



Taxonomy and evolutionary relationships of *Passiflora* subg. *Decaloba* supersect. *Decaloba* sect. *Xerogona* (Passifloraceae): contributions of palynological, morphological and molecular studies

Michaele Alvim Milward-de-Azevedo^{1,4}, Loreta Brandão de Freitas² and Luiza Sumiko Kinoshita³

Received: April 29, 2013. Accepted: November 25, 2013

ABSTRACT

Passiflora subg. *Decaloba* supersect. *Decaloba* sect. *Xerogona* (Passifloraceae) is a tropical and subtropical group comprising 14 species that occur in tropical biomes throughout Latin America, including the Atlantic Forest. The section *Xerogona* comprises herbaceous vines characterized by a lack of petiole glands on their leaves, of bracts and of ocelli on their leaf blades, as well as by their capsular fruits. We analyzed the phylogeny on the basis of morphological characters (including pollen characters) and molecular data. The inferred phylogeny was used in order to characterize, circumscribe and delimit the section and the species. We examined the phylogenetic relationships among the species, evaluating the circumscription of the section on the basis of the *trnL-trnF* intergenic spacer region of chloroplast DNA and the internal transcribed spacer region of nuclear ribosomal DNA. We constructed phylogenetic trees on the basis of the morphological and molecular data. We found that *P.* subg. *Decaloba* supersect. *Decaloba* sect. *Xerogona* appears to be monophyletic only in the molecular analyses. The phylogenetic analyses performed here also indicated that *P.* subg. *Decaloba* is monophyletic.

Key words: *Passiflora*, *Decaloba*, *Xerogona*, phylogenetics

Introduction

Passiflora L. subg. *Decaloba* (DC.) Reich. supersect. *Decaloba* (DC.) MacDougal & Feuillet sect. *Xerogona* (Raf.) Killip (Passifloraceae) is a tropical and subtropical section comprising 14 species (Feuillet & MacDougal 2003) that occur throughout Latin America, principally in Central America but also in other tropical regions and in the Atlantic Forest Biome. The section is characterized by the absence of ocelli on the leaf blades, by the absence of bracts, and by capsular fruits. The species are herbaceous vines with linear-subulate stipules, small flowers (< 4 cm diameter) with one or two series of corolla filaments, a folded operculum, and transversally sulcate seeds.

Presting (1965) initiated palynological studies of Passifloraceae and proposed a phylogeny for the family based on the pollen characters and on the classification of Killip (1938). Milward-de-Azevedo *et al.* (2004, 2010) presented a palynotaxonomic study of the species of *Passiflora* subg. *Decaloba* that contributed to their characterization and cir-

cumscription, as well as to delimiting the genus *Passiflora* in accordance with the classification of MacDougal & Feuillet (2004); pollen characters were found to be informative for the phylogeny of the group in both works. A cladistic analysis of morphological characters, undertaken by Milward-de-Azevedo (2007) to revise *P.* subg. *Decaloba* in Brazil, did not, however, support the classification proposed by Feuillet & MacDougal (2003), because the authors found that *P.* subg. *Decaloba* supersect. *Decaloba* included species from other supersections and therefore was not monophyletic. The evolutionary relationships of the species of *P.* subg. *Decaloba* supersect. *Decaloba* sect. *Xerogona* (*P. capsularis* L., *P. cervii* Milward-de-Azevedo and *P. rubra* L.) have not been well resolved due to their great morphological similarities and to the difficulties encountered in identifying them in their vegetative states, and the section is therefore considered a polytomy (Milward-de-Azevedo 2007).

Recent phylogenetic studies of *Passiflora* (Muschner *et al.* 2003, Yockteng & Nadot 2004, Souza-Chies *et al.* 2005, Hansen *et al.* 2006, 2007) have not supported the

¹ Universidade Federal Rural do Rio de Janeiro, Instituto de Três Rios, Departamento de Ciências Administrativas e do Ambiente, Três Rios, RJ, Brazil

² Universidade Federal do Rio Grande do Sul, Departamento de Genética, Porto Alegre, RS, Brazil

³ Universidade Estadual de Campinas, Instituto de Biologia, Departamento de Biologia Vegetal, Campinas, SP, Brazil

⁴ Author for correspondence: michaelemilward@gmail.com

supersectional, sectional, and series classifications within *P.* subg. *Decaloba* proposed by Reichenbach (1828). Some of the species within this group are associated with each other in complexes in which individual species are difficult to identify because of the blurring of interspecific distinctions and the existence of individuals with intermediate morphological characteristics. In view of these facts, we performed phylogenetic analyses based on morphological and molecular data in order to help clarify the taxonomy of *P.* subg. *Decaloba* supersect. *Decaloba* sect. *Xerogona*, as well as evaluating the circumscription of this section.

Material and methods

We selected 43 vegetative and reproductive morphological characters (Tab. 1) to be scored in a data matrix for computer analysis (Tab. 2). The selection of morphological characters concentrated on those considered relevant to the taxonomy of species of *Passiflora* subg. *Decaloba* supersect. *Decaloba* sect. *Xerogona*. Autapomorphies were not utilized, because they were not informative in establishing interspecific relationships and groupings. The characters were given equal weights and were encoded.

We performed molecular analyses of the DNA sequences of 20 species of *Passiflora* subg. *Decaloba*, nine of them belonging to *P.* subg. *Decaloba* supersect. *Decaloba* sect. *Xerogona*. The samples were derived from collections of young leaves dehydrated in silica gel, available in the Molecular Evolution Laboratory at the Federal University of Rio Grande do Sul, or from herbarium material. The samples were pulverized in liquid nitrogen and stored at -20°C . Voucher specimens were deposited in the Herbarium of the (São Paulo) State University at Campinas (code, UEC) and the Herbarium of the Federal University of Espírito Santo (code, VIES).

We extracted DNA using the method described by Roy *et al.* (1992). The extraction products were confirmed by electrophoresis on a 1% agarose gel, stained with ethidium bromide, and visualized using an ultraviolet transilluminator. Each sample was quantified by comparisons with markers of molecular weight and concentration (Low DNA Mass Ladder; Invitrogen, Carlsbad, CA, USA).

To amplify the samples, we performed polymerase chain reaction in automated thermal cyclers (PTC-200; MJ Research Inc., Foster City, California, USA—Mastercycler; Eppendorf, Hauppauge, NY, USA), using universal (*c* and *f*) primers for the *trnL-trnF* intergenic spacer region of chloroplast DNA (Taberlet *et al.* 1991) and internal transcribed spacer (ITS) primers 92 and 75, as described by Desfeux & Lejeune (1996). Before sequencing, the samples were purified using the protocol described by Dunn & Blattner (1987). The sequencing of the DNA fragments was performed in an automated sequencer (MegaBACE 1000; Amersham Biosciences/GE Healthcare, Piscataway, NJ, USA) in accordance with the manufacturer instructions, and the DYEnamic™

Table 1. Key to the morphological characters used in the cladistic analyses of *Passiflora* subg. *Decaloba* supersect. *Decaloba* sect. *Xerogona* and their respective states.

Habit	
1. Vine:	(0) woody; (1) herbaceous
2. Indumentum of the vegetative organs:	(0) absent; (1) present
Stem	
3. Consistency:	(0) suberous; (1) not suberous
4. Shape:	(0) cylindrical to triangular; (1) semi-cylindrical, flattened or complanate
5. Verrucae:	(0) absent; (1) present
Stipules	
6. Shape:	(0) ovate-auriculate; (1) linear-subulate to falciform
Leaves	
7. Glands on the petiole:	(0) present; (1) absent
8. Shape of the leaf blade:	(0) elliptical, ovate or lanceolate; (1) 2-3 lobate
9. Leaf margin:	(0) entire; (1) dentate
10. Ocelli:	(0) absent; (1) present
Inflorescence	
11. Type of inflorescence:	(0) racemose; (1) monad; (2) dyad; (3) cyme
Bracts	
12. Presence of bracts:	(0) present; (1) absent
13. Shape:	(0) lanceolate; (1) linear-subulate or triangular-subulate; (2) ovate
14. Placement:	(0) verticillate; (1) alternate
Flowers	
15. Hypanthium:	(0) campanulate; (1) patelliform
16. Apices of the sepals:	(0) obtuse; (1) acute
17. Apices of the petals:	(0) obtuse; (1) acute; (2) truncate
18. Number of series of corolla filaments:	(0) ≥ 3 ; (1) 2; (2) 1
19. Shapes of the filaments of the external series and/or of the uniseriate corolla filaments:	(0) liguliform; (1) filiform
20. Apices of the external series of corolla filaments:	(0) acute; (1) dilated to capitate; (2) dolabriform
21. Presence of a membrane uniting the external corolla filaments:	(0) absent; (1) present
22. Shapes of the internal series of corolla filaments:	(0) liguliform; (1) filiform; (2) capillary
23. Apices of the internal series of corolla filaments:	(0) acute; (1) dilated to capitate; (2) 2-3 lobate
24. Apex of the operculum:	(0) denticulate; (1) fimbriate
25. Shape of the limen:	(0) annular; (1) cupuliform; (2) lobulate; (3) cuculiform
26. Length of the androgynophore column:	(0) ≥ 1.5 cm; (1) 0.5-1.5 cm (2) < 0.5 cm
27. Ovary shape:	(0) oval; (1) globose to subglobose; (2) elliptical to fusiform
28. Indumentum of the ovary:	(0) absent; (1) present
Pollen	
29. Pollen grain shape:	(0) spheroidal; (1) prolate spheroidal; (2) oblate spheroidal; (3) subprolate; (4) suboblate; (5) prolate
30. Number of colpi:	(0) 3; (1) 6; (2) 12
31. Aperture type:	(0) colpate; (1) colpporate
32. Number of mesocolpia:	(0) 3; (1) 6
33. Operculum:	(0) present; (1) absent
34. Number of pseudopercula:	(0) 0; (1) 3; (2) 6
35. Secondary operculum:	(0) absent; (1) present
36. Wall:	(0) straight; (1) sinuous
37. Morphological pattern of the lumens:	(0) microreticulate ($< 1 \mu\text{m}$); (1) reticulate (1-3.5 μm); (2) reticulate ($\geq 3.5 \mu\text{m}$)
38. Bacula within the lumina of the reticula:	(0) absent; (1) present
Fruits	
39. Type:	(0) berry; (1) loculicidal capsule
40. Shape:	(0) globose to subglobose; (1) ovate; (2) fusiform
41. Indumentum:	(0) absent; (1) present
Seeds	
42. Shape:	(0) ovate; (1) obovate; (2) elliptical
43. Testa:	(0) foveolate; (1) reticulate; (2) transversally sulcate

Table 2. Character matrix for the cladistic analysis of *Passiflora* subg. *Decaloba* supersect. *Decaloba* sect. *Xerogona*, showing the states of the characters.*

Species	1										2										3										4																			
	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0
<i>P. brevipes</i>	1	1	1	1	0	1	1	0	0	0	1	1	NANA	1	1	0	1	1	1	1	1	2	0	1	1	2	2	1	3	1	1	0	1	1	0	0	1	0	1	2	1	1	2							
<i>P. capsularis</i>	1	1	1	0	0	1	1	1	0	0	1	1	NANA	1	1	1	2	1	0	1	1	NA	0	0	0	1	2	1	1	2	0	0	1	1	1	1	2	1	1	2	1	2	2							
<i>P. cervii</i>	1	1	1	0	0	1	1	1	0	0	1	1	NANA	1	1	1	2	0	1	1	1	NANA	0	1	1	2	1	2	2	1	0	1	1	1	1	2	1	1	2	1	2	2								
<i>P. citrine</i>	1	1	1	0	0	1	1	1	0	0	1	1	NANA	0	1	0	2	1	1	1	0	NANA	1	?	1	2	1	1	1	1	0	0	1	0	0	1	0	1	2	1	1	2								
<i>P. cobanensis</i>	1	1	1	0	0	1	1	0	0	0	1	1	NANA	1	1	0	1	1	1	1	1	2	0	1	1	2	2	1	3	1	1	0	0	1	0	0	1	0	1	2	1	1	2							
<i>P. conzattiana</i>	1	1	1	0	0	1	1	1	0	0	1	1	NANA	1	1	0	2	1	1	1	1	NANA	1	0	2	2	1	5	1	1	0	0	1	0	0	2	0	1	2	1	1	2								
<i>P. costaricensis</i>	1	1	1	0	0	1	1	1	0	0	1	1	NANA	1	?	?	?	?	?	?	?	?	?	?	?	1	?	?	3	1	1	0	0	1	0	0	2	1	1	2	1	2	2							
<i>P. escobariana</i>	0	0	1	0	0	1	1	1	0	0	1	1	NANA	1	?	?	1	1	0	1	1	NANA	1	0	1	2	1	?	?	?	?	?	?	?	?	?	?	?	?	1	2	0								
<i>P. goniosperma</i>	1	1	1	0	0	1	1	1	0	0	1	1	NANA	1	1	0	2	1	0	1	1	NANA	?	?	2	2	1	1	1	1	0	0	1	0	1	2	1	1	2	1	?	?								
<i>P. pusilla</i>	1	1	1	0	0	1	1	1	0	0	1	1	NANA	1	?	?	1	1	0	1	1	2	?	0	0	2	2	1	1	1	1	0	0	1	0	1	2	1	1	2	1	?	?							
<i>P. quinquangularis</i>	1	1	1	0	0	1	1	1	0	0	1	1	NANA	1	1	1	1	1	0	1	1	2	1	1	1	1	2	1	1	2	1	0	0	1	1	0	2	1	1	?	?	?	?							
<i>P. rovirosae</i>	1	0	1	0	0	1	1	1	0	0	3	1	NANA	1	1	1	1	0	0	0	2	2	1	1	1	2	1	1	1	1	1	0	0	0	1	2	1	1	2	1	1	2								
<i>P. rubra</i>	1	1	1	0	0	1	1	1	0	0	1	1	NANA	1	1	1	2	1	0	1	0	NANA	0	1	1	1	1	1	2	1	0	1	1	1	1	2	1	1	1	1	2	2								
<i>P. sanguinolenta</i>	1	1	1	0	0	1	1	1	0	0	1	1	NANA	1	1	?	?	?	?	?	?	?	?	?	?	0	2	1	1	2	1	0	0	1	1	1	2	1	1	2	1	2	2							
<i>P. tenella</i>	1	0	1	0	0	1	1	1	0	0	1	1	NANA	1	?	?	1	1	0	1	1	2	1	0	?	2	2	0	?	?	?	?	?	?	?	?	?	?	?	?	0	?	?							
<i>P. auriculata</i>	1	1	1	0	0	1	0	0	0	1	1	0	1	0	0	1	1	1	1	0	1	1	2	0	0	2	2	1	3	1	1	0	1	0	0	1	1	0	0	0	1	1	2							
<i>P. misera</i>	1	1	1	1	1	1	1	1	0	1	1	0	1	1	1	1	1	1	1	0	1	1	2	0	1	1	2	0	1	2	0	1	1	1	0	0	1	0	0	0	0	1	2							
<i>P. morifolia</i>	1	1	1	0	0	0	0	1	1	0	1	0	1	1	1	1	1	2	1	0	0	NANA	0	0	1	1	1	3	2	1	0	1	0	1	1	2	1	0	0	1	2	0								
<i>P. pohlii</i>	1	1	1	0	0	1	1	1	0	1	1	0	1	0	1	0	1	1	0	0	0	1	1	1	2	1	2	0	3	1	1	1	0	NA	0	0	1	0	0	0	0	2	2							
<i>P. porophylla</i>	1	1	1	0	0	1	1	1	0	1	1	0	1	1	1	1	0	2	0	2	1	NANA	0	0	1	1	1	1	1	1	1	0	NA	0	0	1	0	0	0	0	2	2								
<i>P. suberosa</i> ssp. <i>litoralis</i>	1	1	0	0	0	1	0	A	0	A	A	0	1	1	1	0	NA	1	1	0	0	1	1	1	0	B	1	0	3	2	0	1	1	1	0	0	1	1	0	0	0	1	0							
<i>P. transversalis</i>	1	1	1	1	0	1	1	1	0	1	1	0	1	1	1	1	1	1	1	0	1	1	2	0	2	1	2	1	1	2	1	0	1	0	1	0	1	0	0	0	1	B	2							
<i>P. tricuspis</i>	1	1	1	1	1	1	1	1	0	1	1	0	1	1	1	1	1	1	1	1	0	1	2	0	0	1	2	0	1	2	1	0	1	0	1	1	2	1	0	0	0	2	2							
<i>P. truncate</i>	1	1	1	0	0	1	0	1	0	1	C	0	1	1	1	0	1	1	1	1	0	1	1	0	0	1	2	1	1	1	1	0	1	0	0	0	0	0	0	0	1	2	2							
<i>P. vespertilio</i>	1	1	1	1	0	1	1	1	0	1	1	0	1	1	1	1	1	1	1	1	1	2	0	1	0	1	2	0	1	2	1	0	1	0	1	1	2	1	0	0	0	2	2							

? – no data; NA – data not applicable.

*Multistate characters are indicated by individual upper-case letters: A – (0,1); B – (1,2); C – (1,2,3).

ET terminator sequencing premix kit, with terminal fluorescent markers (Amersham Biosciences/GE Healthcare).

The data for phylogenetic analyses were organized based on morphological data and the ITS and *trnL-trnF* DNA sequences. We also included ITS and *trnL-trnF* sequences deposited in GenBank (Tab. 3). The molecular analyses used the sequences of *Passiflora edulis* Sims, *P. haematostigma* Mart. ex Mast. and *P. ovalis* Vell. ex M. Roem. as external groups. Sequences were visualized with the software Chromas, version 2.0 (Technelysium Pty Ltd., Helensvale, Australia), and alignments were performed with the program MEGA, version 5.01 (Tamura *et al.* 2011), visual adjustments being made as necessary.

Morphological and molecular phylogenetic analyses were conducted using the parsimony method in the PAUP*

program, version 4.0 for Windows (Swofford, 2002). We performed heuristic searches, with the stepwise addition of taxa. For branch rearrangements, we used tree bisection and reconnection. One thousand bootstrap replications were made for each analysis. We performed morphological and molecular phylogenetic analyses with Bayesian interference using the MrBayes program, version 3.0b4 (Ronquist & Huelsenbeck 2003), employing the evolutive models estimated in the jModeltest program, version 0.1.1 (Posada 2008). The models were obtained with the Bayesian information criterion. WE conducted four Markov chain Monte Carlo runs, with 10 million generations in each analysis. The trees were sampled after every 100 generations, and the numbers of generations necessary for each parameter to become stable (i.e., to complete the burn-in period) were

Table 3. Accession numbers of the sequences obtained from GenBank.

Species	ITS	<i>trnL-trnF</i>
<i>Passiflora auriculata</i>	-	HQ901003.1
<i>P. misera</i>	EU258409.1	AY032777.1
<i>P. morifolia</i>	EU258323.1	AY032780.1
<i>P. pohlii</i>	EU258325.1	AY032778.1
<i>P. porophylla</i>	EU258425.1	AY032779.1
<i>P. suberosa</i> ssp. <i>litoralis</i>	-	AY032774.1
<i>P. tricuspis</i>	EU258459.1	AY102396.1
<i>P. edulis</i>	EU258383.1	AY032769.1
<i>P. haematostigma</i>	EU258407.1	AY032773.1
<i>P. ovalis</i>	EU258369.1	AY210978.1

ITS – (sequences of the) internal transcribed spacer region of nuclear of ribosomal DNA; *trnL-trnF* – (sequences of the) *trnL-trnF* intergenic spacer region of chloroplast DNA.

defined after inspection of the log-likelihood values corresponding to those generations using the MrBayes program, with posterior probability being determined for each node. Combined analyses (of morphological and molecular data) were not within the scope of this paper, because not all species were subject to molecular analyses.

Results

Morphological data

Heuristic searches in PAUP* generated the 14 most parsimonious trees with similar topologies. The analysis was based on a consensus tree of parsimony heuristic search (Fig. 1A). Initially, the clades formed by the external groups and by *Passiflora* subg. *Decaloba* were recognized. The latter comprises two distinct clades, the first comprising *P.* subg. *Decaloba* supersect. *Decaloba* sect. *Xerogona* and the second comprising *P.* subg. *Decaloba* supersect. *Decaloba* sect. *Decaloba* and *P.* subg. *Decaloba* supersect. *Cieca*; and the second comprising *P.* subg. *Decaloba* supersect. *Auriculata* and *P.* subg. *Decaloba* supersect. *Truncata* (Fig. 1). *P.* subg. *Decaloba* supersect. *Decaloba* sect. *Xerogona* appears as monophyletic, although there is no statistical support for that. Morphologically, the most basal clade of the section appears to be composed of a complex of the species *P. capsularis*, *P. cervii* and *P. rubra*, which are quite similar species and difficult to distinguish from each other. The species *P. quinquangularis* and *P. sanguinolenta* did not demonstrate variability among the characters evaluated; nor did they demonstrate a significant quantity of synapomorphies in relation to the other species in the section, although these two species can be differentiated by the colors of their flowers (white and pink, respectively), as well as their distinct geographic distributions (Guatemala-El Salvador and Ecuador, respectively). *Passiflora sanguinolenta* is

the only species of *P.* subg. *Decaloba* that has a floral tube and is adapted to pollination by hummingbirds, and the structures of the filaments of its corolla are organized for this type of pollination (Ulmer & MacDougal, 2004). The clade formed by the species *P. escobariana* and *P. tenella* appears to be a sister group to the species *P. rovirosae*, although the last species is taxonomically distinguishable by the shape of its leaves. *Passiflora tenella* appears inserted within the clade of the section *Xerogona* (because it has a capsule fruit), although it is quite similar to the species of *P.* subg. *Decaloba* supersect. *Decaloba* sect. *Decaloba*. The clade composed of the species *P. brevipes* and *P. cobanensis* showed a reversion of the lobate character of the leaves to entire leaves; these species are morphologically quite similar, and there are no diagnostic characters that definitively separate them.

The consensus tree obtained by Bayesian inference (Fig. 1B) demonstrated a topology similar to that seen in the parsimony tree for *Passiflora* subg. *Decaloba* supersect. *Decaloba* sect. *Xerogona*. In this analysis, the most basal clade of the section is composed of the species *P. capsularis*, *P. cervii*, *P. rubra* and *P. morifolia*, the last being representative of *P.* subg. *Decaloba* supersect. *Bryonioides*. It can be assumed that the phylogenetic relationships between *P. morifolia* and the other species of *P.* subg. *Decaloba* supersect. *Decaloba* sect. *Xerogona* in this clade are supported not only by a uniseriate corolla, by pollen grains with sinuous muri and by the absence of ocelli (all of which are synapomorphies) but also by the fact that all have pollen grains with 3 mesocolpia, 3 pseudopercula and 6 secondary opercula.

In the trees obtained using these two methods of analysis based on morphological characters (Fig. 1), it can be seen that *Passiflora* subg. *Decaloba* supersect. *Decaloba* sect. *Xerogona* is divided into two large groups: a more basal group composed of the species *P. capsularis*, *P. cervii* and *P. rubra*; and another group composed of the remaining species.

Molecular data

Among the species of *Passiflora* subg. *Decaloba* supersect. *Decaloba* sect. *Xerogona*, we sequenced nine for the ITS marker and seven for the *trnL-trnF* marker. Sequences of 10 species representing *P.* subg. *Decaloba* and three species representing the other subgenera used as the external group were obtained from GenBank (Tab. 3) and included in the analyses. The markers were analyzed individually. As was expected, the amplified ITS fragments were 401-468 base pairs in size, while the *trnL-trnF* sequences were 199-210 base pairs.

In all of the phylogenetic analyses undertaken using the ITS and *trnL-trnF* markers, *Passiflora* subg. *Decaloba* was found to be monophyletic, with high statistical support (Fig. 2 and 3), as was *P.* subg. *Decaloba* supersect. *Decaloba* sect. *Xerogona*, albeit with low statistical sup-

Taxonomy and evolutionary relationships of *Passiflora* subg. *Decaloba* supersect. *Decaloba* sect. *Xerogona* (Passifloraceae): contributions of palynological, morphological and molecular studies

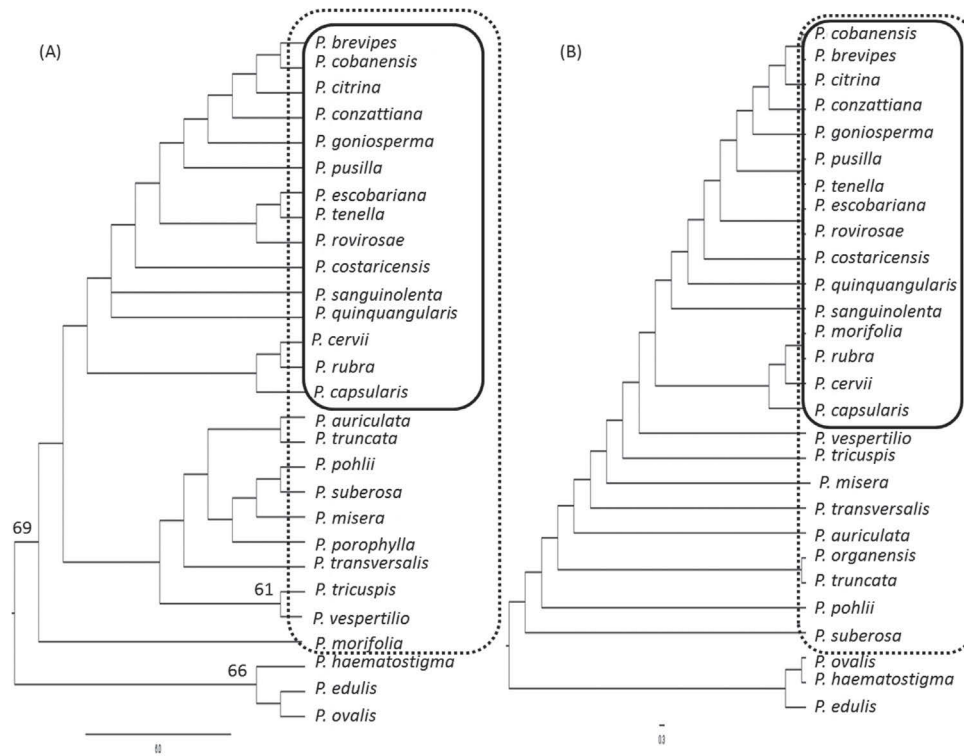


Figure 1. (A) Strict consensus tree of the 14 most parsimonious trees, based on morphological data. Numbers above branches (when higher than 50%) are bootstrap support values based on 1000 replicates. (B) Phylogenetic tree based on morphological data obtained from Bayesian inference.

Continuous line: *Passiflora* subg. *Decaloba* supersect. *Decaloba* sect. *Xerogona*; dotted line: *Passiflora* subg. *Decaloba*.

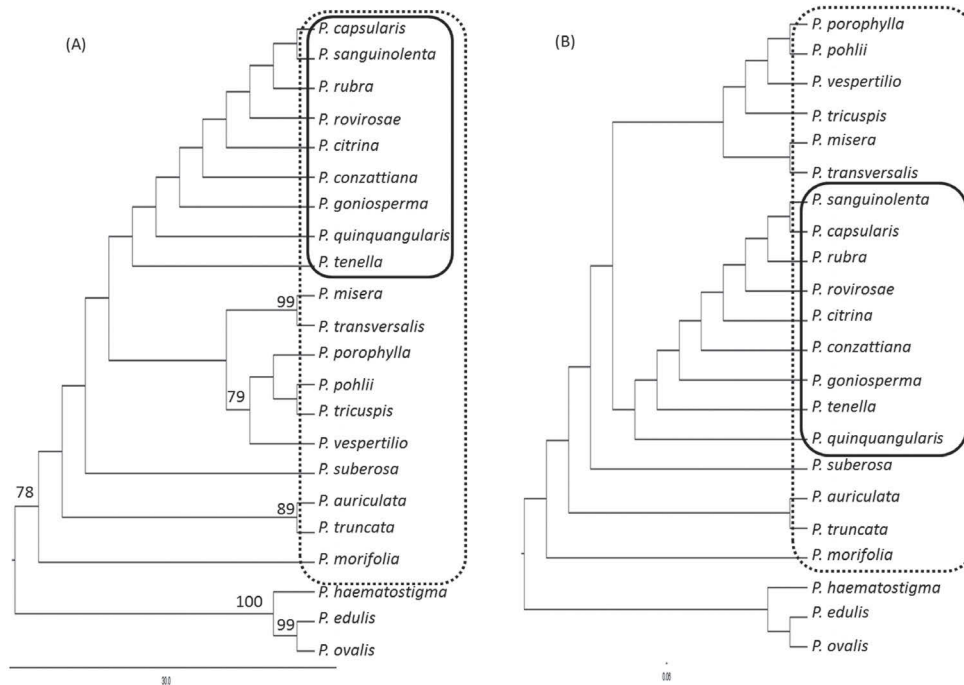


Figure 2. (A) Strict consensus tree of the 75 most parsimonious trees, constructed with the ITS marker. Numbers above branches (when higher than 50%) are bootstrap support values based on 1000 replicates. (B) Phylogenetic tree based on the sequences of the ITS marker obtained by Bayesian inference.

Continuous line: *Passiflora* subg. *Decaloba* supersect. *Decaloba* sect. *Xerogona*; dotted line: *Passiflora* subg. *Decaloba*.

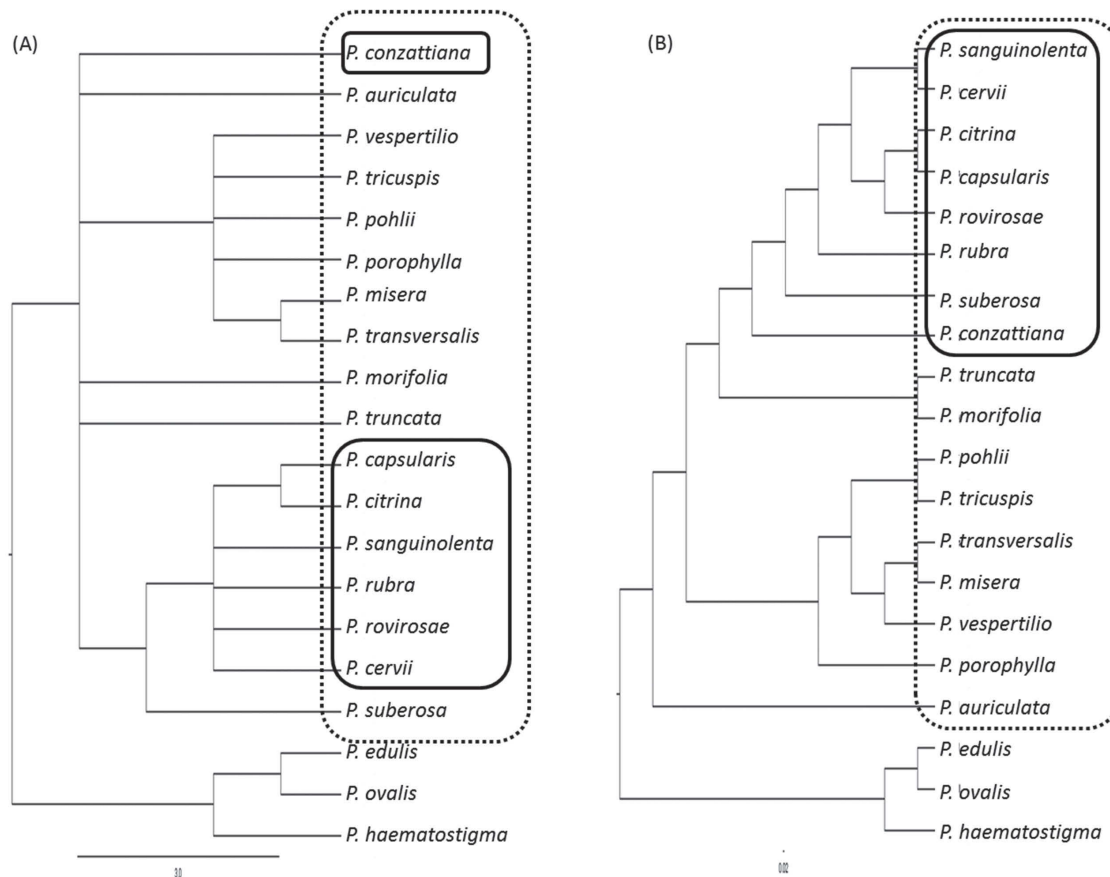


Figure 3. (A) Strict consensus tree of the 69 most parsimonious trees, constructed with the *trnL-trnF* marker. (B) Phylogenetic tree based on the sequences of the *trnL-trnF* region obtained by Bayesian inference.

Continuous line: *Passiflora* subg. *Decaloba* supersect. *Decaloba* sect. *Xerogona*; dotted line: *Passiflora* subg. *Decaloba*.

port. In terms of the ITS marker in the parsimony analysis, *P. tenella*, which is the species morphologically closest to *P.* subg. *Decaloba* supersect. *Decaloba* sect. *Decaloba*, appears at the base of the section (Fig. 2). However, in the analysis of Bayesian inference, *P. quinquangularis*, which is quite similar to and considered by many authors to be a synonym of *P. capsularis*, appeared at the base. These two species (*P. tenella* and *P. quinquangularis*) appeared to be distinct in terms of this marker. *Passiflora capsularis* and *P. sanguinolenta* appeared as closely related species, although their flowers are quite distinct and they have different geographic distributions.

The analyses of *Passiflora* subg. *Decaloba* supersect. *Decaloba* sect. *Xerogona* with the *trnL-trnF* marker did not show it to be monophyletic in the parsimony analyses (Fig. 3A). This marker was informative in the separation of the subgenera but was not capable of revealing the relationships of the supersections and sections within each. The tree obtained by the analysis of Bayesian inference with the *trnL-trnF* marker (Fig. 3B) indicated that the section was monophyletic.

Discussion

The phylogenetic analyses based on morphological characters indicate that *Passiflora morifolia*, placed in *P.* subg. *Decaloba* supersect. *Bryonioides*, belongs to the clade comprising the species *P. capsularis*, *P. cervii* and *P. rubra*, all of which are within *P.* subg. *Decaloba* supersect. *Decaloba* sect. *Xerogona*; the same was observed in the morphological phylogenetic analyses undertaken by Milward-de-Azevedo (2007) for the component taxa of *P.* subg. *Decaloba* occurring in Brazil, due to the synapomorphies demonstrated by these species.

The molecular phylogenetic analyses presented by Muschner *et al.* (2003), Yockteng & Nadot (2004), and Hansen *et al.* (2006) did not support the classifications into supersections, sections, and series. It was seen in the present study that *Passiflora* subg. *Decaloba* is monophyletic, and that *P.* subg. *Decaloba* supersect. *Decaloba* is monophyletic and divided into two sections: *Decaloba* and *Xerogona*. Both of those sections are also monophyletic, corroborating the classifications proposed by Feuillet & MacDougal (2003) for *P.* subg. *Decaloba*; those authors,

however, found that some of the supersections and series did not form monophyletic groups—a finding that was not confirmed for the specimens analyzed in the present study. Zamberlan (2007) determined *P.* subg. *Decaloba* and *P.* subg. *Decaloba* supersect. *Decaloba* to be monophyletic in all of the analyses using ITS markers, as was observed in the present study. However, *P.* subg. *Decaloba* supersect. *Decaloba* displayed two groupings; the first included only species of *P.* subg. *Decaloba* supersect. *Decaloba* sect. *Decaloba*, whereas the second comprised species of *P.* subg. *Decaloba* supersect. *Decaloba* sections *Decaloba* and *Xerogona* and was not monophyletic. In the present work, the sections were considered monophyletic in terms of results of the ITS markers.

In the present study, there were some cases in which our morphological data conflicted with those obtained by phylogenetic analysis, in terms of the divisions into sections within the *Passiflora* subg. *Decaloba* supersect. *Decaloba*, the majority of such analyses have involved only a few species of *P.* subg. *Decaloba* supersect. *Decaloba* sect. *Xerogona*, which would make it more difficult to draw certain distinctions (Muschner *et al.*, 2003; Yockteng & Nadot, 2004; Zamberlan, 2007).

The phylogenetic analyses developed here, using vegetative, floral, palynological, and fruit morphological data, indicated that *Passiflora* subg. *Decaloba* supersect. *Decaloba* sect. *Xerogona* is not monophyletic, because it was found to include *P. morifolia*, which belongs to *P.* subg. *Decaloba* supersect. *Bryonioides*. Our data do not support the classification proposed by Feuillet & MacDougal (2003), who constructed the morphological tree. Nevertheless, our results do demonstrate relative congruency with most of the taxonomic affinities inferred for the taxa studied here and allow inferences to be made concerning some of the phylogenetic lineages of the group.

Hansen *et al.* (2006) noted that none of the published phylogenetic analyses have completely resolved the classification of *Passiflora*, as the series and sections have not been supported. It is probable that phylogenetic analyses, combined with additional morphological and molecular data, will supply more consistent results and present non- or less-conflicting interpretations of the evolution of the *Passiflora* taxa. Within this context, new morphological, anatomical, and biogeographic data concerning pollination vectors would be quite useful in examining this highly diversified group within the flora of Brazil.

Acknowledgments

The authors thank the UNICAMP, CCA/UFES, MN/UF RJ, UFRGS, ITR/UFRRJ and the Missouri Botanical Garden, for the institutional support provided. We are grateful to Dr. Priscilla Menna Zamberlan and to the biologist Luana Castro, for their assistance in the DNA extraction; to Drs. Jeferson N. Fregonezi and André Olmos Simões, for

their assistance with the phylogeny programs; and to Roy Funch, for translating the text. We also thank the staff of the herbaria of the following institutions, for allowing us access to the relevant botanical material: BHCB, ESA, HUEFS, IAC, K, LP, MEXU, PEUFR and VIC. This study received financial support from the Brazilian *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq, National Council for Scientific and Technological Development) and from the *Fundação de Amparo à Pesquisa do Estado de São Paulo* (FAPESP, São Paulo State Research Foundation; Grant no. 2008/56222-3).

References

- Desfeux, C. & Lejeune, B. 1996. Systematics of Euromediterranean *Silene* (Caryophyllaceae): evidence from a phylogenetic analysis using ITS sequence. *Comptes Rendus: Académie des Sciences* 319: 351-358.
- Dunn, I.S. & Blattner, F.R. 1987. Sharons 36 to 40: multi-enzyme, high capacity, recombination deficient replacement vectors with polylinkers and polystuffers. *Nucleic Acids Research* 15: 2677-2698.
- Feuillet, C. & MacDougal, J.M. 2003. A new infrageneric classification of *Passiflora* L. (Passifloraceae). *Passiflora: The Journal & Newsletter of Passiflora Society International* 13(2): 34-38.
- Hansen, A.K.; Gilbert, L.E.; Simpson, B.B.; Downie, S.R.; Cervi, A.C. & Jansen, R.K. 2006. Phylogenetic Relationships and Chromosome Number Evolution in *Passiflora*. *Systematic Botany* 31(1): 138-150.
- Hansen, A.K.; Escobar, L.K.; Gilbert, L.E. & Jansen, R.K. 2007. Paternal, Maternal, and Biparental Inheritance of the Chloroplast Genome in *Passiflora* (Passifloraceae): implications for the phylogenetic studies. *American Journal of Botany* 94(1):42-46.
- Killip, E.P. 1938. The American species of Passifloraceae. *Publication Field Museum of Natural History - Botanical Series* 19(1-2): 1-613.
- MacDougal, J.M. & Feuillet, C. 2004. Systematics. In: Ulmer, T. & MacDougal, J.M. (Eds.). *Passiflora: Passionflowers of the world*. Portland, Oregon: Timber Press.
- Milward-de-Azevedo, M.A. 2007. Revisão taxonômica de *Passiflora* L. subgênero *Decaloba* (DC.) Rchb. (Passifloraceae) no Brasil. Tese de Doutorado, Universidade Federal do Rio de Janeiro/ Museu Nacional / Programa de Pós-graduação em Ciências Biológicas (Botânica).
- Milward-de-Azevedo, M.A.; Gonçalves-Esteves, V. & Baumgratz, J.F.A. 2004. Palinotaxonomia das espécies de *Passiflora* L. subg. *Decaloba* (DC.) Rchb. (Passifloraceae) no Sudeste do Brasil. *Revista Brasileira de Botânica* 27(4): 655-665.
- Milward-de-Azevedo, M.A.; Souza, F.C.; Baumgratz, J.F.A. & Gonçalves-Esteves, V. 2010. Palinotaxonomia de *Passiflora* L. subg. *Decaloba* (DC.) Rchb. (Passifloraceae) no Brasil. *Acta Botanica Brasilica* 24(1): 133-145.
- Muschner, V.C.; Lorenz, A.P.; Cervi, A.C.; Bonatto, S.L.; Souza-Chies, T.T.; Salzano, F.M. & Freitas, L.B. 2003. A First Molecular Phylogenetic Analysis of *Passiflora* (Passifloraceae). *American Journal of Botany* 90(8): 1229-1238.
- Posada, D. & Crandall, K.A. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* 14: 817-818.
- Presting, D. 1965. Zur Morphologie der pollenkörner der Passifloraceae. *Pollen et Spores* 7: 193-247.
- Reichenbach, H.G.L. 1828. *Conspectus regni vegetabilis*. Leipzig, Carl Cnobloch.
- Ronquist, F. & Huelsenbeck, J.P. 2003. MrBayes 3: phylogenetic inference under mixed models. *Bioinformatics* 19: 1572-1574.
- Roy, A.; Frascaria, N.; MacKay, J. & Bousquet, J. 1992. Segregating random amplified polymorphic DNAs (RAPDs) in *Betula alleghaniensis*. *Theoretical and Applied Genetics* 85: 173-180.
- Souza-Chies, T.T.; Yockteng, R. & Nadot, S. 2005. Systématique moléculaire comparée en France et au Brésil du genre *Passiflora* L. (Passifloraceae). *Cahiers Agricultures* 14(2): 209-215.

- Swofford, D. L. 2002. Paup: Phylogenetic usin parsimony (and other methods) 4.0 Beta. Sinauer Associates, Inc. Publishers, Sunderland, Massachussets.
- Taberlet, P.; Gielly, L.; Pautou, G. & Bouvet, J. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. **Plant Molecular Biology** 17: 1105-1109.
- Tamura, K.; Peterson, D.; Peterson, N.; Stecher, G.; Nei, M. & Kumar, S. 2011. MEGA 5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. **Molecular Biology and Evolution** 28: 2731-2739.
- Thiers, B. [continuously updated]. **Index Herbariorum: A global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium**. <http://sweetgum.nybg.org/ih/> (Acesso em 24/09/2013).
- Zamberlan, P.M. 2007. Filogenia de *Passiflora* L. (Passifloraceae): questões infra-subgenéricas. Dissertação de Mestrado, Porto Alegre, Universidade Federal do Rio Grande do Sul.
- Yockteng, R. & Nadot, S. 2004. Phylogenetic relationships among *Passiflora* species based on the glutamine synthetase nuclear gene expressed in chloroplast (ncpGS). **Molecular Phylogenetics and Evolution** 31: 379-396.