

## A MOLECULAR PHYLOGENY OF THE MISTLETOE GENUS *TRIPODANTHUS* (LORANTHACEAE)

Guillermo C. Amico<sup>1,2</sup>, Romina Vidal-Russell<sup>1,2</sup> & Daniel L. Nickrent<sup>2</sup>

<sup>1</sup>Laboratorio Ecotono, Universidad Nacional del Comahue, CRUB, Quintral 1250, 8400 San Carlos de Bariloche, Río Negro, Argentina; gamico@crub.uncoma.edu.ar (author for correspondence).

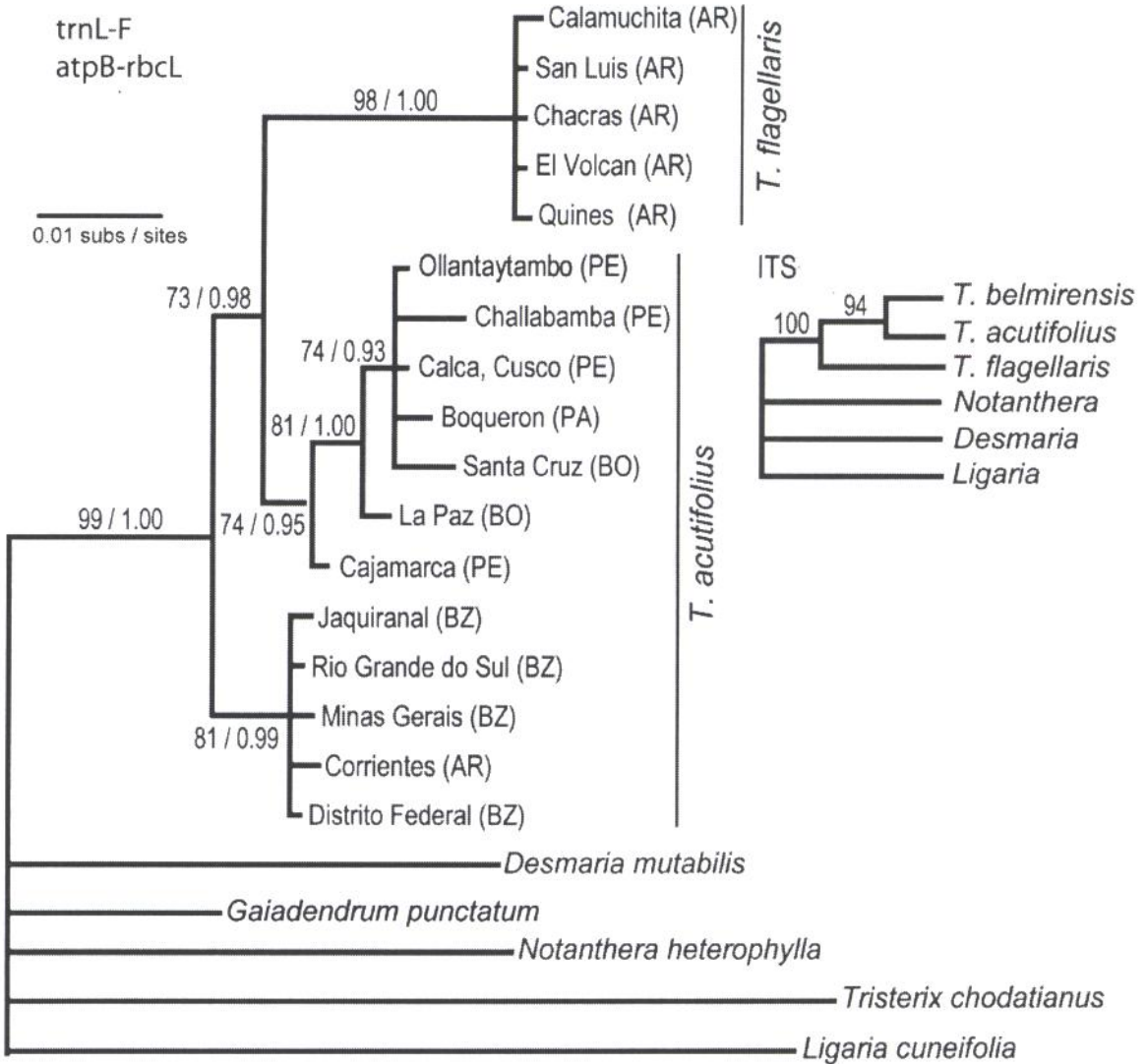
<sup>2</sup>Department of Plant Biology, Southern Illinois University Carbondale, IL 62901-6509, USA.

Loranthaceae is the largest mistletoe family with 73 genera of which 16 are found in South America. *Tripodanthus* plus five more genera, *Gaiadendron*, *Desmaria*, *Ligaria*, *Notanthera*, and *Tristerix*, are considered relictual based on their base chromosome numbers, low number of species, and restricted distributions (Barlow, 1983). *Tripodanthus* is the only relictual genus that has a base number of  $x = 8$ , a number present in 11 of 16 South American genera. Recent molecular phylogenies showed this genus is sister to a clade composed of the remaining  $x = 8$  South American genera (Wilson & Calvin, 2006; Vidal Russell, 2007). *Tripodanthus* consists of three species, *T. acutifolius* (Ruiz & Pavón) Tieghem, *T. flagellaris* (Chamisso & Schlechtendal) Tieghem and the recently described *T. belmirensis* Roldán & Kuijt, all endemic to South America. Only one species, *T. acutifolius*, has a wide distribution. This species is present in the Guiana highlands (Venezuela), and its distribution follows the Andes from Ecuador to Norwest Argentina. It is also found in the east in Bolivia, Paraguay, Uruguay, and south-central Brazil. In Argentina it has a disjunct distribution, being found in the west along the Andes and in the east in the Mesopotamia region (Entre Ríos and Corrientes) (Abbiatti, 1946). The other two species have narrow distributional ranges, with *T. belmirensis* being restricted to Antioquia in Colombia and *T. flagellaris* to central Argentina, Uruguay and southern Brazil. In the Andes these species are found at high elevations (more than 1000 m), but in the eastern part of their distributions they grow at low elevations. As most Loranthaceae, *Tripodanthus acutifolius* is a stem parasite, but it has also been reported as a root parasitic plant. Root parasitism is found in three other Loranthaceae genera: *Nuytsia* of western Australia, *Atkinsonia* of eastern Australia, and *Gaiadendron* of the neotropics. The other two species in *Tripodanthus* have never

been cited as root parasites: *T. flagellaris* is a viney stem parasite and *T. belmirensis* is a shrubby stem parasite. *Tripodanthus* has epicortical roots as do other South American genera. The flowers have six petals with isomorphic stamens and versatile anthers. *Tripodanthus acutifolius* and *T. flagellaris* have small, short-tubular, white to light yellow flowers while *T. belmirensis* has much larger bright red flowers. The objective of the present study is to reconstruct the phylogeny of the genus using molecular markers and to test the monophyly of *T. acutifolius*, a species with a wide distribution.

A total of 18 individuals of *Tripodanthus* representing all species were sampled for this study. Five species of South American loranthids were used as outgroup (Fig. 1). The internal transcribed spacer from the nuclear ribosomal DNA (ITS and 5.8S rDNA), and the chloroplast *atpB-rbcL* intergenic spacer and the *trnL-F* regions were used. DNA extraction, amplification and sequencing protocols followed Amico et al. (2007). Chloroplast region sequences were not obtained for *T. belmirensis*. The ITS region was obtained only for one sample of each species of *Tripodanthus* and three other species as outgroup. Individual sequences were aligned manually using BioEdit. Maximum parsimony (MP) analyses with the branch-and-bound search option were implemented with PAUP\*. Nodal support was assessed using the nonparametric bootstrap (BS) with 100 pseudoreplicates. We also performed Bayesian inference (BI) using MrBayes where the model of sequence evolution was determined using MrModeltest.

Including the outgroup, the aligned positions, variable sites, and parsimony informative sites for the three partitions were as follows: ITS (725, 245, 66), *atpB-rbcL* (715, 120, 42), and *trnL-F* (673, 100, 35). MP analysis of ITS resulted in one most parsimonious tree ( $L = 300$ ) with *T. acutifolius* sister to *T. belmirensis* and this clade



**Fig. 1.** Phylogram obtained from Bayesian inference (BI) of the combined chloroplast regions. Numbers above lines are bootstrap values for maximum parsimony (MP) and BI posterior probabilities. The cladogram to the right was obtained from a MP analysis of the ITS region. Abbreviations: **AR**, Argentina; **BO**, Bolivia; **BZ**, Brazil; **CO**, Colombia; **PA**, Paraguay; **PE**, Peru.

sister to *T. flagellaris* (Fig. 1). MP and BI of both chloroplast regions resulted in trees with congruent topologies, thus they were concatenated into one chloroplast partition. Analysis of this partition yielded 180 equally parsimonious trees ( $L = 262$ ). Both the nuclear and chloroplast partitions provided strong support for a monophyletic ingroup (*Tripodanthus*). Moreover, the three clades obtained from the chloroplast partition did

not coincide with existing species definitions (Fig. 1). The first clade, sister to the others, is composed of the eastern samples of *T. acutifolius* (Brazil and eastern Argentina). *Tripodanthus flagellaris* is strongly supported as monophyletic and is sister to the *T. acutifolius* clade comprising samples from Bolivia, Ecuador, Paraguay and Peru. Thus, these data indicate that *T. acutifolius* as currently defined is paraphyletic. Unfortuna-

tely our current sampling for the ITS region is low, thus we cannot confirm with a nuclear partition the paraphyly found in *T. acutifolius*. The sample of this species used in the ITS analysis derived from western Argentina. Obviously more ITS sequences are needed to test the non-monophyly result obtained using the chloroplast partition. *Tripodanthus acutifolius* shows great morphological variation and it is our intention to compare this variation with clades found using molecular markers. We thank F. Roldán and M. A. García for their help in obtaining material and sequencing some samples used in this study. Thanks also to the MO, MA, and SI herbaria for allowing access to their collections.

## BIBLIOGRAPHY

- Abbiatti, D. 1946. Las Lorantáceas Argentinas. *Rev. Mus. La Plata, N. S., Sec. Bot.* 7:1-110.
- Amico, G. C.; R. Vidal-Russell & D. L. Nickrent. 2007. Phylogenetic relationships and ecological speciation in the mistletoe *Tristerix* (Loranthaceae): the influence of pollinators, dispersers, and hosts. *Am. J. Bot.* 95:558-567.
- Barlow, B. A. 1983. Biogeography of Loranthaceae and Viscaceae, en: D. M. Calder & P. Bernhardt (eds), *The Biology of Mistletoes*, pp 19-45. Academic Press, New York.
- Vidal-Russell, R. 2007. *The first aerial parasites in the sandalwood order (Santalales): molecular phylogenetic and biogeographic investigations*. Ph.D. dissertation, Southern Illinois University Carbondale.
- Wilson, C. A. & C. L. Calvin. 2006. An origin of aerial branch parasitism in the mistletoe family, Loranthaceae. *Am. J. Bot.* 93: 787-796.