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Research Article

A new bivalve host record for the exotic parasite *Perkinsus marinus* in the Gulf of California

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Abstract

Perkinsus marinus belongs to the Chromista Kingdom and is recognized as an opportunistic parasite of mollusks native to the west coast of the Atlantic Ocean. The native range of this species is the Atlantic Ocean, however, it was introduced to the Gulf of California (GC) by a transfaunation of oysters. This microorganism has invaded new niches in the GC and continues to colonize different species of bivalve mollusks. For the first time, *P. marinus* was detected in the mussel *Mytella strigata* from the southeast coast of the GC in 2020 by staining and molecular techniques. The moderate number of cells of this myzozoan in the mussel coincides with the reports for other species of ostreids, venerids, and mytilids in the area, which suggests that it is expanding its geographical range and host species. The invasive potential of the parasite in new environmental niches and species of mollusks with commercial importance elevate the need for a risk assessment.

Key words: Myzozoan, mollusk, Bivalvia, *Mytella strigata*, charrua mussel

Introduction

Perkinus marinus (Mackin, H.M. Owen & Collier) Levine, 1978, is a parasite native to the Atlantic Ocean and distributed from the United States' east coast to Brazil (Remacha-Trivino et al. 2008; Queiroga et al. 2015). It has been found in the soft tissue of some species of bivalve mollusks (Ford 2011), sometimes associated with massive mortalities of oysters in some farms (Smolowitz 2013). This microorganism was introduced to the Pacific Ocean through infected specimens of the Virginia oyster, *Crassostrea virginica* (Gmelin, 1791), that were transported from production facilities on the United States' east coast and the Gulf of Mexico (Cáceres-Martínez and Vázquez-Yeomans 2013) several decades ago. After that, *P. marinus* has been detected in several bivalve species within the Gulf of California (Ek-Huchim et al. 2017), apparently successfully establishing itself outside its native geographic range, that is, as an ecological invader (Ricciardi 2013).

The invasive potential of this parasite refers not only to its easy adaptation in aquatic niches foreign to its origin, such as the Pacific Ocean coast (Cáceres-Martínez et al. 2008, 2016) but also to its ability to inhabit and/or infect different groups of mollusks (Góngora-Gómez et al. 2021). Being a planktonic organism, *P. marinus* distributes along the water column through the movement of sea currents. In addition, all of their life stages are potentially transmissible and infective, either intra- or inter-species, and either horizontally or vertically transmitted (Villalba et al. 2004).

There are already several species of bivalve mollusks from both coasts of the Gulf of California that host this parasite. Enriquez-Espinoza et al. (2010) and Villanueva-Fonseca et al. (2020) reported its presence in farmed or native ostreids such as the Japanese oyster, *Magallana gigas* (Thunberg, 1793) and the pleasure oyster, *Crassostrea corteziensis* (Hertlein, 1951) respectively, while Góngora-Gómez et al. (2020) found the pathogen in wild venerids such as the chocolata clam, *Megapitaria squalida* (G.B. Sowerby I, 1835). Recently, *P. marinus* was documented in the fat horsemussel, *Modiolus capax* (Conrad, 1873) (Góngora-Gómez et al. 2021). Due to its evident dispersal and transmission capacity (Villalba et al. 2004), it is imperative to carry out detection studies on other species of mollusks within the Gulf of California. In this way, it would be possible to take extreme precautionary measures in the handling and transporting of native and cultivated mollusks in the region, given the imminent proliferation of this exotic pathogen.

The objective of the present study aims to evaluate the presence of *P. marinus* in three populations of the charrua mussel, *Mytella strigata* (Hanley, 1843), a bivalve native to the southeastern Gulf of California, by detecting its hypnospores and genetic material in the mollusk tissue. This bivalve is used as a biological monitor to assess the region's health status of coastal lagoons and estuaries (Ruiz-Fernandez et al. 2018), so the detection of the parasite could explain an additional stress factor in the environmental response of the mussel.

Materials and methods

Mussels were collected from three coastal lagoons in the state of Sinaloa, Mexico: Altata Bay (AB) (24°20′–24°35′N and 107°20′–107°55′W), Macapule Lagoon (ML) (25°18′–25°24′N and 108°32′–108°45′W) and El Colorado Bay (CB) (25°43′–25°60′N and 109°23′–109°51′O), in the southeast Gulf of California. Thirty specimens (average shell length = 47.67 ± 3.92 mm, max.–min. = 59.23–32.67 mm) were removed from each lagoon per annual season (N = 120), from summer 2020 to spring 2021. In CB and AB, mussels were attached to mangrove roots, while ML specimens were in the muddy sediment. The bivalves were cleaned *in situ*, placed in plastic containers, and transported to the laboratory for further analysis. During each sampling event, temperature, dissolved oxygen, salinity, pH, chlorophyll *a*, and

suspended water solids were recorded, with the methodology described by Villanueva-Fonseca et al. (2020).

In the laboratory, the live mussels were dissected on ice, and soft tissue was detached from the shells (Cáceres-Martínez and Vázquez-Yeomans 2014). From each specimen, 1 g of pooled tissues (i.e., digestive gland, gills, adductor muscle, gonad, and mantle) was separated to form two subsamples. For the detection of *P. marinus* hypnospores, the first tissue subsample (A) was incubated in Ray's fluid thioglycolate medium (RFTM, Ray 1966) according to OIE standards (2019); the other tissue subsample (B) was stored at −70 °C for confirmatory PCR analysis. Lugol (1 ml) was used for staining and observing the hypnospores under the microscope (10X and 40X), which can be differentiated as dark or bluish-black spherical cells (20–70 µm in diameter) (OIE 2019). The prevalence [(number of organisms with evidence of hypnospores/total organisms) × 100, Thrusfield 2018)] and parasite density (number of hypnospores/g tissue wet weight) (Bush et al. 1997) of *P. marinus* were obtained from tissue subsample A (N = 120). Mussels that were positive for the presence of hypnospores with RFTM (1 in AB, 4 in ML, and 11 in CB) were used for myzozoan confirmation by PCR. All of them were also positive with PCR.

DNA obtained from the digestions and extractions of both hypnospores and soft tissue of mussels was used as target DNA in the PCR electrophoretic reaction (OIE 2019). PerkITS85 (5'-CCG-CTT-TGT-TTG-GAT-CCC-3') and PerkITS750 (5'-ACA-TCA-GGC-CTT-CTA-ATG-ATG-3') oligonucleotides were used to detect any species of *Perkinsus* sp. (Casas et al. 2002), and the specific oligonucleotides PmarITS-70F (5'-CTT-TTG-YTW-GAG-WGT-TGC-GAG-ATG-3') and PmarITS600R (5'-CGA-GTT-TGC -GAG-TAC-CTC-KAG-AG-3') for *P. marinus* (Audemard et al. 2004). Genomic DNA from *Crassostrea corteziensis* infected with *P. marinus* (Villanueva-Fonseca et al. 2020) was used as a positive control, and ultrapure water was added as a negative control.

Results

Except for the temperature for which the higher values were observed in summer and spring for the three sites (> 30 °C), the rest of the environmental parameters showed different seasonal trends in the lagoons (Table 1). For example, the dissolved oxygen and salinity concentrations obtained in summer 2020 showed different values among the three lagoons (5.61, 1.8, and 5.53 mg /L and 22, 42, and 35 PSU, respectively for AB, ML, and CB).

The thioglycolate technique detected dark-colored spherical corpuscles (< 75 µ in diameter) characteristic of *P. marinus* hypnospores in *M. strigata* tissues from the three sampling sites (Figure 1). Analyzing the mussels that were positive for thioglycolate staining, the PCR test confirmed the presence of *P. marinus* in the three populations of *M. strigata* (Figure 2). However, the prevalence varied in each lagoon and by annual season. CB mussels had

Table 1. Temperature (°C), dissolved oxygen (mg/L), salinity (PSU), pH, chlorophyll *a* (Cl*a*, mg/m^3), and total suspended solids (TSS, mg/L) of the water in the three sampling sites (AB = Altata bay, ML = Macapule lagoon, CB = The Colorado bay, Sinaloa, Mexico).

	Summer 2020	Autumn 2020	Winter 2021	Spring 2021
AB				
Temperature	34	29	26.5	30.6
Dissolved oxygen	5.61	3.02	4.61	2.35
Salinity	22	42	35	40
pH	8.05	7.69	8.03	7.99
Cl*a*	8.71	47.97	1.61	6.94
TSS	154.51	189.67	171.36	88.33
ML				
Temperature	31	25.8	21.3	31.1
Dissolved oxygen	1.8	3.64	4.98	3.96
Salinity	42	36	30	36
pH	7.85	8.21	7.81	7.92
Cl*a*	5.48	20.29	6.27	3.03
TSS	65.96	134.26	70.00	30.51
CB				
Temperature	30.9	25	25.1	31.7
Dissolved oxygen	5.53	2.64	3.01	2.33
Salinity	35	40	40	45
pH	8.13	7.84	7.75	7.84
Cl*a*	5.68	1.64	9.20	5.72
TSS	63.99	96.03	17.63	52.41

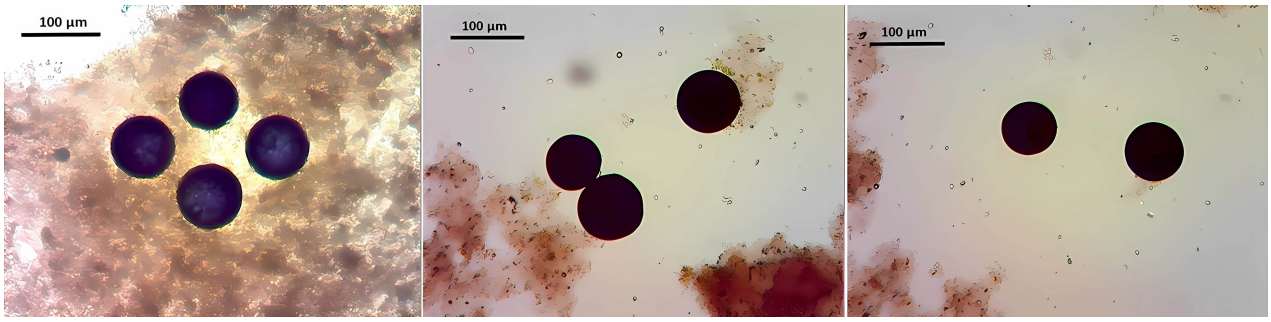

Figure 1. *Perkinsus marinus* hypnospores in *Mytella strigata* tissue from the southeastern Gulf of California (10X). Left = Altata bay, AB; central = Macapule lagoon, ML; right = The Colorado bay, CB.

the highest total prevalence (9.16%); hypnospores were observed in 5/30 and 6/30 mussels for summer 2020 and spring 2021, respectively. Round and dark corpuscles were not detected in fall of 2020 and winter of 2021 (Table 2). In ML, only two mussels were positive for the presence of hypnospores during the fall 2020 and winter 2021 seasons; while for AB, only a single individual had a hyponspore in the fall 2020 sampling. The highest pathogen density (13 hypnospores/g tissue) was obtained from a specimen collected from CB, while the lowest was from a specimen from ML (1 hypnospore/3 g tissue). The mean *P. marinus* density ranged from 4 hypnospores/g tissue in CB to 1 hypnospore/g tissue in ML and AB.

Discussion

Despite the different seasonal patterns that occurred for most of the environmental variables studied in each lagoon, the parasite *P. marinus* was

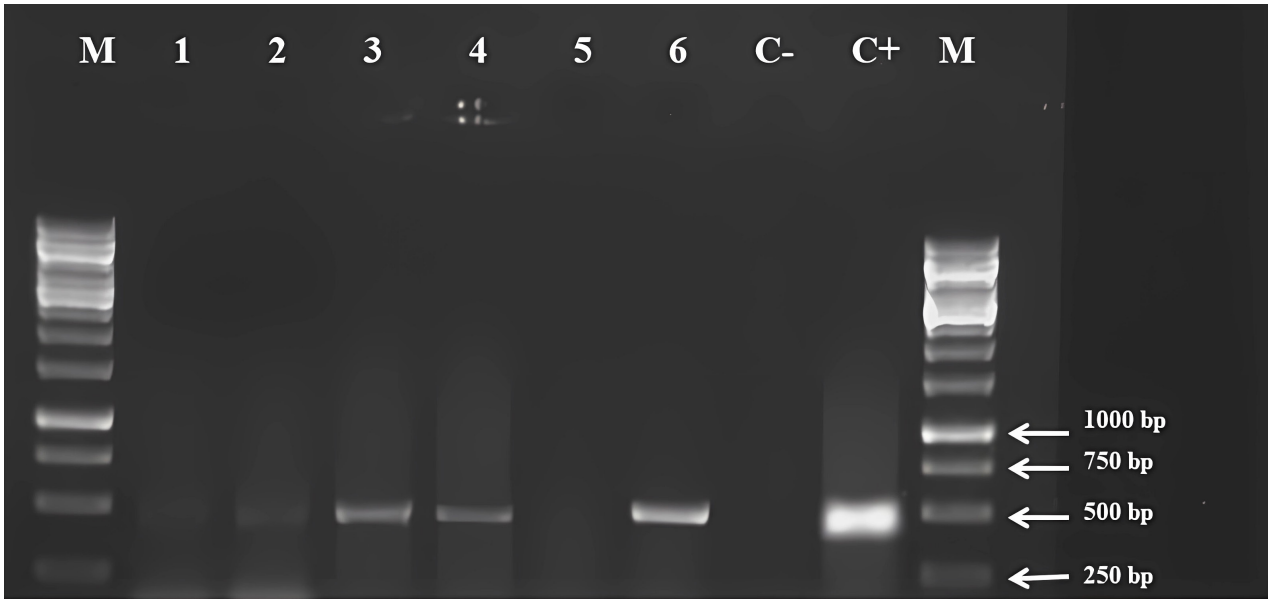

Figure 2. Agarose gel electrophoresis of PCR products of *Perkinsus marinus* in *Mytella strigata* from the three coastal lagoons (CB = El Colorado bay, ML = Macapule lagoon, and AB = Altata bay) at the southeastern Gulf of California. Lanes: M) Molecular weight marker (1kb ladder), 1–3) positive samples of *Mytella strigata* from CB (summer), 4) positive simple of *Mytella strigata* from ML (autumn), 5) negative simple of *Mytella strigata* from ML (winter), 6) positive simple of *Mytella strigata* from AB (autumn), [C-] negative control, [C+] positive control.

Table 2. Annual and seasonal prevalence (%) and parasite density (PD = hypnospores/g tissue) of *Perkinsus marinus* in *Mytella strigata* in the three lagoons (AB = Altata bay, ML = Macapule lagoon, CB = The Colorado bay) at the southeastern Gulf of California.

	Summer 2020	Autumn 2020	Winter 2021	Spring 2021	Total
AB					
Prevalence	0	3.33	0	0	0.83
PD	0	1	0	0	1
ML					
Prevalence	0	6.66	6.66	0	5.55
PD	0	1	1	0	1
CB					
Prevalence	16.66	0	0	20	9.16
PD (max.–min.)	13–3	0	0	1–2	4

detected and confirmed in the tissue of *M. strigata* across all seasons and sites, which demonstrates the physiological plasticity of this microorganism to adapt to wide intervals of environmental conditions, even outside its native geographic range. Such plasticity was documented by Goggin et al. (1990) and Le Peyre et al. (2006) under laboratory conditions. It has been reported that in its native range, the occurrence of *P. marinus* in different ecological niches is mainly conditioned by salinity and water temperature, which become limiting below 12–15 g/L and 20 °C, respectively (Chu and La Peyre 1993; Villalba et al. 2004). However, this parasitoid can survive salinities close to 0 g/L and temperatures down to 10 °C (O'Farrell et al. 2000; Casas et al. 2002). In the particular case of the invasive range of Gulf of California, Navarro-Chávez (2021) detected the pathogen in the black clam, *Chionista fluctifraga* (Sowerby II, 1853), cultivated in the intertidal zone which experienced temperatures and salinities down to 15.9 °C and

25 g/L, respectively. Góngora-Gómez et al. (2020) documented *Perkinsus* sp. in a wild population of the clam *M. squalida*, for which they obtained maximum values of 37 °C for temperature and 40 g/L for water salinity. In this study, the water temperature presented an interval between 21.3 °C obtained in ML (winter 2021) to 34 °C in AB (summer 2020), meanwhile, the higher salinity (45 PSU, spring 2021) was observed in CB and the lowest (22 PSU, summer 2020) in AB. These environmental invasive ranges are within the native values reported in other locations where *P. marinus* has been found (Soniat 1996; Huicab-Pech et al. 2012). Due to these wide tolerance ranges, this parasite can effectively colonize introduced locations, including the three sites studied here on the southeast coast of the Gulf of California.

The parasite prevalence (< 9.16%) and maximum PD (13 hypnospores/g tissue) found in this study suggest that the infective potential of *P. marinus* does not menace the health of mussels in the area, but rather the parasite used the mussel as a host for reproduction (Perkins 1996) or is part of its diet. Thieltges et al. (2013) and Ben-Horin et al. (2015) mention that parasites of free-living stages are part of the prey of filter-feeders, such as bivalves. Similar infection burdens were found for a wild population of the pen shell *Atrina maura* (G. B. Sowerby I, 1835) (Góngora-Gómez et al. 2016), and cultivated C. *corteziensis* (Villanueva-Fonseca et al. 2020) and *M. gigas* (Góngora-Gómez et al. 2019) located near the study area. It is highly relevant that the occurrence of *P. marinus* in several species of mollusks from different locations in the Gulf of California confirms that the pathogen has crossed the four barriers that constitute an "invader" (geographical, environmental, reproductive, and dispersion, Kus Veenvliet 2021). According to its degree of expansion in the area, it is possible that its development is in a lag phase (Pyšek and Richardson 2010). Something similar was reported by Rivol et al. (2004) for the exotic mussel, *Brachidontes pharaonis* (P. Fisher, 1870), in the Red Sea, where it remained inconspicuous for about 120 years, until, in the 1990s, a decline in the population of the native dwarf mussel, *Mytilaster minimus* (Poli, 1795), was detected, which, *B. pharaoins* were displaced it. Specifically, the first record of *P. marinus* in the Mexican coasts of the Pacific Ocean occurred in 2006 when it was detected in the pleasure oyster *C. corteziensis* (Cáceres-Martínez et al. 2008). In that same year, Enríquez-Espinoza et al. (2010) reported massive mortality of *M. gigas* cultivated in Northwest Mexico, which was associated with the presence of the parasite. Since then, there are no information on mortalities in bivalves related to the occurrence of *P. marinus* in this region, which suggests that this parasite maintains a biological association (host-parasite) with the mollusks it invades.

The general focus of *P. marinus* in the aquaculture industry centers on the infectious opportunism that associates it with mortalities of bivalve mollusks (Enríquez-Espinoza et al. 2010); however, the prevalence and PD

obtained for *M. strigata* in the three lagoons of southeastern California suggests that more than a threat, this parasite is a successful colonizer, a non-indigenous species in the process of inhabiting new eco-sites in the region and different species of ostreids, venerids, pinnids, and mytilids. The growing number of bivalve mollusk species in which *P. marinus* has been reported represents a warning signal that must be heralded to avoid a possible negative impact on the fishing and aquaculture industry of these invertebrates. Therefore, this report aims to: 1) provide -for the first time- evidence of the colonization of *P. marinus* in *M. strigata* in the southeastern Gulf of California, 2) notify the community of the invasion of the parasite in the area, with its presence in ecological niches out to its origin and in different species of bivalves native to the Gulf of California, and 3) contribute initial knowledge for the elaboration and consolidation of a risk assessment for *P. marinus*, which is necessary for future control.

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Ethics

The Ethics Committee of the College of Teachers of the IPN-CIIDIR Sinaloa Unit approved this research.

Permits

Mussels were collected, transported, and sacrificed according to the Mexican standard norms (NOM-031-SSA1-1993, Bienes y Servicios. Productos de la pesca. Moluscos bivalvos frescos-refrigerados).

Authors' contribution

Conceptualization: MGU; Methodology: MGU, AMGG; Sampling: AMGG, JAHS: Software: MGU, TEI; Validation: MGU, BPVF, TEI, JACM; Formal analysis: MGU, AMGG; Investigation and writing – original draft: MGU, AMGG, JACM, BPVF, TEI; Writing – review and editing: MGU; Funding acquisition: MGU, AMGG.

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