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# Karyomorphology and GC-rich heterochromatin pattern in *Passiflora* (Passifloraceae) wild species from *Decaloba* and *Passiflora* subgenera

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ABSTRACT

Thirteen wild species of *Passiflora* were analyzed using conventional and CMA/DA/DAPI staining to evaluate the karyotype diversity between and within the subgenus *Decaloba* and *Passiflora*. The karyotypic features indicate that both subgenera have a conserved chromosome number, as reported before for several species. Submetacentric (*sm*) chromosomes were found in species from both subgenera, suggesting that *sm* chromosomes are not restricted to a particular subgenus. The analysis of the karyotypic heterogeneity enabled to distribute the species in three groups, but with no support to phylogenetic and taxonomic levels. The application of fluorochromes allowed for the visualization of $CMA^+/DAPI^-$ blocks, which in our studies always correlated with the occurrence of satellites, showing that occurrence of two chromosome pairs with satellites per cell is a characteristic shared by some species from both subgenera. This feature does not always have relationship with the basic chromosome number. The data found in this study will help to understand the phylogeny, cytotaxonomy, and evolution of the genus *Passiflora* showing that karyotypic variation can be seen between and within the subgenus *Decaloba* and *Passiflora*.



## Introduction

Predominantly tropical, the New World genus *Passiflora* L. (Passifloraceae Juss.) comprises more than 500 species, found in five continents, the Galapagos Islands, and at islands of the Pacific Ocean (Ulmer and MacDougal, 2004; Vanderplank, 2000). Ninety-nine percent of this diversity is found in South America, and Brazil is the representative area of occurrence for the majority of the genetic resources of *Passiflora*, a center of origin of about 200 wild species, with some taxa also almost extinct or in the process of extinction (Bernacci et al., 2005; Herbário IAC, 2013; Vanderplank, 2000; Viana et al., 2003). About 20 species are restricted to the Old World, as a rule in the tropical and sub-tropical regions (Vanderplank, 2000).

*Passiflora* is widely cultivated today for its ornamental flowers, pharmacological properties, and edible fruits. The presence of a corona, colorful flowers, and the varied shapes of the leaves provide the plants with striking beauty. This unique morphology caught the attention of horticulturists already three centuries ago (Abreu et al., 2009; Ulmer and MacDougal, 2004). Since then, wild species and hybrids of *Passiflora* have been used for ornamentation of hedges, pergolas, and gardens in countries of Europe and North America (Vanderplank, 2000). The wild species are important sources of traits of interest to plant breeding, such as resistance to biotic and abiotic factors, as well as the interspecific crosses for the ornamental market (Abreu et al., 2009).

Due to their complex taxonomy, which includes 23 subgenera, in addition to many sections and series, data such as the karyomorphology, chromosome number, and banding can be of great importance in clarifying the systematic position of species and study of plant diversification (Escobar, 1989; Hansen et al., 2006; Killip, 1938; Stebbins, 1950; Stace, 2000). Studies based on conserved nuclear sequences (ITS) and plastid sequences (*trnL-trn*; ncpGS) were carried out aiming at clarifying the taxonomic complex of the genus *Passiflora*, proposing different forms of grouping for some of the representatives of the genus (Muschner et al., 2003; Yockteng and Nadot, 2004).

Most *Passiflora* species are diploid ($2n = 12$ or 18), but $2n = 14, 20, 22, 24, 36, 72$, and 84 were also found (Melo and Guerra, 2003; Melo et al., 2001; Souza et al., 2008). Karyotypic studies already carried out with the genus *Passiflora* enable inferring different basic chromosome numbers for the genus, $x = 6$, $x = 9$, $x = 10$, and $x = 12$; the

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**Table 1**
Investigated *Passiflora* species and their taxonomic position according to the infrageneric classification by Cervi (1997) – species followed by (*) – and Ulmer and MacDougal (2004), chromosome number, geographical origin in Brazil, voucher number and donor.

| Subgenus | Supersection | Section/Series | Species | *n* | 2*n* | Locality (accessions) |
|---|---|---|---|---|---|---|
| *Decaloba* | *Auriculata* | – | *P. ferruginea* Mast. | – | – | Três Irmãos, Acre (398), Instituto Plantarum (IP) |
| | – | – | *P. coriacea* Juss. | 6 (Beal, 1971) | 12 (Snow and MacDougal, 1993; Oliveira and Coleman, 1996; Melo et al., 2001) | Unknown (492), IAC |
| | – | – | *P. micropetala* Mast. | – | – | Unknown (494), IAC |
| *Passiflora* | *Stipulata* | *Granadillastrum* | *P. actinia* Hook. | 9 (Soares-Scott et al., 2001) | 18 (Melo et al., 2001) | Campus da UFPR (427), Dr. Cervi |
| | *Stipulata* | *Kermesinae* | *P. miersii* Mast. | 9 (Snow and MacDougal, 1993) | – | Poços de Caldas, MG (439), IP |
| | *Stipulata* | *Kermesinae* | *P. miersii* Mast. | 9 (Snow and MacDougal, 1993) | – | São Roque de Minas, MG (448), Dr. Mauro Peixoto |
| | – | *Simplicifoliae* | *P. subrotunda* Mast.* | – | – | Fortaleza, CE (452), IP |
| | *Laurifolia* | *Tiliifolia* | *P. serrato-digitata* L. | 9 (Soares-Scott et al., 1999) | – | Unknown (487), Embrapa cerrado |
| | *Coccinea* | – | *P. coccinea* Aubl. | 9 (Oliveira and Coleman, 1996) | 18 (Beal, 1971; Oliveira and Coleman, 1996; Melo et al., 2001) | Monte Alegre do Sul, SP (419), IP |
| | *Coccinea* | – | *P. vitifolia* Kunth. | 9 (Storey, 1950) | 18 (Storey, 1950; Snow and MacDougal, 1993) | Miranda, MG (481), IP |
| | *Passiflora* | *Setacea* | *P. setacea* DC. | 9 (Oliveira and Coleman, 1996) | 18 (Soares-Scott et al., 1999) | Rio de Janeiro, RJ (506), UENF |
| | *Passiflora* | *Setacea* | *P. hatschbachii* Cervi* | – | – | Leopoldina, MG (446), IP |
| | *Passiflora* | *Passiflora* | *P. filamentosa* Cav.* | 9 (Beal, 1969) | 18 (Beal, 1969) | Camacan, BA (513), IP |
| | – | *Serratifoliae* | *P. bahiensis* Klotzsch* | – | – | Camacan, BA (105), Collected |

three last numbers are considered secondary basic numbers (Melo and Guerra, 2003; Melo et al., 2001). However, the understanding of the relationships between those numbers is still uncertain. The two largest subgenera *Decaloba* (DC.) Rchb. and *Passiflora* L. correspond to the subgenera *Plectostemma* and *Grandolla*, according to the old classification by Killip (1938). The subgenus *Decaloba* comprises species with small flowers and fruits, presenting the ancestral basic chromosome number of $x = 6$ or a multiple of it (Snow and MacDougal, 1993). The subgenus *Passiflora* comprises species with large flowers and fruits of economic importance, having $x = 9$ as a basic cytological characteristic (Ulmer and MacDougal, 2004). Cytogenetic differences between the species of groups $x = 6$ and $x = 9$ have been studied; however, the cytological data are more or less limited to the chromosome number, disregarding the chromosome morphology in the comparative analysis between both subgenera (Hansen et al., 2006; Melo et al., 2001). Some analyses conducted with the genus *Passiflora* indicated that the number and position of the satellites, number and length of the chromosomes, and the position of the centromere are probably characteristics of the subgenera and sections (Beal, 1973a,b; Melo et al., 2001; Snow and MacDougal, 1993; Souza et al., 2003; Vieira et al., 2004).

The use of more refined cytogenetic techniques such as banding with CMA (chromomycin $A_3$) and DAPI (4′,6-diamidino-2-phenylindole) fluorochromes allow for the visualization of GC-rich and AT-rich heterochromatin, respectively, which can be useful for evolutionary and cytotaxonomic studies in plant groups. In many cases, the presence of $CMA^+/DAPI^-$ blocks matches the satellites in number and location, and also enables the localization of species-specific markers (Melo et al., 2011). The application of CMA and DAPI fluorochromes in some species of the genus *Passiflora* revealed $CMA^+/DAPI^-$ blocks that always correlate with the presence of satellites. However, in some cases the number of $CMA^+/DAPI^-$ blocks is lower than the number of secondary constrictions, and this fact depends on chromosome length, compaction, and population factors (Melo et al., 2001; Melo and Guerra, 2003; Viana and Souza, 2012). A significant part of the knowledge about karyotypic diversity within subgenera, super-sections, and sections/series is still based on the application of conventional cytogenetic techniques. However, the localization of base-specific heterochromatin and of 45S and 5S DNAr have revealed the karyotypic variability within specific subgenera (Melo et al., 2001; Melo and Guerra, 2003; Viana and Souza, 2012).

The aim of this study was the application of both conventional cytogenetic staining and base-specific fluorochrome for the karyotypic characterization of three species of the subgenus *Decaloba* and ten species of the subgenus *Passiflora*, in order to determine specific karyotypic characteristics restricted to the particular taxonomic levels, contributing to phylogenetic, cytotaxonomic, and evolutionary studies of species of both subgenera.

## Material and methods

### *Plant material*

Thirteen wild species of *Passiflora* representing the subgenera *Decaloba* (3 species) and *Passiflora* (10 species) (Cervi, 1997; Ulmer and MacDougal, 2004) were used in this study. The *Passiflora* species were selected based on their taxonomic positions at the subgenera, supersection, and sections levels (Table 1), and were cultivated at the Active Germplasm Bank (*Banco Ativo de Germoplasma*: BAG-Passifloras), Ilhéus, Bahia, Brazil (14°45′15″S, 39°13′59″W, 40 a.s.l.). The plants were first cultivated in pots, and later propagated by stem cuttings to obtain root tips for the chromosome analyses.

### *Chromosome preparations*

Radicles were pretreated in a 0.002 M 8-hydroxyquinoline solution at room temperature for 1 h, and stored at 8 °C for 21 h, washed twice with distilled water for five minutes, and then fixed in freshly prepared Carnoy 1 fixative [ethanol:acetic acid (3:1, v/v); Johansen, 1940] for at least 3 h at room temperature (RT); and stored at −20 °C until use. The radicles were washed twice for 5 min with distilled water and incubated in 50 μL of enzymatic solution of 2% cellulose and 20% pectinase (w/v) for 60 – 90 min at 37 °C. Roots tips were washed twice for five minutes with distilled water and macerated