

Supplementary materials

Molecular evidence for a new endemic species of *Acartia* (Copepoda, Calanoida) from the Southeast Pacific coast.

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Supplementary Tables

Table S1. Mean general pairwise values of differentiation (Φ_{st}) between populations. Values above the diagonal correspond to nuclear 18s and under diagonal correspond to mitochondrial COI. Statistically significant comparisons (after Bonferroni correction) and 100,000 iterations are shown in bold.

COI/18s

Locality	Antofagasta	Tongoy	Concepcion	Valdivia	Hualaihue	Magallanes
Antofagasta		0.27299	0.21905	0.09107	0.34529	
Tongoy	-0.06660		0.15219	0.09278	0.21413	
Concepcion	0.49874	0.48394		0.12066	0.27556	
Valdivia	0.39977	0.41852	0.05458		0.15680	
Hualaihue	0.02626	0.04501	0.61516	0.52739		
Magallanes	0.65406	0.67874	0.48234	0.54334	0.80898	

Biogeographic zone	Peruvian	Intermediate	Magellanic
Peruvian		0.04409	0.23344
Intermediate	0.42608		0.13725
Magellanic	0.00947	0.43785	

Table S2. Main environmental data conditions at the sites where populations studied were sampled. The mean and annual range for each environmental factor are presented. Annual mean $\pm$ standard deviation ($\pm$ S.D.) are presented for Hualaihue and Magallanes.

Locality	Temperature (°C)		Salinity (psu)		Oxygen (mL L ⁻¹)		CO ₂ (uatm)		Survey water depth (m)
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	
Antofagasta ¹	15.0	14.0-19.0	34.8	34.4-35.0	4.0	1.0-7.0	600.0	360.0-1000.0	15
Tongoy ²	13.4	12.0-18.0	34.6	34.4-34.7	4.8	0.8-8.0	530.0	360.0-710.0	15
Concepcion ³	12.0	11.0-14.0	33.8	32.5-34.5	4.0	2.0-6.0	-	-	10
Valdivia ^{4,5}	11.0	10.5-12.0	31.0	30.0-32.5	5.0	3.0-8.0	-	400.0-1000.0	12
Hualaihue ⁶	12.5 $\pm$ 1.9	9.3-17.8	30.0 $\pm$ 0.9	27.0-32.3	6.1 $\pm$ 0.9	3.7-8.7	542.8 $\pm$ 208.6	171.4-1417.9	5
Magallanes ⁷	8.4 $\pm$ 0.8	-	30.7 $\pm$ 0.3	-	8.9 $\pm$ 0.4	-	414.9 $\pm$ 33.9	-	Surface

The data of environmental conditions were obtained from published studies and the data for Hualaihue and Magallanes come from measurements that are in progress and whose data are in the process of publication.

- 1.- Escribano, R., Daneri, G., Farías, L., Gallardo, V. A., González, H. E., Gutiérrez, D., ... & Braun, M. Biological and chemical consequences of the 1997–1998 El Niño in the Chilean coastal upwelling system: a synthesis. *DEEP-SEA RES PT II* **51**(20-21), 2389-2411 (2004).
- 2.- Saavedra, L. M., Saldías, G. S., Broitman, B. R., & Vargas, C. A. Carbonate chemistry dynamics in shellfish farming areas along the Chilean coast: natural ranges and biological implications. *ICES J. Mar. Sci.* **78**(1), 323-339 (2021).
- 3.- Sobarzo, M., Bravo, L., Donoso, D., Garcés-Vargas, J., & Schneider, W. Coastal upwelling and seasonal cycles that influence the water column over the continental shelf off central Chile. *Prog. Oceanogr.* **75**(3), 363-382 (2007).
- 4.- Garcés-Vargas, J., Ruiz, M., Pardo, L. M., Nuñez, S., & Pérez-Santos, I. Hydrographic features of Valdivia river estuary south-central Chile. *Lat. Am. J. Aquat. Res.* **41**(1), 113-125 (2013).
- 5.- Osma, N., Latorre-Melín, L., Jacob, B., Contreras, P. Y., von Dassow, P., & Vargas, C. A. Response of phytoplankton assemblages from naturally acidic coastal ecosystems to elevated pCO₂. *Front. Mar. sci.* **7**, 323 (2020).
- 6.- Vargas et al. Unpublished data generated from Coastal environmental monitoring, Coastal Social-Ecological Millennium Institute (SECOS), Universidad de Concepción & P. Universidad Católica de Chile, Chile.
- 7.- Giesecke et al. Unpublished data generated from EQM190013 ANID project, Environmental monitoring of fjords and canals of Magallanes region, Universidad Austral de Chile, Chile.
<https://www.starm.cl/>

Table S3. Methodological details of sampling in the studied populations.

Locality	Sampling season	Sampling year	Technique of sampling	Mesh size
Antofagasta	Spring	2015	WP2 with oblique haul	200 μ m
Tongoy	Spring	2015	WP2 with oblique haul	200 μ m
Concepcion	Spring	2012 and 2023	WP2 with oblique haul	200 μ m
Valdivia	Spring	2012	WP2 with oblique haul	200 μ m
Hualaihue	Spring	2022	WP2 with oblique haul	200 μ m
Magallanes	Spring	2022	WP2 with oblique haul	200 μ m

Table S4. Primer details and thermocycler PCR protocols for mitochondrial and nuclear markers

Mitochondrial marker

COI

700bp

Forward LCO1490: 5'-GGTCAACAAATCATAAAGATATTGG-3'

Reverse HCO2198: 5'-TAAACTTCAGGGTGACCAAAAAATCA-3'

Folmer et al. 1994

Thermocycler PCR

protocol

Temperature($^{\circ}$ C)

Time (s)

Initial denaturation

94

60

Denaturation

94

40

Annealing

48

40

32 cycles

Extention

72

40

Final extention

72

60

Final hold

4

∞

Nuclear marker

18s

1800bp

Forward 18A1mod: 5'-CTGGTTGATCCTGCCAGTCATATGC-3'

Reverse 1800mod: 5'-GATCCTTCCGCAGGTTACCTACG-3'

Raupach et al. 2009

Thermocycler PCR

protocol

Temperature($^{\circ}$ C)

Time (s)

Initial denaturation

94

60

Denaturation

94

40

Annealing

54

60

37 cycles

Extention

72

60

Final extention

72

90

Final hold

4

∞

Supplementary Figures

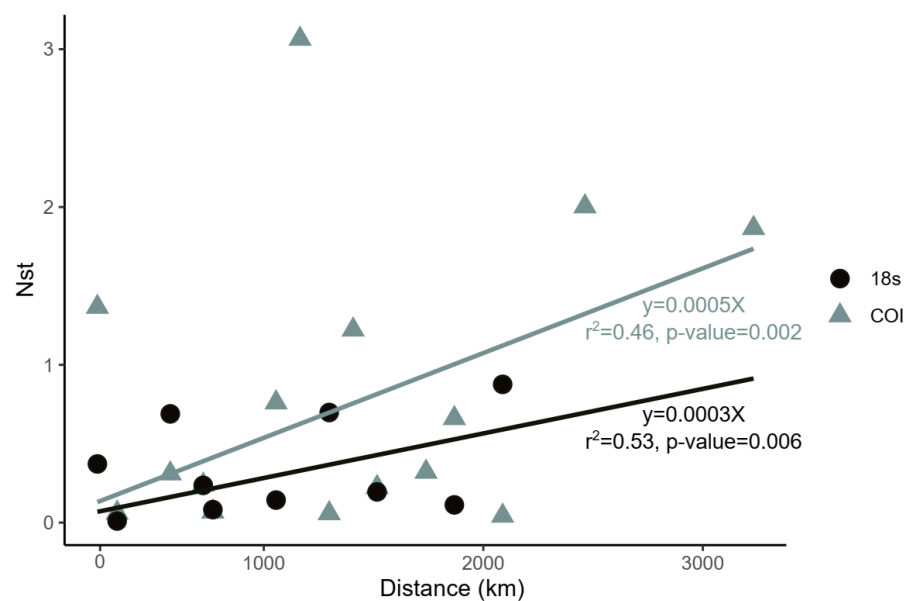


Fig. S1. Relationship between linearized genetic differentiation (N_{st}) and geographic distances among the populations studied along the Peruvian province, Intermediate area and the Magellanic province.

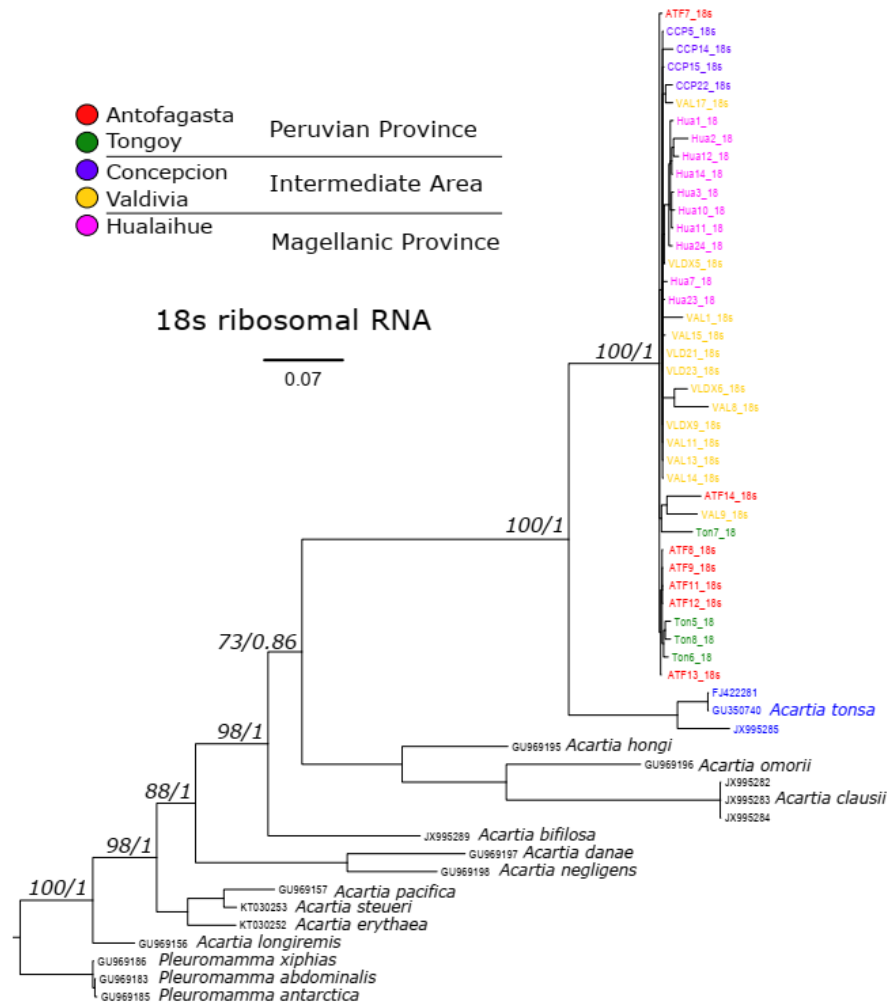


Fig. S2. Bayesian phylogeny performed with 58 sequences of species of genus *Acartia* sp., including 38 sequences obtained from the populations under study (Antofagasta, Tongoy, Concepcion, Valdivia, and Hualaihue). Maximum likelihood / Bayesian posterior probability support values were labeled in each node.