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Natural and cultured populations of the mangrove oyster *Saccostrea palmula* from Sinaloa, Mexico, infected by *Perkinsus marinus*

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ABSTRACT

The mangrove oyster *Saccostrea palmula* coexists with the pleasure oyster *Crassostrea corteziensis* in coastal lagoons of northwest Mexico. Recent discovery of *Perkinsus marinus* infecting the pleasure oyster in the region prompted evaluation of *S. palmula* as an alternative *P. marinus* host. An analysis to determine the possible presence of *P. marinus* in natural and cultured populations of *S. palmula* at four coastal lagoons in Sinaloa, Mexico was carried out during October–November 2010. Tissues from apparently healthy *S. palmula* were evaluated using Ray's fluid thioglycollate method (RFTM), which revealed a *Perkinsus* sp. to be present in all four locations at 6.7–20.0% prevalence. Histopathological analysis of these specimens showed tissue alterations and parasite forms consistent with moderate *P. marinus* infection, which was confirmed by ribosomal non-transcribed spacer (NTS)-based PCR assays on DNA samples from oysters positive by RFTM and histology. DNA sequencing of amplified NTS fragments (307 bp) produced a sequence 98–100% similar to GenBank-deposited sequences of the NTS from *P. marinus*. Fluorescent *in situ* hybridization for *Perkinsus* spp. and *P. marinus* corroborated the PCR results, showing clear hybridization of *P. marinus* in host tissues. This is the first record of *P. marinus* infecting a species from genus *Saccostrea* and the first record of the parasite from coastal lagoons in Sinaloa, Mexico.

1. Introduction

The mangrove oyster *Saccostrea palmula* is distributed from Laguna San Ignacio, Baja California, Mexico to Panama, the Galapagos Islands, Ecuador and Costa Rica. This species occurs on mangrove roots and on rocks exposed at low tides and it presents high morphological variability along the Panamic province (Cruz and Jiménez, 1994; Jiménez, 1994). *S. palmula* coexists with the pleasure oyster *Crassostrea corteziensis*, a larger species, which is distributed in mangrove zones from the Mar de Cortez, Mexico to Peru (Stuardo and Martínez 1975). Morphologicaly *Saccostrea* differs from *Crassostrea* in having the margin crenulated and from *Ostrea* by crenulation present all along the shell's periphery (in *Ostrea* only in the anterior margin near the hinge) (Huber, 2010). Species are clearly distinguishable. In Mexico both species are valued as food by communities along coastal lagoons and support a regional fishery. Reduction in natural populations of *C. corteziensis* has favored development its culture (Góngora-Gómez et al., 2007). Production of *C. corteziensis* now approaches 2000 metric tons/year (Cáceres-Martínez et al., 2010). While there have been some attempts to grow *S. palmula* (Baqueiro, 1984), culture of this oyster is still nascent and generally co-occurs with that of *C. corteziensis*.

Both species play important roles in the ecosystem as filter feeders and show a marked parallelism in life history traits. Their reproductive cycles, for example, are synchronous, with spawning starting in May for *C. corteziensis* and in June for *S. palmula* and continuing in both species to November (Cuevas-Guevara and Martínez-Guerrero, 1979). With the significant pathogen *Perkinsus marinus* recently having been detected infecting *C. corteziensis* in Nayarit (Cáceres-Martínez et al., 2008) and that this pathogen is under the watch of the World Organization of the Animal Health (OIE), the question arose as to whether *S. palmula* would share this particular attribute as well, particularly as *P. marinus* is transmitted horizontally among oysters via the water column (Andrews, 1996) and capable of infecting a range of oyster species (Meyers et al., 1991; Moss et al., 2006). *C. corteziensis* is transported within the region in the context of aquaculture as well as research projects (Chávez-Villalba et al., 2005; Arcos et al., 2009), and disease

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impacts of this activity on other species like *S. palmula* must be considered. Evaluation of the *P. marinus* infection status of natural and culture populations of *S. palmula* was the focus of this project.

2. Materials and methods

2.1. Oysters

Four coastal lagoons in Sinaloa, Topolobampo, La Bocanita, La Reforma, and Cospita (Fig. 1), were sampled in October–November 2010. In Topolobampo, where the aquaculture activity is most concentrated, 30 oysters were sampled from culture arrays. The number of oysters sampled from the culture area in La Bocanita was 15. At La Reforma, where natural populations occur, 15 animals were sampled. Finally, in Cospita, 10 oysters were sampled from natural beds. Oysters were measured by taking the total shell height from the umbo to the distal margin of the shell in mm. One-way ANOVA and Duncańs test for multiple comparisons were used to compare size range values (Zar, 1974). Sample size and oyster size depended on the available organisms. Oysters were shipped alive in a cooler to the laboratory of the Instituto de Sanidad Acuícola for processing.

2.2. Oyster processing and visual diagnostics

All fouling organisms were removed with a hard brush and a stream of seawater. Oysters were placed in a Petri dish, opened, and soft tissues were examined for the presence of abnormalities. For *P. marinus* detection all oysters were screened through induction of hypnospore formation, pieces of rectum, gills and mantle of each oyster were excised and two subsamples were obtained. One subsample was placed in Ray's fluid thioglycollate medium (RFTM, Ray, 1966; Fisher and Oliver, 1996; Kim et al., 2006) and the other was refrigerated for possible confirmation for PCR results. The tissues placed in RFTM were incubated for 8 days in the dark, after which they were stained with Lugol's iodine and observed using light microscopy. The remaining visceral mass of each oyster was removed from the shell and fixed whole in Davidson's fixative (Shaw and Battle, 1957) for at least 24 h. An anterior transverse section including stomach and intestine, digestive gland, gonad, mantle, and gills was processed using standard histological methods and embedded in paraffin. Samples were sectioned and stained with hematoxylin and eosin (Shaw and Battle, 1957). Histological positive controls for *Perkinsus* sp. and *P. marinus* were used for comparison with stained slides.

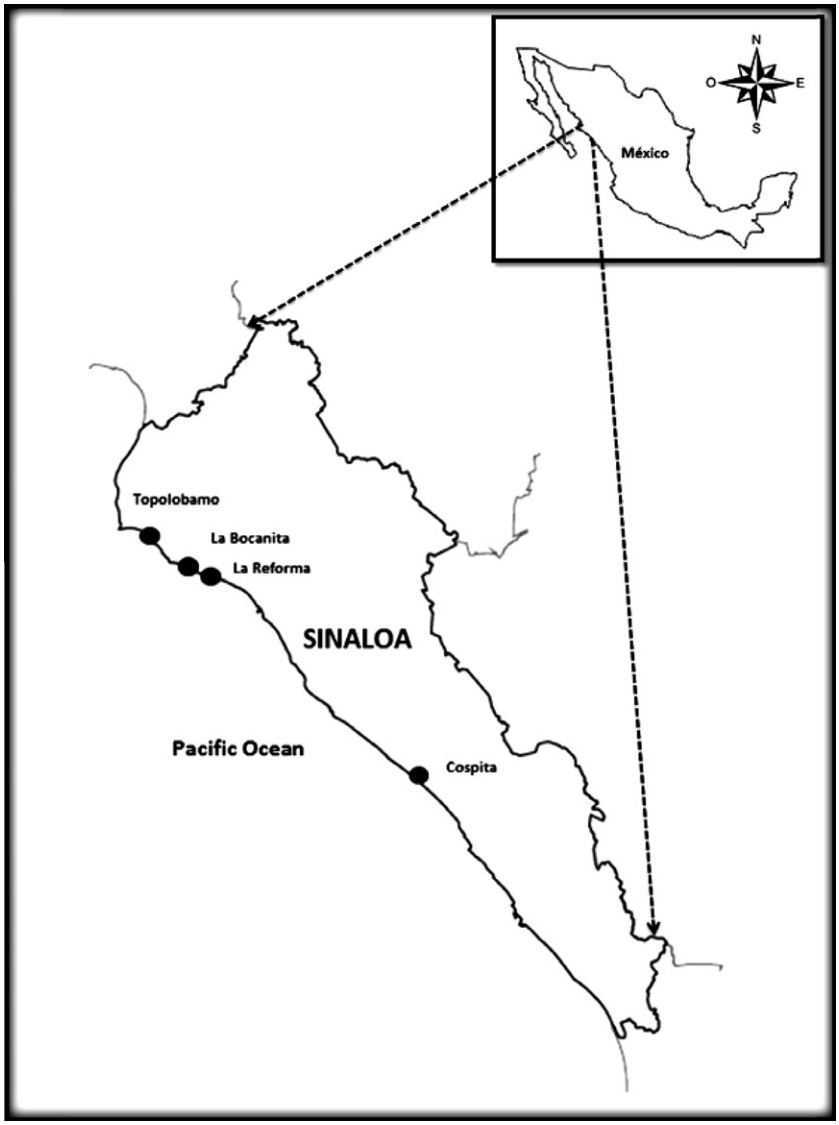

Fig. 1. Map showing sampling localities in the study area.