



Phylogeny, biogeography and divergence times in *Passiflora* (Passifloraceae)

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Abstract

As part of a long-term investigation on the evolution of *Passiflora* L., we investigated the divergence ages of the genus and diversification of its subgenera, relating them with biogeographical and/or historical events, and other characteristics of this taxon. The main aim of the present work was to evaluate the biogeographic distribution of this genus to better understand its evolutionary history. This is the first time that representatives from South American and Old World Passifloraceae genera have been studied as a group comprising a total of 106 widely distributed species, with representative samples of the four suggested subgenera. Seven DNA regions were studied, comprising 7,431 nucleotides from plastidial, mitochondrial and nuclear genomes. Divergence time estimates were obtained by using a Bayesian Markov Chain Monte Carlo method and a random local clock model for each partition. Three major subgenera have been shown to be monophyletic and here we are proposing to include another subgenus in the *Passiflora* infrageneric classification. In general, divergence among the four subgenera in *Passiflora* is very ancient, ranging from ~32 to ~38 Mya, and Passifloraceae seems to follow a biogeographic scenario proposed for several plant groups, originating in Africa, crossing to Europe/Asia and arriving in the New World by way of land bridges. Our results indicated that *Passiflora* ancestors arrived in Central America and diversified quickly from there, with many long distance dispersion events.

Keywords: biogeography, molecular phylogenetics analysis, passionflowers, plant evolution, taxonomic classification.

Introduction

Passiflora L. is the largest genus of the Passifloraceae family, and encompasses more than 500 wild species distributed especially in the Neotropical region (Ulmer and MacDougal, 2004). The majority of these are herbaceous, but there are also shrubs and trees among them. Killip (1938) and MacDougal (1994) asserted that among the Angiosperms no other group presents such a high foliar diversity, and its flowers display ample variation in size and color, with the corona and perianth showing diverse orientation and development. Coevolution with insect pollinators has been suggested as an explanation for these features (MacDougal, 1994). Based on morphology only (especially flower structures) Feuillet and MacDougal (2004) proposed a drastic taxonomic reevaluation of the genus that, according to them, would consist of only four subgenera (*Passiflora*, *Decaloba*, *Astropheia* and

Deidamioides), against the 22 or 23 formerly proposed (Killip, 1938; Escobar, 1989).

The first molecular phylogeny of *Passiflora*, published by Muschner *et al.* (2003), included more than 60 species of *Passiflora* studied for plastidial and nuclear genome markers. They found three clearly defined major clades while the fourth one remained undefined due to the small number of species classified in it. They also found that the monospecific *Tetrastyllis* was part of *Passiflora*. The morphological propositions of Feuillet and MacDougal (2004) are mostly in agreement with our molecular phylogeny. Although some attempts to elucidate the phylogeny of the genus did not agree with our molecular results and the proposition by Feuillet and MacDougal (2004) with respect to the number of subgenera and their composition (*e.g.* Yockteng and Nadot, 2004a; Plotze *et al.*, 2005), a study by Hansen *et al.* (2006) with other molecular sequences from other species in *Passiflora* recovered the four subgenera as monophyletic groups.

Inferences regarding the biogeographic history of tropical angiosperms based on morphology were frequently

very poor, given the difficulty of formulating detailed phylogenetic hypotheses and obtaining adequate estimates of divergence times. For example, biogeographical analyses of the tropical flora attribute transtropical disjunctions at high taxonomic levels to the Gondwana breakup (Raven and Axelrod, 1974; Gentry, 1982, 1993; Barlow, 1990; Burnham and Graham, 1999). This interpretation, however, implies divergence times of 100–90 million years ago (Mya) between the African and Neotropical clades, and even higher values for taxa also found in Southeast Asia. In the absence of an adequate fossil record for key areas like South America (Burnham and Graham, 1999), the controversy between Gondwana breakup explanations and those which rely in more recent long-distance dispersion events for the interpretation of present distribution patterns remains unsettled.

Sequences of plastid, mitochondrial and nuclear DNA have been extensively utilized to study plant (especially Angiosperm) phylogenies (*e.g.* Qiu *et al.*, 1999; Kuzoff and Gasser, 2000; Soltis *et al.*, 2002; APG III 2009). The strategy of combining multiple genes with different functions from the three plant genomes should reduce the phylogenetic noise generated by gene function and/or genome specific phenomena, such as heterogeneity of rates of change, GC-content bias, RNA editing and protein structural constraints (Qiu *et al.*, 1999). Rokas *et al.* (2003) showed that as the number of genes increases in a phylogenetic analysis, the better tree reflects the species' phylogeny. The same type of relationship was examined by Rokas and Carroll (2005), who concluded that for phylogenetic precision the number of genes considered is a more important determinant than the number of taxa examined. However, branch representativeness should also be taken into consideration, and when a large number of taxa is being studied, the ideal number of markers should be decided in cost-benefit terms.

The aims of the present work were: (a) to re-examine the *Passiflora* phylogeny combining markers from the three genomes, thus contributing to taxonomic classification, (b) to test monophyly of the genus and its subgenera, (c) to investigate the divergence time between the main clades, and (d) to evaluate the biogeographic distribution, aiming to better understand its evolutionary history. This is the first time that representatives from South American and Old World Passifloraceae genera are included as an outgroup.

Materials and Methods

Taxon sampling

We investigated a total of 106 species distributed in the four subgenera of Feuillet and MacDougal (2004) and representatives from seven other genera of Passifloraceae

(*Adenia isoalensis*, *Adenia keramanthus*, *Ancystrothyrus sp.*, *Barteria sp.*, *Deidamia sp.*, *Dilkea johannesii*, *Mitostemma brevifilis*, *Paropsia brazzeana* and *Paropsia madagascariensis*), one Malesherbiaceae (*Malesherbia linearifolia*) and one Turneraceae (*Turnera subulata*). These species were utilized as outgroups, all being included in Passifloraceae by the Angiosperm Phylogeny Group APG (2003, 2009). More information about DNA sources and GenBank numbers is provided in Table S1 in Supplementary Material.

DNA extraction, amplification and sequencing

Total DNA was extracted from fresh leaves dried in silica gel or obtained from herbarium material, using the method of Roy *et al.* (1992). Eight DNA regions were sequenced: the *rbcL* and *rps4* genes, *trnL* intron and *trnL-trnF* intergenic spacers from the plastid genome, *nad1* b/c and *nad5* d/e introns from the mitochondrial genome and a partial portion of the *26S* gene from the nuclear ribosomal genome. These regions were amplified with primers 1F and 1460R (Savolainen *et al.*, 2000), *rps45* and *rps43* (Souza-Chies *et al.*, 1997), c, d, e and f (Taberlet *et al.*, 1991), *nad1/2* and *nad1/3* (Duminil *et al.*, 2002), *mt3* and *mt6* (Souza *et al.*, 1991), N-nc26S1 and 1229r (Kuzoff *et al.*, 1998). Sequencing primers were used as listed by these authors except for the *nad1* b/c intron, for which we constructed an internal primer specific for *Passiflora* (5'-ATTCACATAGAGACAGACT).

PCR products were purified using the polyethylene glycol/NaCl precipitation method of Dunn and Blattner (1987). Sequencing was performed on a MegaBace 1000 (GE Health Care) automatic sequencer using the DYEnamic™ ET termination cycle sequencing premix kit (GE Health Care) following the manufacturer's protocol. The sequences were deposited in Genbank (Accession numbers are given in Table S1). The sequences were aligned using Clustal W (Thompson *et al.*, 1994) implemented on Mega5 (Tamura *et al.*, 2011). All alignments were manually adjusted. Regions of ambiguous alignment were excluded from the analyses.

Phylogenetic analyses

The phylogenetic analyses were performed for the seven genetic markers with a Bayesian approach using BEAST 1.7.1. Less than 20% of the alignment corresponded to missing data. Two independent runs of 3 x 10⁷ chains were performed, each with sampling at every 3,000 generations. The parameters used were as follows: a single HKY substitution model with eight gamma categories, a Yule tree prior and a random local clock model for each partition, which is highly recommended in *Passiflora*, since different subgenera present different DNA content (Yotoko *et al.*, 2011). Three calibration points were used. A

fossil seed with age 37 Mya assigned to the genus *Passiflora* (Mai, 1967), also used by Hearn (2006) in an *Adenia* (Passifloraceae) divergence time investigation, was used for an exponential prior for the *Passiflora* clade with a mean of 15 Mya and an offset of 37 Mya. A normal prior with a mean of 48 Mya and standard deviation of 10 Mya was used for the Passifloraceae/Turneraceae divergence and for the root of the phylogeny we used a uniform prior of between 70 and 110 Mya (based on Bell *et al.*, 2010). The software Tracer v1.5 was used to check for convergence after the first 10% of generations had been discounted as burn-in. Maximum-clade-credibility trees were estimated using the program TreeAnnotator, which is part of the Beast package. Statistical support for the clades was determined by assessing Bayesian posterior probabilities.

Results and Discussion

The alignment for all loci totaled 7,431 nucleotides. The numbers of variable and parsimony informative sites

Table 1 - Sequences characterization, alignment size, variable and parsimony informative site numbers.

Marker	Total sites	Variable sites	Parsimony informative
<i>rbcL</i>	1345	354	218
<i>rps4</i>	615	231	146
<i>trnL-trnF</i>	411	158	78
<i>trnL</i> intron	681	204	111
<i>nad1</i>	1704	323	120
<i>nad5</i>	1550	210	75
<i>26S</i>	1125	228	122
All	7431	1708	870

for each marker are shown in Table 1. In Figure 1 we present the phylogenetic relationship and divergence times obtained by the Bayesian approach for the main clades. Diversification ages, geological periods and outstanding events for these clades are shown in Table 2. The full

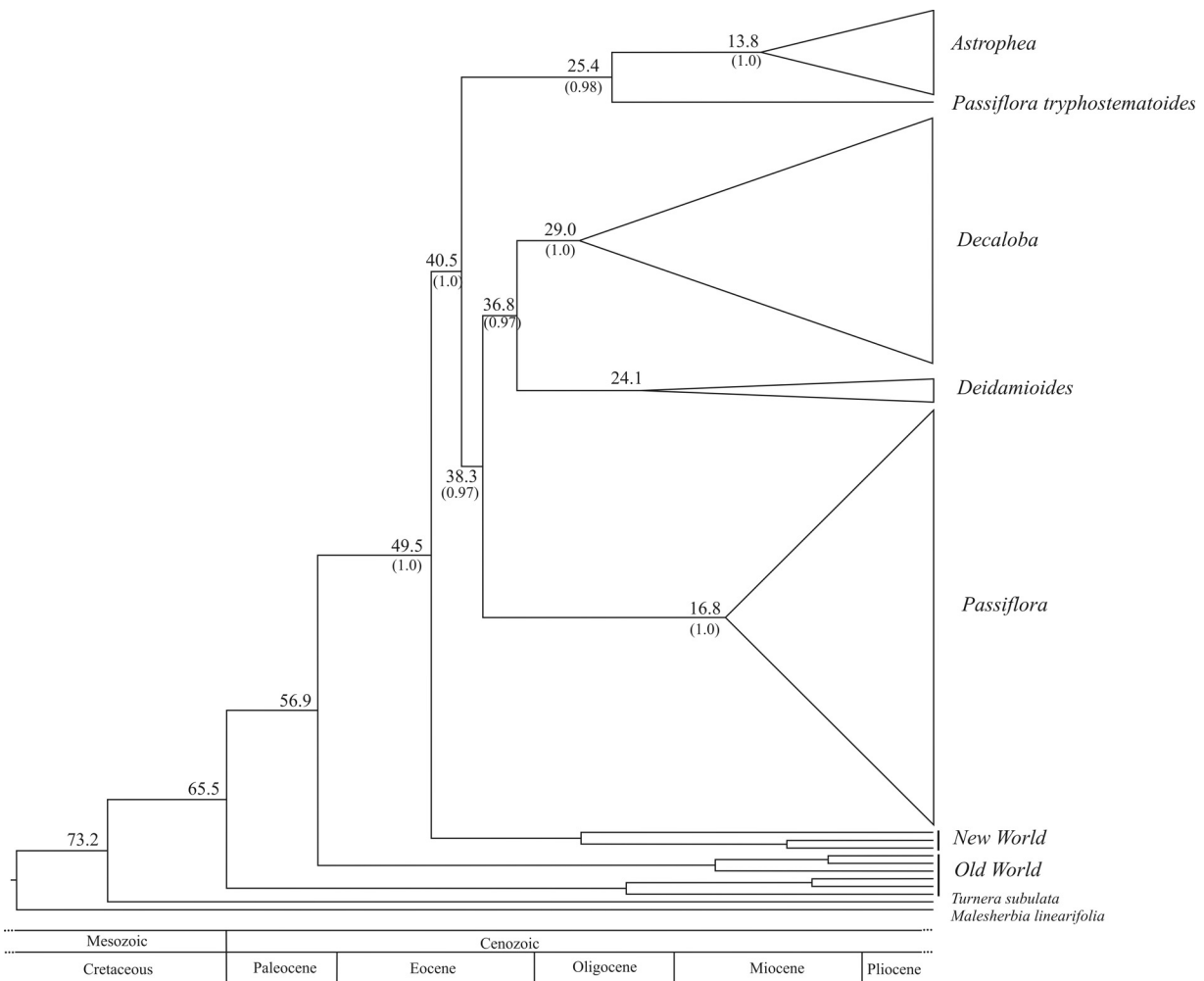


Figure 1 - Phylogenetic relationship and divergence times obtained by the Bayesian approach using seven genetic markers from three plant genomes of *Passiflora* species and related taxa.

non-collapsed branches of the Bayesian tree can be obtained by request from the corresponding author.

In the present analysis, Malesherbiaceae appeared as a sister group of Passifloraceae and Turneraceae, as already proposed by Davis *et al.* (2005) and Krosnick *et al.* (2006), and the divergence time found here (73.2 Mya) is also in agreement with Davis *et al.* (2002). Wikström *et al.* (2001) estimated divergence between Passifloraceae s.s and Turneraceae between 32-36 Mya. We found an older date (65.5 Mya), but note that other authors (*i.e.*, Bremer *et al.*, 2004) also obtained dates older than those reported by Wikström *et al.* (2001) for different groups. A possible explanation for this difference may be that Wikström *et al.* (2001) focused their work on higher taxonomic groups, with a very sparse sample density at lower (below family) taxonomic levels and used a different method to estimate divergence.

Considering that sampling at the molecular level in the present study is similar to other biogeographical analyses (Renner, 2004; Richardson *et al.*, 2004; Bell and Donoghue, 2005; Yuan *et al.*, 2005), the above listed divergence time suggests a post-Gondwanic origin of the Passifloraceae. According to Raven and Axelrod (1974) migration between South America and Africa could have occurred even after the Gondwana breakup at 90-105 Mya. Morley (2003) reviewed the potential world migration routes for the megathermal angiosperms, suggesting that connections between South America and Africa may have existed up to the Oligocene (around 35 Mya). These connections may have been used for stepping stone dispersal across islands of the Rio Grande Rise and the Walvis Ridge, which according to Parrish (1993), were above water southwest of the coast of Africa up until that time, as well as through the Sierra Leone Rise.

Other studies (Wolfe, 1978; Renner *et al.*, 2001; Davis *et al.*, 2002) suggested boreo-tropical migration into southern areas during the Oligocene and Miocene, which

could explain the distribution of plants including Passifloraceae. A possible route to dispersion through Laurasia during the Eocene climatic optimum, which may have supported tropical vegetation, could be the best explanation for many organisms that now have a disjunct distribution in the South American, African, and southeastern Asian tropics (Richardson *et al.*, 2004), such as the Passifloraceae. In this hypothesis, the North Atlantic region was at a thermal maximum between the Eocene/Oligocene (see Wolfe, 1978) and the North Hemisphere was at its warmest period during the Paleocene/Eocene (according to Davies *et al.*, 2004). The land bridges could thus have been warm enough to support plants like Passifloraceae. Given the estimated age of the family, this is a viable route for its migration. As global temperatures dropped during the Oligocene, species might have become extinct in colder regions and expanded their ranges into the warmer south.

Molecular phylogenetic studies have also demonstrated that the role of long-distance dispersals to explain modern distribution patterns may have been underestimated (Renner *et al.*, 2001; Renner, 2004; Yuan *et al.*, 2005). Especially in Passifloraceae, the time frame postulated in the land bridge hypothesis is more plausible than a Gondwana hypothesis because the former allows a larger time window for family evolution and expansion, which would be more favorable for multiple radiations and migrations from South America to Africa and Australia.

Another explanation to the family distribution range is offered by the climate changes that occurred during the late Cretaceous, when the opening of the Tethys Seaway caused a global warming between five and eight degrees (Fluteau, 2003), that would have allowed tropical plants to expand northward. During the following climate cooling, extinctions occurred and many species were restricted to warmer regions in Asia, Africa and the Neotropics. This is congruent with both the Boreo-Tropical and the land bridges hypothesis.

Table 2 - Divergence times, geological periods and outstanding events for the clades presented in Figure 1.

Group	Age	Period	Outstanding events
Malesherbiaceae (Passifloraceae + Turneraceae)	73.2	Cretaceous	Migration from Old to New World through land bridges
Passifloraceae	65.5	Paleocene	
Old and New World genera	49.5	Eocene (E)	Land bridges linking North-Central-South Americas
<i>Passiflora</i> genus	40.5	Eocene (M)	Andes uplifting first stage
(<i>Astrophea</i> + <i>Tryphostematooides</i>) (<i>Passiflora</i> + <i>Decaloba</i> + <i>Deidamioides</i>)	38.3	Eocene (L)	Andes uplifting second stage
(<i>Decaloba</i> + <i>Deidamioides</i>) (<i>Passiflora</i>)	36.8	Eocene (L)	Andes uplifting completed
<i>Decaloba</i> diversification	29.0	Oligocene (L)	Paleo-Orinoco fluvial system
<i>Deidamioides</i> diversification	24.1	Oligocene (L)	
<i>Astrophea</i> diversification	13.8	Miocene (M)	Lake Pebas environment; internal migration/diversification
<i>Passiflora</i> diversification	16.8	Miocene (M)	Lake Pebas environment; internal migration/diversification

The genus *Passiflora* was monophyletic with high support (PP = 1) in this analysis. Three subgenera were equally well supported, but *Passiflora* subg. *Deidamioides* as described by Feuillet and MacDougal (2004) emerged as paraphyletic because *P. tryphostemmatoides* appeared with high support as sister to the *Passiflora* subg. *Astrophea*. *Passiflora tryphostemmatoides* is the type species of a session in the subgenus *Deidamioides* (Feuillet and MacDougal, 2004), but presents unique morphological traits in that group, such that Killip (1938) and Escobar (1989) considered it as the type species of a new subgenus. Although the positioning of *P. tryphostemmatoides* in a separate group was also obtained by Yockteng and Nadot (2004b), our results must be considered with caution because we included only one species of Session Tryphostemmatoides. Yockteng and Nadot (2004b) proposed a different infrageneric classification to *Passiflora*, including eight subgenera. Our results did not support the three extra subgenera (in addition to the four subgenera above plus a clade with *P. tryphostemmatoides*). We therefore suggest a review of the infrageneric classification, including the well supported *Tryphostemmatoides* as a new subgenus.

In general, divergence among the four subgenera in *Passiflora* is very ancient, ranging from ~33 to ~38 Mya (Table 1 and Figure 1). The first divergence event in the *Passiflora* occurred as a split up between the clade *Tryphostemmatoides*+*Astrophea* and the clade *Passiflora*+*Decaloba*+*Deidamioides* (38.3 Mya). In the latter, the two major subclades (*Deidamioides*+*Decaloba* and *Passiflora*) split 36.8 Mya. The subgenera *Deidamioides* and *Decaloba* diverged around 33.5 Mya and *Tryphostemmatoides* and *Astrophea* 25.4 Mya.

The very ancient (~40 Mya) separation of *Astrophea* from the clade *Passiflora*+*Decaloba*+*Deidamioides* could help to explain why the former encompasses species that present the most unusual morphological traits within *Passiflora*, some do not even look much like passionflowers. In *Astrophea* there are species that present tree, shrub or woody vine habits. Similarly, the older divergence of the *Passiflora* subgenus in relation to *Decaloba*+*Deidamioides* is intriguing since many authors have suggested that within the genus the former present ancestral traits in relation to morphology (see Ulmer and MacDougal, 2004, for a review of species description and characteristics) and genetics (Melo and Guerra, 2003 for cytogenetics; Muschner *et al.*, 2006 for organelar inheritance; Yotoko *et al.*, 2011 for genome size evolution). However, the diversification age within *Passiflora* (~16.8 Mya) was much more recent than diversification in *Decaloba* (~29 Mya).

Differences in evolutionary rates between taxa are widespread in plants (Muse, 2000), and can be ascribed to factors intrinsic to each genome type (plastidial, mitochondrial and nuclear) and to extrinsic factors like speciation

dynamics, population size and life history (Bousquet *et al.*, 1992; Muse, 2000; Andreasen and Baldwin, 2001; Barraclough and Savolainen, 2001; Smith and Donoghue, 2009). The *Decaloba* and *Deidamioides* subgenera have longer branches (see Fig. S1) than those of the *Passiflora* and *Astrophea* subgenera, indicating a pattern of accelerated molecular evolution. The mechanisms that could lead to high evolutionary rates in the former subgenera are a generation time that is shorter in *Decaloba* and *Deidamioides* than those in the others, and that they comprise most of the self-compatible species described so far in *Passiflora* (Benson *et al.*, 1975; Ulmer and MacDougal, 2004).

This is the first study that considers *Passiflora* diversification times in detail. Just a few species grow in North America, mainly in Mexico, which could be attributed to the presently unfavorable climate for these species that prefer warmer and moister conditions. Passifloraceae in general seems to follow a biogeographic scenario proposed by other authors for several plant groups (see Antonelli *et al.*, 2009 and Antonelli and Sanmartín, 2011, for more details), with an origin in Africa, crossing to Europe/Asia and arrival in the New World by way of land bridges. Our results indicate that *Passiflora* ancestors arrived in Central America and diversified quickly from there. *Passiflora* subgenera divergence times show the gradual colonization of Americas from north to south. Below we present a more detailed account of this biogeographic scenario.

An alternative hypothesis explaining the disjunct distribution of *Passiflora* (see below) could be by Trans-Pacific dispersion, as suggested for other groups (Sanmartín and Ronquist, 2004). The relationship [(South America, New Zealand) Australia] is the most frequently observed in the flora and fauna of the South Hemisphere and is in conflict with the geologically predicted vicariance patterns (Renner *et al.*, 2000; Winkworth *et al.*, 2002). Sanmartín and Ronquist (2004) documented highly asymmetrical, westward long-distance plant dispersal from South America to New Zealand, against the prevailing wind and oceanic currents (Winkworth *et al.*, 2002). Instead of direct jumps, the dispersal could have occurred in a stepping stone manner along the Antarctic coastline (Renner *et al.*, 2000). This hypothesis is supported by the presence of temperate forests in this area until at least the Pliocene (Swenson and Bremer, 1997; Sanmartín and Ronquist, 2004). This dispersal could have been mediated by the west-flowing East Wind Drift, which runs close to the Antarctic coast, or could have followed the West Wind Drift around Antarctica, involving dispersal first to the sub-Antarctic islands (and/or Australia) and from there to New Zealand (Swenson and Bremer, 1997). Large birds could have contributed to this dispersion, as suggested by Winkworth *et al.* (2002) and Ulmer and MacDougal (2004). Such processes have been proposed for species with characteristics very

similar to those of the *Decaloba* subgenus (Renner *et al.*, 2001; Knapp *et al.*, 2005). This type of dispersion could therefore explain the presence in southeast Asia and Australia of species of a monophyletic session of the *Decaloba* subgenus, as found by Krosnick and Freudenstein (2005).

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Internet Resources

Tracer v1.5 software, <http://beast.bio.ed.ac.uk/Tracer> (accessed in October 22, 2012)

Supplementary Material

The following online material is available for this article:

- Table S1 - DNA sources and GenBank numbers of the *Passiflora* species included in the analysis.

This material is available as part of the online article from <http://www.scielo.br/gmb>.

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Subgenera	Species	Data of collection	GenBank numbers						
			<i>rbcL</i>	<i>rps4</i>	<i>trnL</i>	<i>trnL-trnF</i>	<i>nad1 b/c</i>	<i>nad5 d/e</i>	<i>26S</i>
<i>Astrophea</i>	<i>P. amoena</i> L. K. Escobar	Italy, Ripalta Cremasca, Colection (MV)	DQ123300	DQ123407	DQ123017	DQ123486	DQ123214	DQ123128	DQ122935
	<i>P. arborea</i> Spreng.	Panama, Blois-France-Greenhouse (RY)	DQ123301	DQ123408	DQ123018	DQ123487	DQ123215	DQ123129	DQ122936
	<i>P. candida</i> (P. & E.) Mast.	Italy, Ripalta Cremasca, Colection (MV)	DQ123302	DQ123409	DQ123019	N/A	DQ123216	DQ123130	N/A
	<i>P. ceratocarpa</i> Silveira	Brazil, PA (LCB)	DQ123303	DQ123410	DQ123020	DQ123488	DQ123217	DQ123131	DQ122937
	<i>P. citrifolia</i> (Juss.) Mast.	French Guiana, Ripalta Cremasca, Colection (MV)	DQ123304	AY212311	DQ123021	AY210958	DQ123218	DQ123132	DQ122938
	<i>P. haematostigma</i> Mart. ex Mast.	Guaratuba, PR (ACC)	DQ123305	AY212292	DQ123022	AY032773	DQ123219	DQ123133	DQ122939
	<i>P. kawensis</i> Feuillet	French Guiana, Blois-France-Greenhouse (RY)	DQ123306	DQ123411	DQ123023	DQ123489	DQ123220	DQ123134	DQ122940
	<i>P. lindeniana</i> Tr. & Pl.	Italy, Ripalta Cremasca, Colection (MV)	DQ123307	DQ123412	DQ123024	DQ123490	DQ123221	DQ123135	DQ122941
	<i>P. macrophylla</i> Spruce ex Mast.	Brazil, Ripalta Cremasca, Colection (MV)	DQ123308	AY212313	DQ123025	AY210965	DQ123222	DQ123136	DQ122942
	<i>P. mansoi</i> (Mart.) Mast.	Brazil, MS (ACC)	DQ123309	AY212307	DQ123026	AY102401	DQ123223	DQ123137	DQ122943
	<i>P. pittieri</i> Mast.	Italy, Ripalta Cremasca, Colection (MV)	DQ123310	DQ123413	DQ123027	DQ123491	DQ123224	DQ123138	DQ122944
	<i>P. rhamnifolia</i> Mast.	Brazil, RJ (TSN)	DQ123299	DQ123406	DQ123016	DQ123485	DQ123213	DQ123127	N/A
	<i>Decaloba</i>	<i>P. multiflora</i> L.	Dominica, Ripalta Cremasca, Colection (MV)	DQ123297	DQ123404	DQ123014	AY210967	DQ123211	DQ123125
<i>P. penduliflora</i> Bertero ex DC.		Blois-France-Greenhouse (RY)	DQ123298	DQ123405	DQ123015	DQ123484	DQ123212	DQ123126	DQ122934
<i>P. capsularis</i> L.		Brazil, PR (ACC)	DQ123312	DQ123415	DQ123029	AY032775	DQ123226	DQ123140	DQ122946
<i>P. coriacea</i> Juss.		Colombia, Ripalta Cremasca, Colection (MV)	DQ123313	DQ123416	DQ123030	AY210959	DQ123227	DQ123141	DQ122947
<i>P. guatemalensis</i> Wats.		GenBank numbers	N/A	N/A	DQ087428	DQ087428	N/A	N/A	N/A
<i>P. helleri</i> Peyer		Mexico, Ripalta Cremasca, Colection (MV)	DQ123314	DQ123417	DQ123031	AY210962	DQ123228	DQ123142	DQ122948
<i>P. herbertiana</i> Ker-Gawl.		GenBank numbers	N/A	N/A	AY632736	AY632736	N/A	N/A	N/A
<i>P. holocericea</i> L.		GenBank numbers	N/A	N/A	DQ087426	DQ087426	N/A	N/A	N/A
<i>P. lobbi</i> subsp. <i>ayaucuchoensis</i> Skrabal & Weigend ²		Peru, Kew Botanical Garden (MC)	DQ123315	DQ123419	DQ123032	DQ123493	N/A	N/A	N/A
<i>P. lobbi</i> subsp. <i>obtusiloba</i> (Mast.) Skrabal & Weigend ²		Peru, Kew Botanical Garden (MC)	DQ123316	DQ123418	DQ123033	DQ123494	DQ123229	DQ123143	N/A
<i>P. micropetala</i> Mast.			DQ445924	DQ445930	N/A	DQ445933	DQ832321	DQ857319	DQ445918
<i>P. misera</i> HBK.		Brazil, RS (PASS)	DQ123317	DQ123420	DQ123034	AY032777	DQ123230	DQ123144	DQ122949
<i>P. morifolia</i> Mast. in Mart.		Brazil, RS (PASS)	DQ123318	AY212314	DQ123035	AY032780	DQ123231	DQ123145	DQ122950
<i>P. organensis</i> Gardn.		Brazil, PR (ACC)	DQ123319	DQ123421	DQ123036	AY032779	DQ123232	DQ123146	DQ122951
<i>P. ornithoura</i> Mast.		Guatemala, Ripalta Cremasca, Colection (MV)	DQ123320	DQ123422	DQ123037	AY210968	DQ123233	DQ123147	DQ122952
<i>P. pohlii</i> Mast. in Mart.		Brazil, MG (ACC)	DQ123321	DQ123423	DQ123038	AY032778	DQ123234	DQ123148	DQ122953
<i>P. podlechii</i> Skrabal & Weigend		Peru, Ripalta Cremasca, Colection (MC)	N/A	DQ123403	DQ123013	DQ123483	DQ123210	N/A	N/A
<i>P. punctata</i> L.	Peru, Ripalta Cremasca, Colection (MV)	DQ123322	DQ123424	DQ123039	AY210969	DQ123235	N/A	DQ122954	
<i>P. rufa</i> Feuillet	French Guiana, Ripalta Cremasca, Colection (MV)	DQ123323	AY212315	DQ123041	AY210971	DQ123236	DQ123149	DQ122955	
<i>P. sexflora</i> Juss.	Dominican Republic, Ripalta Cremasca, Colection (MV)	DQ123324	DQ123426	DQ123042	AY210974	DQ123237	DQ123150	DQ122956	

	<i>P. suberosa</i> L.	Brazil, RS (PASS)	DQ123325	DQ123427	DQ123043	AY032774	DQ123238	DQ123151	DQ122957
	<i>P. talamancensis</i> Killip	Costa Rica, Ripalta Cremasca, Colection (MV)	DQ123326	DQ123428	DQ123044	AY210976	DQ123239	DQ123152	DQ122958
	<i>P. tenuiloba</i> Englem.	GenBank numbers	N/A	N/A	AY632744	AY632744	N/A	N/A	N/A
	<i>P. tricuspis</i> Mast. in Mart.	Brazil, SP (MCD)	DQ123327	DQ123429	DQ123045	AY102396	DQ123240	DQ123153	DQ122959
	<i>P. trifasciata</i> Lemaire	Brazil, MG (NFM)	DQ123328	DQ123430	DQ123046	AY210980	N/A	N/A	N/A
	<i>P. truncata</i> Regel	Brazil, SC (ACC)	N/A	DQ123431	DQ123047	AY102390	N/A	N/A	N/A
	<i>P. vespertilio</i> L.	Brazil, PA (LCB)	DQ123329	DQ123432	DQ123048	DQ123495	N/A	N/A	N/A
	<i>P. xiikzodz</i> MacDougal	Italy, Ripalta Cremasca, Colection (MV)	DQ123330	DQ123433	DQ123049	AY210975	DQ123241	DQ123154	DQ122960
	<i>P. lancetillensis</i> MacDougal & Meerman	French Guiana, Ripalta Cremasca, Colection (MV)	DQ123331	AY212312	DQ123050	AY210963	DQ123242	DQ123155	DQ122961
	<i>P. microstipula</i> Gilbert & MacDougal	Mexico, Ripalta Cremasca, Colection (MV)	DQ123332	DQ123434	DQ123051	AY210966	DQ123243	DQ123156	DQ122962
	<i>P. murucuja</i> L.	Blois-France-Greenhouse (RY)	DQ123345	DQ123442	DQ123064	DQ123501	DQ123255	DQ123168	DQ122974
	<i>P. tulae</i> Urban	Puerto Rico, Ripalta Cremasca, Colection (MV)	DQ123346	DQ123443	DQ123065	AY102392	DQ123256	DQ123169	DQ122975
	<i>P. cupraea</i> L.	Bahamas, Ripalta Cremasca, Colection (MV)	DQ123378	DQ123459	DQ123102	DQ123513	DQ123274	DQ123186	DQ122993
	<i>P. tacsonioides</i> Griseb.	Blois-France-Greenhouse (RY)	DQ123379	DQ123461	DQ123103	DQ123514	DQ123275	DQ123187	DQ122995
	<i>P. sanguinolenta</i> Mast.	Ecology & Evolutionary Biology Conservatory, Univ. Connecticut (RY)	N/A	DQ123462	DQ123104	DQ123515	DQ123276	DQ123188	DQ122996
<i>Passiflora</i>	<i>P. racemosa</i> Brot.	Brazil, RJ (FCN)	DQ123311	DQ123414	DQ123028	DQ123492	DQ123225	DQ123139	DQ122945
	<i>P. speciosa</i> Gardn.	Brazil, MS (ACC)	DQ123334	AY212293	DQ123052	AY102402	DQ123244	DQ123157	DQ122963
	<i>P. vitifolia</i> HBK.	Colombia, Ripalta Cremasca, Colection (MV)	DQ123335	DQ123436	DQ123053	AY210977	DQ123245	DQ123158	DQ122964
	<i>P. clathrata</i> Mast.	Brazil, MG (FCN)	DQ123336	DQ123437	DQ123054	DQ123496	DQ123246	DQ123159	DQ122965
	<i>P. foetida</i> L.	Brazil, PE (NFM)	DQ123337	AY212291	DQ123055	AY032763	DQ123247	DQ123160	DQ122966
	<i>P. palmeri</i> var. <i>sublanceolata</i> Killip	Italy, Ripalta Cremasca, Colection (MV)	DQ123338	DQ123438	DQ123056	DQ123497	DQ123248	DQ123161	DQ122967
	<i>P. campanulata</i> Mast.	Brazil, PR (ACC)	DQ123339	AY212317	DQ123057	AY032760	DQ123249	DQ123162	DQ122968
	<i>P. clathrata</i> Mast.	Brazil, MG (FCN)	DQ123336	DQ123437	DQ123054	DQ123496	N/A	DQ123246	DQ123159
	<i>P. setulosa</i> Killip	Brazil, PR (ACC)	DQ123340	AY212297	DQ123058	AY032761	DQ123250	DQ123163	DQ122969
	<i>P. villosa</i> Vell.	Brazil, MG (ACC)	DQ123341	AY212308	DQ123059	AY102403	DQ123251	DQ123164	DQ122970
	<i>P. antioquiensis</i> Karst.	Italy, Ripalta Cremasca, Colection (MV)	DQ123342	DQ123439	DQ123060	DQ123498	DQ123252	DQ123165	DQ122971
	<i>P. trisecta</i> Mast.	Blois-France-Greenhouse (RY)	DQ123343	DQ123440	DQ123061	DQ123499	DQ123253	DQ123166	DQ122972
	<i>P. manicata</i> (Juss.) Pers.	Blois-France-Greenhouse (RY)	DQ123344	DQ123441	DQ123062	DQ123500	DQ123254	DQ123167	DQ122973
	<i>P. actinia</i> Hook	Brazil, RS (PASS)	DQ123347	AY212301	DQ123065	AY032767	DQ123257	DQ123170	DQ122976
	<i>P. alata</i> Curtis	Brazil, RS (PASS)	DQ123348	AY212323	DQ123067	AY032765	DQ123258	DQ123171	DQ122977
	<i>P. ambigua</i> Hemsl.	Brazil, MT (LCB)	DQ123349	DQ123444	DQ123068	DQ123503	DQ123259	DQ123172	DQ122978
	<i>P. amethystina</i> Mikan	Brazil, MG (MCD)	N/A	AY212323	DQ123069	AY102397	N/A	N/A	N/A
<i>P. caerulea</i> L.	Brazil, RS (PASS)	DQ123350	AY212316	DQ123070	AY032772	DQ123260	DQ123173	DQ122979	
<i>P. cincinnata</i> Mast.	Brazil, MS (ACC)	DQ123351	AY212294	DQ123071	AY102400	DQ123261	DQ123174	DQ122980	

<i>P. edmundoi</i> Sacco	Brazil, BA (NFM)	DQ123352	AY212302	DQ123072	AY102399	DQ123262	DQ123175	DQ122981
<i>P. edulis</i> Sims	Brazil, RS (PASS)	DQ123353	AY212303	DQ123073	AY032769	DQ123263	DQ123176	DQ122982
<i>P. eichleriana</i> Mast.	Brazil, RS (PASS)	DQ123354	AY212304	DQ123074	AY102388	N/A	N/A	N/A
<i>P. elegans</i> Mast.	Brazil, RS (PASS)	DQ123355	AY212295	DQ123075	AY032766	DQ123264	DQ123177	DQ122983
<i>P. exura</i>	Italy, Ripalta Cremasca, Colection (MV)	DQ123356	DQ123445	DQ123076	DQ123504	N/A	N/A	N/A
<i>P. gabrielliana</i> sp. new	French Guiana, Ripalta Cremasca, Colection (MV)	DQ123357	AY212319	DQ123077	AY210960	N/A	N/A	N/A
<i>P. galbana</i> Mast.	Brazil, PE (NFM)	DQ123358	DQ123446	DQ123078	AY032770	DQ123265	DQ123178	DQ122984
<i>P. gardneri</i> Mast.	GenBank numbers	DQ445926	DQ445932	N/A	DQ445935	N/A	N/A	N/A
<i>P. garkey</i> Mast.	French Guiana, Ripalta Cremasca, Colection (MV)	DQ123359	AY212320	DQ123079	AY210961	N/A	N/A	N/A
<i>P. incarnata</i> L.	Brazil, SP (BGJ)	DQ123360	AY212306	DQ123080	AY032768	DQ123266	DQ123179	DQ122985
<i>P. ischnoclada</i>	Brazil, PA (LCB)	N/A	DQ123447	DQ123081	DQ123505	N/A	N/A	N/A
<i>P. jilekii</i> Wawra	Brazil, SC (ACC)	DQ123361	AY212318	DQ123082	AY102387	DQ123267	DQ123180	DQ122986
<i>P. kermesina</i> Link & Otto	Brazil, SP (BGJ)	N/A	DQ123448	DQ123083	AY032762	N/A	N/A	N/A
<i>P. maliformis</i> L.	Dominica, Ripalta Cremasca, Colection (MV)	DQ123362	AY212321	DQ123084	AY210964	DQ123268	DQ123181	DQ122987
<i>P. miersii</i> Mast. in Mart.	Brazil, SP (PASS)	DQ123363	DQ123449	DQ123085	AY102395	DQ123269	DQ123182	DQ122988
<i>P. mucronata</i> Lam.	Brazil, PE (MG)	N/A	DQ123450	DQ123086	DQ123506	N/A	N/A	N/A
<i>P. nitida</i> Kunth	Brazil, MT (LCB)	DQ123364	DQ123451	DQ123087	N/A	N/A	N/A	N/A
<i>P. odontophylla</i> Harms ex Glaz.	Brazil, MG (FCN)	DQ123365	DQ123452	DQ123088	DQ123507	N/A	N/A	N/A
<i>P. quadrangularis</i> L.	Brazil, SP (BGJ)	DQ123366	AY212322	DQ123089	AY032764	N/A	N/A	N/A
<i>P. recurva</i> Mast in Mart.	Brazil, MG (ACC)	DQ123367	AY212310	DQ123090	AY102391	N/A	N/A	N/A
<i>P. riparia</i> Mart.	Brazil, PA (LCB)	DQ123368	DQ123453	DQ123091	DQ123508	N/A	N/A	N/A
<i>P. serratifolia</i> L.	Surinam, Ripalta Cremasca, Colection (MV)	DQ123369	DQ123454	DQ123092	AY210973	N/A	N/A	N/A
<i>P. serratodigitata</i> L.	Martinique, Ripalta Cremasca, Colection (MV)	DQ123370	DQ123455	DQ123093	AY210972	N/A	N/A	N/A
<i>P. setacea</i> DC.	Brazil, SP (BGJ)	DQ123371	AY212296	DQ123094	AY102398	N/A	N/A	N/A
<i>P. sidaefolia</i> M. Roemer	Brazil, MG (MCD)	DQ123372	AY212298	DQ123095	AY102394	DQ123270	DQ123183	DQ122989
<i>P. sprucei</i> Mast.	Italy, Ripalta Cremasca, Colection (MV)	DQ123373	DQ123456	DQ123096	DQ123509	DQ123271	DQ123184	DQ122990
<i>P. trintae</i> Sacco	Brazil, BA (TSN)	DQ123375	DQ123457	DQ123098	DQ123510	N/A	N/A	N/A
<i>P. urubicensis</i> Cervi	Brazil, SC (ACC)	N/A	AY212300	DQ123099	AY102393	N/A	N/A	N/A
<i>P. watsoniana</i> Mast.	Brazil, BA (AS)	DQ123376	DQ123458	DQ123100	DQ123511	N/A	N/A	N/A
<i>P. mathewsii</i> (Mast.) Killip	Blois-France-Greenhouse (RY)	DQ123380	DQ123463	DQ123105	DQ123516	DQ123277	DQ123190	DQ122994
<i>P. mixta</i> L. f.	Blois-France-Greenhouse (RY)	DQ123381	DQ123464	DQ123106	DQ123517	DQ123278	DQ123191	DQ122997
<i>P. tripartita</i> var. <i>mollissima</i> (Juss.) Poir.	Blois-France-Greenhouse (RY)	DQ123382	DQ123465	DQ123107	DQ123518	DQ123279	DQ123192	DQ122998
<i>P. trifoliata</i> Cav.	Peru, Kew Botanical Garden (MC)	DQ123383	DQ123466	DQ123108	DQ123519	DQ123280	DQ123193	N/A
<i>P. luetzelburgii</i> Harms	Brazil, BA (TSN)	DQ123384	DQ123467	DQ123109	DQ123520	DQ123281	DQ123194	DQ122999
<i>P. reflexiflora</i> Cav.	Ecuador, Ripalta Cremasca, Colection (MV)	DQ123386	DQ123469	DQ123111	AY210970	DQ123283	DQ123195	DQ123001

	<i>P. umbilicata</i> (Griseb.) Harms	Blois-France-Greenhouse (RY)	DQ123387	DQ123470	DQ123112	DQ123521	DQ123284	N/A	DQ123002
<i>Deidamioides</i>	<i>P. deidamioides</i> Harms	Brazil, SP (MCD)	DQ445925	DQ445931	DQ445920	DQ445934	DQ832322	DQ857320	DQ445919
	<i>P. cirrhiflora</i> Juss.	Italy, Ripalta Cremasca, Colection (MV)	DQ123377	DQ123459	DQ123101	DQ123512	DQ123273	DQ123185	DQ122992
	<i>P. ovalis</i>	Brazil, BA (TSN)	DQ123401	AY216662	DQ123122	AY210978	DQ123295	DQ123207	DQ123010
<i>Tryphostemmatoides</i>	<i>P. tryphostemmatoides</i> Harms	Blois-France-Greenhouse (RY)	DQ123388	DQ123471	DQ123113	DQ123522	N/A	DQ123285	DQ123196
<i>Outgroups</i>	<i>Adenia isoalensis</i>	Kew Botanical Garden (MC)	DQ123389	DQ123472	DQ123115	N/A	DQ123286	DQ123198	DQ123004
	<i>Adenia keramanthus</i>	Kew Botanical Garden (MC)	DQ123390	DQ123473	DQ123114	AY102405	DQ123287	DQ123197	DQ123005
	<i>Ancistrothyrsus</i> sp.	Peru, Kew Botanical Garden (MC)	DQ123391	DQ123474	N/A	DQ123523	N/A	N/A	N/A
	<i>Deidamia</i> sp.	Kew Botanical Garden (MC)	DQ123394	DQ123477	DQ123117	DQ123526	DQ123289	DQ123201	DQ123007
	<i>Dilkea cf johannesii</i> Barb. Rodr.	Peru, Kew Botanical Garden (MC)	DQ123399	DQ123478	DQ123118	DQ123527	DQ123290	DQ123202	DQ123008
	<i>Mitostemma brevifilis</i>	Brazil, MS (ACC)	DQ123400	AY212309	DQ123119	AY102386	DQ123291	DQ123203	DQ123009
	<i>Barteria</i> sp.	Kew Botanical Garden (MC)	DQ123393	DQ123476	DQ123116	DQ123525	N/A	DQ123200	N/A
	<i>Paropsia brazzeana</i>	Africa, Kew Botanical Garden (MC)	DQ123396	DQ123480	DQ123120	DQ123528	DQ123292	DQ123205	N/A
	<i>Paropsia madagascariensis</i>	Kew Botanical Garden (MC)	AF206802	AY216663	DQ123121	AY102404	DQ123293	DQ123206	N/A
	<i>Malesherbia linearifolia</i>	Chile (KG, SK)	DQ123402	DQ123482	DQ123123	DQ123529	DQ123294	DQ123208	DQ123011
<i>Turnera subulata</i>	Brazil, BA (CB)	DQ123398	N/A	DQ123124	DQ123530	DQ123296	DQ123209	DQ123012	