Balsaminaceae in Southeast Asia: systematics, evolution, and pollination biology

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CHAPTER 1

Introduction

1.1. GENERAL INTRODUCTION

Balsaminaceae is a diverse plant family characterized by a huge floral morphological diversity. Its classification has changed many times throughout the taxonomic history of the group, owing to the emphasis on particular diagnostic characters. This suggests that patterns of character evolution are labile and do not contain much phylogenetic signal. This appears particularly prevalent among floral characters, suggesting that floral evolution and pollination ecology may be important drivers of diversification in the family. Further complications in taxonomy arise from the fact that one of the two genera of the family, *Impatiens*, has a very large number of species, and that it is distributed across several continents. This has led to a lack of taxonomic effort, especially those regions where very few studies have been done, despite large species diversity.

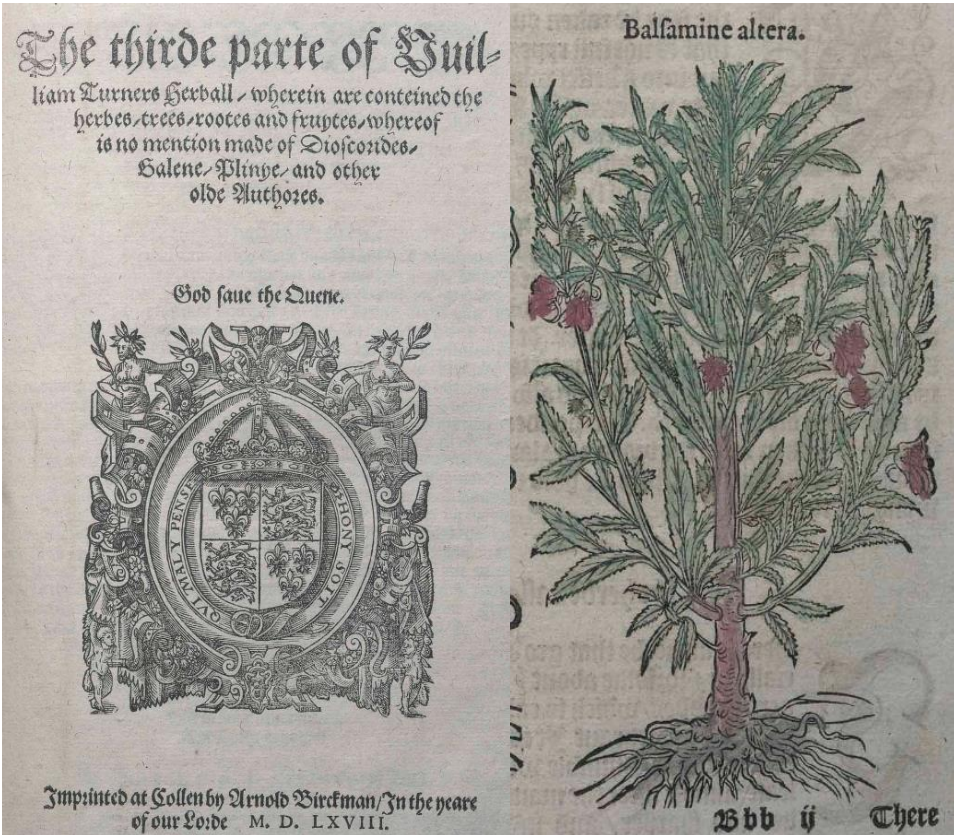
In this thesis, I filled particular gaps in knowledge of Balsaminaceae, focusing on issues related to the Southeast Asian region, including the taxonomic revision of this family in Myanmar (**Chapter 2-4**), molecular phylogeny and morphological character evolution of *Impatiens* sect. *Semeiocardium* (**Chapter 5**), comparative pollination biology of 7 sympatric *Impatiens* species in Thailand (**Chapter 6**), and evolution of corolla symmetry and pollination system of this family (**Chapter 7**).

Balsaminaceae A.Rich (1822) is a medium-sized eudicot family of watery semi-succulent herbs containing the genera *Hydrocera* Blume ex Wight & Arn. (1834) and *Impatiens* Riv. ex L. (1753). Whereas the first genus is monotypic with *H. triflora* Blume ex Wight & Arn. (1934) as a single representative, the latter is a highly diverse and species-rich genus with more than 1000 species (Grey-Wilson, 1980; Yuan *et al.*, 2004; Janssens *et al.*, 2009). Balsaminaceae can be easily identified by their fleshy explosive dehiscent fruits (except for *Hydrocera* which is characterized by a drupe), zygomorphic to asymmetric flowers, lateral united petals, a spurred lower sepal, connate anthers that form a cap over the stigma and serrate to crenate leaves with glands on the lamina base or petiole (Grey-Wilson, 1980; Chen, 2007; Byng, 2014; Ruchisansakun *et al.*, 2018b). Despite these typical characteristics of the family, exceptions can be found for nearly all characters, for example, spurless flowers can be found in some Madagascan and Asian species (Shimizu, 1970; Fischer & Rahelivololona, 2007). Recently, the phylogenetic position of the family was resolved by placing Balsaminaceae in the Ericales (Bremer *et al.*, 2002; APG III, 2009). Most *Impatiens* species occur in one of five informally recognized hotspots of diversity for the genus: tropical Africa, Madagascar, southern India and Sri Lanka, the Sino-Himalayan region and Southeast Asia (Yuan *et al*., 2004). Only a few species are found in Europe, and North and Central America. Similar to the orchid family, Balsaminaceae have a high horticultural value and, informally known as ‘Busy Lizzies’, are amongst the best-selling pot plants worldwide (USDA, 2016). Despite the large number of species in the family, only very few species have made it into cultivation; *I. hawkeri*, *I. walleriana*, and *I. balsamina*. Nevertheless there are still a lot of horticulturally promising species (Morgan, 2007). In contrast, some *Impatiens* species are also renowned for their impact to local environments as strongly invasive species; *I. walleriana, I. parviflora, I. edgeworthii and I. glandulifera* (Čuda *et al*., 2007).

1.2. THE TAXONOMIC HISTORY OF BALSAMINACEAE AND INFRAGENERIC CLASSIFICATION OF *Impatiens*

The name ‘Balsamine’ was used by Turner from the 16th century onwards (Fig. 1.1; Turner, 1568: 15). In 1753, Linnaeus published his Species Plantarum, including 7 species of *Impatiens*. Subsequently, many species have been continuously published. In 1859, based on the intensified study of Indian specimens, Hooker and Thomson described the floral morphology of Balsaminaceae based on 96 *Impatiens* species as well as *Hydrocera triflora* and constructed a key to the known species of that time in their publication ‘Praecursores ad Floram Indicam’. In 1875, Hooker published the Flora of British India including a key with additional samples (123 species and *H. triflora*). Hooker continued working on Balsaminaceae and updated a key for the Indo-Chinese balsams in the Flore Générale L’Indo-Chine for 25 *Impatiens* species and *H. angustifolia* (Hooker, 1911a). From the beginning of the 20th century onwards, other botanists started to become more interested in this family and published several new contributions to the systematics of the Balsaminaceae (e.g. Warburg & Reiche, 1895; Grey-Wilson, 1980a, b, 1989a, b, c, d; Fischer 2002, 2004, 2007). Currently, more than 1000 *Impatiens* species, and one *Hydrocera* species are accepted. Biodiversity studies on Balsaminaceae are ongoing, especially in the informal *Impatiens* hotspots (Fischer & Rahelivololona, 2016; Pusalkar & Singh, 2010; Souvannakhoummane & Suksathan, 2015; Wang *et al*., 2016; Janssen *et al*., 2018). Despite all these regional efforts to improve the worldwide taxonomy of

Impatiens, some areas remain under the radar, even though their geographical position may warrant investigation. An example of such a country is Myanmar.



The thirde parte of Wil-
liam Turners Herball/ wherein are conteined the
herbes/ trees/ rootes and fruytes/ whereof
is no mention made of Dioſcorides/
Galene/ Plinye/ and other
olde Authores.

God ſaue the Quene.

Imprinted at Collen by Arnold Birckman/ In the yeare
of our Lorde M. D. LXVIII.

Balſamine altera.

Bbb ij There

Fig. 1.1. The illustration of Balsamine (*Impatiens*) from Turner's herbal (1568) part 3:15. (Octavo Corp and the Chicago Botanic Library)

The position of Balsaminaceae in the angiosperms has long been contentious. For example, Bentham & Hooker (1883) included Balsaminaceae representatives within the family Geraniaceae. Furthermore, two names were given for the family, Hydroceraceae Blume (1825) and Impatientaceae Barnhart (1895), but were later synonymized to Balsaminaceae. The family has also been put in various orders: Terebinthales (Wettstein, 1924), Sapindales (Engler & Prantl, 1931), Geraniales (Hutchinson, 1959; Cronquist, 1981, 1988; Rouleau, 1981; Takhtajan, 1980; Thorne, 1981, 2000; Goldberg, 1986), and in its own order, Balsaminales Lindl. (1833) (Dahlgren, 1983, 1989; Takhtajan, 1997).

In addition to the difficult positioning of the Balsaminaceae within the angiosperms, its generic delimitation has been problematic and several generic names have been published in the past: *Tytonia* G.Don (1831), *Chrysaea* Nieuwl. & Lunell (1916), *Balsamina* Tourn. ex Scop. (1772), *Semeiocardium* Zoll. (1858), *Petalonema* Peter (1928), *Trimorphopetalum* Baker (1887) and *Impatientella* H.Perrier (1927). Each of these generic names have been put into synonym with either *Hydorcera* or *Impatiens* again afterwards. *Tytonia* was reduced to be synonyms of *Hydrocera*, whereas *Balsamina*, *Chrysaea*, *Impatientella*, *Petalonema*, *Semeiocardium*, and *Trimorphopetalum*, were reduced to be synonyms of *Impatiens*. As such, only *Impatiens* and *Hydrocera* are now accepted as valid names (Grey-Wilson, 1980a,1989).

As can be expected from the above, the subgeneric delimitation within *Impatiens* has remained controversial. The genus *Impatiens* is a strongly diversified genus with highly variable morphological characters that made it difficult to create a proper classification. Nevertheless, some effort has been made to arrive at an infrageneric classification. Specifically, 4 classifications with different subgenera have been proposed (*Caulimpatiens* Warb., *Acaulimpatiens* Warb., *Trimorphopetalum* (Baker) Eb.Fisch., and *Semeiocardium* (Zoll.) Utami). The first two - *Caulimpatiens* and *Acaulimpatiens* – were proposed by Warburg and Reiche in 1895 based on differences in stem structure, leaf arrangement, inflorescence organization and spur length. *Caulimpatiens* was regarded as the major subgenus and was characterized by opposite, whorled, or alternated leaves, and a long stem, while the small subgenus *Acaulimpatiens* was characterized by an alternate phyllotaxy, basally arranged leaves, short rhizomes, and a long peduncle (Warburg & Reiche, 1895). In 2002, Fischer and Rahelivololona established the third subgenus - *Trimorphopetalum* – including Madagascan species with spurless flowers. *Trimorphopetalum* was actually first published by Baker (1887) as a new genus with only one species: *T. dorstenioides* Baker. However, *Trimorphopetalum* Baker was reduced as synonym of *Impatiens*, and *T. dorstenioides* Baker was changed to *I. dorstenioides* (Baker) Warb. (Warburg & Reiche, 1895). The most recently erected subgenus is *Semeiocardium*, representing Southeast Asian species with a 4-locular ovary and connate lateral united petals. In 1858, *Semeiocardium* was initially established as a distinct genus by Zollinger. Zollinger (1858) and later also Backer (1935) distinguished the genus *Semeiocardium* Zoll. from *Impatiens* based on a large connate pair of lateral sepals enveloping the lower sepal and spur, connate lateral united petals, and a 4-locular ovary (Bleeker, 1858; Backer, 1935). In 1989, Grey-Wilson synonymized the genus *Semeiocardium* with *Impatiens* due to the unclear difference between the two genera and the non-exclusive occurrence

Table 1.1. The infrageneric classification of Impatiens based on each system.

Hooker & Thomson, 1859	**Hooker, 1875***	**Warburg & Reiche, 1895**	**de la Bathie, 1934**	**Yu *et al*., 2012**	**Yu *et al*., 2015**	**Akiyama and Ohba (2015a, 2015 b)**
				Clavicarpa	subgenus *Clavicarpa*	
				Calcareimontanae	*Semeiocardium*	
Racemosae	*Axilliflorae*	*Longecalcaratae, Brevicalcaratae*		*Racemus*	*Racemosae*	
					Fasciculatae	
		Brachycentron, Macrocentron		*Longifilamenta, Crena, Laxiflora*	*Impatiens*	
					Tuberosae	
				Scorpioid-cyma	*Scorpioidae*	
Uniflorae, Scapigerae, Oppositifoliae, Subverticillatae, Umbellatae et Capitatae	*Lateriflorae, Epiphyticae, Oppositifoliae and Verticillatae, Subumbellatae*	*Orchimpatiens, Scapimpatiens, Enanthiophyllon, Kathetophyllon, Choniochilon, Microcentron*		*Fusiform*	*Uniflorae*	
			Preimpatiens, Trimorphopetalum, Impatientella			*Sulcatae* *Jurpia* *Urticifoliae*

of character combinations within the genus. In 2009, Utami re-established *Semeiocardium* Zoll. as a subgenus stating that it differs from other balsams in having a 4-locular ovary and connate lateral united petals. In the most recent taxonomic treatment of *Impatiens,* based on results from molecular phylogenetic and morphological analyses, Yu *et al.* (2015) identified two subgenera; subgen. *Clavicarpa* S.X.Yu ex S.X.Yu & Wei Wang and subgen. *Impatiens*.

In the past, many section names have been assigned within the genus *Impatiens* (Table 1.1). In 1859, Hooker & Thomson published an infrageneric classification of *Impatiens*. They classified the genus into 8 sections mainly based on inflorescence types. In addition, Hooker (1875) published 5 more sections in the Flora of British India. In 1895, Warburg & Reiche proposed 14 sections as part of their two subgenera, thereby ignoring Hooker & Thomson's work of 1875. However, they also mainly used phyllotaxy, inflorescence, and spur characters. However, both Hooker's and Warburg & Reiche's classification were neither natural nor practical because of the limited number of species studied at that time as well as the highly variable homoplastic characters they used to base their classification on (Grey-Wilson, 1980). Perrier de la Bathie (1934) proposed 3 sections for the Madagascan *Impatiens* using the presence or absence of a spur and the different types of anther dehiscence. However, this classification was rarely followed by later authors and only appears to apply to Madagascan species. In 2012, Yu designated different section names for *Impatiens*. In 2015, Yu updated his classification by combining it with molecular phylogenetic analyses and previous classifications (Yu *et al*. 2015). Recently, Akiyama and Ohba (2015a, 2015b) published 3 new sections but without substantiation from molecular phylogenetic studies and seemingly not conform with the work of Yu *et al.* (2015).

1.3. MOLECULAR PHYLOGENETIC STUDIES OF BALSAMINACEAE

With the rise of molecular phylogenetic methods to infer phylogenetic relationships, it also became possible to infer the problematic position of Balsaminaceae within the angiosperms. In 1998, the Angiosperm Phylogeny Group (APG, 1998) was the first to confirm that Balsaminaceae belonged to the order Ericales. Later, Balsaminaceae was positioned near the families Marcgraviaceae and Tetrameristaceae, which were altogether referred to as the Balsaminoid Ericales (Soltis *et al*., 2000; Savolainen *et al*., 2000a, b; Schonenberger *et al.*, 2005). All families within the Balsaminoid Ericales have raphides in their parenchyma, which is rarely found in the order Ericales, and thus presumably constitutes a synapomorphy for this clade (Anderberg *et al.*, 2002).

Within the Balsaminoid clade, relationships remain undetermined to date. According to Bremer *et al*. (2002) and Anderberg *et al*. (2002), Marcgraviaceae is the sister group of Balsaminaceae and Tetrameristaceae, whereas Geuten *et al.* (2004) and Janssens *et al*. (2009) demonstrated that Balsaminaceae and Marcgraviaceae are sister groups instead. Micropylar endosperm haustoria are present in both these families and may hence be a synapomorphy, but this has been insufficiently studied in the other taxa in Balsaminoid Ericales (Anderberg *et al*., 2002).

The first molecular study focusing on Balsaminaceae was carried out by Fujihashi *et al*., in 2002 and focused on 25 Eastern Himalayan species that were analysed using *rbcL* and *trnL-F* sequences (Fujihashi *et al*., 2002). Due to the limited sampling and distant outgroup used, namely theTropaeolaceae, only few conclusions could be drawn from this study.

Yuan *et al.* (2004) studied the molecular phylogeny of Balsaminaceae based on nuclear ribosomal Internal Transcribed Spacer (ITS) sequences of 112 species representing both *Impatiens* and *Hydrocera*. They also included good coverage of the whole distribution range of the family with its five hotspots. Their results showed that Balsaminaceae is a monophyletic family. Unfortunately, the position of the genus *Hydrocera* within the family remained unclear as it fell in a basal polytomy with a large *Impatiens* clade and three other *Impatiens* species. Some lineages were resolved and well-supported, such as the monophyletic spurless Madagascan taxa (*Trimorphopetalum*). On the other hand, the resolution among some main lineages remained poor. ITS data are thus not adequate to resolve all phylogenetic relationships within the family and new markers need to be found for this purpose.

In 2006, Janssens *et al*. studied 86 accessions of Balsaminaceae and 6 outgroups using chloroplast *atpB-rbcL* and rDNA ITS sequences. Their study resulted in a well-resolved phylogeny with relatively high support for many nodes. The phylogeny based on *atpB-rbcL* data confirmed that *Hydrocera* was sister to all *Impatiens* species. Fifteen clades of *Impatiens* were identified in their analysis. *Impatiens omeiana* was sister to all other species. The remaining species were divided into two large clades which were both highly supported in the combined ITS and *atpB-rbcL* analyses. By comparing *atpB-rbcL* with the combined ITS and *atpB-rbcL* dataset, only a small number of dissimilar relationships between both phylogenies were present.

To retrieve more informative characters to better infer the interspecific relationships within *Impatiens* species, Janssens *et al*. (2007) tested the suitability of introns from the MADS-box gene family and proposed *ImpDEF1* and *ImpDEF2* as suitable markers for phylogenetic analysis of the Balsaminaceae family. The combined *atpB-rbcL*/*ImpDEF1*/*ImpDEF2* dataset contained 59 accessions and resulted in highly resolved Bayesian and Parsimony consensus trees which were similar to the independently analysed and combined *ImpDEF1*/*ImpDEF2* data sets.

Yu *et al*. (2015) published the most recent infrageneric classification of the genus *Impatiens* by combining morphological data with an improved phylogenetic analysis and integrating the previous classification by Hooker & Thomson (1859) and Warburg & Reiche (1895). According to this classification, *Impatiens* is divided into subgen. *Clavicarpa* S.X.Yu ex S.X.Yu & Wei Wang, characterized by a 4-locular ovary and the presence of a single ovule per locule, and subgen. *Impatiens* S.X.Yu ex S.X.Yu & Wei Wang, characterized by a 5-locular ovary (rarely 4) and the presence of multiple ovules per locule. Within subgen. *Impatiens*, seven sections are delimited (Table 1), mainly based on inflorescence, flower, and fruit characters.

1.4. CHARACTER EVOLUTION OF BALSAMINACEAE

To optimize the taxonomy of a group, studies of character evolution can be used to identify synapomorphies. Interestingly, from a vegetative point of view, there is not so much morphological variation in Balsaminaceae, whereas from a floral morphological point of view there is enormous variation present, especially within the corolla and the calyx. Here we briefly describe the major studies that have dealt with trait characterization within *Impatiens* and *Hydrocera* and their use for inferring relationships between species.

Lens *et al*. (2012) investigated the occurrence of secondary woodiness in the stems of Balsaminaceae species, using a combined wood anatomical and phylogenetic approach. Their study concluded that the degree of secondary woodiness in the stems of *Impatiens* evolved from less woody or clearly herbaceous species towards clearly secondary woody species. The evolution of secondary woodiness in *Impatiens* not only occurred once but multiple times. Another study on vegetative organs of *Impatiens* was carried out by Grey-Wilson (1980a). Based on morphological observations, Grey-Wilson (1980a) proposed an evolutionary trend of a rhizomatous-rootstock system evolving into a more tuberous-root habit. He also proposed a trend of spirally arranged leaves evolving into opposite or verticillate arranged leaves among the African lineages.

Studies that examined evolutionary trends in reproductive organs in Balsaminaceae are much more numerous than the ones carried out on vegetative parts. In 1980, based on morphological data, Grey-Wilson proposed that racemose inflorescences evolved to solitary or fascicled inflorescences, that 5-sepalous flowers evolved to 3-sepalous flowers by loosing the upper lateral sepals, that narvicular lower sepals evolved to bucciniform or saccate lower sepals, that free lateral united petals evolved to connate lateral united petals, and that the enlargement of the lower sepal evolved into diverse nectary-tipped spurs. However, his assumptions were mainly based on data of African species and none of these inferences took an explicit phylogenetic approach into consideration.

Using phylogenetic framework, Yuan *et al.* (2004) suggested that the spurless flowered Madagascan endemic clade evolved once from spurred taxa. This idea was later supported by phylogenetic analyses of Janssens *et al.* (2006, 2007, 2009).

In addition, Yuan *et al.* (2004) suggested that the linear-fusiform capsule and broadly-fusiform-fruit types of Balsaminaceae evolved from a drupe in *Hydrocera* (Janssens *et al.*, 2006).

In terms of palynology, triangular, tri-aperturate pollen grains with reticulate sexine ornamentation is considered to be the ancestral state in *Impatiens* (Janssens *et al.*, 2012). This pollen type evolved into a 4-aperturate, rectangular pollen grain with reticulate sexine ornamentation. Several reversions from 4- to 3-aperturate and changes from reticulate to microreticulate sexine ornamentation were found. The pollen shape is rather variable ranging from circular to quadrangular, elliptic, sub-elliptic and rectangular. In 2015, Yu *et al.* used an improved phylogenetic framework to analyse 46 different morphological characters which they used to set up a new infrageneric classification in *Impatiens*.

Despite previous work on character evolution, several questions remain, especially with regards to the evolution of floral morphology. For example, the connation of both lateral petals and lateral sepals, which was mentioned by Shimizu (1987), is in need of further study in order to better understand the evolution of the *Impatiens* flower. One particularly fascinating character is the presence of various forms of floral symmetry, which varies from zygomorphic to asymmetrical (Kato *et al.*, 1991). Given the different organs involved in the architecture of asymmetrical flowers, it would be useful to study this in a phylogenetic framework, to understand how often, and in which particular sequence, it has evolved.

1.5. POLLINATION BIOLOGY OF BALSAMINACEAE

Pollinator-driven evolution has been proposed to be one of the most important factors causing floral variation and species richness in angiosperm (Darwin, 1877; Stebbins, 1970). Balsaminaceae contains mostly allogamous species that have strongly protandrous flowers in which the stigma becomes receptive after the anthers have been shedded from the receptaculum (Ramasubbu *et al*., 2011; Sreekala *et al*., 2008, 2011; Mohandass, 2013). The lower sepal has a nectar-producing spur, functioning as a reward for animals (Grey-Wilson, 1980). The high level of variation in floral morphology, involving traits such as flower colour, spur length, and nectar production, is strongly suggestive of adaptation to specific pollinators (Bartoš *et al*., 2013; Wang *et al*., 2013), although few studies have implemented a comparative approach to jointly study floral morphology and pollination ecology. Better understanding of the pollination biology of *Impatiens* will most likely provide us with additional insights into the extremely high species number and diverse floral morphology. Moreover, it can contribute towards understanding how pollinators have driven the flower-morphological evolution in other angiosperm lineages.

In the landmark book "*Impatiens* of Africa", Grey-Wilson (1980) identified two types of *Impatiens* flowers, the flat and the funnel types. The "flat type flower" has a shallow lower sepal, a long filiform spur, and an erect, slightly concave, unhooded dorsal petal. Based on a small number of empirical observations, Grey-Wilson (1980) hypothesized that this type of flower would be pollinated by butterflies, which rest on the lower petal and probe the spur for nectar through the narrow opening (Grey-Wilson, 1980). During feeding, pollen may be deposited on the proboscis of the insect. The "funnel type flower" has a deeply navicular or saccate lower sepal and cucullate hooded dorsal petal. The pollinators of this flower type were thought to be (bumble) bees or birds. When bees probe the flower, they have to enter it by squeezing (part of) its body through the floral entrance, which is when their bodies contact anthers and stigma (Grey-Wilson, 1980). However, Grey-Wilson's hypothesis did not come from intensive field observations.

Some empirical pollination studies on *Impatiens* have been done, especially in Asian species (Rust, 1977; Schemske, 1978; Kato, 1988; Wilson, 1995; Tian *et al.*, 2004; Sreekala *et al*., 2008, 2011; Ramasubbu *et al*., 2011; Mohandass, 2013). Nevertheless, only a few studies were conducted in comparative context (Kato *et al.*, 1991), which precludes generalization of associations between floral types and pollinators. The pollination systems of *Impatiens* in Asia appears quite similar to that of North America and *I. noli-tangere* (the only European species) (Knuth, 1898; Rust, 1977; Schemske, 1978), but differs from that of Africa. The majority of studied Asian species is pollinated by bees (Kato *et al*., 1991; Tian *et al*., 2004), while birds and butterflies are thought to be the main pollinators in Africa (Grey-Wilson, 1980; Janecek *et al*., 2015). Moreover, there is still a lack of studies of representative from some major groups of *Impatiens*, for example, from subgen. *Semeiocardium*, and some areas, for example from continental Southeast Asia. In addition, the pollination systems of *Impatiens* has not been studied in the phylogenetic context. This impedes a thorough understanding of the role of pollinators in shaping, what is arguable the category of most variable organs of the large genus *Impatiens*, the flowers (Van der Niet & Johnson, 2012).

1.6. AIMS OF THE THESIS

Based on the above, there are clear gaps in our knowledge of Balsaminaceae systematics in terms of taxonomy, pollination biology, and evolution. Below I outline the aims and objectives of the research done in this thesis:

1) To revise the Balsaminaceae of Myanmar ***(Chapters 2-4)***

Biodiversity studies of Balsaminaceae are ongoing in many countries, especially in Asia. However, the species level taxonomy of this family in Myanmar has not been updated for a long time, even though Myanmar lies at the interface of two hotspots of Balsaminaceae diversity and therefore likely harbors a large number of species. To understand the diversity of Balsaminaceae in Myanmar, a revision is required. My objective is to update the taxonomy of Balsaminaceae using historical and modern collections, partly collected by myself during an extensive expedition to the country. The revision resulted in updated species delimitations, species descriptions, and provides accounts of phenology, ecology and the distribution range of all Myanmar taxa. I also constructed a dichotomous identification key.

2) *To test the validity of the large Southeast Asian sect. Semeiocardium* ***(Chapters 5)***

Recent infrageneric classification of *Impatiens* was published in 2015. However, the status and diagnostic characters of *Semeiocardium* are still problematic. This name has been used to recognize a genus, subgenus, and section, respectively, using a range of different diagnostic characters. All previous molecular phylogenetic studies of Balsaminaceae have included too few samples from Southeast Asia, the main distribution of sect. *Semeiocardium*, to address this problem. In this thesis, I test the monophyly of *I.* subgen. *Semeiocardium* (Zoll.) N. Utami and *I.* sect. *Semeiocardium* (Zoll.) S.X.Yu & Wei Wang, and reconstruct the character evolution of key traits characters to find suitable diagnostic characters, and refine the delimitation of this taxon. I added many Southeast Asian taxa to the phylogeny, including many representatives of the former *I.* subgen. *Semeiocardium*.

3) To study the relationship between pollinator types and floral morphology of Impatiens as well as to identify the relative importance of distinct processes of pollinator-driven floral evolution ***(Chapters 6-7)***

Extensive floral variation of *Impatiens* makes it an ideal study system for understanding the relationship between floral trait variation and pollination systems, and to analyse this in a phylogenetic context. Such a study may reveal how and why the diverse floral morphology of this plant group evolved. I therefore investigated the floral diversity of seven co-flowering *Impatiens* species in Southeast Asia, and evaluated whether this is associated with different pollination systems. I then extended this approach using a literature survey to identify all known pollination systems in *Impatiens*. These data were analysed in a phylogenetic context, to identify the number and direction of shifts in pollination system. Besides this, I also focused on the evolution of highly unusual asymmetrical flowers in the context of pollination biology.