Analysis of pollination syndromes and breeding systems in *Impatiens* (Balsaminaceae)

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Affirmation for the Master's Thesis

I herewith declare, that I have written this thesis independently and myself. I have used no other sources than those listed. I have indicated all places where the exact words or analogous text were taken from sources. I assure that this thesis has not been submitted for examination elsewhere.

Bonn, 30th November 2015

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Contents

Abstract

Pollination syndromes are known as convergent floral traits adapted to particular pollinators. *Impatiens* is one of the most species-rich genus with more than 1000 species characterized by morphologically highly complex flowers, adapted to different groups of pollinators. These characteristics might reflect an adaptation to different ways of pollination under the pollination syndrome concept. In this sense, the Balsaminaceae, which includes two genera: *Hydrocera* with one species, *H. triflora* and *Impatiens*, are a good candidate to test this hypothesis. To achieve this aim we performed a combination of multivariate analyses: a Principal Component Analysis (PCA) and a Cluster Analysis. First, we identified six floral clusters based on morphological and reward traits, and further, in combination with available pollinator data from the literature. From these clusters we identified five pollination syndromes: Long-tongued bees, larger birds, smaller birds, flies and butterflies. All of them show morphological characteristics and reward traits adjusted to the different pollinators. The family presents a different degree of specialization to the different groups of pollinators but a separation of the bird pollination in two clusters, suggest that subgroups of pollinators are present in the family. Additionally, we found a “mixed” group that includes non-specialized and very specialized species. All of them have relative small flowers with low nectar production accessible to more than one group of pollinator, but highly specialized species (*e.g.* to long-proboscis flies) as well. This high adaptation to zoophily is consistent in all the species and reflected in the breeding systems. We defined the breeding systems in the family based on fruit set (%) under two pollination treatments. An autogamy test to evaluate the production of fruits without pollen transfer and a geitonogamy test with hand pollination within the plant to simulate pollen transfer made by pollinators. Additionally, for some species we performed an extra pollination treatment, transferring pollen to a different population. We also evaluated if pollen/ovule ratio (P/O) is a good indicator for the breeding system in the family. The majority of the species are xenogamous or facultative xenogamous, however, autogamy is present as well. The majority of the autogamous, selfing species are fly-pollinated, which might suggest that these group of pollinators are not very efficient. However, these species are cleistogamous (occasionally open flowers) and endemic from Madagascar, suggesting that their cleistogamy condition is more related to their restricted distribution than to pollinators. Almost all groups of pollinators are distributed barely equitant between xenogamous and facultative xenogamous species. Since geitonogamy is a way of selfing, fruit production in both tests, indicate a self-compatibility condition. On the other hand, most of the xenogamous species did not produce fruits under any pollination treatment, suggesting a self-incompatibility condition. If so, it is possible that these species present a high degree of specialization to their pollinators. P/O ratio was useful to differentiate between autogamous selfing and xenogamous species; however, it did not resolve differences in the degree of outcrossing and self-compatibility. Even though floral longevity is strongly related to pollinators, we did not find substantial differences among pollinators groups. Flowers of autogamous species last shorter than the rest but they are influenced by the cleistogamous ones. We emphasize that this family is a good model to address different questions regarding sexual systems in plants. Adding data of distribution, biogeography and phylogeny would help to better understand its evolution and adaptation.

Key words: Balsaminaceae, *Impatiens*, pollination syndromes, breeding systems, P/O ratio, floral longevity.

Chapter 1

General Introduction

1.1 Background

Diversification and expansion of angiosperms in the mid- and late Cretaceous might have been endorsed by flower visiting insects and seed dispersing animals (Berendse and Scheffer 2009). The remarkable floral diversity and pollination mechanisms represent a series of adaptive radiations to different pollen vectors (Stebbins 1970). Many flower traits evolved in a convergent way and specialized according a particular group of pollinator (Fenster *et al.* 2004). This floral specialisation was explained under the concept of pollination syndromes, defined as correlated floral traits (size, shape, position of the flower organs, scent and color) gradual selected by a particular pollinator group (Faegri and van der Pijl 1979).

Typical combinations of floral traits are related to specific pollinators. For instance diurnal, tubular red or orange flowers with exposed stigmas and stamens in the New World are related to hummingbird pollination, whereas odorous, nocturnal flowers, displaying light-coloured corollas are related to bat pollination (*e.g.* Muchhala 2006). Even though the pollination syndrome concept has been successfully demonstrated in several systems (Ackermann and Weigend 2006; Smith *et al.* 2008; Reynolds *et al.* 2009; Große-Veldmann 2012; Murúa and Espíndola 2015), it has also been discussed and questioned by others (Ollerton 1998; Ollerton and Watts 2000; Ollerton *et al.* 2009). This concept is still an ongoing debate, which keep researchers' interest to study more plant groups that will support or reject the concept.

Most of the pollination mechanisms ensure cross-fertilization and a consequent genetic diversity and heterozygosity; but mechanisms that promote self-fertilization have arisen repeatedly as well (Stebbins 1970). Plants have evolved diverse methods to reproduce, known as mating or breeding systems (Richards 1996). These systems are rarely fixed and static (Loveless and Hamrick 1984). They respond to selection pressures, such as flower size, plant size, population size, degree of herkogamy, degree of dichogamy, flower colour, pollination system, and a variety of environmental factors (Loveless and Hamrick 1984).

Among angiosperms, *Impatiens* is one of the most species-rich genus with over 1000 species distributed primarily in the Old World tropics and subtropics (Grey-Wilson 1980b; Fischer 2004). *Impatiens* flowers are very diverse in shape and color. They produce nectar and its protandrous condition forces a separation in time (dichogamy) and space (herkogamy) between production of pollen in anthers and its reception on the stigma. Taking into account all these characteristics, flowers of *Impatiens* are well adapted to pollination by animals (zoophily) (Kato *et al.* 1989; Erpenbach 2006; Kulloli *et al.* 2009; Vervoort *et al.* 2011).

If we follow the pollination syndrome concept, the distinct floral traits in *Impatiens* should lead us to different pollination systems and therefore, to a "dependence" on pollinators to reproduce. In this sense, the genus *Impatiens* is a good candidate to test the pollination syndrome idea relating floral traits with pollinators, and to evaluate how efficient is the pollen transfer among different pollinators and to test if pollinators have an effect on the breeding system of the plant.

1.2 *Impatiens* as a model

1.2.1 Balsaminaceae Family

Balsaminaceae is a monophiletic family of the order Ericales in the basal Asterids and contains two genera: the prolifitic *Impatiens* and monotypic *Hydrocera* with only one species *Hydrocera triflora*(Grey-Wilson 1980b; Yuan *et al.* 2004; Yu *et al.* 2015). These two genera are not very easily distinguished with only vegetative characters, nevertheless *Hydrocera* has free petals and indehiscent berry-like fruit, whereas *Impatiens* has the petals differently united and a characteristic explosive dehiscent capsule fruit (Grey-Wilson 1980) (Figure 1.1).

1.2.2 Distribution

The genus *Impatiens* has more than 1000 species mainly distributed in the Old World tropics and subtropics, with diversity hotspots in tropical Africa, Madagascar, the Western Ghats in SW India and Sri Lanka, the eastern Himalayas, and south-east Asia (Grey-Wilson 1980b; Yuan *et al.* 2004) and high number of local endemics (Yuan *et al.* 2004). However, there are few temperate species occurring in the Northern Hemisphere in Europe, Russia, China and North America; and no native species in South America or Australia (Grey-Wilson 1980b; Yuan *et al.* 2004; Yu *et al.* 2015) (Figure 1.2). *Impatiens* originated in southwest China and started to diversify in the Early Miocene until the Early Pliocene, since then a rapid radiation occured originating 80% of all *Impatiens* lineages (Janssens *et al.* 2009) (Figure 1.2).

1.2.3 Floral diversity

The genus is characterized by its outstanding diversity in flower shape and color (Grey-Wilson 1980b; Yuan *et al.* 2004) making it difficult to classify into subgenera (Janssens *et al.* 2006). Due to the delicate flowers, descriptions of floral characters based on herbarium specimens are usually incomplete or ambiguous (Grey-Wilson 1980b; Yu *et al.* 2015).

The flowers are zygomorphic, with five sepals, one pair of ordinary sepals, other pair strongly reduced and one petal-like lower sepal modified into a spur structure that contains nectar in

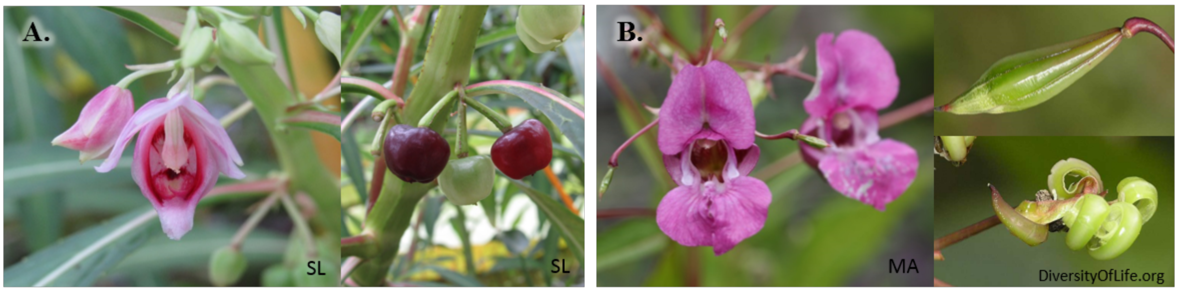

Figure 1.1: **Morphological comparison between the two genera of the Balsaminaceae family**. The species *Hydrocera triflora* has a berry like fruit (A) whereas all known *Impatiens* species have an explosive dehiscent capsule (B). *Impatiens glandulifera* is shown as example. Fotos: Sissi Lozada (SL) and Markus Ackermann (MA).

the majority of the species (Stevens 2001; Yuan *et al.* 2004). The spur exhibits a wide range of variation (Hooker 1882), from shallowly navicular to bucciniform or deeply saccate, to short or long filiform, straight, twisted or curved. In many Malagasy species the spur is completely reduced. Each flower has five petals, one upper dorsal usually hood-like and the other four are united into two lateral pairs. The latter are also very variable morphologically in shape and size (Yuan *et al.* 2004).

The genus present a huge variability in shape and color and has been considered as the "dicots counterpart of orchid" (Yuan *et al.* 2004). The high morphological diversity in *Impatiens* flowers is associated to different pollinator groups (Yu *et al.* 2015). For example, different petal shapes can provide a suitable landing platform or different color patterns can guide them to the spur and nectar and spur length and diameter is adapted to the pollinators' mouthparts. Previous studies with observations in the field identified five pollination modes: ornitophily (birds), sphingophily (moths), melitophily (bees), psycophily (butterflies) and myophily (flies) (Kato *et al.* 1989; Erpenbach 2006; Kulloli *et al.* 2009; Vervoort *et al.* 2011). However, these studies only represent 5% of the species and are strongly geographically biased to temperate and Asian species (see Figure 1.3 for some examples).

The flowers are hermaphrodite but stamens develop earlier than the ovary (protandrous), which confers two well-differentiated phases: male and female. Upon anthesis male phase begins, anthers are fused forming a unit that covers the stigma, start the production of pollen, and ends when filaments break and the whole unit falls off (Grey-Wilson 1980b). The stigma becomes receptive shortly afterwards and the female phase starts taking the same position as occupied by the anthers and therefore receptive to pollen from a separate flower (Figure 1.4) (Grey-Wilson 1980b).

1.2.4 Breeding systems and P/O ratio

Based on Richards (1996), we may expect to have a cross pollination mating system in *Impatiens*, because pollen donation (from anthers) and pollen reception (on stigma) is separated in time (dichogamy) and space (herkogamy) within the flower. The receptivity of the stigma after the anthers have fallen off (protandry) is considered a trait preventing self-pollination (Vervoort *et al.* 2011). Pollinators are an important factor that influence breeding systems

Figure 1.2: **Distribution of the genus *Impatiens*.**The genus is distributed worldwide with no native species in South America and Australia. There are few species in the Palearctic and the majority of species and endemism occur in tropical Africa, Madagascar, southwest India and Sri Lanka, the eastern Himalayas, and south-east Asia. Marks indicate the diversity hotspots (Stevens 2001).

(Charlesworth 2006); and their suitability depend on flower displays and rewards. Thus, reproductive success in *Impatiens* would be different according floral traits, pollinators and breeding system (self-compatibility).

Studies relating pollinator attractiveness and breeding systems have been rarely assessed for the majority of *Impatiens* species (Fischer 2004). In this sense, the aim of the present study is to determine the relation between floral traits and pollinator attractiveness, and their influence on the breeding systems in *Impatiens*. We assessed this objective analysing different floral traits such as morphology, nectar characters to infer pollination modes and fruit set to define breeding systems and relate this data with pollen and ovule number, pollen/ovule ratio, longevity, self-compatibility degree in around 80 species of the genus *Impatiens* and in *Hydrocera triflora*.

Figure 1.3: **Floral diversity of the genus *Impatiens***.The high morphological variability in *Impatiens*, has been related to pollinators and the accessibility of the reward (nectar). For example ***Impatiens noli-tangere*** **(A)** is a typical bee pollinated flower with a funnel shape, a landing platform and colored and wide opening to guide to the spur. ***Impatiens niamniamensis*** **(B)** is a bird pollinated species, flowers have usually vivid colors like red, are very stable, and have a saccate spur containing a lot of nectar. ***Impatiens platypetala*** **(C)** is a butterfly pollinated species with a broad colored frontal display and a long filiform spur. Moth pollinated species like ***Impatiens sodenii*** **(D)** do not differ morphologically from the butterfly ones, but they usually have longer spur and are very aromatic. Some species such as ***Impatiens campanulata*** **(E)** is pollinated for more than one group bees, butterflies and howkmoths. And there are some spurless species like ***Impatiens mandrakae*** **(F)** pollinated by flies, they are colorless, small and with no or few nectar production. Scale bar 0.5 cm and M. Ackermann (MA).

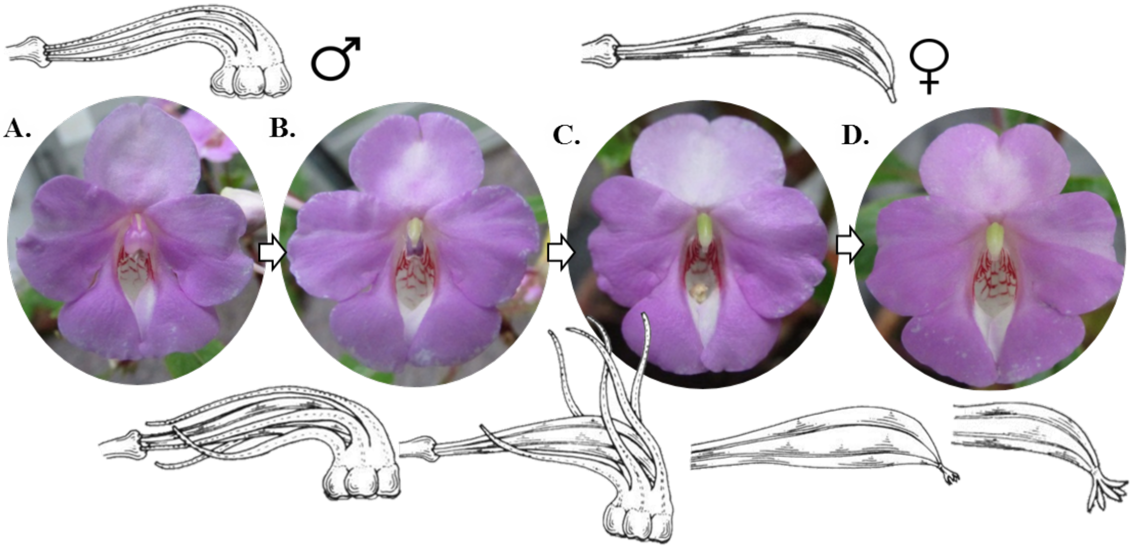

Figure 1.4: **Protandrous flowers of the genus *Impatiens*.** Flowers have five stamens united by the upper part of the filaments and completely cap the gynoecium. When the flower opens starts the male phase, where the five fused anthers produce pollen **(A)**. Later the stamens wilt **(B)** and the filaments break at base and all the unit falls **(C)**. Female phase starts when the ovary is free but not yet receptive, later stigma ripe and become receptive to pollen from other plant **(D)**. Example species: *Impatiens laurenti*. Squemes from Grey-Wilson (1980b).

Chapter 2

Pollination syndromes

2.1 Introduction

The association between floral diversification and pollinator groups has been extensively discussed under the concept of pollination syndromes since the mid-20th century. This idea began with Kölreuter (1761) and Sprengel (1793), who affirmed that floral features promote interactions between plants and pollinators. Later in 1862, Darwin argued that the evolution of floral diversity reflects pollinator-mediated selection. Delpino (1873-1874) proposed two distinct schemes for categorizing flowers according to traits, such as shape, colour, scent and size. Following Delpino's classification Faegri and van der Pijl (1979) categorized angiosperms into "syndromes" according to their pollinators.

The pollination syndrome concept is defined as a suite of floral traits, including rewards, associated with the attraction and utilization of a specific group of animals as pollinators (Vogel 1978; Faegri and van der Pijl 1979; Fenster *et al.* 2004). In this sense, eleven syndromes for plants pollinated by animals are identified based on Faegri and van der Pijl (1979) and Proctor and Yeo's (1973) descriptions: bat (chiropterophily), bee (melitophily), beetle (cantharophily), bird (ornitophily), butterfly (psycophily), carrion fly (sapromyiophily), fly (myiophily), hawkmoth (sphingophily), moth (phalaenophily), non- flying mammal and wasp. Each syndrome is characterized by floral traits, including color, size, morphology, position of the floral organs, floral scent, nectar (presence, composition and quantity) and timing of anthesis (Ollerton *et al.* 2009). Additionally, several subdivisions of the individual groups have been used, such as specialized and non-specialized birds in the Old and New World (Stiles 1978; Cronk and Ojeda 2008).

However, there has been a lot of debate trying to justify whether pollination syndromes do exist or not. Some studies have questioned the pollination syndrome's validity and demonstrated that this hypothesis does not successfully describe the diversity of floral phenotypes and is unreliable in predicting major pollinators (Ollerton 1998; Ollerton and Watts 2000; Ollerton *et al.* 2009). Waser *et al.* (1996) showed that several flowers are visited by many animal species, conferring them a widespread generalisation in pollination systems and making the pollination syndrome classification nonsense. In addition, Ollerton *et al.* (2009) on his global test of pollination syndromes showed that the accuracy of predictions differs across plant families, being more applicable in Fabaceae, Apocynaceae and Asteraceae. These authors argue that floral traits are taxon-specific and adaptations such as floral color, scent chemistry or pollen presentation are only mechanisms of floral evolution and patterns of floral ecology, and not universally recognized patterns to predict traditional pollination syndromes.

Nevertheless, it is important to emphasize that not all floral visitors are pollinators. Stebbins (1970) proposed *the most effective pollinator principle*, which affirms that floral features are adapted to those pollinators that visit flowers most frequently and effectively. Under this assumption, numerous studies support the pollination syndrome concept. Its validity has been demonstrated at different taxonomic levels, for example in *Loasa* (Ackermann and Weigend 2006), *Raphanus*, (Sahli and Conner 2006), *Silene* (Reynolds *et al.* 2009), *Iochroma* (Smith *et al.* 2008), *Calceolaria* (Murúa and Espíndola 2015), *Rhipsalis* (Große-Veldmann 2012), in representative species of Gesneriaceae (Martén-Rodríguez *et al.* 2009) and even at a broader attempts across angiosperms using different techniques, such as phylogenetics and meta-analysis (Smith 2010; Rosas-Guerrero *et al.* 2014). These last comparative studies evaluated the pollination syndrome concept quantitatively, demonstrating that convergent floral evolution is driven by adaptation to the most effective pollinator group.

Until now some effort has been carried out to test pollination syndromes, using different techniques and study systems. We consider the Balsaminaceae a good candidate to further test the validity of pollination syndromes, since the family possess a large number of species (more than 1000) with morphologically very diverse flower types. The flowers differ in shape, size and position of the different organs, coloration, UV pattern and various nectar traits, conferring them adaptation to different pollinators (see Figure 1.3). Additionally, five main pollinator groups have been identified: ornitophily (birds), sphingophily (moths), melitophily (bees), psycophily (butterflies) and myophily (flies) (Kato *et al.* 1989; Erpenbach 2006; Kulloli *et al.* 2009; Vervoort *et al.* 2011). Grey-Wilson (1980b) and Akiyama *et al.* (1985) classified different flower types according to flower morphology. However, this classification seems artificial since several morphological transitions between flower types as well as pollination by different pollinator groups within one species have been reported (Grey-Wilson 1980a,b; Tsukaya 2004). In this sense, it is unclear whether the flowers of the Balsaminaceae form a continuum spanned between different pollination syndromes or whether individual syndromes can clearly be separated from each other.

Therefore, the present study intends to test pollination syndromes in 87 species of *Impatiens* and *Hydrocera triflora*. Accessions representing different floral morphologies were studied to investigate possible differences in flower morphology and different nectar traits in cultivated plants under flower visitor exclusion. In the present study we pretend to respond to the question: Are there pollination syndromes in Balsaminaceae? And if so, which floral traits are the most important to define pollination syndromes and what is the relation to pollinators? In order to achieve this objective, here we present a novel approach using morphological and reward traits to test pollination syndromes with two multivariate analyses, a PCA and a Cluster Analysis. By this, we ensure that the pollinator groups truly explain the high complexity and morphological diversity of Balsaminaceae flowers. In a second step, we combine these results with independent pollinator observation data.

2.2 Methods

2.2.1 Plant material

All species belong to the *Impatiens* collection of the Botanical Garden of the University of Bonn. This collection comprises ca. 180 species, from which 87 species of *Impatiens* plus *Hydrocera triflora* were selected for the present study. Data compilation is the result of the work of different students between 2012-2015 (Jentsch 2013; Redling 2013; Span 2013; Krieger 2014). A standardized methodology was implemented to take the correspondent measurements under controlled conditions in absence of flower visitors.

2.2.2 Floral morphology

We measured six different morphological variables: spur length, sepal length, total length, opening, width and frontal length (see Figure A.1 for details). We took ten individuals per species for these measurements. Additionally, using Adobe Photographshop we measured frontal and lateral display size and we calculated four variables: frontal display, lateral display, total display: frontal+ lateral and quotient: frontal/lateral.

2.2.3 UV-light

Absorption of UV light was assessed in each species. Photographs were taken with normal light and with UV light using a UV filter. Different times of UV expose were used changing the camera obturator. We took photographs in front and lateral views. Presence and distribution of UV patterns were observed and analysed for all species.

2.2.4 Nectar measurement

To determine the amount of nectar we sampled 25 flowers per species during the end of the male phase/beginning of the female phase. Nectar was extracted from the spur using microcapillaries of 1, 5, 10 or 25 ml depending on flower size and nectar amount. For those species without spur we collected nectar from the united lateral petals using the 0.5 ml microcapillaries. For some small species this procedure did not work, so in order to assure the absence of nectar, we tested for the presence of glucose with glucose-testers Combur Test HC adding a drop of distilled water onto the lateral petals' surface. Total sugar concentration was measured with a portable refractometer and the amount of sugar per flower was calculated using the following formula:

$$\text{Sugar amount } [\mu l] = \frac{\text{Sugar } [\%] \times \text{Nectar amount } [\mu l]}{100\ [\%]}$$

2.2.5 Statistical analysis

In order to evaluate how morphological and reward traits are related and how they differ between the *Impatiens* species, we performed a Principal Component Analysis (PCA). This analysis converts correlated variables into a set of values of linearly uncorrelated variables called principal components without losing variance (Jolliffe 2002). Afterwards we selected the variables that explain better the distribution of species given by the PCA "Loadings" and we performed a Spearman correlation test among them. Subsequently, to identify pollination syndromes we performed a UPGMA (Unweighted Pair Group Method with Arithmetic Means) Cluster Analysis based on the Euclidean distances matrix generated by PCA. Cluster Analysis is used to identify groups of items that share similar characteristics (Kaufman and Rousseeuw 2005).

Before analyses of morphological and reward traits among pollinator groups, normality was tested by Shapiro test and homogeneity of variances by Levene test. Since no transformation was able to normalize non-normal data or get homogeneity of variances, we performed non-parametric tests. Thus, to evaluate the effect of the pollinator groups we performed a Non-parametric Kruskal Wallis test and a Pairwise Kruskal Wallis test for multiple comparisons. Means are given with their standard errors. Analyses were performed with a SPSS 17.0 and multivariate analyses were performed with R i386 version 3.0.2 using vegan package (R Development Core Team 2015).

2.3 Results

The three first principal components of the Principal Component Analysis explain 87% of the variance (Proportion of Variance: $PC1 = 0.62, PC2 = 0.14, PC3 = 0.11$) (see Table A.1 for details). On the component 1 there is a positive gradient explained by spur and total flower length spreading larger-flowered species on the right part of the graphic (Loading: Spur length = 0.565; Total length = 0.655); whereas on PC2 there is a positive gradient mostly explained by nectar amount (Loading: Nectar amount = 0.671) (Figure 2.1A). In PC3 sugar concentration is the most important variable but on a negative gradient (Loading: Sugar concentration = -0.780) locating species with higher concentration on the bottom of the figure (Figure 2.1B).

None of the component combinations showed a clear segregation of the species in groups. However, in both graphs we can observe some species, that do not look like the general *Impatiens* species (Figure 2.1 in dotted line). All of them have very small, spurless flowers and produce small amounts or no nectar. To define groups of species with similar characteristics we performed a Cluster Analysis based on Euclidean distances generated by the PCA taking into consideration the same 13 variables (Figure 2.2). In a second step we compiled pollinator records from the literature (Table 2.1) and we defined the clusters based on this pollinator information (Figure 2.2A-F).

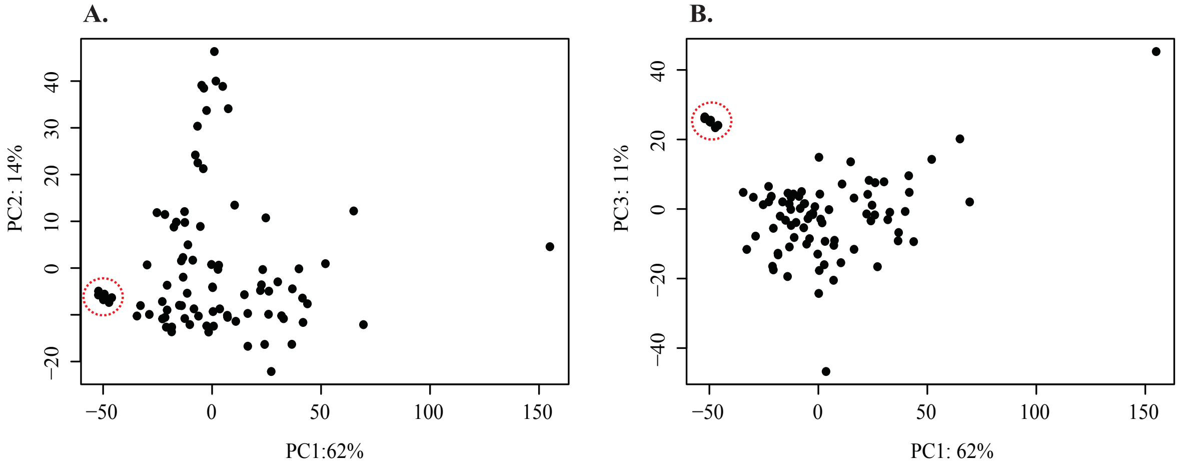

Figure 2.1: **Principal Component Analysis of 87 *Impatiens* species and *Hydrocera triflora* based on morphology and nectar traits.** We combined 13 traits including spur length, sepal length, total length, width, opening, frontal length; display size (frontal, lateral, frontal + lateral, frontal/lateral), nectar amount, sugar amount and sugar concentration. Each point represents a species and the proximity between points indicates similarity among species according the components. The first principle component displays the maximum variability of the data. A. Combination of component 1 and 2 where PC1 is better explained by length and PC2 by nectar amount. B. Plot combination of component 1 and 3 where PC3 is better explained by sugar concentration (negatively). Both combinations (A and B) show a continuum spreading of species differentiating only those without spur (in dotted lines), very small and with very less or no nectar production. Cumulative Proportion: PC1: 62%, PC2: 76%, PC3: 11%.

Table 2.1: **Confirmed pollinators of some *Impatiens* species identified from the literature.** We assumed that the recorded pollinators for some species are the typical pollinators of all species in that clade. Thus, we could identify six pollinator groups. However, the cluster “Long-tongued” bees is not ‘monophyletic‘ since *I. glandulifera* does not cluster with the other species pollinated by long-tongued bees.

CLADE	**CODES**	**SPECIES**	**REFERENCE**
Long-tongued bees	nol	*I. noli-tangere*	Kato (1998)
Larger birds	hia	*I. hians*	Janecek *et al.* (2015)
	nia	*I. niamniamensis*	Cheke and Mann (2008); Janecek *et al.* (2015)
Smaller birds	kei	*I. keilii*	Fischer (2004)
	pau	*I. paucidentata*	Fischer (2004)
Butterflies	tei	*I. teitensis*	Fischer (2004)
	usa	*I. usambarensis*	Grey-Wilson (1980b)
	wal	*I. walleriana*	Grey-Wilson (1980b)
	pla	*I. platypetala*	Kato *et al.* (1991)
Flies	ela	*I. elatostemmoides*	Erpenbach (2006)
	man	*I. mandrakae*	Erpenbach (2006)
Mix, (Short-tongued bees specialized flies, small butterflies)	burto	*I. burtonii*	Janecek pers. comm.
	hoc	*I. hochstetterii*	Potgieter and Edwards (2005)
	bis	*I. bisaccata*	Erpenbach (2006)
	cam	*I. campanulata*	Kulloli *et al.* (2009)
	par	*I. parviflora*	Coombe (1956); Vervoort *et al.* (2011)
Long-tongued bees	gla	*I. glandulifera*	Bahadur (1998); Beerling *et al.* (2012)

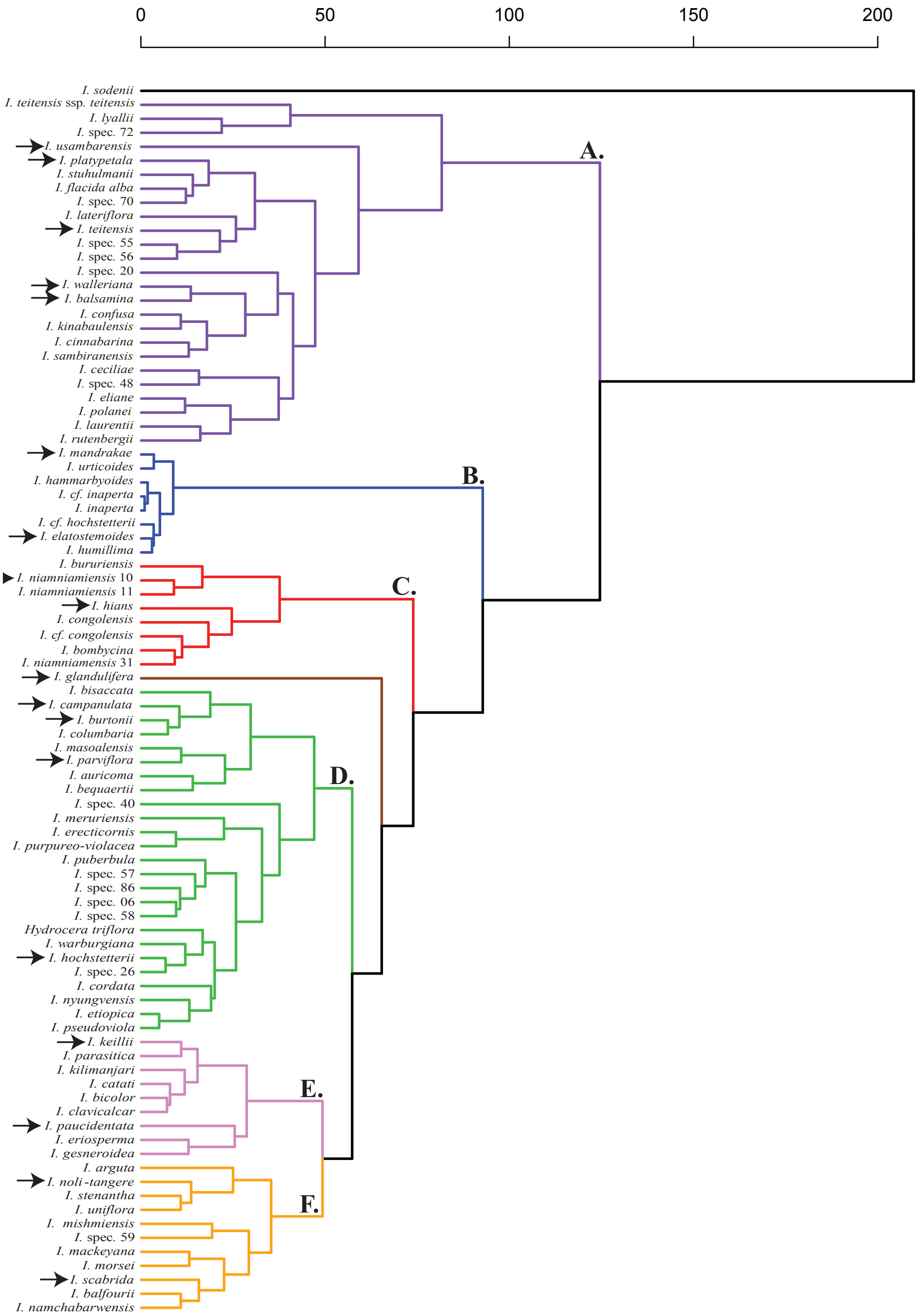

Figure 2.2: **Cluster Analysis based on Euclidean distances generated from the Principal Component Analysis.**.We combined morphometric measures (spur length, sepal length, total length, width, opening, frontal length), display size (frontal, lateral, frontal + lateral, frontal/lateral), nectar and sugar amount and sugar concentration to group the species with similar characteristics. Arrows indicates the species whose pollinators are known from previous studies. Taking into account this information, clusters were colored in and six groups were identified: **A.** Butterflies, **B.** Flies, **C.** Larger birds, **D.** Mix pollinators, **E.** Smaller birds, **F.** Long-tongued bees. It is important to notice that *Impatiens sodenii* and *I. glandulifera* each form their own clusters and because there are no other species to compare them to, they were not included on further analyses.

Table 2.2: **Relation among morphology and reward floral traits.** The variables that explain better the variance of the first three principal components are spur and total length for morphology and nectar amount and sugar concentration for flower rewards. Spearman non-parametric correlation tests were performed in all variables, correlation with $p \leq 0.05$ are shown in bold.

	Spur length	**Total length**	**Nectar amount**
Total length	$R^2 = 0.783$ $\boldsymbol{p < 0.001}$		
Nectar amount	$R^2 = 0.358$ $\boldsymbol{p < 0.001}$	$R^2 = 0.651$ $\boldsymbol{p < 0.001}$	
Sugar concentration	$R^2 = 0.211$ $\boldsymbol{p = 0.049}$	$R^2 = 0.192$ $p < 0.074$	$R^2 = 0.043$ $p < 0.692$

In addition, we performed correlations between the morphological and reward traits and we found a positive correlation between length (spur and total), and nectar amount (Table 2.2). The longer the flower, the more nectar is offered. On the other hand, sugar concentration is only weakly correlated with spur length (Table 2.2). Nectar production is positively correlated with sugar amount, but uncorrelated to sugar concentration (all correlations are shown in Figure A.3).

We identified six pollinator clusters: Long-tongued bees, smaller birds, larger birds, butterflies, flies and a mix group (Figure 2.2). In general, long-tongued bees (Apidae and Anthroporidae) specialize on flowers with deep corolla tubes, with a typical tongue structure usually longer than 5.5 mm can take easily nectar and feed faster from such large flowers than short-tongued bees (Hawkins 1969; Roubik 1992). Butterfly-pollinated species have pink/purple flowers with long and narrow spurs adapted to the animal proboscis. We defined "larger birds" based on the two species pollinated by relatively large sunbirds of the genus *Cyanomitra*, with a length up to 15 cm and weight up to 20 g (Hoyo *et al.* 2008). Unfortunately, the references of the other cluster do not specify the genus of the sunbird. However, *Impatiens humblotiana*, a morphologically similar closely related species of *I. eriosperma* (included in our cluster) is pollinated by the *Souimanga* sunbird (Baillon 1880; Salomonsen 1934; Hawkins 2002). Thus, it is likely that the other species from this cluster are pollinated by similar, small (sun) birds. For these reasons we called the other cluster "smaller birds". The "fly" cluster is based on small flies found as pollinators in *I. elatostemoides* and *I. mandrakae*. Even though the other species of the cluster are very different morphologically, based on the floral size and morphology it is conceivable that they are also pollinated by flies. Finally, the "mixed" cluster is the most heterogeneous one including species pollinated by short-tongued bees, specialized flies or small butterflies or by a combination of these groups.

The butterfly-pollinated species (Figure 2.2A) have salver flowers with a large landing platform formed by the lateral petals. The filiform spurs (3.74 ± 0.48 cm) as well as the entire flowers (5.5 ± 0.5 cm) are significantly longer compared to plants pollinated by other pollinator groups (Figure 2.8A). They present the highest frontal display size (7.02 cm^2) (Figure 2.9B), which is also reflected by the front length (3.48 ± 0.3 cm) and width (3.83 ± 0.31 cm), both significantly different compared to plants pollinated by other pollinator groups (Figure 2.8D-E). UV light show a contrast in front view with a UV adsorption in the center where the opening and the stamens and ovary are located, maybe as a signal leading to the spur with nectar (Figure 2.3). In lateral view petals and spur reflect UV light (Figure 2.3A, C, D). Butterfly-pollinated species produce 7.84 ± 3.96 μl of nectar, containing 2.17 ± 1.14 mg of sugar, and the nectar has a of sugar concentration of 28%.

Figure 2.3: **Example of species that are pollinated by butterflies (Figure 2.2A).** Display of morphometric characteristics and UV light contrast of some butterfly-pollinated species. Photographs were made with normal and UV-light in front (right) and lateral view (left). **A.** *Impatiens usambarensis*, **B.** *I. flaccida-alba*, **C.** I. spec. 36248, **D.** I. spec. 35170. Species have different colors show a wide front display and a long spur that can be a little curved. There is not much UV light absorption, only on the central part close to the stamens. Scale bar: 1 cm. (Photographs: V. Krieger: A, C, D; S. Lozada: B)

Fly-pollinated species (Figure 2.2B) are very small in flower size, displayed by the smallest front length (0.4 ± 0.08 cm), width (0.3 ± 0.06 cm) and opening (0.1 ± 0.03 cm) (Figure 2.8D-F). In consequence, total display size (0.32 cm^2) is significant smaller than in plants pollinated by other pollinator group (Figure 2.9C). Only *Impatiens cf. hochsteteri* has a short spur (0.01 ± 0.03 cm; Figure 2.4D) but no nectar production was detected. The other fly-pollinated species are spurless (Figure 2.4A-C), showing a significantly smaller total flower length (0.3 ± 0.07 cm). Nectar production was detected only in *I. elatostemoides* (0.03 ± 0.02 μl) and *I. mandrakae* (0.02 ± 0.02 μl)(Figure 2.10A, B), but sugar concentration was not possible to quantify due to the small amount of nectar produced. For the other species we assured the absence of sugar by glucose testers (Figure 2.10C). UV light absorption was observed in the central part of the lower and dorsal petals (Figure 2.4A, B), more located on the opening (Figure 2.4C), or covering both (Figure 8D). For the following species *Impatiens inaperta, I. cf inaperta, I. hammarbyoides* and *I. humillima* UV photographs of open flowers were not possible to take because they mostly produced closed flowers (cleistogamous).

Bird-pollinated species have a saccate flower shape, present vivid colors, mostly red and orange (Figure 2.5) and are very stable with thick pedicels. However, they are divided in two groups (Figure 2.2C, E), mainly differentiated by flower size and nectar production. Plants pollinated by "larger birds" have significantly longer sepal (2.74 ± 0.21 cm) (Figure 2.8B), allowing them to keep significantly more nectar (38.01 ± 22.19 μl) than plants pollinated by

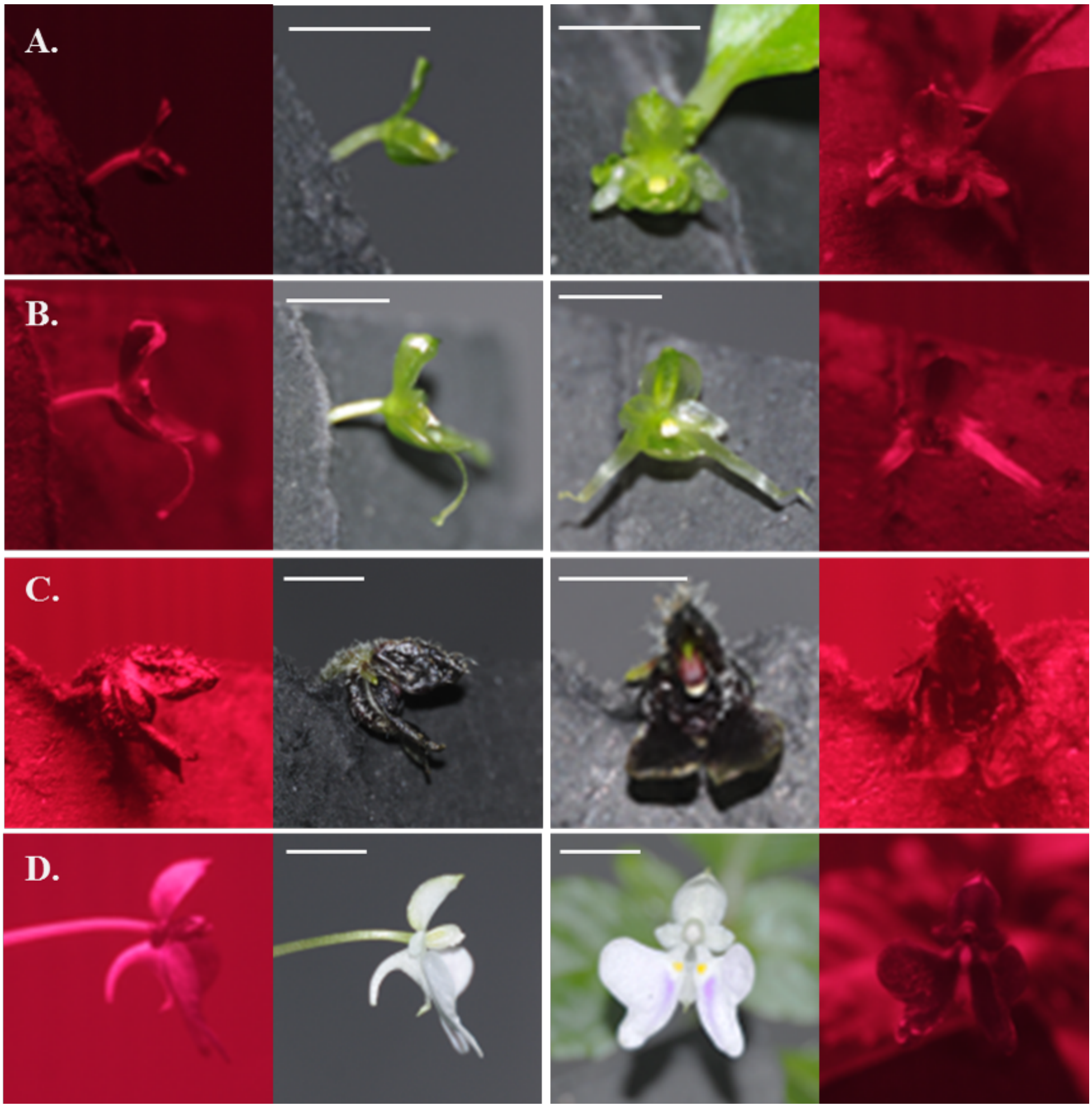

Figure 2.4: **Example of species that are pollinated by flies (Figure 2.2B).** Display of morphometric characteristics and UV light contrast of fly-pollinated species. Photographs were made with normal and UV-light in front (right) and lateral view (left). **A.** *Impatiens elatostemoides*, **B.** *I. mandrakae*, **C.** *I. urticoides*, **D.** *I. cf. hochstetteri*. Species belonging to this cluster are very small and do not present vivid colors. They are spurless (except *I. cf. hochstetteri*) and produce small amounts to no nectar. Some species such as *I. inaperta* are mainly cleistogamous, thus photographs of open flowers were not possible to take. There is UV light absorption in the center especially on the inferior petals. Scale bar: 5 mm (Photographs: V. Krieger: A, C, D; S. Lozada: C).

"smaller birds" (38.01±22.19 μl). These species also offer more sugar (Larger birds 8.37±5.02 mg, smaller birds 3.75±3.13 mg). This pattern however is not reflected in sugar concentration (Larger birds 22±3%; smaller birds 24±4%) (Figure 2.10C). There is a big difference between frontal (Larger birds 2.24 cm^2; smaller birds 1.89 cm^2) and lateral display (Larger birds 3.25 cm^2; smaller birds 2.04 cm^2), but they are not significantly different from long-tongued bees (Front 3.09 cm^2; lateral 3.31 cm^2), and "mix" (Front 3 cm^2; lateral 1.47 cm^2). There is a big contrast in UV light absorption between the lower petals and the opening in the front view, but only reflection in lateral view (Figure 2.5).

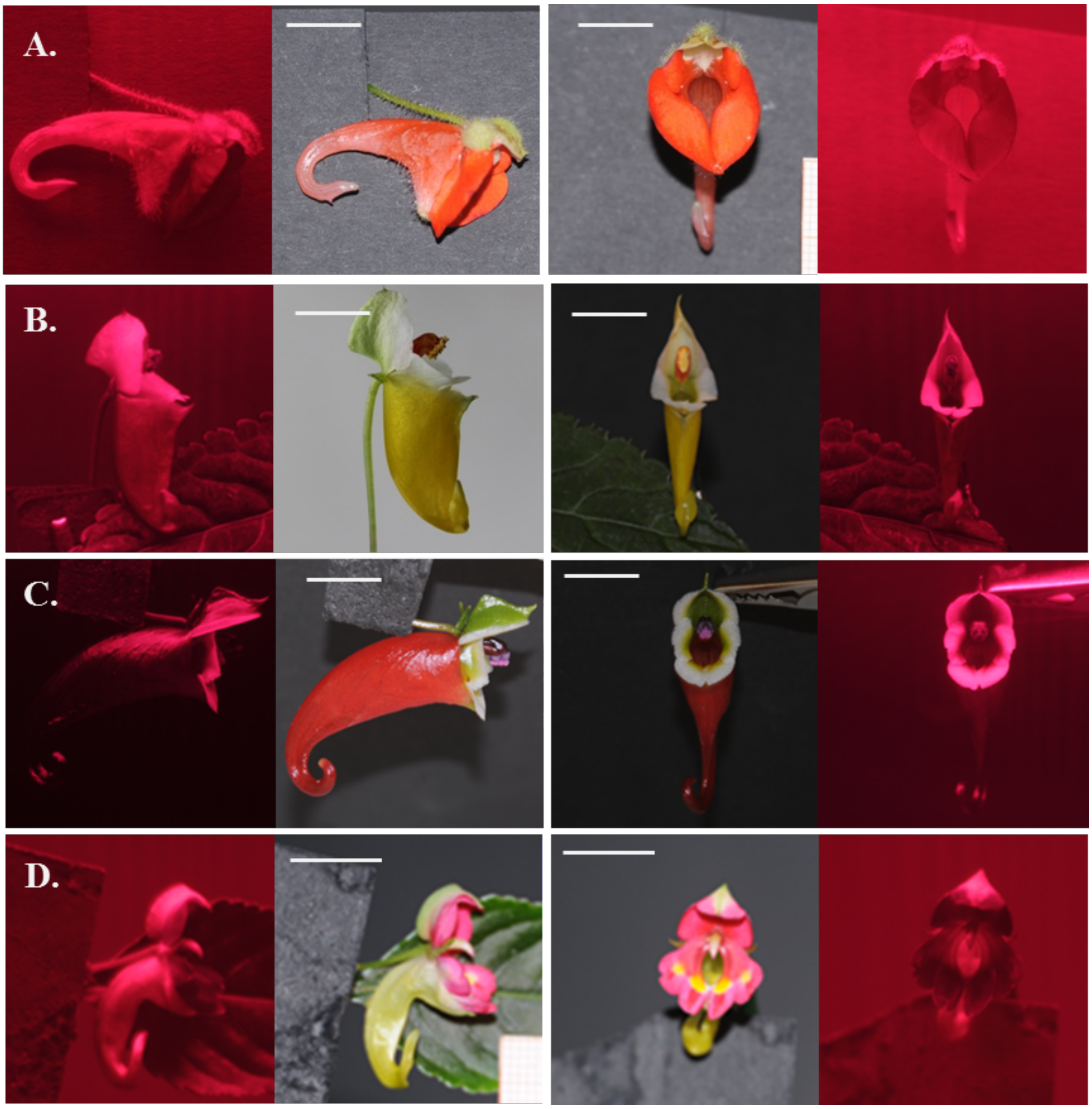

Figure 2.5: **Example of species that are pollinated by larger (A, B) and smaller birds (C, D); (Figure 2.2C, E).** Display of morphometric characteristics and UV light contrast of fly-pollinated species. Photographs were made with normal and UV-light in front (right) and lateral view (left). **A.** *Impatiens bururiensis*, **B.** *I. congolensis*, **C.** *I. paucidentata*, **D.** *I. kilimanjari*. Species have relatively large, vividly colored flowers. They present a small front display and a broad lower sepal containing nectar. There is a UV contrast on the on the lower petal and some species show UV absorption and others UV reflection. Scale bar: 1cm. (Photographs: S. Lozada: A; J. Span: B, C; V. Krieger: D).

Species pollinated by long-tongued bees (Figure 2.2F) have a funnel type flower with a landing platforms built by the lower lateral petals and the spur usually located in straight direction from this platform (Figure 2.6). The opening is significantly larger (0.75 ± 0.1 cm) compared to species pollinated by other pollinator groups, except larger birds (0.41 ± 0.3 cm) (Figure 2.8F). There is UV absorption inside the funnel, which might indicate the nectar location. Nectar production (0.5 ± 0.2 μl) is similar to that in butterfly-pollinated species (0.78 ± 0.4 μl)(Figure 2.10A), but those pollinated by long-tongued bees have the highest sugar concentration ($41 \pm 4\%$) among all groups (Figure 2.10C).

Figure 2.6: **Example of species that are pollinated by long-tongued bees (Figure 2.2F).** Display of morphometric characteristics and UV light contrast of fly-pollinated species. Photographs were made with normal and UV-light in front (right) and lateral view (left). **A.** *Impatiens noli-tangere*, **B.** *I. uniflora*, **C.** *I. arguta*, **D.** *I. morsei*. Species have a funnel flower type with a large opening and a landing platform. There is UV light absorption, only on the central part inside the funnel showing the direction to the spur. Scale bar: 1cm. (Photographs: S. Lozada: A; V. Krieger: B, C, D).

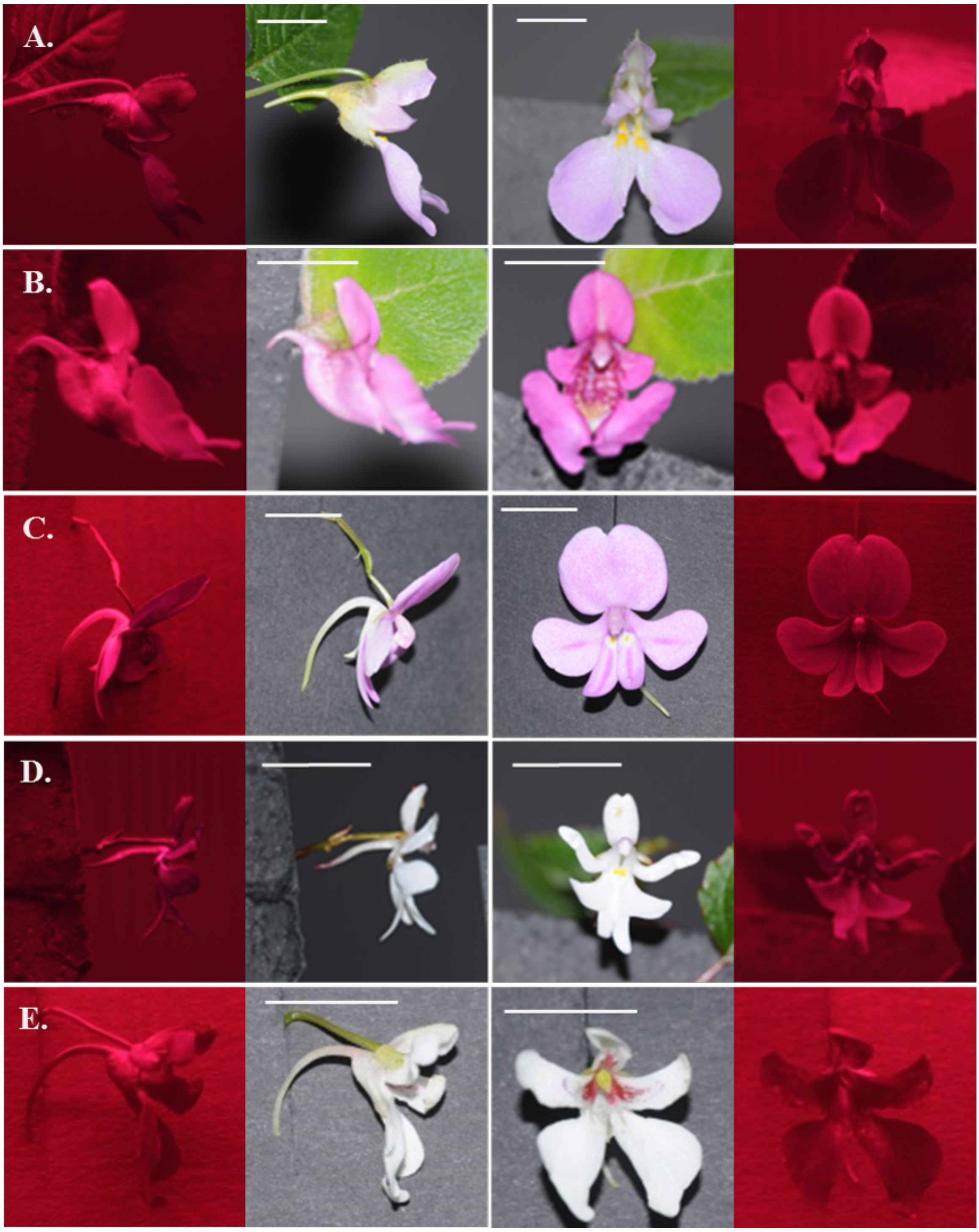

Figure 2.7: **Example of species that are pollinated by the "mixed" group (Figure 2.2D).** Display of morphometric characteristics and UV light contrast of fly-pollinated species. Photographs were made with normal and UV-light in front (right) and lateral view (left). **A.** *Impatiens burtonii*, **B.** *I. columbaria*, **C.** *I. pseudoviola*, **D.** *I. bequaertii*, **E.** *I. pinganoensis*. Species have different colors and shapes. Most of them have small flowers and a short spur. There are different UV light contrasts on both views. Scale bar: 1 cm. (Photographs: V. Krieger: S, B, D; S. Lozada: C, E).

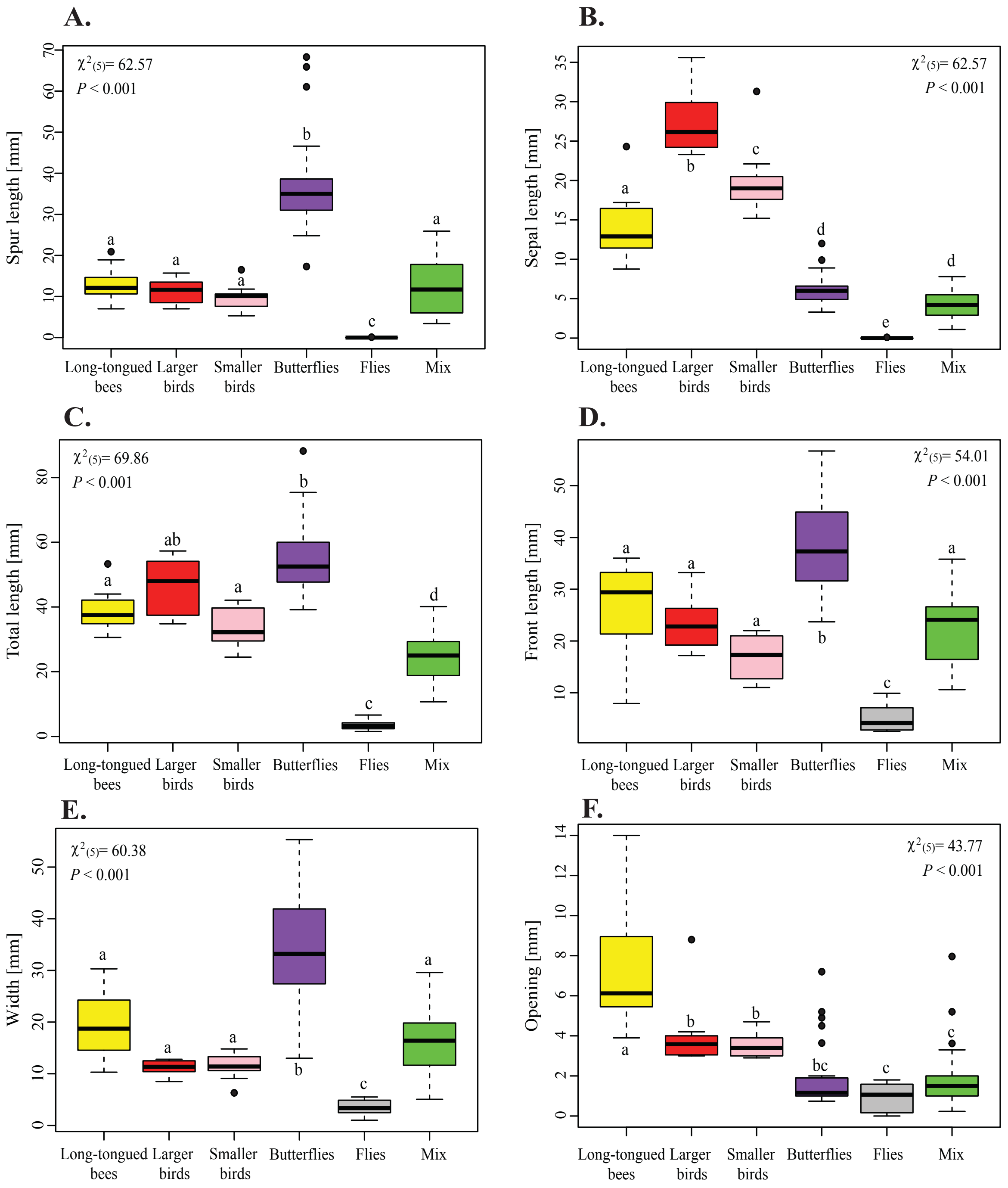

Figure 2.8: **Comparison of morphometric flower measures among pollinator groups.** We tested six morphometric variables: Spur length **(A)**, sepal length **(B)**, total length **(C)**, frontal length **(D)**, width **(E)** and opening **(F)** among the pollinator groups (N: Long-tongued bees: 11, larger birds: 8, smaller birds: 9, butterflies: 25, flies: 8, mix: 25). Variables were analysed by a Kruskal Wallis non-parametric test. Distinct letters indicate significant difference in traits ($p \leq 0.05$; Pairwise Kruskal Wallis test for multiple comparisons).

Species that are pollinated by short-tongued bees, small butterflies, specialized flies or a mixture of these pollinators ("mixed" group; Figure 2.8D) present heterogeneous flower traits. Flowers of individual species are similar to those of plant pollinated by different other groups (Figure 2.7). Nevertheless, all flowers are relatively small, with a total length (2.4 ± 0.3 cm) (Figure 2.8C), nectar amount (0.24 ± 0.15 μl) and sugar amount (0.06 ± 0.03 mg) that differs significantly from all other pollinator groups (Figure 2.10A, B). Photographs show a variable UV absorption in front view, in lower and dorsal petals (Figure 2.7A, E), only in signals close to the center (Figure 2.7C, D) or inside the opening (Figure 2.7B).

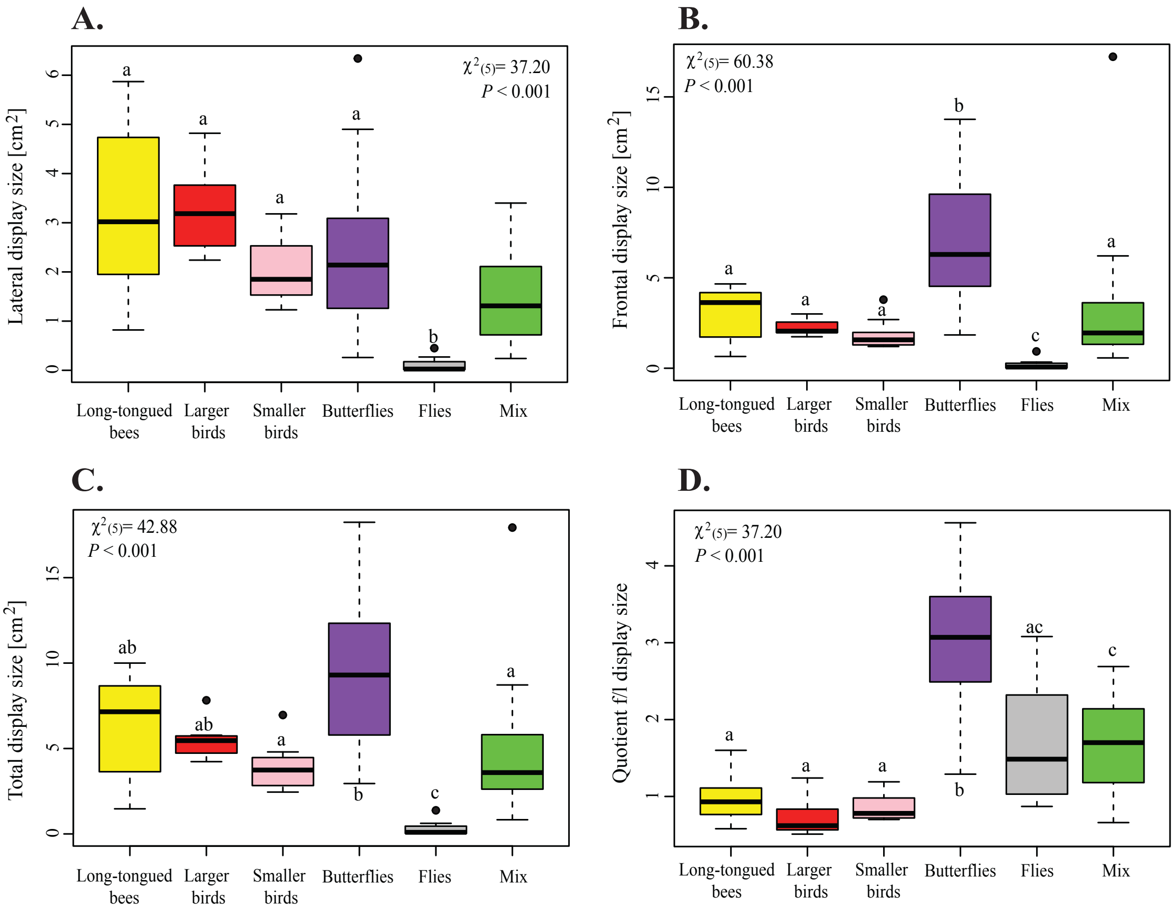

Figure 2.9: **Comparison of display size measures among pollinator groups.** We tested four display variables: lateral **(A)**, frontal **(B)**, total (lateral + frontal) **(C)** and quotient (frontal/lateral) **(D)** among the pollinator groups (N: long-tongued bees: 11, larger birds: 8, smaller birds: 9, butterflies: 25, flies: 8, mix: 25). Variables were analysed by a Kruskal Wallis non-parametric test. Distinct letters indicate significant difference in traits ($p \leq 0.05$; Pairwise Kruskal Wallis test for multiple comparisons).

However, two species *Impatiens sodenii* (sod) and *I. glandulifera* (glan) do not belong to any cluster. *Impatiens sodenii* (Figure A.2A) possesses similar characteristics to those species pollinated by butterflies (Figure 2.3), but with the longest spur recorded 12.84 ± 6 cm and a very large frontal display size, indicating hawkmoth pollination. *Impatiens glandulifera* (Figure A.2B) has the highest sugar concentration measured in this study with 63.8±5 %. Even though previous studies indicate that this latter is pollinated by bees, our cluster analysis located it outside the "long-tongued bee" clade. Since these two species do not have similar species to compare and analyse, were not taken into account for further comparison analyses according pollinator groups.

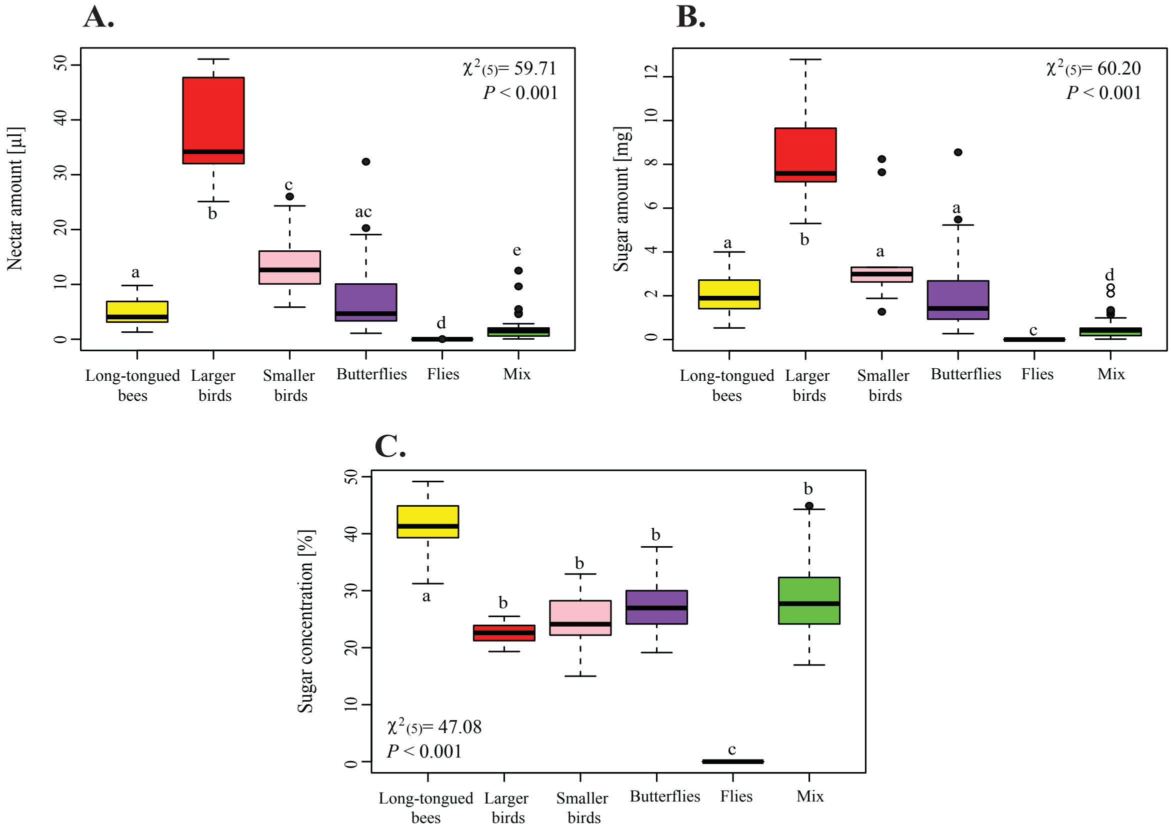

Figure 2.10: **Comparison of nectar measures among pollinator groups** We tested three variables: nectar amount **(A)**, sugar amount **(B)**, sugar concentration **(C)** among the pollinator groups (N: Long-tongued bees: 11, larger birds: 8, smaller birds: 9, butterflies: 25, flies: 8, mix: 25). Variables were analysed by a Kruskal Wallis non-parametric test. Distinct letters indicate significant difference in traits ($p \leq 0.05$; Pairwise Kruskal Wallis test for multiple comparisons).

2.4 Discussion

In this study, we evaluated the existence of pollination syndromes in Balsaminaceae. To achieve this goal we tested the correlation and clustering of floral morphology and nectar as the principal reward offered, in 87 species of *Impatiens* and in *Hydrocera triflora*. Altogether, our results support the existence of pollination syndromes in the family, indicating that a combination of morphological and reward traits can be used to predict different groups of pollinators.

2.4.1 Morphology and nectar traits support pollination syndromes

Flowers adapted to pollination by animals like bees, butterflies, moths, birds, and bats present a highly specialized structure, with all parts precisely adjusted and adapted to animal bodies (Stebbins 1970). However, to define pollination syndromes, rewards are very important floral traits too (Faegri and van der Pijl 1979). Since the main reward offered by *Impatiens* is nectar we included it in the analysis and discuss its importance in relation to floral morphology and pollinators. Reward traits, particularly nectar amount, were essential to define the pollination syndromes in *Impatiens*.

Nectar is related to flower morphology and pollinator attraction. Correlation between nectar production and flower or nectary size have been previously recorded (*e.g.* Witt *et al.* 2013 in Caryophyllaceae, Galetto and Bernardello 2004 in *Ipomoea*), and now confirmed for *Impatiens* (Table 2.2). Additionally, nectar act as a selective mediator to attract pollinators; difference in volume, concentration and chemistry determine a high diversity of nectar consuming animals (Nicolson 2007). This difference, involving nectar amount and sugar concentration has been previously documented, for instance in *Loasa* (Ackermann and Weigend 2006), in *Scrophularia* (Rodríguez-Riano *et al.* 2014), in *Sinningia* (Forcone *et al.* 1997; Perret *et al.* 2001). In *Impatiens* we found a relation between nectar and sugar amount in relation to the body size of the potential pollinators (Figure 2.10A, B), however this tendency was not the same regarding sugar concentration (Figure 2.10C).

Flowers pollinated by long-tongued bees present a typical funnel shape with a big opening and a landing platform allowing the insect to enter the flower to get the nectar (Figure 2.6) (Hatcher 2003). Additionally, these flowers are characterized by having the highest sugar concentration (Figure 2.10C). Despite of eleven morphologically similar *Impatiens* species were consistently cluster inside this group (some examples in Figure 2.6), we found literature supporting that *I. glandulifera* is pollinated by bumblebees and bees as well (Table 2.1); but our cluster analysis located it outside the long-tongued bees group (Figure 2.2). This separation might be explained by the unusual high sugar concentration ($63 \pm 5\%$) found. However, its location is not easy to explain since there are no other species on the cluster to compare with.

Flowers pollinated by butterflies normally possess relatively large landing platforms. Butterflies are equipped with an elongate proboscis and feed from narrow filiform spurs of *Impatiens*. Both, butterfly and moth-pollinated flowers have narrow floral tubes or spurs but differ in color and scent (Ehrlich and Raven 1964). Moth-pollinated flowers usually open at night, have pale-colored petals and a strong floral scent (Faegri and van der Pijl 1979; Johnson and Steiner 2000; Whittall and Hodges 2007). Since we did not include color or scent analysis, there is a possibility that some moth-pollinated species were hidden inside the butterfly cluster. However, we found one species forming its own cluster: *Impatiens sodenii* (Figure A.2A). This species is mainly white flowered and presents the longest spur (12.84 ± 6 cm) and a very large frontal display size (19.5 cm^2) suggesting a hawkmoth-pollinated system. Since few *Impatiens* species with similar characteristics were identified (Grey-Wilson 1980b), to confirm this, it would be necessary to record the pollinator observations and perform scent analysis.

Nectar amount is correlated with the body size of pollinators and sugar concentration is related to their energetic requirements (Nicolson 2007; Witt *et al.* 2013); which could explain the similar amount of nectar found in long-tongued bees and butterflies species but a significantly higher sugar concentration in bee pollinated species (Figure 2.10A, C). Schmitt (1980) argues that higher sugar concentration in bee- compared to butterfly-pollinated species, can be explained by the distinct behaviour while foraging; butterflies thermoregulate by basking and have a relatively low foraging; their energy cost is low; whereas bees invest more energy in foraging.

2.4.2 Bird pollinated species

Flowers adapted for pollination by birds are usually odorless, have vivid colors (red or orange), tubular perianths and abundant and dilute nectar (Stiles 1978; Faegri and van der Pijl 1979). Due to birds diversity in size, morphology and foraging behavior, this syndrome is usually sub-divided according different bird groups or even species (Stiles 1978). In the Old World there is a distinction between plants specialized to sunbirds and plants with a generalized bird pollination (Johnson and Nicolson 2008). Plants pollinated by sunbirds that feed mainly on nectar, produce ca. 10-30 μl of nectar, 15-25% of sugar concentration and high sucrose contents. On the other hand, plants pollinated by a wide range of unspecialized birds produce more nectar (40-100 μl) and very low sugar concentration (8-12%) (Johnson and Nicolson 2008).

Even though there is a distinction in sepal length (Figure 2.8B) and nectar and sugar amount (Figure 2.10A, B) between our two clusters (Figure 2.2C, E), nectar amount and sugar concentration of both groups match the general broad description of adaptation to sunbirds. Additionally, we observe that the flowers of all species are very stable, their pedicels are very resistant and flowers do not fall easily, like the rest of *Impatiens* species. The separation of two bird groups is mainly supported by the difference of nectar amount between the two clusters, which we think fits with the size of pollinators, suggesting a possible sub-division inside this pollinator group in *Impatiens* based on bird size. Nevertheless, in order to confirm this, more work of sunbird species and their preferences on foraging is required.

2.4.3 The spurless from Madagascar: Fly pollinated species?

The so-called "fly" pollinated species (Figure 2.4) are cluster together due to its tiny flower size, the missing or very short spurs (Figure 2.8A) and low amount or absence of nectar (Figure 2.10A). All of them, (excluding *Impatiens cf. hochstetteri*), come from Madagascar. Unfortunately, from these Madagascan species, pollination biology was previously studied only in *I. mandrakae* and *I. elatostemoides* (Table 2.1) and flies belonging to the suborder Nematocera were recorded as pollinators (Erpenbach 2006). However, these two latter species look very different morphologically from the others (Figure 2.4) and are the only ones that produce tiny quantities of nectar, 0.03±0.02 μl in *I. elatostemoides* and 0.02±0.02 μl in *I. mandrakae.*

On the other hand, *Impatiens urticoides* present a peculiar morphology. The tiny, dark chocolate brown flowers with a dark reticulation on lower sepal (Figure 2.4C) are hidden by the pubescent leaves. Since Diptera are usually attracted to flowers by a combination of visual and olfactory stimuli, it is possible that scent are emitted by the flower to attract them (Woodcock and Larson 2014). Maybe these flowers use a deceiving system to attract pollinators due to their morphological, absence of nectar and maybe olfactory characteristics that could remind brood substrate.

It is also known that flowers offer secondary reward like warmth, protection or shelter to flies (Woodcock and Larson 2014). Due to the funnel-shaped flowers and personal observations of flies basking in the tropical rainforest of Madagascar by Abrahamczyk (2015), it is possible that flies use flowers for physical protection from adverse weather conditions. We do not know whether this species offer a reward or use a deceitful way, there are many unsolved questions to investigate more about the ecology of Madagascan species. More intense field observations and detail scent analyses are needed.

During the time of the study, the flowers of *Impatiens hammarbyoides, I inaperta, I. cf. inaperta* and *I. humillima* remained closed, suggesting a cleistogamous condition. It is important to remark that *Impatiens cf. hochstetterii* during the time of study (summer 2015), did not produce open flowers or nectar. However, we cannot classify it as a cleistogamous species because previous observations showed normal flowers. Since it is well know that nectar production and opening are affected by many factors including temperature (Bahadur 1998; Comba *et al.* 1999), we think that the high temperature of last summer affected this species. These did not happen with the previous described cleistogamous species.

2.4.4 Mixed group: generalized species?

Although we found good differentiation into different pollination groups (Figure 2.2), there is one cluster where this relation is not very clear (Table 2.1), the so-called "mixed" group. Species belonging to this cluster have small to medium-sized flowers, with a spur length and width similar to long-tongued bee-pollinated species but a small opening like butterfly-pollinated species (Figure 2.8A, F). Nectar and sugar amount is significant lower than in bee- or butterfly-pollinated species but sugar concentration does not differ from bird- or butterfly-pollinated species (Figure 2.10). These combination of characteristics suggest that these species are not very specialized, but could be adapted to more than one group of pollinators.

In *Impatiens burtonii* (Figure 2.7A) and *I. campanulata* (Figure 1.3E) bees, butterflies and flies were recorded as pollinators (Kulloli *et al.* 2009) (Table 2.1). Taking into account the floral structure adapted to different pollinator we could classified this species as "classical" generalist species following the concept of Ollerton *et al.* (2007). These authors affirm that "generalist" flowers possess open access to the reward (nectar) providing more or less equally good pollination services (ecological and functional generalization). Perhaps we could also include *I. columbaria* (Figure 2.7B), *I. ethiopica*, and *I. briarti* in this category because of their similar floral traits. Since more than one pollinator group was recorded in several other species (*e.g. Impatiens coelotropis* (Sreekala *et al.* 2008b), *I. grandis* (Kulloli *et al.* 2010), *I. henslowiana* (Sreekala *et al.* 2007), it is possible that there are more "classical" generalist species in the genus. It would be interesting to include them on the analysis and see their position on the cluster this could support our hypothesis.

On the other hand, we must be very careful classifying the rest species as "generalist" as well. Even though the majority of them look very similar morphologically, there are some species where only one pollinator group was observed, small bees in *I. bisaccata* (Erpenbach 2006), syrphid flies in *I. parviflora* (Vervoort *et al.* 2011), and long-proboscis flies in *I. pinganoensis*; (B. Ditsch, pers. obs). These species seem to be very specialized and suggest that several other species of this cluster might have a similar degree of specialization.

Certainly, this "mixed" group is very complicated and must be handled carefully. Due to its heterogeneity, we do not consider this cluster as a syndrome. We hypothesise that inside it, there are some "generalist" species adapted to many groups of pollinators as well as some "specialized" ones, such as flies with long proboscis. However, these speculations can only be solved with an increment of taxa sampling and pollinator observations in the field.

2.4.5 UV light and color

Even though several authors affirm that floral color is related to pollinators and therefore important to define pollination syndromes (Daumer 1958; Faegri and van der Pijl 1979; Arikawa *et al.* 1987; Lunau and Maier 1995; Hirota *et al.* 2012), we did not include color into the analysis due to its categorical condition. However, more important than color, are the so-called "nectar guides" which reflect UV light and are very sensitive to the compound eyes of bees and butterflies (Arikawa *et al.* 1987). We observed contrast of UV-light in all groups located in the center between the front petals and the opening. This seems to be well-conserved signals indicating the location of the nectar.

2.5 Conclusion

Our study support the pollination syndrome concept using the Balsaminaceae family as a model. A combination of multivariate analysis (PCA and Cluster Analysis) and the incorporation of morphological and reward trait were essential to elucidate the pollination syndromes in the family. Such combinations seem to very useful for morphologically highly complex flowers, in which it is not clear that the measured morphological traits really reflect adaptations to pollinators. The proposed model can help us to get an idea about the main pollinator group in a data set, in which for some species no pollinator observations are available.

Due to the rapid radiation, species richness and high degree of endemism, Balsaminaceae is a good example of adaptation and specialization. We identified five pollination systems supporting the pollination syndromes concept. However, it is also true that the family present a different degree of specialization and "generalized" species occur as well. In addition, it is also possible that more groups or subgroups of pollinators are present in the family. The present study is the basis for understanding the family but there is still a lot of work to do. We highlight the importance to continue and increase investigations in a broader scale, adding for instance, phytogeography, ecology and phylogenetic information that would help to comprehend the complexity of this family and the reason of its high diversification and adaptation to different environments.

Chapter 3

Breeding Systems

3.1 Introduction

Unlike most animals, plants cannot choose their mates; nevertheless they have diverse methods to reproduce, known as mating or breeding systems (Richards 1996). Mating is the union of gametes, where a male gametophyte (sperm) find a female (egg) and a zygote develops; but there are many mating mechanisms and they vary among plants (Willson 1983). Lower plants, such as terrestrial algae, mosses and ferns, have actively motile sperms that require a film of water to find an egg, whereas seed plants have packaged up their sperm into pollen grains that protect it from desiccation and provide cellular material important for the zygote development (Willson 1983).

In seed plants, both pollination and fertilization are part of the mating, which combined create an effectively infinite array of genetic diversity (Willson 1983; Richards 1996). Pollination is the process, in which pollen is transferred to the female reproductive organs and post-pollination processes include cross-fertilization, ovule fertilization and seed development (Harder and Johnson 2008). Pollen transfer is mediated through abiotic and biotic vectors. Some plants utilise water and release pollen on the surface or underwater, such as Zostera (Willson 1983; Harder and Johnson 2008). Wind pollination species prevails in the gymnosperms but is also common among angiosperms such as Poaceae, Cyperaceae, Juncaceae, Chenopodiaceae and Polygonaceae (Willson 1983). In wind pollinated species, pollen usually is very small in size, present a low density or the addition of air sacs, but especially the number of pollen grains is very high (Willson 1983; Cruden 2000). Other plants use animals as mediators, the most common animal-pollination is by insects but birds and mammals are also important pollinators (Willson 1983).

Albeit most of the pollination mechanisms ensure cross-fertilization; there are also self-fertilization mechanisms in different plants (Stebbins 1970). Selfing plants have the advantage to ensure or increase fruit and seed-set where and when pollination is unreliable (Willson 1983). Lloyd and Schoen (1992) have identified various modes of selfing dividing in two groups: cleistogamous (not opened flowers) and chasmogamous (opened flowers) including, geitonogamy, facilitated and autonomous self-pollination. Self-fertilization is favored under adverse and stressful conditions and self-fertilizing species are usually common as colonizers in environments frequently unsuitable for insect pollinators, and sometimes where density or population size is low (Willson 1983).

The way a plant reproduces depend on many factors, such as flower size and color, population size, number of gametes, longevity, degree of herkogamy and dichogamy, pollination system, and a variety of environmental factors (Loveless and Hamrick 1984; Richards 1996). One way to predict the breeding systems of plant is using the pollen/ovule (P/O) ratio. This idea was proposed by Cruden (1976) who showed that P/O ratio is correlated with breeding systems, with a substantial decrease in P/O from xenogamy to facultative xenogamy to autogamy. He showed that P/O's reflect the likelihood of sufficient pollen grains reaching each stigma to result in maximum seed set. Showing, for example, that P/O ratios of most xenogamous, animal-pollinated species are between 1200:1 and 8000:1, and P/O of wind-pollinated plants are substantially higher than those of animal-pollinated plants independent if they are xenogamous or autogamous.

Consequently, the P/O ratio has been used as predictor of the reproductive system in many groups of plants, for example in Brassicaceae (Preston 1986), Polemoniaceae (Plitmann and Levin 1990), *Solanum* (Mione and Anderson 1992), Caryophylloideae (Jürgens *et al.* 2002) and Araceae (Choteau *et al.* 2006). However, even Cruden (2000) admitted that P/O ratios of some plant groups do not fit into this scheme. There are several exceptions, he mention for instance the P/O ratio of most xenogamous species in Onagraceae or Orchidaceae are less than 500:1, data considered very low with an expected autogamy system, indicating highly efficient pollination systems within these families due to the low energy investment in reward traits. On the other hand, also extremely high (30,000:1- 200,000:1) P/O ratio of xenogamous species exist, for example in Boraginaceae.

However, P/O ratios are not only affected by the sexual system, but also by pollen vectors, pollination mechanisms, and ecological factors (Cruden 2000). Consequently, P/O ratio has further been explored in relation to floral traits such as size, rewards, pollen presentation and pollination mechanisms (Schoen 1982; Plitmann and Levin 1990; Etcheverry and Alemán 2012), life form (Jürgens *et al.* 2002; Alarcón *et al.* 2011; Amela García *et al.* 2015), taxonomic position (Etcheverry and Alemán 2012), among others. Furthermore, a tendency between degree of self-compatibility, P/O and longevity was also identified by Cruden (1976) and confirmed in various taxa (Plitmann and Levin 1990; Jürgens *et al.* 2002; Choteau *et al.* 2006; Amela García *et al.* 2015). Nonetheless, few studies, showed no relation between P/O and self-compatibility (Gallardo *et al.* 1994; Vasek and Weng 1998).

Therefore, the P/O ratio can be used as a hint to elucidate the breeding system of a plant species, but detailed studies on floral and reproductive traits including phenology, patterns of variation in fruit and seed set, and comparisons with closely related species are necessary to find out more about the breeding system of a plant species. Since breeding systems and P/O ratio have not been examined in *Impatiens* in a broader way yet, in the present study, we pretend to answer the following questions: Does pollen/ovule ratio reflect the breeding systems in *Impatiens*? How is the self-compatibility degree in the genus and how it is related to P/O ratio and breeding systems? Do pollinator groups and floral longevity have an effect on P/O ratio and breeding systems?

3.2 Methods

3.2.1 Plant material

All species belong to the *Impatiens* collection of the Botanical Garden of the University of Bonn. This collection comprises ca. 180 *Impatiens* species from which 73 species of *Impatiens* and *Hydrocera triflora* where selected for the present study. Data compilation is the result of the work of different students between 2012-2015 (Jentsch 2013; Redling 2013; Span 2013; Krieger 2014). A standardized methodology was implemented to take the pollen and ovule measurement, flower longevity and pollination experiments under controlled conditions in absence of flower visitors.

3.2.2 Pollen measurement

The stamens of 12 flowers per species were cut off and all five fused stamens of a single flower were transferred into an Eppendorf tube. Anthers were collected before anthesis when pollen sacs still were closed to ensure that all pollen grains are available. Stamens were dried at least 48 hours and then a mixture of 100 μl Glycerol and 100 μl water were added to the dried stamens and mixed for five minutes with a laboratory mixer mill (Retsch MM 200). Due to the fused theca typical of the genus, an extra smashing step with a glass tube was performed. Afterwards the tubes were placed in an ultrasonic bath (Bandelin: Sonorex Rk 52) for 15 minutes to ensure that pollen grains were successfully separated from the anthers and evenly dispersed.

Tubes were vortexed and the number of pollen was counted using a Haemocytometer (Fuchs-Rosenthal counting chamber). The Fuchs-Rosenthal chamber is engraved with a grid consisting of 16 squares of 1 mm^2 each. Five of these squares were chosen randomly and 20 μl of the solution was transferred to the Haemocytometer. The volume of five squares is 1 μl. All pollen grains within the five squares were counted and the total number of pollen grains per flower was calculated. If the density of pollen grains was very high, we added 100-200 μl of 50% Glycerin to the tubes in order to increase the dilution factor. Mean value and standard deviation were calculated.

3.2.3 Ovules counting and P/O calculation

We collected ovaries from 12 advanced female stage flowers. Immediately after sampling, we dissected them under the stereo-microscope and counted the ovules directly. Mean value and standard deviation were calculated. Pollen-ovule ratio was determined by counting the number of pollen grains in an anther, multiplying that number by the number of anthers and dividing by the number of ovules in the ovary following Cruden (1977).

3.2.4 Flower longevity

Initially during the life of a flower the androecium covers the gynoecium (Figure 1.4), which drops before or at the time of perianth abscission. We defined the flowering stages before and after androecium drop as the male and female phases, respectively. We marked ten flowers and recorded the time (in days) of the mentioned phases, as well as the total life span.

3.2.5 Pollinator groups

Pollinator groups were previously identified for the 73 species of *Impatiens* and *Hydrocera triflora*. We performed a combination of multivariate analysis of Principal Component Analysis and Cluster Analysis to defined pollination systems combining morphological and reward traits (see pollination syndromes' results for details). We identified six pollinator groups: Long-tongued bees, smaller birds, larger birds, butterflies, flies and a mixed group. These

Table 3.1: **Classification of breeding systems according to fruit production measured (%).** We evaluated 73 *Impatiens* species plus *Hydrocera triflora*. We marked 20 flowers of each species and observed if they developed into fruits (autogamy test), in addition, we hand pollinated five flowers transferring pollen on the same plant to observe fruit production (geitonogamy test). NA: Not applicable, use for cleistogamous species that do not open, so hand pollination was not possible.

Breeding systems	**Autogamy test**	**Geitonogamy test**
Autogamous selfing	≥80%	>80% or NA
Facultative xenogamous	<80%	≥20%
Xenogamous	<20%	<20%

groups were used as factors to test their effect on pollen number, ovule number, pollen/ovule ratio and flower longevity.

3.2.6 Breeding system experiments

To assess self-compatibility two treatments were performed: (a) Autogamy test: autonomous self-pollination and (b) Geitonogamy test: self-pollination (hand pollination). For the first treatment, we marked and observed 20 un-manipulated flowers per species and recorded the number of mature capsules. For the second one, we transferred pollen from a male-phase flower to five female-phase flowers within the same individual or plant accession, indicating one plant clone. After these treatments, ovaries developing fruits were counted and fruit set was estimated on each species. Taking into account the fruit set in both pollination treatments (autogamy and geitonogamy), we classified the 74 studied species into three breeding systems: Autogamous, facultative xenogamous and xenogamous (Table 3.1).

3.2.7 Crossing Experiment

Of the 74 species used in the breeding system experiment for eight species we had material from two genetically different individuals. Between the two accessions per species we cross-pollinated, involving a transfer of pollen from a male-phase flower of one accession to a female-phase flower of a plant from the other accession. Fruit set was estimated on each species as well. In addition, we search on the literature similar experiments made previously on other *Impatiens* species. This data was compared with other *Impatiens* species of data taken from literature (Table A.2).

3.2.8 UV Photographs

Additionally, we evaluated normal light and UV light photographs for our eight studied species. Photographs were taken with normal light and with UV light using a UV filter. In order to assess UV light adsorption or reflection we changed the obturator of the camera that regulates the entry time of light changing the time of UV exposure. We took photographs in front and lateral views. Presence and location of UV absorption were observed and analysed.

3.2.9 Statistical Analysis

In order to evaluate the effect of breeding systems on pollen number, ovule number and P/O ratio we performed one-way ANOVAs in combination with Scheffe Post-hoc tests for multiple comparisons. Ovule number and P/O ratio were log transformed to achieve normality and homogeneity of variances. To evaluate the effect of pollinator groups on pollen number, ovule number and P/O ratio we performed Non-parametric Kruskal Wallis test and a Pairwise Kruskal Wallis test for multiple comparisons because no transformation was able to normalize non-normal data or get homogeneity of variances. Means are given with their standard errors.

To test if breeding systems or pollinator groups have more effect on pollen number, ovule number and P/O ratio we performed a two-way ANOVA. This analysis allows us also to evaluate if there is a combined effect of both factors on the variables. Since homogeneity of variances was present in the variables corresponding to longevity, we performed an ANOVA for Repeated Measures and a Scheffe Post-hoc for multiple comparisons to test the effect of pollinator groups and breeding systems separately.

Finally we assessed a non-parametric U-Mann Whitney test to evaluate the effect of the pollination treatments of the breeding experiment performed in 20 species. Analyses were performed with a SPSS 17.0 and multivariate analyses were performed with R i386 version 3.0.2 using vegan package (R Development Core Team 2015).

3.3 Results

According to Table 3.1, we classified the 74 studied species into three breeding systems categories: autogamous selfing (7 species), facultative xenogamous (36 species) and xenogamous (32 species), and compared pollen and ovule numbers and P/O ratio among these groups. We found significant lower pollen, ovules and P/O ratio in autogamous selfing, but no difference between facultative xenogamous and xenogamous species (Figure 3.1). Grouping the species according to pollination syndromes, the fly-pollinated species have significantly lower number of pollen and ovules (Figure 3.2A, B). The highest P/O ratio was found in long-tongued bees and the lowest in fly-pollinated species, however this differences are not significant in comparison with the other pollinator groups (Figure 3.2C).

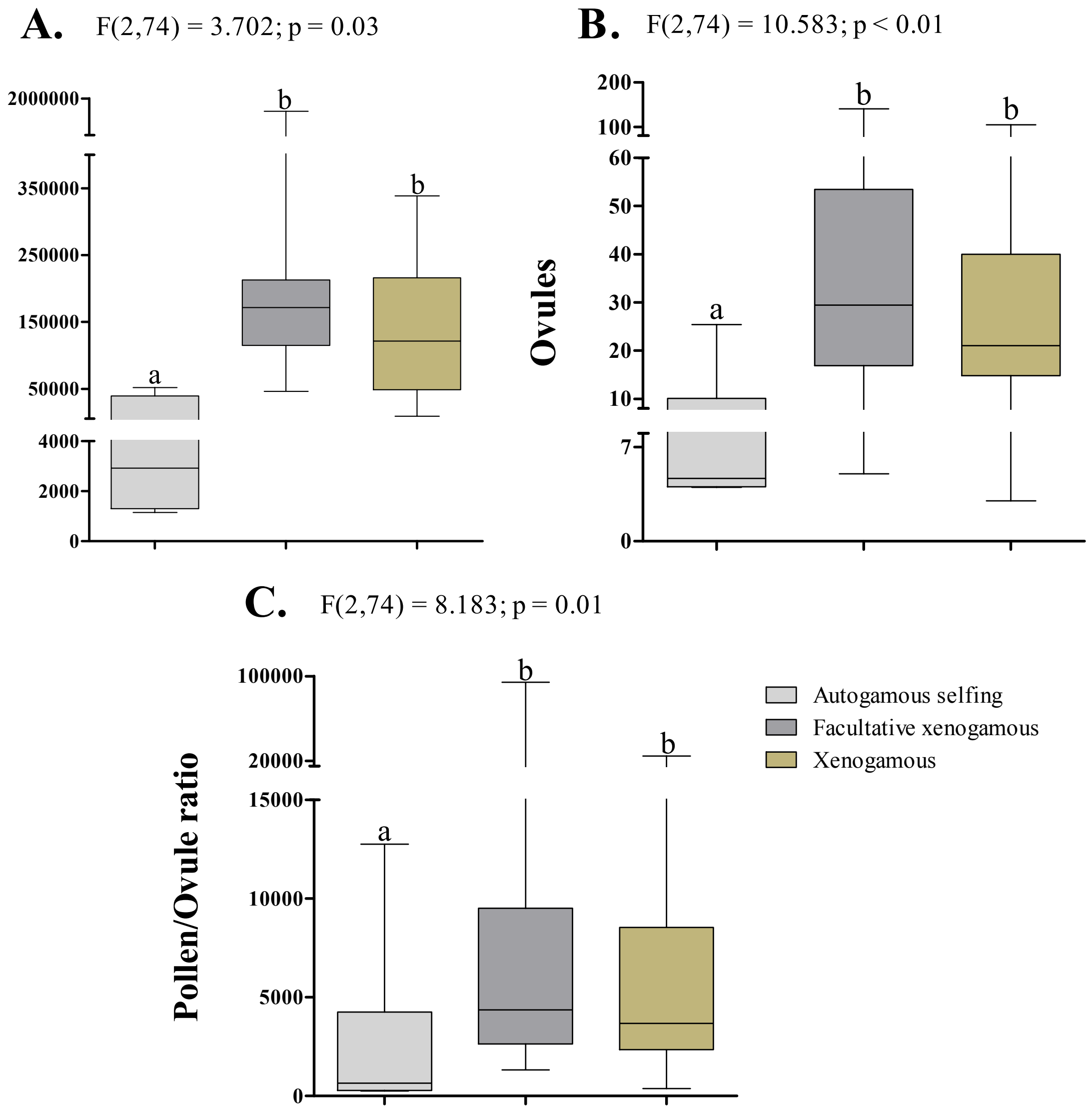

Figure 3.1: **Comparison of pollen and ovule numbers and P/O ratio according to breeding systems.** N= Autogamous selfing: 6, Facultative xenogamous: 36, Xenogamous: 32). Data show F Fisher statistic and p-value by ANOVA test. Distinct letters indicate significant differences between breeding systems ($p \leq 0.05$; Scheffe Post Hoc tests for multiple comparisons). Ovule number and P/O ratio were transformed into log to achieve normality and homogenity of variances.

Combining breeding system and pollinator system, a two-way ANOVA shows that the pollinators have more effect than the breeding system on the three variables pollen number, ovule number and P/O ratio (Table 3.2). In the xenogamous breeding system the six pollination syndromes are present with a dominance of butterfly-pollinated species, while in the facultative xenogamous system species pollinated by the "mixed" group dominate and the fly group is absent. Autogamous selfing is present only in mix and fly pollinated species (Figure 3.3).

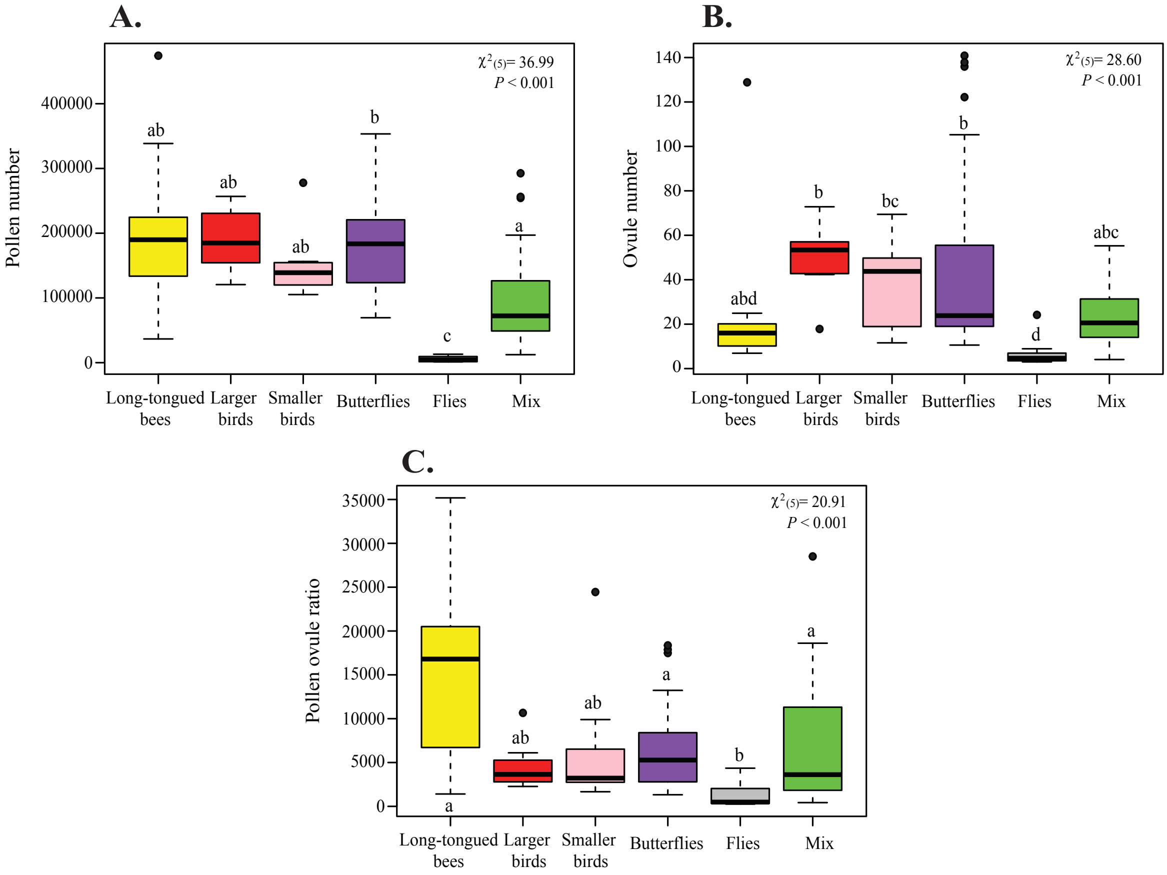

Figure 3.2: **Comparison of pollen and ovule numbers and P/O ratio among pollinator groups.** N total = 72 (excluding *I. glandulifera* and *I. sodenii*) Long-tongued bees: 6, larger birds: 8, smaller birds: 7, butterflies: 23, flies: 7, mix: 22. Variables were analysed by Kruskal Wallis non-parametric tests. Distinct letters indicate significant difference between pollinator groups ($p \leq 0.05$; Pairwise Kruskal Wallis tests for multiple comparisons).

Table 3.2: **Test of effects of breeding systems and pollinator groups on pollen, ovules and P/O ratio.** A two-way ANOVA test was performed to analyse the effect of breeding systems and pollinator groups on pollen number, ovule number and P/O ratio. Data show Fisher statistic and p-value of both factors (Breeding systems and Pollinator group) and its interaction (Breeding system × Pollinator group), $p \leq 0.05$ in bold.

Factor	**Pollen number**	**Log Ovule number**	**Log P/O ratio**
Breeding systems	$\mathrm{F}_{(\mathrm{df})}=0.490_{(13,\ 2)}$	$\mathrm{F}_{(\mathrm{df})}=1.502_{(13,\ 2)}$	$\mathrm{F}_{(\mathrm{df})}=0.100_{(13,\ 2)}$
	$p = 0.615$	$p = 0.231$	$p = 0.905$
Pollinators	$\mathrm{F}_{(\mathrm{df})}=3.838_{(13,\ 5)}$	$\mathrm{F}_{(\mathrm{df})}=4.822_{(13,\ 5)}$	$\mathrm{F}_{(\mathrm{df})}=3.657_{(13,\ 5)}$
	p=0.004	**p=0.001**	**p=0.006**
BS x Pollinators	$\mathrm{F}_{(\mathrm{df})}=0.403_{(13,\ 6)}$	$\mathrm{F}_{(\mathrm{df})}=0.371_{(13,\ 6)}$	$\mathrm{F}_{(\mathrm{df})}=0.456_{(13,\ 6)}$
	$p = 0.874$	$p = 0.895$	$p = 0.838$

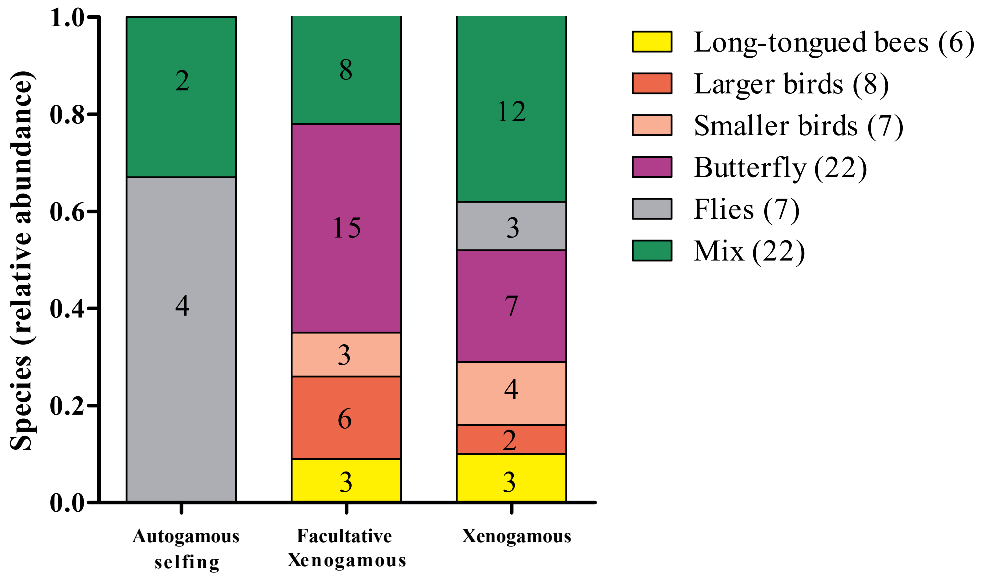

Figure 3.3: **Comparison of relative abundance of species according to pollinator groups and breeding systems.** The graph shows the relative species number distributed in the different breeding systems: Autogamous selfing, facultative xenogamous and xenogamous. Different colors represent pollinator groups with total N in parenthesis. Numbers inside the columns are number species of *Impatiens* corresponding to each pollinator group.

Flower longevity differ significantly among male and female phase, and total days (ANOVA for Repeated Measures $F(1,66) = 193.98, p < 0.001$) and only a significant difference between smaller bird and fly-pollinated species was recorded (Scheffe Post Hoc ANOVA Test, $p = 0.02$) (Figure 3.4A). Similarly, phases differ according breeding systems (Repeated Measure ANOVA $F(1,69) = 226.41, p < 0.001$). Life span is significantly shorter in those species classified as autogamous (Scheffe Post Hoc ANOVA Test, $p < 0.001$) (Figure 3.4B)

In the crossing experiment there was a significant difference among treatments (Kruskal Wallis $H = 9.16$, 3 d.f., $p = 0.010$). In comparison with the autogomous selfing treatment, supplemental hand- pollination resulted in a significant increase in the proportion of flowers setting fruit in almost all species (Figure 3.5B). An increment in fruit set from geitonogamy to xenogamy was recorded in the majority of the species as well; however, this raise was not significant (Figure 3.5A). The three first species: *Impatiens coelotropis*, *I. dalzellii* and *I. elegans* only produce fruits with xenogamy, and their respective references indicate that are self-incompatible (Sreekala *et al.* 2008a, 2014; Kulloli and Sreekala 2009a). In *Impatiens parviflora* 100% of fruit set in all treatments was registered. *Impatiens teitensis* and *I. bisaccata* were the only ones who set fruits on the autogomous selfing treatment (Figure 3.5B).

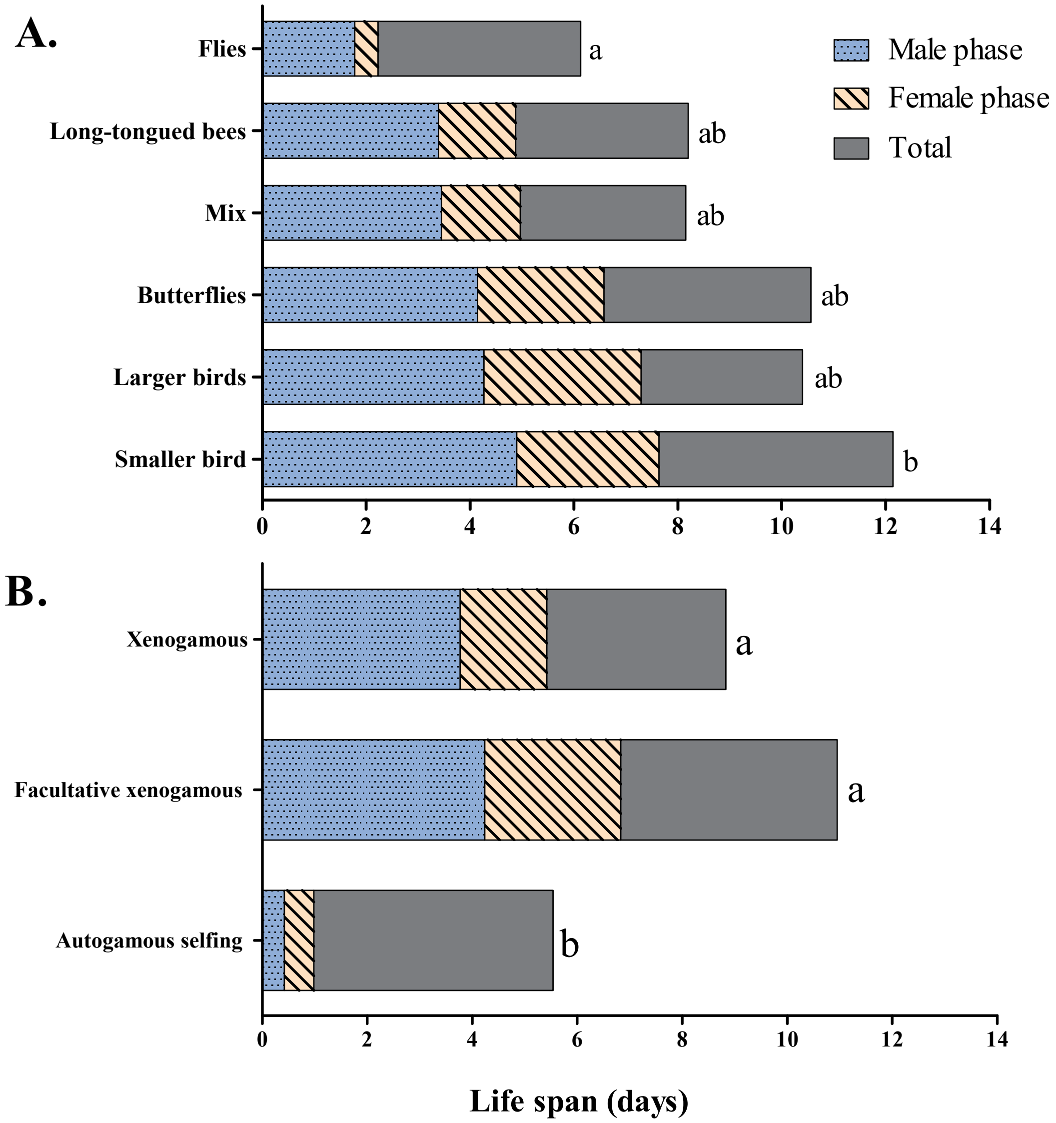

Figure 3.4: **Flower longevity according the different pollinator groups (A) and breeding systems (B)** Life span was measured in days in three time points: Male phase: time from anthesis until fall of stamens; Female phase: time from fall of stamens until fall of flower and Total: time from anthesis until fall of flower. There is significant difference according pollinators and breeding systems. Distinct letters indicate significance difference by ANOVA with Scheffe Post Hoc test for multiple comparisons, $p \leq 0.05$.

Among the eight studied species, *Impatiens scabrida*,*I. balfourii* (Figure 3.6A, B) and *I. glandulifera* (Figure A.2B) present a typical shape for long-tongued bee-pollinated species with a landing platform. *Impatiens* spec. (Figure 2.3C) and *I. teitensis* (Figure 3.6C) have long spurs and UV contrasts in the middle of the flower suggesting a function as nectary guides. There is a total UV absorption in *Impatiens balfourii* (Figure 3.6B) and *I. glandulifera* (Figure A.2B). *Impatiens bisaccata* has a peculiar shape with short two spurs and some kind of landing platform (Figure 3.6D). UV photographs of *Impatiens parviflora* show a strong absorption in the opening which may play a role in pollinator attraction (Figure 3.6E).

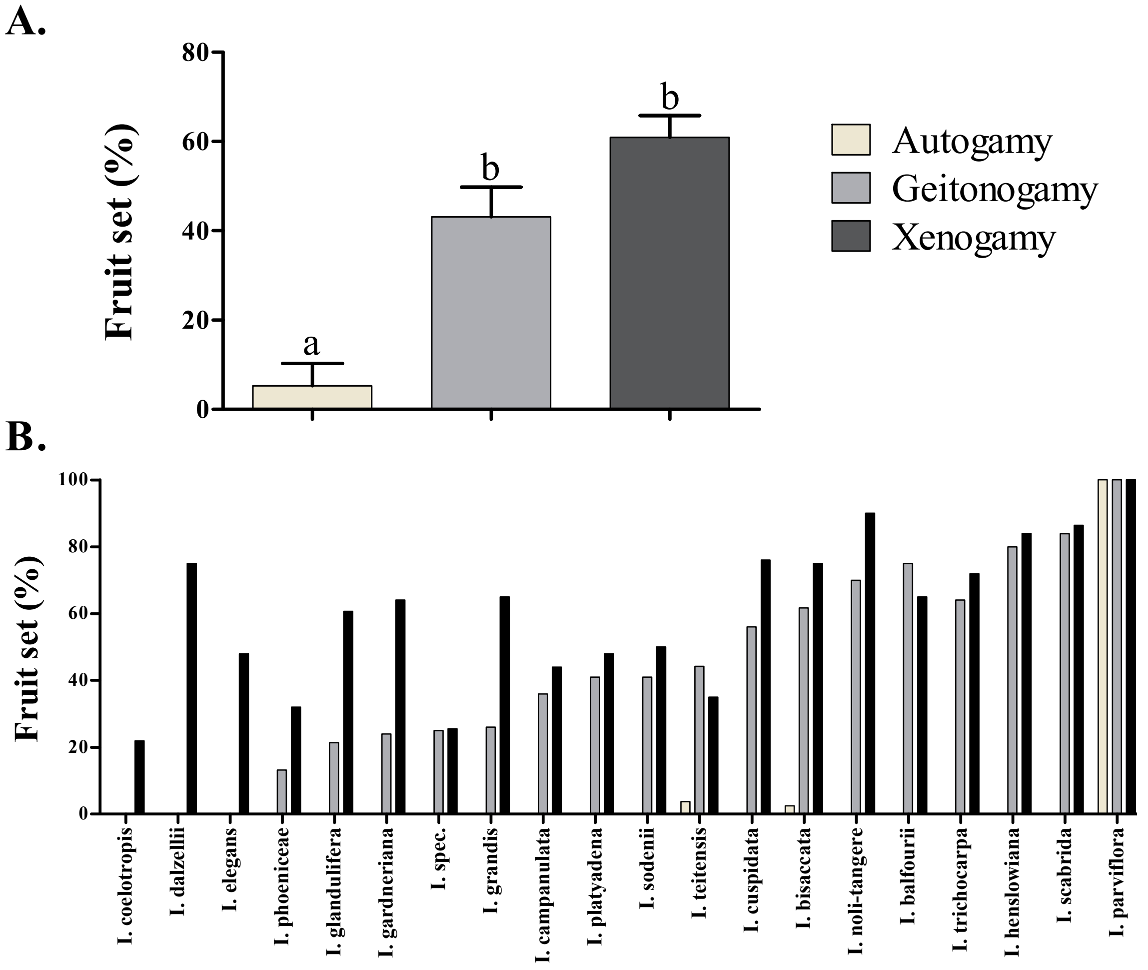

Figure 3.5: **Fruit set in percentage in 20 *Impatiens* species according three pollination treatments: autogamy, geitonogamy and xenogamy.** We combined data from literature with our breeding experiments (8 species). We recorded the number of fruits produced in un-manipulated flowers (autogamy test), in flowers with pollen transfer in the same plant (geitonogamy test) and in flowers with pollen transfer among different populations (xenogamy test). Autogamy test is significant different from the other two treatments ($p \leq 0.05$ by a Kruskal Wallis non-parametric test).

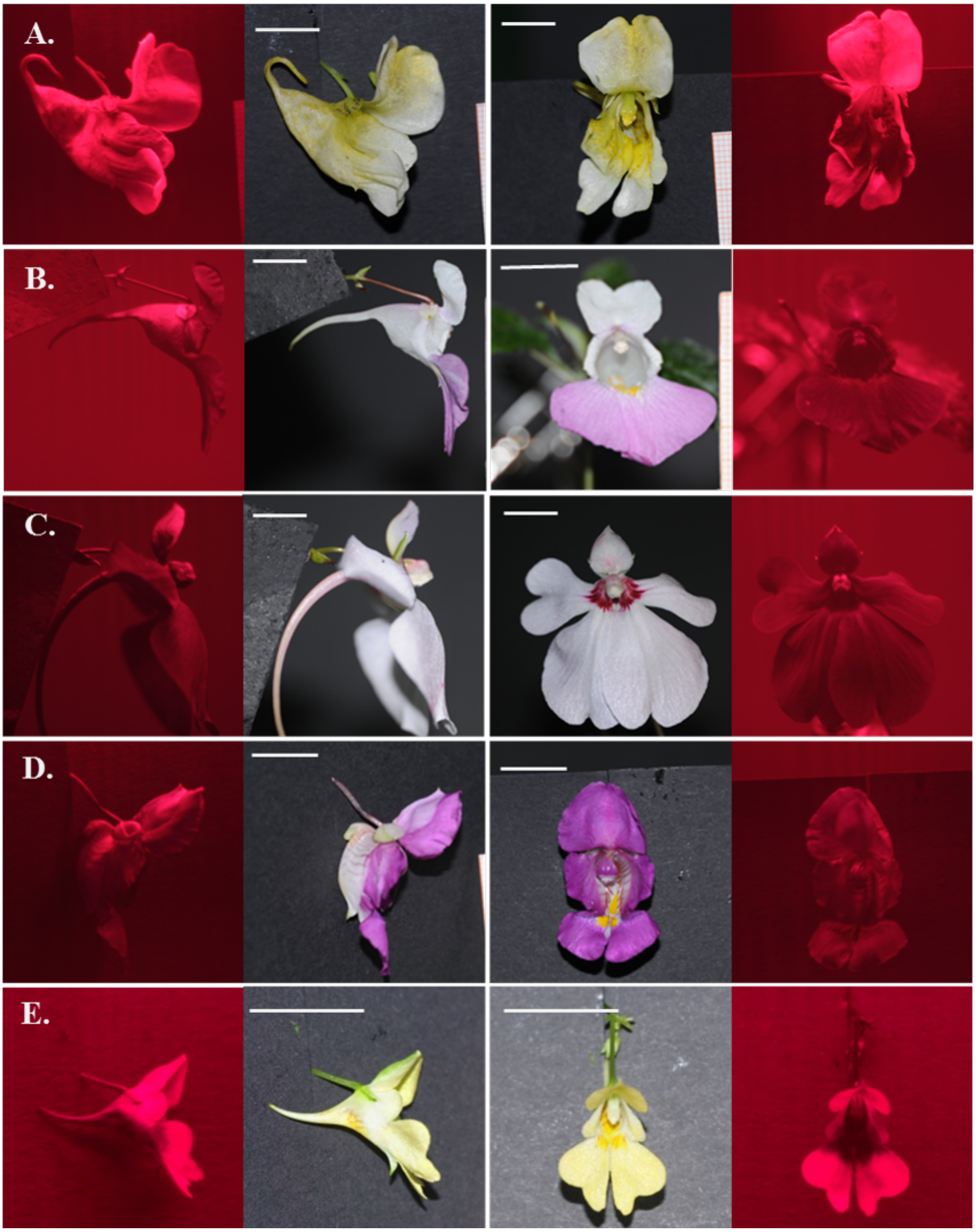

Figure 3.6: **Species used in the breeding experiment.** Display of morphometric characteristics and UV light absorption of species used for the breeding experiment. Photographs were made with normal and UV-light in front (right) and lateral view (left). **A.** *Impatiens scabrida*, **B.** *I. balfourii*, **C.** *I. teitensis*, **D.** *I. bisaccata*, **E.** *I. parviflora*. Species present varied shapes because they belong to different pollination systems: **A, B** are bee-pollinated; **C** is butterfly-pollinated; **D** and **E** is pollinated by the "mix" group, but **E** is pollinated by a specialized flies. Scale bar: 1 cm. (Photographs: V. Krieger: A, B, D; S. Lozada: C, E).

3.4 Discussion

3.4.1 Does P/O ratio reflect breeding systems in *Impatiens*?

Fruit set refers to the percentage of flowering buds that eventually resulted in ripe fruits, conferred as good indicator of successful fertilization. Different fruit set parameters allowed us to identified three mating systems in *Impatiens*: autogamous selfing, xenogamous and facultative xenogamous (Table 3.1). Significant lower pollen, ovule number and P/O ratio were found in autogamous selfing species compared to xenogamous and facultative xenogamous species (Figure 3.1). If we compare different pollinator groups we found only in fly-pollinated species significantly lower P/O ratio (Figure 3.2C). The majority of species ($N = 66$) are xenogamous or facultative xenogamous and the P/O of most of them ($N = 50$) are between 1100 and 9000, matching Cruden's (2000) categorization (1200:1, 8000:1) for xenogamous animal-pollinated species. Nevertheless we expected to have significantly higher P/O ratio in the xenogamous species than the facultative xenogamous (Cruden 1977, 2000; Jürgens *et al.* 2002), but our results only display difference for the autogamous selfing species (2667 ± 4965) and similar data in facultative xenogamous (6837 ± 15976) and xenogamous (6624 ± 6305) (Figure 3.1). These results suggest that P/O ratio might not be a good indicator of breeding systems in *Impatiens*, at least to differentiate between facultative xenogamous and xenogamous species.

In addition, our results showed that differences in these three variables (pollen, ovule number and P/O) are explained better by pollination systems than breeding systems (Table 3.2). Similar results were found for example in *Passiflora*, (Amela García *et al.* 2015) or Fabaceae (Etcheverry and Alemán 2012). It is known that distinct pollinators are important and have an effect on breeding systems (Charlesworth 2006). Similar proportion of species belonging to pollinator groups between facultative xenogamous and xenogamous species (Figure 3.3), suggest that there might be a little difference in pollen transfer efficiency of various animal vectors, as it was proposed by Cruden (2000). No significant difference in fruit set found in 20 species between these systems, support this assumption (Figure 3.6).

3.4.2 A thin line between crossing and selfing

A high variety of ecological, morphological and physiological factors affect how much self-fertilization takes place in a flower and how it occurs (Lloyd and Schoen 1992). In this sense, selfing occurs regularly and involves different modes including autogamy and geitonogamy, plants with this ability present a self-compatibility condition (Lloyd and Schoen 1992). Self-fertilization is widespread among plants (Stebbins 1970; Willson 1983) and *Impatiens* is not the exception. Indeed, selfing (especially geitonogamy) was documented for some species in the genus (*e.g.* Sreekala *et al.* 2007, 2011; Kulloli *et al.* 2009; Ramasubbu *et al.* 2011). On the contrary, a self-incompatibility condition has been rarely reported, only confirmed in *Impatiens dalzellii* (Kulloli and Sreekala 2009a) and *I. elegans* (Sreekala *et al.* 2014). In the present study, all species classified as xenogamous (excluding *I. polanei, I. ceciliae, I. puberbula, I. pingonoensis* and *I. elatostemoides*) did not produce any fruits in both autogamy and geitonogamy tests, which might suggest a self-incompatibility condition. However, in order to confirm this assumption higher sampling and more crossing experiments are required.

3.4.3 Geitonogamy and cleistogamy, selfing condition in *Impatiens*

Geitonogamy is probably the most widespread mode of self-pollination in plants, it has the ecological properties of cross-fertilization and the genetic properties of self-fertilization (Lloyd and Schoen 1992). This mode is almost inevitable in self-compatible species with open flowers with sex organs accessible to potential pollen vectors (chasmogamous flowers), and anthesis at the same time (Willson 1983; Lloyd and Schoen 1992). The majority of our studied species present these conditions, all species identified as facultative xenogamous ($N = 31$), and probably the five xengamous species mentioned above which produced fruits only on the autogamy test. The rest of xenogamous species need compatibility confirmation.

Furthermore, we identified cleistogamy as other mode of self-fertilization inside the autogamous selfing group. Since the cost of pollen and attractants is very low in not opened cleistogamous flowers (Lloyd and Schoen 1992), the lower amount of pollen found in autogamous species (Figure 3.1) might be related to the cleistogamous species present in it. From the total six autonomous selfing species, we identified four as mainly cleistogamous: *Impatiens inaperta*, *I. cf inaperta, I. humillima* and *I. hammarbyoides* (Figure 3.3). These species present the smallest flowers, and all of them are endemic from Madagascar. They are morphologically similar to the xenogamous fly-pollinated species also from Madagascar (Figure 3.3). This suggest that the non-cleistogamous flowers and might have developed from fly-pollinated ancestors (Figure 3.3).

Cleistogamy can be favored under adverse and stressful conditions including pollinators and resources limitation, and often occurs in small-flowered plants that quickly produce seeds without pollinator visits (Lloyd and Schoen 1992; Charlesworth 2006). Consequently, in *Impatiens*, cleistogamy only occurs in the smallest flowered species. The other two autogamous species (*Impatiens warburgiana* and *I. parviflora*) that belong to the "mixed" group are bigger and their morphology and rewards production, suggest that they are well adapted to pollinators. Additionally, cleistogamous species are often closely related to species with more conspicuous flowers that either set seed only after pollinator visits or are self-incompatible (Charlesworth 2006), as confirmed for *Impatiens* as well.

Furthermore, it is important to mention that we observed production of chasmogamous and cleistogamous flowers in *Impatiens* capensis and *I. cf. hochstetteri* (species not included on the study). Presence of both, chasmogamous and cleistogamous flowers was previously recorded in *I. capensis* (Lu 2002; Travers *et al.* 2003) and in *I. pallida* (Schemske 1978), and Wilson (1995) affirms that these species facultatively produce cleistogamous rather than chasmogamous flowers when they are experiencing drought, deep shade or harsh competition. In concordance, cleistogamous flowers were observed only under adverse and stressful conditions (extreme heat) in both cases. Cleistogamy often coexisting with chasmogamy, is a widespread condition occurring at least in 287 species in 56 angiosperm families (Lord 1981). Plants that have this condition are more adapted because they possess both advantages, on the one hand, selfing, that assures reproduction under unfavourable conditions, and on the other, outcrossing that provides the benefits of genetic variation (Stebbins 1970; Schemske 1978; Lord 1981; Lloyd and Schoen 1992; Lu 2002; Charlesworth 2006).

3.4.4 Floral longevity in *Impatiens*

Many factors influence how a plant reproduces, such as flower size mentioned above (Loveless and Hamrick 1984; Richards 1996). Separation of the reproductive organs in time or space is also an important component, because it reduces interference between male and female, conferring pollinators a better manage of pollen removal and delivery among flowers (Richards 1996; Routley *et al.* 2004) . In *Impatiens* it was though that flowers were strongly protandrous due to the well-differentiated male and female phases (Figure 1.4). However, some studies showed that stigma can be receptive before the beginning of the female phase (Kulloli and Sreekala 2009a,b; Ramasubbu *et al.* 2011). This suggest that high self-compatibility condition found in the facultative xenogamous species can be caused not only by pollen transfer within the plant (geitonogamy), but also from pollen of the same flower (autogamous selfing). Nevertheless, to validate this hypothesis studies on stigma receptivity would be needed.

The ability of autonomous self-fertilization should chiefly influence floral longevity, because fewer ovules should remain unfertilized after an extended outcrossing period (Sato 2002). Previous studies showed that outcrossing plants last longer than their selfing relatives, but variation in floral longevity among species with different mating systems remains unexplained (Sato 2002). In *Impatiens* we found a significant shorter floral longevity only in autonomous selfing species (Figure 3.4B), which can be explained by the reduced longevity of cleistogamous species (Schoen and Ashman 1995) characterized in *Impatiens* by absence of male and female phases.

On the other hand, flower longevity is also influenced by different pollination systems (Doom 1997). Plants pollinated by animals benefit from outcrossing but they invest energy and time to maintain flowers attractive to pollinators (Sato 2002). The length of time a flower is open can influence its total number of pollinator visits affecting the amount of pollen a flower receives and the amount of pollen it disseminates (Schoen and Ashman 1995). Nevertheless, even we could identified different pollination syndromes in *Impatiens*, we did not find clear differences in flower longevity among them (Figure 3.4A). In addition, it would be interesting to evaluate how the pollination success affect floral longevity recording time according different pollination experiments.

3.4.5 Crossing pollination in *Impatiens*

The results of our crossing experiments confirm that a genetic self-compatibility system occurs in *Impatiens* and self-incompatibility is very rare. Most important, fruits produced after crossing was significantly higher than fruit set from autogamy test but not from geitonogamy (Figure 3.5). Self-compatibility occurs in the majority of the studied species and seems to be very common way of reproduction in the genus. However, it is important to remark that self-incompatibility also occurs in *Impatiens* (Sreekala *et al.* 2008b; Kulloli and Sreekala 2009a), and since the majority of our xenogamous species showed a tendency to self-incompatibility, it is possible that this condition is present in the genus. More studies are necessary to confirm it.

Previous studies found similar results to ours for *Impatiens parviflora, I. glandulifera* and *I. balfourii* (Vervoort *et al.* 2011; Jacquemart *et al.* 2015). These authors also found that fruit set following autonomous selfing was significantly lower than hand pollination treatments for the latter two species but similar in all treatments for *I. parviflora*. They argue that these characteristics plus a high seed set found in all treatments are associated with invasiveness. To understand better the reproduction success in the genus further studies measuring seed set would be helpful.

In *Impatiens parviflora* we registered 100% of fruit set in all treatments, and we classified it as an autonomous self-pollinated species. Floral traits such as shape and UV light signals, seemed to be adapted to pollen visitors (Figure 3.6). However, no effective pollinators were recorded for this species reflecting its ability to reproduce without pollinators (Vervoort *et al.* 2011). On the other hand, *Impatiens teitensis* and *I. bisaccata* were the only species which produced fruits on the autogamous selfing treatment (Figure 3.5B). It is possible that stigma is receptive before the female phase starts and the flowers self-fertilized or during the experiment pollen from a close flower of the plant caused pollination (geitonogamy).

3.5 Conclusion

Three breeding systems were identified in *Impatiens*: autogamous selfing, facultative xenogamous and xenogamous. P/O ratio in *Impatiens* is useful in determining the general breeding system type, at least to differentiate autogamous selfing and cleistogamous species from the xenogamous. However, it cannot resolve differences in the degree of outcrossing and self-compatibility. Other factors influence P/O ratio as well. The strong link between floral traits and pollinators found in *Impatiens*, has effect on P/O ratio. Since floral longevity is directly related to pollinators, it also has an effect on P/O ratio.

Breeding systems' knowledge give us a clearer idea of how plants evolve, how they develop such different abilities of reproduction. A widespread selfing condition within the genus might explain its broad distribution and the ability to adapt to different environments, even achieving an invasive condition. In parallel, a tendency to self-incompatibility found, could explain the high endemic number of species in *Impatiens*. Its identification is essential to protect them, since a high degree of ecological specialization can make species vulnerable or in danger of extinction.

General conclusion

A rapid radiation in the Balsaminaceae resulted in a high number of species with morphologically complex flowers well-adapted to distinct groups of pollinators. Flowers are designed to be pollinated by animals and consequently, rely on them to reproduce (xenogamy). However, different ways of pollination and reproduction were identified in the family, which might partly explain its broadly distribution but high degree of endemism.

These characteristics make Balsaminaceae a good candidate to test the pollination syndrome concept. This concept is defined as a suite of floral traits, including rewards, associated with the attraction and utilization of a specific group of animals as pollinators. Our results support quantitatively the existence of five pollination syndromes in Balsaminaceae: Long-tongued bees, larger birds, smaller birds, flies and butterflies. Albeit morphological traits are highly variable between species, rewards traits (nectar and sugar measurements) were essential to identify clusters and existing pollinator data to define them as pollination syndromes. However, we also identify a "mixed" group with species adjusted to more than one group of pollinator or highly specialized species to long-proboscis flies, for example. Additionally, a separation of the bird pollination in two clusters, suggest that subgroups of pollinators groups with different degree of specialization might exist inside the identified syndromes.

Certainly, this family has developed different abilities to adapt and deal with different conditions. A specialization to different pollinators occurs and it has an effect on the way of reproduction. Due to the high adaptation of the flowers to animal pollination, a xenogamous breeding system would be expected. Although the majority of the studied species are xenogamous, we also identified some autogamous ones. Our results show that for most of the species, pollinators are needed in order to produce offspring. Furthermore, a self-compatibility condition occurs in autogamous selfing and facultative xenogamous species. On the other hand, most of the xenogamous species showed a tendency to self-incompatibility. Since facultative xenogamous species are geitonogamous, they might be more adapted because the advantages of this breeding system increase the probability to reproduce. On the one hand, they can produce fruits under unfavorable conditions when pollinators are scarce, and on the other, they have the possibility of outcrossing with the benefits of genetic variation as well. Geitonogamy could explain the broad distribution of the family and the ability to adapt to different environments. In this sense, it might be that self-incompatibility in Balsaminaceae is related to restricted distributions and highly specialized pollinators, and a self-compatible condition gave some species the ability to expand and colonize new environments.

Even though we know that floral longevity is strongly related to pollinators, we did not find substantial differences among pollinators groups. Flowers of autogamous species last shorter than the rest but they are influenced by the cleistogamous ones. Pollen/ovule ratio has been extensively used as an indicator of breeding systems in many groups. For Balsaminaceae it is useful in determining the general breeding system type, at least to differentiate autogamous selfing and cleistogamous species from the xenogamous, but it cannot resolve differences in the degree of outcrossing and self-compatibility.

The pollination syndrome concept has been discussed for so long and keeps on motivating scientist to study more groups of plants. Here, on the one hand, we present Balsaminaceae as a good model to test this concept. On the other hand, a novel methodological approach is suggested, suitable for highly complex flowers, where it is not clear whether the morphological traits really reflect the adaptations to the pollinators. This approach can be applied in other groups of plants with similar characteristics. However, we emphasize the need of collection of more pollinator data in the field in order to test the power of prediction of our model.

It is very clear that the cleistogamous and the rest of fly-pollinated species are very peculiar and different from the rest of the *Impatiens*. This group is very complex, but it is also clear that its characteristics are giving by their distribution, all of them endemic from Madagascar. For further investigations, we recommend to focus on these Malagasy species and increase the data set to understand better the pollination mechanism. On the other hand, the separation of the bird-pollinated species suggest a subdivision in the bird syndrome with different degree of specialization. In order to confirm this, more pollinators observation are required. Furthermore, the mixed group must be handled carefully and confirmation of more than one pollinator group are essential as well.

Finally, the tendency of xenogamous species to self-incompatibility might be also related to degree of endemism. In this sense, their identification is essential to protect them, the high degree of ecological specialization could make species vulnerable or in danger of extinction. In general, we highly recommend to keep on investigating the family in a broader scale, adding for instance, phytogeography, ecology and phylogenetic data would help to comprehend the complexity of this family and could explain better the reasons of its high diversification and adaptation to different environments.

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Appendix

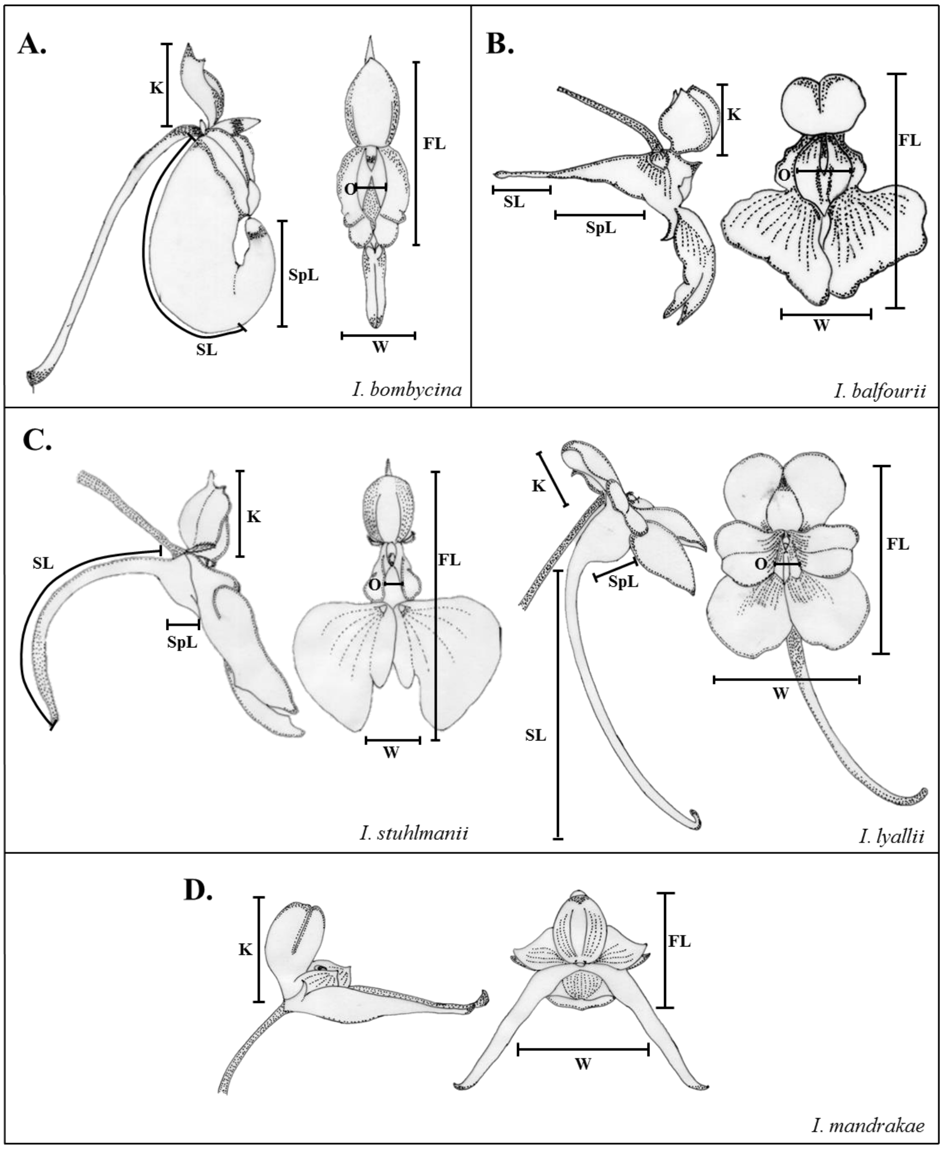


Figure A.1: **Morphological measurements according to different flower types in *Impatiens*.** .We took morphological measurements of all studied *Impatiens* species belonging to one of the following flower types: **A.** Saccatte, **B.** Between saccatte and filiform, **C.** Filiform, **D.** Spurless flowers. We measured a total of six variables on lateral (left) and frontal (right) view. Lateral view: K: "Kappe", SL: Spur length, SpL: Sepal length. Frontal view: O: Opening, W: Width, FL: Frontal length. Drawings show a representative species of each flower type, flowers between saccatte and filiform flower type tend to be very variable on the frontal view, here we show two examples species. Drawings: S. Lozada.

Table A.1: **Confirmed pollinators of some *Impatiens* species identified from the literature.** We assumed that the recorded pollinators for some species are the typical pollinators of all species in that clade. Thus, we could identify six pollinator groups. However, the cluster Long-tongued bee is not "monophyletic" since *I. glandulifera* does not cluster with the other species pollinated by long-tongued bees.

Importance of components					
	Comp. 1	Comp. 2	Comp. 3	Comp. 4	Comp. 5
Standard deviation	31.0222	14.9249	13.4306	8.9936	5.7090
Proportion of Variance	0.6237	0.1427	0.1155	0.0518	0.0208
Cumulative Proportion	0.6237	0.7664	0.8820	0.9338	0.9547
Loadings					
Spur length	0.565	-0.143	0.393	0.205	-0.381
Sepal length		0.483	-0.225		0.402
Total length	0.655	0.317		0.293	0.285
Opening		-0.113	0.168		
Total width	0.330	-0.321	-0.202	-0.452	-0.163
Front length	0.324	-0.205	-0.341	-0.428	0.343
Nectar amount		0.671		-0.441	-0.502
Sugar concentration		-0.780	0.474	-0.365	
Sugar amount		0.150			-0.134
Display size total	0.119			-0.116	0.154
Display frontal	-0.109			-0.116	0.154
Display lateral					
Display quotient				-0.163	

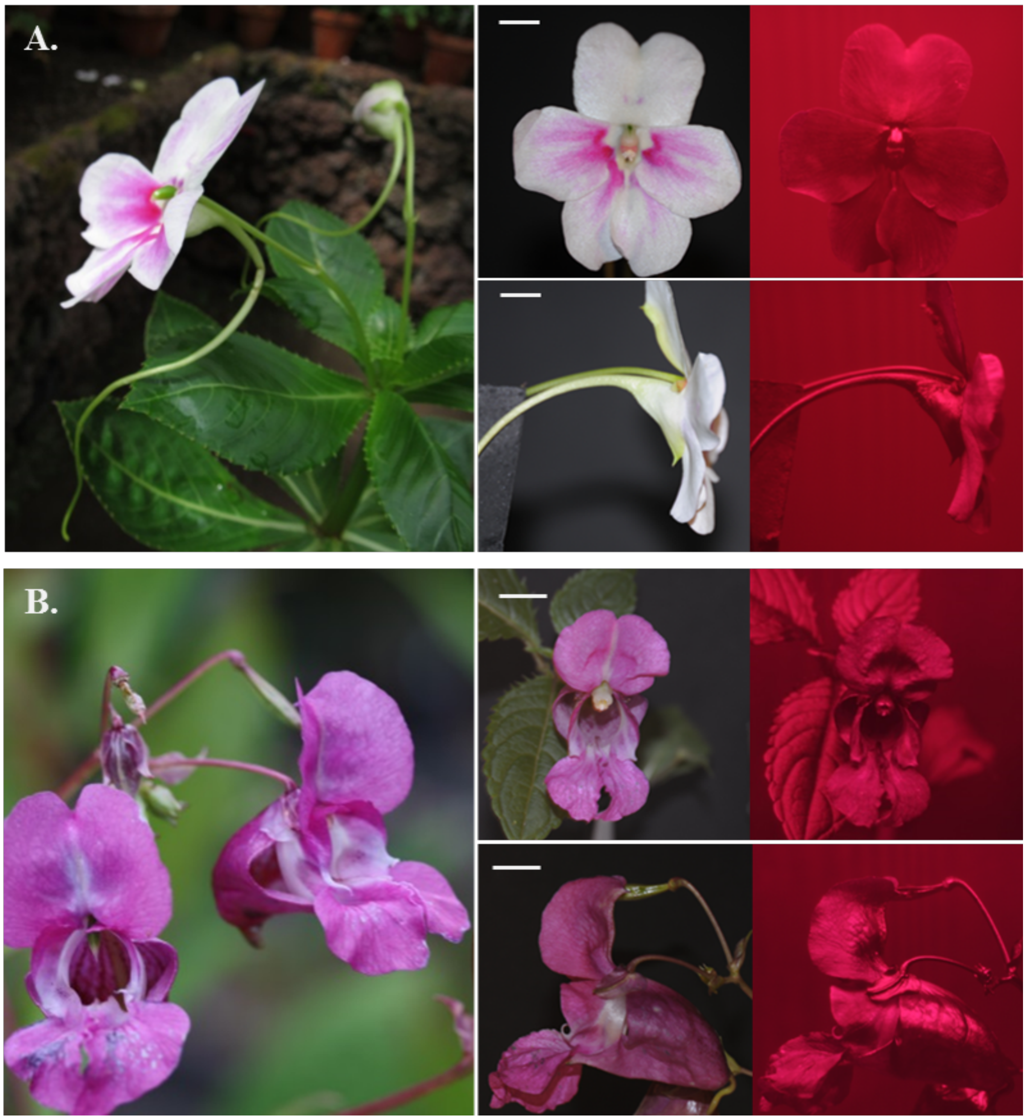
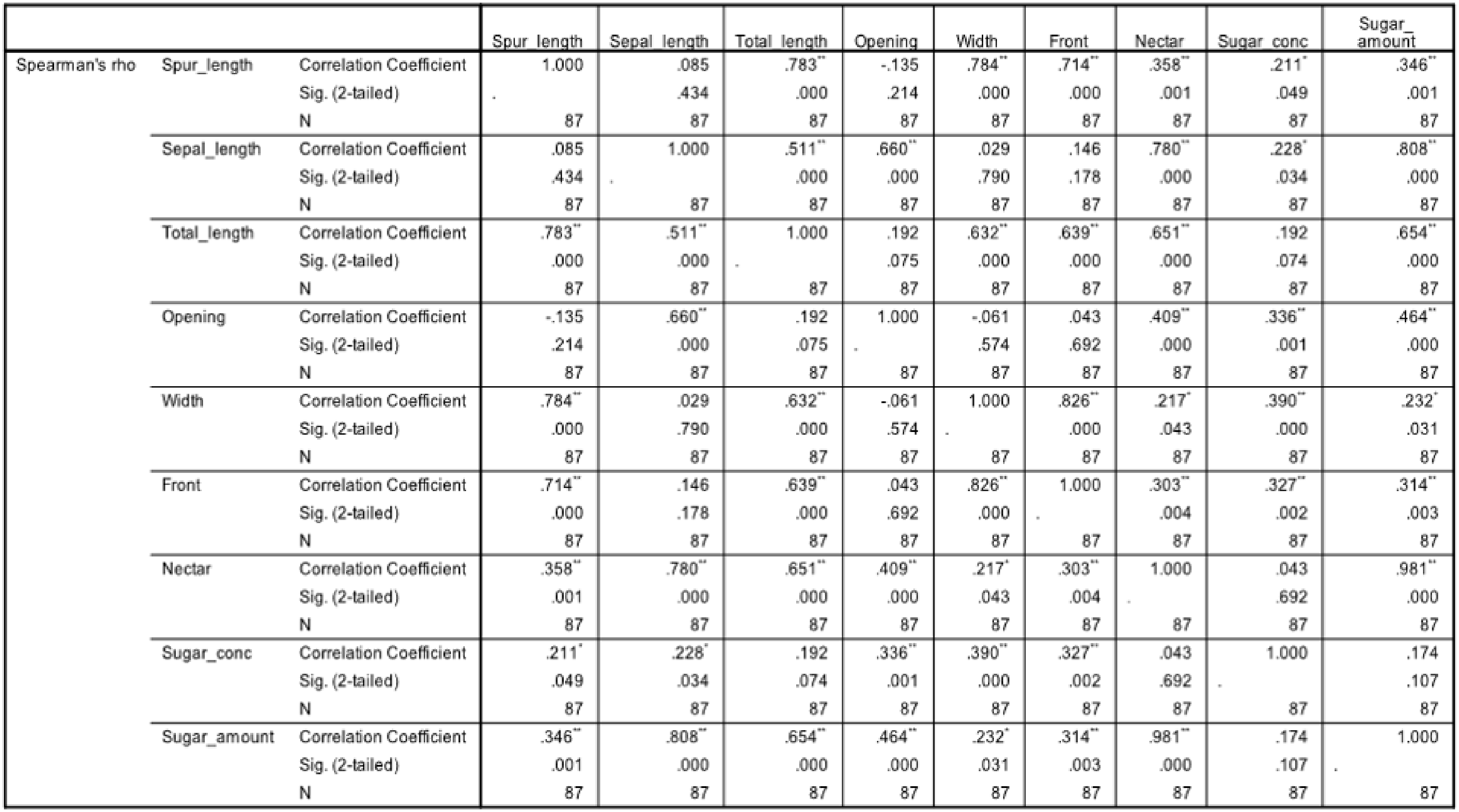


Figure A.2: **Two *Impatiens* species forming their own clusters: *Impatiens sodenii, Impatiens glandulifera.*** These two species are not located in any cluster. A. *Impatiens sodenii* possess the longest spur, suggesting a moth pollination system. B. Impatiens glandulifera was previous studied and bumblebees and bees were identified as the main pollinators. Even though, this species possess the highest sugar concentration and pollen number it ended up outside the "Long-tongued bee" cluster. Scale bar: 1 cm.



			Spur_length	Sepal_length	Total_length	Opening	Width	Front	Nectar	Sugar_conc	Sugar_amount
Spearman's rho	Spur_length	Correlation Coefficient	1.000	.085	.783**	-.135	.784**	.714**	.358**	.211*	.346**
		Sig. (2-tailed)	.	.434	.000	.214	.000	.000	.001	.049	.001
		N	87	87	87	87	87	87	87	87	87
	Sepal_length	Correlation Coefficient	.085	1.000	.511**	.660**	.029	.146	.780**	.228*	.808**
		Sig. (2-tailed)	.434	.	.000	.000	.790	.178	.000	.034	.000
		N	87	87	87	87	87	87	87	87	87
	Total_length	Correlation Coefficient	.783**	.511**	1.000	.192	.632**	.639**	.651**	.192	.654**
		Sig. (2-tailed)	.000	.000	.	.075	.000	.000	.000	.074	.000
		N	87	87	87	87	87	87	87	87	87
	Opening	Correlation Coefficient	-.135	.660**	.192	1.000	-.061	.043	.409**	.336**	.464**
		Sig. (2-tailed)	.214	.000	.075	.	.574	.692	.000	.001	.000
		N	87	87	87	87	87	87	87	87	87
	Width	Correlation Coefficient	.784**	.029	.632**	-.061	1.000	.826**	.217*	.390**	.232*
		Sig. (2-tailed)	.000	.790	.000	.574	.	.000	.043	.000	.031
		N	87	87	87	87	87	87	87	87	87
	Front	Correlation Coefficient	.714**	.146	.639**	.043	.826**	1.000	.303**	.327**	.314**
		Sig. (2-tailed)	.000	.178	.000	.692	.000	.	.004	.002	.003
		N	87	87	87	87	87	87	87	87	87
	Nectar	Correlation Coefficient	.358**	.780**	.651**	.409**	.217*	.303**	1.000	.043	.981**
		Sig. (2-tailed)	.001	.000	.000	.000	.043	.004	.	.692	.000
		N	87	87	87	87	87	87	87	87	87
	Sugar_conc	Correlation Coefficient	.211*	.228*	.192	.336**	.390**	.327**	.043	1.000	.174
		Sig. (2-tailed)	.049	.034	.074	.001	.000	.002	.692	.	.107
		N	87	87	87	87	87	87	87	87	87
	Sugar_amount	Correlation Coefficient	.346**	.808**	.654**	.464**	.232*	.314**	.981**	.174	1.000
		Sig. (2-tailed)	.001	.000	.000	.000	.031	.003	.000	.107	.
		N	87	87	87	87	87	87	87	87	87

Figure A.3: **Multiple correlation matrix taking into account all floral traits.** Data show Spearman Non-parametric Correlation Test. P-values are shown in Sig. (2-tailed) parameter.

Table A.2: **Fruit set in percentage according three pollination treatments.** Autogamy (autonomous selfing), Geitonogamy (pollen transfer within the plant) and Xenogamy (pollen transfer among different populations). Data shows the fruit set in percentage (%), some species were evaluated in this study and other data is presented with the corresponding reference.**A:** Autogamy; **G**: Geitonogamy; **X**: Xenogamy.

Species	**A**	**G**	**X**	**Reference**	**N**
I. scabrida	0.0	83.9	86.4	Present study	20
I. balfourii	0.0	75.0	65.0	Present study	20
I. glandulifera	0.0	21.4	60.7	Present study	20
I. spec.	0.0	25.0	25.6	Present study	20
I. teitensis	3.8	44.2	35.0	Present study	20
I. sodenii	0.0	41.0	50.0	Present study	20
I. bisaccata	2.5	61.7	75.0	Present study	20
I. parviflora	100.0	100.0	100.0	Present study	20
I. noli-tangere	0.0	70.0	90.0	Vervoort et al. 2011	24
I. campanulata	0.0	36.0	44.0	Kulloli *et al.* (2009)	50
I. platyadena	0.0	41.0	48.0	Ramasubbu and Sreekala (2011)	100
I. coelotropis	0.0	0.0	22.0	Sreekala *et al.* (2008b)	50
I. cuspidata	0.0	56.0	76.0	Sreekala *et al.* (2011)	25
I. gardneriana	0.0	24.0	64.0	Kulloli and Sreekala (2010)	50
I. dalzellii	0.0	0.0	75.0	Kulloli and Sreekala (2009a)	60
I. henslowiana	0.0	80.0	84.0	Sreekala *et al.* (2007)	25
I. phoeniceae	0.0	13.2	32.0	Ramasubbu *et al.* (2009)	50
I. grandis	0.0	26.0	65.0	Kulloli *et al.* (2010)	
I. trichocarpa	0.0	64.0	72.0	Kulloli and Sreekala (2009b)	50
I. elegans	0.0	0.0	48.0	Sreekala *et al.* (2014)	50

Table A.3: **Morphology and Display size data**. Six flower morphometric [cm] and four display size measurements [cm^2] were performed in 88 *Impatiens* species and *Hydrocera triflora:* Spur length, sepal length, total length, opening, width and front length . For morphology, data is given by the mean plus standard deviation.

Species	BG NR	Pollinator groups	Worker	n	Spur length	Sepal length	Total length	Opening	Total width	Front length	Display size	Frontal	Lateral	Quotient (F/L)
I. arguta	36166	Long-tongued bees	V. Krieger	11	10.55±1.69	16.91±2.47	44±2.24	7.91±1.3	18.73±4.2	7.91±1.3	8.56	3.63	4.93	0.74
I. auricoma	34154	Mix	V. Krieger	10	4.33±1.41	7.8±6.94	19.2±3.01	1.8±0.45	9±0.82	10.6±1.14	5.68	2.39	3.29	0.73
I. balfourii	36126	Long-tongued bees	V. Krieger	10	18.92±3.07	8.76±3.37	36.68±2.27	11.48±1.69	30.3±1.69	35.9±1.6	5.85	3.6	2.25	1.6
I. balsamina	36195	Butterflies	V. Krieger	10	17.3±2.71	6.1±0.74	39.8±3.12	5.2±0.42	34.7±2.67	30.8±3.08	6.96	4.75	2.21	2.15
I. bequaertii	36126	Mix	A. Redling	10	6±1.41	1.1±0.32	10.7±1.57	0.23±0.09	11±1.56	12.7±1.25	0.86	0.57	0.29	1.97
I. bicolor	33521	Smaller birds	K. Jentsch	10	11.8±1.62	19.7±0.95	30.7±1.95	3.1±1.95	12.45±1.95	22±2.31	3.74	1.57	2.17	0.72
I. bisaccata	36496	Mix	S. Lozada	10	3.4±0.52	5.6±0.97	18.8±1.69	1.9±0.32	19±2.98	35.8±5.47	6.9	4.35	2.56	1.7
I. bombycina	36143	Larger birds	S. Lozada	10	13.6±1.96	27.5±2.27	54.1±4.65	3.1±0.32	10.8±0.63	21.6±1.78	4.23	1.99	2.24	0.88
I. burtonii	11528	Mix	A. Redling	10	10.1±0.57	5.5±0.71	17.2±3.52	3.3±0.48	20.2±3.52	25±2.31	5.81	3.52	2.29	1.54
I. bururiensis	36564	Larger birds	S. Lozada	10	13.4±1.35	23.8±1.23	47.6±2.12	4.2±0.42	10.1±1.1	19.7±2.11	4.77	2.11	2.66	0.79
I. campanulata	36242	Mix	S. Lozada	10	5.5±0.85	4.8±0.63	20.6±1.65	5.2±0.63	15.6±2.01	24.1±2.88	3.18	1.49	1.69	0.88
I. catati	28424	Smaller birds	K. Jentsch	10	10.1±3.25	20.5±4.28	32.2±5.83	4.25±0.42	13.3±1.57	20.5±1.58	4.47	1.95	2.53	0.77
I. ceciliae	28425	Butterflies	S. Lozada	10	35±2.26	5±0	51.3±2.75	4.9±0.57	15.7±2.54	40.3±3.74	9.3	7.83	1.47	5.32
I. cf. congolensis	30441	Larger birds	A. Redling	10	8.6±0.7	24.6±2.27	48.4±3.5	3.8±0.42	12.8±1.48	17.2±2.35	5.54	2.12	3.42	0.62
I. cf. inaperta		Flies	S. Lozada	5	0±0	0±0	2.25±0.35	1.4±0.42	2.6±0.89	2.6±1.52	0.02	0.01	0.01	0.87
I. cinnabarina	34145	Butterflies	V. Krieger	10	35.57±6.81	5±1.22	55.4±6.31	1±0	30.52±2.75	33±3.58	4.16	3.07	1.09	2.81
I. clavicalcar-epifitica	27465	Smaller birds	A. Redling	10	7.8±1.81	17.6±1.78	27.7±1.89	3.4±0.55	10.6±3.6	21.8±2.7	5.92	4.58	1.34	3.42
I. columbaria	13781	Mix	K. Jentsch	10	5.55±0.52	5.18±0.4	18±1.1	1±0	19.82±1.54	24.91±2.3	4.8	1.98	2.82	0.7
I. confusa	27471	Butterflies	A. Redling	11	33.3±12.07	3.3±1.16	47.7±13.07	1.2±0.42	27.4±2.12	23.7±1.57	2.79	1.51	1.28	1.18
I. congolensis	31069	Larger birds	A. Redling	10	8.4±0.7	25.7±0.67	38.8±1.03	3.8±0.35	11.9±0.74	24.6±0.97	4.35	3.18	1.17	2.7
I. cordata	36127	Mix	S. Lozada	10	17.8±9.82	3.1±2.02	30.1±11.79	0.95±0.11	5.05±0.55	24.3±3.56	5.78	1.95	3.83	0.51
I. elatostemmoides	26821	Flies	V. Krieger	10	0±0	0±0	2.8±0.48	0±0	4.95±0.6	4.5±0.71	7.32	6.21	1.11	5.6
I. eliane	36144	Butterflies	S. Lozada	10	43.9±2.02	6±0.82	60.5±2.27	1±0	24.8±1.62	25.8±1.81	0.12	0.09	0.03	3.08
I. erecticornis	32579	Mix	A. Redling	10	22.6±1.58	2.5±0.47	29.6±1.58	1.5±0.47	29.6±1.58	30.5±4.25	8.72	5.32	3.4	1.57
I. eriosperma	35921	Smaller birds	S. Lozada	10	10.3±2.11	18.5±2.72	42.1±2.92	2.9±0.74	14±2.4	21±2.83	4.26	2.69	1.57	1.72
I. ethiopica	36142	Mix	V. Krieger	10	17.3±1.42	4.3±0.48	29.9±1.45	1.9±0.74	21.4±2.07	21±1.05	3.59	1.43	2.16	0.66
I. flacida alba	36244	Butterflies	S. Lozada	10	35.4±3.69	6.5±0.71	55.3±4.47	1±0	37.7±2.21	38.7±2.67	11.89	8.81	3.09	2.85
I. gesneroidea	32578	Smaller birds	V. Krieger	10	16.5±1.58	15.2±1.03	39.7±1.89	3.9±1.1	10.8±2.44	12.7±1.34	2.83	1.31	1.52	0.87
I. glandulifera	28969	Long-tongued bees	A. Redling	10	8.3±0.67	21.3±1.42	29.6±1.17	8.5±1.43	31.3±2.36	48.7±2.91	10.34	4.76	5.58	0.85
I. hammarbyoides	36433	Flies	S. Lozada	10	0±0	0±0	2.5±0.71	1.5±0.71	1±0	2.5±0.71	0.02	0.01	0.01	1.12

Table A.3: continued

I. hians	5606	Larger birds	K. Jentsch	10	7±0.47	35.6±0.97	57.3±2.79	8.8±0.79	12.8±1.32	33.2±2.53	5.38	2.98	2.4	1.24
I. hochstetterii	17872	Mix	A. Redling	10	14.8±2.15	2.7±0.54	18.8±2.15	0.72±0.13	14.3±1.64	16.4±2.22	0.83	0.6	0.24	2.54
I. hochstetterii cf.	36129	Flies	S. Lozada	10	0.1±0.32	0.1±0.32	4.25±2.3	1.8±0.35	3.5±0.71	3.8±0.79	1.38	0.93	0.45	2.06
I. humillima	36426	Flies	S. Lozada	10	0±0	0±0	3.6±0.7	0.73±0.12	3.2±0.63	6.1±0.88	0.07	0.04	0.02	1.71
I. inaperta	27467	Flies	A. Redling	10	0±0	0±0	1.5±0.85	1.67±0.58	2.33±0.58	3±1	0.04	0.02	0.02	0.94
I. keilii	17556	Smaller birds	K. Jentsch	10	7.6±2.59	22.1±1.52	29.5±2.22	3.6±0.57	6.3±0.82	12.5±1.58	3.13	1.29	1.85	0.7
I. kilimanjari	17989	Smaller birds	S. Lozada	10	5.5±0.71	19±2.36	33.5±3.24	3±0.47	14.8±3.01	16.1±0.99	2.45	1.21	1.23	0.98
I. kinabaulensis	34121	Butterflies	V. Krieger	10	27.4±7.03	3.4±0.52	42.9±7.45	1±0	32.8±3.26	25.7±2.67	4.5	3.69	0.81	4.56
I. lateriflora	36131	Butterflies	S. Lozada	10	31±8.99	4.8±0.63	53.8±9.99	7.2±0.42	43.1±4.95	56.7±2.26	17.48	12.58	4.9	2.57
I. laurentii	36132	Butterflies	S. Lozada	10	45.3±3.02	6.4±1.26	67±4.4	4.5±0.53	33.2±2.15	36.7±3.09	8.44	6.29	2.14	2.94
I. lyallii	152	Butterflies	A. Redling	10	65.9±8.66	8.9±1.2	67.7±8.43	1.03±0.36	29±2.83	31.6±4.7	9.49	6.18	3.31	1.87
I. mackeyana ssp zenkeri	6550	Long-tongued bees	A. Redling	10	11.4±0.52	12.5±0.71	39.2±2.49	9.5±0.71	22.1±2.38	29.9±1.6	8.77	4.23	4.54	0.93
I. mandrakae	26822	Flies	V. Krieger	10	0±0	0±0	4.15±0.41	0.32±0.46	4.8±0.42	9.9±0.99	0.29	0.21	0.08	2.58
I. masoalensis	36386	Mix	S. Lozada	10	5.2±1.81	2.9±0.57	15.1±2.38	2±0	9.3±2.41	19.8±1.23	2.64	1.92	0.72	2.69
I. meruensis ssp cruciata	36135	Mix	S. Lozada	10	25.9±1.97	5.5±0.85	40.1±2.64	0.95±0.08	15±2.11	22.5±2.8	2.16	1.32	0.84	1.57
I. mishmiensis	36651	Long-tongued bees	S. Lozada	10	16.1±2.18	24.3±2.87	53.3±2.75	8.4±1.07	16±2.31	29.4±2.22	8.39	4.38	4.01	1.09
I. morsei	28039	Long-tongued bees	A. Redling	12	7±1.81	16±1.41	41.25±5.21	14±1.41	29.42±4.91	36±5.66	9.76	3.89	5.87	0.66
I. namchabarwensis	17994	Long-tongued bees	A. Redling	17	13.18±1.78	10.35±1.73	35.06±4.39	6.12±0.78	26.41±5.41	33.88±6.32	7.15	4.13	3.02	1.37
I. niamniamensis	11521	Larger birds	K. Jentsch	10	13±1.76	23.3±2.31	34.8±2.7	3±0.21	12.2±0.63	28±1.63	4.69	1.74	2.95	0.59
I. niamniamensis	31233	Larger birds	K. Jentsch	10	15.7±1.64	26.6±1.51	36.1±2.02	3±0	10.7±0.82	24±1.83	7.82	3	4.82	0.62
I. niamniamensis	10305	Larger birds	A. Redling	14	10.29±0.83	32.29±5.74	54.14±7	3.36±0.5	8.5±1.91	18.71±2.76	5.69	1.99	3.7	0.54
I. noli-tangere	33527	Long-tongued bees	A. Redling	10	7.4±1.51	17.2±1.99	30.6±4.27	5.7±1.25	10.3±1.49	24.3±2.83	3.24	1.59	1.65	0.97
I. nyngvensis	18406	Mix	V. Krieger	10	16.3±3.15	7.65±1.63	32.5±2.84	3.62±0.68	17.6±1.84	19.1±2.08	2.96	1.27	1.69	0.75
I. parasitica	28428	Smaller birds	A. Redling	10	5.3±0.48	17.2±1.03	24.5±1.08	2.95±0.28	9.1±0.88	11±1.05	2.73	1.2	1.53	0.78
I. parviflora	36893	Mix	A. Redling	10	4±1.33	4.3±3.43	14.1±3.38	2.15±0.34	6.6±0.84	13.2±1.4	1.27	0.74	0.54	1.37
I. paucidentata	4819	Smaller birds	A. Redling	10	10.6±1.43	31.3±0.82	41.5±0.85	4.7±0.48	11.4±0.97	17.3±0.82	6.96	3.79	3.18	1.19
I. platypetala	36569	Butterflies	A. Redling	10	38.6±3.41	6±0.82	39.15±4.85	1.6±0.57	38.55±3.03	43.75±3.86	7.28	5.88	1.4	4.2
I. polanei	34147	Butterflies	T. Joßberger	10	34.52±2.55	7.9±0.74	60±4.99	1±0	21.6±1.61	27.6±1.78	2.95	1.84	1.11	1.66
I. pseudoviola	36595	Mix	S. Lozada	10	19.4±1.65	2.9±0.32	30.7±2.87	0.9±0.11	20.9±1.85	22.3±1.95	3.89	3.05	0.84	3.63
I. puberbula	36758	Mix	S. Lozada	10	11±2	7.4±1.17	27.2±1.69	2.8±0.63	11.6±1.17	29.6±1.96	6.16	4.05	2.11	1.92
I. purpureo-violaceae	12079	Mix	A. Redling	10	22.3±2.31	4.2±0.92	27.3±2.31	1.94±0.87	24.3±1.06	35±2.62	6.09	3.85	2.24	1.72
I. rutenbergii	27463	Butterflies	V. Krieger	10	37.16±10.06	12±2	67.3±8.39	3.64±0.92	41.09±8.75	35.7±3.75	5.73	3.37	2.36	1.43
I. sambiranensis	36428	Butterflies	S. Lozada	14	29.07±7.69	4.79±0.58	47.43±10.26	1.62±0.45	26.14±3.53	35.43±3.69	6.19	4.83	1.36	3.55
I. scabrida	11186	Long-tongued bees	S. Lozada	10	10.7±2.54	12.9±2.47	37.5±6.24	5.2±0.63	22.1±4.48	32.6±1.71	10	4.66	5.34	0.87

Table A.3: continued

I. sodenii	28429	Moth?	V. Krieger	10	128.42±6.74	11±1.6	146.89±8.83	0.96±0.33	48.89±4.85	55.3±3.74	26.19	19.54	6.65	2.94
I. spec.06	17206	Mix	K. Jentsch	10	36.6±0.97	7.6±1.07	55.2±1.87	1±0	55.3±3.8	52.1±3	3.83	2.52	1.31	1.92
I. spec.20	34120	Long-tongued bees	V. Krieger	10	15.6±3.24	1.7±0.67	24.6±3.47	1.5±0.53	17.8±2.39	26.6±2.88	5.79	4.53	1.26	3.6
I. spec.40	36240	Mix	T. Joßberger	10	32.8±1.55	3.3±0.67	51.2±2.25	1±0	33±0.94	32.7±2.58	17.93	17.22	0.71	24.26
I. spec.48	36248	Mix	S. Lozada	10	35±1.63	6.6±1.07	52.5±2.68	1±0	48.3±3.71	46.4±3.44	14.7	10.48	4.21	2.49
I. spec.55	34555	Butterflies	V. Krieger	10	30.2±5.05	8.1±0.88	51.8±6.23	1.16±0.45	32.1±2.23	44.5±3.44	13.29	10.26	3.03	3.39
I. spec.56	34556	Mix	V. Krieger	10	18.24±2.4	3.4±0.52	29.3±3.59	1±0	22.3±0.95	25.6±3.06	12.53	9.62	2.91	3.31
I. spec.57	34557	Butterflies	V. Krieger	10	7.9±1.37	2.3±0.48	18.3±1.06	1.2±0.42	14.4±2.63	26.3±1.83	2.07	1.48	0.59	2.52
I. spec.58	34558	Butterflies	V. Krieger	10	32.5±5.68	6.6±0.97	52.3±6.73	2±0	13±1.41	47.2±4.71	3.17	1.95	1.22	1.6
I. spec.70	35170	Butterflies	S. Lozada	10	11.7±4.42	3±0.67	21.9±4.7	0.9±0.12	12.4±2.41	13.8±1.32	10.83	8.35	2.49	3.36
I. spec.72	27472	Mix	S. Lozada	10	14.85±3.46	3.69±1.25	25±3.68	1±0	17.31±3.5	33.8±3.29	10.4	6.38	4.03	1.58
I. spec.86	33486	Butterflies	A. Redling	10	9±2.33	7.32±2.58	25.1±2.73	1.2±0.38	18.28±3.89	26.75±2.92	5.38	3.62	1.76	2.06
I. spec. gelb 59	36659	Butterflies	S. Lozada	10	68.3±8.64	9.9±1.2	88.2±7.48	1.8±0.63	24.6±2.37	30.1±2.69	3.51	1.86	1.65	1.13
I. pinganoensis	36626	Mix	S. Lozada	10	20.9±5.57	13.8±1.32	43±6.38	4.7±0.48	16.6±3.63	23.8±3.65	1.73	1.18	0.55	2.14
I. stenantha	36164	Long-tongued bees	V. Krieger	10	13.1±4.33	10±3.23	31±5.03	3.9±0.88	13.1±2.64	15.8±1.55	1.47	0.65	0.82	0.79
I. stuhlmanii	16583	Butterflies	A. Redling	10	34.8±2.57	4.9±0.74	52.2±2.9	1±0	41.9±1.2	44.9±2.6	14.53	8.19	6.34	1.29
I. teitensis	27473	Butterflies	A. Redling	10	46.6±5.08	5.2±0.92	53.6±5.08	1.9±0.21	48.1±6.42	39.5±3.75	18.24	13.76	4.48	3.07
I. teitensis ssp teitensis	36189	Butterflies	V. Krieger	10	61.04±4.22	5.2±1.03	75.4±3.5	1.2±0.41	52.56±4.23	52.78±4.12	12.1	10.62	1.48	7.17
I. uniflora	36165	Long-tongued bees	V. Krieger	10	12.1±2.64	12.6±1.96	34.6±2.55	6±1.33	10.3±3.47	18.89±1.76	3.77	1.38	2.39	0.58
I. urticoides	36391	Flies	S. Lozada	10	0±0	0±0	6.6±0.7	0±0	5.5±1.58	8.1±0.74	0.62	0.34	0.27	1.26
I. usambarensis	13386	Butterflies	S. Lozada	10	24.8±3.71	4.9±0.32	46.9±3.75	1±0	45.8±3.08	45.5±2.8	12.33	12.07	0.26	46.42
I. walleriana	36585	Butterflies	S. Lozada	10	24.8±1.14	3.6±0.52	42±1.56	0.74±0.24	39.6±2.22	37.3±4.08	5.06	4.29	0.77	5.6
I. warburgiana	32580	Mix	A. Redling	10	19.5±4.12	2.24±0.41	26.34±3.87	1.3±0.48	16.4±1.43	12.4±1.07	2.62	1.29	1.33	0.97
Hydrocera triflora	35302	Mix	A. Redling	25	6.92±0.41	6.92±0.4	26.28±1.37	7.96±0.84	11.64±2.1	16.44±2.06	4.05	2.09	1.96	1.07

Table A.4: **Nectar, pollen, ovules and P/O ratio data.** Nectar $[\mu l]$ and sugar concentration [%] were measured and sugar amount [mg] calculated. Pollen and ovules were counted in 12 flowers per species and the P/O calculated. A total of 87 *Impatiens* species and *Hydrocera triflora* were analysed. Data is given by the mean plus standard deviation (SD).

Species	BG NR	Pollinator groups	Worker	n	Nectar amount	Sugar concentration	Sugar amount	n	Pollen number	n	Ovule number	P/O ratio
I. arguta	36166	Long-tongued bees	V. Krieger	25	6.56±1.78	41.32±1.86	2.72±0.75	12	474200± 72539.49	12	24.92±2.19	19207.76±3590.36
I. auricoma	34154	Mix	V. Krieger	25	5.5±2.46	24.16±1.77	1.35±0.7	10	125440± 14049.69	12	31.33±2.67	4073.65±667.2
I. balfourii	36126	Long-tongued bees	V. Krieger	25	4.06±1.94	45.44±3.88	1.89±0.99	12	217479.17± 19904.7	12	6.92±2.07	35183.98±14812.34
I. balsamina	36195	Butterflies	V. Krieger	25	3.38±1.27	34.8±3.27	1.18±0.45	12	170466.67± 32483.54	12	15.75±2.18	10976.66±2612.08
I. bequaertii	36126	Mix	A. Redling	25	0.34±0.36	24.24±6.03	0.07±0.04	12	12333.33± 6040.07	12	10.83±1.11	1168.42±613.38
I. bicolor	33521	Smaller birds	K. Jentsch	25	14.39±9.15	22.94±2.08	3.3±2.08	12	105200± 14907.72	12	63.42±5.55	1667±251.55
I. bisaccata	36496	Mix	S. Lozada	25	1.5±0.73	32.32±7.76	0.5±0.34	12	197025± 62221.97	12	37.75±6.48	5344.19±1903.4
I. bombycina	36143	Larger birds	S. Lozada	23	36.03±17.33	21.56±2.77	7.62±3.48	12	210875± 33144.4	12	55.42±2.81	3824.07±690.65
I. burtonii	11528	Mix	A. Redling	25	0.39±0.31	39.44±2.78	0.16±0.13	12	81466.67± 14470.87	12	11.08±2.02	7633.72±2117.82
I. bururiensis	36564	Larger birds	S. Lozada	25	51.08±27.21	24.36±3.28	12.79±7.49	12	250458.33± 61526.22	12	72.83±5.92	3472.81±906.4
I. campanulata	36242	Mix	S. Lozada	25	2.83±1.44	44.91±5.84	1.14±0.69	11	254454.55± 76241.87	12	18.42±1.31	13810.42±4096.34
I. catati	28424	Smaller birds	K. Jentsch	25	10.09±11.47	28.58±4.46	2.99±3.53	12	154541.67± 90961.89	12	16.58±2.68	9898.33±6010.96
I. ceciliae	28425	Butterflies	S. Lozada	25	5.5±2.14	33.68±1.95	1.85±0.74	12	123583.33± 22914.7	12	21.5±2.61	5838.7±1407.32
I. cf. congolensis	30441	Larger birds	A. Redling	25	31.95±21.51	22.88±2.7	6.93±3.48	12	146200± 54049.27	12	58.67±3.73	2511.35±957.09
I. cf. inaperta		Flies	S. Lozada	25	0±0	0±0	0±0	12	1150± 597.72	12	4.5±0.52	255±124.19
I. cinnabarina	34145	Butterflies	V. Krieger	25	4.1±1.72	31.96±3.95	1.35±0.61	12	287583.33± 50561.77	12	16.92±3	17487.71±4555.81
I. clavicalcar-epifitica	27465	Smaller birds	A. Redling	25	6.38±2.53	22.92±3	1.49±0.67	12	167833.33± 23807.54	12	20±1.04	8404.36±1199.69
I. columbaria	13781	Mix	K. Jentsch	25	11±7.13	24.62±3.2	2.75±1.86	12	148225± 11244.16	12	49.75±5.82	3020.75±439.64
I. confusa	27471	Butterflies	A. Redling	25	0.81±1.05	42.78±5.88	0.34±0.41	12	77325± 7849.28	12	53.67±3.7	1446.18±161.71
I. congolensis	31069	Larger birds	A. Redling	25	4.58±4.64	19.14±3.31	0.93±0.94	12	89766.67± 30452.33	12	19±2.66	4844.57±1837.27
I. cordata	36127	Mix	S. Lozada	25	32.37±18.7	23.44±2.53	7.55±4.27	12	162408.33± 62770.18	12	53.5±7.18	3076.83±1203.12
I. elatostemmoides	26821	Flies	V. Krieger	18	1.85±1.08	27.4±3.62	0.5±0.29	12	126458.33± 29540	12	29.58±3.94	4316.91±996.6
I. eliane	36144	Butterflies	S. Lozada	25	0.03±0.02	0±0	0±0	12	9766.67± 3759.92	12	3±0	3255.56±1253.31
I. erecticornis	32579	Mix	A. Redling	25	4.8±1.94	20.46±2.82	0.99±0.46	12	64000± 43831.99	12	55.25±10.2	1167.88±816.96
I. eriosperma	35921	Smaller birds	S. Lozada	25	24.32±13.21	32.92±4.53	8.24±5.47	12	277875± 54018.15	12	11.58±2.15	24446.28±5022.05
I. ethiopica	36142	Mix	V. Krieger	25	1.65±0.43	29.6±1.53	0.49±0.13	12	119000± 29270.68	12	10.83±1.34	11311.55±4186.21
I. flacida alba	36244	Butterflies	S. Lozada	25	4.38±2.01	31.48±2.79	1.42±0.72	12	199750± 55104.65	12	36.92±4.85	5595.12±2176.89
I. gesneroidea	32578	Smaller birds	V. Krieger	25	26.02±21.61	28.24±2.99	7.64±6.99	12	123666.67± 26509.87	12	18.92±1.78	6525.35±1262.09
I. glandulifera	28969	Long-tongued bees	A. Redling	25	5.1±3.92	63.56±5.31	3.27±2.59	12	1449450± 136757.2	12	15.83±2.44	94032.86±19404.59
I. hammarbyoides	36433	Flies	S. Lozada	25	0±0	0±0	0±0	12	4083.33± 1328.59	12	5±0	816.67±265.72

Table A.4: continued

I. hians	5606	Larger birds	K. Jentsch	25	25.11±21.12	22.32±4.4	5.3±4.48	12	181200± 33247.5	12	17.83±4.02	10660.55±3102.53
I. hochstetterii	17872	Mix	A. Redling	25	0.08±0.02	27.79±3.6	0.02±0.01	12	48450± 10607.07	12	14.08±1.83	3533.87±1123.28
I. hochstetterii cf.	36129	Flies	S. Lozada	25	0±0	0±0	0±0	12	4575± 6600.15	12	8.92±0.9	500.97±717.99
I. humillima	36426	Flies	S. Lozada	25	0±0	0±0	0±0	12	1750± 997.72	12	4±1.04	470±299.27
I. inaperta	27467	Flies	A. Redling	25	0±0	0±0	0±0	12	1350± 703.89	12	4.83±0.39	285.83±161.27
I. keilii	17556	Smaller birds	K. Jentsch	25	5.85±3.04	22.2±3.89	1.27±0.6	12	120000± 22951.53	12	43.75±4.33	2746.42±489.24
I. kilimanjari	17989	Smaller birds	S. Lozada	25	8.13±8.55	20.28±5.29	1.88±2.14	12	138958.33± 28996.44	12	44.42±9.55	3218.1±845.39
I. kinabaulensis	34121	Butterflies	V. Krieger	25	1.15±0.7	22.04±4.74	0.27±0.21	12	97550± 36759.45	12	29.33±3.94	3401.7±1270.19
I. lateriflora	36131	Butterflies	S. Lozada	25	3.85±2.2	26.96±4.3	1.05±0.62	12	162208.33± 22000.47	12	122.17±3.49	1327.95±175.75
I. laurentii	36132	Butterflies	S. Lozada	25	2.37±0.79	23.32±3.87	0.55±0.2	12	297458.33± 65388.86	12	48.42±17	6841.62±2947.89
I. lyallii	152	Butterflies	A. Redling	25	12.27±6.49	29.48±3.58	3.73±2.23	12	151316.67± 17114.79	12	19.08±2.91	8071.07±1275.87
I. mackeyana ssp zenkeri	6550	Long-tongued bees	A. Redling	25	7.54±4	35.5±4.28	2.71±1.55	12	178791.67± 10013.4	12	128.83±14.08	1409.46±232.94
I. mandrakae	26822	Flies	V. Krieger	25	0.02±0.02	0±0	0±0	12	13062.5± 2110.16	12	3±0	4354.17±703.39
I. masoalensis	36386	Mix	S. Lozada	25	0.59±0.28	36.71±9.45	0.18±0.14	12	167041.67± 44194.02	12	20.58±3.75	8183.99±1741.78
I. meruensis ssp cruciata	36135	Mix	S. Lozada	25	0.45±0.29	16.96±6.84	0.08±0.07	12	72325± 15716.24	12	12±1.54	28505±15347.35
I. mishmiensis	36651	Long-tongued bees	S. Lozada	25	9.8±3.01	40.48±3.78	4±1.38	12	338541.67± 52562.28	12	20.17±0.94	16802.07±2657.29
I. morsei	28039	Long-tongued bees	A. Redling	25	6.11±4.23	31.24±6.45	1.99±1.57	12	203500± 76562.2	12	11.17±1.03	18330.23±6766.23
I. namchabarwensis	17994	Long-tongued bees	A. Redling	25	1.3±0.73	41.3±4.17	0.53±0.3	12	105400± 18827.06	12	16±2.04	6657.62±1227.36
I. niamniamensis	11521	Larger birds	K. Jentsch	25	50.15±16.5	19.32±3.24	9.65±3.62	12	256825± 51222.16	12	42.33±2.9	6108.07±1378.51
I. niamniamensis	31233	Larger birds	K. Jentsch	25	45.3±24.26	20.9±3.43	9.65±5.44	12	188583.33± 33323.37	12	43.17±5.47	4423.19±876.25
I. niamniamensis	10305	Larger birds	A. Redling	25	32.09±30.88	25.5±4.22	7.48±7.95	10	120610± 13396.06	12	53.33±1.72	2270.01±271.49
I. noli-tangere	33527	Long-tongued bees	A. Redling	25	2.98±1.72	46.76±5.11	1.45±0.94	12	231766.67± 40299.86	12	9.17±0.83	25562.56±5651.9
I. nyngvensis	18406	Mix	V. Krieger	23	9.63±5.34	24.52±2.22	2.39±1.43	12	175466.67± 50542.3	12	49.08±6.22	3614.52±1024.98
I. parasitica	28428	Smaller birds	A. Redling	25	12.64±11.68	24.12±1.78	3.04±2.76	12	156333.33± 10426.92	12	69.42±2.94	2254.47±156.56
I. parviflora	36893	Mix	A. Redling	25	0.06±0.03	44.28±9.29	0.02±0.02	12	51916.67± 15020.94	12	4.08±0.51	12765.97±3581.04
I. paucidentata	4819	Smaller birds	A. Redling	25	16.07±14.66	15±3.94	2.63±2.79	12	118250± 32025.91	12	35.25±6.09	3358.47±684.68
I. platypetala	36569	Butterflies	A. Redling	25	5.38±2.87	30±3.54	1.63±0.92	12	217458.33± 37848.66	12	41.17±2.59	5279.75±839.82
I. polanei	34147	Butterflies	T. Joßberger	25	2.9±0.54	26.44±3.49	0.78±0.19	12	69425± 13772.38	12	19.58±5.04	3888.48±1630.72
I. pseudoviola	36595	Mix	S. Lozada	25	1.26±0.53	29±4.43	0.37±0.16	10	59550± 46444.38	12	31.58±2.81	1832.66±1292.02
I. puberbula	36758	Mix	S. Lozada	25	12.51±12.71	20.8±9.57	2.09±1.41	12	292583.33± 58111.9	12	23.42±1.44	12495.63±2411.25
I. purpureo-violaceae	12079	Mix	A. Redling	25	1.99±1.88	24.16±3.07	0.52±0.57	12	65100± 9381.03	12	26.17±2.62	2507.23±408.04
I. rutenbergii	27463	Butterflies	V. Krieger	25	9±5.13	28.72±3.75	2.68±1.66	12	308600± 41385.64	12	55.5±3	5564.17±700.86
I. sambiranensis	36428	Butterflies	S. Lozada	25	1.09±1.36	25.33±5.9	0.29±0.39	12	184450± 44089.9	12	10.58±1.56	17873.43±5271.68
I. scabrida	11186	Long-tongued bees	S. Lozada	25	4.06±2.77	49.16±10.75	1.88±1.28	11	189883.33± 89718.04	12	20.08±4.06	10864±3941.77

Table A.4: continued

I. sodenii	28429	Moth?	V. Krieger	25	8.81±5.55	10.94±3.21	1±0.72	10	178920± 52545.95	12	61.83±7.18	2874.36±787.03
I. spec.06	17206	Mix	K. Jentsch	25	2.05±0.96	23.42±5.09	0.5±0.27	12	39566.67± 8915.7	12	17.33±1.72	2305.38±584.97
I. spec.20	34120	Long-tongued bees	V. Krieger	18	32.37±12.04	26.2±4.38	8.55±3.73	12	84200± 34735.15	12	21.58±1.08	3902.58±1613.78
I. spec.40	36240	Mix	T. Joßberger	25	4.57±1.35	27.72±4.82	1.27±0.45	10	256140± 113550.15	12	17.67±1.56	14550.68±6386.01
I. spec.48	36248	Mix	S. Lozada	25	4.67±3.24	22.72±4.14	1.14±0.9	12	353375± 72840.46	12	137.83±11.5	2604.17±686.7
I. spec.55	34555	Butterflies	V. Krieger	25	19.08±8.89	27.52±1.66	5.23±2.41	12	312733.33± 53754.95	12	140.83±29.9	2337.63±719.72
I. spec.56	34556	Mix	V. Krieger	25	20.27±13.83	27.64±3.24	5.48±3.25	12	178683.33± 42081.52	12	135.92±13.11	1322.67±321.23
I. spec.57	34557	Butterflies	V. Krieger	25	0.94±0.59	21.8±5.51	0.19±0.11	12	46250± 16540.28	12	18.75±1.48	2470.4±906.49
I. spec.58	34558	Butterflies	V. Krieger	25	1.04±0.6	29±3.86	0.31±0.2	12	57200± 20542.82	12	20.17±2.44	2819.48±884.53
I. spec.70	35170	Butterflies	S. Lozada	25	2.66±1.1	25.56±6.98	0.71±0.39	11	112954.55± 26331.97	12	12±1.88	4310.42±1097.28
I. spec.72	27472	Mix	S. Lozada	25	15.91±7.09	25.72±1.63	4.16±1.98	11	205890.91± 25697.99	11	23.82±1.25	8663.81±1170.19
I. spec.86	33486	Butterflies	A. Redling	25	1.53±0.57	26.76±7.04	0.42±0.21	12	48966.67± 24725.01	12	27.83±2.82	1793.58±955.3
I. spec. gelb 59	36659	Butterflies	S. Lozada	25	3.28±1.68	41.28±4.4	1.37±0.74	12	109075± 25190.08	12	16.25±1.42	6770.61±1774.04
I. pinganoensis	36626	Mix	S. Lozada	25	0.56±0.32	24.24±5.88	0.13±0.07	12	15500± 3586.59	12	36.58±3.2	427.61±116.58
I. stenantha	36164	Long-tongued bees	V. Krieger	25	2.42±2.41	44.32±1.46	1.09±1.1	12	36650± 6554.46	12	11.25±1.76	3331.24±811.88
I. stuhlmanii	16583	Butterflies	A. Redling	25	8.33±5.71	24.16±4.52	2.15±1.53	12	220700± 19211.64	12	80.5±12	2793.29±435.98
I. teitensis	27473	Butterflies	A. Redling	25	10.77±6.01	22.88±3.38	2.54±1.59	8	186300± 23091.37	12	11.17±1.75	18357.17±3792.53
I. teitensis ssp teitensis	36189	Butterflies	V. Krieger	25	10.07±4.53	29.04±2.95	2.98±1.4	12	183566.67± 51370.23	12	14.17±1.4	13228.51±4469.49
I. uniflora	36165	Long-tongued bees	V. Krieger	25	7.22±4.27	38.12±2.8	2.78±1.69	12	158200± 38144.8	12	7.58±1.24	21797.25±7629.84
I. urticoides	36391	Flies	S. Lozada	25	0±0	0±0	0±0	12	9175± 4149.95	12	24.17±2.62	383.92±182.88
I. usambarensis	13386	Butterflies	S. Lozada	25	3.35±1.57	36.6±7.52	1.27±0.68	12	254100± 62058.04	12	105.25±7.58	2444.38±594.49
I. walleriana	36585	Butterflies	S. Lozada	25	2.11±0.55	37.68±6.61	0.79±0.21	10	82600± 25213.31	12	39.75±6.55	1973.16±121.14
I. warburgiana	32580	Mix	A. Redling	25	1.51±0.52	30.6±2.07	0.46±0.16	12	34825± 9880.11	12	25.42±4.5	1414.31±432.12
Hydrocera triflora	35302	Mix	A. Redling	25	1.18±0.36	34.32±4.13	0.41±0.13	12	1449450± 136757.2	12	15.83±2.44	94032.86±19404.59

Table A.5: **Longevity and breeding systems.** Longevity was measured in time in male, female phases and in total. Data is given by the mean plus standard deviation (SD). Three breeding systems were determined: **AS:** Autogamy selfing, **FX:** Facultative xenogamous, **X:** Xenogamous based on fruit set (%) according two pollination treatements(Autogamy and geitonogamy). These data was calculated from 73 *Impatiens* species and *Hydrocera triflora*. Pollination experiments to determin breedng systems were performed by S. Lozada.

Species	BG NR	Pollinator groups	Worker	n	Male phase	Female phase	Total	Breeding system	n	Fruits(A)	Fallen	Fruit set (%)	n	Fruits (G)	Fallen	Fruit set (%)
I. arguta	36166	Long-tongued bees	V. Krieger	12	5.38±3.45	2.56±1.09	7.62±2.78	——	20	0	20	0				
I. auricoma	34154	Mix	V. Krieger	12	3.88±1.04	5.43±3.12	8.79±3.81	——								
I. balfourii	36126	Long-tongued bees	V. Krieger	10	4.72±1.8	3.17±1.93	7.89±0.88	FX	20	1	19	5	5	4	1	80
I. balsamina	36195	Butterflies	V. Krieger	11	3.65±1.08	5.22±1.74	8.87±1.79	AS	20	20	0	100	5	5	0	100
I. bequaertii	36126	Mix	A. Redling	10	6.28±0.61	1.46±0.53	7.34±0.97	X	20	0	20	0	5	0	5	0
I. bicolor	33521	Smaller birds	K. Jentsch	10	2.85±2.74	12.95±7.96	14.09±7.54	X	20	0	20	0	5	0	5	0
I. bisaccata	36496	Mix	S. Lozada	10	2.01±1.59	5.53±3.54	7.55±3.13	FX	20	1	19	5	5	2	3	40
I. bombycina	36143	Larger birds	S. Lozada	10	2.68±0.91	6.16±1.7	8.84±1.55	X	20	0	20	0	5	0	5	0
I. burtonii	11528	Mix	A. Redling	10	3.49±0.86	2.22±0.92	5.71±0.84	FX	20	0	20	0	5	3	2	60
I. bururiensis	36564	Larger birds	S. Lozada	10	2.52±2.43	9.97±3.5	11.92±2.95	FX	20	0	20	0	5	2	3	40
I. campanulata	36242	Mix	S. Lozada	10	1.64±1.37	7.46±3.53	9.1±4.58	X	20	0	20	0	2	0	2	0
I. catati	28424	Smaller birds	K. Jentsch	10	4.11±1.45	4.76±1.47	8.89±1.71	FX	20	0	20	0	5	3	2	60
I. ceciliae	28425	Butterflies	S. Lozada	10	3.12±0.73	9.8±3.64	12.92±3.86	X	20	1	19	5	5	0	5	0
I. cf. congolensis	30441	Larger birds	A. Redling	10	5.27±2.04	11.95±4.82	17.22±4.03	FX	20	0	20	0	5	5	0	100
I. cf. inaperta		Flies	S. Lozada	6	0±0	0±0	5.51±1.89	AS	10	6	4	60	NA	NA	NA	NA
I. cinnabarina	34145	Butterflies	V. Krieger	10	3.47±1.86	6.29±1.74	9.76±0.88	FX	20	0	20	0	5	5	0	100
I. clavicalcar-epifitica	27465	Smaller birds	A. Redling	10	3.21±1.29	1.77±1.51	4.99±1.41	——	20	0	20	0				
I. columbaria	13781	Mix	K. Jentsch	10	2.55±0.88	5.69±2.59	7.61±3.2	X	10	0	10	0	5	0	5	0
I. confusa	27471	Butterflies	A. Redling	10	4.92±2.22	3.65±2	7.96±2.56	——	20	0	20	0				
I. congolensis	31069	Larger birds	A. Redling	10	3.7±1.25	4.89±2.69	8.76±3.29	FX	20	0	20	0	5	1	4	20
I. cordata	36127	Mix	S. Lozada	10	4.78±2.51	5.57±2.75	10.19±2.95	FX	20	0	20	0	5	1	4	20
I. elatostemmoides	26821	Flies	V. Krieger	10	3.5±1.29	6.34±2.47	9.18±2.97	X	20	1	19	5	5	0	5	0
I. eliane	36144	Butterflies	S. Lozada	10	4.06±3.26	5.49±2.19	8.44±4.75	X	16	1	15	6	5	0	5	0
I. erecticornis	32579	Mix	A. Redling	10	4.1±1.71	5.77±2.15	10.15±2.31	——	4	0	4	0				
I. eriosperma	35921	Smaller birds	S. Lozada	10	2.49±1.11	6.98±3.1	9.46±3.23	X	20	0	20	0	5	0	5	0
I. ethiopica	36142	Mix	V. Krieger	10	2.91±1.41	3.06±2.05	5.96±2.44	X	20	0	20	0	5	0	5	0
I. flacida alba	36244	Butterflies	S. Lozada	10	6.13±2.37	8.23±2.85	14.36±3.35	FX	20	1	19	5	5	3	2	60
I. gesneroidea	32578	Smaller birds	V. Krieger	10	4.56±2.44	4.77±2.54	8.46±3.75	FX	20	0	20	0	5	4	1	80
I. glandulifera	28969	Long-tongued bees	A. Redling	10	2.66±1.73	4.23±2.32	6.89±1.38	FX	20	0	20	0	10	3	7	30

Table A.5: continued

I. hammarbyoides	36433	Flies	S. Lozada	0	±0	0±0	0±0	AS		20	0	100	NA	NA	NA	NA
I. hians	5606	Larger birds	K. Jentsch	10	3.12±1	2.59±1.87	5.71±1.63	FX	20	0	20	0	5	1	4	20
I. hochstetterii	17872	Mix	A. Redling	10	2.7±1.37	2.47±0.96	5.18±0.63	X	20	0	20	0	5	0	5	0
I. hochstetterii cf.	36129	Flies	S. Lozada	10	1.85±1.23	15.65±8.35	17.49±8.38	——	20	7	13	35				
I. humillima	36426	Flies	S. Lozada	7	0±0	0±0	5.85±0.67	AS	20	5	15	25	NA	NA	NA	NA
I. inaperta	27467	Flies	A. Redling	10	0.1±0.32	0.4±1.26	4.82±1.53	AS	5	0	5	0	NA	NA	NA	NA
I. keilii	17556	Smaller birds	K. Jentsch	10	10.08±1.76	14.36±3.75	24.44±3.9	FX	20	0	20	0	3	1	2	33
I. kilimanjari	17989	Smaller birds	S. Lozada	10	4.25±1.22	2.57±0.93	6.82±1.59	X	20	0	20	0	5	0	5	0
I. kinabaulensis	34121	Butterflies	V. Krieger	19	4.88±2.22	4.12±3.61	9±3.79	FX	20	2	18	10	5	2	3	40
I. lateriflora	36131	Butterflies	S. Lozada	10	5.44±1.32	9.27±3.89	14.54±2.65	FX	20	1	19	5	5	2	3	40
I. laurentii	36132	Butterflies	S. Lozada	10	7.16±9.26	13.39±5.74	17.52±7.61	FX	20	8	12	40	5	4	1	80
I. lyallii	152	Butterflies	A. Redling	10	5.06±0.65	6.72±2.26	11.73±2.41	X	20	0	20	0	5	0	5	0
I. mackeyana ssp zenkeri	6550	Long-tongued bees	A. Redling	10	3.12±1.89	5.16±1.35	7.1±1.72	——								
I. mandrakae	26822	Flies	V. Krieger	10	5.27±1.7	1.52±0.56	6.2±1.26	X	20	0	20	0	5	0	5	0
I. masoalensis	36386	Mix	S. Lozada	10	3.68±1.91	3.25±1.89	6.51±3.24	X	20	0	20	0	5	0	5	0
I. meruensis ssp cruciata	36135	Mix	S. Lozada	10	0±0	0±0	4.46±3.33	——	20	0	20	0				
I. mishmiensis	36651	Long-tongued bees	S. Lozada	10	3.76±0.66	1.76±0.75	5.25±1.02	X	20	0	20	0	5	0	5	0
I. morsei	28039	Long-tongued bees	A. Redling	5	2.98±1.41	5.16±4.22	9.04±4.06	——	2	0	2	0				
I. namchabarwensis	17994	Long-tongued bees	A. Redling	10	3.09±0.73	3.8±1.14	6.89±0.74	FX	20	0	20	0	5	3	2	60
I. niamniamensis	11521	Larger birds	K. Jentsch	10	5.97±1.45	2.3±1.51	8.27±2.15	X	20	0	20	0	5	0	5	0
I. niamniamensis	31233	Larger birds	K. Jentsch	10	4.92±4.5	10.11±9.53	16.62±4.21	FX	20	0	20	0	5	4	1	80
I. niamniamensis	10305	Larger birds	A. Redling	10	4.9±1.44	9.71±2.7	14.61±2.06	FX	20	0	20	0	5	1	4	20
I. noli-tangere	33527	Long-tongued bees	A. Redling	0	0±0	0±0	0±0	——	15	0	15	0	0			
I. nyngvensis	18406	Mix	V. Krieger	11	4.28±1.88	7.21±3.4	11.49±3.6	FX	20	0	20	0	5	1	4	20
I. parasitica	28428	Smaller birds	A. Redling	10	14.64±2.19	5.8±2.05	20.44±1.28	——								
I. parviflora	36893	Mix	A. Redling	10	1.04±0.69	0.58±0.38	1.9±1.1	AS	20	20	0	100	5	5	0	100
I. paucidentata	4819	Smaller birds	A. Redling	10	5.96±2.12	7.07±2.8	12.81±2.6	X	10	0	10	0	5	0	5	0
I. platypetala	36569	Butterflies	A. Redling	10	2.77±1.03	5.7±1.34	8.46±1.64	X	13	0	13	0	1	0	1	0
I. polanei	34147	Butterflies	T. Joßberger	10	3.79±1.9	2.93±2.6	6.83±2.19	X	20	4	16	20	4	0	4	0
I. pseudoviola	36595	Mix	S. Lozada	6	2.48±0.55	5.84±3.42	7.87±2.83	X	20	0	20	0	5	0	5	0
I. puberbula	36758	Mix	S. Lozada	10	4.02±0.56	6.36±4.02	10.38±3.74	X	20	1	19	5	5	0	5	0
I. purpureo-violaceae	12079	Mix	A. Redling	10	3.65±1.79	4.73±2.13	8.7±1.83	FX	20	3	17	15	5	2	3	40
I. rutenbergii	27463	Butterflies	V. Krieger	11	4.03±1.52	3.68±1.49	7.73±0.87	FX	20	0	20	0	5	4	1	80
I. sambiranensis	36428	Butterflies	S. Lozada	5	1.55±0.83	12.37±1.32	13.92±0.63	——	10	0	10	0				

Table A.5: continued

I. scabrida	11186	Long-tongued bees	S. Lozada	10	2.11±0.95	14.72±7.65	16.83±7.29	FX	20	0	20	0	5	4	1	80
I. sodenii	28429	Moth?	V. Krieger	10	3.61±1.82	4.73±1.63	8.34±1.03	X	20	0	20	0	5	0	5	0
I. spec.06	17206	Mix	K. Jentsch	10	3.41±2.6	6.58±3.41	9.99±3.65	X	20	0	20	0	5	0	5	0
I. spec.20	34120	Long-tongued bees	V. Krieger	15	4.82±0.9	3.71±2.14	8.29±2.35	FX	20	6	14	30	5	1	4	20
I. spec.40	36240	Mix	T. Joßberger	10	4.63±1.6	5.7±2.18	10.34±2.1	FX	20	1	19	5	5	3	2	60
I. spec.48	36248	Mix	S. Lozada	10	2.31±0.8	8.09±2.2	10.39±2.17	FX	20	0	20	0	5	5	0	100
I. spec.55	34555	Butterflies	V. Krieger	10	4.44±1.53	4.52±1.5	8.96±1.61	FX	20	0	20	0	14	4	10	29
I. spec.56	34556	Mix	V. Krieger	10	3.69±2.26	5.96±3.95	9.18±4.17	FX	20	0	20	0	5	1	4	20
I. spec.57	34557	Butterflies	V. Krieger	12	3.54±1.7	4.75±2.53	8.29±1.83	FX	20	0	20	0	5	1	4	20
I. spec.58	34558	Butterflies	V. Krieger	12	3.57±1.87	5.33±2.91	8.9±3.36	X	20	0	20	0	5	0	5	0
I. spec.70	35170	Butterflies	S. Lozada	10	4.07±1.17	11.32±2.64	15.39±2.95	FX	22	3	19	14	5	3	2	60
I. spec.72	27472	Mix	S. Lozada	10	3.19±1.73	7.95±3.71	11.14±2.94	X	20	0	20	0	5	0	5	0
I. spec.86	33486	Butterflies	A. Redling	10	3.17±2.82	11.7±2.62	13.99±5	X	20	0	20	0	5	0	5	0
I. spec. gelb 59	36659	Butterflies	S. Lozada	9	1.47±1.1	7.28±5.08	8.07±5.46	——	20	1	19	5				
I. pinganoensis	36626	Mix	S. Lozada	10	6.08±4.64	7.8±4.15	11.17±3.99	X	18	2	16	11	5	0	5	0
I. stenantha	36164	Long-tongued bees	V. Krieger	13	3.27±0.9	1.76±1.23	4.87±0.77	X	20	0	20	0	5	0	5	0
I. stuhlmanii	16583	Butterflies	A. Redling	10	5.29±2.63	6.09±3.16	11.38±3.13	FX	20	0	20	0	5	3	2	60
I. teitensis	27473	Butterflies	A. Redling	10	3.73±0.84	4.67±1.6	8.4±1.65	FX	20	1	19	5	8	6	2	75
I. teitensis ssp teitensis	36189	Butterflies	V. Krieger	11	4.44±1.48	6.32±1.51	10.76±2.51	FX	10	0	10	0	5	3	2	60
I. uniflora	36165	Long-tongued bees	V. Krieger	11	3.39±1.74	4.08±3.67	7.47±2.94	X	20	0	20	0	5	0	5	0
I. urticoides	36391	Flies	S. Lozada	10	3±1.28	8.03±3.65	8.92±4.67	X	13	0	13	0	2	0	2	0
I. usambarensis	13386	Butterflies	S. Lozada	10	3.93±0.5	3.74±1.63	7.67±1.44	X	20	0	20	0	5	0	5	0
I. walleriana	36585	Butterflies	S. Lozada	10	2.7±1.13	11.8±1.99	14.5±1.89	FX	20	0	20	0	5	1	4	20
I. warburgiana	32580	Mix	A. Redling	10	1.36±1.3	2.37±2.32	3.73±2.32	AS	20	20	0	100	5	4	1	80
Hydrocera triflora	35302	Mix	A. Redling	0	0±0	0±0	0±0	FX	20	12	8	60	5	4	1	80