Evolution of dioecy from monoecy in *Begonia*: The effects of seed shadow handicap

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Cover image: From bottom left continuing clockwise, fleshy fruit of *Begonia guttapila*, dioecious *Begonia guttapila* showing female flower with fleshy fruits, monoecious *Begonia macintyreana* showing both male and female flowers on a single plant, and dioecious *Begonia silletensis* showing female flowers with fleshy fruits. Photos courtesy of Daniel Thomas, Ching-I Peng, and Mark Hughes.

Abstract

Begonia is a diverse, mostly monoecious and self-compatible genus whose species often have small isolated populations potentially likely to become inbred, and would theoretically benefit from dioecy to promote outcrossing. However, only 19 of 1839 *Begonia* species are dioecious, suggesting dioecy may be difficult to evolve. This may be due to the "seed shadow handicap" (SSH), an increase in progeny clustering in dioecious species where seed production is limited to half the individuals, causing increased competition between progeny. To compete with monoecious species, adaptions such as fleshy fruits that promote increased seed dispersal are necessary. To test if the SSH is affecting the evolution of dioecy in *Begonia,* breeding system, fruit type, and extent of occurrence (EOO) data for 628 species were analyzed for phylogenetic signal and correlation across a plastid phylogeny. Results show many more dioecious species have fleshy fruit than expected by chance (P=0.0005), although there was no evidence for phylogenetic correlation between EOO and either breeding system or fruit type. There is some evidence for the SSH limiting evolution of dioecy in *Begonia*, but further work is needed to test other characters potentially correlated with dioecy to better understand the evolution of dioecy within *Begonia*.

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Chapter 1 – Introduction

1.1 *Dioecy in angiosperms*

Dioecy, the separation of unisexual flowers between individuals, is an interestingly rare breeding system within flowering plants. The past research on dioecy has been driven by the attempt to understand the rarity of the condition and its effects on plant evolution. Even though dioecy is currently known in just 5-6% of flowering plants (Renner, 2014), it is recognized to have an impact on population structure, gene flow and subsequent evolution. Many theories, including those of Darwin, have focused on the selective pressure for outcrossing as the driving force behind the isolation of functional male and female organs to separate plants within a population (Darwin, 1876). Dioecy is then considered a substitute for self-incompatibility genes in a breeding population, but a poor one, as it divides the population of seed bearing individuals by 50% (Bawa, 1980). This theory was thought to explain the apparent rarity of dioecy. However, other ecological pressures have been considered in addition to outcrossing, such as resource allocation, sexual selection, pollination syndrome or seed dispersal agents, and recently, the genetic background behind the evolution of dioecy is beginning to be uncovered (Akagi et al., 2014; Boualem et al., 2015).

1.1.2 *Character correlations with dioecy* – Before ecological theories about the evolution of dioecy promotion can be formed, character correlations must be made. Several significant ecological associations with dioecious taxa have been observed and recorded. Dioecious species can be either wind or animal pollinated. The majority of tropical dioecious species are known to be animal pollinated, specifically through entomophily (Bawa, 1980). However, dioecy in more temperate regions is more closely associated with anemophily (Renner and Ricklefs, 1995). Dioecy is thought to increase pollination efficiency as male dioecious flowers are able to focus energy to increase pollen production and female flowers avoid the risk of stigma pollen competition (Bawa, 1980). Those that are entomophilous tend to be visited by small, generalist bees with unspecialized, small, greenish flowers. Muenchow (1987) believed that this association between dioecy and small, greenish, insect pollinated flowers was the strongest character correlation so far observed within dioecious species. With the increase in pollen production per flower seen in dioecious species, it has been observed that more male flowers are produced per plant than female flowers on female plants, which hold a greater attraction specifically for small generalist pollinators (Bawa, 1980). This creates a unidirectional pollination pattern within populations of insects visiting males first then females when the male rewards are exhausted, resulting in a more efficient pollination

(Beach, 1981). This unbalanced pollinator preference may be a strong ecological pressure leading a population towards dioecy.

Dioecious species are often animal dispersed as well, and are weakly associated with fleshy fruits (Muenchow, 1987). Animals are attracted to fleshier fruit and will disperse the seeds after consumption. One reason behind this association is that more energy is available to be allocated to female floral and fruit production in dioecious species than hermaphrodites. In neotropical floras, dioecy is also associated with greater seed production, either in quality or quantity, compared to hermaphroditic taxa, again due to focused resource allocation (Vamosi et al., 2008). The reduction of the seed bearing individuals in a single population places dioecious species at a competitive disadvantage with hermaphrodites or monoecious species (Bawa, 1980), creating evolutionary pressure for dioecious species to have traits that increases female fitness. However, the increase in seed quantity produced per female plant does not necessarily provide an increase in fitness, whereas fleshy fruit and animal dispersal does (Heilbuth, Ilves and Otto, 2001). Without the ability to disperse seed a significant distance from the parent, and only 50% of a population capable of dispersal, the offspring of dioecious individuals will grow in denser populations than a hermaphroditic population with similar dispersal mechanisms and 100% of individuals capable of dispersing seed. These denser populations of dioecious offspring are at a competitive disadvantage compared to their less densely dispersed hermaphroditic neighbors, due to a seed shadow handicap, or increase in local resource competition between individuals (Heilbuth, Ilves and Otto, 2001). The seed shadow handicap would negate any fitness increase produced by an increase in the quantity of seed produced by female dioecious individuals. Animal dispersed fleshy fruit is carried greater distances away from the parent plant, reducing the dioecious seed shadow handicap and increasing the fitness of the entire dioecious population through the greater ability to produce successful offspring. The correlation of dioecy with fleshy fruit then emphasizes the role resource allocation plays in the evolution of dioecy, as it provides dioecious populations with the ability to persist in a competitive environment through an increased dispersal capability.

1.1.3 *Location of dioecious species* – Tropics and islands have high incidence of dioecious species among the unisexual components of their floras (Bawa and Opler, 1975; Sakai et al., 1995). Perhaps correlated with this is the higher frequency of dioecy in woody perennial plants and plants with non-specialized animal pollination. In island systems that rely on long distance dispersal to contribute to the flora, dioecious perennials are hypothesized to have an advantage after colonization. With the seed bearing individuals reduced by 50% and elongated life cycles of woody perennials, there is less urgent need to provide a large quantity of pollinators, or complex pollination among mutualistic partners that have coevolved for many generations (Bawa, 1980). It is also hypothesized that the high frequency of dioecious species in the tropics and on islands is due to the correlation between dioecy and animal dispersed, fleshy fruit. The thick canopy in tropical forests prevents wind and other elements from easily aiding pollination or dispersing seed, causing animal vectors to become very important to tropical plant life cycles (Whitehead, 1969). With an island, most plant species are brought through long distance dispersal via birds and were therefore animal dispersed, perhaps with fleshy fruit. Therefore, dioecious species with fleshy fruit and animal dispersal have a higher probability of being dispersed to an island than a non-dioecious plant without fleshy, bird-dispersed fruits. Dioecious species from Hawaii are more often found on older islands, originating both from the colonist plants and subsequent diversification of non-dioecious lineages (Sakai et al., 1995).

1.1.4 *Evolutionary pathways to dioecy* – The evolution of angiosperm breeding systems has generally been thought of in a linear pathway leading from hermaphroditism, both sexes in a single flower on a single plant, to dioecy, each sex within a single flower on separate plants. The differing paths hypothesized to lead from hermaphroditism to dioecy are categorized as direct, dimorphic or monomorphic (Goldberg et al., 2017). In the direct pathway, hermaphroditism becomes dioecy without an intermediate stage. This can occur through heterostyly, when different floral style lengths exist in a population, such as is seen in the family Rubiaceae (Pailler and Thompson, 1997). Direct evolution from hermaphroditism to dioecy is also hypothesized to occur through heterodichogamy, when bisexual flowers in a population have two different morphs that interchange positions at different times of day, making them functionally unisexual as is seen in *Alpinia* Roxb. (Zingiberaceae) (Renner, 2001).

In the dimorphic pathway, the evolving population consists of both hermaphroditic and unisexual individuals, female (gynodioecious) or less commonly male (androdioecious). The dimorphic pathway requires two different stages of sex isolation, as one type of unisexual flower evolves from hermaphroditic flowers separately from the other. Gynodioecy, the more studied and understood dimorphic breeding system pathway, results from unequal resource allocation to the female floral components, enforced by male sterility mutations (Barrett, 2002). With some individuals of the population producing only female flowers, resources are more focused on producing improved ovules, which when fertilized guarantee offspring produced through outcrossing (Bawa, 1980). To lead to a dioecious population, remaining hermaphroditic individuals must also unequally allocate resources to male components of floral meristems to compensate for the lack of pollen produced from the female plants (Delph, 2009). Although the reverse situation, leading from completely hermaphrodite to androdioecious is also possible, it has been observed that androdioecy perhaps plays a role in the evolution away from dioecy towards hermaphroditism, as is observed in the species *Mercurialis annua* L. (Euphorbiaceae) (Delph, 2009).

The monomorphic pathway to dioecy involves monoecy as an intermediate stage, and both sexes are isolated in a single disruptive evolutionary stage from hermaphroditic flowers (Barrett, 2002). This “single” disruptive evolutionary stage is thought to occur through many gradual genetic mutations and environmental influence (Dorken and Barrett, 2004). Variation on the monomorphic pathway from hermaphroditic individuals to fully monoecious individuals include gynomonoecy (hermaphroditic and female flowers on a single individual), andromonoecy (hermaphroditic and male flowers on a single individual), or trimonoecy (hermaphroditic, female, and male flowers on a single individual) (Torices, Mendez and Gomez, 2011). The monomorphic pathway is currently thought to be the most common pathway to dioecy, though there has been argument in the past regarding the dominance of dimorphic versus monomorphic pathways (Dorken and Barrett, 2004; Goldberg et al., 2017). Despite the currently believed frequency of the monomorphic pathway and the significant general association of monoecy to dioecious lineages (Renner and Ricklefs, 1995), less research has been done on factors affecting the evolution of dioecy from monoecious ancestors than has been done for gynodioecious lineages. Even a study analyzing the potential monomorphic ancestry to the dioecious species *Sagittaria latifolia* Willd. (Alismataceae) proved to demonstrate a more likely dimorphic evolutionary pathway involving gynodioecious populations rather than monoecy (Dorken and Barrett, 2004).

However, studies within the Asteraceae have emphasized the significance of the monomorphic pathway to dioecy and its variations between hermaphroditism and monoecy (Torices, Mendez and Gomez, 2011). Within Asteraceae, monoecy evolved from hermaphroditism through gynomonoecy. Both monomorphic and dimorphic evolutionary pathways to dioecy are present within the family, and it is suggested that, while the monomorphic pathway is more common, the dimorphic pathway occurs more quickly over evolutionary time (Torices, Mendez and Gomez, 2011). The greater length of time involved in the monomorphic pathway to dioecy may reflect the gradual genetic mutations supposedly required to achieve the transition. The monomorphic pathway to dioecy can therefore be considered more complex than the dimorphic pathway and this complexity may account for the difficulty involved in studying the system in close detail. As the Asteraceae was an ideal subject to model the monomorphic pathway to dioecy compared to the dimorphic pathway, a model system is needed to provide a more focused environment to study the monomorphic pathway in greater detail. The genus *Begonia* L., the subject of this study, provides an ideal model lineage for analyzing the monomorphic pathway, as it is a mostly monoecious genus with few dioecious species. *Begonia* is a mega-diverse, widely studied genus, and much pertinent information is available for species that would provide an ideal situation for studying breeding system ecological correlates and genetic background behind the monomorphic pathway.

1.1.5 *Environmental stress influencing sexual selection* – Maintaining genetic diversity through avoidance of inbreeding depression, unequal resource allocation as is seen in the dimorphic pathway, and genetic background are commonly accepted pressures for the development of breeding systems away from hermaphroditism (Barrett, 2002). However, environmental pressures have been observed to cause non-uniform variations in hermaphroditism, leading to uni-sexuality in some instances. Drought, nutrient availability, and light intensity can play a significant role in a population's sex composition (Golenberg and West, 2013). For example, an increase in aridity was correlated with gynodioecy in the genus *Wurmbea* Thunb. (Colchicaceae) and was thought to play a part along with altered pollination patterns in the onset of gender dimorphism within the lineage (Case and Barrett, 2004). Environmental variations in floral breeding system are then seen as an important precursor to the evolution of dioecy. However, variations continue to persist in existing dioecious lineages. Some dioecious species, such as species within the genus *Populus* L. (Salicaceae) have been observed to have rare aberrant individuals, which produce both sexes, and even hermaphroditic flowers (Bawa, 1980). In other dioecious species, sex is determined by age or size of the plant, and varying sexual development is uniform across a single population. For example, some *Arisaema* Mart. (Araceae) species develop only male flowers in early growth stages and are female only at maturity (Bawa, 1980). Uniform variation in sex determination in a population such as *Arisaema* points to a connection between genotype and environmental variation. This connection may be explained by the interaction of plant hormones with floral identity genes. The hormones auxin, gibberellin, cytokinin, and ethylene are known to cause either feminizing or masculinizing effects in floral growth in numerous experiments (Khryanin, 2002; Golenberg and West, 2013). It has been suggested that these hormones can enhance or inhibit a gene's function thereby influencing sex determination (Golenberg and West, 2013). Hormone and other chemical levels in plant meristem tissues are influenced by environmental stress. Therefore, variation in a population's sex determination due to environment can also be related to a species' genetics.

1.1.6 *Ambiguity in breeding system identification* – The effect that environment and growth have on sexual selection can lead to ambiguity in the determination of a definite breeding system. A species that appears to be dioecious may really be monoecious, with a single plant producing both sexes at different times. This can be observed only through prolonged investigation. In this situation, a temporally separated monoecy, sex development can be separated among stages of an individual's life cycle, as in *Arisaema* species, or among sequential breeding seasons in a single year. The latter case can be observed in some species within the genus *Begonia* and causes difficulty in determining dioecy (Smith and Schubert, 1946; Thomas, Ardi and Hughes, 2011 - see below for further discussion). Therefore, for a species to be known as dioecious, populations must be observed for an extended period of time.

1.1.7 *Genetic causes behind dioecy* – In some dioecious lineages, specific genetic backgrounds have been shown to be active in sex determination. Certain dioecious taxa from angiosperm families such as Cannabaceae (*Cannabis* L. and *Humulus* L.), Caryophyllaceae (*Silene* L.), Cucurbitaceae (*Coccinia* Wight & Arn.), and Polygonaceae (*Rumex* L.) have been found to have distinct sex chromosomes that play a part in determining the sex of individual plants (Matsunaga and Kawano, 2001; Renner, 2014). These sex chromosomes typically follow the XY system, where males are heterogametic and females are homogametic, although much variety on that pattern is observed across angiosperm dioecious lineages. In the past, genes from a single sex chromosome have been hypothesized to suppress ovary development and promote anther growth, inducing dioecy through a "two mutations linked on one chromosome" model (Charlesworth and Charlesworth, 1978). More recently, specific genes within both sex and autosomal chromosomes that affect unisexual floral development are beginning to be understood (Renner, 2016). For example, whole genome sequencing, transcriptomes and genetic recombination techniques were utilized to identify an autosomal paralog of a small male-inducing RNA molecule *OGI* on the Y chromosome that suppresses an identified female-inducing gene, *MeGI* within the genome of the persimmon, *Diospyros lotus* L. (Akagi et al., 2014). This persimmon study revealed a potential gene that transforms a bisexual system into a unisexual one. A second study of *Cucumis* L. within the Cucurbitaceae went a step further and found multiple potential gene candidates that may play a part in the evolution from monoecy to dioecy through various allele fixation or heterozygosity. In this particular case, genes identified in the study were associated with several autosomes, as sex chromosome are absent in *Cucumis* (Boualem et al., 2015).

These two cited studies demonstrate that the genetic machinery behind dioecy, in some cases, is more complicated than two gene mutations in a single sex chromosome. Other studies involving hormonal interactions with floral identity genes also demonstrate the lack of simplicity in identifying a set genotype behind dioecy, as the environment can have a great effect on a set genotype, altering the observed sexes in a population (Golenberg and West, 2013). As dioecy has independently evolved many times across flowering plants at different times in evolutionary history, it is likely that differing genetic pathways occur in each dioecious lineage (Renner, 2016; Harkess and Leebens-mack, 2017). As the ever growing field of genomics increases the ability to identify genes, such as those leading the floral sex separation, further pathways leading to the evolution of dioecy will be discovered and provide greater clarity to genetic mechanisms behind this rare breeding system.

1.1.8 *The rarity of dioecy in flowering plants* – More studies are beginning to focus on the rates and directionality of breeding system evolution within flowering plant phylogenies (Käfer et al., 2014; Sabath et al., 2016; Goldberg et al., 2017; Käfer, Marais and Pannell, 2017). These studies take into account the possibility that flowering plant lineages are evolving *away* from dioecy. This perspective adds to the decades of research and thought that focused on the evolutionary pathways leading toward dioecy and may begin to shed light on the rarity of dioecy in flowering plants. Originally, it was thought that dioecy was rare because it was a "dead end" in evolution. Dioecy reduces the seed bearing individuals by 50%, and prevents selfing, thereby maintaining gene flow between individuals (Bawa, 1980). With only half the individuals in a population capable of producing seed, dioecious populations are at a competitive disadvantage with hermaphroditic or monoecious populations. To become competitive, dioecious species must significantly increase fitness of their fruit and seed production (Heilbuth, Ilves and Otto, 2001). It was thought that these characteristics of dioecy slowed lineage diversification, as is suggested in a survey of diversification in New Zealand, where dioecy is prevalent in the flora (Jesson, 2007). However, Käfer et al. (2014) produced a study demonstrating that dioecy has evolved early on in some lineages and promoted diversification. In contrast with these findings, Sabath et al. (2016) found that dioecy doesn't necessarily have a significant affect on diversity rates in lineages where it has evolved, and much research must be done before conclusions can be made regarding evolutionary advantage or disadvantage of dioecy. A later study emphasized that the current rarity of dioecy is likely due to a common tendency for dioecious lineages to "lose" their dioecy and revert back to monoecy or hermaphroditism, as populations that demonstrate relaxed, or "leaky" sexual determination have higher fitness (Käfer, Marais and Pannell, 2017). Future research must begin to focus on specific groups with frequent reversions from dioecy, to elucidate the cause behind the loss.

Therefore, rather than viewing dioecy as a definite driver or hindrance to the evolution of diversity, it may simply be seen as an important, sometimes intermediate stage in flowering plant evolution that has been reached through a great variety of genetic or ecological mechanisms. Analyzing single dioecious lineages will provide clearer answers to the precise cause or influence of dioecy within that single lineage, rather than focusing on the cause or influence of dioecy across all angiosperms. As a model to investigate the pathway from monoecy to dioecy postulated by Bawa (1980), this present study focuses solely on dioecy within the genus *Begonia*, to increase understanding of the evolution of dioecy and potential reasons for its rarity within the genus.

1.2 *Dioecy within Begoniaceae*

Begonia is a mega-diverse tropical genus with unisexual flowers. Of the 1839 currently described species in the genus, only 19, or around 1%, are known to be dioecious (Hughes et al., 2015; this study). The rare cases of dioecy developing within the genus *Begonia* appear to have evolved directly from monoecy through the monomorphic pathway (Goldberg et al., 2017). Begoniaceae, along with the families Cucurbitaceae, Tetramelaceae, and Datiscaceae belong to a distinct clade within the Cucurbitales that is distinguished by high occurrence of unisexual lineages. Phylogenetic studies have shown this clade to be derived from a dioecious ancestor (Zhang et al., 2006). As Begoniaceae is mostly monoecious, it is then inferred that the ancestral lineage to the family transitioned from dioecy to monoecy.

The transition away from dioecy within the history of angiosperm lineages is a common trend (Käfer et al., 2014). The tendency for dioecy to act as an "intermediate", or easily transition into another breeding system, may account for its rarity in extant angiosperms (Käfer, Marais and Pannell, 2017). The initial transition from dioecy to monoecy within the ancestral lineage to Begoniaceae and the current rarity of dioecy in *Begonia* may reflect this angiosperm-wide hypothesis. However, existing data suggests that breeding system evolution within *Begonia* is solely unidirectional, from monoecy to dioecy (Goldberg et al., 2017). The presence of dioecy within *Begonia* may be seen as a reversion to the ancestral breeding system, though the precise mechanism for its evolution within Begoniaceae is not known and may differ from that of its initial evolution within the Cuburbitales. Other than inclusion in the 2017 angiosperm-wide study looking into the directionality of transition in breeding systems, which used a highly unresolved ITS phylogeny and did not include all known dioecious *Begonia* species*,* evolution of *Begonia* breeding systems has not yet been researched in any detail. The genus *Begonia* represents an ideal genus for researching the mechanisms behind the monomorphic pathway and other theories concerning the evolution of dioecy.

Theories regarding the evolution of dioecy within *Begonia* may be formulated by analyzing correlated characters. Species within the genus *Begonia* have a variety of fruit types: dry dehiscent capsules, rain-ballist capsules or fleshy berry-like capsules (Thomas et al., 2011). Fleshy fruit is correlated with dioecy in certain lineages (Bawa, 1980; Renner and Ricklefs, 1995). Fleshy fruit seen in dioecious taxa and not their hermaphroditic relative implies an increase in resource allocation to female reproduction, which is believed to increase population fitness by avoiding the seed shadow handicap, or local resource competition between overcrowded offspring, through increased seed dispersal distance away from the parent (Heilbuth, Ilves and Otto, 2001). Muenchow (1987) suggests that this association is only weakly proved and is influenced by previous phylogenetic association, as dioecious species and fleshy fruit both tend to evolve in understory forest habitats. However, in the case of *Begonia,* there is potential to investigate an unbiased association between dioecy and fleshy fruit as all species occupy similar micro-habitats and both dioecy and fleshy fruit are uncommon within the genus.

As dioecy is thought to be associated with fleshy fruit for the purpose of maintaining competitive ability by diminishing the seed shadow handicap with increased dispersal distance, it should therefore also be associated with larger range sizes. Specifically, animal vectors such as birds and other vertebrates disperse fleshy *Begonia* fruits greater distances than dry capsules with passive distribution mechanisms (Tebbitt et al., 2006). Another argument supporting the potential association between dioecy and large range sizes within *Begonia* involves the maintenance of a population's spatial genetic cohesion due to forced outcrossing. Many *Begonia* species distributions are narrow and restricted to specific wet, shaded environments. The high species diversity of *Begonia* may be a result of these isolated micro-habitats where gene flow is prevented between populations (Hughes, 2002). Isolated populations are also prone to inbreeding, providing sufficient pressure to promote dioecy. If dioecy appears within a lineage with widely dispersed seeds, it allows a *Begonia* species to maintain its genetic identity across a greater distance. This maintenance of a species genetic cohesion is due to forced outcrossing and uninterrupted gene flow imposed by dioecy on populations, as is seen in dioecious *Ficus* L. species (Nazareno, Alzate-Marin and Pereira, 2013). While the maintenance of a population's level of gene flow through dioecy is beneficial in avoiding inbreeding and loss of allele diversity due to drift, it can also be seen as a hindrance to a potential diversification because the process of speciation is slowed. This may be one of many potential reasons as to why only 1% of *Begonia* species are dioecious, though it is not addressed in this study.

It is difficult to confidently identify dioecy within *Begonia* due to observed dichogamy and unstable, or "leaky" dioecy. Dichogamy is the temporal separation of effective male and female flowering on a single plant, typically applied to pollen dispersal and stigma receptivity within a hermaphroditic flower. In the case of monoecious species*,* dichogamy implies male and female unisexual flowers differing in the timing of their appearance. Temporal separation in flowering times has been observed numerous times for species across *Begonia*, creating difficulty in determining sexual system (Smith and Schubert, 1946; Thomas, Ardi and Hughes, 2011). As there are *Begonia* species that are only known from a single collection or have not been observed in the wild for extended periods of time to accurately access flowering, false reports of dioecy are easily made.

Even if a *Begonia* species has been determined to be truly dioecious, there are also occasions where a monoecious plant is observed within a dioecious population due to the tendency for dioecy to be "leaky" and relax whatever mechanism is utilized in restricting a single plant to a single sex. It has been suggested that the monomorphic evolutionary pathway to dioecy is driven by many mutations and gene interactions, and is more affected by environmental variation. Indeed, a recent study within Cucurbitales identified numerous genes interacting to produce a dioecious species from monoecious ancestry (Boualem et al., 2015). Therefore, dioecy that has evolved from monoecy may be more prone to leakiness due to environmental influence rather than from the dimorphic pathway involving gynodioecy (Dorken and Barrett, 2004). This "leaky" tendency is hypothesized to be a leading factor in the common reversions from dioecy recorded within angiosperms (Käfer, Marais and Pannell, 2017).

Understanding the molecular background associated with dioecy may provide insight into this breeding system's unstable pattern. Within the Cucurbitaceae, a closely related family to Begoniaceae, species such as *Coccinia indica* are reported to have distinct XY sex chromosomes (Matsunaga and Kawano, 2001). In families such as Begoniaceae, however, sex chromosomes are not reported. Instead, hormones have been shown to have a significant effect in determining the sex of individuals in *Begonia* species (Khryanin, 2002). For example, an increase in gibberellin concentration was shown to induce induced maleness in floral meristem of horticultural hybrids (Bessler, 1996). The influence of hormones on floral identity genes in response to environmental stress (Golenberg and West, 2013) and lack of sex chromosomes may be one driving factor behind the ambiguity seen within *Begonia* breeding systems.

1.3 Aims of this study

Dioecious breeding systems are thought to benefit populations by preventing loss of genetic diversity due to selfing and inbreeding. The monomorphic pathway involving monoecy is currently thought to be the most common pathway in the evolution of dioecy across angiosperms. As *Begonia* is a highly diverse, mostly monoecious, self-compatible genus that tends to have small isolated populations that are more likely to become inbred, one would expect to see more lineages within the genus becoming dioecious. However, only 19 of 1839 *Begonia* species are reported to be dioecious, suggesting that dioecy may be difficult to evolve and maintain within a lineage. Heilbuth, Ilves and Otto (2001), hypothesize that the rarity of dioecy among angiosperms may be due to the "seed shadow handicap", or an increase in density of dioecious offspring populations, causing individuals to compete with each other for local resources. The seed shadow handicap prevents dioecious species from successfully competing with hermaphroditic and monoecious neighbors. In order for dioecious lineages to remain competitive, adaptions such as animal dispersed fleshy fruit that promote increased seed dispersal distance are necessary. If this were true in the case of *Begonia*, one would expect to see dioecy associated with fleshy fruit and larger range size. To understand the mechanism behind the evolution and rarity of dioecy in the genus *Begonia*, the following questions are put forward:

1. How many times dioecy has independently evolved within *Begonia?*
2. Is fleshy fruit correlated with larger range sizes, implying that animal dispersal is a more effective dispersal method within *Begonia* than dry capsule passive dispersal?
3. Are these transitions to dioecy in *Begonia* correlated with fleshy fruit and/or large range size supporting the hypothesis that larger range sizes achieved through fleshy fruit dispersal are necessary for dioecious species to overcome the seed shadow handicap and remain as competitive as neighboring hermaphroditic or monoecious plant within the environment?

Here, these questions are addressed through plastid phylogeny and character reconstruction. *Begonia* species' breeding system type is compared with fruit type and range size with respect to phylogeny through correlative analyses. Extent of occurrence (EOO) data is used as a proxy for range size. If a correlation exists, further hypotheses regarding the rarity of dioecy within *Begonia* can be made. In addition, the directionality and transition rate of breeding system types will also be examined to re-affirm the conclusion that only unidirectional transitions from monoecy to dioecy occurs within *Begonia* (Goldberg et al., 2017). The resulting information will be the first step leading to a deeper understanding of the evolution of dioecy from monoecy within this mega-diverse tropical genus.

Chapter 2 – Materials & Methods

2.1 *Taxon sampling*

Several *Begonia* taxa were added to the existing molecular dataset constructed by Moonlight et al. (unpubl. data), with *Hillebrandia sandwicensis* Oliv. as the outgroup. Emphasis was placed on potentially dioecious species, which were identified through literature searches. DNA was available for 13 of the 19 known dioecious *Begonia* species. Four of the remaining six species were grafted into subsequent phylogenetic trees using the 'phytools' package in R (Revelle, 2012) based upon known sister species. *Begonia extranea* L.B. Sm. & B.G. Schub. and *B. nemoralis* L.B. Sm. & B.G. Schub. are two New World dioecious species in section *Knesebeckia* that could not be grafted into the dataset due to ambiguity in phylogenetic placement of their sister taxon, *B. incarnata* Link & Otto (Peter Moonlight, RBGE, pers. comm.). A dataset, comprising of 825 accessions representing about 570 *Begonia* species from across the genus, was used for initial phylogenetic analyses. If a species represented by multiple accessions proved to be monophyletic in initial analyses, the extra accessions were then pruned out of molecular dataset by hand in preparation for final phylogenetic analyses and maximum likelihood character reconstruction. Any spelling errors in the dataset were corrected using the R package 'ape' (Paradis et al., 2004). In the final dataset, with 628 accessions, species with paraphyletic or polyphyletic lineages retained multiple representative accessions.

2.2 *DNA extraction, amplification, and sequencing*

DNA extraction, amplification and sequencing followed Thomas et al. (2011), with minor modification. For the several *Begonia* taxa added to the dataset, standard procedures utilizing the Qiagen DNeasy Plant Mini kit were used for genomic DNA extraction from both silica dried and living material. The elution buffer (AE) was preheated to 65˚C before use to maximize the quantity of the DNA eluted from the column in the final 150µl of solution collected from the extraction.

Three chloroplast (cp) non-coding regions, the *ndhA* intron and the *ndhF-rpl32* and *rpl32-trnL* intergenic spacers, were selected for amplification to contribute to the existing *Begonia* molecular dataset. Each polymerase chain reaction (PCR) for the three regions was a total of 25µl, consisting of 10.8µl H_2O, 2.5µl dNTPs (2mM), 5µl Phusion TAQ polymerase 5× buffer, 0.75µl of both forward and reverse primers (10µM), 4µl 5× TBT-PAR, 0.2µl Phusion TAQ polymerase (5u/µl), and 1µl template taken from the 150µl of genomic DNA. The use of TBT-PAR and Phusion polymerase were used to combat PCR inhibitors and polymerase mis-pairing due to repeated strands of nucleotides common in *Begonia* DNA

(Thomas et al., 2011). Primers used for the three regions are as follows. For the ndhA intron, the two primers ndhAx1 (GCYCAATCWATTAGTTATGAAATACC) and ndhAx2 (GGTTGACGCCAMARATTCCA) were used (Shaw et al., 2007). For the ndhF-rpl32 intergenic spacer, ndhFBeg-F(CO57F) (TGGATGTGAAAGACATATTTTGCT) and rpl32Beg-R (CO57R) (TTTGAAAAGGGTCAGTTAATAACAA) were used (Thomas et al., 2011). Finally, for the rpl32-trnL intergenic spacer, trnL(CO54F) (CTGCTTCCTAAGAGCAGCGT) and rpl32-F (CO54R) (CAGTTCCAAAAAAACGTACTTC) were used (Shaw et al., 2007). The PCR temperature profile was as follows: initial template denaturation at 95˚C for four minutes, 35 cycles of denaturation at 94˚C for 30 seconds, primer annealing at 50˚C for 30 seconds, and primer extension at 72˚C for two minutes followed by a final extension step of 72˚C for seven minutes and a hold temperature at 10˚C forever.

PCR products were visualized on a 1% agarose TBE gel with SYBR Safe stain to determine concentration for the sequencing reaction. ExoSAP-IT solution was used remove extra dNTPs and primers to prepare PCR products for sequencing. 5µl of PCR product was mixed with 2µl of ExoSAP-IT and run in the thermocycler for 15 minutes at 37˚C followed by 15 minutes at 80˚C, according to manufacturer's protocol. This solution was then prepared for sequencing through a set of 10µl PCR reactions consisting of 6.18µl H_2O, 2µl 5× BIGDYE mix buffer, 10µl of either the forward or reverse primer (10µM), 0.5µl of BIGDYE mix, and 1µl of PCR template. H_2O and PCR template amounts varied based upon concentration of PCR products shown by the agarose gel. These solutions were run through a temperature profile of denaturing at 95˚C for 30 seconds, annealing at 50˚C for 20 seconds, and primer extension at 60˚C for four minutes for 25 cycles. The samples were then sent for sequencing at the GenePool facilities at the University of Edinburgh for analysis.

2.3 *Phylogenetic analysis*

2.3.1 *Sequence alignment* – Sequences of the three cp regions added to the previous concatenated dataset (Moonlight et al., unpubl. data) were manually aligned in BioEdit (Hall, 1999). The resulting alignment was 5697 base pairs. Gaps in the alignment corresponding to the beginning and end of each of the three cp regions were excluded in BioEdit. Specifically, base pairs 1-74, 1811-1919, and 3416-3595 were exluded, resulting in a 5334 bp alignment. After taxon multiples were taken out, the alignment was 5311 base pairs. This alignment was used for phylogenetic analyses in Random Axelerated Maximum Likelihood (RAxML) and Bayesian Evolutionary Analysis Sampling Trees (BEAST).

2.3.2 *MrBayes* – A preliminary MrBayes analysis was performed on the initial molecular dataset of 5697 base pairs to ascertain the quality of the sequence alignment and note any errors. From the BioEdit alignment fasta file, a nexus file was obtained through the conversion application ALignment Transformation EnviRonment (ALTER) (Glez-Peña et al., 2010). The analysis was run through MrBayes 3.2.6 on the online server CIPRES (Ronquist and Huelsenbeck, 2003; Miller et al., 2010). A Bayesian analysis was performed in MrBayes, including two separate runs with 4 chains each, of 5000000 generations, using a GTR model of DNA evolution with a gamma distribution of rates and a proportion of invariant sites (Moonlight et al., 2015), and sampling every 10000 generations. Stationarity of the Bayesian Markov chain Monte Carlo (MCMC) runs were assumed based on a value less than 0.01 for the average standard deviation of split frequencies between two runs. Trees sampled prior to stationarity were excluded by "burnin" (25% of samples) and the 375 remaining trees were used to construct a majority rule consensus tree with clade credibility values to ascertain quality of the molecular data and sequence alignment.

2.3.3 *RAxML* – For additional preliminary analyses to compare with Mr. Bayes output, phylogenetic analysis was performed in RAxML v.8 (Stamatakis et al., 2008). The alignment was converted to the phylip file format using ALTER website (Glez-Peña et al., 2010) and uploaded to the RAxML blackbox website for analysis. A general time reversible model with gamma distribution of rates (GTR+G) model was specified. A maximum likelihood analysis of the dataset was performed through 100 independent bootstrap analyses and the resulting bipartitioned tree was used in decision making regarding taxa maintained within the final analyses in BEAST.

2.3.4 *BEAST* – To produce a population of time calibrated ultrametric trees, a phylogenetic analysis utilizing BEAST v1.8.4 was performed (Drummond et al., 2012). The data file was prepared for analysis in the BEAST package formatting application, BEAUti. The concatenated data was considered as a single partition and all *Begonia* species were included in a monophyletic ingroup. The site substitution model was set at general time reversible model (GTR), with estimated base frequencies and a gamma site heterogeneity model with four categories. An uncorrelated relaxed clock with lognormal distribution was used. Tree priors included Yule Process Speciation with a random starting tree. A secondary time calibration (24Ma +/- 3.57) derived from a wider taxonomic analysis of Cucurbitales-Fagales was used to calibrate the crown node of *Begonia* (Thomas et al., 2012) within the time of the most recent common ancestor (tmrca) prior. All other priors were left to default. The MCMC prior was set to a 5000000 state chain (or generations). The analysis was performed in two duplicates (to produce a desired 1000000 states) through the online server CIPRES (Miller et al., 2010). The associated log files were analyzed through Tracer v1.6.0 (Rambaut et al., 2014) for quality of the trees produced. The two tree files produced were combined to form a single tree file through the LogCombiner application within BEAST. 2000000 states, or 2000 trees were burned in for each analysis as the MCMC only reached stationarity after the first 2000000 states in each run. The resulting 6000 trees were run in the TreeAnnotator application within BEAST to find the single best tree based upon the log clade credibility statistic. A post-burnin population of 6000 ultrametric trees was used for character analysis in R (R core team, 2017).

2.4 *Character analysis*

2.4.1 *Matrix construction* – A matrix of 628 taxa was produced coding for three characters: breeding system, fruit type, and range size. Breeding system was coded as a binary character representing the two breeding types found in *Begonia*, dioecious – 0 and monoecious – 1. Fruit type was also coded as a binary character, fleshy – 0 and dry – 1. Binary character data was collected from literature references from the Royal Botanic Garden Edinburgh (RBGE) library and various online databases such as Google Scholar and the University of Edinburgh library system. Range size for each *Begonia* species in the tree was represented by extent of occurrence (EOO) measurements, based on a boundary polygon. EOO was calculated using the ConR package in R (Dauby, 2017). Latitude and longitude data of representative herbaria specimens were obtained from the RBGE *Begonia* Database (Hughes et al., 2015), taxonomic revisions of *Begonia* (de Wilde, 2002) and GBIF (2017). Some coordinate points were obtained from external Internet atlases and were evaluated for accuracy based upon expected geographic location (i.e. within political boundaries listed in species descriptions). Ocean cover was excluded within the ConR package in R using a world land shapefile obtained from openstreetmapdata.com. Area of occupancy (AOO) was also calculated within the same R program. EOO and AOO data were graphed to compare values, to ascertain the quality of the calculations (Appendix 3). EOO data was chosen to represent species range size as it was less affected by gaps in specimen collections across each species natural range. Many *Begonia* species are restricted endemics with less than the three representative collections needed to calculate EOO. The default minimum range area of 4km^2, typically used for AOO calculations, was given to the taxa with less than three collections.

2.4.2 *Ancestral State Reconstruction* – Character reconstruction using a maximum likelihood approach for the two discrete characters, breeding system and fruit type, was performed on the population of 6000 ultrametric BEAST trees within Mesquite (Maddison and Maddison, 2015). The characters were traced over the 6000 trees producing likelihood scores for node ancestral states, and were represented on the single best tree with the highest likelihood produced from the BEAST analysis. Maximum likelihood character reconstruction for the logarithm of the continuous character, EOO, was performed on the single best BEAST tree using the R package ‘phytools’ fastAnc and contMap functions and the resulting tree was printed as a fan to visually accommodate the large phylogeny (Revell, 2012). The logarithm of extent of occurrence (log(EOO)) was taken to produce a more normal distribution of the data, due to a significant right skew in the data, as *Begonia* species are mainly narrow endemics, with a few species obtaining exceptionally large ranges. The number, rate and directionality of breeding system transitions were also analyzed on the single best BEAST tree in Mesquite, through stochastic character mapping using the asymmetric 2-parameter model with estimated transition rates. This model allowed the possibility of reversions from dioecy to monoecy within the genus to be analyzed.

2.4.3 Statistical *character analysis* – The log(EOO) values were compared with breeding system and fruit type using simple boxplot and mean calculations in R. Phylogenetic signal of characters was analyzed in the R package ‘caper’ (Orme et al., 2013) with function phylo.d for calculating D, a measure of phylogenetic signal in a binary trait (Fritz & Purvis, 2010) and the package ‘phytools’ with function phylosig to find the lambda statistic and p-value for the phylogenetic significance of the continuous character log(EOO).

Correlative character analyses were also performed in R utilizing the ‘phylolm’ package (Ho and Ane, 2014). To determine if log(EOO) variation across the phylogeny was phylogenetically correlated to fruit type or breeding system, the function phylolm was used with the Brownian Motion model. Phylolm analyzes character correlations through testing the likelihood that breeding system or fruit type can explain the variation of log(EOO) across the phylogeny (logEOO~BS or FT). For each character comparison, a null model in which log(EOO) variation across the phylogeny was not explained by any variable (logEOO~1) was also analyzed to compare AIC scores (Akaike, 1974). The character comparison that better explains the variation of log(EOO) values across the tree would have a lower AIC score and be considered more likely. Through this comparison, a correlation between characters is proved if the variation of log(EOO) across the phylogeny given one of the discrete characters proved more likely than the null. To test correlations between the two discrete characters breeding system and fruit type, comparative functions in phylolm could not be used, as the number of tips in the phylogeny that showed breeding system and fruit type transitions were too low to allow for statistical convergence (or too little information was available in the data). Instead, basic probability and chi-squared tests were used to see if the proportion of observed taxa with both transitions in fruit type (fleshy fruit) and breeding system (dioecy) were higher than the proportion of taxa expected statistically. A chi-squared test with p-value was used to determine the significance of any association between the two variables that was observed.

Chapter 3 – Results and discussion

3.1 *Literature search for characters*

Characters for reconstruction were confirmed through a literature search and references for each potentially dioecious species are indicated in Table 1. Due to the nature of searching for dioecious *Begonia* species in the literature based upon previous knowledge of their existence, it is possible that some dioecious species were overlooked, although this study improved upon that of Goldberg et al. (2017), which only reported 6 dioecious species. During the literature search, in some cases, the breeding system of a species was not mentioned in descriptions within relevant floras or even the original species description. In these instances, a subsequent journal article mentioning the presence of dioecy in the species was cited. When descriptions of dioecy were found in the literature, often they were not confident in claiming the species to be dioecious, unless a species had been grown under supervision for a long period of time to observe the lack of one sex of flower on a single plant. For example, *B. guttapila* D.C. Thomas & Ardi was listed as monoecious in its original publication (Thomas, Ardi and Hughes, 2009), but later found to be dioecious (Thomas, Ardi and Hughes, 2011). There are also some instances of dioecious species, such as *B. biserrata* Lindl. and *B. burkillii* Dunn that demonstrate a “leaky” dioecy on occasion, and produce a monoecious plant.

Ambiguity was also present in defining the fruit type. To confidently claim whether a fruit was fleshy or dry, the live plant must be observed to the mature fruit stage. As this was not possible in this study, species descriptions, herbarium specimens, and photographs were used to determine fruit type. Species descriptions and herbarium specimens did not necessarily demonstrate a fruit’s fleshiness or dryness, so other characters were used to determine fruit type. Typically, if wings were present on the fruit capsule, it was assumed to be dry, such as those fruits of *B. dioica* Buch.-Ham. ex D. Don. However, some species such as those in section Petermannia have winged fruit yet retain a somewhat fleshy pericarp, and were considered fleshy. Another potentially helpful indicator as to whether a fruit is fleshy or dry was dehiscence. Typically, a fleshy, more berry-like fruit would not dehisce. However, dehiscence or indehiscence were not definitively reliable as an indicator of fleshiness or dryness either, as some fleshy fruits were described as dehiscent, such as those of *B. subalpestris* A. Chev.

Lastly, range size (EOO) measurements obtained from searching the *Begonia* database (Hughes et al., 2015), and GBIF (2017) had potential bias toward smaller measurements, as underrepresented specimen collections or incorrect specimen identification could affect the calculation of the EOO. Even with a potential bias toward smaller values, EOOS were assumed to be sufficient in observing evolution patterns.

Table 1. Potentially dioecious *Begonia* species and corresponding references.

Species	Section	References
B. guttapila D.C. Thomas & Ardi	Petermannia	(Thomas, Ardi and Hughes, 2009, 2011)
B. rantemarioensis D.C. Thomas & Ardi	Petermannia	(Thomas, Ardi and Hughes, 2011)
B. mekonggensis Girm. & Wiriad.	Petermannia	(Girmansyah et al., 2009; Thomas, Ardi and Hughes, 2011)
B. sanguineopilosa D.C. Thomas & Ardi	Petermannia	(Thomas, Ardi and Hughes, 2011)
B. torajana D.C. Thomas & Ardi	Petermannia	(Thomas, Ardi and Hughes, 2011)
B. acetosella Craib.	Sphenanthera	(Tebbitt, 2003b; Gu, Peng and Turland, 2007)
B. handelii Irmsch.	Sphenanthera	(Tebbitt, 2003a; Gu, Peng and Turland, 2007)
B. silletensis (A.DC.) C.B. Clarke	Sphenanthera	(Tebbitt, 2002; Gu, Peng and Turland, 2007)
B. aborensis Dunn	Sphenanthera	(‘Decades Kewenses’, 1920; Uddin and Phukan, 2007)
B. burkillii Dunn	Sphenanthera	(‘Decades Kewenses’, 1920; Tebbitt, 2003a)
B. roxburghii (Miq.) A. DC	Sphenanthera	(Tebbitt, 2005)
B. dioica Buch.-Ham. ex D. Don.	Diploclinium	(Thomas, Ardi and Hughes, 2011)
B. wilburi Burt-Utley &Utley	Casparya	(Burt-Utley and Utley, 2012; Burt-Utley, 2014)
B. ursina L.B.Sm. & B.G.Schub	Casparya	(Burt-Utley and Utley, 2012)
B. extranea L.B. Sm. & B.G. Schub.	Knesebeckia	(Smith and Schubert, 1939; Burt-Utley and Utley, 2012)
B. nemoralis L.B. Sm. & B.G. Schub.	Knesebeckia	(Smith and Schubert, 1947; Burt-Utley and Utley, 2012)
B. biserrata Lindl.	Quadriperigonia	(Burt-Utley and McVaughn, 2001; (Burt-Utley and Utley, 2012; Burt-Utley, 2014)
B. subalpestris A. Chev.	Tetraphila	(de Wilde, 2002)
B. meyeri-johannis Engl.	Mezierea	(Plana, 2003, Plana et al., 2006)

3.2 *Dated phylogenetic analysis*

The BEAST analysis of the three cp regions produced 6000 dated ultrametric trees, the best of which, tree number 4365, had a log clade credibility score of -248.96. Time of the most recent common ancestor (tmrca) for the genus *Begonia* (ingroup) was 22.98 million years (Ma) with an estimated sample size of 785 and the root height of the entire tree (tmrca of *Hillebrandia* and *Begonia*) was 26.27 Ma (Fig.1a-c). Dates for the nearest node associated with dioecious taxa within *Begonia* are listen in Table 2. *Begonia meyeri-johannis* Engl., *B. biserrata,* and *B. dioica* have the oldest nearest ancestral nodes, indicating that they have evolved the earliest. *B. wilburi* Burt-Utley &Utley, *B. ursina* Burt-Utley &Utley and *B. subalpestris* have only evolved dioecy within the last half million years. Overall, within the genus *Begonia,* dioecy has evolved several times between 16 million and 0.001 million (1000) years ago.

Table 2. Age, in millions of years, of the nearest ancestral node associated with the appearance of dioecy in the *Begonia* phylogeny. Age is represented through median node height taken from the highest posterior density interval (95% HPD) produced in BEAST.

Section	Species	Height_median (Ma)	Height_95%_HPD (Ma)
Mezierea	*B. meyeri-johannis*	5.4	[1.9, 11.8]
Tetraphila	*B. subalpestris*	0.3	[0.001, 1.6]
Quadriperigonia	*B. biserrata*	6.1	[3.7, 8.6]
Casparya	*B. wilburi, B. ursina*	0.5	[0.3, 0.9]
Sphenanthera	*B. acetosella, B. handelii, B. silletensis, B. aborensis, B. burkillii*	2.3	[1.2, 3.6]
Sphenanthera	*B. roxburghii*	1.8	[0.6, 3.1]
Diploclinium	*B. dioica*	11.3	[7.0, 16.0]
Petermannia	*B. torajana, B. mekonggensis, B. guttapila, B. rantemarioensis, B. sanguineopilosa*	0.9	[0.5, 1.4]

Fig1a

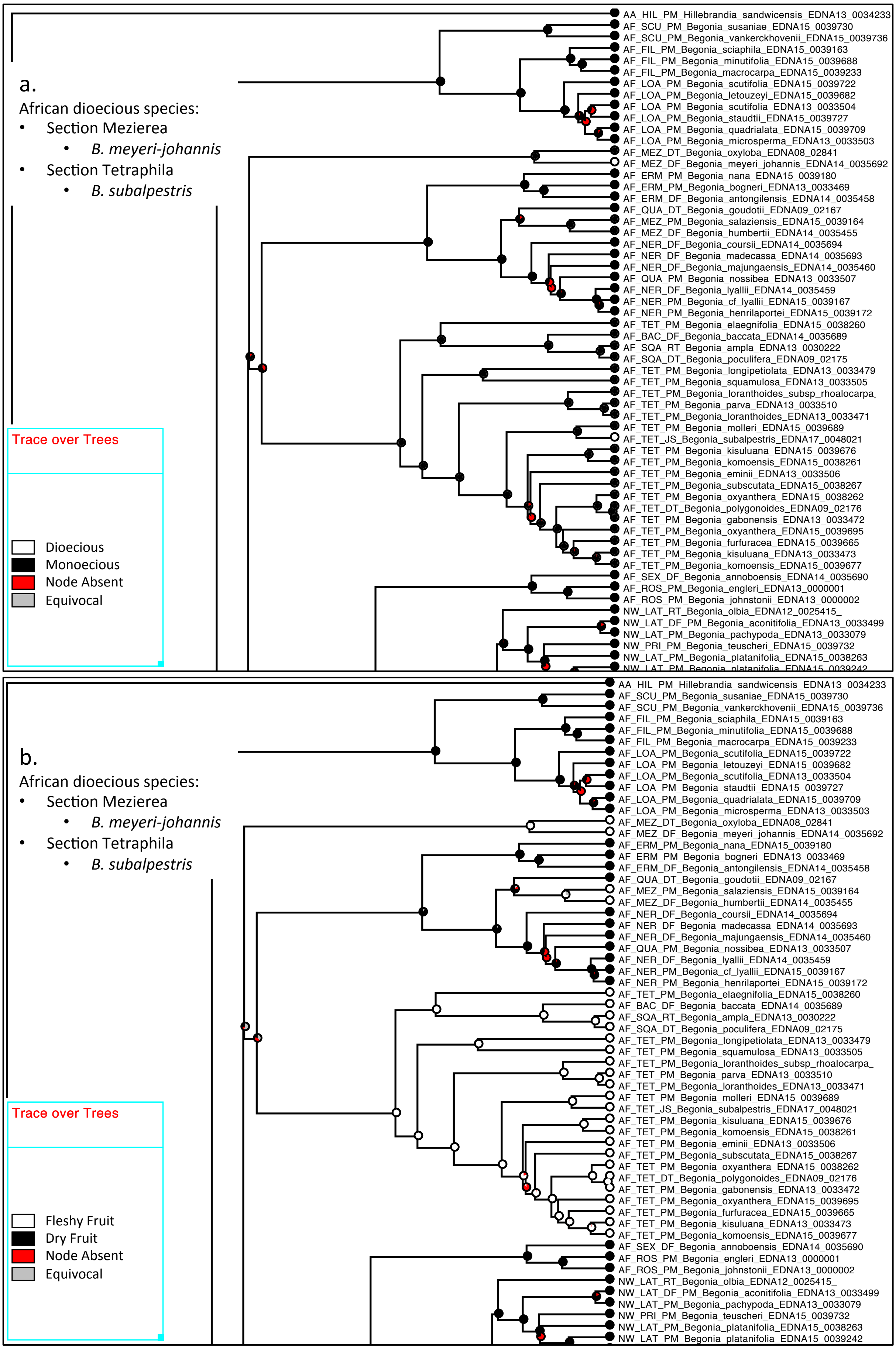
Fig1b

Fig1c

Figure 1a-c. Single best tree obtained from BEAST. Tree is divided into three sections based on geographic region: 1a shows African *Begonia* clades, 1b shows New World *Begonia* clades, and 1c shows Asian clades. This single tree, number 4365, was derived from a population of 6000 dated ultrametric trees with a log clade credibility statistic of -248.96 and is presented with posterior probability support values. Time scale is presented in millions of years beneath each portion of the phylogeny (a-c).

3.3 *Character reconstruction*

3.3.1 *Breeding system* – Dioecy has evolved independently more than once in each tropical region of the world where *Begonia* occurs, as categorized in this study (Africa, Asia, New World). Seven *Begonia* sections have evolved dioecy, each with a single independent origin (Table 1, Fig. 2a, c, e, g, i), excepting section Sphenanthera, which may have two origins of dioecy (Table 2, Fig. 2g), although ancestral states, and phylogenetic structure (Fig.1c within the section are not well enough supported across the 6000 trees to make a firm claim. Assuming section Sphenanthera has two independent origins and without considering the two species in section Knesebeckia, which could not be analyzed here, dioecy has evolved a total of eight times in *Begonia* in seven sections and 17 species (Table 1). Interestingly, at least two transitions from dioecy back to monoecy are potentially evident from stochastic character mapping in the more specious dioecious clades Sphenanthera and Petermannia, as *B. vermeunelii* D.C. Thomas, *B. longifolia* Blume*,* and *B. aptera* Blume are monoecious and nested within dioecious clades. Phylogenetic support within the dioecious clades of Sphenanthera and Petermannia is low (Fig.1c), but when structure is present within the population of trees used for ancestral state reconstruction, ancestral nodes indicate a reversion to monoecy within these two sections. Transition rates from monoecy to dioecy and from dioecy to monoecy within *Begonia* are 0.2506 and 0.0051, respectively. Breeding system transition in *Begonia* is therefore bidirectional between monoecy and dioecy, but biased toward dioecy.


a.
African dioecious species:
- Section Mezierea
  - B. meyeri-johannis
- Section Tetraphila
  - B. subalpestris
Trace over Trees
☐ Dioecious
■ Monoecious
🟥 Node Absent
▨ Equivocal
AA_HIL_PM_Hillebrandia_sandwicensis_EDNA13_0034233
AF_SCU_PM_Begonia_susaniae_EDNA15_0039730
AF_SCU_PM_Begonia_vankerckhovenii_EDNA15_0039736
AF_FIL_PM_Begonia_sciaphila_EDNA15_0039163
AF_FIL_PM_Begonia_minutifolia_EDNA15_0039688
AF_FIL_PM_Begonia_macrocarpa_EDNA15_0039233
AF_LOA_PM_Begonia_scutifolia_EDNA15_0039722
AF_LOA_PM_Begonia_letouzeyi_EDNA15_0039682
AF_LOA_PM_Begonia_scutifolia_EDNA13_0033504
AF_LOA_PM_Begonia_staudtii_EDNA15_0039727
AF_LOA_PM_Begonia_quadrialata_EDNA15_0039709
AF_LOA_PM_Begonia_microsperma_EDNA13_0033503
AF_MEZ_DT_Begonia_oxyloba_EDNA08_02841
AF_MEZ_DF_Begonia_meyeri_johannis_EDNA14_0035692
AF_ERM_PM_Begonia_nana_EDNA15_0039180
AF_ERM_PM_Begonia_bogneri_EDNA13_0033469
AF_ERM_DF_Begonia_antongilensis_EDNA14_0035458
AF_QUA_DT_Begonia_goudotii_EDNA09_02167
AF_MEZ_PM_Begonia_salaziensis_EDNA15_0039164
AF_MEZ_DF_Begonia_humbertii_EDNA14_0035455
AF_NER_DF_Begonia_coursii_EDNA14_0035694
AF_NER_DF_Begonia_madecassa_EDNA14_0035693
AF_NER_DF_Begonia_majungaensis_EDNA14_0035460
AF_QUA_PM_Begonia_nossibea_EDNA13_0033507
AF_NER_DF_Begonia_lyallii_EDNA14_0035459
AF_NER_PM_Begonia_cf_lyallii_EDNA15_0039167
AF_NER_PM_Begonia_henrilaportei_EDNA15_0039172
AF_TET_PM_Begonia_elaegnifolia_EDNA15_0038260
AF_BAC_DF_Begonia_baccata_EDNA14_0035689
AF_SQA_RT_Begonia_ampla_EDNA13_0030222
AF_SQA_DT_Begonia_poculifera_EDNA09_02175
AF_TET_PM_Begonia_longipetiolata_EDNA13_0033479
AF_TET_PM_Begonia_squamulosa_EDNA13_0033505
AF_TET_PM_Begonia_loranthoides_subsp_rhoalocarpa_
AF_TET_PM_Begonia_parva_EDNA13_0033510
AF_TET_PM_Begonia_loranthoides_EDNA13_0033471
AF_TET_PM_Begonia_molleri_EDNA15_0039689
AF_TET_JS_Begonia_subalpestris_EDNA17_0048021
AF_TET_PM_Begonia_kisuluana_EDNA15_0039676
AF_TET_PM_Begonia_komoensis_EDNA15_0038261
AF_TET_PM_Begonia_eminii_EDNA13_0033506
AF_TET_PM_Begonia_subscutata_EDNA15_0038267
AF_TET_PM_Begonia_oxyanthera_EDNA15_0038262
AF_TET_DT_Begonia_polygonoides_EDNA09_02176
AF_TET_PM_Begonia_gabonensis_EDNA13_0033472
AF_TET_PM_Begonia_oxyanthera_EDNA15_0039695
AF_TET_PM_Begonia_furfuracea_EDNA15_0039665
AF_TET_PM_Begonia_kisuluana_EDNA13_0033473
AF_TET_PM_Begonia_komoensis_EDNA15_0039677
AF_SEX_DF_Begonia_annoboensis_EDNA14_0035690
AF_ROS_PM_Begonia_engleri_EDNA13_0000001
AF_ROS_PM_Begonia_johnstonii_EDNA13_0000002
NW_LAT_RT_Begonia_olbia_EDNA12_0025415_
NW_LAT_DF_PM_Begonia_aconitifolia_EDNA13_0033499
NW_LAT_PM_Begonia_pachypoda_EDNA13_0033079
NW_PRI_PM_Begonia_teuscheri_EDNA15_0039732
NW_LAT_PM_Begonia_platanifolia_EDNA15_0038263
b.
African dioecious species:
- Section Mezierea
  - B. meyeri-johannis
- Section Tetraphila
  - B. subalpestris
Trace over Trees
☐ Fleshy Fruit
■ Dry Fruit
🟥 Node Absent
▨ Equivocal
AA_HIL_PM_Hillebrandia_sandwicensis_EDNA13_0034233
AF_SCU_PM_Begonia_susaniae_EDNA15_0039730
AF_SCU_PM_Begonia_vankerckhovenii_EDNA15_0039736
AF_FIL_PM_Begonia_sciaphila_EDNA15_0039163
AF_FIL_PM_Begonia_minutifolia_EDNA15_0039688
AF_FIL_PM_Begonia_macrocarpa_EDNA15_0039233
AF_LOA_PM_Begonia_scutifolia_EDNA15_0039722
AF_LOA_PM_Begonia_letouzeyi_EDNA15_0039682
AF_LOA_PM_Begonia_scutifolia_EDNA13_0033504
AF_LOA_PM_Begonia_staudtii_EDNA15_0039727
AF_LOA_PM_Begonia_quadrialata_EDNA15_0039709
AF_LOA_PM_Begonia_microsperma_EDNA13_0033503
AF_MEZ_DT_Begonia_oxyloba_EDNA08_02841
AF_MEZ_DF_Begonia_meyeri_johannis_EDNA14_0035692
AF_ERM_PM_Begonia_nana_EDNA15_0039180
AF_ERM_PM_Begonia_bogneri_EDNA13_0033469
AF_ERM_DF_Begonia_antongilensis_EDNA14_0035458
AF_QUA_DT_Begonia_goudotii_EDNA09_02167
AF_MEZ_PM_Begonia_salaziensis_EDNA15_0039164
AF_MEZ_DF_Begonia_humbertii_EDNA14_0035455
AF_NER_DF_Begonia_coursii_EDNA14_0035694
AF_NER_DF_Begonia_madecassa_EDNA14_0035693
AF_NER_DF_Begonia_majungaensis_EDNA14_0035460
AF_QUA_PM_Begonia_nossibea_EDNA13_0033507
AF_NER_DF_Begonia_lyallii_EDNA14_0035459
AF_NER_PM_Begonia_cf_lyallii_EDNA15_0039167
AF_NER_PM_Begonia_henrilaportei_EDNA15_0039172
AF_TET_PM_Begonia_elaegnifolia_EDNA15_0038260
AF_BAC_DF_Begonia_baccata_EDNA14_0035689
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AF_SQA_DT_Begonia_poculifera_EDNA09_02175
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AF_TET_PM_Begonia_squamulosa_EDNA13_0033505
AF_TET_PM_Begonia_loranthoides_subsp_rhoalocarpa_
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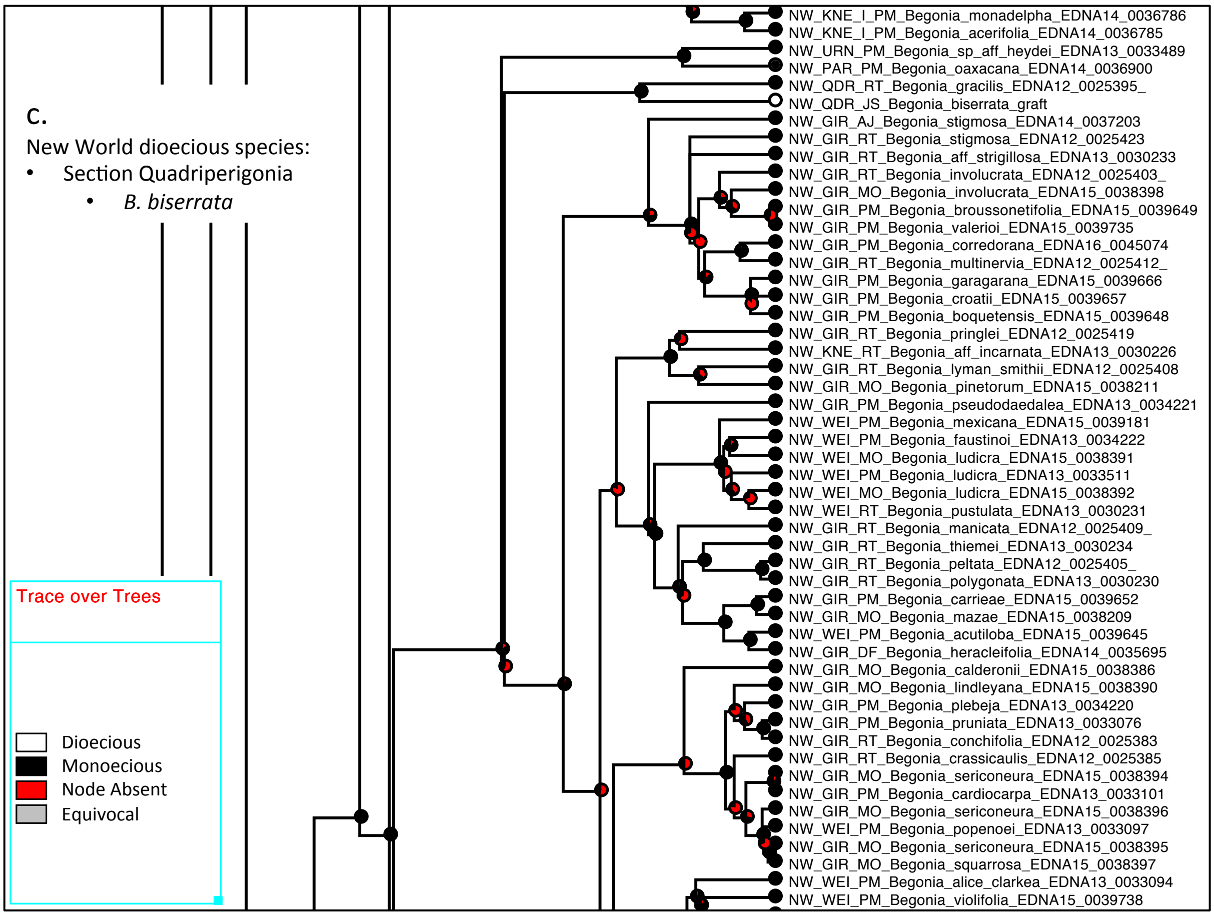
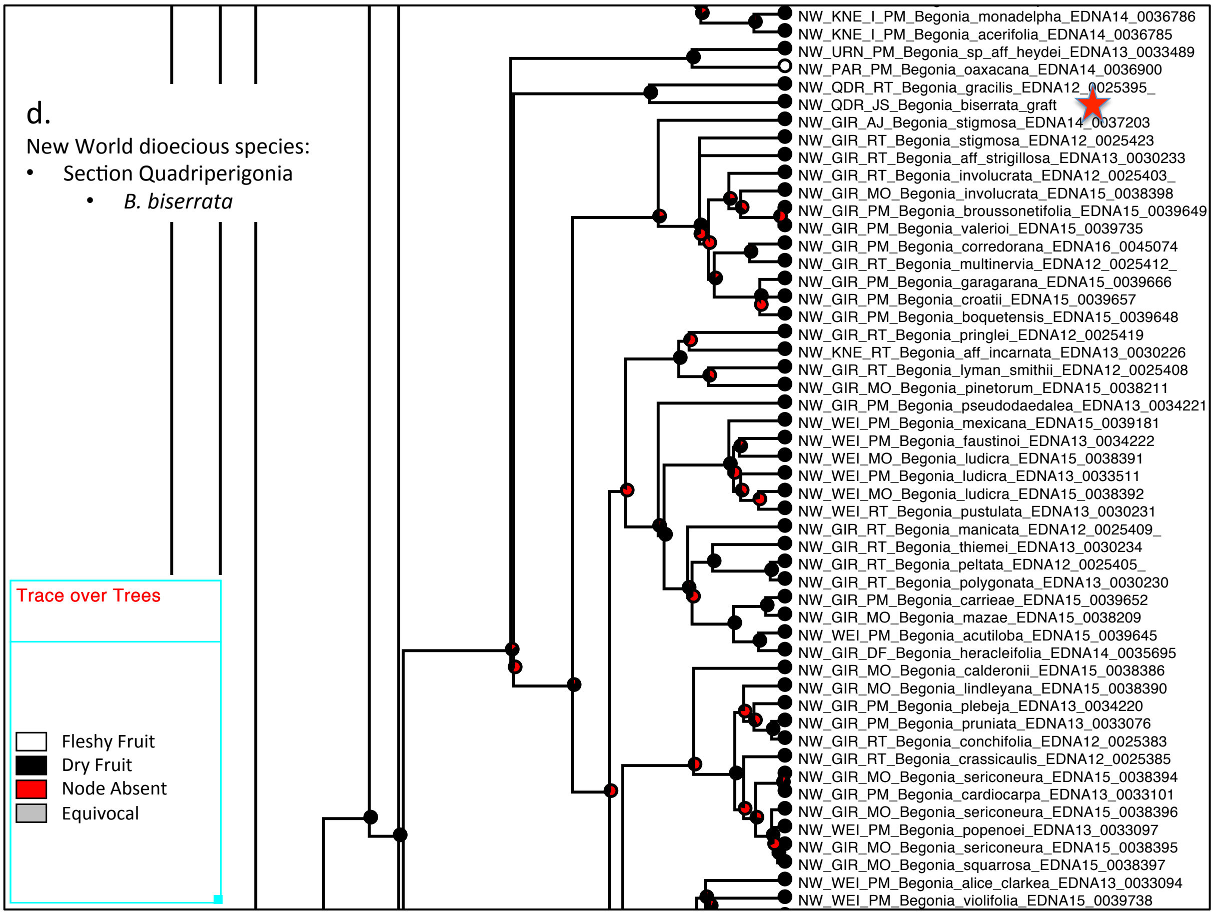
AF_TET_PM_Begonia_loranthoides_EDNA13_0033471
AF_TET_PM_Begonia_molleri_EDNA15_0039689
AF_TET_JS_Begonia_subalpestris_EDNA17_0048021
AF_TET_PM_Begonia_kisuluana_EDNA15_0039676
AF_TET_PM_Begonia_komoensis_EDNA15_0038261
AF_TET_PM_Begonia_eminii_EDNA13_0033506
AF_TET_PM_Begonia_subscutata_EDNA15_0038267
AF_TET_PM_Begonia_oxyanthera_EDNA15_0038262
AF_TET_DT_Begonia_polygonoides_EDNA09_02176
AF_TET_PM_Begonia_gabonensis_EDNA13_0033472
AF_TET_PM_Begonia_oxyanthera_EDNA15_0039695
AF_TET_PM_Begonia_furfuracea_EDNA15_0039665
AF_TET_PM_Begonia_kisuluana_EDNA13_0033473
AF_TET_PM_Begonia_komoensis_EDNA15_0039677
AF_SEX_DF_Begonia_annoboensis_EDNA14_0035690
AF_ROS_PM_Begonia_engleri_EDNA13_0000001
AF_ROS_PM_Begonia_johnstonii_EDNA13_0000002
NW_LAT_RT_Begonia_olbia_EDNA12_0025415_
NW_LAT_DF_PM_Begonia_aconitifolia_EDNA13_0033499
NW_LAT_PM_Begonia_pachypoda_EDNA13_0033079
NW_PRI_PM_Begonia_teuscheri_EDNA15_0039732
NW_LAT_PM_Begonia_platanifolia_EDNA15_0038263
NW_LAT_PM_Begonia_platanifolia_EDNA15_0039242


c.
New World dioecious species:
• Section Quadriperigonia
• B. biserrata
Trace over Trees
Dioecious
Monoecious
Node Absent
Equivocal
NW_KNE_I_PM_Begonia_monadelpha_EDNA14_0036786
NW_KNE_I_PM_Begonia_acerifolia_EDNA14_0036785
NW_URN_PM_Begonia_sp_aff_heydei_EDNA13_0033489
NW_PAR_PM_Begonia_oaxacana_EDNA14_0036900
NW_QDR_RT_Begonia_gracilis_EDNA12_0025395_
NW_QDR_JS_Begonia_biserrata_graft
NW_GIR_AJ_Begonia_stigmosa_EDNA14_0037203
NW_GIR_RT_Begonia_stigmosa_EDNA12_0025423
NW_GIR_RT_Begonia_aff_strigillosa_EDNA13_0030233
NW_GIR_RT_Begonia_involucrata_EDNA12_0025403_
NW_GIR_MO_Begonia_involucrata_EDNA15_0038398
NW_GIR_PM_Begonia_broussonetifolia_EDNA15_0039649
NW_GIR_PM_Begonia_valerioi_EDNA15_0039735
NW_GIR_PM_Begonia_corredorana_EDNA16_0045074
NW_GIR_RT_Begonia_multinervia_EDNA12_0025412_
NW_GIR_PM_Begonia_garagarana_EDNA15_0039666
NW_GIR_PM_Begonia_croatii_EDNA15_0039657
NW_GIR_PM_Begonia_boquetensis_EDNA15_0039648
NW_GIR_RT_Begonia_pringlei_EDNA12_0025419
NW_KNE_RT_Begonia_aff_incarnata_EDNA13_0030226
NW_GIR_RT_Begonia_lyman_smithii_EDNA12_0025408
NW_GIR_MO_Begonia_pinetorum_EDNA15_0038211
NW_GIR_PM_Begonia_pseudodaedalea_EDNA13_0034221
NW_WEI_PM_Begonia_mexicana_EDNA15_0039181
NW_WEI_PM_Begonia_faustinoi_EDNA13_0034222
NW_WEI_MO_Begonia_ludicra_EDNA15_0038391
NW_WEI_PM_Begonia_ludicra_EDNA13_0033511
NW_WEI_MO_Begonia_ludicra_EDNA15_0038392
NW_WEI_RT_Begonia_pustulata_EDNA13_0030231
NW_GIR_RT_Begonia_manicata_EDNA12_0025409_
NW_GIR_RT_Begonia_thiemei_EDNA13_0030234
NW_GIR_RT_Begonia_peltata_EDNA12_0025405_
NW_GIR_RT_Begonia_polygonata_EDNA13_0030230
NW_GIR_PM_Begonia_carrieae_EDNA15_0039652
NW_GIR_MO_Begonia_mazae_EDNA15_0038209
NW_WEI_PM_Begonia_acutiloba_EDNA15_0039645
NW_GIR_DF_Begonia_heracleifolia_EDNA14_0035695
NW_GIR_MO_Begonia_calderonii_EDNA15_0038386
NW_GIR_MO_Begonia_lindleyana_EDNA15_0038390
NW_GIR_PM_Begonia_plebeja_EDNA13_0034220
NW_GIR_PM_Begonia_pruniata_EDNA13_0033076
NW_GIR_RT_Begonia_conchifolia_EDNA12_0025383
NW_GIR_RT_Begonia_crassicaulis_EDNA12_0025385
NW_GIR_MO_Begonia_sericoneura_EDNA15_0038394
NW_GIR_PM_Begonia_cardiocarpa_EDNA13_0033101
NW_GIR_MO_Begonia_sericoneura_EDNA15_0038396
NW_WEI_PM_Begonia_popenoei_EDNA13_0033097
NW_GIR_MO_Begonia_sericoneura_EDNA15_0038395
NW_GIR_MO_Begonia_squarrosa_EDNA15_0038397
NW_WEI_PM_Begonia_alice_clarkea_EDNA13_0033094
NW_WEI_PM_Begonia_violifolia_EDNA15_0039738


d.
New World dioecious species:
• Section Quadriperigonia
• B. biserrata
Trace over Trees
Fleshy Fruit
Dry Fruit
Node Absent
Equivocal
NW_KNE_I_PM_Begonia_monadelpha_EDNA14_0036786
NW_KNE_I_PM_Begonia_acerifolia_EDNA14_0036785
NW_URN_PM_Begonia_sp_aff_heydei_EDNA13_0033489
NW_PAR_PM_Begonia_oaxacana_EDNA14_0036900
NW_QDR_RT_Begonia_gracilis_EDNA12_0025395_
NW_QDR_JS_Begonia_biserrata_graft
NW_GIR_AJ_Begonia_stigmosa_EDNA14_0037203
NW_GIR_RT_Begonia_stigmosa_EDNA12_0025423
NW_GIR_RT_Begonia_aff_strigillosa_EDNA13_0030233
NW_GIR_RT_Begonia_involucrata_EDNA12_0025403_
NW_GIR_MO_Begonia_involucrata_EDNA15_0038398
NW_GIR_PM_Begonia_broussonetifolia_EDNA15_0039649
NW_GIR_PM_Begonia_valerioi_EDNA15_0039735
NW_GIR_PM_Begonia_corredorana_EDNA16_0045074
NW_GIR_RT_Begonia_multinervia_EDNA12_0025412_
NW_GIR_PM_Begonia_garagarana_EDNA15_0039666
NW_GIR_PM_Begonia_croatii_EDNA15_0039657
NW_GIR_PM_Begonia_boquetensis_EDNA15_0039648
NW_GIR_RT_Begonia_pringlei_EDNA12_0025419
NW_KNE_RT_Begonia_aff_incarnata_EDNA13_0030226
NW_GIR_RT_Begonia_lyman_smithii_EDNA12_0025408
NW_GIR_MO_Begonia_pinetorum_EDNA15_0038211
NW_GIR_PM_Begonia_pseudodaedalea_EDNA13_0034221
NW_WEI_PM_Begonia_mexicana_EDNA15_0039181
NW_WEI_PM_Begonia_faustinoi_EDNA13_0034222
NW_WEI_MO_Begonia_ludicra_EDNA15_0038391
NW_WEI_PM_Begonia_ludicra_EDNA13_0033511
NW_WEI_MO_Begonia_ludicra_EDNA15_0038392
NW_WEI_RT_Begonia_pustulata_EDNA13_0030231
NW_GIR_RT_Begonia_manicata_EDNA12_0025409_
NW_GIR_RT_Begonia_thiemei_EDNA13_0030234
NW_GIR_RT_Begonia_peltata_EDNA12_0025405_
NW_GIR_RT_Begonia_polygonata_EDNA13_0030230
NW_GIR_PM_Begonia_carrieae_EDNA15_0039652
NW_GIR_MO_Begonia_mazae_EDNA15_0038209
NW_WEI_PM_Begonia_acutiloba_EDNA15_0039645
NW_GIR_DF_Begonia_heracleifolia_EDNA14_0035695
NW_GIR_MO_Begonia_calderonii_EDNA15_0038386
NW_GIR_MO_Begonia_lindleyana_EDNA15_0038390
NW_GIR_PM_Begonia_plebeja_EDNA13_0034220
NW_GIR_PM_Begonia_pruniata_EDNA13_0033076
NW_GIR_RT_Begonia_conchifolia_EDNA12_0025383
NW_GIR_RT_Begonia_crassicaulis_EDNA12_0025385
NW_GIR_MO_Begonia_sericoneura_EDNA15_0038394
NW_GIR_PM_Begonia_cardiocarpa_EDNA13_0033101
NW_GIR_MO_Begonia_sericoneura_EDNA15_0038396
NW_WEI_PM_Begonia_popenoei_EDNA13_0033097
NW_GIR_MO_Begonia_sericoneura_EDNA15_0038395
NW_GIR_MO_Begonia_squarrosa_EDNA15_0038397
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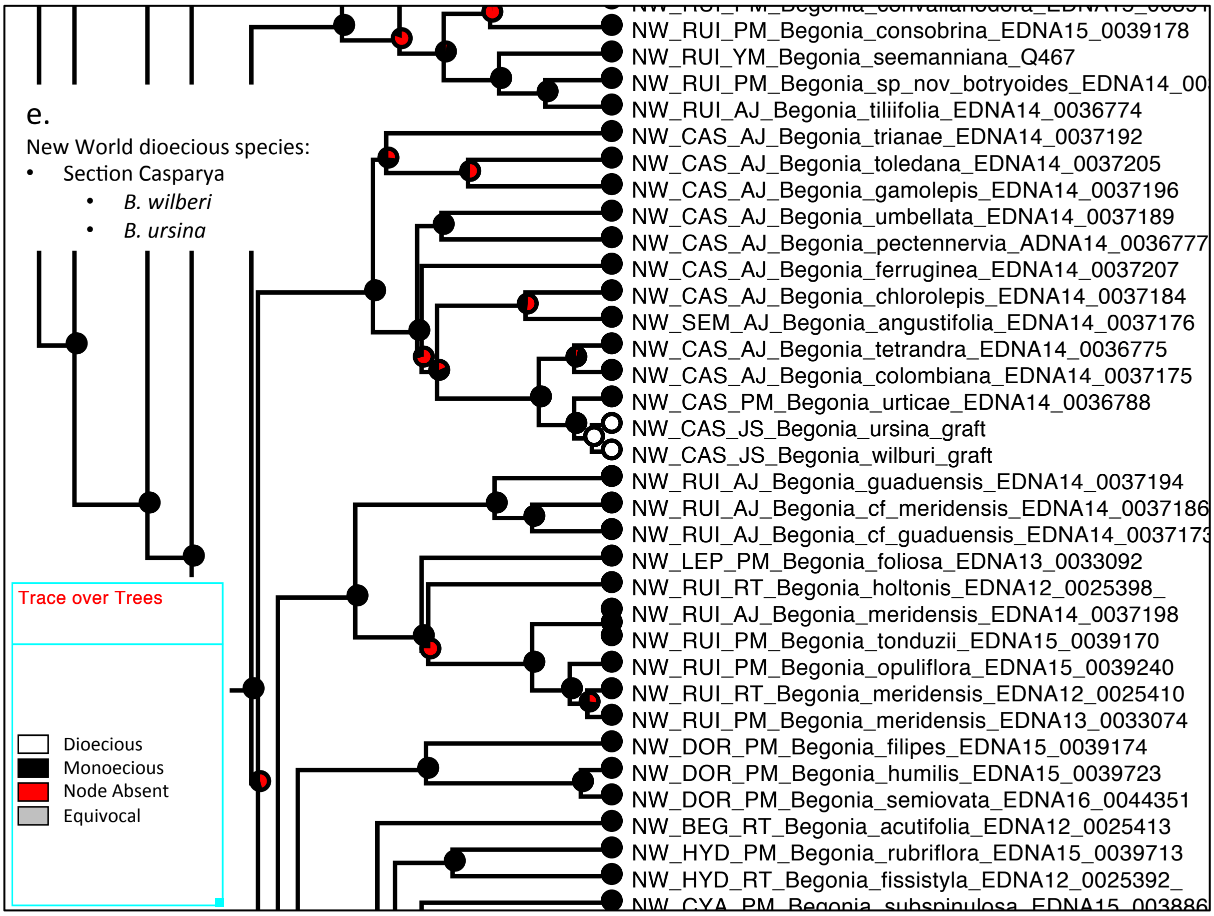
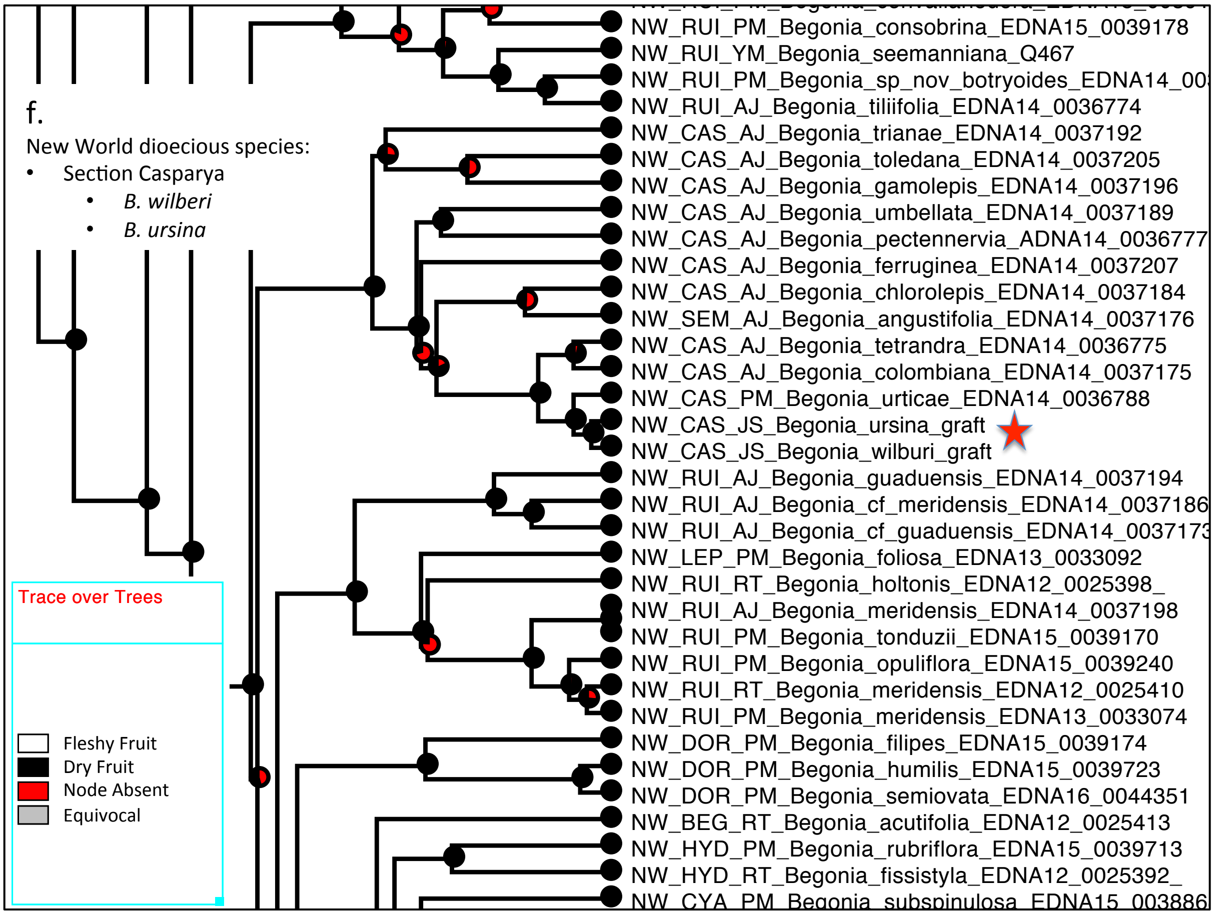
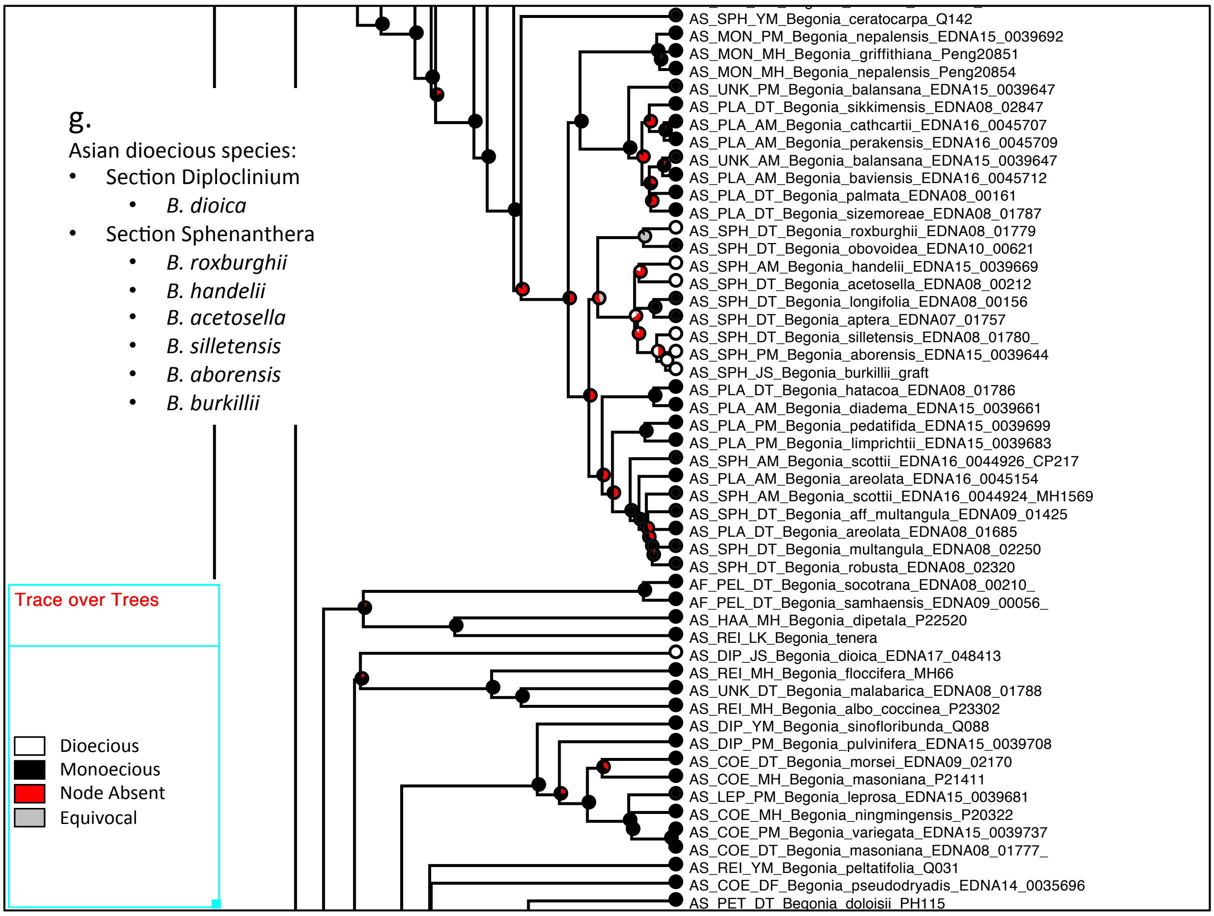
NW_WEI_PM_Begonia_violifolia_EDNA15_0039738


e.
New World dioecious species:
- Section Casparya
  - B. wilberi
  - B. ursina
Trace over Trees
☐ Dioecious
■ Monoecious
🟥 Node Absent
▨ Equivocal
NW_RUI_PM_Begonia_consobrina_EDNA15_0039178
NW_RUI_YM_Begonia_seemanniana_Q467
NW_RUI_PM_Begonia_sp_nov_botryoides_EDNA14_00
NW_RUI_AJ_Begonia_tiliifolia_EDNA14_0036774
NW_CAS_AJ_Begonia_trianae_EDNA14_0037192
NW_CAS_AJ_Begonia_toledana_EDNA14_0037205
NW_CAS_AJ_Begonia_gamolepis_EDNA14_0037196
NW_CAS_AJ_Begonia_umbellata_EDNA14_0037189
NW_CAS_AJ_Begonia_pectennervia_ADNA14_0036777
NW_CAS_AJ_Begonia_ferruginea_EDNA14_0037207
NW_CAS_AJ_Begonia_chlorolepis_EDNA14_0037184
NW_SEM_AJ_Begonia_angustifolia_EDNA14_0037176
NW_CAS_AJ_Begonia_tetrandra_EDNA14_0036775
NW_CAS_AJ_Begonia_colombiana_EDNA14_0037175
NW_CAS_PM_Begonia_urticae_EDNA14_0036788
NW_CAS_JS_Begonia_ursina_graft
NW_CAS_JS_Begonia_wilburi_graft
NW_RUI_AJ_Begonia_guaduensis_EDNA14_0037194
NW_RUI_AJ_Begonia_cf_meridensis_EDNA14_0037186
NW_RUI_AJ_Begonia_cf_guaduensis_EDNA14_003717
NW_LEP_PM_Begonia_foliosa_EDNA13_0033092
NW_RUI_RT_Begonia_holtonis_EDNA12_0025398_
NW_RUI_AJ_Begonia_meridensis_EDNA14_0037198
NW_RUI_PM_Begonia_tonduzii_EDNA15_0039170
NW_RUI_PM_Begonia_opuliflora_EDNA15_0039240
NW_RUI_RT_Begonia_meridensis_EDNA12_0025410
NW_RUI_PM_Begonia_meridensis_EDNA13_0033074
NW_DOR_PM_Begonia_filipes_EDNA15_0039174
NW_DOR_PM_Begonia_humilis_EDNA15_0039723
NW_DOR_PM_Begonia_semiovata_EDNA16_0044351
NW_BEG_RT_Begonia_acutifolia_EDNA12_0025413
NW_HYD_PM_Begonia_rubriflora_EDNA15_0039713
NW_HYD_RT_Begonia_fissistyla_EDNA12_0025392_
NW_CYA_PM_Begonia_subspinulosa_EDNA15_003886


f.
New World dioecious species:
- Section Casparya
  - B. wilberi
  - B. ursina
Trace over Trees
☐ Fleshy Fruit
■ Dry Fruit
🟥 Node Absent
▨ Equivocal
NW_RUI_PM_Begonia_consobrina_EDNA15_0039178
NW_RUI_YM_Begonia_seemanniana_Q467
NW_RUI_PM_Begonia_sp_nov_botryoides_EDNA14_00
NW_RUI_AJ_Begonia_tiliifolia_EDNA14_0036774
NW_CAS_AJ_Begonia_trianae_EDNA14_0037192
NW_CAS_AJ_Begonia_toledana_EDNA14_0037205
NW_CAS_AJ_Begonia_gamolepis_EDNA14_0037196
NW_CAS_AJ_Begonia_umbellata_EDNA14_0037189
NW_CAS_AJ_Begonia_pectennervia_ADNA14_0036777
NW_CAS_AJ_Begonia_ferruginea_EDNA14_0037207
NW_CAS_AJ_Begonia_chlorolepis_EDNA14_0037184
NW_SEM_AJ_Begonia_angustifolia_EDNA14_0037176
NW_CAS_AJ_Begonia_tetrandra_EDNA14_0036775
NW_CAS_AJ_Begonia_colombiana_EDNA14_0037175
NW_CAS_PM_Begonia_urticae_EDNA14_0036788
NW_CAS_JS_Begonia_ursina_graft
NW_CAS_JS_Begonia_wilburi_graft
NW_RUI_AJ_Begonia_guaduensis_EDNA14_0037194
NW_RUI_AJ_Begonia_cf_meridensis_EDNA14_0037186
NW_RUI_AJ_Begonia_cf_guaduensis_EDNA14_003717
NW_LEP_PM_Begonia_foliosa_EDNA13_0033092
NW_RUI_RT_Begonia_holtonis_EDNA12_0025398_
NW_RUI_AJ_Begonia_meridensis_EDNA14_0037198
NW_RUI_PM_Begonia_tonduzii_EDNA15_0039170
NW_RUI_PM_Begonia_opuliflora_EDNA15_0039240
NW_RUI_RT_Begonia_meridensis_EDNA12_0025410
NW_RUI_PM_Begonia_meridensis_EDNA13_0033074
NW_DOR_PM_Begonia_filipes_EDNA15_0039174
NW_DOR_PM_Begonia_humilis_EDNA15_0039723
NW_DOR_PM_Begonia_semiovata_EDNA16_0044351
NW_BEG_RT_Begonia_acutifolia_EDNA12_0025413
NW_HYD_PM_Begonia_rubriflora_EDNA15_0039713
NW_HYD_RT_Begonia_fissistyla_EDNA12_0025392_
NW_CYA_PM_Begonia_subspinulosa_EDNA15_003886


g.
Asian dioecious species:
• Section Diploclinium
• B. dioica
• Section Sphenanthera
• B. roxburghii
• B. handelii
• B. acetosella
• B. silletensis
• B. aborensis
• B. burkillii
Trace over Trees
Dioecious
Monoecious
Node Absent
Equivocal
AS_SPH_YM_Begonia_ceratocarpa_Q142
AS_MON_PM_Begonia_nepalensis_EDNA15_0039692
AS_MON_MH_Begonia_griffithiana_Peng20851
AS_MON_MH_Begonia_nepalensis_Peng20854
AS_UNK_PM_Begonia_balansana_EDNA15_0039647
AS_PLA_DT_Begonia_sikkimensis_EDNA08_02847
AS_PLA_AM_Begonia_cathcartii_EDNA16_0045707
AS_PLA_AM_Begonia_perakensis_EDNA16_0045709
AS_UNK_AM_Begonia_balansana_EDNA15_0039647
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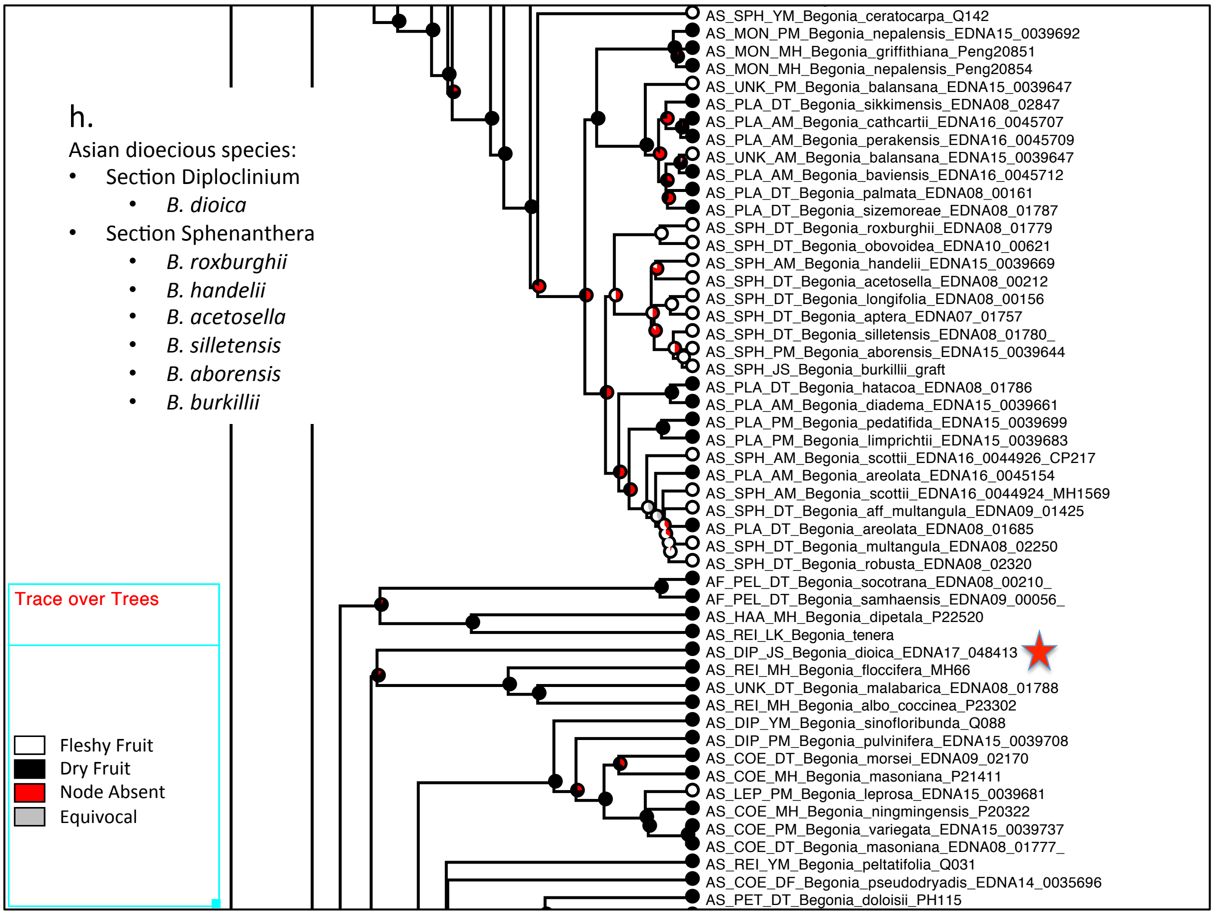
AS_SPH_DT_Begonia_roxburghii_EDNA08_01779
AS_SPH_DT_Begonia_obovoidea_EDNA10_00621
AS_SPH_AM_Begonia_handelii_EDNA15_0039669
AS_SPH_DT_Begonia_acetosella_EDNA08_00212
AS_SPH_DT_Begonia_longifolia_EDNA08_00156
AS_SPH_DT_Begonia_aptera_EDNA07_01757
AS_SPH_DT_Begonia_silletensis_EDNA08_01780_
AS_SPH_PM_Begonia_aborensis_EDNA15_0039644
AS_SPH_JS_Begonia_burkillii_graft
AS_PLA_DT_Begonia_hatacoa_EDNA08_01786
AS_PLA_AM_Begonia_diadema_EDNA15_0039661
AS_PLA_PM_Begonia_pedatifida_EDNA15_0039699
AS_PLA_PM_Begonia_limprichtii_EDNA15_0039683
AS_SPH_AM_Begonia_scottii_EDNA16_0044926_CP217
AS_PLA_AM_Begonia_areolata_EDNA16_0045154
AS_SPH_AM_Begonia_scottii_EDNA16_0044924_MH1569
AS_SPH_DT_Begonia_aff_multangula_EDNA09_01425
AS_PLA_DT_Begonia_areolata_EDNA08_01685
AS_SPH_DT_Begonia_multangula_EDNA08_02250
AS_SPH_DT_Begonia_robusta_EDNA08_02320
AF_PEL_DT_Begonia_socotrana_EDNA08_00210_
AF_PEL_DT_Begonia_samhaensis_EDNA09_00056_
AS_HAA_MH_Begonia_dipetala_P22520
AS_REI_LK_Begonia_tenera
AS_DIP_JS_Begonia_dioica_EDNA17_048413
AS_REI_MH_Begonia_floccifera_MH66
AS_UNK_DT_Begonia_malabarica_EDNA08_01788
AS_REI_MH_Begonia_albo_coccinea_P23302
AS_DIP_YM_Begonia_sinofloribunda_Q088
AS_DIP_PM_Begonia_pulvinifera_EDNA15_0039708
AS_COE_DT_Begonia_morsei_EDNA09_02170
AS_COE_MH_Begonia_masoniana_P21411
AS_LEP_PM_Begonia_leprosa_EDNA15_0039681
AS_COE_MH_Begonia_ningmingensis_P20322
AS_COE_PM_Begonia_variegata_EDNA15_0039737
AS_COE_DT_Begonia_masoniana_EDNA08_01777_
AS_REI_YM_Begonia_peltatifolia_Q031
AS_COE_DF_Begonia_pseudodryadis_EDNA14_0035696
AS_PET_DT_Begonia_doloisii_PH115


h.
Asian dioecious species:
• Section Diploclinium
• B. dioica
• Section Sphenanthera
• B. roxburghii
• B. handelii
• B. acetosella
• B. silletensis
• B. aborensis
• B. burkillii
Trace over Trees
Fleshy Fruit
Dry Fruit
Node Absent
Equivocal
AS_SPH_YM_Begonia_ceratocarpa_Q142
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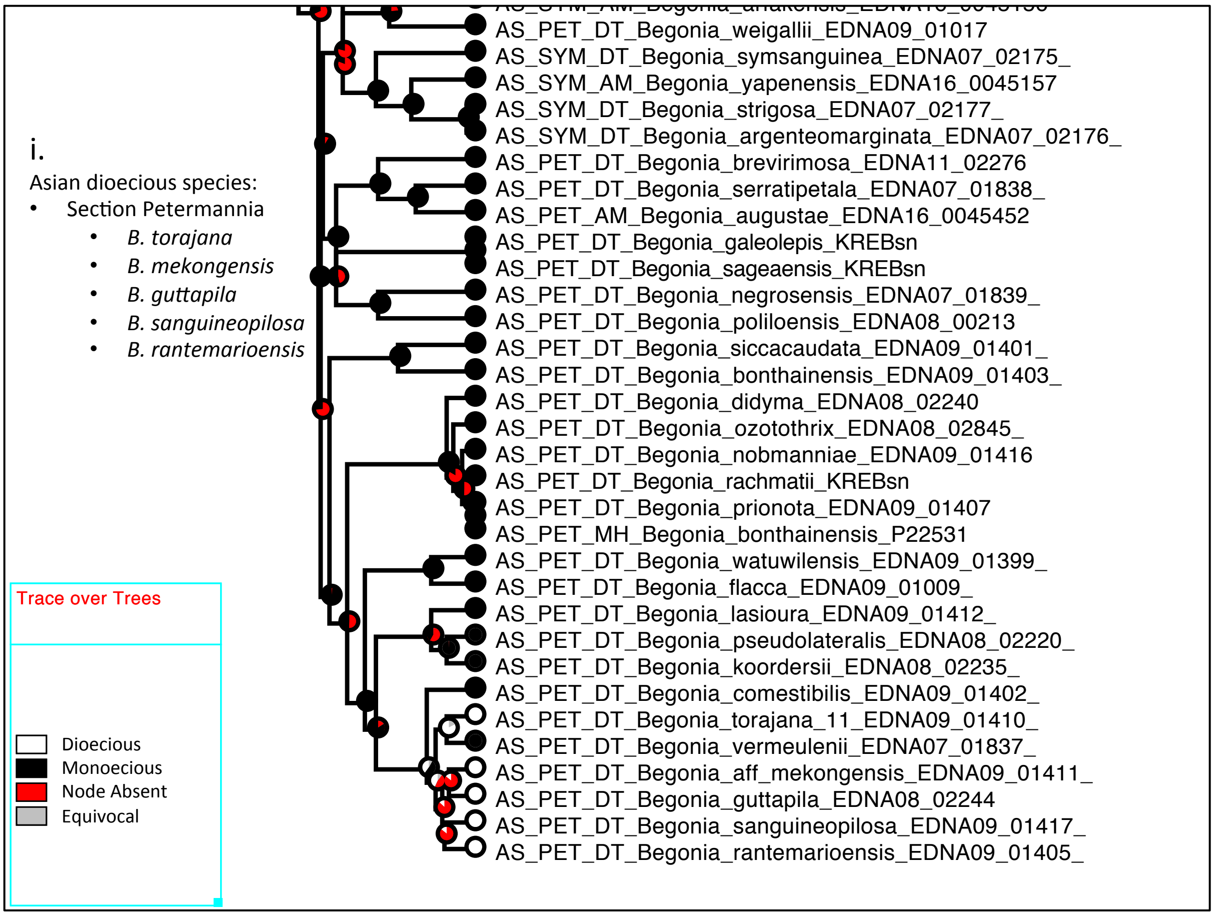
i.
Asian dioecious species:
Section Petermannia
B. torajana
B. mekongensis
B. guttapila
B. sanguineopilosa
B. rantemarioensis
Trace over Trees
□ Dioecious
■ Monoecious
🟥 Node Absent
▨ Equivocal
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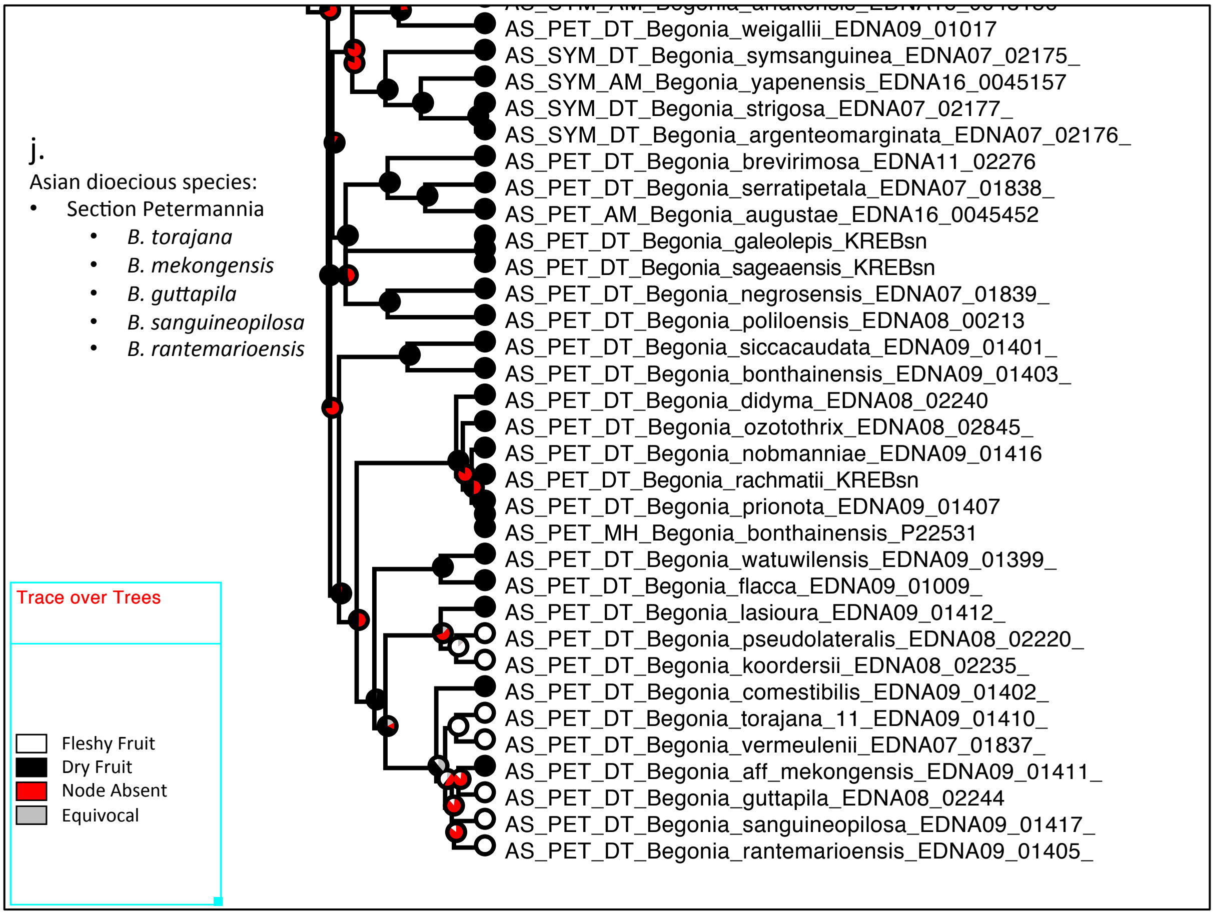
j.
Asian dioecious species:
Section Petermannia
B. torajana
B. mekongensis
B. guttapila
B. sanguineopilosa
B. rantemarioensis
Trace over Trees
□ Fleshy Fruit
■ Dry Fruit
🟥 Node Absent
▨ Equivocal
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AS_PET_DT_Begonia_rantemarioensis_EDNA09_01405_

Figure 2a-j. Maximum likelihood character reconstruction of the discrete characters breeding system and fruit type. Character reconstruction was traced over 6000 ultrametric trees produced from BEAST analysis and presented on tree no. 4365 (log clade credibility statistic -248.96) with ancestral state confidence presented at each node. Only sections of the tree with dioecious clades are depicted. Plates a-b highlight African sections Tetraphila and Mezierea; plates c-d highlight New World section Quadriperigonia; plates e-f highlight New World section Casparya; plates g-h highlight Asian sections Sphenanthera and Diploclinium; and finally, plates i-j highlight Asian section Petermannia. The dioecious species that do not have fleshy fruit in the reconstructions of fruit type are indicated with a star.

3.3.2 *Fruit type and extent of occurrence* – Fleshy fruit has evolved within eight sections of *Begonia* (Fig 2b, d, f, h, j), and often occurs along with dioecy and/or larger EOO, but not always. Fleshy fruit occurs in 12 of the 17 dioecious species analyzed here, and seven of those 12 have large EOOs. The dioecious species that have fleshy fruit but are narrow endemics are *B. torajana* D.C. Thomas & Ardi*, B. guttapila, B. rantemarioensis* D.C. Thomas & Ardi*,* and *B. sanguineopilosa* D.C. Thomas & Ardi within section Petermannia and *B. subalpestris* in Tetraphila. With monoecious species, fleshy fruit occurs in narrow endemics within species in section Sphenanthera, *B. ceratocarpa* S.H. Huang & Y.M. Shui, section Mezierea, *B. salaziensis* (Gaudich.) Warb., section Squamibegonia *B. ampla* Hook.f., and *B. poculifera* Hook.f., and section Baccabegonia, *B. baccata* Hook.f.

Larger EOOs has evolved throughout the *Begonia* phylogeny numerous times (Fig. 3), and only sometimes occurs with fleshy fruit and/or dioecy. Ten of the 17 dioecious species analyzed here have large EOOs. The two species that were not included in phylogenetic analyses in section Knesebeckia, *B. extranea* and *B. nemoralis* are also dioecious with large EOOs. *Begonia extranea* has an EOO of 38653.3 km^2 and *B. nemoralis* has an EOO of 24469.2 km^2. Most species in section Tetraphila, Mezierea, and Sphenanthera have fleshy fruit and larger EOO, yet only *B. subalpestris, B. meyeri-johannis,* and six of 14 Sphenanthera species have evolved dioecy. In addition, *B. koordersii* Warb. ex L.B.Sm. &Wassh.*, B. pseudolateralis* Warb.*, B. leprosa* Hance*,* and *B. oaxacana* A. DC. all have fleshy fruit and large EOOs, but are monoecious. These results suggest that there are other impediments to the evolution of dioecy from monoecy in *Begonia* in addition to seed shadow handicap.

Fig 3

Figure 3. Maximum likelihood character reconstruction of the logarithm of the continuous character extent of occurrence. Character reconstruction was performed on the single best tree from the BEAST analysis in R and is presented as a fan to visualize character change across the phylogeny of 628 taxa. Dioecious taxa are indicated.

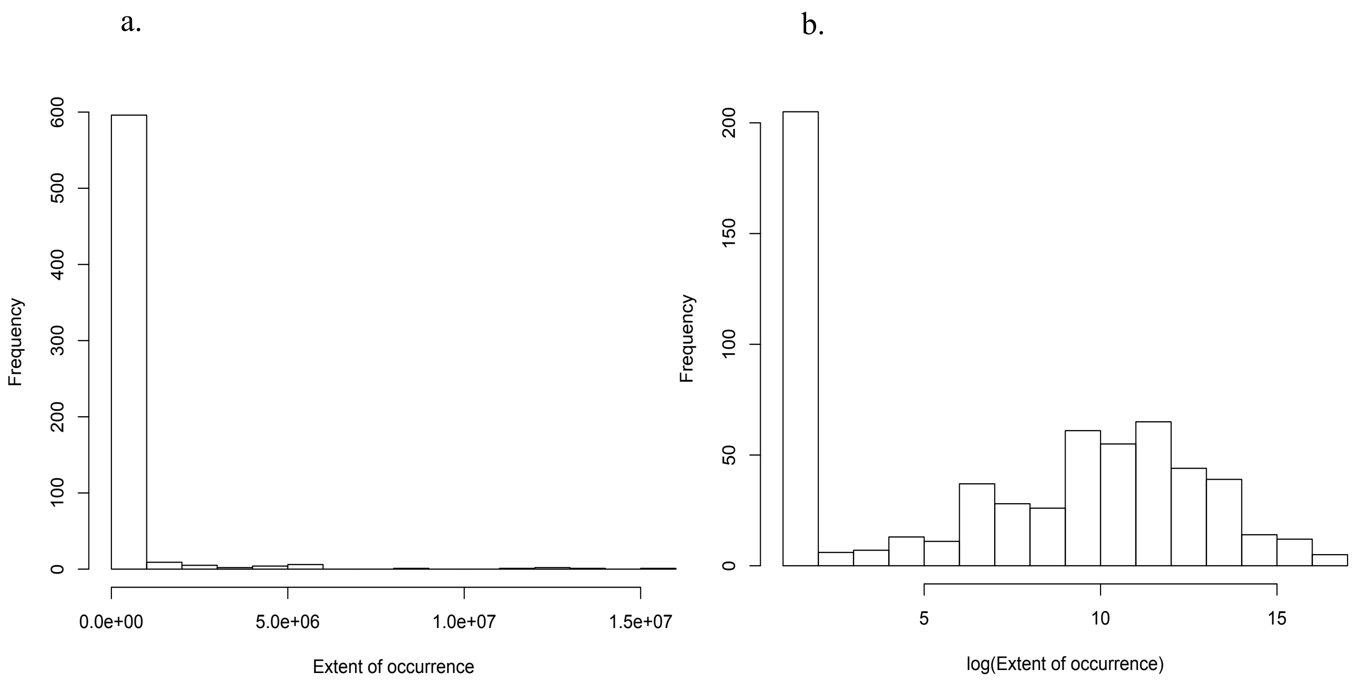

Figure 4a-b. Histogram representation of the continuous character extent of occurrence data (a), and logarithm of extent of occurrence data (b). The logarithm of the data was used to normalize the right-skewed data distribution when running correlative statistic analyses. Extent of occurrence is measured in km^2 on the x-axis.

3.4 *Statistics in R, character patterns and correlations*

Data for the EOO in *Begonia* was extremely right skewed due to the frequency of narrow endemics in the genus and incomplete data (Fig. 4a). To counteract this, the log(EOO) was taken and used in comparative analyses with the two other discrete characters, though the data remained skewed to the right (Fig. 4b). The non-normal distribution of EOO across the dataset must be taken into account when understanding the relationship between the three characters. The relationship between the discrete characters, breeding system and fruit type, and the log of the continuous character EOO was analyzed through box plots (Fig. 5a-b). Comparison of the log(EOO) data and breeding system across *Begonia* taxa show that taxa with either unisexual breeding system have a wide range of EOO values without a distinct association, but the median EOO value for each breeding system category may demonstrate a slight relationship. The median and mean log(EOO) values for dioecious *Begonia* taxa are 11.02 and 8.19, respectively (calculations taken from the non-log EOO dataset being median, 60904 km^2 and mean, 285144 km^2). For monoecious *Begonia* taxa, the median and mean log(EOO) are 8.36 and 7.25, respectively (or from EOO data - 4268 km^2 and 317677 km^2). Median and mean log(EOO) and median EOO values are higher in dioecious taxa than monoecious taxa. Monoecious taxa have a higher mean EOO, but this is due to outlier weedy taxa with very large range sizes (such as *B. humilis* Dryand.). When comparing fruit type to log(EOO) data in *Begonia*, there is a much clearer association between fleshy fruit and larger log(EOO) values. The median and mean log(EOO) values for fleshy fruited *Begonia* taxa are 11.20 and 9.72, respectively (calculations taken from the original EOO dataset being median, 81237 km^2 and mean, 468270 km^2). For dry fruited *Begonia* taxa, the median and mean log(EOO) are 7.85 and 7.06, respectively (or from EOO data - 2576 km^2 and 303122 km^2).

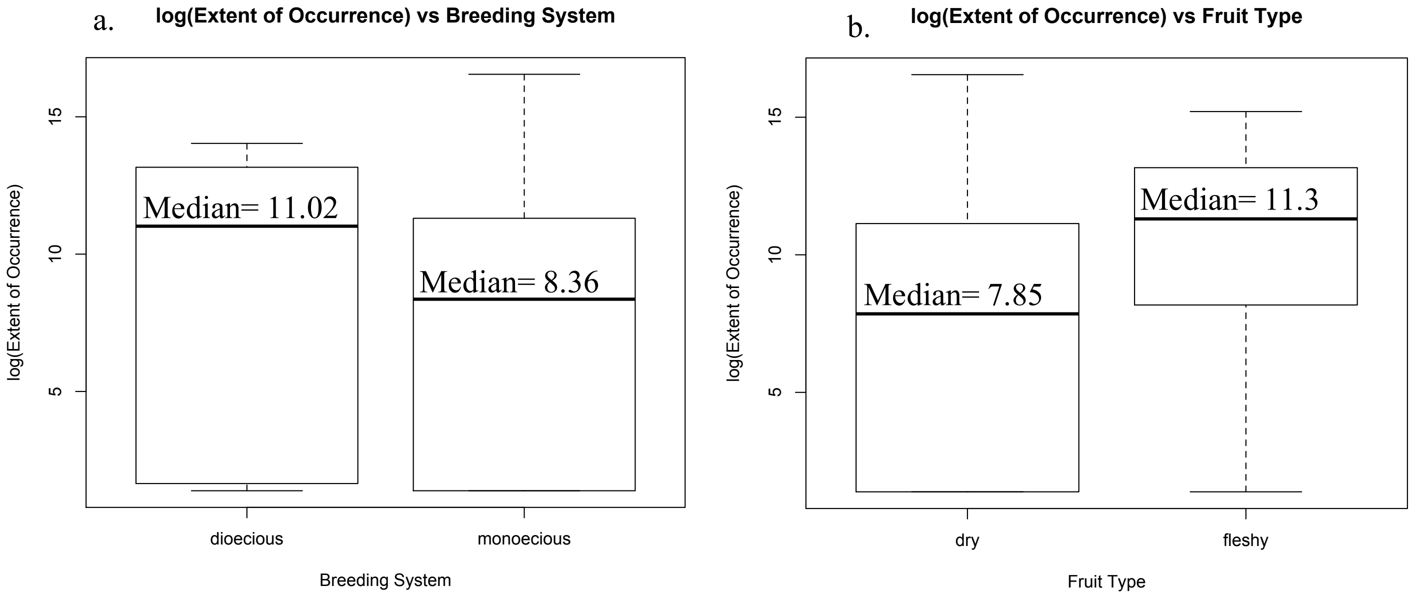

Figure 5a-b. Boxplot representation of breeding system (a) or fruit type (b) with extent of occurrence in *Begonia*. The median logEOO value for each discrete character state is listed above the median line.

Characters were then analyzed with respect to phylogeny. Phylogenetic signal was calculated separately for discrete and continuous characters. For both breeding system and fruit type, the estimated D (E(D)) value was negative, with no chance of E(D) resulting from random phylogenetic structure (Table 3). This indicates that dioecy and fleshy fruit characters cluster on the phylogeny due to phylogenetic conservation rather than by chance. Both discrete characters also had high probability of their phylogenetic clustering being affected by Brownian motion. For EOO and log(EOO), a different statistic test for continuous characters utilizing lambda and P-values was used. Lambda value is high and the P-value was significantly below 0.5 (Table 4), again indicating phylogenetically conserved clustering of EOO on the tree, or that related species tend to have more similar range sizes than expected by chance.

Table 3. D value representation of phylogenetic signal of discrete characters breeding system and fruit type in reference to *Begonia* plastid phylogeny. Statistical test run through the R program caper using the phylo.d and comparative.data functions.

Character	Breeding System	Fruit Type
Estimated D (E(D))	-0.168	-0.484
Probability of E(D) resulting from no (random) phylogenetic structure	0	0
Probability of E(D) resulting from Brownian phylogenetic structure	0.682	0.99

Table 4. Statistical representation of phylogenetic signal of the continuous character extent of occurrence (EOO) in reference to *Begonia* plastid phylogeny. Statistical test run through the R program phytools using the phylosig function.

Character	Extent of Occurrence	Log(Extent of Occurrence)
lambda	0.69	0.64
P-value	8.073e-21	1.63e-35

To confirm a phylogenetic correlation between either discrete character and log(EOO), AIC values were compared from statistic tests analyzing whether either breeding system or fruit type character variation could explain variation seen in log(EOO) across the phylogeny (Table 5). The AIC values were similar for both breeding system (4179.566) and fruit type (4179.204) and higher than the null analysis of log(EOO) values without an explanatory variable (4178.235). This indicates that neither discrete variable explains the variation of log(EOO) seen across the phylogeny, i.e. there is not a correlation between the characters. This result may be affected by the inaccuracies and outliers in the data, as taxa with each discrete character state also had wide ranges of log(EOO) values (Fig. 5a-b). Testing for phylogenetic correlation between breeding system and fruit type was not possible, as there were too few data points for each character (17 dioecious species and 52 fleshy fruit species out of 628 taxa in the dataset) to allow statistical convergence on the phylogeny. Instead, basic probability and chi-squared statistics were used to demonstrate correlation between the two discrete characters, without reference to the phylogeny (Table 6). Twelve of the 17 dioecious *Begonia* species had fleshy fruit, and this is a higher percentage than if 12 species had been chosen randomly from the data. The p-value obtained from the chi-square test was low, further showing significant correlation between breeding system and fruit type.

Table 5. AIC values from correlative character analyses in R program phylolm with continuous character log(extent of occurrence) and breeding system or fruit type.

Character comparison	AIC
logEOO~Breeding System	4179.566
logEOO~Fruit Type	4179.204
logEOO~1 (null)	4178.235

Table 6. Results of statistical analyses between breeding system and fruit type.

Expected % of taxa with both dioecy and fleshy fruit	Observed % of taxa with both dioecy and fleshy fruit	X^2	P-value
(17/628)(52/628)=0.22%	12/628= 1.9%	89.32	0.0005

3.5 *Effect of seed shadow handicap in Begonia*

Though larger EOOs do not correlate with dioecy and/or fleshy fruit with respect to the *Begonia* phylogeny, the hypothesis that the seed shadow handicap effects the evolution of dioecy within *Begonia,* and the need to have widely dispersed seed, is still reasonable. Dioecy is also phylogenetically correlated with fleshy fruit. Sections Sphenanthera, Mezierea, Diploclinium, Quadriperigonia, and Knesebeckia have both dioecy and larger EOOs. Species in sections Sphenanthera and Mezierea (*B. meyeri-johannis*) have both larger EOO and fleshy fruit, while those in sections Diploclinium (*B. dioica*) and Quadriperigonia (*B.* biserrata), despite having dry fruit, still have large EOOs. Even the two Knesebeckia dioecious species, *B. extranea* and *B. nemoralis,* that could not be phylogenetically analyzed here, showed larger EOOs despite having dry fruit. In Diploclinium, Quadriperigonia, and Knesebeckia, larger EOOs may be accomplished through another means than animal dispersed fleshy fruit. However, there must be other impediments to evolving dioecy, or loss in dioecious population fitness compared to monoecy that current dioecious *Begonia* species have overcome, as there are ~370 other species in the dataset that are not narrow endemics, but have remained monoecious.

Though not phylogenetically or statistically tested here, time may also effect the development of larger EOOs, with or without fleshy fruit and the evolution of dioecy. For example, the dioecious species with older nearest nodes tend to have larger range sizes, such as *B. dioica,* with a median node height of 11.3 Ma, and *B. biserrata,* with a median node height of 6.1 Ma. These two dioecious *Begonia* do not have fleshy fruit but have achieved large EOOs over a longer period of time, compared with dioecious *Begonia* such as those in section Sphenanthera, which have similarly large EOOs, but also have fleshy fruit and two nearest nodes of potential origin of dioecy with smaller median heights (i.e. are younger) at 1.8 and 2.3 Ma. Likewise, dioecious *Begonia* species that are narrow endemics, whether with fleshy fruit or not, tend to have speciated more recently. For example, the nearest node to dry-fruited dioecious *B. wilburi* and *B. ursina* has a median height at 0.5 Ma. Similarly, narrow endemic, dioecious, and fleshy-fruited *B. subalpestris* and the species in section Petermannia have median node heights of 0.3 Ma and 0.9 Ma, respectively. It is not unreasonable to hypothesize that the EOOs of these narrow endemic dioecious *Begonias* have the potential to increase over a longer evolutionary time, as is seen in *B. dioica, B. biserrata, B. meyeri-johannis* and the dioecious species in section Sphenanthera.

The lack of phylogenetic correlation between EOO and dioecy or fleshy fruit may be influenced by the right skew of the data (even within the logarithm of EOO data - Fig. 4b), a few extremely widespread weedy monoecious *Begonia* species, (as seen in the long tails of the box plots in Figure 5) and inaccuracies of the data. Fruit type is a more reliable character to score than EOO, perhaps leading to the correlation between fruit type and breeding systems, but not between EOO and breeding system. For example, dioecious species within section Petermannia all had fleshy fruit, but narrow endemic EOO data. The small values calculated for these taxa may be due to under-collecting of Sulawesi *Begonia* species (Mark Hughes, RBGE, pers. comm.). If this is the case, and dioecious *Begonias* in Petermannia have larger EOOs, then only the species in sections Casparya and Tetraphila (3 of 17 species phylogenetically analyzed here) were potentially unaffected by seed shadow handicap and have smaller EOOs.

Chapter 4 – Future work and conclusion

4.1 *Future work*

Several additional hypotheses regarding the evolution of dioecy from monoecy within *Begonia* have been postulated during the course of this work. Further analyses with the range size data presented here and speciation rates within *Begonia* may support the idea that dioecy is rare, as lineages that have evolved dioecy may diversify at a slower rate due to the maintenance of gene flow across a population. Another important link to the evolution of dioecy within *Begonia* may be the evolution of varying forms of monoecy within the genus (molecular background of variation discussed in Twyford et al., 2014). Some of these forms of monoecy include temporally spaced flowering of the two sexes within a single plant, or various arrangements of the unisexual flowers (mixed or separated) among the inflorescences. The effect of sexual selection based upon pollinator preference in *Begonia* may also be significant in the evolution of breeding systems. *Begonia* only have pollen as a reward and attract pollinators to female flowers through male mimicry (Agren and Schemske, 1991). Despite the deceit mechanism, pollinators may show a preference toward the male flowers that offer real reward (Schemske and Agren, 1995) and that may play into sexual selection within the population, influencing sex ratios among individuals. It is hypothesized that this sexual selection and sex ratio bias toward male flowers in populations may provide pressure to evolve toward dioecy (Bawa, 1980). Studies involving pollinator driven sexual selection and sex ratio within *Begonia* populations may provide more insight into the evolution of dioecy.

Another hypothesized evolutionary pressure to develop dioecy is plant size. Larger plants must expend more energy to control flowering times and are therefore more likely to self-fertilize, providing the necessary pressure to evolve dioecy to promote outcrossing (Smith, 1978). Some of the known dioecious *Begonia* species identified in this work have also been observed to have large growth forms. For example, *B. subalpestris,* which is a narrow endemic despite being dioecious, can reach up to 2 meters in height (de Wilde, 2002). The necessity of overcoming the seed shadow handicap may not provide a sufficient explanation as to the evolution of dioecy in *B. subalpestris,* but perhaps large plant size can. A second example; *B. acetosella,* which is a species potentially demonstrating the necessity for dioecy to overcome the seed shadow handicap through fleshy fruit and larger range size to persist within a lineage, may also be pressured to maintain a dioecious breeding system by its large size, as it can also grow into to a 2 meter shrub. Due to timing and difficulty in accurately measuring this character for all species, plant size was not analyzed in this study, but future studies involving the correlation between plant size and dioecy may prove to be insightful.

Polyploidy has also been hypothesized to form a link with dioecy (Barrett, 2002; Ashman, Kwok and Husband, 2013). Polyploidy has been hypothesized to occur at a high rate within the genus *Begonia,* with diploid chromosome numbers ranging from 2n=16 to 2n=104 (Oginuma and Peng, 2002). Therefore, research exploring a link between polyploidy and dioecy in begonia may be enlightening. As new *Begonia* species are described in the future and the genus is further phylogenetically resolved as to involve the *Knesebeckia* dioecious species in analysis, further links to dioecy and patterns of evolution may also be revealed beyond the potential influence of the seed shadow handicap. Further character correlations, such as plant size, monoecy type, sex ratios and polyploidy, and other new dioecious species described may assist in advancing the understanding of evolution of dioecy from monoecy within *Begonia.*

4.2 *Conclusion, the rarity of dioecy in Begonia*

In summary, dioecy has independently evolved within *Begonia* eight times. The seed shadow handicap does not appear to be a hindrance to the evolution of dioecy across the entire genus of *Begonia*, as larger range sizes do not correlate with dioecious species, despite a correlation between fleshy fruit and dioecy. Looking at specific *Begonia* dioecious species, the seed shadow handicap may be a potential pressure influencing dioecy in sections Sphenanthera, Mezierea, Quadriperigonia, Diploclinium, and Knesebeckia, as they have dioecious species with larger range sizes, achieved through fleshy fruit dispersal or other unknown means. The seed shadow handicap is then an assumed impediment in the pathway to dioecy from monoecy in some cases in *Begonia,* suggesting that lineages will not be able to successfully evolve and maintain dioecy without achieving larger range sizes to remain competitive. This may provide one reason why only 1% of *Begonia* species have become dioecious, as many *Begonias* are narrow endemics, and will remain more immediately competitive in their environments if all individuals in a population can produce seed. However, large range size did not correlate with dioecy with respect to the *Begonia* phylogeny, indicating other pressures affecting dioecy. Reasons behind the development of dioecy in species with restricted range sizes, such as those in section Casparya, Tetraphila, and perhaps Petermannia may be related to other dioecy correlates such as large plant size. Other pressures for, or impediments to, evolving dioecy from monoecy in *Begonia* must be further explored in future studies.

References

Agren, J. and Schemske, D. W. (1991) Pollination by Deceit in a Neotropical Monoecious Herb, Begonia involucrata. *Biotropica* **23**: 235–241.

Akagi, T., Henry, I. M., Ryutaro, T. and Comai, L. (2014) A Y-chromosome-encoded small RNA acts as a sex determinant in persimmons. *Science* **346**: 646-650.

Akaike, H. (1974) A new look at the statistical model identification. IEEE *Transactions on Automatic Control* **19**: 716–723.

Anonymous (1920) Decades Kewenses. *Bulletin of Miscellaneous Information* **3**: 109–110.

Ashman, T. L., Kwok, A. and Husband, B. C. (2013) Revisiting the dioecy-polyploidy association: Alternate pathways and research opportunities. *Cytogenetic and Genome Research* **140**: 241–255. doi: 10.1159/000353306.

Barrett, S. C. H. (2002) The Evolution of Plant Sexual Diversity. *Nature Reviews Genetics* **3**: 274–284. doi: 10.1038/nrg776.

Bawa, K. S. and Opler, P. A. (1975) Dioecism in Tropical Forest Trees. *Evolution* **29**: 167–179.

Bawa, K. S. (1980) Evolution of Dioecy in Flowering Plants. *Annual Review of Ecology and Systematics* **11**: 15–39. doi: 10.1146/annurev.es.11.110180.000311.

Beach, J. H. (1981) Pollinator Foraging and the Evolution of Dioecy. *The American Naturalist* **118**: 572–577.

Bessler, B. (1996). Changes in Habit and Sex Expression in Tuberous Begonia Hybrids by Use of GA(3) and Benzylaminopurine, *Gartenbauwissenschaft* **61**: 205-210.

Boualem, A., Troadec, C., Camps, C., Lemhemdi, A., Morin, H., Sari, M.-A., Fraenkel-Zagouri, R., Kovalski, I., Dogimont, C., Perl-Treves, R. and Bendahmane, A. (2015) A cucurbit androecy gene reveals how unisexual flowers develop and dioecy emerges. *Science* **350**: 688–691. doi: 10.1126/science.aac8370.

Burt-Utley, K. & R. McVaugh. (2001) Begoniaceae. In: R. McVaugh, R. & Anderson, W. R. (eds.), *Flora Novo- Galiciana* **3**: 653–695.

Burt-Utley, K. and Utley, J. F. (2012) New species and notes on Begonia (Begoniaceae) from México and Central America. *Phytoneuron* **5**: 1–25.

Burt-Utley, K. (2014) Begoniaceae. In: *Flora Mesoamericana* **2**: 1–102.

Case, A. L. and Barrett, S. C. H. (2004) Environmental stress and the evolution of dioecy: *Wurmbea dioica* (Colchicaceae) in Western Australia. *Evolutionary Ecology* **18**: 145–164. doi: 10.1023/B:EVEC.0000021152.34483.77.

Charlesworth, B. and Charlesworth, D. (1978) A Model for the Evolution of Dioecy and

Gynodioecy. *The American Naturalist* **112**: 975–997.

Darwin, C. (1876) *The effects of cross and self fertilization in the vegetable kingdom*. London: John Murray.

Dauby, Gilles (2017) *ConR: Computation of Parameters Used in Preliminary Assessment of Conservation Status*. R package version 1.2.1. https://CRAN.R-project.org/package=ConR.

Delph, L. F. (2009) Sex Allocation: Evolution to and from Dioecy. *Current Biology* **19**: R249–R251. doi: 10.1016/j.cub.2009.01.048.

Dorken, M. E. and Barrett, S. C. H. (2004) Sex determination and the evolution of dioecy from monoecy in Sagittaria latifolia (Alismataceae). *Proceedings of the Royal Society B: Biological Sciences* **271**: 213–219. doi: 10.1098/rspb.2003.2580.

Drummond, A. J., Suchard, M. A., Xie, D. & Rambaut, A. (2012) Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology And Evolution* **29**: 1969-1973.

Fritz, S. A. & Purvis, A. (2010) Selectivity in mammalian extinction risk and threat types: a new measure of phylogenetic signal strength in binary traits. *Conservation Biology* **24**:1042-1051.

GBIF.org (2017).

Girmansyah, D., Wiriadinata, H., Thomas, D. C. and Hoover, W. S. (2009) Two New Species and One New Subspecies of Begonia (Begoniaceae) From Southeast Sulawesi, Sulawesi, Indonesia. *Reinwardtia* **13**: 69–74.

Glez-Peña, D., Gómez-Blanco, D., Reboiro-Jato, M., Fdez-Riverola, F., & Posada, D. (2010) ALTER: program-oriented format conversion of DNA and protein alignments. Nucleic Acids Research. Web Server issue. ISSN: 0305-1048.

Goldberg, E. E., Otto, S. P., Vamosi, J. C., Mayrose, I., Sabath, N., Ming, R. and Ashman, T. L. (2017) Macroevolutionary synthesis of flowering plant sexual systems. *Evolution* **71**: 898–912. doi: 10.1111/evo.13181.

Golenberg, E. M. and West, N. W. (2013) Hormonal interactions and gene regulation can link monoecy and environmental plasticity to the evolution of dioecy in plants. *American Journal of Botany* **100**: 1022–1037. doi: 10.3732/ajb.1200544.

Gu, C., Peng, C.-I. and Turland, N. (2007) Begoniaceae 秋海棠科. In: *Flora of China*, pp. 313–327.

Hall, T. A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT *Nucleic Acids Symposium Series*, **41**: 95-98.

Harkess, A. and Leebens-mack, J. (2017) A Century of Sex Determination in Flowering Plants. *Journal of Heredity* **108**: 69–77. doi: 10.1093/jhered/esw060.

Heilbuth, J. C., Ilves, K. L. and Otto, S. P. (2001) The consequence of dioecy for seed dispersal: modeling the seed shadow handicap. *Evolution* **55**: 880–888.

Hughes, M. (2002) *Population Structure and Speciation in Begonia L.* PhD Thesis, University of Glasgow, United Kingdom.

Hughes, M., Moonlight, P., Jara, A & Pullan, M. (2015) Begonia Resource Centre. Online database available from http://padme.rbge.org.uk/begonia/ [accessed 2 June 2017].

Jesson, L. K. (2007) Ecological correlates of diversification in New Zealand angiosperm lineages. *New Zealand Journal of Botany* **45**: 35–51. doi: 10.1080/00288250709509701.

Käfer, J., de Boer, H. J., Mousset, S., Kool, A., Dufay, M. and Marais, G. A. B. (2014) Dioecy is associated with higher diversification rates in flowering plants. *Journal of Evolutionary Biology* **27**: 1478–1490. doi: 10.1111/jeb.12385.

Käfer, J., Marais, G. A. B. and Pannell, J. R. (2017) On the rarity of dioecy in flowering plants. *Molecular Ecology* **26**: 1225–1241. doi: 10.1111/mec.14020.

Khryanin, V. N. (2002) Role of Phytohormones in Sex Differentiation in Plants. *Russian Journal of Plant Physiology* **49**: 608–614.

Maddison, W., & Maddison, D. (2015) MESQUITE Version 3.10 (build 765). Available at http://mesquiteproject.org.

Matsunaga, S. and Kawano, S. (2001) Sex Determination by Sex Chromosomes in Dioecious Plants. *Plant Biology* **3**: 481–488.

Miller, M.A., Pfeiffer, W., & Schwartz, T. (2010) "Creating the CIPRES Science Gateway for inference of large phylogenetic trees" in Proceedings of the Gateway Computing Environments Workshop (GCE), New Orleans, LA pp 1 - 8.

Moonlight, P. W., Richardson, J. E., Tebbitt, M. C., Thomas, D. C., Hollands, R., Peng, C. I. and Hughes, M. (2015) Continental-scale diversification patterns in a megadiverse genus: The biogeography of Neotropical Begonia. *Journal of Biogeography* **42**: 137–1149. doi: 10.1111/jbi.12496.

Muenchow, G. E. (1987) Is Dioecy Associated with Fleshy Fruit ? *American Journal of Botany* **74**: 287–293.

Nazareno, A. G., Alzate-Marin, A. L. and Pereira, R. A. S. (2013) Dioecy, more than monoecy, affects plant spatial genetic structure: The case study of Ficus. *Ecology and Evolution* **3**: 3495–3508. doi: 10.1002/ece3.739.

Oginuma, K. and Peng, C. I. (2002) Karyomorphology of Taiwanese Begonia (Begoniaceae): Taxonomic implications. *Journal of Plant Research* **115**: 225–235. doi: 10.1007/s102650200028.

Orme, D., Freckleton, R., Thomas, G., Petzoldt, T., Fritz, S., Isaac N., & Pearse, W. (2013) caper: Comparative Analyses of Phylogenetics and Evolution in R. R package version 0.5.2.

Pailler, T. and Thompson, J. D. (1997) Distyly and Variation in Heteromorphic Incompatibility in Gaertnera vaginata (Rubiaceae) Endemic to La Reunion Island. *American Journal of Botany* **84**: 315–327.

Paradis, E., Claude, J. & Strimmer, K. (2004) APE: analyses of phylogenetics and evolution in R language. *Bioinformatics* **20**: 289-290.

Plana, V. (2003) Phylogenetic Relationships of the Afro-Malagasy Members of the Large Genus Begonia Inferred from trnL Intron Sequences. *Systematic Botany* **28**: 693–704.

Plana, V., Sands, M. S. J., & Beentje, H. J. (2006) *Begoniaceae.* In: Beentje, H. J., & Ghazanfar, S. A. (eds.), *The Flora of Tropical East Africa.* Richmond, Surrey, England, Royal Botanic Gardens, Kew, pp. 4-6.

R Core Team (2017) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Rambaut, A., Suchard, M. A., Xie, D. & Drummond, A. J. (2014) Tracer v1.6, Available from http://beast.bio.ed.ac.uk/Tracer.

Renner, S. S. and Ricklefs, R. E. (1995) Dioecy and Its Correlates in the Flowering Plants. *American Journal of Botany* **82**: 596–606.

Renner, S. S. (2001) How common is heterodichogamy? *Trends in Ecology and Evolution* 16: 595–597. doi: 10.1016/S0169-5347(01)02280-7.

Renner, S. S. (2014) The relative and absolute frequencies of angiosperm sexual systems: Dioecy, monoecy, gynodioecy, and an updated online database. *American Journal of Botany* **101**: 1588–1596. doi: 10.3732/ajb.1400196.

Renner, S. S. (2016) Pathways for making unisexual flowers and unisexual plants: Moving beyond the “Two mutations linked on one chromosome” model. *American Journal of Botany* **103**: 587-589. doi: 10.3732/ajb.1600029.

Revell, L. J. (2012) phytools: An R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution.* **3**: 217-223. doi:10.1111/j.2041-210X.2011.00169.x.

Ronquist, F. & Huelsenbeck, J. P. (2003) MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**: 1572-1574.

Sabath, N., Goldberg, E. E., Glick, L., Einhorn, M., Ashman, T. L., Ming, R., Otto, S. P., Vamosi, J. C. and Mayrose, I. (2016) Dioecy does not consistently accelerate or slow lineage diversification across multiple genera of angiosperms. *New Phytologist* 209: 1290–1300. doi: 10.1111/nph.13696.

Sakai, A. K., Wagner, W. L., Ferguson, D. M. and R., H. D. (1995) Biogeographical and Ecological Correlates of Dioecy in the Hawaiian Flora. *Ecology* **76**: 2530–2543.

Schemske, D. W. and Agren, J. (1995) Deceit Pollination and Selection on Female Flower Size in Begonia involucrata: An Experimental Approach. *Evolution*, **49**: 207–214.

Shaw, J., Lickey, E. B., Schilling, E. E. and Small, R. L. (2007) Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: The Tortoise and the hare III. *American Journal of Botany* **94**: 275–288. doi: 10.3732/ajb.94.3.275.

Smith, J. M. (1978). The Evolution of Sex. Cambridge University Press. Cambridge.

Smith, L. B. and Schubert, B. G. (1939) Plantae mexicanae. *Contributions From the Gray Herbarium of Harvard University* **127**: 20–32.

Smith, L. B. and Schubert, B. G. (1946) The Begoniaceae of Colombia. *Caldasia* **4**: 71–209.

Smith, L. B. and Schubert, B. G. (1947) Some Mexican Begonias. *Contributions From the Gray Herbarium of Harvard University* **165**: 90–94.

Stamatakis, A., Hoover, P., & Rougemont, J. (2008) A Rapid Bootstrap Algorithm for the RAxML Web-Servers, *Systematic Biology*, **75**: 758-771.

Tebbitt, M. C. (2002) Emended Circumscription of Begonia silletensis (Begoniaceae) and Description of a New Subspecies from Yunnan. *Novon* **12**: 133–136.

Tebbitt, M. C. (2003a) Notes on South Asian Begonia (Begoniaceae). *Edinburgh Journal of Botany* **60**: 1–9. doi: 10.1017/S0960428603000015.

Tebbitt, M. C. (2003b) Taxonomy of Begonia longifolia Blume (Begoniaceae) and related species. *Brittonia* **55**: 19–29. doi: 10.1663/0007-196X(2003)055[0019:TOBLBB]2.0.CO;2.

Tebbitt, M. C. (2005) *Begonias; cultivation, identification, and natural history.* Timber Press, Portland.

Tebbitt, M. C., Lowe-Forrest, L., Santoriello, A., Clement, W. L. and Swensen, S. M. (2006) Phylogenetic Relationships of Asian Begonia, with an Emphasis on the

Evolution of Rain-ballist and Animal Dispersal Mechanisms in Sections Platycentrum, Sphenanthera and Leprosae. *Systematic Botany* **31**: 327–336. doi: 10.1600/036364406777585784.

Thomas, D. C., Ardi, W. H. and Hughes, M. (2009) Two New Species of Begonia (Begoniaceae) From Central Sulawesi, Indonesia. *Edinburgh Journal of Botany* **66**: 229-238. doi: 10.1017/S0960428609005320.

Thomas, D. C., Ardi, W. H. and Hughes, M. (2011) Nine New Species of Begonia (Begoniaceae) From South and West Sulawesi, Indonesia. *Edinburgh Journal of Botany* **68**: 225–255. doi: 10.1017/S0960428611000072.

Thomas, D. C., Hughes, M., Phutthai, T., Rajbhandary, S., Rubite, R., Ardi, W. H. and Richardson, J. E. (2011) A non-coding plastid DNA phylogeny of Asian Begonia (Begoniaceae): Evidence for morphological homoplasy and sectional polyphyly. *Molecular Phylogenetics and Evolution* **60**: 428–444. doi: 10.1016/j.ympev.2011.05.006.

Thomas, D. C., Hughes, M., Phutthai, T., Ardi, W. H., Rajbhandary, S., Rubite, R., Twyford, A. D. and Richardson, J. E. (2012) West to east dispersal and subsequent rapid diversification of the mega-diverse genus Begonia (Begoniaceae) in the Malesian archipelago. *Journal of Biogeography* **39**: 98–113. doi: 10.1111/j.1365-2699.2011.02596.x.

Torices, R., Mendez, M. and Gomez, J. M. (2011) Where do monomorphic sexual systems fit in the evolution of dioecy? Insights from the largest family of angiosperms. *New Phytologist* **190**: 234–248. doi: 10.1111/j.1469-8137.2010.03609.x.

Twyford, A. D., Ennos, R. A., White, C. D., Shaukat Ali, M. and Kidner, C. A. (2014) The evolution of sex ratio differences and inflorescence architectures in Begonia (Begoniaceae). *American Journal of Botany* **101**: 308–317. doi: 10.3732/ajb.1300090.

Uddin, A. & Phukan, S. (2007) Notes on *Begonia aborensis* Dunn. – A New record From Assam, India. *Journal of Economic and Taxonomic Botany* **31**: 160-162.

Vamosi, S. M., Mazer, S. J., Cornejo, F. and Mazer, J. (2008) Breeding Systems and Seed Size in a Neotropical Flora : Testing Evolutionary Hypotheses. *Ecology* **89**: 2461–2472.

Whitehead, D. R. (1969) Wind Pollination in the Angiosperms : Evolutionary and Environmental Considerations. *Evolution* **23**: 28–35.

de Wilde, J. J. F. E. (2002) *STUDIES IN BEGONIACEAE VII.* Edited by J. J. F. E. De Wilde. Wageningen: Backhuys Publishers.

Zhang, L. B., Simmons, M. P., Kocyan, A. and Renner, S. S. (2006) Phylogeny of the Cucurbitales based on DNA sequences of nine loci from three genomes: Implications for morphological and sexual system evolution. *Molecular Phylogenetics and Evolution* **39**: 305–322. doi: 10.1016/j.ympev.2005.10.002.

Appendix 1: *Begonia* species, accession, and the plastid molecular regions newly sequenced for this study.

Begonia	EDNA17 accession #	Plastid regions sequenced
subalpestris	0048021	*ndhA,ndhF-rpl32, rpl32-trnL*
dioica	0048413	*ndhA,ndhF-rpl32, rpl32-trnL*
grisea	0048024	*ndhA*
paganuccii	0048025	*ndhA,ndhF-rpl32, rpl32-trnL*
c.f. grandis	0048022	*ndhA,ndhF-rpl32, rpl32-trnL*
sp.	0048023	*ndhA,ndhF-rpl32, rpl32-trnL*

Appendix 2: *Begonia* character state matrix.

Taxon	Breeding system	Extent of occurrence	Fruit type
AA_HIL_PM_Hillebrandia_sandwicensis_EDNA13_0034233	monoecious	500	dry
AF_AUG_DT_Begonia_dregei_EDNA09_02164	monoecious	20718.3	dry
AF_AUG_MH_Begonia_sutherlandii_MH80	monoecious	49857.3	dry
AF_BAC_DF_Begonia_baccata_EDNA14_0035689	monoecious	4	fleshy
AF_ERM_DF_Begonia_antongilensis_EDNA14_0035458	monoecious	42795.1	dry
AF_ERM_PM_Begonia_bogneri_EDNA13_0033469	monoecious	627.9	dry
AF_ERM_PM_Begonia_nana_EDNA15_0039180	monoecious	37852.5	dry
AF_FIL_PM_Begonia_macrocarpa_EDNA15_0039233	monoecious	209790.9	dry
AF_FIL_PM_Begonia_minutifolia_EDNA15_0039688	monoecious	4	dry
AF_FIL_PM_Begonia_sciaphila_EDNA15_0039163	monoecious	4	dry
AF_LOA_PM_Begonia_letouzeyi_EDNA15_0039682	monoecious	17168.2	dry
AF_LOA_PM_Begonia_microsperma_EDNA13_0033503	monoecious	21462.8	dry
AF_LOA_PM_Begonia_quadrialata_EDNA15_0039709	monoecious	774732.9	dry
AF_LOA_PM_Begonia_scutifolia_EDNA13_0033504	monoecious	154954.9	dry
AF_LOA_PM_Begonia_scutifolia_EDNA15_0039722	monoecious	154954.9	dry
AF_LOA_PM_Begonia_staudtii_EDNA15_0039727	monoecious	2254.2	dry
AF_MEZ_DF_Begonia_humbertii_EDNA14_0035455	monoecious	41441.4	fleshy
AF_MEZ_DF_Begonia_meyeri_johannis_EDNA14_0035692	dioecious	756174.8	fleshy
AF_MEZ_DT_Begonia_oxyloba_EDNA08_02841	monoecious	29201.6	fleshy
AF_MEZ_PM_Begonia_salaziensis_EDNA15_0039164	monoecious	4	fleshy
AF_NER_DF_Begonia_coursii_EDNA14_0035694	monoecious	2716.9	dry
AF_NER_DF_Begonia_lyallii_EDNA14_0035459	monoecious	173577.7	dry
AF_NER_DF_Begonia_madecassa_EDNA14_0035693	monoecious	155706.6	dry
AF_NER_DF_Begonia_majungaensis_EDNA14_0035460	monoecious	329656.7	dry
AF_NER_PM_Begonia_cf_lyallii_EDNA15_0039167	monoecious	173577.7	dry
AF_NER_PM_Begonia_henrilaportei_EDNA15_0039172	monoecious	4	dry
AF_PEL_DT_Begonia_samhaensis_EDNA09_00056_	monoecious	4	dry
AF_PEL_DT_Begonia_socotrana_EDNA08_00210_	monoecious	4	dry
AF_QUA_DT_Begonia_goudotii_EDNA09_02167	monoecious	76309.4	dry
AF_QUA_PM_Begonia_nossibea_EDNA13_0033507	monoecious	23503.6	dry
AF_ROS_PM_Begonia_engleri_EDNA13_0000001	monoecious	4	dry
AF_ROS_PM_Begonia_johnstonii_EDNA13_0000002	monoecious	14799.4	dry
AF_SCU_PM_Begonia_susaniae_EDNA15_0039730	monoecious	4	dry

AF_SCU_PM_Begonia_vankerckhovenii_EDNA15_0039736	monoecious	4	dry
AF_SEX_DF_Begonia_annoboensis_EDNA14_0035690	monoecious	4	dry
AF_SQA_DT_Begonia_poculifera_EDNA09_02175	monoecious	4	fleshy
AF_SQA_RT_Begonia_ampla_EDNA13_0030222	monoecious	4	fleshy
AF_TET_DT_Begonia_polygonoides_EDNA09_02176	monoecious	1231608.5	fleshy
AF_TET_JS_Begonia_subalpestris_EDNA17_0048021	dioecious	4	fleshy
AF_TET_PM_Begonia_elaegnifolia_EDNA15_0038260	monoecious	47874.7	fleshy
AF_TET_PM_Begonia_eminii_EDNA13_0033506	monoecious	3364516.9	fleshy
AF_TET_PM_Begonia_furfuracea_EDNA15_0039665	monoecious	1948.8	fleshy
AF_TET_PM_Begonia_gabonensis_EDNA13_0033472	monoecious	12303.6	fleshy
AF_TET_PM_Begonia_kisuluana_EDNA13_0033473	monoecious	2186872.3	fleshy
AF_TET_PM_Begonia_kisuluana_EDNA15_0039676	monoecious	2186872.3	fleshy
AF_TET_PM_Begonia_komoensis_EDNA15_0038261	monoecious	80948.2	fleshy
AF_TET_PM_Begonia_komoensis_EDNA15_0039677	monoecious	80948.2	fleshy
AF_TET_PM_Begonia_longipetiolata_EDNA13_0033479	monoecious	1428513.7	fleshy
AF_TET_PM_Begonia_loranthoides_EDNA13_0033471	monoecious	497904.8	fleshy
AF_TET_PM_Begonia_loranthoides_subsp_rhoalocarpa_EDNA13_0033470	monoecious	497904.8	fleshy
AF_TET_PM_Begonia_molleri_EDNA15_0039689	monoecious	67.7	fleshy
AF_TET_PM_Begonia_oxyanthera_EDNA15_0038262	monoecious	8250.5	fleshy
AF_TET_PM_Begonia_oxyanthera_EDNA15_0039695	monoecious	8250.5	fleshy
AF_TET_PM_Begonia_parva_EDNA13_0033510	monoecious	418149.7	fleshy
AF_TET_PM_Begonia_squamulosa_EDNA13_0033505	monoecious	398129.3	fleshy
AF_TET_PM_Begonia_subscutata_EDNA15_0038267	monoecious	1238980.3	fleshy
AS_ALI_DT_Begonia_alicida_EDNA10_00614	monoecious	27315.2	dry
AS_BAR_DT_Begonia_chloroneura_EDNA09_02162	monoecious	4	dry
AS_BAR_DT_Begonia_cleopatrae_EDNA08_00167	monoecious	4	dry
AS_BAR_DT_Begonia_fenicis_EDNA08_01795	monoecious	188.6	dry
AS_BAR_DT_Begonia_hernandioides_EDNA08_01794	monoecious	4	dry
AS_BAR_MH_Begonia_acuminatissima_R321	monoecious	2930.6	dry
AS_BAR_MH_Begonia_anisoptera_R479	monoecious	4	dry
AS_BAR_MH_Begonia_biliranensis_R311	monoecious	4	dry
AS_BAR_MH_Begonia_blancii_P22545	monoecious	4	dry
AS_BAR_MH_Begonia_calcicola_P20761	monoecious	655.8	dry
AS_BAR_MH_Begonia_camiguinensis_R506	monoecious	4	dry
AS_BAR_MH_Begonia_castilloi_R98	monoecious	4	dry
AS_BAR_MH_Begonia_chingipengii_P23368	monoecious	4	dry
AS_BAR_MH_Begonia_chloroneura_W29015	monoecious	4	dry
AS_BAR_MH_Begonia_cleopatrae_MH25	monoecious	4	dry
AS_BAR_MH_Begonia_copelandii_R238	monoecious	4	dry
AS_BAR_MH_Begonia_culasiensis_R234	monoecious	4	dry
AS_BAR_MH_Begonia_dinglensis_P23859	monoecious	4	dry
AS_BAR_MH_Begonia_elmeri_R319	monoecious	4	dry
AS_BAR_MH_Begonia_elnidoensis_P23508	monoecious	4	dry
AS_BAR_MH_Begonia_gabaldonensis_P23356_2	monoecious	4	dry
AS_BAR_MH_Begonia_gitingensis_R255	monoecious	4	dry
AS_BAR_MH_Begonia_gueritziana_P22311	monoecious	112161.9	dry

AS_BAR_MH_Begonia_gueritziana_P22342	monoecious	112161.9	dry
AS_BAR_MH_Begonia_gutierrezii_EDNA13_0033154	monoecious	4	dry
AS_BAR_MH_Begonia_hernandioides_P21006	monoecious	4	dry
AS_BAR_MH_Begonia_hernandioides_R106	monoecious	4	dry
AS_BAR_MH_Begonia_hughesii_P23466	monoecious	6.7	dry
AS_BAR_MH_Begonia_hughesii_P23475	monoecious	6.7	dry
AS_BAR_MH_Begonia_klemmei_R182	monoecious	4	dry
AS_BAR_MH_Begonia_longiscapa_R298	monoecious	37812.3	dry
AS_BAR_MH_Begonia_longiscapa_R309	monoecious	37812.3	dry
AS_BAR_MH_Begonia_luzonensis_KO30960	monoecious	56454.3	dry
AS_BAR_MH_Begonia_luzonensis_R316	monoecious	56454.3	dry
AS_BAR_MH_Begonia_luzonensis_R420	monoecious	56454.3	dry
AS_BAR_MH_Begonia_manillensis_R256	monoecious	8996.9	dry
AS_BAR_MH_Begonia_merrilliana_P23765	monoecious	4	dry
AS_BAR_MH_Begonia_mindorensis_R326	monoecious	32696.8	dry
AS_BAR_MH_Begonia_obtusifolia_P23828_4	monoecious	4	dry
AS_BAR_MH_Begonia_oxysperma_R213	monoecious	23936.6	dry
AS_BAR_MH_Begonia_rhombicarpa_P23372	monoecious	68592.8	dry
AS_BAR_MH_Begonia_rhombicarpa_P23451	monoecious	68592.8	dry
AS_BAR_MH_Begonia_rhombicarpa_P23855	monoecious	68592.8	dry
AS_BAR_MH_Begonia_rhombicarpa_R419	monoecious	68592.8	dry
AS_BAR_MH_Begonia_rubitae_R356	monoecious	4	dry
AS_BAR_MH_Begonia_rufipila_R265	monoecious	500	dry
AS_BAR_MH_Begonia_subnummarifolia_SUBN	monoecious	4	dry
AS_BAR_MH_Begonia_suborbiculata_R353	monoecious	1091.2	dry
AS_BAR_MH_Begonia_sykakiengii_P23836	monoecious	4	dry
AS_BAR_MH_Begonia_sykakiengii_P23890	monoecious	4	dry
AS_BAR_MH_Begonia_tagbanua_P23472	monoecious	4	dry
AS_BAR_MH_Begonia_taraw_MH109	monoecious	4	dry
AS_BAR_MH_Begonia_tayabensis_R360	monoecious	4	dry
AS_BAR_MH_Begonia_trichocheila_P20764	monoecious	4	dry
AS_BAR_MH_Begonia_wadei_R699	monoecious	57.9	dry
AS_BAR_MH_Begonia_woodii_P23496	monoecious	4	dry
AS_BRA_AM_Begonia_beludruvenea_EDNA16_0044927	monoecious	621.7	dry
AS_BRA_DT_Begonia_barbellata_P33_SBGsn	monoecious	14630.9	dry
AS_BRA_DT_Begonia_bracteata_EDNA08_02252	monoecious	4	dry
AS_BRA_DT_Begonia_resecta_EDNA08_00204	monoecious	4	dry
AS_BRA_DT_Begonia_verecunda_EDNA08_02332	monoecious	458.8	dry
AS_COE_DF_Begonia_pseudodryadis_EDNA14_0035696	monoecious	4	dry
AS_COE_DT_Begonia_masoniana_EDNA08_01777_	monoecious	4	dry
AS_COE_DT_Begonia_morsei_EDNA09_02170	monoecious	10000	dry
AS_COE_MH_Begonia_masoniana_P21411	monoecious	4	dry
AS_COE_MH_Begonia_ningmingensis_P20322	monoecious	10000	dry
AS_COE_PM_Begonia_variegata_EDNA15_0039737	monoecious	10	dry
AS_DIP_AM_Begonia_poilanei_EDNA16_0045155	monoecious	4	dry
AS_DIP_DT_Begonia_grandis_EDNA08_03023_	monoecious	2503673.6	dry

AS_DIP_DT_Begonia_puttii_EDNA10_00622	monoecious	4	dry
AS_DIP_DT_Begonia_rabilii_EDNA10_00624	monoecious	4	dry
AS_DIP_JS_Begonia_dioica_EDNA17_048413	dioecious	520605.3	dry
AS_DIP_LK_Begonia_bryophila_EDNA14_0035340	monoecious	4	dry
AS_DIP_LK_Begonia_flagellaris_1	monoecious	9776.3	dry
AS_DIP_LK_Begonia_flagellaris_2	monoecious	9776.3	dry
AS_DIP_LK_Begonia_picta	monoecious	537387.6	dry
AS_DIP_LK_Begonia_tribenensis	monoecious	6271.1	dry
AS_DIP_MH_Begonia_gigabracteata_Peng22174	monoecious	4	dry
AS_DIP_MH_Begonia_murina_Peng24137	monoecious	4	dry
AS_DIP_MH_Begonia_rubella_CIH6000	monoecious	28694.4	dry
AS_DIP_MH_Begonia_yunnanensis_Peng20491	monoecious	250000	dry
AS_DIP_PM_Begonia_pulvinifera_EDNA15_0039708	monoecious	4	dry
AS_DIP_TP_Begonia_aceroides_EDNA10_00623	monoecious	4	dry
AS_DIP_YM_Begonia_cehengensis_Q234	monoecious	4	dry
AS_DIP_YM_Begonia_lithophila_Q149	monoecious	4	dry
AS_DIP_YM_Begonia_sinofloribunda_Q088	monoecious	4	dry
AS_DIP_YM_Begonia_wilsonii_Q007	monoecious	4	dry
AS_HAA_MH_Begonia_dipetala_P22520	monoecious	48.2	dry
AS_HEE_PM_Begonia_sibthorpoides_EDNA16_0045767	monoecious	4	dry
AS_IGN_DT_Begonia_cucphuongensis_Peng20227	monoecious	152.5	dry
AS_IGN_DT_Begonia_demissa_EDNA10_00617	monoecious	152251.7	dry
AS_JAC_AM_Begonia_droopiae_EDNA16_0044923	monoecious	4	dry
AS_JAC_AM_Begonia_kudoensis_EDNA16_0044922	monoecious	4	dry
AS_JAC_AM_Begonia_nurii_EDNA15_0039693	monoecious	2724.2	dry
AS_JAC_AM_Begonia_nurii_EDNA16_0045448	monoecious	2724.2	dry
AS_JAC_AM_Begonia_olivacea2_EDNA16_0044920	monoecious	4	dry
AS_JAC_AM_Begonia_rajah_EDNA15_0039710	monoecious	4	dry
AS_JAC_AM_Begonia_rajah_EDNA16_0045446	monoecious	4	dry
AS_JAC_AM_Begonia_reginula_EDNA16_0045447	monoecious	125.2	dry
AS_JAC_AM_Begonia_simolapensis_EDNA16_0044921	monoecious	4	dry
AS_JAC_AM_Begonia_speluncae_EDNA15_0039725_	monoecious	898.4	dry
AS_JAC_AM_Begonia_tricopoda_EDNA16_0045152	monoecious	6.7	dry
AS_JAC_DF_Begonia_karangputihensis_EDNA14_0036126_CP53	monoecious	4	dry
AS_JAC_DT_Begonia_goegoensis_EDNA08_01783	monoecious	4	dry
AS_JAC_DT_Begonia_muricata_EDNA08_02254	monoecious	85587.6	dry
AS_JAC_DT_Begonia_sudjanae_EDNA08_01785_	monoecious	4	dry
AS_JAC_LK_Begonia_fluvialis_EDNA12_0025037_MH1489	monoecious	4	dry
AS_JAC_LK_Begonia_karangputihensis_EDNA12_0029749_CP53	monoecious	4	dry
AS_JAC_LK_Begonia_puspitae_EDNA12_0029751_CP134	monoecious	4	dry
AS_JAC_LK_Begonia_rajah_EDNA12_0025040_DED1497	monoecious	4	dry
AS_JAC_LK_Begonia_stictipoda_EDNA12_0029747_CP239	monoecious	16094.1	dry
AS_JAC_LK_Begonia_sublobata_EDNA_0025038_DED1486_OK	monoecious	4	dry
AS_JAC_MH_Begonia_forbesii_P22685	monoecious	4	dry
AS_JAC_MH_Begonia_foxworthyii_P22721	monoecious	824	dry
AS_JAC_MH_Begonia_ignorata_P22725	monoecious	4	dry

AS_JAC_MH_Begonia_speluncae_PP22344	monoecious	898.4	dry
AS_JAC_MH_Begonia_stictopoda_EDNA14_0036123_20070789_MH1409	monoecious	16094.1	dry
AS_JAC_MH_Begonia_tigrina_P22720	monoecious	4	dry
AS_JAC_PM_Begonia_kemumuensis_EDNA13_0034225_20101655_DED1499	monoecious	4	dry
AS_JAC_PM_Begonia_pasamanensis_EDNA13_0034227_20101656_DED1506	monoecious	3328.4	dry
AS_LEP_PM_Begonia_leprosa_EDNA15_0039681	monoecious	109710.2	fleshy
AS_MON_MH_Begonia_griffithiana_Peng20851	monoecious	137821	dry
AS_MON_MH_Begonia_nepalensis_Peng20854	monoecious	159291.6	dry
AS_MON_PM_Begonia_nepalensis_EDNA15_0039692	monoecious	159291.6	dry
AS_PAR_AM_Begonia_pteridiformis_EDNA15_0039236	monoecious	6136.5	dry
AS_PAR_DT_Begonia_elisabethae_EDNA10_00618	monoecious	15474.6	dry
AS_PAR_DT_Begonia_tenuifolia_EDNA08_02248	monoecious	89139.4	dry
AS_PAR_MH_Begonia_martabanica_Peng24184	monoecious	526270.8	dry
AS_PET_AM_Begonia_atricha_EDNA16_0045151	monoecious	248204.7	dry
AS_PET_AM_Begonia_augustae_EDNA16_0045452	monoecious	400688.9	dry
AS_PET_AM_Begonia_dolichocarpa_EDNA16_0045153	monoecious	4	dry
AS_PET_AM_Begonia_erythrogyna_EDNA15_0039662	monoecious	4	dry
AS_PET_AM_Begonia_holttumii_EDNA15_0039670	monoecious	141724.9	dry
AS_PET_AM_Begonia_isoptera_EDNA16_0045150	monoecious	208304.1	dry
AS_PET_AM_Begonia_rubida_EDNA16_0045706	monoecious	4	dry
AS_PET_CK_Begonia_aff_cauliflora_FL065	monoecious	4	dry
AS_PET_CK_Begonia_alabensis_FL072	monoecious	4	dry
AS_PET_CK_Begonia_beryllae_isolate_FL071	monoecious	809.6	dry
AS_PET_CK_Begonia_burbidgei	monoecious	84.2	dry
AS_PET_CK_Begonia_imbricata_isolate_FL075	monoecious	4	dry
AS_PET_CK_Begonia_inobangensis_FL062	monoecious	4	dry
AS_PET_CK_Begonia_mamutensis_isolate_FL029	monoecious	4	dry
AS_PET_CK_Begonia_oblongifolia_isolate_FL014	monoecious	4	dry
AS_PET_CK_Begonia_pendula	monoecious	12180.7	dry
AS_PET_CK_Begonia_vaccinioides_isolate_SNP25535_1	monoecious	4	dry
AS_PET_DF_Begonia_amphioxus_EDNA08_01792	monoecious	4	dry
AS_PET_DF_Begonia_fuscisetosa_EDNA14_0035456	monoecious	89985.9	dry
AS_PET_DT_Begonia_aff_congesta_EDNA09_00060_	monoecious	21586.6	dry
AS_PET_DT_Begonia_aff_mekongensis_EDNA09_01411_	dioecious	477.8	dry
AS_PET_DT_Begonia_baik_Peng24235	monoecious	4	dry
AS_PET_DT_Begonia_baramensis_P34_S09	monoecious	7706.7	dry
AS_PET_DT_Begonia_bipinnatifida_KREBsn	monoecious	1165.1	dry
AS_PET_DT_Begonia_bonthainensis_EDNA09_01403_	monoecious	29.2	dry
AS_PET_DT_Begonia_brevirimosa_EDNA11_02276	monoecious	4	dry
AS_PET_DT_Begonia_bruneiana_NHZ34	monoecious	4	dry
AS_PET_DT_Begonia_capituliformis_EDNA09_01419_	monoecious	1074.5	dry
AS_PET_DT_Begonia_chiasmogyna_EDNA07_00577	monoecious	4	dry
AS_PET_DT_Begonia_chlorosticta_EDNA08_00208_	monoecious	4	dry
AS_PET_DT_Begonia_comestibilis_EDNA09_01402_	monoecious	4	dry
AS_PET_DT_Begonia_corrugata_EDNA09_00057_	monoecious	4	dry

AS_PET_DT_Begonia_cyanescens_S06	monoecious	424.8	dry
AS_PET_DT_Begonia_didyma_EDNA08_02240	monoecious	4	dry
AS_PET_DT_Begonia_doloisii_PH115	monoecious	4	dry
AS_PET_DT_Begonia_flacca_EDNA09_01009_	monoecious	4	dry
AS_PET_DT_Begonia_galeolepis_KREBsn	monoecious	4	dry
AS_PET_DT_Begonia_guttapila_EDNA08_02244	dioecious	4	fleshy
AS_PET_DT_Begonia_hainanensis_Peng19543	monoecious	4	dry
AS_PET_DT_Begonia_harauensis_EDNA09_01011_	monoecious	66.4	dry
AS_PET_DT_Begonia_hekensis_EDNA08_02227	monoecious	4	dry
AS_PET_DT_Begonia_hispidissima_EDNA09_01418	monoecious	1025.1	dry
AS_PET_DT_Begonia_holttumii_Peng22736	monoecious	141724.9	dry
AS_PET_DT_Begonia_inostegia_FL021	monoecious	4	dry
AS_PET_DT_Begonia_jamilahana_NHZ14	monoecious	4	dry
AS_PET_DT_Begonia_joffrei_S15	monoecious	4	dry
AS_PET_DT_Begonia_koordersii_EDNA08_02235_	monoecious	24534.2	fleshy
AS_PET_DT_Begonia_labiensis_NHZ24	monoecious	4	dry
AS_PET_DT_Begonia_laruei_EDNA08_02333	monoecious	8433.7	dry
AS_PET_DT_Begonia_lasioura_EDNA09_01412_	monoecious	4	dry
AS_PET_DT_Begonia_macintyreana_EDNA07_01454	monoecious	4	dry
AS_PET_DT_Begonia_masarangensis_EDNA09_01679_	monoecious	4	dry
AS_PET_DT_Begonia_mendumae_EDNA07_00578_	monoecious	4	dry
AS_PET_DT_Begonia_multijugata_	monoecious	1482.5	dry
AS_PET_DT_Begonia_negrosensis_EDNA07_01839_	monoecious	47226	dry
AS_PET_DT_Begonia_nobmanniae_EDNA09_01416	monoecious	4	dry
AS_PET_DT_Begonia_nothobaramensis_S08	monoecious	4	dry
AS_PET_DT_Begonia_oblongifolia_FL028	monoecious	4	dry
AS_PET_DT_Begonia_ozotothrix_EDNA08_02845_	monoecious	8947	dry
AS_PET_DT_Begonia_papyraptera_NHZ8	monoecious	4	dry
AS_PET_DT_Begonia_poliloensis_EDNA08_00213	monoecious	4	dry
AS_PET_DT_Begonia_prionota_EDNA09_01407	monoecious	4	dry
AS_PET_DT_Begonia_pseudolateralis_EDNA08_02220_	monoecious	216685.7	fleshy
AS_PET_DT_Begonia_rachmatii_KREBsn	monoecious	4	dry
AS_PET_DT_Begonia_rantemarioensis_EDNA09_01405_	dioecious	14.2	fleshy
AS_PET_DT_Begonia_sageaensis_KREBsn	monoecious	4	dry
AS_PET_DT_Begonia_sanguineopilosa_EDNA09_01417_	dioecious	4	fleshy
AS_PET_DT_Begonia_serratipetala_EDNA07_01838_	monoecious	124191.4	dry
AS_PET_DT_Begonia_sibutensis_S11	monoecious	4	dry
AS_PET_DT_Begonia_siccacaudata_EDNA09_01401_	monoecious	97.3	dry
AS_PET_DT_Begonia_stenogyna_S10	monoecious	1221.8	dry
AS_PET_DT_Begonia_stevei_EDNA07_01455	monoecious	4	dry
AS_PET_DT_Begonia_torajana_11_EDNA09_01410_	dioecious	5.2	fleshy
AS_PET_DT_Begonia_varipeltata_EDNA08_02229_	monoecious	28.5	dry
AS_PET_DT_Begonia_varipeltata_uplandform_KREBsn	monoecious	28.5	dry
AS_PET_DT_Begonia_vermeulenii_EDNA07_01837_	monoecious	4	fleshy
AS_PET_DT_Begonia_watuwilensis_EDNA09_01399_	monoecious	4	dry
AS_PET_DT_Begonia_weigallii_EDNA09_01017	monoecious	180149.6	dry

AS_PET_MH_Begonia_bonthainensis_P22531	monoecious	29.2	dry
AS_PET_MH_Begonia_palawanensis_P23453	monoecious	593.7	dry
AS_PET_MH_Begonia_paracauliflora_Peng22309_Sabah	monoecious	4	dry
AS_PET_MH_Begonia_racemosa_EDNA14_0036125	monoecious	18087.5	dry
AS_PET_PM_Begonia_racemosa_EDNA13_0034226	monoecious	18087.5	dry
AS_PLA_AM_Begonia_areolata_EDNA16_0045154	monoecious	360314.2	dry
AS_PLA_AM_Begonia_baviensis_EDNA16_0045712	monoecious	977.8	dry
AS_PLA_AM_Begonia_cathcartii_EDNA16_0045707	monoecious	932864.4	dry
AS_PLA_AM_Begonia_diadema_EDNA15_0039661	monoecious	4	dry
AS_PLA_AM_Begonia_koksunii_EDNA16_0045708	monoecious	4	dry
AS_PLA_AM_Begonia_perakensis_EDNA16_0045709	monoecious	448273.7	dry
AS_PLA_AM_Begonia_rhoephila_EDNA15_0039711	monoecious	193279	dry
AS_PLA_DT_Begonia_abdullahpieei_Peng22727	monoecious	4	dry
AS_PLA_DT_Begonia_areolata_EDNA08_01685	monoecious	360314.2	dry
AS_PLA_DT_Begonia_decora_EDNA08_00158_	monoecious	311.3	dry
AS_PLA_DT_Begonia_hatacoa_EDNA08_01786	monoecious	530693	dry
AS_PLA_DT_Begonia_palmata_EDNA08_00161	monoecious	1677316.4	dry
AS_PLA_DT_Begonia_pavonina_EDNA08_00157	monoecious	13.1	dry
AS_PLA_DT_Begonia_sikkimensis_EDNA08_02847	monoecious	492089.8	dry
AS_PLA_DT_Begonia_sizemoreae_EDNA08_01787	monoecious	4	dry
AS_PLA_DT_Begonia_smithiae_EDNA10_00615	monoecious	4	dry
AS_PLA_DT_Begonia_venusta_EDNA08_00159_	monoecious	10046.7	dry
AS_PLA_DT_Begonia_versicolor_EDNA08_00209	monoecious	10000	dry
AS_PLA_MH_Begonia_dux_Peng23565	monoecious	4	dry
AS_PLA_PM_Begonia_limprichtii_EDNA15_0039683	monoecious	252711.4	dry
AS_PLA_PM_Begonia_pedatifida_EDNA15_0039699	monoecious	4	dry
AS_PLA_PM_Begonia_thomsonii_EDNA16_0045769	monoecious	177500.2	dry
AS_REI_DT_Begonia_brandisiana_EDNA10_00616	monoecious	4	dry
AS_REI_DT_Begonia_hymenophylla_EDNA10_00613	monoecious	74427	dry
AS_REI_LK_Begonia_tenera	monoecious	2653.3	dry
AS_REI_MH_Begonia_albo_coccinea_P23302	monoecious	1643.2	dry
AS_REI_MH_Begonia_floccifera_MH66	monoecious	4	dry
AS_REI_YM_Begonia_peltatifolia_Q031	monoecious	4	dry
AS_RID_MH_Begonia_kingiana_P21226	monoecious	7571.9	dry
AS_SPH_AM_Begonia_handelii_EDNA15_0039669	dioecious	1240472.9	fleshy
AS_SPH_AM_Begonia_scottii_EDNA16_0044924_MH1569	monoecious	47537.2	fleshy
AS_SPH_AM_Begonia_scottii_EDNA16_0044926_CP217	monoecious	47537.2	fleshy
AS_SPH_DT_Begonia_acetosella_EDNA08_00212	dioecious	837201.4	fleshy
AS_SPH_DT_Begonia_aff_multangula_EDNA09_01425	monoecious	577002	fleshy
AS_SPH_DT_Begonia_aptera_EDNA07_01757	monoecious	81524.8	fleshy
AS_SPH_DT_Begonia_longifolia_EDNA08_00156	monoecious	4017717.4	fleshy
AS_SPH_DT_Begonia_multangula_EDNA08_02250	monoecious	577002	fleshy
AS_SPH_DT_Begonia_obovoidea_EDNA10_00621	monoecious	6484.6	fleshy
AS_SPH_DT_Begonia_robusta_EDNA08_02320	monoecious	334383	fleshy
AS_SPH_DT_Begonia_roxburghii_EDNA08_01779	dioecious	548171.4	fleshy
AS_SPH_DT_Begonia_silletensis_EDNA08_01780_	dioecious	428121.7	fleshy

AS_SPH_JS_Begonia_burkillii_graft	dioecious	140398.8	fleshy
AS_SPH_PM_Begonia_aborensis_EDNA15_0039644	dioecious	60904.2	fleshy
AS_SPH_YM_Begonia_ceratocarpa_Q142	monoecious	10	fleshy
AS_SYM_AM_Begonia_arfakensis_EDNA16_0045156	monoecious	8693	dry
AS_SYM_AM_Begonia_yapenensis_EDNA16_0045157	monoecious	4	dry
AS_SYM_DT_Begonia_argenteomarginata_EDNA07_02176_	monoecious	8438.9	dry
AS_SYM_DT_Begonia_strigosa_EDNA07_02177_	monoecious	59781.2	dry
AS_SYM_DT_Begonia_symsanguinea_EDNA07_02175_	monoecious	38162.3	dry
AS_UNK_AM_Begonia_balansana_EDNA15_0039647	monoecious	90036.7	fleshy
AS_UNK_DT_Begonia_malabarica_EDNA08_01788	monoecious	12602.3	dry
AS_UNK_PM_Begonia_balansana_EDNA15_0039647	monoecious	90036.7	fleshy
AS_UNK_PM_Begonia_boisiana_Q214	monoecious	1862.9	dry
NW_AST_PM_Begonia_grisea_EDNA15_0039668	monoecious	300687.5	dry
NW_AST_PM_Begonia_kuhlmannii_EDNA13_0033071	monoecious	2513.6	dry
NW_AST_RT_Begonia_petastifolia_EDNA12_0025418	monoecious	100631	dry
NW_AUS_MH_Begonia_micranthera_1_EDNA13_0033515	monoecious	391121.1	dry
NW_AUS_PM_Begonia_boliviensis_EDNA14_0036862	monoecious	108755.1	dry
NW_AUS_PM_Begonia_boliviensis_Navarez_EDNA14_0036896	monoecious	108755.1	dry
NW_AUS_PM_Begonia_boliviensis_Vallegrande_EDNA14_0036897	monoecious	108755.1	dry
NW_AUS_PM_Begonia_cinnabarina_EDNA14_0036868	monoecious	22423.3	dry
NW_AUS_PM_Begonia_clarkei_EDNA15_0038862	monoecious	443127.7	dry
NW_AUS_PM_Begonia_clarkei_EDNA15_0038864	monoecious	443127.7	dry
NW_AUS_PM_Begonia_froebelii_EDNA14_0036885	monoecious	13351.9	dry
NW_AUS_PM_Begonia_germaineana_EDNA14_0036864	monoecious	4	dry
NW_AUS_PM_Begonia_heliantha	monoecious	4	dry
NW_AUS_PM_Begonia_krystofii_EDNA14_0036860	monoecious	418.2	dry
NW_AUS_PM_Begonia_micranthera_subsp_micranthera_EDNA14_0036861	monoecious	391121.1	dry
NW_AUS_PM_Begonia_pearcei_EDNA15_0039239	monoecious	2564.4	dry
NW_AUS_PM_Begonia_sp_nov_chrysantha_EDNA14_0036869	monoecious	10	dry
NW_AUS_PM_Begonia_sp_nov_phantasma_EDNA14_0036866	monoecious	3927.5	dry
NW_AUS_PM_Begonia_sp_nov_phantasma_EDNA14_0036871	monoecious	3927.5	dry
NW_AUS_PM_Begonia_tomiana_EDNA14_0036865	monoecious	4	dry
NW_BEG_DF_Begonia_bissei_EDNA14_0036183	monoecious	4	dry
NW_BEG_PM_Begonia_capensis_EDNA15_0039179	monoecious	4	dry
NW_BEG_PM_Begonia_domingensis_EDNA15_0040376	monoecious	1729.1	dry
NW_BEG_PM_Begonia_dominicalis_EDNA13_0033085	monoecious	420.6	dry
NW_BEG_PM_Begonia_nitida_EDNA15_0040377	monoecious	710.3	dry
NW_BEG_PM_Begonia_obliqua_EDNA15_0039241	monoecious	420.6	dry
NW_BEG_PM_Begonia_per_dusenii_EDNA15_0039701	monoecious	1343422.2	dry
NW_BEG_PM_Begonia_plumieri_EDNA15_0039703	monoecious	4809.5	dry
NW_BEG_PM_Begonia_rotundifolia_EDNA15_0040379	monoecious	4	dry
NW_BEG_PM_Begonia_suaveolens_EDNA15_0040380	monoecious	4	dry
NW_BEG_RT_Begonia_acutifolia_EDNA12_0025413	monoecious	6716.8	dry
NW_BEG_RT_Begonia_cubensis_EDNA12_0025386	monoecious	4	dry
NW_BEG_RT_Begonia_minor_EDNA12_0025411	monoecious	710.3	dry
NW_BEG_RT_Begonia_odorata_EDNA12_0025414	monoecious	4	dry

NW_BIF_PM_Begonia_bifurcata_EDNA16_0044327	monoecious	8017.8	dry
NW_CAS_AJ_Begonia_chlorolepis_EDNA14_0037184	monoecious	5682	dry
NW_CAS_AJ_Begonia_colombiana_EDNA14_0037175	monoecious	320.9	dry
NW_CAS_AJ_Begonia_ferruginea_EDNA14_0037207	monoecious	38547.8	dry
NW_CAS_AJ_Begonia_gamolepis_EDNA14_0037196	monoecious	385.5	dry
NW_CAS_AJ_Begonia_pectennervia_ADNA14_0036777	monoecious	1804.6	dry
NW_CAS_AJ_Begonia_tetrandra_EDNA14_0036775	monoecious	18944	dry
NW_CAS_AJ_Begonia_toledana_EDNA14_0037205	monoecious	18927.9	dry
NW_CAS_AJ_Begonia_trianae_EDNA14_0037192	monoecious	4	dry
NW_CAS_AJ_Begonia_umbellata_EDNA14_0037189	monoecious	3572.4	dry
NW_CAS_JS_Begonia_ursina_graft	dioecious	4	dry
NW_CAS_JS_Begonia_wilburi_graft	dioecious	1274.5	dry
NW_CAS_PM_Begonia_urticae_EDNA14_0036788	monoecious	1199930.3	dry
NW_CRE_PM_Begonia_cremnophila_EDNA14_0036870	monoecious	1723.7	dry
NW_CRE_PM_Begonia_speculum_EDNA16_0044346	monoecious	4	dry
NW_CRE_PM_Begonia_urubambensis_EDNA16_0044370	monoecious	4268	dry
NW_CYA_PM_Begonia_altoperuviana_EDNA15_0038883	monoecious	115701.4	dry
NW_CYA_PM_Begonia_bracteosa_EDNA15_0038880	monoecious	86377.6	dry
NW_CYA_PM_Begonia_brevicordata_EDNA15_0038882	monoecious	4	dry
NW_CYA_PM_Begonia_cyathophora_EDNA15_0038859	monoecious	806.9	dry
NW_CYA_PM_Begonia_lophoptera_EDNA15_0038867	monoecious	49042.7	dry
NW_CYA_PM_Begonia_lophoptera_EDNA16_0044367	monoecious	49042.7	dry
NW_CYA_PM_Begonia_subciliata_EDNA16_0044357	monoecious	38948.8	dry
NW_CYA_PM_Begonia_subspinulosa_EDNA15_0038868	monoecious	1375.5	dry
NW_CYA_PM_Begonia_viridiflora_EDNA16_0044360	monoecious	14452.8	dry
NW_DON_PM_Begonia_jairii_EDNA15_0039231	monoecious	5.00E+06	dry
NW_DON_PM_Begonia_saxicola_EDNA15_0039719	monoecious	876310.2	dry
NW_DON_PM_Begonia_ulmifolia_EDNA13_0033089	monoecious	5118535.5	dry
NW_DON_PM_Begonia_ulmifolia_EDNA13_0033483	monoecious	5118535.5	dry
NW_DON_RT_Begonia_ulmifolia_EDNA12_0025425_	monoecious	5118535.5	dry
NW_DOR_PM_Begonia_filipes_EDNA15_0039174	monoecious	2925405.8	dry
NW_DOR_PM_Begonia_humilis_EDNA15_0039723	monoecious	15357642.7	dry
NW_DOR_PM_Begonia_semiovata_EDNA16_0044351	monoecious	5236103.2	dry
NW_EPH_AJ_Begonia_fischeri_EDNA14_0037193	monoecious	12192139.7	dry
NW_EPH_PM_Begonia_cucullata_EDNA15_0039658	monoecious	13121015.4	dry
NW_EPH_PM_Begonia_descoleana_EDNA15_0039660	monoecious	129109.6	dry
NW_EPH_PM_Begonia_exigua_EDNA16_0045210	monoecious	59545.2	dry
NW_EPH_PM_Begonia_hirtella_EDNA16_0044375	monoecious	12063937.5	dry
NW_EPH_PM_Begonia_mollicaulis_EDNA13_0033084_	monoecious	4	dry
NW_EPH_PM_Begonia_schmidtiana_EDNA13_0033095	monoecious	586401	dry
NW_EPH_PM_Begonia_subvillosa_EDNA13_0033486	monoecious	582818.8	dry
NW_EUP_PM_Begonia_aff_veitchii_EDNA14_0036793	monoecious	31313.2	dry
NW_EUP_PM_Begonia_anemoniflora_EDNA16_0044365	monoecious	6450.4	dry
NW_EUP_PM_Begonia_anemoniflora_EDNA16_0044366	monoecious	6450.4	dry
NW_EUP_PM_Begonia_geraniifolia_EDNA16_0044326	monoecious	120013.4	dry
NW_EUP_PM_Begonia_octopetala_EDNA14_0036883	monoecious	402680.7	dry

NW_EUP_PM_Begonia_octopetala_EDNA14_0036886	monoecious	402680.7	dry
NW_EUP_PM_Begonia_octopetala_EDNA15_0040539	monoecious	402680.7	dry
NW_EUP_PM_Begonia_pleiopetala_EDNA15_0038873	monoecious	114279.6	dry
NW_EUP_PM_Begonia_pleiopetala_EDNA15_0040541	monoecious	114279.6	dry
NW_EUP_PM_Begonia_pleiopetala_EDNA16_0044379	monoecious	114279.6	dry
NW_EUP_PM_Begonia_pleiopetala_EDNA16_0044380	monoecious	114279.6	dry
NW_EUP_PM_Begonia_polypetala_EDNA16_0044328	monoecious	1893.9	dry
NW_EUP_PM_Begonia_tumbezensis_EDNA14_0036874	monoecious	39003.5	dry
NW_EUP_PM_Begonia_weberbaueri_EDNA15_0040543	monoecious	2097.5	dry
NW_GAE_PM_Begonia_corallina_EDNA15_0039655	monoecious	100	dry
NW_GAE_PM_Begonia_dichroa_EDNA13_0033075	monoecious	4	dry
NW_GAE_PM_Begonia_edmundoi_EDNA13_0033068	monoecious	6	dry
NW_GAE_PM_Begonia_lunaris_EDNA15_0039684	monoecious	192.7	dry
NW_GAE_PM_Begonia_macduffieana_EDNA13_	monoecious	4	dry
NW_GAE_PM_Begonia_maculata_EDNA13_0033069	monoecious	4728276.1	dry
NW_GAE_PM_Begonia_pseudolubbersii_EDNA13_0033073	monoecious	4	dry
NW_GAE_PM_Begonia_salicifolia_EDNA15_0039717	monoecious	4	dry
NW_GAE_PM_Begonia_undulata_EDNA13_0033513	monoecious	4	dry
NW_GAE_RT_Begonia_lubbersii_EDNA12_0025406	monoecious	4	dry
NW_GIR_AJ_Begonia_stigmosa_EDNA14_0037203	monoecious	36644.4	dry
NW_GIR_DF_Begonia_heracleifolia_EDNA14_0035695	monoecious	749081.6	dry
NW_GIR_MH_Begonia_nelumbifolia_P20879	monoecious	906780.5	dry
NW_GIR_MO_Begonia_aff_fusca_EDNA15_0038388	monoecious	99095	dry
NW_GIR_MO_Begonia_calderonii_EDNA15_0038386	monoecious	189083	dry
NW_GIR_MO_Begonia_calzadae_EDNA15_0038206	monoecious	271.7	dry
NW_GIR_MO_Begonia_involucrata_EDNA15_0038398	monoecious	137298.6	dry
NW_GIR_MO_Begonia_lindleyana_EDNA15_0038390	monoecious	51319.4	dry
NW_GIR_MO_Begonia_mazae_EDNA15_0038209	monoecious	858.9	dry
NW_GIR_MO_Begonia_motozintlensis_EDNA15_0038210	monoecious	200.2	dry
NW_GIR_MO_Begonia_pinetorum_EDNA15_0038211	monoecious	87122.9	dry
NW_GIR_MO_Begonia_sartorii_EDNA15_0038212	monoecious	123451.1	dry
NW_GIR_MO_Begonia_sericoneura_EDNA15_0038394	monoecious	814777.7	dry
NW_GIR_MO_Begonia_sericoneura_EDNA15_0038395	monoecious	814777.7	dry
NW_GIR_MO_Begonia_sericoneura_EDNA15_0038396	monoecious	814777.7	dry
NW_GIR_MO_Begonia_sousae_EDNA15_0038213	monoecious	346.9	dry
NW_GIR_MO_Begonia_squarrosa_EDNA15_0038397	monoecious	13577.3	dry
NW_GIR_PM_Begonia_barkeri_EDNA13_0033500	monoecious	973.5	dry
NW_GIR_PM_Begonia_boquetensis_EDNA15_0039648	monoecious	449032.8	dry
NW_GIR_PM_Begonia_breedlovei_EDNA13_0033474	monoecious	50918.5	dry
NW_GIR_PM_Begonia_broussonetifolia_EDNA15_0039649	monoecious	11679.7	dry
NW_GIR_PM_Begonia_cardiocarpa_EDNA13_0033101	monoecious	82986.5	dry
NW_GIR_PM_Begonia_carrieae_EDNA15_0039652	monoecious	4	dry
NW_GIR_PM_Begonia_corredorana_EDNA16_0045074	monoecious	65125.1	dry
NW_GIR_PM_Begonia_croatii_EDNA15_0039657	monoecious	495.1	dry
NW_GIR_PM_Begonia_garagarana_EDNA15_0039666	monoecious	17337.7	dry
NW_GIR_PM_Begonia_karwinskyana_EDNA13_0033490	monoecious	3993.2	dry

NW_GIR_PM_Begonia_lyniceorum_EDNA13_0033481	monoecious	8038.4	dry
NW_GIR_PM_Begonia_mariti_EDNA13_0033488	monoecious	368.1	dry
NW_GIR_PM_Begonia_multistamniea_EDNA13_0034223	monoecious	528	dry
NW_GIR_PM_Begonia_plebeja_EDNA13_0034220	monoecious	575540.6	dry
NW_GIR_PM_Begonia_pruniata_EDNA13_0033076	monoecious	4	dry
NW_GIR_PM_Begonia_pseudodaedalea_EDNA13_0034221	monoecious	4	dry
NW_GIR_PM_Begonia_squarrosa_EDNA15_0039726	monoecious	13577.3	dry
NW_GIR_PM_Begonia_valerioi_EDNA15_0039735	monoecious	65125.1	dry
NW_GIR_RT_Begonia_aff_barkeri_EDNA13_0030223	monoecious	973.5	dry
NW_GIR_RT_Begonia_aff_strigillosa_EDNA13_0030233	monoecious	203943.5	dry
NW_GIR_RT_Begonia_carolineifolia_EDNA12_0025381	monoecious	30.3	dry
NW_GIR_RT_Begonia_conchifolia_EDNA12_0025383	monoecious	49702.4	dry
NW_GIR_RT_Begonia_crassicaulis_EDNA12_0025385	monoecious	12309.1	dry
NW_GIR_RT_Begonia_hydrocotylifolia_EDNA12_0025399	monoecious	8307.6	dry
NW_GIR_RT_Begonia_involucrata_EDNA12_0025403_	monoecious	137298.6	dry
NW_GIR_RT_Begonia_lyman_smithii_EDNA12_0025408	monoecious	4	dry
NW_GIR_RT_Begonia_manicata_EDNA12_0025409_	monoecious	191910	dry
NW_GIR_RT_Begonia_multinervia_EDNA12_0025412_	monoecious	70646.7	dry
NW_GIR_RT_Begonia_peltata_EDNA12_0025405_	monoecious	50411.5	dry
NW_GIR_RT_Begonia_polygonata_EDNA13_0030230	monoecious	4	dry
NW_GIR_RT_Begonia_pringlei_EDNA12_0025419	monoecious	108.7	dry
NW_GIR_RT_Begonia_stigmosa_EDNA12_0025423	monoecious	36644.4	dry
NW_GIR_RT_Begonia_thiemei_EDNA13_0030234	monoecious	75442.8	dry
NW_GOB_AJ_Begonia_geminiflora_EDNA14_0037177	monoecious	4	dry
NW_GOB_AJ_Begonia_maurandiae_EDNA14_0037191	monoecious	75641.7	dry
NW_GOB_PM_Begonia_rubrotincta_EDNA16_0044331	monoecious	52143.1	dry
NW_GOB_PM_Begonia_tropaeolifolia_EDNA16_0044341	monoecious	159574.8	dry
NW_HYD_PM_Begonia_rubriflora_EDNA15_0039713	monoecious	4	dry
NW_HYD_RT_Begonia_fissistyla_EDNA12_0025392_	monoecious	563.3	dry
NW_KNE_I_PM_Begonia_acerifolia_EDNA14_0036785	monoecious	486934.7	dry
NW_KNE_I_PM_Begonia_acerifolia_EDNA14_0036872	monoecious	486934.7	dry
NW_KNE_I_PM_Begonia_cf_erythrocarpa_EDNA16_0044329	monoecious	486934.7	dry
NW_KNE_I_PM_Begonia_erythrocarpa_EDNA15_0038870	monoecious	486934.7	dry
NW_KNE_I_PM_Begonia_monadelpha_EDNA13_0033104	monoecious	51832.8	dry
NW_KNE_I_PM_Begonia_monadelpha_EDNA14_0036786	monoecious	51832.8	dry
NW_KNE_I_PM_Begonia_monadelpha_EDNA16_0044339	monoecious	51832.8	dry
NW_KNE_I_PM_Begonia_velata_EDNA16_0044323	monoecious	936.4	dry
NW_KNE_III_AJ_Begonia_ludwigii_EDNA14_0036778	monoecious	10877.5	dry
NW_KNE_III_PM_Begonia_albomaculata_EDNA16_0044334	monoecious	286865.5	dry
NW_KNE_III_PM_Begonia_albomaculata_EDNA16_0044352	monoecious	286865.5	dry
NW_KNE_III_PM_Begonia_albomaculata_EDNA16_0044358	monoecious	286865.5	dry
NW_KNE_III_PM_Begonia_arrogans_EDNA16_0044374	monoecious	18869.3	dry
NW_KNE_III_PM_Begonia_chemillenensis_EDNA16_0044373	monoecious	1462.6	dry
NW_KNE_III_PM_Begonia_lugonis_EDNA15_0039232	monoecious	4	dry
NW_KNE_III_PM_Begonia_maynensis_EDNA16_0044336	monoecious	784921.8	dry
NW_KNE_III_PM_Begonia_maynensis_EDNA16_0044387	monoecious	784921.8	dry

NW_KNE_III_PM_Begonia_parcifolia_EDNA14_0036873	monoecious	21975.1	dry
NW_KNE_III_PM_Begonia_piurensis_EDNA16_0044324	monoecious	15874.5	dry
NW_KNE_III_PM_Begonia_serotina_EDNA14_0036877	monoecious	33374.3	dry
NW_KNE_III_PM_Begonia_thyrsoidea_EDNA15_0038869	monoecious	36440.5	dry
NW_KNE_III_PM_Begonia_wollnyi_EDNA14_0036901	monoecious	4278300.8	dry
NW_KNE_PM_Begonia_barkleyana_EDNA16_0045768	monoecious	5675.7	dry
NW_KNE_RT_Begonia_aff_incarnata_EDNA13_0030226	monoecious	122105.7	dry
NW_KOL_DF_Begonia_thelmae_EDNA14_0035457	monoecious	4	dry
NW_KOL_PM_Begonia_jaguarensis_EDNA15_0039675	monoecious	4318.4	dry
NW_LAT_DF_PM_Begonia_aconitifolia_EDNA13_0033499	monoecious	23064.3	dry
NW_LAT_DF_PM_Begonia_aconitifolia_EDNA14_0036895	monoecious	23064.3	dry
NW_LAT_PM_Begonia_pachypoda_EDNA13_0033079	monoecious	4	dry
NW_LAT_PM_Begonia_platanifolia_EDNA15_0038263	monoecious	4	dry
NW_LAT_PM_Begonia_platanifolia_EDNA15_0039242	monoecious	4	dry
NW_LAT_RT_Begonia_olbia_EDNA12_0025415_	monoecious	4	dry
NW_LEP_PM_Begonia_foliosa_EDNA13_0033092	monoecious	982360.3	dry
NW_LUT_AJ_Begonia_lutea_EDNA14_0037171	monoecious	179902.2	dry
NW_MIC_PM_Begonia_elachista_EDNA16_0045213	monoecious	4	dry
NW_PAR_PM_Begonia_oaxacana_EDNA14_0036900	monoecious	357679.5	fleshy
NW_PIL_AJ_Begonia_buddleiifolia_EDNA14_0037195	monoecious	1324900.1	dry
NW_PIL_MO_Begonia_glandulifera_EDNA15_0038389	monoecious	874.2	dry
NW_PIL_PM_Begonia_mariannensis_EDNA15_0039234	monoecious	7.6	dry
NW_PRI_DF_Begonia_hoehneana_EDNA14_0035461	monoecious	749.1	dry
NW_PRI_JS_Begonia_paganuccii_EDNA17_0048025	monoecious	4	dry
NW_PRI_MH_Begonia_tomentosa_EDNA13_0033514	monoecious	67.4	dry
NW_PRI_PM_Begonia_acida_EDNA13_0033494	monoecious	10537.1	dry
NW_PRI_PM_Begonia_bufoderma_EDNA15_0039650	monoecious	4	dry
NW_PRI_PM_Begonia_callosa_EDNA15_0039651	monoecious	9328.6	dry
NW_PRI_PM_Begonia_catharinensis_EDNA15_0039653	monoecious	48316.7	dry
NW_PRI_PM_Begonia_coccinea_EDNA13_0033072	monoecious	66577.8	dry
NW_PRI_PM_Begonia_cornitepala_EDNA15_0039656	monoecious	5000.3	dry
NW_PRI_PM_Begonia_curtii_EDNA15_0039659	monoecious	20254.3	dry
NW_PRI_PM_Begonia_dichotoma_EDNA13_0033090	monoecious	3193122.1	dry
NW_PRI_PM_Begonia_epipsila_EDNA13_0033475	monoecious	140.9	dry
NW_PRI_PM_Begonia_fluminensis_EDNA13_0033484	monoecious	4	dry
NW_PRI_PM_Begonia_friburgensis_EDNA15_0039663	monoecious	416.5	dry
NW_PRI_PM_Begonia_gardneri_EDNA15_0039667	monoecious	50816.5	dry
NW_PRI_PM_Begonia_gehrtii_EDNA13_0033492	monoecious	4	dry
NW_PRI_PM_Begonia_hookeriana_EDNA15_0039671	monoecious	5638.3	dry
NW_PRI_PM_Begonia_hugelii_EDNA15_0039672	monoecious	68931.9	dry
NW_PRI_PM_Begonia_insularis_EDNA15_0039171	monoecious	4	dry
NW_PRI_PM_Begonia_itaguassuensis_EDNA15_0039673	monoecious	308478	dry
NW_PRI_PM_Begonia_itatiensis_EDNA15_0039674	monoecious	2587.5	dry
NW_PRI_PM_Begonia_jocelinoi_EDNA13_0033476	monoecious	51869.9	dry
NW_PRI_PM_Begonia_listada_EDNA13_0033512	monoecious	4	dry
NW_PRI_PM_Begonia_moyesii_EDNA15_0039690	monoecious	4	dry

NW_PRI_PM_Begonia_obscura_EDNA13_0033098	monoecious	4	dry
NW_PRI_PM_Begonia_odetiantha_EDNA13_0033081	monoecious	4	dry
NW_PRI_PM_Begonia_olsoniae_EDNA15_0039694	monoecious	27322.3	dry
NW_PRI_PM_Begonia_paleata_EDNA15_0039696	monoecious	155023.6	dry
NW_PRI_PM_Begonia_parilis_EDNA15_0039697	monoecious	22337.8	dry
NW_PRI_PM_Begonia_pilgeriana_EDNA15_0039702	monoecious	584	dry
NW_PRI_PM_Begonia_pluvialis_EDNA15_0039704	monoecious	131.7	dry
NW_PRI_PM_Begonia_polyandra_EDNA15_0039237	monoecious	20630.2	dry
NW_PRI_PM_Begonia_princeps_EDNA15_0039706	monoecious	4	dry
NW_PRI_PM_Begonia_pulchella_EDNA15_0039707	monoecious	58347	dry
NW_PRI_PM_Begonia_reniformis_EDNA13_0033093	monoecious	1670041.8	dry
NW_PRI_PM_Begonia_rigida_EDNA15_0039712	monoecious	64	dry
NW_PRI_PM_Begonia_rufa_EDNA15_0039714	monoecious	128600.7	dry
NW_PRI_PM_Begonia_rufoserica_EDNA15_0039715	monoecious	4	dry
NW_PRI_PM_Begonia_salesopolensis_EDNA15_0039716	monoecious	1278.3	dry
NW_PRI_PM_Begonia_sanguinea_EDNA13_0033493	monoecious	19718.5	dry
NW_PRI_PM_Begonia_scharffiana_EDNA15_0039721	monoecious	4	dry
NW_PRI_PM_Begonia_soli_mutata_EDNA13_0033102	monoecious	4	dry
NW_PRI_PM_Begonia_subacida_EDNA15_0039728	monoecious	9151.5	dry
NW_PRI_PM_Begonia_sylvestris_EDNA15_0039731	monoecious	1810.3	dry
NW_PRI_PM_Begonia_teuscheri_EDNA15_0039732	monoecious	4	dry
NW_PRI_PM_Begonia_valida_EDNA13_0033091	monoecious	4	dry
NW_PRI_PM_Begonia_venosa_EDNA13_0033100	monoecious	4	dry
NW_PRI_RT_Begonia_acetosa_EDNA12_0025374	monoecious	8256.1	dry
NW_PRI_RT_Begonia_angularis_EDNA12_0025376_	monoecious	317595.7	dry
NW_PRI_RT_Begonia_arborescens_var_confertflora_EDNA12_0025377_	monoecious	2300.8	dry
NW_PRI_RT_Begonia_bradei_EDNA12_0025378	monoecious	7918.3	dry
NW_PRI_RT_Begonia_capanemae_EDNA12_0025380	monoecious	50007.7	dry
NW_PRI_RT_Begonia_dentatiloba_EDNA12_0025387	monoecious	15296.4	dry
NW_PRI_RT_Begonia_dietrichiana_EDNA12_0025388_	monoecious	21996.1	dry
NW_PRI_RT_Begonia_echinosepala_EDNA12_0025389_	monoecious	105974.4	dry
NW_PRI_RT_Begonia_hispida_var_cucullifera_EDNA12_0025397_	monoecious	68960.1	dry
NW_PRI_RT_Begonia_juliana_EDNA12_0025404_	monoecious	1257.4	dry
NW_PRI_RT_Begonia_paranaensis_EDNA12_0025417_	monoecious	12085.8	dry
NW_PRI_RT_Begonia_scharffii_EDNA12_0025421	monoecious	434.3	dry
NW_QDR_JS_Begonia_biserrata_graft	dioecious	313619.4	dry
NW_QDR_RT_Begonia_gracilis_EDNA12_0025395_	monoecious	621491	dry
NW_ROS_PM_Begonia_rossmanniae_EDNA15_0040383	monoecious	931456.2	dry
NW_RUI_AJ_Begonia_cf_guaduensis_EDNA14_0037173	monoecious	5023290.1	dry
NW_RUI_AJ_Begonia_cf_meridensis_EDNA14_0037186	monoecious	228695.4	dry
NW_RUI_AJ_Begonia_guaduensis_EDNA14_0037194	monoecious	5023290.1	dry
NW_RUI_AJ_Begonia_meridensis_EDNA14_0037198	monoecious	228695.4	dry
NW_RUI_AJ_Begonia_tiliifolia_EDNA14_0036774	monoecious	98004.9	dry
NW_RUI_PM_Begonia_consobrina_EDNA15_0039178	monoecious	16850.7	dry
NW_RUI_PM_Begonia_convallariodora_EDNA15_0039177	monoecious	287665.8	dry
NW_RUI_PM_Begonia_gesnerioides_EDNA16_0044355	monoecious	4	dry

NW_RUI_PM_Begonia_meridensis_EDNA13_0033074	monoecious	228695.4	dry
NW_RUI_PM_Begonia_obtecticaulis_EDNA16_0044361	monoecious	12019.6	dry
NW_RUI_PM_Begonia_opuliflora_EDNA15_0039240	monoecious	4	dry
NW_RUI_PM_Begonia_peruviana_EDNA14_0036799	monoecious	448273.7	dry
NW_RUI_PM_Begonia_sp_nov_botryoides_EDNA14_0036878	monoecious	97497.9	dry
NW_RUI_PM_Begonia_tonduzii_EDNA15_0039170	monoecious	78543.6	dry
NW_RUI_RT_Begonia_holtonis_EDNA12_0025398_	monoecious	282546.1	dry
NW_RUI_RT_Begonia_meridensis_EDNA12_0025410	monoecious	228695.4	dry
NW_RUI_YM_Begonia_seemanniana_Q467	monoecious	3552.1	dry
NW_SCH_PM_Begonia_digitata_EDNA15_0039176	monoecious	269049.3	dry
NW_SCH_PM_Begonia_parviflora_EDNA16_0044344	monoecious	2119659.4	dry
NW_SCH_PM_Begonia_semidigitata_EDNA15_0039165	monoecious	22958.9	dry
NW_SCH_RT_Begonia_luxurians_EDNA12_0025407	monoecious	36300.6	dry
NW_SEM_AJ_Begonia_angustifolia_EDNA14_0037176	monoecious	93	dry
NW_SOL_PM_Begonia_radicans_EDNA13_0033070	monoecious	320792.8	dry
NW_SOL_PM_Begonia_solananthera_EDNA13_0033077	monoecious	137287	dry
NW_SOL_RT_Begonia_integerrima_EDNA12_0025402	monoecious	54882.4	dry
NW_THY_PM_Begonia_bullatifolia_EDNA15_0039680	monoecious	4	dry
NW_THY_PM_Begonia_santos_limae_EDNA13_0033491	monoecious	9001.1	dry
NW_TRA_PM_Begonia_fulvosetulosa_EDNA15_0039664	monoecious	4	dry
NW_TRA_PM_Begonia_herbacea_EDNA13_0033099	monoecious	16905.7	dry
NW_TRA_PM_Begonia_lanceolata_EDNA15_0039679	monoecious	5285.1	dry
NW_TRE_PM_Begonia_fruticosa_EDNA15_0039173	monoecious	752129.5	dry
NW_TTR_RT_Begonia_egregia_EDNA12_0025391_	monoecious	4	dry
NW_URN_PM_Begonia_sp_aff_heydei_EDNA13_0033489	monoecious	122682.8	dry
NW_WAG_PM_Begonia_fagifolia_EDNA13_0033096	monoecious	4	dry
NW_WAG_PM_Begonia_glabra_EDNA13_0033482	monoecious	11088978.8	dry
NW_WAG_PM_Begonia_polygonifolia_EDNA15_0039705	monoecious	26198.2	dry
NW_WAG_PM_Begonia_smilacina_EDNA15_0039724	monoecious	11460.5	dry
NW_WAG_RT_Begonia_convolvulacea_EDNA12_0025384	monoecious	8417260.8	dry
NW_WEI_MO_Begonia_ludicra_EDNA15_0038391	monoecious	14932.5	dry
NW_WEI_MO_Begonia_ludicra_EDNA15_0038392	monoecious	14932.5	dry
NW_WEI_PM_Begonia_acutiloba_EDNA15_0039645	monoecious	4	dry
NW_WEI_PM_Begonia_alice_clarkea_EDNA13_0033094	monoecious	4	dry
NW_WEI_PM_Begonia_almedana_EDNA13_0034224	monoecious	40.4	dry
NW_WEI_PM_Begonia_copeyana_EDNA15_0039654	monoecious	873.3	dry
NW_WEI_PM_Begonia_faustinoi_EDNA13_0034222	monoecious	497.3	dry
NW_WEI_PM_Begonia_ludicra_EDNA13_0033511	monoecious	14932.5	dry
NW_WEI_PM_Begonia_mexicana_EDNA15_0039181	monoecious	4	dry
NW_WEI_PM_Begonia_popenoei_EDNA13_0033097	monoecious	6839.1	dry
NW_WEI_PM_Begonia_violifolia_EDNA15_0039738	monoecious	1170.2	dry
NW_WEI_RT_Begonia_imperialis_EDNA12_0025400_	monoecious	8579.4	dry
NW_WEI_RT_Begonia_purpusii_EDNA12_0025420	monoecious	292.1	dry
NW_WEI_RT_Begonia_pustulata_EDNA13_0030231	monoecious	16518.2	dry

***Appendix 3**:* Extent of occurrence (EOO) and Area of occupancy (AOO) data comparison through bar charts. The first chart shows solely EOO, the second AOO, and the third AOO graphed on top of EOO for comparison. Taxa represented on the x-axis differ based upon the scale chosen to show data values for convenience.