

An integrative approach to understand species delimitation in *Petunia*

Ana Lúcia Anversa Segatto, Maikel Reck-Kortmann, Caroline Turchetto, Loreta Brandão de Freitas

UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

Abstract

Plants are considered to be plastic organisms, not only due to the phenotypic plasticity they usually present but also because they can hybridize frequently. Thereby, it is difficult to determine if the morphological variation seen, in what is considered one plant species, is morphological plasticity, hybrid organisms, or local adaptation. Taking this into account, simulated datasets have shown that the most used methods of species delimitation are negatively influenced by gene flow and incomplete lineage sorting. The long corolla tube clade of *Petunia*, formed by *Petunia exserta*, *Petunia secreta* and three subspecies of *Petunia axillaris*, presents taxa with morphological variation, genetic sharing, and disjoint distribution that make it a good system to study the effects of population phenomena in species delimitation. In this work, our objective was to clarify the evolutionary relationships among long corolla tube taxa, including canonical, non-canonical and geographic disjointed individuals within each taxon. To do this, we sequenced eight nuclear regions, five plastid DNA markers, and genotyped seven microsatellite loci. The phylogenetic relationships among the lineages were estimated using the Bayesian Inference as implemented in BEAST 1.7, and species delimitation was conducted using the Program BPP 3.4. The software Structure 2.3 was used to perform clustering analysis based on microsatellite data. The supertree showed better resolution than the species tree. *Petunia* species previously described as belonging to the long tube clade formed a monophyletic group in the supertree and were distributed in two main subgroups. Multiple runs of BPP with different MCMC parameters and guide trees gave similar results; the posterior probabilities values were higher considering eight species. In contrast, microsatellite markers analyses indicated the occurrence of two genetic components. The long corolla tube clade of *Petunia* encompasses markedly different taxa, regarding morphology and life habits. The multigene and multimarker approaches used here to disentangle the evolutionary relations among these taxa confirmed their genetic identity; however, it did not agree with the current taxonomic classification. The observed scenario possibly involves a complex interaction of different environmental, phenotypic, and genetic phenomena, which is similar to what is proposed to a great number of species. Understanding the relationship between genetic diversity and other variability sources is extremely important to the preservation of evolutionary lineages, mainly in front of the global environmental changes, and can contribute to understanding how the taxa identity is maintained. Incongruence between different data sources may be the key to understand the evolutionary history and new Bioinformatics approaches are necessary to deal with that.

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