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Detection of *Paragonimus mexicanus* (Trematoda) metacercariae in crabs from Oaxaca, Mexico

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ABSTRACT

Metacercariae of *Paragonimus mexicanus* were collected in crabs *Tehuana guerreroensis* (Rathbun, 1933) in the municipality of Putla, Oaxaca, Mexico. Metacercariae were found in 20.8% of the crabs collected, with an average of 1.9 metacercarie per crab. Stained metacercariae showed the specific characteristics of *P. mexicanus* by morphology and sequencing a fragment of the 28S ribosomal gene obtained by PCR. These findings reveal that *T. guerreroensis* is an intermediate host for *P. mexicanus*; this new report is relevant considering the potential risk of transmission in the states of Oaxaca and Guerrero, Mexico.

1. Introduction

Paragonimiasis is a food-borne parasitic disease of humans, other mammals, molluscs and crustaceans; it is caused by flukes of the genus *Paragonimus* spp. Human paragonimiasis is distributed across three continents: South and Central America, East Asia and West Africa. Typical symptoms of human paragonimiasis include fever, cough, eosinophilia and hemoptysis, which together can be misdiagnosed as tuberculosis. Praziquantel is the drug of choice for paragonimiasis; however, other drugs have been used (Keiser and Utzinger, 2010). *Paragonimus* spp. use snails as first intermediate hosts and decapod crustaceans (crabs and freshwater shrimp) as second intermediate hosts.

Infection occurs when man ingests either raw or uncooked crustaceans infected with metacercariae or meat from paratenic hosts. More than 30 species of the genus *Paragonimus* have been described; of these, 10 species have been reported to infect humans. *Paragonimus mexicanus* is located in México, Colombia, Perú, Ecuador, Costa Rica, Panamá and Guatemala. In México, this parasite has been found in wild animals from Colima, Chiapas, Hidalgo, Michoacán, Nayarit, Puebla, San Luis Potosi, Tabasco, Yucatán, Veracruz and State of México (Lamothe-Argumedo, 1985). Several crustacean species of the family Pseudothelphusidae and one of the family Trichodactylidae serve as second intermediate hosts (Blair et al., 1999).

At least 13 genera and 48 species of the family Pheudothelphusidae have been described in México, as summarized elsewhere (Rodríguez and Magalhães, 2005). *P. mexicanus* metacercariae have been found in crustaceans such as *Pseudothelphusa dilatata* (Lamothe-Argumedo et al., 1977), *Ptychophallus tristani* and *P. costaricensis* (Brenes et al., 1980; Mongue et al., 1985), *Odontothelphusa maxillipes* and *Raddaus tuberculatus* (Lamothe-Argumedo, 1984), *Pseudothelphusa americana belliana*, *P. nayaritae* and *P. terrestris* (Lamothe-Argumedo, 1995), all of which serve as second intermediate hosts. In many regions of Mexico, as in the state of Oaxaca, people traditionally include wild crabs and other crustaceans as part of their diet. Improper cooking of these crabs represents a potential risk for infection due to ingestion of *Paragonimus* spp. metacercariae. The aim of this study is to isolate and evaluate the presence of *P. mexicanus* from crabs collected in the state of Oaxaca, Mexico.

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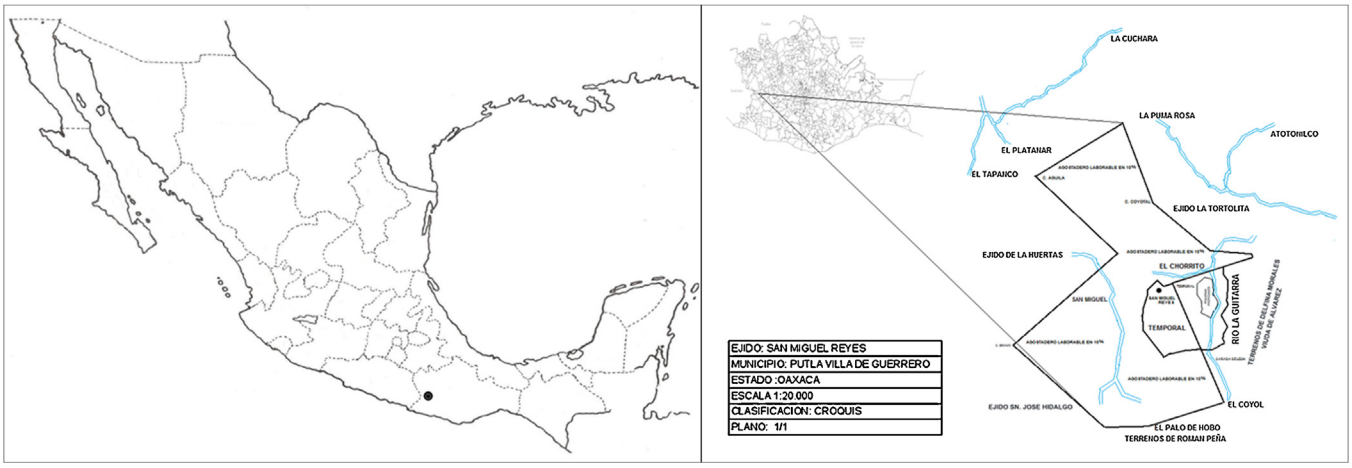

Fig. 1. Map of Mexico country and localization of Putla Villa de Guerrero, state of Oaxaca, Mexico.

2. Materials and methods

2.1. Source of crabs

Crabs were collected at San Miguel Reyes, municipality of Putla Villa de Guerrero, State of Oaxaca, Mexico (97°55′45″ West and, 17°01′33″ North at an altitude of 783 m.a.s.l.) (Fig. 1). A total of 120 crabs (Fig. 2) of various sizes were collected between February 16th and March 13th 2011, in small freshwater streams. Water temperature at the time of capture was 21 °C; pH was 7.0. Crabs were placed in plastic containers and transported alive to the laboratory.

2.2. Isolation of metacercariae

In the laboratory, crabs were allowed to acclimatize for two days and then weighed and measured with a caliper. Afterwards, crabs were killed by thermal shock (2 °C for 10 min) fixed in 5% formalin and then placed in plastic containers. Crabs were then dissected: they were cut in half from the cephalothorax in longitudinal section in order to extract the gills, digestive system and ontocele. This material was placed in Petri dishes with saline, the tissue was finelly sliced with scalpel and carefully examined under a stereo microscope in search of *Paragonimus* spp. When found, metacercariae were rinsed in saline and preserved in 96% ethanol until used. Individual metacercaria were observed under the microscope.

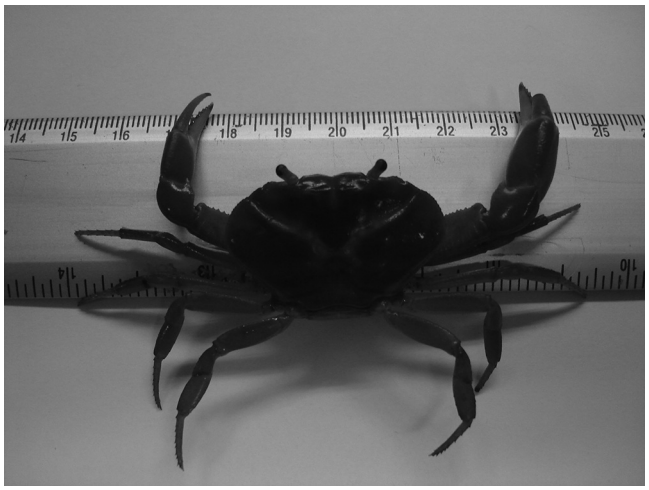

Fig. 2. Image of crab *Tehuana guerreroensis* collected in Oaxaca, Mexico.

2.3. DNA extractions from metacercariae

DNA was extracted from approximately 5 mg of metacercariae. DNA was purified using the DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA, USA) and its concentration was measured using a nanoDrop 2000 (Thermo Scientific Waltham MA, USA). DNA from uninfected crabs maintained and bred in the laboratory was used as negative control. All DNA samples were stored at −20 °C until use.

2.4. PCR assay

The DNA extracted was amplified in a 25-μl reaction well using 100 ng of DNA template, 0.8 μM each of forward and reverse primers (final concentration), and Master Mix (Roche). Amplification was run in a Tc-3000 (TECHNE)®. Cycling was performed as follows: initial DNA denaturing (94 °C, 5 min), followed by 30 cycles each of denaturing (92 °C, 30 s), annealing (61 °C, 30 seg), extension (72 °C, 1 min). The final extension was 72 °C for 4 min. The primers 28S-F 5′-GAGGGTGAAAGGCCCGTGGG-3′ and 28S-R 5′-ACGCATGCACACACCTCRAGCCG-3′ were designed in a conservative region bracketing a variable region of approximately 630 bp of the 28 S rRNA. Amplicons obtained were analyzed in 1.5% agarose gel stained with ethidium under UV light. Positive and negative controls were always included.

2.5. Sequencing and BLAST

The PCR product was sequenced in both strands in the Unit of Proteogenomics, UNAM, Juriquilla, Mexico. The sequences were viewed in the Chromas program Lite and refined manually. The final sequence was analyzed by BLAST in the NCBI server.

2.6. Identification of crabs

Adult crabs were sacrificed by thermal shock, fixed in 5% formalin for 5 days and then preserved in glass jars with 70% ethanol. Specimens were sexed and identified at the family and genus levels based on external morphological features using a Nikon SMZ1000 stereo microscope. Males were dissected and the gonopod was extracted to allow the specific identification by taxonomic keys (Rodríguez and Smalley, 1696; Rodríguez, 1982). The updated species name was taken from criteria of Alvarez and Villalobos (1994).