

Progress Report

Title: Conservation of ancient lineages in ecologically dynamic scenarios

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Project Summary

The design of conservation strategies of endangered conifers is challenging. Experimental approaches may threaten natural populations and/or take long periods because they are slow-growing. Genetic information is widely used to detect significant units in conservation but the relevance of neutral markers has been debated. Yet, genome scans of functional diversity have great potential for the conservation of wild populations and species. Monotypic genera of conservation concern *Austrocedrus*, *Fitzroya*, and *Pilgerodendron* are ancient conifers endemic to temperate forests of South America.

The aim of the project was to combine genomic approaches with the climatic niche and drought experimental approaches to assess climate-adjusted current and future conservation and restoration action of key conifers of conservation concern *Austrocedrus*, *Fitzroya*, and *Pilgerodendron* which are ancient monotypic taxa endemic to temperate forests of South America.

Project Progress

Currently, we have reached a project completion rate of 75% of the project.

Courses taken: I participated in the XV Latin American Workshop on Genetics for Conservation at Puerto Williams, Chile (<https://regenec.org/taller/sep2022/>) organized by the REGENEC (Latin American Genetics Conservation Net), which has enhanced my ability to understand and improve data analysis and results interpretation.

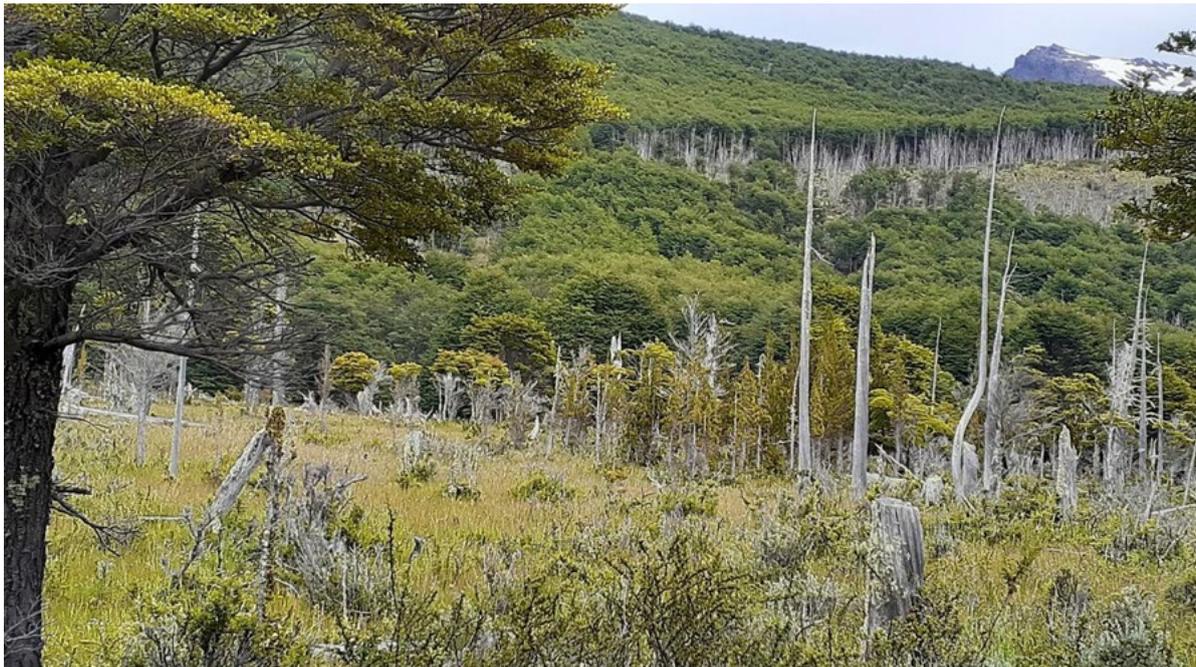
Fieldwork: We collected fresh foliage samples at a total of 21 sites, including 12 sites with alerce (*Fitzroya cupressoides*), 5 with Guaitecas cypress (*Pilgerodendron uviferum*), and 4 sites in mixed forests with both species. Field trips to natural populations involved complex Logistics given the inaccessible location of sampling locations as well as permit requests to authorities of protected areas either National or Provincial Parks. Samples of *Austrocedrus chilensis* consisted of seeds and leaves from adult trees that were collected from contrasting dry (DF) and humid (HF) forests along a steep but short precipitation gradient.

Study on *Pilgerodendron uviferum*, common name 'Guaitecas cypress'

Four of the Guaitecas cypress sites are located in the Santa Cruz province. One of these sites exhibited adult trees enclosed within Los Glaciares National Park, with reproductive structures, while nearby juveniles were not enclosed and might be vulnerable to invasive cattle like "baguales". Additionally, within the same park, two sectors at Lake Roca with well-preserved peat bogs featured reproductive structures and juveniles, surrounded by native forest.



Los Glaciares National Park. Vulnerable population.



Los Glaciares National Park. Lago Roca site, panoramic view of Guaitecas cypress (small light green trees) population surrounded by native forest.

The other two Guaitecas cypress sites are within private lands. In Santa Cruz, no reproductive structures were observed, and some adult trees displayed partial mortality within a natural forest predominantly composed of coihues (*Nothofagus dombeyi*). In the Corcovado site, the population was in excellent conditions, with reproductive structures, fencing, and surrounded by Cordilleran cypress (*Austrocedrus chilensis*) forest.



Estancia El Condor private site in Santa Cruz province.

Study on *Fitzroya cupressoides*, common name 'alerce'

As for Alerce sites, all populations displayed reproductive structures. The largest trees were found in the ANPRALE-protected area, with diameters at breast height reaching up to 2 meters.

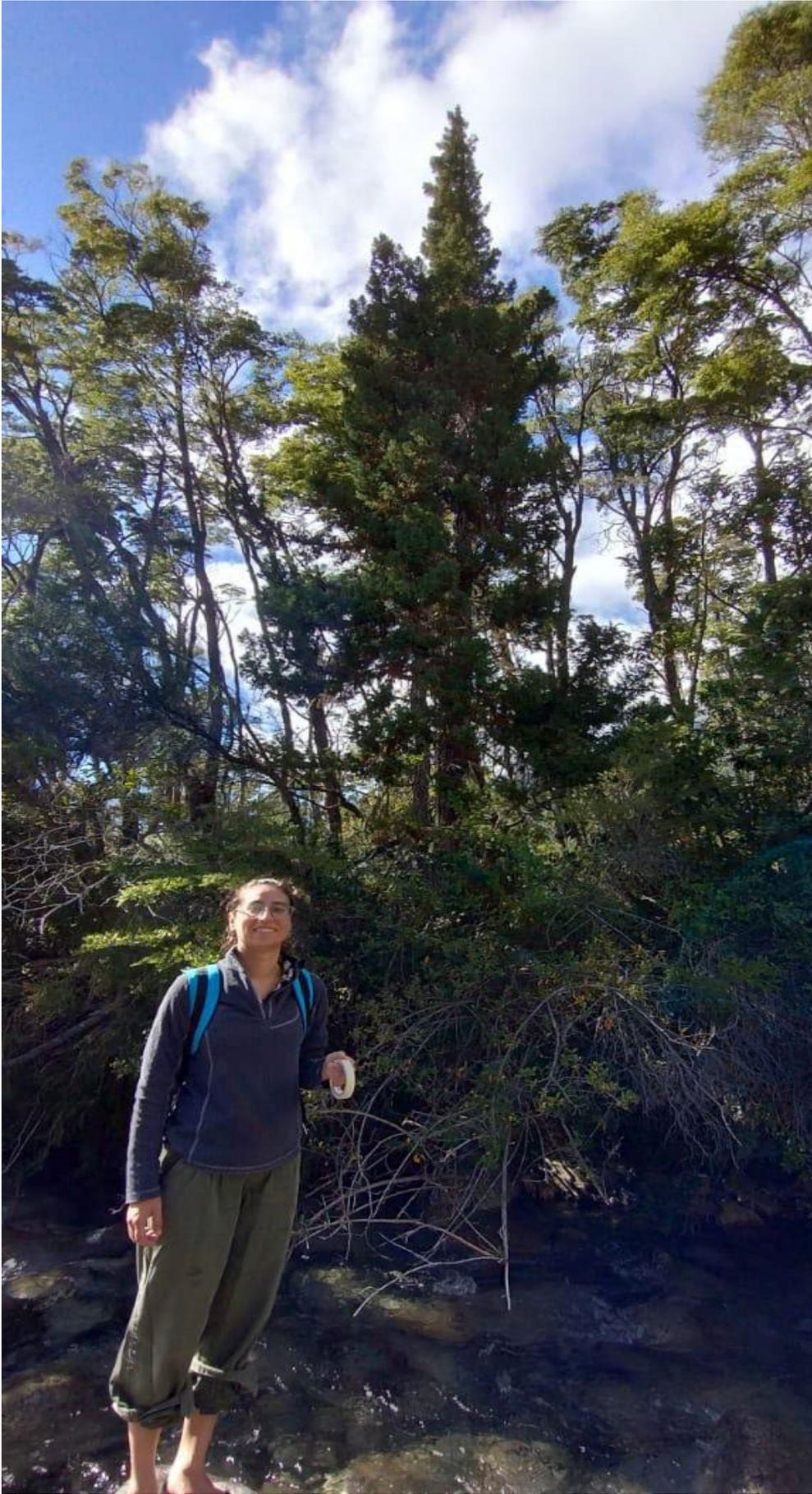
Acces to this remote area was facilitated from private owner at Lago Escondido, which supported our research providing lodging and transport. Given their interest in our work, they created a video explaining our study and its significance (<https://www.instagram.com/tv/CbYjVv2AcNM/>).



Corcovado private site in Chubut. Natural population in good conditions.



Adult individual of Alerce. Lago Escondido site.



Individuals of riparian Alerce in Lago Puelo National Park.

Three peat bogs in excellent conservation conditions, with reproductive structures and juveniles, were identified as sites where both species coexist within native forest surroundings.



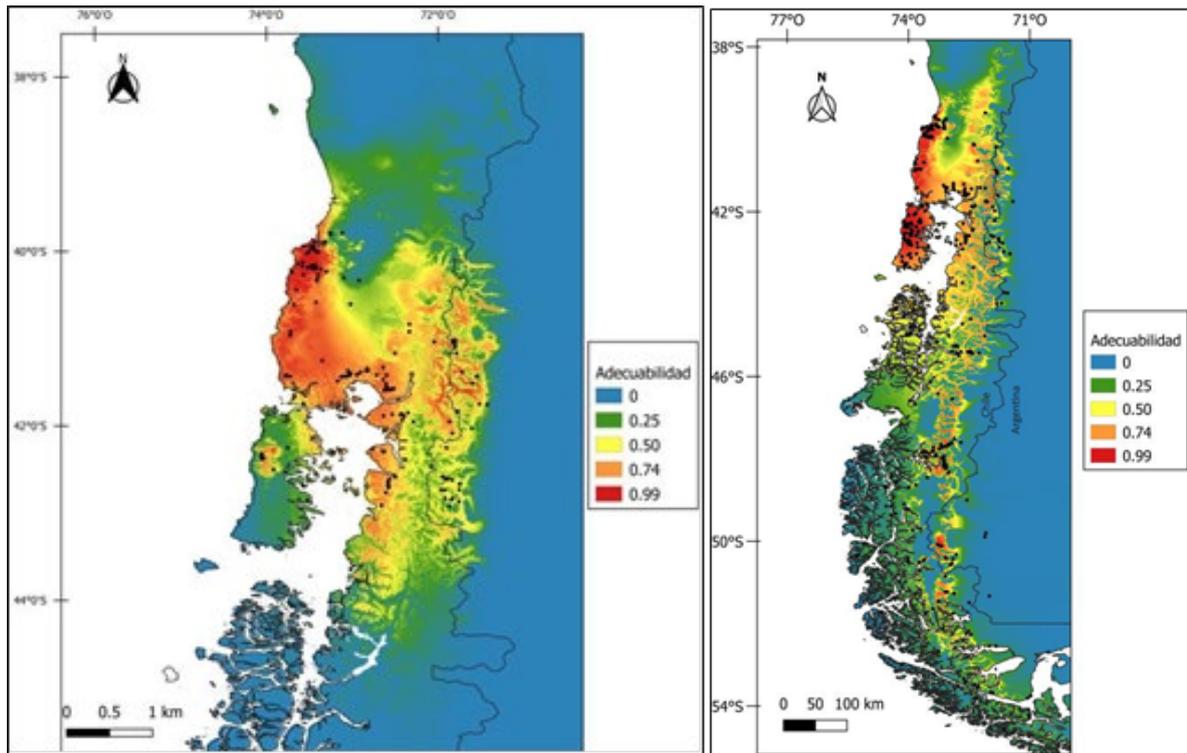
Private site in Cordon Serrucho, Rio Negro province. Forest where the tree species of Cupressaceae coexist.



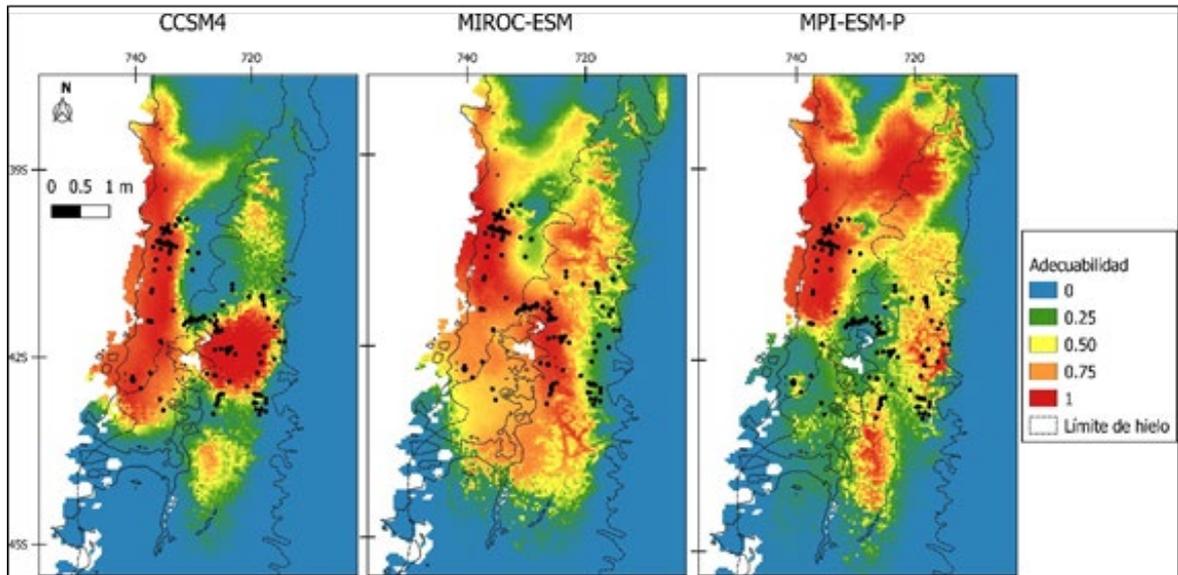
Peatbog of Puerto Blest, in Nahuel Huapi National Park. Place where the two species coexist.

Ecological niche models in *Pilgerodendron* and *Fitzroya*

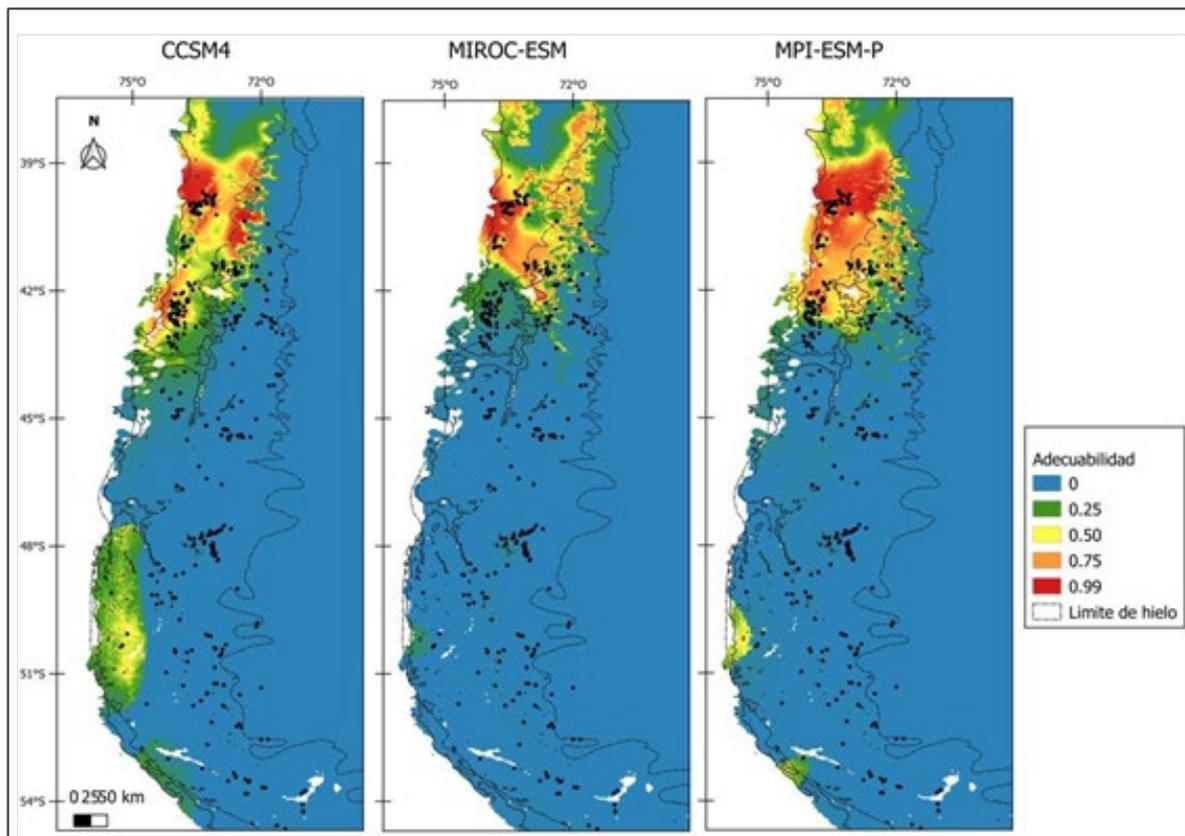
We conducted ecological niche modelling for *Pilgerodendron* and *Fitzroya*, assessing the degree of overlap, and projected their distributions into the past, where we can see that there was an expansion from the last glacial maximum (LGM) to the present. The degree of niche overlap, assessed using the Schoener's D index, yields an observed value of equivalence $D=0.204$, suggesting that the species have differentiated ecological niches. Future projections are underway.



a) b)
 Current distribution models of a) alerce and b) Guaitecas cypress.



Niche modeling distribution of alerce during the last glacial maximum with three different scenarios.



Niche modeling distribution of Guaitecas cypress during the last glacial maximum with three different scenarios.

Study on *Austrocedrus chilensis*, common name 'cipres'

The hypothesis tested in that plants inhabiting contrasting physical conditions might develop local adaptations overriding the homogenizing effects of gene flow. Hypotheses of local adaptation on phenotypic, genomic, and environmental variation under extreme precipitation regimes were analyzed on cipres. We combined common garden experiments on progeny and genotype-to-environment association analysis. Samples consisting of seeds and leaves from adult trees were collected from contrasting dry (DF) and humid (HF) forests along a steep but short precipitation gradient. Seeds were germinated and seedlings were grown under common gardens for 24 months. DNA was extracted from 75 randomly selected trees from DF and HF, and genotyped by sequencing to obtain single nucleotide polymorphisms (SNPs). Seedlings from HF outgrew DF ones suggesting genetically-based differences. Twenty-four outlier SNP loci differed between DF and HF, whereas neutral genes (3,242) showed high levels of admixture.

Eight out of the 24 outlier SNPs aligned with transcripts, half of them related to drought stress responses, and PCA identified four precipitation-related marker-climate associations. Quantitative and genomic traits suggested that natural selection maintains divergence under contrasting climatic conditions regardless high gene flow. This underscores the importance of dry forests as reservoirs of drought-tolerant variants to cope with forecasted climate change.

Regarding the common garden experiment, greenhouse-grown seedlings from both humid and dry forests (seeds collected from adult trees that presented genomic differences explained above) were maintained under controlled conditions and where survival and

growth patterns were monitored. A set of those plants were subjected to a water stress experiment, while another set was kept under irrigation as control. After two months of treatment physiological responses of seedlings were measured. The stomatal conductance, relative soil humidity, water potential and photosystem damage. The results were presented at the Ecology meeting (RAE 2023).



Austrocedrus humid forest site.



Austrocedrus dry forest site.



Seedlings from dry and humid sites growing under common garden conditions.

Presentation of results at scientific meetings. Posters are enclosed as pdf files:

Diaz, D.G., R.R. Ripa, P. Mathiasen & A.C. Premoli. 2023. Diversidad genómica y su relación con factores climáticos en Ciprés de Guaitecas. RAE XXX, 17-20 octubre, Bariloche.

Fasanella, M., Varela, S., Souto, C.P., Tripaldi, A. Premoli, A.C. & T. Kitzberger. 2023. Mecanismos fisiológicos de aclimatación a la sequía en plántulas de Austrocedrus. RAE XXX, 17-20 octubre, Bariloche.

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