Journal of Invertebrate Pathology 105 (2010) 254–260

Contents lists available at ScienceDirect

Journal of Invertebrate Pathology

journal homepage: www.elsevier.com/locate/jip

Host–parasite relationship of the geoduck *Panopea abbreviata* and the green alga *Coccomyxa parasitica* in the Argentinean Patagonian coast

Nuria Vázquez [a,*], Francisco Rodríguez [b], Cristián Ituarte [c], Javier Klaich [a], Florencia Cremonte [a]

[a] Centro Nacional Patagónico (CONICET), Boulevard Brown 2915, U9120ACD Puerto Madryn, Chubut, Argentina
[b] Instituto Español de Oceanografía, Apdo. 1552, 36200 Vigo, España, Spain
[c] Museo Argentino de Ciencias Naturales (CONICET), Av. Ángel Gallardo 470, C1405DJR Buenos Aires, Argentina

ARTICLE INFO

Article history:
Received 4 December 2009
Accepted 21 July 2010
Available online 27 July 2010

Keywords:
Panopea abbreviate
Coccomyxa parasitica
Condition index
Green alga

ABSTRACT

The association of the geoduck *Panopea abbreviata* and the green alga *Coccomyxa parasitica* is described. The identity of the green alga was confirmed by molecular studies; the alga was found within the hemocytes that infiltrate the connective tissue of the geoduck siphons. Cytological characteristics of hemocytes were not altered by algal infection; very often the algae were seen enveloped by a digestive vacuole within the hemocyte cytoplasm, evidencing diverse degrees of resorption. Connective cells of siphons were rarely infected by *C. parasitica*. The mean prevalence of *C. parasitica* was higher (82%) in San Matías Gulf (42°00′S, 65°05′W) than in San José Gulf (45%) (40°32′S, 64°02′W); except for spring, when the two locations showed no differences in prevalences (80%). Independently of location, season and host size, infected geoducks showed lower condition index values than uninfected ones. Regarding other bivalve species, only one specimen of the razor clam *Ensis macha* was found infected, and none of the oysters *Ostrea puelchana* and *Pododesmus rudis* and scallop *Aequipecten tehuelchus* was parasitized by the green alga.

1. Introduction

The geoduck *Panopea abbreviata* (Valenciennes, 1839) (Hiatellidae) is endemic from the Southwestern Atlantic, occurring between 23°S and 48°S (Scarabino, 1977). This species is a large and long-lived bivalve which is found deeply buried, up to 70 cm in soft muddy sediments; only the tips of the siphons remain exposed above the sediment surface (Morsan and Ciocco, 2004). Nevertheless, this species represents a valuable potential fishery resource; the exploitation of their Atlantic populations started only in the recent years (Ciocco, 2000).

During a survey of the health status of *P. abbreviata* in Northern Patagonian gulfs (Argentina), some geoducks showed the tips of siphons to be green colored, resembling infections by the intracellular green algae of the genus *Coccomyxa* (Chlorococcales: Coccomyxaceae) described by Rodríguez et al. (2008) in *Mytilus edulis*.

The genus *Coccomyxa* includes both free-living marine and freshwater planktonic species (Guiry et al., 2005), epiphytic (Lamenti et al., 2000), symbiotic with lichens (Lohtander et al., 2003) and protozoans (Hoshina and Imamura, 2008), and parasitic in starfish (Mortensen and Rosenvinge, 1933), scallops (Naidu and South, 1970; Stevenson and South, 1974), and mussels (Boraso de Zaixso and Zaixso, 1979; Bala, 1995; Gray et al., 1999). *C. parasitica* was first described by Stevenson and South (1974), infecting the giant scallop *Placopecten magellanicus* (Pectinidae) from Newfoundland, Canada. It was also reported in *M. edulis chilensis* from Patagonia, Argentina (Boraso de Zaixso and Zaixso, 1979; Bala, 1995) and from Malvinas (Falkland) Islands (Gray et al., 1999). However, the identification of the alga in the last mentioned studies was only supported by morphological features. Rodríguez et al. (2008) confirmed the presence of *C. parasitica* infecting *M. edulis chilensis* in the North Sea and Malvinas (Falkland) Islands, based on data from molecular studies, histopathology, ultrastructure, and pigments of the green alga.

In the present study, the presence of *C. parasitica* in *P. abbreviata* from Northern Patagonian gulfs is reported for the first time, and the alga was characterized based on both morphological and molecular data (small subunit ribosomal RNA (SSU rRNA) sequencing). Furthermore, the seasonal and geographical variations of prevalence and the effects of the green alga on the condition index of geoducks were studied.

2. Materials and methods

2.1. Sample collection and processing

During 2007, 60 geoducks (*P. abbreviata*) were seasonally collected at 15 m depth at Puerto Lobos (42°00′S, 65°05′W), San

* Corresponding author. Fax: +54 2965 451543.
E-mail address: nuria@cenpat.edu.ar (N. Vázquez).

0022-2011/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.jip.2010.07.005

Matías Gulf ($n = 240$), and approximately 50 geoducks at Punta Conos (40°32′S, 64°02′W), San José Gulf ($n = 210$) (Fig. 1). Each sampling season was carried out in January, April, July and October for both locations. Additionally, during 2006 and 2007, 682 oysters [*Ostrea puelchana* d'Orbigny, 1842 (Ostreidae)], 133 jingle-shell oysters [*Pododesmus rudis* Broderip, 1834 (Anomiidae)] were collected at Puerto Lobos and Playa Fracasso (42°25′S, 64°07′W), 480 razor clams [*Ensis macha* (Molina, 1782) (Solenidae)] at Playa Fracasso and 180 scallops [*Aequipecten tehuelchus* (d'Orbigny, 1846) (Pectinidae)] at Punta Conos (Fig. 1). The bivalves were transported to the laboratory and maintained in aquaria with aerated seawater at 13 °C for 24 h until processing.

Maximum shell length of each specimen was measured; shell and flesh were weighed separately to calculate the condition index (wet flesh weight to shell weight ratio). Soft parts of all bivalves were macroscopically examined for signs of algal infection, as the extent of green color, presumed to indicate algal colonization or infection. Infection intensity, based on the extension of the green area on the siphons, was graded as: uninfected: absence of green coloration; slightly infected: green color confined up to 1 cm from the tip of the siphons; moderately infected: green color reaching up to 3 cm from the tip of the siphon; heavily infected: a dark green color exceeding the 3 cm from the tip of the siphons.

2.2. Histological processing

Small pieces of siphonal tissues showing green coloration as well as pieces of nondiscolored siphonal tips (5 × 10 mm) were obtained, and one oblique transverse 5 mm thick section of 180 geoducks, containing gill, digestive gland, mantle, nephridia and gonad was excised. The siphonal tissues and the oblique transverse sections were fixed in Davidson's fixative (Shaw and Battle, 1957), dehydrated in an ethanol series and embedded in Paraplast® and Historesin® Leica. Histological sections (5 μm thick) were stained either with Harris or Mayer's hematoxylin and eosin, and observed under a light microscope.

2.3. Transmission electron microscopy

Small pieces of siphonal tissues showing green coloration (5 × 10 mm) were fixed in cold 2.5% glutaraldehyde with 4% formalin (from paraformaldehyde) in 0.2 M cacodylate buffer at pH 7.2 for 1 h. After rinsing in cacodylate buffer, samples were postfixed in 1% osmium tetroxide in the same buffer at 4 °C, rinsed in 0.2 M cacodylate buffer, dehydrated in an ascending ethanol series (70–100%), and transferred to Spurr's resin via propylene oxide. Infiltration was performed in Spurr's resin. Ultrathin sections were double stained with uranyl acetate and lead citrate, and examined either in a Jeol 1200 EX II and a Philips EM301 transmission electron microscopes (TEM).

2.4. DNA extraction and SSU rRNA sequencing

Pieces of tissues from two geoducks showing green coloration were cut off and preserved in ethanol 96% until molecular analysis was performed. A green supernatant obtained after grinding tissues with a spatula was pipetted in an Eppendorf microtube and centrifuged (5000 rpm, 5 min) using a table-top minicentrifuge. A greenish pellet mixed with tissue debris was collected. The supernatant was centrifuged again (12,000 rpm, 10 min) and a second dark green pellet was obtained. Both pellets were rinsed in 100 μl of N-cetyl N,N,N,-trimethylammonium bromide (CTAB)

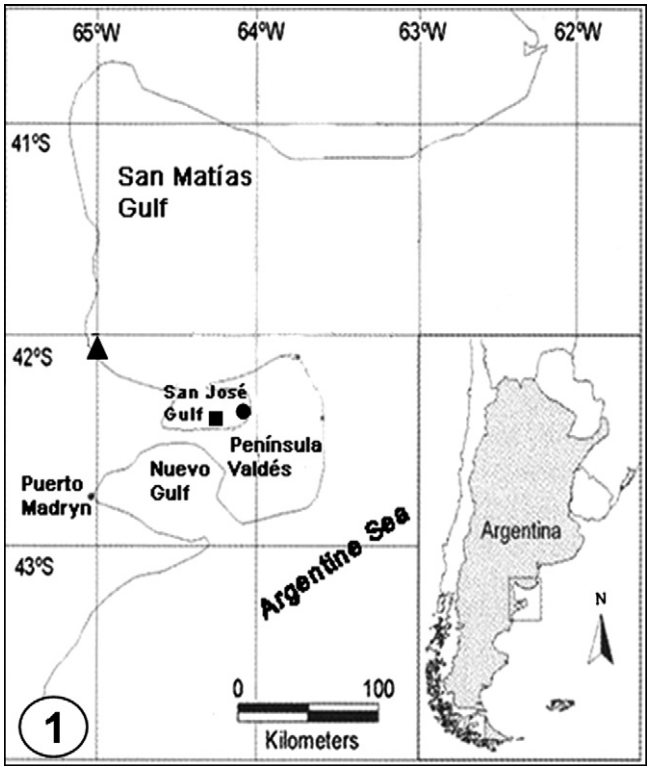

Fig. 1. Collection sites. References: ▲ = Puerto Lobos, ● = Punta Conos, ■ = Playa Fracasso.

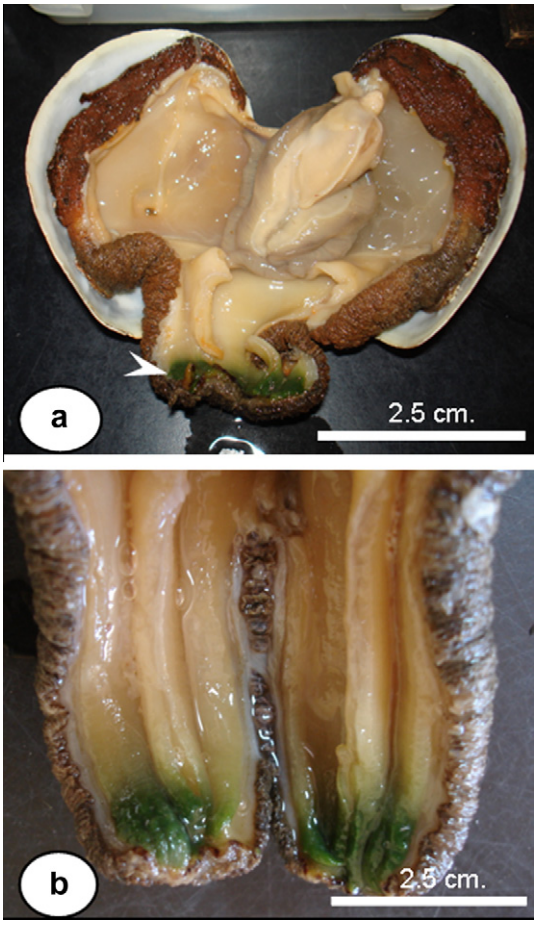

Fig. 2. *Panopea abbreviata*. Dissected clam (a) and detail of siphons (b) showing a moderate intensity of infection (arrow head).