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<u>Taxonomy, Systematics, Biogeography and Pollination Biology of *Impatiens* L. (Balsaminaceae) in Eastern Africa</u>

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摘 要

Abstract

The Balsaminaceae, commonly known as the Balsam family, primarily comprises annual and perennial herbs known for their remarkable floral diversity. This family includes economically and ecologically significant species that inhabit tropical and subtropical regions in tropical Africa, Madagascar, southern India, the eastern Himalayas, and Southeast Asia, particularly in high-humidity environments such as forests and near streams. The family contains two genera: the monotypic Hydrocera Blume and the large genus *Impatiens Linnaeus*, which contains over 1,000 species. The *Impatiens* species produce flowers that exhibit a remarkable diversity in colour, shape, spur size, and the two fused lateral petals. This diversity is thought to be influenced by pollinators; however, a more comprehensive analysis of the pollinator groups associated with this genus is still needed. In addition, the extraordinary morphological variability, higher species diversity, and broad geographic distribution of *Impatiens* species have often complicated taxonomy and evolutionary studies within this genus. Although several studies have addressed the taxonomy and phylogenetic position of the genus *Impatiens* in the Balsaminaceae family using morphological, morphometric, cytogenetic, and molecular techniques, their evolutionary relationships have remained unclear, resulting in inconsistencies at the classification level. Conflicting hypotheses have also been proposed regarding the origin, diversification, and identification of the genus *Impatiens* species obscuring our understanding of their dispersion and current geographic distribution. However, in recent years, several broad-scale studies of phylogenetic relationships within *Impatiens* using chloroplast and nuclear sequences have provided important insights into the systematics, biogeography, and evolutionary relationship in the genus *Impatiens*. However, the results were inconsistent, and many studies were unsuccessful in inferring evolutionary relationships with fully resolved phylogenies. Alternatively, the use of complete plastomes and nuclear ribosomal DNA (nrDNA) sequences data has been widely used as they include useful phylogenetic information that can be used to study evolutionary relationships at different taxonomic levels and resolve difficult problems in plant phylogenetics. However, so far, the complete plastomes and nuclear ribosomal DNA (nrDNA) sequences of only a few *Impatiens* species have been sequenced and the data accumulated are still insufficient for the clarification of the phylogenetic relationships within the genus.

The genus *Impatiens* is among the most species-rich genera in tropical Africa. The *Impatiens* flora of tropical Africa comprises more than 131 species, primarily concentrated in Afromontane forests and highland areas in west, central and East Africa. This exceptional species diversity and the wide distribution of the genus provide an opportunity to explore the role that tropical rainforests have played in generating high *Impatiens* species diversity and endemism. However, there is very limited knowledge regarding the various drivers of species diversification within this large genus on the continent, and the taxonomy and phylogenetic relationships of *Impatiens* species in tropical Africa remain poorly understood. This is largely because the African *Impatiens* lacks modern complete taxonomic revision and many investigations of species delimitation relied heavily on morphological features, with molecular data often not sought or disregarded. This limited information hinders potential evolutionary pattern recognition as well as the exploration of inter- and intra-species plastid genome diversity. In this study, we aim to use a variety of approaches to analyze the relative importance of various drivers of species diversification in the species-rich genus *Impatiens* L. from Eastern Africa. This group is ideal for such a study given the narrow endemism of many *Impatiens* species, the sky-island biogeography of several taxa and the striking diversity of inflorescences that are associated with different pollinators. We will review the taxonomy of the genus *Impatiens* using an integrative approach that combines morphological, morphometric and molecular data. In addition, the complete plastomes and nuclear ribosomal DNA (nrDNA) sequences will be assembled based on high-throughput paired-end sequences to obtain greater resolution and support than had been possible using traditional approaches employing sequences for just a few genes to investigate the phylogenetic relationships of the genus *Impatiens* of East Africa species within the broader context of the sub-family Balsaminaceae. Based on complete plastomes and nuclear ribosomal DNA (nrDNA) sequences, this study aims to reconstruct a robust phylogenetic tree and explore historical biogeography and clade diversification in the genus *Impatiens*.

We will also investigate the reproductive ecology and pollination syndromes of several representative *Impatiens* species through studies of pollinator behaviour and measurement of floral morphology to identify distinct patterns associated with specific pollinator groups and reveal how different *Impatiens* species have evolved floral traits to attract particular pollinators. Furthermore, the quantitative and qualitative macro- and micromorphological features of seeds and capsules of *Impatiens* will be comprehensively investigated to determine the potential systematic significance value of the seed and fruit morphology and anatomy characters for infrageneric delimitation. Finally, data resulting from this study will be integrated into analyses of the drivers of taxonomic and morphological diversification in *Impatiens*. These findings obtained from this study will provide the molecular basis for the classification and molecular taxonomic criteria for the genus *Impatiens* (at least in East Africa), which should aid in more objective classification within this genus. Moreover, the newly developed markers will be useful for understanding the species delimitation of *Impatiens* and closely related species.

Keywords: Balsaminaceae, *Impatiens*, Pollination, East Africa, Chloroplast genomes, Evolution, phylogeny, Taxonomy, Biogeography, Tropical Africa

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Chapter 1: Introduction

1.1 Overview of the family Balsaminaceae

The Balsaminaceae (commonly known as the Balsam family) is a family of dicotyledonous plants that comprise water-semi-succulent annual or perennial herbs, undershrub, and shrubs, sometimes with tubers or rhizomes [1]. The family comprises over 1,000 species, but only two genera are recognized [2,3]. The two genera recognized under this family include the monotypic genus *Hydrocera* Blume ex Wight & Arnott n. (1834: 140) and *Impatiens* Linnaeus (1753:937) [4,5]. Three other genera, *Petalonema* Peter, *Trimorphopetalum* Baker *Semeiocardium* Zollinger, and *Impatientella* Perrier were earlier included in the Balsaminaceae. However, the three genera were confirmed synonyms of the *Impatiens Linnaeus* genus (i.e. *Impatiens* L.) [6,7]. *Hydrocera* Blume ex Wight and Arnott is a monotypic genus with only one species *Hydrocera triflora* while the *Impatiens* Linnaeus is the largest genus in the family Balsaminaceae with about 1,121 species [2,4,8]. The genus *Hydrocera* is a semi-aquatic herb native to lowland areas stretching from India, Sri Lanka, Southern China, Indo-China, Thailand, Peninsular Malaysia to Indonesia [5] while the genus *Impatiens* is mainly distributed in highlands and mountains in the tropics and sub-tropics of Asia, Africa, Madagascar, India, Europe and North America, and is absent from Australia and South America [2]. The species of *Impatiens* are characterized by having zygomorphic flowers, with enormous diversity both in corolla color and morphology, that's why it was regarded as the "dicot counterpart of the orchid" [9,10]. Due to their diverse colors, unique flower shapes, and long flowering period, many *Impatiens* plants are cultivated as ornamental worldwide, and some have long been utilized in traditional medicine for their medicinal properties and cosmetics [11,12]. Conversely, some *Impatiens* species, such as *Impatiens walleriana, Impatiens parviflora, Impatiens edgeworthii, Impatiens capensis, Impatiens balfourii and Impatiens glandulifera,* are well-known for their invasive nature and their negative effects on local ecosystems [13,14].

1.2 Position of the family Balsaminaceae

The systematic position of the family Balsaminaceae has always been much debated, and historically most traditional classifications based on morphological character have considered Balsaminaceae to be closely related to Tropaeolaceae and Geraniaceae families [15], while some important taxonomists treated Balsaminaceae as a separate order, i.e. the Balsaminales, or classified as a member of the order Geraniales of former Rosidae, as it was thought to be closely related to garden nasturtiums (Tropaeolum) [16]. However, the employment of molecular phylogenetics in recent years has contributed tremendously to our current understanding of evolutionary history and the problematic position of Balsaminaceae within the angiosperms. The molecular phylogenetic analyses indicated that Balsaminaceae belongs to the order Ericales [17,18]. Both plastid (*rbcL*) and nuclear (18S rDNA) or combined sequence data showed that Balsaminaceae form a monophyletic group (balsaminoid clade) with Tetrameristaceae (including Pellicieraceae) and Marcgraviaceae [19,20] (Figure 1-1). This knowledge of the position of Balsaminaceae within the angiosperms helps in choosing suitable outgroups for the phylogenetic analyses of the Balsaminaceae family.

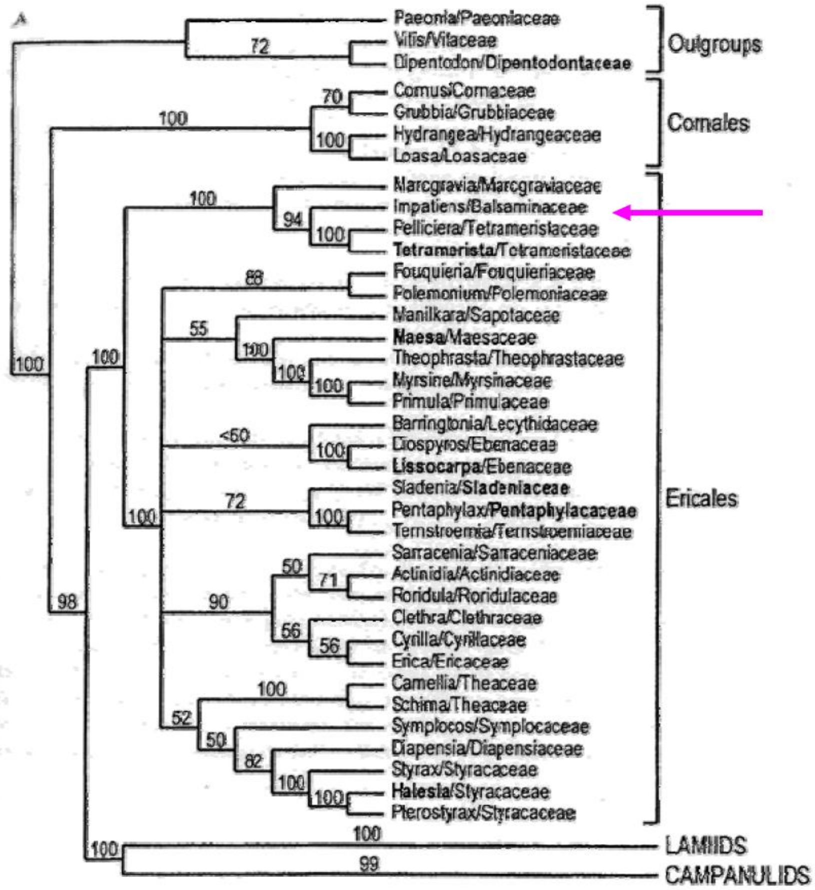

Figure 1-1 phylogenetic tree shows the position of Balsaminaceae and its relationships with the other families [20]

1.3 The genus Hydrocera

1.3.1 Habitats and Distribution

The genus Hydrocera is a semi-aquatic herb that grows in still or stagnant, shallow water in rice fields, wetlands and swampy areas with the lower part of the stem submerged into about 70 cm of water [21].

1.3.2 Morphology

Plant Structure: The portion of the stem that is above water is leafy, unbranched, and standing upright, while the submerged section is thick and spongy by nature, and lacks leaves and rooting at the lower nodes [21].

Flower Description: Morphologically, the two genera are almost similar, however, they possess two basic differences in flower and fruit structure that clearly distinguish them. The flower of Hydrocera exhibits a resupinate orientation, having rotated 180 degrees. Its basic structure comprises four whorls: five sepals, five petals, five stamens, and a five-carpelled ovary. The sepals and petals are distinct and separate from one another, with all sepals being prominent and sharing the same coloration as the petals [21]. The outer pair of lateral sepals are positioned below the upper lateral sepals, being large and projecting downward and backwards to completely encase the lower sepal and spur. The lower sepal features a broad mouth and has a navicular shape in side view, narrowing into a short, stout, incurved spur that serves both to store nectar and support the lateral petals. The five petals are large and noticeable, with the dorsal petal forming a partial hood that shields the androecium. The lateral petals are arranged in two pairs; an upper pair and a lower pair. The upper pair primarily functions to attract pollinators, while the lower pair provides support for visiting insects; they overlap slightly yet remain distinct. The five stamens are fused at their upper parts, encircling the gynoecium completely. The anthers point downward, functioning like a brush to contact pollinators. The ovary is formed from five fused carpels, each containing a single ovule [21].

Fruits: The fruit is a fleshy, indehiscent pseudoberry that is globose, more or less five-sided, and five-loculed, with one seed per locule [21].

1.4 The Genus *Impatiens*

1.4.1 Habitat and Distribution

Impatiens species can establish and grow in various habitats, including lowlands, moist forests, forest clearing, swamps, stream and river sides, ditches, spray zones of waterfalls and cascades, as well as in forest edges or paths [21,22]. They are rarely found near still or stagnant water. Most species are terrestrial, while a few are epiphytic [2,23]. The genus *Impatiens* L. is the largest and most widespread genus in the family Balsaminaceae comprising approximately 1,121 species [24–26]. *Impatiens* are mainly distributed in the Old World tropics and subtropics of Asia, Africa, Madagascar, and the northern temperate regions [3,27]. On the other hand, native *Impatiens* species are absent from South America and Australia [28]. The *Impatiens* species are mainly concentrated in five major biodiversity hotspots: tropical Africa ca.131 spp.[2,27–32]; Madagascar ca.260 spp.[33–35]; southern India and Sri Lanka (ca. 220 spp.); the eastern Himalayas (ca. 120 spp.), and Southeast Asia in its broad sense including Myanmar, Thailand, southwest China, the Indochina peninsula, and the Malesian archipelago, ca. 250 spp., [36]. The northern temperate zones of Europe, Russia, North America, and northern China are occupied by very few species of *Impatiens* [3]. There are 7 native species in North America with two species reaching Central America (Mexico: *Impatiens mexicana* Rydb., Costa Rica: *Impatiens turrialbana* J.D.Sm.) 1 native species in Europe (*Impatiens nolitangere* L.) and 1 native species in boreal Eurasia (*Impatiens parviflora* DC.).

1.4.2 Morphology

Plant Structure: *Impatiens* are primarily annual or perennial herbs, characterized by thick, fleshy, and often semi-translucent stems, which are rarely woody [2]. The leaves tend to be membranous and can appear almost transparent when dried. Some species possess rhizomes or tubers [23].

Flower Structure: The flowers of *Impatiens* display remarkable diversity in shape and colour. They are zygomorphic and typically undergo a 180-degree twist, resulting in a resupinate appearance [21] . Most species feature three sepals: two small lateral sepals and one modified lower sepal that forms a nectary-tipped spur. Some species may possess five sepals, including two pairs of small lateral sepals along with the petaloid lower sepal [2]. All *Impatiens* species have five petals, with one upper dorsal petal often resembling a hood. The other four lower petals are combined into two lateral pairs [2]. The extensive diversity in floral morphology, particularly in the spur shape and the configuration of the lateral petals, plays a crucial role in the classification [37].

Lateral Sepals: Most *Impatiens* species have two lateral sepals that can be narrow-lanceolate or broadly ovate. Some species possess four lateral sepals, with the inner pair being linear and smaller than the outer pair [2]. This configuration, though not common, is found in various regions, including Africa, China, Vietnam, and Madagascar [2,38,39] .

Lower Sepal: The lower sepals of *Impatiens* exhibit a wide range of forms and sizes, from shallowly navicular to deeply saccate. The variations in spur shape are often linked to the pollination and evolutionary process at work within the genus [2]. Notably, some endemic species from Madagascar lack a spur altogether [35,40].

Lateral Petals: The four lateral petals which are typically fused together into two lateral pairs, commonly known as the lateral united petals. In some species, the petals are large in filiform-spurred species and small in the bucciniform or saccate-spurred species [2]. The size and shape of these petals vary among species, influencing their adaptation to specific pollination strategies.

Dorsal Petal: The dorsal petal is flat and rounded in many of the filiform-spurred species with a crest behind or distinctly cucullate, forming a hood over the top of androecium [2]. The shape and position of the dorsal petal are often associated with the type of pollinator and flower color.

The gynoecium and androecium: The androecium is rather uniform featuring five stamens with flat filaments. The upper parts of the filaments and anthers form a cap over the superior gynoecium, which consists of five fused carpels containing numerous ovules.

Fruits: The fruit is typically an elongated, linear, or broadly fusiform capsule that explosively releases many seeds. The ovary and fruit may be glabrous or variously pubescent and this is generally a good taxonomic character.

1.5 *Impatiens* of Africa

1.5.1 General Overview of African *Impatiens*

Within the family Balsaminaceae, the genus *Impatiens* L. is of particular interest because it is the largest and most widespread genus in the family and is also the only genera within this family that occurs in Africa. The main diversity centres of the genus *Impatiens* within the continent are located in tropical Africa and Madagascar [24,41], with poor species representation in Tropical Africa compared to Madagascar. Currently, Madagascar has the highest *Impatiens* species richness with about 260 species, and almost all the native species of Madagascar are endemic [35]. In Tropical Africa, the genus *Impatiens* is one of the most prolific genera in terms of the number of taxa and population size [42]. About 131 *Impatiens* species occur in tropical Africa [2,26–29,31,32,43–45], of which a large number of its species are listed as threatened species (48 taxa within the region are listed in the Red Data Book) [46]. The first intensive taxonomic revision of African *Impatiens* was prepared by [2], and about 110 species were enumerated in his monograph. However, after his publication, several new species have been published across Africa [27–30,32,43–45,47–55], increasing the number of *Impatiens* species in this region to more than 141[26]. However, these numbers certainly will change, as new species have been described and studied in various parts of the continent.

In general, the *Impatiens* species in tropical Africa are mainly concentrated in almost six mountain areas in the regions: Eastern Tanzania (in Uluguru/Ukaguru Mountains); Eastern Zaire (Democratic Republic of the Congo), West Uganda, Rwanda, and Burundi (in Ruwenzori and neighbouring Mts); Mountains of Nigeria, Cameroon, and Gabon; Mountains and highlands of South Tanzania and North Malawi, Mountains of South Malawi and Eastern Rhodesia (Zimbabwe)/West Mozambique; Usambara Mountains and Northern Tanzania (in Mountains Meru and Kilimanjaro) [2]. In the continent, there are three broad centres of species diversity which are found in the western African mountains, mainly in the Cameroon Highlands (28 species), and in Eastern Africa primarily in the Eastern Arc mountains of Tanzania with the Kenya Highlands (27 species) and the Albertine Rift (23 species) [27,31,45,49,56]. The genus exhibits one prominent feature within continental Africa: the highest degree of endemism of its constituent species [2]. The geographical distribution of *Impatiens* in the continent is very localized and endemic, whereby most of the species are endemic to one particular country or are restricted to several regions, and no African species occur outside the continent [2,7]. However, within the African continent, only two species occur in all West, East, and Central African regions, four species are shared in common between the East and Central African regions, and five species occur between the Central and West African regions. In Africa, most species of *Impatiens* flower over a long period, but the main flowering period is shortly after the onset of the rainy season (s) and most seeds are also set during this time [2].

1.5.2 Eastern African *Impatiens* L.

Regionally, Eastern Africa (EA) is endowed with a rich assemblage of diverse plant species including many endemic taxa [57,58], and harbors two hotspots, among the three distinct diversity hotspots of the genus *Impatiens* L. in tropical Africa [2,27,45,59]. In East Africa, about 104 species of this genus occur within the region [26], of which more than 50 species are endemic to the area [2,27,43,44,50,56,60]. In this region, *Impatiens* diversity and endemism are mainly concentrated in two areas viz., Eastern Arc Mountains (EAM) of Tanzania and the Southern Rift, the Albertine Rift (AR) [2,27,45,59]. These two centers of *Impatiens* diversity in Eastern Africa are found along one of the most prominent Afromontane regions on the earth, the Eastern Afromontane Biodiversity Region (EABR), which is more than 7,000 kilometers long and transects Saudi Arabia and Yemen in the Middle East, to Zimbabwe and Mozambique in southern Africa [61,62].

The Eastern Afromontane Biodiversity Region (EABR), is a series of montane “sky islands” extending from the Asir Mountains of southwest Saudi Arabia and the highlands of Yemen in the north; along the Eastern Rift branches including the Ethiopian Highlands, the Kenyan and Tanzanian Highlands and the Eastern Arc Mountains (EAM); and the Western Rift branch including the Albertine Rift Mountains (ARM), the Southern Montane “islands” and distant outliers in the Chimanimani Highlands of eastern Zimbabwe, Malawi and Gorongosa of western Mozambique [56,61]. These East African mountains are often referred to as biological "sky islands" because the plant species they harbor frequently have their closest relatives not in the lower elevations or surrounding lowlands, but in far-distance regions of the world [63]. These mountains are mainly of volcanic origin and lie widely scattered across the wide plateau of east Africa, several reaching altitudes between 3500 and 6000 m. The highlands in the EABR provide an excellent example of a highly isolated system, where interaction between historical and contemporary processes has formed a flora rich in local endemics [64]. EABR houses one of the highest concentrations of threatened endemic species globally which has earned it recognition as the Eastern Afromontane Biodiversity Hotspot [65]. Indeed, East Africa boasts one of the highest concentrations of endemic flora and fauna globally, with over 80% of the flora in the Eastern Afromontane Biodiversity Region (EABR) being endemic to the massifs of East Africa [64,66–68].

Among the best-known, the strictly Afromontane species and the most important genera with higher species endemism in the East Africa mountains are the members of the Balsaminaceae family (*Impatiens* L.) [56]. The *Impatiens* L. is one of the most species-rich genera of tropical Afromontane areas of East Africa, with more than 100 species of this genus occurring across the eastern African region and about 50 species are endemic to the mountains located within the Eastern Afromontane Biodiversity Region [26,56]. Although the *Impatiens* genus is widespread across the Afromontane areas across the EA region, most of these East African *Impatiens* are often restricted to specific geographic areas or even individual mountain peaks, leading to a high degree of local endemism [2,32,50,62,69–71]. The unusual distribution pattern and high richness of *Impatiens* species in the EABR provide an ideal model system for exploring the processes that gave rise to the higher genus endemism in the EABR.

Chapter 2 Literature Review

2.1 Taxonomic and phylogenetic studies of the family Balsaminaceae

2.1.1 Historical review of the Balsaminaceae taxonomy

The taxonomic history of the family Balsaminaceae began when the *Impatiens* species first appeared in print in 'Turner's Herbal (1568) as Balsamina, and it was later formally named *Impatiens balsamina* by Carolus Linnaeus (1753). In his publication ("Species Plantarum"), Linnaeus (1753) described six other species of Balsaminaceae, including *Impatiens triflora* L. (1753: 938), in the present day known as *Hydrocera triflora*. This was later regarded as a monotypic genus. This early research on Balsaminaceae focused primarily on species found in Asia and then gradually began to include species from other parts of the world such as Africa and Europe. For example, in 1859, [73] conducted an intensive study of Indian specimens, documenting the floral morphology of Balsaminaceae based on 96 *Impatiens* species and Hydrocera triflora, and they created a key for the known species at that time in their work *Praecursores ad Floram Indicam*. In 1875, Hooker published the Flora of British India, which included a key featuring additional specimens (123 *Impatiens* species and H. triflora) [74]. Continuing his research on Balsaminaceae, Hooker later updated a key for the Indo-Chinese balsams in Flore Générale L'Indo-Chine, which encompassed 25 *Impatiens* species and *H. angustifolia* [75].

Following these publications, the botanists became more interested in the Balsaminaceae family, from the beginning of the 20th century onwards and published numerous studies on the systematics of Balsaminaceae. As a result, several new contributions have been continuously provided, and various novel species have been described across different regions, especially in the *Impatiens* diversity hotspots [2,7,23,28–30,33,34,43,44,47–49,52,71,76–84]. Currently, approximately 1,121 species of *Impatiens* and the monospecific *Hydrocera triflora* species are recognised [26].

Being the largest genus in the Balsaminaceae family, the genus *Impatiens* L. is often viewed as one of the most challenging groups from a taxonomic standpoint [2,85] and has been declared a " terror to botanists" by various botanists [2,85]. This is mainly due to the large number of species accompanied by cosmopolitan distribution, small and endemic areas of occurrences, extremely high diversity of floral morphological features within this genus, and convergent evolution of certain morphological characters, which made it difficult to conduct comparative research on all of them [3,9,12,86]. In addition, the genus contains semi-succulent stems and fleshy leaves, and the extremely delicate and fragile nature of its flowers makes it challenging to determine a species when specimens are pressed conventionally without detailed descriptions or drawings of its floral morphology [2,9]. In addition, the capsules and seeds of *Impatiens* species display significant diversity and are often regarded as important morphological characteristics for resolving classification challenges [87,88]. However, due to the explosive nature of mature seed pods, seeds or fruits are frequently absent from herbarium specimens. Thus, many early publications describing floral, stem and fruit characteristics based on herbarium specimens may be incomplete or ambiguous [9,89]. Due to the diverse and complicated morphological characters, no comprehensive infrageneric classification is available for the genus *Impatiens*.

2.1.2 Morphology-based infrageneric classification of Balsaminaceae

Despite being among the most difficult genus to classify taxonomically, several studies have attempted to arrive at an infrageneric classification of genus *Impatiens* based entirely on morphological characters. [73] published the first comprehensive global study of the *Impatiens* and classified the genus into 8 sections mainly based on differences in inflorescence type and leaf organization. In their publication, they recognized only eight African taxa. However, they stated that "numerous species will yet be detected in Madagascar and tropical Africa" [73]. The only comprehensive global infrageneric classification ever made until now is that of [76] who classified *Impatiens* into two subgenera (i.e. subgenus *Impatiens* Warb. and subgenus *Acaulimpatiens* Warb.) based on the leaves appearing as the basic or top position of the stem, thereby ignoring the work conducted by [73]. These two subgenera comprised 14 sections (subgenus *Acaulimpatiens*, two sections, and subgenus *Impatiens*, 12 sections) mainly segregated based on the arrangement of the leaves, the number of the flowers on the inflorescences and the proportion of the length of the flower and spur [76]. However, both Hooker's and Warburg & Reiche's classifications were neither natural nor practical because of the limited number of species studied at that time as well as the highly variable homoplastic characters they used to base their classification on [2]. Thus, his classification was not followed by later authors.

For Madagascar, [90] proposed 3 sections for the Madagascan *Impatiens* using the presence or absence of a spur and the different types of anther dehiscence. The proposed sections included: 1) sect. *PreImpatiens* (=sect. *Impatien*s) (flowers with conspicuous spur), 2) sect. *Trimorphopetalum* (flowers without spur, anthers dehiscent apically), and 3) the monotypic sect. *Impatientella* (flowers without spur, anthers dehiscent laterally), the latter two being endemic to Madagascar. However, this classification was rarely followed by later authors and only appears to apply to Madagascan species. Recently, [40] elevated Perrier de la Bâthie's sect. *Impatiens* with spurs and sect. *Trimorphopetalum* without spurs to subgeneric level. The monotypic sect. *Impatientella* now contains only the spurless and entirely cleistogamous species, *Impatiens inaperta* (H. Perrier).

The first modern and the most widely used classification of the genus *Impatiens* during recent decades is the important continental-level revision of tropical African taxa conducted by [2]. In his work, [2] recognized six informal infrageneric groups for the African species only for practical diagnosis. While [76] searched for a comprehensive scheme in which every species is included in an artificial section, [2] did not intend to group species according to their natural affinities at all. He searched for natural species aggregates in *Impatiens* that could not be divided into discrete lineages [2]. Generally, he proposed the classification of African *Impatiens*, based on the presence of peduncle and the shape of lower sepal. In his revision, he grouped the *Impatiens* species of tropical Africa into six informal intrageneric groups based on these characteristics for practical diagnosis only.

Group one was defined by primarily opposite or verticillate leaves, with a peduncle present, very reduced or absent, lower sepal bucciniform, or constricted into a filiform spur. Group two features leaves that are spirally arranged with a peduncle present and exceeding one cm in length, exhibiting lower sepal navicular and abruptly constricting into a filiform spur considerably longer than the length of the lower sepal. Group three consists of leaves spirally arranged with peduncle absent or sometimes very reduced to a length of less than 5mm. The flowers are solitary or in fascicles at the leaf axils and spur filiform, curved or incurved, and always considerably longer than the length of the lower sepal. In group four, the leaves are spirally arranged with peduncle absent or very reduced and never more than 5mm long although there are some exceptions. Lower sepals are abstractly constricted into short filiform spurs equal in length or shorter than the length of the lower sepal. Group five showcases leaves that are spirally arranged with peduncle present and inflorescence flowered. Their sepals are saccate to buccinirom, gradually or abruptly constricted into a curved or incurved spur. Finally, group six encompasses leaves spirally arranged and flowers are solitary or fascicled. Peduncles are absent or very reduced and never more than 5mm long. Lower sepal bucciniform or saccate, gradually± abruptly constricted into a curved or incurved spur. Despite the efforts made to classify the African *Impatiens* into different groups, Grey-Wilson's study highlighted the challenges of further subdividing the species into sections, as essential taxonomic traits are unevenly distributed among them. In addition, although [2] work provided invaluable and testable data for the genus *Impatiens* especially those from continental Africa, the major relationships in the *Impatiens* remained obscure.

For Chinese *Impatiens* species, [91] provided an identification key primarily based on the shape of the capsule, inflorescences, the number of lateral sepals, and the shape of the perianth; however, this key did not clearly distinguish between groups. Floral morphology, including the shape of the lateral sepals, spur shape, flower color, size, and hairiness, is essential for differentiating closely related species. For instance, *I. omeiana* Hook.f. features yellow flowers with a narrowly infundibuliform lower sepal and an involute stout spur, while *I. wilsonii* Hook.f. ex Eb. Fisch. has white flowers with a saccate lower sepal and a very short curved spur. *I. siculifer* Hook.f. displays navicular lateral sepals, whereas *I. stenantha* Hook.f. has ovate-oblong lateral sepals. Additionally, *I. imbecilla* Hook.f. is characterized by small pink flowers with puberulous pedicels and bracts, while *I. faberi* Hook.f. features larger violet flowers with glabrous pedicels and bracts.

Therefore, although aggregates of closely related species within the genus *Impatiens* can be consistently identified using the morphological classifications provided by these previous authors, all these studies were mainly based on gross morphological data, and they also failed to test any evident synapomorphies uniting these infrageneric groups, and monophyly. In addition, most of these previous studies conducted in *Impatiens* have largely been regional and used descriptive traditional taxonomic approaches. Thus, given that new species of *Impatiens* are being described and redescribed from various tropical and sub-tropical regions of the world, more work is required to be conducted to give more investigation and confirmation about the classification of the Balsaminaceae family.

2.1.3 Molecular phylogenetic studies in the Balsaminaceae family

2.1.3.1 Sequencing of Chloroplast and Nuclear Markers

Improvements in sequencing technologies have enabled the analysis of chloroplast and nuclear DNA markers. Recently, the analysis of these markers has also been utilised to determine the relationships of *Impatiens* species. Indeed, many researchers have employed molecular phylogenetic analysis to examine the taxonomy of *Impatiens* species, and have proposed new taxonomic systems based on the phylogenetic relationships of species within the family Balsaminaceae. [92], conducted the first molecular phylogenetic analysis of the Balsaminaceae family focusing on 25 Eastern Himalayan species that were analyzed using *rbcL* and *trnL-F* sequences. However, due to limited sampling, and their use of a distant outgroup (Tropaeolum, Tropaeolaceae), only a few insights into infrageneric relationships could be drawn from this study. Subsequently, several other molecular phylogenetic trees that included low-density worldwide taxon sampling of *Impatiens* have been published [3,9,12,93,94].

The work by [9], utilized nuclear ribosomal internal transcribed spacer (ITS) sequences from 111 species including *Impatiens* and *Hydrocera*. The study also achieved comprehensive coverage of the entire distribution range of the family, including its five biodiversity hotspots. The findings from this study confirmed that the Balsaminaceae is a monophyletic family and that the species of African *Impatiens* are not monophyletic but are derived from at least two independent colonizations of the continent. However, in this study, the phylogenetic position of the genus *Hydrocera* within the family remained ambiguous, as it was placed in a basal polytomy alongside a large clade of *Impatiens* and three additional *Impatiens* species. In addition, some lineages were resolved and well-supported, such as the monophyletic group of spurless Madagascan taxa (*Trimorphopetalum*). Conversely, the resolution among several major lineages was still inadequate and offered limited taxonomic implications. For example, four African species, *I. fischeri*, *I. flanaganae*, *I. teitensis*, *I. rothii*, and the Indian *I. repens* were grouped as a weakly supported clade that nested out of the other African species but grouped with Southeast Asian clades. Therefore, ITS data alone was insufficient for clarifying all phylogenetic relationships within the family Balsaminaceae, indicating a need for new genetic markers for further analysis [9].

In 2006, [3], investigated 86 accessions of Balsaminaceae and six outgroup species using chloroplast atpB-rbcL and rDNA ITS sequences, leading to a well-defined phylogeny with robust support for numerous nodes. Their results established that *Hydrocera* is closely related to all *Impatiens* species and identified 15 distinct clades within the genus, with *Impatiens omeiana* positioned as a sister to all other species. The remaining taxa were grouped into two major clades that received strong support from the combined ITS and atpB-rbcL analyses, showing only slight inconsistencies when comparing the atpB-rbcL dataset with the combined dataset. However, in this study, some species such as *I. fischeri* Warb., *I. flanaganae* Hemsl., and *I. teitensis* Grey-Wilson formed a weakly supported clade, while the placement of African species *I. tinctoria* A. Rich. was not resolved [3].

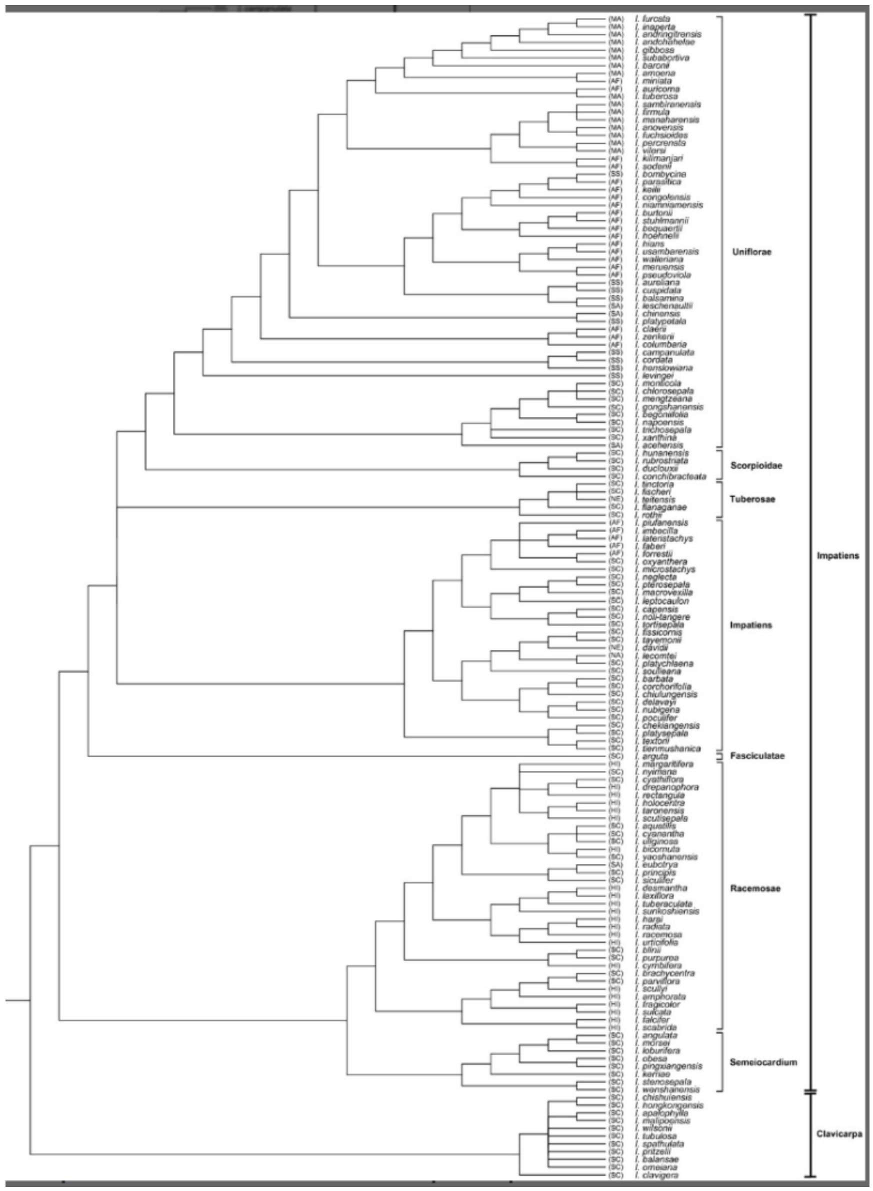

Figure 2-1 **The phylogenetic tree inferred from maximum likelihood (ML) and Bayesian based on a combined dataset of ITS and atpB-rbcL sequences**[12]

To further clarify interspecific relationships among *Impatiens*, [93], explored the phylogenetic utility of two *APETALA3 (AP3)/DEFICIENS (DEF)* paralogues (*ImpDEF1* and *ImpDEF2*) in the genus *Impatiens* (Balsaminaceae). *AP3*/*DEF* is a MADS-box transcription factor that is involved in petal and stamen floral organ identity. [93] sequenced introns 4 and 5 of both paralogues across 59 species of *Impatiens*, revealing significant sequence variation and intron length variability. Phylogenetic analyses of these loci, both separately and in combination, resulted in well-supported Bayesian and parsimony consensus trees, with the combined nuclear (*ImpDEF1*/*ImpDEF2*) and chloroplast data (*atpB-rbcL*) providing a robust evolutionary hypothesis for the genus. The study highlights the advantages of using *AP3*/*DEF* introns over more conserved chloroplast markers like *atpB-rbcL*, which evolve too slowly for fine-scale phylogenetic resolution. The study also reveals the presence of differential evolutionary rates between the two paralogues, with some species showing variation in the K-domain region, and discusses the functional implications of intron length variation in *ImpDEF2*. The results confirm that *AP3*/*DEF* genes, despite their rapid evolution, can be valuable for phylogenetic studies at the infrageneric level, providing insights into the evolutionary relationships within *Impatiens*.

On the other hand, the research conducted by [95], demonstrated that integrating morphological and molecular data can enhance the resolution of certain phylogenetically challenging lineages within *Impatiens*. This approach also offers a clearer understanding of the diagnostic significance of the morphological traits that earlier taxonomists used to formulate their perspectives on infrageneric relationships.

The most recent molecular intrageneric study on *Impatiens* by [12] integrated morphological characters with advanced molecular phylogenetic analyses, utilizing nuclear (ITS) and plastid (*atpB–rbcL* and *trnL–F*) sequence data, while also incorporating the morphological classifications established by [73] and [76]. The classification by [12] led to the division of *Impatiens* into two subgenera: *I*. subgenus *Clavicarpa* S.X.Yu ex S.X.Yu and Wei Wang, characterized by a four-locular ovary and one ovule per locule, and *I*. subgenus *Impatiens*, characterized by a five-locular ovary (rarely four) and many ovules per locule, but some relationships within the *Impatiens* subgenera was not very well resolved (Figure 2-1). The taxonomic difficulties are probably due to the existence of a large number of intermediate groups and taxa within the genus [2]. In their classification, the subgenera *Impatiens* was further subdivided into seven sections based mainly on inflorescence, flower, and fruit characters: *Semeiocardium, Racemosae, Fasciculatae, Impatiens, Tuberosae, Scorpioidae, and Uniflorae* [12] (Figure 2-1). In this study, the African species of *Impatiens* were primarily distributed amongst three clades: two of these are part of a big clade that comprises *I*. sect. *Uniflorae* are characterized by short fusiform capsules and lack of peduncles, whereas some African species are members of the smaller clade which comprises the *I*. sect. *Tuberosae*, are characterized by four lateral sepals with inner two fully developed, lower petals more than three times the length of the upper petals and clavate capsules. Madagascan species form a monophyletic clade and are nested inside the largest African clade within *Uniflorae* section [12].

Although the new schematics incorporating molecular phylogenies have helped clarify the phylogenetic relationships of some *Impatiens* species, all previously published molecular systematics and phylogeny studies on this genus have either included only a small number of samples from clearly defined regional sources, been geographically biased (with most species investigated restricted to Asian countries [92,95]), focused on limited taxon sampling outside Asia, especially from continental Africa [3,9,12] or relied on restricted genetic sampling involving no more than three loci [3,9,12,93]. As a result, the relationships among many species within the genus *Impatiens* remain unclear. Thus, an analysis with extensive taxon sampling and phylogenomic data which provides dense locus sampling is critically important to test hypotheses and monopoly of sections that were proposed in these studies based on molecular datasets with more limited sampling and to provide a framework for comparative analyses. This is particularly important for understudied but yet important centres of genus *Impatiens* diversity regions like Tropical Africa. Given that tropical Africa is a crucial region for understanding the origin and relationships of *Impatiens* species, there is a pressing need for further investigation to determine the monophyly and evolutionary history of the sect. *Uniflorae* and sect. *Tuberosae,* which contains Tropical African *Impatiens* by using extensive taxon sampling together with morphological as well as phylogenomic data to determine their accurate phylogenetic position.

2.1.3.2 Chloroplast DNA (cp) Genome and Nuclear ribosomal DNA (nrDNA)

Due to the diversity of floral and morphological characters and frequent hybridization and polyploidization, the genus *Impatiens* is considered one of the most taxonomically and phylogenetically challenging plant groups [2,77,96,97]. The use of traditional species classification based on morphology tends to be unstable and unreliable and can lead to misclassification if convergent evolution occurs, where unrelated species evolve similar traits as a result of influence from environmental conditions. The absence of suitable DNA fragments and polymorphic genetic markers for phylogenetic analysis has hindered the establishment of a dependable phylogeny, contributing to ongoing controversies in taxonomic classification that complicate our understanding of the diversification and evolution of genus *Impatiens* [3,9,12,93,94].

Previous molecular studies in the genus *Impatiens* have utilised plastid protein-coding genes *matK*, *rbcL*, and *trnK* and the intergenic regions *atpB-rbcL* and *trnL-trnF* [3,12,98], nuclear ribosomal internal transcribed spacer (ITS) [9], and inter-simple sequence repeat (ISSR) markers [99] and various DNA loci have provided additional insights into the taxonomy, biogeography and phylogeny of *Impatiens* species, yet their discriminatory power has proven to be very low for recently originated groups via rapid speciation [100–102]. Next-generation sequencing has expanded the plant barcodes using substantial high-throughput sequencing technologies to enhance discrimination efficiency[101,102]. Complete plastomes and nuclear ribosomal DNA (nrDNA) sequences, which provide more comprehensive data compared to standard barcodes, can significantly enhance the resolution of phylogenetic analyses [100,101]. Despite their importance, only a limited number of empirical studies of large and taxonomically difficult plant genus have evaluated their effectiveness in phylogenetic studies and species identification [103,104].

Compared with the most frequently used and predicted genus-specific DNA barcodes, the cp genome contains more variations with a significantly higher resolution of phylogenies hence offering valuable insights for taxonomic classification and phylogenetic reconstruction due to the sequence divergence observed between different plant species and individuals [105]. Using the entire chloroplast genome as a super-barcode represents a novel strategy that may overcome the limitations associated with conventional two-locus barcoding [106]. Traditional barcoding typically depends on sequence variation in two specific regions of the chloroplast genome (i.e. *matK* and *rbcL*) which often proves inadequate for accurate species delimitation [107].

The genus *Impatiens* is among the most challenging groups of flowering plants where species identification and classification are difficult [2]. Some species of the genus *Impatiens* originated via an allopolyploid event through hybridization, and thus, it could be among the factors that cause intense conflicts between the nuclear and plastid phylogenies [96,97,108]. Previous molecular studies based on few plastid and nuclear genes and with relatively limited taxon sampling did not yield a well-resolved infrageneric phylogeny of *Impatiens* [3,9,12,93,94]. These studies established a phylogenetic framework for the genus *Impatiens*; however, it is still a big challenge for species identification in such a complex genus.

Due to the lack of a well-resolved phylogenetic tree within the Basalm family [25,109,110], based on the whole chloroplast genome, reconstructed a highly robust phylogeny tree, and investigated the divergence times, biogeographic history, and diversification rate of the genus *Impatiens*. However, their research only used a few *Impatiens* species particularly from the Asia region hence resulting in the resolution of the relationship within the *Impatiens* subgenus and sections with limited support [109–111]. In addition, their results were also not sufficient to provide information for the clarification of the infra-familial relationships of the family. Generally, while the NCBI database contains complete chloroplast genomes of approximately a thousand plant species, only a few (91) complete chloroplast genome sequences are available for the genus *Impatiens.* In addition, Complete plastomes and nuclear ribosomal DNA (nrDNA) sequences for almost all species in most regions with higher *Impatiens* diversity including Africa are still lacking. The absence of Complete plastomes and nuclear ribosomal DNA (nrDNA) sequences for most species within the genus *Impatiens* significantly hinders our understanding of the molecular evolution and phylogeny of the genus *Impatiens*. Furthermore, this limitation also restricts our ability to explore genetic information and conduct thorough analyses of interspecific relationships within the genus. Therefore, it is crucial to obtain the complete plastomes and nrDNA sequences of the *Impatiens* for identifying species and reconstructing phylogenetic relationships within the genus.

2.2 Taxonomic and phylogenetic studies Africa *Impatiens*

Research on the genus *Impatiens* in continental Africa has been piecemeal, beginning with their description and classification in the publication of [73]. [73] published the first comprehensive global study of the genus *Impatien*s, where they recognized only eight African taxa. Subsequently, [112] cataloged 17 *Impatiens* species from Tropical Africa. Among the published species, ten were sourced from the high-altitude areas of the Cameroon region, whereas only four were recorded from the mountains of East Africa, which were still almost unknown at the time. In 1895, significant advancements in the understanding of African *Impatiens* species were made when Warburg described 47 species from the East African mountains, followed by the publication of an additional eight species, of which two were from East Africa and six from West Africa [76]. This marked a notable expansion of knowledge regarding the diversity of *Impatiens* in tropical Africa. In his subsequent publication, Warburg provided a comprehensive classification key for all known African species of the genus *Impatiens* (i.e. the classification key of approximately 46 species at that time). After 1895, an extensive collection of *Impatiens* specimens from tropical Africa continued to be gathered and numerous new species were published, especially from the Congo State and southern East Africa, it became essential to conduct a comprehensive revision of all available material. As a result, the *Impatiens* of Africa were brought to the notice of the world by [113] in his classic paper entitled “Balsaminaceae Africanae”, which contained mainly a comprehensive key to the species of *Impatiens* found in Africa, particularly focusing on their morphological characteristics and classifications. Afterwards, [114] presented the genus *Impatiens* of the tropical African continent more systematically than his predecessor and recognized 85 species of *Impatiens* of the African continent. The work of [114] was however fulfilling what was already proposed almost fifty years ago by [73] who only recognized only eight African taxa however, they stated that “numerous species will yet be detected in Madagascar and tropical Africa” [73]. After Gilg’s day, the botanical impetus on African *Impatiens* slowed down, however, documentation of several country floras and floristic inventories continued. During this time multiple writers generally produced significant floristic works that included the Balsaminaceae family in their publications: Carrisso, L. Wittnich, “Conspectus Florae Angolensis”, Wilczek&Schulze, “Balsaminaceae, Flore du Congo Belge et du Ruanda-Urundi”, N.Halle , “Flore du Gabon”, and Launert , “Balsaminaceae Flora Zambesiaca”, Launert, E. & Gonçalves, M.L. “Balsaminaceae Flora de Moçambique Junta de Investigaçoes de Ultramar”. Additionally, major important contributions to Eastern Africa *Impatiens* (particularly the Tanganyika) that are especially noteworthy came from G. M. Schulze, who largely confined himself to publishing accounts of new *Impatiens* species of this country [115–117]. The current understanding of *Impatiens* in Tanganyika is largely attributable to Schulze’s foundational work, which provided critical insights into the taxonomy of this genus in the country. All these taxonomic past endeavours of these authors resulted in the deposition of a large number of collections in various herbaria in Africa and

Europe. The only major study of the entire genus for the African continent was that of [2] which was primarily based on field visits and assessment of herbarium specimens and described 110 *Impatiens* species. However, up to now critical systematics, phylogenetic relationships among species and infrageneric classification have not been dealt with within this genus in the tropical Africa After [2] publication.

2.3 Pollinators and pollination biology of Balsaminaceae

Plant-pollinator interactions play a crucial role in the evolution of most angiosperms at both micro- and macroevolutionary scales [118]. Despite the emerging evidence that pollinators can strongly influence evolutionary processes, many questions on the reproductive biology of many plants remain to be fully answered. Generally, research on the reproductive biology of plants has been essential for understanding its systematics and evolutionary biology [119,120]. The reproductive biology of numerous plant species in temperate and neotropical regions has been extensively studied. The findings have revealed that many flowers with long-tubed or spurred corollas are primarily visited by long-tongued bumblebees and hawkmoths in temperate areas, while in the Neotropics, they are predominantly pollinated by euglossine bees, hummingbirds, and hawkmoths [121–123]. In contrast, numerous deep flowers, exist in the paleotropical region, however, detailed analyses of their pollination syndromes have yet to be conducted. *Impatiens* L. is one of the most species-rich genera of angiosperms belonging to the family Balsaminaceae and contains more than 1,121 species worldwide. The floral diversity in this genus is extremely high. The *Impatiens* contain mostly allogamous species with strongly protandrous flowers in which male (stamens) and female (pistils) organs mature at greatly different times (i.e. The male organ matures when the flower is at anthesis, and the female organ matures several days after the male organ) [124]. The lower sepal of *Impatiens* has a nectar-producing spur, functioning as a reward for animals [2].

Flowers of the large genus *Impatiens* are characterized by extensive variation in various traits such as flower colour, spur length, and nectar production which strongly suggests an adaptation to specific pollinators [124]. Despite this huge diversity in floral morphology in *Impatiens*, very few comparative studies so far have used an integrative approach to investigate the relationship between floral morphology diversity and pollination ecology within the Balsaminaceae family. Among the largest barriers to understanding the role of pollinators in the diversification of floral morphology is the lack of detailed studies of pollination ecology, particularly those that catalog not only the range of visitors but their effectiveness as pollinators. However, few researchers have conducted detailed studies on the pollination ecology of *Impatiens* species [125–131]. These studies found that some *Impatiens* in temperate zones are mainly pollinated by bumblebees and hummingbirds [132–134]. On the other hand, in the palaeotropics, there are more *Impatiens* species than in the temperate zone. Few detailed studies on the pollination of *Impatiens* in some palaetropical regions such as Asia (i.e. Sumatra, Japan, Southeast Asia, Korea, and China) have documented hawk moths, bees, and butterflies as effective pollinators for the species in the region [37,125,129,135,136].

Conversely, tropical Africa is one of the diversity hotspots for balsams, but detailed studies on pollination studies for the genus *Impatiens* are generally lacking [137]. Generally, pollinators for this genus in the continent in most cases are inferred by pollination syndromes [2]. In general, [2], classified tropical African *Impatiens* species into several pollination syndromes. The analysis of his study focused on how the combination of flower type, flower color, and adaptations relate to different pollinator types [2]. In his monograph, [2] described two distinct types of *Impatiens* flowers: the flat type and the funnel type. The flat type features a shallow lower sepal, a long filiform spur, and an upright, slightly concave dorsal petal that lacks a hood. Based on limited empirical evidence, Grey-Wilson proposed that this flower type is likely pollinated by butterflies, which perch on the lower petal and reach into the spur for nectar through the narrow opening. During this process, pollen can adhere to the butterfly's proboscis. In contrast, the funnel type is characterized by a deeply boat-shaped lower sepal and a hooded dorsal petal. This flower type is believed to attract (bumble) bees or birds. When bees access the flower, they must navigate through the floral entrance, causing their bodies to come into contact with the anthers and stigma. However, it is important to note that Grey-Wilson's hypothesis was not based on extensive field studies. Furthermore, the pollination systems proposed by [2] on African *Impatiens* have not been examined within a phylogenetic framework, which hinders a comprehensive understanding of how pollinators influence the evolution of the flowers which is arguably the most variable structures in the extensive genus *Impatiens*.

Therefore, studies are needed to provide a framework for comparative analyses of floral diversity and pollinator preferences and assess the utility of pollination syndromes in this genus in tropical Africa. Understanding the evolutionary transitions of floral features in the genus *Impatiens* will lay the foundation for future studies and elucidate the genetic machinery controlling transitions in their phenotype. Furthermore, it will yield valuable insights into the remarkable species diversity and varied floral morphology observed in this genus and enhance our understanding of how pollinators have influenced floral morphological evolution across other angiosperm lineages.

Despite the longstanding recognition of pollination syndromes of *Impatiens* as a framework to understand the relationship between floral traits and pollinator groups, significant gaps remain in the empirical validation of this concept within the genus. The first gap necessitates a combination of analyses of floral traits and detailed observations to distinguish between visitors and pollinators, thereby confirming the link between floral diversity and specialization for different functional pollinator groups. The second gap requires the use of a phylogenetic approach to test hypotheses about the role of pollinators in floral evolution and adaptation. Since the genus *Impatiens* contains a large number of species, well-known phylogeny as well as the remarkable diversity of pollination systems, thus it makes it an ideal group for testing the role of pollinators in floral diversification and then map pollination systems onto the phylogeny to analyze pathways of floral evolution. Although the phylogenetic relationships between genera in *Impatiens* are well-supported [12], species-level relationships within sections remain poorly understood [110,138]. In addition, to date, no comprehensive phylogenetic analysis of African *Impatiens* spanning from the diversity of floral size, color, morphology, form, scent, and rewards has yet been conducted. Therefore, studies are needed to provide a framework for comparative analyses of floral diversity and pollinator preferences and assess the utility of pollination syndromes in this genus in the region. Understanding the evolutionary transitions of floral features in the genus *Impatiens* will lay the foundation for future studies and elucidate the genetic machinery controlling transitions in their phenotype.

2.4 Historical Biogeography

In general, the origin and diversification of the genus *Impatiens* L. are still largely unknown, and there is an ongoing debate about whether the disjunctive distribution of the primary diversity centers of Balsaminaceae across the paleotropical regions results from the fragmentation of Gondwana or more recent dispersal events [9]. Understanding the evolutionary history of high biodiversity in mountain regions and how clades originated and diversified has been the focus of intense interest of many scientists [139,140]. Several hypotheses have been proposed regarding the processes leading to the current distribution of the Balsaminaceae family, and several migration paths have been inferred based on extant stands. [141] proposed that *Impatiens* originated in the Himalayan region, dispersing to other areas based on species diversity and karyological evidence. Conversely, [2] based on karyological studies hypothesized that Balsaminaceae originated in western Gondwana during the Paleogene, around 50 million years ago, spreading to Southeast Asia via Madagascar and India following the collision of the Indian plate with Laurasia approximately 45 million years ago. He dismissed the idea of overland migration between Africa and India, citing the notable similarities among species in Africa, Madagascar, and southern India and the presumed sister relationship between Balsaminaceae and Tropaeolaceae. However, karyological studies alone are not enough to test the hypothesis, thus molecular phylogenetic studies can hopefully give more insights.

ITS phylogeny of *Impatiens* proposed by [9] revealed that the species in northern temperate regions are likely the result of recent radiations from Southeastern Asia and nearby Sino-Himalayan regions. However, it remains uncertain whether the disjunct distribution of major diversity centers across paleotropical regions is due to ancient vicariant events stemming from Gondwana's fragmentation or more recent dispersal events [9]. Other studies, however, noted that rapid radiation of the *Impatiens* genus occurred during the Pliocene and Pleistocene, [94] and argued that the genus originated in southwest China and started to diversify in the Early Miocene (albeit with a relatively low net diversification rate), which coincided with the global cooling of the Earth’s climate and subsequent glacial oscillations. Recently, [142] presented a global phylogeny of *Impatiens* using ITS sequences to explore biogeographic implications, supporting the conclusions proposed by [9] regarding the genus's origin. Additionally, [143] examined the biogeographic history of *Impatiens* by employing a combined ITS and *atpB-rbcL* Bayesian phylogeny with the S-DIVA method, suggesting that China may be an ancestral region for the genus. The distribution patterns of modern Balsaminaceae indicate that the family's diversification likely began in South or Southeast Asia rather than Africa, as *Hydrocera* is exclusively found in these regions [9,99,143]. If we assume that *Impatiens* originated in Southeast Asia and later radiated to Africa and Madagascar via India, this scenario could also account for the significant species similarities observed among India, Africa, and Madagascar, as [2] noted.

While these findings provide valuable insights into the origin and evolutionary radiation of *Impatiens*, the molecular phylogenetic and biogeographic data for Balsaminaceae, derived from ITS sequences [9] and chloroplast *atpB–rbcL* spacer sequences [3], include only a limited number of *Impatiens* samples from Tropical Africa [3] included 16 species and [9] included 20 species. This limitation presents a gap in the current understanding of the phylogeny and evolutionary history of the diversification in the Balsaminaceae family, particularly the role of the diversification centres such as Afromontane forests of tropical Africa such as Eastern Afromontane Biodiversity Region (EABR) which contains higher diversity and endemism of the genus *Impatiens* [56] in shaping taxon dispersal and in situ diversification, due to inadequate sampling of *Impatiens* taxon from these mountains and few genetic markers used to form molecular phylogenetic inference [9,94]. Thus, this further highlights the need for increased taxon and gene sampling in *Impatiens* to resolve relationships within the genus and infer the spatio-temporal evolution within the Balsaminaceae family.

2.5 Morphological taxonomic characters for studies in Balsaminaceae

Numerous morphological studies have been conducted on the Balsaminaceae family, with most previous classifications relying heavily on gross morphology. Key taxonomic characters in this family include fruit shape, phyllotaxis, leaf shape, and inflorescence [2,37,87,90,91,144]. Among these, floral morphology is the most critical character, while seed and fruit morphology are also important characteristics that can be used to determine species relationships in the taxonomically complex and economically important genus *Impatiens*.

2.5.1 Floral Morphology and Evolution

The fascinating diversity in floral morphology makes it an excellent subject for studying floral evolution. [2] proposed a set of hypotheses regarding the evolution of floral morphology based on his research on African *Impatiens*. He suggested that the ancestral *Impatiens* flower was characterized by a posterior sepal lacking a spur and by free floral parts of equal size. Similar to the developments seen in Orchidaceae [145], the process of resupination likely played a crucial role in the evolution of the modern, highly zygomorphic *Impatiens* flower. The 180° twist of the pedicel during resupination positions the posterior sepal abaxially, which is believed to facilitate the growth of a relatively heavy nectar-producing spur, thus initiating zygomorphy in *Impatiens* [2]. According to Grey-Wilson, resupination occurs after the spur on the posterior sepal has formed. In contrast to the pronounced zygomorphy observed in *Impatiens*, zygomorphic traits are less evident in Hydrocera. The dorsal petal, positioned outside the other petals, offers protection during the bud stage. The remaining petals have fused into two lateral pairs, which not only attract pollinators but also provide a landing platform (except in the case of hawkmoths) and facilitate access to the lower sepals, where the nectary is located at the tip of the spur. The transition to zygomorphic flowers and the process of resupination appear to be closely linked to pollinator interactions, with these evolutionary changes aimed at enhancing efficient cross-pollination.

However, although there are a lot of assumptions regarding the floral evolution in the genus *Impatiens*, the majority of species within the family have yet to be explicitly studied for their pollination biology. [2] examined African species and found that out of 109 morphologically diverse *Impatiens* species, 58 are pollinated by butterflies, 3 by moths, 27 by birds, and 21 by bees. He analyzed how the recombination of various floral traits and colors adapts to different pollinators, proposing specific floral characteristics for each group: erect or hood-like dorsal petals, shallow lower petals, and filiform spurs with pink, mauve, or purple flowers for butterflies; hood-like dorsal petals, shallow lower petals, and white flowers for moths; hood-like dorsal petals, deeply navicular or bucciniform lower sepals, incurved spurs, and white, yellow, or pink flowers for bees; and hood-like dorsal petals, broadly saccate lower sepals, incurved spurs, and pink or mauve flowers for large solitary bees. For birds, the characteristics include hood-like dorsal petals, shallow or bucciniform lower petals, filiform or incurved spurs, and red flowers. However, this classification was based on hypothesis and no detailed studies have been carried out to confirm these assumptions.

Research has suggested a close relationship between pollinators, floral morphology, and angiosperm diversification [2,37,146] in *Impatiens*, if multiple independent gains of floral traits are common, the complex specialization of floral structures (i.e. shapes and sizes of spurs and the morphology of the fused lateral petals) may be mediated by pollinators and thus exhibit high homoplasy. However, phylogenetic assessments of these diverse floral types and their associated pollination syndromes are lacking for the Balsaminaceae.

2.5.2 Seed and Fruit Morphology

Although the taxonomic significance of seed and fruit morphology differs among plant groups, these structures demonstrate considerably less phenotypic plasticity compared to many vegetative and floral traits, and seed coat and fruit morphology are little affected by environmental conditions [147]. Therefore, the importance of other morphological features such as fruit and seed morphological characters has long been recognized as they can also provide valuable information and reliable evidence for the classification of species and a better understanding of interspecific relationships [24,73]. Despite their significance in the classification of *Impatiens*, the most notable contradictions arise from the insufficient sampling and the scarcity of herbarium specimens that include mature fruits and seeds [148]. In most cases, the capsule cannot easily be studied from herbarium specimens (because most of the time they are either immature or have already dehisced during pressing) and fruit is often not produced by plants growing under artificial conditions such as found in greenhouses and “botanical gardens” [2]. This is why descriptions of fruits and seeds are seldom included alongside the taxonomic descriptions of *Impatiens* species [2]. However, compared to fruit morphology, the seed coat ornamentation of *Impatiens* has received considerable attention since the work of [73], although comprehensive surveys remain lacking. Previous studies have examined seed and capsule morphology within the Balsaminaceae family. These include various research on seed morphology by [2] on 55 African species, as well as similar investigations have also been conducted in China and Thailand [87,88,149–155].

In the study conducted by [152] on 12 *Impatiens* species from Mt. Omei in southwest China, scanning electron microscopy (SEM) was employed to analyze seed morphology, revealing two distinct types of seed surface characteristics. The first type, represented by *I. wilsoni*, lacked noticeable surface differentiation, while the second type featured scabrous seeds with prominent cell elevations, found in the other 11 species examined. The study also correlated these seed types with pollen characteristics, noting that species with the first type of seeds exhibited 3-colpate pollen grains, whereas those with scabrous seeds had 4-colpate pollen grains. These findings indicate that seed morphology can serve as a valuable taxonomic character at the species level within the genus. In contrast, [156] examined the seed coat of 20 *Impatiens* species from Thailand, focusing on their classification and ecology. However, detailed information regarding the seed coat was limited, with only brief descriptions provided for three species. Notably, *I. chinensis* L. was recognized for its smooth seeds, *I. platypetala* Lindl. for its flat seeds covered in long hairs, and *I. violaeflora* Hook.f. for possessing two types of projections, large and small. In addition, the study conducted by [2] classified tropical African *Impatiens* seeds into five main categories: (I) **Smooth seeds**, which are generally large; (II) **Warted seeds**, which are medium-sized with a "warted" epidermis; (III) **Short-haired seeds**, which are medium-sized and covered, or partially covered, with short, hair-like protuberances; (IV) **Long-haired seeds**, characterized by small or large seeds with few to many, generally long, helical "hair-like" structures; and (V) **Seeds with club-shaped appendages**, covered or partly covered by stiff, "club-like" projections, featuring a network of thickenings and small, regularly spaced "windows," as seen in *I. gesneroidea*.

Consequently, compared to seeds, few taxonomists have studied the fruit in any detail, and it is assumed that the capsule is structurally quite uniform throughout the genus. However, [85] was the first one to study the fruit of the genus *Impatiens* and divided the genus into two major groups: the first group was fruit capsules short and swollen in the middle, and the second group contained fruit capsule terete or clavate [85]. However, little evidence was found to support Hooker's two fruit types, but fruit shape remained one of the important diagnostic features for separating closely related groups within the genus *Impatiens*. For example, *I. tinctoria* and *I. stuhlmannii* complexes of Africa generally contain similar floral and vegetative features, but the former has elongated fruit while the latter has short ovate fruit [2].

Apart from fruit shape, very few taxonomists have documented differences between the dehiscence mechanism found in various groups of *Impatiens*, but this must be mainly because there has been very little material has been available for this kind of study. Most herbarium specimens do not include mature fruit, and when they. do, it has been so damaged during pressing, that it is why usually mode of *Impatiens* dehiscence is usually overlooked [2].

Consequently, for many species of *Impatiens*, there is no information on the capsule whatsoever. This becomes evident when one undertakes a detailed study of the fruit characters presented in Hooker's Flora of British India in which for many species no fruit was available and so only a tentative placement in Series A or Series B could be made [73]. However, the fact that Hooker studied the fruit, tends to make one believe that the fruit is well-understood and offers no challenges. In Fact, [157] carried out a study that was based on greenhouse observation of *Impatiens* and found out that not all *Impatiens* dehiscence in the same way. In this study, they categorized fruit dehiscence into two types (Figure 2-2). Type l dehiscence begins at the midpoint of one suture, and proceeds outward, eventually causing the fruit to double over. The placenta and much of the seed are trapped until the fruit abscises several, days later. In Type II capsules, all five valves split at the sutures beginning at the Base and proceeding almost to the apex. The seed and capsule are thrust violently away from the parent plant. This is the type of fruit, that has been thought to characterize *Impatiens*, but this only occurs in only some relatively advanced phylogenetic lines. Thus, based on fruit dehiscence observation it seems that fruit is very much in need of further study as they seem to be of adequate taxonomic importance because of their large number of species for which fruit characteristics have never been observed, and it would not be surprising to find additional fruit types.

Therefore, a critical and comprehensive study of seed and capsule morphology as well as their manner of dehiscence of the African *Impatiens* species in the light of their phylogenetic relationships is needed. The information obtained will determine the usefulness of seed, capsule, and manner of dehiscence of *Impatiens* characters for taxonomic identification at specific and intraspecific levels and assess if these traits correspond with the phylogenic pattern.

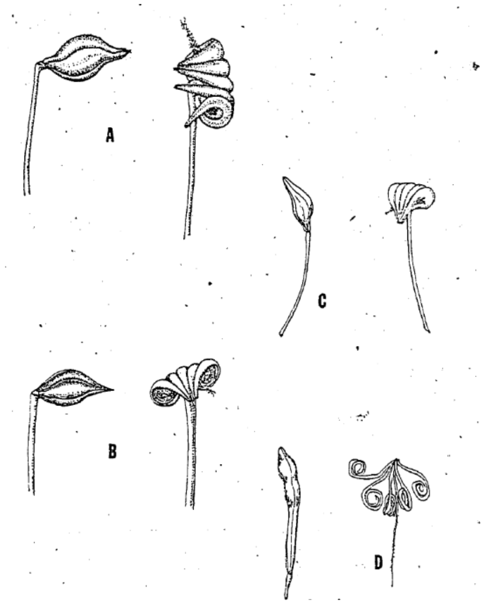

Figure 2-2 Mode of fruit Dehiscence: A. eg *I. flaccida* Arn Type I capsule, B. eg *I. niamnimensis* Gilg, Type I capsule, C. eg I. kleinii W.& A., Type I capsule, D.eg I. parviflora DC. Type II capsule

2.6 Problem Statement

Based on the above, it is clear that there is a significant gap in our knowledge of African *Impatiens* systematics regarding taxonomy, evolution, phylogeny, pollination biology, and biogeography. The genus *Impatiens*, a prominent component of the Balsaminaceae family, exhibits considerable diversity across Africa, yet its systematic understanding remains incomplete. Current taxonomic frameworks often rely on traditional morphological characteristics that are insufficient for accurately delineating species boundaries, resulting in misclassifications and obscured evolutionary relationships among African *Impatiens*. Although previous studies, including those by [12], [9] and [2] have emphasized the complexity of *Impatiens* taxonomy and evolution, they have been limited by a lack of comprehensive molecular data and insufficient sampling of African *Impatiens*. The reliance on morphological traits, particularly in a group characterized by high species diversity and convergent evolution, highlights the need for integrating modern molecular techniques to establish more robust phylogenetic frameworks. Additionally, the absence of complete chloroplast genome sequences for African *Impatiens* species further complicates efforts to clarify evolutionary lineages, especially for endemic taxa in this region.

Moreover, the ecological interactions of *Impatiens* species, particularly their pollination biology, have not been adequately studied. While some species are known to exhibit diverse pollination syndromes, there is a notable lack of detailed investigations into their specific pollinators and the ecological and evolutionary implications of these interactions. This knowledge gap is critical, as pollination mechanisms are closely linked to evolutionary processes, influencing floral traits, reproductive success, and speciation. Without a thorough understanding of these interactions, we risk overlooking important evolutionary dynamics that shape the diversity of *Impatiens* in Africa. To address these interconnected gaps, we will focus on East Africa as a case study. This region presents a unique opportunity to explore the diversity and evolutionary dynamics of *Impatiens* species within a specific ecological and geographical context because the region harbours the highest diversity and endemism of this genus in tropical Africa [56].

2.7 Justification of Study

This project will generate information that will help to advance our understanding of the systematics of the genus *Impatiens* in Tropical Africa, using an integrative approach, combining morphological analysis of *Impatiens* species and molecular phylogenetics. By updating species descriptions and resolving existing taxonomic ambiguities, the study will help to clarify species boundaries and evolutionary histories, which are essential for developing effective conservation strategies. Understanding the taxonomy and the evolution and diversification of the genus evolutionary relationships of *Impatiens* species in this region is crucial for effective conservation strategies, as current taxonomic frameworks often rely on inadequate morphological traits that may obscure true species boundaries and evolutionary histories. By integrating modern molecular techniques, this study aims to provide greater clarity in the classification of *Impatiens*, addressing significant knowledge gaps that hinder our understanding of the genus diversity. Additionally, the investigation of pollination biology is vital, as pollination mechanisms are closely linked to floral traits and reproductive success, influencing speciation processes. This research will explore specific pollinator interactions, elucidating how ecological relationships drive evolutionary changes within *Impatiens* species. Furthermore, the study will examine the morphological characteristics of seeds and fruits, which are essential for resolving taxonomic relationships and understanding evolutionary adaptations. As human activities increasingly threaten biodiversity, the findings from this research will contribute critical data necessary for the formulation of informed conservation strategies and sustainable management practices. Ultimately, this study not only aims to enhance our understanding of *Impatiens* diversity, evolution, diversification and ecology in East Africa but also seeks to provide broader implications for the field of plant systematics, offering insights that could inform similar research in other taxonomic groups facing analogous challenges.

2.8 Research Objectives

2.8.1 Main objective

This research project aims to employ both phylogenomic and taxonomic methods to examine tropical African *Impatiens*, with a special focus on Eastern African species. Both molecular and morphological investigations will be carried out to infer the evolutionary relationships and biogeographic histories of the genus *Impatiens* in tropical Africa with a special focus on *Impatiens* from East Africa. Despite the considerable number of authors who have dealt with tropical African *Impatiens*, to date there are no comprehensive and systematic reviews on this genus that have recently been carried out in the continent since that of [2], however, some sporadic publications are available [45,158]. Because tropical Africa is one of the most important regions of biodiversity and centers of endemism of the genus *Impatiens*, these knowledge gaps need to be filled. Therefore, this study will conduct a comprehensive and systematic study of tropical African *Impatiens* focusing on the Eastern Africa region to generate updated information on the taxonomy, biogeography, pollination biology, and evolution of the genus in the region based on modern principles of phylogenomic and the accumulated information since Grey-Wilson's previous morphology-based classification from 1980 [2]. The phylogeny of the genus *Impatiens* will then be constructed using significantly extended taxon sampling that will include representatives of two Sections that include African *Impatiens* (sections *Uniflorae* and

Tuberosae) and expand the existing dataset based on Complete Chloroplast DNA (cp) Genome and Nuclear ribosomal DNA (nrDNA).

2.8.2 Specific objectives

To improve the checklist of the *Impatiens* of Eastern Africa and resolve their phylogenetic relationships and taxonomic complex groups as well as assess the evolution and biogeographic history of *Impatiens* occurring in tropical Africa the proposed project aims at;

1) To revise the genus *Impatiens* of Tropical Africa using both morphological and molecular approaches and address uncertainties in tropical Africa, with a specific focus on the Eastern Africa region. This will involve performing morphometric analyses, updating species delimitations and descriptions, creating a diagnostic key, and developing distribution maps for the species in the region. The integrative approach will allow for a more comprehensive and accurate assessment of the phylogeny of *Impatiens* species, shedding light on their evolutionary history and genetic relationships.
2) To review and expand the family-wide phylogenetic study that was recently conducted by [12] by utilizing morphological data together with single-copy gene SNPs, complete chloroplast (cp) genome data, nuclear ribosomal DNA (nrDNA) data and extensive taxon sampling to elucidate a well-supported phylogeny of *Impatiens* species from the Eastern African region and those from adjacent regions and continents for future evolutionary studies.
3) To test the monophyly of *Impatiens* sections *Uniflorae* and *Tuberosae*, and reconstruct the character evolution of key traits for these sections, identifying suitable diagnostic characters, and refining the taxonomic delimitation of these sections.
4) To identify effective pollinators of *Impatiens* by analyzing the morphological characteristics of flowers and their respective flower visitors. This will also involve testing whether floral traits (i.e. morphology, color, scent, size, and rewards) can accurately predict the various functional groups of pollinators within the genus *Impatiens*, using both field-collected data and literature sources. Additionally, the study will investigate morphological changes associated with pollination

syndromes across the genus to determine if inflorescence traits are constrained by the phylogenetic relationships of African *Impatiens* species and whether these traits evolved in a correlated manner. Finally, we aim to assess if differing rates of diversification are linked to traits associated with pollination syndromes.

5) To explore and compare the spatial genetic structure and reconstruct the biogeographic history of *Impatiens* species by describing the distribution of genetic variation across populations within the Afromontane forests of Africa. This will include examining the impact of Pleistocene climatic fluctuations on population ranges, particularly concerning the "mountain-forest bridge" hypothesis and the role of isolated "sky islands." Ultimately, this will provide insights into the biogeography of Afromontane plant species with significant species diversity and endemism, given that the genus *Impatiens* is closely associated with high endemism and dominates these Afromontane Forest ecosystems.
6) To investigate possible hybridization and introgression and test hypotheses of interspecific gene flow events within the genus *Impatiens*
7) To investigate the long-term evolutionary impact of hybridization within the genus *Impatiens* by identifying hybrid specimens, comparing relative rates of species formation with parent species, and reconstructing historical and geographical ranges.
8) To analyze the quantitative and qualitative macro- and micromorphological traits of seeds and capsules from East African *Impatiens* species, assessing the utility of seed and carpological characteristics for taxonomic identification at both specific and intraspecific levels, while examining the congruence of seed and capsule morphological traits with phylogenetic patterns.

2.9 Research Questions

1) How can the integrative approach of using both morphological and molecular phylogenetic methods reshape our understanding of species relationships within the African *Impatiens*, and what implications does this have for its classification and conservation?

2) What are the phylogenetic relationships among species within the African *Impatiens* as revealed by integrated molecular and morphological data, and how do these relationships inform our understanding of their evolutionary history?
3) Are the sections *Uniflorae* and *Tuberosae* of *Impatiens* monophyletic, and what key traits can be identified to refine the taxonomic delimitation of these sections through character evolution analysis?
4) What are the key pollinators of the species of East African *Impatiens*? What morphological traits of *Impatiens* flowers are most effective in predicting pollinator functional groups, and how do these traits vary in relation to phylogenetic relationships and pollination syndromes across the genus?
5) How does the spatial genetic structure of *Impatiens* species within Afromontane forests reflect the impact of Pleistocene climatic fluctuations, particularly in relation to the "mountain-forest bridge" hypothesis and isolated "sky islands"?
6) How do the macro- and micromorphological traits of seeds and capsules from East African *Impatiens* species contribute to taxonomic identification, and what congruence exists between these traits and phylogenetic patterns
7) How can hybridization and introgression lead to the formation of *Impatiens* species complexes in the East African Region?
8) How have historical climatic fluctuations and geographical barriers influenced the speciation and distribution patterns of *Impatiens* species in Eastern Africa, as revealed through complete chloroplast genome sequence data?

Chapter 3 Materials and Methods

3.1 Study Area

The study will be conducted within the Eastern Africa region, which encompasses a diverse array of Afromontane forests and highlands that are critical for exploring the systematics, taxonomy, evolution and biogeography of *Impatiens* L. (Balsaminaceae). The Eastern Africa region includes high-altitude montane forests, grasslands, and wetlands, characterized by unique climatic conditions and a rich diversity of flora and fauna[67,159] . Key areas of focus will include the Afromontane forests and highlands located within East African countries (i.e. Tanzania, Kenya, Rwanda, Burundi, Ethiopia, DRC Congo and Uganda) (Figure 3-1). The Eastern Africa region contains two centres of *Impatiens* species diversity and endemism within tropical Africa such as the Eastern Arc Mountains, which stretch from northeastern Tanzania to southeastern Kenya, and the Albertine Rift and both are renowned for their endemic species and significant ecological importance [56] . Generally, these Afromontane forests and highlands within the East Africa region harbor a variety of *Impatiens* species, many of which are endemic and adapted to specific microhabitats [2,56]. The varied topography, ranging from steep slopes to valley floors, along with a wide range of altitudes and moisture gradients, contributes to high levels of biodiversity and complex ecological interactions. By conducting field surveys and sampling in these critical areas, the study aims to capture the taxonomic as well as ecological and evolutionary dynamics of *Impatiens*, and provide insights into their origin and diversification patterns and conservation needs within this biodiverse region.

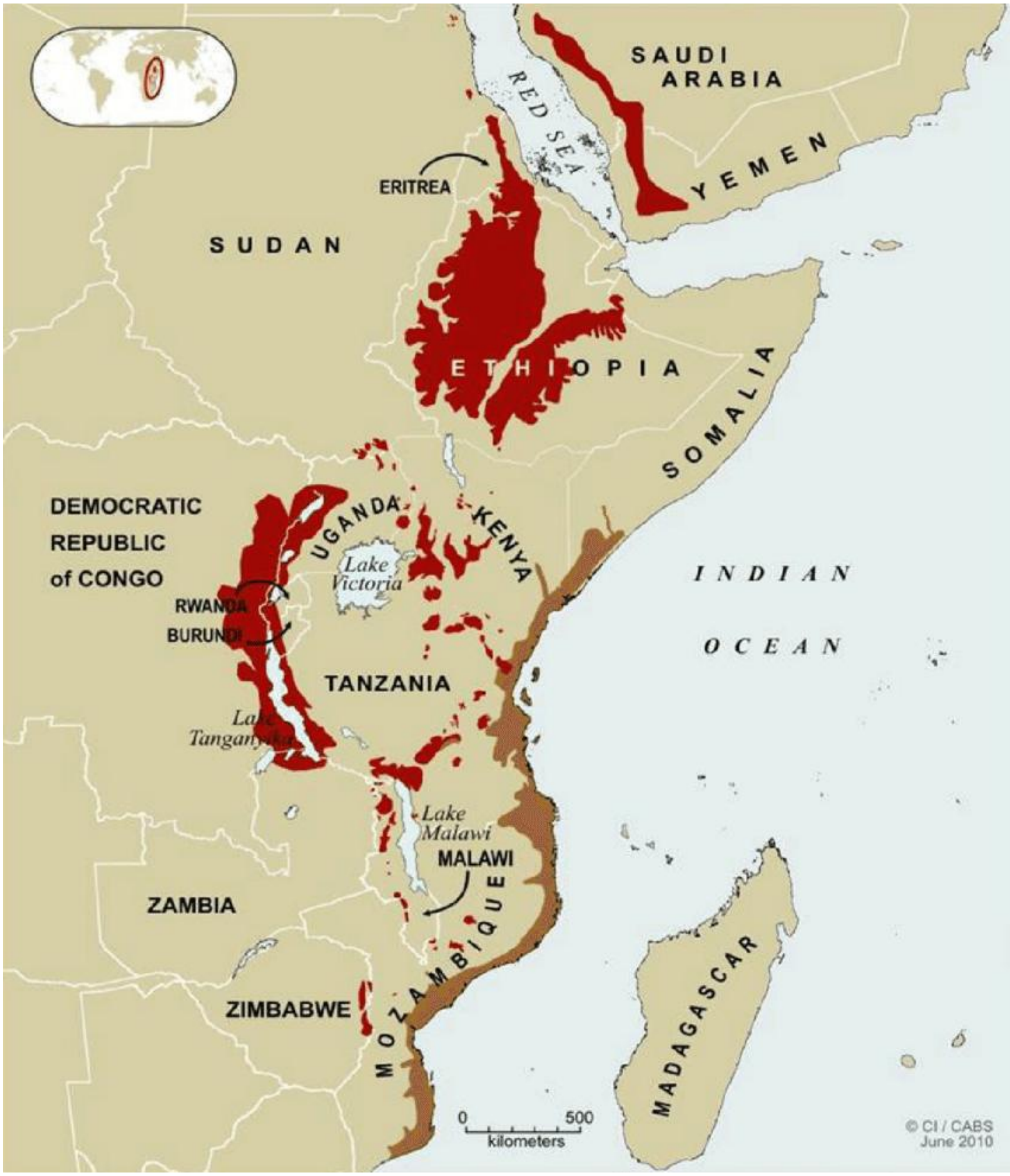

Figure 0-1 The map showing the Afromontane forests and highlands along the Eastern Afromontane hotspot (in red)

3.2 Data collection

3.2.1 Taxonomic and Morphological Data Collection

3.2.1.1 Literature Review and Herbarium Samples

A comprehensive review of existing literature related to the genus *Impatiens* will be conducted, focusing on taxonomic revisions, species descriptions, and distribution data. Herbarium collections from various institutions will be investigated to examine historical and contemporary specimens, noting morphological characteristics crucial for species identification.

3.2.1.2 Field Data Collections

Data collection for this study will be prioritized in under-sampled areas within the East African countries located along the Eastern African Afromontane biogeographic region (EABR) (i.e. Tanzania, Kenya, Rwanda, Burundi, Ethiopia, DRC Congo and Uganda). During this period, live specimens, including herbarium samples, flowers, mature fruits, and seeds, will be collected. Morphological traits will be documented to provide essential taxonomic information. Appropriate collection permits will be used, and ethical guidelines for plant collection will be adhered to.

3.2.1.3 Floral Component Documentation

Floral components (e.g., petals, sepals) will be carefully flattened and secured onto sturdy paper using herbarium-grade adhesives to preserve maximum diagnostic information. This method will minimise damage and preserve the integrity of the specimens, allowing for detailed morphological analysis.

3.2.2 Genetic Data Collection

Fresh leaves will be collected from multiple individuals of each species to ensure genetic diversity. The leaves will be immediately placed in silica gel within sealable plastic bags to facilitate rapid drying, which is critical for preserving DNA quality. Each sample will be labelled with collection data (e.g., location, date, collector's name) for traceability and future reference.

3.2.3 Pollinators and Pollination Biology Data Collection

3.2.3.1 Flower Morphometry and Signals

Functional and morphometric traits will be measured using calipers or digital measuring tools. Key traits to measure will include spur length, total flower length, flower width, and dorsal petal length. Standardized frontal and lateral photographs of flowers will be captured to analyze visual signals, ensuring consistent lighting and background for accurate pixel count analysis.

3.2.3.2 Pollinator Observations

Systematic observations of pollinator activity will be conducted during peak flowering periods. Observation points will be set up 2.5 to 3.5 meters from the subject plants to minimize disturbance. The frequency of visits, duration of each visit, and identification of pollinator species will be recorded using field guides and expert consultation. Data collection will span 2 to 3 days for each species across two to four populations.

3.2.3.3 Nectar Sampling

Nectar samples will be collected from about ten flowers per population during morning hours when insect activity is minimal. Nectar volume will be measured using a calibrated micropipette, and samples will be stored in Eppendorf tubes for subsequent laboratory analysis via paper chromatography to determine sugar composition.

3.2.3.4 Insect Behavior Documentation

High-quality photographs of visiting insects will be captured to document behaviour and interactions with flowers. Insect specimens will be collected using nets and preserved in 70% ethanol for morphological analysis. Proboscis length and other relevant features will be measured to assess adaptation to floral traits.

3.2.3.5 Functional Group Classification and Visitation Rates

Visitor Species Categorization Observed pollinators will be classified into functional groups based on established methodologies from the literature [2]. Functional groups may include bees, birds, butterflies, and day-flying hawkmoths. Visitation rates will be calculated by dividing the total number of visits by the total observation hours for each *Impatiens* species.

3.2.3.6 Interspecific Pollinator Movement Observation

Pollinator visitation patterns among *Impatiens* species growing in close proximity (1 to 5 meters apart) will be observed. Interspecific pollen flow will be recorded by monitoring the movement of pollinators between different species, noting any potential for hybridization or shared pollinator networks.

3.2.4 Pollinator Effectiveness Assessment

3.2.4.1 Flower Bud Covering

Before flowering, selected flower buds will be covered in the evening with 2 mm netting to exclude visitors while allowing natural environmental conditions. Once flowers open, the netting will be removed, and visitor interactions will be documented, timing each visit with a stopwatch for accurate duration measurements.

3.2.4.2 Video Recordings of Feeding Durations

Video cameras will be set up to record feeding durations of bird visitors, particularly those that hover between flower visits. The footage will be analyzed to obtain precise data on mean feeding times and behaviour patterns, helping to assess pollinator effectiveness.

3.2.5 Ecological and Environmental Data Collection

Comprehensive ecological data, including species distribution, altitude, habitat type, and growth forms will also be collected during fieldwork. GPS devices will be used for accurate location tracking, and field data will be supplemented with existing literature and herbarium records to enhance the contextual understanding of *Impatiens* species distribution and ecology.

3.3 Expected Outcome

1) A systematic review of the genus *Impatiens* in tropical Africa will be conducted, focusing on Eastern Africa. This will include updated species delimitations and descriptions, a diagnostic key, and detailed distribution maps for the region's species.
2) Improved understanding of the monophyly of *Impatiens* sections *Uniflorae* and *Tuberosae* will be achieved through the expanded phylogenetic study, which will provide insights into character evolution, suitable diagnostic characters, and refined taxonomic delimitations.

3) Identification of effective pollinators of *Impatiens* will be realized by analyzing floral morphological characteristics and their relationships with flower visitors. This will lead to insights into how floral traits can predict the functional groups of pollinators and an understanding of the evolutionary changes linked to pollination syndromes across the genus.
4) Phylogenetic relationships among *Impatiens* species from Eastern Africa and adjacent regions will be elucidated through the utilization of complete chloroplast genome data, improving our comprehension of species relationships.
5) Insights into the spatial genetic structure and biogeographic history of *Impatiens* species will be provided, highlighting the impact of Pleistocene climatic fluctuations on population ranges and contributing to a better understanding of the biodiversity and endemism within Afromontane Forest ecosystems.
6) The long-term evolutionary impact of hybridization and introgression within the genus *Impatiens* will be investigated, which will identify hybrid specimens and compare rates of species formation, enhancing our understanding of species evolution and distribution patterns.
7) Analysis of macro- and micromorphological traits of seeds and capsules from East African *Impatiens* species will generate important information regarding the utility of these characteristics for taxonomic identification while examining their congruence with phylogenetic patterns.

3.4 Proposed Project Timeline

Table 0-1 Proposed Timeline

Activity	**2025**												**2026**											
Activity	**J**	**F**	**M**	**A**	**M**	**J**	**J**	**A**	**S**	**O**	**N**	**D**	**J**	**F**	**M**	**A**	**M**	**J**	**J**	**A**	**S**	**O**	**N**	**D**
Reviewing data collection methods																								
Research Permits Application																								
Data collection																								
Data Analysis																								
Framework Development																								
Chapter 1 (write, comments & Edits)																								
Chapter 2 (write, comments & Edits)																								
Chapter 3 (write, comments & Edits)																								
Chapter 4 (write, comments & Edits)																								
Chapter 6 (write, comments & Edits)																								
Chapter 7 (write, comments & Edits)																								
Chapter 8 (write, comments & Edits)																								
Full proofreading/edits																								
Final Plagiarism check																								
Final submission																								

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