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Abstract The present study contributes to a better understanding of major palynological trends in *Impatiens*, one of the largest genera in angiosperms, and evaluates the phylogenetic usefulness of pollen morphological characters in the genus. The pollen morphological diversity of 115 Asian species of the genus was investigated using light microscopy and scanning electron microscopy and analyzed in a phylogenetic framework using Bayesian inference and Bayesian posterior character mapping as well as parsimony character optimization. Despite the rather young age of *Impatiens* and its extremely rapid radiation, a striking palynological variability could be observed. The ancestral pollen type in *Impatiens* is a triangular, tri-aperturate pollen grain with reticulate sexine ornamentation, which subsequently evolved into the main *Impatiens* pollen type: a 4-aperturate, rectangular pollen grain with a reticulate sexine ornamentation. The most recently diversified Asian clades are characterized by several reversions from 4-aperturate to 3-aperturate and changes in sexine ornamentation from reticulate to microreticulate sexine ornamentation. Also differences in pollen view can be observed amongst the most recently diversified Asian lineages ranging from circular, quadrangular, elliptic to sub-elliptic and rectangular. Although some pollen morphological characters seem to be homoplasious, others help to improve the resolution of some phylogenetically problematic lineages in the genus.

Keywords character evolution; character mapping; *Impatiens*; pollen morphology; total evidence approach

Supplementary Material Figures S1–S6 and Tables S1–S3 are available in the Electronic Supplement to the online version of this article (http://www.ingentaconnect.com/content/iapt/tax).

INTRODUCTION

Impatiens L. is a highly diverse genus (>1000 spp.) consisting of annual and perennial herbs (Grey-Wilson, 1980a; Janssens & al., 2009b). Representatives of the genus are generally distributed in the Old World tropics and subtropics above an altitude of 500 m (Yuan & al., 2004). Only few species grow in temperate regions of Europe, North America and northern China. No indigenous species occur in Australia and South America. The highest diversity of *Impatiens* species is found in Africa, Madagascar, the Sino-Himalayan region, South India and Sri Lanka, and Southeast Asia (Toppin, 1920; Shimizu, 1979; Grey-Wilson 1980a, 1989; Bhaskar & Razi, 1981; Chen, 2001; Rahelivololona & al., 2003). *Impatiens* diverged from its monospecific sister genus *Hydrocera* in the late Eocene while initial diversification only started in the Early Miocene (Janssens & al., 2009b). Its centre of origin is assumed to be in southwest China from where the genus gradually dispersed into neighbouring areas and subsequently spread towards adjacent continents or subcontinents. Since the early Pliocene, *Impatiens* rapidly diversified into the large genus as we know it today. The high species diversity in *Impatiens* is reflected in the hypervariable floral morphology in which the spurred sepal and the lateral petals show extreme variability (Yuan & al., 2004; Caris & al., 2006; Janssens & al., 2006, 2008, 2010). Despite this large flower morphological diversity, the vegetative morphology of *Impatiens* is conserved, always having glandular-toothed leaves and a fleshy semi-succulent stem. High levels of convergent evolution on flower morphology (Yuan & al., 2004; Janssens & al., 2006, 2010) is probably the main reason why it has been so difficult in the past to divide the genus into natural groups based on macromorphological data only (Hooker & Thomson, 1859; Warburg & Reiche, 1895; Hooker, 1905).

Since Erdtman's (1952) comprehensive pollen morphological study on angiosperms, including *Impatiens* pollen, several palynological studies have been carried out that focused on the pollen morphology of *Impatiens* (Narayana & Sayeeduddin, 1959; Huynh, 1966, 1968; Bhaskar & Razi 1973, 1979; Tara & Namboodri, 1974; Bhaskar & al., 1975; Moore & Webb, 1978; Grey-Wilson, 1980b; Lu, 1991; Perveen & Qaiser, 2001; Janssens & al., 2005, 2009a, 2011; Vinckier & al., 2012). Despite these studies, the pollen morphology of *Impatiens* is still underexamined due to the large size of the genus. Huynh's (1968) light microscopic study is the only one to include both a large and geographically balanced *Impatiens* sample. All other studies have either focused on a specific geographic region or have only treated a limited number of species. Although Huynh (1968) showed that reticulate rectangular pollen grains with four apertures are the main pollen type in *Impatiens*, he also observed large variation in shape, aperture number and ornamentation of the tectum. This palynological variation was confirmed by studies on Indian species by Bhaskar & Razi (1973) and Bhaskar & al. (1975). In 1980, Grey-Wilson was the first to stress the possible value of pollen characters to tackle taxonomical questions in *Impatiens* as he used pollen morphological data to detect possible hybrids and to optimize his species aggregation hypothesis on African *Impatiens* (Grey-Wilson, 1980a, b). Whereas Grey-Wilson's scanning electron microscopic study was focused on the African representatives of the genus, Janssens & al. (2005) were the first to investigate the pollen morphology of a broad taxonomic and geographic *Impatiens* sample using scanning and transmission electron microscopy. Their results, however, contained only eleven African and eight Asian species and as a result they were unable to infer palynological evolution of Asian *Impatiens* species.

The aim of the present study is to provide a comprehensive pollen morphological description of the Asian representatives of *Impatiens* based on light microscopy (LM) and scanning electron microscopy (SEM). In addition, palynological synapomorphies are being detected for the different Asian *Impatiens* clades and the phylogenetic signal of various pollen morphological characters is assessed. Furthermore, palynological evolutionary trends in Balsaminaceae are inferred by combining present and previous palynological observations with molecular sequence data using Bayesian posterior mapping. Finally, we want to illustrate the importance of a 'total evidence approach' using molecular and morphological data in order to infer complex evolutionary relationships that could not be resolved using solely molecular or morphological data.

■ MATERIALS AND METHODS

Pollen morphological analysis. — In total 124 Asian *Impatiens* species were examined in this study. Material has been obtained from herbarium specimens at the National Botanic Garden Belgium (BR), the National Herbarium of the Netherlands, Leiden Branch (L), and the Herbarium of Neuchâtel (NEU) (Appendix). LM preparations were made in glycerin jelly and immediately visualized without further treatment.

For SEM, dried flowers or mature buds were rehydrated for 48 h in Agepon wetting agent (1 : 200, Agfa Gevaert, Leverkusen, Germany). Prior to critical-point drying (CPD 030, Balzers, Liechtenstein), pollen material was dehydrated in a graded acetone series (30%–50%–70%–100%). The critical-point-dried anthers were mounted on stubs with double-sided adhesive tape and pollen grains were removed from the locules with a fine cactus needle to facilitate observation of the inner locule wall. Removed pollen grains were accumulated on the same stub for further observations. The stubs were sputter coated with gold (SPI-MODULE Sputter Coater, SPI Supplies, West Chester, Pennsylvania, U.S.A.). We used a Jeol JSM-6400 microscope at 15 kV for morphological observations. Comparative size measurements of pollen were made from digital SEM-micrographs using Carnoy v.2.0 (Schols & al., 2002) and Macnification v.1.0 (www.orbicule.com).

Pollen terminology follows the *Glossary of Pollen and Spore Terminology* (Punt & al., 2007; http://www3.bio.uu.nl/palaeo/glossary/glos-lit.htm). The terminology of pollen shape in polar view follows Reitsma (1970) while terms for shape classes in equatorial view were adopted from Erdtman (1971).

Molecular phylogenetic analysis. — A recent molecular phylogenetic study of *Impatiens* (Janssens & al., 2006) was extended with additional species from India, China, the Himalayan region and Southeast Asia. Total genomic DNA was extracted using a modified version of the hot CTAB protocol (Janssens & al., 2007). Primers and temperature profiles used for the amplification of *atpB-rbcL* follow Janssens & al. (2006). Amplification reactions were carried out on a GeneAmp PCR system 9700 (Applied Biosystems, Foster City, California, U.S.A.). Nucleotide sequences were determined by the Macrogen sequencing facility (Macrogen, Seoul, Korea). MUSCLE v.3.6 (Edgar, 2004) was used for the initial alignment of the DNA sequences, followed by manual adjustment in MacClade v.4.05 (D.R. Maddison & Maddison, 2002). GenBank accession numbers and voucher information are listed in the Appendix. The chloroplast data matrix has been submitted to TreeBase (S12527).

Indels present in the *atpB-rbcL* data matrix were coded as separate characters following the simple indel coding method (Simmons & Ochoterena, 2000). Non-informative or ambiguous gaps were not coded. In order to investigate the phylogenetic value of palynological characters in *Impatiens*, we additionally combined the pollen morphological characters from species investigated in this study and the study of Janssens & al. (2005) with the gap-coded *atpB-rbcL* data matrix. Three datasets have been analyzed using Bayesian inference (BI): (1) molecular sequence data, (2) pollen morphological data, and (3) molecular sequence data and pollen morphological characters combined. The most appropriate substitution model was determined for each of the datasets using a series of likelihood ratio tests as implemented in ModelTest v.3.06 (Posada & Crandall, 1998). The standard discrete model was used for the analysis of the morphological dataset (Ronquist & Huelsenbeck, 2003). Bayesian analyses were conducted with MrBayes v.3.1 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003). 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.

Character optimization. — In order to hypothesize the ancestral palynological state for each node in the phylogeny, 13 pollen morphological characters were analyzed using Parsimony character optimization and Bayesian posterior character mapping. In total, seven discrete characters—pollen shape in equatorial view, shape in polar view (amb), aperture number, aperture type, presence of a margo, sexine ornamentation pattern, presence of granules in the lumina)—and six continuous characters—length of the polar axis (P), length of the longest equatorial diameter (E_{long}), length of the shortest equatorial diameter (E_{short}), aperture size, muri width, lumina size)—were analyzed (Table 1; Table S1). Continuous characters were coded following Thiele's gap weighting method as implemented in

MorphoCode v.1.10 (Schols & al., 2004). The number of possible character states for continuous characters was set at three. For the African lineages, we used the ancestral pollen states as determined in Janssens (2008). Both continuous and discrete characters were considered as unordered with weight one.

Bayesian posterior character mapping was conducted with SIMMAP v.1.0 (Bollback, 2006) using the last 5000 topologies of the Bayesian analysis as input data. Hyperparameters defining the mean (E) and standard deviation (SD), which accommodate the substitution rate parameter θ, were calculated following the approach of Couvreur & al. (2010). A flat prior was used for the bias rate parameter *I* in all analyses. Due to the significant influence of E(θ) and SD(θ) on the overall outcome of each analysis, they were independently selected for each character using the "number of realizations sampled from priors" function as implemented in SIMMAP (Couvreur & al., 2010). The mean E(θ) value was optimized for each character, whereas the SD(θ) was set at 2. The topology used for visualization of the stochastic character mapping results was one of the 5000 sampled Bayesian topologies used as input data. This topology was chosen to infer the same phylogenetic relationships as for the 50% majority-rule Bayesian consensus tree but with additional resolution for the unresolved lineages of the consensus topology. Node constraint numbers and topology are presented in Fig. S1.

Simple parsimony reconstruction was conducted with Mesquite v.2.75 (W.P. Maddison & Maddison, 2010) using the same topology as for Bayesian stochastical mapping.

■ RESULTS

Pollen grains examined in this study are in general colpate with a reticulate sexine pattern. We can provide a comprehensive description of the SEM data arranged according to the following pollen features (size, shape, apertures, sexine ornamentation, orbicules and pollen threads) and with reference to specific specimens if required (see Table S2 for a summary).

Pollen size. — Based on SEM graphs, pollen grains of Asian *Impatiens* species are small to medium-sized. The polar axis (P) ranges from 10.9 µm (*I. barbata*) to 24.5 µm (*I. forrestii*) and is on average 17.4 µm long, whereas the long equatorial diameter (E_{long}) varies between 21.0 µm (*I. oppositifolia*) and 48.1 µm (*I. mengtzseana*) being on average 31.5 µm. The short equatorial diameter (E_{short}) of the pollen from Asian species varies between 12.1 µm (*I. oppositifolia*) and 37.8 µm (*I. chevalieri*) and is 18.8 µm on average. The largest pollen grains were observed in *I. viscida* (P: 24.8 µm, E_{long}: 43.2 µm, E_{short}: 22.7 µm) and *I. mengtzseana* (P: not available, E_{long}: 48.1 µm, E_{short}: 23.4 µm), while the smallest grains are present in *I. leptoceras* (P: 11.4 µm, E_{long}: 22.8 µm, E_{short}: 13.7 µm) and *I. oppositifolia* (P: 11.4 µm, E_{long}: 21.0 µm, E_{short}: 12.1 µm). Intraspecific size variation is very limited (based on data from three specimens).

Pollen shape (shape in equatorial view). — In order to determine pollen shape we used the ratio polar axis/long equatorial diameter. A wide range of pollen shapes is present within Asian *Impatiens* species, varying from peroblate and oblate to suboblate and spheroidal. Of all Asian species examined, the majority is peroblate (47%) or oblate (41%) in shape, with suboblate and spheroidal pollen being present in a much smaller number of species (8% and 4%, respectively).

Shape in polar view. — Polar view varies from rectangular over elliptic to quadrangular, circular and triangular shapes (Fig. 1A–O). Of all Asian species examined, the majority is rectangular (51%; Fig. 1K–O) in polar view, with elliptic pollen being present in 29% of the species (Fig. 1H–I). In comparison to elliptic and rectangular pollen grains, there is only a small number of Asian species with triangular (Fig. 1A), quadrangular (Fig. 1E–G), sub-elliptic (Fig. 1J) or circular (Fig. 1B–D) pollen (1%, 4%, 6%, and 9%, respectively). Sub-elliptic grains in polar view are here defined as elliptic in shape

Table 1. Characters and character states used in the combined molecular-palynological analysis.

Character	Character state					
	0	1	2	3	4	5
P (mean)	<18 µm	18–30 µm	>30 µm			
E_{long} (mean)	<31 µm	31–46 µm	>46 µm			
E_{short} (mean)	<19 µm	19–36 µm	>36 µm			
Lumina size (mean)	<1.35 µm	1.35–4.1 µm	>4.1 µm			
Muri width (mean)	<0.4 µm	0.4–0.8 µm	>0.8 µm			
Aperture length (mean)	<6.2 µm	6.2–11 µm	>11 µm			
Shape	Peroblate	Oblate	Suboblate	Spheroidal		
No. of apertures	3	4				
Polar view	Triangular	Rectangular	Elliptic	Sub-elliptic	Circular	Quadrangular
Sexine pattern	Reticulate	Microreticulate	Gemmate			
Granules	Absent	Present				
Margo	Absent	Present				
Aperture type	Colpus	Porus				

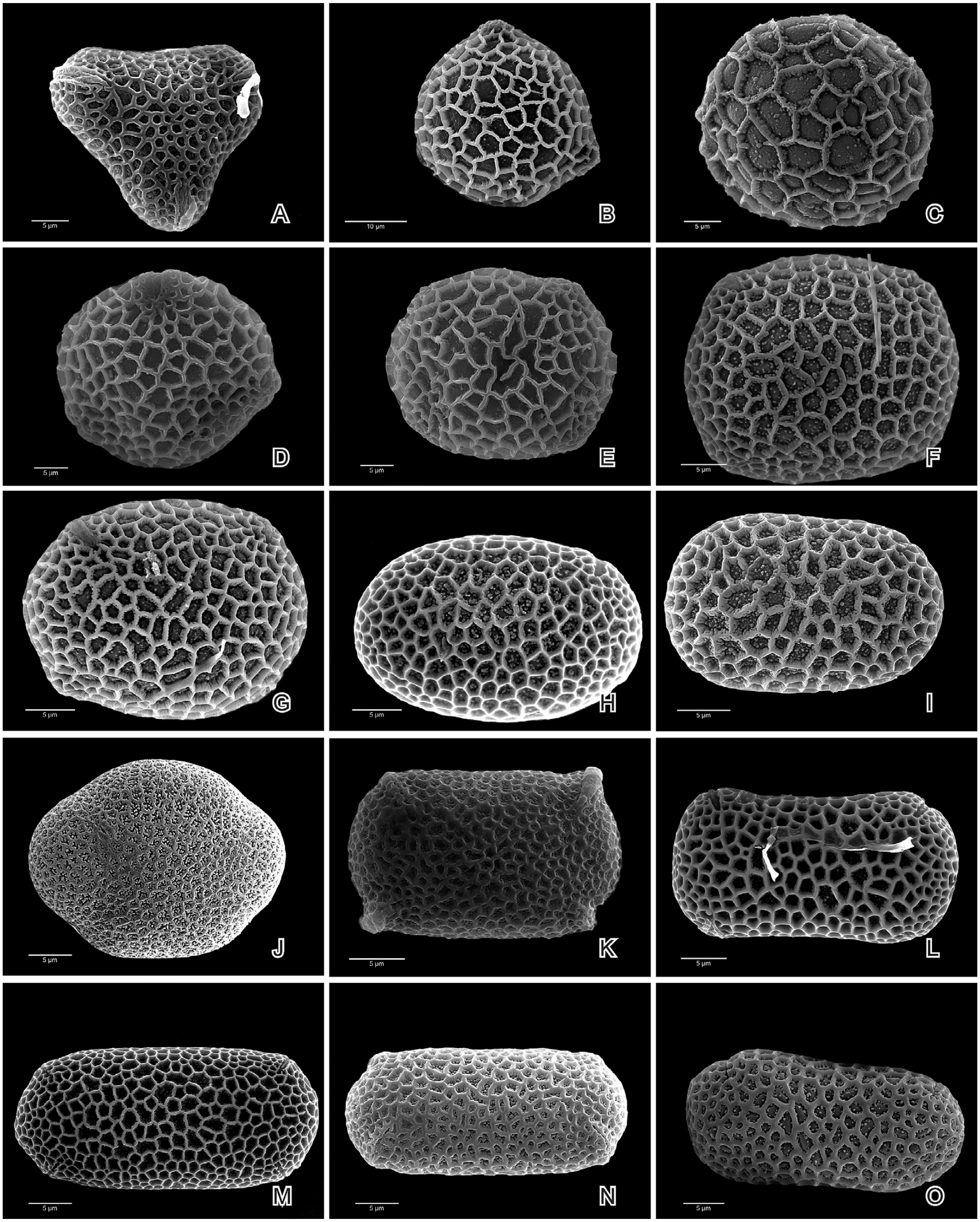

Fig. 1. Polar view of pollen of Asian *Impatiens* (SEM). **A,** *I. omeiana*, triangular 3-colpate pollen grain; **B,** *I. eberhardtii*, triangular to circular pollen grain; **C,** *I. leptopoda*, circular 4-colpate pollen grain; **D,** *I. flaccida*, circular 3-colpate pollen grain; **E,** *I. flaccida*, quadrangular 4-colpate pollen grain; **F,** *I. leschenaultii*, quadrangular 4-colpate pollen grain; **G,** *I. pseudochinensis*, quadrangular 4-colpate pollen grain; **H,** *I. subaequalis*, elliptic 4-colpate pollen grain; **I,** *I. balfourii*, elliptic 4-colpate pollen grain; **J,** *I. poculifer*, sub-elliptic 4-colpate pollen grain; **K,** *I. fragicolor*, rectangular 4-colpate pollen grain; **L,** *I. racemulosa*, rectangular 4-colpate pollen grain; **M,** *I. elongata*, rectangular 4-colpate pollen grain; **N,** *I. napoensis*, rectangular 4-colpate pollen grain; **O,** *I. ruiliensis*, rectangular 4-colpate pollen grain.

with four slightly sunken apertures. The pollen grains appear swollen with slight indents at apertural sites (Fig. 1J). Interestingly, species with variable aperture number (except for *I. repens*) are characterized by different polar views. For example, 4-aperturate pollen of *I. flaccida* (Fig. 1E) and *I. oncidioides* pollen is quadrangular in polar view, whereas the 3-aperturate pollen morph of *I. flaccida* (Fig. 1D) and the 5-aperturate pollen morph of *I. oncidioides* are circular in polar view. It is clear from our data that the aperture configuration greatly defines pollen shape, so number of apertures and polar view should be considered as dependent characters.

Apertures. — Within the Asian *Impatiens* species investigated, apertures are simple and generally consist of an ectocolpus (Fig. 2A–D). The presence of ectopori, as observed in some African *Impatiens* species (Janssens & al., 2005; Janssens, 2008) is rarely encountered in Asian species (*I. eberhardtii*; Fig. 2C). In general, Asian species have pollen grains with four apertures situated at the angles of the polar outline (angulaperturate). Of all Asian *Impatiens* species investigated, only *I. javensis*, *I. omeiana* (Fig. 1A), *I. eberhardtii* (Fig. 1B), *I. chevalieri* and *I. stocksii* are characterized by 3-aperturate pollen. In *I. flaccida*, we observed both 3- and 4-aperturate pollen within the same specimen (Fig. 1D–E). Furthermore, in *I. repens* and *I. oncidioides* we observed both 4- and 5-aperturate pollen in which the proportion of 4-aperturate pollen largely outnumbers the grains with five apertures.

The colpus ends are acute or obtuse (Fig. 2A–D) and colpus membranes are always smooth. Longest ectocolpi are observed in *I. omeiana* (14.26 µm) and *I. forrestii* (13.58 µm), whereas the shortest ones are observed in *I. appendiculata* (2.35 µm) and *I. salicifolia* (3.18 µm). On average, ectocolpi are 6.55 µm in length.

Sexine ornamentation. — In general, sexine ornamentation in Asian *Impatiens* species is reticulate (Fig. 3A–O). A microreticulate ornamentation pattern is observed in *I. noli-tangere*, *I. chungtienensis*, *I. uliginosa*, *I. langbianensis*, *I. napoensis*, *I. soulieana*, *I. fimbriata*, *I. walkeri* and *I. oppositifolia*. *Impatiens gongshanensis* has a microreticulate sexine ornamentation with supratectal microspines (Fig. 3P). In contrast to all other Asian species observed, *I. cristata* is characterized by a gemmate sexine pattern (Fig. 3Q). Some species have a microreticulate to perforate sexine pattern at the mesocolpia (e.g., *I. latifolia*, *I. cuspidata*, *I. acuminata* and *I. flaccida*; Fig. 3A). In addition, most Asian *Impatiens* species are characterized by a gradual decrease in lumen size towards the apertures whereas others have a distinct microreticulate margo (Table S2).

Despite the fact that nearly all Asian *Impatiens* have a reticulate sexine ornamentation, we observed several differences among this ornamentation type that might allow species identification. Although the majority of Asian species is homobrochate reticulate, also heterobrochate pollen is present. In several species we detected lumina that were not fully surrounded by muri resulting in connected/fused neighbouring lumina (Fig. 3A). The average lumen diameter within Asian *Impatiens* species varies from 0.49 µm (*I. gongshanensis*; Fig. 3P) to 4.66 µm (*I. leptopoda*; Fig. 1C), being on average 1.74 µm. The width and shape of the muri varies strongly amongst Asian species. Muri vary from slender with a tapering top edge

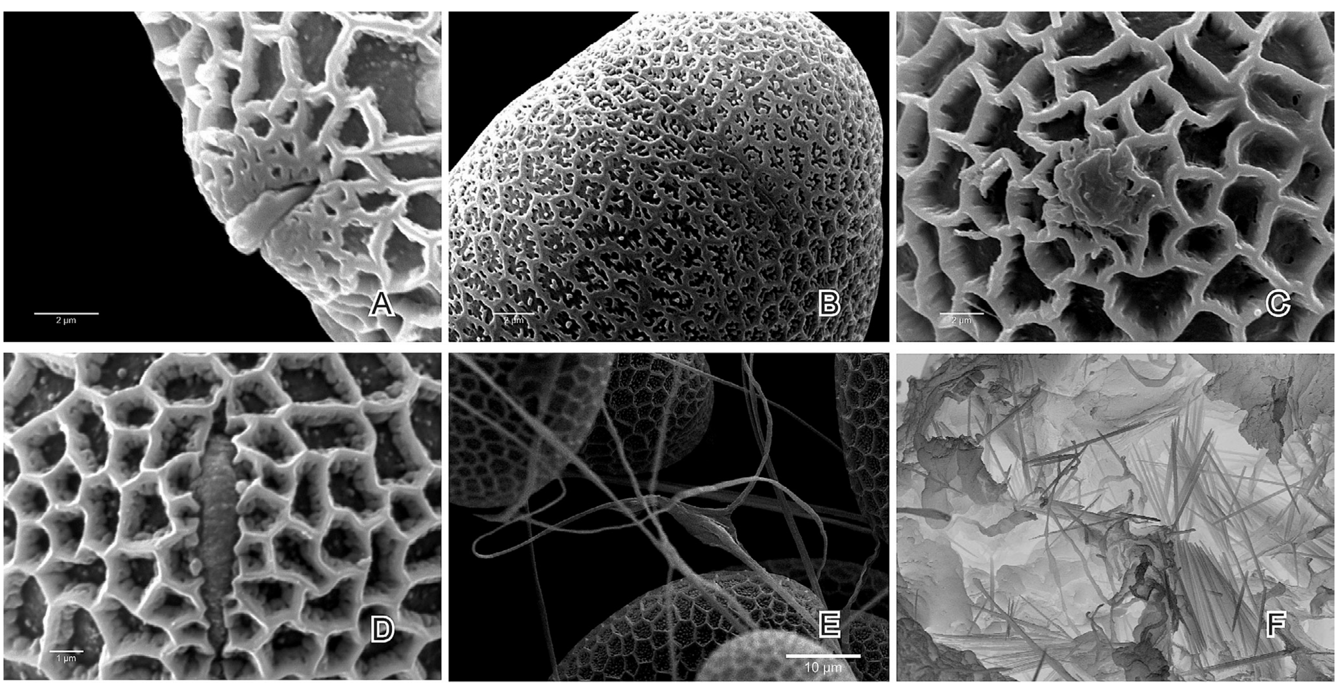

Fig. 2. SEM of apertures **(A–D)** and the anther locule **(E–F)** of pollen grains of Asian *Impatiens*. **A,** *I. flaccida*, detailed view of a colpus with microreticulate margo; **B,** *I. poculifer*, detailed view of an enlarged colpus **C,** *I. eberhardtii*, detailed observation of a porus; **D,** *I. leschenaultii*, detailed view of a colpus without margo; **E,** *I. namchabarwensis*, pollen threads; **F,** *I. omeiana*, calcium oxalate raphides.

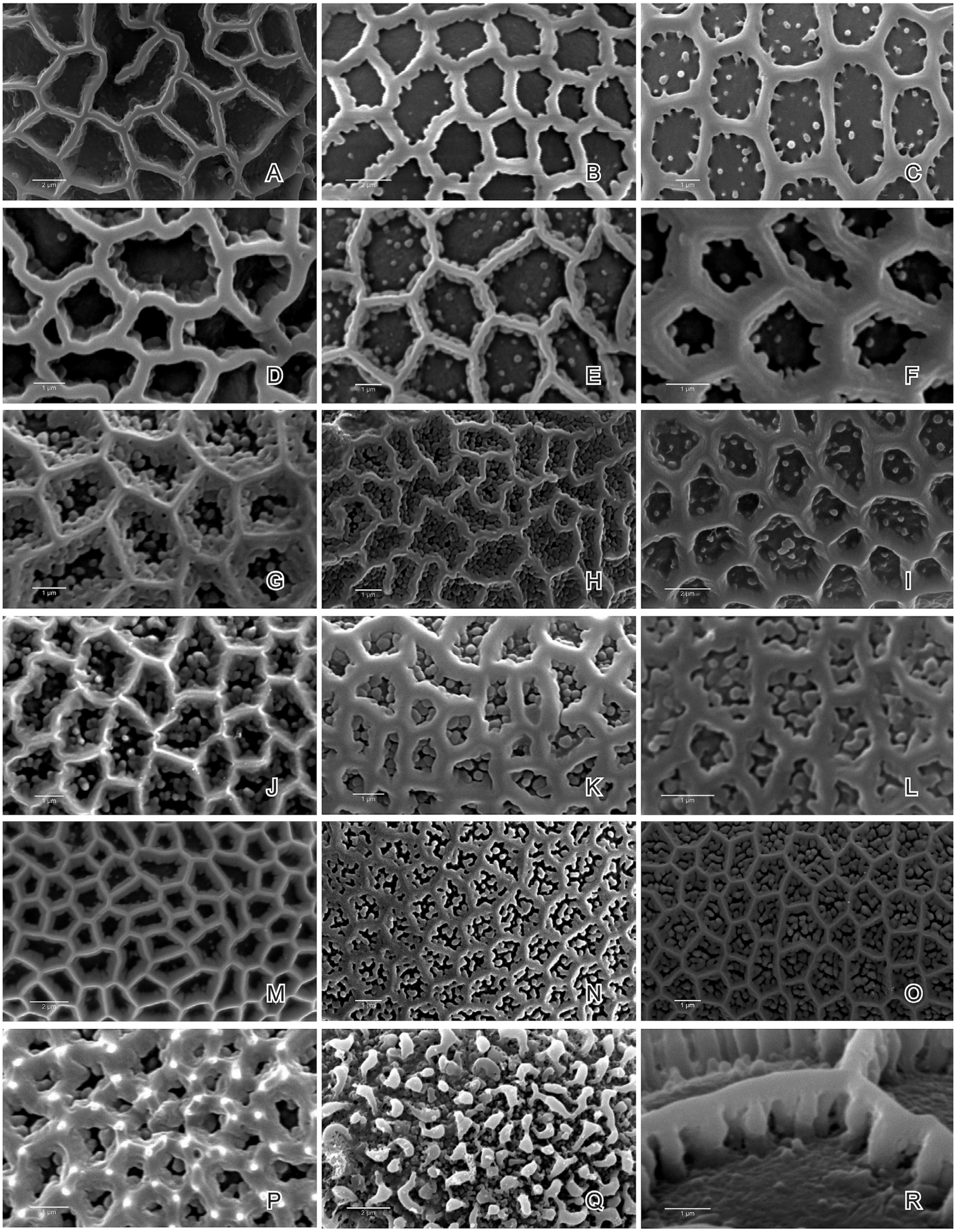

Fig. 3. SEM of sexine ornamentation of Asian *Impatiens*. **A–O,** Reticulate sexine ornamentation. **A,** *I. flaccida*; **B,** *I. dolichoros*; **C,** *I. ruiliensis*; **D,** *I. infirma*; **E,** *I. leschenaultii*; **F,** *I. holocentra*; **G,** *I. rubrostriata*; **H,** *I. nyimani*; **I,** *I. grandis*; **J,** *I. subaequalis*; **K,** *I. napoensis*; **L,** *I. chimiliensis*; **M,** *I. siculifer*; **N,** *I. poculifer*; **O,** *I. chrysantha*; **P,** *I. gongshanensis*, microreticulate sexine ornamentation with supratectal spines; **Q,** *I. cristata*, gemmate sexine ornamentation; **R,** *I. chevalieri*, detail of foot layer, columellae and tectum of reticulate sexine pattern.

over tapering with a rounded top edge to broad or flattened muri (Fig. 3A–R). On average, muri of Asian *Impatiens* are 0.47 µm in width. Except for *I. apsotis*, *I. aureliana*, *I. chevalieri* (Fig. 3R), *I. diepenhorstii*, *I. drepanophora*, *I. eberhardtii*, *I. flavida*, *I. salicifolia*, *I. sarawakensis* and *I. siamensis*, all other Asian *Impatiens* have granules (free-standing columellae) in their lumina. Granules can be solitary, interconnected or fused with neighbouring muri.

Orbicules. — Orbicules are absent from all species investigated. The tapetal membrane on the inner locule wall is smooth without any patterned sporopollenin deposition.

Pollen threads. — Pollen threads (sensu Vogel & Cocucci, 1988) were observed in the majority of species examined (Fig. 2E). These fine cellulosic fibers are often branched and form a dense network covering the anther slits, thereby preventing the pollen mass from falling out (Vogel & Cocucci, 1988). We only observed pollen threads in mature anthers.

Phylogenetic signal of pollen morphological characters. — The aligned molecular dataset contains 879 characters of which 203 are variable and 126 parsimony-informative. By gapcoding the molecular dataset, 28 additional characters were obtained. The palynological dataset consists of 13 parsimony-informative characters. Bayesian analysis of the *atpB-rbcL* dataset produced a well-resolved topology with moderate to high support values (Fig. 4). Not surprisingly, analysis of the pollen morphological dataset alone resulted in a completely unresolved tree topology (not shown). By adding the 13 palynological characters to the molecular dataset, a slight improvement of resolution could be observed with an overall increase of support values for most nodes (Fig. 4). Analysis of the combined molecular-palynological dataset revealed the following additional phylogenetic relationships which were not found in the molecular analysis: (1) *I. aquatilis* sister species to *I. uliginosa*; (2) *I. radiata* sister to *I. desmantha* and *I. racemosa*; (3) *I. textori* sister to the remainder of the North American–Southwest Chinese clade 4; (4) *I. namchabarwensis* sister to *I. arguta* and *I. lingzhiensis*; (5) *I. langbianensis* sister to *I. chevalieri* and *I. eberhardtii*; (6) *I. latifolia* sister to *I. cuspidata* and *I. flaccida*; (7) the Southwest Chinese clade 7 sister to a large group of species containing South Indian clade 8, Southeast Asian clade 10, South Indian–Southeast Asian clade 12, West African clade 9 (here represented by *I. mackeyana* subsp. *claeri*) and Afro-Malagasy clade 11 (here represented by *I. burtonii*); (8) *I. aureliana* sister to *I. langbianensis*, *I. chevalieri* and *I. eberhardtii*. However, the relationship between *I. dolichoceras* and *I. drepanophora* that was produced by the molecular dataset could only be recovered with very low support. No discrepancies were detected between the molecular and combined molecular-palynological analyses.

Character evolution and ancestral states. — The average number of transformations and the average number of state-to-state transformations for every character are given in Table 2. Posterior probabilities and parsimony values for the ancestral states of the 13 palynological characters for each node are provided in Fig. S1 and Table S3.

Bayesian character mapping and parsimony optimization generally resulted in a similar ancestral character state per node (Table S3). Character optimization across the broadly sampled single gene phylogeny of Asian *Impatiens* revealed that some palynological characters are rather homoplasious (length of the shortest equatorial diameter, aperture size, aperture type, presence of granules in the lumina, muri width, and lumina size) whereas others are systematically informative characters—length of the polar axis (Fig. S2), length of the longest equatorial diameter (Fig. S3), pollen shape, polar view (Fig. S4), aperture number (Fig. S5), presence of a margo (Fig. S6).

■ DISCUSSION

Pollen morphology of Asian *Impatiens*. — Pollen of Asian *Impatiens* species is in general medium-sized. The character ‘long equatorial axis length’ which also determines the size of the pollen can be considered of phylogenetic importance as pollen with a large ‘long equatorial axis’ (>31 µm) is derived from pollen with a shorter ‘long equatorial axis’ (<31 µm). Interestingly, the monophyletic lineage containing clades 6 to 12 (Fig. 4), which is characterized by pollen with a large ‘long equatorial axis’ is also characterized by fusiform fruits, whereas the Asian lineages with linear fruits have in general pollen with a shorter ‘long equatorial axis’ (Yuan & al., 2004). Apertures are nearly always simple colpi. In contrast to the African species where pori are more frequent (Grey-Wilson, 1980b), a porus was only observed twice in Asian *Impatiens* (*I. eberhardtii*, *I. chevalieri*) and may be regarded a synapomorphy for this South Indian lineage. Pollen with four apertures is by far the most common condition, combinations with three or five apertures occur less frequently. Compared to the African species, only a small proportion of 3-aperturate pollen is present among the Asian species (the South Indian *I. eberhardtii* and *I. chevalieri*, and the basal *I. omeiana*). Three-aperturate pollen is absent from Sino-Himalayan species, confirming Huynh’s (1968) observations. In this study, no commonly 5-aperturate *Impatiens* is found, and also Huynh (1968) noticed only two 5-aperturate Asian species (*I. lauterbachii*, *I. goughii*). Despite a rather constant aperture number within *Impatiens*, some Asian specimens show a remarkable plasticity in aperture number, either from three to four or from four to five apertures. In some cases the polar view transforms together with a shift in apertural configuration (*I. flaccida*, *I. oncidioides*). On the other hand, within a single *I. repens* specimen, a small number of pollen grains is characterized by an additional aperture at the mesocolpium, thereby increasing the number of apertures from four to five but without changing its overall polar outline. In general, Asian *Impatiens* pollen grains are rectangular or elliptic in outline, with only a small fraction being circular, triangular, quadrangular or subelliptic. Compared with African *Impatiens*, Asian *Impatiens* species are characterized by a much smaller ratio of circular and quadrangular pollen.

Although pollen of Asian *Impatiens* species is mainly reticulate, some species are characterized by other sexine ornamentation patterns. A microreticulate sexine pattern is present in two South Indian and one Southeast Asian species, yet

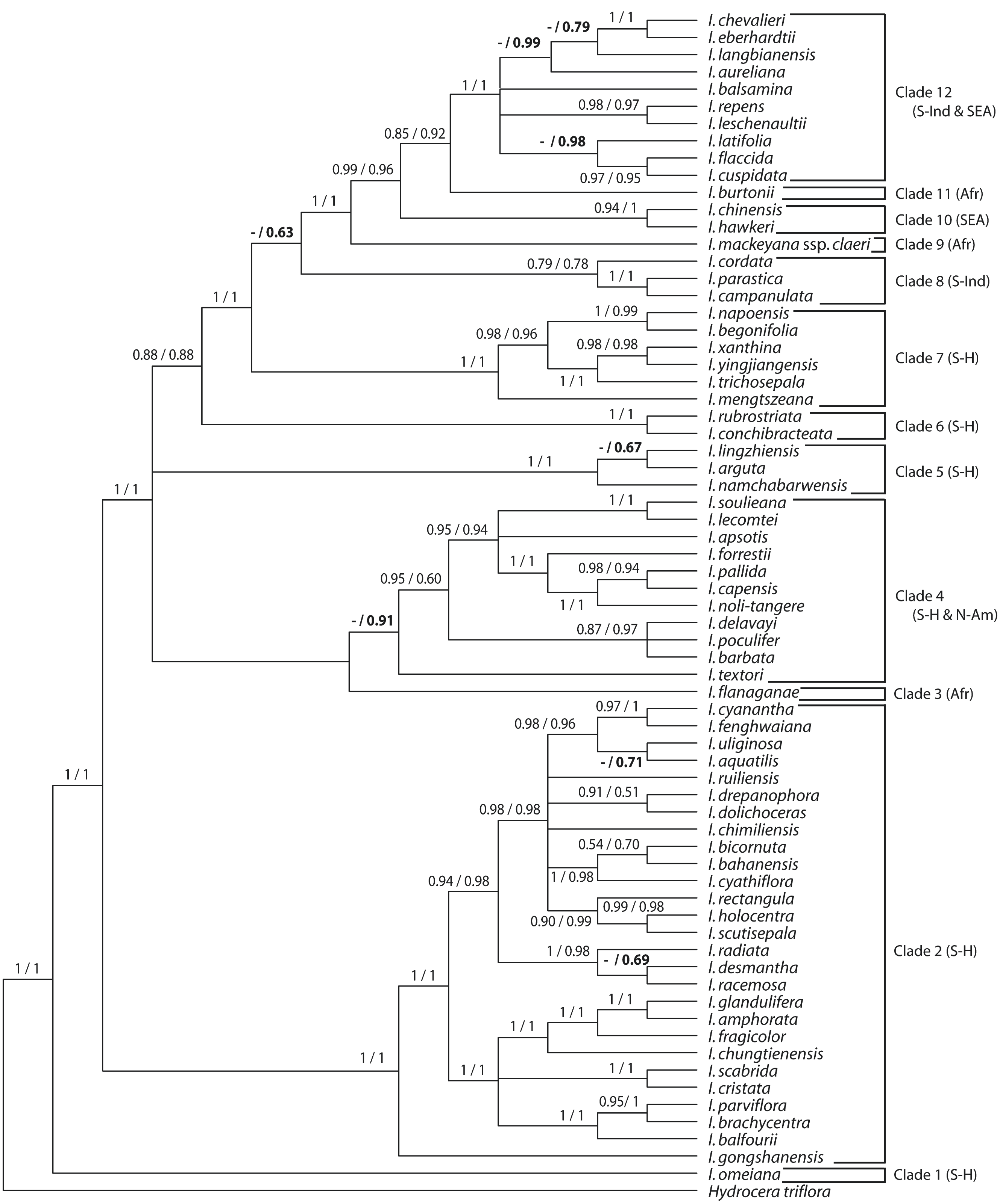

Fig. 4. Bayesian consensus topology of the molecular and combined molecular-palynological analysis. Specific clades are numbered 1 through 12. The first number on a branch represents BPP of the molecular analysis whereas the second number indicates BPP of the combined molecular-palynological analysis. Support values in bold indicate an improved resolution of that node when using the total evidence approach. — Afr, Africa; N-Am, North-America; S-H, Sino-Himalaya; S-Ind, South India; SEA, Southeast Asia.

Table 2. Average total and between-state number of transformations. *E(T)* = mean substitution rate.

Site	No. character states	Bias	Rate	Average no. of total transformations	Average number of transformations from																													
					0⇩1	0⇩2	0⇩3	0⇩4	0⇩5	1⇩0	1⇩2	1⇩3	1⇩4	1⇩5	2⇩0	2⇩1	2⇩3	2⇩4	2⇩5	3⇩0	3⇩1	3⇩2	3⇩4	3⇩5	4⇩0	4⇩1	4⇩2	4⇩3	4⇩5	5⇩0	5⇩1	5⇩2	5⇩3	5⇩4
Length P (*E(T)* = 4)	3	0.33	4.19	13.50	7.70	0.70	–	–	–	3.29	1.29	–	–	–	0.23	0.27	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Length E_{long} (*E(T)* = 4.5)	3	0.33	4.32	18.00	9.45	0.63	–	–	–	6.05	1.34	–	–	–	0.39	0.18	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Length E_{short} (*E(T)* = 4.5)	3	0.33	4.47	20.20	10.10	0.54	–	–	–	5.52	2.43	–	–	–	1.04	0.59	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Lumina size (*E(T)* = 4.5)	3	0.33	4.49	24.10	6.36	2.57	–	–	–	8.86	2.21	–	–	–	1.38	2.75	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Muri width (*E(T)* = 4.5)	3	0.33	4.50	26.70	7.88	0.63	–	–	–	14.40	2.55	–	–	–	0.36	0.88	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Aperture size (*E(T)* = 4.5)	3	0.33	4.49	28.60	11.50	1.86	–	–	–	10.40	0.93	–	–	–	1.82	2.02	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Pollen Shape (*E(T)* = 4.5)	4	0.25	4.46	23.40	6.59	0.25	0.29	–	–	7.59	3.48	2.96	–	–	0.11	0.59	0.68	–	–	0.21	0.52	0.11	–	–	–	–	–	–	–	–	–	–	–	–
# Ap. (*E(T)* = 7)	2	0.40	7.05	2.030	3.86	–	–	–	–	3.20	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Polar View (*E(T)* = 4.5)	6	0.17	4.46	21.50	1.30	0.36	0.16	0.23	0.20	0.18	4.07	1.09	0.09	0.09	0.13	2.89	0.16	3.05	1.96	0.23	2.02	1.04	0.05	0.11	0.11	0.07	1.05	0.11	0.05	0.11	0.11	0.43	0.07	0.00"
Sexine pattern (*E(T)* = 4)	3	0.33	4.18	14.30	3.14	0.30	–	–	–	8.64	1.55	–	–	–	0.25	0.41	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Granules (*E(T)* = 4)	3	0.33	3.77	11.30	3.61	0.46	–	–	–	5.59	1.14	–	–	–	0.27	0.18	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Margo (*E(T)* = 2)	2	0.65	1.84	3.98	1.82	–	–	–	–	2.16	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

most of the Asian representatives with microreticulate sexine ornamentation are distributed in the Sino-Himalayan region. *Impatiens cristata* is pollen-morphologically unique within the genus as it is so far the only species with a gemmate sexine ornamentation. Although Huynh (1966) observed spinulate pollen grains in *I. javensis*, the specimen we examined of the same species was characterized by reticulate pollen. In 1975, Bhaskar & al. also demonstrated that it is possible for a species to be characterized by two ornamentation types (*I. acaulis*: reticulate and granulate).

Systematic value of pollen morphological characters. — The overall radiation of *Impatiens* is known to have occurred very rapidly (Janssens & al., 2009b) and therefore one might assume that palynological characters are rather uniform amongst the rapidly diversifying lineages of *Impatiens*. Huynh (1966, 1968) was the first to observe some variability in pollen morphological characters within *Impatiens* yet also emphasized the main occurrence of 4-aperturate rectangular pollen with a reticulate sexine ornamentation. Grey-Wilson (1980b) noticed rather large pollen morphological variation amongst the African *Impatiens* species. He used the palynological data of his study to infer taxonomic relationships among African species but stated that the taxonomic value of most pollen morphological features might have been obscured by multiple hybridization events. Yet, the convergent evolution of certain pollen morphological characters (e.g., number of apertures) might as well be explained by the plasticity of these characters indicating that they are not all under the same selective pressure (Furness & Rudall, 2004). Here, we will discuss the evolution of several key pollen morphological characters in *Impatiens*.

By optimizing the length of both polar axis and long equatorial diameter, we notice a trend towards larger pollen grains in the more derived clades of *Impatiens* (Figs. S2–S3). For the long equatorial diameter, a significant increase in length (>32 μm) was observed in the monophyletic lineage containing clades 6 to 12 (Fig. 4), with additionally a few reversals towards smaller pollen grains (*I. chinensis*, *I. balsamina*, *I. eberhardtii*). Within the Asian *Impatiens* species investigated, only *I. mengtszeana* was characterized by large pollen (>45 μm). Similarly, an increase in length of the polar axis was observed. However, this enlargement occurred in the lineage containing clades 8 to 12, with one additional reversal (*I. chinensis*). Clades 7 and 8 form a polytomy with clades 9 to 12 in the molecular analysis, yet the combined palynological-molecular analysis addresses clade 8 as sister to clades 9 to 12, though without any support (Fig. 4). This palynological character might therefore provide morphological support for this unresolved relationship in *Impatiens*. In contrast to the length of both polar axis and long equatorial diameter, the length of the short equatorial diameter is rather homoplasious.

Pollen shape is a rather homoplasious character in *Impatiens*, as may be expected. Peroblate and oblate pollen is rather randomly distributed amongst the Asian lineages and multiple switches from peroblate to oblate and vice versa have occurred. Suboblate and spheroidal pollen grains only evolved from an ancestral oblate pollen grain. Spheroidal pollen is only present in the more recently diversified South Indian and Southeast Asian clade 12 and the Southeast Asian clade 10. An oblate pollen shape is the plesiomorphic condition in *Impatiens*, a character state which also occurs in sister genus *Hydrocera*.

Regarding the small number of Asian species with a different aperture number than four (*I. chevalieri*, *I. eberhardtii* and *I. omeiana* have three apertures), it is difficult to make statements about the putative homoplasious nature of this character. Especially since Furness & Rudall (2004) regarded this character as having a rather high level of plasticity for some genera and in some Asian *Impatiens* species two different aperture numbers occur (*I. flaccida*, *I. repens*, *I. oncidioides*). Recent molecular analyses (Janssens & al., 2006, 2007), however, show that *I. chevalieri* and *I. eberhardtii* are sister taxa and therefore the 3-aperturate pollen of both species has evolved in their most recent common ancestor (MRCA; Fig. S5). Although 4-aperturate pollen is most abundantly present in Asian *Impatiens*, the ancestral *Impatiens* pollen grain has three apertures, a feature which is also characteristic for the sister genus *Hydrocera*.

A triangular polar view is plesiomorphic in *Impatiens* and occurs only in the most basal taxon of the genus (*I. omeiana*) and in *Hydrocera* (Fig. S4). The most common shape in polar view throughout the Asian lineages is the rectangular polar view. This polar view is typical for both early and recently diversified Southwest Chinese lineages (clades 2, 4, 5, 6 and 7). A rather large number of Southwest Chinese species from clade 6, however, is characterized by a distinct sub-elliptic polar view (*I. delavayi*, *I. barbata*, *I. poculifer*, *I. soulieana*, *I. lecomtei*, *I. forrestii*), which seems to have evolved from the rectangular outline. In contrast, species from the Eurasian–North American clade (*I. noli-tangere*, *I. pallida*, *I. capensis*) that fall amidst the Southwest Chinese species of clade 4 have a rectangular outline. An elliptic polar view seems to have originated in the MRCA of the more recently diverged South Indian–Southeast Asian clade 12 and the Southeast Asian clade 10 (and also the Afro-Malagassy clade 11). It is unclear whether the MRCA of the more early diversified South Indian clade 8 was characterized by a rectangular or a more elliptic polar outline. Additionally, in the early-diversified Southwest Chinese clade 2 an elliptic polar view independently evolved several times from a rectangular polar view. Although a spheroidal amb is most commonly present in the more recently diversified clades 10 and 12, it is also characteristic for *I. brachycentra*, which belongs to the early diversified Sino-Himalayan clade 2. Quadrangular outlines are only present in *I. leschenaultii* and *I. flaccida*, two South Indian taxa of the recently diversified South Indian–Southeast Asian clade 12.

By mapping sexine ornamentation pattern onto the topology obtained in this study, it is clear that this character appears to be rather homoplasious. Reticulate sexine ornamentation is plesiomorphic in *Impatiens* and lumen size varies considerably among clades or even between closely related species. Because the delimitation of a microreticulate or reticulate ornamentation pattern has been determined according to the average lumen diameter (microreticulate sexine pattern <1 μm and reticulate sexine pattern ≥1 μm; Punt & al., 2007), species with a rather similar lumen diameter measuring either just above 1 μm or just below 1 μm would be characterized by a different ornamentation type. This issue might explain why the presence of a microreticulate sexine character state is randomly scattered over the *Impatiens* topology.

Although rarely present in *Impatiens*, a few South Indian species with a reticulate ornamentation pattern are characterized by a microreticulate margo (*I. latifolia*, *I. flaccida*, *I. cuspidata*). According to recent molecular analyses (Janssens & al., 2006, 2007, 2009b) these species are closely related, thereby suggesting that the presence or absence of a margo might be a taxonomically useful character within Asian *Impatiens* (Fig. S6).

Even though absence or presence of granules in the lumina might provide interesting taxonomic information in *Impatiens*, this character can only be correctly interpreted by ultrastructural TEM data. Janssens & al. (2005) illustrated that the formation of the tectum in *Hydrocera triflora* (and probably also in *Impatiens omeiana*) occurs through the merging of granules (considered to be free-standing columellae) that are present on the foot layer. SEM photographs of *Hydrocera triflora* and *I. omeiana*, however, show lumina without granula. In contrast, a granula-lacking species like *I. hawkeri* hardly has any tectum left and on the foot layer no granules are present. Until more developmental ultrastructural data are available for *Impatiens* pollen grains, the interpretation of granule density in the lumina and their relation to the tectal muri should be made with caution.

Optimizing lumen size, muri width and aperture length onto the *Impatiens* topology completely lacked congruence and can be considered as characters without systematic value for *Impatiens*.

Phylogenetic signal of pollen morphological characters. — Combining the pollen data with the molecular sequences increases the resolution between and within the major Asian lineages. In addition, Bayesian posterior probabilities remain stable or increase for most of the branches whereas only few lineages show a decrease in support value. A possible explanation for a decline in support value may be the homoplasious nature of some of the palynological characters, thereby obscuring the phylogenetic signal in some lineages. Nevertheless, it is clear that a total evidence approach in which molecular and palynological data are combined has proven useful in resolving problematic relationships in a large and systematically difficult genus like *Impatiens*.

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Appendix. List of species names, species provenance and locality, voucher information of the specimens used for the pollen morphological analysis, voucher information of the specimens used for the molecular analysis if differing from voucher specimens used for the pollen morphological study (underlined), *atpB-rbcL* GenBank accession.

I. acuminata Benth.; Sino-Himalaya, Himalaya; *W.N. Koelz 23345* (L). ***I. amphorata*** Edgew.; Sino-Himalaya, Himalaya; *C. d'Alleizette 1002A* (L); B. de Retz 5566 (BR); HE647186. ***I. appendiculata*** Arn.; South India–Sri Lanka, Sri Lanka; *A.J.G.H. Kostermans 24159A* (L). ***I. apsotis*** Hook. f.; Sino-Himalaya, China; *Y.-M. Yuan CN2k2-159* (NEU); DQ147810. ***I. aquatilis*** Hook. f.; Sino-Himalaya, China; *Y. Song CNY017* (NEU); DQ147811. ***I. arguta*** Hook. f. & Thomson; Sino-Himalaya, Himalaya; *F. Kingdon-Ward* 6035 (L); DQ147812. ***I. arriensii*** (Zoll.) T. Shimizu; Sino-Himalaya, China; *A. Stolz 515* (L). ***I. aureliana*** Hook. f.; Sino-Himalaya, China; *Y.-M. Yuan CN2k1-56* (NEU); DQ147814. ***I. bahanensis*** Hand.-Mazz.; Sino-Himalaya, China; *Y.-M. Yuan CN2k-30* (NEU); HE647187. ***I. balfourii*** Hook. f.; Sino-Himalaya, Himalaya; *R. Morgan NE1* (NEU); DQ147817. ***I. balsamina*** L.; South India–Sri Lanka, South India, *Janssens SJ001* (LV); DQ147816. ***I. barbata*** Comber; Sino-Himalaya, China; *Y.-M. Yuan CN2k2-178* (NEU); DQ147818. ***I. beccari*** Hook. f. ex Dunn; Southeast Asia, Sumatra; *C.N.A. de Voogd 2885* (L). ***I. begoniifolia*** S. Akiyama & H. Ohba; Sino-Himalaya, China; *Y.-M. Yuan CN2k1-51* (NEU), DQ147819. ***I. bella*** Hook. f. & Thomson; Sino-Himalaya, Himalaya; *W.N. Koelz 25200* (L). ***I. benthamii*** Steenis; Sino-Himalaya, Himalaya; *T.R. Chand 1742* (L). ***I. bicornuta*** Wall.; Sino-Himalaya, Himalaya; *J.F. Duthie s.n.* (L); DQ147821. ***I. brachycentra*** Kar. & Kir.; Sino-Himalaya, Himalaya; *J.F. Duthie 23976* (L); HE647188. ***I. burtonii*** Hook. f. var. ***burtonii***; Africa, Kenya; Knox 2803 (LV); FJ826885. ***I. campanulata*** Wight; South India–Sri Lanka, South India; *R. Morgan s.n.* (LV); DQ147822. ***I. capensis*** Meerb.; North America; Janssens SJ009 (LV); DQ147823. ***I. chevalieri*** Tardieu; Southeast Asia, Vietnam; *Y. Song & N.L. Phuong 2004-07* (NEU); HE647189. ***I. chiangdaoensis*** Shimizu, Thailand; Southeast Asia; *E. Hennipman 3191* (L). ***I. chimiliensis*** Comber; Sino-Himalaya, China; *Y.-M. Yuan CN2k-51* (NEU); DQ147824. ***I. chinensis*** L.; Sino-Himalaya, China; *A.F.G. Kerr 13757* (L); Yuan CN2k1-49 (NEU); DQ147825. ***I. chrysantha*** Hook. f.; Sino-Himalaya, Himalaya; *Inayat 25127A* (L). ***I. chungtienensis*** Y.L. Chen; Sino-Himalaya, Himalaya; *Y.-M. Yuan CN2k2-204* (NEU); DQ147826. ***I. ciliifolia*** Grey-Wilson; South India–Sri Lanka, Sri Lanka; *A.J.G.H. Kostermans 25471* (L). ***I. conchibracteata*** Y.L. Chen & Y.Q. Lu; Sino-Himalaya, China; *G. Hao 427* (NEU); DQ147829. ***I. cornucopia*** Franch.; Sino-Himalaya, China; *Sino-Amer. Bot. Exped. 448* (BR). ***I. crassicaudex*** Hook. f.; Sino-Himalaya, Himalaya; *Sino-Amer. Bot. Exped. 304* (BR). ***I. cristata*** Wall.; Sino-Himalaya, Himalaya; *Y.-M. Yuan & X.J. Ge CN2k3-15* (NEU); HE647190. ***I. cuspidata*** Wight & Arn.; South India–Sri Lanka, South India; *R. Morgan s.n.* (LV); DQ147832. ***I. cyanantha*** Hook. f.; Sino-Himalaya, China; *Y.-M. Yuan CN2k1-84* (NEU); DQ147833. ***I. cyathiflora*** Hook. f.; Sino-Himalaya, China; *P. Chassot & Y.-M. Yuan 99-29* (NEU); DQ147834. ***I. cyclocoma*** Miq.; Southeast Asia, Java; *de Voogd s.n.* (L). ***I. davidi*** Franch.; Sino-Himalaya, China; *Y.-M. Yuan CN2k-09* (NEU); DQ147835. ***I. delavayi*** Franch.; Sino-Himalaya, China; *P. Chassot & Y.-M. Yuan 99-154* (NEU); DQ147836. ***I. desmantha*** Hook. f.; Sino-Himalaya, China; *Y.-M. Yuan CN2k2-30* (NEU); DQ147837. ***I. dicentra*** Franch. ex Hook. f.; Sino-Himalaya, China; *Sino-Amer. Guizhou Bot. Exped. 582* (BR). ***I. diepenhorstii*** Miq.; Southeast Asia, Sumatra; *A.H.G. Alston 14054* (L). ***I. diversifolia*** Heyne; South India–Sri Lanka, India; *R.F. Hohenacker 275* (L). ***I. dolichoceras*** E. Pritz ex Diels; Sino-Himalaya, China; *Y.-M. Yuan CN2k1-80* (NEU); HE647191. ***I. drepanophora*** Hook. f.; Sino-Himalaya, China; *Y.-M. Yuan CN2k1-41* (NEU); DQ147838. ***I. duthiei*** Hook. f.; South India–Sri Lanka, Sri Lanka; *P. Vermeulen s.n.* (L). ***I. eberhardtii*** Tardieu; Southeast Asia, Vietnam; *Y. Song & N.L. Phuong 2004-10* (NEU); DQ147839. ***I. edgeworthii*** Hook. f.; Sino-Himalaya, Himalaya; *H.P. Nooteboom 3034* (L), 89.2005 (UC); DQ147840. ***I. elongata*** Arn.; South India–Sri Lanka, Sri Lanka; *C.F. van Beusekom & R.J. van Beusekom 15613* (L). ***I. eubotrya*** Miq.;

Appendix. Continued.

Southeast Asia, Sumatra; *W. Meijer 6065* (L). ***I. fenghwaiana*** Y.L. Chen; Sino-Himalaya, China; *Y.-M. Yuan CN2k1-78* (NEU); HE647192. ***I. fimbriata*** Hook. f.; Sino-Himalaya, Himalaya; *T.R. Chand 1866* (L). ***I. flaccida*** Arn.; South India–Sri Lanka, South India; *L.H. Cramer 5233* (L); *FB/S3925* (BR); DQ147845. ***I. flanaganae*** Hemsl.; Africa, South Africa; 19860179 (E); DQ147846. ***I. flavida*** Hook. f. & Thomson; Sino-Himalaya, Himalaya; T.R. Chand 3336 (L). ***I. forrestii*** Hook. f. & W.W. Sm.; Sino-Himalaya, China; *Y.-M. Yuan CN2k-79* (NEU); DQ147847. ***I. fragicolor*** C. Marquand & Airy Shaw; Sino-Himalaya, Himalaya; *Y.-M. Yuan & X.J. Ge CN2k3-14* (NEU); HE647193. ***I. fruticosa*** Leschen. ex DC; South India–Sri Lanka, South India; *Wight 329* (L). ***I. furcillata*** Hemsl. ex Forb. & Hemsl.; Sino-Himalaya, China; (BR). ***I. gongshanensis*** Y.L. Chen; Sino-Himalaya, China; *Y.-M. Yuan CN2k1-27* (NEU); HE647194. ***I. grandis*** Heyne; South India–Sri Lanka, India; *N. Wallich 4759* (BR). ***I. holocentra*** Hand.-Mazz.; Sino-Himalaya, China; *Y.-M. Yuan CN2k-54* (NEU); HE647195. ***I. hypophylla*** Makino; Sino-Himalaya, Japan; *M. Togashi 10048* (L). ***I. infirma*** Hook. f.; Sino-Himalaya, China; *Y.-M. Yuan CN2k2-60* (NEU). ***I. javensis*** Steud.; Southeast Asia, Java; *M.M.J. van Balgooy 5232* (L). ***I. kleinii*** Wight. & Arn.; South India–Sri Lanka, South India; *H.F. Mooney 8620* (L). ***I. laevigata*** Wall.; Sino-Himalaya; *W.N. Koelz 30264* (L). ***I. langbianensis*** Tardieu; Southeast Asia, Vietnam; *Y. Song & N.L. Phuong 2004-08* (NEU); HE647196. ***I. latifolia*** L.; South India–Sri Lanka, South India; *R.S. Rao 20076* (L), R. Morgan s.n. (LV), DQ147854. ***I. lecomtei*** Hook. f.; Sino-Himalaya, China; *Y.-M. Yuan CN2k2-202* (NEU), DQ147855.***I. leptoceras*** DC.; Sino-Himalaya, Himalaya; *T.R. Chand 2305* (L). ***I. leptopoda*** Arn.; South India–Sri Lanka, Sri Lanka; *A.J.G.H. Kostermans 25080* (L). ***I. leschenaultii*** Wall.; South India–Sri Lanka, South India; *K.N. Subramanian 629* (L), R. Morgan s.n. (LV), DQ147856. ***I. linearis*** Arn.; South India–Sri Lanka, Sri Lanka; *L.H. Cramer 5225* (L). ***I. linghziensis*** Y.L. Chen; Sino-Himalaya, China; *Y.-M. Yuan & X.J. Ge CN2k3-81* (NEU); HE647197. ***I. mackeyana*** Hook. f. subsp. ***claeri*** (N. Hallé) Grey-Wilson; Africa, Cameroon; Fischer NE21 (NEU), DQ147857. ***I. macrophylla*** Gardn. ex Hook. f.; South India–Sri Lanka, Sri Lanka; *A.J.G.H. Kostermans 24608* (L). ***I. masonii*** Hook. f.; Southeast Asia, Myanmar; *Maxwell s.n.* (L). ***I. mathildae*** Chiov.; Sino-Himalaya, Himalaya; *R.H. 20837* (L). ***I. mengtszeana*** Hook. f.; Sino-Himalaya, China; *E. Hennipman 3356* (L), *Yuan CN2k1-38* (NEU); DQ147858. ***I. mirabilis*** Hook. f.; Southeast Asia, Malaysia; *M.M.J. van Balgooy 2361* (L). ***I. nalampoonii*** Shimizu; Southeast Asia, Thailand; *T. Shimizu, H. Koyama & A. Nalampoon 12* (L). ***I. namchabarwensis*** R.J. Morgan, Y.M. Yuan & X.J. Ge; Sino-Himalaya, Himalaya; *CN2k3-70* (NEU); HE647198. ***I. napoensis*** Y.L. Chen; Sino-Himalaya, China; Y.-M. *Yuan CN2k1-61* (NEU); DQ147861. ***I. noei*** Craib; Southeast Asia, Thailand; *A.F.G. Kerr 10861* (L). ***I. noli-tangere*** L.; Temperate Eurasia; *Janssens SJ007* (LV); DQ147863. ***I. nyimani*** C. Marquand & Airy Shaw; Sino-Himalaya, Himalaya; *F. Kingdon-Ward 6067* (L). ***I. omeiana*** Hook. f.; Sino-Himalaya, China; *2002.0214* (UC); DQ147864. ***I. oncidioides*** Ridley ex. Hook. f.; Southeast Asia, Malaysia; *B.C. Stone 15110* (L). ***I. oppositifolia*** L.; South India–Sri Lanka, South India; *A. Kostermans 27999* (BR). ***I. pallida*** Nutt.; North America; *Janssens SJ008* (LV). ***I. parasitica*** Bedd.; South India–Sri Lanka, South India; *R. Morgan s.n.* (BR); HE647199. ***I. parviflora*** DC.; Sino-Himalaya, Himalaya; *Janssens SJ004* (LV); DQ147866. ***I. poculifer*** Hook. f.; Sino-Himalaya, China; *Y.-M. Yuan CN2k2-209* (NEU); DQ147870. ***I. pseudochinensis*** Shimizu; Southeast Asia, Thailand; *T. Smitinand, H. Sleumer & al. 4768* (L). ***I. psittacina*** Hook. f.; Southeast Asia, Thailand; *T. Shimizu & M. Hutoh T10228* (L). ***I. putii*** Craib; Southeast Asia, Thailand; *T. Shimizu, N. Fukuoka & al. s.n.* (BR). ***I. pallida*** Nutt.; North America; *Janssens SJ008* (LV); DQ147813. ***I. racemosa*** DC.; Sino-Himalaya, Himalaya; *M. Togashi 671* (L), *De Haas 2620* (L); DQ147873. ***I. racemulosa*** Wall.; Sino-Himalaya, Himalaya; *W.N. Koelz 30676* (L). ***I. radiata*** Hook. f.; Sino-Himalaya, China; *W.N. Koelz 31254* (L), *Yuan CN2k-77* (NEU); HE647200. ***I. rectangula*** Hand.-Mazz.; Sino-Himalaya, China; *Yuan CN2k1-26* (NEU); DQ147874. ***I. repens*** Moon; South India–Sri Lanka, South India; *Hortus Leiden 17953* (L). ***I. rubrostriata*** Hook. f.; Sino-Himalaya, China; Y.-M. Yuan CN2k1-44 (NEU), DQ147876. ***I. ruiliensis*** S. Akiyama & H. Ohba; Sino-Himalaya, China; *Y.-M. Yuan CN2k1-66* (NEU), HE647201. ***I. salicifolia*** Hook. f. & Thomson; Sino-Himalaya, Himalaya; *W.N. Koelz 28802* (L). ***I. sarawakensis*** T. Shimizu; Southeast Asia, Borneo; T. Shimizu, *N. Fukuoka & al. s.n.* (L.). ***I. scabrida*** DC.; Sino-Himalaya, Himalaya; *F. Billiet & J. Leonard 6510* (BR), *941314* (DBG); HE647202. ***I. scutisepala*** Hook. f.; Sino-Himalaya, China; *Y.-M. Yuan CN2k-56* (NEU); HE647203. ***I. siamensis*** T. Shimizu; Southeast Asia, Thailand; *A.J.G.H. Kostermans 1425* (L). ***I. siculifer*** Hook. f.; Sino-Himalaya, China; *Sino-Amer. Guizhou Bot. Exped. 471* (BR). ***I. singgalangensis*** Grey-Wilson; Southeast Asia, Sumatra; *V. Schiffner 2183* (L). ***I. soulieana*** Hook. f.; Sino-Himalaya, China; *Y.-M. Yuan CN2k2-163* (NEU); DQ147880. ***I. spirifer*** Hook. f. & Thomson; Sino-Himalaya, Himalaya; *W.N. Koelz 26035* (L). ***I. stenantha*** Hook. f.; Sino-Himalaya, Himalaya; *W.N. Koelz 31125* (L). ***I. stocksii*** Hook. f. & Thomson; South India–Sri Lanka, South India; *J.E. Stocks & J.S. Law s.n.* (L). ***I. subaequalis*** Craib; Southeast Asia, Thailand*; K. Larsen, T. Smitinand & E. Warncke 1624* (L). ***I. textori*** Miq.; Sino-Himalaya, Japan; *Kanno & al. 1114* (TUS). ***I. thomsonii*** Hook. f.; Sino-Himalaya, Himalaya; *F. Billiet & J. Leonard 6566* (BR). ***I. trichosepala*** Y.L. Chen; Sino-Himalaya, China; *Y.-M. Yuan CN2k1-68* (NEU); DQ147885. ***I. uliginosa*** Franch.; Sino-Himalaya, China; *Y.-M. Yuan CN2k2-173* (NEU); DQ147887. ***I. viscida*** Wight; South India–Sri Lanka, South India; *R. Morgan s.n.* (LV); DQ147891. ***I. walkeri*** Hook. f. ex Arn.; South India–Sri Lanka, Sri Lanka; *J. Dransfield 2318* (L). ***I. xanthina*** Comber; Sino-Himalaya, China; *Y.-M. Yuan CN2k1-15* (NEU); DQ147893. ***I. yingjiangensis*** S. Akiyama & H. Ohba; Sino-Himalaya, China; *Y.-M. Yuan CN2k1-55* (NEU); DQ147894.