

Functional Connectivity of the California Bulrush (*Schoenoplectus californicus*) in Central-Western Wetlands in Mexico

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ABSTRACT

Wetlands are natural links between terrestrial and aquatic ecosystems maintaining biodiversity at regional and landscape levels. Wetland conservation in Mexico could benefit from understanding functional connectivity for aquatic plants. This is an ongoing research project aimed at identifying the landscape factors that determine gene flow and genetic diversity of *Schoenoplectus californicus* in the highland wetlands of Central-Western Mexico. Between 10 to 30 leaf tissue samples from *S. californicus* individuals were collected in natural and artificial wetlands in the states of Michoacán, Guanajuato, and Jalisco for genomic DNA extraction. A total of sixteen nuclear microsatellite markers developed for *S. americanus* will be transferred and genotyped to estimate genetic diversity and differentiation and to statistically test their relationship with landscape elements. To date, 419 individuals from 27 sites have been sampled from 11 wetlands. Six out of 16 microsatellites have been transferred. The results of this work will guide the design of wetland management and conservation strategies at the landscape scale, particularly in anthropogenic landscapes where wetlands are at high risk.

RESUMEN

Los humedales actúan como conectores naturales entre los ecosistemas terrestres y acuáticos, y mantienen la biodiversidad a escalas regional y del paisaje. La conectividad funcional ha sido poco estudiada en plantas acuáticas a pesar de su potencial para informar la conservación de humedales en la región neotropical. Esta investigación en curso tiene como objetivo identificar los elementos del paisaje que determinan el flujo de genes y la diversidad genética de *Schoenoplectus californicus* en los humedales del Centro-Occidente de México. Se colectarán entre 10 a 30 muestras de tejido foliar de individuos de *S. californicus* en humedales naturales y artificiales en los estados de Michoacán, Guanajuato y Jalisco para extraer ADN genómico. Un total de dieciséis microsatélites nucleares desarrollados para *S. americanus* serán transferidos y genotipados para estimar la diversidad y diferenciación genética, y para

probar estadísticamente su relación con los elementos del paisaje. Al presente se han registrado 27 sitios de colectas, discriminados en 11 humedales independientes, obteniendo un total de 419 individuos. Se han transferido 6 de los 16 microsatélites con buena amplificación y especificidad. Los resultados de este trabajo guiarán el diseño de estrategias de manejo y conservación de humedales a escala de paisaje, particularmente en paisajes antrópicos donde los humedales están en gran riesgo.

INTRODUCTION

Freshwater wetlands act as natural connectors between terrestrial and aquatic ecosystems and sustain biodiversity and human well-being by supplying ecological services (Schofield et al. 2018; Lobato-de Magalhães et al. 2020). Freshwater wetlands are vulnerable to many factors, such as changes in land use, pollution, and climate change, and recent estimates of wetland loss report that since 1700, 21% of the world's wetlands have been lost. (Fluet-Chouinard et al. 2023). Maintaining functional connectivity of freshwater wetlands is of high importance for preserving biodiversity and ecosystem functions (Babu 2023). For plants, functional connectivity involves the effective dispersal of seeds and pollen between habitat patches, their interaction with dispersal vectors, the landscape elements, and the environment (Auffret et al. 2017).

The study of functional connectivity through genetic methods allows the identification of genetically isolated and depauperate populations, as well as the landscape elements that facilitate or restrict gene flow. For example, de la Luz Perez-Garcia et al. (2023) characterized the genetic diversity of a woody bamboo (*Guadua velutina* Londoño & L.G. Clark) in the rivers of the Huasteca Potosina in Mexico and found that the river network functions as the primary corridor for gene flow. Moreover, Lobato-de Magalhães et al. (2020) assessed the functional connectivity of the yellow floating heart (*Nymphoides fallax* Ornduff) in geographically isolated temporary wetlands in central Mexico and found that connectivity was associated with genetic diversity when wetlands were within 5 km, while forest cover sustains gene flow. Thus, conducting studies on functional connectivity using genetic data in aquatic plants is crucial for the conservation planning of wetlands in human-modified landscapes (Lozada-Gobilard et al. 2021).

Mexico's wetlands are home to a wide variety of aquatic plants (Lobato-de Magalhães et al. 2020), of which *Schoenoplectus californicus* (C.A. Mey.) Soják (Cyperace-

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ae) is a characteristic plant of the central-western wetlands. Native from Southern California to Patagonia (Tropicos 2023), it is a perennial, polyploid sedge (with 32 to 68 chromosomes) that reproduces sexually and vegetatively (Tena-Flores et al. 2014; Kettenring et al. 2019). The species has been used for construction materials, handicrafts, and phytoremediation, and as a nesting site and food source for migratory birds (Tiner 2014; Hidalgo-Cordero et al. 2020). Wetlands in Central-Western Mexico face significant threats, including declining water levels, contamination from untreated wastewater, the presence of livestock, and the proliferation of invasive plant species. These factors jeopardize the ecological and economic benefits that these wetlands provide to local communities. Hence, it is imperative to study functional connectivity in this region to develop effective conservation strategies and identifying hub populations of *S. californicus* necessary for preserving genetic diversity and functional connectivity in the Central-Western Mexico wetland system.

Therefore, the objective of this research is to assess the genetic diversity and functional connectivity of *S. californicus* in freshwater wetlands in Central-Western Mexico. Specifically, our questions are: (1) Do large and well-connected wetlands harbor higher genetic diversity for *S. californicus*? (2) Do landscape elements explain functional connectivity of *S. californicus*?

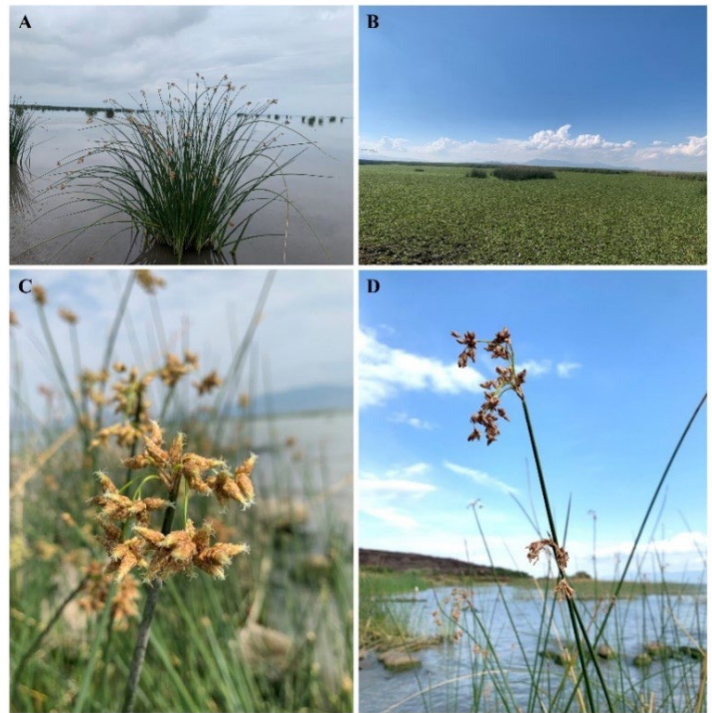


Figure 1. Photographs of the species *Schoenoplectus californicus* in (A) Lake Chapala, Jalisco, (B) Lake Cuitzeo, Michoacán, where the presence of the invasive species *Pontederia crassipes* (syn. *Eichhornia crassipes*) is evident, (C–D) and close-up of some specimens where the inflorescence of the study species is observed.

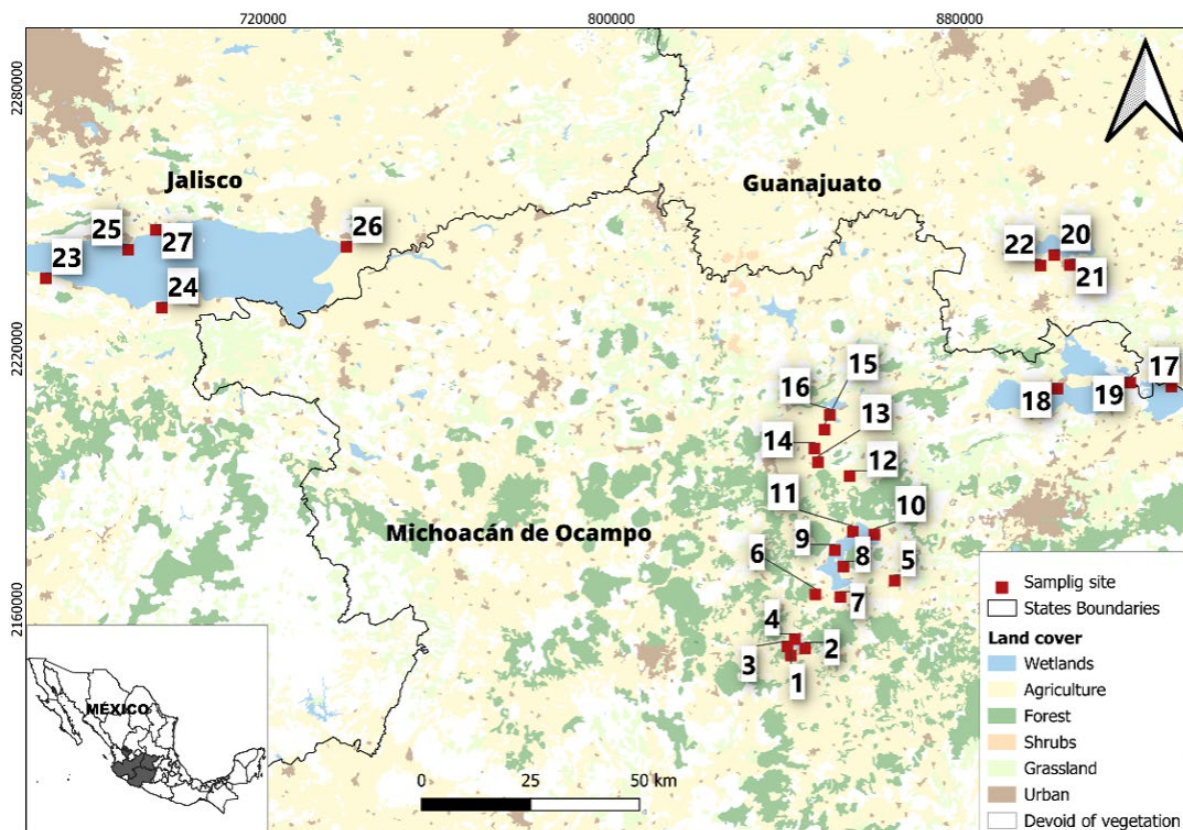


Figure 2. Geographic distribution of *S. californicus* populations in the freshwater wetlands of Central-Western Mexico, with land use and vegetation characterization. The red dots represent collection sites. 1-4: Zirahuén Lagoon; 5,6,8-11: Pátzcuaro Lake; 7: El Jagüey Lake; 12: Tunguitiro Lagoon; 13: Bellas Fuentes; 14: Hierbabuena; 15: Laguna; 16: Copándaro; 17-19: Cuitzeo Lake; 20-22: Yuriria Lake; 23-27: Chapala Lake.

MATERIAL AND METHODS

The study area includes natural and man-made freshwater wetlands in Michoacán, Guanajuato, and Jalisco states (Figure 2). A systematic sampling approach was employed considering between 10 to 30 individuals per site. Individuals were collected from both the edge and center of each wetland to ensure genetic variability within each. Leaf tissue samples were collected and stored in silica gel. Botanical vouchers were deposited on the IEB INECOL Herbarium.

Genomic DNA was extracted using the CTAB technique (Wagner et al. 1987). Sixteen microsatellite markers specific to *Schoenoplectus americanus* will be transferred to *S. californicus* (Blum et al. 2005). Microsatellite products will be observed by 1% agarose gel electrophoresis. Methods to amplify and genotype microsatellites will follow the protocols of Lobato-de Magalhães et al. (2020). All genetic analysis procedures will be performed at the Laboratory of Genetics and Molecular Biology of the Centro Regional del Bajío Instituto de Ecología, Pátzcuaro, Mexico.

We will calculate genetic diversity parameters (e.g., H_E , H_O , F_{IS} , F_{ST} , population-specific F_{ST}) using SPAGeDi V.1.5a, which allows the use of codominant polyploid data. To evaluate genetic structure, we will perform a Bayesian clustering algorithm implemented in STRUCTURE V.2.3.4, and a multivariate discriminant analysis (DAPC) using *adegenet* in R V.4.2.1.

Connectivity network analyses will be performed using a graph theory approach to estimate wetland connectivity by parameterizing several structural connectivity indices that consider the geographical distance among wetlands (e.g., Hanski index, integral connectivity index, connectivity probability index) using *igraph* in R V.4.2.1. Site variables considered will be wetland size, perimeter, depth, and the 19 bioenvironmental variables (Worldclim) and presence of agriculture around the wetland. These site parameters and the wetland connectivity indices will be related against estimates of genetic diversity and population-specific F_{ST} using linear regression approaches. To evaluate the effects of landscape elements on gene flow, we will construct several landscape layers using the aridity index (CGIARCSI), forest and agriculture covers, the hydrological-network, and slope. These landscape layers will be optimized using a resistance-surface approach in *ResistanceGA* and their association with estimates of F_{ST} will be evaluated through a model selection approach using linear mixed effects models with maximum likelihood population effects (MPLE).

WORK TO DATE AND PRELIMINARY FINDINGS

We have sampled 27 sites with the presence of *S. californicus* within 11 wetlands (Figure 2). Chapala and Cuitzeo lakes (266 km) are the wetlands with the greatest distance

between them. The wetlands with the shortest distance are Bellas Fuentes and Hierbabuena with 3 km. Wetlands like Lake Pátzcuaro, Cuitzeo, and Chapala have been distinguished by high anthropogenic pressure in their surroundings and the presence of invasive aquatic plants such as the species *Pontederia crassipes* Mart., commonly known as “water lily”. DNA extractions have been carried out for all sampling sites, and six nuclear microsatellites from the species *S. americanus* have been transferred, showing specificity and good amplification for *S. californicus*.

Changes in land use and vegetation can impact the composition and structure of the landscape, which in turn can influence the flow of organisms and their genes (Echeverría et al. 2014). Land use and vegetation in our study area have been heavily modified, with agriculture being the primary anthropogenic activity covering extensive areas of land around the wetlands under investigation in the states of Michoacán, Guanajuato, and Jalisco.

EXPECTATIONS

The study on the functional connectivity of *Schoenoplectus californicus* in freshwater wetlands in Central-Western Mexico will allow us to characterize the patterns of genetic diversity and structure for this aquatic plant. It will also result in the development of a functional connectivity model that integrates environmental, genetic, and landscape data. This landscape genetics approach as a conservation tool may contribute to the formulation of conservation actions to protect an ecosystem that is rapidly disappearing.

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