UNIVERSIDAD AUTÓNOMA DE BAJA CALIFORNIA
FACULTAD DE CIENCIAS MARINAS
INSTITUTO DE INVESTIGACIONES OCEANOLÓGICAS
POSGRADO EN OCEANOGRAFÍA COSTERA

IDENTIFICACIÓN DEL LIMITE SUR DE DISTRIBUCIÓN DEL OSTION NATIVO *OSTREA LURIDA* USANDO DOS MARCADORES DE ADN MITOCONDRIAL: 16S Y COI

TESIS

QUE PARA CUBRIR PARCIALMENTE LOS REQUISITOS NECESARIOS PARA OBTENER EL GRADO DE

MAESTRO EN CIENCIAS EN OCEANOGRAFÍA COSTERA

PRESENTA

ALFONSO FERREIRA SARRACINO

ENSENADA, BAJA CALIFORNIA, A 14 DE NOVIEMBRE DE 2024

FACULTAD DE CIENCIAS MARINAS
INSTITUTO DE INVESTIGACIONES OCEANOLÓGICAS
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PRESENTA

Alfonso Ferreira Sarracino

Aprobada por:

Dra. Alicia Abadía Cardoso
Director de Tesis

Dr. Julio Lorda Solorzano
Co-director de Tesis

Miguel Tripp Valdez

Dr. Miguel Tripp Valdez
Sinodal

Asunto: Voto aprobatorio sobre trabajo
de tesis de grado de Maestría

Dra. Ivone Giffard Mena
Coordinadora de Investigación y
Posgrado, F.C.M.
Presente

Estimada Dra. Giffard:

Me dirijo a usted en mi calidad de **Director de Tesis** encargado de revisar la tesis de Maestría presentada por el estudiante **Alfonso Ferreira Sarracino**, como parte de los requisitos para obtener el grado de **Maestro en Ciencias** en Oceanografía Costera.

Tras llevar a cabo una revisión minuciosa y exhaustiva del trabajo mencionado, es mi deber informarle que he emitido mi **Voto Aprobatorio** sobre la tesis titulada:

Identificación del límite sur de distribución del ostión nativo *Ostrea lurida* usando dos marcadores de ADN mitocondrial: 16s y COI.

He realizado esta revisión con el compromiso de asegurar que el trabajo cumple con los estándares de calidad y excelencia académica requeridos por nuestro programa de posgrado. Después de un análisis detenido, he llegado a la conclusión de que el trabajo de tesis satisface plenamente estos criterios y representa una contribución valiosa al campo de la Oceanografía Costera.

El trabajo exhibe una sólida base teórica, una metodología rigurosa y una presentación coherente de los hallazgos. Las referencias bibliográficas están actualizadas y pertinentes, y las figuras y tablas son claras y respaldan eficazmente los argumentos del texto. La sección de conclusiones proporciona un resumen sólido de los resultados y sus implicaciones, la referencias y citas están actualizadas y son pertinentes.

Ensenada, B. C., a 14 de junio de 2024

Atentamente,

Dra. Alicia Abadía Cardoso
Directora de Tesis

c.c.p. Expediente

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Atentamente,

Miguel Tripp Valdez

Dr. Miguel Tripp Valdez
Sinodal

c.c.p. Expediente

Prólogo

El presente escrito es la tesis desarrollada, en formato de borrador para artículo científico, del estudiante **Alfonso Ferreira Sarracino**, durante el período de agosto de 2021 a mayo de 2024, para cubrir parcialmente los requisitos de la **Maestría en Ciencias** del Posgrado en **Oceanografía Costera** de la **Universidad Autónoma de Baja California**.

La intención final del presente trabajo es someterlo a la revista de investigación: **Journal of Shellfish Research**, cuyo factor de impacto es de **1.3** y que sea revisado por pares para que eventualmente sea publicado.

Resumen

El Ostión Olympia (*Ostrea lurida*) es la única ostra nativa de la costa del Pacífico oriental, que abarca Canadá, Estados Unidos y la península de Baja California en México. Históricamente abundantes, las poblaciones de *O. lurida* ha disminuido significativamente debido a la sobreexplotación, la contaminación y la destrucción de hábitats. Esta disminución, junto con la naturaleza críptica de la especie, requiere el uso de herramientas moleculares para una identificación y evaluación de distribución precisas. En este estudio, se analizaron 274 ostras de siete sitios a lo largo de la costa de Baja California utilizando dos marcadores de ADN mitocondrial: 16S y Citocromo Oxidasa subunidad I (COI).

Nuestros hallazgos confirmaron la presencia de seis especies de ostras dentro de la familia *Ostreidae: Ostrea lurida, Ostrea conchaphila, Saccostrea palmula, Crassostrea gigas, Crassostrea sikamea* y *Crassostrea corteziensis*. Notablemente, *O. lurida* solo se encontró en los sitios de San Quintín, contradiciendo informes previos de su presencia en Guerrero Negro. Esta discrepancia sugiere una contracción del rango, posiblemente debido a las Olas de Calor Marino Extremas (EMH) entre 2013 y 2018. Sin embargo, la insuficiencia o mala dirección de los esfuerzos de muestreo también podría explicar la ausencia de *O. lurida en* otras zonas.

Además, el estudio destacó la presencia de *Saccostrea palmula* en Guerrero Negro, indicando una posible extensión del rango hacia el norte debido al calentamiento de las aguas. *Crassostrea corteziensis*, una especie nativa del Golfo de California se encontró en El Coyote, alineándose con su preferencia por temperaturas más cálidas.

Estos resultados subrayan el papel crítico de las herramientas moleculares en la evaluación precisa de las distribuciones de especies, especialmente para especies crípticas como *O. lurida*. Los hallazgos enfatizan la necesidad de esfuerzos de conservación específicos para proteger y restaurar las poblaciones del Ostión Olympia en medio de condiciones ambientales cambiantes y la competencia entre especies.

Identification of the southern distribution range of the native Olympia oyster *Ostrea lurida* using two mtDNA markers: 16S and COI

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Keywords: *Ostrea lurida*, Olympia oyster, *Ostrea conchaphila*, molecular markers, native oyster, oyster distribution

Abstract

The Olympia oyster (*Ostrea lurida*) is the only native oyster of the eastern Pacific coast, spanning Canada, the United States, and the Baja California peninsula in Mexico. Historically abundant, *O. lurida* populations have significantly declined due to overharvesting, pollution, and habitat destruction. This decline, coupled with the species' cryptic nature, prompts using molecular tools for accurate identification and distribution assessment. In this study, 274 oysters from seven sites along the Baja California coast were analyzed using two mitochondrial DNA markers: 16S and Cytochrome Oxidase Subunit I (COI). Our findings confirmed the presence of six oyster species within the *Ostreidae* family: *Ostrea lurida*, *Ostrea conchaphila, Saccostrea palmula, Crassostrea gigas, Crassostrea sikamea*, and *Crassostrea corteziensis*. Notably, *O. lurida* was only found at the San Quintín sites, contradicting previous reports of its presence in Guerrero Negro. This discrepancy suggests a range contraction potentially due to Extreme Marine Heatwaves (EMH) between 2013 and 2018. However, insufficient or poorly directed sampling efforts could also explain the absence of *O. lurida* in other zones. Additionally, the study highlighted the presence of *Saccostrea palmula* in Guerrero Negro, indicating a possible northward range extension due to warming waters*. Crassostrea corteziensis*, a species native to the Gulf of California, was found at El Coyote, aligning with its preference for warmer temperatures. These results underscore the critical role of molecular tools in accurately assessing species distributions, especially for cryptic species like *O. lurida*. The findings emphasize the need for targeted conservation efforts to protect and restore Olympia oyster populations amidst changing environmental conditions and interspecies competition.

1. Introduction

Oysters play a crucial role as foundation species, creating habitats for various estuarine species and offering essential ecosystem services to human communities (Ridlon et al., 2021). These services encompass the enhancement of water quality, elimination of excessive nutrients from coastal ecosystems, the creation of hard-substrate biogenic reefs, and the stabilization or formation of neighboring habitats like salt marshes and seagrass beds (Baggett et al., 2015; Grabowski et al., 2012; Ruesink et al., 2005).

The Olympia oyster (*Ostrea lurida,* Carpenter, 1864) is the only native oyster of the tempered west coast of Canada, United States, and Baja California, Mexico. This small oyster was abundant throughout its historic distribution and was harvested and consumed by native communities for thousands of years (Barrett, 1963). This, along with other anthropogenic impacts such as extensive harvesting by modern Americans (Baker, 1995), pollution, and habitat destruction led to a significant decline in the populations. The growing demand and decline in water quality resulted in many natural populations along its range being depleted by 1930 (Baker, 1995; Conte, 1996). Once the fisheries collapsed, attention was shifted to the introduction of several oysters from the Atlantic and the west Pacific (Carlton, 1992).

Because of the rapid growth and great adaptability of the Pacific oyster, *Crassostrea gigas*, it quickly became the oyster of choice for aquaculture worldwide and the region, limiting the harvesting of the native oyster to Oregon and Washington, as it became much less profitable (Polson & Zacherl, 2009).

The distribution of *Ostrea lurida* has been debated over the years. Carpenter (1864) first described its distribution from what is now British Columbia, to the northern part of Baja California (Polson et al., 2009). There have been other reports of a broader range ranging from Sitka, Alaska, USA to Cabo San Lucas, Baja California Sur, Mexico, however, these were not confirmed by Polson et al. (2009) (Fig. 1). Later, Raith et al. (2015) would *locate O. lurida* individuals in Guerrero Negro, Baja California.

Also, *O. lurida* taxonomy has been controversial due to the cryptic nature of the species and its sister species *O. conchaphila*, which made them difficult to identify based merely on morphological characters. However, the use of molecular markers has enabled scientists to identify previously cryptic taxa and resolve taxonomic disputes (Knowlton, 1993; Simison and Lindberg, 1999). Hartlein (1959) proposed an overlapping and possible hybridization zone between southern California and Baja California Sur, and Harry (1985) proposed *Ostrea lurida* as a junior synonym of *Ostrea conchaphila.* This led to misuse and confusion in the literature due to the indiscriminate use of either name to refer to oysters in northern Mexico (Raith et al., 2015). Polson and colleagues (2009) were motivated by this contestation and analyzed oysters from western North America using mitochondrial 16S ribosomal DNA (16S) and cytochrome oxidase subunit III (COIII) markers. Their results showed only *O. lurida* in Hartlein's proposed area of overlap and depicted *O. lurida* and *O. conchaphila* as reciprocally monophyletic sister species. Later, Raith et al. (2015) tested Polson's sister species hypothesis using another molecular marker, cytochrome oxidase subunit I (COI) and arrived at the same conclusion, resolving *O. lurida* and *O. conchaphila* as sister taxa. They also identified a broader range for both species, further north for *O. conchaphila* (Laguna San Ignacio, Baja California Sur) and further south for *O. lurida* (Guerrero Negro, Baja California).

In Mexico, very little is known about the native Olympia oyster and there are only a few shell midden records (Laylander & Moore, 2007; Moore, 1999).

Historically, the fishery industry in Baja California focused on the exploitation of other marine species such as lobster, clam, abalone, among others. Nevertheless, a decrease in their populations due to precarious management and overfishing led local authorities and researchers to introduce the exotic Pacific oyster (*Crassostrea gigas*) in Bahia San Quintin

(Islas-Olivares, 1975), near to native Olympia oyster reefs. At that time, Bahia San Quintin was believed to be the southernmost point of *O. lurida* distribution, up until Raith et al. (2015) found the species in Guerrero Negro, just north of Punta Eugenia a major biogeographic breakpoint, which separates Californian from Surian biota (Valentine, 1966). This motivated us to identify the southern end of Olympia oyster distribution, as Raith's (2015) results suggest that Punta Eugenia acts as a boundary between *O. lurida* and its sister species from warmer waters, *O. conchaphila.*

2. Materials and methods

2.1 Sampling

During two consecutive summers, July 2019 and August 2020, 274 oysters were collected from 3 different sites along the Baja California peninsula; these sites are divided into 7 subsites (from north to south: San Quintin (n=73): Molino Viejo and Cultivo San Quintín (MOLV, CULTSQ); Guerrero Negro (n=96): Laguna Manuela, El Chaparrito and Faro Viejo (LAGMANU, CHAPA, FAROV); Mulege (n=105): La Bocana and El Coyote (BOCANA, COYOTE) (Table 1).

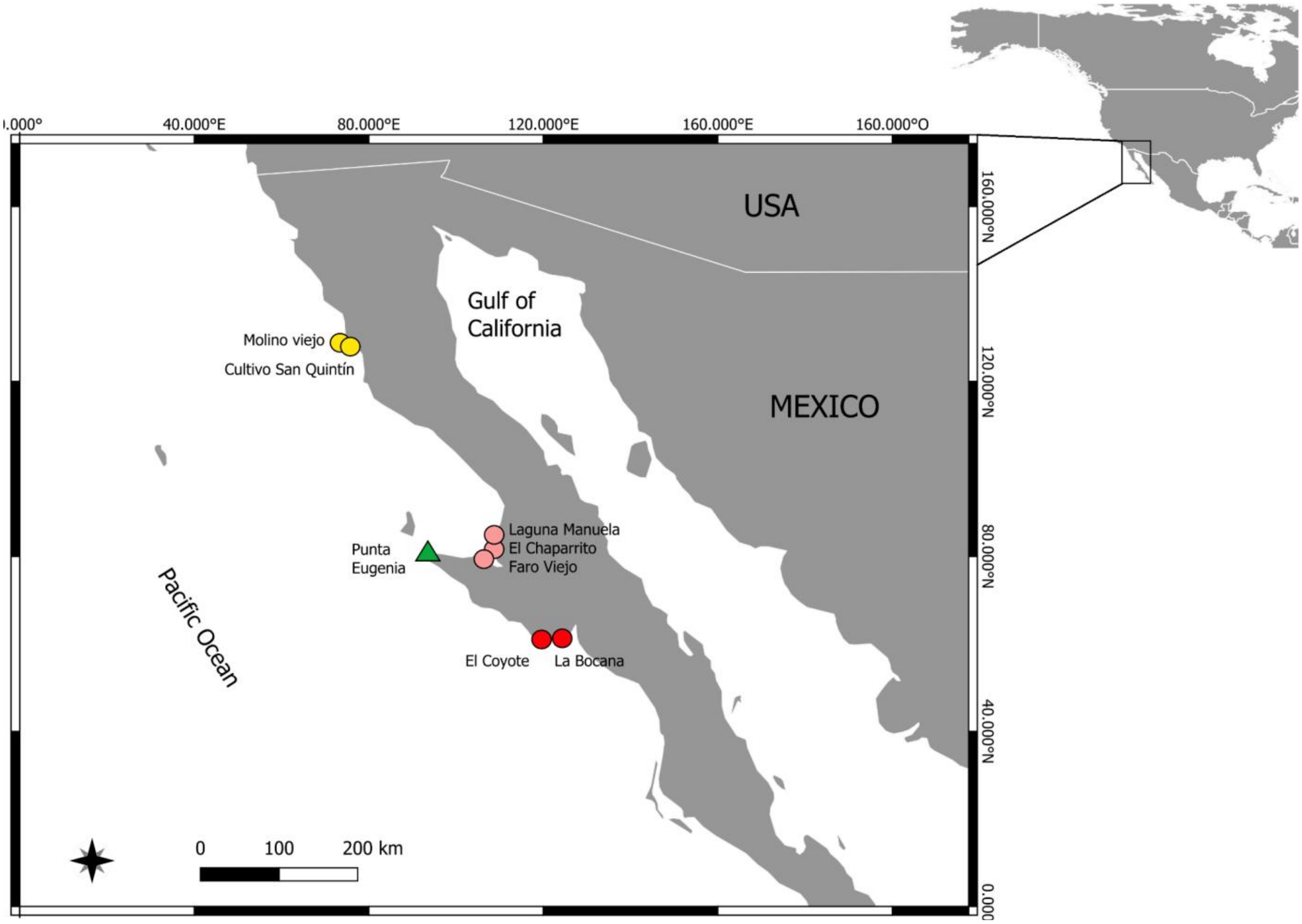

Fig.1. Sampling sites along the Baja California peninsula.

Zone	Sampling site	Site ID	Latitude (N)	Longitude (W)	No. samples
San Quintin	Molino Viejo	MOLV	30°29'05.5"	115°58'40.8"	37
San Quintin	Cultivo San Quintín	CULTSQ	30°27'05.8"	116°01'05.4"	36
Guerrero Negro	Laguna Manuela	LAGMANU	28°14'57.2"	114°05'01.1"	6
Guerrero Negro	Chaparrito	CHAPA	27°53'50.8"	114°07'09.6"	43
Guerrero Negro	Faro Viejo	FAROV	28°02'10.5"	114°07'09.6"	47
Mulege	La Bocana	BOCANA	26°46'54.1"	113°39'57.7"	82
Mulege	El Coyote	COYOTE	26°48'31.9"	113°28'12.2"	23

Table 1. Zones and sites with their respective site ID´s, coordinates, and number of samples.

Each oyster was labeled, preserved in 95% ethanol, and immediately stored in a cooler at -5°C upon arrival at the laboratory where they were stored at -20°C. All oysters were dissected to collect the gills and then preserved in 95% ethanol at -20°C.

2.2 DNA extraction and polymerase chain reaction (PCR)

Genomic DNA extraction and purification were performed using a KingFisher purification kit (ThermoFisher Scientific) following the manufacturers' specifications. Approximately 20 mg of gill tissue was taken from each specimen.

A fragment of 550 bp of the 16s mitochondrial DNA (mtDNA) gene was PCR amplified in 15 µL reactions using primers 16Sar (5’ - CGC CTG TTT ATC AAA AAC AT - 3’) and 16Sbr (5’ - GCC GGT CTG AAC TCA GAT CAC GT - 3’) (Palumbi, 1996). Each reaction included 1.5 µL of Qiagen 10X buffer (with 3mM $MgCl_2$), 1.2 µL of dNTP's (2.5 µM), 0.6 µL of primers (0.3 µL forward / 0.3 µL reverse), 1 µL of genomic DNA template, 0.12 µL of Taq polymerase (Qiagen, Valencia, CA), and sterile deionized water to bring the reaction volume to 15 µL per reaction. Also, a fragment of 500 bp of the Cytochrome Oxidase subunit I mtDNA gene was amplified in 20µL reactions using primers LCOI490 (5′ - GGT CAA CAA ATC ATA AAG ATA TTG G - 3′) and HCO2198 (5′ - TAA ACT TCA GGG TGA CCA AAA AAT CA - 3′) (Folmer et al., 1994). Each reaction included 2 µL of Qiagen 10X buffer (with 3mM MgCl2), 1.6 µL of dNTP's (2.5 µM), 2 µL of primers (1 µL forward / 1 µL reverse), 1 µL of genomic DNA template, 0.32 µL of Taq polymerase (Qiagen, Valencia, CA), and sterile deionized water to bring the reaction volume to 20 µL per reaction. There were several cases where the COI gene failed to be amplified using the LCOI490 primer. This was resolved with the use of a different primer, veneroid-LCO (5'-YAG NAC YAA TCA TAA AGA TAT TGG - 3'; E. M. Pilgrim, unpublished).

Reactions were carried out in an Eppendorf 5333 mastercycler. For the 16S gene, the initial denaturation was carried out at 94 °C for 3 minutes; continuing at 94°C for 1 minute, followed by an annealing temperature of 56°C for 1 minute, and an extension temperature of 72°C for 1 minute for 30 cycles. Once the cycles were finished, the temperature was maintained at 72 °C for 5 minutes, followed by a hold at 12 °C until the samples were removed to storage at -20 °C. For the COI gene, the initial denaturation started at 94 °C for 3 minutes; continuing at 94°C for 1 minute, followed by an annealing temperature of 46°C for 1 minute and an extension temperature of 72°C for 1 minute for 30 cycles. Once the cycles were finished, the temperature was maintained at 72 °C for 5 minutes, followed by a hold at 12 °C until the samples were removed to storage at -20 °C. PCR products were observed with agarose-gel electrophoresis (1.5%), ethidium bromide staining, and UV illumination to confirm the successful amplification of the mtDNA marker.

2.3 DNA Sequencing alignment and analyses

Products were cleaned and sequenced commercially at Eton Bioscience Inc. in San Diego, CA. All sequences were aligned using the software Geneious (Biomatters Ltd. 2017. Geneious, version 10.2.6. Available from https://www.geneious.com) using the MUSCLE algorithm (Edgar, 2004) and manually. Consensus trees were constructed using the Neighbor-Joining method with Tamura-Nei genetic distance model, as well as bootstrap analyses (200 bootstrap replicates).

3. Results

DNA extraction was successful for all individuals. For the 16S marker, 261 sequences were obtained, whereas for COI, only 229; this was due to recurrent problems using Folmer´s LCOI410 primer, which didn´t amplify for oysters of the *Ostrea* genus. This issue was finally resolved by pairing Folmer´s HCO2198 with Veneroid-LCO (See methods). The DNA sequence alignments of 16S and COI were 380 and 434 sites respectively. In total, 224 specimens with both 16s and COI sequences.. The resulting phylogenetic trees confirm the presence of *Ostrea lurida, Ostrea conchaphila, Saccostrea palmula, Crassostrea gigas, Crassotrea sikamea* and *Crassostrea corteziensis,* all belonging to the family Ostreidae. *Ostrea lurida* was collected at the two sites that conformed the San Quintin zone (Fig. 2), nevertheless, despite multiple surveys, we were not able to locate any individuals from Guerrero Negro which Raith et al. (2015) suggested as the southernmost point of the species' current distribution range. *Ostrea conchaphila*, was only found in the sites below Punta Eugenia, El Coyote, and La Bocana, north to the previously documented northern range lìmit of the species reported by Raith (2015) at Laguna San Ignacio. *Crassostrea gigas* was the most prevalent species throughout all locations, being found at all sites, with a lesser presence in the south, where a single specimen was found in La Bocana. A single individual of *Crassostrea sikamea* was collected from Guerrero Negro, while 5 individuals of *Crassostrea corteziensis* were in La Bocana.

Neighbor-Joining (NJ) trees were generated for the combined and separate 16S and COI datasets, which included an array of oyster sequences from GenBank.

In our 16s only results, there's an evident distinction between four lineages: *Ostrea, Saccostrea, Crassostrea,* and *Striostrea*. The same results can be observed on the COI only tree, the only distinction is the lack of resolution of the later marker to distinguish *Ostrea lurida* from *Ostrea conchaphila*; also, the COI gene hasn't been sequenced for *Crassostrea corteziensis*, so these individuals grouped with *Crassostrea gigas*. *Ostrea lurida* was the most abundant at both San Quintín sites but found on any other. Its sister species was found at Estero La Bocana and Estero El Coyote, yet, no oysters from the *Ostrea* genus were found in Guerrero Negro.

Table 2. Number of oysters found at each site.

Sampling site	# of oysters found					
	O. lurida	*O. conchaphila*	*S. palmula*	*C. gigas*	*C. corteziensis*	*C. sikamea*
Molino Viejo	26	0	0	11	0	0
Cultivo San Quintín	31	0	0	5	0	0
Laguna Manuela	0	0	4	2	0	0
Chaparrito	0	0	1	42	0	0
Faro Viejo	0	0	0	46	0	1
La Bocana	0	0	81	1	0	0
El Coyote	0	7	11	0	5	0

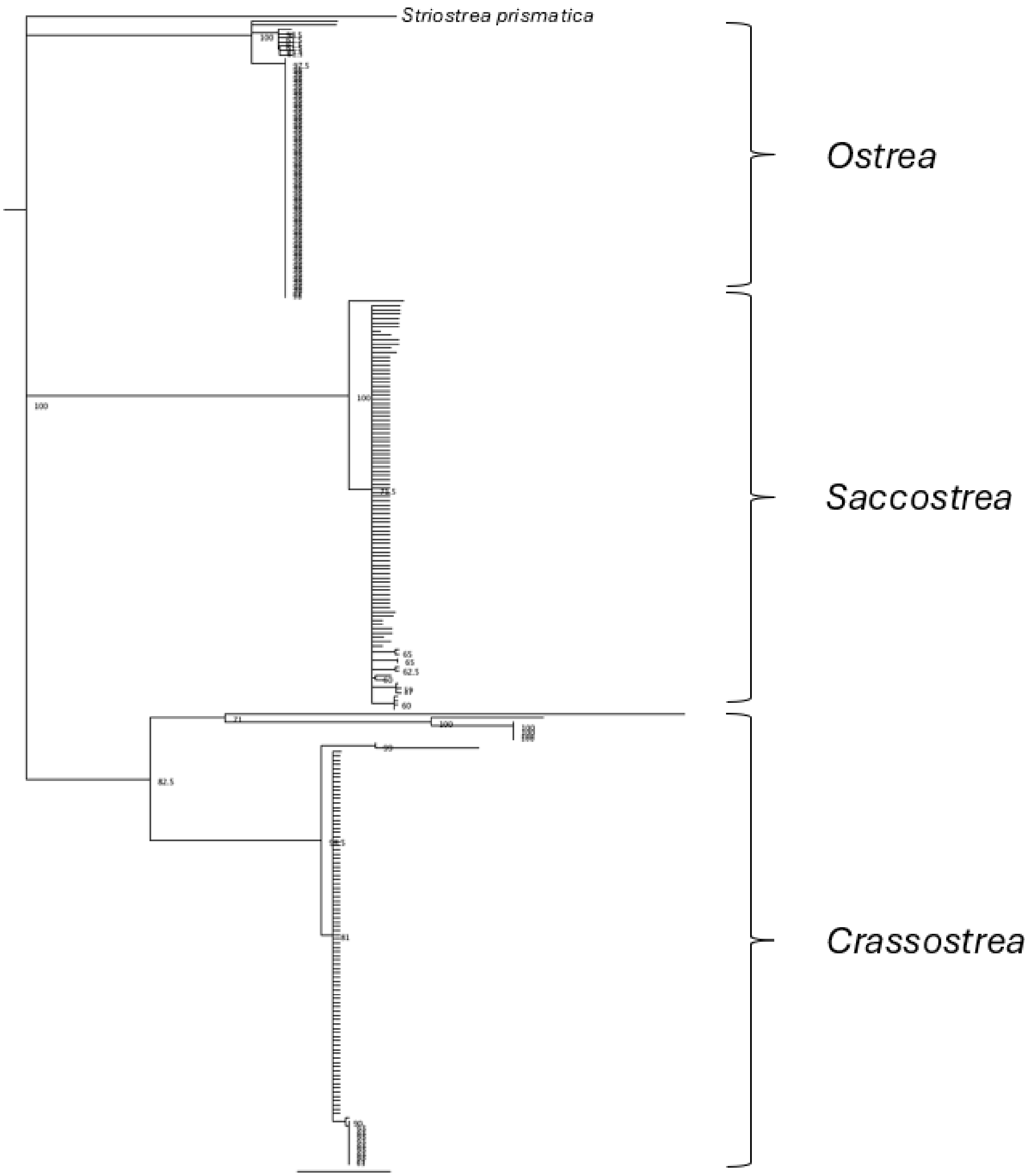

Fig. 4. Best neighbor-joining tree found for 16S only data set including all 16S sequences for this study and available sequences from GenBank, with *Striostrea prismatica* as outgroup. *Ostrea, Saccostrea* and *Crassostrea* sections are differentiated

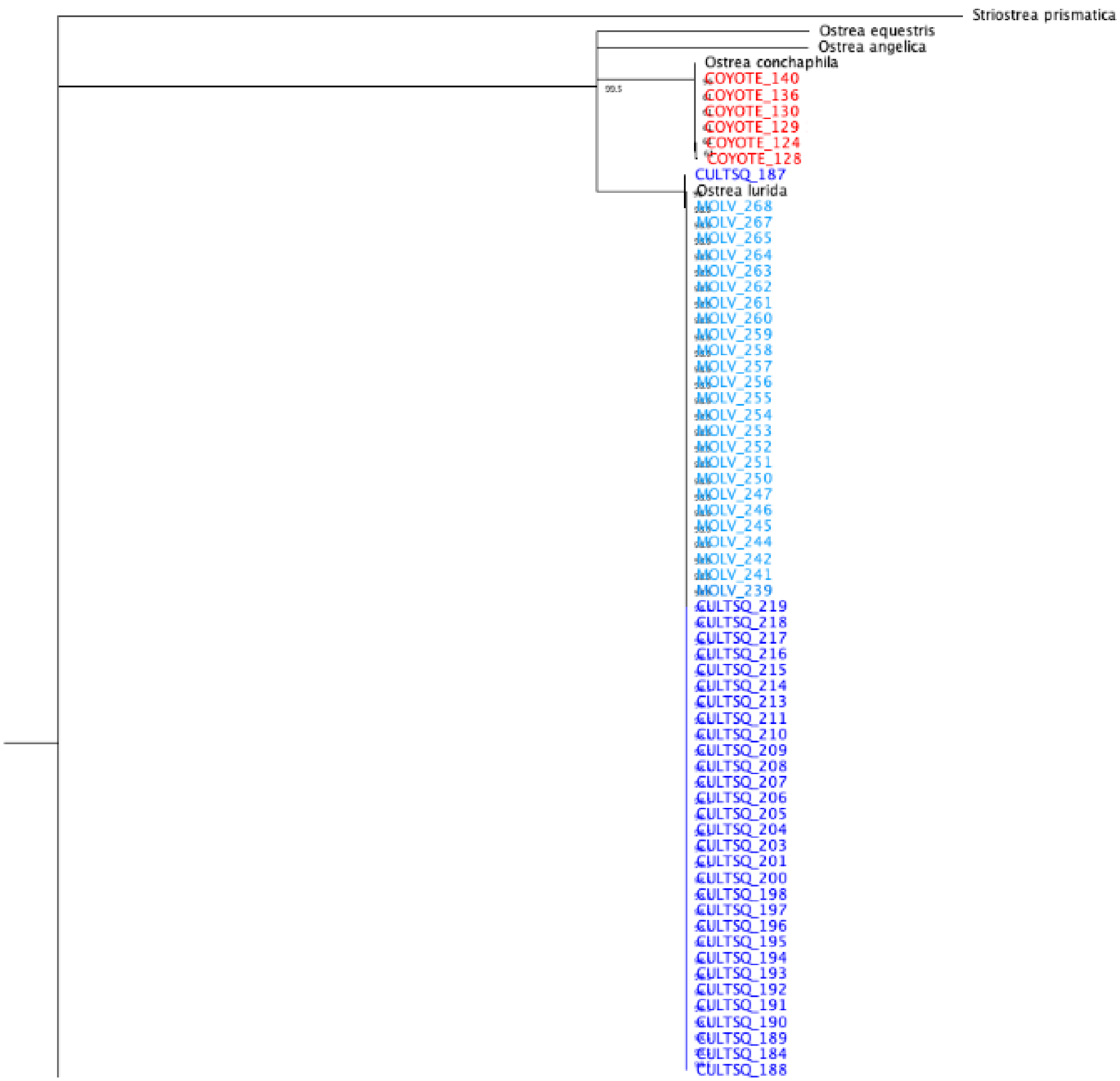

Fig. 3B. Best 16S Neighbor-joining tree as in figure 3A but showing detail for the *Saccostrea* portion. Bootstrap values> 50% are shown, based on 200 bootstrap replicates.

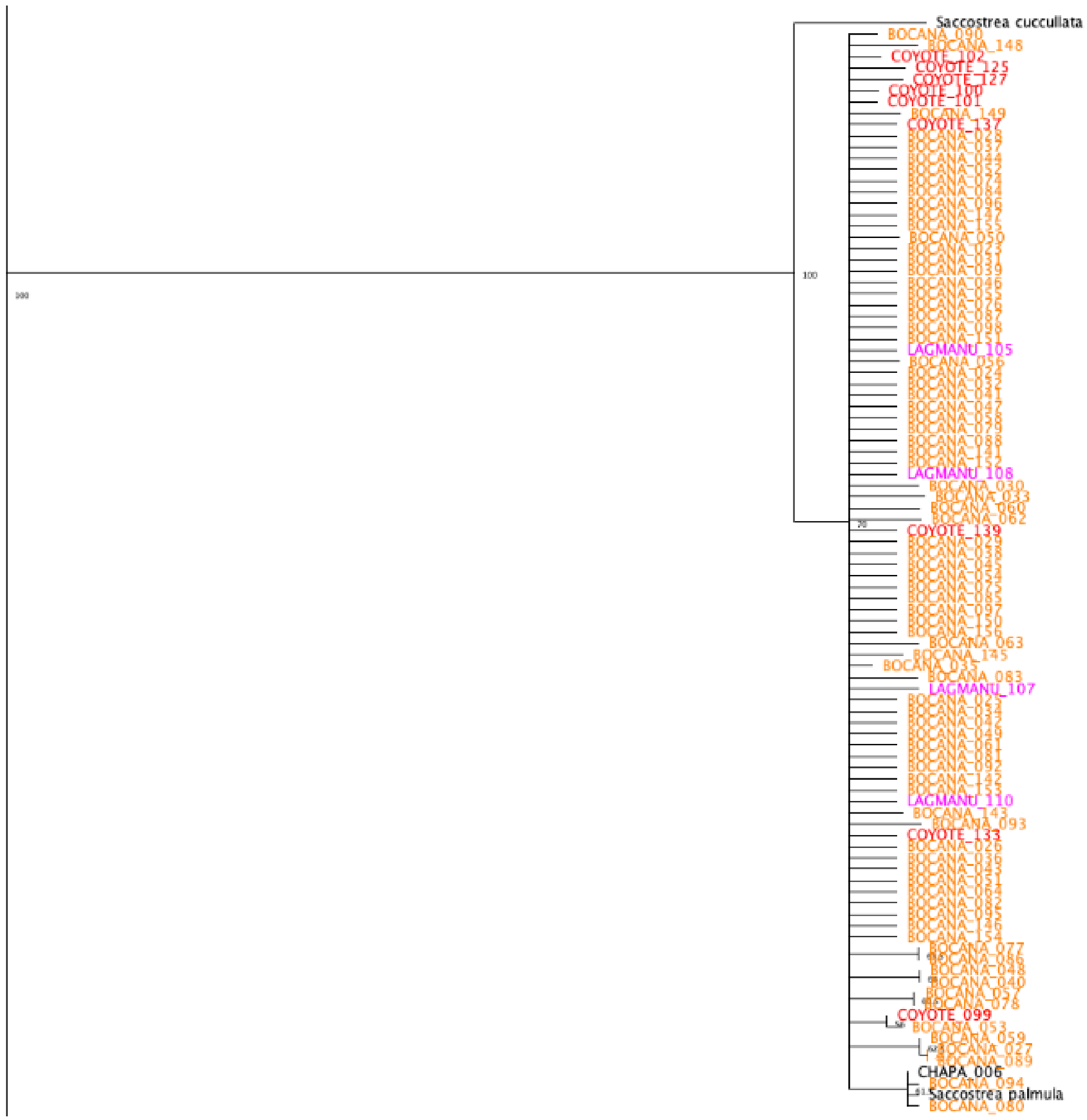

Fig. 5. Best 16S Neighbor-joining tree as in figure 3A but showing detail for the Saccostrea portion. Bootstrap values> 50% are shown, based on 200 bootstrap replicates.

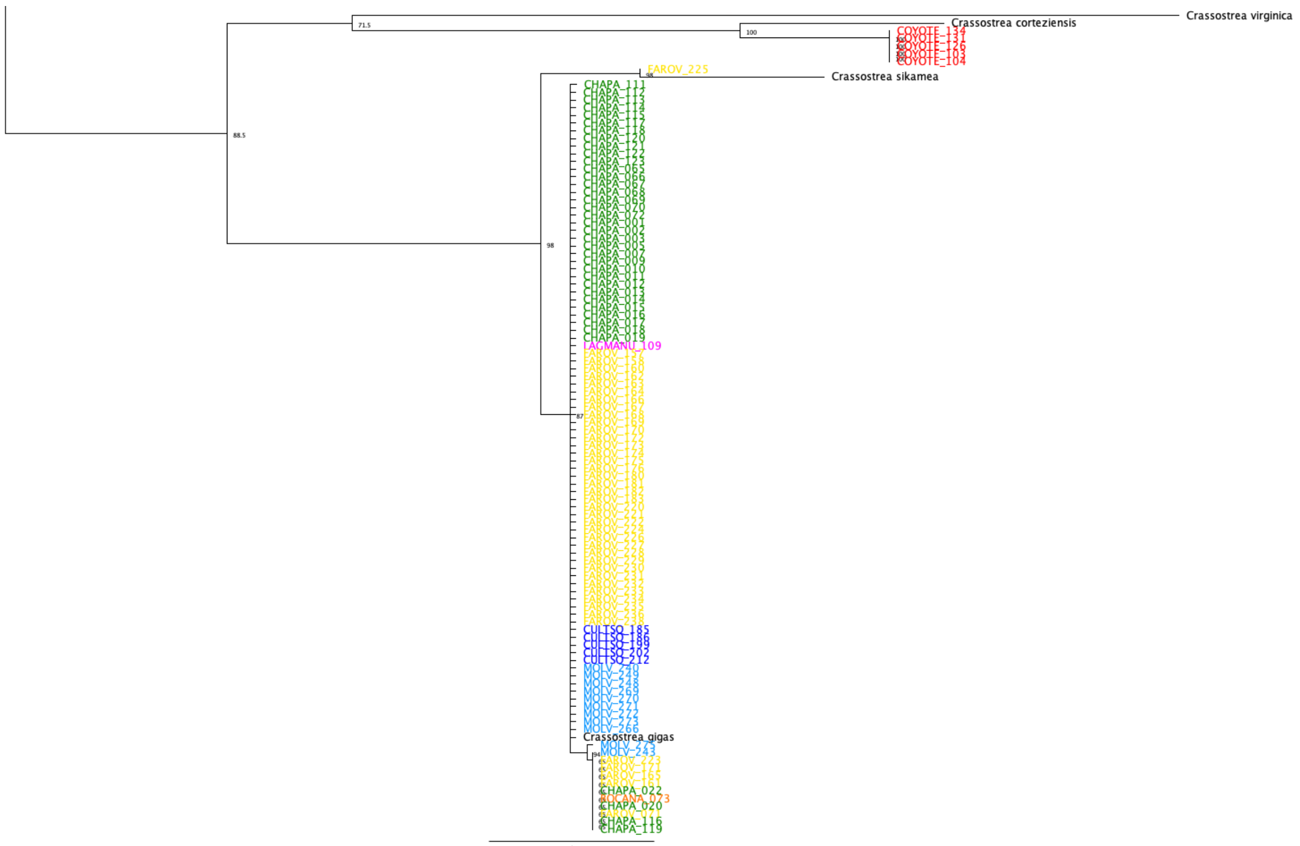

Fig. 6. Best 16S Neighbor-joining tree as in figure 3A but showing detail for the Crassostrea portion. Bootstrap values> 50% are shown, based on 200 bootstrap replicates.

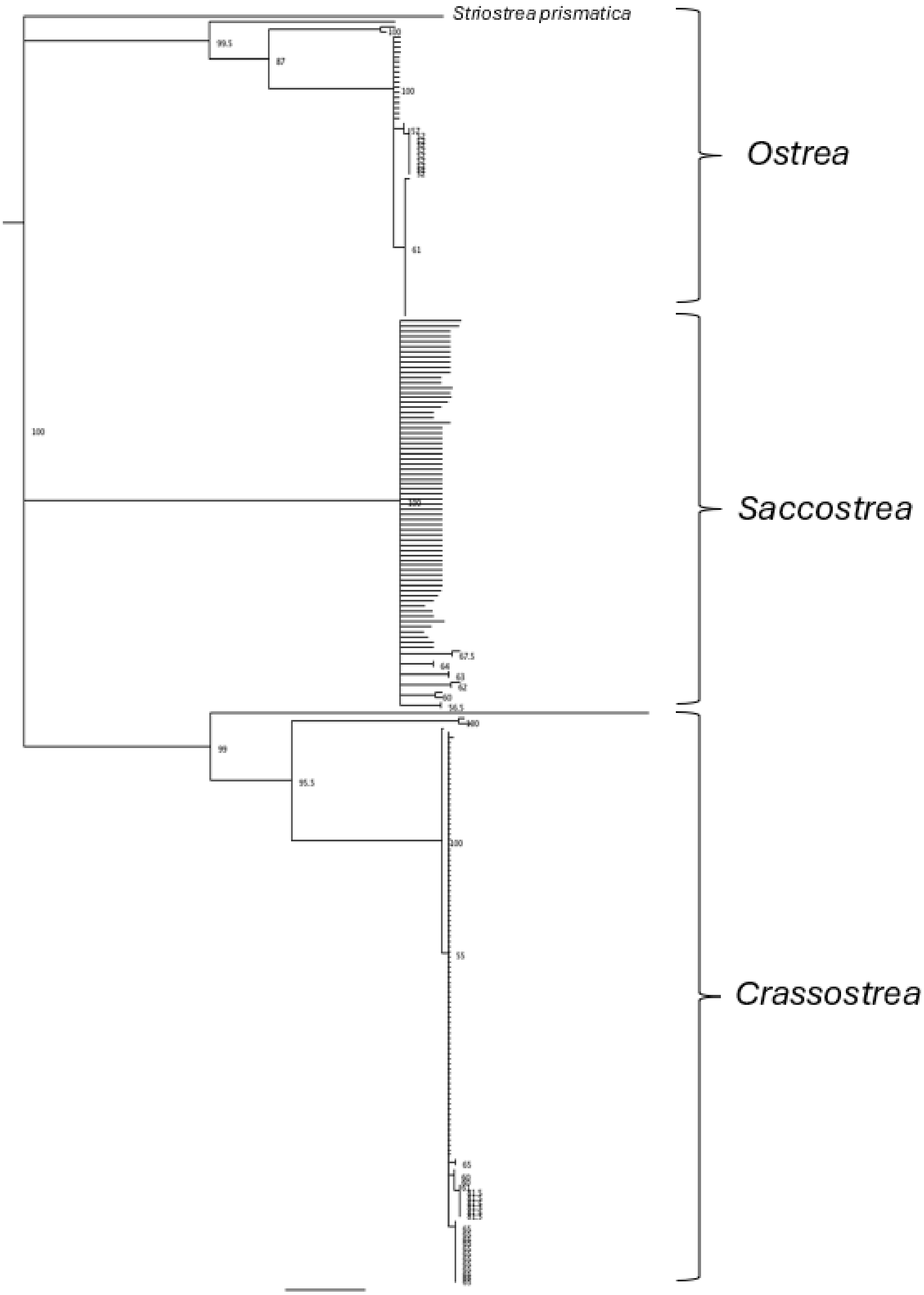

Figure. 7. Best neighbor-joining tree found for COI only data set including all COI sequences for this study and available sequences from GenBank, with *Striostrea prismatica* as outgroup. *Ostrea, Saccostrea* and *Crassostrea* sections are differentiated.

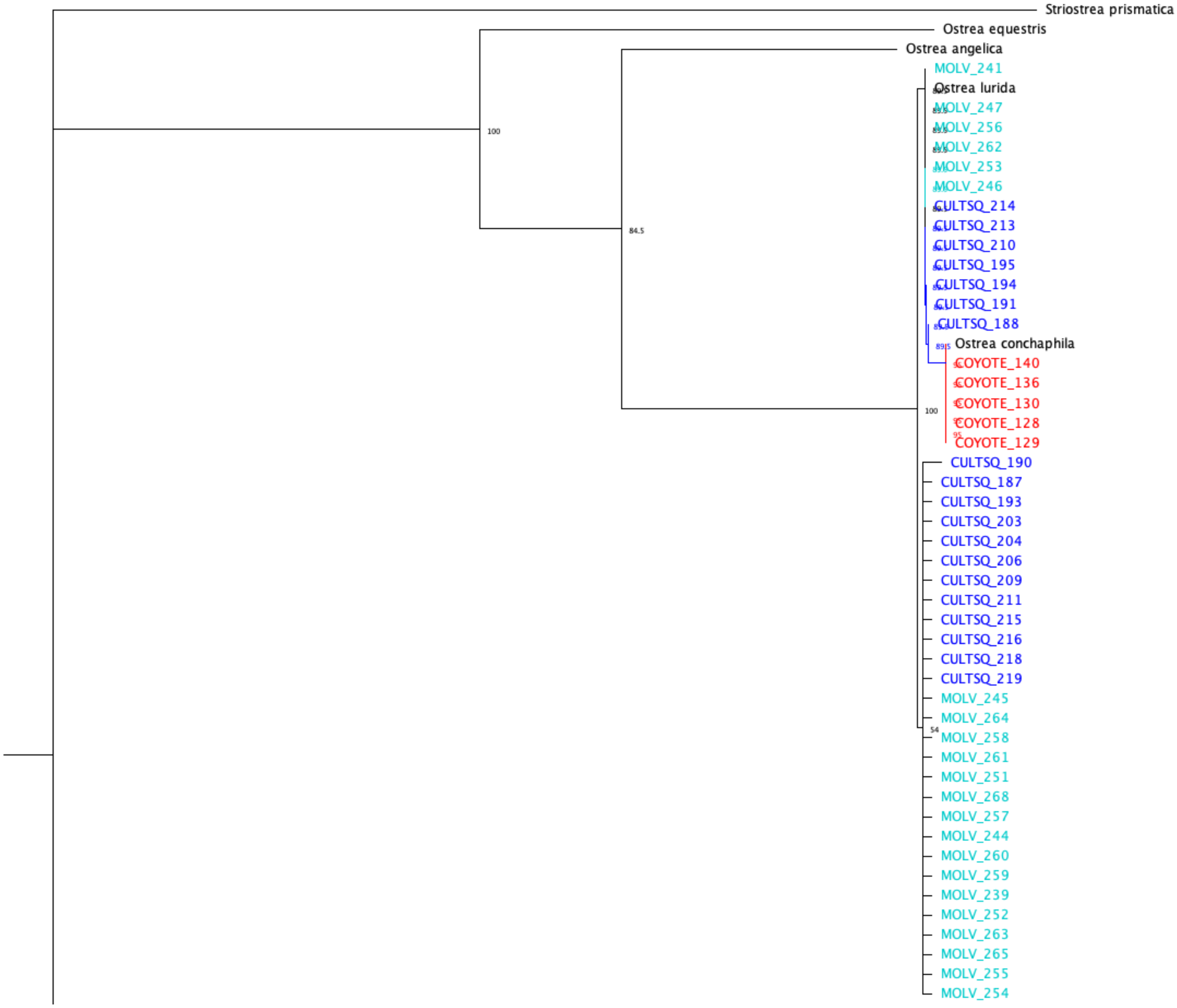

Figure. 8. Best COI Neighbor-joining tree as in figure 4A but showing detail for the *Ostrea* portion. Bootstrap values> 50% are shown, based on 200 bootstrap replicates.

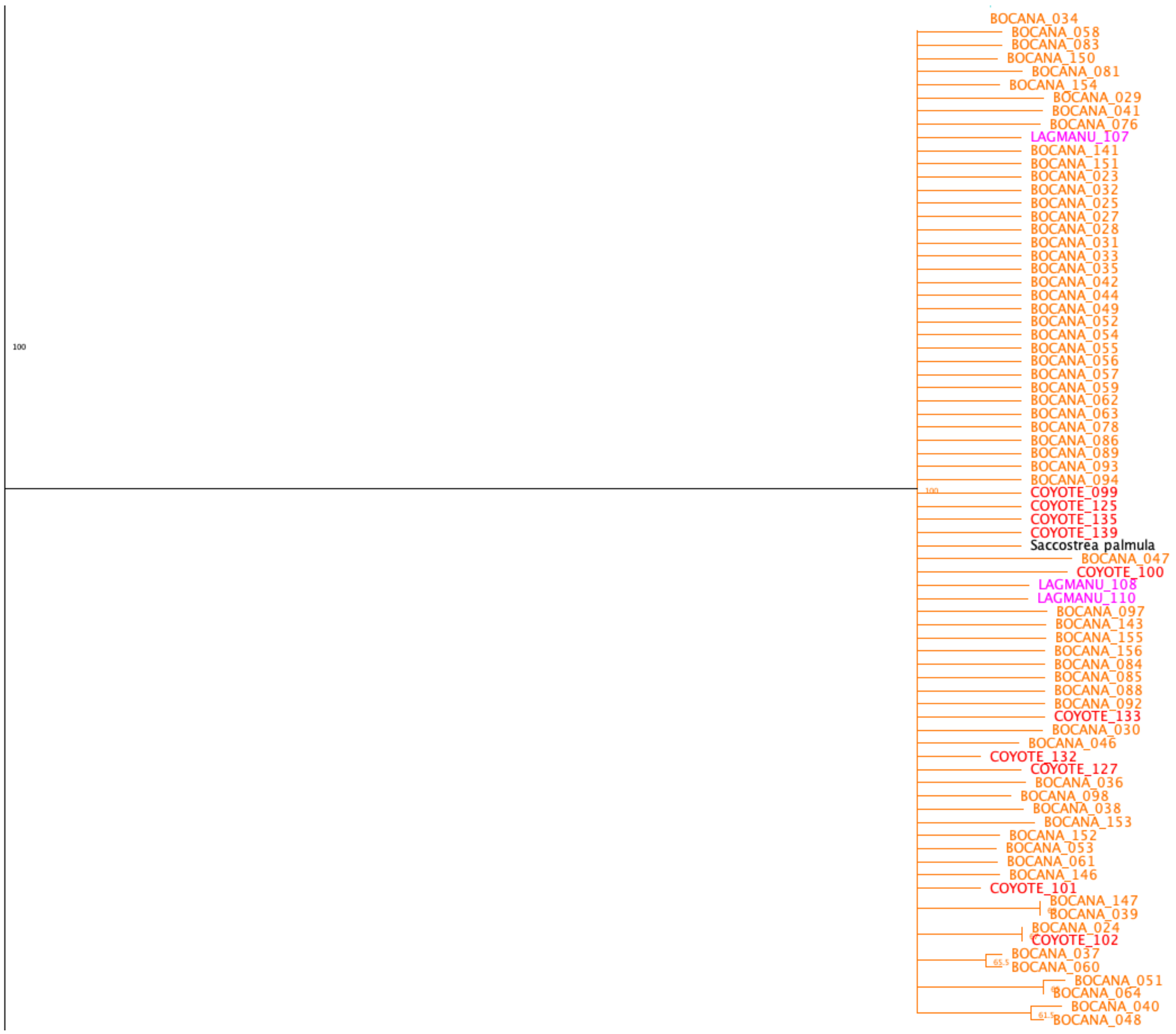

Figure. 9. Best COI Neighbor-joining tree as in figure 4A but showing detail for the *Saccostrea* portion. Bootstrap values> 50% are shown, based on 200 bootstrap replicates.

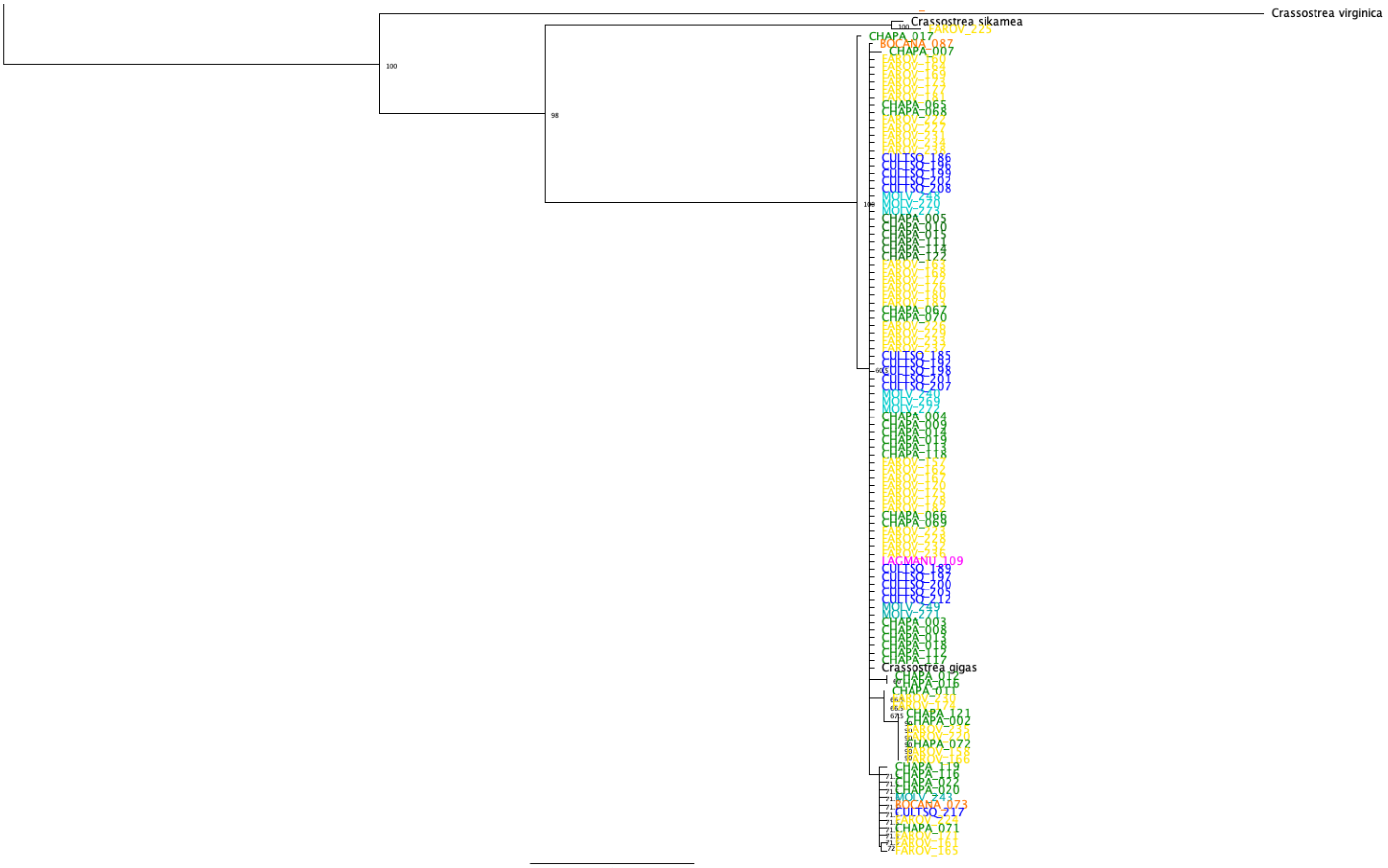

Figure. 10. Best COI Neighbor-joining tree as in figure 4A but showing detail for the *Crassostrea* portion. Bootstrap values> 50% are shown, based on 200 bootstrap replicates.

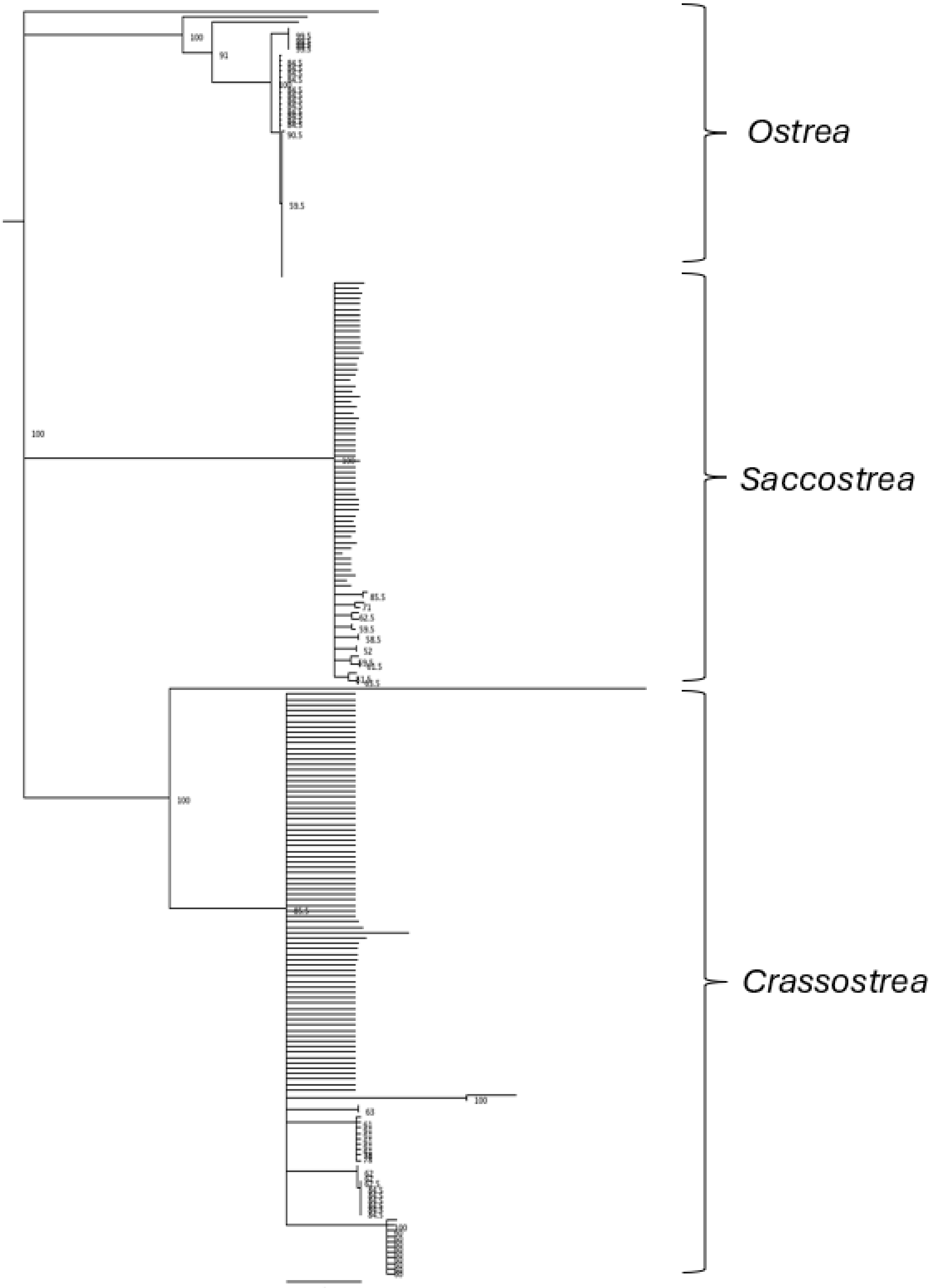

Figure. 11. Best neighbor-joining tree found for the concatenated sequences and available sequences from GenBank, with *Striostrea prismatica* as outgroup. *Ostrea, Saccostrea* and *Crassostrea* sections are differentiated.

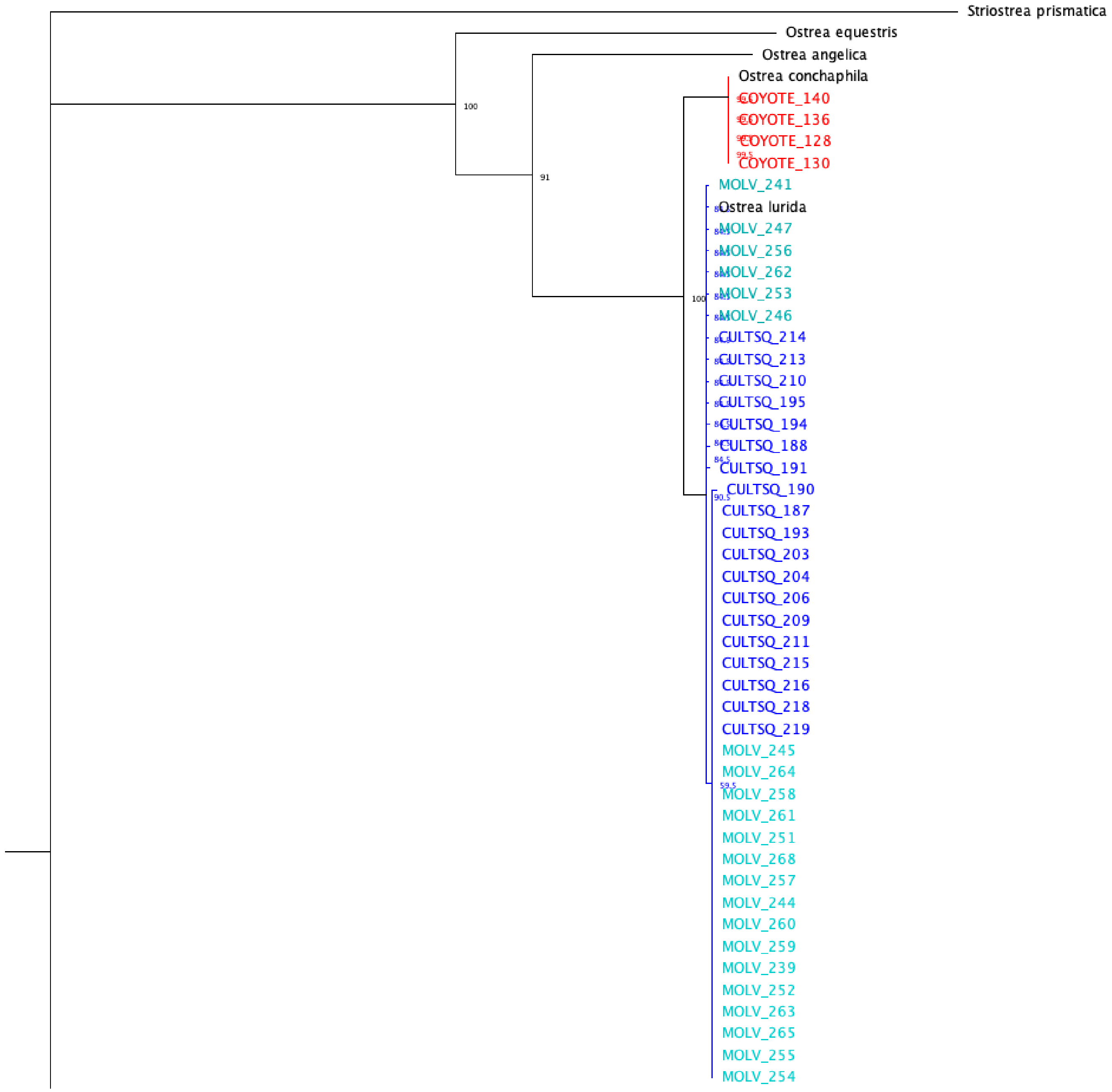

Figure. 12. Best concatenated Neighbor-joining tree as in figure 5A but showing detail for the *Ostrea* portion. Bootstrap values> 50% are shown, based on 200 bootstrap replicates.

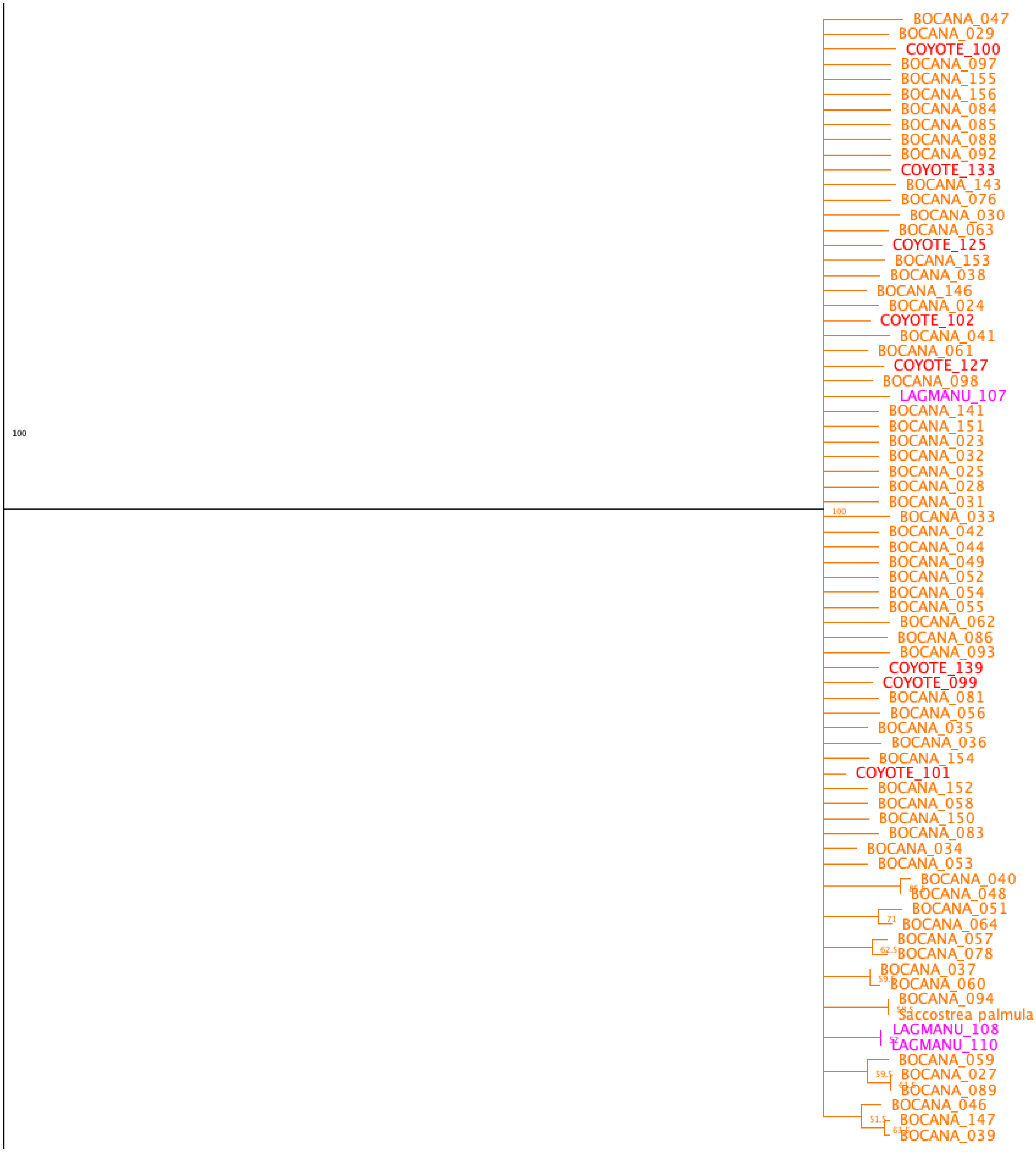

Figure. 13. Best concatenated Neighbor-joining tree as in figure 5A but showing detail for the *Saccostrea* portion. Bootstrap values> 50% are shown, based on 200 bootstrap replicates.

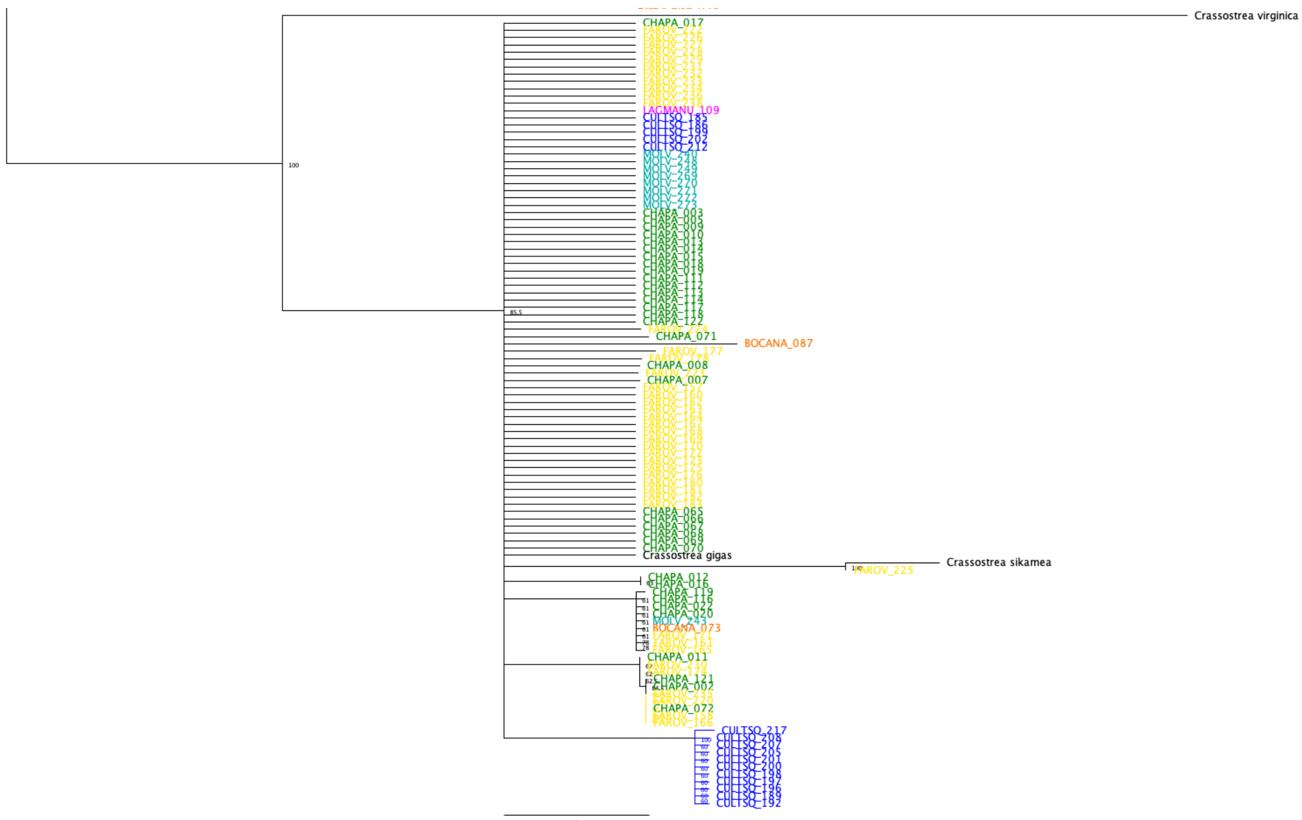

Figure. 14. Best concatenated Neighbor-joining tree as in figure 5A but showing detail for the *Crassostrea* portion. Bootstrap values> 50% are shown, based on 200 bootstrap replicates.

The 16s only results of this study show that *Crassostrea gigas* is present at all sites, except Mulege, also, five individuals grouped with 16s sequences extracted from genebank identified as *Crassostrea corteziensis* and a single individual grouped with *Crassostrea sikamea*.

The combined dataset of individuals that represented both genes, using *Striostrea prismatica* as an outgroup, showed a high bootstrap support for *Ostrea lurida* and *Ostrea conchaphila* as sister taxa, confirming Raith et al. (2015) results.

All individuals from this study group under Saccostrea were identified as *Sacosstrea palmula*, and, just as Polson (2009) described, these oysters posed morphological distinctions between them.

4. **Discussion**

Our analyses depict *Ostrea lurida* and *Ostrea conchaphila* as sister taxa, as Polson (2009) and Raith (2015) noted. Raith reported no evidence of sympatry regarding the distribution of *Ostrea lurida* and *Ostrea conchaphila*. They noted a distributional break at Punta Eugenia where *Ostrea conchaphila* is present south of Punta Eugenia and continues its distribution further north into the Gulf of California, whereas *Ostrea lurida* was present north of Punta Eugenia, at Guerrero Negro. Nevertheless, despite having sampled in Guerrero Negro in three different years (2020, 2021, and 2023), *Ostrea lurida* was not found at this site.

Between 2013 and 2015, the northeastern Pacific experienced the largest marine heatwave ever recorded (Di Lorenzo & Mantua, 2016). A large-scale warm water anomaly known as the 'Blob' was the result of a persistent atmospheric ridge (Bond et al., 2015). Following the dissipation of the Blob, an El Niño-Southern Oscillation (ENSO) event occurred in 2016. The convergence of these phenomena created an unprecedented warm water anomaly lasting three years, during which multiple marine heatwaves (MHWs) occurred (Lonhart et al., 2019).

Long-lasting MHW and ENSO events intensify the northward flow of coastal currents and sustain exceptionally high temperatures, assisting in the migration of coastal marine species from lower to higher latitudes (Sanford et al., 2019). Lonhart and collaborators documented 29 species that responded to the warm water anomalies between 2013 and 2018 from central California to the west coast of the California Peninsula. Only one of the observed changes was cataloged as a range contraction, where *Medialuna californiensis* seems to be missing from a significant part of its southern distribution along the tip of the Baja California peninsula. A similar case could be ensuing with *Ostrea lurida*, as Raith samples were collected before the major heatwaves, our results could suggest a contraction of the species distribution range. Water temperature is a critical factor that directly affects the reproductive cycle of the Olympia oyster, which requires a minimum of 12.5 °C to reproduce but prefers a temperature between 14 °C and 16 °C to do so (Roper & Hopkins, 1937). Range Contractions are expected to become more frequent this century (cita), nevertheless, they don't receive as much attention as range shift expansions/extensions and have proven to be harder to monitor (cita). The lack of evidence is a significant problem since the absence of a species could be attributed to sampling limitations and error. Additionally, as the population declines near its southern range, the remaining individuals become scattered and scarce, making detection more challenging (Lonhart et al., 2019). Furthermore, the presence an oyster species native to the Gulf of California in Guerrero Negro, *Saccostrea palmula,* further support the theory that, due to tropicalization effects, species from warmer climates are moving northward and settling in sites where they hadn´t previously occurred. Lonhart and collaborators (2019) found seven species that showed this behavior of expansion: Three were crabs (*Achelous xantusii, Malacoplax californiensis,* and *Uca princeps), two mollusks (Aplysia vaccaria and Lobatus galeatus),* one urchin (*Arbacia stellata*), and one fish *(Alphestes immaculatus)*. The Gulf of California oyster was observed in very low quantities (*Saccostrea Palmula n=5*).

Another factor that could potentially be at play is the widespread presence of the extremely invasive Pacific oyster (*Crassostrea gigas)* at the species' southern range. The Pacific oyster is currently the most cultured bivalve around the globe (FAO, 2009). It was first introduced to North America in Puget Sound, Washington in 1902 after the collapse of the Olympia oyster (*Ostrea lurida*) populations and failed attempts to stock *Crassostrea virginica* (Xavier Kirby, 2004). Its first introduction to Mexican territory took place in 1975 (Islas-Olivares, 1975) in San Quintín, Baja California in a joint operation between researchers from Universidad Autónoma de Baja California and local authorities, and only four years later, at the end of 1979, *C. gigas* was introduced in Laguna Manuela, roughly 40 km north of Guerrero Negro (Islas-Olivares et al., 1982), followed by the introduction of another Asian oyster, *Crassostrea sikamea*, in the near Guerrero Negro Lagoon. Part of the success of the Pacific oyster is due to its plasticity of adaptation to different environments and its capacity to tolerate and live in a wide temperature range (Padilla, 2010; Rico-Villa et al., 2009). Oysters act as ecosystem engineers, altering the habitat (Crooks & Crooks, 2002), creating dense biogenic structures, and filtering the water column (Padilla, 2010; Ruesink et al., 2005). Pacific oysters are notably prominent among the most impactful invaders of marine ecosystems; their potential ability to create living shorelines exceeds that of the smaller, native Olympia oyster (Crooks et al., 2015) and could compete for resources, potentially displacing it from its habitat. *Ostrea lurida* has never been a species of commercial interest in the area due to its small size, which can take several years to reach 5 cm (Couch et al., 1989), and it would take between 2000 and 2500 individuals to fill a gallon of shucked meat, while the Pacific oyster only requires around 120 individuals to fill the same amount (Steele, 1964). These differences make the pacific oyster a much more sought-after species for the local fisheries as the economic benefits are greater and require less labor than that required for Olympia oyster culturing. Furthermore, the presence an oyster species native to the Gulf of California in Guerrero Negro, *Saccostrea* *palmula,* further support the theory that, due to tropicalization effects, species from warmer climates are moving northward and settling in sites where they hadn´t previously occurred.

Conclusions

This study suggests a contraction in the southern range limit of the Olympia oyster with its current confirmed presence extending only as far south as San Quintín, Mexico. Our repeated sampling efforts in Guerrero Negro, Mexico, historically considered part of Ostrea lurida's range, yielded no evidence of the species despite the presence of suitable habitat and several sampling efforts. This coincides with a period of unprecedented warming in the northeastern Pacific due to recent marine heat waves (MHWs). Conversely, an oyster species native to the Gulf of California (Saccostrea palmula) was found in Guerrero Negro, potentially reflecting a northward expansion of warm-adapted species in response to tropicalization. The absence of *Ostrea lurida* in Guerrero Negro could also be attributed to competition with the invasive Pacific oyster (Crassostrea gigas), introduced in the late 1970s. *Crassostrea gigas* is known for its wider temperature tolerance and ability to outcompete native species for resources. Further studies are needed to definitively confirm the contraction of Ostrea lurida's range and disentangle the potential roles of MHWs and invasive species on its decline. To achieve this, a more robust sampling effort specifically designed to detect the presence of Ostrea lurida across its historical range is crucial. Monitoring programs established for this purpose will be essential to understand the population status and future persistence of Ostrea lurida.

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