

Non-coding chloroplast regions analysis within the Orchidaceae family in Southern Ecuador

Análisis de regiones del cloroplasto no codificante dentro de la familia Orchidaceae en el sur de Ecuador

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ABSTRACT

Non-coding regions of the chloroplast genome offer interesting levels of nucleotide variation which are very useful for molecular genetics, population and phylogenetic analysis. The family Orchidaceae is represented by ca. 500 species in Southern Ecuador. In order to determine the genetic variability present in members of this family belonging to the genera *Cyrtochilum*, *Masdevallia*, *Epidendrum*, *Polystachya*, *Stelis* and *Zelenchoa*, we have analyzed four chloroplastic intergenic spacers: atpH - atpI, trnL - trnF, trnF - ndhJ and rps16 - trnQ. All these markers have shown high richness in simple sequence repeats (SSR), indels and substitutions. They resulted to be useful for species identification, phylogenetic analysis and population structure studies. Moreover the information provided by this analysis suggests that the endemic species *Masdevallia deformis* must be considered vulnerable and conservation strategies need to be adopted for its protection.

Keywords: Chloroplast intergenic spacers, conservation, genetic variability, Orchidaceae, Southern Ecuador.

RESUMEN

Regiones del genoma del cloroplasto no codificante ofrecen niveles interesantes de variación de nucleótidos que son muy útiles para la genética molecular, la población y el análisis filogenético. La familia Orchidaceae está representada por casi 500 especies en el sur de Ecuador. Con el fin de determinar la variabilidad genética presente en los miembros de esta familia pertenecientes a los géneros *Cyrtochilum*, *Masdevallia*, *Epidendrum*, *Polystachya*, *Stelis* y *Zelenchoa*, se han analizado cuatro espaciadores intergénicos cloroplásticos: atpH - atpI, trnL - TRNF, trnF - ndhJ y rps16 - trnQ. Todos estos marcadores han demostrado una alta riqueza en simples repeticiones de secuencia (SSR), indeles y sustituciones. Ellos resultaron ser útiles para la identificación de las especies, el análisis filogenético y los estudios de estructura de la población. Además la información proporcionada por este análisis sugiere que las especies endémicas *deformis* *Masdevallia* deben ser considerados vulnerables y deben adoptarse estrategias de conservación para su protección.

Palabras clave: espaciadores intergénicos, conservación, variabilidad genética, Orchidaceae, sur de Ecuador.

The Orchidaceae family encloses members with the most beautiful reproductive organs within Plantae kingdom. This monophyletic family is one of the largest with 765 genera and approximately 26500 species worldwide distributed (World Checklist of Selected Plant Families).

Orchids exhibit a large panoply of adaptation, ecological and morphological patterns which explain their capacity to colonize all land habitats. They constitute one of the most interesting biological models to assess evolutionary, phylogenetic and ecological studies.

Orchids represent around 25% of plants in Ecuador and they are the most important family of vascular plants in this country.¹ One third of species of this group is endemic. About 500 members of the Orchidaceae family have been described for the Southern Ecuadorian sierra.² In Ecuador the deforestation index is particularly high.³ Epiphytic orchids are sensitive to this kind of anthropogenic activity.⁴

The southern province of Zamora-Chinchipec in Ecuador will be the focus of an intensive mining activity with harmful ecological consequences for its territory and neighbouring provinces. In this context a genetic approach to evaluate variability in the Orchi-

daceae family in Southern Ecuador, in order to establish levels of biological resistance to habitat modification, is a priority.

The chloroplast genome has demonstrated to be a source of important information for population and conservation genetics, phylogenetic inference and species identification (bar-coding) studies.⁵⁻⁷

Plastid genome shows a very conserved size, structure, gene content and arrangement of genes within terrestrial plants.⁷ The most common structural pattern of this genome consist of two inverted repeats (IR) zones (25 Kb each one) separated by a long single copy (LSC) and a short single copy (SSC) regions respectively. The latter two accumulate mutations at a higher rate than the rest of the organelle genome.⁸

Seven orchids chloroplast genomes have been recently sequenced: *Phalaenopsis Aphrodite*,⁹ *Oncidium sp.* Gower Ramsey,¹⁰ *Rhyzantella gardneri*,¹¹ *Neottia nidus-avis*,¹² *Erycina pusilla*,¹³ *Corallorhiza striata*¹⁴ *Cypripedium macranthos* and *Dendrobium officinale*.¹⁵

Chloroplastic regions with phylogenetical signal were identified around 1990's.¹⁶ Moreover, Shaw has developed interesting

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markers for the non-coding region (2005.2007). Scarcelli et al.¹⁷ reported a set of oligonucleotides useful to assess monocotyledons chloroplast variability.

In the present study we used four plastid markers: trnQ-rps16, atpH-atpI, trnL-ndhJ¹⁷ and trnL (UAA)3'exon-trnF(GAA)¹⁶ to study genetic diversity, phylogenetic relationships and chloroplast molecular evolution within members of the Orchidaceae family distributed in the South of Ecuador. The species analyzed were: *Cyrtorchilum myanthum*, *Epidendrum parviflorum*, *Epidendrum madsenii*, *Masdevallia deformis*, *Masdevallia gnoma*, *Stelis patinaria*, *Polystachya stenophylla* and *Zelenchoa onusta*.

With the exception of *C. myanthum* trnL-trnF studied region, this is the first report about variability within chloroplast intergenic spacers in members of Orchidaceae family distributed in Southern Ecuador.

About one hundred individuals belonging to the species *C. myanthum*, *E. parviflorum*, *M. deformis*, *M. persicina*, *M. gnoma*, *S. patinaria*, *P. stenophylla* and *Z. onusta* were sampled in the provincias of Loja and Zamora Chinchipe and analyzed using the four intergenic spacers afore described.

Sequence editing, alignment, coding sequences translation and Megablast searches were performed with Geneious 4.7.5 software package (Biomatters Ltd). All sequences here obtained were deposited in GenBank. Phylograms based on SNP data were constructed using the BioNJ algorithm with the Jukes-Cantor distance, as implemented in Seaview version 4.¹⁸

Our results revealed that all chloroplastic loci here analyzed resulted rich in indels of varied size. An important sequence length variation among genera at all loci was also observed. Moreover, simple sequence repeats (SSRs), including mono-, di- and tetranucleotidic microsatellites and Single Nucleotide Polymorphisms (SNPs) provided a significant source of polymorphism in these plastid regions.

The non-coding chloroplastic regions here studied revealed high variability at intra- and interspecific level, as well as marked structural differences among Orchidaceae genera. All analyzed sequences provided a high phylogenetical signal. These markers were very efficient to distinguish species, so they could be considered as potential barcodes. The scarce genetic variability detected for the species *Masdevallia deformis* (Figure .1) suggests that protective measures must be adopted in order to conserve this endemic component of the biodiversity in southern Ecuador.

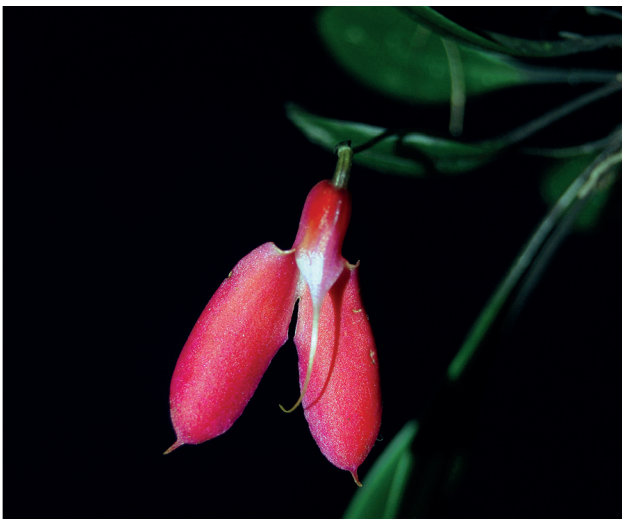


Figure 1. *Masdevallia deformis*

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