Study of the genetic diversity of genus *Passiflora* L. (Passifloraceae) and its distribution in Colombia

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Diversity of Colombian Passifloraceae: biogeography and an updated list for conservation

Colombian Passifloraceae were listed, gathering and georeferencing 3,930 records, from herbaria, literature and field observations. It includes 167 species, 165 of them native, which is equivalent to 27% of the family. Forty-two produce an edible fruit, and nine are commercially cultivated. Our list brings more details on species distribution and presents 26 species new to Colombia. Most of the 58 endemic species, including 36 narrow endemics, are Andean species of subgenera *Tacsonia* and *Decaloba*.

Applying the IUCN criteria, 70% of the species appear threatened and three extinct. When compared with other regions, the Andes of Colombia and Ecuador constitute the center of *Passiflora* diversity, whose elevational distribution shows a small peak below 500 m, and two higher ones at 1000-2,000 and 2,500-3,000 m. This pattern corresponds to divergent adaptive trends among infrageneric divisions, subgenus *Tacsonia* contributing markedly to the highest peak.

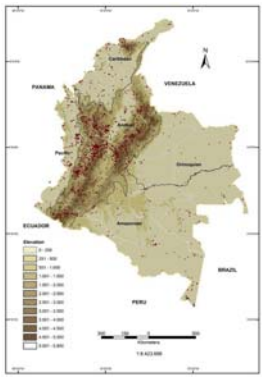
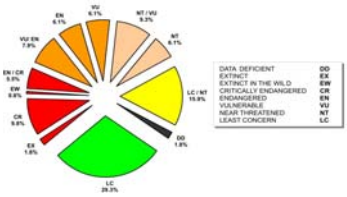


Figure 1. Map of distribution of Passifloraceae specimens for 3,930 collections in the five biogeographic regions in Colombia. Points on the map represent sites of collection.

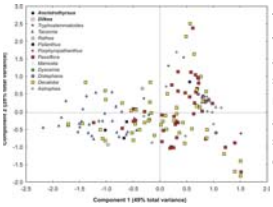


Most common species thrive in disturbed habitats, such as borders of roads, cultivated plots, and secondary forest. Colombia may still harbor many unknown species in poorly explored departments. The urgent task of conserving this threatened richness must target the conservation of these resources as well as their habitat. Both aspects may be combined if Passifloraceae can be used as an indicator of biodiversity in the Andean region, which seems justified by their multiple ecological interactions with many organisms.

Figure 2. Percentual number of the threat status of 165 Passifloraceae native species under the IUCN criteria.

Distribution, diversity and *in situ* conservation of Colombian Passifloraceae

Analysis was made of 3,930 records of 165 wild Passifloraceae to assess the distribution of their diversity in Colombia, identify collection gaps, and explore their potential as indicator species. Despite variable collecting density among and within biogeographic regions, the Andean region clearly presents a higher species richness, particularly in the central coffee growing zone.



The analysis on 19 climatic variables showed that the two principal variance components, explaining 77% of the total, are respectively associated with temperature and precipitation, without influence of seasonality. Distribution parameters allow recognizing more than 36 narrow endemics. Prediction of species distribution showed nine areas with very high richness (predicted sympatry of 41 to 54 species) in the Andean region, three of which correspond to collection gaps.

Figure 3. Distribution of Passifloraceae species in the PCA principal plane for climatic variables.

Their striking correspondence with coffee growing zone ecotopes imposes a strategy integrating agricultural and environmental management at the landscape level for preserving this threatened richness as well as a region of particular importance for the country. Both aspects may be combined if Passifloraceae can be used as an indicator of biodiversity in this region, which seems justified by their diversity and characteristics, including multiple ecological interactions with many organisms.

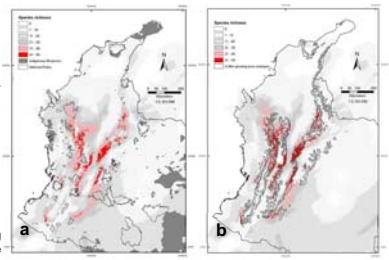
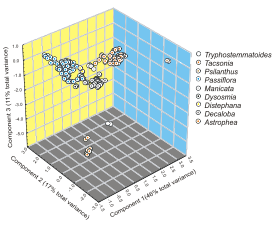


Figure 4. Distribution of protected areas (**a**) and coffee growing zone ecotopes (**b**) in Colombia, showing poor correspondence with areas of high Passifloraceae diversity.

A phenetic analysis of morphological diversity in the genus *Passiflora* L.

Morphological variation was studied in 124 accessions from eight subgenera and 60 species among the most common cultivated and wild species of genus *Passiflora*, using the analysis of variance components and principal component analysis (PCA) on 43 quantitative traits, and *neighbor joining* cluster analysis on 84 qualitative traits.



Twenty-four quantitative traits showing high variation among subgenera were selected. The three principal components of variation are associated with (i) flower length; (ii) flower width and bract shape; and (iii) peduncle branching, stem width and leaf length. The projection of accessions in the resulting tridimensional space consistently separates subgenera.

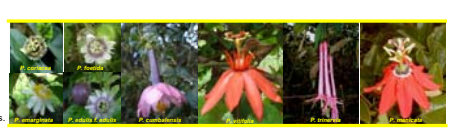


Figure 5. Tridimensional plot of the scores of *Passiflora* accessions.

A similar selection of 32 qualitative traits, and four categorized quantitative traits, allowed classifying our sample consistently. Most discriminating characters include size of stems and leaves, presence of tendrils, number and distribution of extrafloral nectaries, dimensions and general shape of bracts, width and length of flowers and corona complexity.

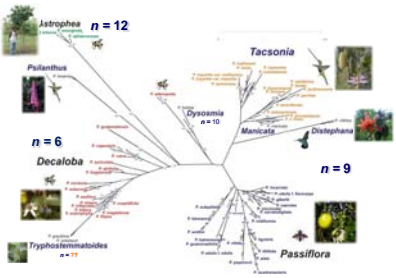
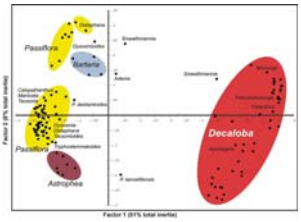


Figure 5. Cluster analysis obtained with 32 qualitative traits. Distances of Sokal & Michener.

Our results support seven of the eight Killip's (1938) subgenera of our sample, but no infrasubgeneric classifications. However, the new classification of subgenus *Decaloba* by Feuillet & MacDougal (2003) was partly supported. They converge on many points with previous phylogenetic results obtained with DNA sequences, although the latter group subgenera *Tacsonia* and *Distephana* with subgenus *Passiflora*.

Chloroplast and mitochondrial DNA variation in the genus *Passiflora* L. (Passifloraceae) as revealed by PCR-RFLP

The chloroplast and mitochondrial DNA diversity of 213 genotypes belonging to 151 *Passiflora* species and 15 subgenera recognized by Killip was studied by PCR-RFLP, identifying 280 haplotypes for cpDNA and 372 for mtDNA. The principal co-ordinate analysis on cpDNA data allowed visualizing a strong separation of subgenera *Apodogyne*, *Decaloba*, *Murucuja*, *Pseudomurucuja* and *Psilanthus* (constituting the "*Decaloba* group"), while the neighbor-joining cluster analysis showed three well-supported clusters within *Passiflora*, corresponding to the three major divisions of the taxonomy proposed by Feuillet & MacDougal.



The first one, named the "*Passiflora* group", includes subgenera *Calopathanthus*, *Deidamioides*, *Distephana*, *Dysosmia*, *Dysosmioides*, *Manicata*, *Passiflora*, *Tacsonia*, and *Tacsonioides*, with a very loose substructure and considerable intraspecific variation. The second one includes subgenus *Astrophea*, and the third is the '*Decaloba* group'. The outgroup species, take an undefined position among the *Passiflora* clusters.

Figure 6. Principal co-ordinates on cpDNA data estimated with 268 PCR-RFLP marker.

The phenogram from mtDNA data separates four moderately supported clusters. As for cpDNA, a first one corresponds to the '*Decaloba* group'. The other are different, as subgenera *Astrophea* and *Tryphostemmatoides* appear integrated within the '*Passiflora* group', while subgenus *Tacsonia* forms a uniform distinct cluster, close to another one comprising species of *Passiflora* series *Kermesinae*, *Simplicifoliae*, *Lobatae*, and *Menispermifoliae*.

The analyses of cpDNA and mtDNA give different pictures of the *Passiflora* diversity, in the position of the outgroup, the relative position of four subgenera, and the relationships between species, which we attribute to different rates of evolution and modes of transmission of the chloroplastic and mitochondrial genomes, combined with reticulate evolution in the genus.

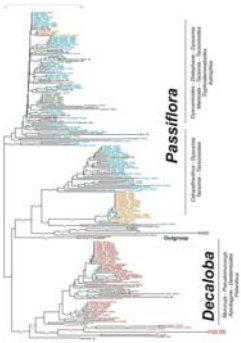


Figure 7. Phenogram derived from on mtDNA data illustrating the distribution of the different *Passiflora* subgenera studied.

Conclusions

Breeding programs aimed at producing interspecific hybrids involving the cultivated species of *Passiflora* should therefore focus on the species belonging to the same clade as subgenus *Passiflora*. According to our results, the '*Decaloba* clade' and subgenus *Astrophea* do not constitute interesting genetic resources for passion fruit breeding.

These results constitute potentially crucial inputs for the development of a coherent strategy for the conservation and use of these genetic resources. Studies of *Passiflora* diversity in the Andean countries, and the maps presented here, will be used in future prospecting and identifying sites for *in situ* conservation, and more generally guiding government conservation strategies.

Acknowledgements

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