander | T11 (PBZT) | 2 Dep. 1999 | Tsaratanana, Madagascar | |
| *I. glandulifera* Arn. | Y.-M. Yuan | JBE1 | | Western Himalaya origin, cult. Bot. Gard. Neuchâtel | |
| *I. gongshanensis* Y. L. Chen | Y.-M. Yuan | CN2k1-27 | 19 Aug. 2001 | Qiqi,Gongshan, Yunnan, China | 2100 |
| *I. hawkeri* W.Bull, ex Gard. | K. Geuten & S. Janssens | SJ006(LV) | | New Guinea origin, cult. in University of Leuven | |
| *I.henryi* E.Pritz.ex Diels | Y.-M. Yuan | 2004-67 | 17 Aug. 2004 | Mt. ShenNongJia, Hubei, China | 2310 |
| *I. henslowiana* Arn. | E. Fischer | EF10 | | Sri Lanka origin, cult. Bot. Gard. Koblenz Univ | |
| *I. hians* Hook. f. | Y. Song | 19991311 | 27 Sep. 2003 | West Africa origin, cul. In Bot. Zurich | |
| *I. hoehnelii* T. C. E. Fries | E. Fischer | EF9 | | African origin, cult. Bot. Gard. Koblenz Univ. | |

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| *I. holocentra* Hand.-Mazz. | Y.-M. Yuan | CN2k-54 | 3 Aug. 2000 | Under the Xishaofang, Yunnan, China | 2700 |
| *I. hookeriana* Arn. | Bot. Gard. UC Davis | 75.0276 | | Sri Lanka origin, cult. Bot. Gard. UC, Davis | |
| *I. imbecilla* Hook.f. | Y.-M. Yuan | 2004-30 | 6 Aug. 2004 | Xixiangchi, Mt. Emei, Sichuan, China | 2070 |
| *I. imbecilla* Hook.f. | G. Hao | Hao 426 | 18 Aug. 2002 | Yixiantian to Hongchunping, Mt. Emei, Sichuan, China | |
| *I. inaperta* H. Perrier | Y.-M. Yuan | JBE2 | | Madagascar origin, cult. Bot. Gard. Neuchâtel | |
| *I. infirma* Hook.f. | Y.-M. Yuan | CN2k2-60 | 6 Aug. 2002 | Shagong, Xiangcheng, Sichuan, China | 2950 |
| *I. javensis* Steud. | A. Favre | AF56 | | Gunung Gede, West Java, Indonesia | |
| *I. keilii* Gilg. | E. Fischer | EF11 | | African origin, cult. Bot. Gard. Koblenz Univ | |
| *I. kerriae* Craib | P. Chassot | 99-238 | 30 Oct. 1999 | Dui Suthep,Qingmai, Thailand | |
| *I. kilimanjari* Oliver | JMG | JMG 94613 (BR) | | Africa | |
| *I. langbianensis* Tardieu | Y. Song & N. L. Phuong | 2004-08 | 18 Aug. 2004 | Liangbian Mt , Dalat, Vietnam | |
| *I. lateristachys* Y.L.Chen et Y.Q.Lu | Y.-M. Yuan | 2004-33 | 7 Aug. 2004 | Xixiangchi to Leidongping, Mt. Emei Sichuan, China | 2150 |
| *I. latifolia* L. | Ray Morgan s. n. | | 12 Nov. 2002 | Inida origin, cult. by Ray Morgen, UK | |
| *I. lecomtei* Hook. f. | Y.-M. Yuan | CN2k2-202 | 23 Aug. 2002 | Behind Ludian, Lijiang, Yunnan, China | 2900 |
| *I. leschenaultii* Wall. | Ray Morgan s. n. | | 12 Nov. 2002 | India Origin, cult. by Ray Morgen, UK | |
| *I. levingei* Gamble ex Hook. f. (I. clavicornu Turcz.) | Ray Morgan s. n. | | 12 Nov. 2002 | India Origin, cult. UK | |
| *I. lingzhiensis* Y. L. Chen | Y.-M. Yuan & X.-J. Ge | CN2k3-81 | 27 Sep. 2003 | Zhamu, Bomi, Tibet, China | 2735 |
| *I. lucorum* Hook.f. | Y.-M. Yuan | 2004-34 | 7 Aug. 2004 | Xixiangchi to Leidongping, Mt. Emei, Sichuan, China | 2150 |
| *I. lyallii* Baker | Y.-M. Yuan & M. Ravokatra | YMG8 | 10 Mar. 2005 | Mt. Amber, Madagascar | 1216 |
| *I. malcomberi* Eb.Fisch. & Raheliv. | Y.-M. Yuan & M. Ravokatra | YMG13 | 11 Mar. 2005 | Grand Lac, Mt. Amber, Madagascar | 1433 |
| *I. manaharensis* Baill. | S. Wohlhauser & et. al. | WCL 1 (PBZT) | | Madagascar | |
| *I. mandrakae* Eb.Fisch. & Raheliv. | Y.-M. Yuan & M. Ravokatra | YMG38 | 22 Mar. 2005 | Mandraka, Madagascar | 1206 |
| *I. mannii* Hook.f. | K. Geuten | Carvalho 4534 | 1990 | Habarium, National Botanic Garden of Belgium, Africa origin | |
| *I. margaritifera* Hook. f. | Y.-M. Yuan & X.-J. Ge | CN2k3-26 | 24 Sep. 2003 | Duoxiongla, Paiqu, Miling, Tibet, China | 4200 |
| *I. mengtseana* Hook. f. | Y.-M. Yuan | CN2k1-38 | 24 Aug. 2001 | Longling, Yunnan, China | 1580 |
| *I. meruensis* Gilg. | Ray Morgan s. n. | | 12 Nov. 2002 | Tanzania origin, cult. by Ray Morgen, UK | |
| *I. microcentra* Hand.-Mazz. | Y.-M. Yuan | CN2k-50 | 3 Aug. 2000 | Gongshan to Dulongjiang, Yunnan, China | 2800 |
| *I. miniata* Grey-Wilson | M.E. Rahelivololona, S. Wohlhauser & M. Callmander | T1 (PBZT) | 27 Nov. 1999 | Tsaratanana, Madagascar | 1100 |
| *I. monticola* Hook. f. | G. hao | Hao 425 | 18 Aug. 2002 | Yixiantian to Hongchunping, Mt. Emei, Sichuan, China | |

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| *I. namchabawensis* Morgan, Y.-M. Yuan and X.-J. Ge | Y.-M. Yuan & X.-J. Ge | CN2k3-70 | 29 Sep. 2003 | Vicinity of 113K, Lengduo, Medog, Tibet, China | 930 |
| *I. napoensis* Y. L. Chen | Y.-M. Yuan | CN2k1-61 | 27 Aug. 2001 | Longchuan, Yunnan, China | 1450 |
| *I. niamniamensis* Gilg. | E. Fischer | EF13 | | Africa origin, cult. Bot. Gard. Koblenz Univ | |
| *I. nolitangere* L. | Y.-M. Yuan | CN2k-86 | 19 Aug. 2001 | Helong country, laoyeling, Jilin, China | 1300 |
| *I. nubigena* W.W.Smith | Y.-M. Yuan | 2004-4YM | July. 2004 | Songpan, Sichuan, China | |
| *I. omeiana* Hook.f. | Y.-M. Yuan | 2004-21 | 5 Aug. 2004 | Sichuan, China | |
| *I. oncidioides* Ridley, ex Hook. F. | A. Favre | AF14 | 3 Jul. 2003 | Gunung Beremba, Cameron Highlands, Malayan Peninsula | |
| *I. oxyanthera* Hook. f. | Y. Song | CN S008 | 3 Sep. 2000 | Sichuan, China | |
| *I. pallida* Nutt. | Eric (J. Grant x. n.) | Eric2004-01 | 19 Jul. 2004 | New York, USA | |
| *I. parasitica* Bedd. | Ray Morgan s. n. | | 12 Nov. 2002 | India origin, cult. by Ray Morgen, UK | |
| *I. parviflora* DC: | M. Ronikier | | 1 Jun. 2001 | Poland | |
| *I. percrenata* H. Perrier | M. E. Rahelivololona, P. Druard, Jerome & I. Fiadana | And 3 (PBZT) | 15 Nov. 2000 | Andohahela, Madagascar | |
| *I. platychlaena* Hook.f. | Y. Song | CN S009 | 3 Sep. 2000 | Caopengsi, Mt. Emei,Sichuan, China | |
| *I. platypetala* Lindl. | Ray Morgan s. n. | | 12 Nov. 2002 | Bali, Indinesia origin, cult. by Ray Morgen, UK | |
| *I. poculifer* Hook. f. | Y.-M. Yuan | CN2k2-209 | 24 Aug. 2002 | Yunnan, China | 3000 |
| *I. poilanei* Tardieu | Y. Song & N. L. Phuong | 2004-09 | 18 Aug. 2004 | Dalta, Vietnam | |
| *I. pritzelii* Hook.f. | Y. Song | CNjf011 | 14 Jul. 2001 | Jinfoshan,bitanyougu, Sichuan, China | |
| *I. pseudoviola* Gilg. | RBG Edinburgh | 19680124 (E) | 18 Jul. 2001 | central Africa | 2533 |
| *I. psittacina* Hook.f._ | A. Favre | AF12 | Aug. 2003 | Chiang Dao, Thailand | 1800 |
| *I. puberula* DC. | A. Favre | AF5 | 12 Oct. 2003 | Darjeeleing , India | 2200 |
| *I. purpurea* Hand.-Mazz. | Y. Song | CNY007 | 23 Aug. 2000 | Dali,cangshan,Yunnan, China | |
| *I. racemosa* DC | Y.-M. Yuan & X.-J. Ge | CN2k3-29 | 24 Sep. 2003 | Tibet, China | 3265 |
| *I. radiata* Hook. f. | Y.-M. Yuan | CN2k-77 | 11 Aug. 2000 | Dali, zhonghesi, Yunnan, China | 2650 |
| *I. rectangula* Hand.-Mazz. | Y.-M. Yuan | CN2k1-26 | 19 Aug. 2001 | Qiqi,Gongshan, Yunnan, China | 2100 |
| *I. repens* Moon. | | Leiden, 17953(L) | | South India origin, cul. Nat. Bot. Gard. | |
| *I. rostellata* Franch. | Y.-M. Yuan | 2004-28 | 6 Aug. 2004 | Yuxiansi, Mt. Emei, Sichuan, China | 1550 |
| *I. rothii* Hook. f. | P. Binggeli | | 8 Nov. 2001 | Ethiopia | |
| *I. rubrostriata* Hook. f. | Y.-M. Yuan | CN2k1-44 | 24 Aug. 2001 | Gaoligongshan,Tengchun, Yunnan, China | 2097 |
| *I. ruiliensis* S.Akiyama et H.Ohba | Y.-M. Yuan | CN2k1-66 | 27 Aug. 2001 | Ruili, Yunnan, China | 1620 |
| *I. sambiranensis* H. Perrier | M. E. Rahelivololona, S. Wohlhauser & M. Callmander | T4 (PBZT) | 29 Nov. 1999 | Tsaratanana, Madagascar | |

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| *I. scabrida* DC. | Ray Morgan s. n. | NE6 | 5 May. 2005 | West Himalaya origin, cult. by Ray Morgan, UK | |
| *I. scabrida* DC. | S. Janssens | 941314 (DBG) | | West Himalaya origin, Holden arboretum | |
| *I. scutisepala* Hook. f. | Y.-M. Yuan | CN2k-56 | 4 Aug. 2000 | Under the Xishaofang, Yunnan, China | 2500 |
| *I. siculifer* Hook. f. | Y.-M. Yuan | CN2k-80 | 11 Aug. 2000 | Dali cangshan, Yunnan, China | 2650 |
| *I. sodenii* Engl. & Warb. ex Engl. | E. Fischer | EF15 | | Kenya origin, cult. Bot. Gard. Koblenz Univ | |
| *I. soulieana* Hook. f. | Y.-M. Yuan | CN2k2-163 | 15 Aug. 2002 | Paomashan, Kangding, Sichuan, China | 2580 |
| *I. sp200416_nov* | Y.-M. Yuan | 2004-16YM | 2 Aug. 2004 | Mt. Qingcheng shan, Sichuan, China | 1000 |
| *I. sp200439* | Y.-M. Yuan | 2004-39 | 8 Aug. 2004 | Tianquan, Sichuan, China | 1350 |
| *I. sp200456* | Y.-M. Yuan | 2004-56 | 15 Aug. 2004 | Taihe, Jiansi, Hubei, China | 1500 |
| *I. sp200472* | Y.-M. Yuan | 2004-72 | 20 Aug. 2004 | Zhengba, Zhengping, Shanxi, China | 1440 |
| *I. sp200475* | Y.-M. Yuan | 2004-75 | 21 Aug. 2004 | Mt. BaiChiShan, Chengkou, Chongqing, China | 1500 |
| *I. sp200476* | Y.-M. Yuan | 2004-76 | 21 Aug. 2004 | Mt. BaiChiShan, Chengkou, Chongqing, China | 1500 |
| *I. spnovATibet* | Y.-M. Yuan & X.-J. Ge | 2003-65 | 26 Sep. 2003 | Er-Hao-Qiao, Medog, Tibet, China | 1080 |
| *I. spnovBTibet* | Y.-M. Yuan & X.-J. Ge | 2003-61 | 26 Sep. 2003 | Nearby Hanmi, Medog, Tibet, China | 2100 |
| *I. spThai3* | A. Favre | AF10 | Aug. 2003 | Khao Luang Mt., South East Thailand | |
| *I. spThai5* | A. Favre | AF12 | Aug. 2003 | Doi Suthep, Chiang Mai, Thailand | |
| *I. spWoLong* | A. Favre | AF 41 | 21 Sep. 2004 | Wolong, Sichuan, China | |
| *I. spYMG005* | Y.-M. Yuan & M. Ravokatra | YMG5 | 10 Mar. 2005 | Lac de Mahasariaka, Madagascar | 1183 |
| *I. spYMG007* | Y.-M. Yuan & M. Ravokatra | YMG7 | 10 Mar. 2005 | Mt. Amber, Madagascar | 1216 |
| *I. spYMG009* | Y.-M. Yuan & M. Ravokatra | YMG9 | 10 Mar. 2005 | Mt. Amber, Madagascar | 1324 |
| *I. spYMG010* | Y.-M. Yuan & M. Ravokatra | YMG10 | 10 Mar. 2005 | Mt. Amber, Madagascar | 1324 |
| *I. spYMG012* | Y.-M. Yuan & M. Ravokatra | YMG12 | 11 Mar. 2005 | Grand Lac, Mt. Amber, Madagascar | 1433 |
| *I. spYMG018* | Y.-M. Yuan & M. Ravokatra | YMG18 | 13 Mar. 2005 | Grand Lac, Mt. Amber, Madagascar | 1324 |
| *I. stenosepala* Pritz. ex Diels | M.-L. Wang | CNsh001 | 10 Aug. 2000 | Taibaishan, Shaanxi, China | |
| *I. stuhlmannii* Warb. | E. Fischer | EF17 | | African origin, cult. Bot. Gard. Koblenz Univ | |
| *I. subabortiva* H.Perrier | M. E. Rahelivololona, S. Wohlhauser & M. Callmander | T6 (PBZT) | 1 Dep. 1999 | Tsaratanana, Madagascar | |
| *I. taronensis* Hand.-Mazz. | Y.-M. Yuan | CN2k-57 | 4 Aug. 2000 | Under Xishaofang, Yunnan, China | 2600 |
| *I. tayemonii* Hayata | Z.-Y. Jiang | Taiwan2 | 1973 | Nan Tou Guan Wu, Taiwan, China | |
| *I. teitensis* Grey-Wilson | E. Fischer | EF18 | | African origin, cult. Bot. Gard. Koblenz Univ | |

Appendix

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| *I. tenerrima* Y.L.Chen | Y.-M. Yuan | 2004-63 | 17 Aug. 2004 | ShenNongJia, Hubei, China | 2600 |
| *I. textori* Miq. | M. Kanno & al. | 1114 (TUS) | 17 Sep. 2001 | Japan | |
| *I. tinctoria* Grey-Wilson | Ray Morgan s. n. | | 14 Nov. 2003 | East Africa origin, cult. by Ray Morgan, UK | |
| *I. translucida* Eb.Fisch. & Raheliv. | Y.-M. Yuan & M. Ravokatra | YMG55 | 27 Mar. 2005 | Vatoharanana Camp, Madagscar | 1000 |
| *I. trichosepala* Y. L. Chen | Y.-M. Yuan | CN2k1-68 | 28 Aug. 2003 | Huangcaoba, Longling, Yunnnan, China | 1260 |
| *I. tuberosa* H.Perrier | E. Fischer | EF19 | | Madagascar origin, cult. Bot. Gard. Koblenz Univ | |
| *I. turrialbana* Donn. Sm. | Cascante & Sanchez | Cascante & Sanchez 285 | 4 Sep. 2004 | Costa Rica | |
| *I. uliginosa* Franch. | Y.-M. Yuan | CN2k2-173 | 18 Aug. 2002 | Hongdi, Huaping, Yunnan, China | 2150 |
| *I. undulata* Y. L. Chen et Y. Q. Lu | Y.-M. Yuan | 2004-26 | 6 Aug. 2004 | Xianfengsi, Mt. Emai, Sichuan, China | 1400 |
| *I. uniflora* Hayata | Z.-Y. Jiang | Taiwan1 | 1973 | Taiwain, China | |
| *I. urticifolia* Wall. | Y.-M. Yuan & X.-J. Ge | CN2k3-45 | 24 Sep. 2003 | Nearby Lage, Medog, Tibet, China | 3265 |
| *I. usambarensis* Grey-Wilson | E. Fischer | EF20 | | African origin, cult. Bot. Gard. Koblenz Univ | |
| *I. vilersi* Costantin & Poisson | M. E. Rahelivololona, P. Druard, Jerome & Fiadana, | And 2 (PBZT) | 15 Nov. 2000 | Andohahela, Madagascar | 690 |
| *I. vittata* Franch | Y.-M. Yuan | 2004-15YM | 1 Aug. 2004 | Qing Cheng Shan, Sichuan, China | 1000 |
| *I. walleriana* Hook.f. | O. Phaehler & M. Schnell | I08 (NEU) | 7 Aug. 2001 | Kenya, Afica | 22 |
| *I. xanthina* Comber | Y.-M. Yuan | CN2k1-15 | 16 Aug. 2001 | Cikai,Gongshan, Yunnan, China | 1620 |
| I. yingjiangensis S.Akiyama & H. Ohba | Y.-M. Yuan | CN2k1-55 | 27 Aug. 2001 | Yingjiang, Yunnan, China | 1435 |
| *I. zenkeri* Warb. | E. Fischer | EF21 | | African origin, cult. Bot. Gard. Koblenz Univ | |
| *Hydrocera triflora* Wight & Arn. | Y. Song, A. Favre & N. L. Phuong | 2004-04 | 10 Aug. 2004 | from Thôt Not to Can tho,Mekong Delta, Vietnam | |
| *Marcgravia polyantha* Delp. | Jard. Bot. Nat. Belgique | FB/S3781 (BR) | | sample from botanical Garden, origin unknown | |
| *Norantea guianensis* Aubl. | Jard. Bot. Nat. Belgique | | | sample from botanical Garden, origin unknown | |
| *Pelliciera sp.* | Pennington & al. | 586 (K) | | Costa Rica | |
| *Souroubea sp.* | BG Univ. Utrecht | 76GR00102 (U) | | Peru | |
| *Tetramerista sp.* | Coode s. n. (K) | | | Brunei Darussalam | |
| *Marcgravia Maguire* de roon | | FB/S3708 (BR) | | French Guyana, cult. Net. Bot. Gard. Meise | |

# Paper I

# Seedcoat Micromorphology of *Impatiens* (Balsaminaceae) from China

Song Y., Yuan Y.-M. and Küpfer P.

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# Seedcoat micromorphology of *Impatiens* (Balsaminaceae) from China

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Seeds of 38 species of *Impatiens* mostly from south-western China were observed by scanning electron microscopy. High diversity was found in seedcoat micromorphology. Based on the structure and ornamentation of epidermal cells of the seedcoat, particularly the pattern of the arrangement of the cells and the degree of elevation of the anticlinal cell walls, four morphological types can be distinguished, viz, laevigate, granulate, reticulate and protrusive. The laevigate type and granulate type are unique, each occurring in only one species. The other two types are common and can be further divided into subdivisions according to the shape of the epidermal ornamentation of the seedcoat. Descriptions of seed size, shape and seedcoat types are summarized for the genus. Taxonomic and phylogenetic implications of the seedcoat micromorphology are also discussed, in comparison with the available gross morphological and molecular data. © 2005 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2005, **149**, 195–208.

ADDITIONAL KEYWORDS: evolution – SEM – seeds – taxonomy.

## INTRODUCTION

The Balsaminaceae has two genera, *Hydrocera* with only one species that occurs in the Indo-Malaysian countries, and *Impatiens* with over 900 species occurring mostly in tropical and subtropical regions of the Old World (Grey-Wilson, 1980). Recent studies have been devoted to the morphology, cytology, taxonomy and molecular systematics of this family (Chen, 2001; Fischer & Rahelivololona, 2002, 2003; Song, Yuan & Küpfer, 2003; Yuan *et al*., 2004). Classification of *Impatiens* remains a strong challenge as the traditionally favourite characters, such as floral morphology and structure, are extremely variable (Grey-Wilson, 1980). The search for new taxonomic characters for *Impatiens* is therefore urgent.

The importance of seed morphology for classification has long been recognized (Hooker & Thomson, 1860). Recent examples of successful applications of seed morphology include studies on *Veronica* (Martinez-Ortega & Rico, 2001), Massonieae (Pfosser *et al*., 2003), *Aeschynanthus* (Mendum *et al*., 2001), *Phyllocladus* (Bobrov, Melikian & Yembaturova, 1999), Caryophyllaceae (Yildiz, 2002) and Brassicaceae (Abdel Khalik & Van der Maesen, 2002). However, seed morphology of Balsaminaceae has been observed for only 15 species (Shimizu, 1979; Lu & Chen, 1991). Two types of seedcoat micromorphology, laevigate and scabrous, have been described in *Impatiens*. The seed morphology of the genus was shown to provide useful taxonomic characters at species level, highly correlated with flower and pollen morphology (Shimizu, 1979; Lu & Chen, 1991). These studies, however, described only the main types of seedcoat, and it was not possible to elucidate the overall variation in seedcoat micromorphology and its implementation for taxonomy, due to insufficient sampling. Detailed observations on more species and a systematic overall evaluation of seedcoat micromorphology in *Impatiens* are still necessary. South-western China is one of the most important diversification centres of *Impatiens*. Scanning electron microscopy (SEM) observations on seeds of 38 species of *Impatiens*, mostly from south-

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western China, were therefore carried out to elucidate the systematic value of seedcoat micromorphological characters and, furthermore, to detect evolutionary trends among seedcoat microcharacters and their correlations with other traits.

## MATERIAL AND METHODS

Seeds used for observations were collected in the field during the summers and autumns of 2000 and 2003. Two species of the western Himalayan origin were collected from a botanic garden. The origins of the samples and vouchers, together with information on seed size, shape, colour and length/width ratio, are shown in Table 1. Seed sizes were measured from five seeds randomly chosen. Voucher specimens are deposited in the Herbarium of the Botanical Institute of the University of Neuchâtel (NEU). For scanning electron microscopy, dried mature seeds were mounted on stubs, using double-sided adhesive tape, and were coated with about 2.3 nm of gold with a BALTEC SCD 005 sputter coater. Coated seeds were then examined and photographed with the Philips ESEM-FEG XL30 scanning electron microscope.

## OBSERVATIONS

Morphological characteristics of the seeds such as size, shape, colour and structure of the seedcoat, as summarized in Table 1, were found to be highly variable within *Impatiens* and provided a set of useful taxonomic characters for most species analysed. Seeds range from 1.3–1.5 × 0.6–0.9 mm (length × width) (*I. xanthina* Comber) to 4.1–4.5 × 2.0–2.1 mm (*I. parviflora* DC.) in size, and are globose, ovate, ellipsoid, subellipsoid or long ellipsoid in outline. Their colour varies from yellow, beige, grey, to various shades of brown, to black. The 38 *Impatiens* species observed also show wide variation in seedcoat micromorphological patterns. Based on the structure and ornamentation of the epidermal cells of the seedcoat, four principal morphological types can be distinguished: laevigate, granulate, reticulate and protrusive; the latter two types can be further divided into several subdivisions.

### LAEVIGATE TYPE (FIG. 1)

Found only in *I. chinensis* L., this type of seedcoat is characterized by an almost smooth surface without obvious sculpture (Fig. 1A). Seeds are 1.7–2.0 × 1.4–1.7 mm, globose, and shining black in colour. The cuticular pattern is fine and dense, rarely with fine hairs and granules on the cell surface that can be seen only at high magnification (Fig. 1B).

### GRANULATE TYPE (FIG. 2)

Seen only in *I. balsamina* L., this type of seedcoat is characterized by granulate protrusions evenly covering the entire surface (Fig. 2A). Seeds are 2.2–3.1 × 1.4–1.7 mm, subellipsoid and grey-brown in colour. In detail, each granule is a bud-like protrusion with radial pleats (Fig. 2B).

### RETICULATE TYPE (FIGS 3–20)

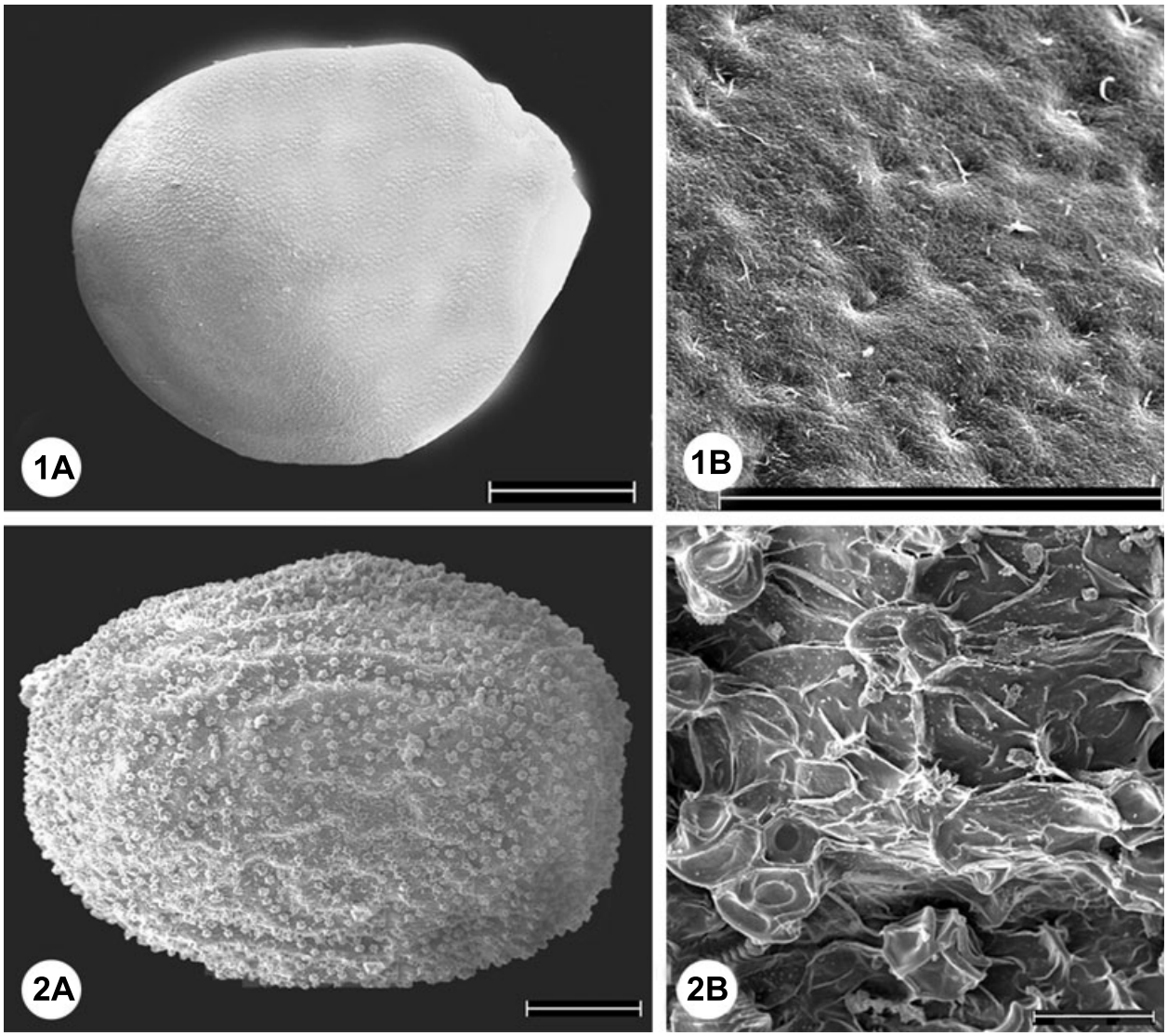
The outer periclinal wall of the epidermal cells of the seedcoat bulges, or becomes more or less bullate, forming a reticulate ornamentation with varied shapes and sizes of the meshes. This is the most common type observed. Based on the extent of the projection and the arrangement of the bullate epidermal cells, the following four subtypes can be distinguished.

*Fine reticulate subtype (Figs 3–9)*

The outer periclinal walls of all the epidermal cells of the seedcoat are evenly, but only slightly bulged. Eight of the species observed belong to this group, i.e. *I. dolichoceras* E. Pritz. ex Diels (Fig. 4), *I. gongshanensis* Y. L. Chen (Fig. 3), *I. holocentra* Hand.-Mazz., *I. forrestii* Hook. f. et W. W. Sm. (Fig. 5), *I. infirma* Hook. f. (Fig. 6), *I. noli-tangere* L. (Fig. 7), *I. rostellata* Franch. (Fig. 8) and *I. scabrida* DC. (Fig. 9). The seeds of this category vary in size and shape: 1.5–3.8 × 0.8–2.4 mm, round, ovate or ellipsoid, yellowish brown to dark brown in colour. *Impatiens gongshanensis* (Fig. 3A) and *I. holocentra* have comparatively small seeds (1.5–2.0 × 0.8–1.4 mm) with subcircular or ocellate testa cells (Fig. 3B). *Impatiens dolichoceras* has rhombic cells on the flat seedcoat surface with undulate pits inside (Fig. 4A, B). The seed of *I. forrestii* has a round shape (Fig. 5A) and the curvature of the periclinal wall of the seedcoat cells make up an undulated pattern (Fig. 5B). Cuticle granules can be seen under higher magnification (Fig. 5C). The seed of *I. infirma* has irregular elevations along the anticlinal walls of the epidermal cells (Fig. 6A, B). *Impatiens noli-tangere* has narrow and ellipsoid seeds with ornamentations similar to *I. infirma* (Fig. 7A, B). The seeds of *I. rostellata* are narrow and ellipsoid, with nearly round or polygonal testa cells. The anticlinal walls of the testa cells are curved or slightly undulate (Fig. 8A, B). *Impatiens scabrida* has ovate seeds with stellate-undulate pits on the seedcoat (Fig. 9A). Fine cuticle dusts can be seen under high magnification (Fig. 9B).

*Colliculate subtype (Figs 10–15)*

In comparison with the above fine reticulate subtype, the colliculate subtype was assigned to those seeds that have seedcoat cells with higher inflated or bulged outer periclinal walls. The epidermal cells with inflated or bulged outer periclinal walls are evenly




**Figures 1–2.** SEM micrographs of seedcoat of *Impatiens*. Fig. 1. Laevigate type. *I. chinensis*. Fig. 2. Granulate type. *I. balsamina*. A, entire seed. Scale bars = 0.5 mm. B, detail of seedcoat. Scale bars = 0.05 mm.

distributed across the entire seedcoat and are densely reticulate. Seeds of the following species fall into this category: *I. aquatilis* Hook. f. (Fig. 10A, B), *I. chungtienensis* Y. L. Chen (Fig. 11A, B), *I. cyanantha* Hook. f., *I. fenghwaiana* Y. L. Chen (Fig. 12A, B), *I. pseudo-kingii* Hand.-Mazz., *I. purpurea* Hand.-Mazz. (Fig. 13A–C), *I. radiata* Hook. f., *I. rectangula* Hand.-Mazz., *I. apsotis* Hook. f. (Fig. 14A, B) and *I. soulieana* Hook. f. (Fig. 15A, B). Seeds are 1.5–3.4 × 0.8–2.3 mm, ovate, subellipsoid to subspheroid, yellowish brown to dark grey in colour. Dense pits are seen on the inflated outer periclinal walls of epidermal cells of the seedcoats of *I. aquatilis* and *I. fenghwaiana* (Figs 10B, 12B). The seeds of *I. radiata* and *I. rectangula* (not shown) are very similar to those of *I. purpurea* (Fig. 13A), being 1.8–2.4 × 1.3–1.7 mm, subspheroid, yellowish brown to dark brown in colour, and their seedcoats have undulate surfaces with round aculeate protrusions covering the entire testa (Fig. 13B). Fine cuticular granules can be seen all over the surface (Fig. 13C). *Impatiens apsotis* and *I. soulieana* have similar seedcoats with irregular polygonal or rounded cells. Fine cuticle dust covers the entire surface (Figs 14, 15).

*Carinate subtype (Figs 16,17)*

The basic ornamentation of this subtype is very similar to the above. However, some of the seedcoat

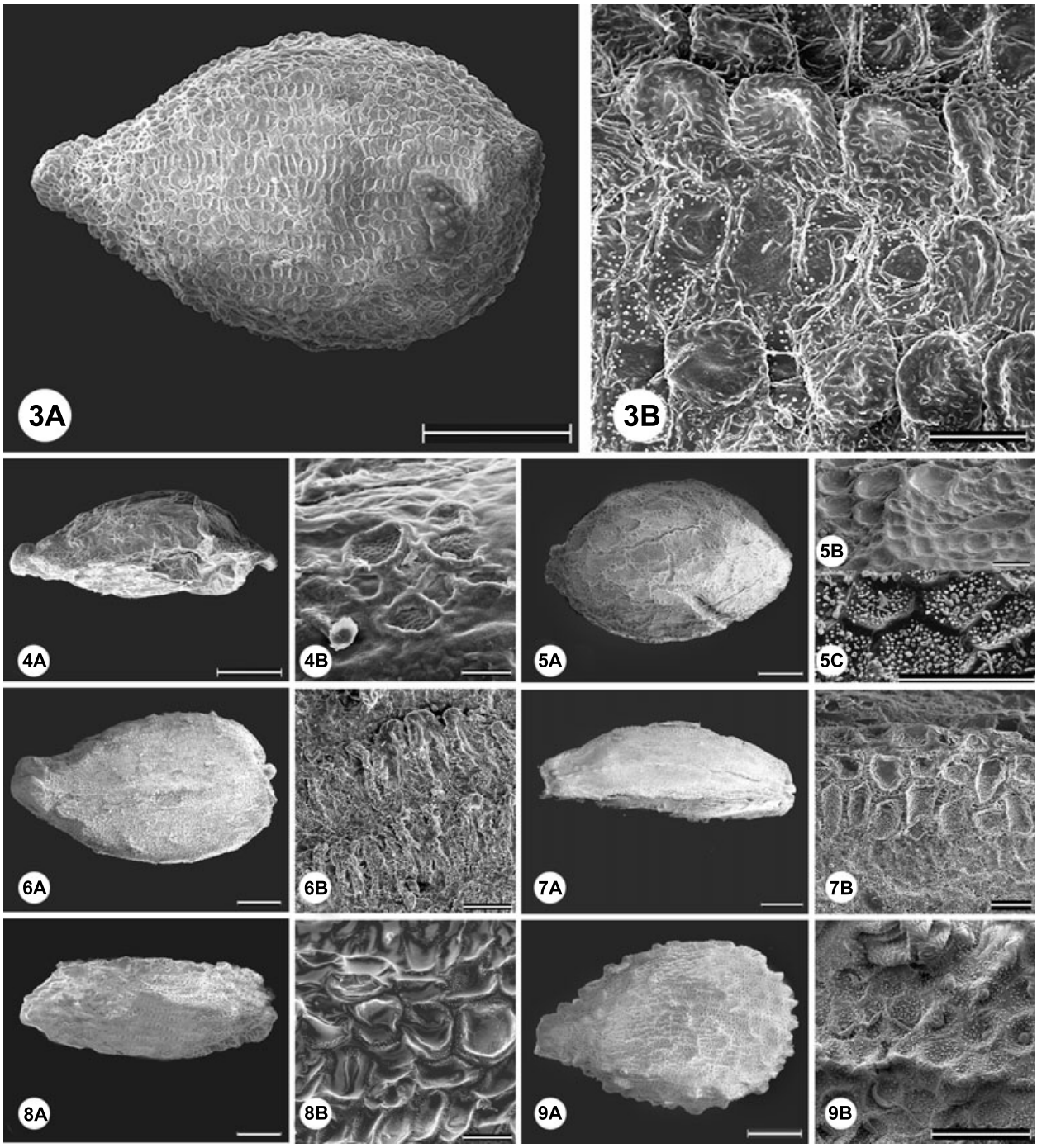
**Table 1.** List of the *Impatiens* species observed, the origin and voucher of the samples, and the seed characteristics (L/W*, Length/Width; †Type refers to the types and subtypes described in the text)

| | | | Seed characters | | | | | |
|---|---|---|---|---|---|---|---|---|
| Species | Origin | Voucher | Colour | Shape | Size (mm) | L/W* radio | Type† | Figure |
| *I. arguta* Hook. fl. et Thomson | Yunnan, China | CN2k-85 | black brown | subspheroid | 2.5–3.0 × 1.9–2.2 | 1.35 | 3.4 | 18 |
| *I. apsotis*. Hook. f. | Sichuan, China | CN2k2–159 | light brown-yellowish brown | ellipsoid | 2.0–2.2 × 1.2–1.3 | 1.62 | 3.2 | 14 |
| *I. aquatilis* Hook. f. | Yunnan, China | CN-Y017 | brown | subovate | 2.8–3.4x.1.8–2.2 | 1.55 | 3.2 | 10 |
| *I. aureliana* Hook. f. | Yunnan, China | CN2k1–56 | maroon-brown | subellipsoid | 1.8–2.8 × 1.1–1.8 | 1.47 | 4.3 | 27 |
| *I. bahanensis* Hand.-Mazz. | Yunnan, China | CN2k-30 | grey-brown | subellipsoid | 2.2–3.1 × 1.4–1.7 | 1.87 | 4.1 | 22 |
| *I. balfourii* Hook. f. | Himalayan origin, Bot. Gard. Neuch., Switzerland | NE1 | beige to grey | subellipsoid | 2.1–3.0 × 1.6–2.2 | 1.40 | 3.4 | 19 |
| *I. balsamina* L. | Yunnan, China | CN2k1-GSh | maroon–brown | subellipsoid | 2.2–2.9 × 1.5–2.2 | 1.30 | 2 | 2 |
| *I. begoniifolia* S. Akiyama et H. Ohba | Yunnan, China | CN2k1–51 | brown | subellipsoid | 1.7–2.2 × 1.1–1.6 | 1.46 | 4.3 | 25 |
| *I. chinensis* L. | Yunnan, China | CN2k1–49 | shiny black | globose | 1.7–2.0 × 1.4–1.7 | 1.27 | 1 | 1 |
| *I. chungtienensis* Y. L. Chen | Yunnan, China | CN2k2–179 | dark-grey | subellipsoid | 3.1–3.8 × 1.6–1.8 | 2.06 | 3.2 | 11 |
| *I. cyanantha* Hook. f. | Yunnan, China | CN2k1–84 | grey brown | subovate | 2.9–3.1 × 1.8–2.3 | 1.50 | 3.2 | |
| *I. cyathiflora* Hook. f. | Yunnan, China | CN2k-74 | yellowish brown | subellipsoid | 1.9–2.5 × 1.0–1.4 | 1.69 | 4.1 | |
| *I. davidi* Franch. | Fujian, China | CN2k-09 | yellow | ellipsoid | 2.1–2.2 × 1.1–1.5 | 1.50 | 4.1 | 21 |
| *I. delavayi* Franch. | Yunnan, China | CN2k-76 | yellowish brown | ellipsoid | 2.2–3.0 × 1.3–1.6 | 1.80 | 3.3 | 16 |
| *I. dolichoceras* E. Pritz. ex Diels | Guangxi, China | CN2k1–80 | yellowish brown | oblate ovate | 2.0–2.8 × 1.1–1.6 | 1.92 | 3.1 | 4 |
| *I. drepanophora* Hook. f. | Yunnan, China | CN2k-20 | dark-brown | subellipsoid | 2.2–2.9 × 1.3–1.7 | 1.67 | 4.1 | |
| *I. fenghwaiana* Y. L. Chen | Guangxi, China | CN2k1–78 | brown | ovate | 2.3–2.5 × 1.3–1.7 | 1.50 | 3.2 | 12 |

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| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *I. forrestii* Hook. f. et W. W. Sm. | Yunnan, China | CN2k-79 | dark-brown | globose | 2.2–2.9 × 2.0–2.4 | 1.18 | 3.1 | 5 |
| *I. gongshanensis* Y. L. Chen | Yunnan, China | CN2k-23 | dark-brown | ovate | 1.6–2.0 × 0.8–1.4 | 1.58 | 3.1 | 3 |
| *I. holocentra* Hand.-Mazz. | Yunnan, China | CN2k-54 | brown | ovate | 1.5–1.8 × 1.0–1.1 | 1.60 | 3.1 | |
| *I. infirma* Hook. f. | Sichuan, China | CN2k2–162 | black brown | long ovate | 3.1–3.8 × 1.9–2.1 | 1.85 | 3.1 | 6 |
| *I. mengtszeana* Hook. f. | Yunnan, China | CN2k1–60 | green-brown | spheroid | 1.4–1.9 × 1.0–1.5 | 1.31 | 4.3 | 26 |
| *I. noli-tangere* L. | Jilin, China | CN2k-86 | yellowish brown | long ellipsoid | 2.9–3.2 × 1.2–1.5 | 2.21 | 3.1 | 7 |
| *I. parviflora* DC. | Bern, Switzerland | Bern1 | beige–dark grey | ellipsoid | 4.1–4.5 × 2.0–2.1 | 2.10 | 3.4 | 20 |
| *I. poculifer* Hook. f. | Yunnan, China | CN2k2–209 | brown | subspheroid | 2.9–3.1 × 1.6–2.0 | 1.50 | 3.3 | 17 |
| *I. pseudo-kingii* Hand.-Mazz. | Yunnan, China | CN2k-31 | yellow | ovate | 1.5–2.0 × 0.8–1.0 | 2.13 | 3.2 | |
| *I. purpurea* Hand.-Mazz. | Yunnan, China | CN-Y007 | yellowish brown | subspheroid | 2.1–2.2 × 1.4–1.7 | 1.31 | 3.2 | 13 |
| *I. radiata* Hook. f. | Yunnan, China | CN2k-77 | dark brown | subspheroid | 2.1–2.4 × 1.3–1.6 | 1.47 | 3.2 | |
| *I. rectangula* Hand.-Mazz | Yunnan, China | CN2k1–17 | grey-brown | subspheroid | 1.8–2.0 × 1.3–1.6 | 1.33 | 3.2 | |
| *I. rostellata* Franch. | Sichuan, China | CN2k2–167 | blackish brown | oblate ellipsoid | 2.2–3.0 × 1.3–1.8 | 1.87 | 3.1 | 8 |
| *I. ruiliensis* S. Akiyama et H. Ohba | Yunnan, China | CN2k1–50 | dark yellow | subspheroid | 1.6–2.0 × 1.2–1.6 | 1.38 | 4.2 | 24 |
| *I. scabrida* DC. | Himalayan origin, Bot. Gard. Neuch., Switzerland | NE6 | dark yellow | ovate | 2.1–2.8 × 1.4–2.0 | 1.33 | 3.1 | 9 |
| *I. siculifer* Hook. f. | Yunnan, China | CN2k-80 | brown | ellopsoid | 2.2–3.0 × 1.4–2.1 | 1.50 | 4.1 | 23 |
| *I. soulieana* Hook. f. | Sichuan, China | CN2k2–163 | yellowish brown | subspheroid | 2.5–3.1 × 1.7–2.0 | 1.67 | 3.2 | 15 |
| *I. trichosepala* Y. L. Chen | Yunnan, China | CN2k1–68 | brown | subspheroid | 1.3–1.6 × 0.9–1.2 | 1.36 | 4.3 | |
| *I. walleriana* Hook. f. | African origin, Cult. Yunnan, China | CN-Y040 | yellowish brown | subspheroid | 1.7–2.0 × 1.2–1.4 | 1.46 | 4.4 | 28 |
| *I. xanthina* Comber | Yunnan, China | CN2k-17 | yellowish brown | ellipsoid | 1.3–1.5 × 0.6–0.9 | 1.75 | 4.4 | 29 |
| *I. yingjiangensis* S. Akiyama et H. Ohba | Yunnan, China | CN2k1–55 | light brown | spheroid | 1.4–1.7 × 1.1–1.4 | 1.25 | 4.3 | |

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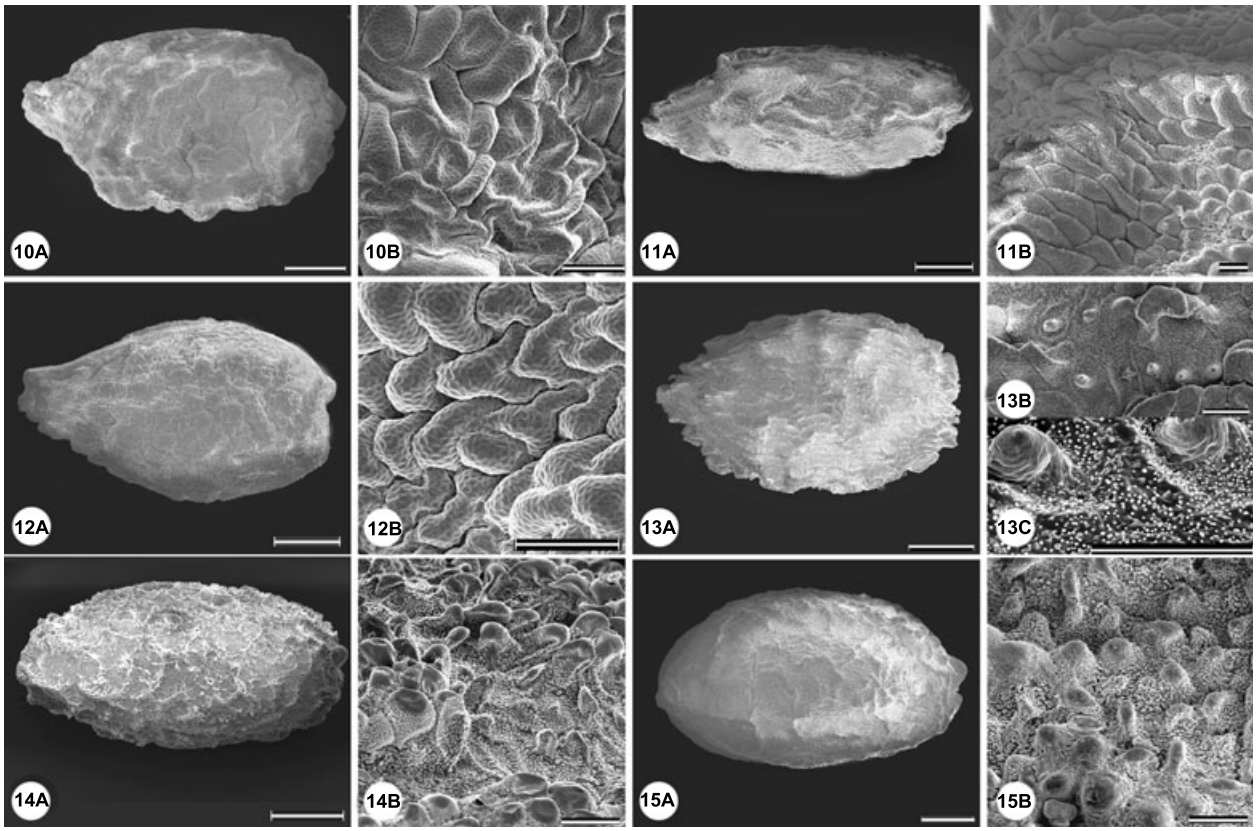



**Figures 3–9.** SEM micrographs of seedcoat of *Impatiens*: reticulate type – fine reticulate subtype. Fig. 3. *I. gongshanensis.* Fig. 4. *I. dolichoceras.* Fig. 5. *I. forrestii.* Fig. 6. *I. infirma.* Fig. 7. *I. noli-tangere.* Fig. 8. *I. rostellata.* Fig. 9. *I. scabrida.* A, entire seed. Scale bars = 0.5 mm. B, C, details of seedcoat. Scale bars = 0.05 mm.

epidermal cells are elevated significantly higher and arranged in ridges. This subtype is observed only in *I. delavayi* Franch. (Fig. 16A, B) and the closely related species, *I. poculifer* Hook. f. (Fig. 17A, B). Seeds are 2.2–3.1 × 1.3–2.0 mm, ellipsoid to subspheroid, yellowish brown to brown in colour. Both species also have a fine circle sculpture on the surface of the epidermal cells (Figs 16B, 17B).

*Striate subtype (Figs 18–20)*

The elevated epidermal cells are arranged in clear lines, as observed in *I. arguta* Hook. fl. et Thomson




**Figures 10–15.** SEM micrographs of seedcoat of *Impatiens*: reticulate type – colliculate subtype. Fig. 10. *I. aquatilis.* Fig. 11. *I. chungtienensis.* Fig. 12. *I. fenghwaiana.* Fig. 13. *I. purpurea.* Fig. 14. *I. apsotis.* Fig. 15. *I. soulieana.* A, entire seed. Scale bars = 0.5 mm. B, C, details of seedcoat. Scale bars = 0.05 mm.

(Fig. 18), *I. balfourii* Hook. f. (Fig. 19) and *I. parviflora* (Fig. 20). Seeds of these species are comparatively large, 2.1–4.5 × 1.6–2.2 mm, subspheroid to subellipsoid, black brown, beige to grey in colour. *Impatiens arguta* has rugose elevated cells on the seedcoat surface as pleats (Fig. 18A–C). *Impatiens balfourii* has colliculate or corrugate epidermal cells (Fig. 19A, B), while *I. parviflora* has elevated epidermal cells regularly arranged in lines isolated by nonelevated cells. Fine cuticular granules and pits are seen on the periclinal walls of the epidermal cells of the latter two species (Figs 19B, 20B).

### Protrusive type (Figs 21–29)

Some of the epidermal cells of the seedcoat protrude significantly to form projections of varied shapes. According to the shapes of the projections, the following four subtypes can be recognized.

*Digitiform subtype (Figs 21–23)*

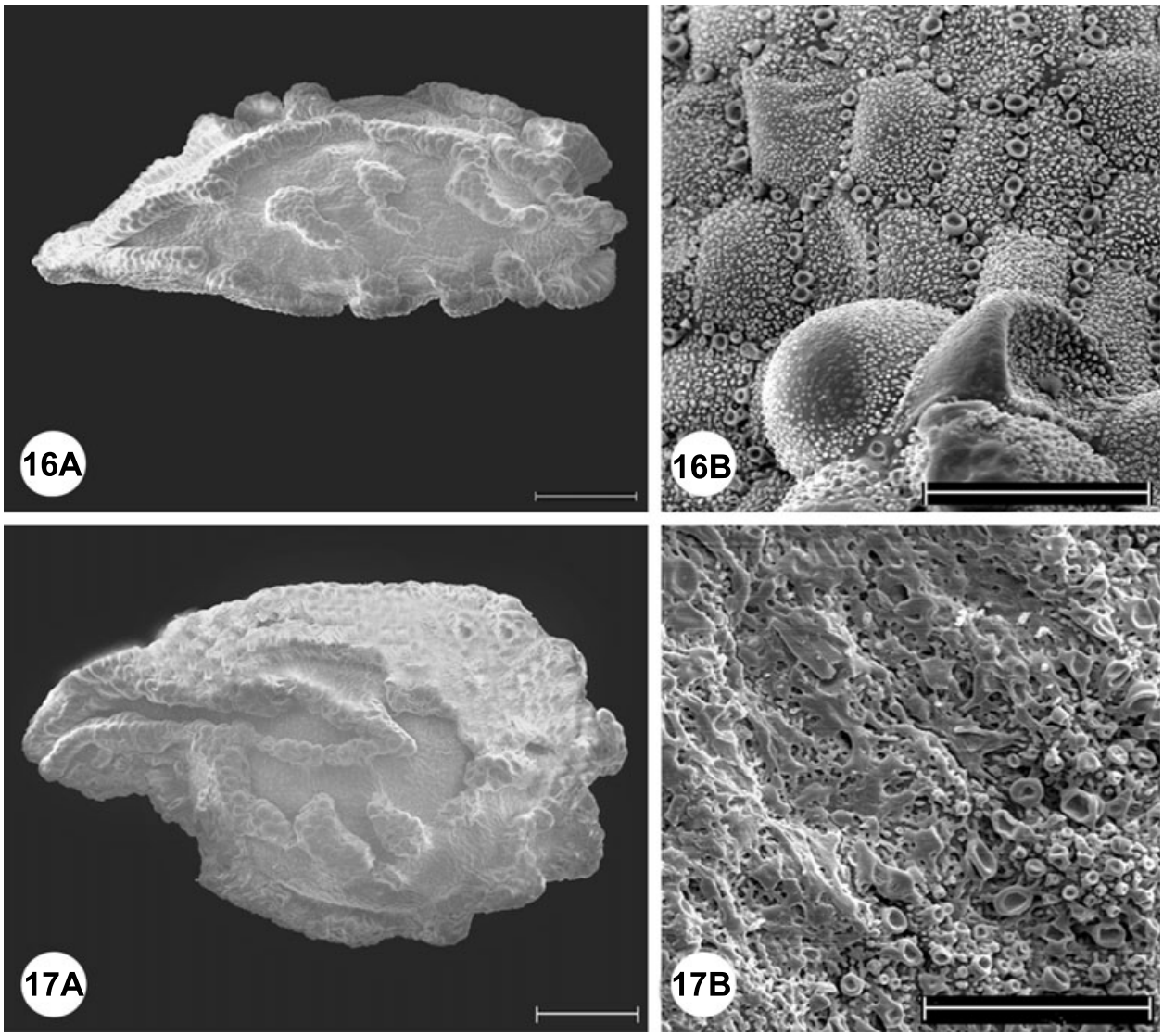
The protrusive epidermal cells of the seedcoat are finger-like, as shown by the species *I. davidi* Franch. (Fig. 21A), *I. bahanensis* Hand.-Mazz. (Fig. 22A), *I. drepanophora* Hook. f., *I. cyathiflora* Hook. f. and *I. siculifer* Hook. f. (Fig. 23A). Seeds are 1.9–3.1 × 1.0–2.1 mm, ellipsoid to subspheroid, yellow to brown in colour. In *I. davidi*, the protrusions have flat tops and numerous cuticular granules on their lateral surface (Fig. 21B), whereas in the other species of this group, the protrusions have numerous pits (Figs 22B, 23B).

*Clustered subtype (Fig. 24)*

As seen in *I. ruiliensis* S. Akiyama et H. Ohba, several spine-shaped projections cluster together (Fig. 24A). Seeds are 1.6–2.0 × 1.2–1.6 mm, subspheroid, and dark yellow in colour. Both the protrusive and flat epidermal cells have conspicuous pits (Fig. 24B).

*Squamalate subtype (Figs 25–27)*

The protrusions are scale-like. Squamalate protrusions sparsely but evenly cover the entire seedcoat, or they are denser near the hilum region. *Impatiens begoniifolia* S. Akiyama et H. Ohba (Fig. 25A), *I. yingjiangensis* S. Akiyama et H. Ohba, *I. mengtszeana* Hook. f. (Fig. 26A), *I. trichosepala* Y. L. Chen and *I. aureliana* Hook. f. (Fig. 27A) show




**Figures 16–17.** SEM micrographs of seedcoat of *Impatiens*: reticulate type – carinate subtype. Fig. 16. *I. delavayi.* Fig. 17. *I. poculifer.* A, entire seed. Scale bars = 0.5 mm. B, detail of seedcoat. Scale bars = 0.05 mm.

this kind of seedcoat. Seeds are 1.3–2.8 × 0.9–1.8 mm, spheroid, ellipsoid and subellipsoid, light to dark brown in colour. Cuticular granules are found mostly on the nonprotrusive cells, while pits are conspicuous on the squamalate projections (Figs 25B, 26B, 27B).

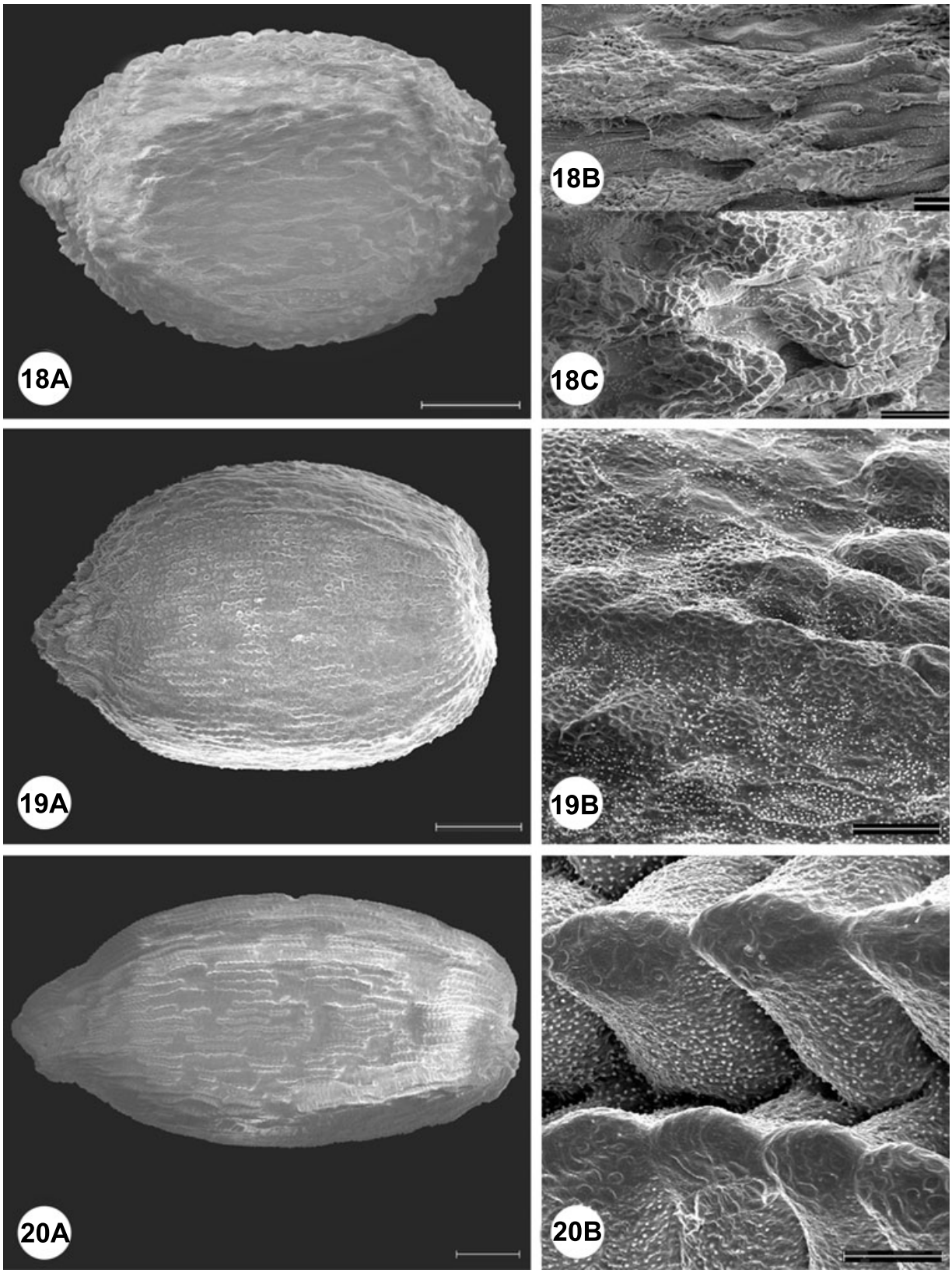
*Cristate subtype (Figs 28, 29)*

This subtype is similar to the above squamalate form, but the protrusions are more complex and are crest-like. Two species belong to this group: *I. walleriana* Hook. f. (Fig. 28A) and *I. xanthina* (Fig. 29A). Seeds are 1.3–2.0 × 0.6–1.4 mm, subspheroid to ellipsoid, and yellowish brown in colour. The seedcoat of *I. walleriana* is sparsely covered with cristate protrusions that have a reticulate sculpture, and fine cuticular granules densely cover the nonprotrusive epidermal cells (Fig. 28B). The protrusions of *I. xanthina* are spirally thickened, but no cuticular granules were seen (Fig. 29B).

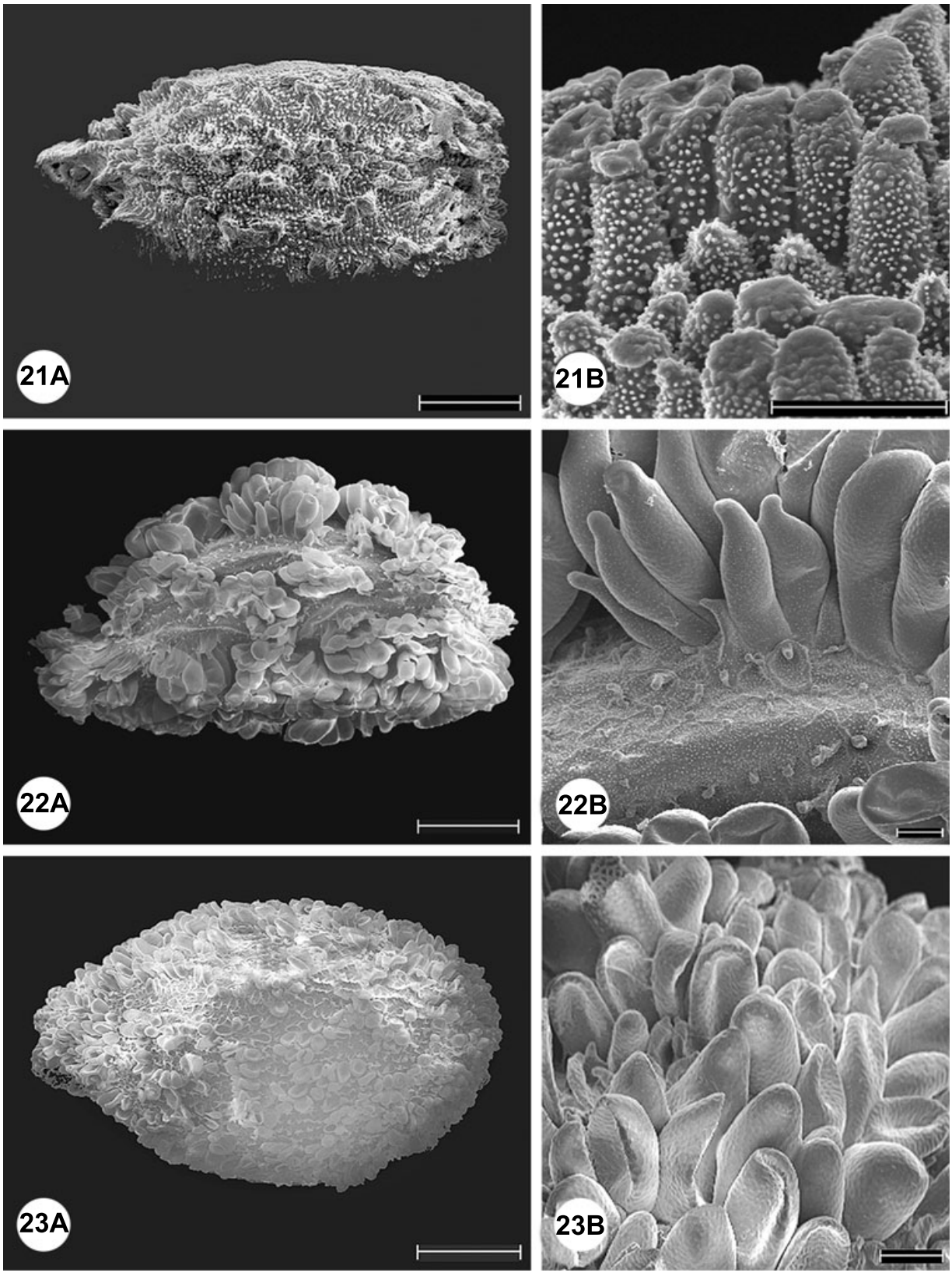
## DISCUSSION

### Diversity of seedcoat micromorphology in *Impatiens*

This examination of *Impatiens* species from China and other regions has revealed considerable diversity in seed size, shape, colour and seedcoat micromorphology. Four main types of seedcoat, laevigate, granulate, reticulate and protrusive, and detailed subdivisions of




**Figures 18–20.** SEM micrographs of seedcoat of *Impatiens*: reticulate type – striate subtype. Fig. 18. *I. arguta.* Fig. 19. *I. balfourii.* Fig. 20. *I. parviflora.* A, entire seed. Scale bars = 0.5 mm. B, C, details of seedcoat. Scale bars = 0.05 mm.




**Figures 21–23.** SEM micrographs of seedcoat of *Impatiens*: protrusive type – digiform subtype. Fig. 21. *I. davidi.* Fig. 22. *I. bahanensis.* Fig. 23. *I. siculifer.* A, entire seed. Scale bars = 0.5 mm. B, detail of seedcoat. Scale bars = 0.05 mm.

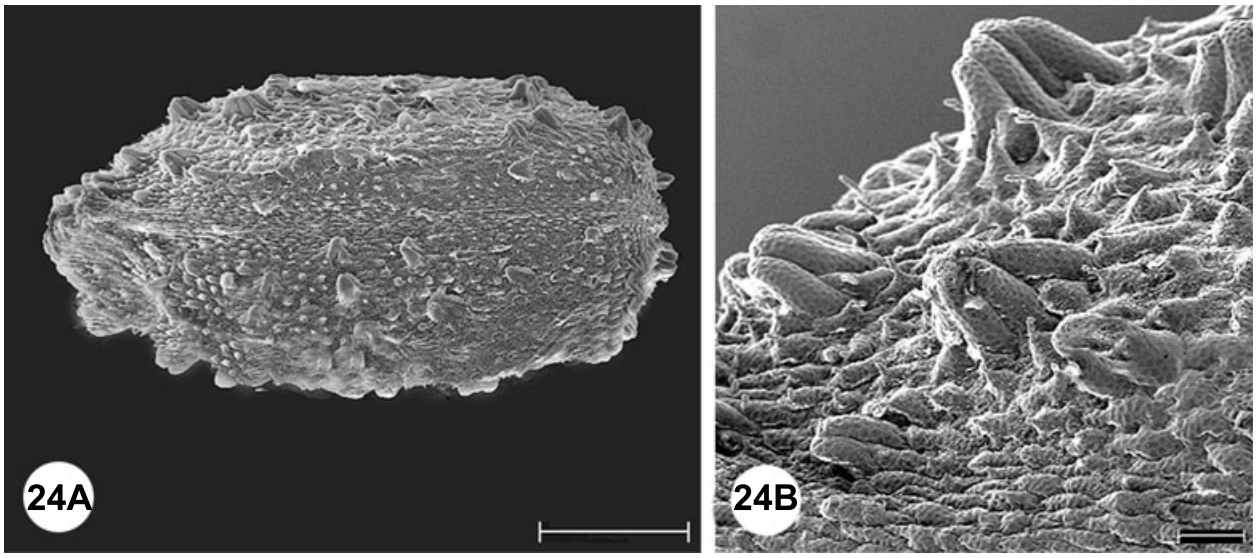
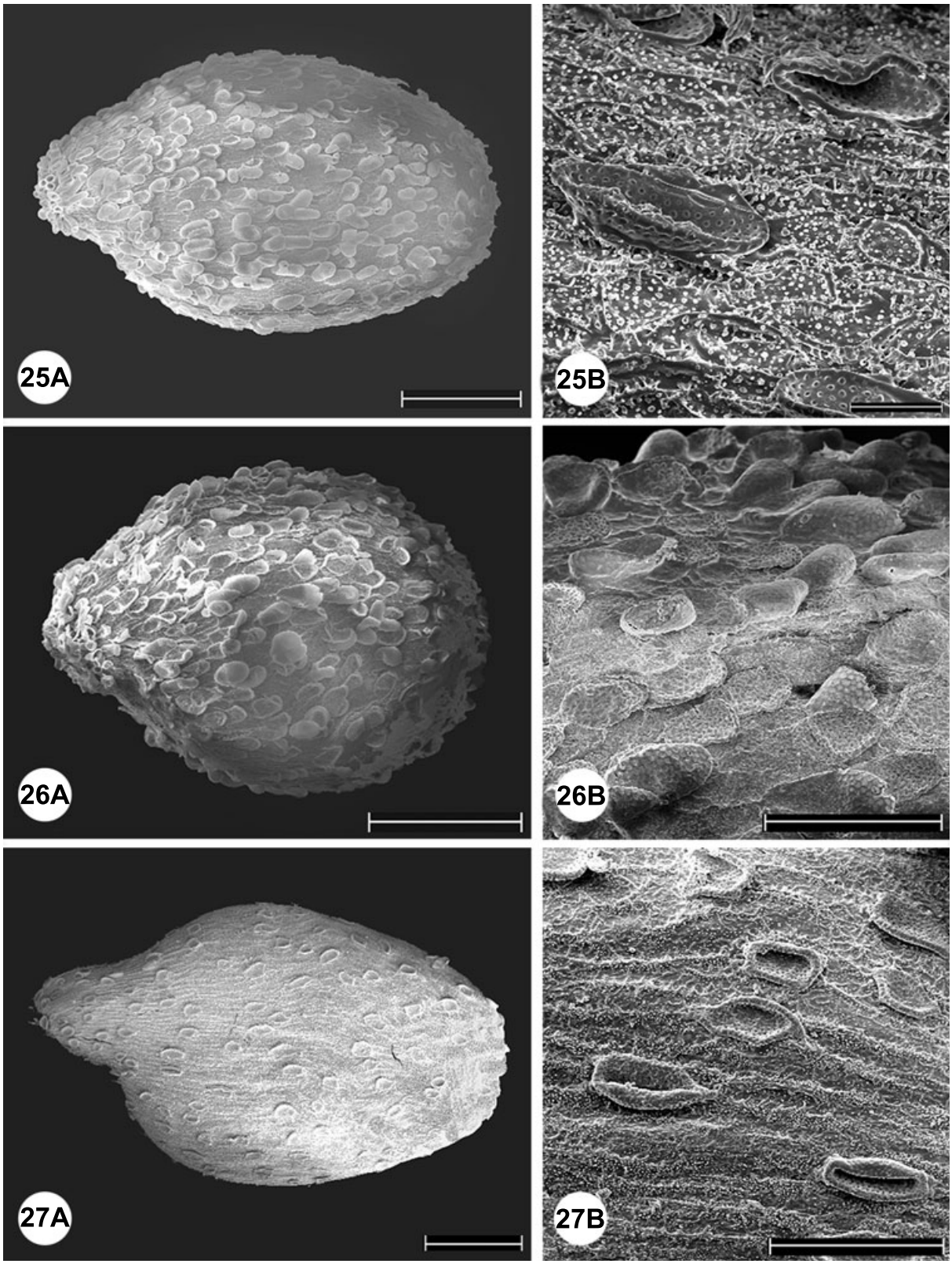



**Figure 24.** SEM micrographs of seedcoat of *Impatiens*: protrusive type – clustered subtype. Fig. 24. *I. ruiliensis.* A, entire seed. Scale bar = 0.5 mm. B, detail of seedcoat. Scale bar = 0.05 mm.

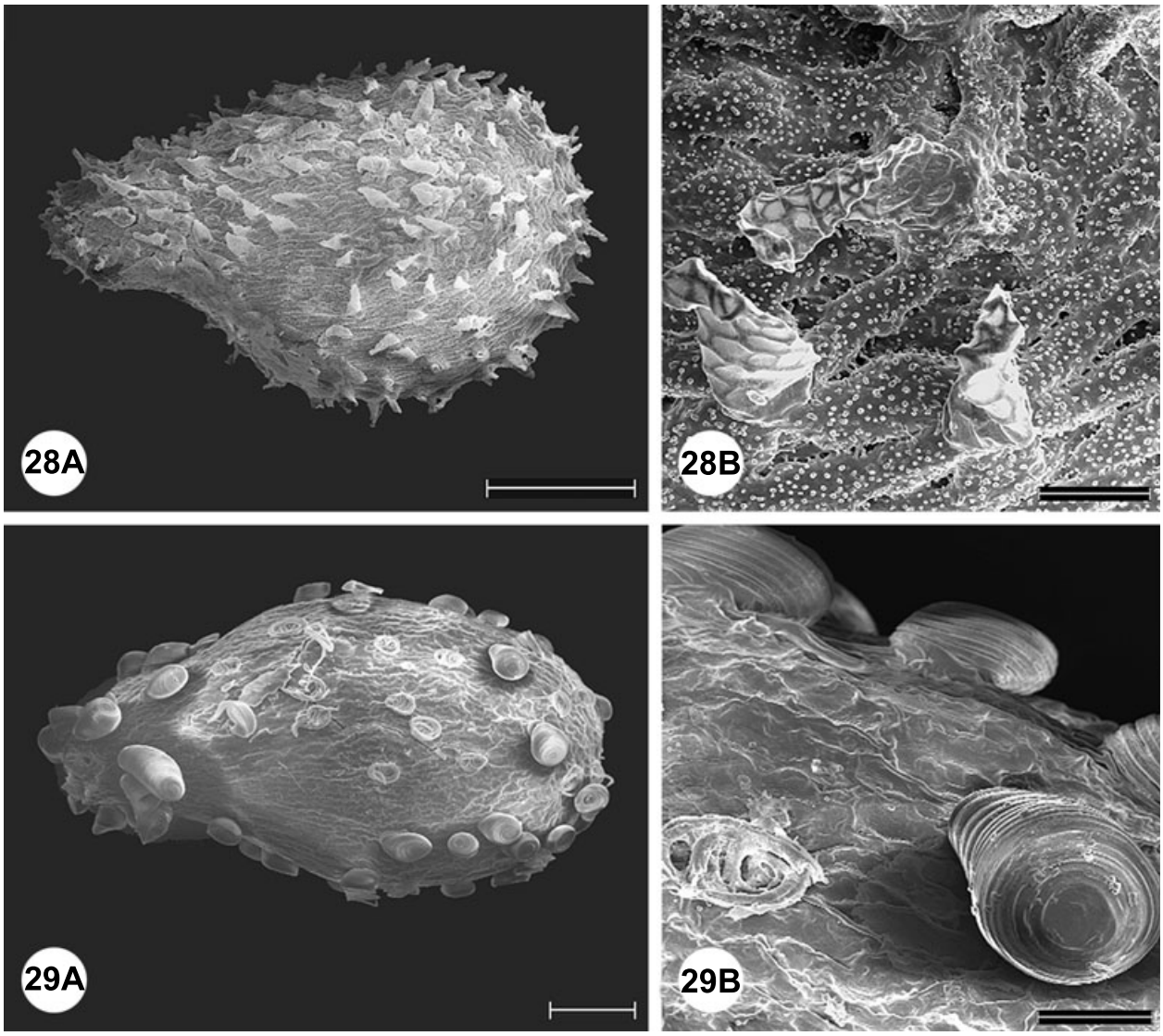
the reticulate and protrusive types, were distinguished according to the ornamentation of the seedcoat. The granulate type in *I. balsamina*, carinate subtype in *I. delavayi* and *I. poculifer*, striate subtype in *I. arguta*, *I. balfourii* and *I. parviflora*, digitiform subtype in *I. davidi*, *I. bahanensis*, *I. drepanophora*, *I. cyathiflora* and *I. siculifer*, and clustered subtype in *I. ruiliensis* were observed for the first time. Lu & Chen (1991) studied 12 species collected from Mt Omei, China. One species, *I. wilsonii* Hook. f., was described as having the laevigate seedcoat, which was found only in *I. chinensis* in this study and by Shimizu (1979). However, the micrographs of the species (Lu & Chen, 1991: plate 1, figs 1–6) appeared to represent the fine reticulate seedcoat we defined here. The remaining 11 species were described as having scabrous seedcoats, without further detailed classification of the types. The micrographs of these species show that they correspond to our reticulate and colliculate subtypes, except in *I. monticola* Hook. f. The seedcoat of *I. monicola* was described as having sieve-like discs that, in fact, correspond to our squamalate subtype of the protrusive category. Shimizu (1979) reported that the seed of the Thailand *I. violaeflora* Hook. f. has two types of projections, big and minute. The micrographs show that the morphology of the seedcoat of this species is very similar to the cristate subtype we observed in *I. walleriana* (Africa origin, cultivated in Yunnan, China): the big projections are the cristate sculpture while the minute projections seem to be the fine cuticular granules. In gross morphology, *I. violaeflora* is very similar to *I. aureliana* (distributed mainly in south-western China and Burma), which has a squamalate subtype of seedcoat. This suggests that the cristate and squamalate sculpture may have a similar origin. Our observations here on a limited number of species have revealed high diversity in seedcoat micromorphology. Nevertheless, we should note that the majority of the species of *Impatiens* remain to be studied. Further examination of more species, particularly of the African, Indian and Madagascan species, may reveal further diversity and seedcoat types. At that point, it will be possible to establish a more comprehensive scheme of terminology and classification of seedcoat micromorphology.

## Phylogenetic and taxonomic implications of seedcoat micromorphology

Previous studies have suggested possible taxonomic implications of seedcoat micromorphological characters (Shimizu, 1979; Lu & Chen, 1991). Our present observations on more species confirmed the possibility that types or subtypes of seedcoat can be diagnostic or indicative of phylogenetic relationships. For example, the granulate seedcoat is unique to *I. balsamina* within the species observed so far. *Impatiens delavayi* and *I. poculifer*, both with a unique carinate seedcoat, are very similar to each other in gross morphology (Chen, 2001). The species with a squamalate subtype seedcoat, including *I. aureliana*, *I. begoniifolia*, *I. mengtszeana*, *I. trichosepala* and *I. yingjiangensis*, and the species with a cristate subtype seedcoat represented by *I. xanthina* and *I. walleriana*, all characterized by having small and globose seeds, are limited to a lineage of species that have globose or broadly




**Figures 25–27.** SEM micrographs of seedcoat of *Impatiens*: protrusive type – squamalate subtype. Fig. 25. *I. begoniifolia.* Fig. 26. *I. mengtszeana.* Fig. 27. *I. aureliana.* A, entire seed. Scale bars = 0.5 mm. B, detail of seedcoat. Scale bars = 0.05 mm.




**Figures 28–29.** SEM micrographs of seedcoat of *Impatiens*: protrusive type – cristate subtype. Fig. 28. *I. walleriana.* Fig. 29. *I. xanthina.* A, entire seed. Scale bars = 0.5 mm. B, detail of seedcoat. Scale bars = 0.05 mm.

fusiform (length/diameter = 4) capsules (Grey-Wilson, 1980). Interestingly, species of this lineage were found to have lower basic chromosome numbers ($x$ = 8, 7, 6) (Song *et al*., 2003), and a molecular phylogenetic study based on the sequences of the internal transcribed spacers of ribosomic DNA confirmed the monophyly of the lineage (Yuan *et al*., 2004). Thus, the squamalate and cristate sculpture of seedcoats seems to be associated with the fruit shape. In addition to capsule shape, these species are very similar to each other in other aspects of morphology, e.g. all species have relatively large flowers *c.* 3.5–4 cm long, obtuse stigmata, and are slightly pubescent on the upper surface of the leaves. The only exceptions amongst the observed species are the two species with a somewhat specialized seedcoat sculpture, *I. balsamina* with a granulate seedcoat and *I. chinensis* with a laevigate seedcoat. These two species have globose or broadly fusiform capsules, but do not have a squamalate or cristate sculpture on their seedcoats.

Of the various seedcoat types, the reticulate is the most common, as shown by 18 observed species. These species occupied relatively basal positions on the molecular phylogenetic tree (Yuan *et al*., 2004), suggesting that the reticulate seedcoat might represent a comparatively ancestral primitive type. For example, *I. arguta* is one of the most basal species in the phylogenetic tree and has a striate type of seedcoat. On the other hand, the species with complex sculptures, such as squamalate and cristate, on their seedcoats

occurred in the more apical branches of the phylogenetic tree. Thus, they may represent more advanced types.

Based on the samples mainly collected from one of the most important diversity centres, our observations have confirmed high diversity of seedcoat micromorphology in *Impatiens* and allowed development of a preliminary scheme to describe such diversity. Some important correlations among the micromorphological characters of the seedcoat and gross morphological characters have been identified. The sculpture on seedcoats thus offers a set of characters useful for the taxonomy of the genus. However, species from other diversity centres, such as southern India, tropical Africa and Madagascar, are needed for a similar study, that will allow widely applicable conclusions to be drawn. Meanwhile, studies on the developmental variation of seedcoat sculpture, which may provide insights into the better understanding of the evolutionary relationships among different types of sculpture, are also urgently needed.

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## REFERENCES

**Abdel Khalik K, Van der Maesen LJG. 2002.** Seed morphology of some tribes of Brassicaceae (implications for taxonomy and species identification for the flora of Egypt). *Blumea* **47:** 363–383.

**Bobrov AVFC, Melikian AP, Yembaturova EY. 1999.** Seed morphology, anatomy and ultrastructure of *Phyllocladus* L. C. & A-Rich. ex Mirb. (Phyllocladaceae (Plig.) Bessey) in connection with the generic system and phylogeny. *Annals of Botany* **83:** 601–618.

**Chen Y-L. 2001.** Balsaminaceae. In: Chen Y-L, ed. *Flora Reipublicae Popularis Sinicae*, Tomus 47 (2). Beijing: Science Press, 1–243.

**Fischer E, Rahelivololona ME. 2002.** New taxa of *Impatiens* (Balsaminaceae) from Madagascar. I. *Adansonia* **24:** 271–294.

**Fischer E, Rahelivololona ME. 2003.** New taxa of *Impatiens* (Balaminaceae) from Madagascar. II. A collection from Masoala Peninsula. *Adansonia* **25:** 17–31.

**Grey-Wilson C. 1980.** *Impatiens of Africa*. Rotterdam: A. A. Balkema.

**Hooker JD, Thomson T. 1860.** Praecursores ad floram Indicam. *Journal of the Linnean Society* **4:** 106–157.

**Lu Y-Q, Chen Y-L. 1991.** Seed morphology of *Impatiens* L. (Balsaminaceae) and its taxonomic significance. *Acta Phytotaxonomica Sinica* **29:** 252–257.

**Martinez-Ortega MM, Rico E. 2001.** Seed morphology and its systematic significance in some *Veronica* species (Scrophulariaceae) mainly from the Western Mediterranean. *Plant Systematics and Evolution* **228:** 15–32.

**Mendum M, Lassnig P, Weber A, Christie F. 2001.** Testa and seed appendage morphology in *Aeschynanthus* (Gesneriaceae): phytogeographical patterns and taxonomic implications. *Botanical Journal of the Linnean Society* **135:** 195–213.

**Pfosser M, Wetschnig W, Ungar S, Prenner G. 2003.** Phylogenetic relationships among genera of *Massonieae* (Hyacinthaceae) inferred from plastid DNA and seed morphology. *Journal of Plant Reservation* **116:** 115–132.

**Shimizu T. 1979.** A comment on the limestone flora of Thailand, with special reference to *Impatiens*. *Acta Phytotaxonomica et Geobotanica* **30:** 180–188.

**Song Y, Yuan Y-M, Küpfer P. 2003.** Chromosomal evolution in Balsaminaceae with cytological observations on 45 species from Southeast Asia. *Caryologia* **56:** 463–481.

**Yildiz K. 2002.** Seed morphology of Caryophyllaceae species from Turkey (North Anatolia). *Pakistan Journal of Botany* **34:** 161–171.

**Yuan Y-M, Song Y, Geuten K, Rahelivololona E, Wohlhauser S, Fisher E, Smets E, Küpfer P. 2004.** Phylogeny and biogeography of Balsaminaceae inferred from ITS sequences. *Taxon* **53:** 391–403.

# Paper II

# Chromosomal evolution in Balsaminaceae, with cytological observations on 45 species from Southeast Asia

Song Y., Yuan Y.-M. and Küpfer P.

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# Chromosomal evolution in Balsaminaceae, with cytological observations on 45 species from Southeast Asia

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**Abstract** - Balsaminaceae consists of two genera, *Hydrocera* with only one species *H. triflora* and *Impatiens* with over 900 species. The chromosome number of *H. triflora* was consistently reported as 2*n*=16 or *n*=8. The somatic chromosome numbers vary greatly from 2*n*=6 to 2*n*=66 in *Impatiens*. In order to provide more complete information to understand the chromosomal evolution and cytogeography of Balsaminaceae, we counted chromosome numbers for 45 species of *Impatiens* from southwest China and the adjacent areas. Chromosome numbers were confirmed for 11 species, and numbers different from previous reports were found for two species. 32 species were examined for the first time, and the numbers 2*n*=12, 14, 16, 18, 20, 40, 54 (or the relevant gametic numbers) were found. The number *n*=27 found in *I. pseudokingii* is new for the family. The number 2*n*=18, mostly involving a bimodal karyotype with one pair of chromosomes conspicuously longer than others, is predominant among the species studied. Considering all the available chromosomal data, x=7, 8, 9, 10 are the most frequent basic numbers of the family. Previous authors have suggested x=7 or x=8 to be ancestral. Based on the present data, we suppose that x=8, x=9, or x=10 are all possible candidates of the ancestral basic numbers in *Impatiens*. Geographic distributions of the most frequent basic numbers show interesting patterns: x=7 and 8 occur in Africa, x=7, 8, 10 in Southern India and Sri Lanka, x=7, 9, 10 in the Himalayas, x=7, 8, 9, 10 in Southeast Asia, and x=10 in northern Asia, Europe and North America.

**Key words**: Balsaminaceae; *Hydrocera*; *Impatiens*; chromosome numbers; cytogeography.

## INTRODUCTION

Balsaminaceae is a diversified family with two genera: *Hydrocera* and *Impatiens*. Other generic names such as: *Petalonema, Semeiocardium* and *Impatientella* are confirmed to be synonyms of *Impatiens* (GREY-WILSON 1980a; RAO *et al*. 1986). *Hydrocera*, has only one species, *H. triflora* (L.) Wight et Arn., a semi-aquatic herb native to the Indo-Malesian countries. It can be readily distinguished from *Impatiens* by its five free petals and indehiscent berry. *Impatiens* is a large genus with annual or perennial species distributed primarily in the highlands and mountains of the Old-World tropical and subtropical regions. GREY-WILSON (1980a) estimated conservatively the total number of species to be around 850, but it perhaps has more, as many new species are still being described from different regions such as southwestern China and Madagascar (e. g. AKIYAMA *et al*. 1995, 1996b; FISCHER and

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Table 1 – The chromosome numbers observed, and the origins of materials and voucher specimens. The chromosome numbers reported for the first time are marked with "*", the number different from previous reports is marked with "!", and the numbers confirmed some of the previous reports but different from other reports are marked with "◊".

| Taxon | Coll. No. | Origin and altitude | Chromosome Number |
|---|---|---|---|
| *I. acehensis* C. Grey-Wilson | AF1 | Gunung Sinabung, Indonisia | $2n = 14$* |
| *I. aquatilis* Hook. f. | cn2k1-75 | Kunming, Yunnan, China, 2100m | $n = 9$* |
| *I. apsotis* Hook. f. | cn2k2-159 | Kangding, Sichuan, China, 2915m | $n = 9$* |
| *I. arguta* Hook. f. et Thomas. | cn2k-52 | Gongshan, Yunnan, China, 2600 m | $n = 10$◊ |
| *I. aureliana* Hook. f. | cn2k1-63 | Longchuan, Yunnan, China, 1700m | $n = 6$ |
| *I. bahanensis* Hand.-Mazz. | cn2k-30 | Gongshan jidu, Yunnan, China, 2550m | $n = 9$* |
| *I. bicornuta* Wall. | cn2k-55 | Gongshan, Yunnan, China, 2600m | $2n = 18$◊ |
| *I. chimiliensis* Comber | cn2k-51 | Gongshan, Yunnan, China, 3500m | $2n = 18$* |
| *I. chinensis* L. | cn2k1-49 | Tengchuan, Yunnan, China, 1630m | $n = 8$ |
| *I. chungtienensis* Y. L. Chen | cn2k2-179 | Zhongdian, Yunnan, China, 2700m | $n = 9$* |
| *I. crassicaudex* Hook. f. | cn2k2-104 | Batang, Sichuan, China, 3800m | $n = 9$* |
| *I. cyathiflora* Hook. f. | cn2k1-74 | Kunming, Yunnan, China, 2100m | $2n = 18$* |
| *I. delavayi* Franch. | cn2k-76 | Dali, Yunnan, China, 2650m | $2n = 20$* |
| | cn2k2-14 | Zhongdian, Yunnan, China, 3160m | $n = 10$* |
| *I. desmantha* Hook. f. | cn2k2-30 | Zhongdian, Yunnan, China, 3410m | $n = 9$* |
| *I. dolichoceras* Pritz. ex Diels | cn2k1-80 | Nanning, Guangxi, China, 1519m | $2n = 18$* |
| *I. drepanophora* Hook. f. | cn2k1-39 | Longling, Yunnan, China, 1580m | $n = 9$! |
| | cn2k-16 | Fugong, Yunnan, China, 1335m | $n = 9$! |
| | cn2k-20 | Gongshan, Yunnan, China, 1433m | $n = 9$! |
| *I. eubotrya* Miq. | AF2 | Gunung Merapi, Indonisia, 2502m | $2n = 18$! |
| *I. fenghwaiana* Y. L. Chen | cn2k1-78 | Nanning, Guangxi, China, 1519m | $2n = 18$* |
| *I. gongshanensis* Y. L. Chen | cn2k-26 | Gongshan, Yunnan, China, 2040m | $n = 10$* |
| *I. holocentra* Hand.-Mazz. | cn2k-54 | Gongshan, Yunnan, China, 2700m | $n = 9$* |
| *I. imbecilla* Hook. f. | Hao 426 | Omei Mt., Sichuan, China | $2n = 20$* |
| *I. infirma* Hook. f. | cn2k2-60 | Xiangcheng, Sichuan, China, 2950m | $n = 9$* |
| *I. lecomtei* Hook. f. | cn2k2-202 | Lijiang, Yunnan, China, 2900m | $2n = 18$* |
| *I. mengtszeana* Hook. f | cn2k1-60 | Longchuan. Yunnan, China, 1450m | $n = 8, 9$ |
| | cn2k2-214 | Jiangcheng, Yunnan, China, 1100m | $n = 8$ |
| *I. microcentra* Hand.-Mazz. | cn2k-50 | Gongshan, Yunnan, China, 2800m | $n = 9$* |
| *I. napoensis* Y. L. Chen | cn2k1-61 | Longchuan, Yunnan, China, 1450m | $n = 8$* |
| *I. oxyanthera* Hook. f. | 00-s008 | Omei Mt., Sichuan, China | $n = 7$* |
| *I. poculifer* Hook. f. | cn2k2-209 | Weixi, Yunnan, China, 3000m | $2n = 20$* |
| *I. principis* Hook. f. | 01-yjp006 | Lushui, Yunnan, China | $n = 9$* |
| *I. pseudokingii* Hand.-Mazz. | cn2k-29 | Gongshan, Yunnan, China, 2550m | $n = 27$* |
| *I. radiata* Hook. f. | cn2k1-43 | Tengchun, Yunnan, China, 2097m | $2n = 18$◊ |
| *I. rectangula* Hand.-Mazz. | cn2k-19 | Gongshan, Yunnan, China, 1433m | $n = 9$* |
| | cn2k-28 | Gongshan, Yunnan, China, 2050m | $n = 9$* |
| | cn2k1-17 | Gongshan, Yunnan, China, 1580m | $2n = 18$* |
| *I. rostellata* Franch. | cn2k2-167 | Shimian, Sichuan, China, 2245m | $n = 10$* |
| *I. rubrostriata* Hook. f. | cn2k1-44 | Tengchun, Yunnan, China, 2097m | $2n = 20$◊ |
| *I. scutisepala* Hook. f. | cn2k-56 | Gongshan, Yunnan, China, 2500m | $n = 9$* |
| *I. siculifer* Hook. f. | cn2k2-215 | Jiangcheng, Yunnan, China, 1200m | $n = 9$ |
| *I. stenantha* Hook. f. | 01-yjp008 | Lushui, Yunnan, China | $n = 9$ |
| *I. taronensis* Hand.-Mazz. | cn2k-57 | Gongshan, Yunnan, China, 2600m | $2n = 18$* |
| *I. tenuibracteata* Y. L. Chen | 01-yjp003 | Lushui, Yunnan, China | $n = 9$* |
| *I. tortisepala* Hook. f. | cn2k2-166 | Shimian, Sichuan, China, 2245m | $n = 10$* |
| *I. trichocepala* Y. L. Chen | cn2k1-68 | Longling, Yunnan, China, 1260m | $n = 7$* |
| *I. uliginosa* Franch. | 99-174 | Binchuan, Yunnan, China, 2200m | $n = 9$ |
| | cn2k2-173 | Huaping, Yunnan, China, 2150m | $n = 9$ |
| *I. uniflora* Hayata | AF3 | Taiwan, China, 2565m | $n = 20$* |
| *I. xanthina* Comber | cn2k1-35 | Gongshan, Yunnan, China, 2070m | $n = 7$* |
| *I. yingjiangensis* S. Akiyama et H. Ohba | cn2k1-55 | Yingjiang, Yunnan, China, 1435m | $n = 8$ |

RAHELIVOLOLONA 2002). Five conspicuous diversification centres in the paleotropical regions can be recognized: tropical Africa ca. 109 spp. (GREY-WILSON 1980a); Madagascar ca. 120 spp. (FISCHER and RAHELIVOLOLONA 2002); southern India and Sri Lanka ca. 150 spp., the eastern Himalayas ca. 120 spp., and Southeast Asia area in its broad sense (including Burma, Thailand, southwest China, Indochina peninsula, and the Malesia archipelagos) ca. 250 spp. High proportion of endemic species are found in these centres. For example, as many as 91% of the southern Indian species are endemic (CHATTERJEE 1940; RAO *et al.* 1986). On the contrary to the paleotropical areas, only a few species of *Impatiens* are represented in the temperate areas in northern Asia, Europe and North America. There is notably no native species in South America and Australia.

Chromosomal variation contributes to the processes of species evolution (HONG 1990; STEBBINS 1971). Knowledge of chromosomal evolution in Balsaminaceae stands as an important aspect in understanding the extraordinary diversity, endemism, and biogeography of the family. About 157 species of *Impatiens* from most of the distribution areas have been karyologically studied (Table 2) prior to our present study. Some important observations include SMITH (1934), KHOSHOO (1955a, b, 1956, 1957, 1966), JONES and SMITH (1966), BHASKAR (1976, 1980), LARSEN (1981), ZINOV'EVA-STAHEVITCH and GRANT (1984,1985), RAO *et al.* (1986), GOVINDARAJAN and SUBRAMANIAN (1986), AKIYAMA *et al.* (1992, 1993, 1996a), SUGAWARA *et al.* (1994, 1997), etc. The following somatic chromosome numbers have been reported: $2n$ = 6, 8, 10, 12, 14, 15, 16, 17, 18, 19, 20, 24, 26, 28,30, 32, 34, 36, 40, 44, 48, 50, 56, 66. The lowest chromosome number $2n$ = 6 was found in *I. latifolia* and *I. leschenaultii* from southern India (GOVINDARAJAN and SUBRAMANIAN 1986), and the highest number $2n$ = 66 was found in *I. mooreana* from New Guinea (JONE and SMITH 1966). The most frequent chromosome numbers encountered so far are $2n$ = 16 (55 taxa), $2n$ = 18 (49 taxa), $2n$ = 20 (45 taxa), $2n$ = 14 (42 taxa), $2n$ = 12 (18 taxa) and $2n$ = 32 (10 taxa). The other numbers are represented by less than 5 taxa. As to the evolution of the basic chromosome numbers, KHOSHOO (1957) proposed that 7 and/or 10 were the ancestral basic numbers of *Impatiens*. JONES and SMITH (1966) and AKIYAMA *et al.* (1992) suggested x = 7 to be the ancestral type from which the other numbers were derived by mainly ascending dysploidy, whereas RAO *et al.* (1986) suggested that x = 8 might be the original basic number from which the other numbers, lower or higher, were derived by both descending and ascending dysploidy.

So far, the available karyological studies on *Impatiens* were mostly carried out on Indian and African species. Although the mainland Southeast Asia and the adjacent Sino-Himalayan area represent the biggest diversification centre of the family, only a few studies were done on the species growing in these regions. For example, about 220 *Impatiens* species are so far recognized from China (CHEN 2001), highly concentrated in the southwestern part (ca. 90 spp. in Yunnan Province), but chromosome numbers have been counted for 21 species only (SUGAWARA *et al.* 1994, 1997). The chromosome data for the majority of species are still not available. In the present paper, we present the results of our recent observations on chromosome numbers of Chinese and other Asian species, in order to provide complementary karyological information for this family. By incorporating our present results into a thorough survey of previous studies, a review of the chromosome numbers and the possible evolutionary relationships among different basic numbers are also presented.

## MATERIALS AND METHODS

Most of the species observed were collected from southwest China. The species examined, their origin and voucher specimen are listed in Table 1, together with the corresponding chromosome numbers. Voucher specimens were deposited in the herbarium of the University of Neuchâtel, Switzerland (NEU).

Chromosome number determinations were made from flower buds fixed in Carnoy fluid (99% ethanol - glacial acetic acid, 3:1 v/v). After washing thoroughly with 70% ethanol, the flower buds were stained in alcohol-HCL-carmine (SNOW 1963) for about 48 hrs at 60°C. Then the stained flower buds were heated in 45% acetic acid in a ceramic cup for 2-3 minutes. The anthers or young ovaries were then squashed in the standard way and observed using a light microscope. Chromosome numbers were counted from the meiosis of pollen mother cells, or from mitosis in pollen or ovary somatic cells. Drawings were made with a camera lucida apparatus using temporary slides.

## RESULTS

52 samples representing 45 species of *Impatiens* collected from southwest China, Taiwan and Sumatra were examined for their chromosome numbers. The results are shown in Table 1. The gametic numbers $n = 6, 7, 8, 9, 10, 20, 27$ or the corresponding somatic numbers were found (Fig. 1 A through I). The number $n = 27$, new for the Balsaminaceae, was found in *I. pseudokingii* (Fig. 1G). The number $n = 9$ (or $2n = 18$) appeared most frequently in 26 species observed, followed by the number $n = 10$ (or $2n = 20$) which was counted in 8 species. The numbers $n = 7$ (or $2n = 14$) and $n = 8$ (or $2n = 16$) were found in 4 species respectively, and both $n = 6$ and $n = 27$ were found only in one species respectively. The chromosome numbers for 32 species are reported here for the first time (see the species marked with an asterisk in Table 1). For seven species, *I. aureliana* $n = 6$ (Fig 1A), *I. chinensis* $n = 8$, *I. mengtszeana* $n = 8$ and 9 (Fig.1H-I), *I. siculifer* $n = 9$, *I. stenantha* $n = 9$, *I. uliginosa* $n = 9$, and *I. yingjianensis* $n = 8$, our results confirmed all previous reports for them (SUGAWARA *et al.* 1994, 1997; AKIYAMA *et al.* 1996a; RAO *et al.* 1986; LARSEN 1981; STAHEVITCH 1995). For four species our results confirmed some previous

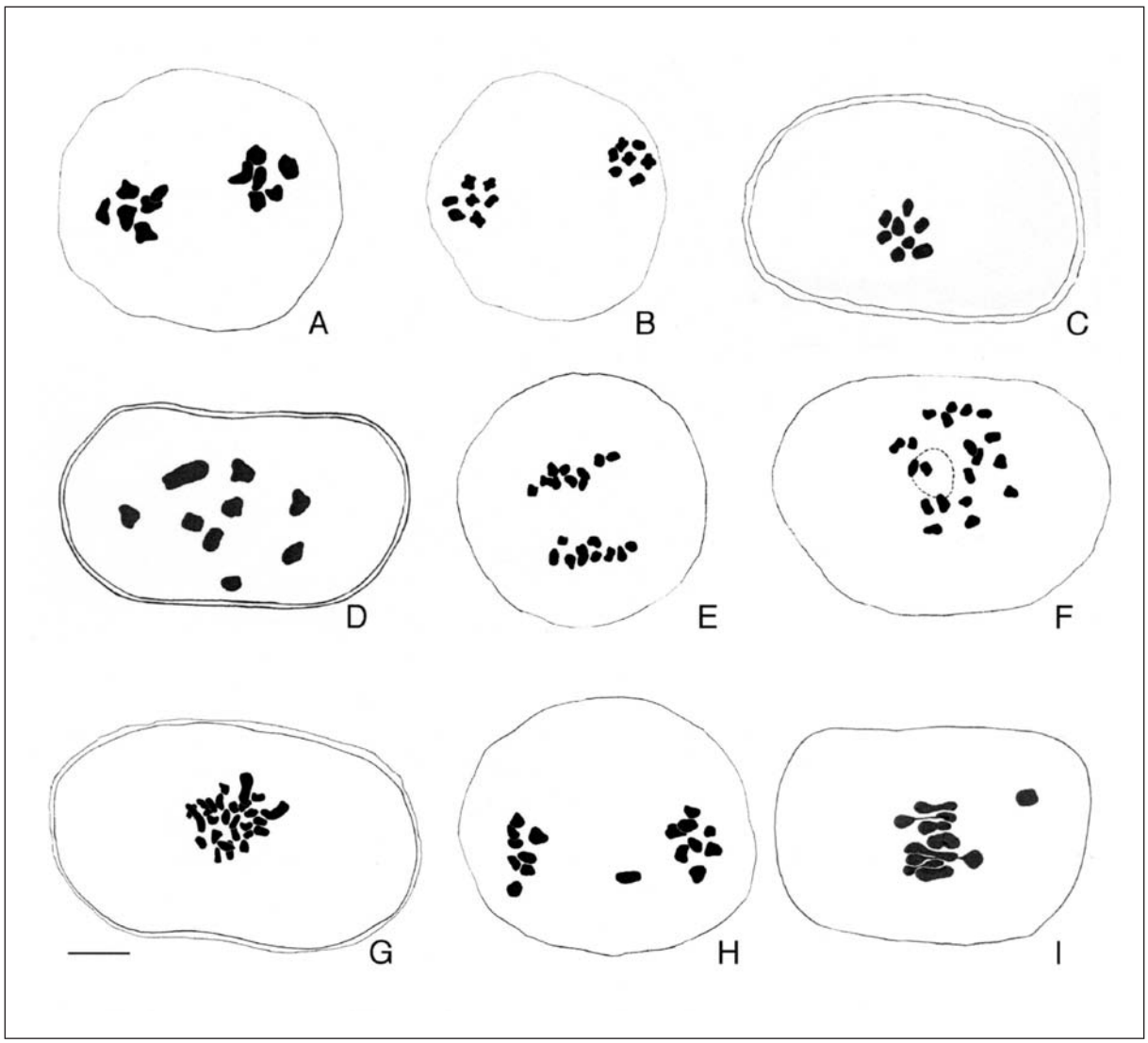



Fig. 1 – Drawings of chromosomes of selected species. A: meiosis of *I. aureliana* ($n = 6$); B: meiosis of *I. trichosepala* ($n = 7$); C: pollen mitosis of *I. nopoensis* ($n = 8$); D: pollen mitosis of *I. radiata* ($n = 9$); E: meiosis of *I. arguta* ($n = 10$); F: meiosis of *I. uniflora* ($n = 20$); G: pollen mitosis of *I. pseudokingii* ($n = 27$); H and I: meiosis of *I. mengtseana* showing one unpaired chromosome and the unbalanced segregation ($2n = 16 + 1$). Bar 5 μm.

reports but were different from some other reports: *I. arguta* (present result $n = 10$, previous reports $n = 6$, 9, or 10) (Fig. 1E), *I. bicornuta* (present $2n = 18$, previous $n = 8$ or $2n = 18$), *I. radiata* (present $2n = 18$, previous $n = 10$ or $2n = 18$) (Fig. 1D), and *I. rubrostriata* (present $2n = 20$, previous $2n = 20$ or 40). A different number, $2n = 18$, was found for *I. eubotrya* from Sumatra which was previously reported as $2n = 28$, and a different number $n = 9$ was found for *I. drepanophora* which was previously reported as $n = 10$. The number $n = 27$, new for the family, was found in *I. pseudokingii*, with three chromosomes conspicuously longer than others in the pollen mitoses suggesting the species as a hexaploid. Intraspecific variation was found in *I. mengtszeana* only, where unbalanced gametes with the numbers of $n = 8$ and $n = 9$ were observed from the mitosis of pollen cells in one population (Fig. 1H-I), whereas only $n = 8$ was observed in another population.

Most of our observations were made on meiosis of pollen mother cells. Therefore, we do not obtain much of karyomorphological data. Nevertheless, noticeably almost all the species we observed with $n = 9$ or $2n = 18$ showed a bimodal karyotype structure: one pair of chromosomes (or one in gamete) is distinctly longer than other chromosomes, for examples, *I. drepanophora* (Fig. 2D), *I. holocentra* (Fig. 2B), *I. radiata* (Fig. 1D), *I. rectangula* (Fig. 2C), etc. In the possible hexaploid *I. pseudokingii* ($n = 27$), three distinctively longer chromosomes were found in its gamete (Fig. 1G). Such bimodal karyotype structure seems to be specific to the species with basic number x = 9. All the species we observed with basic number x = 9 show such karyotype, except only *I. apsotis*, which has 9 chromosomes with similar size in its gametes. All the species we observed with other chromosome numbers do not show such bimodal structure, where all the chromosomes are more or less the same size, for example, *I. delavayi* (Fig. 2A).

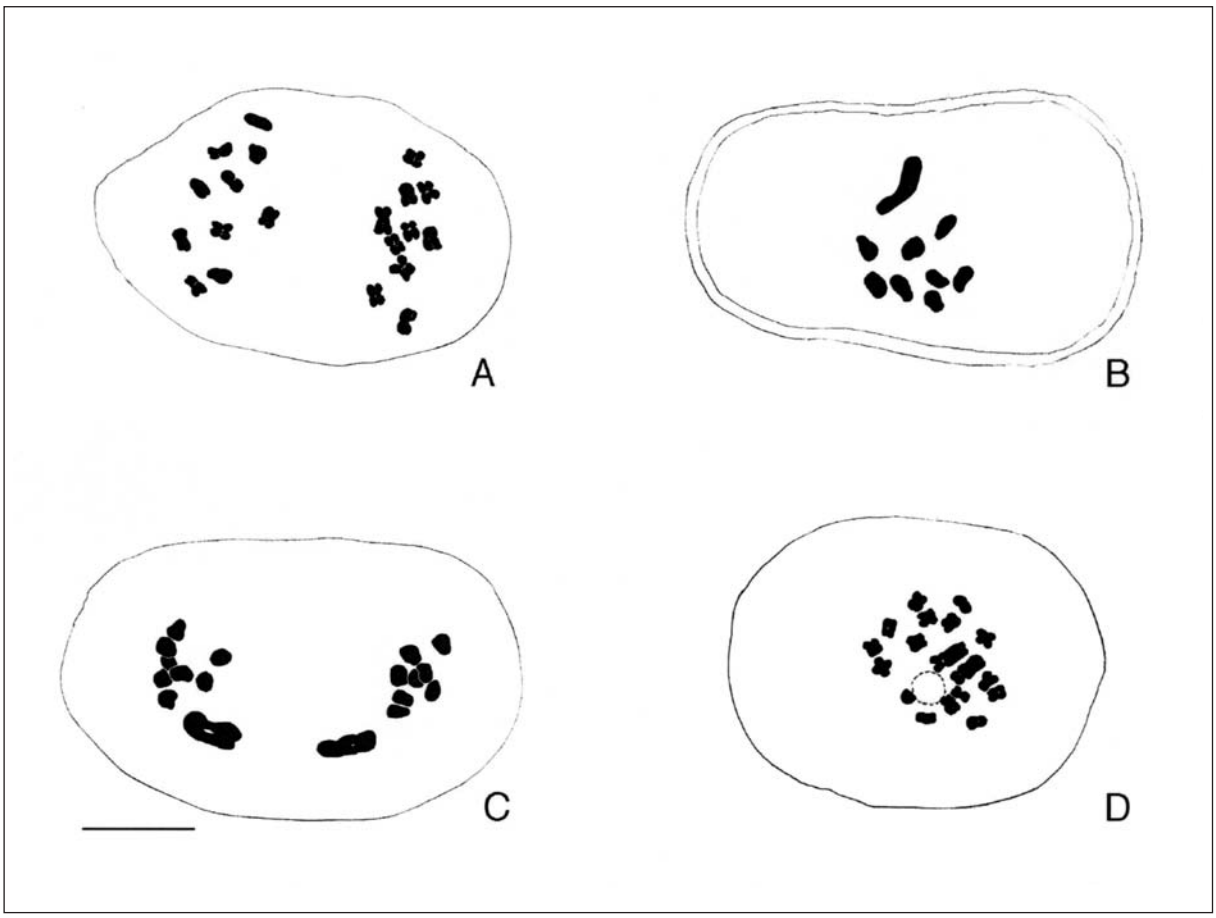



Fig. 2 – Comparison of non-bimodal and bimodal karyotypes. A: regular non-bimodal karyotype of *I. delavayi* ($n = 10$); B: bimodal karyotype of *I. holocentra* (pollen mitosis, $n = 9$); C: bimodal karyotype of *I. rectangula* (meiosis, $n = 9$); D: bimodal karyotype of *I. drepanophora* (mitosis, $2n = 18$). Bar 5 μm.

## DISCUSSIONS

*Variation and evolution of chromosome numbers in Impatiens*

Our present study contributed new documentation of chromosome numbers for 32 species from one of the most important, yet poorly studied, diversification centres of *Impatiens*. While our observations confirmed previous reports for seven species, some differences from previous reports were also revealed. We observed $n = 9$ with one long chromosome in *I. eubotrya*, but it was reported as $2n = 28$ (OKADA *et al.* 1989). The number $n = 10$ was reported for *I. drepanophora* from India (SARKAR *et al.* 1975), but all our samples collected from three localities in southwest China had the same number of $n = 9$ or $2n = 18$ (Fig. 2D). We observed $n = 10$ in *I. arguta* (Fig. 1E), which is the same as the report of SUGAWARA *et al.* (1997), but different from the results of AKIYAMA *et al.* (1996a) and SHIMIZU (1984) of $n = 9$ and $2n = 18$, and also different from the report of CHATTERJEE and SHARMA (1970) of $n = 6$. For *I. bicornuta* we observed $2n = 18$ for the samples collected from southwest China, the same as the results of AKIYAMA *et al.* (1992) and WAKABAYASHI (1992), but different from the report of MALLA *et al.* (1978) with $n = 8$ for samples collected from Nepal. For *I. radiata*, we observed $n = 9$ or $2n = 18$ (Fig. 2C) from southwest China, the same as the results of AKIYAMA *et al.* (1992, 1996a) and SUGAWARA *et al.* (1997), but SARKAR *et al.* (1975) reported $n = 10$ from India. In *I. mengtszeana* $2n = 16$ (LARSEN 1981; AKIYAMA *et al.* 1996a; SUGAWARA *et al.* 1997) or both $2n = 16$ and $2n = 17$ (SUGAWARA *et al.* 1994) were found. In our observation, only $n = 8$ was found in one population, but unbalanced separation of gametes with $n = 8$ and 9 were observed in the pollen mitosis from another population (Fig 1H-I). These different numbers found for a species could be due to true intraspecific variation (as in *I. mengtszeana*), but it might be also the results of misidentifications of different authors, since the determination of species is a well-known difficulty in *Impatiens*.

Combining our present results with all previous reports, chromosome numbers are available for 189 (out of over 900) species of *Impatiens* (Table 2). The distribution of the documented

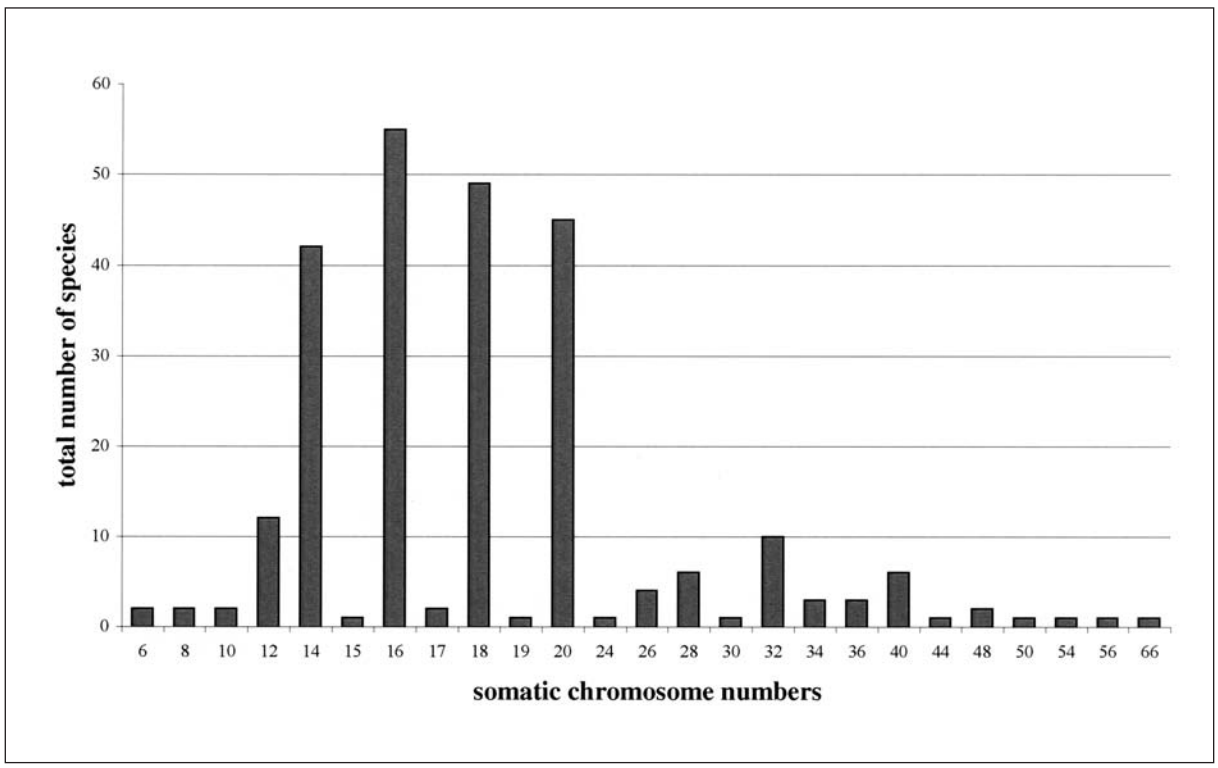



Fig. 3 – Frequency of species with different chromosome numbers based on the available chromosome data including our present results. Species with more than one chromosome numbers were counted repeatedly. The unusual numbers $2n = 15$, 17, and 19 are intraspecific variations occasionally observed.

chromosome numbers is presented in Fig 3. As can be seen from the figure, *Impatiens* shows a wide range of chromosome number variation of $2n$ = 6, 8, 10, 12, 14, 15, 16, 17, 18, 19, 20, 24, 26, 28, 30, 32, 34, 36, 40, 44, 48, 50, 54, and 66. However, the most frequent numbers are $2n$ = 14, 16, 18, and 20, that take the major part (78%) of the species observed. The unusual numbers $2n$ = 15, 17, and 19, which were observed occasionally, represent the intraspecific aneuploid variations as observed in *I. mengtszeana* (present study; SUGAWARA *et al*. 1994). They may also result from hybridisation of parents with different basic numbers. It is not known yet whether these numbers are stable in natural populations.

Except for these few aneuploid cases, *Impatiens* shows a typical dysploid variation. Most of the species can be considered as diploids with the basic numbers of x = 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, etc., and some higher numbers such as $2n$ = 32,

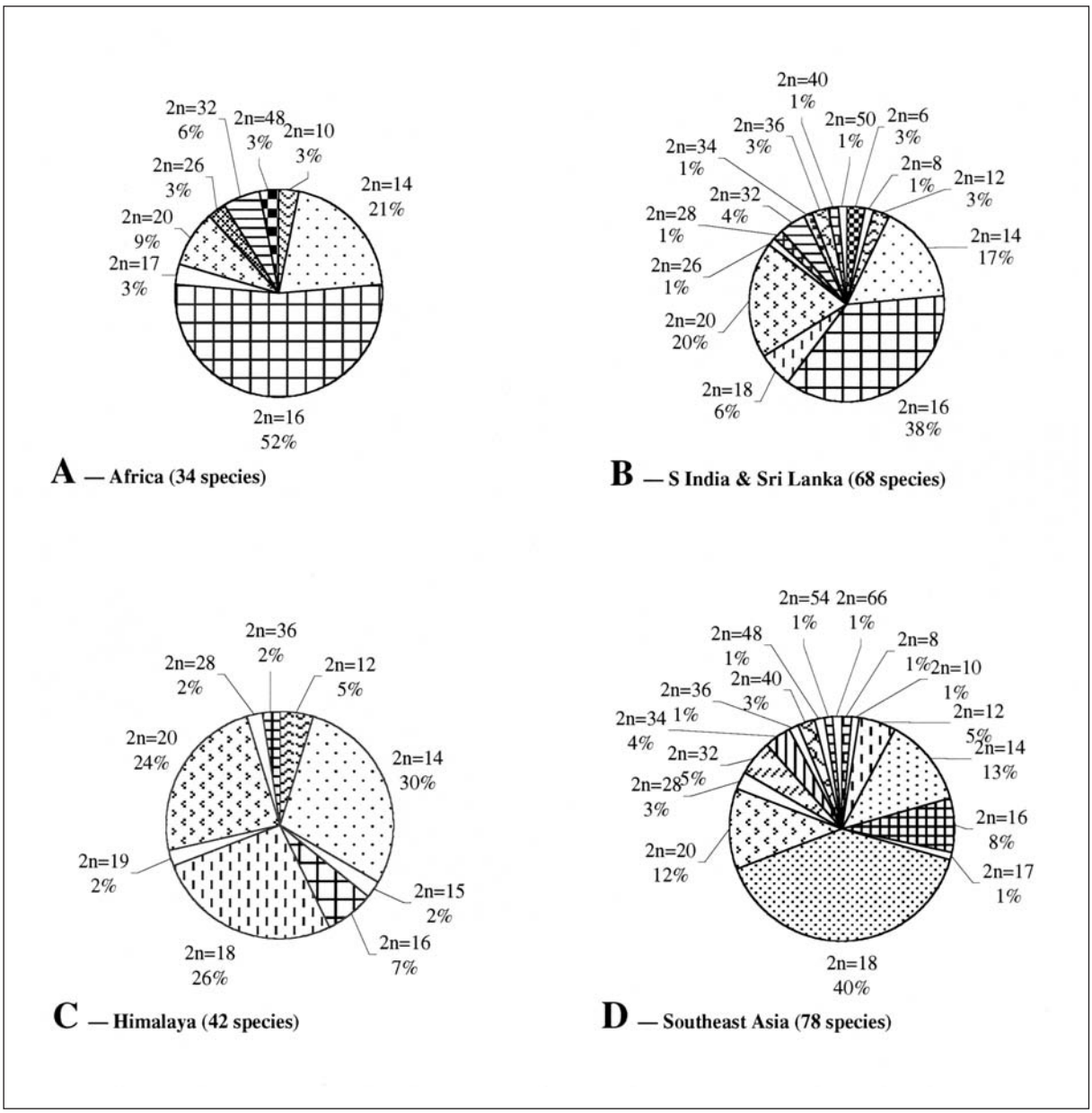



Fig. 4 – Partition of species numbers with different somatic chromosome numbers observed from the hotspots of species diversity of *Impatiens*. Total species number counted for each area is indicated on the figure. Species with more than one chromosome numbers were counted repeatedly. A: Africa; B: southern India and Sri Lanka; C: the Himalayas; D: Southeast Asia in broad sense including southwest China.

36, 40 etc. can be considered as polyploid of these lower basic numbers. Higher numbers such as 2*n* = 34, 44, 54, and 66 can be explained either as diploid with higher secondary basic numbers, or dysploid at polyploid levels. Different hypotheses have been proposed regarding the evolution of the basic numbers through dysploidy in conjunction with polyploidy in *Impatiens*. JONES and SMITH (1966) and AKIYAMA *et al.* (1992) suggested x = 7 to be the ancestral type, from which the other numbers were derived mainly by ascending dysploidy, whereas RAO *et al.* (1986) suggested evolution of x = 7, 9 and 10 from the basic number x = 8 through both descending and ascending dysploidy. Considering the chromosome number of *Hydrocera triflora* (consistently reported as 2*n* = 16), the sister group of *Impatiens*, the suggestion of RAO *et al.* (1986) sounds reasonable. However, the possibility of the basic numbers x = 9 and 10 being ancestral in *Impatiens* cannot be ruled out yet. Chromosome numbers alone may not be able to offer a solid solution to the evolution of the basic numbers, particularly concerning the polarity of the dysploidy. Phylogenetic studies may hopefully bring an insight on these questions. Yet, the mechanism of the dysploidy is not well understood. Meiosis involving irregular segregation, unequal translocation, centric fusion and centric fission are all possible causes of dysploid variations (STEBBINS 1971).

Polyploidy holds certain importance in the chromosomal evolution in *Impatiens*. In particular, it may play an important role in the natural hybridisations reported in this genus (GREY-WILSON 1980b; MERLIN and GRANT 1985). Some polyploid numbers such as 2*n* = 32, 36, and 40 can be simply considered as originated via autoploidization of diploid species with basic numbers x = 8, 9, 10, as in e. g. the African species *I. schlechteri* and *I. niamniamensis* 2*n* = 32 (JONES and SMITH 1966), *I. linearisepala* 2*n* = 36 (SUGAWARA *et al.* 1997), and *I. rubrostriata* 2*n* = 20 and 40 (present result and SUGAWARA *et al.* 1994, 1997) from southwest China. The high numbers as shown by *I. coelotropis* 2*n* = 34 (ZINOV'EVA-STAHEVICH and GRANT 1982, 1984) from southern India, *I. mirabilis n* = 17 (JONES and SMITH 1966) from Malaysia, *I. mooreana* 2*n* = 66 (JONES and SMITH 1966), and *I. pseudokingii n* = 27 (present results) could have resulted from hybridization, and therefore, can be considered as secondary dibasic polyploids. For example, one may

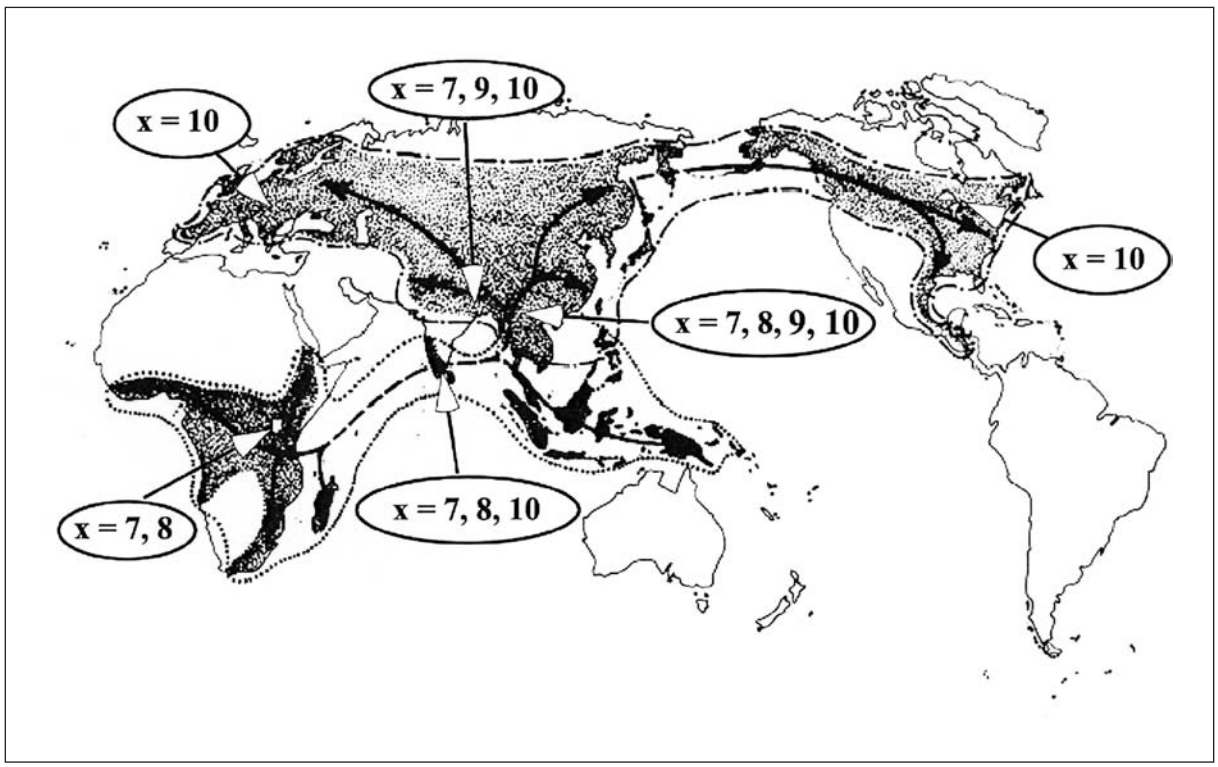



Fig. 5 – Distribution of the most frequent basic chromosome numbers and the migration routes proposed by GREY-WILSON (1980a).

consider $2n = 34$ ($n = 17$) as a result of hybridisation of parent species of $n = 7$ and $n = 10$, or $n = 8$ and $n = 9$ respectively. Dysploid variation at polyploid level can also result in such high secondary basic numbers, e. g. descending dysploidy from $2n = 4x = 36$ can bring about the same number $2n = 34$. Further phylogenetic studies may supply independent and more robust test of these hypotheses.

*Karyotype structure*

Most *Impatiens* species have symmetrical karyotypes, i. e. all the chromosomes of a species are similar to each other in size and are mostly metacentric or submetacentric (LEVAN *et al.* 1964). Noticeably, *Impatiens* species with $2n = 18$ (or $n = 9$) from the Sino-Himalayan area, Indochina and Southeast Asia show special bimodal karyotypes: one pair (one in gamete) of chromosomes is distinctly longer than other chromosomes. SMITH (1934) first noticed this phenomenon in *I. glandulifera* (*I. roylei*) from the Himalayas. AKIYAMA *et al.* (1992) found that such bimodal karyotypes are characteristic of the species with $n = 9$, predominantly in central and eastern Nepal. In our present observations, almost all the species with $n = 9$ or $2n = 18$ showed such bimodal karyotypes (Fig. 2B-D), with exception of only one species, *I. apsotis*, which has 9 chromosomes of similar size in its gametes. We did not observe species with any other number showing the bimodal karyotype. However, SUGAWARA *et al.* (1994) reported such bimodal karyotypes for species with $2n = 12, 20$ from China.

Asymmetrical bimodal karyotypes can result from symmetrical karyotypes via unequal translocations (STEBBINS 1971). KHOSHOO (1957) suggested that the bimodal karyotype with x = 9 was derived from a symmetrical karyotype with x = 10 by translocation of the essential material of one chromosome onto one of the arms of another chromosome followed by the loss of one centromere. At present, there is no clear evidence to indicate the origin of the bimodal karyotype in species of *Impatiens* with x = 9. If we accept the assumption of x = 8 as the ancestral basic number for *Impatiens* as suggested by RAO *et al.* (1986), an alternative hypothesis has to be proposed to explain the origin of the bimodal karyotypes related to the basic number x = 9. Such an alternative hypothesis to explain the origin of the bimodal karyotypes via ascending dysploidy is not available yet.

*Cytogeography of Impatiens*

Chromosome number variations in *Impatiens* show interesting geographical patterns, that strongly related to the centres of species diversity and endemism. Of the five hotspots of species diversity, karyological data are almost blank for Madagascar. We made a brief partition of the available chromosome data for the other diversification centres according to the origin of the samples. The results are shown in Fig. 4. Most African species have the chromosome number of $2n = 16$ (shown by 52% of species observed) or $2n = 14$ (21%), other numbers such as $2n = 10, 20, 32$ occurred less frequently, and the number $2n = 18$ has never been found (Fig. 4A). Southern India and Sri Lanka, particularly the Western Ghats host a lot of *Impatiens* species with high proportion of endemics. Most species from this region have the chromosome number of $2n = 16$ (38%), followed by $2n = 20$ (20%) and $2n = 14$ (17%) (Fig. 4B). The lowest chromosome number in *Impatiens*, $2n = 6$, occurred only in this region. JONES and SMITH (1966) believed that the Himalayas represent the centre of origin of *Impatiens* and that species diversified and radiated from an ancestral stock of $2n = 2x = 14$, but KHOSHOO (1966) suggested that the chromosome number $2n = 2x = 20$ was more typical of the Himalayan species. The available data today show three main basic numbers, x = 7, 9, 10, with similar frequencies for the Himalayan species: $2n = 14$ (30%), $2n = 18$ (26%), and $2n = 20$ (24%) (Fig. 4C). The region of the broad Southeast Asia including mainland Southeast Asia, southwest China, Indochina peninsula, and Malesia harbours species mostly with $2n = 18$ (40%), $2n = 14$ (13%), $2n = 20$ (12%), and $2n = 16$ (8%) (Fig. 4D). All native species in northern Asia, Europe and North America have the number of $2n = 2x = 20$ only.

Based on the pronounced similarity of species occurring in Africa, Madagascar, and Southern India, GREY-WILSON (1980a) believed that *Impatiens* originated in west Gondwana in the Paleogene and spread to Southeast Asia through the Indian subcontinent. He further suggested that the spreading drought during the Neogene caused the isolation of *Impatiens* species in Africa, Madagascar, southern India and Sri Lanka and Southeast Asia. Subsequently, from Southeast Asia and the adjacent Himalayan area, *Impatiens* further diversified into two lineages, one lineage radiating to the temperate Eurasian areas and North America, another radiating in the tropical and subtropical areas. Moreover, he pro-

Table 2 – Chromosome numbers documented for the family Balsaminaceae including present results.

| Taxon | Chrom. No. | | Origin | References |
|---|---|---|---|---|
| | *n* | 2*n* | | |
| *Hydrocera triflota* Wight et Arn. | | 16 | S India | GOVINDARAJAN and SUBRAMANIAN 1986 |
| | | 16 | S India | RAO *et al.* 1986 |
| | 8 | | S India | AYYANGAR *et al.* 1987 |
| *Impatiens acaulis* Arn. | 10 | | Karnataka, India | BHASKAR 1976 |
| | 10 | | Udipi, India | BHASKAR 1980 |
| *I. acaulis* Arn. var. *granulata* Bhask. | 9 | | S India | BHASKAR 1980 |
| | 8, 9 | | Agumbe, India | BHASKAR 1980 |
| *I. acehensis* C. Grey-Wilson | | 14 | Sumatra, Indonesia | present study |
| *I. agumbeyana* V. Bhaskar et B. A. Razi | 8 | | Karnataka, India | BHASKAR 1976 |
| | 8 | | Agumbe, India | BHASKAR 1980 |
| *I. alboflava* Miq. | | 14 | W Sumatra | OKADA 1989 |
| *I. aliciae* C. E. C. Fischer var. *bababudensis* Bhask. | 8 | | Karnataka, India | BHASKAR 1976 |
| *I. amphorata* Edgew. | 7 | 14 | Kashmir | KHOSHOO 1957 |
| | 7 | | W Himalaya | FEDOROV 1969 |
| *I. amplexicaulis* Edgew. | | 20 | Himalaya | FEDOROV 1969 |
| *I. aquatilis* Hook. f. | 9 | | Yunnan, China | present study |
| *I. apsotis* Hook. f. | 9 | | Sichuan, China | present study |
| *I. arguta* Hook. f. et Thoms. | 6 | | E Himalaya | CHATTERJEE and SHARMA 1970 |
| | 9 | | E Himalaya | SHIMIZU 1984 |
| | | 18 | Himalaya | AKIYAMA *et al.* 1996a |
| | | 20 | Yunnan, China | SUGAWARA *et al.* 1997 |
| | 10 | | Yunnan, China | present study |
| *I. assurgens*Baker | 5 | | Tanzania | GIL and CHINNAPPA 1977 |
| *I. aureliana* Hook. f. | | 12 | Yunnan, China | SUGAWARA *et al.* 1994 |
| | | 12 | Yunnan, China | AKIYAMA *et al.* 1996a |
| | 6 | | Yunnan, China | present study |
| *I. auricoma* Baill. | | 16 | Africa | ARISUMI 1987 |
| *I. bahanensis* Hand.-Mazz. | 9 | | Yunnan, China | present study |
| *I. balfourii* Hook. f. | 7 | 14 | Himalaya | FEDOROV 1969 |
| | 7 | | Cult. USA | CHINNAPPA and GILL 1974 |
| | | 14 | Cult. France | ZINOV'EVA-STAHEVITCH and GRANT 1982 |
| | | 14 | W Himalaya | ZINOV'EVA-STAHEVITCH and GRANT 1984 |
| | | 14 | Cult. Mediterrtanean region | CHESHMEDYIEV 1994 |
| *I. balsamina* L. | | 14 | ? | FEDOROV 1969 |
| | 5, 6, 7 | | S India | RAO 1975 |
| | | 14 | Cult. Beijing, China | GAO and ZHANG 1984 |
| | | 44 | S India | GOVINDARAJAN 1985 |
| | | 44 | S India | GOVINDARAJAN and SUBRAMANIAN 1986 |
| | | 14 | Maharashtra, India | ZINOV'EVA-STAHEVITCH and GRANT 1982 |
| | 7 | 14 | Maharashtra, India | ZINOV'EVA-STAHEVITCH and GRANT 1984 |
| *I. balsamina* L. var. *arcuata* Wall. | | 44 | S India | GOVINDARAJAN and SUBRAMANIAN 1986 |
| *I. balsamina* L. var. *azaleiflora* | 7 | 14 | Maharashtra, India | ZINOV'EVA-STAHEVITCH and GRANT 1984 |
| | | 14 | S India | RAO *et al.* 1986 |
| | 7 | | S India | AYYANGAR *et al.*1987 |
| *I. balsamina* L. var. *bicolor* | | 14+2b | ? | RAGHUVANSHI and MAHAJAN 1985 |
| *I. balsamina* L. var. *camelliaeflora* | 7 | 14 | Maharashtra, India | ZINOV'EVA-STAHEVITCH and GRANT 1984 |
| | | 14 | S India | RAO *et al.* 1986 |
| | 7 | | S India | AYYANGAR *et al.*1987 |
| *I. balsamina* L. var. *coccinea* (Wall.) Hook. f. | 7 | 14 | S India | ZINOV'EVA-STAHEVITCH and GRANT 1984 |
| | | 14 | S India | RAO *et al.* 1986 |
| | 7 | | S India | AYYANGAR *et al.*1987 |
| *I. balsamina* L. var. *rosea* Hook. f. | | 14, 28 | Maharashtra, India | ZINOV'EVA-STAHEVITCH and GRANT 1982 |
| | 7 | 14 | S India | ZINOV'EVA-STAHEVITCH and GRANT 1984 |
| *I. balsamina* L. var. *vulgaris* | | 14 | S India | RAO *et al.* 1986 |
| | 7 | | S India | AYYANGAR *et al.* 1987 |
| *I. barberi* Hook. f. | 8 | | Karnataka, India | BHASKAR 1976 |
| | 8 | | S India | BHASKAR 1980 |
| *I. begonifolia* S. Akiyama et H. Ohba | | 16 | Yunnan, China | SUGAWARA *et al.* 1994 |
| | | 16 | Himalaya | AKIYAMA *et al.* 1996a |
| *I. bella* Hook. f. et Thoms. | | 14 | Himalaya | FEDOROV 1969 |
| *I. bicornuta* Wall. | 8 | | Nepal | MALLA *et al.* 1978 |
| | | 18 | Nepal | AKIYAMA *et al.* 1992 |
| | | 18 | ? | WAKABAYASHI 1992 |
| | | 18 | Yunnan, China | present study |

| Taxon | Chrom. No. | | Origin | References |
|---|---|---|---|---|
| | *n* | *2n* | | |
| *I. biflora* Walt. | | 20 | N America ? | Smith 1934 |
| | 10 | | ? | Fedorov 1969 |
| | 10 | | N America | Chinnappa and Gill 1974 |
| *I. brachycentra* Kar. et Kir. | | 14 | Himalaya | Fedorov 1969 |
| | 7 | | Kashmir, India | Bhat *et al.* 1975 |
| *I. burtonii* Hook. f. | 8 | 16 | Congo | Jones and Smith 1966 |
| | | 16 | Cameroon | Gadella 1977 |
| | | 14 | Cameroon | Gadella 1982 |
| *I. campanulata* Wight | 10 | 20 | Paini Hills, India | Jones and Smith 1966 |
| | 10 | 20 | Travancore, India | Zinov'eva-Stahevitch and Grant 1984 |
| | 10 | | S India | Zinov'eva-Stahevitch and Grant 1985 |
| | | 20 | S India | Rao *et al.* 1986 |
| | | 18 | India | Arisumi 1987 |
| *I. capensis* Meerb. | | 14 | ? | Fedorov 1969 |
| | | 20 | Manitoba, Canada | Löve and Löve 1982 |
| | | 20 | Montreal, Canada | Zinov'eva-Stahevitch and Grant 1982 |
| | 10 | | Ottawa, Canada | Mulligan 1984 |
| | | 20 | Québec, Canada | Zinov'eva-Stahevitch and Grant 1984 |
| *I. chiangdaoensis* Shimizu | | 12 | Thailand | Larsen 1981 |
| | 6 | 12 | Thailand | Shimizu 1979 |
| *I. chimiliensis* Comber | | 18 | Yunnan, China | present study |
| *I. chinensis* L. | | 16 | S India | Rao *et al.* 1986 |
| | 8 | | Yunnan, China | present study |
| *I. chungtienensis* Y. L .Chen | 9 | | Yunnan, China | present study |
| *I. cinnabarina* C. Grey-Wilson | | 16 | Tanzania | Zinov'eva-Stahevitch and Grant 1982 |
| | | 16 | Tanzania | Zinov'eva-Stahevitch and Grant 1984 |
| *I. clavicornu* Turcz. | 8 | | Doddabetta, India | Bhaskar 1980 |
| | | 14 | S India | Govindarajan and Subramanian 1986 |
| | | 14 | S India | Rao *et al.* 1986 |
| | 7 | | S India | Ayyangar *et al.* 1987 |
| *I. clavigeroides* S. Akiyama, H. Ohba et S. K. Wu | | 34 | Yunnan, China | Sugawara *et al.* 1997 |
| *I. coelotropis* Fisch. | | 34 | Kerala, India | Zinov'eva-Stahevitch and Grant 1982 |
| | | 34 | Kerala, India | Zinov'eva-Stahevitch and Grant 1984 |
| | 17 | | Kerala, India | Zinov'eva-Stahevitch and Grant 1985 |
| *I. congolensis* G. M. Schulze et R. Wilczek *var. longicarata* G. M. Schulze et R. Wilczek | 24 | 48 | Congo | Jones and Smith 1966 |
| *I. corchorifolia* Franch. | | 18 | Yunnan, China | Sugawara *et al.* 1997 |
| *I. cordata* Wight | 10 | | Tamil Nadu, India | Bhaskar 1976 |
| | | 20 | Kerala, India | Zinov'eva-Stahevitch and Grant 1982 |
| | | 20 | Travancore, India | Zinov'eva-Stahevitch and Grant 1984 |
| | | 20 | S India | Rao *et al.* 1986 |
| *I. crassicaudex* Hook. f. | 9 | | Yunnan, China | present study |
| *I. cuspidata* Wight et Arn. | | 14 | Tamil Nadu, India | Zinov'eva-Stahevitch and Grant 1982 |
| | 7 | 14 | Tamil Nadu, India | Zinov'eva-Stahevitch and Grant 1984 |
| | | 14 | Tamil Nadu, India | Zinov'eva-Sttahevitch and Grant 1985 |
| | | 14 | S India | Govindarajan and Subramanian 1986 |
| | | 14 | S India | Rao *et al.* 1986 |
| *I. cyathiflora* Hook. f. | | 18 | Yunnan, China | present study |
| *I. cymbifera* Hook. f. | | 18, 19 | Nepal | Akiyama *et al.* 1992 |
| *I. dalzellii* Hook. f. et Thoms. | | 16 | Maharashtra, India | Zinov'eva-Stahevitch and Grant 1982 |
| | | 16 | Maharashtra, India | Zinov'eva-Stahevitch and Grant 1984 |
| *I. delavayi* Franch. | 10 | 20 | Yunnan, China | present study |
| *I. dendricola* C. E. C. Fischer | 7 | | Karnataka, India | Bhaskar 1976 |
| | 7 | | Tadiandamol, India | Bhaskar 1980 |
| *I. desmantha* Hook. f. | 9 | | Yunnan, China | present study |
| *I. diepenhorstii* Miq. | | 28 | W Sumatra | Okada *et al.* 1989 |
| *I. digitata* Warb | 10 | | Tanzania | Gill and Chinnappa 1977 |
| *I. discolor* DC. | | 20 | Nepal | Akiyama *et al.* 1992 |
| *I. dolichoceras* E. Pritz. ex Diels | | 18 | Guangxi, China | present study |
| *I. drepanophora* Hook. f. | 10 | | India | Sarkar *et al.* 1975 |
| | 9 | | Yunnan, China | present study |
| *I. ecalcarata* Blank. | 10 | | N America | Chinnappa and Gill 1974 |
| *I. edgeworthii* Hook. f. | 6 | 12 | Himalaya | Fedorov 1969 |
| | 7 | | Kashmir | Gohil *et al.* 1981 |
| | 7 | | Kashmir | Jee *et al.* 1989 |

| Taxon | Chrom. No. | | Origin | References |
|---|---|---|---|---|
| | *n* | 2*n* | | |
| *I. elegans* Bedd. | | 20 | Travancore, India | ZINOV'EVA-STAHEVITCH and GRANT 1984 |
| *I. eubotrya* Miq. | | 28 | Sumatra, Indonesia | OKADA *et al.* 1989 |
| | | 18 | Sumatra, Indonesia | present study |
| *I. exlis* Hook. f. | | 28 | ? | WAKABAYASHI 1992 |
| | | 28 | Nepal | AKIYAMA *et al.* 1992 |
| | | 28 | Himalaya | AKIYAMA *et al.* 1993 |
| *I. falcifera* Hook. f. | 8 | | Nepal | MALLA *et al.* 1975 |
| | | 14 | Nepal | AKIYAMA *et al.* 1992 |
| | | 14 | ? | WAKABAYASHI, 1992 |
| *I. fenghwaiana* Y. L. Chen | | 18 | Guangxi, China | present study |
| *I. firmula* Baker | | 14 | Madagascar ? | FEDOROV 1969 |
| *I. flaccida* Arn. | 7 | 14 | Mauritius | JONES and SMITH 1966 |
| | | 14 | Sri. Lanka | ZINOV'EVA-STAHEVITCH and GRANT 1982 |
| | 7 | 14 | Sri. Lanka | ZINOV'EVA-STAHEVITCH and GRANT 1984 |
| | 7 | 14 | Sri Lanka | ZINOV'EVA-STAHEVITCH and GRANT 1985 |
| | | 14 | India | ARISUMI 1987 |
| *I. flanaganae* Hemsl. | | 16 | S Africa | ZINOV'EVA-STAHEVITCH and GRANT 1982 |
| | | 16 | S Africa | ZINOV'EVA-STAHEVITCH and GRANT 1984 |
| *I. fruticosa* Leschen. ex DC. | | 16 | S India | RAO *et al.* 1986 |
| *I. furcillata* Hemsl.ex Forb. et Hemsl. | | 20 | ? | FEDOROV 1969 |
| *I. gadutensis* | | 14 | W Sumatra | OKADA *et al.* 1989 |
| *I. gardneriana* Wight ex Hook. f. | | 16 | Kerala, India | ZINOV'EVA-STAHEVITCH and GRANT 1982 |
| | | 16 | Kerala, India | ZINOV'EVA-STAHEVITCH and GRANT 1984 |
| *I. gongshanensis* Y. L. Chen | 10 | | Yunnan, China | present study |
| *I. glandulifera* Royle | | 18 | Himalaya | SMITH 1934 |
| | | 18, 20 | Himalaya | FEDOROV 1969 |
| | 9, 10 | | Cult. Kew | JONES and SMITH 1966 |
| | 10 | | N America | CHINNAPPA and GILL 1974 |
| | | 18 | Czechoslovakia? | JAVURKOVA 1979 |
| | | 18 | Mediterranean region? | CHESHMEDYIEV 1994 |
| | | 18 | Austria | DOBES *et al.* 1997 |
| *I. gordonii* Horne | | 16 | Seychelles | ZINOV'EVA-STAHEVITCH and GRANT 1982 |
| | 8 | 16 | Seychelles | ZINOV'EVA-STAHEVITCH and GRANT 1984 |
| | | 16 | Seychelles | ZINOV'EVA-STTAHEVITCH and GRANT 1985 |
| | 8 | | Seychelles | MERLIN and GRANT 1985 |
| *I. goughii* Wight | | 16 | S India | RAO 1973 |
| | 10 | | Kerala, India | BHASKAR 1976 |
| | | 32 | Tamil Nadu, India | ZINOV'EVA-STAHEVITCH and GRANT 1982 |
| | | 32 | Tamil Nadu, India | ZINOV'EVA-STAHEVITCH and GRANT 1984 |
| *I. grandis* Heyne | 20 | | Tamil Nadu, India | BHASKAR 1976 |
| | | 36 | Coonoor, India | ZINOV'EVA-STAHEVITCH and GRANT 1984 |
| | | 40 | Sri Lanka; S India | ZINOV'EVA-STAHEVITCH and GRANT 1984 |
| *I. hamata* Warb. | 7 | | Tanzania | GILL and CHINNAPPA 1977 |
| *I. harlandii* Dranfield | | 12 | Borneo | ZINOV'EVA-STAHEVITCH and GRANT 1984 |
| *I. hawkeri* W. Bull ex Gard. | | 48 | South Sea Islands | JONES and SMITH 1966 |
| | 24 | | NE New Guinea | ZINOV'EVA-STAHEVITCH and GRANT 1982 |
| | 24 | | NE New Guinea | ZINOV'EVA-STAHEVITCH and GRANT 1984 |
| *I. hensloviana* Arn. | 8 | | Kerala, India | BHASKAR 1976 |
| | | 16 | S India | GOVINDARAJAN 1985 |
| | | 16 | S India | GOVINDARAJAN and SUBRAMANIAN 1986 |
| *I. herbicola* Hook. f. | 7 | | Karnataka, India | BHASKAR 1976 |
| *I. hochstetteri* Warb. | | 16 | Kenya | JONES and SMITH 1966 |
| *I. holocentra* Hand.-Mazz. | 9 | | Yunnan, China | present study |
| *I. hookeriana* Arn. | | 40 | India | JONES and SMITH 1966 |
| | | 18 | Tamil Nadu, India | ZINOV'EVA-STAHEVITCH and GRANT 1982 |
| | | 16 | S India | RAO *et al.* 1986 |
| | | 36 | India | ARISUMI 1987 |
| | | 50 | Sri Lanka | STAHEVITCH 1995 |
| *I. hypophylla* Makino | | 20 | ? | TAGAWA *et al.* 1997 |
| *I. hypophylla* Makino *var. microhypophylla* Hara | | 20 | ? | TAGAWA *et al.* 1997 |
| *I. imbecilla* Hook. f. | | 20 | Sichuan, China | present study |
| *I. infirma* Hook. f. | 9 | | Sichuan, China | present study |
| *I. irvingii* Hook. f. ex Oliv. | | 14 | Cameroon | MORTON 1993 |
| *I. junghuhnii* Miq. | | 14 | W Sumatra | OKADA *et al.* 1989 |
| *I. kamtilongensis* Toppin | | 14 | Yunnan, China | SUGAWARA *et al.* 1997 |

| Taxon | Chrom. No. | | Origin | References |
|---|---|---|---|---|
| | *n* | *2n* | | |
| *I. keilii* Grig. | 7 | | Tanzania | GILL and CHINNAPPA 1977 |
| | | 16 | Africa | ARISUMI 1987 |
| *I. kilimanjari* Oliver | 13 | | Tanzania | GILL and CHINNAPPA 1977 |
| *I. kleinii* Wight et Arn. | | 16 | Maharashtra, India | ZINOV'EVA-STAHEVITCH and GRANT 1982 |
| | | 16 | Maharashtra, India | ZINOV'EVA-STAHEVITCH and GRANT 1984 |
| *I. latifolia* L. | 3 | | S India | RAO 1975 |
| | | 6 | S India | GOVINDARAJAN and SUBRAMANIAN 1986 |
| | | 6 | S India | RAO *et al.* 1986 |
| | 3 | | S India | AYYANGAR *et al.*1987 |
| *I. lawsoni* Hook. f. | 10 | | Karnataka, India | BHASKAR 1976 |
| | 10 | | Charmadi Ghat, India | BHASKAR 1980 |
| *I. lecomtei* Hook. f. | | 18 | Yunnan, China | present study |
| *I. lenta* Hook. f. | 8 | | Kerala, India | BHASKAR 1976 |
| *I. leptopoda* Arn. | | 8 | Sri Lanka | STAHEVITCH 1995 |
| *I. leschenaultii* Wall. | | 6 | S India | BHASKAR and RAZI 1972-1973 |
| | 3 | | S India | RAO 1975 |
| | | 6 | Tamil Nadu, India | ZINOV'EVA-STAHEVITCH and GRANT 1982 |
| | 3 | 6 | Tamil Nadu, India | ZINOV'EVA-STAHEVITCH and GRANT 1984 |
| | 3 | | Tamil Nadu, India | ZINOV'EVA-STAHEVITCH and GRANT 1985 |
| | | 6 | S India | GOVINDARAJAN and SUBRAMANIAN 1986 |
| | | 6 | S India | RAO *et al.* 1986 |
| | 3 | | S India | AYYANGAR *et al.*1987 |
| *I. levingei* Gamble ex Hook. f. | | 16 | Tamil Nadu, India | ZINOV'EVA-STAHEVITCH and GRANT 1982 |
| | 8 | 16 | Tamil Nadu, India | ZINOV'EVA-STAHEVITCH and GRANT 1984 |
| | 8 | 16 | Tamil Nadu, India | ZINOV'EVA-STAHEVITCH and GRANT 1985 |
| *I. linearifolia* Warb. | | 32 | New Guinea | ZINOV'EVA-STAHEVITCH and GRANT 1984 |
| *I. linearisepala* S. Akiyama, H. Ohba et S. K. Wu | | 36 | Yunnan, China | SUGAWARA *et al.* 1997 |
| *I. longiloba* Craib | | 18 | Thailand | SHIMIZU *et al.* 1984 |
| *I. luchunensis* S. Akiyama, H. Ohba et S. K. Wu | | | | |
| *I. maculata* Wight | | 18 | Yunnan, China | SUGAWARA *et al.* 1997 |
| | 10 | | Tamil Nadu, India | BHASKAR 1976 |
| | | 20 | Kerala, India | ZINOV'EVA-STAHEVITCH and GRANT 1982 |
| | | 20 | Travancore, India | ZINOV'EVA-STAHEVITCH and GRANT 1984 |
| | | 20 | S India | GOVINDARAJAN and SUBRAMANIAN 1986 |
| | | 20 | S India | RAO *et al.* 1986 |
| *I. maguanensis* S. Akiyama, H. Ohba et S. K. Wu | | 18 | Yunnan, China | SUGAWARA *et al.* 1997 |
| *I. mathildae* Chiov. | | 14 | Himalaya ? | FEDOROV 1969 |
| *I. mengtzeana* Hook. f. | | 16 | Thailand | LARSEN 1981 |
| | | 16, 17 | Yunnan, China | SUGAWARA *et al.* 1994 |
| | | 16 | Himalaya | AKIYAMA *et al.* 1996a |
| | | 16 | Yunnan, China | SUGAWARA *et al.* 1997 |
| | 8, 9 | | Yunnan, China | present study |
| *I. meruensis* Gilg | | 16 | Kenya | OGIUMA and TOBE 1991 |
| *I. microcentra* Hand.-Mazz. | 9 | | Yunnan, China | present study |
| *I. mirabilis* Hook. f. | 17 | | Malaysia | JONE and SMITH 1966 |
| *I. modesta* Wight | 8, 9, 16 | | Tamil Nadu, India | BHASKAR 1976 |
| | 8, 9, 16 | | Naduvattam, India | BHASKAR 1980 |
| *I. mooreana* Schlechter | | 66 | New Guinea | JONES and SMITH 1966 |
| *I. mysorensis* Roth. | 7 | | Karnataka, India | BHASKAR 1976 |
| *I. nalampoonii* Shimizu | | 32 | Thailand | SHIMIZU 1979 |
| *I. napoensis* Y. L .Chen | 9 | | Yunnan, China | present study |
| *I. niamniamensis* Gilg | | 32 | Uganda | JONES and SMITH 1966 |
| | | 32 | Uganda | ZINOV'EVA-STAHEVITCH and GRANT 1982 |
| | 16 | 32 | Tropical W Africa | ZINOV'EVA-STAHEVITCH and GRANT 1984 |
| | 16 | | Uganda | ZINOV'EVA-STAHEVITCH and GRANT 1985 |
| | | 32 | Africa | ARISUMI 1987 |
| *I. nolitangere* L. | | 20, 40 | ? | FEDOROV 1969 |
| | 10 | | N America | CHINNAPPA and GILL 1974 |
| | | 12 | Russia | GVNINIANIDZE and AVAZNELI 1982 |
| | | 20 | Japan | NISHIKAWA 1990 |
| | | 20 | Mediterranean region? | CHESHMEDYIEV 1994 |
| | | 20 | Russian Far East | RUDYKA 1995 |
| *I. occultans* Hook. f. | | 18 | Nepal | AKIYAMA *et al.* 1992 |
| *I. omisssa* Hook. f. | | 28 | S India | RAO *et al.* 1986 |
| *I. oncidioides* Ridley ex Hook. f. | | 14 | SE Asia | HELLMAYR *et al.* 1994 |

| Taxon | Chrom. No. | | Origin | References |
|---|---|---|---|---|
| | *n* | *2n* | | |
| *I. oppositifolia* L. | | 16, 32 | Mahabaleshwar, India | ZINOV'EVA-STAHEVITCH and GRANT 1982 |
| | | 16 | Mahabaleshwar, India | ZINOV'EVA-STAHEVITCH and GRANT 1984 |
| *I. orchioides* Bedd. | | 18 | S India | GOVINDARAJAN 1985 |
| | | 18 | S India | GOVINDARAJAN and SUBRAMANIAN 1986 |
| *I. oxyanthera* Hook. f. | 7 | | Sichuan, China | present study |
| *I. pallida* Nutt. | | 20 | N America | SMITH 1934 |
| | | 20, 24? | ? | FEDOROV 1969 |
| | 10 | | N America | CHINNAPPA and GILL 1974 |
| | | 20, 30 | Montréal, Canada | ZINOV'EVA-STAHEVITCH and GRANT 1982 |
| | 10 | 20 | Montréal, Canada | ZINOV'EVA-STAHEVITCH and GRANT 1984 |
| *I. pallidiflora* Hook. f. | 13 | | Kerala, India | BHASKAR 1976 |
| | | 16 | S India | RAO *et al.* 1986 |
| *I. parasitica* Bedd. | 9 | | ? | BHASKAR 1975 |
| | 10 | | Kerala, India | BHASKAR 1976 |
| | | 20 | Kerala, India | ZINOV'EVA-STAHEVITCH and GRANT 1982 |
| | 7, 9, 10 | | Travancore, India | ZINOV'EVA-STAHEVITCH and GRANT 1984 |
| | 7 | | Kerala, India | ZINOV'EVA-STAHEVITCH and GRANT 1985 |
| *I. parviflora* DC. | | 20, 24, 26 | ? | FEDOROV 1969 |
| | 13 | | N America | CHINNAPPA and GILL 1974 |
| | 13 | | Montreal, Canada | ZINOV'EVA-STAHEVITCH and GRANT 1982 |
| | 13 | | NE Asia | ZINOV'EVA-STAHEVITCH and GRANT 1984 |
| *I. parvifolia* Bedd. | 10 | | Kerala, India | BHASKAR 1976 |
| *I. phoenicea* Bedd. | 10 | | Tamil Nadu, India | BHASKAR 1976 |
| | | 16 | S India | GOVINDARAJAN 1985 |
| | | 16 | S India | GOVINDARAJAN and SUBRAMANIAN 1986 |
| *I. petersiana* Gilg ex Grignan | | 16 | Africa ? | FEDOROV 1969 |
| *I. plaghuhnii* | | 16 | W Sumatra | OKADA *et al.* 1989 |
| *I. platypetala* x *aurantiaca* Steen | | 8 | New Guinea | ARISUMI 1987 |
| *I. platypetala* Lindl. | | 14 | ? | FEDOROV 1969 |
| | 7 | 14 | Java, Indonesia | ZINOV'EVA-STAHEVITCH and GRANT 1984 |
| | 7 | | Java, Indonesia | ZINOV'EVA-STAHEVITCH and GRANT 1985 |
| *I. platypetala* Lindl. ssp. *aurantiaca* x spp. Indet. | | 12 | ? | ZINOV'EVA-STAHEVITCH and GRANT 1984 |
| *I. platypetala* Lindl. ssp. *aurantiaca* (Teysm.ex Kds.) Steen. | | 14 | Celebes, Indonesia | ZINOV'EVA-STAHEVITCH and GRANT 1984 |
| | 7 | | Celebes, Indonesia | ZINOV'EVA-STAHEVITCH and GRANT 1985 |
| *I. platypetala* Lindl. ssp. *nematoceras* (Miq) Steen. | 8 | 16 | Indonesia | ZINOV'EVA-STAHEVITCH and GRANT 1982 |
| | 8 | | Indonesia | ZINOV'EVA-STAHEVITCH and GRANT 1985 |
| | | 16 | Indonesia | ZINOV'EVA-STAHEVITCH and GRANT 1984 |
| *I. poculifer* Hook. f. | | 20 | Yunnan, China | present study |
| *I. porphyrea* Toppin | | 18 | Yunnan, China | SUGAWARA *et al.* 1997 |
| *I. pradhani* Hara | | 18 | Nepal | AKIYAMA *et al.* 1992 |
| *I. principis* Hook. f. | 9 | | Yunnan, China | present study |
| *I. pseudokingii* Hand.-Mazz. | 27 | | Yunnan, China | present study |
| *I. pseudoviola* Gilg | | 16 | Kenya | JONES and SMITH 1966 |
| | 8 | 16 | Kenya | ZINOV'EVA-STAHEVITCH and GRANT 1982 |
| | 8 | 16 | Tropical E Africa | ZINOV'EVA-STAHEVITCH and GRANT 1984 |
| *I. psittaciana* Hook. f. | | 34 | Thailand | SHIMIZU 1979 |
| *I. psychadelphoides* Launert | | 32 | Mozambique | JONES and SMITH 1966 |
| *I. puberula* DC. | 14 | | Nepal | MALLA *et al.* 1977A |
| | | 20 | Nepal | AKIYAMA *et al.* 1992 |
| | | 20 | ? | WAKABAYASHI 1992 |
| *I. pulcherrima* Dalzell | | 12 | Maharashtra, India | ZINOV'EVA-STAHEVITCH and GRANT 1982 |
| | 6 | 12 | Maharashtra, India | ZINOV'EVA-STAHEVITCH and GRANT 1984 |
| | 6 | | Maharashtra, India | ZINOV'EVA-STAHEVITCH and GRANT 1985 |
| *I. pusilla* Heyne | 8 | | Kerala, India | BHASKAR 1976 |
| | | 16 | S India | RAO *et al.* 1986 |
| *I. racemosa* DC. | 9 | | Himalaya | CHATTERJEE and SHARMA 1970 |
| | 10 | | Nepal | MALLA *et al.* 1977B |
| | | 18 | Thailand | SHIMIZU *et al.* 1984 |
| | | 18 | Himalaya | AKIYAMA *et al.* 1992 |
| | | 18 | Yunnan, China | SUGAWARA *et al.* 1994 |
| | | 18 | Himalaya | AKIYAMA *et al.* 1996a |
| | | 18 | Yunnan, China | SUGAWARA *et al.* 1997 |

| Taxon | Chrom. No. | | Origin | References |
|---|---|---|---|---|
| | *n* | *2n* | | |
| *I. radiata* Hook. f. | 10 | | India | Sarkar *et al.* 1974 |
| | | 18 | Nepal | Akiyama *et al.* 1992 |
| | | 18 | Himalaya | Akiyama *et al.* 1996a |
| | | 18 | Yunnan, China | Sugawara *et al.* 1997 |
| | | 18 | Yunnan, China | present study |
| *I. rectangula* Hand.-Mazz. | 9 | 18 | Yunnan, China | present study |
| *I. repens* Moon | | 14 | Sri Lanka | Jones and Smith 1966 |
| | | 14 | Sri Lanka | Zinov'eva-Stahevitch and Grant 1982 |
| | 7 | 14 | Sri Lanka | Zinov'eva-Stahevitch and Grant 1984 |
| | 7 | | Sri Lanka | Zinov'eva-Stahevitch and Grant 1985 |
| | | 14 | India | Arisumi 1987 |
| *I. rostellata* Franch. | 10 | | Sichuan, China | present study |
| *I. rubromaculata* Warb. | | 16 | Kenya | Jones and Smith 1966 |
| *I. rubrostriata* Hook. f. | | 20 | Yunnan, China | Sugawara *et al.* 1994 |
| | | 40 | Yunnan, China | Sugawara *et al.* 1997 |
| | | 20 | Yunnan, China | present study |
| *I. ruiliensis* S. Akiyama et H. Ohba | | 18 | Yunnan, China | Sugawara *et al.* 1994 |
| | | 18 | Yunnan, China | Akiyama *et al.* 1996a |
| | | 18 | Yunnan, China | Sugawara *et al.* 1997 |
| *I. sarcantha* Hook. f. ex Ridley | 16 | | SE Asia | Hellmayr *et al.* 1994 |
| *I. scabrida* DC. | | 14 | Himalaya ? | Smith 1934 |
| | | 14, 20 | Himalaya | Fedorov 1969 |
| | 6, 7 | | Cult. Kew, UK | Jones and Smith 1966 |
| | 8 | | Himalaya | Sharma and Ghosh 1976 |
| | | 14 | Himalaya | Zinov'eva-Stahevitch and Grant 1982 |
| | 7 | 14 | Himalaya | Zinov'eva-Stahevitch and Grant 1984 |
| | | 14 | Nepal | Akiyama *et al.* 1992 |
| | | 14 | ? | Wakabayashi 1992 |
| *I. scapiflora* Heyne | 6, 10 | | Karnataka, India | Bhaskar 1976 |
| | 8 | | Kerala, India | Bhaskar 1976 |
| | 6 | | Koyanad, India | Bhaskar 1980 |
| | 7, 8 | | Neilliambudi, India | Bhaskar 1980 |
| | 8 | | Devicolam, India | Bhaskar 1980 |
| | 10 | | Jodpala, India | Bhaskar 1980 |
| *I. scapiflora* Heyne var. *pseudoacaulis* Bhaskar | 10 | | Shevaroys, India | Bhaskar 1980 |
| | 10, 16, 20 | | Naduvattam, India | Bhaskar 1980 |
| *I. schlechteri* Warb. | | 32 | New Guinea | Jones and Smith 1966 |
| | 16 | 32 | New Guinea | Zinov'eva-Stahevitch and Grant 1984 |
| *I. scullyi* Hook. f. | | 18 | Nepal | Akiyama *et al.* 1992 |
| | | 18 | ? | Wakabayashi 1992 |
| *I. scutisepala* Hook. f. | 9 | | Yunnan, China | present study |
| *I. serrata* Benth. | | 14 | Himalaya | Fedorov 1969 |
| | | 14, 15 | Nepal | Akiyama *et al.* 1992 |
| | | 15 | ? | Wakabayashi 1992 |
| *I. siculifer* Hook. f. | | 18 | Yunnan, China | Sugawara *et al.* 1994 |
| | 9 | | Yunnan, China | present study |
| *I. sodenii* Engl. et Warb. ex Engl. | | 16 | Kenya | Zinov'eva-Stahevitch and Grant 1982 |
| | 8 | 16 | Tropical E Africa | Zinov'eva-Stahevitch and Grant 1984 |
| | 8 | | Kenya | Zinov'eva-Stahevitch and Grant 1985 |
| | | 16 | S India | Rao *et al.* 1986 |
| *I. stenantha* Hook. f. | | 18 | Nepal | Stahevitch 1995 |
| | 9 | | Yunnan, China | present study |
| *I. stocksii* Hook. f. et Thoms. | 7 | | Bababudangiri, India | Bhaskar 1980 |
| *I. sulcata* Wall. | | 18 | Nepal | Akiyama *et al.* 1992 |
| | 10 | 20 | Himalaya | Fedorov 1969 |
| *I. sunkoshiensis* S. Akiyama, H. Ohba et M. Wakabayashi | | 18 | Nepal | Akiyama *et al.* 1992 |
| *I. talangensis* | | c.60 | W Sumatra | Okada *et al.* 1989 |
| *I. talbotii* Hook. f. | 6 | | Karnataka, India | Bhaskar 1976 |
| *I. tangachee* Bedd. | 10 | | India ? | Zinov'eva-Stahevitch and Grant 1985 |
| *I. taronensis* Hand.-Mazz. | | 18 | Yunnan, China | present study |
| *I. tenella* Heyne | | 16 | S India | Govindarajan and Subramanian 1986 |
| | | 16 | S India | Rao *et al.* 1986 |
| *I. tenuibracteata* Y. L. Chen | 9 | | Yunnan, China | present study |

| Taxon | Chrom. No. | | Origin | References |
|---|---|---|---|---|
| | *n* | 2*n* | | |
| *I. textori* Miq. | | 20 | Korea | LEE 1967 |
| | | 20 | ? | FEDOROV 1969 |
| *I. thomsoni* Hook. f. | | 14, 20 | Himalaya | FEDOROV 1969 |
| | | 12, 16 | Himalaya | SHARMA and GHOSH 1976 |
| *I. tomentosa* Heyne | | 16 | S India | RAO *et al.* 1986 |
| *I. tongbiguanensis* S. Akiyama et H. Ohba | | 18 | Yunnan, China | SUGAWARA *et al.* 1994 |
| | | 18 | Yunnan, China | AKIYAMA *et al.* 1996a |
| *I. tortisepala* Hook. f. | 10 | | Sichuan, China | present study |
| *I. trichocepala* Y. L. Chen | 7 | | Yunnan, China | present study |
| *I. tripetala* Roxb. | | 14 | Himalaya | FEDOROV 1969 |
| | 8 | | Meghalaya, India | SARKAR *et al.* 1980 |
| *I. uguenensis* Warb. | | 16 | Kenya | JONES and SMITH 1966 |
| | | 16 | Africa | ARISUMI 1987 |
| *I. uliginosa* Franch. | | 18 | Yunnan, China | SUGAWARA *et al.* 1994 |
| | | 18 | Yunnan, China | SUGAWARA *et al.* 1997 |
| | 9 | | Yunnan, China | present study |
| *I. ulugurensis* Warb | 8 | | Tanzania | GILL and CHINNAPPA 1977 |
| *I. umbellata* Heyne | | 20 | S India | RAO *et al.* 1986 |
| *I. uncinata* Wight | 8 | | Tamil Nadu, India | BHASKAR 1976 |
| | | 14 | S India | RAO *et al.* 1986 |
| *I. uniflora* Hayata | 20 | | Taiwan, China | present study |
| *I. urticifolia* Wall. | | 18 | Nepal | AKIYAMA *et al.* 1992 |
| | | 36 | Himalaya | AKIYAMA *et al.* 1993 |
| *I. usambarensis* C. Grey-Wilson | 8 | | Tanzania | MERLIN and GRANT 1985 |
| | | 16 | Tanzania | ZINOV'EVA-STAHEVITCH and GRANT 1982 |
| | | 16 | Tanzania | ZINOV'EVA-STAHEVITCH and GRANT 1984 |
| *I. verticillata* Wight | | 16 | S India | RAO *et al.* 1986 |
| | | 14 | India | ARISUMI 1987 |
| *I. violaeflora* Hook. f. | | 10, 12 | Chiangmai, Thailand | LARSEN 1981 |
| *I. viscida* Wight | | 16 | Tamil Nadu, India | ZINOV'EVA-STAHEVITCH and GRANT 1982 |
| | 8 | 16 | Tamil Nadu, India | ZINOV'EVA-STAHEVITCH and GRANT 1984 |
| | 10 | | Tamil Nadu, India | ZINOV'EVA-STAHEVITCH and GRANT 1985 |
| | | 16 | S India | RAO *et al.* 1986 |
| *I. viscosa* Bedd. | 10 | | S India | BHASKAR and RAZI 1972-1973 |
| | | 16 | S India | RAO 1973 |
| | | 32 | Kerala, India | ZINOV'EVA-STAHEVITCH and GRANT 1984 |
| *I. walleriana* Hook. f. | | 16 | ? | FEDOROV 1969 |
| | | 16 | Africa ? | SMITH 1934 |
| | | 16 | Tanzania | JONES and SMITH 1966 |
| | 10 | | Tanzania | GILL and CHINNAPPA 1977 |
| | 8 | 16 | Tropical East Afria | ZINOV'EVA-STAHEVITCH and GRANT 1984 |
| | 8 | 16 | ? | ZINOV'EVA-STAHEVITCH and GRANT 1985 |
| | 8 | | E Africa | MERLIN and GRANT 1985 |
| | | 16 | S India | RAO *et al.* 1986 |
| | | 16 | S India | GOVINDARAJAN and SUBRAMANIAN 1986 |
| | | 16 | Africa | ARISUMI 1987 |
| *I. wuchengyihii* S.Akiyama, H. Ohba et S. K. Wu | | 14 | Yunnan, China | SUGAWARA *et al.* 1997 |
| *I. xanthina* Comber | 7 | | Yunnan, China | present study |
| *I. yingjiangensis* S. Akiyama et H. Ohba | | 16 | Yunnan, China | SUGAWARA *et al.*, 1994 |
| | | 16 | Yunnan, China | AKIYAMA *et al.*, 1996a |
| | 8 | | Yunnan, China | present study |
| *I. zombensis* Baker | | 16, 17 | Malawi, Africa | STAHEVITCH 1995 |

posed a migration routes based on the above hypothesis. When the most frequent basic chromosome numbers of the diversity centers are mapped onto GREY-WILSON's migration map (Fig. 5), we can see that the basic numbers x = 7, 8 are predominantly shared by species from Africa, southern India, Sri Lanka and Southeast Asia, while the basic number x = 10 is predominantly distributed in the northern hemisphere, and the basic number x = 9 is shared only by the species from Himalayas and Southeast Asia. However, this pattern does not necessarily support GREY-WILSON's (1980a) hypothesis about the origin and migration of *Impatiens*, because assuming a Southeast Asian origin of *Impatiens* and subsequent radiations to all other areas including southern India, Sri Lanka, Africa and Madagascar (thus all the African and Madagascan

species are derived from the Asian ancestors) can equally result in a such pattern. Traditional karyological studies alone may not be able to serve as a test of these alternative hypotheses. However, assuming GREY-WILSON's hypothesis implies an ascending dysploidy as chromosomal evolution, as higher basic numbers such as x = 9 or x = 10 are either infrequent or completely missing from Africa. Conversely, assuming a Southeast Asian origin of *Impatiens* implies more descending dysploidy. Our ongoing molecular phylogenetic studies on the family will hopefully give more insights on these controversies.

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## REFERENCES

AKIYAMA S., OHBA H., SUGAWARA T., YANG Y.P. and MURATA J., 1995 – *Notes on Impatiens (Balsaminaceae) from South-western Yunnan, China*. J. Jap. Bot., 70: 95-106.

AKIYAMA S., OHBA H.and WAKABAYASHI M., 1993 – *Correlation between flower form and chromosome number in the Himalayan Impatiens*. p. 254, in «XV International Botanical Congress», Abstracts.

AKIYAMA S., OHBA H. and WU S.K., 1996a – *Cytotaxonomic comparison of Impatiens distributed in Nepal Himalaya and Yunnan*. In: "International Symposium on Floristic Characteristics and Diversity of East Asian Plants", Abstracts p138. Botanical Society of China, Kunming.

–, 1996b – *Further notes of Impatiens (Balsaminaceae) from Yunnan China. Bull.* Nation. Sci. Mus., B (Tokyo) 22: 135-144.

AKIYAMA S., WAKABAYASHI M. and OHBA H., 1992 – *Chromosome evolution in Himalayan Impatiens (*Balsaminaceae*)*. Bot. J. Linn. Soc., 109: 247-257.

ARISUMI T., 1987 – *Cytology and morphology of ovule culture-derived interspecific Impatiens hybrids*. J. Amer. Soc. Hort. Sci., 112: 1026-1031.

AYYANGAR K.R., RAO R.V.S. and SAMPATHKUMAR. R., 1987 – *An insight into the meiotic profile and behaviour in some members of Balsaminaceae*. Cytologia, 52: 809-812.

BHAT B.K., BAKSHI S.K. and KAUL M.K., 1975 – *In IOPB Chromosome number reports XLIX*. Taxon, 24: 501-516.

BHASKAR V., 1975 – *Studies in Balsaminaceae. PH. D. thesis*, University of Mysore, India.

–, 1976 – *In IOPB chromosome number reports LI*. Taxon, 25: 155-164.

–, 1980 – *Chromosome drift in Impatiens L. (Balsaminaceae).* Cytologia, 45: 233-239.

BHASKAR V. and RAZI B.A., 1972-1973 – *Studies in South Indian Balsaminaceae I: Chromosome numbers in some species of Impatiens L.* J. Mysore Univ. Sect., B25: 115-122.

CHATTERJEE D., 1940 – *Studies on the endemic flora of India and Burma*. Jour. Roy. As. Soc. Bengal, Sci., 5: 19-67.

CHATTERJEE A. and SHARMA A.K., 1970 – *Chromosome study in Geraniales*. Nucleus (Calcutta), 13: 179-200.

CHEN Y.L., 2001 – *Flora Relpublicae Popularis Sinicae.* Tomus 47(2). Science Press, Beijing.

CHESHMEDYIEV V., 1994 – *Mediterranean chromosome number reports* 4: 313-366.

CHINNAPPA C.C. and GILL L.S., 1974 – *Chromosome numbers from pollen in some North American Impatiens (Balsaminaceae)*. Can. J. Bot., 52: 2637-2639.

DOBES C., HAHN B. and NOTAWETZ W., 1997 – *Chromosomenzahlen zur Gefässpflanzen Flora österreichs*. Linzer Biol. Beitr., 29 : 5-43.

FEDOROV A. A., 1969 – *Chromosome Numbers of Flowering Plants*. USSR Acad. Sci. Leningrad.

FISCHER E. and RAHELIVOLOLONA M.E., 2002 – *New taxa of Impatiens (Balsaminaceae) from Madagascar I*., Adansonia sér. 3, 24: 271-294.

GADELLA T.W J., 1977 – *In IOPB chromosome number reports LVI*. Taxon, 26: 257-274.

–, 1982 – *In IOPB Chromosome number reports LXXVI*. Taxon, 31: 595-596.

GAO L. and ZHANG Z., 1984 – *The chromosome numbers of some seed plants*. Chin. Bull. Bot., 2: 43-44.

GILL L.S. and CHINNAPPA C.C., 1977 – *Chromosome numbers from herbarium sheets in some Tanzanian Impatiens L. (Balsaminaceae).* Caryologia, 30: 375-379.

GOHIL R.N., RAINA R. and ASHRUF M., 1981 – *Chromosome number reports LXXII.* Taxon, 30: 697.

GOVINDARAJAN T., 1985 – *Karyotype analysis in four species of genus Impatiens Riv*. Ex Linn. Proc. Indian Sci. Congr. Assoc., 72 (3-VI): 129.

GOVINDARAJAN T. and SUBRAMANIAN. D., 1986 – *Karyotaxonomy of South Indian Balsaminaceae*. Cytologia, 51: 107-116.

GREY-WILSON C., 1980a – *Impatiens of Africa*. A.A. Balkema., Rotterdam, Netherlands.

–, 1980b – *Hybridization in African Impatiens. Studies in Balsaminaceae: 2*. Kew Bull., 34: 689-722.

GVNIANIDZE Z.I. and AVAZNELI A.A., 1982 – *Khromosomnye chisla nekotorykh predstavitelej vysokogornykg floristi-cheskikh kimpleksov Kvkaya*. Soobsc. Acad. Nauk Gruzinsk. SSR (Tbilisi) 106: 577-580.

HELLMAYR E.M., KIEHN M. and WEBER., 1994 – *Chromosome numbers of Malayan rain-forest angiosperms*. Betrage zur Biologie Pflanzen, 68: 51-71.

HONG D.Y., 1990 – *Plant Cytotaxonomy.* p. 120, Science Press, Beijing.

JAVURKOVA V., 1979 – *In IOPB chromosome number reports LXIV*. Taxon, 28: 400-401.

JEE V., DHAR U. and KACHROO P., 1989 – *Cytogeography of some endemic taxa of Kashmir Himalayan*. Proc. Indian Natl. Sci. Acad., Part B, Biol. Sci., 55: 177-184.

JONES K. and SMITH J.B., 1966 – *The cytogeography of Impatiens L.* (Balsaminaceae). Kew Bull., 20: 63-72.

KHOSHOO T.N., 1955a – *Cytology of Impatiens*. Current Sci., 24: 423-424.

–, 1955b – *Biosystematies of the Sisymbrium irio complex*. Nature (London), 176: 608.

–, 1956 – *Chromosomes from herbarium sheets of Impatiens*. Stain Technol., 31: 31-33.

–, 1957 – *Cytology of some Impatiens species*. Caryologia, 10: 55-74.

–, 1966 – *Cytology of pollen with particular reference to Impatiens and Allieae*. Proc. Indian Acad. Sci., Seat. B, 63: 35-45.

LARSEN K., 1981 – *Chromosome numbers in Impatiens from Thailand*. Nordic J. Bot., 1: 43-44.

LEE Y.N., 1967 – *Chromosome number of flowering plants in Korea*. J. Korean Res. Inst. Ewha Women's Univ., 11: 455-478.

LEVAN A., Fredga K., Sandberg A.A., 1964 – *Nomenclature for centrimeric position on chromosomes*. Hereditas, 52: 201-220.

LÖVE A. and LÖVE. D., 1982 – *In IOPB Chromosome number reports LXXV*. Taxon, 31: 344-360.

MALLA S.B., BHATTARAI S., GORKHALI M. and SINGH M.P., 1975 – *In IOPB chromosome number reports XLIX*. Taxon, 24: 501-516.

–, 1977a – *In IOPB chromosome number reports LVII*. Taxon, 26: 443-452.

MALLA S.B., BHATTARAI S., GORKHALI M., SAIJU H. and KAYASTHA M., 1977b – *In IOPB chromosome number reports LVII*. Taxon, 26: 557-565.

–, 1978 – *In IOPB Chromosome number reports LXII*. Taxon, 27: 519-535.

MERLIN C.M. and GRANT W.F., 1985 – *Hybridization studies in the genus Impatiens*. Canad. J. Bot., 64: 1069-1074.

MORTON J.K., 1993 – *Chromosome number and polyploidy in the flora of Cameroon Mountain*. Opera Bog. 121: 159-172.

MULLIGAN G.A., 1984 – *Chromosome numbers of some plants native and naturalised in Canad*. Naturaliste Canad., 111: 447-449.

NISHIKAWA T., 1990 – *Chromosome counts of flowering plants of Hokkaido (13)*. Hokkaido Univ. Educ., Sec. 2B, 40: 19-30.

OGIUMA K. and TOBE H., 1991 – *Karyomorphology of two species of Impatiens from Kenya*. Acta Phytotax. Geobot., 42: 67-71.

OKADA H., 1989 – *Chromosome counts of some plants collected from west Sumatra*. In M. Hotta (ed.), "Diversity and Plant-Animal Interaction in Equatorial Rain Forests", Report of the 1987-1988 Sumatra Research, 16: 11-14. Ovv. Pap. Kagoshima Univ. Res. Center S. Pacific.

RAGHUVANSHI S.S. and MAHAJAN S., 1985 – *Morphology of the pachytene chromosomes of Impatiens balsamina L. var. bicolor*. Cytologia, 50: 233-239.

RAO R.V.S., 1973 – *Further studies on the cytology of South Indian Balsaminaceae*. Proc. Indian Sci. Congr., 3: 317.

–, 1975 – *On the chromosome counts from the pollen grains of Impatiens*. Proc. Indian Sci. Congr. Assoc., 62: 120.

RAO R.V.S., AYYANGAR K.R. and SAMPATHKUMAR R., 1986 – *On the karyological characteristics of some members of Balsaminaceae*. Cytologia, 51: 251-260.

RUDYKA E.G., 1995 – *Chromosome numbers in vascular plants from the Southern part of the Russian Far East*. Bot. Zhurn. (St. Petersburg), 80: 87-90.

SARKAR A.K., DATTA N. and CHATTERJEE U., 1980 – *In Chromosome number reports LXVII*. Taxon, 29: 360-361.

SARKAR A.K., DATTA N. and RAYCHOWDHURY M., 1974 – *Balsaminaceae. IOPB chromosome number reports XLVI*. Taxon, 23: 810.

SARKAR A.K., DATTA N., RAYCHOWDHURY M. and DAS S., 1975 – *In IOPB chromosome reports L*. Taxon, 24: 671-678.

SHARMA A.K. and GHOSH A., 1976 – *Cytotaxonomical studies in Bambusae*. Res. Bull. Univ. Calcutta, 3: 51-52.

SHIMIZU T., 1979 – *A comment on the limestone flora of Thailand, with special reference to Impatiens*. Acta Phytotax. Geobot., 30: 180-187.

–, 1984 – *Chromosome numbers of the genus Impatiens L.* Bull. Bot. Soc. Nagano, 17: 19-25.

SHIMIZU T., KONTA F. KOYAMA H. and SHIMIZU M., 1984 – *Contribution to the Southeast Asia. VII. Taxonomy and phytogeography of some temperate species in Thailand (3)*. Acta Phytotax. Geobot., 35: 37-43.

SMITH F.H., 1934 – *Prochromosomes and chromosome structure in Impatiens*. Proc. Amer. Phil. Soc., 74: 193-215.

SNOW R., 1963 – *Alcoholid-hydrochloric acid-carmine as a stain for chromosomes in squash preparations*. Stain Techn., 38:9-13.

STAHEVITCH A.E., 1995 – *IOPB chromosome data 9.* Newslett. International Organanization of Plant Biosystimatics (Zürich), 24: 23.

STEBBINS G.L., 1971 – *Chromosomal Evolution in Higher plants.* Edward Arnold Ltd., London.

SUGAWARA T., AKIYAMA S., MURATA. J. and YANG Y.P., 1994 – *Karyology of ten species of Impatiens (Balsaminaceae) from SW.Yunnan, China*. Acta Phytotax. Geobot., 45: 119-125.

SUGAWARA T., AKIYAMA S., YANG Y.P. and MURATA. J., 1997 – *Karyological characteristics of Impatiens (Balsaminaceae) in Yunnan, China*. Acta Phytotaxonomica Geobot., 48: 7-14.

TAGAWA K., ARAKI H. and MAKI M., 1997 – *Chromosome numbers of Impatiens hypophylla var. hypophylla and var. microhypophylla*. Journal of Phytogeography and Taxonomy, 45: 32-34.

WAKABAYASHI M., 1992 – *Kozansyokubutsu no Sensyokutai no Tokusei*. Journal of Phytogeography and Taxonomy, 46: 36-42.

ZINOV'EVA-STAHEVITCH A.E. and GRANT. W.F., 1982 – *In IOPB chromosome number reports LXXIV*. Taxon, 31: 365-366.

–, 1984 – *Chromosome numbers in Impatiens (Balsaminaceae)*. Canad. J. Bot., 62: 2630-2635.

–, 1985 – *Aberrant microsporogensis Impatiens L. (Balsaminaceae) and its bearing on the taxonomy of the genus*. Cytologia, 50: 825-836.

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# Paper III

# Phylogeny and biogeography of Balsaminaeae inferred from ITS sequences

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# Phylogeny and biogeography of Balsaminaceae inferred from ITS sequences

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Sequences of the internal transcribed spacers (ITS) of nuclear ribosomal DNA were acquired for 112 species of Balsaminaceae worldwide and five species of its closest relatives Marcgraviaceae and Tetrameristaceae. Phylogenetic analyses applying parsimony and distance estimates confirmed the monophyly of Balsaminaceae and suggest the monophyly of *Impatiens*. Within *Impatiens*, a few clades are recognized with strong support. Two of the most important clades are the spurless Madagascan endemic group, and the one comprising species with broadly fusiform fruits and the basic chromosome number $x = 8$, that shows a Southeast Asia, southern India, Africa, and Madagascar connection. Despite recognition of several strongly supported small lineages, ITS data alone could not resolve relationships among most of the lineages with confident support values. ITS phylogenies are therefore of limited taxonomic value for *Impatiens*. However, ITS phylogenies do reveal that extant *Impatiens* species are of Southeast Asian origin, from where dispersals to boreal Eurasia and North America, to central Asia and eastern Europe via the Himalayas, and to India and Africa have occurred. The Madagascan *Impatiens* show an African origin. Molecular phylogenies suggest the ancestral basic chromosome number to be $x = 10$, and the spurred flowers and elongated linear fruits to be plesiomorphic states in *Impatiens*. A predominantly descending dysploid chromosome evolution, following dispersal of the clade with broadly fusiform fruits from Southeast Asia to India, Africa, and Madagascar, is also suggested.

KEYWORDS: Balsaminaceae, biogeography, chromosome evolution, ITS, phylogeny.

## INTRODUCTION

Balsaminaceae are a medium-sized family with two genera, *Hydrocera* and *Impatiens*. Other generic names published for Balsaminaceae, such as *Petalonema* Peter, *Semeiocardium* Zoll. and *Impatientella* H. Perrier, are confirmed to be synonyms of *Impatiens* (Grey-Wilson, 1989a; Rao & al., 1986). *Hydrocera* has only one species, *H. triflora*, a semi-aquatic herb native to the Indo-Malesian countries. Morphologically, it can be easily distinguished from *Impatiens* by its five free petals and the indehiscent berry-like fruit. *Impatiens* is a highly diversified genus with over 900 species distributed primarily in the highlands and mountains of the Old World tropics and subtropics with five conspicuous diversity hotspots: tropical Africa, (ca. 109 spp.; Grey-Wilson, 1980b), Madagascar (ca. 120 spp.; Fischer & Rahelivololona, 2002), southern India and Sri Lanka (ca. 150 spp.), the eastern Himalayas (ca. 120 spp.), and Southeast Asia in its broad sense (including Burma, Thailand, southwest China, the Indochina peninsula, and the Malesian archipelagos, ca. 250 spp.). Many new species are still being described from these regions (e.g., Chen, 2000; Shimizu, 2000; Fischer & Rahelivololona, 2002; Fischer & al., 2003; Huang & al., 2003). High proportions of local endemism are associated with these hotspots, for example, as many as 91% of the southern Indian species are endemic (Rao & al., 1986), and almost all the native species of Madagascar are endemic. Contrary to paleotropical areas, only a few *Impatiens* species are found in temperate areas of the northern hemisphere. There are no native species in South America or Australia. Some *Impatiens* species are of horticultural importance, e.g., the popularly grown flowers, the "Balsam", *I. balsamina*, the "Busy Lizzie", *I. walleriana*, and the "New Guinea Hybrid *Impatiens*", *I. hawkeri*.

*Impatiens* is a well-known example of a taxonomically difficult group (Hooker & Thompson, 1859; Grey-Wilson, 1980b). The delicate yet hypervariable structure

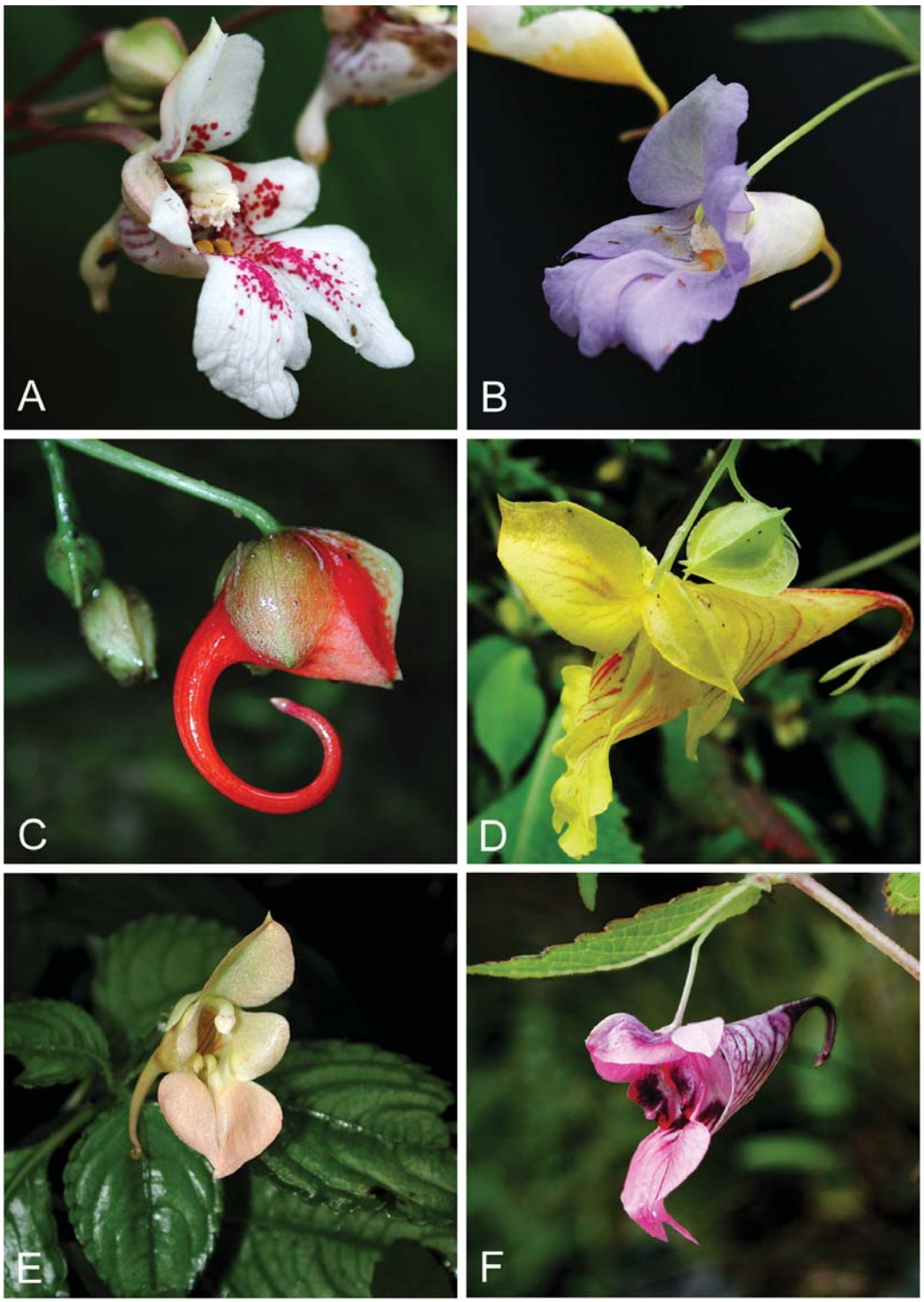



**Fig. 1.** ***Impatiens*** **shows tremendous diversity in floral colour and morphology, and can be considered as the "dicots counterpart of orchid". Its flower is zygomorphic and resupinate through twisting of the pedicel. Each flower has three sepals, two of which are reduced lateral sepals and one petal-like lower sepal that is modified into a nectary-tipped spur that exhibits a wide range of variation in form and size, from shallowly navicular to bucciniform or deeply saccate, short or long filiform, straight, twisted or curved. Each flower has five petals. One upper dorsal petal is usually hood-like. The other four lower petals are united into two lateral pairs, yet each retains two unequal lobes. The united lateral petals are also extremely variable in shape and size, associated with different pollinators as they provide a suitable landing platform and entrance guide for pollinators to the spur and nectar. The five stamens are united by the upper part of the filaments and completely cap the gynoecium, which has five fused carpels. Shown here are examples of flowers of** ***Impatiens*** **of different colours. A,** ***I. campanulata***; **B,** ***I. arguta***; **C,** ***I. yingjiangensis***; **D,** ***I. soulieana***; **E,** ***I. mengtseana***; **F,** ***I. delavayi***.

and fragile nature of its flowers (Fig. 1) make it almost impossible to determine a species when specimens are pressed conventionally without detailed descriptions or drawings of its floral morphology (Grey-Wilson, 1980c). Many early publications based on herbarium specimens are therefore incomplete or misleading. The only global infrageneric classification of *Impatiens* was the early work of Warburg & Reiche (1895), which recognized 14 sections. However, this classification was considered neither natural nor practical (Grey-Wilson, 1980b). Thus a reliable infrageneric classification is still missing. Although aggregates of closely allied species can be recognized, it is hard to unravel the phylogenetic relationships among the species aggregates on the basis of gross morphology. These difficulties have necessarily limited most studies on *Impatiens* so far to be regional and purely descriptive taxonomic treatments (e.g., Perrier de la Bathie, 1934, 1948; Humbert, 1956; Chen, 1978; Grey-Wilson, 1980a, 1985, 1989a; Akiyama & al., 1992a, 1995, 1996; Akiyama & Ohba, 2000; Fischer & Rahelivololona, 2002).

*Impatiens* shows a wide range of chromosome number variation of $2n$ = 6, 8, 10, 12, 14, 15, 16, 17, 18, 19, 20, 24, 26, 28, 30, 32, 34, 36, 40, 44, 48, 50, 54, and 66 (Song & al., 2003). The most frequent numbers are $2n$ = 14, 16, 18, and 20, that take the major part (78%) of the species observed. Different hypotheses have been proposed regarding the evolution of the basic numbers through dysploidy in conjunction with polyploidy in *Impatiens*. Jones & Smith (1966) and Akiyama & al. (1992b) suggested $x$ = 7 to be the ancestral type, from which the other numbers were derived mainly by ascending dysploidy, whereas Rao & al. (1986) suggested evolution of $x$ = 7, 9 and 10 from the basic number $x$ = 8 through both descending and ascending dysploidy. Phylogenetic studies may offer independent test on the evolution of the basic chromosome numbers.

Balsaminaceae show an interesting distributional pattern. While *Hydrocera* is an Indo-Malesian genus, *Impatiens* basically exhibits prominent African-Malagasy/Indo-Himalayan-Southeast Asian phytogeographic connection, since its diversity centers are all located along this chain. Whereas the species in northern temperate regions probably represent recent radiations from Southeastern Asia and the adjacent Sino-Himalayan areas, it is still questionable whether the disjunctive distribution of the main diversity centers across the paleotropical regions represents ancient vicariant events resulting from the fragmentation of Gondwana, or more recent dispersals. Jones & Smith (1966) suggested that *Impatiens* originated in the Himalayan region and dispersed to other areas based on species diversity and karyological data. On the contrary, Grey-Wilson (1980b) formulated an hypothesis suggesting that Balsaminaceae originated in western Gondwana in the Paleogene ca. 50 million years ago (Ma), and subsequently spread to Southeast Asia through Madagascar and India at the time of or after the Indian plate collided with Laurasia (ca. 45 Ma). He rejected the possibility of an overland migration between Africa and India. These conclusions were based on the pronounced similarity of species among Africa, Madagascar, and southern India, as well as on the assumed sister relationship between Balsaminaceae and Tropaeolaceae. The distributional patterns of extant Balsaminaceae, however, suggest that the diversification of the family probably started in South or Southeast Asia instead of Africa, as *Hydrocera* occurs only in these regions. Assuming a Southeast Asian origin of *Impatiens* and a subsequent radiation to Africa and Madagascar through India can equally result in the pronounced similarity of species among India, Africa, and Madagascar that was correctly recognized by Grey-Wilson (1980b). Obviously, the competing biogeographic hypotheses can be tested by using the phylogenies of the species from the main diversification centers.

Despite the conspicuous diversity and the interesting distributional patterns shown by Balsaminaceae, few molecular phylogenetic studies have been made on this family. Several studies aimed at addressing phylogenetic relationships at the family level or above have included limited representative species (not more than five) of Balsaminaceae. These studies have confirmed Balsaminaceae as a member of the order Ericales at the base of the asterids (Morton & al., 1996, 1997; Soltis & al., 2000; Albach & al., 2001; Anderberg & al., 2002; Bremer & al., 2002; Geuten & al., 2004), instead of the order Geraniales of former Rosidae as traditionally considered (Cronquist, 1981). Recently, 25 species from the eastern Himalayan area have been subjected to a molecular phylogenetic study using chloroplast *rbcL* and *trnL-F* sequences (Fujihashi & al., 2002). Besides its inappropriate analysis (e.g., too distant outgroups from rosids were used), this study is sketchy due to its limited sampling, and the resulted phylogenies are incapable of addressing the questions about overall phylogeny and biogeography of the whole family as mentioned above. Global phylogenetic relationships within the family remain unknown, and the more general problems such as diversification patterns of floral and vegetative morphology and historical biogeography of the family need to be addressed through more comprehensive global studies of the entire family. Here we conducted a comprehensive molecular phylogenetic study on Balsaminaceae by using nucleotide sequence data of internal transcribed spacer regions of nuclear ribosomal DNA. Through the molecular phylogenies we intended to further examine the morphological and karyological evolution, as well as the historical biogeography, of the family.

## MATERIALS AND METHODS

**Ingroup sampling and outgroup choice. —** We sampled both genera of Balsaminaceae, *Hydrocera* and *Impatiens*. As far as possible, samples were selected to maximize representation of the whole distribution range and the diversity of *Impatiens*. Representatives from all the hot-spots of species diversity of the genus were sampled. Representatives of the closely related families, Marcgraviaceae and Tetrameristaceae sensu APG (2003), were sampled as outgroups, following the results revealed by the recent molecular phylogenetic studies on large scope (Soltis & al., 2000; Albach & al., 2001; Anderberg & al., 2002; Geuten & al., 2004). The species, origin of samples, voucher information, and GenBank sequence accessions are listed in the Appendix (see online version of *Taxon*). Our data matrix includes 117 taxa in total, of which five taxa of Tetrameristaceae and Marcgraviaceae were used as outgroups. A reduced analysis limited to Balsaminaceae (112 taxa in total) was also conducted, to reveal possible consequences caused by high divergence among the three families. In the latter case, *Hydrocera triflora* was considered as an outgroup.

**DNA extraction, PCR amplification and sequencing. —** Total DNA was extracted from fresh, silica-gel dried, or herbarium leaves with the CTAB method of Doyle & Doyle (1987) or the DNeasy Plant Mini Kit (QIAGEN AG, Basel). The ITS fragment was amplified via standard PCR in 25 µl reaction volume as described in Yuan & al. (2003). Successfully amplified DNA fragments were purified prior to sequencing using the QIAquick™ PCR purification kit (QIAGEN AG, Basel) following the manufacturer's protocol. Cycle sequencing reactions were performed using the dye-terminator chemistry as implemented in the ABI PRISM® BigDye™ Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems) in a Biometra thermal cycler. The sequencing products were cleaned using the Ethanol/Sodium Acetate precipitation method and then analyzed on an ABI310 automated sequencer (Applied Biosystems). Automation-generated base-calls were edited manually against the electropherograms using the software Sequence Navigator (Applied Biosystems, Foster City, U.S.A.). Alternatively, some sequences were obtained via the methods described in Geuten & al. (2004).

**Sequence alignment. —** The obtained ITS sequences were initially aligned with Clustal X applying the default parameters (Thompson & al., 1997) and then manually adjusted for indels otherwise not properly recognized by Clustal. Careful manual adjustment was necessary, and three regions that seem to correspond to loops in secondary rRNA structure (Denduangboripant & Cronk, 2001) involve high alignment ambiguity. These regions (103 sites in the aligned data matrix) were then excluded from subsequent phylogenetic analyses. The gaps of aligned ITS sequences were considered as missing data.

**Maximum parsimony (MP) analysis. —** For MP analysis, the dataset was analyzed with heuristic searches by using PAUP* v4.0b10 (Swofford, 2000). Characters were equally weighted and unordered. Branch collapse option was set to collapse if minimum length was zero. Heuristic searches were conducted in three steps. First search was made to obtain an empirical tree length. Subsequently, heuristic searches were conducted for 1000 replicates of random addition of sequences, with TBR branch swapping, ACCTRAN, MULTREES option on, STEEPEST DESCENT option off, and from each replicate a maximum of 100 trees saved. Finally, a TBR branch swapping was conducted on all the best trees found in the previous step to save a maximum of 100,000 trees. The optimal trees kept by this swapping were then filtered out and a consensus was calculated. Relative clade support was evaluated by bootstrap analyses (Felsenstein, 1985). Bootstrap values were calculated by using 500 replicates of heuristic searches, with random sequence addition, TBR branch swapping, MULTREES options on, the STEEPEST DESCENT option off, and a maximum of 1000 trees saved for each replicate.

**Neighbor-joining (NJ) analysis. —** As a comparison to MP analysis, a distance analysis applying NJ optimality criteria (Saitou & Nei, 1987) was also conducted on both complete and reduced data matrices by using PAUP* v4.0b10 (Swofford, 2000). The NJ analyses applied maximum likelihood distance estimates based on the model and parameters suggested by Modeltest (3.06) using an arbitrarily chosen MP tree and the Akaike information criterion (Akaike, 1974; Posada & Crandall, 1998). Bootstrap values were obtained from 1000 replicate NJ analyses.

**Character-state optimization and biogeographic analyses. —** Selected characters, such as presence of flower spur, shape of fruit, and the basic chromosomal numbers, have been considered as unordered binary or multi-state characters and were optimized onto the molecular phylogeny to examine their evolution by using MacClade version 3.08 (Maddison & Maddison, 1992). The geographic distribution was also examined in the same way to trace the historical biogeography of Balsaminaceae. The NJ tree based on the reduced data set was used for the tracing. Arbitrarily chosen MP trees were also compared for the tracing. Chromosomal data were based on Song & al. (2003), and for species that have no chromosome number report the basic number was considered as missing. Seven areas of endemism

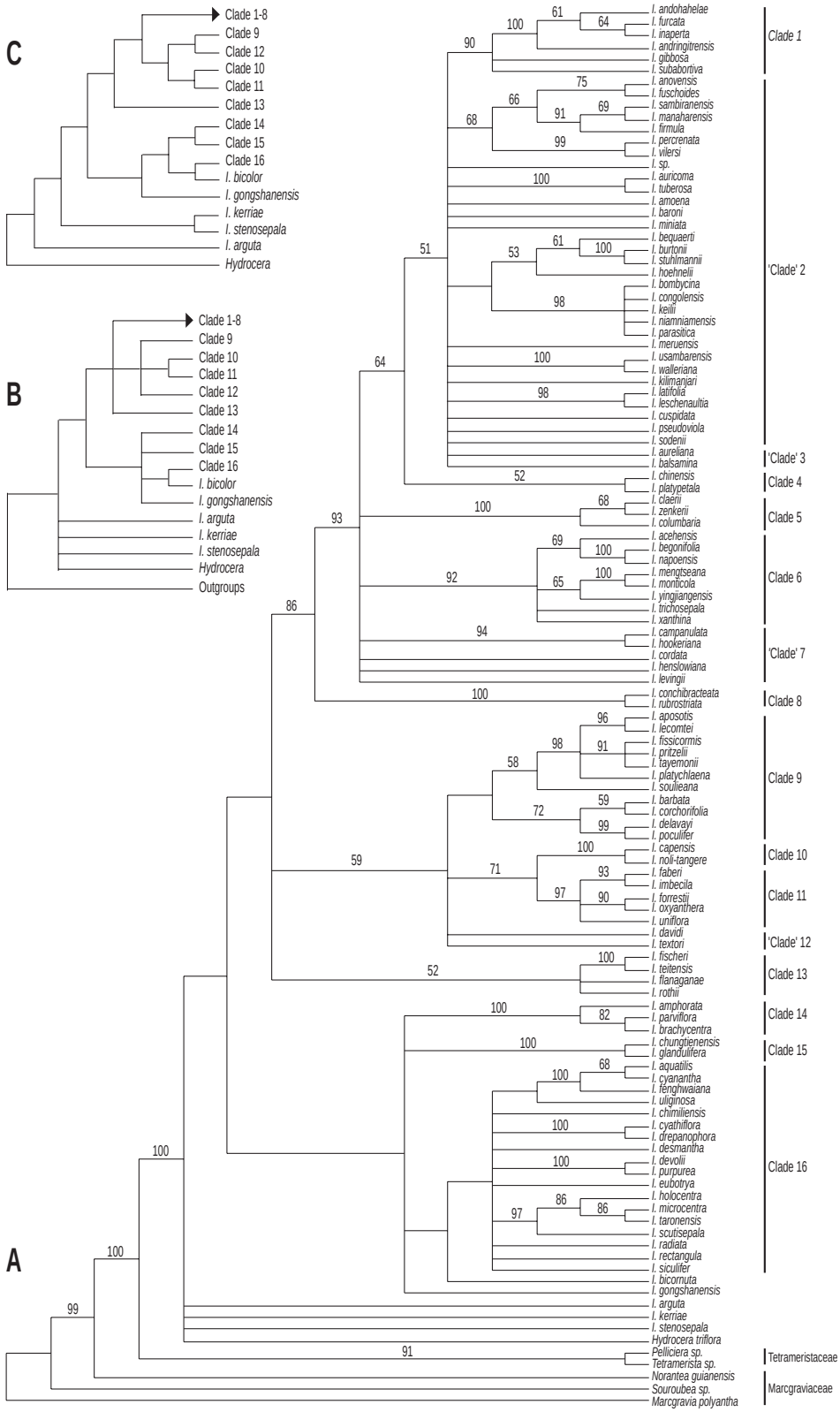



**Fig. 2. Parsimony analyses. A, the strict consensus of the most parsimonious trees retained from MP analyses on the complete ITS data set (length = 2928, CI = 0.34 including autapomorphies, CI = 0.30 excluding autapomorphies, RI = 0.66). Numbers above the branches are bootstrap values supporting the corresponding branch when greater than 50%. B, a simplified dendrogram of the strict consensus shown in A. C, a simplified dendrogram of the strict consensus of the most parsimonious trees retained from MP analyses on the reduced ITS dataset where the divergent outgroups were removed.**

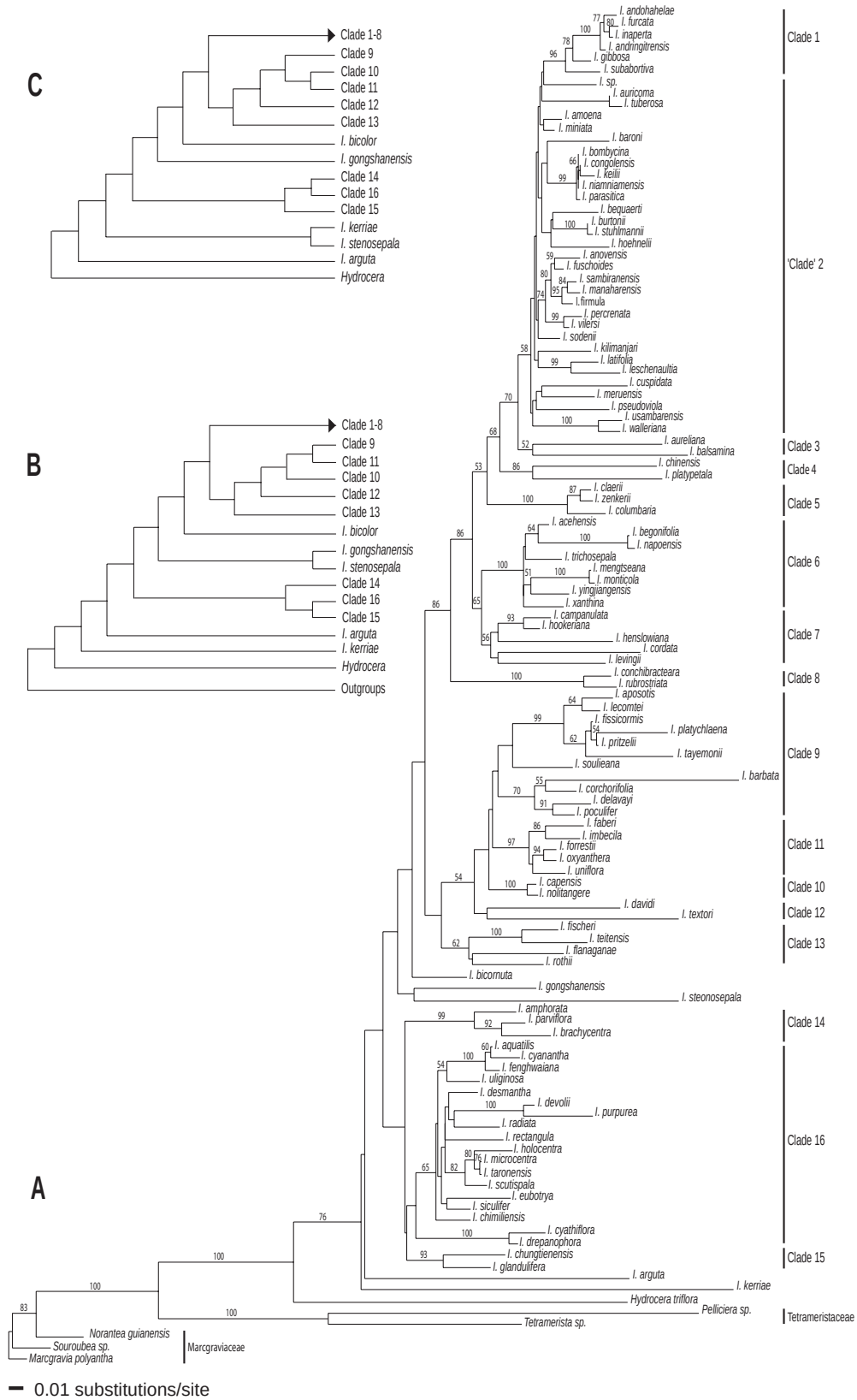



**Fig. 3. Neighbor-joining analyses: A, phylogram of the neighbor-joining tree based on maximum likelihood distance estimates of the complete ITS dataset. Numbers above the branches are bootstrap values supporting the corresponding branch when greater than 50%. B, a simplified dendrogram of the strict consensus shown in A. C, a simplified dendrogram of the neighbor-joining tree based on the reduced ITS dataset.**

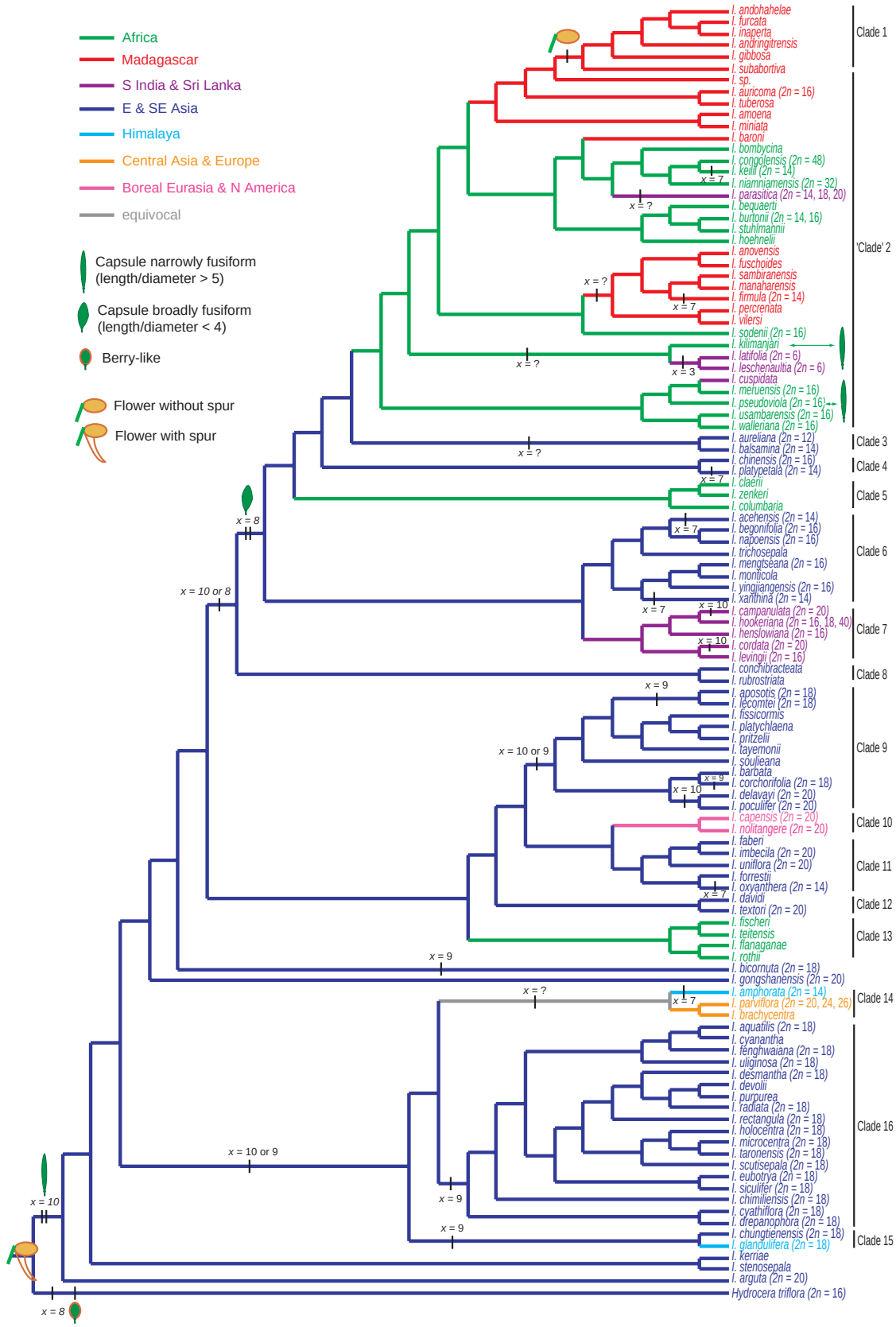



**Fig. 4. Parsimonious optimization of the distribution centers of endemism, basic chromosome numbers, and selected morphological characters onto the NJ tree generated from the reduced ITS dataset. Vertical bars indicate positions of character state changes. Chromosome numbers when available are shown after the species names.**

were designated on the basis of species diversity: (1) Africa, (2) Madagascar, (3) South India and Sri Lanka, (4) the Himalayas, (5) East and Southeast Asia, comprising Japan, Taiwan and the other Southeast Asian islands, the mainland Southeast Asian countries, and the area of the southern and southwestern China, (6) Central Asia and Europe, and (7) boreal Eurasia and North America. Wide distributions over more than two areas of endemism were counted as polymorphisms.

## RESULTS

**Sequence characteristics. —** All the sequences are newly acquired and have been submitted to GenBank (Appendix). No evidence of paralogous ITS sequences was found, because all PCR products were resolved as a single band and no double peaks were encountered in sequencing. The length of the complete unaligned ITS fragments of the ingroup taxa ranged from 579 to 655 base pairs.

The full ITS dataset including ingroup and outgroup taxa consisted of 783 characters, of which 103 (13.2%) involved alignment ambiguity and were excluded from phylogenetic analyses, 180 (23.0%) are constant, 121 (15.4%) are variable but uninformative, and 379 (48.4%) are potentially informative. This dataset resulted in pairwise maximum likelihood distance estimate ranging from 0 (*I. bombycina* vs. *I. congolensis*, *I. bombycina* vs. *I. niamniamensis*, *I. congolensis* vs. *I. niamniamensis*) to 0.840 (*I. platychlaena* vs. *Pelliciera* sp.) among all taxa, and 0 to 0.542 (*I. kerriae* vs. *Hydrocera triflora*) among the ingroup taxa. The alignments were submitted to TreeBASE and are also available from the corresponding author.

**ITS phylogeny. —** MP analysis of the ITS dataset including all outgroup and ingroup taxa resulted in an unknown number of equally parsimonious trees (61,443 trees kept) of 2928 steps, consistency index (CI) = 0.34 including autapomorphies, CI = 0.30 excluding autapomorphies, and retention index (RI) = 0.66. The strict consensus of the optimal trees kept by heuristic searches and the bootstrap clade support (when greater than 50%) are shown in Fig. 2A. The MP strict consensus tree was moderately resolved. Despite the relative high sequence divergence, the resolution among the main lineages is poor or receives less significant support, while some lineages of closely related taxa are better resolved with strong supports. The internal branches connecting the main lineages are conspicuously shorter in comparison to the long terminal branches, suggesting a high proportion of autapomorphic variations of the sequences. The monophyly of Balsaminaceae is highly supported (bootstrap value 100%). The genus *Hydrocera* fell in a basal polytomy with the main *Impatiens* clade and several other *Impatiens* species, viz., *I. arguta*, *I. kerriae*, and *I. stenosepala*.

We assigned numbers (clades 1 through 16) to the resolved lineages of *Impatiens* (Fig. 2A). In addition, a group of closely related non-monophyletic taxa were also referred as a clade, e.g., "clade 2", for convenience of illustration and discussion. Noticeably, the spurless Madagascan endemic taxa were resolved as a highly supported (90%) monophyletic clade (clade 1). Together with a few species from Southeast Asia (clades 3, 4 and 6), most of the sampled African, Madagascan, and southern Indian species formed a strongly supported clade (93%), consisting of the clades 1 through 7, whereas only four African species, *I. fischeri*, *I. flanaganae*, *I. teitensis*, *I. rothii*, and the Indian *I. repens* grouped together as a weakly supported clade (52%, clade 13) that nested out of the other African species but grouped with Southeast Asian clades. The North American species, *I. capensis*, showed close affinity with the boreal temperate species, *I. nolitangere* (100%, clade 10), and both together grouped with some Southeast Asian clades (9, 11, and 12). The eastern European species, *I. parviflora*, and the central Asian species, *I. brachycentra*, grouped together with the western Himalayan species, *I. amphorata*, as a strongly supported clade (100%, clade 14), and these three species together showed affinity to some Southeast Asian and Himalayan clades (Fig. 2A).

The general-time-reversible model with invariable sites and rate heterogeneity (i.e., GTR+I+Γ model) was suggested to best fit the ITS data based on an arbitrarily chosen MP tree (data not shown). NJ tree was reconstructed using maximum likelihood distance estimates applying the inferred model and its parameters. The topology of the neighbor-joining tree (Fig. 3A) highly resembles that of the strict consensus of the MP analyses. The monotypic genus *Hydrocera* was resolved as sister to the monophyletic *Impatiens* with moderate bootstrap support (76%). The main lineages, e.g., the clades 1 through 16 of *Impatiens*, remained almost the same as revealed by MP analyses with similar clade supports. The minor differences between NJ and MP analysis results involve different positions (Figs. 2A and 3A) of *I. bicornuta*, *I. gonshanensis*, *I. stenosepala*, clade 10 (consisting of *I. capensis* and *I. nolitangere*), and clade 15 (consisting of *I. chungtienensis* and *I. glandulifera*). However, these different resolutions did not receive significant bootstrap support in either analysis.

To determine if the relatively divergent outgroups had any potential influence on results of the phylogenetic analysis, we conducted both MP and NJ analyses on a reduced dataset that included only Balsaminaceae. The topology of the trees based on the reduced datasets strongly resembles that of trees generated from the com-

plete datasets, as shown in the simplified dendrograms (Figs. 2C, 3C). While the main topology of the trees remained the same, the positions of *I. arguta*, *I. bicornuta*, *I. gongshanensis*, *I. kerriae*, *I. stenosepala*, and clade 12 consisting of *I. davidi* and *I. textori* were slightly altered. However, none of these different resolutions receives significant support.

**Character-state mapping. —** Two morphological characters, occurrence of flower spur and shape of fruit, and the basic chromosome numbers were optimized onto the ITS tree generated from NJ analyses on the reduced dataset (Fig. 4). Parsimonious optimization suggests that linear-fusiform capsule, spurred flower, and basic chromosome number $x = 10$ represent the plesiomorphic states within *Impatiens*. Despite that the relationships among basal clades of the NJ tree were poorly supported, it is noteworthy that the important character-state changes suggested by the optimization correspond well to a few strongly supported nodes. For example, the broad-fusiform fruit was suggested as having evolved from linear-fusiform only once, which corresponds to the transition of basic chromosome number from $x = 10$ to $x = 8$, and the node of these transitions were highly supported by both MP and NJ analyses (bootstrap value 85–93%). The spurless flower was also suggested as a unique synapomorphic character-state for the Madagascan endemic clade 1 which received strong MP and NJ supports (90–96%). Optimization using arbitrarily chosen MP trees did not reveal any deviation of the above conclusions.

**Biogeographic analysis. —** Fitch parsimony optimization of centers of endemism as a multi-state character infers Southeast Asia as the ancestral area for extant Balsaminaceae (Fig. 4). *Impatiens* species in other areas are inferred to have dispersed from Southeast Asia. Two to three dispersals from Southeast Asia to Africa are suggested: one dispersal resulted in clade 13 consisting of *I. fischeri*, *I teitensis*, etc., and another one or two dispersals involved divergence of other African species. The Madagascan *Impatiens* are inferred as having dispersed from Africa, whereas the *Impatiens* species from southern India and Sri Lanka seem to have had two origins: some were dispersed from Southeast Asia (clade 7), and others from Africa (within the assemblage "clade" 2). The central Asian and European *Impatiens* were dispersed from Southeast Asia via the Himalayas, whereas the boreal Eurasian and North American *Impatiens* are resolved as being dispersed from Southeast Asia. Optimization using the NJ tree or arbitrarily chosen MP trees revealed the same pattern.

## DISCUSSION

**Phylogeny of Balsaminaceae. —** Recent studies, using cpDNA markers to address phylogenetic relationships at family level or above, have revealed the monophyly of Balsaminaceae and its inclusion in the order Ericales of the basal asterids (Morton & al., 1996, 1997; Soltis & al., 2000; Albach & al., 2001; Anderberg & al., 2002; Bremer & al., 2002; Geuten & al., 2004). Our present results based on nuclear ITS data are highly in agreement with these studies in concluding the monophyly of Balsaminaceae (bootstrap value 100% in both MP and NJ analyses).

At first glance, *Hydrocera triflora*, the only species of the genus, looks very much like an *Impatiens* species, in particular *I. balsamina* (Grey-Wilson, 1980d). Due to its unique floral (five free petals) and fruit (indehiscent and berry-like) morphology, *Hydrocera* is almost always considered as a genus distinct from *Impatiens* in modern botanical publications (e.g., Grey-Wilson, 1985, 1980d; Chen, 2001), despite that in some classic works such as Linnaeus' *Species Plantarum* it was considered as a species of *Impatiens*. Nevertheless, its phylogenetic relationships with the different lineages of *Impatiens* have never been explicitly studied. Since the floral morphology of *Impatiens* is highly diversified, the unique floral morphology of *H. triflora* does not necessarily ensure it as a distinct genus rather than a specialized ingroup of *Impatiens*. Our present results, however, allow the recognition of *Hydrocera* as a distinct genus sister to *Impatiens*. In the strict consensus of the MP trees, *H. triflora* falls into a basal polytomy consisting of *I. arguta*, *I. kerriae*, and *I. stenosepala*, and the clade comprising all the other *Impatiens* species (Fig. 2A), but in the NJ tree all *Impatiens* species are shown to be monophyletic with moderate support (76%) and sister to *Hydrocera* (Fig. 3A).

**Implications of the molecular phylogenies on infrageneric classification of *Impatiens*. —** *Impatiens* is taxonomically considered as one of the most difficult genera of angiosperms, primarily due to the delicate yet hypervariable structure and fragile nature of its flowers that are extremely difficult to examine in dried specimens if prepared conventionally (Grey-Wilson, 1980b, c). There is so far no comprehensive infrageneric classification available for the genus. The only global attempt was the early work of Warburg & Reiche (1895), in which 14 sections were recognized. However, this classification was considered neither natural nor practical (Grey-Wilson, 1980b), and was thus not followed by later authors. The only modern general treatment for the genus is the important revision of the African taxa by Grey-Wilson (1980b), which recognized six informal infrageneric groups for the African species for practical

diagnosis only. Perrier de la Bathie (1934) proposed three sections for the Madagascan *Impatiens* based on floral morphology: sect. *Preimpatiens* (= sect. *Impatiens*) (flowers with conspicuous spur), sect. *Trimorphopetalum* (flowers without spur, anthers dehiscent apically), and the monotypic sect. *Impatientella* (flowers without spur, anthers dehiscent laterally), the latter two being endemic to Madagascar. Fischer & Rahelivololona (2002) recently elevated sect. *Trimorphopetalum* to subgeneric level. The monotypic sect. *Impatientella* contains only the spurless and entirely cleistogamous species, *I. inaperta*. Our results support monophyly of the Madagascan endemic spurless species (Figs 2A and 3A: clade 1), and show that the cleistogamous *I. inaperta* is closely related to *I. furcata* of sect. *Trimorphopetalum* (or subgen. sensu Fischer & Rahelivololona, 2002). However, our molecular phylogenies show that this monophyletic clade is the most derived within *Impatiens*, and recognizing it as a distinct taxonomic identity makes the rest of the genus (taxonomically defined as section or subgenus *Impatiens*) paraphyletic. Apart from the highly supported spurless clade, molecular phylogenies also revealed a highly supported clade comprising clades 1 through 7 as shown in Figs. 2A and 3A. This clade is supported by several non-molecular characters as well, e.g., broad fusiform fruit and basic chromosome number principally $x = 8$, which have taxonomic importance.

Despite recognition of the clades mentioned above and several strongly supported small lineages (e.g., clades 5–6, 8, 10–11, 13–15), our molecular phylogenies do not resolve relationships among the lineages with confidence, and therefore offer limited taxonomic implications. Nevertheless, despite the low bootstrap support (52–64%), the African clade 13 (Figs. 2A and 3A), which nested apart from other African species, corresponds well to a species aggregate recognized by Grey-Wilson (1980b). Further phylogenetic studies, e.g., with additional molecular data from cpDNA or single-copy nuclear DNA, may improve the resolution and clade supports of the molecular phylogenies.

As pointed out by Grey-Wilson (1980b), clusters or aggregates of species are more or less clearly defined by gross morphology and geographic distribution, but the relationships among these distinguishable aggregates of species cannot be defined with confidence. Molecular phylogenies seem to show a similar pattern: small clades are recognized with strong support, but relationships among those clades are unresolved or resolved without significant support. The wide distribution of the genus, and the high morphological diversity associated with localized endemism (linked to distinguishable aggregates of species) suggest that active diversification and rapid radiation have been prevalent in the evolution of *Impatiens*. Molecular data reveal evidence of such rapid evolution, by the relatively high sequence divergence, the noticeable difficulties in sequence alignment of certain regions, the high proportion of autapomorphic variations, and the conspicuous short internal branches connecting the main branches.

**Biogeographic implications of the molecular phylogeny. —** Balsaminaceae show an interesting mainly paleotropical distributional pattern, with several diversity hotspots disjunctively located in tropical Africa, Madagascar, southern India and Sri Lanka, the eastern Himalayas, and Southeast Asia. While Jones & Smith (1966) suggested that *Impatiens* originated in the Himalayan region, Grey-Wilson (1980b) formulated an hypothesis suggesting that Balsaminaceae originated in western Gondwana and spread to Southeast Asia through Madagascar and India. From Southeast Asia and the adjacent Sino-Himalayan area, *Impatiens* diversified secondarily into two lineages: one lineage radiating to the temperate Eurasian areas and North America, and the other radiating to tropical and subtropical areas of Southeast Asian islands. Meanwhile, Grey-Wilson (1980b) rejected the possibility of an overland migration between Africa and India.

Parsimonious optimization of the centers of endemism (Africa, Madagascar, southern India and Sri Lanka, the Himalayas, Central Asia and Europe, Southeast Asia, and Boreal Eurasia and North America) onto the molecular phylogenies reveal Southeast Asia as the ancestral area of extant *Impatiens* (Fig. 4). The boreal species *I. nolitangere* and North American species *I. capensis* are suggested as having dispersed from Southeast Asia. The central Asian and European species, such as *I. brachycentra* and *I. parviflora*, may have been dispersed from Southeast Asia through the Himalayas. Africa seems to have been colonized at least twice from Southeast Asia: once by a colonizer with elongated linear-fusiform fruits that led to the small clade 13, and at another time by a colonizer with broad fusiform fruits that gave rise to the others (Fig. 4). As our sampling of Indian species is still insufficient, it is not known if the colonization of Africa might have been achieved via India as stepping-stones. Nevertheless, as far as the species we sampled from India and Sri Lanka are concerned, some species (such as those of clade 7) showed Southeast Asian connection and some showed African connections (e.g., *I. cuspidata*, *I. latifolia*, *I. leschensultia*, and *I. parasitica*). Madagascan *Impatiens* shows clearly an African origin.

The historical biogeography of *Impatiens* suggested by molecular phylogenies is, in fact, in concordance with the conspicuous species similarity among Africa, Madagascar, southern India, and Southeast Asia noticed by Grey-Wilson (1980b). This similarity is shown mainly by the species with broad fusiform fruits and the basic

chromosome number of $x = 8$ (clades 1 through 7 in Fig. 4). However, our present results suggest a Southeast Asian origin of *Impatiens* and dispersals to Africa and Madagascar, whereas on the contrary, Grey-Wilson (1980b) suggested an opposite direction of dispersal from an assumed west Gondwanan (African) origin. It is worth mentioning that the present biogeographic pattern largely depends on a few nodes with strong bootstrap supports. These include the node comprising all Balsaminaceae (bootstrap 100%), the node comprising clades 1 through 8 (bootstrap 82–86%), and the node comprising clades 1 through 7 (bootstrap 85–97%) (Figs. 2A and 3A).

**Morphological and chromosomal evolution.** — The fascinating floral diversity of *Impatiens* makes it an ideal subject for studying floral evolution. The greatest variation of floral morphology is seen in the lower sepal, its spur, and the lateral united petals, which seem related to different pollinators and breeding systems. Grey-Wilson (1980b) has proposed hypothetic schemes for floral evolution, e.g., loss of the upper pair of lateral sepals resulted in the evolution of a flower type with three sepals from flower types with five sepals, elaboration of the lower sepal into a nectary-tipped spur of various shapes and colors, fusion of the two lateral petals into a united pair, with reduction in size of the upper petal, and elaboration of the lower petal. Our results do not yet offer comprehensive inferences on floral evolution, due to poor resolution and weak support for many internal branches. Our ongoing studies seek more insights on these questions by acquiring more molecular data from different loci. Nevertheless, our phylogenies do bring insight on evolution of some floral traits. For example, the loss of a floral spur seen in some Madagascan endemics appears to have occurred only once (Fig. 4). Floral characters related to cleistogamy shown by *I. inaperta* have been used to diagnose section *Impatientella* (Perrier de la Bathie, 1934). Recent studies show that facultative cleistogamy is also regularly observed in the North American *I. capensis* (Antlfinger, 1986; Paoletti & Holsinger, 1999; Lu, 2002). In our molecular trees, these two species are far isolated from each other, which suggests that the same cleistogamous breeding system originated independently.

The characters of fruits have been considered of phylogenetic importance in Balsaminaceae, particularly the shape of capsules in *Impatiens* (Grey-Wilson, 1980b). When traced with our molecular phylogenies, the elongated linear or narrowly fusiform fruit was shown to be plesiomorphic, and the global or broadly fusiform fruit was shown to be derived from the linear type, while a few reversals also occurred (Fig. 4). The elongated linear type of fruit occurs mostly in Southeast Asian, Himalayan, and North-temperate species, whereas the broadly fusiform type of fruit is more characteristic of African, Madagascan and southern Indian species.

*Impatiens* shows a wide range of chromosome number variation, but the most frequent numbers are $2n = 14$, 16, 18, and 20 (Song & al., 2003). Thus the major part of species show the basic chromosome numbers $x = 7$, 8, 9, and 10. This series of basic chromosome numbers suggests a typical dysploid evolutionary relationship among them. Different hypotheses have been proposed to interpret the evolution of the basic numbers. Jones & Smith (1966) and Akiyama & al. (1992b) suggested $x = 7$ to be the ancestral type, from which the other numbers were derived by ascending dysploidy, whereas Rao & al. (1986) suggested evolution of $x = 7$, 9 and 10 from the basic number $x = 8$ through both descending and ascending dysploidy. Interestingly, preliminary optimization of the incomplete chromosomal data (data are missing for most Madagascan species) onto the molecular phylogenies reveals $x = 10$ to be the most likely ancestral base number in *Impatiens*, with other base numbers being derived mainly through descending dysploidy (Fig. 4). Consistent with the transition of elongated linear to broadly fusiform fruits, the optimization suggested a transition of base chromosome number from $x = 10$ to $x = 8$ for the same clade. Considering geographic distribution, there was probably a predominantly descending dysploidy following dispersal of the clade with broadly fusiform fruits to India, Africa, and Madagascar. As an extreme case, the lowest basic chromosome number, $x = 3$, was found in two Indian species, *I. latifolia* and *I. leschenaultii*. Interestingly, these two species are confirmed to have African affinity.

Our present paper has presented the first detailed phylogenetic study on Balsaminaceae with a worldwide sampling, and it has given insight on the evolution and biogeography of the family. Despite poor resolution or weak support among main lineages of *Impatiens*, we have established evolutionary patterns of some important morphological traits, basic chromosome numbers, and geographical origins. The evolutionary history of *Impatiens* apparently involved rapid diversification and extensive range expansion particularly in the Old World tropics and subtropics.

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## LITERATURE CITED

**Akaike, H.** 1974. A new look at the statistical model identification. *IEEE Trans Autom. Contr.* 19: 716–723.

**Akiyama, S. & Ohba, H.** 2000. Inflorescences of the Himalayan species of *Impatiens* (Balsaminaceae). *J. Jap. Bot.* 75: 226–240.

**Akiyama, S., Ohba, H., Sugawara, T., Yang, Y.-P. & Murata, J.** 1995. Notes on *Impatiens* (Balsaminaceae) from southwestern Yunnan, China. *J. Jap. Bot.* 70: 95–106.

**Akiyama, S., Ohba, H. & Suzuki, M.** 1992a. Notes on east Himalayan species of *Impatiens*, I. *J. Jap. Bot.* 67: 187–193.

**Akiyama, S., Ohba, H. & Wu, S.-K.** 1996. Further notes of *Impatiens* (Balsaminaceae) from Yunnan, China. *Bull. Nation. Sci. Mus., B (Tokyo)* 22: 135–144.

**Akiyama, S., Wakabayashi, M. & Ohba, H.** 1992b. Chromosome evolution in Himalayan *Impatiens* (Balsaminaceae). *Bot. J. Linn. Soc.* 109: 247–257.

**Albach, D. C., Soltis, P. S., Soltis, D. E. & Olmstead, R. G.** 2001. Phylogenetic analysis of asterids based on sequences of four genes. *Ann. Missouri Bot. Gard.* 88: 163–212.

**Anderberg, A. A., Rydin, C. & Källersjö, M.** 2002. Phylogenetic relationships in the order Ericales s. l.: analyses of molecular data from five genes from the plastid and mitochondrial genomes. *Amer. J. Bot.* 89: 677–687.

**Antlfinger, A. E.** 1986. Field germination and seedling growth of chasmogamous and cleistogamous progeny of *Impatiens capensis* (Balsaminaceae). *Amer. J. Bot.* 73: 1267–1273.

**APG.** 2003. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG II. *Bot. J. Linn. Soc.* 141: 399–436.

**Bremer, B., Bremer, K., Heidari, N., Erixon, P., Olmstead, R. G., Anderberg, A. A., Källersjö, M. & Barkhordarian, E.** 2002. Phylogenetics of asterids based on 3 coding and 3 non-coding chloroplast DNA markers and the utility of non-coding DNA at higher taxonomic levels. *Mol. Phylog. Evol.* 24: 274–301.

**Chen, Y.-L.** 1978. Notulae de genere *Impatiens* L. florae sinicae. *Acta Phytotaxon. Sin.* 16: 36–55.

**Chen, Y.-L.** 2000. Three new species of *Impatiens* L. from China. *Acta Phytotaxon. Sin.* 38: 557–562.

**Chen, Y.-L.** 2001. Balsaminaceae, *Flora Reipublicae Popularis Sinicae*, Tomus 47 (2). Science Press, Beijing.

**Cronquist, A.** 1981. *An Integrated System of Classification of Flowering Plants.* Columbia Univ. Press, New York.

**Denduangboripant, J. & Cronk, Q. C. B.** 2001. Evolution and alignment of the hypervariable *arm1* of *Aeschynanthus* (Gesneriaceae) ITS2 nuclear ribosomal DNA. *Mol. Phylog. Evol.* 20: 163–172.

**Doyle, J. & Doyle, J. L.** 1987. A rapid DNA isolation method for small quantities of fresh tissues. *Phytochem. Bull.* 19: 11–15.

**Felsenstein, J.** 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783–791.

**Fischer, E. & Rahelivololona, M. E.** 2002. New taxa of *Impatiens* (Balsaminaceae) from Madagascar. I. *Adansonia* 24: 271–294.

**Fischer, E. Wohlhauser, S. & Rahelivololona, M. E.** 2003. New taxa of *Impatiens* (Balsaminaceae) from Madagascar. II. A collection from Masoala Peninsula. *Adansonia* 25: 17–31.

**Fujihashi, H., Akiyama, S. & Ohba, H.** 2002. Origin and relationships of the Sino-Himalayan *Impatiens* (Balsaminaceae) based on molecular phylogenetic analysis, chromosome numbers and gross morphology. *J. Jap. Bot.* 77: 284–295.

**Geuten, K., Smets, E., Schols, P., Yuan, Y.-M., Janssens, S., Küpfer, P. & Pyck, N.** 2004. Conflicting phylogenies of balsaminoid families and the polytomy in Ericales: combining data in a Bayesian framework. *Molec. Phylogen. Evol.* 31: 711–729.

**Grey-Wilson, C.** 1980a. *Impatiens* in Papuasia, Studies in Balsaminaceae: I. *Kew Bull.* 34: 661–688.

**Grey-Wilson, C.** 1980b. Impatiens *of Africa.* A. A. Balkema, Rotterdam.

**Grey-Wilson, C**. 1980c. *Hydrocera triflora*, its floral morphology and relationships with *Impatiens*. Studies in Balsaminaceae: V. *Kew Bull.* 35: 213–219.

**Grey-Wilson, C.** 1980d. Notes on collecting *Impatiens* (Balsaminaceae). *Fl. Males. Bull.* 33: 3435–3436.

**Grey-Wilson, C.** 1985. Balsaminaceae. Pp. 76–120 in: Dassanayake, M. D. & Fosberg, F. R. (eds.), *A Revised Handbook to the Flora of Ceylon.* Amerind Publ. Co., Pvt. Ltd., New Delhi.

**Grey-Wilson, C.** 1989a. A revision of Sumatran *Impatiens*. Studies in Balsaminaceae: VIII. *Kew Bull.* 44: 67–105.

**Grey-Wilson, C.** 1989b. *Semeiocardium* Zoll.; is it a good genus? Studies in Balsaminaceae: IX. *Kew Bull.* 44: 107–113.

**Hooker, J. D. & Thompson, T.** 1859. Praecursores ad floram Indicam. Balsaminaceae. *Bot. J. Linn. Soc.* 4: 106–157.

**Huang, S.-H., Shui, Y.-M. & Chen, W.-H.** 2003. New taxa of *Impatiens* from Yunnan. *Acta Bot. Yunnan.* 25: 261–280.

**Humbert, H.** 1956. Contributions a l'étude de la flore de Madagascar et des Comores (fascicule 5). *Notul. Syst. (Paris)* 15: 113–134.

**Jones, K. & Smith, J. B.** 1966. The cytogeography of *Impatiens* L. (Balsaminaceae). *Kew Bull.* 20: 63-72.

**Lu, Y.-Q.** 2002. Why is cleistogamy a selected reproductive strategy in *Impatiens capensis* (Balsaminaceae)? *Biol. J. Linn. Soc.* 75: 543–553.

**Maddison, W. P. & Maddison, D. R.** 1992. *MacClade:*

*Analysis of Phylogeny and Character Evolution, version 3.0*. Sinauer, Sunderland, Massachusetts.

**Morton, C. M., Chase, M. W., Kron, K. A. & Swensen, S. M.** 1996. A molecular evaluation of the monophyly of the order Ebenales based upon *rbcL* sequence data. *Syst. Bot.* 21: 567–586.

**Morton, C. M., Mori, S. A., Prance, G. T., Karol, K. G. & Chase, M. W.** 1997. Phylogenetic relationships of Lecythidaceae: a cladistic analysis using *rbcL* sequence and morphhological data. *Amer. J. Bot.* 84: 530–540.

**Paoletti, C. & Holsinger, K. E.** 1999. Spatial patterns of polygenic variation in *Impatiens capensis*, a species with an environmentally controlled mixed mating system. *J. Evol. Biol.* 12: 689–696.

**Perrier de la Bathie, H.** 1934. Les *Impatiens* de Madagascar. *Arch. Bot. Mém.* 7: 1–124.

**Perrier de la Bathie, H.** 1948. Révision des *Impatiens* de Madagascar et des Comores. *Mem. Acad. Sci.* 67: 1–16.

**Posada, D. & Crandall, K. A.** 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* 14, 817–818.

**Rao, R. V. S., Ayyangar, K. R. & Sampathkumar, R.** 1986. On the karyological characteristics of some members of Balsaminaceae. *Cytologia* 51: 251–260.

**Saitou, N. & Nei, M.** 1987. The neighbor-joining methods: a new method for reconstructing phylogenetic trees. *Molec. Biol. Evol.* 4: 406–425.

**Shimizu, T.** 2000. New species of the Thai *Impatiens* (Balsaminaceae): 2. *Bull. Nation. Sci. Mus., B (Tokyo)* 26: 35–42.

**Soltis, D. E., Soltis, P. S., Chase, M. W., Mort, M. E., Albach, D. C., Zanis, M., Savolainen, V., Hahn, W. H., Hoot, S. B., Fay, M. F., Axtell, M., Swensen, S. M., Prince, L. M., Kress, W. J., Nixon, K. C. & Farris, J. S.** 2000. Angiosperm phylogeny inferred from 18S rDNA, *rbcL*, and *atpB* sequences. *Bot. J. Linn. Soc.* 133: 381–461.

**Song, Y., Yuan, Y.-M. & Küpfer, P.** 2003. Chromosomal evolution in Balsaminaceae, with cytological observations on 45 species from Southeast Asia. *Caryologia* 56: 463–481.

**Swofford, D. L.** 2000. *PAUP* Phylogenetic Analysis Using Parsimony (*and other methods)*, version 4.0b10. Sinauer Associates, Sunderland, Massachusetts.

**Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G.** 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.* 24: 4876–4882.

**Warburg, O. & Reiche, K.** 1895. Balsaminaceae. Pp. 383–392 in: Engler, H. G. A. & Prantl, K. A. E. (eds.), *Die Natürlichen Pflanzenfamilien*, Teil 3, Abteil. 5. Wilhelm Engelmann, Leipzig.

**Yuan, Y.-M., Wohlhauser, S., Möller, M., Chassot, P., Mansion, G., Grant, J., Küpfer, P. & Klackenberg, J.** 2003. Monophyly and relationships of the tribe Exaceae (Gentianaceae) inferred from nuclear ribosomal and chloroplast DNA sequences. *Molec. Phylog. Evol.* 28: 500–517.

**Appendix. Origin of plant material, voucher, and GenBank accession number of the species sampled.**

| Taxon | Origin | Voucher | GenBank acc. | Clade |
|---|---|---|---|---|
| *I. acehensis* C. Grey-Wilson | Sumatra, Indonesia | *Favre* s.n. (NEU) | AY348739 | Clade 6 |
| *I. amoena* H. Perrier | Antsatrotro, Madagascar | *Wohlhauser, Callmander & Rakotomamonjy SW 61422* (PBZT) | AY348795 | "Clade" 2 |
| *I. amphorata* Edgew. | W Himalaya | *B. de Retz 5566* (BR) | AY348740 | Clade 14 |
| *I. andohahelae* Eb. Fisch. & Rahelivololona | Andohahela, Madagascar | *Rahelivololona, Druard, Jerome & Fiadana And6* (PBZT) | AY348741 | Clade 1 |
| *I. andringitrensis* H. Perrier | Ambatofitorahana, Madagascar | *Rahelivololona Ambatofitorahana-2* (PBZT) | AY348742 | Clade 1 |
| *I. anovensis* H. Perrier | Ambanizana, Magadascar | *Rahelivololona, Saola & Scenario Ambanizana-11* (PBZT) | AY348743 | "Clade" 2 |
| *I. aposotis* Hook. f. | Sichuan, China | *Yuan CN2k2-159* (NEU) | AY348744 | Clade 9 |
| *I. aquatilis* Hook. f. | Yunnan, China | *Song CNY017* (NEU) | AY348745 | Clade 16 |
| *I. arguta* Hook. f. & Thoms. | Yunnan, China | *Yuan CN2k-74* (NEU) | AY348746 | |
| *I. aureliana* Hook. f. | Yunnan, China | *Yuan CN2k1-56* (NEU) | AY348747 | Clade 3 |
| *I. auricoma* Baill. | Comores origin, cult. Bot. Gard. Koblenz Univ. | *Fischer NE1* (NEU) | AY348748 | "Clade" 2 |
| *I. balsamina* L. | cult. Guanzhou, China | *Yuan CN2k1-06* (NEU) | AY348749 | Clade 3 |
| *I. barbata* Comber | Yunnan, China | *Yuan CN2k2-178* (NEU) | AY348750 | Clade 9 |
| *I. baroni* Baker | Ranomafana, Madagascar | *Rahelivololona, Jacky & Remi RNM-4* (PBZT) | AY348751 | "Clade" 2 |
| *I. begoniifolia* S. Akiyama & H. Ohba | Yunnan, China | *Yuan CN2k1-51* (NEU) | AY348752 | Clade 6 |
| *I. bequaerti* De Wild. | Congo origin, cult. Bot. Gard. Koblenz Univ. | *Fischer NE2* (NEU) | AY348753 | "Clade" 2 |
| *I. bicornuta* Wall. | Yunnan, China | *Yuan CN2k-55* (NEU) | AY348754 | |
| *I. bombycina* W. Lobin & E. Fischer | African origin, cult. Bot. Gard. Koblenz Univ. | *Fischer NE3* (NEU) | AY348755 | "Clade" 2 |
| *I. brachycentra* Kar. & Kir. | central Asia | *Burtt 3834* (E) | AY348756 | Clade 14 |
| *I. burtonii* Hook. f. | Kenya origin, cult. Bot. Gard. Koblenz Univ. | *Fischer NE4* (NEU) | AY348757 | "Clade" 2 |
| *I. campanulata* Wight | South India origin, cult. by Ray Morgan, UK | *Ray Morgan s. n.* (NEU) | AY348758 | Clade 17 |
| *I. capensis* Meerb. | Quebec, Canada | *Küpfer s. n.* (NEU) | AY348759 | Clade 10 |
| *I. chimiliensis* Comber | Yunnan, China | *Yuan CN2k-51* (NEU) | AY348760 | Clade 16 |
| *I. chinensis* L. | Yunnan, China | *Yuan CN2k1-49* (NEU) | AY348761 | Clade 4 |
| *I. chungtienensis* Y. L. Chen | Yunnan, China | *Yuan CN2k2-204* (NEU) | AY348762 | Clade 15 |
| *I. claeri* N. Halle | Gabon origin, cult. Bot. Gard. Koblenz Univ | *Fischer NE5* (NEU) | AY348763 | Clade 5 |
| *I. columbaria* J. J. Bos | Gabon origin, cult. Bot. Gard. Koblenz Univ | *Fischer NE6* (NEU) | AY348764 | Clade 5 |
| *I. conchibracteata* Y. L. Chen & Y. Q. Lu | Sichuan, China | *Hao 427* (NEU) | AY348765 | Clade 8 |
| *I. congolensis* G. M. Schulze & R. Wilczek | Congo origin, Africa, cult. Bot. Gard. Koblenz Univ. | *Fischer NE7* (NEU) | AY348766 | "Clade" 2 |
| *I. corchorifolia* Franch. | Yunnan, China | *Chassot & Yuan 99-173* (NEU) | AY348767 | Clade 9 |
| *I. cordata* Wight | South India origin, cult. by Ray Morgan, UK | *Ray Morgan s. n.* (NEU) | AY348768 | Clade 7 |
| *I. cuspidata* Wight & Arn. | South India origin, cult. by Ray Morgan, UK | *Ray Morgan s. n.* (NEU) | AY348769 | "Clade" 2 |
| *I. cyanantha* Hook. f. | Yunnan, China | *Yuan CN2k1-84* (NEU) | AY348770 | Clade 16 |
| *I. cyathiflora* Hook. f. | Yunnan, China | *Chassot & Yuan 99-29* (NEU) | AY348771 | Clade 16 |
| *I. davidi* Franch. | Fujian, China | *Yuan CN2k-09* (NEU) | AY348772 | Clade 12 |
| *I. delavayi* Franch. | Yunnan, China | *Chassot & Yuan 99-154* (NEU) | AY348773 | Clade 9 |
| *I. desmantha* Hook. f. | Yunnan, China | *Yuan CN2k2-30* (NEU) | AY348774 | Clade 16 |
| *I. devolii* T. C. Huang | Taiwan, China | *Jiang s. n.* | AY348775 | Clade 16 |
| *I. drepanophora* Hook. f. | Yunnan, China | *Yuan CN2k1-41* (NEU) | AY348776 | Clade 16 |
| *I. eubotrya* Miq. | Sumatra, Indonesia | *Favre s.n.* (NEU) | AY348777 | Clade 16 |
| *I. faberi* Hook. f. | Sichuan, China | *Song S007* (NEU) | AY348778 | Clade 11 |
| *I. fenghwaiana* Y. L. Chen | Guangxi, China | *Yuan CN2k1-78* (NEU) | AY348779 | Clade 16 |
| *I. firmula* Baker | Ranomafana, Madagascar | *Rahelivololona, Jacky & Remi RNM 4* (PBZT) | AY348780 | "Clade" 2 |
| *I. fischeri* Warb. | Kenya | *Odile Phaehler & Magali Schnell I-01* (NEU) | AY348781 | Clade 13 |
| *I. fissicornis* Maxim. | Shaanxi, China | *Wang SH-003* (NEU) | AY348782 | Clade 9 |
| *I. flanaganae* Hemsl. | Africa | RBG Edinburgh 19860179 (E) | AY348783 | Clade 13 |
| *I. forrestii* Hook. f. ex W. W. Smith | Yunnan, China | *Yuan CN2k-79* (NEU) | AY348784 | Clade 11 |
| *I. fuchsioides* H. Perrier | Tsaratanana, Madagascar | *Rahelivololona ROR 395* (PBZT) | AY348785 | "Clade" 2 |
| *I. furcata* H.Perrier | African origin, cult. Bot. Gard. Koblenz Univ | *Fischer NE8* (NEU) | AY348786 | Clade 1 |
| *I. gibbosa* H. Perrier | Tsaratanana, Madagascar | *Rahelivololona, Wohlhauser & Callamander T11* (PBZT) | AY348787 | Clade 1 |
| *I. glandulifera* Arn. | Western Himalaya origin, cult. Bot. Gard. Neuchâtel | *Yuan JBE1* (NEU) | AY348788 | Clade 15 |
| *I. gongshanensis* Y. L. Chen | Yunnan, China | *Yuan CN2k1-27* (NEU) | AY348789 | |
| *I. henslowiana* Arn. | Sri Lanka origin, cult. Bot. Gard. Koblenz Univ | *Fischer NE10* (NEU) | AY348790 | Clade 7 |
| *I. hoehnelii* T. C. E. Fries | African origin, cult. Bot. Gard. Koblenz Univ. | *Fischer NE9* (NEU) | AY348792 | "Clade" 2 |
| *I. holocentra* Hand.-Mazz. | Yunnan, China | *Yuan CN2k-54* (NEU) | AY348793 | Clade 16 |
| *I. hookeriana* Arn. | Sri Lanka origin, cult. Bot. Gard. UC, Davis | *Bot. Gard. UC Davis 75.0276* | AY348794 | Clade 7 |
| *I. imbecilla* Hook. f. | Sichuan, China | *Hao 426* (NEU) | AY348796 | Clade 11 |
| *I. inaperta* H.Perrier | Madagascar origin, cult. Bot. Gard. Neuchâtel | *Yuan JBE2* (NEU) | AY348797 | Clade 1 |
| *I. keilii* Gilg. | African origin, cult. Bot. Gard. Koblenz Univ | *Fischer NE11* (NEU) | AY348798 | "Clade" 2 |

**Appendix. Continued.**

| Taxon | Origin | Voucher | GenBank acc. | Clade |
|---|---|---|---|---|
| *I. kerriae* Craib | Qingmai, Thailand | *Chassot 99-238* (NEU) | AY348799 | |
| *I. kilimanjari* Oliver | Africa | *JMG 94613* (BR) | AY348800 | "Clade" 2 |
| *I. latifolia* L. | Inida origin, cult. by Ray Morgen, U.K. | *Ray Morgan s.n.* (NEU) | AY348801 | "Clade" 2 |
| *I. lecomtei* Hook. f. | Yunnan, China | *Yuan CN2k2-202* (NEU) | AY348802 | Clade 9 |
| *I. leschenaultii* Wall. | India Origin, cult. by Ray Morgen, U.K. | *Ray Morgan s.n.* (NEU) | AY348803 | "Clade" 2 |
| *I. levingei* Gamble ex Hook. f. | India Origin, cult. U.K. | *Ray Morgan s.n.* (NEU) | AY348804 | Clade 7 |
| *I. manaharensis* Baill. | Madagascar | *Wohlhauser & al. WCL 1* (PBZT) | AY348805 | "Clade" 2 |
| *I. mengtseana* Hook. f. | Yunnan, China | *Yuan CN2k1-38* (NEU) | AY348806 | Clade 6 |
| *I. meruensis* Gilg. | Tanzania origin, cult. by Ray Morgen, U.K. | *Ray Morgan s.n.* (NEU) | AY348807 | "Clade" 2 |
| *I. microcentra* Hand.-Mazz. | Yunnan, China | *Yuan CN2k-50* (NEU) | AY348808 | Clade 16 |
| *I. miniata* Grey-Wilson | Tsaratanana, Madagascar | *Rahelivololona, Wohlhauser & Callmander T1* (PBZT) | AY348809 | "Clade" 2 |
| *I. monticola* Hook. f. | Sichuan, China | *Hao 425* (NEU) | AY348810 | Clade 6 |
| *I. napoensis* Y. L. Chen | Yunnan, China | *Yuan CN2k1-61* (NEU) | AY348811 | Clade 6 |
| *I. niamniamensis* Gilg. | Africa origin, cult. Bot. Gard. Koblenz Univ | *Fischer NE13* (NEU) | AY348812 | "Clade" 2 |
| *I. noli-tangere* L. | Jilin, China | *Yuan CN2k1-86* (NEU) | AY348813 | Clade 10 |
| *I. oxyanthera* Hook. f. | Sichuan, China | *Song S008* (NEU) | AY348814 | Clade 11 |
| *I. parasitica* Bedd. | India origin, cult. by Ray Morgen, U.K. | *Ray Morgan s.n.* (NEU) | AY348815 | "Clade" 2 |
| *I. parviflora* DC: | Poland | *Ronikier s.n.* | AY348816 | Clade 14 |
| *I. percrenata* H. Perrier | Andohahela, Madagascar | *Rahelivololona, Druard, Jerome & Fiadana And 3* (PBZT) | AY348817 | "Clade" 2 |
| *I. platychlaena* Hook.f. | Sichuan, China | *Song S009* (NEU) | AY348818 | Clade 9 |
| *I. platypetala* Lindl. | Bali, Indinesia origin, cult. by Ray Morgen, U.K. | *Ray Morgan s.n.* (NEU) | AY348819 | Clade 4 |
| *I. poculifer* Hook. f. | Yunnan, China | *Yuan CN2k2-209* (NEU) | AY348820 | Clade 9 |
| *I. pritzelii* Hook.f. | Sichuan, China | *Song jf011* (NEU) | AY348821 | Clade 9 |
| *I. pseudoviola* Gilg. | central Africa | RBG Edinburgh 19680124 (E) | AY348822 | "Clade" 2 |
| *I. purpurea* Hand.-Mazz. | Yunnan, China | *Song Y007* (NEU) | AY348823 | Clade 16 |
| *I. radiata* Hook. f. | Yunnan, China | *Yuan CN2k-77* (NEU) | AY348824 | Clade 16 |
| *I. rectangula* Hand.-Mazz. | Yunnan, China | *Yuan CN2k1-26* (NEU) | AY348825 | Clade 16 |
| *I. rothii* Hook. f. | Ethiopia | *Pierre Binggeli s. n.* (NEU) | AY348827 | Clade 13 |
| *I. rubrostriata* Hook. f. | Yunnan, China | *Yuan CN2k1-44* (NEU) | AY348828 | Clade 8 |
| *I. sambiranensis* H. Perrier | Tsaratanana, Madagascar | *Rahelivololona, Wohlhauser & Callamander T4* (PBZT) | AY348829 | "Clade" 2 |
| *I. scutisepala* Hook. f. | Yunnan, China | *Yuan CN2k-56* (NEU) | AY348830 | Clade 16 |
| *I. siculifer* Hook. f. | Yunnan, China | *Yuan CN2k-80* (NEU) | AY348831 | Clade 16 |
| *I. sodenii* Engl. & Warb. ex Engl. | Kenya origin, cult. Bot. Gard. Koblenz Univ. | *Fischer NE15* (NEU) | AY348832 | "Clade" 2 |
| *I. soulieana* Hook. f. | Sichuan, China | *Yuan CN2k2-163* (NEU) | AY348833 | Clade 9 |
| *I. stenosepala* Pritz. ex Diels | Shaanxi, China | *Wang Sh001* (NEU) | AY348835 | |
| *I. stuhlmannii* Warb. | African origin, cult. Bot. Gard. Koblenz Univ. | *Fischer NE17* (NEU) | AY348836 | "Clade" 2 |
| *I. subabortiva* H.Perrier | Tsaratanana, Madagascar | *Rahelivololona, Wohlhauser & Callmander T6* (PBZT) | AY348837 | Clade 1 |
| *I. taronensis* Hand.-Mazz. | Yunnan, China | *Yuan CN2k-57* (NEU) | AY348838 | Clade 16 |
| *I. tayemonii* Hayata | Taiwan, China | *Zhengyu Jiang T2* (NEU) | AY348839 | Clade 9 |
| *I. teitensis* Grey-Wilson | African origin, cult. Bot. Gard. Koblenz Univ. | *Fischer NE18* (NEU) | AY348840 | Clade 13 |
| *I. textori* Miq. | Japan | *Kanno & al. 1114* (TUS) | AY348841 | Clade 12 |
| *I. trichosepala* Y. L. Chen | Yunnnan, China | *Yuan CN2k1-68* (NEU) | AY348843 | Clade 6 |
| *I. tuberosa* H.Perrier | Madagascar origin, cult. Bot. Gard. Koblenz Univ. | *Fischer NE19* (NEU) | AY348844 | "Clade" 2 |
| *I. uliginosa* Franch. | Yunnan, China | *Yuan CN2k2-173* (NEU) | AY348845 | Clade 16 |
| *I. uniflora* Hayata | Taiwain, China | *Zhengyu Jiang T1* | AY348846 | Clade 11 |
| *I. usambarensis* Grey-Wilson | African origin, cult. Bot. Gard. Koblenz Univ. | *Fischer NE20* (NEU) | AY348847 | "Clade" 2 |
| *I. vilersi* Costantin & Poisson | Andohahela, Madagascar | *Rahelivololona, Druard, Jerome & Fiadana And 2* (PBZT) | AY348848 | "Clade" 2 |
| *I. walleriana* Hook.f. | Kenya | *Odile Phaehler & Magali Schnell I08* (NEU) | AY348849 | "Clade" 2 |
| *I. xanthina* Comber | Yunnan, China | *Yuan CN2k1-15* (NEU) | AY348850 | Clade 6 |
| *I. yingjiangensis* S.Akiyama & H. Ohba | Yunnan, China | *Yuan CN2k1-55* (NEU) | AY348851 | Clade 6 |
| *I. zenkeri* Warb. | African origin, cult. Bot. Gard. Koblenz Univ. | *Fischer NE21* (NEU) | AY348852 | Clade 5 |
| *I.* sp. | Tsaratanana, Madagascar | *Rolland Ranaivojaona, & Roll Tsaratanana 1* (PBZT) | AY348834 | "Clade" 2 |
| *Hydrocera triflora* Wight & Arn. | Sri Lanka | *Robyns 7260, 0249369* (L) | AY348853 | |
| *Marcgravia polyantha* Delp. | sample from botanical Garden, origin unknown | Jard. Bot. Nat. Belgique FB/S3781 (BR) | AY348854 | |
| *Norantea guianensis* Aubl. | sample from botanical Garden, origin unknown | Jard. Bot. Nat. Belgique FB/S3779 (BR) | AY348855 | |
| *Pelliciera* sp. | Costa Rica | *Pennington & al. 586* (K) | AY348856 | |
| *Souroubea* sp. | Peru | BG Univ. Utrecht 76GR00102 (U) | AY348857 | |
| *Tetramerista* sp. | Brunei Darussalam | *Coode s.n.* (K) | AY348858 | |

# Paper IV

# Phylogenetics of *Impatiens* and *Hydrocera* (Balsaminaceae) using chloroplast atpB-rbcL spacer sequences

Janssens S., Geuten K., Yuan Y.-M., Song Y, Küpfer P. and Smets E.

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# Phylogenetics of *Impatiens* and *Hydrocera* (Balsaminaceae) Using Chloroplast *atpB-rbcL* Spacer Sequences

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**ABSTRACT.** Balsaminaceae are a morphologically diverse family with ca. 1,000 representatives that are mainly distributed in the Old World tropics and subtropics. To understand the relationships of its members, we obtained chloroplast *atpB-rbcL* sequences from 86 species of Balsaminaceae and five outgroups. Phylogenetic reconstructions using parsimony and Bayesian approaches provide a well-resolved phylogeny in which the sister group relationship between *Impatiens* and *Hydrocera* is confirmed. The overall topology of *Impatiens* is strongly supported and is geographically structured. *Impatiens* likely originated in South China from which it colonized the adjacent regions and afterwards dispersed into North America, Africa, India, the Southeast Asian peninsula, and the Himalayan region.

Balsaminaceae are annual or perennial herbs with flowers that exhibit a remarkable diversity. The family consists of more than 1,000 species (Grey-Wilson 1980a; Clifton 2000), but only two genera are recognized. *Impatiens* is a very large genus of over 1,000 species, while *Hydrocera triflora* is the only member of the genus *Hydrocera*. Though the two genera are very similar, several features distinguish them. *Hydrocera* has five free sepals and five free petals, while *Impatiens* usually has three free sepals and always five petals, four of which are united into two lateral pairs. In addition, *Hydrocera* produces an indehiscent pseudo-berry (Grey-Wilson 1980b; Raghuveer et al. 1993), whereas *Impatiens* have an explosively dehiscent capsule. *Impatiens* is mainly distributed in montane areas in the Old World tropics and subtropics (Grey-Wilson 1980a; Yuan et al. 2004) and *Hydrocera triflora* has a distributional area confined to the lowlands of Indo-Malaysia (Grey-Wilson 1980b; Ramadevi and Narayana 1989). A high diversity of *Impatiens* species is found in tropical Africa, Madagascar, South India, the eastern Himalayas, Southeast Asia, and Southwest China (Grey-Wilson 1980a). The temperate regions in Europe, North America, and northern China are inhabited by only a few species. Indigenous *Impatiens* species are absent from South America and Australia.

Despite the diversity of floral morphological features within this genus, taxonomic studies on Balsaminaceae have encountered several difficulties, leaving numerous relationships within *Impatiens* unresolved (Hooker 1875; Warburg and Reiche 1895; Hooker 1905). Until now, only two molecular surveys have been carried out to unravel the relationships within the Balsaminaceae. Fujihashi et al. (2002) published the first molecular phylogeny of *Impatiens*. However, the use of *Tropaeolum* as an outgroup and the limited taxon sampling (25 representatives) resulted in limited insights in the intrageneric relationships of *Impatiens*. The most recent molecular intrageneric study on Balsaminaceae utilized Internal Transcribed Spacer (ITS) sequences and provided new phylogenetic insights in *Impatiens* (Yuan et al. 2004). In this study, we follow up the ITS study with chloroplast *atpB-rbcL* sequence data and propose a preliminary reconstruction of the intrageneric relationships in *Impatiens*.

## MATERIALS AND METHODS

***Taxon Sampling.*** Representatives of both *Impatiens* and *Hydrocera* were included in our sampling. We chose our sampling to represent the whole geographic and taxonomic diversity of the family. Fresh material, silica dried specimens, and herbarium specimens of 86 *Impatiens* species are used in this molecular study. Recent molecular results confirmed a well supported clade of Tetrameristaceae and Marcgraviaceae with Balsaminaceae (Morton et al. 1996; Savolainen et al. 2000; Soltis et al. 2000; Albach et al. 2001; Anderberg et al. 2002; Bremer et al. 2002; APG 2003; Geuten et al. 2004). Therefore, we used representatives of Marcgraviaceae (*Marcgravia umbellata*, *M. maguire*, *Norantea guianensis*, *Souroubea* sp.) and Tetrameristaceae (*Pelliciera rhizoporae*) as the outgroups. The list of taxa with authorities, localities, voucher numbers and accession numbers is shown in Appendix 1.

***Molecular Protocols.*** Total genomic DNA was extracted using a modified version of the hot CTAB protocol (Saghai-Maroof et al. 1984; Doyle and Doyle 1987). The lysis buffer was described in Chase and Hills (1991) with 3% PVP-40 that was added to bind more phenolic compounds. The aqueous phase was extracted three times with chloroform-isoamylalcohol (24/1 v/v). After an ethanol-salt precipitation (1/10 volume Natrium-Acetate 3M and 2 vol. ethanol 100%) and subsequent centrifugation, the pellet was washed two times and air-dried. The pellet was dissolved in 10mM TrisHCl (pH 8.5) and stored at 4°C (K. Geuten, in mss.)

Specific primers for the *atpB-rbcL* spacer in Balsaminaceae were designed: IMP-*atpB* (5′-ACATCTAGTACCGGACCAATGA-3′) and IMP-*rbcL* (5′-AACACCAGCTTTGAATCCAA-3′). The temperature profile consisted of 2 min initial denaturation at 94°C and 30 cycles of 1 min denaturation at 94°C, 30s primer annealing at 51°C, and 30s extension at 72°C. Amplification reactions were carried out on a GeneAmp PCR system 9700 (Applied Biosystems). Cycle sequencing reactions were performed as in Geuten et al. (2004).

***Data Matrices and Alignment.*** *atpB-rbcL* sequences were initially aligned with CLUSTALX applying the default parameters

for gap opening and gap extension. Further adjustment of the preliminary aligned data matrix was performed manually using MacClade 4.05 (Maddison and Maddison 2002). For the parsimony-based analyses, we coded the indels in the *atpB-rbcL* spacer as separate characters, following the 'simple indel coding' method (Simmons and Ochoterena 2000). Non-informative or ambiguous gaps were not coded. Both indel coded and non-coded data matrices were analyzed using maximum parsimony. The ITS matrix of Yuan et al. (2004) was used for a combined analysis. The same regions as described in the ITS manuscript and 77 characters were removed from the combined data matrix. Species that were absent in the nuclear or the chloroplast data set were excluded to avoid phylogenetic incongruence caused by the presence of missing data. All sequences were submitted to GenBank (Appendix 1) and the data sets and the representative trees are deposited in TreeBASE (study accesion S1378).

***Phylogenetic Analysis.*** The *atpB-rbcL* dataset was analyzed both separately and in combination with ITS. A partition homogeneity test (implemented in PAUP*4.0b10a) was used to determine whether the datasets were providing different signal in the combined analysis.

Maximum parsimony (MP) analyses were conducted using PAUP* 4.0b10a (Swofford 2002). Heuristic searches were conducted with tree-bisection reconnection (TBR) branch swapping on 10,000 random addition replicates, with five trees held at each step. Characters were equally weighted and character states were specified to be unordered. Non-parametric bootstrap analysis (MP-BS) was carried out to calculate the relative support for individual clades found in the parsimony analysis (Felsenstein 1985). For each of the 500 bootstrap replicates, a heuristic search was conducted with identical settings as in the original heuristic analysis.

The best fitting substitution model for Bayesian analysis was selected using a series of likelihood ratio tests as implemented in ModelTest 3.06 (Posada and Crandall 1998). A Bayesian analysis was conducted with MrBayes 3b4 (Huelsenbeck and Ronquist 2001). Four chains (one cold, three heated), initiated from a random starting tree were run for 10 million generations. Every 500 generations, a tree was sampled from the chain for a total of 20,000 trees. Due to the burn-in, 10,000 sample points were discarded until stationarity was established among the chains. PAUP* 4.0b10 was used to calculate a 50% majority-rule consensus tree and to report the posterior probabilities for each clade. Posterior probabilities have been shown to overestimate branch support, so they will be interpreted with caution (Suzuki et al. 2003).

***Character Evolution and Biogeographical Implementation.*** Cytological and morphological characters such as pollen type, capsule shape and number of sepals were mapped together with geographic distribution on the *atpB-rbcL* tree using MacClade 4.05 (results not shown).

## RESULTS

**atpB-rbcL *Spacer.*** The aligned data matrix contains 961 characters from which 277 (29%) are variable. Of the total variable positions, 154 (56%) are parsimony informative. A total of 46 indels were found, of which 21 (46%) were insertions and 25 deletions (54%). Only 25 gaps were found to be potentially informative and were included as separate characters.

Parsimony analysis of the non-gapcoded data matrix resulted in 6407 most parsimonious trees. The strict consensus tree is well resolved (Fig. 1), especially among the most early diversified branches. Bootstrap analysis shows relatively high support for many of the lineages. Although the use of the coded indels did not change the general topology, we noticed an overall increase of the bootstrap support for almost every lineage and a reduction in the number of shortest trees to 1531 (Fig. 1). The Bayesian consensus tree is well resolved and most of the lineages have support percentages of 95% or higher (Fig. 2).

Both Bayesian analysis and MP highly support the monophyly of the family (BS: 100 / BPP: 100). With respect to the outgroup chosen, our data confirm the sistergroup relationship between *Impatiens* and *Hydrocera* with very high support value (BS: 100 / BPP: 100). Clade 1 (BS: 98 / BPP: 100) contains only one species (*I. omeiana*) and is sister to all the other *Impatiens* species. The remaining representatives of *Impatiens* are divided into two large clades, which are both highly supported (clade I—BS: 95 / BPP: 100; clade II—BS: 92 / BPP: 100). The first large group comprises three lineages, two of which are moderately supported (clades 3, 4). Clade 4 (BS: 70 /BPP: 100) is sister to clade 3 and consists mainly of endemic South Chinese species. Clade 3 includes species that are mainly distributed in the Himalaya and the more temperate regions of Eurasia (BS: 82 / BPP: 95).

Clade II is a more heterogeneous collection of species with a distribution in Asia, Africa, and Madagascar. Most of the clades in this heterogeneous group are highly supported by both MP and Bayesian analysis, but the relationships among them are not well resolved. It is worth noting that almost every well-supported lineage in this second group has a specific area of distribution (Fig. 1). Clade 6 comprises mainly South Chinese species including two North American species. Bayesian analysis shows that this Chinese-American clade is sister to a small African lineage (clade 5), which is isolated from the other African species. However, the Bayesian support value for this relationship is low (BPP: 74), whereas the MP bootstrap value does not support this relationship.

Two other groups of South Chinese species are resolved in clade 7 (BS: 94 / BPP: 100) and clade 9 (BS: 97 / BPP: 100). Clade 9 is part of a large group (clade III) comprising mainly African species and species from Madagascar, Southeast Asia, and South India. Within clade III, two South Indian lineages show no affinity to each other (clade 8 and clade 15). Clade 8 comprises South Indian species, while clade 15 comprises mainly species from South India and Vietnam (BS: 84 / BPP: 100). Clade 15 includes also one species from Myanmar and Yunnan, China. The relationships within the latter group remain mostly unresolved. A West African clade (clade 10—BS: 97 / BPP: 100), a Central African clade (clade 13—BS: 94 / BPP: 100), an East African clade (clade 14—BS: 99 / BPP: 100) and a Southeast Asian clade (clade 11—BS: 79 / BPP: 100) are resolved within clade III as well. Madagascan species (clade 12) are resolved as a highly supported monophyletic group (BS: 87 / BPP: 100). Both Bayesian analyses and MP point out that the Madagascan clade might be sister to *I. meruensis* from Eastern Africa.

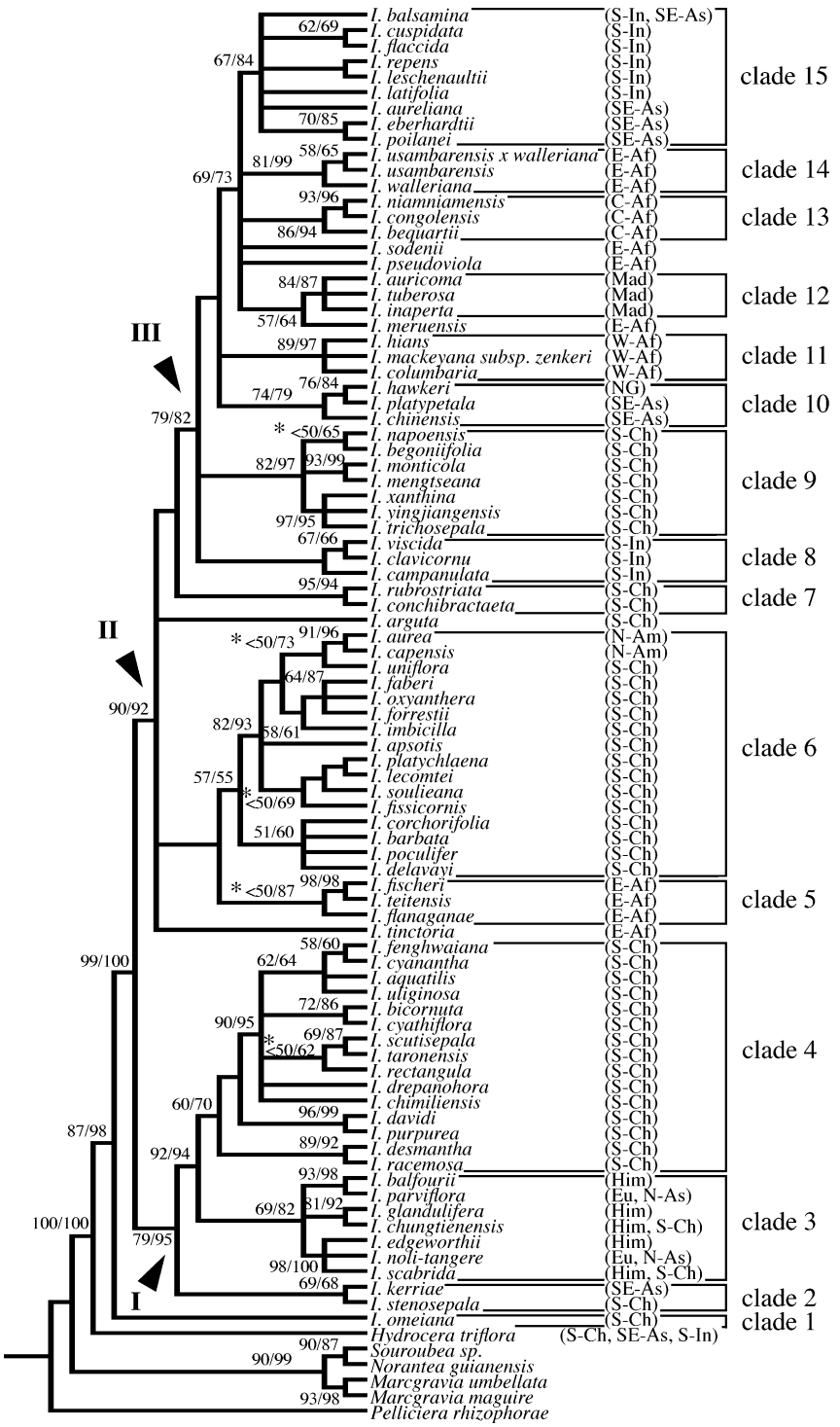



FIG. 1. Strict consensus tree from the parsimony analysis based on *atpB-rbcL* spacer data. Roman figures are assigned to deeper internal nodes that are highly supported, whereas specific clades are numbered 1 through 15. The first number on the branch always represents bootstrap support of the non-gapcoded data matrix and the second number always indicates bootstrap support of the gapcoded data matrix. Branches that collapse in the non-gapcoded consensus tree have been indicated by an asterisk. Distribution areas are shown after the species name (C-Af: Central Africa, E-Af: East Africa, Eu: Europe, Him: Himalaya, Mad: Madagascar, N-Am: North America, N-As: North Asia, NG: New Guinea, S-Ch: South China, SE-As: South East Asia, S-In: South India, W-Af: West Africa).

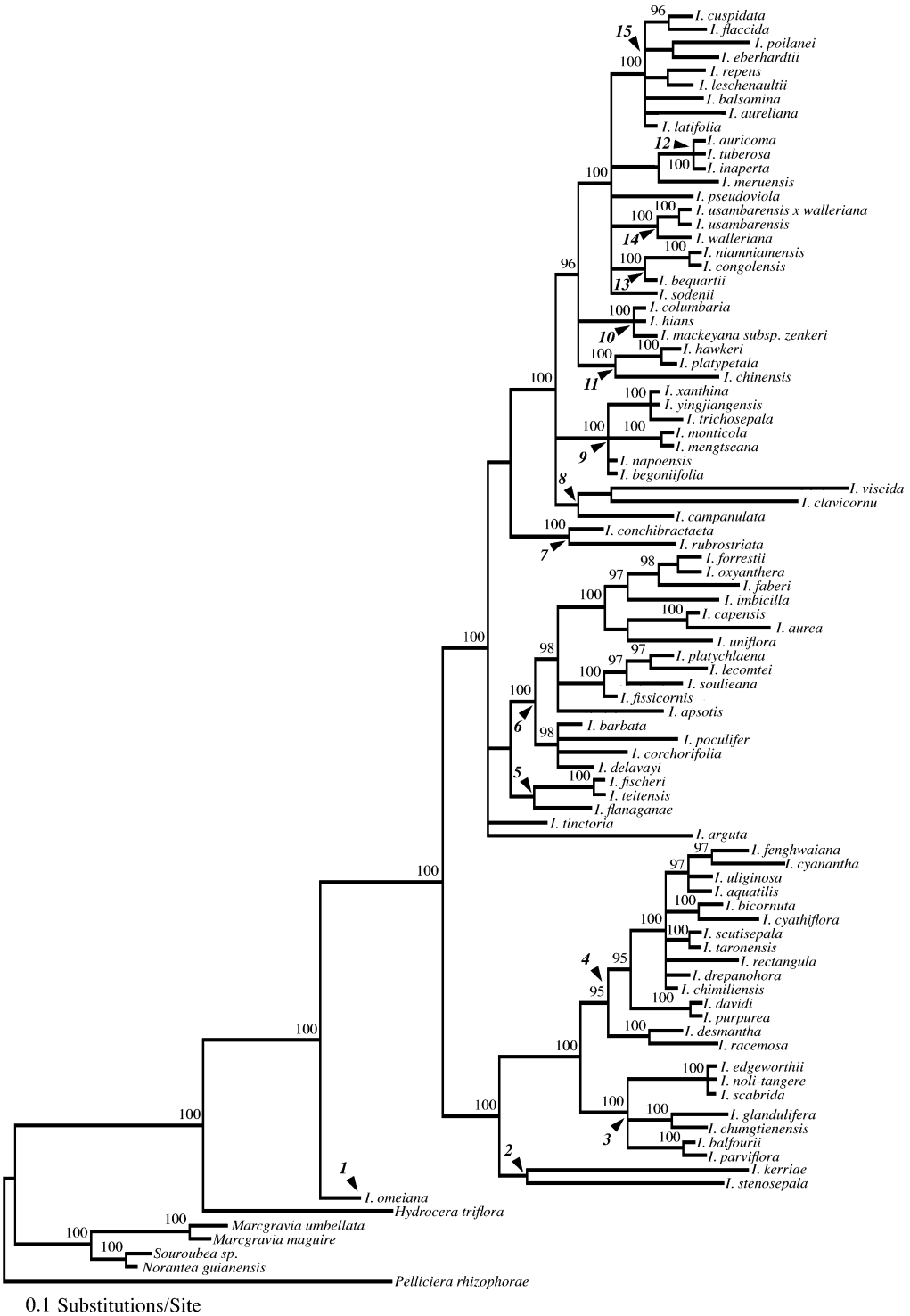



FIG. 2. Bayesian consensus phylogram based on the branch length of the complete *atpB-rbcL* dataset. Numbers on the branch indicate posterior probabilities higher than 95%.

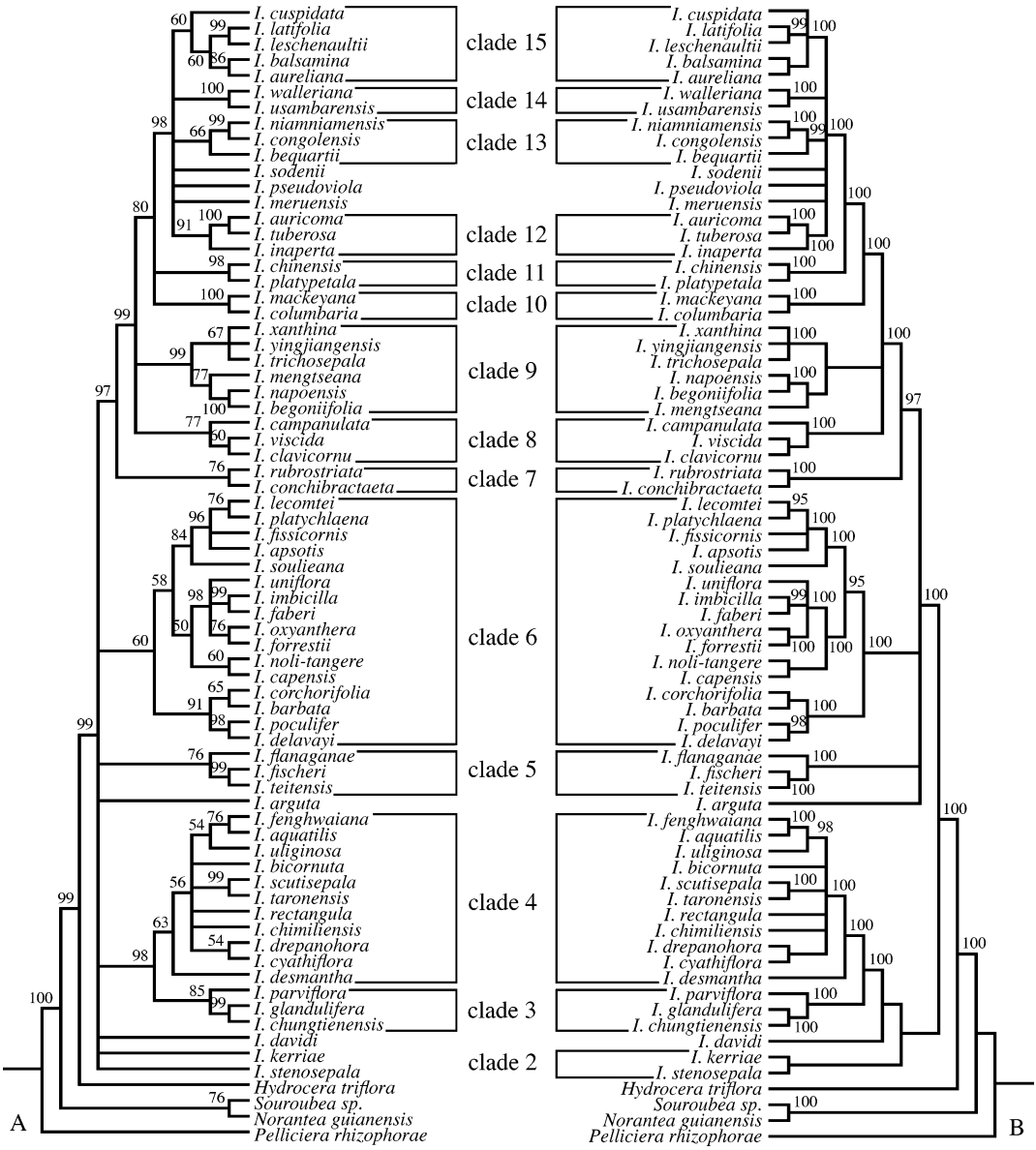



FIG. 3. Phylogenetic trees based on combined ITS and *atpB-rbcL* spacer data. A. Strict consensus tree from the parsimony analysis. Bootstrap values are present above the branches. B. Bayesian consensus cladogram. Numbers above the branch indicate posterior probabilities. Only posterior probabilities higher than 95% are shown.

However, this relationship is only moderately supported (BS: 64 / BPP: 92).

***Combined ITS and* atpB-rbcL.** The combined data matrix contains 72 species and 1722 characters from which 610 (35 %) are variable. Of the total variable positions, 417 (68%) are parsimony informative. The partition homogeneity test found no significant difference between ITS and *atpB-rbcL* spacer partitions of the combined dataset ($p>0.05$). The parsimony consensus tree produced by combining the data sets (Fig. 3A) is mainly in agreement with the consensus tree produced by the chloroplast analysis. However, bootstrap support values are remarkably lower for the majority of the branches. Additionally, the earliest diverged lineages of *Impatiens* are collapsed, showing *I. arguta*, *I. kerriae*, *I. stenosepala*, *I. davidi*, and the main *Impatiens* clade as one basal polytomy. Bayesian analysis of the combined data matrix resulted in greater resolution (Fig. 3B) than the parsimony bootstrap analysis of the combined data (Fig. 3A).

In comparing *atpB-rbcL* with the combined ITS and *atpB-rbcL* dataset, we notice a small number of incon-

gruent relationships between both phylogenies. One of the largest dissimilarities is found in the position of *I. noli-tangere*. According to the combined analysis (Fig. 3), *I. noli-tangere* is sister to *I. capensis* (clade 6), but with only chloroplast data (Figs. 1, 2) *I. noli-tangere* forms a well-supported clade with *I. edgeworthii* and *I. scabrida* (clade 6). A second incongruence is found in the position of *I. cyathiflora* (clade 4). The combined analysis shows a sistergroup relationship between *I. drepanophora* and *I. cyathiflora* (Fig. 3), whereas *I. cyathiflora* is sister to *I. bicornuta* in the chloroplast phylogeny (Figs. 1, 2). Another difference between the combined and chloroplast phylogenies is the position of *I. uniflora* and *I. imbicilla* (clade 6). In the chloroplast phylogeny (Figs. 1, 2), *I. uniflora* is sister to the North American lineage, while in the combined analysis (Fig. 3) *I. uniflora* is located in a trichotomy with *I. imbicilla* + *I. faberi* and *I. forrestii* + *I. oxyanthera*. In the combined analysis, *I. imbicilla* is sister to *I. faberi*, whereas chloroplast data group *I. imbicilla* as sister to a polytomy consisting of *I. faberi*, *I. oxyanthera*, and *I. forrestii*.

## Discussion

Phylogenetic analysis of chloroplast *atpB-rbcL* spacer data provides a well supported tree that we regard as a good hypothesis of evolutionary relationships in Balsaminaceae. Although the chloroplast and combined data phylogenies resemble each other closely, we noticed some minor differences, which are also present when the *atpB-rbcL* phylogeny is compared with only the nuclear one. According to Grey-Wilson (1980c) many *Impatiens* species originated through hybridization. Therefore, we assume that these minor dissimilarities are probably caused by the occurrence of reticulate evolution. However, since ITS is susceptible to segregation, concerted evolution, and recombination, which causes an ITS phylogeny sometimes to be inaccurate or to reflect an incomplete species tree (Alvarez and Wendel 2003), we must be cautious with our conclusions about hybridization in *Impatiens*.

We prefer to use the plastid phylogeny instead of the combined ITS/*atpB-rbcL* dataset for further discussion. The combined data hypothesis is less resolved with lower support values, which could be caused by the very high substitution rate of ITS1 and ITS2, even for the greater taxon sampling used in Yuan et al. (2004), resulting in the suppression of the phylogenetic signal of the *atpB-rbcL* dataset (Mansion and Struwe 2004; Schneeweiss et al. 2004).

***Chloroplast Phylogeny in Comparison to Global Intrageneric Classification.*** Due to hybridization events, rapid speciation, and the occurrence of transitional character states (Grey-Wilson 1980a, c; Merlin and Grant 1985; Shimizu et al. 1996; Tsukaya 2004), normally important taxonomic characters seem to be rather randomly distributed throughout the whole genus. Consequently, the use of morphological data to divide the genus *Impatiens* into natural groupings has caused many difficulties in the past. Warburg and Reiche (1895) presented the only global intrageneric classification. Due to its artificial nature, the established subgenera and sections of this early classification were hardly used in later morphological and molecular studies. In fact, even the authors recognized their classification as 'not natural'. Together with previously published molecular data (Yuan et al. 2004), this study confirms the artificiality of their attempt. For example, according to Warburg and Reiche's (1895) classification, *I. latifolia* (sect. *Enantiophyllon*, subg. *Caulimpatiens*), *I. balsamina* (sect. *Microcentron*, subg. *Caulimpatiens*) and *I. flaccida* (sect. *Macrocentron*, subg. *Caulimpatiens*) belong to three different sections, but our analysis clearly illustrates that each of these species are part of the same south Indian lineage (clade 15). Moreover, Warburg and Reiche (1895) suggested that *I. repens* and *I. glandulifera* belong to the same section (sect. *Microcentron*, subg. *Caulimpatiens*). According to our results, *I. glandulifera* is part of a more basal clade in *Impatiens* (clade 3), while *I. repens* is found in the more derived, south Indian clade together with *I. flaccida*, *I. balsamina*, and *I. latifolia* (clade 15).

***Phylogenetic Relationships within Balsaminaceae.*** Although many earlier studies at family level or above showed that Balsaminaceae are part of the basal clade in Ericales, the use of only very few *Impatiens* species in those surveys could not provide a definite answer regarding the monophyly of the family (Savolainen et al. 2000; Soltis et al. 2000; Albach et al. 2001; Anderberg et al. 2002; Bremer et al. 2002; APG 2003; Geuten et al. 2004). Yuan et al. (2004) included adequate taxon sampling to test and affirm the monophyly of Balsaminaceae. This conclusion was strongly confirmed by our study (BS: 100 / BPP: 100).

Based on differences in floral morphology, anatomy, and habitat, many botanists regarded *Hydrocera* as a distinct genus in Balsaminaceae (Warburg and Reiche 1895; Venkateswarlu and Lakshinarayana 1957; Grey-Wilson 1980b; Ramadevi and Narayana 1989; Raghuveer et al. 1993). However, it could have been possible that *Hydrocera* was nested within the genus *Impatiens*. Yuan et al. (2004) recognized both genera as two distinct lineages, but the strict consensus of the ITS trees could not give a decisive answer regarding the phylogenetic relationship between the genera. Nevertheless, our results illustrate with very high support (BS: 100 / BPP: 100) that *Hydrocera* is sister to *Impatiens*.

***Phylogenetic Relationships within* Impatiens*, a Biogeographical Interpretation.*** Although the *atpB-rbcL* dataset refines our knowledge of the phylogenetic relationships in *Impatiens*, it remains very difficult to find morphological synapomorphies for the different clades that we find in the *atpB-rbcL* tree. In contrast, many of

the clades can be easily associated with a specific area of distribution. It is therefore interesting to compare the available biogeographical data with our molecular phylogeny (Fig. 1).

Biogeographical optimization onto the chloroplast phylogeny illustrates that *Impatiens* probably originated in South China. The North American species form a strongly supported clade that is nested within a South Chinese lineage (clade 6). Previous studies already suggested that the North American species (*I. capensis* and *I. aurea*) are derived from an East Asian ancestor (Grey-Wilson 1980a; Yuan et al. 2004).

Yuan et al. (2004) concluded that the African species are not monophyletic. Both nuclear and chloroplast trees illustrate that the African continent has been colonized at least twice. One of the dispersals resulted in a small clade that is characterized by long-linear fruits and the presence of five sepals (clade 5), whereas the other group of African species has only three sepals and more fusiform capsules. However, due to the uncertain phylogenetic position of the African species *I. tinctoria* (five sepals with long-linear fruits), and the West African lineage (clade 10), it is not possible to conclude with certainty how many times the African continent has been colonized. New Guinean species are closely related to those of China, which is consistent with findings of Grey-Wilson (1980d).

The Madagascan species form a highly supported clade (clade 12), originated from an African ancestor (Fig. 1). Due to insufficient sampling, it is not possible to conclude with confidence that the invasion of Madagascar happened from a single colonization event or through multiple invasions. South India was colonized at least twice, certainly once by an East Asian ancestor (clade 8), another time most likely by an ancestor with African affinities (clade 15), confirming the assumption of Yuan et al. (2004).

***The Implication of the Molecular Phylogeny for Character Evolution.*** One of the most important taxonomic characters in *Impatiens* is the shape of the dehiscent fruit capsule. Grey-Wilson (1980a) delimited a group of northern species with long-linear fruits, a group with broad fusiform fruits belonging to a group of southerly oriented species. Chloroplast spacer data do not provide evidence for the delimitation of these two lineages in *Impatiens*, as suggested by Grey-Wilson (1980a). Moreover, our analysis supports the results of Yuan et al. (2004), which showed that long-linear capsules are plesiomorphic whereas broadly fusiform capsules are a synapomorphy for a large clade consisting of mainly African, Madagascan, and Indian species (clade III). Grey-Wilson's correlation between fruit type and geographic distribution turned out to be correct.

Karyological data offer interesting insights into the molecular phylogeny of *Impatiens*. According to numerous karyological studies, the majority of species in *Impatiens* possess one of the following haploid chromosome numbers $n=7$, $n=8$, $n=9$, $n=10$ (Song et al. 2003). Several different hypotheses have been suggested regarding the ancestral haploid chromosome number in *Impatiens*. Koshoo (1957) assumed that n=7 or $n=10$ were basic chromosome numbers in *Impatiens*. On the contrary, Rao et al. (1986) suggested $n=8$ as ancestral number, whereas Jones and Smith (1966) and Akiyama et al. (1992) proposed $n=7$ to be the basic chromosome number. Based on their molecular data, Yuan et al. (2004) suggested that $n=10$ might be the ancestral chromosome number in *Impatiens*. Due to a lack of karyological data for some taxa it is not possible to provide a definite answer about the basic chromosome number in *Impatiens*. However, our phylogeny points out that $n=10$ might indeed be ancestral, yet the possibility of $n=9$ as ancestral chromosome number should not be ruled out.

Several botanists have repeatedly emphasized the extremely high degree of floral variation in *Impatiens* (Hooker 1875; Grey-Wilson 1980a; Akiyama and Ohba 2000; Yuan et al. 2004). Most likely, this high floral diversity is correlated with the evolution of an ancestral *Impatiens* flower with separate and equal perianth organs into the zygomorphic *Impatiens* flower (Grey-Wilson 1980a). One of the trends in this global developmental pattern is the loss of the upper pair of lateral sepals, resulting in a calyx with three sepals (Grey-Wilson 1980a). Although anterolateral sepals are generally absent in *Impatiens*, they are still present in some species as small, rudimentary organs. In others, they occur only in an early developmental stage and disappear later (P. Caris, in mss.). Eleven species in our sample are known to have five sepals in mature flowers and according to our results these species are scattered all over the molecular phylogeny. Regarding our molecular phylogeny and the floral ontogenetic study of P. Caris (in mss.), the occurrence of five sepals is plesiomorphic in *Impatiens*. Consequently, the loss of the anterolateral pair is not a synapomorphy for the genus.

Pollen characters are useful for the recognition of broad evolutionary trends in *Impatiens* (Grey-Wilson 1980d; Janssens et al. 2005). Comparing our molecular phylogeny with previous palynological studies (Huynh 1968; Grey-Wilson 1980d; Lu 1991), various trends in the evolutionary development of the pollen can be observed. Tricolpate pollen grains with a triangular, oblate shape and elongated colpi are the plesiomorphic pollen type in Balsaminaceae. This kind of pollen is observed in *Hydrocera triflora* and some endemic *Impatiens* on Mount Omei in China (Lu 1991; Janssens et al. 2005). Lu (1991) suggested that these Chinese species consisting of *Impatiens omeiana* and its 3-colpate, triangular shaped allies are closely related to *Hydrocera triflora*. Our molecular data confirm this

hypothesis since *I. omeiana* is resolved as sister to the remaining species in *Impatiens*. Furthermore, a trend from triangular 3-colpate pollen to rectangular 4-colpate pollen is observed in the major *Impatiens* clade that is sister to *I. omeiana*. However, in several lineages the rectangular 4-colpate pollen type reverses again to 3-colpate pollen (Huynh 1968; Grey-Wilson 1980d).

We believe that a more extensive sampling and additional data from faster evolving DNA sequences will be necessary to obtain an even better view on the evolution and the biogeography of more recently diverged taxa. Additionally, more extensive morphological studies are needed in order to better comprehend the subclades that are established by molecular data. It is obvious that a more thorough biogeographical survey with dating of the internal nodes will play an important role in the understanding of the evolution and the family.

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## LITERATURE CITED

AKIYAMA, S. and H. OHBA. 2000. Inflorescences of the Himalayan species of *Impatiens* (Balsaminaceae). *Journal of Japanese Botany* 75: 226–240.

———, M. WAKABAYASHI, and H. OHBA. 1992. Chromosome evolution in Himalaya *Impatiens* (Balsaminaceae). *Botanical Journal of the Linnean Society* 109: 247–257.

ALBACH, D. C., P. S. SOLTIS, D. E. SOLTIS, and R. G. OLMSTEAD. 2001. Phylogenetic analysis of asterids based on sequences of four genes. *Annals of the Missouri Botanical Garden* 88: 163–212.

ALVAREZ, I. and J. F. WENDEL. 2003. Ribosomal ITS sequences and plant phylogenetic inference. *Molecular Phylogenetics and Evolution* 29: 417–434.

ANDERBERG, A. A., C. RYDIN, and M. KALLERSJÖ. 2002. Phylogenetic relationship in the order Ericales s.l.: analyses of molecular data from five genes from the plastid and mitochondrial genomes. *American Journal of Botany* 89: 677–687.

ANGIOSPERM PHYLOGENY GROUP. 2003. An update of the angiosperm phylogeny group classification for the orders and families of flowerings: APG II. *Botanical Journal of the Linnean Society* 141: 399–436.

BREMER, B., K. BREMER, N. HEIDARI, P. ERIXON, R. G. OLMSTEAD, A. A. ANDERBERG, M. KÄLLERSJÖ, and E. BARKHORDARIAN. 2002. Phylogenetics of asterids based on 3 coding and 3 non-coding DNA at higher taxonomic levels. *Molecular Phylogenetics and Evolution* 24: 274–301.

CHASE, M. W. and H. H. HILLS. 1991. Silica gel: an ideal material for field preservation of leaf samples for DNA studies. *Taxon* 40: 215–220.

CLIFTON, R. 2000. *Geraniales species checklist series: vol. 6. Geraniaceae Bentham and Hooker, tribe VII Balsamineae*. Dover: The Geraniaceae Group.

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

FELSENSTEIN, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783–791.

FUJIHASHI, H., S. AKIYAMA, and H. OHBA. 2002. Origin and relatioships of the Sino-Himalayan *Impatiens* (Balsaminaceae) based on molecular phylogenetic analysis, chromosome numbers and gross morphology. *Journal of Japanese Botany* 77: 284–295.

GEUTEN, K., E. SMETS, P. SCHOLS, Y.-M. YUAN, S. JANSSENS, P. KÜPFER, and N. PYCK. 2004. Conflicting phylogenies of balsaminoid families and the polytomy in Ericales: combing data in a Bayesian framework. *Molecular Phylogenetics of Evolution* 31: 711–729.

GREY-WILSON, C. 1980a. *Impatiens of Africa*. Rotterdam: Balkema.

———. 1980b. *Hydrocera triflora*, its floral morphology and relationship with *Impatiens*. Studies in Balsaminaceae. *Kew Bulletin* 34: 221–227.

———. 1980c. Hybridisation in African *Impatiens*. Studies in Balsaminaceae. *Kew Bulletin* 34: 689–722.

———. 1980d. *Impatiens* in Papuasia. Studies in Balsaminaceae. *Kew Bulletin* 34: 661–687.

HOOKER, J. D. and T. THOMSON. 1859. Praecursores ad floram Indicam. Balsaminaceae. *Botanical Journal of the Linnean Society* 4: 106–157.

HOOKER, J. D. 1875. *Flora of British India*. Kent: L. Reeve.

———. 1905. An epitome of the British Indian species of *Impatiens*. *Records of the Botanical Survey of India* 4: 1–58.

HUELSENBECK, J. and F. RONQUIST. 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17: 754–755

HUYNH, K.-L. 1968. Morphologie du pollen des Tropaeolacées et des Balsaminacées I & II. *Grana Palynologica* 8: 88–184 and 277–516.

JANSSENS, S., F. LENS, S. DRESSLER, K. GEUTEN, E. SMETS, and S. VINCKIER. 2005. Palynological variation in balsaminoid Ericales. II. Balsaminaceae, Tetrameristaceae, Pellicieraceae and general conclusions. *Annals of Botany* 96: 1061–1073.

JONES, K. and J. SMITH. 1966. The cytogeography of *Impatiens* L. (Balsaminaceae). *Kew Bulletin* 20: 63–72.

KHOSHOO, T. 1957. Cytology of some *Impatiens* species. *Caryologia* 10: 55–74.

LU, Y.-Q. 1991. Pollen morphology of *Impatiens* L. (Balsaminaceae) and its taxonomic implications. *Acta Phytotaxonomica Sinica* 29: 352–357.

MADDISON, D. and W. MADDISON. 2002. MacClade. Sunderland: Sinauer Associates.

MANSION, G. and L. STRUWE. 2004. Generic delimitation and phylogenetic relationships within the subtribe Chroniinae (Chironieae: Gentianaceae), with special reference to *Centaurium*: evidence from nrDNA and cpDNA sequences. *Molecular Phylogenetics and Evolution* 32: 951–977.

MERLIN, C. M. and W. F. GRANT. 1985. Hybridization studies in the genus *Impatiens*. *Canadian Journal of Botany* 64: 1069–1074.

MORTON, C. M., M. W. CHASE, K. A. KRON, and S. M. SWENSEN. 1996. A molecular evaluation of the monophyly of the order Ebenales based upon *rbcL* sequence data. *Systematic Botany* 21: 567–586.

POSADA, D. and K. A. CRANDALL. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* 14: 817–818

RAGHUVEER, M., L. L. NARAYANA, and B. S. M. DUTT. 1993. Dehiscent fruit of *Hydrocera triflora* (Linn). Wt. and Arn. (Balsaminaceae): its anatomy and dispersal. *Rheedea* 3: 12–14.

RAMADEVI, D. and L. L. NARAYANA. 1989. Floral anatomy of Balsaminaceae. *Plant Science Research In India* 12: 707–713.

RAO, R., K. AYYANGAR, and R. SAMPATHKUMAR. 1986. On the karyological characteristics of some members of Balsaminaceae. *Cytologia* 51: 251–260

SAGHAI-MAROOF, M. A., K. A. SOLIMAN, R. A. JORGENSON, and R. W. ALLARD. 1984. Ribosomal DNA spacer length polymorphism in Barley: Mendelian inheritance, chromosal location and population dynamics. *Proceedings of the National Academy of Science, USA* 8: 8014–8018.

SAVOLAINEN, V., M. W., CHASE, S. B. HOOT, C. M. MORTON, D. E. SOLTIS, C. BAYER, M. F. FAY, A. Y. DE BRUIJN, S. SULLIVAN, and Y. L. QUI. 2000. Phylogenetics of flowering plants based on combined analysis of plastid *atpB* and *rbcL* gene sequences. *Systematic Biology* 49: 306–362.

SCHNEEWEISS, G. M., A. COLWELL, J.-M. PARK, C.-G. JANG, and T. F. STUESSY. 2004. Phylogeny of holoparasitic *Orobanche* (Orobanchaceae) inferred from nuclear ITS sequences. *Molecular Phylogenetics and Evolution* 30: 465–478.

SHIMIZU, T., T. TAKAO, N. UTAMI, and A. TAKAO. 1996. Anatomy of floral organs and its taxonomic significance in the genus *Impatiens* (Balsaminaceae), with special reference to subgen. *Acaulimpatiens*. *Phytomorphology* 46: 253–266.

SIMMONS, M. P. and H. OCHOTERENA. 2000. Gaps as characters in sequence-based phylogenetic analyses. *Systematic Biology* 49: 369–381.

SOLTIS, D. E., P. S. SOLTIS, M. W. CHASE, M. E. MORT, D. C. ALBACH, M. ZANIS, V. SAVOLAINEN, W. H. HAHN, S. B. HOOT, M. F. FAY, M. AXTELL, S. M. SWENSEN, L. M. PRINCE, W. J. KRESS, K. C. NIXON, and J. S. FARRIS. 2000. Angiosperm phylogeny inferred from 18S rDNA, *rbcL*, and *atpB* squences. *Botanical Journal of the Linnean Society* 133: 381–461.

SONG, Y., Y.-M. YUAN, and P. KÜPFER. 2003. Chromosomal evolution in Balsaminaceae with cytological observations on 45 species from Southeast Asia. *Caryologia* 56: 463–481.

SUZUKI, Y., G. V. GLAZKO, and M. NEI. 2002. Overcredibility of molecular phylogenies obtained by Bayesian phylogenetics. *Proceedings of the National Academy of Science, USA* 99: 16138–16143.

SWOFFORD, D. L. 2002. PAUP*. Phylogenetic analysis using parsimony (*and other methods). Version 4. Sunderland: Sinauer Associates.

TSUKAYA, H. 2004. Gene flow between *Impatiens radicans* and *I. javensis* (Balsaminaceae) in Gunung Pangrango, central Java, Indonesia. *American Journal of Botany* 91: 2119–2123.

VENKATESWARLU, J. and L. LAKSHINARAYANA. 1957. A contribution to the embryology of *Hydrocera triflora* Wt. and Arn. *Phytomorphology* 7: 194–203.

WARBURG, O. and K. REICHE. 1895. Balsaminaceae. Pp. 583–592 in *Planzenfamilien*, eds. A. Engler and K. Prantl. Berlin: Dunckel and Humblott.

YUAN, Y. -M., Y. SONG, K. GEUTEN, E. RAHELIVOLOLONA, S. WOHLHAUSER, E. FISCHER, E. SMETS, and P. KÜPFER. 2004. Phylogeny and biogeography of Balsaminaceae inferred from ITS sequence data. *Taxon* 53: 391–403.

## APPENDIX 1

Accession numbers, voucher data and origin of plant material for taxa included in the DNA analyses.

*Impatiens apsotis* Hook.f.: China, Sichuan, *Yuan CN2k2–159* (NEU): DQ147810 *atpB-rbcL,* AY348744 ITS. *Impatiens aquatilis* Hook.f.: China, Yunnan, *Song CNY017* (NEU): DQ147811 *atpB-rbcL,* AY348745 ITS. *Impatiens arguta* Hook.f. and Thoms.: China, Yunnan, *Yuan CN2k–74* (NEU): DQ147812 *atpB-rbcL,* AY348746 ITS. *Impatiens aurea* Muhl.: North American origin, cult. Holden arboretum, *Janssens SJ008* (LV): DQ147813 *atpB-rbcL*. *Impatiens aureliana* Hook.f.: China, Yunnan, *Yuan CN2k1–56* (NEU): DQ147814 *atpB-rbcL,* AY348747 ITS. *Impatiens auricoma* Baill.: Comores origin, cult. Bot. Gard. Marburg, *Janssens SJ003* (LV): DQ147815 *atpB-rbcL,* AY348748 ITS. *Impatiens balsamina* L.: Indian origin, cult. Kruidtuin Leuven, *Janssens SJ001* (LV): DQ147816 *atpB-rbcL,* AY348749 ITS. *Impatiens balfourii* Hook.f.: Himalayan origin, cult. Denver Bot. Gard., *33051 (DBG)*: DQ147817 *atpB-rbcL*. *Impatiens barbata* Comber: China, Yunnan, *Yuan CN2k2–178* (NEU): DQ147818 *atpB-rbcL,* AY348750 ITS. *Impatiens begoniifolia* S. Akiyama and H. Ohba: China, Yunnan, *Yuan CN2k1–51* (NEU): DQ147819 *atpB-rbcL,* AY348752 ITS. *Impatiens bequartii* De Wild.: Congo origin, cult. Bot. Gard. Koblenz. Univ., *Fischer NE1* (NEU): DQ147820 *atpB-rbcL,* AY348753 ITS. *Impatiens bicornuta* Wall.: China, Yunnan, *Yuan CN2k–55* (NEU): DQ147821 *atpB-rbcL,* AY348754 ITS. *Impatiens campanulata* Wight: South Indian origin, cult. by Ray Morgan, U.K., *Ray Morgan s.n.* (LV): DQ147822 *atpB-rbcL,* AY348758 ITS. *Impatiens capensis* Meerb.: North American origin, cult. Holden Arboretum, *Janssens SJ009* (LV): DQ147823 *atpB-rbcL,* AY348759 ITS. *Impatiens chimiliensis* Comber: China, Yunnan, *Yuan CN2k–51* (NEU): DQ147824 *atpB-rbcL,* AY348760 ITS. *Impatiens chinensis* L.: China, Yunnan, *Yuan CN2k1–49* (NEU): DQ147825 *atpB-rbcL,* AY348761 ITS. *Impatiens chungtienensis* Y.L. Chen: China, Yunnan, *Yuan CN2k2–204* (NEU): DQ147826 *atpB-rbcL,* AY348762 ITS. *Impatiens clavicornu* Turcz.: South Indian origin, cult. by Ray Morgan, U.K., *Ray Morgan s.n.* (LV): DQ147827 *atpB-rbcL,* AY348804 ITS. *Impatiens columbaria* J.J. Bos: African origin, cult. Nat. Bot. Gard. Meise, *FB/S2966* (BR): DQ147828 *atpB-rbcL,* AY348764 ITS. *Impatiens conchibracteata* Y.L. Chen: China, Yunnan, *Hao 427* (NEU): DQ147829 *atpB-rbcL,* AY348765 ITS. *Impatiens congolensis* G.M. Schulze and R. Wilczek: African origin, cult. Bot. Gard. Koblenz Univ., *Fischer NE7* (NEU): DQ147830 *atpB-rbcL,* AY348766 ITS. *Impatiens corchorifolia* Franch.: China, Yunnan, *Chassot and Yuan 99–173* (NEU): DQ147831 *atpB-rbcL,* AY348767 ITS. *Impatiens cuspidata* Wight and Arn.: South Indian origin, cult. by Ray Morgan, U.K., *Ray Morgan s.n.* (LV): DQ147832 *atpB-rbcL,* AY348769 ITS. *Impatiens cyanantha* Hook.f.: China, Yunnan, *Yuan CN2k1–84* (NEU): DQ147833 *atpB-rbcL,* AY348770 ITS. *Impatiens cyathiflora* Hook.f.: China, Yunnan, *Chassot and Yuan 99–29* (NEU): DQ147834 *atpB-rbcL,* AY348771 ITS. *Impatiens davidi* Franch.: China, Fujian, *Yuan CN2k–09* (NEU): DQ147835 *atpB-rbcL,* AY348772 ITS. *Impatiens delavayi* Franch.: China, Yunnan, *Chassot and Yuan 99–154* (NEU): DQ147836 *atpB-rbcL,* AY348773 ITS. *Impatiens desmantha* Hook.f.: China, Yunnan, *Yuan CN2k–30* (NEU): DQ147837 *atpB-rbcL,* AY348774 ITS. *Impatiens drepanophora* Hook.f.: China, Yunnan, *Yuan CN2k1–41* (NEU): DQ147838 *atpB-rbcL,* AY348776 ITS. *Impatiens eberhardtii* Tardieu: Vietnam, Anam, *Song s.n.* (NEU): DQ147839 *atpB-rbcL*. *Impatiens edgeworthii* Hook.f.: Himalayan origin, cult. Univ. California Bot. Gard. Berkeley, *89.2005* (UC): DQ147840 *atpB-rbcL*. *Impatiens faberi* Hook.f.: China, Sichuan, *Song S007* (NEU): DQ147841 *atpB-rbcL,* AY348778 ITS. *Impatiens fenghwaiana* Y.L. Chen: China, Guangxi, *Yuan CN2k–41* (NEU): DQ147842 *atpB-rbcL,* AY348779 ITS. *Impatiens fischeri* Warb.: Africa, *Menn. and Bar.-Kui. 284* (U): DQ147843 *atpB-rbcL,* AY348781 ITS. *Impatiens fissicornis* Maxim.: China, Shaanxi, *Wang SH-003* (NEU): DQ147844 *atpB-rbcL,* AY348782 ITS. *Impatiens flaccida* Arn.: South Indian origin, cult. Nat. Bot. Gard. Meise, *FB/S3925* (BR): DQ147845 *atpB-rbcL*. *Impatiens flanaganae* Hemsl: African origin, cult. Roy. Bot. Gard. Edinburgh, *19860179* (E): DQ147846 *atpB-rbcL,* AY348783 ITS. *Impatiens forrestii* Hook.f. ex W.W. Smith: China, Yunnan, *Yuan CN2k–79* (NEU): DQ147847 *atpB-rbcL,* AY348784 ITS. *Impatiens glandulifera* Arn.: Belgium, Leuven, *Janssens SJ002* (LV): DQ147848 *atpB-rbcL,* AY348788 ITS. *Impatiens hians* Hook.f.: African origin, cult. Bot. Gard. Berlin, *Schwerdtfeger 9492a* (B): DQ147849 *atpB-rbcL*. *Impatiens hawkeri* W. Bull: New Guinean origin, *Janssens SJ006* (LV): DQ147850 *atpB-rbcL*. *Impatiens imbecilla* Hook.f.: China, Sichuan, *Hao 426* (NEU): DQ147851 *atpB-rbcL,* AY348796 ITS. *Impatiens inaperta* H. Perrier: Madagascar, *JBE2* (NEU): DQ147852 *atpB-rbcL,* AY348797 ITS. *Im-*

*patiens kerriae* Craib: Thailand, Qingmai, *Chassot 99–238* (NEU): DQ147853 *atpB-rbcL,* AY348799 ITS. *Impatiens latifolia* L.: South Indian origin, cult. by Ray Morgan, U.K., *Ray Morgan s.n.* (LV): DQ147854 *atpB-rbcL,* AY348801 ITS. *Impatiens lecomtei* Hook.f.: China, Yunnan, *Yuan CN2k2–202* (NEU): DQ147855 *atpB-rbcL,* AY348802 ITS. *Impatiens leschenaultii* Wall.: South Indian origin, cult. by Ray Morgan, U.K., *Ray Morgan s.n.* (LV): DQ147856 *atpB-rbcL,* AY348803 ITS. *Impatiens mackeyana* subsp. *zenkeri* Hook.f.: African origin, cult. Bot. Gard. Koblenz Univ., *Fischer NE21* (NEU): DQ147857 *atpB-rbcL,* AY348852 ITS. *Impatiens mengtseana* Hook.f.: China, Yunnan, *Yuan CN2k1–38* (NEU): DQ147858 *atpB-rbcL,* AY348806 ITS. *Impatiens meruensis* Gilg.: Tanzanian origin, cult. by Ray Morgan, U.K., *Ray Morgan s.n.* (LV): DQ147859 *atpB-rbcL,* AY348807 ITS. *Impatiens monticola* Hook.f.: China, Sichuan, *Hao 425* (NEU): DQ147860 *atpB-rbcL,* AY348810 ITS. *Impatiens napoensis* Y.L. Chen: China, Yunnan, *Yuan CN2k1*-61 (NEU): DQ147861 *atpB-rbcL,* AY348811 ITS. *Impatiens niamniamensis* Gilg.: African origin, cult. Nat. Bot. Gard. Meise, *FB/S2590* (BR): DQ147862 *atpB-rbcL,* AY348812 ITS. *Impatiens noli-tangere* L.: Temperate origin, cult. Kruidtuin Leuven, *Janssens SJ007* (LV): DQ147863 *atpB-rbcL,* AY348813 ITS. *Impatiens omeiana* Hook.f.: China, Sichuan, cult. Univ. California Bot. Gard. Berkeley, *2002.0214* (UC): DQ147864 *atpB-rbcL. Impatiens oxyanthera* Hook.f.: China, Sichuan, *Song S008* (NEU): DQ147865 *atpB-rbcL,* AY348814 ITS. *Impatiens parviflora* DC.: Belgium, Leuven, *Janssens SJ004* (LV): DQ147866 *atpB-rbcL,* AY348816 ITS. *Impatiens platychlaena* Hook.f.: China, Sichuan, *Song S009* (NEU): DQ147867 *atpB-rbcL,* AY348818 ITS. *Impatiens platypetala* Lindl.: Bali, Indonesian origin, cult. by Ray Morgan, U.K., *Ray Morgan s.n.* (LV): DQ147868 *atpB-rbcL,* AY348819 ITS. *Impatiens poilanei* Tardieu: Vietnam, Anam, *Song s.n.* (NEU): DQ147869 *atpB-rbcL. Impatiens poculifer* Hook.f.: China, Yunnan, *Yuan CN2k2–209* (NEU): DQ147870 *atpB-rbcL,* AY348820 ITS. *Impatiens pseudoviola* Gilg.: African origin, cult. Roy. Bot. Gard. Edinburgh, *19680124* (E): DQ147871 *atpB-rbcL,* AY348822 ITS. *Impatiens purpurea* Hand.-Mazz.: China, Yunnan, *Song Y007* (NEU): DQ147872 *atpB-rbcL,* AY348823 ITS. *Impatiens racemosa* DC.: China, Xizang, *De Haas 2620* (U): DQ147873 *atpB-rbcL. Impatiens rectangula* Hand.-Mazz.: China, Yunnan, *Yuan CN2k1–26* (NEU): DQ147874 *atpB-rbcL,* AY348825 ITS. *Impatiens repens* Moon: Indian origin, cult. Nat. Bot. Gard. Leiden, *17953* (L): DQ147875 *atpB-rbcL. Impatiens rubrostriata* Hook.f.: China, Yunnan, *Yuan CN2k1–44* (NEU): DQ147876*atpB-rbcL,* AY348828 ITS. *Impatiens scabrida* DC.: Himalayan origin, cult. Holden arboretum, *941314* (DBG): DQ147877 *atpB-rbcL. Impatiens scutisepala* Hook.f.: China, Yunnan, *Yuan CN2k–56* (NEU): DQ147878 *atpB-rbcL,* AY348830 ITS. *Impatiens sodenii* Engl. and Warb.ex Engl.: African origin, cult. Roy. Bot. Gard. Edinburgh, *19660477* (E): DQ147879 *atpB-rbcL,* AY348832 ITS. *Impatiens soulieana* Hook.f.: China, Sichuan, *Yuan CN2k2–163* (NEU): DQ147880 *atpB-rbcL,* AY348833 ITS. *Impatiens stenosepala* Pritz. ex Diels: China, Shaanxi, *Wang SH-001* (NEU): DQ147881 *atpB-rbcL,* AY348835 ITS. *Impatiens taronensis* Hand.-Mazz.: China, Yunnan, *Yuan CN2k–57* (NEU): DQ147882 *atpB-rbcL,* AY348838 ITS. *Impatiens teitensis* Grey-Wilson: African origin, cult. Bot. Gard. Koblenz Univ., *Fischer NE18* (NEU): DQ147883 *atpB-rbcL,* AY348840 ITS. *Impatiens tinctoria* A.Rich: African origin, cult. Nat. Bot. Gard. Meise, *2002–1249–61* (BR): DQ147884 *atpB-rbcL. Impatiens trichosepala* Y.L. Chen: China, Yunnan, *Yuan CN2k1–68* (NEU): DQ147885 *atpB-rbcL,* AY348843 ITS. *Impatiens tuberosa* H. Perrier: Madagascan origin, cult. Bot. Gard. Univ. Kopenhagen, *Janssens SJ005* (LV): DQ147886 *atpB-rbcL,* AY348844 ITS. *Impatiens uliginosa* Franch.: China, Yunnan, *Yuan CN2k2–173* (NEU): DQ147887 *atpB-rbcL,* AY348845 ITS. *Impatiens uniflora* Hayata: China, Taiwan, *Zhengyu Jiang T1* (NEU): DQ147888 *atpB-rbcL,* AY348846 ITS. *Impatiens usambarensis* Grey-Wilson: African origin, cult. Bot. Gard. Koblenz Univ., *Fischer NE20* (NEU): DQ147889 *atpB-rbcL,* AY348847 ITS. *Impatiens usambarensis* Grey-Wilson x *walleriana* Hook.f.: African origin, cult. Roy. Bot. Gard. Edinburgh, *19821569* (E): DQ147890 *atpB-rbcL. Impatiens viscida* Wight: South Indian origin, cult. by Ray Morgan, U.K., *Ray Morgan s.n.* (LV): DQ147891 *atpB-rbcL,* AY34768 ITS. *Impatiens walleriana* Hook.f.: African origin, cult. Nat. Bot. Gard. Meise, *S3926* (BR): DQ147892 *atpB-rbcL,* AY348849 ITS. *Impatiens xanthina* Comber: China, Yunnan, *Yuan CN2k1–15* (NEU): DQ147893 *atpB-rbcL,* AY348850 ITS. *Impatiens yingjiangensis* S.Akiyama and H.Ohba: China, Yunnan, *Yuan CN2k1–55* (NEU): DQ147894 *atpB-rbcL,* AY348851 ITS. *Hydrocera triflora* Wight and Arn.: Sri Lanka, *Robyns 7260, 0249369* (BR): DQ147895 *atpB-rbcL,* AY348853 ITS. *Marcgravia maguire* de Roon: French Guyana, cult. Nat. Bot. Gard. Meise, FB/S3780 (BR): DQ147896 *atpB-rbcL. Marcgravia umbellata* L.: South American origin, cult. Nat. Bot. Gard. Meise, *FB/S3782* (BR): DQ147897 *atpB-rbcL. Norantea guianensis* Aubl.: South American origin, cult. Nat. Bot. Gard. Meise, *FB/S3779* (BR): DQ147898 *atpB-rbcL,* AY348855 ITS. *Pelliciera rhizophorae* Planch. and Triana: Costa Rica, *Pennington et al. 586* (K): DQ147899 *atpB-rbcL,* AY348856 ITS. *Souroubea* sp.: Peru, *76GR00102* (U): DQ147900 *atpB-rbcL,* AY348857 ITS.

# Paper V

# Phylogenetic analysis of Balsaminaceae based on one nuclear and two chloroplast markers

Song Y., Yuan Y.-M., Janssens S., Smets E. and Küpfer P.

Manuscript

# PHYLOGENETIC ANALYSIS OF BALSAMINACEAE BASED ON ONE NUCLEAR AND TWO CHLOROPLAST MARKERS


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## ABSTRACT

To confirm the phylogeny of Balsaminaceae and understand better its biogeography and floral evolution, a phylogenetic analysis of combined nuclear ribosomal ITS and chloroplast trnL-trnF and atpB-rbcL from 182 species of Balsaminaceae worldwide were conducted. Five species of its closest relatives Marcgraviaceae and Tetrameristaceae were chosen as outgroups. Phylogenetic reconstructions using Neighbor-joining method based on individual and combined sequence data provide similar but not identical phylogeny topology. Combined analysis with Bayesian inference resulted in a better resolved topology similar to the NJ trees. Combined molecular phylogeny confirmed the monophyly of Balsaminaceae and *Impatiens*. The phylogenetic relationships within *Impatiens* are associated with geographic distribution, less defined by gross morphology. When morphological, palynological, karyological and biogeographical characters were mapped and integrated to the combined Bayesian tree, it is suggested that Balsaminaceae are of mainland Southeast Asian origin, from where dispersals to Southeast Asian islands, to North America, to central Asia and eastern Europe via the Himalayas, and to India and Africa have occurred. Madagascan *Impatiens* shows a clear African origin. Both x=8 or 10 are the most likely ancestral base chromosome number, and the spurred flowers, narrowly fusiform fruits, 4 lateral sepals, 3-colpate pollen and reticulate type seedcoat are plesiomorphic characters in *Impatiens*. The diverse and complicate specialization of floral structures of Balsaminaceae is pollinator-mediated, and therefore highly homoplasious.



**Key words**: atpB-rbcL; Balsaminaceae; biogeography; chromosome number; ITS; floral morphology evolution; molecular phylogeny; trnL-trnF


## INTRODUCTION

Balsaminaceae are a medium-sized family comprising only two genera, the monotypic *Hydrocera* and the prolific *Impatiens*. The two genera are easily distinguished from each other: *Hydrocera* has free petals and an indehiscent 'berry-like' fruit, whereas *Impatiens* has the petals variously united, and a characteristic, rather explosive, dehiscent fruit capsule. *Hydrocera,* with only one species, *H. triflora* is restricted to the Indo-Malesian countries, while *Impatiens,* with over 1000 species, highly clustered in tropical and subtropical montane regions of the Old World. Its has five 'hotspots' of species diversity: tropical Africa, Madagascar, southern India and Sri Lanka, the eastern Himalayas, and Southeast Asia area in its broad sense (including Burma, Thailand, southwest China, Indochina peninsula, and the Malesia archipelagos). There are a few *Impatiens* species in temperate areas of the northern hemisphere, but there is no native species in South America and Australia.

The understanding of phylogenetic relationships among angiosperms has greatly increased in the last few years, in particular as a result of analyses of molecular data accumulated from multiple regions (Clausing & Renner, 2001). In Balsaminaceae, to reveal the phylogenetic relationship within this family with molecular methods, a few studies have been made (Fujihashi et al., 2002; Yuan et al., 2004; Janssens et al., 2006). Fujihashi (2002)'s work is

sketchy due to its limited sampling and unsuitable analysis using too distant outgroups. Yuan et al. (2004)'s study of molecular phylogeny of Balsaminaceae based on internal transcribed spacer (ITS) including 112 Balsaminaceae species worldwide confirmed the monophyly of Balsaminaceae and suggested the monophyly of *Impatiens*. The results revealed that extant *Impatiens* are of Southeast Asia origin and from where dispersals to the other regions. But ITS data alone could not resolve relationships among most of the lineages with confident support values. The chloroplast atpB-rbcL sequences from 86 Balsaminaceae species were obtained and analyzed separately and combined with ITS data in Janssens et al. (2006)'s study. The results support the monophyly of Balsaminaceae and *Impatiens* from ITS phylogeny of Yuan et al. (2004)'s study, and have better resolved evolutionary relationships in Balsaminaceae. This result revealed *Impatiens* likely originated in South China. But this study has less samples. Extensive taxa and additional gene from other DNA sequences was necessary to obtain a better resolved molecular phylogeny.

*Impatiens* has high diversity in floral morphology, wide chromosomal variation, extensive distribution in paleotropical regions. All this made the Balsaminaceae an idea model to study floral evolution and paleotropical biogeography. Combining morphological data sets for phylogenetic analysis will help to understand their evolutionary history and rules (Miyamoto & Fitch, 1995).

## MATERIALS AND METHODS

***Taxon sampling and outgroup choice—***The species included in this study are listed in Table 1. Both of the two genera are collected. *Hydrocera* was collected in Vietnam and *Impatiens* were collected in East and Southeast Asia, Madagascar, Africa, Himalayas, southern India and Sri Lanka, North America, central Asia and Europe to maximize the morphological variation. A total of 182 species are included in this study as representatives of the ingroup taxa. Among them, 93 species were sampled from East and Southeast Asia; 31 from Madagascar; 25 from Africa; 15 from Himalayas; 10 from southern India and Sri Lanka; 5 from North America; 1 from Central Asia, Europe and Boreal Eurasia respectively. Three species of Marcgraviaceae and two species of Tetrameristaceae were sampled as outgroup since it was revealed these two family are closed related with Balsaminaceae by recently molecular phylogenetic studies (Morton et al., 1996, 1997; APG, 1998; Soltis et al., 2000; Albach et al., 2001; Anderberg et al., 2002; Bremer et al., 2002; Geuten et al., 2004). Voucher specimens are deposited in the herbarium of the University of Neuchâtel (NEU).

***Molecular markers—***Three molecular markers were used in this study, i.e. internal transcribed spacers of nuclear ribosomal DNA (ITS1, 5.8S and TIS2), and noncoding regions of chloroplast DNA (trnL intron, trnL-trnF spacers and atpB-rbcL spacer) (Table2).

**Table 2 The names and sequences of primers used**

| Primer | Primer sequence | Reference |
|---|---|---|
| **ITS** | | White et al., 1990 |
| ITS 5 | 5'-GGAAGTAGAAGTCGTAACAAGG-3' | |
| ITS 4 | 5'-TCCTCCGCTTATTGATATGC-3' | |
| ***trn*LF** | | Taberlet et et al., 1991 |
| trnLF C | 5'-CGAAATCGGTAGACGCTACG-3' | |
| trnLF D | 5'-GGGGATAGAGGGGACTTGA -3' | |
| trnLF E | 5'-GGTTCAAGTCCCTCTATCCCC -3' | |
| trnLF F | 5'-ATTTGAACTGGTGACACGAG-3' | |
| **atpB-rbcL** | | Hoot et al, 1995 |
| atpB-rbcL F | 5'-GAATCCAACACTTGCTTTAGTCTCT-3' | |

| | | |
|---|---|---|
| atpB-rbcL R | 5’ AGA AGT AGT AGG ATT GAT TCT CAT A -3’ | |

***DNA extraction, amplification and sequencing—***Total DNA was extracted from fresh silica gel dried leaf tissue (Chase & Hills, 1991), or herbarium leaves was made using the CTAB procedure of Doyle and Doyle (1987) or the DNeasy Plant Mini Kit (QIAGEN AG, Basel). Double-stranded DNA was directly amplified by standard PCR for each fragment analyzed. Reaction volumes were 25µl containing 2.5µl 10 x PCR buffer (with 1.5 mM $MgCl_2$), 0.5µl 10 mM dNTPs, 0.5µl of 10 mM each forward and reverse primers, 0.2µl (1U) HotStar Taq DNA polymerase (Qiagen AG, Basel), 19.8µl $H_2O$, and 1µl (ca. 10-20 ng) genomic DNA. PCRs were performed in a Biometra thermocycler (biometra, Göttingen, Germany) with the following parameters: 1 cycle of 15 min at 95˚C for the activation of the HotStar Taq DNA polymerase, followed by 35 cycles of 45s at 94˚C, 45s at 55˚C, 1 min at 72˚C, with a final extension period of 10 min at 72˚C. But the annealing temperature was reduced to 50˚C when amplifying atpB-rbcL. Successfully amplified DNA fragments were purified prior to sequencing using the QIAquick™ PCR purification kit (QIAGEN AG, Basel) following the manufacturer’s protocol. Cycle sequencing reactions were performed with one of the same primers for PCR, using the dye-terminator chemistry as implemented in the ABI PRISM BigDye™ Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems) in Biometra thermal cycler. The cycle sequencing products were cleaned using the Ethanol/Sodium acetate precipitation method and then analyzed on an ABI310 automated sequencer (Applied Biosystems). Automation-generated base-calls were subsequently checked manually against the electropherograms using the software Sequence Navigator (Applied Biosystems, Foster City, USA).

***Sequence alignment—***The sequences were aligned with Clustal X (Thompson et al., 1997) and subsequently hand-adjusted for indels otherwise not recognized. The alignment does not involve severe ambiguities, therefore it was not necessary to use secondary structure for alignment. The gaps of aligned sequences were considered as missing data. The data matrix is available from the authors on request.

***Data combination and congruence testing—***To assess the level of congruence between the ITS, trnLF and atpB-rbcL data sets, each data set was analyzed independently to see if they produced a similar topology. The real issues are how to determine the source of conflict between data sets, and more important, if there are any evolutionary explanations for any differences between data sets (Mansion & Struwe, 2004). Following the conditional data combination approach (Huelsenbeck et al., 1996), the incongruence length difference (ILD) test of Farris et al. (1995) (with 100 replicates and same settings as for other analyses) were implemented in PAUP* 4.0b10 (Swofford, 2000) to assess the character congruence between the respective nrDNA and cpDNA data sets. Following Cunningham (1997), p value of 0.01 is taken as a significance criterion for this test.

***Phylogenetic analyses—***Neighbor-joining (NJ) analysis**:** A distance analysis applying NJ optimality criteria (Saitou & Nei, 1987) was conducted on both separated and combined ITS, trnLF, atpB-rbcL data sets using PAUP*v4.0b10 (Swofford, 2000). The number of nucleotide substitutions per site was estimated by Kimura’s (1980) two-parameter method and used to estimate the genetic distance. The reliability of the clades was assessed using bootstrapping with PAUP* (Felsenstein, 1985) performed with 1000 replicates. Only two species of Marcgraviaceae were used as outgroups for analyses involving trnLF data due to limited availability of sequence data for this fragment.

Bayesian inference: Bayesian inference (Rannala & Yang, 1996; Mau et al., 1999) was performed using Mr. Bays v. 3.1 (Ronquist & Huelsenbeck, 2003). Data were partitioned into functional categories (ITS, trnLF and atpB-rbcL). The most optimal model of sequence evolution for each of these partitions was selected using Modeltest version 2.2 (Nylander, 2004). A combined dataset of all partitions, including gaps, was analyzed applying separate model and parameters estimated separately for each individual partition. Two million generations were run with trees and parameters sampled for every 100 generations, Runs were repeated twice to confirm results. Based on inspection of the likelihood scores for each generation, all sample points that occurred before stationary of negative log likelihood scores achieved were discarded as part of the burn-in period (Huelsenbeck & Bollback, 2001). Nodes with posterior probability values $\geqslant$ 0.95 were retained in the 50% majority rule consensus tree.

***Character state optimization and biogeographic analyses*—**A total of 182 ingroup and two outgroup species were used in all analyses. Evolution of structural characters was examined in two steps using MacClade version 3.08 (Maddison & Maddison, 1992): (1) molecular data were used to create a topology onto which morphological characters were mapped; (2) five morphological characters (presence of flower spur, fruit shape, number of lateral sepals, type of pollen, and type of seedcoat micromorphology), basic chromosome number and geographic distribution (Table (3) were traced onto the combined Bayesian tree. The other flower morphological characters (shape and form of the spur, lateral petals and dorsal petals) (Table 3) were integrated with molecular phylogeny.

**Table 3 Morphological, palynological, karyological and biogeographical characters states used in the optimization**

| No. | Character and state descriptions |
|---|---|
| 1. | Presence of spur: N: no spur; Y: with spur |
| 2. | Shape of fruits: 1: capsule broadly fusiform; 2: capsule narrowly fusiform; 3: berry like |
| 3. | Number of lateral sepals: 2: two lateral sepals; 4: four lateral sepals |
| 4. | Pollen morphology: 3: 3-colpate pollen type; 4: 4-colpate pollen type |
| 5. | Basic chromosome number: 1: x=7; 2: x=8; 3: x=9; 4: x=10; 5: x=5; 6: x=6; 7: x=17; 8: x=13; 9: x=3 |
| 6. | Species distribution area: 1: Madagascar; 2: Africa; 3: South India & Sri Lanka; 4: East & Southeast Asia (comprising Japan, Taiwan & the other Southeast Asian islands, the Southeast Asian countries & the area of southwestern China); 5: North America & Boreal Eurasia; 6: the Himalayas & Tibet; 7: Central Asia & Europe |
| 7. | Shape of the spur: 1: filiform; 2: navicular; 3: saccate; 4: almost no spur |
| 8. | Shape of the lateral petals: Lateral petals united: 1-3: 1: lower/upper > 2times, 1A : lower part narrow; 1B: lower part broad; 1C : lower part 2 split; 2: lower/upper 1~2times; 3: lower<upper; 4: lateral petals almost seperated |
| 9. | Shape of the dorsal petals: 1: flat or slightly concave; 2: hooklike |
| 10. | Seedcoat micromorphology: 1: laevigate; 2: granulate; 3: reticultate: 3A: fine reticulate; 3B: colliculate; 3C: carinate; 3D: striate; 4: protrusive: 4A: digitiform; 4B: clustered; 4C: squamalate; 4D: cristate |

## RESULTS

***Sequence characteristics*—**The length of the complete unaligned fragments of the ingroup taxa varied from 579 to 655 base pairs for ITS, from 866 to 998 base pairs for trnLF, from 657 to 762 base pairs for atpB-rbcL. The full ITS dataset including 187 sequences of ingroup and outgroup taxa had 809 aligned characters, of which 92 (11.4%) involved alignment ambiguity and were excluded from phylogenetic analyses, 183 (22.6%) were constant, 422

(52.2%) were informative when considered with the criteria of parsimony. The full trnLF dataset including 184 sequences of ingroup and outgroup taxa had 1243 aligned characters, of which 652 (52.4 %) were constant, 355 (28.6 %) were informative, and in some species e.g. *I. cyathiflora*, *I. delavayi* and *I. barbata*, there were big deletion among the sequences. The full atpB-rbcL dataset including 187 sequences of ingroup and outgroup taxa had 961 aligned characters, of which 120 (12.5%) involved alignment ambiguity and were excluded from phylogenetic analyses, 457 (47.6 %) were constant, 230 (23.9%) were informative. The sequence showed higher divergence in ITS (from 0% - 38.7%) than in trnLF (from 0% - 10.5%) and in atpB-rbcL (from 0% - 13.6%). Combined data matrix covered 184 taxa (including the 2 outgroup species) and 3019 characters, of which 212 (7%) were excluded from analyses, 1309 (43.4%) were constant, 1000 (33.1 %) were informative.

**Table 4 Sequences characteristics of the ITS, trnLF and atpB-rbcL, separately and combined for 182 Balsaminaceae and 5 outgroups used in this study, got from parsimony methods.**

| **Nucleotide sites** | **ITS** | **trnL-trnF** | **atpB-rbcL** | **Combined data** |
|---|---|---|---|---|
| No. total aligned positions | 809 (92 exclude, 11.4%) | 1243 | 961(120 exclude, 12.5%) | 3019 (212 exclude, 7%) |
| No. constant | 183 (22.6%) | 652 (52.4%) | 457 (47.6 %) | 1309 (43.4 %) |
| No. autaponorphic | 112 (13.8%) | 236 (19.0%) | 154 (16.0%) | 498 (16.5%) |
| No. parsimony informative | 422 (52.2%) | 355 (28.6%) | 230 (23.9 %) | 1000 (33.1 %) |
| Longest sequences | *I. kerriae* Craib | *I. bicornuta* Wall. | *I. scabrida* DC & *I. edgeworthii* Hook.f. | |
| Shortest sequences | *I. chinensis* L. | *I. cyathiflora* Hook. f. | *I. ecalcarata* Collett & Hemsl. | |

***Separate ITS, trnLF, and atpB-rbcL phylogeny*—**NJ analysis on the ITS dataset including 182 ingroup and 5 outgroup taxa was conducted using the Kimura-2-parameter method. The same method used with trnLF dataet included 182 ingroup and 2 ourgtoup, and with atpB-rbcL dataset included 182 ingroup and 5 outgroup. The topologies of the three separate analyses are similar with each other (results not shown). ITS NJ tree is moderately resolved, the resolution among the main lineages is poor or receives less significant support, but some lineages of closely related taxa are better resolved with strong support. trnLF NJ tree is moderately resolved as well, like ITS NJ tree, poor resolution among the main lineages and better resolution among small lineages. AtpB-rbcL NJ tree is generally poorly resolved except three basal split and some small lineage were well supported. The three NJ trees based on separate genes confirmed the monophyly of Balsaminaceae and *Impatiens* with high bootstrap support (BS) 100%. In all of the three topologies, three main groups were retained and each contains almost the same species. However, several clades changed their position in different NJ trees.

***Assessment of congruence*—**The partition homogeneity test (PHT) was performed on a combined data set of 184 sequences (with constant and ambiguous characters excluded), indicate incongruence between the ITS, trnLF and atpB-rbcL data sets (p=0.01). The ILD test has been criticized by several authors (e.g. Dolphin et al., 2000; Yoder et al., 2001; Darlu & Lecoinre, 2002). It has been suggested that PHT p values greater than p=0.01 reflect congruent data sets that, if combined, will either improve or will not negatively affect phylogenetic accuracy (Cunningham et al., 1998). Even in some cases, tests of detecting highly significant incongruence ($p < 0.001$) through combining the incongruent partitions actually increased phylogenetic accuracy (Templeton, 1983; Rodrigo, 1993). Nevertheless, a

comparison of the cladograms obtained for each separate partition suggests a general congruence in topologies and branch supports. Therefore, all data sets were combined and were subjected to a combine analysis.

***Combined ITS, trnLF, and atpB-rbcL phylogeny*—**Fig. 1 is the NJ tree using Kimura-2-parameter based on combined data sets. It is in general congruent with the NJ trees based on individual gene. For Bayesian analysis, the model GTR + I + G was selected for partitions ITS, trnLF and atpB-rbcL respectively by Modeltest 2.2. Fig. 2 is the 50% majority-rule consensus tree using Bayesian method with the combined data sets. Based on this tree, numbers (A through V) were assigned to the resolved lineages of *Impatiens*. In addition, a group of closely related non-monophyletic taxa were also referred as a clade, e.g. "clade F", for convenience of illustration and discussion. Combined Bayesian topology is almost identified with combined NJ tree except the position of clade D and E which are close to group II in Bayesian tree but close to group I and III respectively in NJ tree. Bayesian analysis of the combined data matrix resulted in greater resolution than the Neighbor-joining analysis of the combined data. Most of the lineages are well resolved with Bayesian posterior probabilities (BPP) of 0.95 or higher. The consensus tree resulting from Bayesian analysis of the combined dataset is considered as the preferred topology and used for discussions unless mentioned otherwise.

Both Bayesian analysis and NJ analysis highly support the monophyly of the family and the sister relationship between *Hydrocera* and *Impatiens* with very high support value (BPP: 1.0 /BS: 100%). Within *Impatiens*, the most basal clade A, including *I. omeiana* (southwest Chinese species), *I. clavigera* (Vietnam species), and *I. sp200416* (a new species from Mt. QingCheng in southwest china), splits from the others with a strong support (BPP: 1.0). The remaining representatives of *Impatiens* are divided into three large groups in the same way as in separate ITS, trnLF and atpB-rbcL NJ tree.

Group I (clade C) staying in the basal position of the tree, was well supported (BPP: 1.0). It comprises all the Himalayan and Tibet species (except *I. puberula*), some Southwest Chinese species, one European species *I. parviflora*, and one central Asian species *I. brachycentra*. *Impatiens parviflora* and *I. brachycentra* are closely grouped together (BPP: 1.0), then grouped with three Himalayan species (BPP: 1.0), these species showed affinity to other Himalayan and Southeast Asian species in clade C. *Impatiens gongshanensis* (southwest Chinese species) split from clade B (*I. kerriae* and *I. psittacina*, both from Thailand and *I. stenosepala*, from southwest China) is close to Group I. Clade B is close to Group I and *I. gongshanensis.*

Group II (F, G) staying in the middle position of the tree, was well supported (BPP: 1.0). It comprises five North American species, one boreal species (*I. nolitangere*) and some southwest Chinese species. The North American species were grouped with the boreal species *I. nolitangere* (BPP: 1.0), then grouped with another seven Chinese species to form clade G (BPP: 1.0). Clade G showed the affinity to the other southwest Chinese species which formed clade F in Gourp II. It is worth noting that clade E including five East African species and clade D including one Southwest Asian species *I. arguta* and two Himalayan species *I. lingyhiensis* and *I. namchabarwensis* are close to group II, but do not belong to group II on Bayesian tree. But in combined NJ tree, clade E is close to Group III, but no significant bootstrap support; clade D is in the basal position of the whole combined NJ tree with high support (BS: 95%).

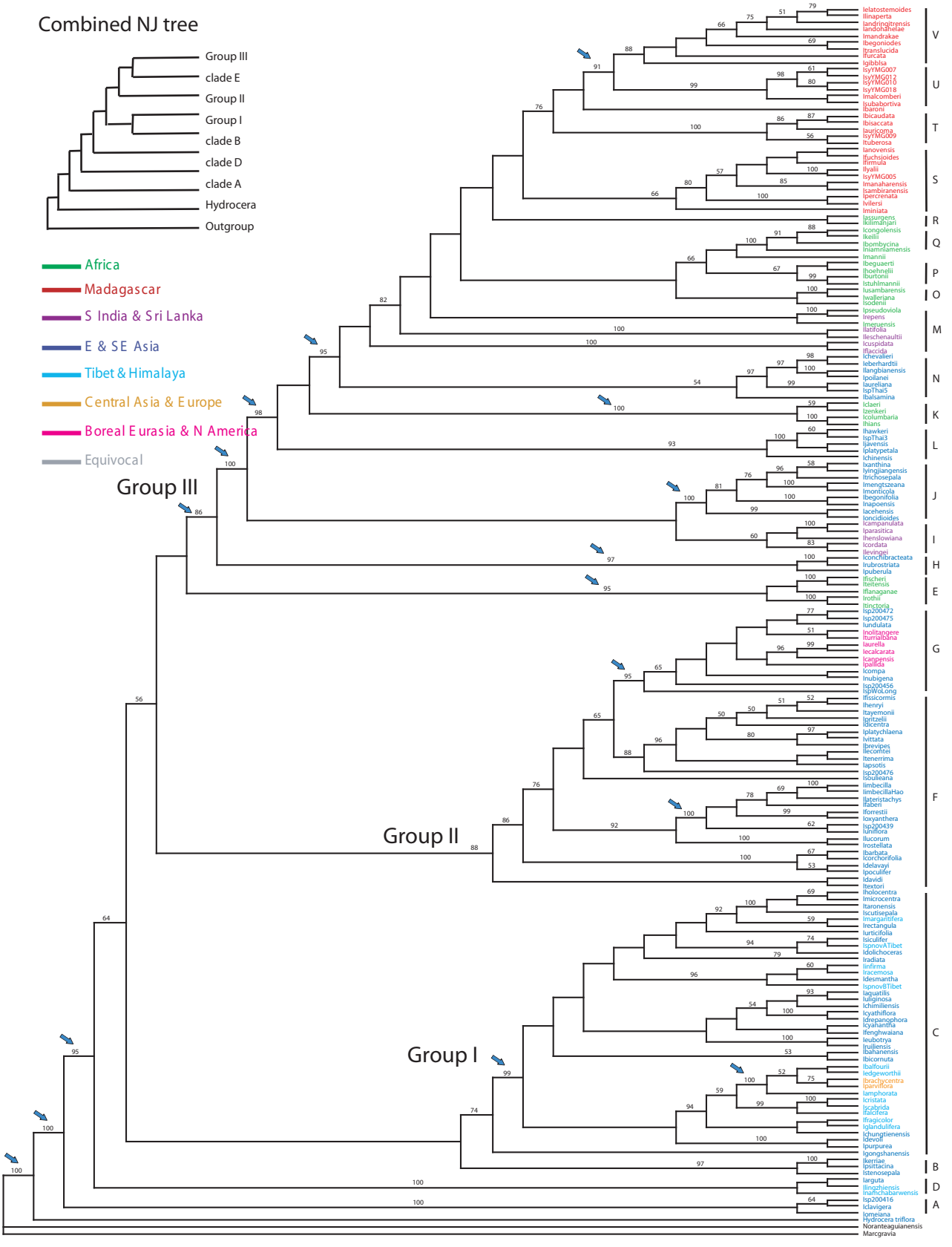
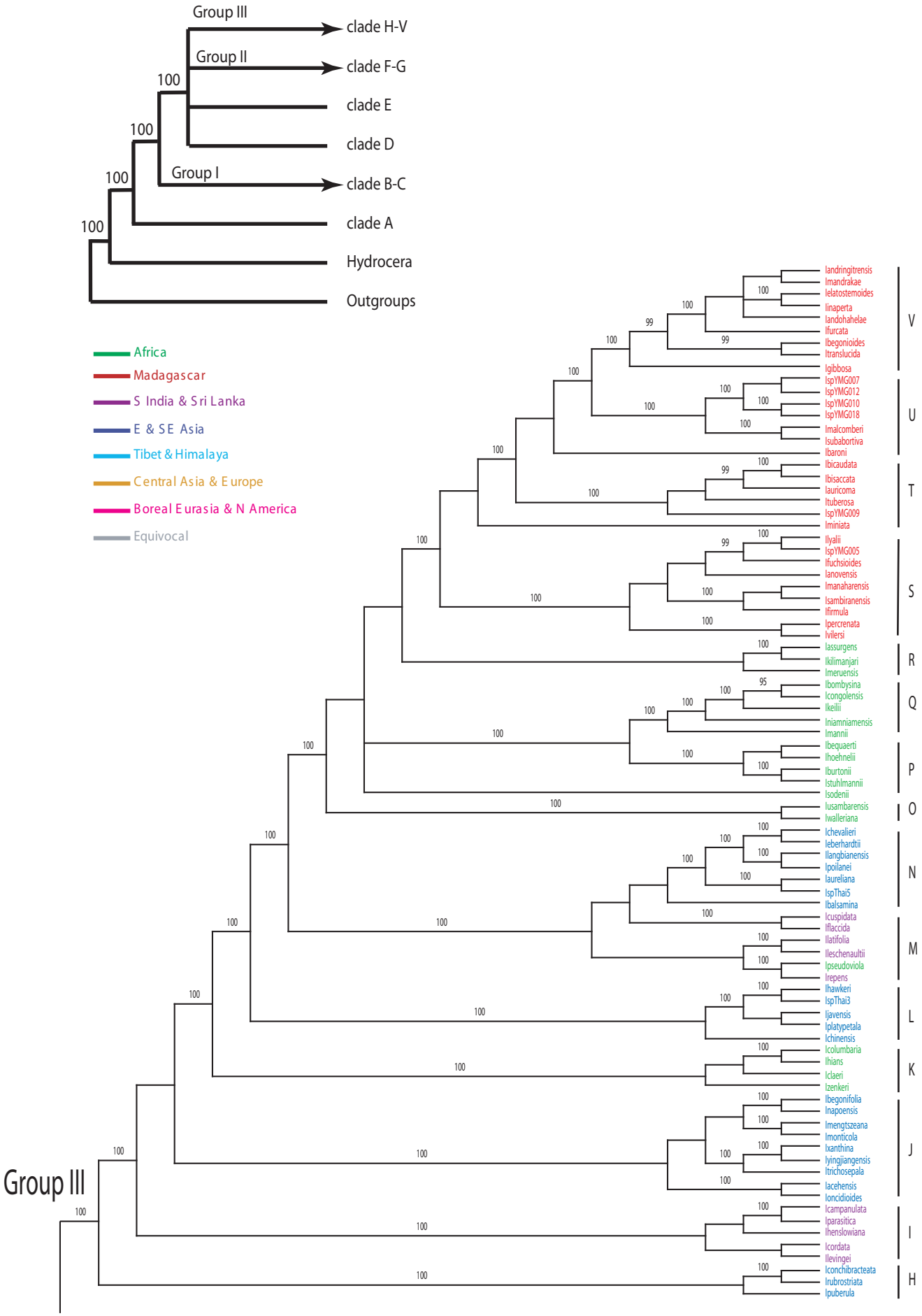



Fig. 1 The phylogram of the Neighbor-joining tree based on Kimura-2-parameter of the combined ITS, trnLF, and atpB-rbcL (including 182 ingroup and 2 outgroup). Numbers above the branches are bootstrap values supporting the corresponding branch when greater than 50%. Species name with different color indicate different sampling places: Red-Madagascar; Green-Africa; Violet-South India & Sri Lanka; Blue-East & Southeast Asia; Light blue-Tibet & Himalaya; Yellow-Central Asia & Europe; Pink-Boreal Eurasia & N. America; Grey-Equivocal (same color indicate for Fig. 2-3).


Group III
clade H-V
Group II
clade F-G
clade E
clade D
Group I
clade B-C
clade A
Hydrocera
Outgroups
Africa
Madagascar
S India & Sri Lanka
E & SE Asia
Tibet & Himalaya
Central Asia & Europe
Boreal Eurasia & N America
Equivocal
landringitrensis
lmandrakae
lelatostemoides
linaperta
landohahelae
lfurcata
lbegonioides
ltranslucida
lgibbosa
lspYMG007
lspYMG012
lspYMG010
lspYMG018
lmalcomberi
lsubabortiva
lbaroni
lbicaudata
lbisaccata
lauricoma
ltuberosa
lspYMG009
lminiata
llyalii
lspYMG005
lfuchsioides
lanovensis
lmanaharensis
lsambiranensis
lfirmula
lpercrenata
lvilersi
lassurgens
lkilimanjari
lmeruensis
lbombysina
lcongolensis
lkeilii
lniamniamensis
lmannii
lbequaerti
lhoehnelii
lburtonii
lstuhlmannii
lsodenii
lusambarensis
lwalleriana
lchevalieri
leberhardtii
llangbianensis
lpoilanei
laureliana
lspThai5
lbalsamina
lcuspidata
lflaccida
llatifolia
lleschenaultii
lpseudoviola
lrepens
lhawkeri
lspThai3
ljavensis
lplatypetala
lchinensis
lcolumbaria
lhians
lclaeri
lzenkeri
lbegonifolia
lnapoensis
lmengtszeana
lmonticola
lxanthina
lyingjiangensis
ltrichosepala
lacehensis
loncidioides
lcampanulata
lparasitica
lhenslowiana
lcordata
llevingei
lconchibracteata
lrubrostriata
lpuberula
V
U
T
S
R
Q
P
O
N
M
L
K
J
I
H
Group III

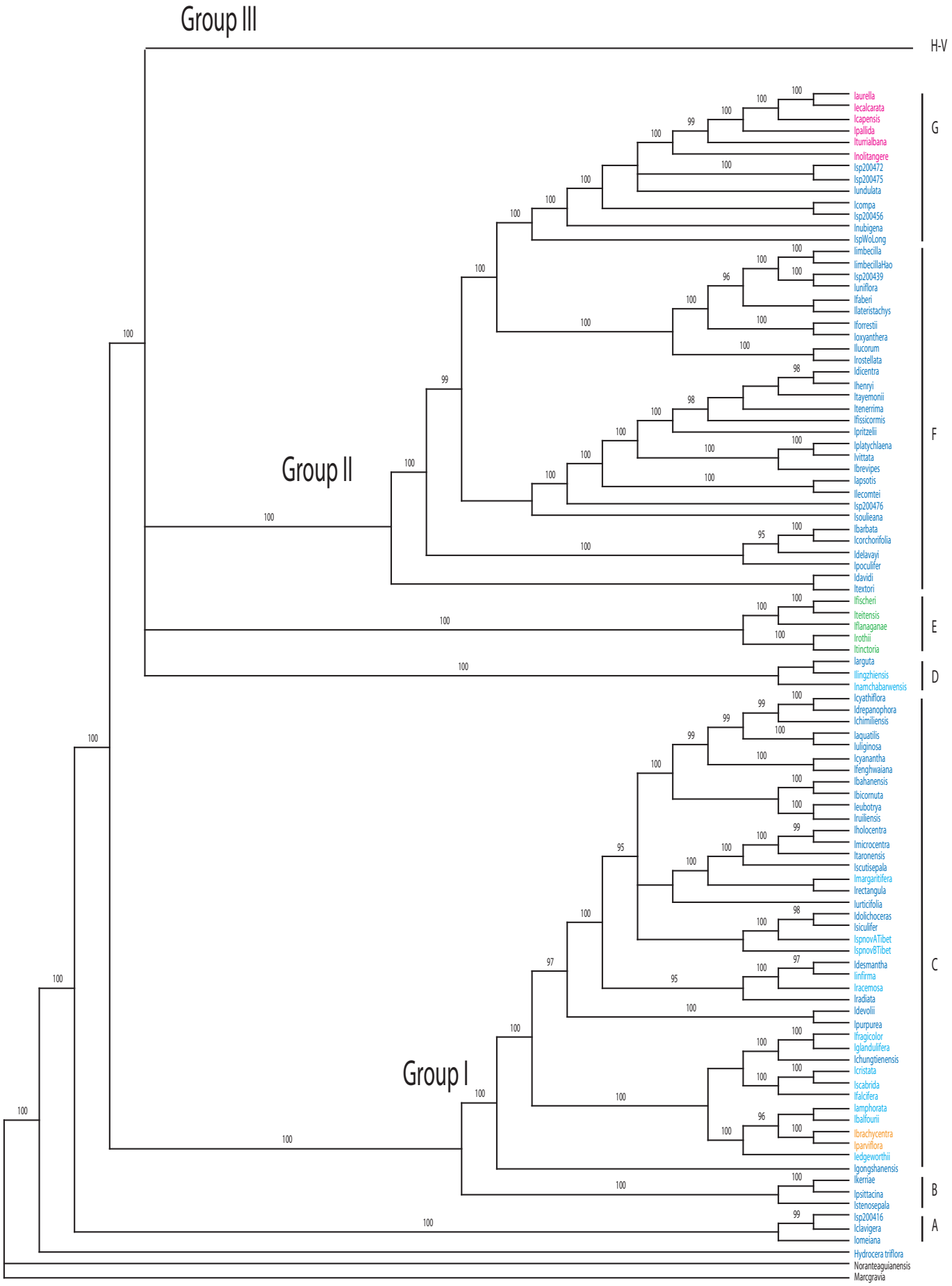



Fig. 2 Bayesian consensus cladogram of the combined ITS, trnLF, and atpB-rbcL (including 182 ingroup and 2 outgroup). Numbers above the branches indicate posterior probabilities higher than 95%.

Group III (clades H-V) staying in the terminal position of the tree, was well supported (BPP: 1.0). This group is a heterogeneous collection of Madagascan, African, southern Indian and Southeast Asian species. Almost every well-supported lineage in this group is strongly correlated with a specific area of distribution. Madagascan species were all grouped together (clade S, T, U, V, BPP: 1.0), appearing the most terminal position of the whole tree. The spurless Madagascan endemic taxa (clade U, V) were resolved as being monophyletic with a high support (BPP: 1.0). African species are not monophyletic. But African species in this group were clustered together and close to Madagascan species except that clade K (four West African species) was nested out and grouped with Southeast Asian species. One African species *I. pseudoviola* grouped with Indian species in clade M. South Indian species occurred in two lineages (clade I, M) which show no immediate affinity to each other, but showed close connections with Southeast Asian species, e.g. clade M was grouped with clade N (Southeast Asian species) with high support (BPP:1.0). Other Southeast Asian speceis in clade H, J, L were well supported respectively (BPP:1.0) and fell within this group.

## DISCUSSION

***Similarity and congruence of different phylogeny—***The NJ tree based on individual and combined datasets are mainly congruent with each other and with the Bayesian tree based on combined datasets. They are similar in topology: all phylogenies have the same three large groups and every group contains almost the same species. But they have different position of several clades and slightly changes inside each group. Only Bayesian tree is well supported in most of lineages. In fact, even in the same well supported lineage, e.g. in combined NJ tree and combined Bayesian tree, bootstrap values did not always correlate well with the posterior probabilities, and were often considerably lower. This irregularity was noted by Huelsenbeck et al. (2002) and has been identified in a number of other studies utilizing these two analytical methods (e.g. Soltis et al., 2002; Simmons et al., 2003; Erixon et al., 2003).

The present phylogenies based on three genes with NJ and Bayesian method have no conflicts with the previous study of phylogeny of Balsaminnaceae based on 112 ITS sequences (Yuan. et al., 2004), and with the study based on 86 chloroplast atpB-rbcL spacer sequences (Janssens et al. 2006), but still several clades appeared in the different position of the tree. For example, in the present Bayesian combined tree, all the Madagascar species were clustered together and appearing in the terminal position of the whole tree, but on the ITS tree (Yuan et al., 2004), some African species were nested into the Madagascar species, then showed in the terminal position of the tree, and on the atpB-rbcL tree (Janssens et al., 2006), three Madagascan species were nested among African species and didn't show in the terminal position of the tree.

The minor dissimilarities among the phylogenetic tree of Balsaminaceae can be explained in two ways: 1). Nuclear ribosomal DNA and chloroplast DNA provide different amount of information and noise. The non-coding fast-evolving ITS sequences are more variable and more homoplasious than the chloroplast markers (Dolphin et al., 2000; Barker & Lutzoni, 2002; Dowton & Austin, 2002), and contain phylogenetic information adequate for addressing relationships within more terminal parts of the trees, while plastid data brought resolutions for deeper level branches. Some molecular data of ITS was affected by long-branch attraction and showed the wrong combining of the species from different ancestral (Hendy & Penny, 1989; Bergsten, 2005). It is suggested that multiple independent DNA fragments can yield the gene trees that represent the true phylogeny relationship (Miyamoto & Fitch, 1995; Sang, 2002). Thus, the present combined tree can improve the phylogeny information. 2). Different

tree reconstruction methods might have different accuracy. The accuracy of Neighbor-joining method is influenced by the number of taxa, sample size, sequence differences etc. It is showed that increasing taxon number correlates with a slight decrease in phylogenetic study (Rokas & Carroll, 2005). Bayesian method is a practical alternative to the other methods, because it has advantages over the other methods in terms of ability to use complex models of evolution, different models for separate partition, ease of interpretation of the results, and computational efficiency (Huelsenbeck, 2002; Nylander et al., 2004). Thus, this method can produce more accurate phylogeny.

***Phylogenetic relationships within Balsaminaceae—***Some recent molecular analyses indicated that Balsaminaceae belong to the order Ericales and are part of basal clade of it (Morton et al., 1996; Savolainen et al., 2000; Soltis et al., 2000; Alhach et al., 2001; Anderberg et al., 2002; Bremer et al., 2002; APG, 1998; Geuten et al., 2004). Yuan et al. (2004) and Jassens et al. (2006) confirmed the monophyly of Balsaminaceae with ITS phylogeny and atpB-rbL phylogeny respectively. Our present study strongly confirmed this conclusion once again. (BS: 100% in Fig. 1/BPP:1.0 in Fig. 2).

The monotypic *Hydrocera* is regarded as a distinct genus in Balsaminaceae because of its unique floral structure and indehiscent fruit (Grey-Wilson, 1980c, 1989). Yuan et al. (2004) recognized *Hydrocera* as a distinct genus sister to *Impatiens*, but the relationship between two genera in the strict consensus ITS trees is not so clear. Jassens et al. (2006) illustrate that *Hydrocera* is sister to *Impatiens* with high support. In present study, all the phylogeny based on separate gene or combined datasets showed strong support (BS:100% in Fig. 1/BPP:1.0 in Fig. 2) of this sister relationship between these two genera.

***Phylogenetic relationships within Impatiens, a biogeographical interpretation—***The present molecular phylogeny showed that many of the clades are associated with a specific area of distribution but it is very difficult to find morphological synapomorphies for the different clades revealed by molecular phylogeny. Different type of flower can appear in the same clade or the same type of flowers can appear in the different clades. But it is interesting to compare the available biogeography data with the molecular phylogeny.

Biogeographical optimization onto the combined Bayesian phylogeny illustrates the region of origin and dispersal route in this family. It revealed that mainland Southeast Asia is the ancestral area of extant *Impatiens*. The boreal species *I. nolitangere* and North American species (clade G) are suggested as having dispersed from mainland Southeast Asia. The central Asian species *I. brachycentra* and European species *I. parviflora* may have been dispersed from mainland Southeast Asia through the Himalayas. Africa seems to have been colonized at least twice by ancestors from mainland Southeast Asia: once led to the clade E (East African species, possessing long-linear fruits, flower with five sepals, large seeds with reticulate seedcoat) through a dispersal directly from mainland Southeast Asia, and at another one by a colonizer with broad fusiform fruits that gave rise to the others (Clade K, O-R) through a dispersal from mainland Southeast Asia probably via India subcontinent. However, it is to be further confirmed if the colonization of Africa might have been achieved via India as stepping-stones, as our present sampling of India species is still insufficient. Nevertheless, as far as the species sampled from South India and Sri Lanka are concerned, some species (clade I) showed Southeast Asian connection and some (clade M) showed African connections. Thus, it is suggested that south India was colonized at least twice, once by a mainland Southeast Aisa ancestor (clade I), and another time most likely by an ancestor with

African affinities (clade M). Madagascan *Impatiens* shows clearly an African origin and the colonization event might be only once.

Several hypothesis of original center of Balsaminaceae were suggested by different authors: Jones and Smith (1966) and Khoshoo (1966) suggested that *Impatiens* originated in the Himalayan region. Their hypothesis was mainly based on species diversity and karyological data. Bhaskar (1981) thought that south India contains most of the primitive or phylogenetically old species and Western Ghats is the places of origin of *Impatiens* considering the concentration of diploids, primitive radial pollen grains and shrubby habit. Grey-Wilson (1980b) formulated a hypothesis suggesting that Balsaminaceae originated in West Gondwana and spread to Southeast Asia through Madagascar and India. From Southeast Asia and the adjacent Sino-Himalayan area, *Impatiens* diversified secondarily into two lineages: one lineage radiating to the temperate Eurasian areas and North America, and another radiating to the tropical and subtropical areas in Southeast Asian islands. Meanwhile, he rejected the possibility of an overland migration between Africa and India.

Our biogeographical interpretation is mainly in concordance with the previous conclusion indicated by molecular phylogeny. ITS phylogeny (Yuan et al., 2004) revealed that Southeast Asia is the original place and from where dispersals to boreal Eurasia and North America, to central Asia and eastern Europe via the Himalayas, and to India and Africa have occurred, and atpB-rbcL phylogeny (Jassens et al., 2006) reveals that South China is the original place and the similar dispersal route to the other regions like ITS phylogeny. Our results based on the combined Bayesian tree with more taxa included reveal that mainland Southeast Asia is the original place and similar dispersal route as the other two conclusion. But our conclusion is in contrary with Grey-Wilson(1980b)'s suggestion of dispersal from an assumed West gondwanan (African) origin and spread to Southeast Asia and the adjacent Sino-Himalayan area.

Bayesian inference was well applied to the biogeography and the identification of past dispersal patterns (Nepokroeff et al., 2003; Sanmartin & Ronquist, 2004; Waters & Roy, 2004). Extensive dispersal patterns across the paleotropical regions were also well documented in other families such as Melastomataceae. The study on the historical biogeography of this family revealed that it initially diversified in the tropical forest north of the Tethys sea, from where it spread throughout Eurasia and to North America. They also entered South America, and from where one clade (*Melastomeae*) reached Africa by long distance dispersal. From Africa, this clade further spread to Madagascar, India, and Indochina (Renner et al., 2001). *Exacum* (Gentianaceae) represents another example (Yuan et al., 2005). The ancestral area reconstruction suggests that *Exacum* originated in Madagascar. One clade colonized Sri Lanka and southern India via long-distance dispersals, from where it dispersed extensively to Southeast Asia. These examples showed that similar geographical distribution patterns in the paleotropical regions may have very different historical origins. The present molecular study revealed Balsaminaceae are of mainland Southeast Asia origin, from where extensive overland dispersals to boreal Eurasia and North America, to central Asia and eastern Europe via the Himalayas, and to India and Africa via the Himalayas have happened. The historical biogeography of Balsaminaceae shows additional patterns and thus can contribute to a better comprehension of the historical biogeography across the paleotropical regions.

***The implication of the molecular phylogeny for character evolution—***It is proved that Bayesian statistical methods enable the study of character evolution (Ronquist, 2004). In

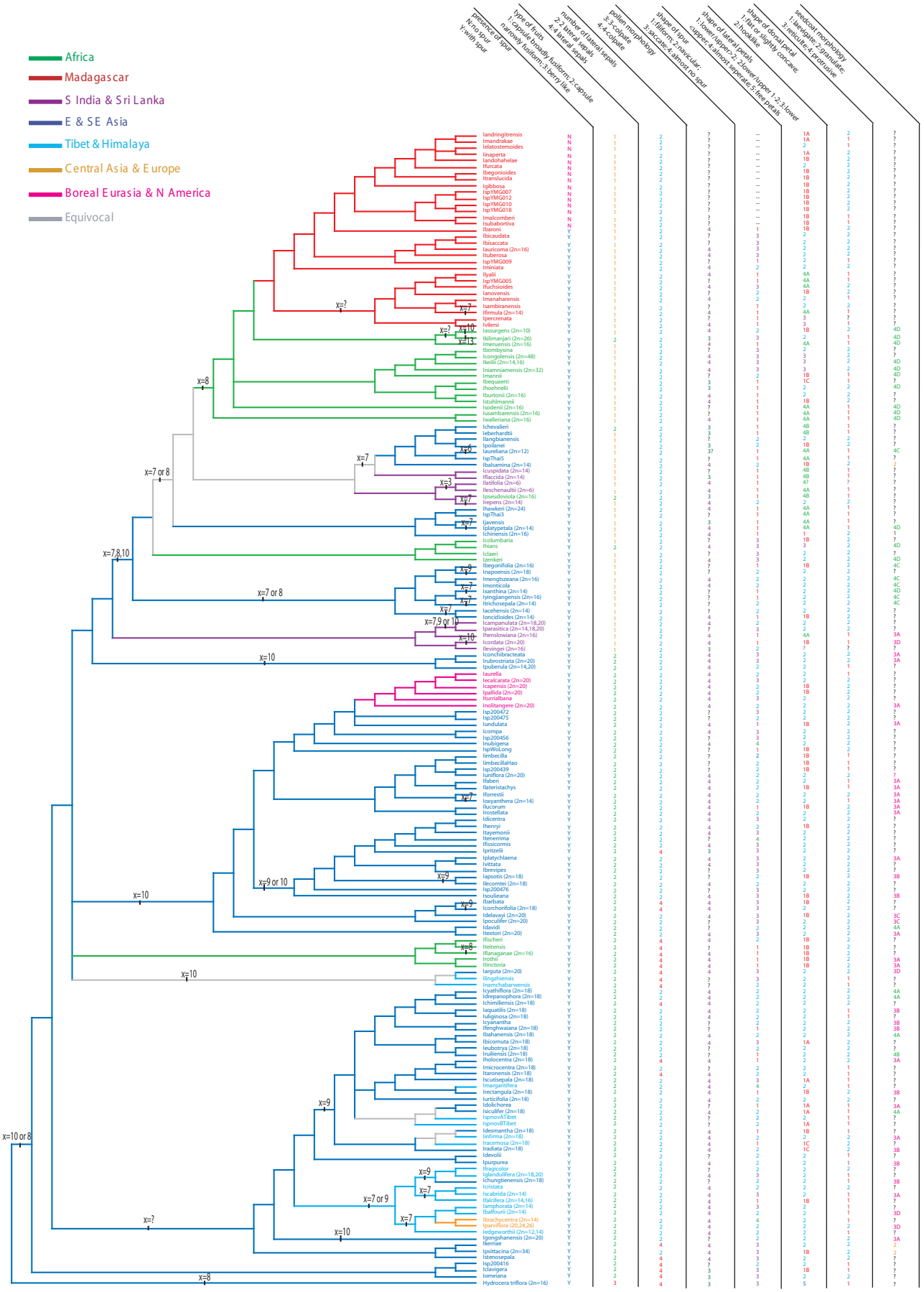



Fig. 3 Parsimonious optimization of the distribution centers of endemism, basic chromosomal numbers, and selected morphological characters onto the Bayesian combined tree. Vertical bars indicate positions of character state changes. Chromosome numbers when available are shown after the species names.

order to better understand the evolution of karyology, floral morphology and other morphology of Balsaminaceae, basic chromosomal number, five morphological features were optimized on molecular tree and the other flower morphological characters (shape and form of the spur, lateral petals and dorsal petals) were integrated with molecular phylogeny.

Several hypotheses about the basic chromosome number and evolution trends in Balsaminaceae were proposed by different authors: Khoshoo (1957) assumed that x=7 or 10 were basic chromosome numbers in *Impatiens*; Jones and Smith (1966) and Akiyama et al. (1992b) suggested x=7 to be the ancestral type, from which the other numbers were derived mainly by ascending dysploidy; whereas, Rao et al. (1986) suggested x=8 as the ancestral number and the evolution of x=7, 9 and 10 is from x=8 through both descending and ascending dysploidy. Song et al. (2003) proposed that x=8, 9 or 10 are all possible to be the ancestral basic chromosomal number only based karyological study. Yuan et al. (2004) revealed x=10 to be the most likely ancestral base number in *Impatiens* , with other base numbers being derived mainly through descending dysploidy after tracing this character onto ITS phylogeny. Jassens et al. (2006) pointed out that x=10 might indeed to ancestral, yet the possibility of x=9 should not be ruled out after tracing this character onto atpB-rbcL phylogeny. Tracing all the available chromosomal number onto the molecular phylogeny, it suggested both x=8 or 10 are the most likely ancestral base number in *Impatiens* (Fig. 3). The evolution of chromosome basic number in *Impatiens* might be: from x=10 derived to the other numbers mainly by descending dysploidy or from x=8 derived to the other numbers by both descending and ascending dysploidy. Since some important chromosomal information of the species on the molecular tree is still missing, e.g. the most basal clade A (*I. omeiana*, *I. clavigera* and *I. sp200416*), Madagascan species, and the chromosome number of many other species is still not available (only 86 of 182 species of Balsaminaceae were known in this study), it is still impossible to say which number is the base ancestral number in *Impatiens* for sure.

Fruit shape is one of the most important taxonomic characters in Balsaminaceae. *Hydrocera* has unique indehiscent berry-like fruits, while *Impatiens* has two types of dehiscent capsules: narrowly fusiform (length/diameter >5) and broadly fusiform (length/diameter ≤ 4). Tracing this character on the molecular phylogeny (Fig. 3), it is showed that narrowly fusiform fruit is plesiomorphic and the broadly fusiform fruit are derived from the linear type, though there are several reversals. This conclusion is congruent with Yuan et al. (2004) and Jassens et al. (2006). Considering the distribution area related with the fruits shape, the present results roughly support Grey-Wilson's (1980b) division about North group with long-linear fruits and South group with broadly fusiform fruits. He mentioned that there are overlaps between these two groups, in the molecular tree, it is seen that southwest Chinese *Impatiens* has both of the two type fruits.

*Hydrocera* and most of *Impatiens* have one lower sepal which developed into a nectary-tipped spur. But there are some species endemic to Madagascar that have no spur at all and they were called '*Trimorphopetalum*' group (Perrier de la Bathie, 1934). This group takes almost half of the species of the Madagascar *Impatiens* and showed a big diversification. On the molecular phylogeny, 15 spurless species were grouped together (clade U, V) with strong support (BPP: 1.0) (Fig. 2), and stay in the most advanced position of phylogeny. It suggested this is the derived character in *Impatiens* flower. The evolutionary trend is from spurred flower to spurless flower (Fig. 3). The study of the comparison of spurred flower and nonspurred flower in *Aquilegia* was made to reveal that at what developmental stage the morphology of spurred versus nonspurred taxa diverge (Hoddges, 1997). In Balsaminaceae,

the occurrence of spurless *Impatiens* is still a mystery, why they are the derived flower type and how they adapt to the habitat in Madagascar? The future study about floral ontogeny of *Impatiens* could ascertain the origin and development of spurless *Impatiens* and might give some insight to the questions.

Number of lateral sepals is the key character for the classification of Balsaminaceae (e.g. Perrier de la Bathie, 1949; Grey-Wilson, 1980b; Akiyama et al., 1992a,b, 1995; Akiyama & Ohba, 2000; Fischer & Rahelivololona, 2002, 2003, 2004). *Hydrocera* has 1 lower sepal and 4 free lateral sepals. Most *Impatiens* have 1 lower sepal and 2 lateral sepals, but some *Impatiens* have 1 lower sepal and 4 lateral sepals which form two lateral pairs in the flowers that exists in different diversity centers. Grey-Wilson (1980b) suggested an important trend of floral evolution in *Impatiens* is the loss of upper pair of lateral sepals resulting the evolution of a flower type from 5 sepals to 3 sepals. Though species possessing 4 lateral sepals are minority in this genus, but still present a plesiomorphic state in *Impatiens.* Among 182 Balsaminaceae species used for the present molecular study, 19 species have 4 lateral sepals in mature flowers, 12 of them are Southeast Asian species (clade A ,B, C, D, F), 2 of them are Himalayan species (clade D) and 5 of them are East African species (clade E). After tracing this character onto the molecular phylogeny, it is confirmed that five sepals is plesiomorphic in *Impatiens*, the evolutionary trend is from 4 lateral sepals flower to 2 lateral sepals flower. This important floral morphology character is parallel evolution from different distribution centre (Fig. 3).

Pollen characters are useful for the recognition of broad evolutionary trends in *Impatiens* (Grey-Wilson 1980a; Janssens et al. 2005). *Hydrocera triflora* was found to have tricolpate pollen grains with a triangular, oblate shape and elongated colpi and was thought to be plesiomorphic pollen type in Balsaminaceae (Lu 1991; Janssens et al., 2005). Lu (1991) reported *I. omeiana* and *I. wilsoni* from Mount Omei in southwest China and confirmed *I. clavigera* (southeast Chinese species) have the same pollen type like *Hydrocera triflora*. She suggested that these Chinese species with 3-colpate pollen are very close to *Hydrocera triflora*. The present molecular phylogeny showed that *I. omeiana* and *I. clavigera* were grouped together and resolved as sister to the remaining species of *Impatiens*, they exist just between *Hydrocera* and other *Impatiens.* This confirmed the hypothesis of Lu (1991). The most common pollen type of *Impatiens* is 4-colpate type, but few 3-colpate type were reported in African, Indian, Southeast Asian species as well (Huynh, 1968; Lu 1991; Perveen et al., 2001; Jassens et al., 2005). From all the available pollen data of Balsaminaceae, there is information about 114 species (among 184 species used in present study) on pollen colpate type. Tracing this character on the molecular phylogeny (Fig. 3), it is concluded that the evolution trend is from triangular 3-colpate pollen to rectangular 4-colpate pollen in the lineage of *Impatiens*, but there are reverses in several lineages. This confirmed that 3-colpate pollen grains with a triangular, oblate shape and elongated colpi are the ancestral pollen type in Balsaminaceae. It supports the atpB-rbcL phylogeny (Jassens 2006).

Previous study of seedcoat micromorphology revealed that it has a high diversity among the *Impatiens* species (Shimizu, 1979; Grey-Wison, 1980b; Lu & Chen, 1991; Utami & Shimizu, 2005; Song et al., 2005). The seedcoat information of the species used in our study was chosen to be matched into the four types first (division see Table 3), then traced on the molecular phylogeny (Fig. 3). It showed that species with reticulate type are more common in *Impatiens* and they occurred relatively basal position on the molecular phylogenetic tree. This suggested that the reticulate type is comparatively ancestral primitive type. The species with complex sculptures, such as squamalate and cristate, occurred in the more apical branches of

the phylogenetic tree and they might represent more advanced type. There are reversals: *I. davidi*, *I. cyathiflora*, *I. drepanophora*, *I. bahanensis*, *I. ruiliensis*, *I. siculifer* which have protrusive seedcoat appearing the comparatively basal position on the phylogenetic tree. Two Thailand species *I. kerriae* and *I. psittacina* having granulate seedcoat (Utami & Shimizu, 2005), appeared the very basal position of the phylogeny tree (clade B). From the molecular phylogeny, it is indicated that the evolutionary trend is from reticulate type to protrusive type. But the information about the seedcoat micromorphology of this family is still insufficient (only 67 of 182 species known on the present molecular tree), more species need to be studied to confirm this conclusion.

The extremely high floral diversity of *Impatiens* makes it a good candidate to study the floral evolution. Its diversity is mainly embodied in the diversity of the shape and size of the spur and the two united lateral petals, but the correlations among the different floral parts have remained unclear. Lower sepals, lateral petals, dorsal petals were divided into different types (Table 3) and these characters were integrated with our molecular phylogeny (Fig. 3). It is found that there is no regular correlation between floral characters and the phylogeny of the species in the general sense. For example, *I. lyallii* (Madagarcar speices, in clade S), *I. sodenii* (African species, in clade P), *I. aureliana* (southwest Chinese species, in clade N), *I. javensis* (Indonesian species, in clade L), and *I. flacida* (south Indian species, in clade M), all have similar flower morphology with long filiform spur, almost separate lateral petals and flat dorsal petal, but they come from different regions and belong to different clades in the molecular tree (Fig. 2). Similarly, *I. auricoma* (Madagascan species, in clade T), *I. niamniamensis* (African species, in calde Q), *I. parasitica* (south Indian species, in clade I), *I. conchibracteata* (southwest Chinese species, in clade H), *I. ecalcarata* (North American species, in clade G), all have similar flower morphology with a saccate spur, but they come from different region and nested in different clades in the molecular tree (Fig. 2).

However, in some small aggregates of species, different floral characters are associated with localized endemism. In Madagascan group (clade S-V) and African group (clade O-R), it showed that the trend is from filiform spur to saccate spur or no spur. This confirms the hypothesis of Grey-Wilson (1980b) about the evolutionary processes of the lower sepals within African species.

The rich variation in the floral structure is linked to the pollinators of the species (Grey-Wilson, 1980b). Bees, bumble-bees, butterflies, moths, and hawkmoth were reported and observed as pollinators in *Impatiens* (Grey-Wilson, 1980b; Yuan & Boehler s. n.). Though a few pollinators were observed, it is still suggested, in Balsaminaceae, floral parts may well function in an integrated manner and pollinators mediated the evolution of the flower. Therefore, there must be conspicuous parallel and convergent evolution in floral morphology, i.e. floral morphology is not always a reliable indication of phylogenetic relationships.

Floral morphology evolution has been most intensively studied in group such as the Orchidaceae (Johnson et al., 1998; Aceto et al., 1999) and the Polemoniaceae (Grant & Grant, 1965). In the study of phylogeny and evolution of *Orchis* and allied genera based on ITS DNA variation (Aceto et al., 1999), the results indicate that floral morphology is highly flexible and current generic and infrageneric limits are artificial. Similar with *Impatiens*, various traits pertaining to floral morphology may be interpreted as a result of ecological convergence related to pollinator-mediated selection; such characters can undergo drastic modifications without correspondingly dramatic genetic changes. The study on floral characters, phylogeny and pollinator shifts of the twin spurred orchid genus *Satyrium*,

represents another similar example, which showed the floral characters reflected pollinator class more than the phylogeny of the orchid species (Van der Niet, 2006).

## LITERATURE CITED


ACETO, S., P. CAPUTO, S. COZZOLINO, L. GAUDIOM, AND A. MORETTI. 1999. Phylogeny and evolution of *Orchis* and allied genera based on ITS DNA variation: morphological gaps and molecular continuity. *Molecular Phylogenetic Evolution* 13: 67-76.

AKIYAMA, S., H. OHBA, AND M. SUZUKI. 1992a. Notes on east Himalayan species of *Impatiens*: 1. *Japanese Botany* 67: 187-193.

AKIYAMA, S., M. WAKABAYASHI, AND H. OHBA. 1992b. Chromosome evolution in Himalayan *Impatiens (*Balsaminaceae*). Botanical Journal of the Linnean Society* 109: 247-257.

AKIYAMA, S., H. OHBA, T. SUGAWARA, Y.-P. YANG, AND J. MURATA. 1995. Notes on *Impatiens* (Balsaminaceae) from southwestern Yunnan, China. *Journal of Japanese Botany* 70: 95-106.

ALBACH, D. C., P. S. SOLTIS, D. E. SOLTIS, AND R. G. OLMSTEAD. 2001. Phylogenetic analysis of asterids based on sequences of four genes. *Annals of the Missouri Botanical Garden* 88: 163–212.

ANDERBERG, A. A., C. RYDIN, AND M. KÄLLERSJÖ. 2002. Phylogenetic relationships in the order Ericales s.l.: analyses of molecular data from five genes from the plastid and mitochondrial genomes. *American Journal of Botany* 89: 677–687.

APG. 1998. An ordinal classification for the families of flowering plants. *Annals of the Missouri Botanical Garden* 85: 531–553.

BARKER, F. K., AND F. M. LUTZONI. 2002. The utility of the incongruence length difference test. *Systematic Biology* 51: 625-637.

BERGSTEN, J. 2005. A review of long-branch attraction. *Cladistics* 21: 163-193.

BHASKAR, V. 1981. The genus *Impatiens* L. in south India: endemism and affinities. *Indian Forester* 107: 368-376.

BREMER, B., K. BREMER, N. HEIDARI, P. ERIXON, R. G. OLMSTEAD, A. A. ANDERBERG, M. KÄLLERSJÖ, AND E. BARKHORDARIAN. 2002. Phylogenetics of asterids based on 3 coding and 3 non-coding chloroplast DNA markers and the utility of non-coding DNA at higher taxonomic levels. *Molecular Phylogenetic Evolution* 24: 274-301.

CHASE, M. W., AND H. H. HILLS. 1991. Silica-gel: An ideal material for field preservation of leaf samples for DNA studies. *Taxon* 40: 215-220.

CLAUSING, G., AND S. S. RENNER. 2001. Molecular phylogenetics of Melastomataceae and Memecylaceae: implications for character evolution. *American Journal Botany* 88: 486-498.

CUNNINGHAM, C. W. 1997. Can three incongruence tests predict when data should be combined? *Molecular Biology and Evolution* 14, 733-740.

CUNNINGHAM, C. W., K. E. OMLAND, AND T. H. OAKLEY. 1998. Reconstructing ancestral character states: a critical reappraisal. *Trends in Ecology and Evolution* 13: 361-366.

DARLU, P., AND G. LECOINTRE. 2002. When does the incongruence length difference test fail? *Molecular Biology and Evolution* 19:432-437.

DOLPHIN, K., R. BELSHAW, C. D. L. ORME, AND D. L. J. QUICKE. 2000. Noise and incongruence: interpreting results of the incongruence length difference test. *Molecular Phylogenetic Evolution* 17, 401–406.

DOWTON, M., AND A. D. AUSTIN. 2002. Increased congruence does not necessarily indicate increased phylogenetic accuracy--the behavior of the incongruence length difference test in mixed-model analyses. *Systematic Biology* 51: 19-31.

DOYLE J. J., AND J. L. DOYLE. 1987. A rapid DNA isolation method for small quantities of fresh tissues. *Phytochemical Bulletin* 19: 11–15.

ERIXON P., B. SVENNBLAD, T. BRITTON, AND B. OXELMAN. 2003. Reliability of Bayesian posterior probabilities and bootstrap frequencies in phylogenetics. *Systematic Biology* 52: 665-673.

FARRIS J. S., M. KÄLLERSJÖ, A. G. KLUGE, AND C. BULT. 1995. Testing significance of incongruence. *Cladistics* 10: 315-319.

FELSENSTEIN, J. 1985**.** Confidence limits on phylogenied: an approach using the bootstrap. *Evolution* 39: 783-791.

FISCHER, E. AND M. E. RAHELIVOLOLONA. 2002. New taxa of *Impatiens* (Balsaminaceae) from Madagascar. I. *Adansonia* 24: 271–294.

FISCHER, E., AND M. E. RAHELIVOLOLONA. 2004. New taxa of *Impatiens* (Balsaminaceae) from Madagascar. III. *Adansonia* 26:37–52.

FISCHER, E., S. WOHLHAUSER, AND M. E. RAHELIVOLOLONA. 2003. New taxa of *Impatiens* (Balsaminaceae) from Madagascar. II. A collection from Masoala Peninsula. *Adansonia* 25: 17–31.

FUJIHASHI, H., S. AKIYAMA, AND H. OHBA. 2002. Origin and relationships of the Sino-Himalayan *Impatiens* (Balsaminaceae) based on molecular phylogenetic analysis, chromosome numbers and gross morphology. *Journal of Japanese Botany* 77: 284-295.

GEUTEN, K., E. SMETS, P. SCHOLS, Y.-M. YUAN, S. JANSSENS, P. KÜPFER, AND N. PYCK. 2004. Conflicting phylogenies of balsaminoid families and the polytomy in Ericales: combining data in a Bayesian framework. *Molecular Phylogenetic Evolution* 31: 711–729.

GRANT, V., AND K. A. GRANT. 1965. Flower pollination in the phlox family. Columbia University Press, New York.

GREY-WILSON C. 1980a. *Impatiens* in Papuasia, Studies in Balsaminaceae: I. *Kew Bulletin* 34: 661–688.

GREY-WILSON C. 1980b. *Impatiens* of Africa. A. A. Balkema, Rotterdam.

GREY-WILSON C. 1980c. Notes on collecting *Impatiens* (Balsaminaceae). *Flower Malesiana Bulletin* 33: 3435–3436.

HENDY, M. D., AND D. PENNY. 1989. A framework for the quantitative study of evolutionary trees. *Systematic Zoology* 38: 297-309.

HODDGES, S. A. 1997. Floral nectar spurs and diversification. *Int. J. Plant Sci*. 158: 581-588.

HUELSENBECK, J. P. 2002. Potential applications and pitfalls of Bayesian inference of phylogeny. *Systematic Botany* 51: 673-688.

HUELSENBECK, J. P., AND J. P. BOLLBACK. 2001. Emperical and hierarchical Bayesian estimation of ancestral states. *Systematic Botany* 50: 351-366.

HUELSENBECK, J. P., J. J. BULL, AND C. W. CUNNINGHAM. 1996. Combining data in Phylogenetic analysis. *Tree* 11: 152-157.

HUELSENBECK, J. P., LARGET B., R. E. MILLER, AND F. RONQUIST. 2002. Potential Applications and Pitfalls of Bayesian Inference of Phylogeny. *Systematic Biology* 51: 673-688.

HUYNH, K.-L. 1968. Morphologie du pollen des Tropaeolacées et des Balsaminacées I and II. *Grana Palynologica* 8: 88-184 and 277-516.

JANSSENS, S., K. GEUTEN, Y.-M. YUAN, Y. SONG, P. KÜPFER, AND E. SMETS. 2006. Phylogenetics of *Impatiens* and *Hydrocera* (Balsaminaceae) using chloroplast atpB-rbcL spacer sequences. *Systematic Botany* 31: 171-180.

JANSSENS, S., F. LENS, S. DRESSLER, K. GEUTEN, E. SMETS, AND S. VINCKIER. 2005. Phynological variation in balsaminoid Ericales. II. Balsaminaceae, Tetrameristaceae, Pellicieraceae and general conclusions. *Annals of Botany* 96: 1061-1073.

JOHNSON, S. D., P. LINDER, AND K. E. STEINER. 1998. Phylogeny and radiation of pollination systems in *Disa* (Orchidaceae). *American Journal of Botany* 85: 402-411.

JONES, K., AND J. B. SMITH. 1966. The cytogeography of *Impatiens* L. (Balsaminaceae). *Kew Bulletin* 20: 63-72.

KHOSHOO, T. N. 1957. Cytology of some *Impatiens* species. *Caryologia* 10: 55-74.

KHOSHOO, T. N. 1966. Cytology of pollen with particular reference to *Impatiens* and Allieae. *Proceedings of the Indian National Science Academy part*. B, 63: 35-45.

KIMURA, M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* 16: 111-120.

KLUGE, A. G., AND J. S. FARRIS. 1969. Quantitative phyletics and the evolution of anurans. *Sysematic Zoology* 18: 1-32.

LU, Y.-Q. 1991. Pollen morphology of *Impatiens L.* (Balsaminaceae) an dits taxonomic implications. *Acta Phytotaxonomica Sinica* 29: 352-357.

LU, Y.-Q., AND Y-L. CHEN. 1991. Seed morphology of *Impatiens* L. (Balsaminaceae) and its taxonomic significance. *Acta Phytotaxonomica Sinica* 29: 252–257.

MADDISON, D. R., AND W. P. MADDISON. 2000. MacClade 4: analysis of phylogeny and character evolution, version 4.0. Sinauer Associates, Sunderland, Massachusetts, USA.

MANSION, G., AND L. STRUWE. 2004. Generic delimitation and phylogenetic relationships within the subtribe Chironiinae (Chironieae: Gentianaceae), with special reference to *Centaurium*: evidence from nrDNA and cpDNA sequences. *Molecular Phylogenetic Evolution* 32: 951-977.

MAU, B., M. A. NEWTON, AND B. LARGET. 1999. Bayesian phylogenetic inference via Markov chain Monte Carlo methods. *Biometric* 55: 1-12.

MIYAMOTO, M. M., AND M. W. FITCH. 1995. Testing species phylogenies and phlogenetic methods with congruence. *Systematic Botany* 44: 64 -76.

MORTON, C. M., M.W. CHASE, K.A. KRON, AND S. M. SWENSEN. 1996. A molecular evaluation of the monophyly of the order Ebenales based upon rbcL sequence data. *Systematic Botany* 21: 567-586.

NEPOKROEFF, M., K. J. SYTSMA, W. L. WAGNER, AND E. A. ZIMMER. 2003. Reconstructing ancestral patterns of colonization and dispersal in the Hawaiian understory tree genus *Psychotria* (Rubiaceae): a comparison of parsimony and likelihood approaches. *Systematic Botany* 52: 820-838.

NYLANDER, J. A. A. 2004. MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.

PERRIER DE LA BATHIE, H. 1934. Les *Impatiens* de Madagascar. *Archives de Botanique Mémoire N.1* 7: 1-124.

PERVEEN, A., AND M. QAISER. 2001. Pollen flora of Pakistan-XXVI. Balsamincaceae. *Turkish Journal of Botany* 25: 35-38.

RANNALA, B., AND Z.-H. YANG. 1996. Probability distribution of molecular evolutionary trees: A new method of phylogenetic inference. *Journal of Molecular Evolution* 43: 304-311.

RAO, R. V. S., K. R. AYYANGAR, AND R. SAMPATHKUMAR. 1986. On the karyological characteristics of some members of Balsaminaceae. *Cytologia* 51: 251--260.

RENNER, S. S., G. CLAUSING, AND K. MEYER. 2001. Historical biogeography of Melastomataceae: the roles of Tertiary migration and long-distance dispersal. *American Journal of Botany* 88: 1290-1300.

RODRIGO, A. G., M. KELLY-BORGES, P. R. BERGOQUIST, AND P. L. BERGQUIST. 1993. A randomisation test of the null hypothesis that two cladograms are sample estimates of a parametric phylogenetic tree. *New Zealand Journal of Botany* 31:257-268.

ROKAS, A., AND S. B. CARROLL. 2005. More genes or more taxa? the relative contribution of gene number and taxon number to phylogenetic accuracy. *Molecular Biology and Evolution* 22: 1337-1344.

RONQUIST, F. 2004. Bayesian inference of character evolution. *Trends in Ecology and Evolution* 19: 475-481.

RONQUIST, F., AND J. P. HUELSENBECK. 2003. Mr. Bayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572-1574.

SAITOU, N., AND M. NEI. 1987. The neighbor-joining methods: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* 4: 406–425.

SANG, T. 2002. Utility of low-copy nuclear gene sequences in plant phylogenetics. *Critical Reviews in Biochemistry and Molecular Biology* 37: 121-147.

SANMARTIN, I., AND RONQUIST, F. 2004. Southern hemisphere biogeography Inferred by event-based models: plant versus animal patterns. *Systematic Botany* 53: 216-243.

SAVOLAINEN, V., M. W. CHASE, S. B. HOOT, C. M. MORTON, D. E. SOLTIS, C. BAYER, M. F. FAY, A. Y. BRUJUN, S. SULLIVAN, AND Y. L. QUI. 2000. Phylogenetics of flowering plants based on combined analysis of plastid atpB and rbcL gene sequences. *Systematic Botany* 49: 306-362.

SHIMIZU, T. 1979. The lectures on the flora of Thailand. *Acta Phytotax. Geobot.* 30: 180-187.

SIMMONS, M. P., K. M. PICKETT, AND M. MIYA. 2003. How meaningful are Bayesian support values? *Molecular Biology and Evolution* 21: 188-199.

SOLTIS, D. E., P. S. SOLTIS, AND M. W. CHASE. 1999. Angiosperm phylogeny inferred from multiple genes as a tool for comparative biology. *Nature* 402: 402-404.

SONG, Y., Y.-M. YUAN, AND P. KÜPFER. 2003. Chromosomal evolution in Balsaminaceae with cytological observations on 45 species from Southeast Asia. *Caryologia* 56: 463-481.

SWOFFORD, D. L. 2000. PAUP* Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4. Sinauer Associates, Sunderland, Massachusetts.

TABERLET P., L. GIELLY, G. PANTOU AND J. BOUVER. 1991. Universal primers for amplification of three non-coding regions of plastid DNA. *Plant Mol. Biol.* 17: 1105-1105.

TEMPLETON, A. R. 1983. Phylogenetic inference from restriction endonuclease cleavage site maps with particular reference to the humans and apes. *Evolution* 37:221-244.

UTAMI, N., AND T. SHIMIZU. 2005. Seed morphology and classification of *Impatiens* (Balsaminaceae). *Blumea* 50: 447-456.

VAN DER NIET, T. 2006. Systematics and radiation patterns of *Satyrium* Sw. (Orchidaceae). Phd-thesis: 80. University of Zurich.

WATERS, J. M., AND M. S. ROY. 2004. Out of Africa: The train to Australasia. *Systematic Biology* 53: 18-24.

YODER, A. D., J. A. IRWIN, AND B. PAYSEUR. 2001. Failure of the ILD to determine data combinability for slow loris phylogeny. *Systematic Biology* 50: 408-424.

YUAN, Y.-M., Y. SONG, K. GEUTEN, E. RAHELIVOLOLONA, S. WOHLHAUSER, E. FISCHER, E. SMETS, AND P. KÜPFER. 2004. Phylogeny and biogeography of Balsaminaceae inferred from ITS sequence data. *Taxon* 53: 391–403.

YUAN, Y.-M., S. WOHLHAUSER, M. MÖLLER, J. KLACKENBERG, M.W. CALLMANDER, AND P. KÜPFER. 2005. Phylogeny and Biogeography of *Exacum* (Gentianaceae): A disjunctive distribution in the Indian ocean basin resulted from long distance dispersal and extensive radiation. *Systematic Biology* 54: 21-34.

Table1. Origin of plant material, voucher, and GeneBank accession number of the species sampled.

| **Taxon** | **Origin** | **Voucher** | **GenBank Accesion No.** | | | **Clade** |
|---|---|---|---|---|---|---|
| | | | ITS | trnL-F | atpB-rbcL | |
| *I. acehensis* C.Grey-Wilson | Sumatra, Indonesia | Favre AF1 (NEU) | AY348739 | | | Clade J |
| *I. amphorata* Edgew. | West Himalaya | B. de Retz 5566 (BR) | AY348740 | | | Clade C |
| *I. andohahelae* Eb. Fisch. & Rahelivololona | Andohahela, Madagascar | Rahelivololona, Druard, Jerome & Fiadana And6 (PBZT) | AY348741 | | | Clade V |
| *I. andringitrensis* H. Perrier | Ambatofitorahana, Madagascar | Rahelivololona Ambatofitorahana-2 (PBZT) | AY348742 | | | Clade V |
| *I. anovensis* H. Perrier | Ambanizana, Madagascar | Rahelivololona, Saola & Scenario Ambanizana-11 (PBZT) | AY348743 | | | Clade S |
| *I. aposotis* Hook. f. | Sichuan, China | Yuan CN2k2-159 (NEU) | AY348744 | | DQ147810 | Clade F |
| *I. aquatilis* Hook. f. | Yunnan, China | Yuan CN2k2-173 (NEU) | AY348745 | | DQ147811 | Clade C |
| *I. arguta* Hook. f. & Thoms. | Yunnan, China | Yuan CN2k-74 (NEU) | AY348746 | | DQ147812 | Clade D |
| *I. assurgens* Baker | Zambia, Afica | Dessein 707 | | | | Clade R |
| *I. aureliana* Hook. f. | Yunnan, China | Yuan CN2k1-56 (NEU) | AY348747 | | DQ147814 | Clade N |
| *I. aurella* Rydb. | Washington, USA | Zika 20125 | | | | Clade G |
| *I. auricoma* Baill. | Comores origin, cult. Bot. Gard. Koblenz Univ. | Fischer NE1 (NEU) | AY348748 | | DQ147815 | Clade T |
| *I. bahanensis* Hand.-Mazz. | Yunnan, China | Yuan CN2k-30 (NEU) | | | | Clade C |
| *I. balfourii* Hook.f. | West Himalaya | Ray Morgan s. n. (NEU) | | | | Clade C |
| *I. balsamina* L. | cult. Guanzhou, China | Yuan CN2k1-06 (NEU) | AY348749 | | DQ147816 | Clade N |
| *I. barbata* Comber | Yunnan, China | Yuan CN2k2-178 (NEU) | AY348750 | | DQ147818 | Clade F |
| *I. baroni* Baker | Ranomafana, Madagascar | Rahelivololona, Jacky & Remi RNM-4 (PBZT) | AY348751 | | | Clade T |
| *I. begoniifolia* S. Akiyama & H. Ohba | Yunnan, China | Yuan CN2k1-51 (NEU) | AY348752 | | DQ147819 | Clade J |
| *I. begonioides* Eb.Fisch. & Raheliv. | Vatoharanana Camp, Madagascar | Yuan & Ravokatra YMG56 (NEU) | | | | Clade V |
| *I. bequaerti* De Wild. | Congo origin, cult. Bot. Gard. Koblenz Univ. | Fischer NE2 (NEU) | AY348753 | | DQ147820 | Clade P |
| *I. bicaudata* H.Perrier | Cascade sacrie, Madagascar | Yuan & Ravokatra, YMG4 (NEU) | | | | Clade T |
| *I. bicornuta* Wall. | Yunnan, China | Yuan CN2k-55 (NEU) | AY348754 | | DQ147821 | Clade C |
| *I. bisaccata* Warb | Joffreville, Madagascar | Yuan & Ravokatra YMG3 (NEU) | | | | Clade T |
| *I. bombycina* W. Lobin | African origin, cult. Bot. Gard. | Fischer NE3 (NEU) | AY348755 | | | Clade Q |

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| & E. Fischer | Koblenz Univ. | | | | |
| *I. brachycentra* Kar. & Kir. | central Asia | Burtt 3834 (E) | AY348756 | | Clade C |
| *I. brevipes* Hook.f. | Sichun, China | Yuan 2004-37 (NEU) | | | Clade F |
| *I. burtonii* Hook. f. | Kenya origin, cult. Bot. Gard. Koblenz Univ. | Fischer NE4 (NEU) | AY348757 | | Clade P |
| *I. campanulata* Wight | South India origin, cult. by Ray Morgan, UK | Ray Morgan s. n. (NEU) | AY348758 | DQ147822 | Clade I |
| *I. capensis* Meerb. | Quebec, Canada | Küpfer s. n. (NEU) | AY348759 | DQ147823 | Clade G |
| *I. chevalieri* Tardieu | Dalat, Vietnam | Song & Phuoong, 2004-07 (NEU) | | | Clade N |
| *I. chimiliensis* Comber | Yunnan, China | Yuan CN2k-51 (NEU) | AY348760 | DQ147824 | Clade C |
| *I. chinensis* L. | Yunnan, China | Yuan CN2k1-49 (NEU) | AY348761 | DQ147825 | Clade L |
| *I. chungtienensis* Y. L. Chen | Yunnan, China | Yuan CN2k2-204 (NEU) | AY348762 | DQ147826 | Clade C |
| *I. claeri* N. Halle | Gabon origin, cult. Bot. Gard. Koblenz Univ | Fischer NE5 (NEU) | AY348763 | | Clade K |
| *I. clavigera* Hook. f. | Sapa, Vietnam | Favre AF23 (NEU) | | | Clade A |
| *I. columbaria* J. J. Bos | Gabon origin, cult. Bot. Gard. Koblenz Univ | Fischer NE6 (NEU) | AY348764 | DQ147828 | Clade K |
| *I. compa* Hook. f. | Hubei, China | Yuan 2004-58 (NEU) | | | Clade F |
| *I. conchibracteara* Y. L. Chen & Y. Q. Lu | Sichuan, China | Hao 427 (NEU) | AY348765 | DQ147829 | Clade H |
| *I. congolensis* G. M. Schulze & R. Wilczek | Congo orin, Africa, cult. Bot. Gard. Koblenz Univ. | Fischer NE7 (NEU) | AY348766 | DQ147830 | Clade Q |
| *I. corchorifolia* Franch. | Yunnan, China | Chassot & Yuan 99-173 (NEU) | AY348767 | DQ147831 | Clade F |
| *I. cordata* Wight | South India origin, cult. by Ray Morgan, UK | Ray Morgan s. n. (NEU) | AY348768 | DQ147891 | Clade I |
| *I. cristata* Wall. | Tibet, China | Yuan & Ge CN2k3-15 (NEU) | | | Clade C |
| *I. cuspidata* Wight & Arn. | South India origin, cult. by Ray Morgan, UK | Ray Morgan s. n. (NEU) | AY348769 | DQ147832 | Clade M |
| *I. cyanantha* Hook. f. | Yunnan, China | Yuan CN2k1-84 (NEU) | AY348770 | DQ147833 | Clade C |
| *I. cyathiflora* Hook. f. | Yunnan, China | Chassot & Yuan 99-29 (NEU) | AY348771 | DQ147834 | Clade C |
| *I. davidi* Franch. | Fujian, China | Yuan CN2k-09 (NEU) | AY348772 | DQ147835 | Clade F |
| *I. delavayi* Franch. | Yunnan, China | Chassot & Yuan 99-154 (NEU) | AY348773 | DQ147836 | Clade F |
| *I. desmantha* Hook. f. | Yunnan, China | Yuan CN2k2-30 (NEU) | AY348774 | DQ147837 | Clade C |
| *I. devolii* T. C. Huang | Taiwan, China | Jiang s. n. | AY348775 | | Clade C |
| *I. dicentra Franch.ex* Hook.f. | Hubei, China | Yuan 2004-62 (NEU) | | | Clade F |
| *I. dolichoceras* Pritz. ex | Guangxi, China | Yuan CN2k1-80 (NEU) | | | Clade C |

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| Diels | | | | | |
| *I. drepanophora* Hook. f. | Yunnan, China | Yuan CN2k1-41 (NEU) | AY348776 | DQ147838 | Clade C |
| *I. eberhardtii* Tardieu | Dalta, Vietnam | Song & Phuong 2004-10 (NEU) | | DQ147839 | Clade N |
| *I. ecalcarata* Collett & Hemsl. | Oregon, USA | Zika 20068 | | | Clade G |
| *I. edgeworthii* Hook.f. | Nepal | Ray Morgan s. n. | | DQ147840 | Clade C |
| *I. elatostemmoides* H.Perrier | Ambatovy, Madagascar | Yuan & Ravokatra YMG30 | | | Clade V |
| *I. eubotrya* Miq. | Sumatra, Indonesia | Favre AF2 (NEU) | AY348777 | | Clade C |
| *I. faberi* Hook. f. | Sichuan, China | Song S007 (NEU) | AY348778 | DQ147841 | Clade F |
| *I. falcifera* Hook.f. | West Himalaya | Ray Morgan s. n. (NEU) | | | Clade C |
| *I. fenghwaiana* Y. L. Chen | Guangxi, China | Yuan CN2k1-78 (NEU) | AY348779 | DQ147842 | Clade C |
| *I. firmula* Baker | Ranomafana, Madagascar | Rahelivololona, Jacky & Remi RNM 4 (PBZT) | AY348780 | | Clade S |
| *I. fischeri* Warb. | Kenya | Odile Phaehler & Magali Schnell, I-01 (NEU) | AY348781 | DQ147843 | Clade E |
| *I. fissicornis* Maxim. | Shaanxi, China | Wang SH-003 (NEU) | AY348782 | DQ147844 | Clade F |
| *I. flacida* Arn. | South India origin, Cult. United Kingdom, Richmond, Royal H.B. Kew | Meise, FB/S3925(BR) | | DQ147845 | Clade M |
| *I. flanaganae* Hemsl. | Africa | 19860179 (E) | AY348783 | DQ147846 | Clade E |
| *I. forrestii* Hook. f. ex W. W. Smith | Yunnan, China | Yuan CN2k-79 (NEU) | AY348784 | DQ147847 | Clade F |
| *I. fragicolor* Marq. et Airy-Shaw | Tibet, China | Yuang & Ge CN2k3-14 (NEU) | | | Clade C |
| *I. fuchsioides* H. Perrier | Tsaratanana, Madagascar | Rahelivololona ROR 395 (PBZT) | AY348785 | | Clade S |
| *I. furcata* H.Perrier | African origin, cult. Bot. Gard. Koblenz Univ | Fischer NE8 (NEU) | AY348786 | | Clade V |
| *I. gibbosa* H. Perrier | Tsaratanana, Madagascar | Rahelivololona Wohlhauser & Callamander T11 (PBZT) | AY348787 | | Clade V |
| *I. glandulifera* Arn. | Western Himalaya origin, cult. Bot. Gard. Neuchâtel | JBE1 (NEU) | AY348788 | DQ147848 | Clade C |
| *I. gongshanensis* Y. L. Chen | Yunnan, China | Yuan CN2k1-27 (NEU) | AY348789 | | Clade C |
| *I. hawkeri* W.Bull, ex Gard. | New Guinea origin, cult. in University of Leuven | Geuten & Janssens SJ006 (LV) | | DQ147850 | Clade L |
| *I.henryi* E.Pritz.ex Diels | Hubei, China | Yuan 2004-67 (NEU) | | | Clade F |
| *I. henslowiana* Arn. | Sri Lanka origin, cult. Bot. Gard. Koblenz Univ | Fischer NE10 (NEU) | AY348790 | | Clade I |
| *I. hians* Hook. f. | West Africa cul. In Bot. | Song 19991311 | | | Clade K |

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| | Zurich | | | | |
| *I. hoehnelii* T. C. E. Fries | African origin, cult. Bot. Gard. Koblenz Univ. | Fischer NE9 (NEU) | AY348792 | | Clade P |
| *I. holocentra* Hand.-Mazz. | Yunnan, China | Yuan CN2k-54 (NEU) | AY348793 | | Clade C |
| *I. imbecilla* Hook.f. | Sichuan, China | Yuan 2004-30 (NEU) | | | Clade F |
| *I. imbecilla* Hook.f. | Sichuan, China | Hao 426 (NEU) | AY348796 | DQ147851 | Clade F |
| *I. inaperta* H.Perrier | Madagascar origin, cult. Bot. Gard. Neuchâtel | JBE2 (NEU) | AY348797 | DQ147852 | Clade V |
| *I. infirma* Hook.f. | Sichuan, China | Yuan CN2k2-60 (NEU) | | | Clade C |
| *I. javensis* Steud. | West Java, Indonesia | Favre AF56 | | | Clade L |
| *I. keilii* Gilg. | African origin, cult. Bot. Gard. Koblenz Univ | Fischer NE11 (NEU) | AY348798 | | Clade Q |
| *I. kerriae* Craib | Qingmai, Thailand | Chassot 99-238 (NEU) | AY348799 | DQ147853 | Clade B |
| *I. kilimanjari* Oliver | Africa | JMG 94613 (BR) | AY348800 | | Clade R |
| *I. langbianensis* Tardieu | Dalat, Vietnam | Song & Phuong 2004-08 (NEU) | | | Clade N |
| *I. lateristachys* Y.L.Chen et Y.Q.Lu | Sichuan, China | Yuan 2004-33 (NEU) | | | Clade F |
| *I. latifolia* L. | Inida origin, cult. by Ray Morgen, UK | Ray Morgan s. n. (NEU) | AY348801 | DQ147854 | Clade M |
| *I. lecomtei* Hook. f. | Yunnan, China | Yuan CN2k2-202 (NEU) | AY348802 | DQ147855 | Clade F |
| *I. leschenaultii* Wall. | India Origin, cult. by Ray Morgen, UK | Ray Morgan s. n. (NEU) | AY348803 | DQ147856 | Clade M |
| *I. levingei* Gamble ex Hook. f. | India Origin, cult. UK | Ray Morgan s. n. (NEU) | AY348804 | DQ147827 | Clade I |
| *I. lingzhiensis* Y. L. Chen | Tibet, China | Yuan & Ge CN2k3-81 (NEU) | | | Clade D |
| *I. lucorum* Hook.f. | Sichuan, China | Yuan 2004-34 (NEU) | | | Clade F |
| *I. lyallii* Baker | Mt. Amber, Madagascar | Yuan & Ravokatra YMG8 | | | Clade S |
| *I. malcomberi* Eb.Fisch. & Raheliv. | Grand Lac, Mt. Amber, Madagascar | Yuan & Ravokatra YMG13 | | | Clade U |
| *I. manaharensis* Baill. | Madagascar | Wohlhauser & et. al. WCL 1 (PBZT) | AY348805 | | Clade S |
| *I. mandrakae* Eb.Fisch. & Raheliv. | Mandraka, Madagascar | Yuan & Ravokatra YMG38 | | | Clade V |
| *I. mannii* Hook.f. | Habarium, National Botanic Garden of Belgium, Africa origin | Geuven Carvalho 4534 | | | Clade P |
| *I. margaritifera* Hook. f. | Tibet, China | Yuan CN2k3-26 (NEU) | | | Clade C |
| *I. mengtseana* Hook. f. | Yunnan, China | Yuan CN2k1-38 (NEU) | AY348806 | DQ147858 | Clade J |

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| *I. meruensis* Gilg. | Tanzania origin, cult. by Ray Morgen, UK | Ray Morgan s. n. (NEU) | AY348807 | DQ147859 | Clade R |
| *I. microcentra* Hand.-Mazz. | Yunnan, China | Yuan CN2k-50 (NEU) | AY348808 | | Clade C |
| *I. miniata* Grey-Wilson | Tsaratanana, Madagascar | Rahelivololona, Wohlhauser & Callmander T1 (PBZT) | AY348809 | | Clade T |
| *I. monticola* Hook. f. | Sichuan, China | Hao 425 (NEU) | AY348810 | DQ147860 | Clade J |
| *I. namchabawensis* Morgan, Y.-M. Yuan and X.-J. Ge | Tibet, China | Yuan & Ge CN2k3-70 (NEU) | | | Clade D |
| *I. napoensis* Y. L. Chen | Yunnan, China | Yuan CN2k1-61 (NEU) | AY348811 | DQ147861 | Clade J |
| *I. niamniamensis* Gilg. | Africa origin, cult. Bot. Gard. Koblenz Univ | Fischer NE13 (NEU) | AY348812 | DQ147862 | Clade Q |
| *I. nolitangere* L. | Jilin, China | Yuan CN2k-86 (NEU) | AY348813 | DQ147863 | Clade G |
| *I. nubigena* W.W.Smith | Sichuan, china | Yuan 2004-4YM (NEU) | | | Clade F |
| *I. omeiana* Hook.f. | Sichuan, China | Yuan 2004-21 (NEU) | | | Clade A |
| *I. oncidioides* Ridley, ex Hook. F. | Malayan Peninsula | Favre AF14 (NEU) | | | Clade J |
| *I. oxyanthera* Hook. f. | Sichuan, China | Song S008 (NEU) | AY348814 | DQ147865 | Clade F |
| *I. pallida* Nutt. | New York USA | Eric2004-01 (NEU) | | | Clade G |
| *I. parasitica* Bedd. | India origin, cult. by Ray Morgen, UK | Ray Morgan s. n. (NEU) | AY348815 | | Clade I |
| *I. parviflora* DC: | Poland | Ronikier s. n. | AY348816 | DQ147866 | Clade C |
| *I. percrenata* H. Perrier | Andohahela, Madagascar | Rahelivololona, Druard, Jerome & Fiadana And 3 (PBZT) | AY348817 | | Clade S |
| *I. platychlaena* Hook.f. | Sichuan, China | Song S009 (NEU) | AY348818 | DQ147867 | Clade F |
| *I. platypetala* Lindl. | Bali, Indinesia origin, cult. by Ray Morgen, UK | Ray Morgan s. n. (NEU) | AY348819 | DQ147868 | Clade L |
| *I. poculifer* Hook. f. | Yunnan, China | Yuan CN2k2-209 (NEU) | AY348820 | DQ147870 | Clade F |
| *I. poilanei* Tardieu | Dalta, Vietnam | Song & Phuong 2004-09 (NEU) | | DQ147869 | Clade N |
| *I. pritzelii* Hook.f. | Sichuan, China | Song jf011 (NEU) | AY348821 | | Clade F |
| *I. pseudoviola* Gilg. | central Africa | 19680124 (E) | AY348822 | DQ147871 | Clade M |
| *I. psittacina* Hook.f._ | Chiang Dao, Thailand | Favre AF12 (NEU) | | | Clade B |
| *I. puberula* DC. | Darjeeleing , India | Favre AF5 | | | Clade H |
| *I. purpurea* Hand.-Mazz. | Yunnan, China | Song Y007 (NEU) | AY348823 | DQ147872 | Clade C |
| *I. racemosa* DC | Tibet, China | Yuan & Ge CN2k3-29 (NEU) | | | Clade C |
| *I. radiata* Hook. f. | Yunnan, China | Yuan CN2k-77 (NEU) | AY348824 | | Clade C |
| *I. rectangula* Hand.-Mazz. | Yunnan, China | Yuan CN2k1-26 (NEU) | AY348825 | DQ147874 | Clade C |

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| *I. repens* Moon. | South India origin, cul. Nat. Bot. Gard. | Leiden 17953(L) | | DQ147875 | Clade M |
| *I. rostellata* Franch. | Sichuan, China | Yuan 2004-28 | | | Clade F |
| *I. rothii* Hook. f. | Ethiopia | Pierre Binggeli s. n. (NEU) | AY348827 | | Clade E |
| *I. rubrostriata* Hook. f. | Yunnan, China | Yuan CN2k1-44 (NEU) | AY348828 | DQ147876 | Clade H |
| *I. ruiliensis* S.Akiyama et H.Ohba | Yunnan, China | Yuan CN2k1-66 (NEU) | | | Clade C |
| *I. sambiranensis* H. Perrier | Tsaratanana, Madagascar | Rahelivololona, Wohlhauser & Callamander T4 (PBZT) | AY348829 | | Clade S |
| *I. scabrida* DC. | West Himalaya | Ray Morgan s. n. (NEU) | | | Clade C |
| *I. scabrida* DC. | West Himalaya origin, Holden arboretum | Janssens 941314 (DBG) | | DQ147877 | Clade C |
| *I. scutisepala* Hook. f. | Yunnan, China | Yuan CN2k-56 (NEU) | AY348830 | DQ147878 | Clade C |
| *I. siculifer* Hook. f. | Yunnan, China | Yuan CN2k-80 (NEU) | AY348831 | | Clade C |
| *I. sodenii* Engl. & Warb. ex Engl. | Kenya origin, cult. Bot. Gard. Koblenz Univ | Fischer NE15 (NEU) | AY348832 | DQ147879 | Clade R |
| *I. soulieana* Hook. f. | Sichuan, China | Yuan CN2k2-163 (NEU) | AY348833 | DQ147880 | Clade F |
| *I. sp200416_nov* | Mt. QINGCHENG, Sichuan, China | Yuan 2004-16YM (NEU) | | | Clade A |
| *I. sp200439* | Sichuan, China | Yuan 2004-39 (NEU) | | | Clade F |
| *I. sp200456* | Hubei, China | Yuan 2004-56 (NEU) | | | Clade F |
| *I. sp200472* | Shanxi, China | Yuan 2004-72 (NEU) | | | Clade F |
| *I. sp200475* | Chongqing, China | Yuan 2004-75 (NEU) | | | Clade F |
| *I. sp200476* | Chongqing, China | Yuan 2004-76 (NEU) | | | Clade F |
| *I. spnovATibet* | Tibet, China | Yuan & Ge 2003-65 (NEU) | | | Clade C |
| *I. spnovBTibet* | Tibet, China | Yuan & Ge 2003-61 (NEU) | | | Clade C |
| *I. spThai3* | East Thailand | Favre AF10 (NEU) | | | Clade L |
| *I. spThai5* | Chiang Mai,Thailand | Favre AF12 (NEU) | | | Clade N |
| *I. spWoLong* | Sichuan, China | Favre AF 41 (NEU) | | | Clade F |
| *I. spYMG005* | Lac de Mahasariaka, Madagascar | Yuan & Ravokatra YMG5 (NEU) | | | Clade S |
| *I. spYMG007* | Mt. Amber, Madagascar | Yuan & Ravokatra YMG5 (NEU) | | | Clade U |
| *I. spYMG009* | Mt. Amber, Madagascar | Yuan & Ravokatra YMG5 (NEU) | | | Clade T |
| *I. spYMG010* | Mt. Amber, Madagascar | Yuan & Ravokatra YM10 (NEU) | | | Clade U |
| *I. spYMG012* | Grand Lac, Mt. Amber, Madagascar | Yuan & Ravokatra YM12 (NEU) | | | Clade U |
| *I. spYMG018* | Grand Lac, Mt. Amber, Madagascar | Yuan & Ravokatra YM18 (NEU) | | | Clade U |

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|---|---|---|---|---|---|
| *I. stenosepala* Pritz. ex Diels | Shaanxi, China | Wang Sh001 (NEU) | AY348835 | DQ147881 | Clade B |
| *I. stuhlmannii* Warb. | African origin, cult. Bot. Gard. Koblenz Univ | Fischer NE17 (NEU) | AY348836 | | Clade P |
| *I. subabortiva* H.Perrier | Tsaratanana, Madagascar | Rahelivololona, Wohlhauser & Callmander T6 (PBZT) | AY348837 | | Clade U |
| *I. taronensis* Hand.-Mazz. | Yunnan, China | Yuan CN2k-57 (NEU) | AY348838 | DQ147882 | Clade C |
| *I. tayemonii* Hayata | Taiwan, China | Jiang T2 (NEU) | AY348839 | | Clade F |
| *I. teitensis* Grey-Wilson | African origin, cult. Bot. Gard. Koblenz Univ | Fischer NE18 (NEU) | AY348840 | DQ147883 | Clade E |
| *I. tenerrima* Y.L.Chen | Hubei, China | Yuan 2004-63 (NEU) | | | Clade F |
| *I. textori* Miq. | Japan | Kanno & al. 1114 (TUS) | AY348841 | | Clade F |
| *I. tinctoria* Grey-Wilson | East Africa | Ray Morgan s. n. (NEU) | | | Clade E |
| *I. tinctoria* Grey-Wilson | African origin, cult. Nat. Bot. Gard. Meise | Janssens 2002-1249-61 (BR) | | DQ147884 | Clade E |
| *I. translucida* Eb.Fisch. & Raheliv. | Vatoharanana Camp, Madagscar | Yuan and Ravokatra YMG55 (NEU) | | | Clade V |
| *I. trichosepala* Y. L. Chen | Yunnnan, China | Yuan CN2k1-68 (NEU) | AY348843 | DQ147885 | Clade J |
| *I. tuberosa* H.Perrier | Madagascar origin, cult. Bot. Gard. Koblenz Univ | Fischer NE19 (NEU) | AY348844 | DQ147886 | Clade T |
| *I. turrialbana* Donn. Sm. | Costa Rica | Cascante & Sanchez 285 (NEU) | | | Clade G |
| *I. uliginosa* Franch. | Yunnan, China | Yuan CN2k2-173 (NEU) | AY348845 | DQ147887 | Clade C |
| *I. undulata* Y. L. Chen et Y. Q. Lu | Sichuan, China | Yuan 2004-26 (NEU) | | | Clade F |
| *I. uniflora* Hayata | Taiwain, China | Jiang T1 | AY348846 | DQ147888 | Clade F |
| *I. urticifolia* Wall. | Tibet, China | Yuan & Ge CN2k3-45 (NEU) | | | Clade C |
| *I. usambarensis* Grey-Wilson | African origin, cult. Bot. Gard. Koblenz Univ | Fischer NE20 (NEU) | AY348847 | DQ147889 | Clade O |
| *I. vilersi* Costantin & Poisson | Andohahela, Madagascar | Rahelivololona, Druard, Jerome & Fiadana, And 2 (PBZT) | AY348848 | | Clade R |
| *I. vittata* Franch | Sichuan, China | Yuan 2004-15YM (NEU) | | | Clade F |
| *I. walleriana* Hook.f. | Kenya | Odile Phaehler & Magali Schnell I08 (NEU) | AY348849 | | Clade O |
| *I. walleriana* Hook.f. | African origin, cult. Nat. Bot. Gard. Meise | Janssens S3926 (BR) | | DQ147892 | |
| *I. xanthina* Comber | Yunnan, China | Yuan CN2k1-15 (NEU) | AY348850 | DQ147893 | Clade J |
| I. yingjiangensis S.Akiyama & H. Ohba | Yunnan, China | Yuan CN2k1-55 (NEU) | AY348851 | DQ147894 | Clade J |

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| *I. zenkeri* Warb. | African origin, cult. Bot. Gard. Koblenz Univ | Fischer NE21 (NEU) | AY348852 | DQ147857 | Clade K |
| *Hydrocera triflora* Wight & Arn. | Mekong Delta, Vietnam | Song, Favre & Phuong 2004-04 (NEU) | AY348853 | DQ147895 | |
| *Marcgravia polyantha* Delp. | sample from botanical Garden, origin unknown | FB/S3781 (BR) | AY348854 | | |
| *Norantea guianensis* Aubl. | sample from botanical Garden, origin unknown | FB/S3779 (BR) | AY348855 | DQ147898 | |
| *Pelliciera sp.* | Costa Rica | Pennington & al. 586 (K) | AY348856 | DQ147899 | |
| *Souroubea sp.* | Peru | 76GR00102 (U) | AY348857 | DQ147900 | |
| *Tetramerista sp.* | Brunei Darussalam | Coode s. n. (K) | AY348858 | | |
| *Marcgravia Maguire* de roon | French Guyana, cult. Net. Bot. Gard. Meise | FB/S3708 (BR) | | DQ147896 | |