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Author: Ruchisansakun, S.

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

Issue Date: 2018-09-19

CHAPTER 8

Conclusions

In this thesis, I present the results of my studies on taxonomy, systematics, and evolution of Balsaminaceae with a specific focus on Southeast Asian taxa. **In Chapter 1**, I reviewed the literature on taxonomy, molecular phylogenetics, and pollination biology of Balsaminaceae. **In Chapters 2-4**, I presented the results of a revision of Balsaminaceae of Myanmar, including the description of 4 new species. **In Chapter 5**, I combined results from molecular phylogenetic and morphological analyses to test the monophyly of *Impatiens* sect. *Semeiocardium* and to identify suitable diagnostic characters. **Chapter 6** features a study of the pollination biology of 7 co-flowering *Impatiens* species in Thailand, conducted to understand the associations between floral morphological diversity and pollination systems. Building on this, in **Chapter 7**, I combined pollination data with molecular phylogenetic analyses, to study distinct processes of pollinator-driven floral evolution of Balsaminaceae. Below I summarise the most important findings of each chapter and provide an outlook for future studies.

How many species of Balsaminaceae occur in Myanmar? How can they be identified and how do their morphology, ecology, and distribution differ?

Based on a taxonomic revision of the Balsaminaceae of Myanmar (**Ruchisansakun et al., 2018b, see Chapter 2**), using ca 1500 herbarium specimens and material I collected during an expedition to the country, I concluded that at least 66 species of Balsaminaceae are found in Myanmar: *H. triflora* and 65 *Impatiens* species. Four *Impatiens* species, *I. tanintharyiensis*, *I. decurva*, *I. oblongata*, *I. hartnolliae*, were newly described, and their phylogenetic position was shown (**Ruchisansakun et al., 2017, 2018a, see Chapters 3 & 4**). Twenty species were found to be new records for the country, seventeen names were typified, and seven species names were synonymized. Species and subspecies were treated by section cf. Yu et al. (2015) and an identification key was provided. For each species, a description of the morphology, phenology, ecology and distribution range was provided. Within the genus *Impatiens*, *I.* sect. *Uniflorae* is the most abundant with 35 species, followed by 18 species in *I.* sect. *Racemosae*, 8 species in *I.* sect. *Semeiocardium*, and 1 species in *I.* sect. *Impatiens*, *I.* sect. *Fasciculatae*,

and *I. sect. Scorpioidae*, reflecting the representation of these sections within the genus. Balsaminaceae are distributed throughout most of Myanmar, although they are concentrated in montane areas with only few species distributed in lowland areas. Most species in Myanmar have a terrestrial or lithophytic growth form, and no epiphytic taxa are known from this country.

Is the named taxon Semeiocardium monophyletic and should it be treated as subgenus or section? What are suitable morphological characters to diagnose members of Semeiocardium?

Throughout the taxonomic history of Balsaminaceae, many different infrageneric classifications of *Impatiens* have been published (see Chapter 1). The most recent, global classification was provided by Yu *et al.* (2015) and based on combining molecular and morphological data. Molecular phylogenetic results are often in disagreement with classifications based on morphological characters and these analyses may lead to updated taxon delimitation. In **Ruchisansakun *et al.* 2015 (see Chapter 5)**, I focused on *I. sect. Semeiocardium*, which is particularly abundant in Southeast Asia. I implemented phylogenetic analyses of DNA sequences from the nuclear (ITS) and plastid (*atpB-rbcL*) genomes, and included many samples from Southeast Asia. I clarified the status of *Semeiocardium*, a taxon recognized as a distinct genus, subgenus, or section in previous taxonomic treatments. I found that *Impatiens* subgen. *Semeiocardium* is not monophyletic and the definition of *I. sect. Semeiocardium* is ambiguous. I also reconstructed the evolution of several morphological characters. The previously used characters of *I. sect. Semeiocardium*. (Zoll.) S.X.Yu & Wei Wang are not adequate to identify each member of the monophyletic clade. Instead, I identified a new character – the presence of connate lateral united petals – as a taxonomically useful character to distinguish a monophyletic clade within *I. sect. Semeiocardium* and I suggested separating the clade from its sister clade comprising *I. stenosepala* and its allies.

Is diversity in floral morphology of Impatiens from Southeast Asia associated with different, specialised pollination systems?

Floral morphological diversity in *Impatiens* is tremendous (which may partly explain the taxonomic confusion by *Impatiens* taxonomists who each used different characters, including floral ones, in their treatments). The morphological variation is generally associated with the presence of different, specialised pollination systems. However, comparative field studies have been lacking so far. I found that floral variation among sympatric *Impatiens* species is associated with differences in

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functional pollinator groups and divergent use of the same pollinator (**Ruchisansakun *et al.* 2016, see Chapter 6**). The flowers of *I. muscicola* are small, spurless, and do not produce nectar. Experimental evidence showed that this species relies on autonomous self-pollination for reproduction. This species was the only one for which I found no significant difference in fruit and seed set between flowers from which insects were excluded compared to open-pollinated control flowers. Other species have spurred flowers which produce nectar. These species were visited by several animals, including bees, birds, butterflies and hawkmoths, but not all were effective pollinators. This conclusion is based on observations of the interaction between floral and pollinator morphology. Birds, for instance, only robbed the nectar but never pollinated any species in my study. Besides autonomous self-pollination, two animal pollination systems were found in the studied species, and floral characters significantly differed among all three pollination systems. Bee-pollinated species (*I. psittacina*, *I. kerriae*, *I. racemosa* and *I. daraneenae*) had flowers with short spurs and large floral chambers with a wide entrance. Bimodally bee- and lepidopteran-pollinated species (*I. chiangdaoensis* and *I. santisukii*) had long spurs and a small floral chamber with a narrow entrance. Within bee-pollinated species, I found floral variation that is indicative of species-specific pollen-placement on bee-bodies. Curiously, I confirmed a previous discovery that some species have asymmetrical corollas. *Impatiens kerriae* and *I. daraneenae* have asymmetric corollas which cause pollen placement on the left-hand side of the upper thorax and wing of the bee, and the right legs of the bee, respectively. The mechanism of floral asymmetry differed between these species. The floral morphology of *Impatiens psittacina* results in pollen placement on the dorsal thorax under the wings of bumble bees. I also studied nectar properties but did not find any significant difference in volume and sugar concentration among different pollination systems.

How do pollination systems and floral symmetry evolve?

In **Chapter 7**, I investigated the evolution of pollination systems, partly based on the findings of Chapter 6. To understand the evolution of pollination systems of the entire genus, I first expanded the scope to include as many species as were sampled in the phylogeny. These mostly included Asian species, but also species from other continents. The pollination systems of *Impatiens* can be categorised by floral morphology, consistent with the results from Chapter 6. However, spurless small-flowered species, autogamous in the study from Chapter 6, may be fly-pollinated in some cases. Moreover, African species, characterised by red flowers with a large floral entrance and short spur, are bird-pollinated. I further

categorized the corolla symmetry for each species. Data on the pollination system and floral symmetry were then analysed in a phylogenetic context. Ancestral character state reconstruction showed that bee-pollination is ancestral in Balsaminaceae and *Impatiens*. However, for corolla symmetry, the ancestral state of *Impatiens* and Balsaminaceae depends on the coding of the symmetry of the polymorphic species *Hydrocera triflora*. The pollination system shifted around 40 times: most of these shifts were from bee-pollination to bimodal pollination (bees and lepidoptera), or to autogamous/fly-pollination. The reverse shifts occurred only from bimodal pollination to bee pollination. These pollinator shifts were concentrated in a clade of *I. sect. Uniflorae* which includes the sampled species from Africa and Madagascar. Corolla symmetry shifted between zygomorphic and asymmetric around 20 times. In contrast to shifts among pollination systems, shifts in floral symmetry were concentrated in other clades, except *I. sect. Uniflorae* and *I. sect. Tuberosae*, including species which are ancestrally-bee-pollinated and mainly distributed in Asia.

Based on these results, I concluded that pollinator-driven evolution in *Impatiens* appears to occur through both pollinator shifts and divergent use of the same pollinator. Shifts in the pollination system appear mostly associated with colonisation of new areas, out of Asia, where bumble bees are not present. On the other hand, shifts in floral symmetry occur within areas where bumble bees may be the most abundant and available pollination niche. Pollinator-driven evolution does not appear to be the only process responsible for explaining morphological variation.

Future studies

My work on the taxonomic revision of Balsaminaceae in Myanmar has helped to clarify the status of some problematic species, especially in Thailand and neighbouring countries. However, to understand the diversity of Balsaminaceae of the entire continental Southeast Asia, taxonomic revision of the family in areas which have not received attention for more than 100 years needs to be prioritized. Such areas include countries such as Cambodia, Laos, Vietnam. Probably many new species can be discovered in these countries, which could help to optimise our phylogenetic insights if *Impatiens*.

The infrageneric classification of *Impatiens* is also in need of improvement. Some previous sections seem to be distinctively different from others and easy to identify. Studies which combine molecular phylogenetic analyses with morphological analyses of species in those sections, can answer whether those sections are monophyletic. To avoid taxonomic confusion, I do not recommend

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publishing any further sections, or other infrageneric taxa, without phylogenetic evidence or based on samples of a limited geographical area. Additionally, to reconstruct a new identification key, I recommend to use only characters which can be observed in the field.

Floral morphology can partially predict the pollination systems of *Impatiens*. For improved understanding and accuracy, field pollination observation of specific species still needs to be done; for example, it is unclear which pollination system occurs in *Impatiens* species with large flowers where the anthers and stigma are covered by fused petals. In some cases, it can be difficult to determine whether a visiting animal is an effective pollinator, like the visitation by day-flying hawkmoths. This may lead to an overestimation of the number of pollinating agents. In that case single-visit experiments could be useful to determine the effectiveness of different types of floral visitors.