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New locations and parasitological findings for the invasive shrimp *Palaemon macrodactylus* in temperate southwestern Atlantic coastal waters

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ABSTRACT: This paper presents new records that considerably expand the geographical range of the invasive shrimp *Palaemon macrodactylus* in Argentina to include new estuarine areas on the Argentine south Atlantic coast: Bahía Samborombón, and the Bahía Blanca and Río Negro estuaries. The latter 2 locations are the southernmost reports for this species. The epibiotic barnacle *Balanus amphitrite* and a microphallid metacercaria *Odhneria* sp. are reported for the first time in *P. macrodactylus*. The prevalence of white spot syndrome virus (WSSV) was 10% in the Bahía Blanca estuary. Our results strongly suggest that WSSV is spreading in crustacean populations in the Argentine Sea and that *P. macrodactylus* plays a role in the ecology of parasite infections in this environment.

KEY WORDS: Oriental shrimp · *Palaemon macrodactylus* · Parasites · Barnacles · Microphallidae · White spot syndrome virus · Argentina

INTRODUCTION

The oriental shrimp *Palaemon macrodactylus* Rathbun, 1902, is an invasive crustacean in Argentina that has been widely dispersed outside of its native range in the coasts of Japan, Korea, and northern China (Rathbun 1902, Newman 1963, Spivak et al. 2006). The species was first reported in Argentina in the Mar del Plata harbor by Spivak et al. (2006). Although there have not been any new reports of *P. macrodactylus* in Argentina since then, it was postulated that the species has a great potential to move beyond natural dispersal barriers and to become established and prolific (González-Ortegón et al. 2007). Moreover, Spivak et al. (2006) suggested the Mar del Plata port as an entrance site and possible dispersion center for *P. macrodactylus*.

Among others, the 'enemy release' hypothesis posits that in their native range, populations are regulated by enemies, but these enemies are reduced in number or absent from populations outside their natural dispersal range, thereby allowing introduced species to escape regulatory forces and become invasive (Hierro et al. 2005). Recent research has recognized the role of parasites (both macro- and microparasites) as such enemies and has documented lower numbers of parasites occurring in invasive host species in invaded, compared to natural, ranges (Torchin et al. 2001, Torchin & Mitchell 2004). Consequently, the study of the parasites present in an invasive species in a new environment is an important first step to better understanding the factors that favor or impede successful biological invasions.

The objectives of the present study were 2-fold: (1) to assess the expansion of *Palaemon macrodactylus* in areas outside its known distributional range in Argentina, and (2) to investigate the presence of helminths, white spot syndrome virus (WSSV), and epibionts in this invasive shrimp.

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MATERIALS AND METHODS

Shrimp were sampled between March 2007 and March 2011 at 4 sites in Argentina: the Río Salado flood relief channel (Site 1: 35° 50′ S, 57° 25′ W; n = 49) and Tapera de López (Site 2: 36° 19′ S, 56° 46′ W; n = 46) in Bahía Samborombón, the Bahía Blanca estuary (Site 3: 38° 44′ S, 62° 22′ W; n = 110), and the Río Negro estuary (Site 4: 41° 1′ S, 62° 47′ W; n = 1). The first 3 sites are located in the Buenos Aires province, and the fourth site is in the Río Negro province (Fig. 1). Specimens were collected using cast nets and minnow traps baited with fish meat. The specimens were transported alive to the laboratory and kept in small aquaria with seawater. Some ovigerous females were kept alive until their eggs hatched, and the resulting larvae were fixed in 10% formalin for morphological studies.

The shrimp were studied alive, killed by freezing, and fixed in 10% formalin until examination for parasites using a stereomicroscope. A total of 20 specimens from Bahía Blanca estuary were examined fresh for the presence of white spots suggestive of WSSV. These specimens were then preserved in 96% ethanol and tested for WSSV using nested PCR. DNA was extracted from the gills using DNeasy kits (Quiagen). PCR amplification was conducted using illustra PuReTaq Ready-To-Go™ PCR Beads (GE Healthcare). Details of the primers and PCR procedures used are described by Martorelli et al. (2010). Unless otherwise stated, throughout the present study, we refer to parasites and pathogens as simply ‘parasites’.

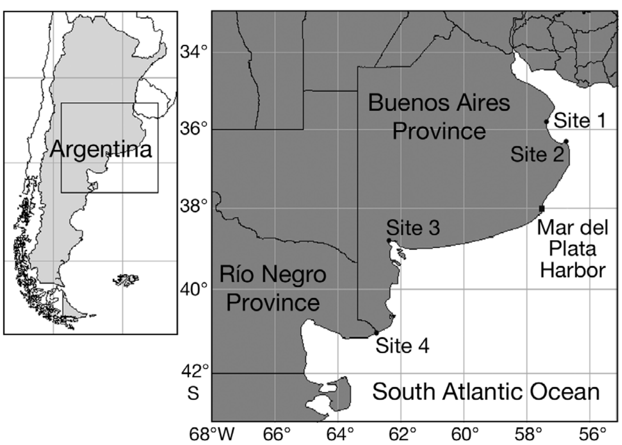

Fig. 1. *Palaemon macrodactylus* sample sites. Site 1: Río Salado flood relief channel. Site 2: Tapera de López. Site 3: Bahía Blanca estuary. Site 4: Río Negro estuary. Mar del Plata Port: first reported site of *P. macrodactylus* in Argentina

We deposited voucher specimens from each sampling area in the invertebrate collection of the La Plata Museum (voucher number): Río Salado (26753) and Tapera de López in Bahía Samborombón (26754), Bahía Blanca estuary (26755), and Río Negro estuary (26756). We also deposited one specimen with an epibiont from Tapera de López (26757) and zoea larvae from Bahía Blanca estuary (26758).

RESULTS

The present paper presents the southernmost report of *Palaemon macrodactylus* off the southwestern Atlantic coast. We also report for the first time a helminth parasite and an epibiotic exotic crustacean in *P. macrodactylus* and provide new data on the prevalence of WSSV in *P. macrodactylus* from the Argentine Sea. *P. macrodactylus* individuals, including females bearing eggs with viable embryos, were found at all of the study sites (Fig. 1).

Three types of biological interactions — with helminths, epibionts, and one virus — were identified in *Palaemon macrodactylus*. Among the helminths, a digenean metacercaria (Microphallidae) was found encysted in the cephalothorax of one specimen (Table 1) and was tentatively identified as *Odhneria* sp. (Fig. 2). Among the epibionts, the barnacle *Balanus amphitrite* Darwin, 1854, was found on the exoskeleton of shrimps (Table 1, Fig. 3). Some individuals had melanized cuticular lesions (Table 1, Fig. 4). A total of 25% (5 of 20) of *P. macrodactylus* from the Bahía Blanca estuary that were examined fresh had WSSV-like cuticular spots. The spots measured 1 to 3 mm in diameter. Also, 10% (2 of 20) of the specimens tested through PCR were positive for WSSV, but only one (0.05%) of these had white spots on fresh examination (Table 1, Fig. 5).

DISCUSSION

In Argentina, *Palaemon macrodactylus* has been previously reported only in the Mar del Plata port (Spivak et al. 2006). To date, that first report and the one in Lake Mannering in Australia (Buckworth 1979) were the 2 most southern records for the species. Therefore, this work greatly expands the current distributional range of the species along the Argentine Atlantic coast and represents the southernmost record for this species. The new locations reported here indicate the great capacity of the species to adapt to a range of environments around the world

Table 1. Prevalence of conditions investigated in *Palaemon macrodactylus* in Argentina. F: ovigerous females; M: males; WSSV: white spot syndrome virus; na: not applicable; ne: not examined; 95% CI: 95% confidence interval for prevalence. See Fig. 1 for site locations and description. Water temperatures from the National Hydrographic Service (www.hidro.gov.ar)

Site	Water temp. (annual range, °C)	Sample size	Carapace length (range, mm)	Prevalence (%) and 95% CI			
				Odhneria sp.	*Balanus amphitrite*	Melanized cuticular lesions	WSSV
1	13–23	F: 65 M: 29	12–16 21–26	0	6.4 (2.7–13.5)	6.4 (2.7–13.5)	ne
2	11–23	F: 30 M: 16	14–17 18–24	0	8.7 (2.9–20.9)	15.2 (7.3–28.5)	ne
3	4–23	F: 80 M: 30	16–29 12–16	0.9 (~0.0–5.5)	0	1.8 (~0.0–6.8)	10.0 (1.6–31.3)
4	4–24	F: 1 M: 0	19 na	0	0	0	ne

Fig. 2. *Odhneria* sp. Experimental unencysted metacercariae. Scale bar = 0.15 mm

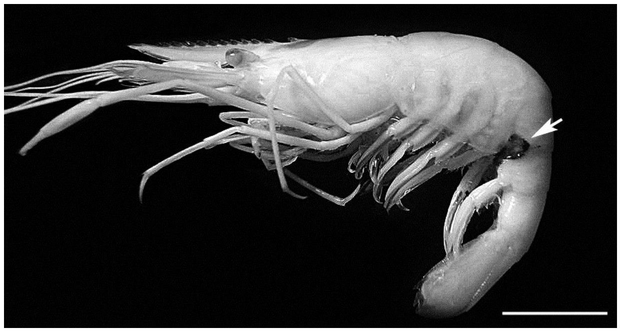

Fig. 4. Melanized cuticular lesions in posterior abdominal region of *Palaemon macrodactylus* (arrow). Scale bar = 10 mm

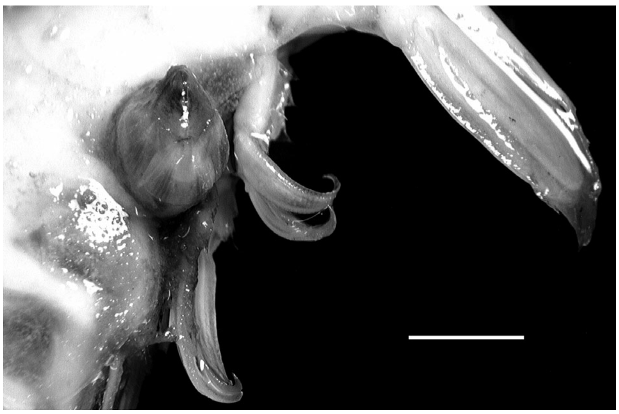

Fig. 3. Barnacle *Balanus amphitrite* fixed in the tail region of *Palaemon macrodactylus* from Bahía Samborombón. Scale bar = 2 mm

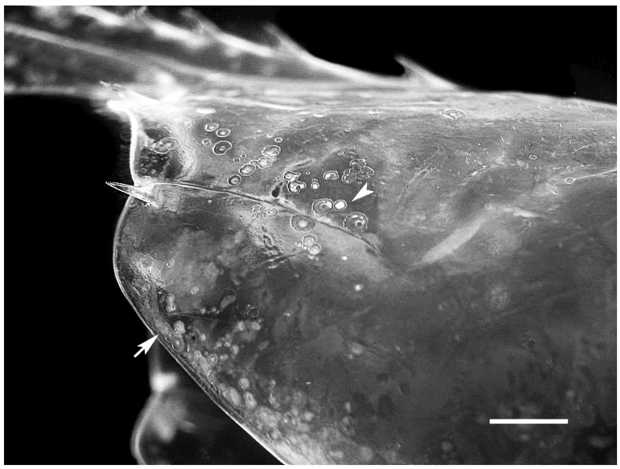

Fig. 5. White spot on surface of the cephalothorax from a WSSV-positive *Palaemon macrodactylus* (arrows). Scale bar = 1.5 mm

and confirm early predictions that *P. macrodactylus* would rapidly expand in Argentina (Spivak et al. 2006).

Palaemon macrodactylus may have been introduced in the Mar del Plata harbor from the Pacific via discharged ballast water (Wasson et al. 2001). As Spivak et al. (2006) predicted, larvae may have been dispersed both north and south of the initial entrance point and then colonized brackish water habitats.

The main diagnostic morphological characteristics of the studied specimens agreed with those reported by Rathbun (1902), Newman (1963), d'Udekem d'A-

Fig. 6. Two-day-old laboratory-reared zoea of *Palaemon macrodactylus*. Arrow: hook-shaped dorsal spine. Scale bar = 0.2 mm

coz et al. (2005), González-Ortegón & Cuesta (2006), and Elder & Fuller (2012). The carapace of the ovigerous females collected in the Bahía Blanca and Bahía Samborombón estuaries was longer than that reported by Spivak et al. (2006) for females in Mar del Plata (12–29 mm vs. 16.5–19 mm). Live specimens of *Palaemon macrodactylus* showed a whitish longitudinal dorsal stripe similar to that reported by d'Udekem d'Acoz et al. (2005). Laboratory-reared zoea also showed the typical hook-shaped dorsal spine in the third abdominal segment (Fig. 6) (González-Ortegón & Cuesta 2006).

The digenean metacercaria reported here was remarkably similar to that of *Odhneria* sp. reported by Alda et al. (2011). A heteroxenic life cycle, like the one described for the genus *Odhneria* (Stunkard 1979), makes it unlikely that metacercarial infections are accidental in *Palaemon macrodactylus*. Rather, the infections would be the consequence of this genus having host specificities ranging between stenoxenous (phylogenetically related hosts) and euryxenous (ecologically related hosts). Also, the role of *P. macrodactylus* as a second intermediate host in the life cycle of *Odhneria* spp. would increase the likelihood that this helminth successfully completes its life cycle in the studied habitats. *Odhneria* spp. has been previously reported as metacercaria larva in the hemocoel and muscle of the grapsid crabs *Neohelice granulata* (Dana, 1851) and *Cyrtograpus angulatus* Dana, 1851 (Alda et al. 2011) and as adults of *Odhneria odhneri* Travassos, 1921, in the intestines of Olrog's Gull *Larus atlanticus* Olrog, 1958, from the Bahía Blanca estuary (La Sala et al. 2009). Palaemonid shrimp are preyed upon by Kelp gulls *Larus dominicanus* Lichtenstein, 1823 (Petracci et al. 2004), and by Olrog's gulls (L. F. La Sala pers. comm.) in the Bahía Blanca estuary; therefore, *P. macrodactylus* most probably serves as an intermediate host for the trophic transmission of helminth parasites to their final vertebrate hosts.

The morphological characteristics of the barnacles reported in the present work agree with those of the introduced striped barnacle *Balanus amphitrite*. This species has been previously reported as a fouling organism in Argentina (Elías & Vallarino 2001, Spivak 2003).

In marine invasions, larval stages introduced via ballast water lack parasites that can infect adult stages (Lafferty & Kuris 1996). During a parasitological survey conducted on >50 individuals of *Palaemon macrodactylus* from the Mar del Plata area between 2004 and 2005, no helminths were found in this species (S. R. Martorelli pers. comm.). These early negative parasitological results support the hypothesis that *P. macrodactylus* arrived in Argentina as helminth-free larvae and then acquired a role as a suitable intermediate host at least in the Bahía Blanca estuary, where this species seems to play a role in the trophic transmission of larval stages of microphallids, such as *Odhneria* spp., to their definitive hosts.

In our study, indirect evidence of bacterial infections, such as melanized cuticular lesions, were observed in some individuals. The cause of these lesions could not be determined; however, they were possibly associated with bacterial infections, viral infections, or healing of physical injuries (Lightner 1996, Noga et al. 2000).

The presence of white spots on the cuticular tissue could be due to its abnormal development leading to tissue thinning and calcium deposition. These lesions were similar to those observed in WSSV-infected *Artemesia longinaris* Bate, 1888 and *Palae-*

mon macrodactylus (Martorelli et al. 2010). Despite this, in our study, the presence of cuticular lesions correlated very poorly (0.05%) with infection by WSSV as evidenced using PCR.

Other pathogens, such as bacteria, can cause similar lesions in the carapace (e.g. bacterial white spot syndrome) (Wang et al. 2000), thus explaining the high rate of white spot-positive individuals that tested negative for WSSV through PCR.

The present study is the second report of WSSV-infected shrimp in the Bahía Blanca estuary, thus adding relevant data about the epidemiology of this virus (Martorelli et al. 2010). According to Martorelli et al. (2010), WSSV may have been introduced in Argentina by WSSV-infected *Palaemon macrodactylus*, some vector carried in ballast water, some infected crustaceans carried by natural currents from Brazil, or through infected seafood imported from other countries.

Our results suggest that WSSV, also present in *Artemesia longinaris* and *Cyrtograpsus angulatus* (Martorelli et al. 2010), is spreading in the crustacean populations of the Argentine Sea.

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