ÌNDICE

RESUMO

As espécies do género *Impatiens* L. (Balsaminaceae) em São Tomé e Príncipe são sujeitas a uma revisão sistemática. As quatro espécies referenciadas até ao momento *I. buccinalis* Hooker.f, *I. manteroana* Exell e *I. thomensis* Exell (endémicas para o território) e *I. balsamina* L. (cultivada, nativa da Índia), são descritas taxonomicamente com base no acréscimo de colheitas disponível (incluindo colheitas realizadas no âmbito deste estudo). As quatro espécies foram sujeitas a análises morfométricas e uma chave dicotómica foi produzida. O pólen destas espécies foi também analisado por microscopia óptica e electrónica de varrimento e uma chave dicotómica para o pólen foi elaborada. As mesmas espécies foram analisadas do ponto de vista molecular ao nível das regiões ITS e trnL-F. A possível origem híbrida de *I. thomensis* a partir de um cruzamento de *I. buccinalis* x *I. balsamina*, foi investigada. O estatuto de *I. tamsiana* Exell (= ***I. balsamina***) foi avaliado.

I. wallerana Hooker.f., uma cultivada nativa da África Centro-oriental foi assinalada pela primeira vez para o território. Por ser tratar de uma cultivada, apenas com uma colheita e sem referências prévias na bibliografia, esta espécie não foi abrangida no tratamento taxonómico ou palinológico tendo apenas sido considerada para efeitos de análise molecular.

A posição taxonómica das três endémicas de STP é mantida. Os resultados da análise ITS indicam que *I. thomensis* e *I. buccinalis* podem ser taxa irmanados formando um grupo monofilético. Os dados da análise combinada ITS/trnL-F apontam, com algumas reservas, para o facto de *I. manteroana* poder formar um grupo monofilético com as espécies de São Tomé. Tanto os dados da análise morfométrica como os dados moleculares demonstram a ausência de relação entre a introduzida *I. balsamina* e as três endémicas de São Tomé não

suportando assim a hipótese de origem híbrida de *I. thomensis* avançada por outros autores. Este trabalho suporta a classificação, por outros autores, de *I. tamsiana* como um sinónimo de *I. balsamina*.

CAPÍTULO 1   Introdução e Objectivos

1.1 INTRODUÇÃO

1.1.1 O género *Impatiens* L. (Balsaminaceae)

A família Balsaminaceae DC. contém dois géneros actualmente reconhecidos: o género monotípico *Hydrocera* Blume e o prolífico género *Impatiens* L.

O género *Impatiens*, encontra-se maioritariamente distribuído pelas zonas montanhosas das regiões tropicais e sub-tropicais do Velho-Mundo (África, Sudoeste Asiático, Índia, Sul da China e Japão), com algumas espécies a estenderem-se à zona temperada do Norte da Europa, territórios da ex-U.S.S.R., China e América do Norte.

Yuan *et al.* (2004), com base em estudos moleculares da região ITS (Internal Transcribed Spacer), demonstraram a monofilia da família Balsaminaceae e o facto de as espécies Africanas de *Impatiens* não serem monofiléticas mas derivarem de pelo menos duas colonizações independentes do continente. Janssens *et al.* (2006), usando sequências cloroplastídicas *atpB-rbcL*, confirmaram a monofilia da família e a sua provável origem no Sul da China a partir de onde o género terá radiado. Estes resultados são consistentes com o hipotético caminho de migração do género em África proposto por Grey-Wilson (1980a), tendo como base as zonas montanhosas da África Oriental, estendendo-se depois para Oeste.

O número de espécies de *Impatiens* está estimado entre 850 (Grey-Wilson, 1980a) e 1000 (Janssens *et al.*, 2006). Grey-Wilson (1980a), na sua revisão dos *Impatiens* africanos, aponta um número de 109 espécies nativas para o continente (excluindo Madagáscar). No entanto, este número tem crescido de ano para ano fruto da descrição de novas espécies e

de revisões taxonómicas (e.g. Grey-Wilson, 1980c; Hallé & Louis, 1989; Boss, 1991; Fischer, 1997; Cheek & Fischer, 1999; Frimodt-Møller & Grey-Wilson, 1999).

As espécies de *Impatiens* africanas são elementos típicos da flora hidrófita do continente e a grande maioria cresce em áreas de floresta ou de pastagens de altitude, com um regime intenso, sazonal ou não, de pluviosidade. A maioria dos taxa são portanto espécies de montanha e muito poucas espécies se encontram abaixo dos 800 m de altitude, sendo que estas últimas são as que, naturalmente, apresentam distribuição mais alargada. Para além disso, a maioria das espécies encontra-se, normalmente, restringida a pequenas áreas geográficas (normalmente uma montanha ou cadeia de montanhas). Esta distribuição parece resultar dos sucessivos períodos de seca a que a floresta tropical Africana foi sujeita desde o Oligoceno (há cerca de 27 m.a.), que terão restringido as espécies de *Impatiens* (com elevados requerimentos em termos de regime hídrico), a áreas mais favoráveis (Grey-Wilson, 1980a). Um bom exemplo, entre outros em África, são o Monte Camarões e as zonas montanhosas do vizinho Gabão (zonas particularmente ricas em espécies endémicas), a partir das quais se terá processado uma nova expansão do género aquando do reaparecimento de um clima pautado por uma maior pluviosidade.

O género *Impatiens* é assim caracterizado pela existência de um grande número de endemismos que parecem indiciar uma evolução recente e activa fazendo com que muitos *taxa* possam ser vistos como neo-endémicos (Grey-Wilson, 1980a). A elevada diversidade do género e sua distribuição geográfica bem como a grande (e por vezes bizarra) variabilidade de tipos de inflorescência tornam difícil a divisão do género em grupos naturais (Grey-Wilson, 1980b). O mesmo autor (1980a, 1980b) refere que, pelo menos no que concerne às espécies africanas, a difícil definição de fronteiras entre grupos se deve à

existência de intermediários, possivelmente de origem híbrida. Muitas vezes estes intermediários parecem preencher vazios entre *taxa* muito diferentes morfologicamente.

Outro dos factores que tem contribuído para um menor esclarecimento das relações inter-específicas dentro do género está relacionado com as dificuldades de observação e estudo de material de herbário (muitas vezes mal preparado ou conservado). As espécies de *Impatiens* apresentam normalmente flores delicadas, com estruturas florais (particularmente pétalas) que, quando espalmadas perdem frequentemente a sua posição original e se tornam tão finas que a sua posterior dissecação representa um verdadeiro desafio à paciência exigindo um cuidado particular no seu tratamento. Grey-Wilson (1980a), no final da sua monografia, apresenta algumas indicações úteis quanto à análise de material de herbário.

1.1.2 *Impatiens* de São Tomé e Príncipe

Quatro espécies de *Impatiens* foram dadas até ao momento para o arquipélago de São Tomé e Príncipe (STP).

Hooker (1864), descreveu *I. buccinalis* Hooker.f. (Fig. 1 A,B), a primeira espécie endémica a ser assinalada para a Ilha de São Tomé. Exell (1944), no seu Catálogo das Plantas Vasculares de STP (o trabalho taxonómico de referência para o estudo dos *Impatiens* de STP), descreveu mais três endémicas: *I. manteroana* Exell (endémica para a Ilha do Príncipe, Fig. 1 C,D), *I. thomensis* Exell (endémica para a Ilha de São Tomé. Figs. 1 E, 2 A) e *I. tamsiana* Exell, supostamente endémica para a Ilha de São Tomé, mais tarde reconhecida por Grey-Wilson (1980a) como um sinónimo para *I. balsamina* L.. Exell, na mesma obra, assinalou ainda a existência de *I. balsamina* (Fig. 1 F, 2 B) em STP, uma espécie introduzida originária da Índia, presente em ambas as ilhas e usada como

ornamental em alguns jardins, colhida pela primeira vez por Chevalier (n.c. 14372) em 1905. Grey-Wilson (1980a), refere-se a *I. balsamina* como estando naturalizada em STP, no entanto, um recente trabalho de campo levado a cabo por mim, em ambas as ilhas, não revelou a presença desta espécie no estado selvagem mas apenas em alguns (poucos) jardins. Este trabalho regista uma quinta espécie - *I. wallerana* Hooker.f.. Este *taxon*, cultivado e nativo da África Centro-Oriental, foi colhido num jardim em Nova Moka (Soares M. 46, COI; Paiva 1600, COI). Por ser uma cultivada, apenas com uma localização de colheita e sem referências prévias na bibliografia esta espécie não foi abrangida no tratamento taxonómico ou palinológico tendo apenas sido considerada para efeitos de análise molecular.

Mais recentemente a monografia sobre os *Impatiens* africanos de Grey-Wilson (1980a), abordou as três endémicas de STP e a relação destas com as restantes espécies africanas do mesmo género. Outros trabalhos de referência incluem Exell (1956, 1973a) e ainda Sobrinho (1959), Figueiredo (1994) e Joffroy (2000).

Em termos de palinologia, Huynh (1968a), abordou brevemente a morfologia dos grãos de pólen de três das cinco espécies de *Impatiens* ocorrentes em STP: *I. tamsiana* (= ***I. balsamina***), *I. buccinalis* e *I. manteroana*. No entanto este autor baseou a sua análise apenas num exemplar por espécie, o que faz com que os seus dados, apesar de informativos, devam ser encarados com algumas reservas.

Apesar de nos últimos anos vários estudos se terem dedicado a questões de filogenia do género *Impatiens* a nível mundial (Fujihashi et al., 2002; Yuan et al., 2004; Janssens et al., 2006; Janssens *et al.*, 2007), incluindo nalguns casos espécies africanas, nenhum destes trabalhos incluiu qualquer das três endémicas de STP.

No que se refere à sua origem, Grey-Wilson (1980a), avança a hipótese das espécies de *Impatiens* de STP derivarem de uma migração do género que teria como ponto de radiação o Monte Camarões, passando pela ilha de Bioko. Exell (1958), no entanto, referindo-se à colonização das ilhas de STP, adiantou a hipótese de estas terem poderem ter atingido condições favoráveis de colonização mais cedo do que o pico Clarence (na ilha de Bioko) e o Monte Camarões devido ao abrandamento da actividade vulcânica. STP teria então funcionado como um refúgio para uma série de espécies (que poderiam entretanto ter-se extinto no continente), antes dos dois outros picos se terem tornado suficientemente inactivos para providenciar um habitat adequado.

Em termos de endemismo, as ilhas de São Tomé e do Príncipe são as que maior taxa de endemismo apresentam dentro das ilhas do Golfo da Guiné, com, respectivamente 15,4% e 9,9% (Exell, 1973a).

O mesmo autor (1973b), baseando-se numa análise das floras do Sul da Nigéria, Camarões, Bioko, Annobon e São Tomé e Príncipe e através de um Quociente de Afinidade, concluiu também que as ilhas de São Tomé e do Príncipe terão recebido independentemente as suas quotas de espécies colonizadoras.
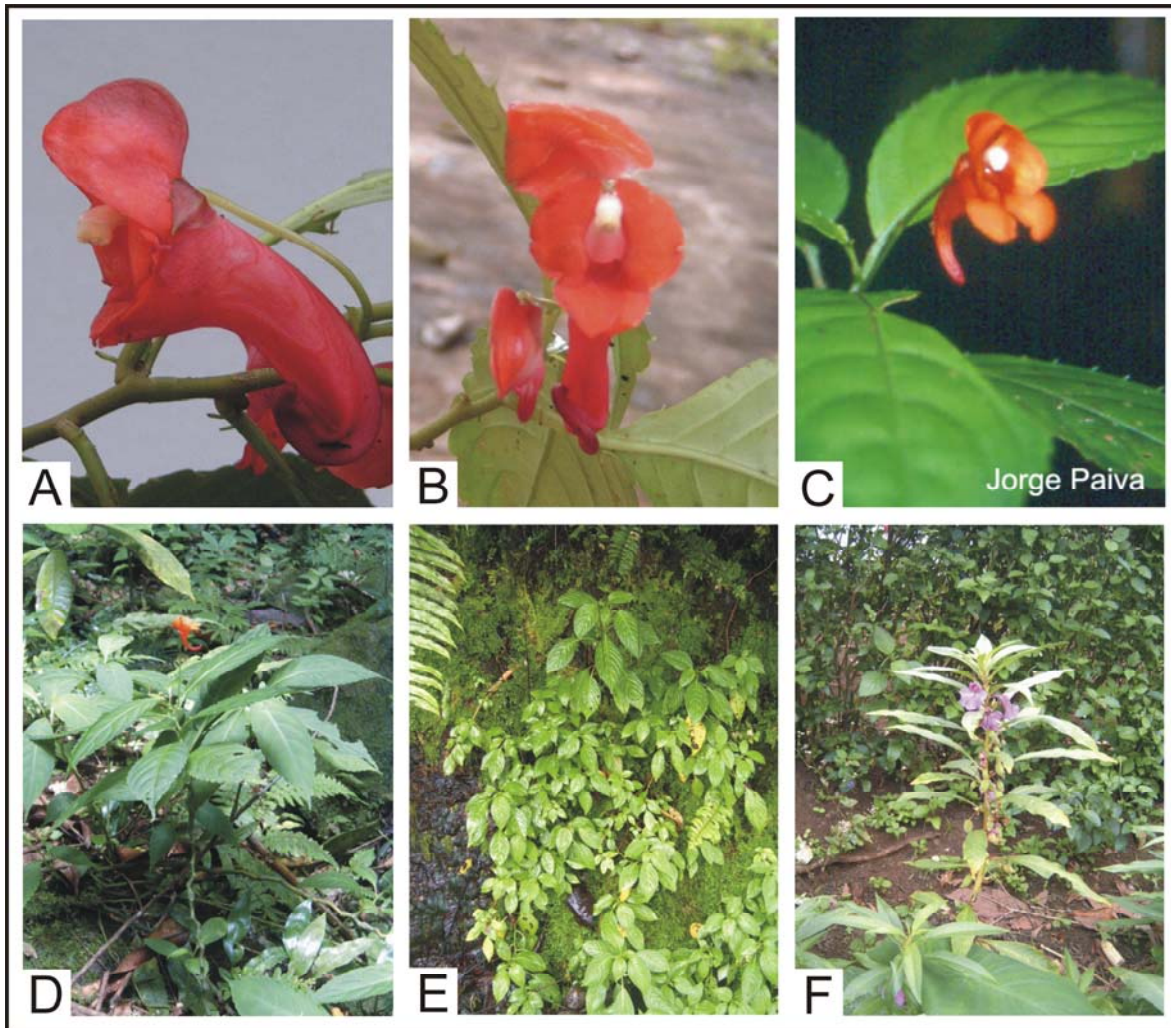
Figura 1. Imagens das quatro espécies de *Impatiens* abrangidas por este

estudo. A,B - *I. buccinalis*. C,D – *I. manteroana*. E – *I. thomensis*. F – *I.*
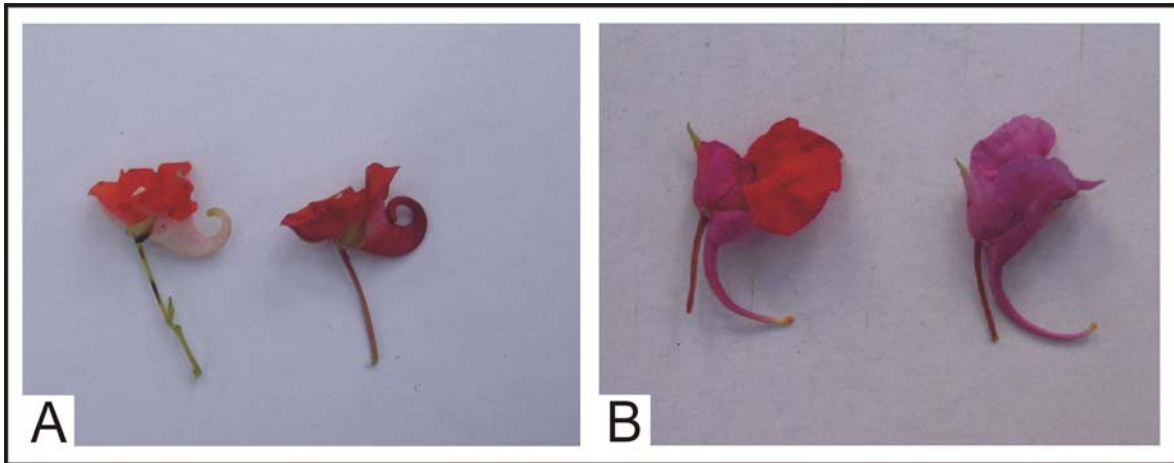
balsamina.

Figura 2. Pormenor das flores de A) *I. thomensis* e B) *I. balsamina*. Em ambos os casos, as duas flores pertenciam ao mesmo exemplar.

1.1.3 Descrição da área de estudo

O arquipélago de São Tomé e Príncipe (STP), é constituído por um conjunto de ilhas vulcânicas. Estas ilhas derivam de actividade vulcânica ocorrida durante o Terciário, e integram-se numa linha de actividade vulcânica quase extinta que se estende na direcção NE-SW a partir das terras altas dos Camarões (Mts. Kupe e Manengouba) até à Ilha de Santa Helena (Furon 1963; Piper & Richardson 1972).

O arquipélago de STP é constituído por duas ilhas principais: Ilha de São Tomé e Ilha do Príncipe (abrangidas por este estudo) e por uma série de ilhéus (Rolas, Cabras, Tinhosa Grande, Tinhosa Pequena, Caroço ou Boné de Jóquei e Bombom).

A Ilha do Príncipe (1º32'N-1º43'N, 7º20'E-7º28'E), encontra-se a cerca de 210 km a Su-sudoeste da Ilha de Bioko e a 220 km de distância do continente. Esta ilha tem o seu ponto mais elevado no Pico do Príncipe a 948 m de altitude. A Ilha de São Tomé (0º25'N-0º01'S,

6º28'E-6º45'E), situa-se a 150 km a Su-sudoeste do Príncipe e a cerca de 255 km da costa Noroeste do Gabão. Tem o seu ponto mais alto no Pico Grande, a 2024 m de altitude. Há evidências (Exell, 1973b; White, 1984), de que as ilhas de São Tomé e do Príncipe nunca estiveram ligadas entre si ou ao continente ao contrário da Ilha de Bioko (Exell, 1944). É portanto seguro admitir que toda a colonização biológica das ilhas se tenha, em última análise, feito por via de dispersão a longa distância com base em ventos, correntes marítimas, aves e mamíferos voadores e/ou intervenção humana. Esta ter-se-á verificado apenas a partir do séc. XV, aquando da chegada dos primeiros navegadores Portugueses, uma vez que o arquipélago de São Tomé e Príncipe se encontrava desabitado até à altura.

Em termos do coberto vegetal, Exell (1944) propôs uma classificação da vegetação de STP, mais tarde ligeiramente modificada por Monod (1960). De acordo com esta classificação, a vegetação da Ilha de São Tomé pode ser divida em quatro regiões, definidas pela ocorrência de espécies características. Apresenta-se a seguir uma breve descrição de cada uma das regiões:

1. Região Litoral (Dunas e Mangal) – Esta região ocupa uma pequena área espacial da ilha e, de acordo com Exell, é provável que a floresta se estendesse até à costa antes do estabelecimento dos colonos, o que de resto é ainda visível nalgumas partes da ilha. A região de mangal é ainda mais reduzida estando restringida principalmente à zona Sul da Ilha.

2. Floresta densa húmida de baixa altitude (0-800 m) – Esta floresta estava já, à altura do trabalho de Exell (1944), bastante degradada devido, principalmente, ao cultivo do cacau, do café e de outras culturas menores. A descrição da vegetação típica desta região foi baseada quer em colheitas antigas quer nos trabalhos de Júlio Henriques. Dada a assimetria

verificada na ilha em termos de pluviosidade, a zona norte da ilha recebe menos precipitação apresentando assim uma vegetação tipo savana, com predominância de gramíneas e ocorrência de embondeiros (*Adansonia digitata* L.). Existem indicações de que o abandono das plantações de cacau e coqueiros tem promovido o crescimento de um coberto florestal secundário tornando-se por vezes difícil discernir os limites entre as manchas de floresta primordial (camadas "obó") e as decorrentes de culturas abandonadas ("capoeira") (UICN, 1991).

3. Floresta densa húmida de montanha (800-1400 m) – A partir dos 800 m de altitude, onde a cultura do cacau deixa de ser viável, a floresta de montanha parece estar ainda relativamente intacta. Esta zona é caracterizada por humidade e precipitação bastante elevadas e uma acentuada descida da temperatura mínima.

4. Floresta de nevoeiro (1400-2024 m) – Esta zona é caracterizada por temperaturas mais baixas, maior precipitação e intensa formação de nevoeiros.

A vegetação da Ilha do Príncipe apresenta semelhanças com a de São Tomé. Apesar de possuir áreas de mangal, esta ilha não apresenta zonas de savana. A floresta assemelha-se à floresta de baixa altitude da Ilha de São Tomé. Algumas áreas mais elevadas, no entanto, apresentam uma vegetação de carácter mais montanhoso. A floresta de nevoeiro encontra-se ausente excepção feita ao cume do Pico do Príncipe que, por se encontrar frequentemente encoberto, apresenta algumas espécimes que podem indiciar uma tendência para esse tipo de vegetação (Figueiredo, 1995).

1.2 OBJECTIVOS

Este trabalho teve como objectivo principais:

1. Levar a cabo uma revisão Sistemática do género *Impatiens* em STP face ao crescente número de colheitas disponíveis. Proceder a análises morfométricas, descrever taxonomicamente as quatro espécies ocorrentes em STP e elaborar uma chave dicotómica.

2. Abordar as principais questões envolvendo o género *Impatiens* em STP: o estatuto de *I. tamsiana* (= *I. balsamina*), e a possível origem híbrida de *I. thomensis* com *I. buccinalis* e *I. balsamina* como potenciais progenitores, proposta por Grey-Wilson (1980a, 1980b).

3. Descrever palinologicamente o género *Impatiens* em STP apresentando pela primeira vez uma descrição do pólen de *I. thomensis*. Elaborar uma chave dicotómica para o pólen. Avaliar o interesse da palinotaxonomia no género em STP.

4. Recorrendo a dados moleculares (ITS e trnL-F), tentar elucidar as relações filogenéticas entre as espécies de *Impatiens* de STP e a das ilhas e continente adjacentes. Disponibilizar em bases de dados internacionais de livre acesso as sequências moleculares obtidas.

Capítulo 2

Systematics of the *Impatiens* L. (Balsaminaceae) in São Tomé e Príncipe

Abstract

A contribution to the knowledge of the genus *Impatiens* (Balsaminaceae) in São Tomé e Príncipe (STP) is presented. Principal Component Analysis and Cluster Analyses (UPGMA) were performed to elucidate the relationships between the four *Impatiens* species given up to now to the archipelago, namely the possible hybrid origin of *Impatiens thomensis* Exell from the crossing of *I. buccinalis* Hook.f. x *I. balsamina* L. Two datasets were analyzed, one comprising morphological data concerning stem and leaves and the other including the previous dataset plus flower, fruit and seed information. The analyses of both datasets resulted in a good delimitation of the four species as well as a clear separation of *I. balsamina* from the three STP endemics not supporting its relation to either *I. buccinalis* or *I. thomensis* as advanced by other authors. Phenetic analysis revealed a close relationship between the three STP endemics. The four species are described and a dichotomous key is presented. Pollen morphology is also described and was statistically analyzed. A description of the pollen morphology of I. thomensis is given for the first time.

A key with pollen characters is presented. The presence of *I. wallerana* Hook.f., a cultivated species native to East Africa is registered for STP.

KEY WORDS: *Balsaminaceae, Impatiens, systematics, palynology.*

INTRODUCTION

Impatiens L. (Balsaminaceae), is mainly a tropical or sub-tropical genus occurring particularly on highlands and mountainous habitats in most of the tropical areas from Africa, India, South-west Asia, China and Japan with some species being found in temperate zones of Europe, and North America (Grey-Wilson 1980a). The number of *Impatiens* species is estimated to be between 850 (Grey-Wilson 1980a) and 1000 (Janssens *et al.* 2006). Grey-Wilson (1980a), in its revision of the African *Impatiens*, estimates a number of 109 species native to the continent (excluding Madagascar), but this number has since increased due to the description of new species and taxonomic changes.

Four species of *Impatiens* were given, up to now, to São Tomé e Príncipe (STP). *Impatiens buccinalis* Hook.f. and *I. thomensis* Exell, both endemic to the island of São Tomé; *I. manteroana* Exell, endemic to the island of Príncipe and *I. balsamina* L., a cultivated species native to India. A fifth species, *I. wallerana* Hook.f. is given for Island of São Tomé. This *taxa* was only collected at one location – Nova Moka, 23.i.2006, *Soares M*. 46, *Paiva* 1600 (COI). The fact this species is cultivated, native to East Africa and known only from a single collection site in STP (a garden), lead us to exclude this taxa from the present analysis.

Exell (1944) described a third endemic species (to the Island of Príncipe), as *I. tamsiana* Exell. This species was latter considered to be a synonym of *I. balsamina* L. (Grey-Wilson 1980a).

The STP *Impatiens* have been dealt taxonomically before, main works including Exell (1944) and Grey-Wilson's (1980a) monographic work on African *Impatiens*.

The molecular phylogeny of the three endemics has been addressed by Soares *et al.* (unpublished data).

In terms of palynological studies, Huynh (1968a, 1968b), briefly studied three STP *Impatiens* species, namely *I. balsamina* (=*I. tamsiana* Exell), *I. buccinalis* and *I. manteroana*, but he used a very limited dataset for each species and though his results are informative they are not representative.

Grey-Wilson (1980b), while not dealing exclusively with pollen issues, used pollen as a support for an investigation of hybridization in African *Impatiens*. Based on the overall morphological characters of *I. buccinalis*, *I. balsamina* and *I. thomensis,* especially the leaf shape, inflorescence type and floral morphology, he advanced the hypothesis of an hybrid origin of *I. thomensis* with the first two species as the putative parents.

By the time Exell published is Catalogue of Vascular Plants of S. Tomé (1944), only a few *Impatiens* collections were available. Since then, the number of available collections more than triplicated.

In the present study a taxonomic revision is made. Phenetic analyses were performed to elucidate the relationships between the three endemics and between these and the cultivated *I. balsamina* and to shade some light on the possible hybrid origin of *I. thomensis*.

An analysis of pollen morphology of the three endemic species and of *I. balsamina* was conducted. The taxonomic value of pollen characters was evaluated.

MATERIAL AND METHODS

Material

This study was based on herbarium specimens from the herbaria COI, LISC, LISU, P and PO (abbreviations follow Holmgren et al. 1990). A complete list of the specimens studied is presented in Appendix 1. For *I. balsamina*, only specimens collected in STP were considered. Pollen was obtained from herbarium specimens.

Morphology

Leaf characters were measured directly on herbarium specimens. Flower characters were measured after dissection of the flowers. The dissection of flower parts was achieved by hydrating the flowers in cold water (to which some few drops of liquid detergent where previously added), for some minutes. The flowers where then slightly boiled for less than one minute (so as to prevent the flowers from becoming pulpy), letting them rest in warm water for a few minutes. Although Grey-Wilson (1980a), dissuaded the use of this procedure, the reduced boiling time applied resulted in perfectly usable flowers. Flowers were then dissected and parts carefully laid on cards covered with a transparent slide with the help of a small brush. Flower parts were observed under a WILD M5 stereomicroscope adapted with a drawing tube and drawn. Measurements where conducted over the drawings (see Fig. 1 for a detailed description of measurements taken from sepals and petals). Figure 2 presents the floral structure of the four *Impatiens* species studied.

The data were analysed using NTSYSpc© 2.1 (Rohlf 1994). Cluster Analysis (CA) and Principal Component Analysis (PCA) were used as methods of analysis. Since the variables analyzed were discrete and continuous, data were log_{10} transformed. For CA the data matrices were standardized and distance matrices computed using the average taxonomic distance. Clustering was achieved using UPGMA. Matrices of cophenetic values were produced and correlations between these and the original matrices calculated as measure of goodness of fit (r).

For PCA the data matrices were standardized and matrices of correlations among the variables were calculated. The first three eigenvectors were extracted. The standardized data were projected onto these vectors.

The reduced number of specimens available and the availability of usable material dictated restrictions mainly on the dissection of flower material as well as on the amount of fruits and seeds available. In order not to lose the overall available information, two datasets were produced for numerical analysis, the first set comprising information on stem, leafs and glands and the second set including all the records containing the previous information as well as, at least, some information on flower, fruit and/or seed (see Table 1 for a description of the characters used for both analysis).
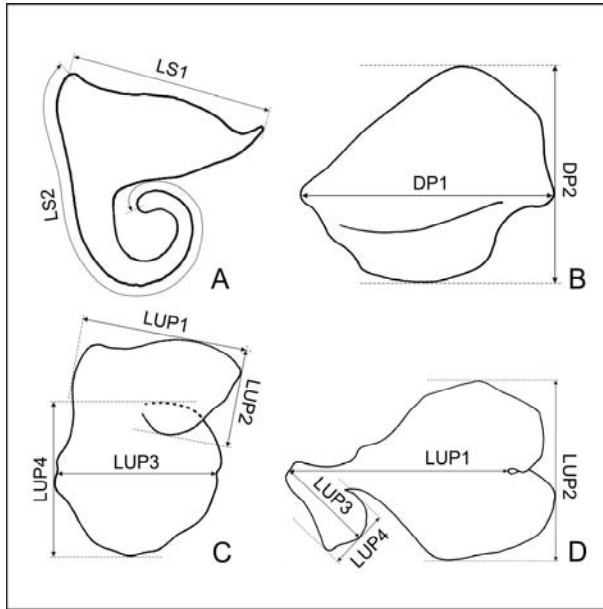
Figure 1. Measures taken from floral material. A – Lower sepal; B – Dorsal

petal; C – Lateral united petals (*I. buccinalis*, *I. manteroana* and *I. thomensis*);

D- Lateral united petals (*I. balsamina*)

Character	Description	Dataset 1	Dataset 2
1	Stem – pubescence (1 – glabrous; 2 – pubescent)	+	+
2	Maximum length of petiole	+	+
3	Petiole – position of glands (1 – along the petiole; 2 – near base of leaf)	+	+
4	Petiole – maximum length glands	+	+
5	Leaf – pubescence on upper surface (1 – glabrous; 2 – slightly pubescent)	+	+
6	Leaf – pubescence on lower surface (1 – glabrous; 2 – slightly pubescent)	+	+
7	Leaf margin (1- crenate 2- serrate)	+	+
8	Leaf margin – number of teeth	+	+
9	Maximum length of the lamina	+	+
10	Maximum width of the lamina	+	+
11	Leaf - position of greatest width (1- base to ½; 2 – ½; 3- ½ to apex)	+	+
12	Leaf – position of glands (1 – at the extremity of teeth; 2 – in between teeth)	+	+
13	Leaf – Maximum length of the glands	+	+
14	Leaf – base (1- cuneate; 2 – attenuate; 3 – obtuse; 4 – cuneate and obtuse)	+	+
15	Leaf – number of main lateral veins	+	+
16	Leaf – average distance beetween main lateral veins	+	+
17	Maximum length of peduncle	-	+
18	Thickness of peduncle (measured at ½ length)	-	+
19	Peduncule-pubescence (1-glabrous; 2-pubescent)	-	+
20	Maximum length of the bract	-	+
21	Maximum width of the bract	-	+
22	Maximum length of lateral sepals	-	+
23	Maximum width of lateral sepals	-	+
24	Lower sepal - LS1 (see Fig. 1A)	-	+
25	Lower sepal – LS2 (see Fig. 1A)	-	+
26	Lower spepal – pubescence (1-glabrous; 2-pubescent)	-	+
27	Dorsal petal – DP1 (see Fig. 1B)	-	+
28	Dorsal petal – DP2 (see Fig. 1B)	-	+
29	Lateral united petals – LUP1 (see Fig. 1C,D)	-	+
30	Lateral united petals – LUP2 (see Fig. 1C,D)	-	+
31	Lateral united petals – LUP3 (see Fig. 1C,D)	-	+
32	Lateral united petals – LUP4 (see Fig. 1C,D)	-	+
33	Androecium – Maximum length of filament	-	+
34	Maximum length of the gineceu	-	+
35	Maximum width of the gineceu	-	+
36	Ovary-pubescence (1-glabrous; 2-pubescent)	-	+
37	Fruit – pubescence (1 – glabrous; 2 – pubescent)	-	+
38	Maximum length of the fruit	-	+
39	Maximum width of the fruit	-	+
40	Fruit – number of seeds	-	+
41	Maximum length of seed	-	+
42	Maximum width of seed	-	+

Table 1. Characters used for the analysis of the species of *Impatiens* in STP
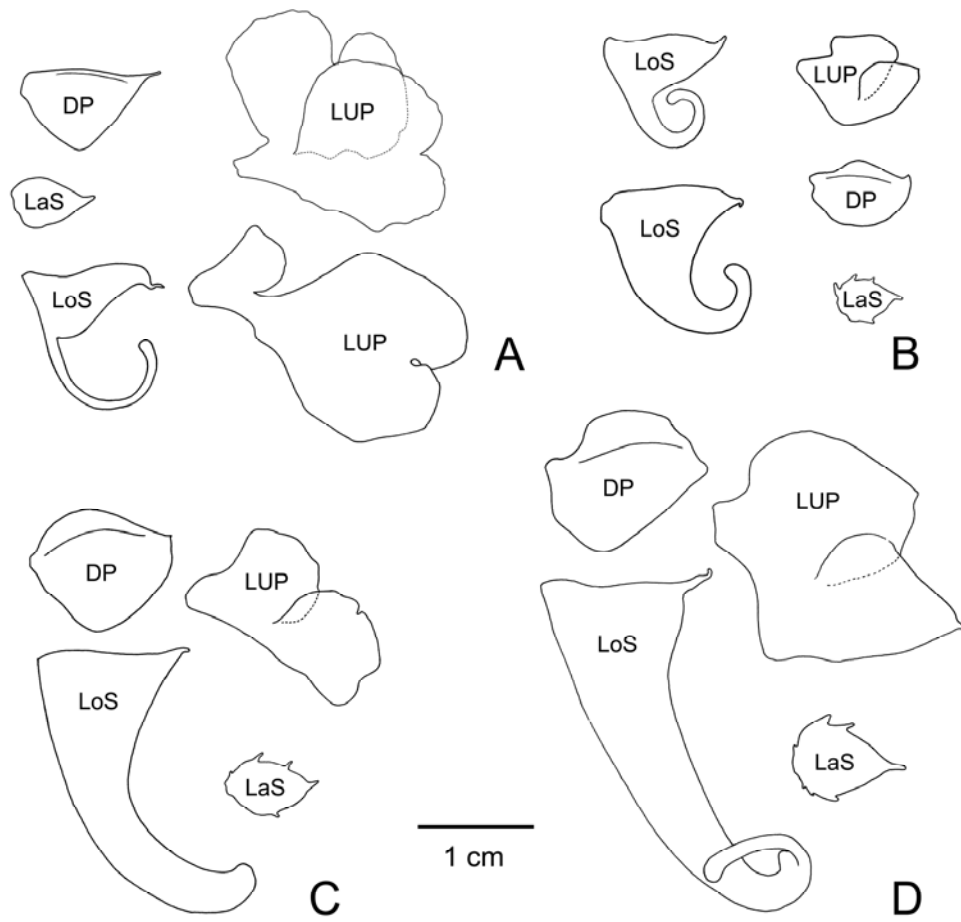
and their inclusion in the datasets.

Figure 2. Floral structure of the four *Impatiens* species considered in this study. A – *I. balsamina*; B – *I. thomensis*; C – *I. manteroana*; D – *I. buccinalis*. DP - Dorsal petal; LaS – Lateral sepal; LoS - Lower sepal; LUP – Lateral united petals.

Pollen material was obtained from herbarium specimens, nevertheless some of the specimens were very old and poor both in usable flowers and in pollen.

The acetolysis procedure followed an adapted version of that proposed by Erdtman (1960), with an acetolysis mixture of 1:18 and a bath at 90ºC for 9 minutes.

When subjected to acetolysis, the pollen of *Impatiens* tends to collapse (to different extents, depending on the species), due to its structure and shape. The same problem has been previously reported by Huynh (1968a) and Janssens (2005). To overcame this problem several approaches were tried. A slight ebullition in water (1–2 minutes) prior to the acetolysis, as proposed by Huynh (1968a), proved to be successful. The best solution proved to be a combination of two techniques. Pollen was first boiled for 1–2 minutes in water, submitted to the acetolysis procedure and then, for the Scanning Electronic Microscope (SEM) samples, subjected to the Critical Point Drying technique.

For light microscopy (LM), the pollen was then included in silicone oil and observed at x 1000 under a Leitz Laborlux S microscope. Two characters were measured – the length of the polar axis (P) and the equatorial diameter (E). P/E was then calculated.

For SEM, after being subjected to the Critical Point Drying technique, the pollen was poured onto carbon discs previously fixed to stubs. An Ion Sputter JEOL JFC-1100 (1200 V, 6 mA, 10m) was used for the metallization of the pollen grains which were coated by about a 30 nm of gold/palladium film. Observations were made in a JEOL JSM 5400 SEM.

SEM photographs were digitized and several measurements were performed using CARNOY® 2.0 (Schols *et al.*, 2002). Measurements were performed on a 10 μm square frame located at the top centre of the grain (in polar view), with the sides of the square

being aligned with the longitudinal axis of the grain. The characters measured were: number, area, perimeter and longest axis of lumina, and muri thickness.

Statistical analysis included a one way Anova followed by a Tukey's HSD test (Zar, 1996), performed with the software package Statistica® 4.1 for Macintosh.

Pollen terminology followed that proposed by Punt *et al.* (2007).

RESULTS

Morphology

Analysis of the first dataset

For the first dataset (stem, leafs and glands), 70 OTUs were considered (see Appendix 1). Sixteen characters were measured (Table 1). CA resulted in a phenogram where three main clusters can be recognized (Fig. 3). The first cluster corresponds to *I. balsamina*, clearly separated from the three STP endemics. The second cluster groups *I. buccinalis* and *I. manteroana* while the third cluster corresponds to *I. thomensis*. Cophenetic correlation produced a value of r = 0.92. The cluster grouping *I. buccinalis* and *I. manteroana* is divided into two main subclusters, one grouping six specimens identified as *I. manteroana* (white arrow on Fig. 3), and the other grouping specimens of both species. Two OTUs identified as *I. buccinalis* appear outside the main cluster grouping that species in this analysis (black arrow on Fig. 3).
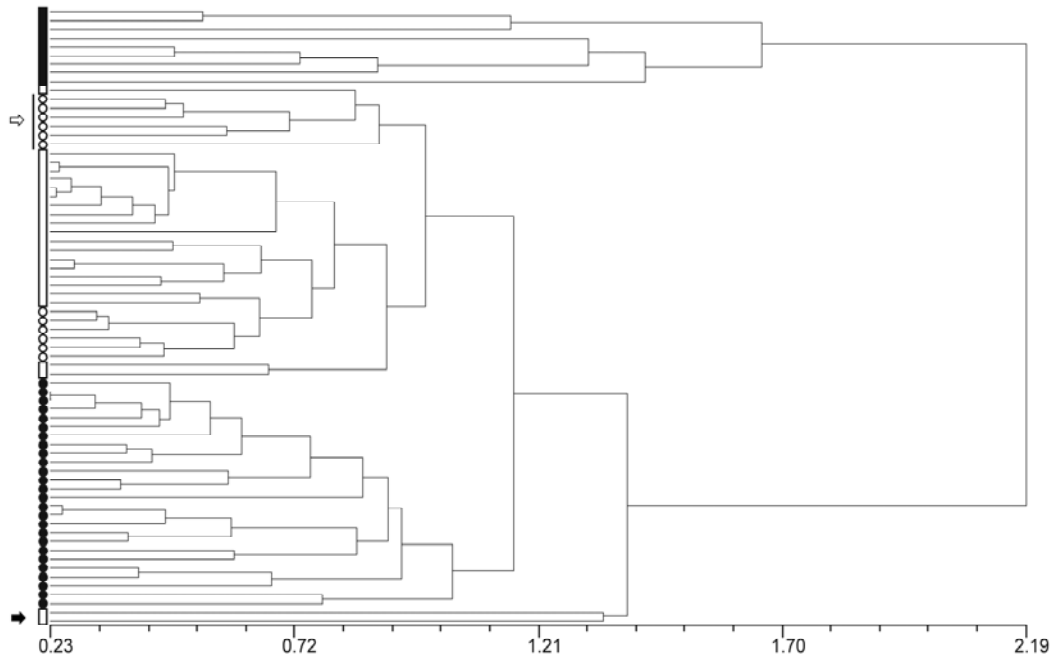
Figure 3. Phenogram from the Cluster Analysis for the first dataset. *I. balsamina* (■), *I. buccinalis* (□), *I. manteroana* (○) and *I. thomensis* (●).

The PCA results for the same data matrix also showed a separation of the four species (Fig. 4). The two axes accounted for 61.18 % of the variation (the first component accounts for 43.95 % of the variation and the second for 17.24 % - Tab. 2). For component loading along the first three axes see Appendix 2. *Impatiens balsamina* is clearly separated from the three STP endemics. *Impatiens thomensis* is closer but clearly isolated from the other two endemics. *Impatiens buccinalis* and *I. manteroana* show a close proximity and some introspection. The specimen Exell 574 (*I. tamsiana* = **I. balsamina**), is marked with a surrounding white square in Fig. 4.

Axis	Eingenvalue	Percentage of variance explained	Cumulative percentage of variance explained
1	7.03120951	43.95	43.95
2	2.75829459	17.24	61.18
3	1.76460569	11.03	72.21

Table 2. Results of the PCA for the first dataset. Eingenvalues, percentage of total variance explained by each axis and cumulative percentage along the three first axes.
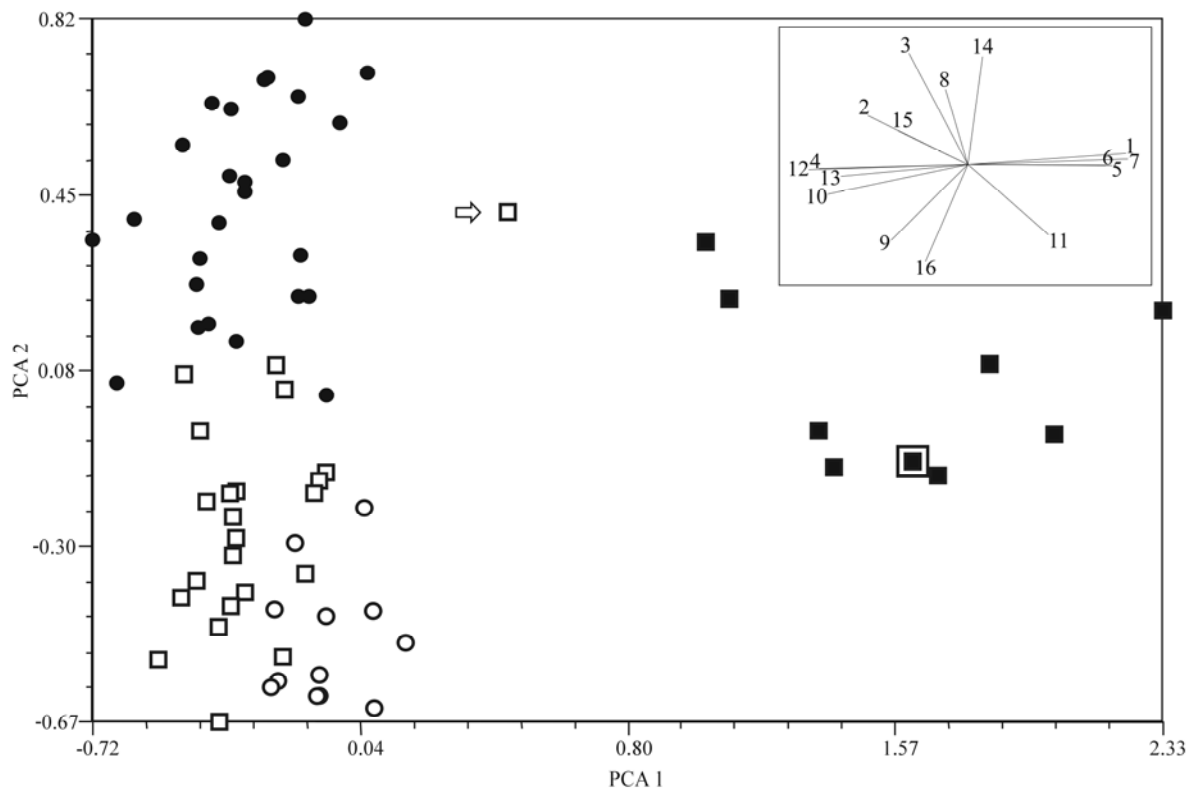

Figure 4. PCA results for the first dataset, showing the projection of the OTUs onto the first two axes. Enclosed, the character vector diagram. *I. balsamina* (■), *I. buccinalis* (□), *I. manteroana* (O) and *I. thomensis* (•).

Analysis of the second dataset

The second dataset included the characters used in the first dataset plus flower, fruit and seed in a total of 42 characters (Tab. 1). Forty-eight OTUs were analysed (see Appendix 1). The CA resulted in a phenogram where three main clusters can be recognized (Fig. 5). *Impatiens balsamina* remains isolated and separated from the three STP endemics in this analysis. *Impatiens buccinalis* and *I. manterona* form a cluster where two subclusters can be recognized corresponding to each of the species, exception made to two OUT's identified as *I. manteroana* which appear mixed with the *I. buccinalis* OUT's. *Impatiens thomensis* appears as the third cluster. Cophenetic correlation produced a value of r = 0.94.
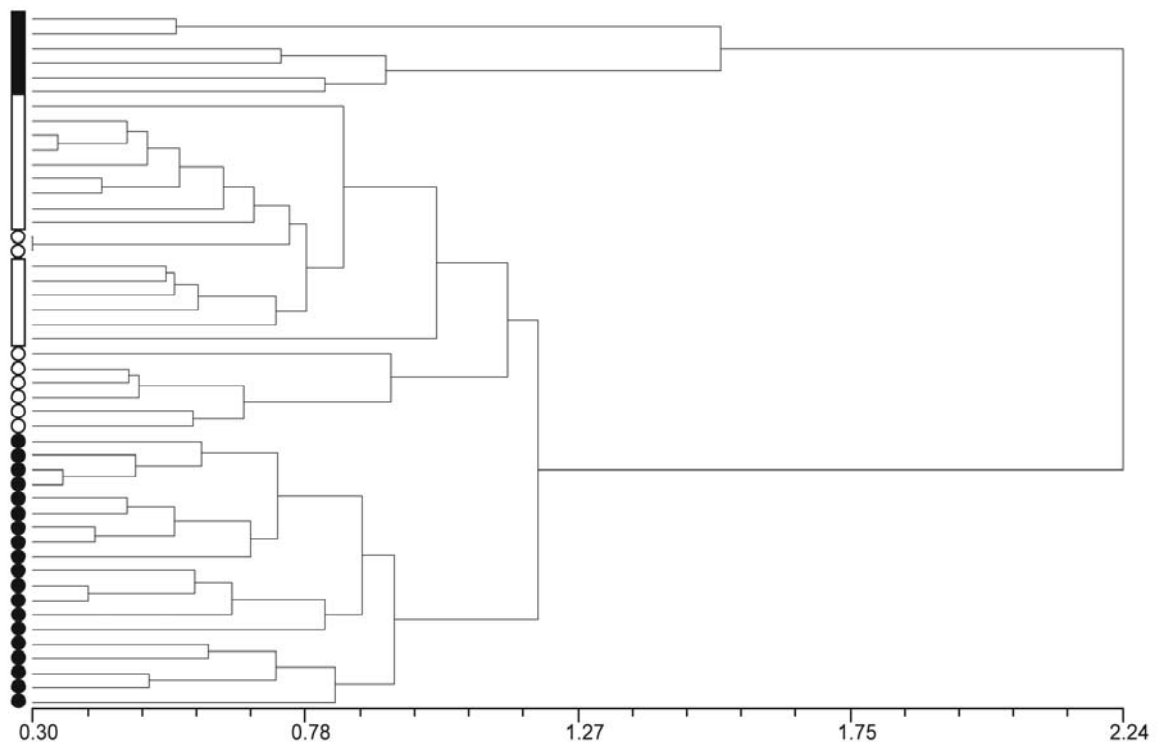

Figure 5. Phenogram from the Cluster Analysis for the second dataset. *I. balsamina* (■), *I. buccinalis* (□), *I. manteoana* (○) and *I. thomensis* (●).

The PCA results for the same data matrix also show a separation of the four species (Fig. 6). The two axes accounted for 70.51 % of the variation (the first component accounts for 40.10 % of the variation and the second for 30.41 % - see Tab. 3). For component loading along the first three axes see Appendix 3. *Impatiens balsamina* remains clearly separated from the three STP endemics in this analysis. *Impatiens manteroana* is positioned halfway between *I. buccinalis* and *I. thomensis*. Three of the *I. manteroana* OTU's group closely to *I. thomensis*. A white square surrounds the specimen Exell 574 (*I. tamsiana* = **I. balsamina**). This specimen is clearly separated from the three STP endemics and groups with the *I. balsamina* specimens.

Axis	Eingenvalue	Percentage of variance explained	Cumulative percentage of variance explained
1	16.84339335	40.10	40.10
2	12.77042051	30.41	70.51
3	4.91795490	11.71	82.22

Table 3. Results of the PCA for the second dataset. Eingenvalues, percentage of total variance explained by each axis and cumulative percentage along the three first axes.

Figure 6. PCA results for the second dataset, showing the projection of the

OTUs onto the first two axes. Enclosed, the character vector diagram. *I.*

balsamina (■), *I. buccinalis* (□), *I. manteroana* (○) and *I. thomensis* (●).

Palynology

Tab. 4 presents the One Way ANOVA results. Descriptive statistics for the four species are summarized on Tab. 5.

The general description of the pollen of *Impatiens* in STP and description of the pollen of each of the four species present in the islands are provided here.

The pollen of the four species falls into the four-colpate with a square or squarish equator and the four-colpate grains with a rectangular or oblong equator group. The differences in pollen size and in the reticulum sculpture allow a clear separation between the three endemics. *Impatiens thomensis* and *I. balsamina* share a significant amount of features nevertheless the grain shape allows a clear separation of the two species.

Statistical analysis

The statistical analysis of the palynological data showed differences between the four species in all the variables considered (Tab. 4). The Tukey's HSD test (Tab. 5) analysis revealed the statistical relationships between the species. *Impatiens buccinalis* and *I. thomensis* present only similarities at P level . *Impatiens manteroana* and *I. thomensis* have similarities in area of the lumina. *Impatiens buccinalis* and *I. manteroana* are statistically similar to *I. balsamina* respectively in the P/E ratio and number of lumina per 10 μm square frame. *Impatiens thomensis* and *I. balsamina* proved to be statistically similar in the perimeter and longest axis of lumina and width of the muri. None of the species presented similarities regarding the equatorial diameter (E).

Palynological description of the genus Impatiens in STP

Pollen grains subisopolar, slightly concave on one face, more or less convex on the opposite face; usually with a bilateral symmetry; rectangular in polar view (Fig. 7A, E, G, J); peroblate to oblate spheroidal, P/E = 0.4–0.8 (0.5 ± 0.07); P = 18.0–38.0 (25.7 Mean ± 4.28 Standard Deviation) μm; E = 32.2–75.0 (50.5 ± 9.72) μm; 4-zono-colpate

angulaperturate; Colpi 0.8–12.0 μm long, thin (0.5–0.75 μm); sculpture reticulate, homobrochate or heterobrochate, muri 0.22–0.98 (0.52 ± 0.16) μm thick, sometimes incomplete causing coalescence of adjacent lumina; lumina typically pentagon or hexagon shaped sometimes polygonal concave, size of lumina varies considerably between species and throughout the grains, being smaller around the apertures and larger on the centre of the apocolpium; longest axis of lumina 0.21–4.23 (1.26 ± 0.63) μm; exine ca. 0.62 μm thick, sexine ca. 3/4 x the exine, tectum more or less granulate to pilate, columellae ca. 1/2 x the tectum, nexine ca. 1/4 x the exine.

DICHOTOMOUS KEY TO SPECIES

1.   Mean E = 61.46 ± 1.12* μm; grains slightly contracted at between 1/3 to 1/2 of the length when in polar view (Fig. 7E) …………..………………….…...……..…… *I. buccinalis*

        Mean E < 61.46 ± 1.12 μm; grains not contracted when in polar view ….…..….. 2

2.   Mean muri thickness = 7.3 ± 0.08 μm. Grains usually asymmetrical in polar view, presenting a hump to one of the lateral sides, and truncated towards one of the ends in equatorial view (Fig. 7G) …………………………...……………....…….. *I. manteroana*

        Mean muri thickness < 7.3 ± 0.08 μm. Grains usually symmetrical in polar view .. 3

3.   Mean P = 28.45 ± 0.5; grains usually prolate to oblate spheroidal in equatorial view ………………………………………………………………………………….. *I. thomensis*

         Mean P = 19.6 ± 0.26; grains prolate in equatorial view ………............. *I. balsamina*

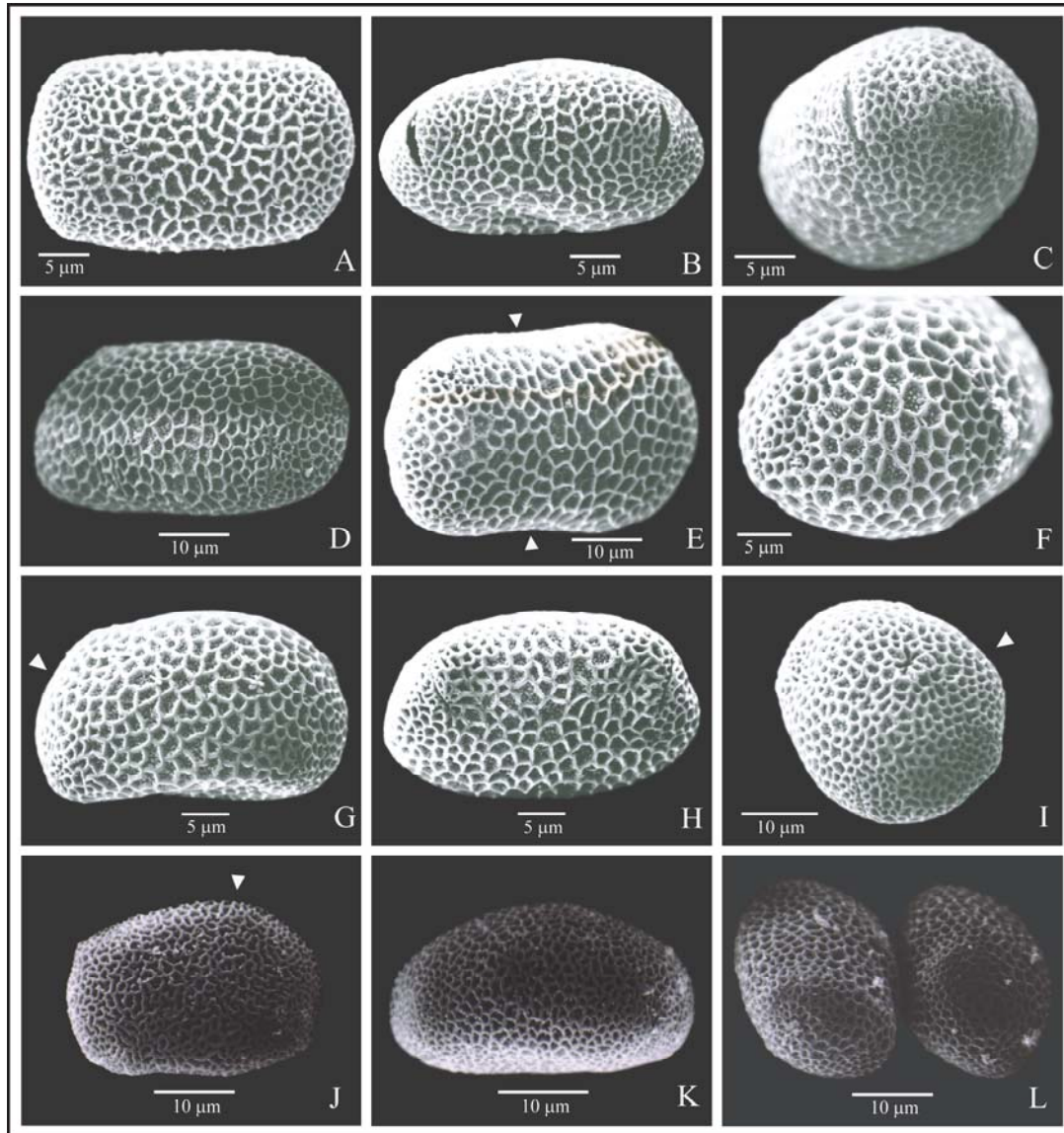   * Confidence limits for mean (α=0.05).

Figure 7. SEM photographs of pollen grains. A-C *I. balsamina*. A) Grain in polar view. Clearly visible the homobrochate *reticulum* showing some concave polygon shaped lumina; B) Equatorial view; C) Mesocolpium view. D-F *I. buccinalis*. D) Equatorial view from above at a 45º angle towards the mesocolpium; E) Polar view at a 45º angle from the side and above. Note the slightly contraction (white arrows) on each side of the grain; F) Mesocolpium view. G-I *I. manteroana*. G) Polar view at a 45º angle from the side and above. Grains truncated towards one of the ends (white arrow); H) Equatorial view slightly from beneath; I) Mesocolpium view at a 45º angle from above. Note the asymmetry of the grain with a projecting bump on the right side of the grain (white arrow). J-L *I. thomensis*. J) Polar view. The asymmetry of the grain is clearly visible, very similar to that presented by *I. manteroana* (white arrow); K) Equatorial view. The accentuated subisopolar characteristic of this grains is more evident in this species then in any other of the *Impatiens* studied; L) Mesocolpium view.
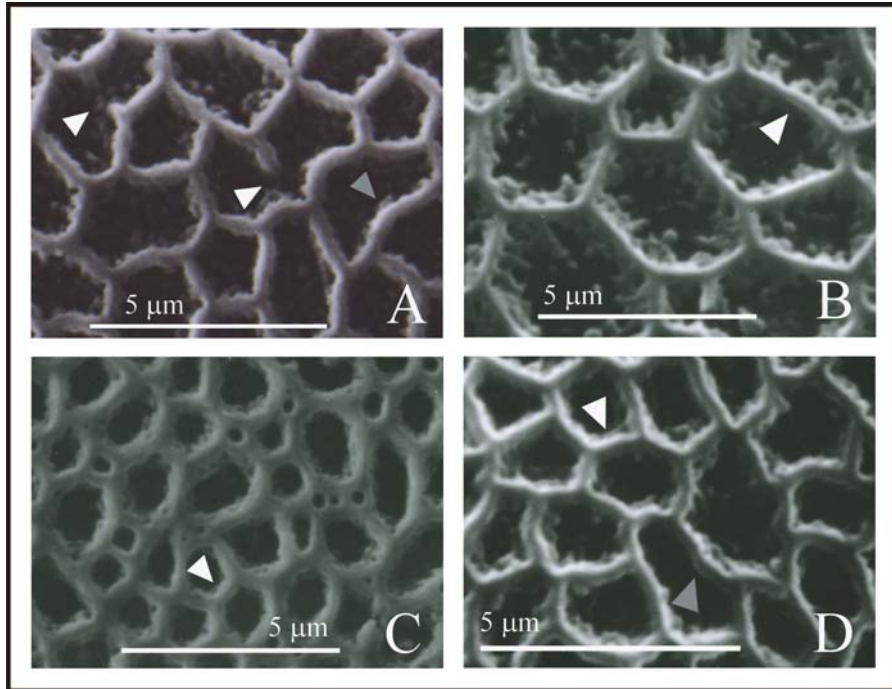
Figure 8. SEM photographs of pollen grains showing details of reticulum. A) *I. balsamina*. Coalescence between the adjacent lumina due to incomplete formation of the muri (white arrow). Concave polygon shape of lumina and detail of rectangular muri (grey arrow). B) *I. buccinalis*. Obtuse muri (white arrow). C) *I. manteroana*. Heterobrochate reticulum. Detail showing rectangular shape of muri (white arrow). D) *I. thomensis*. Obtuse muri shape (white arrow). Concave polygon shape of lumina (grey arrow).

Impatiens balsamina: Pollen grains bilateral. P = 18.0–23.5 (19.60 *Mean* ± 1.19 *Standard Deviation*) μm; E = 35.0–44.5 (41.18 ± 1.71) μm; P/E = 0.4–0.6 (0.48 ± 0.04) μm. Sexine reticulate homobrochate. Lumina in some cases adopting a concave polygon shape, sometimes not completely surrounded by the muri causing coalescence between adjacent lumina (Fig. 8A). Muri obtuse to sharply crested (Fig. 8A)

Impatiens buccinalis: Pollen grains bilateral, slightly contracted on both sides (more or less at 1/3 to 1/2 of the length), when viewed in polar view (Fig. 7E). P = 20.0–38.0 (28.77 ± 2.58) μm; E = 53.0–75.0 (61.46 ± 5.07) μm; P/E = 0.4–0.6 (0.47 ± 0.04) μm. Sexine reticulate homobrochate. Muri typically obtuse (Fig. 8B).

Impatiens manteroana: Pollen grains asymmetrical in polar view (Fig. 7I). P = 18.0–30.0 (23.17 ± 2.86) μm; E = 38.0–52.5 (44.64 ± 3.19) μm; P/E = 0.4–0.7 (0.52 ± 0.05) μm. Sexine reticulate heterobrochate. Muri rectangular (Fig. 8C). A negligible number of grains (less than 0.1 %) were found to be tricolpate.

Impatiens thomensis: Oblate to oblate spheroidal or prolate. Pollen grains asymmetrical in polar view (Fig. 7J). P = 22.3–34.3 (28.45 ± 2.28) μm; E = 32.2–58.7 (46.90 ± 4.22) μm; P/E = 0.5–0.8 (0.61 ± 0.08) μm. Sexine reticulate homobrochate. Muri commonly obtuse (rarely sharply crested), sometimes becoming sharply crested towards the equatorial line, occasionally not complete causing coalescence of adjacent lumina (Fig. 8D). Lumina in some cases adopting a concave polygon shape (Fig. 8D). A negligible number of grains (less than 0,1 %) were found to be tricolpate.

	Between SS	Df	Within SS	df	*F*
P	1583.699	3	5.363476	320	295.27
E	6455.623	3	14.18979	320	454.95
P/E	0.3472107	3	0.002847	320	121.96
Area of lumina*	40.69813	3	1.631585	392	24.94
Perimeter of lumina*	63.87164	3	2.753841	392	23.19
Longest axis of lumina*	8.316439	3	0.3304836	392	25.16
Number of lumina per 10 μm square	737.2738	3	113.381	24	6.50
Width of the muri	1.3716	3	0.0058	200	236.48

Table 4. One Way ANOVA results for the pollen analysis.

* Values correspond to lumina on a 10 μm side square frame located at the top centre

of the grain in polar view.

	n	*I. balsamina*	*I. buccinalis*	*I. manteroana*	*I. thomensis*
P	81	19.60 ± 1.19 (18.0 to 23.5)	28.77 ± 2.58 (20.0 to 38.0)	23.17 ± 2.86 (18.0 to 30.0)	28.45 ± 2.28 (22.3 to 34.3)
E	81	41.18 ± 1.71 (35.0 to 44.5)	61.46 ± 5.07 (53.0 to 75.0)	44.64 ± 3.19 (38.0 to 52.5)	46.90 ± 4.22 (32.2 to 58.7)
P/E	81	0.48 ± 0.04 (0.4 to 0.6)	0.47 ± 0.04 (0.4 to 0.6)	0.52 ± 0.05 (0.4 to 0.7)	0.61 ± 0.08 (0.5 to 0.8)
Area of lumina*	99	1.19 ± 0.52 (0.33 to 2.89)	2.36 ± 2.02 (0.10 to 11.66)	0.91 ± 0.86 (0.04 to 4.33)	1.24 ± 1.19 (0.05 to 6.12)
Perimeter of lumina*	99	3.98 ± 1.08 (2.08 to 7.15)	4.97 ± 2.12 (1.08 to 12.51)	3.01 ± 1.46 (0.66 to 8.13)	3.80 ± 1.79 (0.78 to 9.52)
Longest axis of lumina*	99	1.24 ± 0.35 (0.65 to 2.47)	1.64 ± 0.79 (0.28 to 4.23)	0.94 ± 0.49 (0.21 to 2.27)	1.20 ± 0.58 (0.23 to 3.20)
Number of lumina per 10 μm square frame	7	47 ± 6 (40 to 56)	30 ± 10 (16 to 41)	54 ± 16 (30 to 78)	41 ± 9 (30 to 58)
Width of the muri	51	0.38 ± 0.05 (0.22 to 0.50)	0.56 ± 0.07 (0.43 to 0.7)	0.73 ± 0.11 (0.44 to 0.98)	0.40 ± 0.05 (0.27 to 0.5)

Table 5. Descriptive statistics for the four *Impatiens* species analyzed (units in

μm except for number of lumina). Mean ± Standard Deviation (Range in

Parenthesis). Tukey´s HSD test results are represented by shaded areas

(representing similarities between means).

* Values correspond to lumina on a 10 μm side square frame located at the top centre

of the grain in polar view.

TAXONOMIC TREATMENT

DICHOTOMOUS KEY TO SPECIES

1.   Annual herb, pubescent. Leaves finely pubescent on upper and/or lower surface, margin serrate, gland-tipped appendages located at the apex of the teeth. Peduncle and lower sepal pubescent or finely pubescent; fruit pubescent ………..…...…….. *I. balsamina*

        Perennial, glabrous, very rarely slightly pubescent. Leaves glabrous, margin crenate, gland-tipped appendages at the tooth base, adaxially. Peduncule, lower sepal and fruit glabrous 2

2.   Petiole 1.75–7.9 cm long, petiole glands located near the base of the lamina. Flowers red, scarlet or pink, sometimes red with lower sepal pink. Lower sepal deeply navicular, abruptly constricted into a spiraled or incurved filiform spur (Fig. 2B), rarely slightly bucciniform……………………………...……..... *I. thomensis* (endemic to São Tomé)

        Petiole 0.7–4.3 cm long, petiole glands located along the entire length of petiole. Flowers red. Lower sepal bucciniform to saccate (Fig. 2 C,D) ………………….…...… 3

3.   Lower sepal bucciniform to saccate, slightly incurved with rounded tip (Fig. 2C). Petiole 1–4.3 cm long; lamina 9.4–14.9 x 3.1–4.9 cm; leaf base cuneate; main lateral veins 5–8 pairs. .... *I. manteroana* (endemic to Príncipe)

        Lower sepal deeply saccate and abruptly constricted into a filiform spur, the tip of which swollen, with a protuberant appendix at the base of the spur (Fig. 2D). Petiole 0.7–3.5 cm long; lamina 6.9–17.6 x 3.1–6.5 cm; leaf base usually cuneate sometimes cuneate to obtuse; main lateral veins 7–11 pairs. …………..……. *I. buccinalis* (endemic to São Tomé)

Impatiens buccinalis Hook.f., Journ. Proc. Linn. Soc. 7: 187 (1864) & in Oliver, Fl. Trop. Afr. 1: 299 (1868); Henriques, Bol. Soc. Brot. 10: 105 (1893) & Bol. Soc. Brot. 27: 190 (1917); Warburg in Engler, Bot. Jahrb. Syst. 22: 47 (1897); Gilg in Engler, Bot. Jahrb. Syst. 43: 106 (1909); Chevalier, Sudania, 2: 47 (1914); Exell, Cat. Vasc. Pl. St. Tomé 125 (1944), Suppl. Cat. Vasc. Pl. S. Tomé: 13 (1956) & Bull. Brit. Mus. (Nat. Hist.) Bot. 4: 342 (1973); Grey-Wilson, *Impatiens* of Africa: 212 (1980); Joffroy, Etude des plantes endémiques de São Tomé et Príncipe, Travail de fin d'études, Université Libre de Bruxelles: annex. 10 (2000). Type: São Tomé e Príncipe, São Tomé, *Mann* 1089 (K holotype, B isotype).

HERB, UNDERSHRUB OR SHRUB 1.2–2(3) m high, erect, glabrous, very rarely slightly pubescent, perennial. LEAVES spirally arranged, glabrous on both surfaces, the petiole 0.7–3.5 cm long, petiole glands 0.17–0.37 cm long, located along the entire length of the petiole; lamina 6.9-17.6 x 3.1–6.5 cm, elliptic to elliptic-lanceolate rarely ovate-lanceolate, the apex acuminate, the base usually cuneate sometimes cuneate and obtuse; main lateral veins 7–11 pairs; margin crenate with short 0.1-0.3 cm long gland-tipped appendages at the tooth base, adaxially. INFLORESCENCE a 2–3(–5) flowered raceme, flowers red. PEDUNCLE 2.04–2.95 cm long, slender, glabrous. LATERAL SEPALS 0.63–0.91 x 0.5–0.77 cm, ovate, shortly apiculate, teeth small, (2–)3 on the upper margin, rarely 4, (1–)2 on the lower margin, rarely absent. LOWER SEPAL glabrous, 1.12–2.18 cm deep, 3.05–6.14 cm long, deeply saccate, abruptly constricted into a more or less incurved, filiform spur, swollen at the tip, with a protuberant appendix at the inner base of the spur. DORSAL PETAL 1.21–1.84 x 1.01–1.69 cm, folded, cucullate, with a pronounced crest above. LATERAL UNITED PETALS, upper petal 1.01–1.58 x 0.45–0.79 cm; lower petal 1.08–

1.73 x 0.44–1.16 cm. OVARY glabrous, 0.62–1.06 x 0.12–0.23 cm. FRUIT glabrous, c. 1.4 x 0.19 cm. SEED unknown.

Distribution: Endemic to the Island of São Tomé.

Habitat: Humid and shaded places, sometimes forming dense populations, primary and secondary forest, along the margin of pathways. Known to flower in I, IV, VI-VIII, IX-XI and to fruit in IV, VI-VIII, XI. Altitudinal range 800–1500 m.

Vernacular names: "Camarões", "Camarões da Austrália" (Portuguese)

Conservation status: Not endangered (Joffroy, 2000).

Representative collections:

Monte Café – Pico, 1905, *Chevalier* 14287 (P). Monte Café – Pico, 1905, *Chevalier* 14305 (P). Monte Café, 1200 m alt., 17.i.1949, *Espírito Santo* 168 (BM, COI, LISC). Monte Café, Esperança, 1318 m alt., 13.viii.1959, *Espírito Santo* 4023 (COI, LISC). Monte Café, Esperança, 1200 m alt., 17.vi.1968, *Espírito Santo* 4612 (LISC). Macambrará, ca. 1300 m alt., 2.xi.1932, *Exell* 195 (BM, COI). Calvário - Bom Sucesso, 1280 m alt., 30.vii.1999, *Joffroy G.* 110 (BRLU). Lat. N 00º 18' Long. E 06º 36'. Beira do caminho a caminho de Chamiço, 820 m alt., 27.viii.1999, *Joffroy G.* 180 (BRLU, LISC). Bom Sucesso - Lagoa Amélia, 1300 m alt., 29.xii.1994, *Lejoly* 94/522 (BRLU). Entre Macambrará e Zampalma, 1150 m alt., 30.ix.1997, *Lejoly* 97/325 (BRLU, LISC). Island of São Tomé, 1200 m alt., vii.1861, *Mann* 1089 (K, B). Zona Ecológica Lat. 0º 16' 30" N long. 6º 35' 20" E, entre

Lagoa Amélia e Calvário, 1400 m alt., 6.vii.1994, *Matos C.* 7539 (BRLU, LISC). Zona Ecológica lat. 0º 15' 55" N; long. 6º 33' 30" E, entre Estação Sousa e Pico, 1700 m alt., 7.vii.1994, *Matos C.* 7550 (BRLU, LISC). Zona Ecológica Lat. N 00º 17'; Long. E 06º 35'. Entre Bom Sucesso e Calvário. sentier 2, entre km. 1.3 e 2.0, 1325-1350 m alt., 13.iv.1994, *Matos C. & K. Van. Essche (CMVE)* 7340 (BRLU, LISC). Zona Ecológica Lat. N 00º 15' Long. E 06º 34'. Entre Charuto e Maria Pires, 800-850 m alt., 24.iv.1994, *Matos C. & K. Van. Essche (CMVE)* 7430 (BRLU, LISC). Bom Sucesso, 1080 m alt., vi.1885, *Moller* 291 (COI, LISC, LISU). Próximo dos morros no Monte Café, 800 m alt., vi.1885, *Moller* 342,367 (COI). Lagoa Amélia e Bacia do Rio Contador, 1250-1400 m alt., vii.1885, *Moller* 419 (COI). Bacia do Rio Contador, 1250 m alt., vii.1885, *Moller* 593 (COI). "?" da roça Macambrará, 1250 m alt., 1885, *Moller* 872 (COI). Perto da Lagoa Amélia, ca. 1500 m alt., 15.viii.1956, *Monod* 11719 (COI). Lagoa Amélia, 1350-1380 m alt., 15.viii.1956, *Monod* 11773 (COI). Ilha de São Tomé, 1400 m alt., 1.iv.1998, *Oliveira* 617 (BRLU). Monte Café. Entre S. Luís e Chamiço, 900-1000 m alt., 8.x.1992, *Paiva* 466 (COI). Monte Café. S. Luís, 900 m alt., 21.ix.1995, *Paiva* 884 (COI). Monte Café. Entre S. Luís e Chamiço, 900 m alt., 2.vii.1996, *Paiva* 992 (COI). Entre São Luís e Chamiço, ca. 900 m alt., 3.ii.2006, *Paiva* 1858 (COI). *Quintas* 872 (Z). Lagoa Amélia, 1400 m alt., vi.1888, *Quintas* 1319 (COI). Lagoa Amélia, 1957, Rose 217 (P). Entre Lagoa Amélia e Nova Moka, 8.xi.1954, *Rozeira* 112 (PO). Andados cerca de 1500 m de São Luís em direcção a Chamiço, ca. 900 m alt., 3.ii.2006, *Soares M.* 148 (COI).

Impatiens manterona Exell, Cat. Vasc. Pl. St. Tomé: 125, Fig.4 (1944); Exell & Rozeira, Comun. 6th C.I.A.O. 3: 81 (1956); Exell, Bull. Brit. Mus. (Nat. Hist.) Bot. 4: 342 (1973); Grey-Wilson, *Impatiens* of Africa: 213 (1980); Joffroy, Etude des plantes endémiques de São Tomé et Príncipe, Travail de fin d'études, Université Libre de Bruxelles: annex. 11 (2000). Type: São Tomé e Príncipe, Príncipe, Pico Papagaio, ca. 620 m alt., *Exell* 682 (BM holotype, B, BR, COI isotypes).

HERB, perennial, glabrous, up to 0.5 m high. LEAVES spirally arranged, glabrous on both surfaces, the petiole 1–4.3 cm long, petiole glands located along the entire length of the petiole; lamina 9.4–14.9 x 2.95–4.9 cm, elliptic-lanceolate or oblanceolate, base cuneate, apex acuminate; main lateral veins 5–8 pairs; margin crenate with gland-tipped appendages at the tooth base, adaxially. INFLORESCENCE a 2–3 flowered axillary raceme; flowers red. BRACTS 0.14 x 0.07 cm, entire, lanceolate, acute. PEDUNCLE c. 1.36 cm long, slender, glabrous. LATERAL SEPALS 0.4–0.78 x 0.28–0.55 cm, ovate, acute, teeth small, 2 on the upper margin, (0–)1 on the lower margin. LOWER SEPAL glabrous, 0.76–0.91 cm deep, 1.91–1.97 cm long, bucciniform to saccate, curved or incurved, the tip rounded. DORSAL PETAL 0.68–1.2 x 0.63–1.01cm, folded, cucullate, with a crest above, sometimes pronounced and highest towards the peduncle. LATERAL UNITED PETALS, upper petal 0.6–0.96 x 0.3–0.81 cm; lower petal 0.7–1.22 x 0.34–0.64 cm. OVARY glabrous. FRUIT, fusiform, glabrous. SEED unknown.

Distribution: Endemic to the island of Príncipe.

Habitat: Primary rainforest, among wet rocks in shaded humid places, forming dense and continuous populations sometimes from 200 m upwards. Known to flower in V, VIII, IX-X, XII and to fruit in VIII, IX-X, XII. Altitudinal range 200-948 m.

Conservation status: Not endangered (Joffroy, 2000).

Representative collections:

Pico Papagaio, ca. 620 m alt., 27.xii.1932, *Exell* 682 (B, BM, BR, COI). Pico do Príncipe, 600 m alt., 2.ix.1999, *Joffroy G.* 213 (BRLU). Morro do Leste, 660 m alt., 5.ix.1999, *Joffroy G.* 225 (BRLU). Pico Papagaio. Lat. 01º 37' 00" N; Long. 07º 23' 40" E, 400 m alt., 16.v.1996, *Matos C.* 7746 (LISC). Face W do Pico, 30.viii.1956, *Monod* 12167 (COI). Pico Papagaio, 400-620 m alt., 11.x.1992, *Paiva* 579, 586 (COI). Monte Papagaio, 680 m alt., 14.viii.2002, *Paiva* 1445 (COI). Pico do Príncipe, 948 m alt., 17.viii.2002, *Paiva* 1477 (COI). Na floresta da encosta do Pico A Mesa, 29.i.2006, *Paiva* 1726 (COI). Pico do Príncipe, 600-800 m alt., 28.ix.1954, *Rozeira* 948 (PO). No cume do Pico A Mesa, 528 m alt., 29.i.2006, *Soares M.* 119 (COI). Na face NE do Pico A Mesa, descendo para o Rio Macóia, ca. 350 m alt., 29.i.2006, *Soares M.* 127 (COI). Na descida da face NE do Pico A Mesa, na direcção do Rio Macóia, ca. 300 m alt., 29.i.2006, *Soares M.* 128 (COI). Na descida da face NE do Pico A Mesa, junto às margens húmidas do Rio Macóia, ca. 200 m alt., 29.i.2006, *Soares M.* 129 (COI).

Impatiens thomensis Exell, Cat. Vasc. Pl. S. Tomé: 127, Fig.6 (1944), Exell & Rozeira, Comun. 6th C.I.A.O. 3: 82 (1956); Exell, Bull. Brit. Mus. (Nat. Hist.) Bot. 4: 342 (1973); Grey-Wilson, *Impatiens* of Africa: 214 (1980); Figueiredo, Garcia de Orta, Ser. Bot., 12 (1-2): 121 (1994) & Fontqueria 44: 48 (1996); Joffroy, Etude des plantes endémiques de São Tomé et Príncipe, Travail de fin d'études, Université Libre de Bruxelles: annex. 11 (2000). Type: São Tomé e Príncipe, São Tomé, between Vanhulst and Jamar, ca. 830 m, *Exell* 281 (BM holotype; B, BR, COI isotypes)

HERB, UNDERSHRUB OR SHRUB, perennial, glabrous, up to 1 m high. LEAVES spirally arranged, glabrous on both surfaces, the petiole 1.75–7.9 cm long, petiole glands located along the entire length of the petiole; lamina 6–14.4 x 2.87–7.65 cm, elliptic-lanceolate or ovate-lanceolate rarely obovate, the base cuneate, obtuse or both, the apex acuminate; main lateral veins 6–11 pairs; margin crenate with gland-tipped appendages at the tooth base, adaxially. INFLORESCENCE a 2–5 flowered axillary raceme; flowers red, scarlet or pink, sometimes red with lower sepal pink. PEDUNCLE 1.28–2.24 cm long, slender, glabrous. LATERAL SEPALS 0.47–0.7 x 0.24–0.45 cm, ovate, acute, teeth small, 2(–3) on the upper margin, (0–)2 on the lower margin, sometimes absent. LOWER SEPAL glabrous, 0.88–1.47 cm deep, 1.51–2.62 cm long, deeply navicular, abruptly constricted into a spiraled or incurved filiform spur, sometimes slightly bucciniform, slightly swollen at the tip. DORSAL PETAL 0.76–1.16 x 0.52–0.76 cm, folded, cucullate, with a crest above, sometimes wide, highest at the middle of the petal. LATERAL UNITED PETALS, upper petal 0.7–1.22 x 0.34–0.64 cm; lower petal 0.6–0.96 x 0.3–0.81 cm. OVARY 0.4–0.5 x c. 0.1 cm, glabrous. FRUIT glabrous. SEEDS 6, c. 0.11 x 0.07 cm.

Distribution: Endemic to the island of São Tomé.

Habitat: Primary and secondary forest, along the paths, in shaded and humid places, waterfall walls, river margins, rocky walls with pouring water. Known to flower in III, IV, VI-VIII, IX-XII and to fruit in III, IV, VII-VIII, IX-XI. Altitudinal range 10-1700 m.

Conservation status: Not endangered (Joffroy, 2000).

Representative collections: Santa Catarina, 30.vii.1959, *Espírito Santo* 3960 (COI, LISC). Santa Catarina, 28.iii.1968, *Espírito Santo* 4354 (LISC). Entre Santa Catarina e Cadão, 23.iii.1973, *Espírito Santo* 5158 (COI, LISC). São Nicolau, cascata, 700 m alt., 10.iv.1973, *Espírito Santo* 5177 (COI, LISC). No caminho de pé-posto de Macambrará a Jamar, ca. 830m alt., 7.xi.1932, *Exell* 281 (B, BM, BR, COI). No caminho de pé-posto de Macambrará a Jamar, ca. 830m alt., 23.xi.1932, *Exell* 433 (BM, COI). Rio Paga Fogo, junto à costa, 10.ix.2002, *Figueiredo E.* 255 (LISC). Estrada Monte Café - São Nicolau, perto da queda-de-água, ca. 870 m alt., 31.x.1993, *Figueiredo E. & Arriegas P.I.* 113 (K, LISC). Lat. N 00º 17'; E 06º 37'. Caminho em direcção ao Calvário, 1275 m alt., 28.vii.1999, *Joffroy G.* 83 (BRLU, LISC). Zona Ecológica, Lat. 0º 15'55" N; long. 6º 33' 30" E. Entre Estação Sousa e Pico, 1700 m alt., 7.vii.1994, *Matos C.* 7549 (BRLU, LISC). Z.E. Lat. 00º 16' N; Long. 06º 29' E. Santa Catarina, 10 m alt., 16.vii.1994, *Matos C.* 7589 (BRLU, LISC). Z.E. Lat. 00º 23' N; Long. 06º 37' E. Roça D.ª Amélia, Santa Catarina, 500 m alt., 17.vii.1994, *Matos C.* 7596 (BRLU, LISC). Z.E. Lat. N 00º 16' 00"; long. E 06º 34' 50". Nos arredores de Calvário, 1450 m alt., 26.x.1994, *Matos C.* 7701 (BRLU, LISC). Cascata de S. Nicolau, 28.viii.1956, *Monod* 12038 (COI). Entre Santa Clotilde e Santa

Catarina. Numa barreira da estrada, 13.x.1992, *Paiva* 665 (COI). Santa Catarina. S. Manuel. Numa barreira húmida da estrada, 13.ix.1995, *Paiva* 721 (COI). Cascata de S. Nicolau, 19.ix.1995, *Paiva* 837 (COI). Cascata de S. Nicolau, 31.vii.1996, *Paiva* 948 (COI). Entre Macambrará e Zampalma, 1300 m alt., 5.ix.1996, *Paiva* 1085 (COI). Santa Catarina, 7.ix.1996, *Paiva* 1170 (COI). Santa Catarina. Junto à entrada de uma mina húmida ("Vulcão"), 13.xii.1996, *Paiva* 1232 (COI). Cascata de S.Nicolau, 17.vii.1998, *Paiva* 1277 (COI). Cascata de Bombaim, 24.viii.2002, *Paiva* 1509 (COI). Entre Santa Catarina e o Rio Água de Azeitonas, 10 m alt., 25.i.2006, *Paiva* 1655 (COI). Entre Santa Catarina e o Rio Água de Azeitonas, 10 m alt., 25.i.2006, *Soares M.* 82 (COI). Calvário, 16.viii.1997, *Stévart* 54 (BRLU, BR). Bombaim, 1957, *Rose* 161 (P). Cascata de S. Nicolau, no fundo, 8.x.1954, *Rozeira* 558 (PO).

Impatiens balsamina L., Sp. Pl. 938 (1753); Chevalier, Sudania, 2: 49 (1914); A.W. Exell, Cat. Vasc. Pl. St. Tomé 125 (1944) & Bull. Brit. Mus. (Nat. Hist.) Bot. 4: 342 (1973); Sobrinho, L.G., Garcia de Orta 7 (1): 91 (1959); Grey-Wilson, *Impatiens* of Africa: 214 (1980).

　Impatiens tamsiana Exell, Cat. Vasc. Pl. St. Tomé: 127 (1944) & Bull. Brit. Mus. (Nat. Hist.) Bot. 4: 342 (1973). Type: São Tomé e Príncipe, Príncipe, Terreiro Velho, ca. 220, *Exell* 574 (BM holotype, COI isotype).

HERB, annual, pubescent, up to 1.5 m high. LEAVES spirally arranged, the petiole 0.4–2.4 cm long, glabrous, petiole glands sessile, located along the entire length of the petiole; lamina 5.3–11.5 x 1.22–3.15 cm, narrowly elliptic or oblanceolate, normally lanceotate

when young, base cuneate, apex acute, pubescent on both surfaces, very rarely glabrous; lateral veins 5–8 pairs; margin serrate with gland-tipped appendages at the apex of the teeth. FLOWERS epedunculate, solitary or in fascicles of 2, rarely 3, white or pink, sometimes pink with lateral united petals red. PEDUNCLE c. 1.26 cm long, slender, slightly pubescent. LATERAL SEPALS c. 0.28 x 0.19 cm, ovate, apex acute. LOWER SEPAL c. 1.23 cm deep, c. 2.39 cm long, deeply navicular, abruptly constricted into an incurved filiform spur, glabrous. DORSAL PETAL c. 1.22 x 0.71 cm, folded, cucullate, the apex extending into a c. 0.6 cm long pointed appendage. LATERAL UNITED PETALS, the upper petal sometimes considerably smaller, about 1/3 of the lower or both united petals of about the same size the upper petal 0.87–2 x 0.55–2.03 cm, the lower petal 1.94–2.43 x 1.62–1.71 cm. OVARY, fusiform, densely pubescent. FRUIT 0.9–1.32 x 0.13–0.5 cm, fusiform, densely pubescent. SEEDS 13, c. 0.22 x 0.17 cm.

Distribution: Introduced species native to India. Cultivated on both islands.

Habitat: Cultivated, gardens. The type locality of *I. tamsiana* (=**I. balsamina**), was recently visited by one of the authors that found no traces of its presence in the area. In fact, in a recent field survey to both islands, *I. balsamina* was never found in the wild but always restricted to a few gardens. Known to flower and fruit in I, IV, VII, X, XII. Altitudinal range 10-700 m.

Vernacular names: "Balsamina", "Melindres" (Portuguese)

Representative collections:

Island of São Tomé: Boa Entrada, vii.1905, *Chevalier* 14372 (P). Água d'Agó, 29.vii.1968, *Espírito Santo* 4672 (LISC). Quinta da Graça, 600 m alt., 10.iv.1973, *Espírito Santo* 5180 (COI, LISC). Missão de Santo António de Onango, i.1934, *Gomes e Sousa* 1652, 1668 (unknown). Monte Café, 700 m alt., 14.x.1956, *Lains e Silva H.* 131 (LISU). Aldeia de Monte Café, ca. 700 m alt., 23.i.2006, *Paiva* 1583 (COI). Junto da entrada da sede da Roça Monte Café, 26.x.1954, *Rozeira* s.n. (PO). Na aldeia de Monte Café, junto à estrada, 700 m alt., 23.i.2006, *Soares M.* 32 (COI). *Island of Príncipe*: Terreiro Velho, ca. 220 m alt., 12.xii.1932, *Exell* 574 (BM, COI). Cidade de Santo António do Príncipe, ca. 10 m alt., 27.i.2006, *Paiva* 1690 (COI). Cidade de Santo António do Príncipe, Rua Ôa (Rua da casa da cooperação Portuguesa), 10 m alt., 27.i.2006, *Soares M.* 105 (COI).

DISCUSSION

Palynology

In the present study, pollen characters possess taxonomical value since they allow a clear separation of the four species in study. The pollen of the four *Impatiens* species studied here follows the general pattern of the 4-colpate with rectangular or oblong equator group (Grey-Wilson 1980b). The four species in study presented a medium to large pollen size. *Impatiens buccinalis* can be clearly separated from the other species by its noticeably larger pollen grains. *Impatiens manteroana* presents an asymmetrical pollen grain in polar view, with a hump projecting to one of the lateral sides, and a more or less evident

truncated end on one side in equatorial view, making it more or less easily separated from the remaining two species. The pollen of this species presents the thickest muri of all four species. The distinction between *I. thomensis* and *I. balsamina* is not immediately obvious. Their pollen presents relatively similar size and a quite similar structure of the reticulum. Nevertheless, pollen grains from *I. balsamina* tend to be isopolar in equatorial view while those from *I. thomensis* generally present a more pronounced subisopolar shape.

I. buccinalis, by its noticeably larger pollen grains is only comparable to *I. volkensii* Warb. (Huynh, 1968b), an endemic species from Tanzania which is the largest species of *Impatiens* in Africa (Grey-Wilson, 1980a). This relation is purely based on the size of the grain since both species are considerably large for *Impatiens*. Huynh (1968b) also refers the similarity between the pollen of *I. manteroana* and that of *I. niamniamensis* Gilg (an ubiquitous species present throughout most of tropical Africa, also in Cameroon and Bioko), and *I. congolensis* G.M. Schulze & R. Wilczek (Congo). This is particularly acceptable namely on what regards *I. niamniamensis* due to the distribution of the species and is in accordance with the aggregate proposed by Grey-Wilson (1980a) for this species.

Morphology

Phenetic analysis produced similar results for both datasets. In the first dataset, the cluster grouping *I. buccinalis* and *I. manteroana* is divided into two main subclusters, one grouping specimens of both species, and the other grouping six specimens identified as *I. manteroana* (white arrow on Fig. 3). This six specimens shared leaves with less main lateral veins, longer distances between veins and smaller leaf glands. Interestingly, 5 out of 6 of the specimens of this subcluster were collected at or very near Pico Papagaio with one

being collect at Pico A Mesa (in the SW part of the island). This segregation was not verified when floral material was included in the analysis. This might indicate that the specimens of Pico Papagaio form a particular population within the island which is interesting given the small size of Príncipe and the fact that the species was detected from 200 m upwards and that there is no evident geographic obstacle between the area of Pico Papagaio and the remaining areas of the island. In the same analysis, two OTUs identified as *I. buccinalis* appear outside the main cluster grouping that species (black arrow on Fig. 3). One of these OTUs (specimen Paiva 992), is an atypical specimen since it has a slight pubescence of the stem which is extremely unusual for this species. The second OTU (Moller 342,367), presents leaves with a relatively smaller maximum length of the lamina, smaller average distance between main lateral veins and an atypical leaf base (cuneate and obtuse against the general cuneate base trend for the species). PCA results for the same dataset produced similar results in what regards the three endemics. A with arrow on Fig. 4 signs the position of the specimen Paiva 992, midway between the STP endemics and *I. balsamina*.

The CA for the second dataset (with the inclusion of floral characters) helped to separate *I. manteroana* from *I. buccinalis* but maintained the close relationship between the two species. The PCA results for this dataset however brought *I. thomensis* and *I. manteroana* closer together which can probably be explained by the relatively similar sizes of the floral parts measured in comparison with those of *I. buccinalis*.

I. balsamina appeared clearly isolated from the three STP endemics, based on both vegetative and floral characters. A study of the molecular phylogeny of these taxa (Soares *et al.*, unpublished data), also supports this result. This does not agree with Grey-Wilson's (1980a, 1980b) suggestion of a possible hybrid origin of *I. thomensis* with *I. buccinalis* and

I. balsamina as putative parents. This author based is hypothesis in the fact that *I. thomensis* appears to occupy a unique position within the red flower balsams since that, apart from the red flower, the flower seems to be a reminiscence of *I. balsamina*. He considered *I. thomensis* to be an intermediary between the two assumed parents. Nevertheless, there is no similarity between the inflorescence of the two STP endemics and *I. balsamina* since the first have flowered axillary racemes and the latter epedunculate flowers, solitary or in fascicules. Another character mentioned by Grey-Wilson (1980b) as support of the possible hybrid origin of *I. thomensis* was the leaf shape which in this species, according to the author, seemed to fall halfway between *I. buccinalis* and *I. balsamina*. The results of the present phenetic analysis and a thorough observation of all the available collections contradict this assumption.

The fact that some specimens of *I. thomensis* present a lower sepal intermediate between the deeply navicular, abruptly constricted into a spiraled or incurved filiform spur and a more bucciniform shape with an incurved filiform spur, indicates a closer proximity to the other two endemics. In fact, apart from the apparent similarities between the lower sepal general structure of *I. thomensis* and *I. balsamina* (deeply navicular and abruptly constricted into a more or less incurved filiform spur), no other character supports any relationship between the two species.

Grey-Wilson (1980a, 1980b) also considered *I. balsamina* as being possibly naturalized in STP, using this as an additional argument for the possible hybrid origin of *I. thomensis*. In a recent field survey conducted by one of the authors, *I. balsamina* was only found restricted to a few gardens. There is no evidence, to our knowledge, supporting the idea that this species is naturalized in STP.

Another interesting remark concerning *I. balsamina,* is the fact that two recent collections of this species, one in São Tomé (Soares M. 32, COI) and the other one in Príncipe (Soares M. 105, COI), both in residential gardens, showed that this species is highly variable, most certainly due to the fact of being a cultivated species. Most striking variation occurred at the Lateral United Petals (LUPs) since both specimens presented a fully developed upper LUP, about the same size as the lower one (Fig. 2A) which goes against the description presented by Grey-Wilson (1980a) for this species. Furthermore, the specimen Soares M. 32 presented, on the same individual, flowers completely pink and flowers pink with red LUPs. Regarding the size of the LUPs it is worth mentioning the tremendous difficulty one can expect while dissecting dried flowers of *I. balsamina*. In fact, due to the size and fragility of the LUPs, dissection must be carried out with increased care and patience since the two LUPs tend to fold by the middle and closely adhere to one another. Giving both the small thickness of the petals and their transparency, it is easy first to break one of the petals near their junction and/or, second, to overlook the presence of a fully developed upper LUP. Nevertheless, other specimens (e.g. Espírito Santo 1580), presented an upper LUP about one third the size of the lower, in accordance with Grey-Wilson's (1980a) description for *I. balsamina*. This intra-specific variation might explain the description by Exell of *I. tamsiana* (= **I. balsamina**). In fact, the drawings of this species presented by Exell (1944), show the two LUPs of about the same size, similar to the specimens mentioned above. Our phenetic analysis consistently grouped the specimen Exell 574 with the remaining *I. balsamina* specimens. In our opinion, Grey-Wilson's decision to consider *I. tamsiana* as a synonym of *I. balsamina* is correct.

Acknowledgements

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Appendix 1 - List of OTUs analyzed and their use in the datasets.

Collector	Number	Species	Dataset 1	Dataset 2	Palynology
Chevalier	14372	*I. balsamina*	+	-	-
Espírito Santo	168	*I. buccinalis*	+	+	+
Espírito Santo	3960	*I. thomensis*	+	+	+
Espírito Santo	4023	*I. buccinalis*	+	+	-
Espírito Santo	4354	*I. thomensis*	+	-	-
Espírito Santo	4612	*I. buccinalis*	+	+	-
Espírito Santo	4672	*I. balsamina*	+	+	+
Espírito Santo	5158	*I. thomensis*	+	+	-
Espírito Santo	5177	*I. thomensis*	+	+	-
Espírito Santo	5180	*I. balsamina*	+	+	+
Exell	195	*I. buccinalis*	+	+	-
Exell	281	*I. thomensis*	+	+	-
Exell	433	*I. thomensis*	+	+	-
Exell	574	*I. balsamina*	+	+	+
Exell	682	*I. manteroana*	+	+	+
Figueiredo	255	*I. thomensis*	+	+	+
Figueiredo & Arriegas	113	*I. thomensis*	+	-	-
Gomes & Sousa	1652	*I. balsamina*	+	-	-
Gomes & Sousa	1668	*I. balsamina*	+	+	-
H. Lains & Silva	131	*I. balsamina*	+	-	-
Joffroy G.	180	*I. buccinalis*	+	-	-
Joffroy G.	83	*I. thomensis*	+	+	-
Lejoly	97/325	*I. buccinalis*	+	-	+
Matos C.	7539	*I. buccinalis*	+	+	-
Matos C.	7549	*I. thomensis*	+	+	-
Matos C.	7550	*I. buccinalis*	+	+	-
Matos C.	7589	*I. thomensis*	+	+	+
Matos C.	7596	*I. thomensis*	+	+	-
Matos C.	7701	*I. thomensis*	+	+	+
Matos C.	7746	*I. manteroana*	+	+	+
Matos C. & Van. Essche	7340	*I. buccinalis*	+	+	-
Matos C. & Van. Essche	7430	*I. buccinalis*	+	+	+
Moller	291	*I. buccinalis*	+	+	-
Moller	342, 367	*I. buccinalis*	+	+	-
Moller	419	*I. buccinalis*	+	+	+
Moller	593	*I. buccinalis*	+	+	-
Moller	872	*I. buccinalis*	+	+	-
Monod	11719	*I. buccinalis*	+	-	-
Monod	11773	*I. buccinalis*	+	-	-
Monod	12038	*I. thomensis*	+	-	-
Monod	12167	*I. manteroana*	+	+	-
Paiva	466	*I. buccinalis*	+	+	+
Paiva	579	*I. manteroana*	+	-	-
Paiva	586	*I. manteroana*	+	+	+
Paiva	665	*I. thomensis*	+	+	-
Paiva	721	*I. thomensis*	+	+	-
Paiva	837	*I. thomensis*	+	-	-
Paiva	884	*I. buccinalis*	+	-	+
Paiva	948	*I. manteroana*	+	+	-
Paiva	992	*I. buccinalis*	+	-	-
Paiva	1085	*I. thomensis*	+	+	-
Paiva	1170	*I. thomensis*	+	+	-
Paiva	1232	*I. thomensis*	+	+	+
Paiva	1277	*I. thomensis*	+	-	-

Paiva	1445	*I. manteroana*	**+**	**+**	-
Paiva	1477	*I. manteroana*	**+**	**+**	**+**
Paiva	1509	*I. thomensis*	**+**	-	-
Quintas	1319	*I. buccinalis*	**+**	-	-
Rose	161	*I. thomensis*	**+**	**+**	-
Rozeira	112	*I. buccinalis*	**+**	-	-
Rozeira	558	*I. thomensis*	**+**	-	-
Rozeira	948	*I. thomensis*	**+**	-	-
Soares M.	32	*I. balsamina*	**+**	**+**	-
Soares M.	82	*I. thomensis*	**+**	**+**	-
Soares M.	105	*I. balsamina*	**+**	**+**	-
Soares M.	119	*I. manteroana*	**+**	-	-
Soares M.	127	*I. manteroana*	**+**	-	-
Soares M.	128	*I. manteroana*	**+**	**+**	-
Soares M.	129	*I. manteroana*	**+**	**+**	-
Soares M.	148	*I. buccinalis*	**+**	**+**	-

Appendix 2 – Results of PCA for the 1st dataset. Component loading along the three first axes.

Variable	Axis 1	Axis 2	Axis 3
1	0.9196	0.0780	0.1840
2	-0.5886	0.3481	0.3635
3	-0.3430	0.7896	0.0847
4	-0.8605	-0.0316	-0.1165
5	0.8412	-0.0098	0.1085
6	0.8005	-0.0036	0.1538
7	0.9315	0.0388	0.2570
8	-0.1294	0.5348	0.6438
9	-0.4486	-0.5391	0.6766
10	-0.8181	-0.2127	0.3009
11	0.4763	-0.4999	0.0256
12	-0.9315	-0.0388	-0.2570
13	-0.7443	-0.0838	-0.2490
14	0.0882	0.7686	0.0315
15	-0.4006	0.2340	0.4620
16	-0.2467	-0.6922	0.4127

Appendix 3 - Results of PCA for the 2nd dataset. Component loading along the three first axes.

Variable	Axis 1	Axis 2	Axis 3
1	0.9531	0.2067	0.1933
2	-0.4901	0.3615	-0.2506
3	-0.4825	0.6322	0.1642
4	-0.7955	-0.5255	0.0630
5	0.6056	0.2270	0.3666
6	0.6056	0.2270	0.3666
7	0.9531	0.2067	0.1933
8	0.1173	0.5842	-0.1412
9	-0.0014	-0.1622	-0.7163
10	-0.7444	-0.2383	-0.1546
11	0.4706	-0.1161	-0.3462
12	-0.9531	-0.2067	-0.1933
13	-0.8015	-0.2795	-0.0378
14	-0.1277	0.4497	0.5916
15	0.1045	-0.0634	-0.2089
16	-0.0659	-0.0314	-0.5165
17	-0.3736	-0.7945	-0.2168
18	0.1487	-0.6989	0.0234
19	0.9624	0.2167	0.2236
20	-0.1571	-0.5707	0.5290
21	0.1957	-0.5001	0.7738
22	-0.3448	-0.8481	-0.0422
23	-0.2602	-0.9349	0.0671
24	0.1745	-0.8465	-0.2780
25	0.1540	-0.9775	-0.2148
26	0.9473	0.2004	0.1742
27	0.3674	-0.6677	-0.2030
28	-0.0382	-0.9342	0.1778
29	0.4532	-0.8171	0.0441
30	0.5474	-0.5185	0.1735
31	0.7073	-0.6072	0.2999
32	0.6236	-0.5702	0.2349
33	-0.4662	-0.9287	0.5511
34	-0.3950	-0.8605	0.4699
35	0.2913	-0.8187	-0.0553
36	0.9539	0.2070	0.1904
37	1.0045	0.2355	0.1648
38	0.4699	-0.4701	0.0879
39	0.8676	0.1491	0.2464
40	1.0605	-0.3432	-0.7976
41	1.1060	-0.3767	-0.4668
42	1.1389	-0.3716	-0.4529

Capítulo 3

Phylogenetic origin of *Impatiens* L. (*Balsaminaceae*) of São Tomé e Príncipe

SUMMARY - Phylogenetic relationships within species of *Impatiens L.* endemic to São Tomé e Príncipe (Gulf of Guinea), and their relationship to species from the continent and adjacent islands, were evaluated using sequence data from *ITS*, *trnL-F*. *Impatiens thomensis Exell*, *I. manteroana Exell* and *I. buccinalis Hook.f.* (São Tomé e Príncipe endemics) consistently grouped with species present in West-Central Africa assembling together a monophyletic group, which seems to support the theory that their presence on the islands is due to one (or more) dispersal events from a nearby continental area. The phylogenetic relationships between these three species are not completely resolved. The *ITS* sequence analysis indicates that none of these species are closely related to *I. balsamina L.*, a cultivated species that previous authors have suggested as a putative parent to *I. thomensis*.

KEY WORDS - *Balsaminaceae*, Gulf of Guinea, *Impatiens*, *ITS*, phylogeny, São Tomé e Príncipe *, trnL-F*.

INTRODUCTION

The prolific and highly diversified genus *Impatiens L.* (*Balsaminaceae*) is mainly distributed throughout the mountain areas of the Old-World tropical and subtropical regions. The number of *Impatiens* species is estimated to be over 1000 (Grey-Wilson 1980b; Janssens *et al.* 2006).

Grey-Wilson (1980b), in its revision of the African *Impatiens*, sets the figure of 109 species native to the continent, but the number of African species has since increased due to the description of new species and taxonomic changes.

Four *Impatiens* species have been recorded for the islands of São Tomé e Príncipe, in the Gulf of Guinea : *I. manteroana Exell* (endemic to Príncipe), *I. buccinalis Hook.f.* and *I. thomensis Exell* (both endemic to São Tomé), and *I. balsamina L.*, a species native to India, recorded in both islands and used as an ornamental in some gardens. Grey-Wilson (1980b) refers to *I. balsamina* as being naturalized in São Tomé e Príncipe.

The study of *Impatiens* poses some well-known challenges. The fragility of floral material and the consequent difficulties in analyzing herbarium material and the diversity in floral morphological features, with many intermediate specimens that appear to bridge the gap between taxa, makes any attempt to naturally group species within the genus a very difficult task (Grey-Wilson 1980b). The high level of both hybridization and endemism within the genus (Grey-Wilson 1980b) also contribute to this fact.

In recent years the study of *Balsaminaceae* has been approached at molecular level by several authors. Fujihashi *et al.* (2002) published the first molecular phylogeny of *Impatiens* but the limited number of taxa sampled and the use of too distant outgroups limited the definition of intrageneric relationships. Yuan *et al.* (2004), using Internal

Transcribed Spacer (*ITS*) brought new phylogenetic insights on the family, namely the monophyly of *Balsaminaceae* and the fact that the African species of *Impatiens* are not monophyletic, with the African continent being colonized at least twice. Janssens *et al.* (2006), using Chloroplast *atpB-rbcL* Spacer Sequences, confirmed the monophyly of the family and outlined a probable origin of *Impatiens* in the South of China from where the radiation of the genus would have occurred. Janssens *et al.* (2007), evaluated the phylogenetic utility of the *AP3*/*DEF K*-domain and proved the value of these genes as a source of characters for phylogenetic studies at infrageneric level in *Impatiens*.

Although the species of *Impatiens* endemic to São Tomé e Príncipe were not included in either of the referred studies, their morphology has been investigated by other authors (Exell 1944; Grey-Wilson 1980b). The latter author placed the three species of Impatiens endemic to São Tomé e Príncipe in his *I. hians Hook.f.* aggregate, a west African species complex centered on Cameroon. He also hypothesized the hybrid origin of *I. thomensis,* with *I. buccinalis* and *I. balsamina* as putative parents, based mainly on floral morphology. The present study aims to investigate the phylogeny of the species of *Impatiens* endemic to São Tomé e Príncipe and the relationship between these species and *I. balsamina*.

MATERIALS AND METHODS

Plant material and DNA extraction: Total DNA was extracted from herbarium material or silica-gel dried leaves. Five species of *Impatiens* were sequenced for *ITS* and nine for *trnL-F* including, in both cases, the three species endemic to São Tomé e Príncipe; additional sequences were obtained from GeneBank (see Appendix). Four sequences from three taxa (*I. mackeyana Hook.f.* subsp. *mackeyana, I. mackeyana* subsp. *zenkeri (Warb.) Grey-*

Wilson and *I. mannii Hook.f.*) were obtained from DNA extracted from herbarium material. DNA was extracted from c. 0.2 - 0.25 g silica gel-dried plant leaves, using the 2X CTAB method (Doyle & Doyle 1987). DNA was further purified by QIAquick column purification according to the manufacturer protocol (Qiagen Ltd).

Gene amplification and sequencing: the *trnL-F* region was amplified using primers c, d, e and f (Taberlet *et al.* 1991) with the standard protocol. The *ITS* region was amplified using primers four and five (White *et al.* 1990). Amplification products were purified using the QIAquick column purification system according to the manufacturer´s protocol (Qiagen Ltd). Modified dideoxy cycle sequencing with dye terminators run on ABI 310 automated sequencer (Applied Biosystems Inc.) according to the manufacturer's protocols was used to sequence the amplification products directly. The sequences were edited and assembled using BioEdit 7.0.5 (Hall 1999).

Sequence alignment and phylogenetic analysis: for both *ITS* and *trnL-F* an initial alignment was performed for five sequences using ClustalX (Thompson *et al.* 1997) . Subsequent sequences were aligned by eye. Data were analysed with the parsimony algorithm of the software package PAUP* version 4.0b10 (Swofford 1998). Heuristic searches were conducted on the separate and combined *ITS* and *trnL-F* datasets. Trees were initiated via stepwise addition with 1000 replicates of random taxon entry, saving 10 optimal trees per replicate. All trees produced in the 1000 replicates were saved and then swapped to completion using tree bisection-reconnection (TBR) as the swapping algorithm. To assess internal support in each of the above analyses, 1000 bootstrap replicates were performed using simple taxon addition and TBR branch swapping. A tree limit of 10 trees per replicate

was set to reduce time spent swapping on large numbers of equally parsimonious trees. *Impatiens chinensis L.* was used as an outgroup for *ITS* analysis and *I. uniflora Hayata, I. bicornuta Wall.* and *I. racemosa DC.* were used as an outgroup for *trnL-F* and combined analysis.

RESULTS

trnL-F

Sequences of the 14 taxa included in the *trnL-F* analysis ranged in length from 391 bp (*I. hoehnelii T.C.E.Fr.*) to 937 bp (*I. manteroana*). The aligned *trnL-F* matrix consists of 1064 bp. 59 characters were excluded because of the excess of missing data at the edges of the matrix; of the remaining 1005 characters, 102 (10.1%) were variable. Potentially informative characters (34) accounted for 3.4 % of all *trnL-F* sites and 33.3 % of variable sites. *Impatiens hians* had the largest indel (38bp). Heuristic searches resulted in 39 trees of 106 steps. One of the 39 most parsimonious trees is shown in Fig. 1. The consistency index (CI) was 0.97 and the retention index (RI) was 0.95. The African species were grouped in two main clades. Clade 1 (BS: 82%) comprises East African species. *I. balsamina* is included in this species group. Clade 2 (BS: 69%) consists of species present in the area of the Gulf of Guinea with some species extending into Central Tropical Africa.
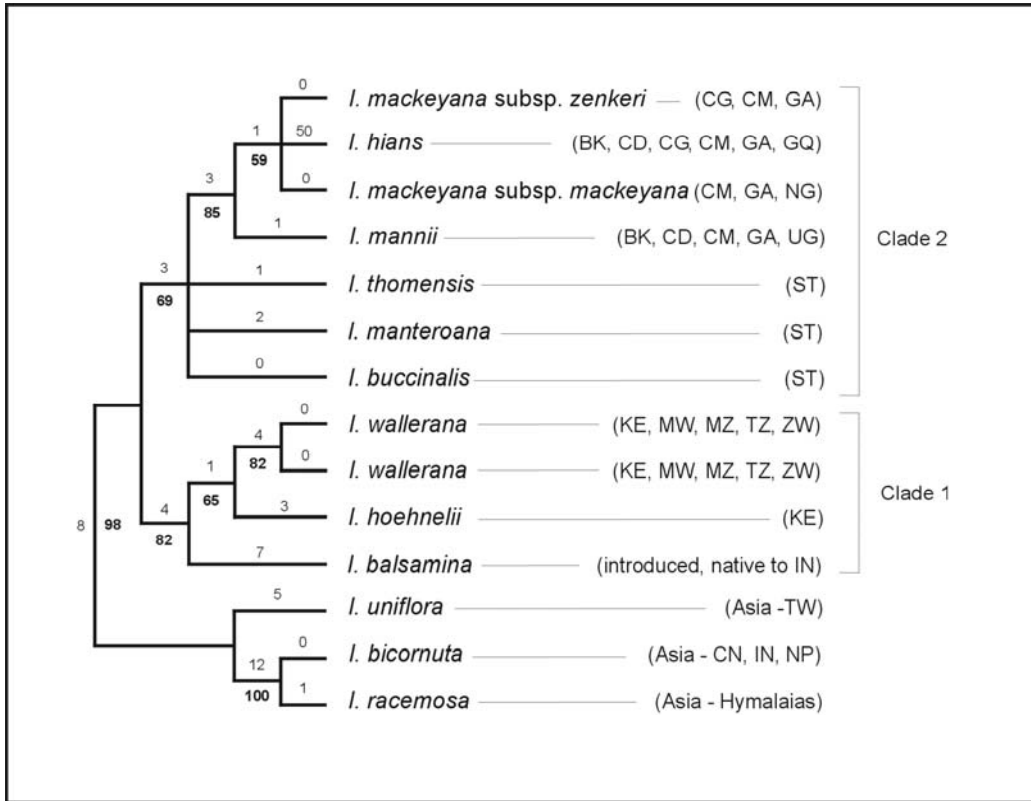
Fig. 1 - One of the 39 *trnL-F* most parsimonious trees. Numerals above branches represent branch length, Bootstrap percentages (BP) over 50 are indicated below branches. Distribution areas are shown after the species name (BK – Bioko, CD – Congo (Kinshasa), CG – Congo (Brazzaville), CM – Cameroon, CN – China, GA – Gabon, GQ – Equatorial Guinea , IN – India, KE – Kenya, MW – Malawi, MZ – Mozambique, NG – Nigeria, NP – Nepal, ST – São Tomé e Príncipe, TW – Taiwan, TZ – Tanzania, UG – Uganda, ZW – Zimbabwe).

ITS

The aligned *ITS* matrix with 26 taxa had 806 characters of which 146 were excluded. Sequences ranged from 597 bp (*I. chinensis*) to 737 bp (*I. buccinalis*), Of the 660 included

characters, 268 were variable (40.6%). Parsimony informative characters (161) accounted for 24.4% of all *ITS* sites and for 60% of variable sites. Heuristic searches resulted in 194 trees of 511 steps. One of the 194 most parsimonious trees is shown in Fig. 2. The consistency index (CI) was 0.72 and the retention index (RI) was 0.78. The trees were not very well resolved (Fig. 2) and most of the branches collapsed in the strict consensus tree. Six clades were identified. Clade 1 (BS: <50 %) and clade 2 (BS: 100 %) group species mostly from eastern Africa. The presence of *I. hians* (AY348791) a more western species, in clade 2 is unexpected. This may be due to a misidentification of the corresponding voucher. Clade 3 (BS: <50 %), with the exception of *I. hoehnelii*, groups species with a wide range with a common distribution around Central Africa. Clade 4 (BS: 100 %) corresponds to Grey-Wilson's (1980b) *I. gomphophylla Baker f.* aggregate. Interestingly, the three species share a more or less pronounced epyphytism. The two species in Clade 5 (BS: 100 %) have been placed by Grey-Wilson (1980b) in different aggregates. Clade 6 (BS: 100 %) is fairly consistent biogeographically. With the exception of *I. tinctoria A.Rich.,* which is mostly an East African species, all the species have an area of occurrence that includes the Gulf of Guinea, including the three São Tomé e Príncipe endemics.
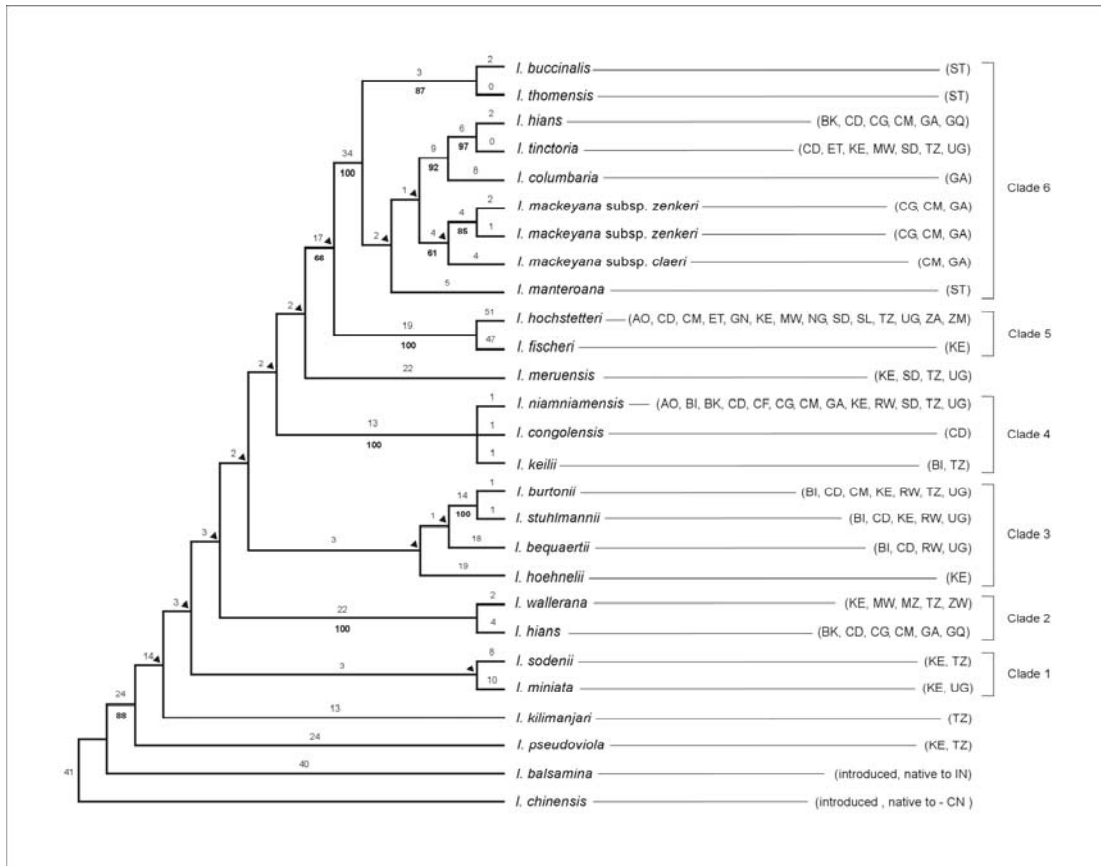
Fig. 2 - One of the 194 *ITS* most parsimonious trees. Numerals above branches represent branch length, Bootstrap percentages (BP) over 50 are indicated below branches, and an arrow indicates those clades that collapsed in the strict consensus tree. Distribution areas are shown after the species name (AO – Angola, BI- Burundi, BK – Bioko, CD – Congo (Kinshasa), CF – Central African Republic, CG – Congo (Brazzaville), CM – Cameroon, CN – China, ET – Ethiopia, GA – Gabon, GN – Guinea, GQ – Equatorial Guinea, IN – India, KE – Kenya, MW – Malawi, MZ – Mozambique, NG – Nigeria, RW – Rwanda, SD – Sudan, SL – Sierra Leone, ST – São Tomé e Príncipe, TZ – Tanzania, UG – Uganda, ZA – South Africa, ZM – Zambia, ZW – Zimbabwe).

Combined *ITS* and *trnL-F*

For the combined analysis only 14 taxa common to both *ITS* and *trnL-F* matrices were included, the final matrix had 1788 bp excluding ends of both matrices. The heuristic searches resulted in three trees of 278 steps. The consistency index (CI) was 0.93 and the retention index (RI) was 0.87. Combined *trnL-F* and *ITS* data (Fig. 3), originated two main clades. Clade 1 (BS: 93 %) included the East African species and *I. balsamina*. Clade 2 (BS: 68 %) aggregated the West and Central African species.
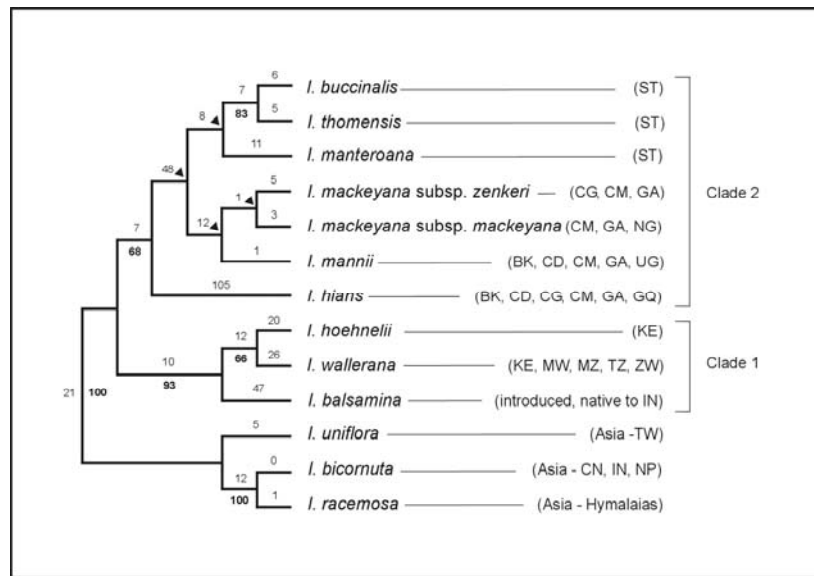

Fig. 3 - One of the three most parsimonious trees obtained in the combined *ITS* and *trnL-F* analysis. Numerals above branches represent branch length, Bootstrap percentages (BP) over 50 are indicated below branches, and an arrow indicates those clades that collapsed in the strict consensus tree. Distribution areas are shown after the species name (BK – Bioko, CD – Congo (Kinshasa), CG – Congo (Brazzaville), CM – Cameroon, CN – China, GA – Gabon, GQ – Equatorial Guinea , IN – India, KE – Kenya, MW – Malawi, MZ – Mozambique, NG – Nigeria, NP – Nepal, ST – São Tomé e Príncipe, TW – Taiwan, TZ – Tanzania, UG – Uganda, ZW – Zimbabwe).

DISCUSSION

The islands of São Tomé e Principe are derived from volcanic activity that took place during the Tertiary along a NE-SW line of fractures from the Cameroon highlands to St. Helena (Furon 1963; Piper & Richardson 1972). Príncipe is 210 km away from both yhe island of Bioko and the continent and 135 km away from São Tomé which in turn is more or less 200 km off the northwestern coast of Gabon. There is evidence (Exell 1973), supporting the theory that geologically the islands of São Tomé e Príncipe were never connected to the African continent, unlike the neighbouring island of Bioko (Exell 1944). Therefore, their floras must have originated from species dispersed by wind, sea currents, birds or by human intervention.

Exell (1973) pointed out the high levels of similarity between the floras of both São Tomé and of Príncipe and the ones of Bioko and Annobon and the neighbouring African countries. He also inferred from his analysis that both São Tomé e Príncipe received their independent share of colonizing species through time.

Both the *trnL-F* and *ITS* data analyses consistently grouped the *Impatiens* endemic to São Tomé e Príncipe with species present in West-Central Africa, namely with *I. mackeyana* subsp. *zenkeri* (Cameroon, N. Gabon, W. Congo (Brazzaville), *I. mackeyana* subsp. *claeri (N.Hallé) Grey-Wilson* (S.E. Cameroon, C. & E. Gabon), *I. mannii* (S. & W. Cameroon, Bioko, Gabon, E. Congo (Kinshasa), W. Uganda), *I. hians* (Cameroon, Bioko, Equatorial Guinea, Gabon, E. Congo (Brazzaville), W. Congo (Kinshasa) and *I. columbaria J.J.Bos* (Gabon). The bootstrap values (100%) for Clade 6 in the *ITS* tree support this statement. This is consistent with the analysis made by Exell (1973). It is simultaneously consistent with the hypothetical pathway of migration of the genus in Africa proposed by Grey-

Wilson (1980b), at least with regard to the Gulf of Guinea area, with a radiation point centered in Cameroon spreading westwards into Nigeria (up to Guinea), southwards into Gabon (going as far as the north of Angola), and southwestwards into Bioko, Príncipe and São Tomé.

The relationship between the three *Impatiens* endemic to São Tomé e Príncipe is not clearly resolved by this analysis. The 87% bootstrap value for the clade of *I. buccinalis* and *I. thomensis* in the *ITS* tree indicates that these two São Tomé endemics may be sister taxa and may form a monophyletic group. Based on the combined analyses, *I. manteroana,* from Príncipe, may form a monophyletic group with the species from São Tomé. Nevertheless, the reduced number of taxa included in this analysis reduced the support of this group which was also not supported by the independent ITS tree.

When floral morphology is considered, while *I. buccinalis* (São Tomé) and *I. manteroana* (Príncipe) share the saccate structure of the lower sepal, *I. thomensis* has a more navicular shaped lower sepal ending in a filiform spur which indicates adaptation to different pollinators. The two species endemic to São Tomé are very different both in the size of the flowers, and in their altitudinal range and habitat. *I. thomensis* can be found from sea level up to 1700 m but always creeping up humid walls with constant pouring water, while *I. buccinalis* is a true montane species, ranging from 800-1700 m in altitude and typically a forest perennial upright species.

A scenario of two different colonization events for the genus *Impatiens* in São Tomé and in Príncipe, cannot be ruled out by the results of our analysis as the position of the latter species remains unresolved in the consensus trees.

Concerning the hypothesized hybrid origin of *I. thomensis* from *I. buccinalis* and *I. balsamina*, advanced by Grey-Wilson (1980a; 1980b), this work shows that, at least based

on *trnL-F* and *ITS* data, there is no evidence for such descendent hybrid origin. As mentioned above, *I. thomensis* and *I. buccinalis* occupy very distinct habitats and partially different altitudinal ranges. Also, both species are perennial while *I. balsamina* is an annual species. On the island of São Tomé *I. balsamina* occupies a short intermediate altitudinal range (600-700m). The idea that this species could have became naturalized in São Tomé e Príncipe is possibly derived from the description of *I. tamsiana* by Exell based on a collection from a plantation in Príncipe. *I. tamsiana* was considered later as a synonym of *I. balsamina* (Grey-Wilson 1980b). The type locality of *I. tamsiana* was recently visited by one of the authors that found no traces of the presence of *I. balsamina*. In fact, in a recent field survey to both islands, *I. balsamina* was never found in the wild but always restricted to some few gardens. There is no evidence, to our best knowledge, that supports the idea that this species is naturalized in STP. There seems to be no support either from field evidence or from molecular data for *I. thomensis* being an hybrid from a crossing of *I. buccinalis* with *I. balsamina*. A recent palynological analysis of the four STP *Impatiens* species supports this conclusion (Soares M. *et al.* unpubl. data).

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APPENDIX

List of investigated taxa with accession numbers. Voucher information is given for new sequences.

Taxon	*ITS*	*trn L-F*	Voucher
Impatiens balsamina L.	-	EF649982	Soares M. 32 (COI)
Impatiens buccinalis Hook.f.	EF649983	EF649978	Soares M. 09/2002 (COI)
Impatiens hians Hook.f.	EF649985	EF649977	Chase 17146 (K)
Impatiens mackeyana Hook.f. subsp. mackeyana	-	EF649979	Parnentier & Esono 2913 (BRLU, LISC)
Impatiens mackeyana Hook.f. subsp zenkeri (Warb.) Grey-Wilson	EF649984	EF649974	Parnentier & Esono 3403 (BRLU, LISC)
Impatiens mannii Hook.f.	-	EF649980	Parnentier 3996 (BRLU, LISC)
Impatiens manteroana Exell	EF649986	EF649975	Paiva 1445 (COI)
Impatiens thomensis Exell	EF649987	EF649976	Soares M. 82 (COI); Figueiredo 255 (LISC)
Impatiens wallerana Hook.f.	-	EF649981	Soares M. 46 (COI)
Impatiens balsamina L.	AY348749	-	-
Impatiens bequaerti De Wild.	AY348753	-	-
Impatiens bicornuta Wall.	-	AB043644	-
Impatiens burtonii Hook.f.	AY348757	-	-
Impatiens capensis Thumb. non Meerb.		AF396205	
	AF396238	AF396206	-
		AJ430870	
Impatiens chinensis L.	AY348761	-	-
Impatiens columbaria J.J.Bos	AY348764	-	-
Impatiens congolensis Schulze & Wilczek	AY348766	-	-
Impatiens fischeri Warb.	AY348781	-	-
Impatiens hians Hook.f.	AY348791	K17146	-
Impatiens hoehnelii T.C.E.Fr.	AY348792	AB043642	-
Impatiens keilii Gilg	AY348798	-	-
Impatiens kilimanjari Oliv.	AY348800	-	-
Impatiens mackeyana Hook.f. subsp claeri (N.Hallé) Grey-Wilson	AY348763	-	-
Impatiens mackeyana Hook.f. subsp zenkeri (Warb.) Grey-Wilson	AY348852	-	-
Impatiens meruensis Gilg.	AY348807	-	-
Impatiens miniata Grey-Wilson	AY348809	-	-
Impatiens niamniamensis Gilg.	AY348812	-	-
Impatiens pseudoviola Gilg.	AY348822	-	-
Impatiens racemosa D.C.	-	AB043645	-
Impatiens sodenii Engl. & Warb. ex Engl.	AY348832	-	-
Impatiens stuhlmannii Warb.	AY348836	-	-
Impatiens tinctoria A.Rich.	AY348842	-	-
Impatiens uniflora Hayata	-	AB043661	-
Impatiens walleriana Hook.f.	AY348849	AF303469	-

Capítulo 4   Discussão

A posição taxonómica de cada uma das três endémicas é mantida. No entanto, os resultados do presente trabalho permitem uma nova avaliação das relações entre as três espécies. O facto de *I. thomensis* apresentar, ainda que raramente e nunca antes descrita, uma sépala inferior ligeiramente buciniforme, contrária à tendência do género (sépala inferior profundamente navicular e abruptamente contraída num esporão filiforme, espiralado ou encurvado), coloca-a mais próxima das restantes endémicas, e pode ser reveladora de algum vestígio de ancestralidade entre as três espécies. Os dados moleculares, ainda que de análise ponderada, parecem reforçar esta tendência mostrando uma íntima ligação entre as três endémicas. Os dados de ITS indicam que *I. buccinalis* e *I. thomensis* podem ser taxa irmanados formando um grupo monofilético. Por outro lado, a análise combinada (ITS/trnL-F), apesar do reduzido número de taxa disponíveis para análise (que convida a uma leitura com reservas), aponta para o facto de *I. manteroana* formar um grupo monofilético com as endémicas de São Tomé. Tanto as análises de ITS como de trnL-F, consistentemente agrupam as *Impatiens* endémicas de STP com espécies presentes na África Centro-Ocidental, nomeadamente com *I. mackeyana* subsp. *zenkeri* (Warb.) Grey-Wilson (Camarões, N. Gabão, W. Congo (Brazzaville), *I. mackeyana* subsp. *claeri (N.Hallé) Grey-Wilson* (S.E. Camarões, C. & E. Gabão), *I. mannii* Hook.f. (S. & W. Camarões, Bioko, Gabão, E. Congo (Kinshasa), W. Uganda), *I. hians* Hook.f. (Camarões, Bioko, Guiné Equatorial, Gabão, E. Congo (Brazzaville), W. Congo (Kinshasa)) e *I. columbaria J.J.Bos* (Gabão). Estes resultados são consistentes com o hipotético percurso de migração do género em África proposto por Grey-Wilson (1980a), pelo menos no que se refere à área do Golfo da Guiné com um ponto de radiação centrado nos Camarões, estendendo-se para Oeste até à Nigéria (chegando à Guiné), para Sul até ao Gabão (descendo até ao Norte de Angola) e para Sudoeste até às ilhas de Bioko e São Tomé e

Príncipe. No entanto, o ainda reduzido número disponível de sequências genéticas de espécies de *Impatiens* africanos não permite definir com clareza a relação precisa entre as três endémicas de STP e as restantes espécies do continente e ilhas adjacentes. Dado que tanto as ilhas do Príncipe e de São Tomé se encontram a aproximadamente a mesma distância do continente e que a primeira se encontra a igual distância da ilha de Bioko e do continente, devemos considerar a existência de dois cenários hipotéticos para a colonização de STP pelas espécies de *Impatiens*: uma migração a partir das terras altas dos Camarões (um importante ponto de radiação do género de acordo com Grey-Wilson, 1980a) com a ilha de Bioko a actuar como ponte e uma colonização a partir da costa continental do Gabão (cujas terras altas são também consideradas por Grey-Wilson (1980a) um importante ponto de radiação). Como Exell (1973b) referiu, as ilhas de São Tomé e do Príncipe terão recebido a sua quota independente de colonizadores. Considerando os dados morfológicos e moleculares deste trabalho, as três endémicas parecem partilhar uma ancestralidade comum, descendendo provavelmente de apenas uma linha de colonização e não duas.

Em termos de palinologia, as três endémicas de STP pertencem ao grupo dos grãos 4-colpados com equador rectangular ou oblongo. As diferenças na forma e tamanho do grão e estrutura do retículo mostram que, pelo menos no que toca às espécies consideradas neste estudo, o polén tem valor taxonómico. Algumas comparações com outras espécies podem ser estabelecidas com base em estudos prévios. Huynh (1968a), compara o pólen de *I. buccinalis* com o de *I. volkensii* Warb., uma endémica da Tanzânia e a maior espécie de *Impatiens* Africana (Grey-Wilson, 1980a). No entanto parece-me que esta comparação só pode ser circunstancial e baseada apenas numa comparação dos tamanhos dos grãos, uma característica certamente relacionada com as dimensões de ambas as espécies, e não em

qualquer relação directa entre os dois *taxa*. O mesmo autor (1968a) refere também a semelhança entre os grãos de *I. manteroana* e os de *I. niamniamensis* Gilg (uma espécie ubíqua distribuía por grande parte da África Tropical, incluindo Camarões e a Ilha de Bioko) e *I. congolensis* G.M. Schulze & R. Wilczek (Congo e R.D. do Congo). A possível relação com *I. niamniamensis* é concordante com o agregado de espécies proposto por Grey-Wilson (1980a).

Quanto ao pólen de *I. thomensis*, algumas considerações, ainda que muito reservadas, podem ser feitas no que concerne à sua possível relação com *I. balsamina* num cenário de possível origem híbrida com esta última como uma das progenitoras tal como proposto por Grey-Wilson (1980a). Este autor (1980b), descreve duas características para o pólen de espécies de conhecida origem híbrida: a) frequentemente ocorrem entre espécies pertencentes a um dos dois grupos de pólen de *Impatiens* africanos (3-colpados com equador triangular x 4-colpados com equador rectangular ou oblongo); b) grãos 5-colpados ou multi-colpados pertencem exclusivamente a plantas de origem híbrida conhecida. Nenhum dos dois casos se verificou neste estudo. Por um lado, *I. buccinalis* e *I. balsamina*, os dois supostos progenitores, apresentam ambos grãos 4-colpados com equador rectangular ou oblongo, por outro, nenhum grão de *I. thomensis* observado se mostrou penta ou multi-colpado ocorrendo apenas, numa proporção negligenciável, grãos 3-colpados. Embora os dados de palinologia não permitam, pelo seu valor, rejeitar com certeza a origem híbrida de *I. thomensis*, apontam, em conjunto com os restantes resultados deste trabalho, para a rejeição da hipótese da origem híbrida desta espécie.

Quanto à possível relação de *I. balsamina* com as endémicas de STP (nomeadamente *I. buccinalis* e *I. thomensis*), tanto a análise morfométrica como a molecular colocam esta

cultivada numa posição claramente distanciada das três endémicas de STP, não mostrando qualquer relação entre aquela e estas. O facto da análise morfométrica, quer com dados de material não floral, quer com estes, ter apresentado os mesmo resultados quanto à posição de *I. balsamina* relativamente às três endémicas suporta a ideia de que a hipótese da origem híbrida de *I. thomensis* a partir de um cruzamento de *I. buccinalis* x *I. balsamina*, avançada por Grey-Wilson (1980a, 1980b) não tem qualquer consistência. Este autor baseou a sua hipótese no facto de *I. thomensis* apresentar uma posição única entre as restantes balsamináceas de flores vermelhas dado que, exceptuando a cor, a forma da sépala inferior parece ser remanescente da flor de *I. balsamina*. Na verdade, foi maioritariamente com base na estrutura floral que este autor baseou a sua hipótese pois, de facto, *I. thomensis*, pela sua estrutura de sépala inferior, parece posicionar-se como intermediário entre as duas espécies assumidas como potenciais progenitores. O mesmo autor considerou igualmente a forma da folha e tipo de inflorescência para suportar a sua ideia de que *I. thomensis* se posicionaria a meia distância entre os dois supostos progenitores. Embora concorde, de uma forma geral, com a apreciação relativamente à questão da morfologia floral, não posso no entanto concordar com as referências às restantes características. *Impatiens buccinalis* e *I. thomensis* apresentam uma inflorescência do tipo racemo axilar enquanto que *I. balsamina* apresenta flores sésseis solitárias ou em fascículos. Quanto à forma da folha, uma análise abrangente do material colhido em STP não coloca *I. thomensis* numa posição intermédia entre aquela e *I. buccinalis*.

As evidências de campo reforçam também a ausência de relação entre *I. balsamina* e *I. thomensis*. Apesar de algumas colheitas mais antigas posicionarem *I. balsamina* em habitats como terrenos cultivados e floresta aberta, e mesmo considerando o facto de, sendo uma introduzida, a sua colheita poder ter sido negligenciada por vários colectores, num

recente trabalho de campo conduzido por mim em ambas as ilhas, esta espécie nunca foi encontrada no estado selvagem mas apenas restrita a alguns jardins, contrariando assim a ideia avançada por Grey-Wilson (1980a) de que a espécie se encontraria possivelmente já naturalizada no arquipélago. Por outro lado é de admitir que a sua introdução na ilha seja relativamente recente dado por um lado a sua origem asiática e por outro o facto das ilhas de STP se encontrarem desabitadas à chegada dos Portugueses no séc. XV.

Outra das questões analisadas envolve o exemplar Exell 754 (*I. tamsinana* = ***I. balsamina***). Apesar de este exemplar não ter sido incluído na análise molecular os resultados da análise morfométrica mostram que este espécime agrupa claramente com os restantes espécimes de *I. balsamina* analisados. Embora não tenha tido acesso ao holótipo de *I. tamsiana*, o isótipo foi no entanto analisado por mim. Não foram encontradas razões que contradigam a escolha de Grey-Wilson (1980a) em ter considerado *I. tamsiana* um sinónimo de *I. balsamina*. No entanto, os exemplares colhidos em STP mostram uma grande variabilidade no que respeita particularmente à forma das pétalas laterais unidas (PLU) sendo que alguns dos exemplares analisados possuíam PLU's em que tanto a pétala superior e inferior se apresentavam aproximadamente de igual tamanho, contrariando a descrição apresentada por Grey-Wilson (1980a) que descrevia a pétala superior com um tamanho 1/3 mais pequeno que o apresentado pela pétala inferior. Na verdade, um dos exemplares analisados (Espírito Santo 5180) apresenta uma forma das PLU's coincidente com a descrição apresentada por Grey-Wilson, mas os restantes exemplares analisados apresentam PLU's com ambas as pétalas de igual tamanho. De realçar também o exemplar Soares M. 32 (COI), que apresentava, no mesmo indivíduo flores completamente rosa e flores rosa com PLU's vermelhas (Fig. 2B). Estes factos demonstram a variabilidade desta espécie, fruto muito provavelmente do facto

de ser cultivada e poderão explicar a descrição de *I. tamsiana* (= ***I. balsamina***) por Exell em 1944, uma vez que o exemplar Exell 574, com base no qual a descrição da espécie foi feita, apresenta ambas as PUL's de tamanho aproximado, semelhantes aos exemplares colhidos mais recentemente por mim em jardins de São Tomé e do Príncipe. A decisão de Grey-Wilson (1980a) de considerar *I. tamsiana* um sinónimo de *I. balsamina* parece-me, pois, fundamentada.

Perspectivas futuras

O crescente interesse na filogenia do género *Impatiens* trará á luz, com toda a certeza, em anos próximos um maior número de sequências moleculares de *Impatiens* africanas. A inclusão das sequências obtidas neste estudo em futuros trabalhos permitirá ter uma ideia mais clara do posicionamento das três endémicas de STP em relação às suas congéneres continentais e de ilhas adjacentes.

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