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NOTE

Preliminary evidence of the demographic history of *Xystreurys rasilis* (Pleuronectiformes: Paralichthyidae) in the southwestern Atlantic as inferred from mtDNA

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ABSTRACT. *Xystreurys rasilis* (Jordan, 1891) is a flounder species from the Southwestern Atlantic, particularly abundant in the high-salinity waters of the Buenos Aires Coastal Ecosystem and San Matías Gulf. It primarily feeds on benthic invertebrates and utilizes local frontal systems as breeding and nursery grounds. Analysis of a 406 bp fragment of the mitochondrial control region revealed high haplotype ($h = 0.994$) and nucleotide ($\pi = 0.022$) diversities. Genetic divergence between Necochea and San Antonio Oeste was low and not significant, with 18 unique haplotypes and no evident phylogeographic structure. Demographic analyses indicated long-term population stability, with the most recent common ancestor estimated at ~ 148,000 years ago. The results do not provide evidence for the existence of two discrete genetic units. Nevertheless, it is possible that the species experienced historical isolation followed by secondary contact between different lineages of the flounder *X. rasilis*.

Key words: Genetic diversity, Last Glacial Maximun, flatfish.

Evidencia preliminar de la historia demográfica de *Xystreurys rasilis* (Pleuronectiformes: Paralichthyidae) en el Atlántico Sudoccidental según se infiere del ADNmt

RESUMEN. *Xystreurys rasilis* (Jordan, 1891) es una especie de lenguado del Atlántico Sudoccidental, particularmente abundante en las aguas salinas del Ecosistema Costero Bonaerense y del Golfo San Matías. Se alimenta principalmente de invertebrados bentónicos y utiliza los sistemas frontales locales como áreas de reproducción y crianza. El análisis de un fragmento de 406 pb de la región control mitocondrial reveló una alta diversidad haplotípica ($h = 0{,}994$) y nucleotídica ($\pi = 0{,}022$). La divergencia genética entre Necochea y San Antonio Oeste fue baja y no significativa, con 18 haplotipos únicos y sin estructura filogeográfica evidente. Los análisis demográficos indicaron estabilidad poblacional a largo plazo, con el ancestro común más reciente estimado en ~ 148.000 años. Los resultados no aportan evidencia de la existencia de dos unidades genéticas discretas. No obstante, es posible que la especie haya sufrido un aislamiento histórico seguido de contacto secundario entre distintos linajes del lenguado *X. rasilis*.

Palabras clave: Diversidad genética, Último Máximo Glacial, lenguado.

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Xystreurys rasilis (Jordan, 1890) (Pleuronectiformes: Paralichthyidae) is a flounder species distributed in the southwestern Atlantic between 23° S and 47° S (Fabré and Díaz de Astarloa 1996; Díaz de Astarloa and Munroe 1998), ranging from Rio de Janeiro (Brazil) to San Jorge Gulf (Argentina). *Xystreurys rasilis* is a small- to medium-sized flounder (20 cm) that feeds exclusively on benthic invertebrates, with small crabs constituting its primary prey (Díaz de Astarloa and Munroe 1998; Cousseau and Perrotta 2013). This species is of commercial importance, yet it represents about 6% of the flounder landings in the region (Fabré and Díaz de Astarloa 2001; Díaz de Astarloa 2002; Rico 2010). *Xystreurys rasilis* is most abundant in high-salinity waters at 50-100 m in the Buenos Aires Coastal Ecosystem (BCE) (Díaz de Astarloa 2002; Díaz de Astarloa and Fabré 2003) and at 50-90 m in San Matías Gulf (SMG) (Perier and Di Giácomo 2002) (Figure 1). According to the IUCN, *X. rasilis* is currently classified as a species of Least Concern (Riestra and Díaz de Astarloa 2020).

Within the BCE, two groups have been identified: one north of 36° S and another south of 37° S, the latter being considerably more abundant (Fabré et al. 2001). In addition, parasitological evidence indicates that at least two stocks of *X. rasilis* inhabit the coastal and shelf waters of the BCE: a northern group between 34° S and 38° S, and a southern group between 38° S and 41° S, in the area known as 'El Rincón' (39° S-41° S) (Alarcos and Timi 2013). These stocks have been proposed as discrete management units to promote the sustainable exploitation of the resource (Alarcos and Timi 2013). Seasonal migrations have also been reported: in autumn, individuals move to deeper waters for feeding and growth, while in spring, mature specimens migrate toward the coast and further south (Díaz de Astarloa 2002). Both groups converge south of Mar del Plata (38° S) to reproduce (Fabré et al. 2001). In these areas, variations in water masses generate temperature and salinity fronts with key biological functions (Acha et al. 2004), acting as breeding and nursery grounds for several fish species by enhancing larval retention (Piola and Rivas 1997). In addition, Alarcos and

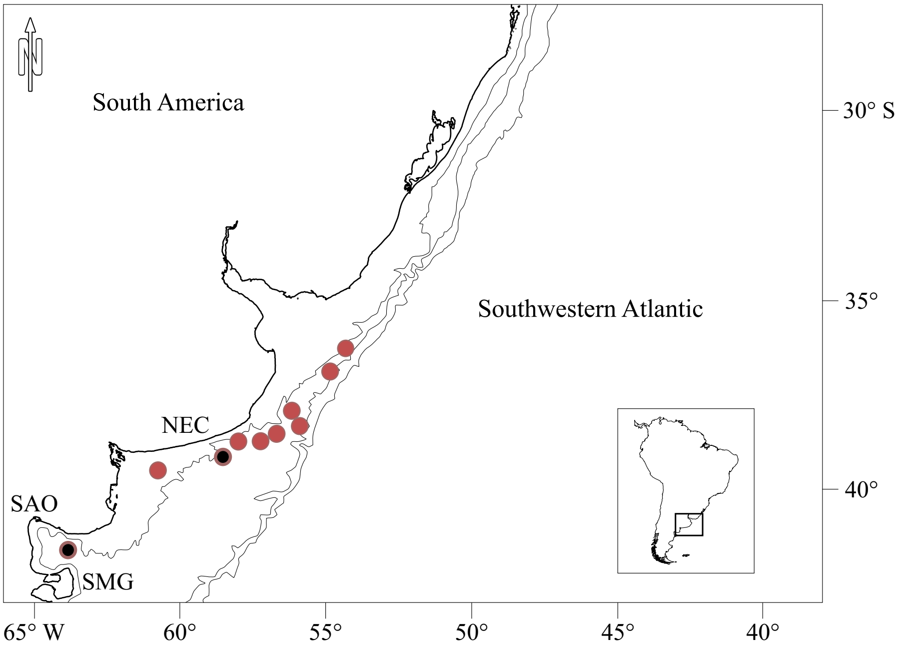

Figure 1. Main concentrations of *Xystreurys rasilis* in the Buenos Aires Coastal Ecosystem (modified from Fabré and Díaz de Astarloa 1996; Díaz de Astarloa and Fabré 2003), showing sampling sites (black circles). Lines on the continental shelf indicate depths of 50, 100, and 200 m. Necochea (NEC) and San Antonio Oeste (SAO) in San Matías Gulf (SMG).

Timi (2013) suggested incorporating samples from southern areas of 'El Rincón' to assess whether the southern stock moves toward these latitudes during the warm season. The spawning area for this stock could be located at SMG, a north Patagonian gulf located in the southern limit of 'El Rincón' (Figure 1). This hypothesis is supported by the observed increase in the relative abundance and peak reproductive activity of *X. rasilis* in this gulf during the warm season (Perier and Di Giácomo 2002).

The aim of this study was to test the hypothesis that samples from Necochea ('El Rincón') and San Antonio Oeste (SMG) constitute distinct genetic units. Samples of *X. rasilis* were obtained from landings of the Argentine coastal fleet in the coastal waters off Necochea (NEC; 38° 40′ S, 58° 37′ W; 12 specimens) and from San Antonio Oeste (SAO) in SMG (41° 30′ S, 64° 15′ W; 7 specimens) (Figure 1). Muscle tissue samples were preserved in ethanol. Amplification of a mitochondrial control region fragment was performed using the universal primers developed by Lee et al. (1995): A (5' TTCCACCTCTAACTCCCAAAGCTAG 3') and E (5' CCTGAAGTAGGAACCAGATG 3'), following the protocols described by Fainburg et al. (2022). Sequences were manually edited using Proseq v.2.91 (Filatov 2002) and aligned using ClustalX2 (Larkin et al. 2007). *Xystreurys rasilis* sequences of the control region were deposited in GenBank (access no: PP506082- PP506065).

Haplotype diversity (*h*) and nucleotide diversity (π) for the mitochondrial control region fragment were estimated using DnaSP (Librado and Rozas 2009). Haplotype networks were constructed using the Maximum Parsimony method with Haplotype Viewer (Greg B. Ewing –http://www.cibiv.at/~greg/haploviewer.shtml). Genetic divergence between populations (F_{ST}) was estimated with 10,000 permutations in ARLEQUIN (Excoffier and Lischer 2010). Deviations from neutrality were assessed with Tajima's D (Tajima 1983) and Fu's F (Fu 1997) using DnaSP (Ramos-Onsins and Rozas 2002). Demographic history was further investigated through mismatch distributions, which represent the distribution of pairwise differences among haplotypes (Rogers and Harpending 1992). This approach can discriminate between sudden population expansion and long-term stability. Expansion times and demographic changes in effective population size, weighted by generation time (Ne t), were inferred using the Bayesian Skyline Plot model in BEAST 1.7 (Drummond et al. 2012). Analyses were performed under a relaxed molecular clock with an intra-specific substitution rate of 6%/Ma, as reported for marine fishes (see Fainburg et al. 2022, 2025). To account for uncertainty, results are also presented assuming mutation rates of 4%/Ma and 8%/Ma. Three independent Markov Chain Monte Carlo (MCMC) runs of 10 million steps each were performed, with trees and parameters sampled every 1,000 steps. Convergence was verified in Tracer, ensuring Effective Sample Sizes > 200. Only one representative run is shown, with 95% confidence intervals also reported (Drummond et al. 2012).

A 406-bp fragment of the mitochondrial control region was sequenced in *Xystreurys rasilis* from Necochea (N = 12) and San Antonio Oeste (N = 7). Haplotype diversity was extremely high ($h = 0.994$), and nucleotide diversity was also elevated at both sampling sites ($\pi = 0.022$) (Table 1). Tajima's D was negative but not significant, whereas Fu's F test yielded a negative and highly significant value (Table 1), suggesting population expansion. Genetic divergence between NEC and SAO was low and not statistically significant ($F_{ST} = 0.050$, $p = 0.105$). Among the 19 individuals of *X. rasilis* analyzed, 18 distinct haplotypes were identified: 11 in NEC and 7 in SAO with one haplotype shared by two individuals from NEC (Figure 2). The haplotype network differed markedly from those reported for other flatfish species of the region (Fainburg et al. 2025), as no haplotypes were shared among populations, all haplotypes were separated by multiple mutational steps, and no clear phylogeographic structure was detected (Figure 2). *Xystreurys rasilis* exhibited a demographic signal consistent with a bimodal pairwise mismatch distribution (Figure

Table 1. Genetic variability and demographic parameters of the Control Region in *Xystreurys rasilis* (406 bp). n = number of individuals; Nh = number of haplotypes; *h* = haplotypic diversity; π = nucleotidic diversity; sd = standard deviation; *D* = Tajima's D; *F* = Fu's F. *P < 0.05; **P < 0.01. NEC: Necochea; SAO: San Antonio Oeste

Site	n	Nh	*h* ± sd	π ± sd	*D*	*F*
NEC	12	11	0.985 ± 0.040	0.023 ± 0.002	-0.847	-3.053*
SAO	7	7	1.000 ± 0.076	0.019 ± 0.003	-0.765	-2.042*
Total	19	18	0.994 ± 0.019	0.022 ± 0.002	-1.262	-9.157**

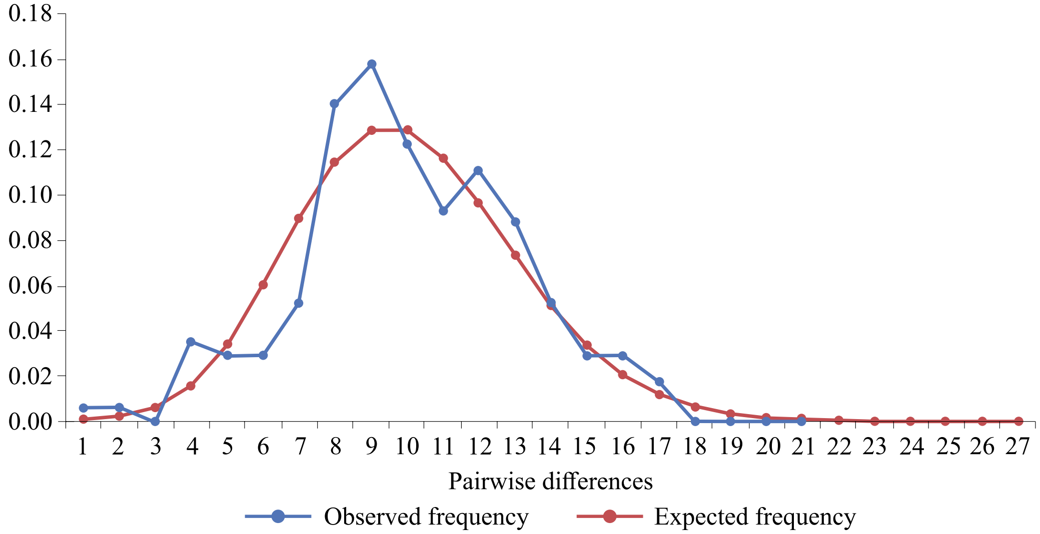

Figure 2. Mismatch distribution based on the mitochondrial control region of *Xystreurys rasilis*.

3), a pattern typically explained as evidence for two distinct lineages. In addition, fishes showing high haplotype and nucleotide diversities suggest a large and stable population with an extended evolutionary history, possibly resulting from historical isolation followed by secondary contact between differentiated allopatric lineages (Grant and Bowen 1998). Coalescent analyses further suggested a deep evolutionary history, consistent with long-term population stability with the most recent common ancestor dating to ~ 148,000 years before present assuming a mutation rate of 6%/Ma (98,000-222,000 years before present considering mutation rates of 4%/Ma and 8%/Ma respectively), accompanied by a gradual increase in effective population size over this period (Figure 4).

Xystreurys rasilis appears to have been little affected by the last glacial maximum (LGM) around 18.000-23.000 years ago, exhibiting high genetic variability, large effective population sizes, and an evolutionary history indicative of habitat stability. However, the haplotype network showed that most individuals differ by five to seven mutational steps, corresponding to the 2% (π) sequence divergence observed among haplotypes pairs (Table 1). This network seems to support the occurrence of secondary contact between at least two old genetic lineages, possibly driven by the seasonal migrations

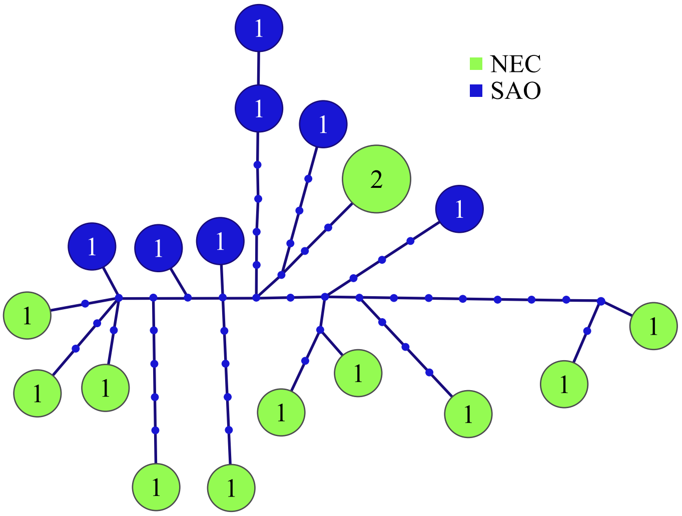

Figure 3. Mitochondrial DNA haplotype networks based on the control region of *Xystreurys rasilis* from the Buenos Aires Coastal Ecosystem, including samples from Necochea (NEC) and San Antonio Oeste (SAO). Circle size represents haplotype frequency, and each line denotes a mutational step (dots) separating haplotypes.

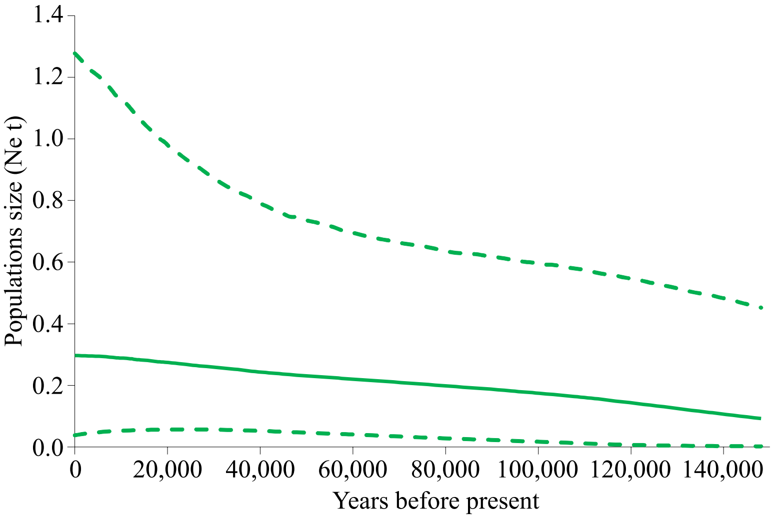

Figure 4. Bayesian Skyline Plot based on the mitochondrial control region of *Xystreurys rasilis* showing the demographic history of the species. The y-axis represents the product of effective population size (Ne) and generation time (t). Dashed lines indicate 95% confidence intervals.

of *X. rasilis* and the post-LGM coastline habitats evolution. This pattern suggests that individuals from 'El Rincón' and the San Matías Gulf may have been historically isolated yet exhibit significant contemporary connectivity.

This genetic evidence should be interpreted cautiously given the limited sample size; nevertheless, we must assume that these results, using only a mitochondrial marker, need to be further analyzed with a codominant marker such as microsatellite loci or genomic SNP's (such as RAD-seq technology).

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Data availability

DNA sequences generated in this study were submitted to GenBank. The control region sequences of *X. rasilis* were deposited in GenBank (access no: PP506082- PP506065).

Conflict of interest

The authors declare no competing interests.

Ethical approval

The authors declare that no ethical approval was required for the writing of this article.

Author contributions

Leandro A. Fainburg: conceived and designed the experiment; generated data; conducted the primary data analysis; and contributed to the writing of the manuscript. David E. Sabadin: helped in data collection and wrote the initial draft of the manuscript. Pedro Fernández Iriarte: agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved; drafted the final version of the manuscript. All authors reviewed and approved the manuscript.

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