UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

INSTITUTO DE BIOCIÊNCIAS

DEPARTAMENTO DE GENÉTICA

PROGRAMA DE PÓS-GRADUAÇÃO EM GENÉTICA E BIOLOGIA MOLECULAR

Avaliação do marcador nuclear ITS para estudos evolutivos de espécies do gênero *Passiflora* L. (Passifloraceae)

GIOVANNA CÂMARA GIUDICELLI

Orientadora: Prof.ª Dr.ª Loreta Brandão de Freitas

Porto Alegre, março de 2015.

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Dissertação submetida ao Programa de Pós-graduação em Genética e Biologia Molecular da Universidade Federal do Rio Grande do Sul como requisito parcial para a obtenção do grau de Mestre em Genética e Biologia Molecular.

Orientadora: Prof.ª Dr.ª Loreta Brandão de Freitas

Porto Alegre, março de 2015.

"Provavelmente, pensa a morte, houve um tempo em que todos os seres vivos eram uma cousa só, mas depois, a pouco e pouco, com a especialização, acharam-se divididos em cinco reinos, a saber, as móneras, os protistos, os fungos, as plantas e os animais, em cujo interior, aos reinos nos referimos, infindas macroespecializações e microespecializações se sucederam ao longo das eras, não sendo portanto nada de estranhar que, em meio de tal confusão, de tal atropelo biológico, algumas particularidades de uns tivessem aparecido repetidas noutros."

(As intermitências da morte, José Saramago)

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RESUMO

O gênero pantropical *Passiflora* L. possui cerca de 520 espécies que apresentam grande diversidade de caracteres florais e foliares, o que contribui para a complexidade taxonômica do grupo. A atual revisão infragenérica do gênero baseia-se em dados morfológicos e ecológicos e divide *Passiflora* em quatro subgêneros. Os espaçadores internos transcritos do DNA ribossomal nuclear (ITS1 e ITS2) são utilizados em estudos filogenéticos em grupos vegetais desde a década de 1990 e se tornaram um dos marcadores mais utilizados para estas inferências. Em *Passiflora*, foram conduzidos estudos com diferentes abordagens utilizando estas regiões, demonstrando a contribuição deste marcador para estudos evolutivos em espécies do gênero. Muitos estudos têm sido realizados em grupos vegetais considerando o uso da estrutura secundária das regiões ITS para aprimorar os alinhamentos das sequências e, consequentemente, possibilitando uma melhor inferência filogenética. Porém, a conservação das estruturas secundárias sugere a existência de uma pressão seletiva para que estas estruturas se mantenham preservadas, o que pode comprometer as inferências filogenéticas. Além da utilidade em estudos filogenéticos de plantas, as sequências dos ITS também apresentam potencial para ser usado como DNA *barcoding* em diferentes grupos vegetais.

Para avaliar o uso potencial das estruturas secundárias das sequências de ITS1 e ITS2 para incrementar os alinhamentos destas sequências para estudos evolutivos em espécies de *Passiflora* e determinar sua capacidade em distinguir espécies do gênero, foram analisadas 222 espécies dos quatro subgêneros de *Passiflora*. As análises de modelagem mostraram que as regiões de ITS1 e ITS2 estão sob pressão seletiva para manter suas estruturas secundárias e que a conservação de motivos específicos pode aprimorar os alinhamentos de *Passiflora* e, consequentemente, as análises filogenéticas neste gênero. Nossos resultados também mostraram o potencial destas sequências como DNA *barcoding*, uma vez que elas foram capazes de distinguir mais 50% das espécies dos subgêneros, diferentemente de outros marcadores. Como em outros estudos envolvendo ITS, aqui também foi demonstrada a utilidade deste marcador nas análises evolutivas envolvendo espécies de *Passiflora*.

ABSTRACT

Pantropical genus *Passiflora* L. comprises about 520 species that exhibit great diversity of flower and leaf characters, which contributes to the taxonomic complexity of the group. The current infrageneric classification of the genus is based on morphological and ecological data and divided *Passiflora* in four subgenera. The nuclear ribosomal DNA internal transcribed spacers (ITS1 and ITS2) are used for phylogenetic studies in plant groups since the 1990s and became one of the most used markers for these inferences. In *Passiflora*, several studies were conducted with different approaches using these regions, demonstrating the contribution of this marker for evolutionary studies in the genus. Many studies have been carried out on plant groups considering the use of the secondary structure of ITS region to improve alignments of sequences and consequently providing better phylogenetic inference. However, secondary structures conservation suggests the existence of a selective pressure for these structures remain preserved, which can compromise phylogenetic inferences. Besides the use in phylogenetic studies of plants, the sequences of ITS also have potential to be used as DNA barcoding in different plant groups.

To evaluate the potential use of ITS1 and ITS2 secondary structures to improve alignments of these sequences for evolutionary studies in *Passiflora* species and determine the effectiveness of these sequences to distinguish species from genus, 222 species from all four *Passiflora* subgenera were analyzed. Secondary structure analysis shown that ITS1 and ITS2 regions are under selective constrains to maintain their secondary structures and also specific conserved motifs could be useful to improve *Passiflora* alignments, and consequently the phylogenetic analysis of this genus. Our results also demonstrate the potential use of ITS1 and ITS2 sequences for DNA barcoding studies, as well as those sequences were able to distinguish more than 50% of species, differently of other markers. As in other studies involving this marker, here was also demonstrated the utility of ITS in evolutionary analyzes involving *Passiflora* species.

1. INTRODUÇÃO

1.1 O gênero Passiflora

Passiflora L., o gênero mais especioso da família Passifloraceae, é composto por cerca de 520 espécies e 400 híbridos produzidos artificialmente (Ulmer & MacDougal 2004). O gênero apresenta distribuição pantropical, com espécies que ocorrem no Novo e Velho Mundo (Deginani 2001), e seu centro de diversidade se estende pela América Central e, principalmente, América do Sul (Ulmer & MacDougal 2004). No Brasil, ocorrem cerca de 140 espécies nativas (Cervi 2006). *Passiflora* apresenta uma das maiores gamas de variação em estruturas florais e vegetativas observadas entre as Angiospermas (Killip 1938), o que pode ser resultado de relações coevolutivas entre suas espécies e respectivos polinizadores (MacDougal 1994).

A grande diversidade de caracteres florais únicos, formatos foliares e variação dos tipos de nectários extraflorais observada em *Passiflora* contribuem para a complexa taxonomia do gênero (Krosnick & Freuddenstein 2005). Killip (1938) dividiu o grupo em 22 subgêneros, baseado principalmente na morfologia floral das espécies, sendo posteriormente adicionado mais um subgênero a esta classificação (Escobar 1989).

A revisão taxonômica infragenérica de *Passiflora* realizada por Feuillet & MacDougal (2003), baseada em caracteres morfológicos e ecológicos, propôs o reagrupamento das espécies em apenas quatro subgêneros: *Astrophea* (DC.) Mast., *Deidamioides* (Harms) Killip, *Decaloba* (DC.) Rchb. e *Passiflora* L. Trabalhos de sistemática filogenética realizados posteriormente com marcadores moleculares distintos e diferentes quantidades e composições de espécies corroboraram total (Muschner *et al*. 2003, 2012; Hansen *et al*. 2006, 2007) ou parcialmente (Yockteng & Nadot 2004) a nova classificação infragenérica do gênero.

1.2 Espaçadores Internos Transcritos (ITS)

O DNA ribossomal nuclear (rDNA) envolve uma das maiores famílias multigênicas dos genomas eucariotos e consiste de múltiplas cópias organizadas *en tandem* em grandes arranjos denominados região organizadora nucleolar (NOR) e comumente distribuídas em diferentes *loci* (Hillis & Dixon 1991; Parkin & Butlin 2004). As unidades de repetição do rDNA são compostas por três genes (18S, 5.8S e 26S), entre os quais estão localizados os espaçadores internos transcritos (ITS1 e ITS2), e delimitadas pelos

espaçadores externos transcritos (ETS) e espaçadores intergênicos (IGS). O gene que codifica o RNA ribossomal (rRNA) 5S também representa uma classe de DNA repetitivo, embora não faça parte do arranjo que compõe os outros genes (Hillis & Dixon 1991; Buckler *et al*. 1997). As regiões gênicas do arranjo são altamente conservadas em organismos eucarióticos, enquanto as sequências de ITS podem apresentar variações por mutações de ponto e grandes eventos de inserção/deleção (indels) (Baldwin *et al*. 1995; Álvarez & Wendel 2003).

O pré-RNA resultante da transcrição das unidades de repetição é processado no núcleo da célula para que ocorra a liberação dos rRNAs maduros que formarão os ribossomos citoplasmáticos (Baldwin *et al*. 1995). Para isso, as regiões dos ITS sofrem clivagens específicas durante a maturação das subunidades ribossomais e da região gênica 5.8S e, portanto, não são incorporadas aos ribossomos maduros (Hillis & Dixon 1991; Rosselló *et al*. 2007). As regiões de ITS desempenham um importante papel no processamento dos rRNAs. Estudos constataram que deleções de porções centrais do ITS1 inibem a maturação da subunidade menor dos rRNAs (Musters *et al*. 1990), enquanto deleções na porção 5' terminal do ITS2 impedem a maturação da subunidade maior do rRNA e deleções na região 3' terminal do ITS2 reduzem significativamente a eficiência deste mesmo processo (Sande *et al*. 1992). A maturação dos rRNAs e o processo de *splicing* são dependentes da estrutura secundária do ITS, o que torna necessária a existência de uma forte pressão seletiva para que as sequências e estruturas dessa região sejam mantidas relativamente conservadas (Hillis & Dixon 1991; Mai & Coleman 1997; Goel *et al*. 2002).

As múltiplas cópias que compões o arranjo do rRNA não evoluem de forma independente, mas sim em concerto (Arnheim *et al*. 1980; Baldwin *et al*. 1995). Esse fenômeno possibilita que as cópias se tornem semelhantes às cópias de outros indivíduos e até mesmo outras espécies, mantendo um alto grau de similaridade dentro da família gênica e afetando a variabilidade da região (Hillis & Dixon 1991; Buckler *et al*. 1997). A evolução em concerto foi observada na maioria das famílias gênicas repetitivas estudadas até o momento e ocorre principalmente através dos mecanismos de conversão gênica e permuta desigual entre as unidades de repetição (Baldwin *et al*. 1995; Liao 1999; 2003). A taxa destes dois processos, associada aos eventos de recombinação e mutação, afeta diretamente o impacto da evolução em concerto (Parkin & Butlin 2004).

A taxa de homogeneização das cópias de ITS e seus níveis de variabilidade dependem de diferentes fatores, como a quantidade de cópias e o número de *loci* e cromossomos onde estas cópias podem ser encontradas, além da localização do *locus* ao longo do cromossomo e as taxas de *crossing-over* entre os cromossomos (Arheim *et al*. 1980; Wendel *et al*. 1995; Quijada *et al*. 1998). Estudos de diferentes grupos de angiospermas sugerem que taxas de homogeneização e níveis de polimorfismo podem variar entre diferentes grupos (Razafimandimbison *et al*. 2004).

Entretanto, em alguns casos o rDNA não evolui em concerto (Wissemann & Ritz 2005; Harpke & Peterson 2006), não havendo, portando, homogeneização entre as cópias. Além disso, algumas das cópias podem divergir, tornando-se pseudogenes (Álvarez & Wendel 2003; Bailey *et al*. 2003). A não-homogeneização das cópias e a presença de pseudogenes podem ser identificadas através do alto conteúdo AT nas sequências e menor estabilidade termodinânima da estrutura do RNA (Mayol & Rosselló 2001). A ocorrência de altas taxas de substituições nucleotídicas e grandes eventos de indels em posições que deveriam ser conservadas também são indicadores que devem ser levados em consideração na identificação da falha na evolução em concerto, bem como a estrutura secundária do ITS, que nestes casos se encontra alterada em relação às cópias homogeneizadas (Harpke & Peterson 2006). No estudo de Melo & Guerra (2003), os autores sugerem que a localização cromossômica do rDNA em espécies de *Passiflora* pode ser responsável pela baixa uniformidade das cópias de ITS observada neste gênero, a exemplo do que foi sugerido para *Gossypium* L. (Wendel *et al*. 1995), explicando, assim, pelo menos parcialmente, a grande variabilidade genética encontrada em *Passiflora* para este marcador.

As sequências de ITS são utilizadas em estudos filogenéticos em grupos vegetais desde a década de 1990 (Hamby & Zimmer 1992; Baldwin 1992; 1993) e se tornaram um dos marcadores mais utilizados para realizar inferência filogenéticas, apesar de estudos questionarem a neutralidade do marcador por esta região estar sob pressão seletiva (Hillis & Dixon 1991; Edger *et al*. 2014). O uso difundido de ITS se explica pela disponibilidade de *primers* universais, herança biparental do marcador e os consideráveis níveis de variação genética para estudos infragenéricos (Álvarez & Wendel 2003). Além destes fatores, o ITS também se destaca devido ao grande número de cópias e o tamanho

moderado de suas sequências, que facilitam o isolamento e amplificação das regiões mesmo a partir de materiais de herbário (Feliner & Rosselló 2007).

Em relação ao gênero *Passiflora*, diversos estudos já foram conduzidos incluindo a região de ITS com diferentes ênfases: Muschner *et al*. (2003) e Krosnick & Freuddenstein (2005) realizaram estudos de filogenia molecular para o gênero baseados nas sequências de ITS combinados a marcadores plastidiais. Nos trabalhos de Lorenz-Lemke *et al*. (2005) e Koehler-Santos *et al*. (2006) foi estudada a diversidade populacional de espécies de *Passiflora* utilizando o ITS como marcador genético. As sequências de ITS também foram usadas por Mäder *et al*. (2010) para avaliar padrões de variação intra e interespecífica em espécies de *Passiflora*, a fim de testar a utilidade do marcador para estudos filogeográficos no gênero. O estudo conduzido por Cazé (2012) valeu-se destas sequências para caracterizar a distribuição da variabilidade genética em uma espécie de *Passiflora* da Mata Atlântica e identificou as hipóteses de rios como barreiras e refúgios interferindo nos padrões filogeográficos de espécies de distribuição ao nível do mar como válidas. Cazé *et al*. (2013) utilizaram o ITS e marcadores plastidiais para confirmar a existência de duas espécies de *Passiflora* que haviam sido reagrupadas, enquanto Ramaiya *et al*. (2014) estudaram características morfológicas e relações filogenéticas entre espécies de *Passiflora* através da análise da diversidade genética e comparação de sequências de ITS. Todos estes estudos demonstram a contribuição deste marcador para estudos evolutivos em espécies de *Passiflora*, a exemplo do que já foi observado para outras espécies e gêneros de plantas (Goel *et al*. 2002; Harpke & Peterson 2006; Kan et *al*. 2007; Queiroz *et al*. 2011). À exceção do trabalho pioneiro de Muschner *et al.* (2003), os demais trabalhos envolveram o estudo de espécies em subgêneros determinados. Uma justificativa para a não comparação entre subgêneros baseada neste marcador é a dificuldade em alinhar as sequências, decorrente da grande diversidade observada entre espécies de subgêneros diferentes (Zamberlan 2007).

Muitos estudos têm sido realizados em grupos vegetais considerando a estrutura secundária das regiões ITS. Liu & Schardl (1991) relataram a existência de uma sequência altamente conservada na região central do ITS1, sugerindo sua provável relação com o processo de *splicing*, enquanto outros estudos sugerem a existência de uma estrutura secundária comum para o ITS2 (Hershkovitz & Zimmer 1996; Mai & Coleman 1997, Shultz *et al*. 2005). Análises da estrutura secundária permitem aperfeiçoar o alinhamento

das sequências primárias (Gottschling *et al*. 2001, Wolf *et al*. 2005), uma vez que a conservação da estrutura possibilita a identificação de homologias em sequências de difícil alinhamento (Goertzen *et al*. 2003). Consequentemente, a estrutura secundária proporciona informações que aprimoram as reconstruções filogenéticas (Tippery & Les 2008; Keller *et al*. 2010). Entretanto, a conservação das estruturas secundárias do ITS sugere a existência de uma pressão seletiva para que estas estruturas se mantenham preservadas, o que pode comprometer as inferências filogenéticas, como observado em Brassicaceae (Edger *et al*. 2014), dando argumento para aqueles que consideram esta região inadequada para análises filogenéticas ou taxonômicas.

Além da utilidade em estudos filogenéticos de plantas, o ITS também apresenta potencial para ser usado como DNA *barcoding* (Chase *et al*. 2005; Kress *et al*. 2005; Taberlet *et al*. 2007), permitindo a identificação de espécies vegetais. O uso desta técnica foi primeiramente descrito por Hebert *et al*. (2003) e se baseia na análise de sequências curtas e padronizadas de DNA como ferramenta para identificar e descrever organismos a exemplo de um código de barras espécie-específico. Embora bem estabelecida para outros organismos, em plantas a utilização do DNA *barcoding* ainda é problemática (Chase *et al*. 2005; Kress *et al*. 2005; Fazekas *et al*. 2009). Li *et al*. (2011) amplificaram e sequenciaram quatro regiões potenciais de DNA *barcoding* para espécies vegetais, dentre elas o ITS, e constataram que este marcador apresenta, em geral, maior poder de discriminação do que os outros por eles testados. Os autores também apuraram a alta eficiência do ITS2 quando considerado sozinho, embora esta eficiência seja menor que a obtida com a região completa. Outros estudos também sugerem o uso do ITS2 como DNA *barcoding* em plantas (Chen *et al*. 2010; Shi *et al*. 2011; Han *et al*. 2013).

O ITS apresenta resultados promissores como DNA *barcoding* em diferentes famílias vegetais (Gao *et al*. 2010a, b; Pang *et al*. 2010; Alvez *et al*. 2013, Zhang *et al*. 2014), o que justifica e comprova a recomendação do seu uso pelo grupo internacional de pesquisa CBOL (Consortium for the Barcode Of Life), que procura estimular estudos científicos nesta área. O trabalho publicado pelo grupo (CBOL Plant Working Group 2009), assim como os de Li *et al*. (2011) e Holingsworth *et al*. (2011) enfatizaram o uso do marcador ITS por seu maior poder discriminatório quando comparado às regiões plastidiais comumente usadas em estudos de DNA *barcoding*.

A existência de filogenias robustas para *Passiflora* e a contribuição do ITS em trabalhos que envolvem o gênero possibilitam o estudo da evolução molecular do marcador usando espécies de *Passiflora*. O ITS é um marcador molecular altamente variável e com grande informação disponível. Sua estrutura secundária conservada permite inferências sobre a evolução molecular da região e o bom sinal filogenético para o gênero possibilita estudos de análise filogenética e DNA *barcoding*. *Passiflora* apresenta taxonomia complexa e muitas de suas espécies ainda não foram incluídas em trabalhos filogenéticos. Assim, a possibilidade de usar o ITS como único marcador permitiria o sequenciamento de poucos pares de bases e uma resposta eficaz à posição filogenética das espécies, que poderiam ser incluídas à medida que fossem sendo obtidas a fim de completar lacunas na filogenia.

2. OBJETIVOS

2.1 Objetivo geral

O objetivo geral deste trabalho foi estudar o potencial discriminatório dos espaçadores internos transcritos (ITS) usando espécies de *Passiflora* e avaliar a utilidade desta região como indicador de posicionamento filogenético e identificação das espécies do gênero.

2.2 Objetivos específicos

Os objetivos específicos do trabalho foram:

(1) Caracterizar as sequências de ITS em *Passiflora*;

(2) Determinar a estrutura secundária das sequências de ITS em espécies de *Passiflora* e avaliar sua contribuição para o alinhamento das sequências de espécies dos diferentes subgêneros;

(3) Avaliar o potencial de uso de regiões do ITS como DNA *barcoding* em *Passiflora*, possibilitando a criação de um sistema que permita a identificação das espécies e das relações evolutivas entre elas.

3. ESTRUTURA E ORGANIZAÇÃO DA DISSERTAÇÃO

Esta dissertação foi dividida em capítulos para descrever o conjunto de experimentos que levaram ao alcance dos objetivos propostos e foram assim distribuídos:

Capítulo 1:

"Secondary structure of nrDNA Internal Transcribed Spacers as a useful tool in phylogenetic studies"

Giovanna C. Giudicelli, Geraldo Mäder, Gustavo A. S. Arias, Priscilla M. Zamberlan, Sandro L. Bonatto, Loreta B. Freitas

Este artigo está em preparação para ser submetido à revista *BMC Evolutionary Biology* e contempla os objetivos específicos 1 e 2 da dissertação.

Capítulo 2:

"Efficiency of ITS sequences for DNA barcoding in *Passiflora* (Passifloraceae)"

Giovanna C. Giudicelli, Geraldo Mäder, Loreta B. Freitas

Este artigo está publicado na revista *International Journal of Molecular Sciences* e contempla os objetivos específicos 1 e 3 da dissertação.

4. CAPÍTULO 1: Secondary structure of nrDNA Internal Transcribed Spacers as a useful tool in phylogenetic studies

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Artigo em preparação para ser submetido à revista *BMC Evolutionary Biology*.

5. CAPÍTULO 2: Efficiency of ITS sequences for DNA barcoding in Passiflora (Passifloraceae)

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Article

Efficiency of ITS Sequences for DNA Barcoding in *Passiflora* (Passifloraceae)

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Abstract: DNA barcoding is a technique for discriminating and identifying species using short, variable, and standardized DNA regions. Here, we tested for the first time the performance of plastid and nuclear regions as DNA barcodes in *Passiflora*. This genus is a largely variable, with more than 900 species of high ecological, commercial, and ornamental importance. We analyzed 1034 accessions of 222 species representing the four subgenera of *Passiflora* and evaluated the effectiveness of five plastid regions and three nuclear datasets currently employed as DNA barcodes in plants using barcoding gap, applied similarity-, and tree-based methods. The plastid regions were able to identify less than 45% of species, whereas the nuclear datasets were efficient for more than 50% using "best match" and "best close match" methods of TaxonDNA software. All subgenera presented higher interspecific pairwise distances and did not fully overlap with the intraspecific distance, and similarity-based methods showed better results than tree-based methods. The nuclear ribosomal internal transcribed spacer 1 (ITS1) region presented a higher discrimination power than the other datasets and also showed other desirable characteristics as a DNA barcode for this genus. Therefore, we suggest that this region should be used as a starting point to identify *Passiflora* species.

Keywords: rDNA internal transcribed spacer; plant DNA barcoding; phylogenetic signal; *Passiflora*

1. Introduction

DNA barcoding is a method that involves species identification and discrimination using short, variable, and standardized DNA regions [1,2]. A DNA sequence is considered to be helpful as a barcode when it conforms to three basic criteria: (i) meaningful genetic variability at the species level to enable species discrimination; (ii) a short sequence length to facilitate DNA extraction and amplification; and (iii) conserved flanking regions for the development of universal primers across highly divergent taxa [3–5].

In animal genomes, the most accepted sequence used as a DNA barcode is the mitochondrial cytochrome oxidase I gene (COI). However, studies in plants show that the insufficient variability of this region caused by its low mutation rate, has led to the search for alternative barcoding regions [3,6,7]. As a result, many different plastid loci and combinations of these loci have been proposed as promising DNA barcoding in plants [3,8]. In studies comparing different markers, some observed that each group presents distinct plastid loci or combinations of loci as an ideal barcode [9–12], whereas others highlight the challenges with the use of plastid data for some groups [13–15]. Therefore, many researchers have accepted that multiple markers may be necessary to obtain appropriate species discrimination [16,17].

In addition to plastid markers, the nuclear ribosomal internal transcribed spacer (ITS) region has also been indicated as a barcoding region [3,6,18–20]. Despite the problems associated with this marker [21,22], it has been shown to perform better when compared with either coding or noncoding plastid markers [23–28]. Many studies have also compared the discriminatory power revealed by the ITS region in its entirety with ITS2 [29–32], proposing the use of ITS2 as an alternative barcode to the entire ITS region due to the difficulty in amplifying and directly sequencing the entire region. In spite of this, the ITS1 region has rarely been tested as a DNA barcode in plants [33]. Comparisons between ITS1 and ITS2 in 10 major groups of eukaryotes suggest that ITS1 represents a better barcode than ITS2 for eukaryotic species [34].

Passiflora L., the largest genus in Passifloraceae, comprises more than 520 species largely distributed in the Neotropical region [35,36], with just a few species occurring in the Old World [37]. The wide diversity of floral and vegetative features contributes to the large diversity and complex taxonomy of this genus [38].

The *Passiflora* genus was initially divided into 22 [39] or 23 [40] subgenera based on floral morphology. The current infrageneric taxonomy [41] regrouped the species into four subgenera: *Astrophea* (DC.) Mast, *Decaloba* (DC.) Rchb, *Deidamioides* (Harms) Killip, and *Passiflora*. Subsequent phylogenetic studies performed using distinct molecular markers and different amounts and proportions of species recovered well-supported clades corresponding partially [42] or fully [43–46] to this infrageneric classification.

Despite the ecological and economic importance of *Passiflora* species, molecular markers have only recently been utilized in genetic studies of this genus. In addition, both basic genetic researches related to population studies and pre-breeding programs remain scarce for most *Passiflora* species (for a review, see [47]). Considering the number of *Passiflora* species and the increasing use of these species as a resource for ornamental, medicinal, and food purposes, a simple source of genetic markers to identify the different species is necessary.

Several studies in *Passiflora* have been conducted utilizing the ITS region for different proposes [36,38,43,48–51]. These studies demonstrate the phylogenetic signal of ITS in *Passiflora* and the subsequent contribution of this marker in clarifying the evolutionary relationships between and within species of the genus. Although the results were not based on the DNA barcoding concept, they did indicate a potential role for the ITS region in resolving species identification and differentiation in *Passiflora*.

In this study, we evaluate the potential utility of ITS regions for identifying and discriminating *Passiflora* species based on a representative sample consisting of approximately 40% of the genus. The applicability and effectiveness of different regions (ITS1 and ITS2) in discriminating species across *Passiflora* were studied for the first time. Because the plastid genes *rbcL* and *matK* have been suggested as the standard barcode for land plants [5,8], sequences of these markers available in GenBank were also tested as candidates for DNA barcodes in *Passiflora*, as were other markers commonly used in barcoding studies with sufficient sequences available in GenBank for this analysis, such as *trnH-psbA* and the *trnL* (UAA) intron [52]. The main goals of this study were as follows: (i) to test different standard barcode regions in *Passiflora*; (ii) to compare the effectiveness of the ITS1, ITS2, and ITS1+2 regions as barcoding candidates for *Passiflora*, selecting the region most suitable for distinguishing species in this genus; and (iii) to compare different methods of evaluating barcodes in plants.

2. Results

2.1. Sequence Characteristics

The results for analyses of *rbcL*, *matK*, *trnH-psbA*, and the *trnL* (UAA) intron showed that these markers present low interspecific variability in *Passiflora* (Supplementary Table S1). Indeed, they were only able to identify less than 45% of *Passiflora* species using the TAXONDNA software and criteria previously described, and they also presented low discrimination power between subgenera. Based on these results, these markers sequences were not included in our further analyses.

The sequence characteristics of the ITS regions evaluated in this study are summarized in Table 1. The ITS1 alignment length was always greater than that of ITS2 within each subgenus. The subgenus *Decaloba* presented the longest alignment length for both datasets, whereas shorter alignment lengths for ITS1 and ITS2 were observed in subgenera *Astrophea* and *Deidamioides*, respectively. *Decaloba* also had the highest percentage of variable and informative sites, in addition to the highest overall Kimura-2-Parameters distance (K2P) compared to the other subgenera. *Astrophea* showed lowers values of variable and informative characters, whereas *Deidamioides* (ITS1 and ITS1+2) and *Passiflora* (ITS2) presented lower overall K2P distances. ITS1 commonly presented a higher percentage of variable and informative sites compared to ITS2, except for *Deidamioides*.

Table 1. Characteristics of each internal transcribed spacer (ITS) dataset presented per subgenus.

Subgenus	Barcode Region	*N* Individuals	*N* Species	*N* Singletons	Alignment Length (bp)	Variable Characters (%)	PI Characters (%)	Overall K2P (%)
Astrophea	ITS1	53	16	12	291	32.99	22.68	8.8
	ITS2	53	16	12	237	28.27	16.46	7.2
	ITS1+2	53	16	12	528	30.87	19.89	8.0
Decaloba	ITS1	314	134	85	359	76.88	65.46	24.7
	ITS2	314	134	85	258	72.87	56.59	14.0
	ITS1+2	314	134	85	617	75.20	61.75	19.7
Deidamioides	ITS1	101	8	3	301	40.53	24.92	4.8
	ITS2	101	8	3	226	44.25	25.22	5.8
	ITS1+2	101	8	3	527	42.13	25.05	5.3
Passiflora	ITS1	287	64	46	292	55.48	39.73	8.8
	ITS2	287	64	46	249	52.21	30.92	3.8
	ITS1+2	287	64	46	541	53.97	35.67	6.5

ITS, ribosomal DNA internal transcribed spacer; BP, base pairs; PI, parsimony informative; K2P, pairwise genetic distance Kimura-2-Parameters.

2.2. Assessment of Barcoding Gap

The relative distribution of the frequencies of K2P distances was calculated for the three ITS datasets for all *Passiflora* subgenera using TAXONDNA software, and the pairwise intra- and inter-specific genetic distances showed a similar pattern for all subgenera and datasets. To illustrate the observed patterns, the ITS1 results are shown in Figure 1, and the results for ITS2 and ITS1+2 are presented in Figures S1 and S2. The interspecific distance was higher in all subgenera and did not fully overlap with the intraspecific distance. Therefore, the barcoding gap was identified for all datasets and subgenera.

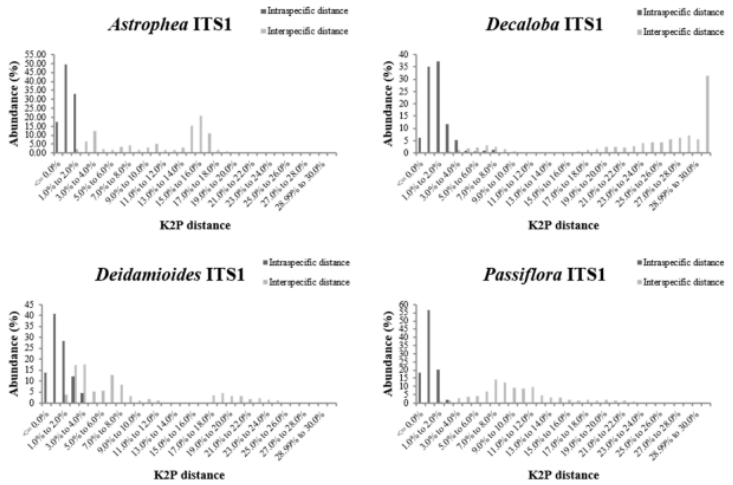

Figure 1. Relative abundance of intra- and inter-specific Kimura-2-Parameter pairwise distance considering the ITS1 dataset in subgenera *Astrophea*, *Decaloba*, *Deidamioides*, and *Passiflora*.

2.3. "*Best Match*" *and* "*Best Close Match*" *Analyses*

The results of similarity tests performed in TAXONDNA software are shown in Table 2. In the subgenus *Astrophea*, the same success rate of species identification (74%) was observed for the three datasets based on both TAXONDNA functions: BM and BCM. The other subgenera presented higher values of correct identification when BM was selected compared to BCM. The lowest discriminatory powers were obtained using ITS2 in the subgenera *Decaloba* (BM: 51%; BCM: 50%) and *Passiflora* (BM: 55%; BCM: 51%); nevertheless, more than 50% of species were correctly identified. The three datasets recovered the same percentage of correctly identified species in subgenera *Astrophea* (BM and BCM: 74%) and *Deidamioides* (BM: 96%; BCM: 95%); in contrast, ITS1+2 showed the best results in the subgenus *Decaloba* (BM: 65%; BCM: 64%), and ITS1 performed better in the subgenus *Passiflora* (BM: 82%; BCM: 78%). The highest rates of correct identification were observed in *Deidamioides* and the lowest values in *Decaloba*. Comparing the results of the BM and BCM options, we observed that BCM presented a lower discriminatory power than BM, most likely because BCM is a more stringent analysis.

Table 2. DNA barcoding performance evaluated based on similarity methods per ITS dataset per subgenus.

Subgenus	Barcode Region	*N* Individuals	BM (%)			BCM (%)				Threshold (%)
			Correct	Ambiguous	Incorrect	Correct	Ambiguous	Incorrect	No Match	
Astrophea	ITS1	53	73.58	5.66	20.75	73.58	3.77	5.66	16.98	1.51
	ITS2	53	73.58	3.77	22.64	73.58	3.77	9.43	13.20	2.97
	ITS1+2	53	73.58	3.77	22.64	73.58	3.77	5.66	16.98	1.92
Decaloba	ITS1	314	63.05	4.13	32.80	61.78	3.82	23.24	11.14	3.77
	ITS2	314	50.95	16.87	32.16	50.31	16.55	25.15	7.96	3.46
	ITS1+2	314	64.64	1.27	34.07	64.01	1.27	23.88	10.82	3.43
Deidamioides	ITS1	101	96.03	0	4.96	95.04	0	0	4.95	2.98
	ITS2	101	96.03	0	4.96	95.04	0	0	4.95	5.41
	ITS1+2	101	96.03	0	4.96	95.04	0	0	4.95	3.56
Passiflora	ITS1	287	81.53	1.74	16.72	78.39	1.39	5.57	14.63	1.83
	ITS2	287	54.70	28.57	16.72	50.87	27.87	9.40	11.84	1.19
	ITS1+2	287	81.18	1.74	17.07	77.00	1.39	4.52	17.07	1.28

BM, best match, and BCM, best close match (according to [53]) obtained in TaxonDNA software.

2.4. Tree-Based Methods

The evaluation of barcode sequences based on phylogenetic trees was estimated according to the correct assignment of individuals (Table 3) in their respective subgenus or species group, respectively. Considering the phylogenetic method, BI recovered the highest value of species monophyly, except for ITS2 in *Deidamioides*, for which NJ performed better (63% of species correctly identified); this last result was due to the identification of one extra species with the NJ method compared to BI. Comparing the datasets within each subgenus and tree-based method, the highest discriminatory power was observed when ITS1+2 was used in all cases, except for *Deidamioides*. In this subgenus, ITS2 performed better using a NJ approach, and ITS1 recovered the same percentage of correctly identified species as ITS1+2 using the ML method. Although BI performed slightly better with the ITS1 dataset in the subgenus *Astrophea* (89%) and with the ITS1 and ITS1+2 datasets in the subgenus *Passiflora* (97% and 98%, respectively), the other results showed differences among the methods.

Table 3. Comparison of tree-based (NJ, ML, and BI) and similarity (BM and BCM) methods performance for different ITS datasets and subgenera. The highest values of percentage of correct individuals' identification for each subgenus and barcode region are shown in bold.

Subgenus	Barcode Region	*N* Individuals	Correct Identifications (%)				
			NJ	ML	BI	BM	BCM
Astrophea	ITS1	53	45.28	22.64	**88.68**	73.58	73.58
	ITS2	53	35.85	13.21	50.94	**73.58**	**73.58**
	ITS1+2	53	49.06	49.06	56.60	**73.58**	**73.58**
Decaloba	ITS1	314	23.25	22.58	33.76	**63.05**	61.78
	ITS2	314	21.34	15.29	29.30	**50.95**	50.31
	ITS1+2	314	31.85	33.76	38.22	**64.64**	64.01
Deidamioides	ITS1	101	11.88	11.88	**97.03**	96.03	95.04
	ITS2	101	29.70	2.97	28.71	**96.03**	95.04
	ITS1+2	101	28.71	27.72	**98.02**	96.03	95.04
Passiflora	ITS1	287	28.92	7.67	65.85	**81.53**	78.39
	ITS2	287	14.29	2.44	24.04	**54.70**	50.87
	ITS1+2	287	33.80	33.10	68.64	**81.18**	77.00

NJ, Neighbor-Joining; ML, Maximum Likelihood; BI, Bayesian inference; BM, best match; BCM, best close match.

2.5. Statistical Analysis

The results obtained using the BM and BCM similarity methods were significantly better than those acquired using phylogenetic trees (Table 3). The analysis performed using SPSS showed that the three ITS datasets worked equally well for the subgenera *Astrophea* and *Deidamioides*. For these subgenera, analyses conducted with the BI and BM and BCM similarity methods gave better discrimination when using these barcode loci. In the subgenus *Decaloba*, ITS1+2 performed better than other datasets and was as effective as ITS1 in the subgenus *Passiflora*. For these two subgenera, BM and BCM outperformed the tree-based methods.

3. Discussion

Our work includes sequences obtained from many different studies through their GenBank records. Therefore, we believe that all of them were based on correctly identified plant species, but as we are not able to identify resulting mistakes, we included at least two different sequences from different sources in our analyses. In our study, the three ITS datasets studied presented equally efficient results as potential barcodes in the subgenera *Astrophea* and *Deidamioides* as did ITS1 and ITS1+2 for the subgenus *Passiflora* and ITS1+2 for the subgenus *Decaloba*. One also must consider the steps of DNA isolation, PCR amplification, and sequencing when choosing a DNA barcode [8]; in this case, the ITS region has proved to be a suitable marker in *Passiflora* studies [36,38,43,48–51]. Neither ITS1 nor ITS2 alone were perfect to distinguish all samples in this study. *Astrophea* and *Deidamioides* subgenera presented a lower rate of variable and informative sites than *Passiflora*, while in *Decaloba* these rates are higher than in the other three subgenera. These results were expected, considering the complexity of *Passiflora* and *Decaloba* subgenera, and directly reflected on the performance of ITS1 and ITS2 as barcode marker in each subgenus. For example, *Decaloba* presented the highest rates of variable and informative sites and this is the likely reason why the rate of species discrimination is higher in this subgenus when ITS1 and ITS2 are concatenated. Even though both markers presented higher rates of species discrimination in all four *Passiflora* subgenera, ITS1 commonly presented a higher number of variable and parsimoniously informative sites for all analyzed species, although this difference was not significant. Therefore, we suggest that ITS1 itself could be the first option for DNA barcode in *Passiflora*, though ITS2 should not be discarded.

The ITS region does not always present high rates of species discrimination, and different plastid markers have already been proposed instead of ITS for several plant groups, especially *matK* (for example, *Holcoglossum*; [13]) and two combinations of plastid loci (as *Lamium*; [11]). Indeed, ITS sequences alone have been reported to be insufficient in other plants, with the combination of ITS and plastid loci being proposed [54,55]. The ITS2 region has been indicated as a DNA barcode for some plant groups [29–31,56]. Here, we demonstrate high rates of species discrimination based on ITS data for the *Passiflora* genus, as shown in other studies [19,23].

However, there are few studies comparing the individual performances of ITS1 and ITS2. ITS1 showed superior performance to ITS2 and several plastid regions analyzed in *Salvia* species [33], whereas [34] suggest that ITS1 should be tested first in species discrimination studies for taxonomic groups where ITS1 is known to perform better than ITS2.

In our study, the similarity-based methods generally outperformed the tree-based methods. The statistics of BM and BCM options are commonly used in plant barcoding studies to evaluate the rate of species identification [11,14,28,55]. These two similarity-based methods presented high rates of species discrimination in the *Passiflora* genus, with at least half of the species being correctly assigned. In fact, the BM and BCM results were considerably higher than those obtained for the tree-based methods NJ and ML and slightly better than those of BI, except for the subgenus *Decaloba*, for which the BI tree-based method discriminated less than 38% of species.

4. Experimental Section

4.1. Taxon Sampling

ITS1, ITS2, and ITS1+2 loci were selected as barcoding candidates. The sampling obtained (Supplementary Table S2) from GenBank included 1034 accessions from 222 *Passiflora* species representative of all four subgenera: *Astrophea* (16 spp.), *Decaloba* (134 spp.), *Deidamioides* (8 spp.), and *Passiflora* (64 spp.). On average, we analyzed four individuals per species. The number of taxa represents approximately 43% of the species richness of the *Passiflora* genus. Some of the plastid sequences tested were also obtained from GenBank (Supplementary Table S3) and included 191 accessions of 122 species for *rbcL*, 47 sequences of 22 species for *matK*, 63 accessions of 30 species for *trnH-psbA*, and 346 sequences of 185 species for the *trnL* (UAA) intron. Supplementary Table S4 includes primer sequences and references for all analyzed ITS.

4.2. Data Analysis

Due to its well-conserved nature, the 5.8S gene region was removed from any sequence so that the ITS1 and ITS2 regions could be analyzed separately and concatenated. The analyses were performed in each subgenus separately due to the large genetic variability observed among them. Therefore, for each marker and subgenus, sequences were automatically aligned using ClustalX [57], visually inspected, and manually adjusted using MEGA6 [58]. These software programs were also used for testing plastid sequences, but in these analyses, all four *Passiflora* subgenera were aligned together due to the reduced variability compared to the ITS region.

We evaluated the effectiveness of ITS1, ITS2, and their combination (ITS1+2) as barcodes using three different methods.

4.2.1. Genetic Distance-Based Method

The barcoding gap is a measure of the effective barcode locus and is present when the minimum K2P interspecific distance is larger than the maximum intraspecific distance [5,8,11]. To estimate the barcoding gap, the TAXONDNA software [53] was used to calculate genetic distance over sequence pairs between and within species based on the K2P nucleotide substitution model. To estimate the presence of any barcoding gaps, histograms of distance *vs.* abundance were generated to evaluate whether the interspecific distances were larger than the intraspecific distances.

4.2.2. DNA Sequence Similarity-Based Method

To estimate the potential of the ITS regions to identify species accurately, we measured the proportion of correct identification using a method based on a direct comparison of DNA sequences. The SpeciesIdentifier program from the TAXONDNA software package compares each sequence with all others present in the dataset and groups sequences based on their pairwise genetic distances, determining whether two sequences are likely to be conspecific. We used the “best match” (BM) and “best close match” (BCM) software functions to evaluate the proportion of successful identifications based on the K2P distance as a model. The “best match” analysis establishes the closest match for a

given sequence. The identification is considered correct if both compared sequences were from the same species and incorrect if the sequences did not belong to the same species. Two or more equally good results classify the sequence as ambiguous. The "best close match" option is more stringent because it depends on 95% pairwise distance threshold calculated by the "pairwise summary" function. Results above threshold are classified as "no match", and the remaining queries below the threshold were analyzed as in the "best match" criteria [53].

4.2.3. Tree-Based Method

This analysis evaluates the proportion of monophyletic species in phylogenetic trees to assess marker discriminatory performance as a potential barcode [11,26,28]. Therefore, three different phylogenetic methods were selected for these analyses: Neighbor-Joining (NJ), maximum likelihood (ML), and Bayesian inference (BI). NJ and ML trees were constructed in MEGA using the K2P distance as a model of substitution, and running 1000 bootstrap replicates to assess the relative support for the branches. BI trees were constructed in BEAST1.8 [59] using the HKY substitution model with four gamma categories and a Yule tree prior, and 107 chain lengths were performed. The first 1000 trees were discarded as "burn in". Species were considered correctly identified if the individuals formed a monophyletic group in the trees with a bootstrap value higher than 80% or a posterior probability greater than 0.80; these values are more stringent that those used by [26] and [60] and minimize spurious relationships due to low genetic variability in datasets. We conducted statistical analyses to evaluate the discriminatory power of each potential barcode with a two-way ANOVA test followed by a *post-hoc* Student–Newman–Keuls (SNK) test for pairwise comparisons ($p \leq 0.05$) using the PASW Statistics18 software [61].

5. Conclusions

Our results show that ITS1 and ITS2 presents all the desired characteristics of a DNA barcode in *Passiflora*, such as the highest rate of discrimination and fulfillment of amplification and sequencing requirements. However, there is no ideal barcode for plants. Plastid regions were initially proposed for DNA barcoding studies [8,9] and have since been commonly used [10,54,62]. The ITS region does not always present a higher rate of species discrimination than plastid markers, though many studies indicate ITS regions as being useful for recovering high rates of correctly assigned species [24,26]. The combination of ITS and plastid loci may be chosen as the best option for some groups [25,27], and ITS2 alone is indicated as a DNA barcode for other groups [32,63]. However, ITS1 has been poorly evaluated for this purpose. Recently, it was suggested that ITS1 should be tested first as DNA barcoding when it presents better results than ITS2 for the studied taxonomic group [34]. We found that this is especially true for *Passiflora* species, and we suggest that the ITS1 region should be used as a starting point to identify species and subgenera in this highly diverse genus.

Supplementary Materials

Supplementary materials can be found at http://www.mdpi.com/1422-0067/16/04/7289/s1.

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Author Contributions

Loreta Brandão de Freitas conceived and designed research; Giovanna Câmara Giudicelli and Geraldo Mäder analyzed data and wrote the paper; Loreta Brandão de Freitas critically reviewed the manuscript. Collectively the group is interested in investigating evolutionary process and plant speciation.

Conflicts of Interest

The authors declare no conflict of interest.

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Supplementary Information

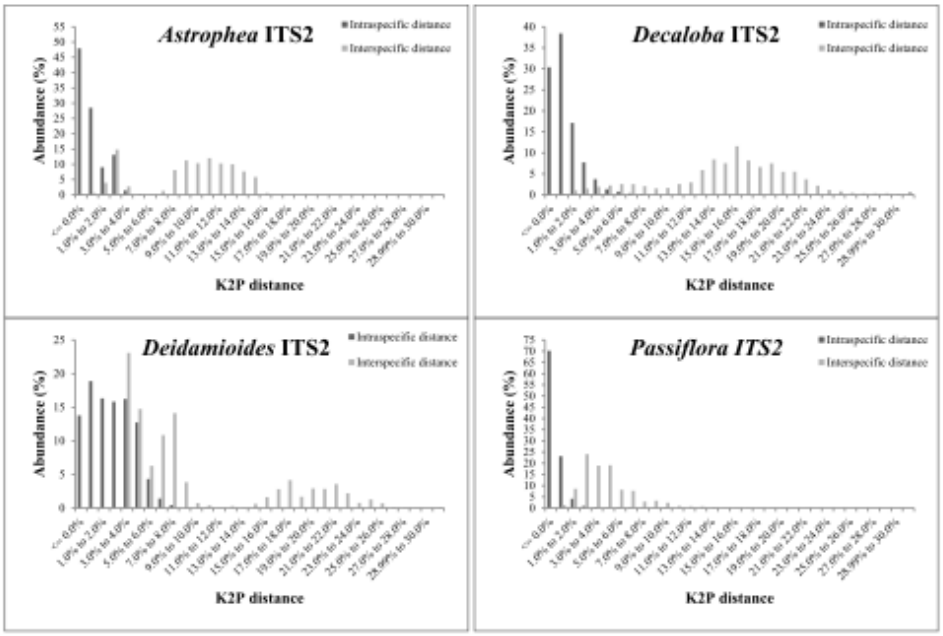

Figure S1. Relative abundance of intra- and inter-specific Kimura-2-Parameter pairwise distance considering the ITS2 dataset in subgenera *Astrophea*, *Decaloba*, *Deidamioides*, and *Passiflora*.

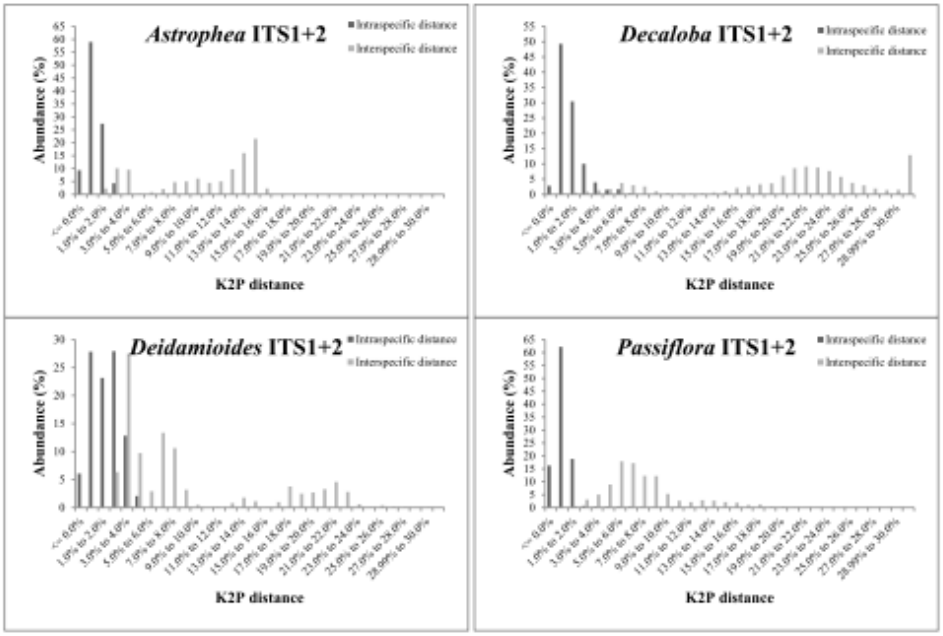

Figure S2. Relative abundance of intra- and inter-specific Kimura-2-Parameter pairwise distance considering the ITS1+2 dataset in subgenera *Astrophea*, *Decaloba*, *Deidamioides*, and *Passiflora*.

Table S1. Results for "best match" (BM) and "best close match" (BCM) analyses of TaxonDNA software for Plastid Marker *matK*, *rbcL*, *trnL-psbA*, and *trnL* intron (UAA).

Barcode Region	*N* Individuals	BM, *N* (%)			BCM, *N* (%)				
		C	A	I	C	A	I	No Match	Threshold, %
matK	47 (22 sp.)	38.29	42.55	19.14	38.29	42.55	19.14	0	14.49
rbcL	191 (122 sp.)	36.64	23.56	39.79	36.64	23.56	37.17	2.61	1.49
trnH-psbA	63 (30 sp.)	44.44	17.46	38.09	42.85	17.46	19.04	20.63	2.20
trnL intron (UAA)	346 (185 sp.)	18.78	49.13	32.08	11.84	43.35	2.60	42.19	0.0
rpoB	Four sequences available	Analysis not performed due to low availability of sequences							
rpoC1	Six sequences available								
atpF-atpH	Two sequences available								
psbK-psbI	No sequences available								

BM, best match; BCM, best close match; C, correct; A, ambiguous; I, incorrect.

Table S2. GenBank Access numbers for ITS sequences per *Passiflora* subgenera.

Astrophea ITS1		*Astrophea* ITS2	
Species	**GenBank Access**	**Species**	**GenBank Access**
P. amoena	KP769869 [a]	*P. amoena*	KP769917 [a]
P. arborea	JX470767 [b]	*P. arborea*	JX470767 [b]
P. candida	DQ521279 [c]	*P. candida*	DQ521279 [c]
P. ceratocarpa	KP769870 [a]	*P. ceratocarpa*	KP769918 [a]
P. citrifolia	AY210939 [d]	*P. citrifolia*	AY210920 [d]
	AY632707 [e]		AY632707 [e]
P. haematostigma	EU258395—EU258408 [f]	*P. haematostigma*	EU258395—EU258408 [f]
	EU907230—EU907234 [g]		EU907230—EU907234 [g]
	AY032835 [d]		AY032794 [d]
P. jussieu	JX470768 [b]	*P. jussieu*	JX470768 [b]
P. kawensis	KP769871 [a]	*P. kawensis*	KP769919 [a]
P. lindeniana	KP769872 [a]	*P. lindeniana*	KP769920 [a]
P. macrophylla	EU907225—EU907230 [g]	*P. macrophylla*	EU907225—EU907230 [g]
	AY210944 [d]		AY210925 [d]
	DQ458062 [h]		DQ458062 [h]
P. mansoi	AY102361 [d]	*P. mansoi*	AY102381 [d]
P. pittieri	DQ995476 [h]	*P. pittieri*	DQ995476 [h]
P. pyrrhantha	JX470771 [h]	*P. pyrrhantha*	JX470771 [h]
P. rhamnifolia	KP769873-KP769884 [a]	*P. rhamnifolia*	KP769921-KP769932 [a]
P. sphaerocarpa	JX470769 [h]	*P. sphaerocarpa*	JX470769 [h]
P. tina	JX470770 [h]	*P. tina*	JX470770 [h]

[a] Sequences from Giudicelli *et al*. (in prep); [b] Krosnick, S.E.; Porter-Utley, K.E.; MacDougal, J.M.; Jørgensen, P.M.; McDade, L.A. New insights into the evolution of *Passiflora* subgenus *Decaloba* (Passifloraceae): Phylogenetic relationships and morphological synapomorphies. *Syst. Bot*. **2013**, *38*, 692–713; [c] Hearn, D.J. Adenia (Passifloraceae) and its adaptative radiation: Phylogeny and growth form diversification. *Syst. Bot.* **2006**, *31*, 805–821; [d] Muschner, V.C.; Lorenz, A.P.; Cervi, A.C.; Bonatto, S.L.; Souza-Chies, T.T.; Salzano, F.M.; Freitas, L.B. A first molecular phylogenetic analysis of *Passiflora* (Passifloraceae). *Am. J. Bot.* **2003**, *90*, 1229–1238; [e] Krosnick, S.E.; Freudenstein, J.V. Monophyly and floral character homology of old world *Passiflora* (Subgenus *Decaloba*: Supersection *Disemma*). *Syst. Bot.* **2005**, 30, 139–152; [f] Mäder, G.; Zamberlan, P.M.; Fagundes, N.J.R.; Magnus, T.; Salzano, F.M.; Bonatto, S.L.; Freitas, L.B. The use and limits of ITS data in the analysis of intraspecific variation in *Passiflora* L. (Passifloraceae). *Genet. Mol. Biol.* **2010**, *33*, 99–108; [g] Mäder, G.; Magnus, T.; Lorenz-Lemke, A.P.; *et al*. ITS subgenera and intraspecific variability in Brazilian *Passiflora*: Understandin molecular evolution. Unpublished; [h] Krosnick, S.E.; Ford, A.; Freudenstein, J.V. Resolving the phylogenetic position of *Hollrungia* and *Tetrapathaea*: The end of two monotypic genera in Passifloraceae. Unpublished.

Table S2. *Cont.*

Decaloba ITS1		*Decaloba* ITS2	
Species	**GenBank Access**	**Species**	**GenBank Access**
P. adenopoda	AY632702 [a]	*P. adenopoda*	AY632702 [a]
P. allantophylla	DQ458069 [b]	*P. allantophylla*	DQ458069 [b]
P. altebilobata	DQ458078 [b]	*P. altebilobata*	DQ458078 [b]
P. anadenia	JX470833 [c]	*P. anadenia*	JX470833 [c]
P. apetala	JX470822 [c]	*P. apetala*	JX470822 [c]
P. apoda	JX470779 [c]	*P. apoda*	JX470779 [c]
P. aurantia	DQ521280 [d]	*P. aurantia*	DQ521280 [d]
	AY632704 [a]		AY632704 [a]
P. auriculata	AF454804 [e]	*P. auriculata*	AF454804 [e]
	DQ284532 [f]		DQ284532 [f]
P. berteroana	JX470780 [c]	*P. berteroana*	JX470780 [c]
P. bicornis	JX470836 [c]	*P. bicornis*	JX470836 [c]
P. bicrura	JX470834 [c]	*P. bicrura*	JX470834 [c]
P. biflora	DQ521281 [d]	*P. biflora*	DQ521281 [d]
	AF454805 [e]		AF454805 [e]
	AY632705 [a]		AY632705 [a]
	JX470837 [c]		JX470837 [c]
P. boendery	JX470823 [c]	*P. boendery*	JX470823 [c]
P. bryonioides	JX470796 [c]	*P. bryonioides*	JX470796 [c]
P. calcicola	JX470813 [c]	*P. calcicola*	JX470813 [c]
P. capsularis	EU258327—EU258351 [g]	*P. capsularis*	EU258327—EU258351 [g]
	EU907235—EU907250 [h]		EU907235—EU907250 [h]
	AY032837 [i]		AY032796 [i]
	JX470806 [c]		JX470806 [c]
P. chelidonea	JX470838 [c]	*P. chelidonea*	JX470838 [c]
P. chrysosepala	JX470839 [c]	*P. chrysosepala*	JX470839 [c]
P. cinnabarina	AY632706 [a]	*P. cinnabarina*	AY632706 [a]
P. citrina	DQ458083 [b]	*P. citrina*	DQ458083 [b]
	JX463165 [c]		JX463165 [c]
P. cobanensis	JX470807 [c]	*P. cobanensis*	JX470807 [c]
P. cochinchinensis	DQ458080 [b]	*P. cochinchinensis*	DQ458080 [b]
	DQ087422		DQ087422
	AY632714 [a]		AY632714 [a]
P. colimensis	JX470797 [c]	*P. colimensis*	JX470797 [c]
P. complanata	JX470827 [c]	*P. complanata*	JX470827 [c]
P. coriacea	AF454807 [e]	*P. coriacea*	AF454807 [e]
	AY210940 [i]		AY210921 [i]
	DQ238786 [f]		DQ238786 [f]
	JX463147 [c]		JX463147 [c]
	JX470790 [c]		JX470790 [c]
P. cubensis	JX470814 [c]	*P. cubensis*	JX470814 [c]
P. cuneata	JX470840 [c]	*P. cuneata*	JX470840 [c]
P. cupiformis	AY632708 [a]	*P. cupiformis*	AY632708 [a]

Table S2. *Cont.*

Decaloba ITS1		*Decaloba* ITS2	
Species	**GenBank Access**	**Species**	**GenBank Access**
P. cupraea	AY210941 [i]	*P. cupraea*	AY210922 [i]
	JX470815 [c]		JX470815 [c]
P. dolichocarpa	JX470798 [c]	*P. dolichocarpa*	JX470798 [c]
P. eberhardtii	DQ458073 [b]	*P. eberhardtii*	DQ458073 [b]
	JX470778 [c]		JX470778 [c]
P. ekmanii	JX470835 [c]	*P. ekmanii*	JX470835 [c]
P. escobariana	JX470808 [c]	*P. escobariana*	JX470808 [c]
P. exsudans	JX470799 [c]	*P. exsudans*	JX470799 [c]
P. geminiflora	DQ458075—DQ458076 [b]	*P. geminiflora*	DQ458075—DQ458076 [b]
P. gilbertiana	JX470824 [c]	*P. gilbertiana*	JX470824 [c]
P. gracilis	JX470800 [c]	*P. gracilis*	JX470800 [c]
P. guatemalensis	DQ087419 [f]	*P. guatemalensis*	DQ087419 [f]
P. hahnii	JX470777 [c]	*P. hahnii*	JX470777 [c]
P. helleri	AY210942 [i]	*P. helleri*	AY210923 [i]
	DQ458082 [b]		DQ458082 [b]
P. henryi	AY632710 [a]	*P. henryi*	AY632710 [a]
P. herbertiana	AY632711 [a]	*P. herbertiana*	AY632711 [a]
P. hirtiflora	JX470841 [c]	*P. hirtiflora*	JX470841 [c]
P. hollrungii	DQ458081 [b]	*P. hollrungii*	DQ458081 [b]
P. holosericea	DQ087417 [f]	*P. holosericea*	DQ087417 [f]
	JX470781 [c]		JX470781 [c]
P. ichthyura	JX470842 [c]	*P. ichthyura*	JX470842 [c]
P. ilamo	JX470825 [c]	*P. ilamo*	JX470825 [c]
P. inca	JX463163 [c]	*P. inca*	JX463163 [c]
P. indecora	JX470843 [c]	*P. indecora*	JX470843 [c]
P. intricata	JX470844 [c]	*P. intricata*	JX470844 [c]
P. jianfengensis	DQ458077 [b]	*P. jianfengensis*	DQ458077 [b]
P. jugorum	AY632712 [a]	*P. jugorum*	AY632712 [a]
P. juliana	JX463152—JX463154 [c]	*P. juliana*	JX463152—JX463154 [c]
	JX470791 [c]		JX470791 [c]
P. karwinskii	JX470801 [c]	*P. karwinskii*	JX470801 [c]
P. kwangtungensis	KF207865 [j]	*P. kwangtungensis*	KF207865 [j]
P. lancearia	JX470845 [c]	*P. lancearia*	JX470845 [c]
P. lancetillensis	AY210943 [i]	*P. lancetillensis*	AY210924 [i]
P. lancifolia	JX463158 [c]	*P. lancifolia*	JX463158 [c]
	JX470792 [c]		JX470792 [c]
P. leptoclada	JX470846 [c]	*P. leptoclada*	JX470846 [c]
P. leschenaultii	DQ458079 [b]	*P. leschenaultii*	DQ458079 [b]
P. litoralis	JX463107 [c]	*P. litoralis*	JX463107 [c]
	JX463109 [c]		JX463109 [c]
	JX463112—JX463118 [c]		JX463112—JX463118 [c]
	JX463123—JX463126 [c]		JX463123—JX463126 [c]
	JX463133—JX463134 [c]		JX463133—JX463134 [c]

Table S2. *Cont.*

Decaloba ITS1		*Decaloba* ITS2	
Species	**GenBank Access**	**Species**	**GenBank Access**
P. lobata	AF454808 [e]	*P. lobata*	AF454808 [e]
	JX463164 [c]		JX463164 [c]
	JX470802 [c]		JX470802 [c]
P. lobbii	JX463162 [c]	*P. lobbii*	JX463162 [c]
P. lobbii subsp. *ayacuchoensis*	JX470782 [c]	*P. lobbii* subsp. *ayacuchoensis*	JX470782 [c]
P. lutea	DQ006022 [k]	*P. lutea*	DQ006022 [k]
P. maestrensis	JX470816 [c]	*P. maestrensis*	JX470816 [c]
P. mcvaughiana	JX463148—JX463149 [c]	*P. mcvaughiana*	JX463148—JX463149 [c]
P. membranacea	AY632701 [a]	*P. membranacea*	AY632701 [a]
P. mexicana	AY632713 [a]	*P. mexicana*	AY632713 [a]
P. micropetala	KP769908 [l]	*P. micropetala*	KP769956 [l]
	JX470847 [c]		JX470847 [c]
P. microstipula	DQ458066 [m]	*P. microstipula*	DQ458066 [m]
P. misera	EU258409—EU258413 [g]	*P. misera*	EU258409—EU258413 [g]
	AY032838 [i]		AY032797 [i]
	JX470848 [c]		JX470848 [c]
P. moluccana var. *glaberrima*	DQ284536 [f]	*P. moluccana* var. *glaberrima*	DQ284536 [f]
P. monadelpha	DQ087418 [f]	*P. monadelpha*	DQ087418 [f]
	JX470783 [c]		JX470783 [c]
P. morifolia	EU258323—EU258324 [g]	*P. morifolia*	EU258323—EU258324 [g]
	AY032842 [i]		AY032801 [i]
	DQ284533 [f]		DQ284533 [f]
P. multiflora	AY210945 [i]	*P. multiflora*	AY210926 [i]
	AY632715		AY632715
P. munchiquensis	JX470784 [c]	*P. munchiquensis*	JX470784 [c]
P. murucuja	AY648559 [n]	*P. murucuja*	AY648559 [n]
	JX470817 [c]		JX470817 [c]
P. oblongata	JX470818 [c]	*P. oblongata*	JX470818 [c]
P. obtusifolia	JX463150—JX463151 [c]	*P. obtusifolia*	JX463150—JX463151 [c]
	JX470793 [c]		JX470793 [c]
P. occidentalis	JX470849 [c]	*P. occidentalis*	JX470849 [c]
P. orbiculata	JX470819 [c]	*P. orbiculata*	JX470819 [c]
P. organensis	EU258414—EU258426 [g]	*P. organensis*	EU258414—EU258426 [g]
	AY032839 [i]		AY032798 [i]
P. ornithoura	JX470826 [c]	*P. ornithoura*	JX470826 [c]
P. pallida	DQ458084 [b]	*P. pallida*	DQ458084 [b]
	JX463127—JX463132 [c]		JX463127—JX463132 [c]
	JX463135—JX463142 [c]		JX463135—JX463142 [c]
P. papilio	DQ458074 [b]	*P. papilio*	DQ458074 [b]
P. pardifolia	JX470850 [c]	*P. pardifolia*	JX470850 [c]
P. pavonis	JX470831 [c]	*P. pavonis*	JX470831 [c]

Table S2. *Cont.*

Decaloba ITS1		*Decaloba* ITS2	
Species	**GenBank Access**	**Species**	**GenBank Access**
P. pedicellaris	JX470776 [c]	*P. pedicellaris*	JX470776 [c]
P. pendens	JX470803 [c]	*P. pendens*	JX470803 [c]
P. penduliflora	KP769909 [i]	*P. penduliflora*	KP769957 [i]
	JX463166 [c]		JX463166 [c]
	JX470820 [c]		JX470820 [c]
P. perakensis	DQ087423 [f]	*P. perakensis*	DQ087423 [f]
P. perfoliata	JX463167 [c]	*P. perfoliata*	JX463167 [c]
	JX470821 [c]		JX470821 [c]
P. pilosa	JX470804 [c]	*P. pilosa*	JX470804 [c]
P. podlechii	KP769910 [i]	*P. podlechii*	KP769958 [i]
	JX463161 [c]		JX463161 [c]
P. pohlii	EU258325 [g]	*P. pohlii*	EU258325 [g]
	AY032840 [i]		AY032799 [i]
P. punctata	AY210946 [i]	*P. punctata*	AY210927 [i]
	JX470851 [c]		JX470851 [c]
P. pusilla	JX470809 [c]	*P. pusilla*	JX470809 [c]
P. rovirosae	KP769911 [i]	*P. rovirosae*	KP769959 [i]
	JX470810 [c]		JX470810 [c]
P. rubra	AY032836 [i]	*P. rubra*	AY032795 [i]
	AY632716 [a]		AY632716 [a]
	JX470811 [c]		JX470811 [c]
P. rufa	AY210948 [i]	*P. rufa*	AY210929 [i]
	JX470789 [c]		JX470789 [c]
P. rugosissima	JX470828 [c]	*P. rugosissima*	JX470828 [c]
P. sagasteguii	JX470785 [c]	*P. sagasteguii*	JX470785 [c]
P. sandrae	JX470852 [c]	*P. sandrae*	JX470852 [c]
P. sanguinolenta	KP769912 [i]	*P. sanguinolenta*	KP769960 [i]
	JX470812 [c]		JX470812 [c]
P. sexflora	AY210949 [i]	*P. sexflora*	AY210930 [i]
	JX463168[c]		JX463168 [c]
	JX470829—JX470830 [c]		JX470829—JX470830 [c]
P. sexocellata	JX463143—JX463146 [c]	*P. sexocellata*	JX463143—JX463146 [c]
P. siamica	DQ458212—DQ458216 [b]	*P. siamica*	DQ458212—DQ458216 [b]
	DQ087424 [f]		DQ087424 [f]
	AY632717 [a]		AY632717 [a]
P. sicyoides	JX470805 [c]	*P. sicyoides*	JX470805 [c]
P. sodiroi	JX470786 [c]	*P. sodiroi*	JX470786 [c]
P. solomonii	JX470787 [c]	*P. solomonii*	JX470787 [c]
P. suberosa	AY032841 [i]	*P. suberosa*	AY032800 [i]
	AF454806 [e]		AF454806 [e]
	AY632718 [a]		AY632718 [a]
P. suberosa var. *suberosa*	JX463108 [c]	*P. suberosa* var. *suberosa*	JX463108 [c]

Table S2. *Cont.*

Decaloba ITS1		*Decaloba* ITS2	
Species	**GenBank Access**	**Species**	**GenBank Access**
	JX463110—JX463111 [c]		JX463110—JX463111 [c]
	JX463119—JX463122 [c]		JX463119—JX463122 [c]
P. tacanensis	JX470794 [c]	*P. tacanensis*	JX470794 [c]
P. talamancensis	AF454809 [e]	*P. talamancensis*	AF454809 [e]
P. tatei	JX470853 [c]	*P. tatei*	JX470853 [c]
P. telesiphe	JX470854 [c]	*P. telesiphe*	JX470854 [c]
P. tenella	JX470832 [c]	*P. tenella*	JX470832 [c]
P. tenuiloba	AY632719 [a]	*P. tenuiloba*	AY632719 [a]
	JX463159—JX463160 [c]		JX463159—JX463160 [c]
P. tonkinensis	DQ087425 [f]	*P. tonkinensis*	DQ087425 [f]
P. transversalis	KP769913 [l]	*P. transversalis*	KP769961 [l]
P. tricuspis	EU258455—EU258460 [g]	*P. tricuspis*	EU258455—EU258460 [g]
	AY102348 [i]		AY102368 [i]
	JX470855 [c]		JX470855 [c]
P. trifasciata	KP769885 [l]	*P. trifasciata*	KP769933 [l]
P. truncata	AY102354 [i]	*P. truncata*	AY102374 [i]
	JX470788[c]		JX470788 [c]
P. tuberosa	JX470856 [c]	*P. tuberosa*	JX470856 [c]
P. tulae	AY102352 [i]	*P. tulae*	AY102372 [i]
P. urnifolia	EU258461—EU258465 [g]	*P. urnifolia*	EU258461—EU258465 [g]
	JX470857 [c]		JX470857 [c]
P. vespertilio	KP769916 [l]	*P. vespertilio*	KP769964 [l]
	JX470858 [c]		JX470858 [c]

Table S2. *Cont.*

Decaloba ITS1		*Decaloba* ITS2	
Species	**GenBank Access**	**Species**	**GenBank Access**
P. viridescens	JX470859 [c]	*P. viridescens*	JX470859 [c]
P. viridiflora	JX463155—JX463157 [c]	*P. viridiflora*	JX463155—JX463157 [c]
P. wilsonii	DQ458072 [b]	*P. wilsonii*	DQ458072 [b]
	DQ087426		DQ087426
P. xiizkodz	AY210950 [i]	*P. xiizkodz*	AY210931 [i]
	DQ238786 [n]		DQ238786 [n]
	JX463102—JX463106 [c]		JX463102—JX463106 [c]
	JX470795 [c]		JX470795 [c]
P. xiizkodz subsp. *itzensis*	JX463101 [c]	*P. xiizkodz* subsp. *itzensis*	JX463101 [c]
P. xishuangbannaensis	DQ458071 [b]	*P. xishuangbannaensis*	DQ458071 [b]

[a] Krosnick, S.E.; Freudenstein, J.V. Monophyly and floral character homology of old world *Passiflora* (Subgenus *Decaloba*: Supersection *Disemma*). *Syst. Bot.* **2005**, *30*, 139–152; [b] Krosnick, S.E.; Freudenstein, J.V. Phylogenetic relationships among the Old World species of *Passiflora* L. (Subgenus *Decaloba*: Supersection *Disemma*). Unpublished; [c] Krosnick, S.E.; Porter-Utley, K.E.; MacDougal, J.M.; Jørgensen, P.M.; McDade, L.A. New insights into the evolution of *Passiflora* subgenus *Decaloba* (Passifloraceae): phylogenetic relationships and morphological synapomorphies. *Syst. Bot.* **2013**, *38*, 692–713; [d] Hearn, D.J. *Adenia* (Passifloraceae) and its adaptative radiation: Phylogeny and growth form diversification. *Syst. Bot.* **2006**, *31*, 805–821; [e] Ossowski, A.M.; Hunter, F.F. Coevolution of *Heliconius* spp. and *Passiflora* spp.: A phylogenetic comparison. Unpublished; [f] Krosnick, S.E.; Freudenstein, J.V. Patterns of anomalous floral development in the Asian *Passiflora* (subgenus *Decaloba*: supersection *Disemma*). *Am. J. Bot.* **2006**, *93*, 620–636; [g] Mäder, G.; Zamberlan, P.M.; Fagundes, N.J.R.; Magnus, T.; Salzano, F.M.; Bonatto, S.L.; Freitas, L.B. The use and limits of ITS data in the analysis of intraspecific variation in *Passiflora* L. (Passifloraceae). *Genet. Mol. Biol.* **2010**, *33*, 99–108; [h] Mäder, G.; Magnus, T.; Lorenz-Lemke, A.P.; *et al*. ITS subgenera and intraspecific variability in Brazilian *Passiflora*: Understandin molecular evolution. Unpublished; [i] Muschner, V.C.; Lorenz, A.P.; Cervi, A.C.; Bonatto, S.L.; Souza-Chies, T.T.; Salzano, F.M.; Freitas, L.B. A first molecular phylogenetic analysis of Passiflora (Passifloraceae). *Am. J. Bot.* **2003**, *90*, 1229–1238; [j] Krosnick, S.E.; Xun-Lin, Y.; Deng, Y. The rediscovery of *Passiflora kwangtungensis* Merr. (subgenus *Decaloba* supersection *Disemma*): A critically endangered Chinese endemic. *PhytoKeys* **2013**, *23*, 55–74; [k] Kress, W.J.; Wurdack, K.J.; Zimmer, E.A.; Weigt, L.A.; Janzen, D.H. Use of DNA barcodes to identify flowering plants. *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 8369–8374; [l] Sequences from Giudicelli *et al*. (in prep); [m] Krosnick, S.E.; Ford, A.; Freudenstein, J.V. Resolving the phylogenetic position of *Hollrungia* and *Tetrapathaea*: The end of two monotypic genera in Passifloraceae. Unpublished; [n] Kay, E.E. Floral Evolutionary Ecology of Passiflora: subgenera Murucuia, Pseudomurucuja and Astephia. Unpublished; [o] Muschner, V.C.; Lorenz-Lemke, A.P.; Vecchia, M.; Bonatto, S.L.; Salzano, F.M.; Freitas, L.B. Differential organellar inheritance in *Passiflora* (Passifloraceae) subgenera. Unpublished.

Table S2. *Cont.*

***Deidamioides* ITS1**		***Deidamioides* ITS2**	
Species	**GenBank Access**	**Species**	**GenBank Access**
P. arbelaezii	DQ521278 [a]	*P. arbelaezii*	DQ521278 [a]
	AY632703 [b]		AY632703 [b]
P. cirrhiflora	DQ458063 [c]	*P. cirrhiflora*	DQ458063 [c]
P. contracta	KF196619—KF196691 [d]	*P. contracta*	KF196619—KF196691 [d]
P. deidamioides	EU907257—EU907265 [e]	*P. deidamioides*	EU907257—EU907265 [e]
P. discophora	DQ458061 [c]	*P. discophora*	DQ458061 [c]
	JX470772 [f]		JX470772 [f]
P. gracillima	JX470773 [f]	*P. gracillima*	JX470773 [f]
P. obovata	DQ458064 [c]	*P. obovata*	DQ458064 [c]
P. ovalis	KF196601—KF196618 [d]	*P. ovalis*	KF196601—KF196618 [d]

[a] Hearn, D.J. *Adenia* (Passifloraceae) and its adaptative radiation: Phylogeny and growth form diversification. *Syst. Bot.* **2006**, *31*, 805–821; [b] Krosnick, S.E.; Freudenstein, J.V. Monophyly and floral character homology of old world *Passiflora* (Subgenus *Decaloba*: Supersection *Disemma*). *Syst. Bot.* **2005**, *30*, 139–152; [c] Krosnick, S.E.; Ford, A.; Freudenstein, J.V. Resolving the phylogenetic position of *Hollrungia* and *Tetrapathaea*: The end of two monotypic genera in Passifloraceae. Unpublished; [d] Cazé, A.L.R.; Mäder, G.; Bonatto, S.L.; Freitas, L.B. A molecular systematic analysis of *Passiflora ovalis* and *Passiflora contracta* (Passifloraceae). *Phytotaxa* **2013**, *132*, 39–46; [e] Mäder, G.; Magnus, T.; Lorenz-Lemke, A.P.; *et al.* ITS subgenera and intraspecific variability in Brazilian *Passiflora*: Understandin molecular evolution. Unpublished; [f] Krosnick, S.E.; Porter-Utley, K.E.; MacDougal, J.M.; Jørgensen, P.M.; McDade, L.A. New insights into the evolution of *Passiflora* subgenus *Decaloba* (Passifloraceae): Phylogenetic relationships and morphological synapomorphies. *Syst. Bot.* **2013**, *38*, 692–713.

Table S2. *Cont.*

Passiflora ITS1		*Passiflora* ITS2	
Species	**GenBank Access**	**Species**	**GenBank Access**
P. actinia	AY032832 [a]	*P. actinia*	AY032791 [a]
	AY542629—AY542644 [b]		AY219264—AY219279 [b]
	AY219240—AY219255 [b]		AY542658—AY542673 [b]
P. acuminata	KP769886 [c]	*P. acuminata*	KP769934 [c]
P. alata	AY032826 [a]	*P. alata*	AY032785 [a]
	AY858145—AY858229 [d]		AY858263—AY858347 [d]
	AF454800 [e]		AF454800 [e]
P. ambigua	AF454801 [e]	*P. ambigua*	AF454801 [e]
P. amethystina	EU258307—EU258309 [f]	*P. amethystina*	EU258307—EU258309 [f]
	AY102347 [a]		AY102367 [a]
P. ampullacea	AY632720 [g]	*P. ampullacea*	AY632720 [g]
P. caerulea	EU258310—EU258316 [f]	*P. caerule\a*	EU258310—EU258316 [f]
	AY032824 [a]		AY032782 [a]
	AF454802 [e]		AF454802 [e]
P. campanulata	AY032829 [a]	*P. campanulata*	AY032788 [a]
P. cerasina	KP769887 [c]	*P. cerasina*	KP769935 [c]
P. chrysophylla	KP769906 [c]	*P. chrysophylla*	KP769954 [c]
P. cincinnata	EU258353—EU258358 [f]	*P. cincinnata*	EU258353—EU258358 [f]
	DQ344629 [h]		DQ344629 [h]
	AY102363 [a]		AY102383 [a]

Table S2. *Cont.*

Passiflora ITS1		*Passiflora* ITS2	
Species	**GenBank Access**	**Species**	**GenBank Access**
P. coccinea	KP769888 [c]	*P. coccinea*	KP769936 [c]
P. edmundoi	EU258370 [f]	*P. edmundoi*	EU258370 [f]
	EU258373—EU258374 [f]		EU258373—EU258374 [f]
	AY102351 [a]		AY102371 [a]
P. edulis	EU258375—EU258384 [f]	*P. edulis*	EU258375–EU258384 [f]
	AY032831 [a]		AY032790 [a]
	JX470774 [i]		JX470774 [i]
	AF454803 [e]		AF454803 [e]
P. eichleriana	EU258317—EU258319 [f]	*P. eichleriana*	EU258317—EU258319 [f]
	AY102346 [a]		AY102366 [a]
P. elegans	AY032833 [a]	*P. elegans*	AY032792 [a]
	AY542645—AY542657 [b]		AY219280—AY219286 [b]
	AY219256—AY219262 [b]		AY542674—AY542686 [b]
P. foetida	DQ521376 [j]	*P. foetida*	DQ521376 [j]
	EU258385—EU258390 [f]		EU258385—EU258390 [f]
	EU258393—EU258394 [f]		EU258393—EU258394 [f]
	AY032834 [a]		AY032793 [a]
	DQ238783 [h]		DQ238783 [h]
	DQ458053 [k]		DQ458053 [k]
	DQ499117 [l]		DQ499117 [l]
	JQ723359 [m]		JQ723359 [m]
P. gabrielliana	AY210953 [a]	*P. gabrielliana*	AY210934 [a]
P. galbana	AY032843 [a]	*P. galbana*	AY032784 [a]
P. garckei	AY210952 [a]	*P. garckei*	AY210933 [a]
P. glandulosa	KP769907 [c]	*P. glandulosa*	KP769955 [c]
P. hatsbachii	KP769889 [c]	*P. hatsbachii*	KP769937 [c]
P. incarnata	DQ344630 [h]	*P. incarnata*	DQ344630 [h]
P. ishnoclada	KP769890 [c]	*P. ishnoclada*	KP769938 [c]
P. jervensis	KP769891 [c]	*P. jervensis*	KP769939 [c]
P. jilekii	EU258320—EU258321 [f]	*P. jilekii*	EU258320—EU258321 [f]
	AY102360 [a]		AY102380 [a]
P. kermesina	AY032825 [a]	*P. kermesina*	AY032783 [a]
P. laurifolia	KP769892 [c]	*P. laurifolia*	KP769940 [c]
P. loefgrenii	KP769893 [c]	*P. loefgrenii*	KP769941 [c]
P. luetzelburi	KP769894 [c]	*P. luetzelburi*	KP769942 [c]
P. maliformis	AY210956 [a]	*P. maliformis*	AY210937 [a]
P. mathewsii	KP769895 [c]	*P. mathewsii*	KP769943 [c]
P. mendoncaei	AY102358 [a]	*P. mendoncaei*	AY102378 [a]
P. menispermifolia	AF454795 [e]	*P. menispermifolia*	AF454795 [e]
P. miersii	EU258322 [f]	*P. miersii*	EU258322 [f]
	EU907266—EU907269 [n]		EU907266—EU907269 [n]
	AY102350 [a]		AY102370 [a]

Table S2. *Cont.*

Passiflora ITS1		*Passiflora* ITS2	
Species	**GenBank Access**	**Species**	**GenBank Access**
P. mixta	KP769896 [c]	*P. mixta*	KP769944 [c]
P. mucronata	AY210951 [a]	*P. mucronata*	AY210932 [a]
P. mucugensis	KP769897 [c]	*P. mucugensis*	KP769945 [c]
P. nitida	KP769898 [c]	*P. nitida*	KP769946 [c]
P. odontophylla	KP769899 [c]	*P. odontophylla*	KP769947 [c]
P. oerstedii	AF454797 [e]	*P. oerstedii*	AF454797 [e]
P. palmeri	DQ238784 [h]	*P. palmeri*	DQ238784 [h]
P. pilosicorona	KP769900 [c]	*P. pilosicorona*	KP769948 [c]
P. platyloba	AF454798 [e]	*P. platyloba*	AF454798 [e]
P. quadrangularis	AY032827 [a]	*P. quadrangularis*	AY032786 [a]
	AY636107 [g]		AY636107 [g]
	AF454799 [e]		AF454799 [e]
P. racemosa	KP769901 [c]	*P. racemosa*	KP769949 [c]
P. recurva	AY102349 [a]	*P. recurva*	AY102369 [a]
P. reflexiflora	AY210947 [a]	*P. reflexiflora*	AY210928 [a]
P. serratifolia	AY210954 [a]	*P. serratifolia*	AY210935 [a]
P. serratodigitata	AY636108 [g]	*P. serratodigitata*	AY636108 [g]
	AY210957 [a]		AY210938 [a]
P. setacea	AY102356 [a]	*P. setacea*	AY102376 [a]
P. setulosa	AY032828 [a]	*P. setulosa*	AY032787 [a]
P. sidiifolia	EU258435—EU258445 [f]	*P. sidiifolia*	EU258435—EU258445 [f]
	AY102353 [a]		AY102373 [a]
P. speciosa	AY102362 [a]	*P. speciosa*	AY102382 [a]
P. sprucei	KP769902 [c]	*P. sprucei*	KP769950 [c]
P. temuifila	EU258446—EU258454 [f]	*P. temuifila*	EU258446—EU258454 [f]
P. trifoliata	KP769903 [c]	*P. trifoliata*	KP769951 [c]
P. trintae	KP769914 [c]	*P. trintae*	KP769962 [c]

Table S2. *Cont.*

Passiflora ITS1		*Passiflora* ITS2	
Species	**GenBank Access**	**Species**	**GenBank Access**
P. tripartita	KP769904 [c]	*P. tripartita*	KP769952 [c]
P. trisecta	KP769905 [c]	*P. trisecta*	KP769953 [c]
P. umbilicata	KP769915 [c]	*P. umbilicata*	KP769963 [c]
P. urubiciensis	EU258326 [f]	*P. urubiciensis*	EU258326 [f]
	AY102355 [a]		AY102375 [a]
P. villosa	EU258391—EU258392 [f]	*P. villosa*	EU258391—EU258392 [f]
	EU258466—EU258469 [f]		EU258466—EU258469 [f]
	AY102357 [a]		AY102377 [a]
P. vitifolia	AF454796 [e]	*P. vitifolia*	AF454796 [e]

[a] Muschner, V.C.; Lorenz, A.P.; Cervi, A.C.; Bonatto, S.L.; Souza-Chies, T.T.; Salzano, F.M.; Freitas, L.B. A first molecular phylogenetic analysis of Passiflora (Passifloraceae). *Am. J. Bot.* **2003**, *90*, 1229–1238; [b] Lorenz-Lemke, A.P.; Muschner, V.C.; Bonatto, S.L.; Cervi, A.C.; Salzano, F.M.; Freitas, L.B. Phylogeographic inferences concerning evolution of Brazilian *Passiflora actinia* and *P. elegans* (Passifloraceae) based on ITS (nrDNA) variation. *Ann. Bot.* **2005**, *95*, 799–806; [c] Sequences from Giudicelli *et al*. (in prep); [d] Koehler-Santos, P.; Lorenz-Lemke, A.P.; Muschner, V.C.; Salzano, F.M.; Freitas, L.B. Evolutionary implications of the intrapopulation diversity of *Passiflora alata*. Unpublished; [e] Ossowski, A.M.; Hunter, F.F. Coevolution of *Heliconius* spp. and *Passiflora* spp.: A phylogenetic comparison. Unpublished; [f] Mäder, G.; Zamberlan, P.M.; Fagundes, N.J.R.; Magnus, T.; Salzano, F.M.; Bonatto, S.L.; Freitas, L.B. The use and limits of ITS data in the analysis of intraspecific variation in *Passiflora* L. (Passifloraceae). *Genet. Mol. Biol.* **2010**, *33*, 99–108; [g] Krosnick, S.E.; Freudenstein, J.V. Monophyly and floral character homology of old world *Passiflora* (Subgenus *Decaloba*: Supersection *Disemma*). *Syst. Bot.* **2005**, *30*, 139–152; [h] Muschner, V.C.; Lorenz-Lemke, A.P.; Vecchia, M.; Bonatto, S.L.; Salzano, F.M.; Freitas, L.B. Differential organellar inheritance in *Passiflora* (Passifloraceae) subgenera. Unpublished; [i] Krosnick, S.E.; Porter-Utley, K.E.; MacDougal, J.M.; Jørgensen, P.M.; McDade, L.A. New insights into the evolution of *Passiflora* subgenus *Decaloba* (Passifloraceae): phylogenetic relationships and morphological synapomorphies. *Syst. Bot.* **2012**, *38*, 692–713; [j] Hearn, D.J. *Adenia* (Passifloraceae) and its adaptative radiation: Phylogeny and growth form diversification. *Syst. Bot.* **2006**, *31*, 805–821; [k] Krosnick, S.E.; Ford, A.; Freudenstein, J.V. Resolving the phylogenetic position of *Hollrungia* and *Tetrapathaea*: The end of two monotypic genera in Passifloraceae. Unpublished; [l] Wright, S.; Keeling, J.; Gillman, L. The road from santa Rosalia: A faster tempo of evolution on tropical climes. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 7718–7722; [m] Thulin, M.; Razafimandimbison, S.G.; Chafe, P.; Heidari, N.; Kool, A.; Shore, J.S. Phyloheny of the Turneracea clade (Passifloraceae): Trans-Atlantic disjunctions and two new genera in Africa. *Taxon* **2012**, *61*, 308–323; [n] Mäder, G.; Magnus, T.; Lorenz-Lemke, A.P.; *et al*. ITS subgenera and intraspecific variability in Brazilian *Passiflora*: Understandin molecular evolution. Unpublished.

Table S3. GenBank access numbers for Plastid Markers *matK*, *rbcL*, *trnL-psbA*, and *trnL* intron (UAA).

Plastid Marker	Species	GenBank Access
matK	*P. adenopoda*	AY271608 [1]
	P. ambigua	JQ588571 [2]
	P. aurantioides	AB536631 [3]
	P. bicornis	JQ588572–JQ588574 [2]
	P. biflora	AY271610 [1]
		EU017067 [4]
		GU135122 [5]
		JQ588575–JQ588578 [2]
	P. caerulea	HM850927 [6]
	P. capsularis	AY271611 [1]
	P. cf. *wilsonii*	HG004937 [7]
	P. ciliata	JX661956 [8]
	P. coccinea	EF135577 [9]
	P. coriacea	AY271609 [1]
	P. costaricensis	JQ588579–JQ588580 [2]
	P. menispermifolia	JQ588581 [2]
	P. murucuja	AY271612 [1]
	P. ornithoura	AY271613 [1]
	P. platyloba	JQ588582 [2]
		KJ751095 [10]
	P. quadrangularis	AB233808 [11]
		FM179937 [12]
		GQ248176 [2]
		KJ751079–KJ751081 [10]
		KJ751085 [10]
		KJ751087 [10]
		KJ751090 [10]
		KJ751092 [10]
		KJ751096–KJ751100 [10]
	P. sexflora	AY271614 [1]
	P. suberosa	DQ401363 [13]
		GU266608 [14]
	P. talamancensis	AY271615 [1]
	P. tetrandra	AB536650 [3]
	P. tulae	AY271616 [1]

Table S3. *Cont.*

Plastid Marker	Species	GenBank Access
rbcL	*P. actinia*	DQ123347 [15]
		HQ900845 [16]
	P. alata	DQ123348 [15]
		HQ900846 [16]
	P. ambigua	DQ123349 [15]
		JQ593081–JQ593084 [17]
	P. amoena	DQ123301 [15]
	P. antioquiensis	DQ123342 [15]
	P. arborea	DQ123300 [15]
	P. aurantioides	AB536553 [18]
	P. auriculata	DQ445921 [15]
		HQ900847 [16]
	P. bicornis	JQ593085–JQ593087 [17]
	P. biflora	EU017122 [19]
		GU135279 [20]
		JQ593088– JQ593092 [17]
	P. caerulea	DQ123350 [15]
		HM850239 [21]
		HQ900848 [16]
	P. campanulata	DQ123339 [15]
		HQ900849 [16]
	P. candida	DQ123302 [15]
	P. capparidifolia	HQ900850 [16]
	P. capsularis	DQ123312 [15]
		HQ900851 [16]
	P. cerasina	HQ900852 [16]
	P. ceratocarpa	DQ123303 [15]
	P. cerradensis	HQ900853 [16]
	P. ciliata	JX664062 [22]
	P. cincinnata	DQ123351 [15]
	P. cirrhiflora	DQ123377 [15]
	P. citrifolia	DQ123304 [15]
	P. clathrata	DQ123336 [15]
	P. coccinea	DQ123333 [15]
		HQ900854 [16]
	P. coriacea	DQ123313 [15]
	P. costaricensis	JQ593093–JQ593094 [17]
	P. cuprea	DQ123378 [15]
	P. deidamioides	DQ445925 [15]
		HQ900855 [16]
	P. edmundoi	DQ123352 [15]
		HQ900856 [16]

Table S3. *Cont.*

Plastid Marker	Species	GenBank Access
rbcL	*P. edulis*	DQ123353 [15]
		GQ436714 [23]
		HG765072 [24]
		HQ900857 [16]
	P. eichleriana	DQ123354 [15]
		HQ900858 [16]
	P. elegans	DQ123355 [15]
	P. exura	DQ123356 [15]
	P. foetida	DQ123337 [15]
		HQ900859 [16]
		KF425764 [25]
	P. gabrielliana	DQ123357 [15]
	P. galbana	DQ123358 [15]
		HQ900860 [16]
	P. garckei	DQ123359 [15]
	P. gardneri	HQ900861 [16]
	P. gilbertii	DQ445922 [15]
		HQ900862 [16]
	P. haematostigma	DQ123305 [15]
	P. hatschbachii	HQ900863 [16]
	P. helleri	DQ123314 [15]
	P. incarnata	DQ123360 [15]
		EF590556 [26]
		GQ248664 [27]
		HF565321 [28]
		HG765070–HG765071 [24]
		HQ900864 [16]
	P. iodocarpa	HQ900865 [16]
	P. ishnoclada	HQ900866 [16]
	P. jilekii	DQ123361 [15]
		HQ900867 [16]
	P. kawensis	DQ123306 [15]
	P. lancetillensis	DQ123331 [15]
	P. leptoclada	DQ445923 [15]
		HQ900869 [16]
	P. ligularis	HQ900870 [16]
	P. lindeniada	DQ123307 [15]
	P. lobbii subsp. ayaucuchoensis	DQ123315 [15]
	P. lobbii subsp. obtusiloba	DQ123316 [15]
	P. loefgrenii	HQ900871 [16]
	P. luetzelburgii	DQ123384 [15]
	P. lutea	DQ006111 [29]
	P. macrophylla	DQ123308 [15]
	P. maliformis	DQ123362 [15]
	P. manicata	DQ123344 [15]

Table S3. *Cont.*

Plastid Marker	Species	GenBank Access
rbcL	*P. mansoi*	DQ123309 [15]
	P. mathewsii	DQ123380 [15]
	P. mendoncaei	DQ123385 [15]
	P. menispermifolia	JQ593095–JQ593096 [17]
	P. micropetala	DQ445924 [15]
		HQ900872 [16]
	P. microstipula	DQ123332 [15]
	P. miersii	DQ123363 [15]
		HQ900873 [16]
	P. misera	DQ123317 [15]
		HQ900874 [16]
	P. mixta	DQ123381 [15]
	P. morifolia	DQ123318 [15]
		HQ900875 [16]
	P. mucronata	HQ900876 [16]
	P. multiflora	DQ123297 [15]
	P. murucuja	DQ123345 [15]
	P. nitida	DQ123364 [15]
		HQ900878 [16]
	P. odontophylla	DQ123365 [15]
	P. organensis	DQ123319 [15]
		HQ900877 [16]
	P. ornithoura	DQ123320 [15]
	P. ovalis	DQ123401 [15]
	P. palmeri	DQ123338 [15]
		HQ900879 [16]
	P. penduliflora	DQ123298 [15]
	P. picturata	HQ900880 [16]
	P. pilosicorona	HQ900881 [16]
	P. pittieri	DQ123310 [15]
	P. platyloba	HQ900882 [16]
		JQ593097–JQ593099 [17]
	P. pohlii	DQ123321 [15]
		HQ900883 [16]
	P. punctata	DQ123322 [15]
	P. quadrangularis	AB233912 [30]
		DQ123366 [15]
		EF590557 [26]
		GQ248665 [27]
		L01940 [31]
	P. racemosa	DQ123311 [15]
		HQ900884 [16]
	P. recurva	DQ123367 [15]
	P. reflexiflora	DQ123386 [15]
	P. rhamnifolia	DQ123299 [15]

Table S3. *Cont.*

Plastid Marker	Species	GenBank Access
rbcL	*P. riparia*	DQ123368 [15]
	P. rufa	DQ123323 [15]
	P. serratifolia	DQ123369 [15]
	P. serratodigitata	DQ123370 [15]
		HQ900885 [16]
	P. setacea	DQ123371 [15]
	P. setulosa	DQ123340 [15]
	P. sexflora	DQ123324 [15]
	P. sidiifolia	DQ123372 [15]
		HQ900886–HQ900887 [16]
	P. speciosa	DQ123334 [15]
	P. sprucei	DQ123373 [15]
	P. suberosa	DQ123325 [15]
		HQ900888 [16]
	P. subrotunda	HQ900889 [16]
	P. tacsonioides	DQ123379 [15]
	P. talamancensis	DQ123326 [15]
	P. tenuifila	DQ123374 [15]
	P. tetrandra	AB536572 [18]
	P. tricuspis	DQ123327 [15]
		HQ900890 [16]
	P. trifasciata	DQ123328 [15]
	P. trifoliata	DQ123383 [15]
	P. trintae	DQ123375 [15]
	P. tripartita	DQ123382 [15]
	P. trisecta	DQ123343 [15]
	P. truncata	HQ900891 [16]
	P. tryphostemmatoides	DQ123388 [15]
	P. tulae	DQ123346 [15]
		HQ900892 [16]
	P. umbilicata	DQ123387 [15]
	P. urubiciensis	HQ900893 [16]
	P. vespertilio	DQ123329 [15]
		HQ900894 [16]
	P. villosa	DQ123341 [15]
	P. vitifolia	DQ123335 [15]
		HQ900895 [16]
		JQ593100–JQ593102 [17]
	P. watsoniana	DQ123376 [15]
		HQ900896 [16]
	P. xiikzodz	DQ123330 [15]

Table S3. *Cont.*

Plastid Marker	Species	GenBank Access
trnH-psbA	*P. actinia*	AY032807 [32]
		AY219288–AY219299 [33]
	P. alata	AY032808 [32]
	P. biflora	GU135451 [34]
	P. caerulea	AY032816 [32]
		AY220135 [35]
	P. campanulata	AY032812 [32]
	P. capsularis	AY032822 [32]
	P. cincinnata	DQ238756 [36]
	P. coriacea	DQ238763 [36]
	P. edulis	AY032811 [32]
	P. elegans	AY032806 [32]
		AY219300–AY219310 [33]
	P. foetida	AY032814 [32]
		DQ238759 [36]
		AY220136 [35]
	P. galbana	AY032817 [32]
		AY220137 [35]
	P. haematostigma	AY032819 [32]
	P. incarnata	EF590722 [37]
		GQ248361 [38]
		DQ238757 [36]
		AY032810 [32]
	P. jilekii	AY220138 [35]
	P. kermesina	AY032815 [32]
	P. lutea	DQ006208 [39]
	P. misera	AY032804 [32]
	P. morifolia	AY032805 [32]
	P. organensis	AY032803 [32]
	P. palmeri	DQ249919 [36]
	P. pohlii	AY032802 [32]
	P. quadrangularis	EF590723 [37]
		AY032809 [32]
		GQ248362 [38]
	P. rubra	AY032821 [32]
	P. setulosa	AY032818 [32]
	P. sidiifolia	AY220139 [35]
	P. sprucei	DQ249920 [36]
	P. suberosa	AY032820 [32]
	P. temuifila	AY032813 [32]
		AY220140 [35]
	P. xiikzodz	DQ238762 [36]

Table S3. *Cont.*

Plastid Marker	Species	GenBank Access
trnL (UAA) intron	*P. actinia*	HQ900949 [40]
		DQ123065 [41]
	P. acuminata	DQ123066 [41]
	P. adenopoda	AY632727 [42]
	P. alata	AF454778 [43]
		HQ900950 [40]
		DQ123067 [41]
	P. alnifolia	JX470862 [44]
	P. ambigua	AF454779 [43]
		DQ123068 [41]
	P. amethystina	DQ123069 [41]
	P. amoena	DQ123017 [41]
	P. ampullacea	AY632745 [42]
	P. anadenia	JX470863 [44]
	P. antioquiensis	DQ123060 [41]
	P. apoda	JX470864 [44]
	P. arbelaezii	AY632728 [42]
	P. arborea	JX470865 [44]
		DQ123018 [41]
	P. aurantia	AY632729 [42]
	P. auriculata	AF454780 [43]
		HQ900951 [40]
		DQ284534 [45]
	P. bicornis	JX470866 [44]
	P. biflora	AF454781 [43]
		JX470867 [44]
		AY632730 [42]
	P. boenderi	JX470868 [44]
	P. bryonioides	JX470869 [44]
	P. caerulea	AF454784 [43]
		HQ900952 [40]
		DQ123070 [41]
	P. campanulata	HQ900953 [40]
		DQ123057 [41]
	P. candida	DQ123019 [41]
	P. capparidifolia	HQ900954 [40]
	P. capsularis	HQ900955 [40]
		DQ123029 [41]
	P. cerasina	HQ900956 [40]
	P. ceratocarpa	DQ123020 [41]
	P. cerradensis	HQ900957 [40]
	P. cf. *viridescens*	JX470914 [44]
	P. chelidonea	JX470870–JX470871 [44]
	P. chrysosepala	JX470872 [44]
	P. cincinnata	DQ123071 [41]

Table S3. *Cont.*

Plastid Marker	Species	GenBank Access
trnL (UAA) intron	*P. cinnabarina*	AY632731 [42]
	P. cirrhiflora	DQ123101 [41]
	P. citrifolia	AY632732 [42]
		DQ123021 [41]
	P. clathrata	DQ123054 [41]
	P. cobanensis	JX470873 [44]
	P. coccinea	HQ900958 [40]
	P. contracta	KF196437–KF196509 [46]
	P. coriacea	AF454782 [43]
		DQ123030 [41]
	P. cubensis	JX470875 [44]
	P. cupiformis	AY632733 [42]
	P. cupraea	JX470876 [44]
		DQ123102 [41]
	P. deidamioides	HQ900959 [40]
	P. eberhartii	JX470877 [44]
	P. edmundoi	HQ900960 [40]
		DQ123072 [41]
	P. edulis	AF454783 [43]
		HQ900961 [40]
		JX470878 [44]
		DQ123073 [41]
	P. eichleriana	HQ900962 [40]
		DQ123074 [41]
	P. elegans	DQ123075 [41]
	P. escobariana	JX470879 [44]
	P. exsudans	JX470880 [44]
	P. exura	DQ123076 [41]
	P. filipes	AY632734 [42]
	P. foetida	HQ900963 [40]
		JQ723387 [47]
		DQ123055 [41]
	P. gabrielliana	DQ123077 [41]
	P. galbana	HQ900964 [40]
		DQ123078 [41]
	P. garckei	DQ123079 [41]
	P. gardineri	HQ900965 [40]
	P. gilbertii	HQ900966 [40]
	P. gilbertiana	JX470881 [44]
	P. gracillima	JX470882 [44]
		DQ458091 [48]
	P. guatemalensis	JX470883 [44]
	P. haematostigma	DQ123022 [41]
	P. hatschbachii	HQ900967 [40]
	P. helleri	DQ123031 [41]

Table S3. *Cont.*

Plastid Marker	Species	GenBank Access
trnL (UAA) intron	*P. henryi*	AY632735 [42]
	P. herbertiana	AY632736 [42]
	P. hirtiflora	JX470885 [44]
	P. ichthyura	JX470886 [44]
	P. incarnata	AY756890 [49]
		HQ900968 [40]
		DQ123080 [41]
	P. indecora	JX470938 [44]
	P. iodocarpa	HQ900969 [40]
	P. ischnoclada	HQ900970 [40]
		DQ123081 [41]
	P. jilekii	HQ900971 [40]
		DQ123082 [41]
	P. jugorum	AY632737 [42]
	P. jussieu	JX470943 [44]
	P. karwinskii	JX470887 [44]
	P. kawensis	DQ123023 [41]
	P. kermesina	HQ900972 [40]
		DQ123083 [41]
	P. lancearia	JX470888 [44]
	P. lancetillensis	DQ123050 [41]
	P. leptoclada	HQ900973 [40]
		JX470889 [44]
	P. ligularis	HQ900974 [40]
	P. lindeniana	DQ123024 [41]
	P. lobata	AF454787 [43]
	P. lobbii ayaucuchoensis	DQ123032 [41]
	P. lobbii obtusiloba	DQ123033 [41]
	P. loefgrenii	HQ900975 [40]
	P. luetzerburgii	DQ123109 [41]
	P. lutea	JX470890 [44]
	P. macrophylla	DQ123025 [41]
	P. maestrensis	JX470891 [44]
	P. maliformis	DQ123025 [41]
	P. manicata	DQ123062 [41]
	P. mansoi	DQ123026 [41]
	P. mathewsii	DQ123105 [41]
	P. membranacea	AY632726 [42]
	P. mendoncaei	DQ123110 [41]
	P. menispermifolia	AF454785 [43]
	P. mexicana	AY632738 [42]
	P. micropetala	HQ900976 [40]
	P. microstipula	DQ123051 [41]
	P. miersii	HQ900977 [40]
		DQ123085 [41]

Table S3. *Cont.*

Plastid Marker	Species	GenBank Access
trnL (UAA) intron	*P. misera*	HQ900978 [40]
		JX470892 [44]
		DQ123034 [41]
	P. mixta	DQ123106 [41]
	P. molissima	AF454788 [43]
	P. moluccana	AY632739 [42]
	P. morifolia	HQ900979 [40]
		DQ123035 [41]
	P. mucronata	HQ900980 [40]
		DQ123086 [41]
	P. multiflora	AY632740 [42]
		DQ123014 [41]
	P. murucuja	JX470894 [44]
		AY632747 [42]
		DQ123063 [41]
	P. nitida	HQ900982 [40]
		DQ123087 [41]
	P. oblongata	JX470895 [44]
	P. obtusifolia	JX470896 [44]
	P. odontophylla	DQ123088 [41]
	P. oerstedii	AF454786 [43]
	P. organensis	HQ900981 [40]
		DQ123036 [41]
	P. ornithoura	DQ123037 [41]
	P. ovalis	DQ123122 [41]
		KF196419–KF196436 [46]
	P. palmeri	HQ900983 [40]
		DQ123056 [41]
	P. penduliflora	JX470898 [44]
		DQ123015 [41]
	P. perfoliata	JX470899 [44]
	P. picturata	HQ900984 [40]
	P. pilosicorona	HQ900985 [40]
	P. pittieri	AF454789 [43]
		DQ123027 [41]
	P. platyloba	AF454790 [43]
		HQ900986 [40]
	P. podlechii	DQ123013 [41]
	P. pohlii	HQ900987 [40]
		DQ123038 [41]
	P. porphyretica	JX470939 [44]
	P. punctata	DQ123039 [41]
	P. pusilla	JX470900 [44]
	P. pyrrhantha	JX470901 [44]

Table S3. *Cont.*

Plastid Marker	Species	GenBank Access
trnL (UAA) intron	*P. quadrangularis*	AF454791 [43]
		DQ123089 [41]
	P. racemosa	HQ900988 [40]
		DQ123028 [41]
	P. recurva	DQ123090 [41]
	P. reflexiflora	DQ123111 [41]
	P. rhamnifolia	DQ123016 [41]
	P. riparia	DQ123091 [41]
	P. rovirosae	DQ123040 [41]
	P. rubra	AY632741 [42]
	P. rufa	JX470902 [44]
		DQ123041 [41]
	P. rugosissima	JX470903 [44]
	P. sagasteguii	JX470904 [44]
	P. sandrae	JX470940 [44]
	P. sanguinolenta	JX470905 [44]
		DQ123104 [41]
	P. serratifolia	DQ123092 [41]
	P. serratodigitata	HQ900989 [40]
		DQ123093 [41]
	P. setacea	DQ123094 [41]
	P. setulosa	DQ123058 [41]
	P. sexflora	JX470906 [44]
		DQ123042 [41]
	P. siamica	AY632742 [42]
	P. sidiifolia	HQ900990–HQ900991 [40]
		DQ123095 [41]
	P. sodiroi	JX470907 [44]
	P. solomonii	JX470908 [44]
	P. speciosa	DQ123052 [41]
	P. sphaerocarpa	JX470909 [44]
	P. sprucei	DQ123096 [41]
	P. suberosa	AF454792 [43]
		HQ900992 [40]
		AY632743 [42]
		DQ123043 [41]
	P. subrotunda	HQ900993 [40]
	P. tacanensis	JX470910 [44]
	P. tacsonioides	DQ123103 [41]
	P. talamancensis	AF454793 [43]
		DQ123044 [41]
	P. tatei	JX470941 [44]
	P. tenuifila	DQ123097 [41]
	P. tenuiloba	AY632744 [42]
	P. tetrandra	AY632746 [42]

Table S3. *Cont.*

Plastid Marker	Species	GenBank Access
trnL (UAA) intron	*P. tica*	AF461415 [43]
	P. tina	JX470911 [44]
	P. tricuspis	HQ900994 [40]
		DQ123045 [41]
	P. trifasciata	DQ123046 [41]
	P. trifoliata	DQ123108 [41]
	P. trintae	DQ123098 [41]
	P. tripartita	DQ123107 [41]
	P. trisecta	DQ123061 [41]
	P. truncata	HQ900995 [40]
		DQ123047 [41]
	P. tryphostemmatoides	DQ123113 [41]
	P. tulae	HQ900996 [40]
		JX470912 [44]
		DQ123064 [41]
	P. umbilicata	DQ123112 [41]
	P. urnifolia	JX470942 [44]
	P. urubiciensis	HQ900997 [40]
		DQ123099 [41]
	P. vespertilio	HQ900998 [40]
		JX470913 [44]
		DQ123048 [41]
	P. villosa	DQ123059 [41]
	P. vitifolia	AF454794 [43]
		HQ900999 [40]
		JX470915 [44]
		DQ123053 [41]
	P. watsoniana	HQ901000 [40]
		DQ123100 [41]
	P. xiikzodz	JX470916 [44]
		DQ123049 [41]

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Science 315 (5820), 1812; 10: Palhares RM. Barcode identification of medicinal plants and comparison with chemical identification. Unpublished; 11: Tokuoa T, Tobe H (2006) Phylogenetic analyses of Malpighiales using plastid and nuclear DNA sequences, with particular reference to the embryology of Euphorbiaceae sens. Str. J. Plant Res. 119, 599-616; 12: Worberg A, Alford MH, Quandt D, Borsch T (2009) Huerteales sister to Brassicales plus Malvales, and newly circumscribed to include Dipentodon, Gerrardina, Huertea, Perrottetia, and Tapiscia. Taxon 58, 468-478; 13: Qiu YL, Li L, Hendry TA *et al*. Reconstructing the Basal Angiosperm Phylogeny: Evaluating Information Content of the Mitochondrial Genes. Unpublished; 14: Hilu KW, Black C, Diouf D, Burleigh JG (2008) Phylogenetic signal in matK vs. trnK: a case study in early diverging eudicots (angiosperms). Mol. Phylogenet. Evol. 48, 1120-1130; 15: Muschner VC, Lorenz-Lemke AP, Cervi AC, Bonatto S, Freitas LB. Phylogenetic relationships among *Passiflora* (Passifloraceae) species: a new taxonomic proposal. Unpublished; 16: Yotoko KS, Dornelas MC, Togni PD, *et al*. (2011) Does variation in genome sizes reflect adaptive or neutral processes? New clues from *Passiflora*. PLoS ONE 6: E18212; 17: International Barcode of Life (iBOL). Unpublished; 18: Tokuoka T. Molecular phylogenetic analysis of Passifloraceae *sensu lato* (Malpighiales) based on plastid and nuclear DNA sequences. Unpublished; 19: Jansen RK, Cai Z, Raubeson LA, *et al*. (2007) Analysis of 81 genes from 64 plastid genomes resolves relationships in angiosperms and identifies genome-scale evolutionary patterns. Proceedings of the National Academy of Sciences of United States of America. 104, 19369-19374; 20: Abbott JR, Neubig KM, Whitten WM, Williams NH. DNA barcoding the flora of Florida: Invasive species. Unpublished; 21: Schaefer H, Hardy OJ, Silva L, Barraclough TG, Savolainen V (2011). Testing Darwin's naturalization hypothesis in the Azores. Ecol. Lett. 14, 389-396; 22: Xi Z, Ruhfel BR, Schaefer H, *et al*. (2012) Phylogenomics and a posteriori data partitioning resolve the Cretaceous angiosperm radiation Malpighiales. Proc. Natl. Acad. Sci. U.S.A. 109, 17519-17524; 23: Chen S, Yao H, Han J, *et al*. (2010) Validation of the ITS2 region as a novel DNA barcode for identifying medicinal plant species. PLoS ONE 5, E8613; 24: Laiou A, Mandolini LA, Piredda R, Bellarosa R, Simeone MC (2013) DNA barcoding as a complementary tool for conservation and valorisation of forest resources. Zookeys 365, 197-213; 25: Saritha KV, Khedar GD, Hanumanth Kumar G, Tiknaik AD, Ughade BR. Direct submission; 26: Kress J, Erickson DL (2007) A two-locus global DNA barcode for land plants: the coding rbcL gene complements the non-coding trnH-psbA spacer region. PLoS ONE 2, E508; 27: CBOL Plant Working Group. A DNA Barcode for Land Plants. Unpublished; 28: Cornara L, Borghesi B, Canali C, *et al*. (2013) Smart drugs: green shuttle or real drug? Int. J. Legal Med. 127, 1109-1123; 29: Kress WJ, Wurdack KJ, Zimmer EA, Weigt LA, Janzen DH (2005). Use of DNA barcodes to identify flowering plants. Proc. Natl. Acad. Sci. U.S.A. 102, 8369-8374; 30: Tokuoa T, Tobe H (2006) Phylogenetic analyses of Malpighiales using plastid and nuclear DNA sequences, with particular reference to the embryology of Euphorbiaceae sens. Str. J. Plant Res. 119, 599-616; 31: Albert VA, Williams SE, Chase MW (1992) Carnivorous plants: phylogeny and structural evolution. Science 257 (5076), 1491-1495 (1992); 32: Muschner VC, Lorenz AP, Scherer NM, *et al*. Comparative Phylogenetic Analysis of Nuclear and Plastid Sequences in *Passiflora* (Passifloraceae). Unpublished; 33: Lorenz-Lemke AP, Muschner VC, Bonatto SL, Cervi AC, Salzano FM, Freitas LB (2006) Phylogeographic inferences concerning evolution of Brazilian *Passiflora actinia* and *P. elegans* (Passifloraceae) based on ITS (nrDNA) variation. Ann. Bot. 95: 799-806; 34: Abbott JR, Neubig KM, Whitten WM, Williams NH. DNA barcoding the flora of Florida: Invasive species. Unpublished; 35: Lorenz AP, Muschner VC, Bonatto SL, Salzano FM, Freitas LB. Molecular evidence for the origin of *Passiflora elegans* (Passifloraceae) from southern Brazil Unpublished; 36: Muschner VC, Lorenz-Lemke AP, Vecchia M, Bonatto SL, Salzano FM, Freitas LB (2006) Differential organellar inheritance in *Passiflora* (Passifloraceae) subgenera. Genetica 128, 449-453; 37: Kress J, Erickson DL (2007) A two-locus global DNA barcode for land plants: the coding rbcL gene complements the non-coding trnH-psbA spacer region. PLoS ONE 2, E508; 38: CBOL Plant Working Group. A DNA Barcode for Land Plants. Unpublished; 39: Kress WJ, Wurdack KJ, Zimmer EA, Weigt LA, Janzen DH (2005). Use of DNA barcodes to identify

flowering plants. Proc. Natl. Acad. Sci. U.S.A. 102, 8369-8374; 40: Yotoko KS, Dornelas MC, Togni PD, *et al*. (2011) Does variation in genome sizes reflect adaptive or neutral processes? New clues from *Passiflora*. PLoS ONE 6: E18212; 41: Muschner VC, Lorenz-Lemke AP, Cervi AC, Bonatto S, Freitas LB. Phylogenetic relationships among *Passiflora* (Passifloraceae) species: a new taxonomic proposal. Unpublished; 42: Krosnick SE, Freudenstein JV (2005) Monophyly and floral character homology of old world *Passiflora* (Subgenus *Decaloba*: Supersection *Disemma*). Systematic Botany, 30, 139-152; 43: Ossowski AM, Hunter FF. Coevolution of *Heliconius* spp. and *Passiflora* spp.: A phylogenetic comparison. Unpublished; 44: Krosnick SE, Porter-Utley KE, MacDougal JM, Jørgensen PM, McDade LA (2013) New insights into the evolution of *Passiflora* subgenus *Decaloba* (Passifloraceae): phylogenetic relationships and morphological synapomorphies. Systematic Botany, 38, 692-713; 45: Krosnick SE, Freudenstein JV (2006) Patterns of anomalous floral development in the Asian *Passiflora* (subgenus *Decaloba*: supersection *Disemma*). Am. J. Bot., 93, 620-636; 46: Cazé ALR, Mäder G, Bonatto SL, Freitas LB (2013) A molecular systematic analysis of *Passiflora ovalis* and *Passiflora contracta* (Passifloraceae). Phytotaxa, 132, 39-46; 47: Thulin M, Razafimandimbison SG, Chafe P, Heidari N, Kool A, Shore JS (2012) Phyloheny of the Turneracea clade (Passifloraceae): Trans-Atlantic disjunctions and two new genera in Africa. Taxon, 61, 308-323; 48: Krosnick SE, Ford A, Freudenstein JV. Resolving the phylogenetic position of *Hollrungia* and *Tetrapathaea*: The end of two monotypic genera in Passifloraceae. Unpublished; 49: Alford, MH. Phylogeny, character evolution, and classification of the Flacourtiaceae/Salicaceae complex. Unpublished.

Table S4. Primer sequences and references for studied ITS sequences.

Article Reference	Primers Used for ITS Sequences	Primer Reference
Cazé, A.L.R.; Mäder, G.; Bonatto, S.L.; Freitas, L.B. A molecular systematic analysis of *Passiflora ovalis* and *Passiflora contracta* (Passifloraceae). *Phytotaxa* **2013**, *132*, 39–46.	5' AAGGTTTCCGTAGGTGAAC 3' and 5' TATGCTTAAACTCAGCGGG 3'	Desfeux & Lejeune (1996)
Giudicelli *et al*. (in prep)	5' AAGGTTTCCGTAGGTGAAC 3' and 5' TATGCTTAAACTCAGCGGG 3'	Desfeux & Lejeune (1996)
Hearn, D.J. Adenia (Passifloraceae) and its adaptative radiation: Phylogeny and growth form diversification. *Syst. Bot.* **2006**, *31*, 805–821.	N18S (5' AGGAGAAGTCGTAACAGG 3') and C26A (5' GTTTCTTTTCCTCCGCT 3')	Modified from Wen and Zimmer (1996)
Kay, E.E. Floral Evolutionary Ecology of Passiflora: subgenera Murucuia, Pseudomurucuja and Astephia. Unpublished.	GenBank information: unpublished	
Koehler-Santos, P.; Lorenz-Lemke, A.P.; Muschner, V.C.; Bonatto, S.L.; Salzano, F.M.; Freitas, L.B. Molecular genetic variation in *Passiflora alata* (Passifloraceae), na invasive species in southern Brazil. Biological Journal of the Linnean *Society* **2006**, *88*, 611–630.	5' AAGGTTTCCGTAGGTGAAC 3' and 5' TATGCTTAAACTCAGCGGG 3'	Desfeux & Lejeune (1996)
Kress, W.J.; Wurdack, K.J.; Zimmer, E.A.; Weigt, L.A; Janzen, D.H. Use of DNA barcodes to identify flowering plants. *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 8369–8374.	Primers 5 (5' GGAAGTAAAAGTCGTAACAAGG 3') and 4 (5' TCCTCCGCTTATTGATATGC 3')	White *et al*. (1990)
Krosnick SE, Ford A, Freudenstein JV. Resolving the phylogenetic position of *Hollrungia* and *Tetrapathaea*: The end of two monotypic genera in Passifloraceae. Unpublished.	GenBank information: unpublished	
Krosnick, S.E.; Freudenstein, J.V. Monophyly and floral character homology of old world *Passiflora* (Subgenus *Decaloba*: Supersection *Disemma*). *Syst. Bot.* **2005**, *30*, 139–152.	Primers 5 (5' GGAAGTAAAAGTCGTAACAAGG 3') and 4 (5' TCCTCCGCTTATTGATATGC 3')	White *et al*. (1990)

Table S4. *Cont.*

Article Reference	Primers Used for ITS Sequences	Primer Reference
Krosnick, S.E.; Freudenstein, J.V. Patterns of anomalous floral development in the Asian *Passiflora* (Subgenus *Decaloba*: Supersection *Disemma*). *Am. J. Bot.* **2006**, *93*, 620–636.	Primers 5 (5' GGAAGTAAAAGTCGTAACAAGG 3') and 4 (5' TCCTCCGCTTATTGATATGC 3')	White *et al*. (1990)
Krosnick, S.E.; Freudenstein, J.V. Phylogenetic relationships among the Old World species of *Passiflora* L. (Subgenus *Decaloba*: Supersection *Disemma*). Unpublished.	GenBank information: unpublished	
Krosnick, S.E.; Porter-Utley, K.E.; MacDougal, J.M.; Jørgensen, P.M.; McDade, L.A. New insights into the evolution of *Passiflora* subgenus *Decaloba* (Passifloraceae): Phylogenetic relationships and morphological synapomorphies. *Syst. Bot.* **2013**, *38*, 692–713.	Primers 5 (5' GGAAGTAAAAGTCGTAACAAGG 3') and 4 (5' TCCTCCGCTTATTGATATGC 3')	White *et al*. (1990)
Krosnick, S.E.; Xun-Lin, Y.; Deng, Y. The rediscovery of *Passiflora kwangtungensis* Merr. (subgenus *Decaloba* supersection *Disemma*): A critically endangered Chinese endemic. *PhytoKeys* **2013**, *23*, 55–74.	Primers 5 (5' GGAAGTAAAAGTCGTAACAAGG 3') and 4 (5' TCCTCCGCTTATTGATATGC 3')	White *et al*. (1990)
Lorenz-Lemke, A.P.; Muschner, V.C.; Bonatto, S.L.; Cervi, A.C.; Salzano, F.M.; Freitas, L.B. Phylogeographic inferences concerning evolution of Brazilian *Passiflora actinia* and *P. elegans* (Passifloraceae) based on ITS (nrDNA) variation. *Ann. Bot.* **2005**, *95*, 799–806.	5' AAGGTTTCCGTAGGTGAAC 3' and 5' TATGCTTAAACTCAGCGGG 3'	Desfeux & Lejeune (1996)
Mäder, G.; Zamberlan, P.M.; Fagundes, N.J.R.; Magnus, T.; Salzano, F.M.; Bonatto, S.L.; Freitas, L.B. The use and limits of ITS data in the analysis of intraspecific variation in *Passiflora* L. (Passifloraceae). *Genet. Mol. Biol.* **2010**, *33*, 99–108.	5' AAGGTTTCCGTAGGTGAAC 3' and 5' TATGCTTAAACTCAGCGGG 3'	Desfeux & Lejeune (1996)
Muschner, V.C.; Lorenz, A.P.; Cervi, A.C.; Bonatto, S.L.; Souza-Chies, T.T.; Salzano, F.M.; Freitas, L.B. A first molecular phylogenetic analysis of *Passiflora* (Passifloraceae). *Am. J. Bot.* **2003**, *90*, 1229–1238.	5' AAGGTTTCCGTAGGTGAAC 3' and 5' TATGCTTAAACTCAGCGGG 3'	Desfeux & Lejeune (1996)

Table S4. *Cont.*

Article Reference	Primers Used for ITS Sequences	Primer Reference
Muschner, V.C.; Lorenz-Lemke, A.P.; Vecchia, M.; Bonatto, S.L.; Salzano, F.M.; Freitas, L.B. Differential organellar inheritance in *Passiflora* (Passifloraceae) subgenera. *Genetica* **2006**, *128*, 449–453.	5' AAGGTTTCCGTAGGTGAAC 3' and 5' TATGCTTAAACTCAGCGGG 3'	Desfeux & Lejeune (1996)
Ossowski, A.M.; Hunter, F.F. Coevolution of *Heliconius* spp. and *Passiflora* spp.: A phylogenetic comparison. Unpublished.	GenBank information: unpublished	
Thulin M, Razafimandimbison, S.G.; Chafe, P.; Heidari, N.; Kool, A.; Shore, J.S. Phyloheny of the Turneracea clade (Passifloraceae): Trans-Atlantic disjunctions and two new genera in Africa. *Taxon* **2012**, *61*, 308–323.	P17F (5' CTACCGATTGAATGGTCCGGTGAA 3') and 26S–82R (5' TCCCGGTTCGCTCGCCGTTACTA 3')	Alejandro *et al*. (2005)
Wright, S.; Keeling, J.; Gillman, L. The road from santa Rosalia: A faster tempo of evolution on tropical climes. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 7718–7722.	CY1 (5' TACCGATTGAATGATCCGGTGAAG 3') and CY3 (5' CGCCGTTACTAGGGGAATCCTTGT 3')	C. G. Yong, personal communication

6. CONSIDERAÇÕES FINAIS

As regiões de ITS apresentam potencial para auxiliar inferências filogenéticas quando apenas a estrutura primária dos marcadores é considerada (Baldwin *et al*. 1992; 1995; Hsiao *et al*. 1994; Li *et al*. 2010) e também em estudos em que sua estrutura secundária é analisada (Gottschling *et al*. 2001; Goertzen *et al*. 2003; Tippery & Les 2008). Além disso, as regiões de ITS têm sido amplamente avaliadas como potenciais marcadores em estudos de DNA *barcoding* (Chase *et al*. 2005; Kress *et al*. 2005; Yang *et al*. 2012; Zhang *et al*. 2014). Considerando o extenso uso dos marcadores ITS em trabalhos envolvendo o gênero *Passiflora* (Muschner *et al*. 2003; Krosnick & Freudenstein 2005; Lorenz-Lemke *et al*. 2005; Koehler-Santos *et al*. 2006; Mäder *et al*. 2010; Cazé *et al*. 2013; Krosnick *et al*. 2013), o objetivo deste estudo foi analisar as estruturas secundárias dos espaçadores internos transcritos (ITS1 e ITS2) e seu potencial para auxiliar o alinhamento das sequências de espécies do gênero *Passiflora*, proporcionando melhores filogenias em estudos futuros, além estimar o poder discriminatório deste marcador para estudos de DNA *barcoding* envolvendo espécies do gênero.

As filogenias dos quatro subgêneros de *Passiflora*, obtidas no primeiro capítulo desta dissertação, estão de acordo com as obtidas em outros estudos do gênero (Mushner *et al.* 2003; Krosnick *et al.* 2013). A alta variabilidade das sequências de ITS observada entre os subgêneros dificulta a obtenção de um alinhamento contendo todas as espécies do gênero e torna necessária a abordagem por subgênero. As análises da estrutura secundária dos ITS1 e ITS2, obtidas no primeiro artigo, sugerem o potencial uso destas sequências como ferramentas que permitem aprimorar o alinhamento, como já observado em outros estudos (Wolf *et al.* 2005; Keller *et al.* 2010), uma vez que estas análises possibilitam o reconhecimento de homologias entre sequências através das características das estruturas secundárias, como o número e posição dos *hairpins*.

As análises do primeiro capítulo sugerem que as sequências de ITS estão sob pressão seletiva para manter suas estruturas secundárias, provavelmente em decorrência das funções exercidas durante o processo de *splicing* (Musters *et al.* 1990; Sande *et al.* 1992), como observado por Edger *et al.* (2014) para a família Brassicaceae. Este resultado questiona a neutralidade do marcador e, consequentemente, sua utilidade em estudos filogenéticos. Entretanto, o ITS é um marcador molecular bastante variável que têm se mostrado útil para resolver filogenias em diferentes grupos vegetais e níveis hierárquicos

(Zhou *et al.* 2008; Sharma *et al.* 2012), incluindo *Passiflora* (Muschner *et al.* 2003; Krosnick *et al.* 2013), obtendo as mesmas topologias que com outros marcadores.

A possibilidade de usar apenas os ITS em estudos filogenéticos envolvendo espécies de *Passiflora* permitiria uma resposta eficaz através do sequenciamento de poucos pares de base, o que auxiliaria na resolução da complexa taxonomia do grupo. Entretanto, sabe-se que estudos envolvendo apenas um marcador comumente levam a erros na estimativa de relações filogenéticas (Maddison 1997), o que pode ser contornado usando múltiplos marcadores que apresentem diferentes padrões de herança (Hills 1995; Edwards 2009). Entretanto, o marcador ITS apresenta muitas vantagens, como sua herança biparental, a universalidade de seus *primers* e a grande variabilidade da região, o que torna o uso do marcador tão difundido. Além disso, o grande número de cópias e pequeno tamanho das regiões facilita a amplificação das sequências e permite o uso de amostras parcialmente degradadas ou antigas, como as de herbário (Álvarez & Wendel 2003).

Em *Passiflora*, além do potencial das estruturas secundárias das regiões ITS, os resultados obtidos no segundo capítulo desta dissertação sugerem a importância destas sequências em trabalhos de DNA *barcoding*, como já observado em outros grupos vegetais (Zhang *et al*. 2012; Alves *et al*. 2013; Krawczyl *et al*. 2014). Estas análises refletem a utilidades de ambas as sequências, ITS1 e ITS2, em estudos que objetivam discriminar espécies, e sugerem o ITS1 como um ponto inicial para espécies de *Passiflora*, como sugerido por Wang *et al*. (2014) para outros grupos.

As sequências plastidiais de *Passiflora* apresentam poucos polimorfismos, o que dificulta a discriminação das espécies e prejudica seu uso não apenas em abordagens de DNA *barcoding*, mas também em estudos filogenéticos. Apesar das possíveis questões sobre a neutralidade das sequências de ITS, este marcador tem se mostrado útil para resolver diferentes questões em *Passiflora*, o que reflete sua importância em trabalhos do gênero.

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