Latin American Journal of Aquatic Research, 48(1): 150-155, 2020
DOI: 10.3856/vol48-issue1-fulltext-2339

Short Communication

A new record of a tiger shrimp *Penaeus monodon* Fabricius, 1798 breeding female in the coast of Campeche, Mexico

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ABSTRACT. The tiger shrimp *Penaeus monodon* is native to the Indo-West Pacific Ocean, covering the east coast of Africa, the Arabian Peninsula, Southeast Asia, Taiwan, China, the Sea of Japan, New Guinea and Australia. A new report was recorded on the capture in the wild of a female tiger shrimp *P. monodon* in conditions of reproductive maturity on the coast of Campeche in the Gulf of Mexico. The specimen was captured by a trawler operating near the coast of Campeche, in front of Carmen Island. The specimen was identified using dichotomous keys, additionally corroborated with analysis of the sequence of a fragment of 650 base pairs (bp) of the mitochondrial DNA (mtDNA) gene cytochrome oxidase I (COI), and compared with the sequence of *P. monodon* reported in GenBank. The specimen was deposited in the National Collection of Crustaceans of the Institute of Biology of the UNAM. Histological analysis of the gonads revealed that the organism was in a reproductive condition as ovaries contained oocytes in an advanced development state. The molecular data (sequenced fragments F and R) were identical with the COI sequence of *P. monodon* deposited in GenBank, thus confirming the presence of *P. monodon* from the coasts of Isla del Carmen, Campeche.

Keywords: *Penaeus monodon*; tiger shrimp; genetic identification; introduced species; Gulf of Mexico

The giant or tiger shrimp *Penaeus monodon* inhabits the coast of Australia, Southeast Asia, south Asia and eastern Africa (Dore & Frimodt, 1987; Pérez-Farfante & Kensley, 1997). This species matures and reproduces only in tropical marine habitats and spends its larval, juvenile, adolescent and sub-adult stages in estuaries, coastal lagoons or mangroves. In nature, it shows a marked nocturnal activity, burying itself in the bottom substrate during the day and emerging at night to search for food as a benthic consumer. Under natural conditions like other penaeid shrimps, tiger shrimp is an efficient predator more than an omnivorous or detritivorous scavenger (FAO, 2005-2019). *Penaeus monodon* is a species that has been cultivated for more than a century to provide food and sustenance economic income for coastal human populations in some Asian countries such as Indonesia, Philippines, Taiwan Province of China, Thailand and Vietnam. Research on its breeding has been conducted since 1970 through monoculture techniques in small ponds that were developed at the Tungkang Marine laboratory in Taiwan, Province of China, and partially at the Institut Français de Recherche pour l'Exploitation de la Mer (IFREMER; Pacific Ocean Center). Between 1980 and 1987 there has been an explosive boom of small-scale intensive farms in Taiwan due to commercial success in the development of formulated diets and due to the increase of the shrimp production for exportation to Japan (FAO, 2005-2019). The tiger shrimp has been reported in other areas outside its natural geographical

Corresponding editor: Ingo Wehrtmann

distribution: in the west of Africa from Senegal to northern Angola (Knott *et al*., 2012), in South America from Colombia to Brazil (Fausto-Filho, 1987; Coelho *et al*., 2001; Santos & Coelho, 2002; Aguado & Sayegh, 2007; Altuve *et al*., 2008; Gómez-Lemos & Campos, 2008; Cintra *et al*., 2011), Cuba, Puerto Rico and the Dominican Republic in the Caribbean (Knott *et al*., 2012; Giménez-Hurtado *et al*., 2013). In Mexican coasts, there exist reports by Morán-Silva *et al*. (2014) and Wakida-Kusonoki *et al*. (2013, 2016a, 2016b).

The presence of *Penaeus monodon* in southeastern United States of America was due to the escape of organisms from aquaculture areas, while in the South American countries it is mentioned that in addition to escapes from the cultivation areas, it was due to the transport and introduction of ballast waters by intercontinental vessels (Franklin, 2002; Atencio *et al*., 2006). The present work reports the finding of a female tiger shrimp off the coast of Isla del Carmen, corroborating a series of previous records reported by Wakida *et al*. (2013). In the present study, in addition to the species morphological identification, for the first time, there was included the species identification confirmed by genetic evidence as well as the reproductive condition for the captured specimen.

The tiger shrimp specimen was collected on 17 October 2015 by fishermen aboard an artisanal shrimp trawler boat operating near the coast of Campeche off Isla del Carmen (18º43'17.72" N; 91º 55'32.0"W). The specimen was placed on ice and transported for physical inspection to the "El Carmen" station of the Instituto de Ciencias del Mar y Limnología (ICMyL) at Universidad Nacional Autónoma de México (UNAM). The sex of the organism was determined by examining the gonads and registered the total length in mm (TL), carapace length (CL) and total weight in g (TW). Tissue samples were obtained from the sixth abdominal segment for the genetic identification of the organism, which was transported to the Genetics Laboratory of the ICMyL-UNAM, in Mexico City. The entire organism was preserved in 70% ethanol and sent to the Department of Comparative Biology of the Faculty of Sciences-UNAM, where the histological analysis of the gonads was performed. Finally, the specimen was deposited in the Crustacean National Collection of the Instituto de Biología-UNAM and was assigned the catalog number CNCR 30877.

The genomic DNA was extracted from muscle tissue using the Phenol-Chloroform protocol described by Sambrook *et al.* (1989). Universal primers LCO1490: 5'-GGGGTCAACAAATCATA AAGATA TTGGGG-3' and HCO2198: 5'-TAAACTTCAGGG GGGG GGTGACCAAAAAATCA-3' reported by Folmer *et al*. (1994) were employed to amplify a fragment of 650 base pairs (bp) from the mitochondrial DNA (mtDNA) region of the cytochrome oxidase I (COI). The PCR reactions were made in 15 µL of final volume which consisted of 10 to 100 ng of DNA, 1X of DreamTaq buffer (1.5 μL^{-1}), 200 µM $MgCl_2$, 0.15 µM of each forward and reverse primers and 0.016 U μL^{-1} of DreamTaq Polimerasa. The cycling conditions consisted of denaturalization at 95°C for 60 s, followed by 35 cycles of 95°C for 60 s, annealing temperature of 52°C for 90 s, extension temperature of 72°C for 60 s and a final extension step at 72°C for 5 min. PCR products were visualized on 1% agarose gels and purified with the QIAquick® (Qiagen) kit and sent for sequencing to the Sequencing Unit at the Instituto de Biología-UNAM using both, forward and reverse primers. Both forward and reverse sequences obtained from the mtDNA-COI region were edited using Bioedit. A consensus sequence was generated using the contig option in Bioedit to corroborate each nucleotide position of the sequenced fragment. The consensus sequence was compared against the sequences available on the GenBank database (http://www.ncbi.nlm.nhi.gov) using a BLAST (Basic Local Alignment Search Tool) search to corroborate the identity of the specimen.

A portion of ovarian tissue from the 2nd and 3rd abdominal segments was extracted to perform the histological procedure. The tissue was post-fixed with 10% neutral formaldehyde and further immersed in Davidson's fixative for an additional period of 72 h. The tissue was washed for 3 h in running water and dehydrated through graded alcohols. Inclusion was carried out in paraplast using a melting point of 56-58ºC and cuts obtained with a thickness of 5 µm, which were stained with the Hematoxylin-Eosin technique. Cuts were finally mounted with synthetic resin (Bell & Lightner, 1988). Additionally, fresh samples of oocyte were taken and stained using the acetorcein technique (Martínez-Gómez *et al*., 2005).

The collected organism corresponded to a female with a total length of 345 mm, a carapace length of 122 mm and a total weight of 230 g (Fig. 1a). For species identification, the external morphological characters proposed by Dall *et al*. (1990) and Pérez-Farfante & Kensley (1997) were fully corroborated. The specimen was a closed-type telic with the fifth pair of pereiopods without exopods, horizontally straight hepatic carina, and gastro-orbital carina occupying the posterior half of the distance between the hepatic spine and the postorbital margin of the carapace (FAO, 2005-2019).

The consensus sequence obtained from both sequenced fragments F and R resulted in a 678 bp fragment for the mtDNA-COI region, which when compared to the GenBank database resulted in a 100%

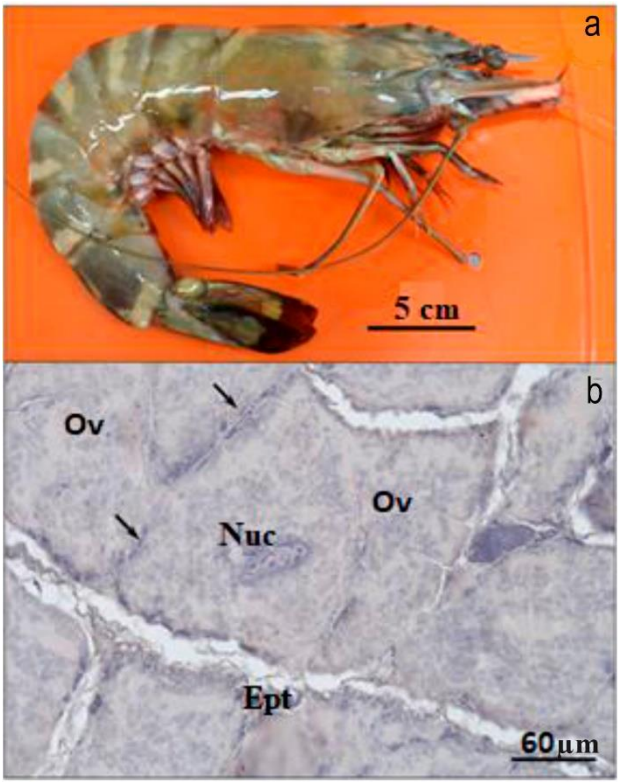

Figure 1. a) *Penaeus monodon* collected in front of Punta de las Disciplinas at the coast of Campeche, Mexico, b) ovaries' histological section of the specimen where oocytes are observed in an advanced stage of development. Ov, ovocites; Nuc, nuclei; Ept; epithelial tissue surrounding oocytes packages; arrows: follicular cells; flattened shape refers to terminal stages of maturation and little space within the ovary that are close to spawning.

of identity to the COI sequence of *Penaeus monodon* (Access NºKF604892.1) with a coverage of 100% of the sequenced bases (Fig. 2).

The two ovaries were well developed. From the histological examination, the tissue showed oocytes in advanced development (Bell & Lightner, 1988; Alfaro-Montoya *et al*., 2015) with histological characteristics typical for reproducing females, indicating that it was a female with reproductive capacity (Fig. 1b).

The tiger shrimp *P. monodon* is a decapod crustacean originated in the tropical zone of the Indo-Pacific, displaying a limited distribution to the western Pacific and the east coast of Africa. This decapod shrimp has presented wide dissemination mainly for aquaculture purposes explaining its presence in the American continent, specifically in Panama and the Dominican Republic, in 1976 and 1985, respectively (Welcome, 1988). There have also been records of *P. monodon* along the coast of the United States of America (Fuller *et al*., 2014), which has been attributed to the involuntary release of farms into the Gulf of Mexico due to the storms and hurricanes present in the region (Altuve *et al*., 2008). Considering that in Mexico the tiger shrimp are not used in breeding farms, it is probable that the presence of the species in Mexican waters is due to the existence of a migration route between the northern Gulf of Mexico, where the species has previously been established in the wild (Knott *et al*., 2012). The displacement of organisms outside their natural geographical distribution area is one of the most significant components of global changes induced by human activities (Altuve *et al*., 2008). The introduction of species of different origins in specific habitats is the cause of major alterations in natural ecosystems leading to loss of biodiversity. Rodríguez (2001) mentioned that when invasive species reach an environment in which they lack natural predators, they become a real risk impacting biodiversity since native species have no defense against invaders (predators, pathogens, competitors or parasites), which become a new and sudden selective force. Also, most of the time, invasive species carry associated parasites, as is the case of the copepod, *Pseudodiaptomus trihamatus*, which was supposedly introduced into Brazilian waters as a host of the tiger shrimp (Henriques *et al*., 2006). Other factors that may be involved in the reduction of wild populations is when the arrival of invasive species leads to an imbalance in ecosystems due to the competition they represent for native species as they promote the creation of new niches, affecting the ecological dynamics of an ecosystem and causing new competition stress in some species in the impacted area(s) (Knott *et al*., 2012; Alfaro-Montoya *et al*., 2015). Some consequences of this could be the decline of populations, including the species' genetic diversity (Grosholz, 2002; Gallardo *et al*., 2015). This last may affect the adaptive response capacity of populations, especially when these are reduced in size because of the competition. Decapod crustaceans are well-adapted organisms due to their anatomy, osmotic resistance and high fecundity, which allow them to successfully colonize new areas and establish new populations rapidly (Rodríguez & Suárez, 2001). The presence of numerous reports for this species on the coasts of the Gulf of Mexico raises the question about whether this species has reached a population size that can impact local populations of other shrimp species. An analysis of this question would be possible through the continuous monitoring of the species in the Gulf of Mexico, and by increasing the sampling efforts. Similarly, other research topics need to be investigated, such as the analysis of parasites present in collected organisms determining their trophic level, the dispersal dynamics of their larvae and postlarvae, their presence in coastal zones and their entrance capability into coastal lagoons.

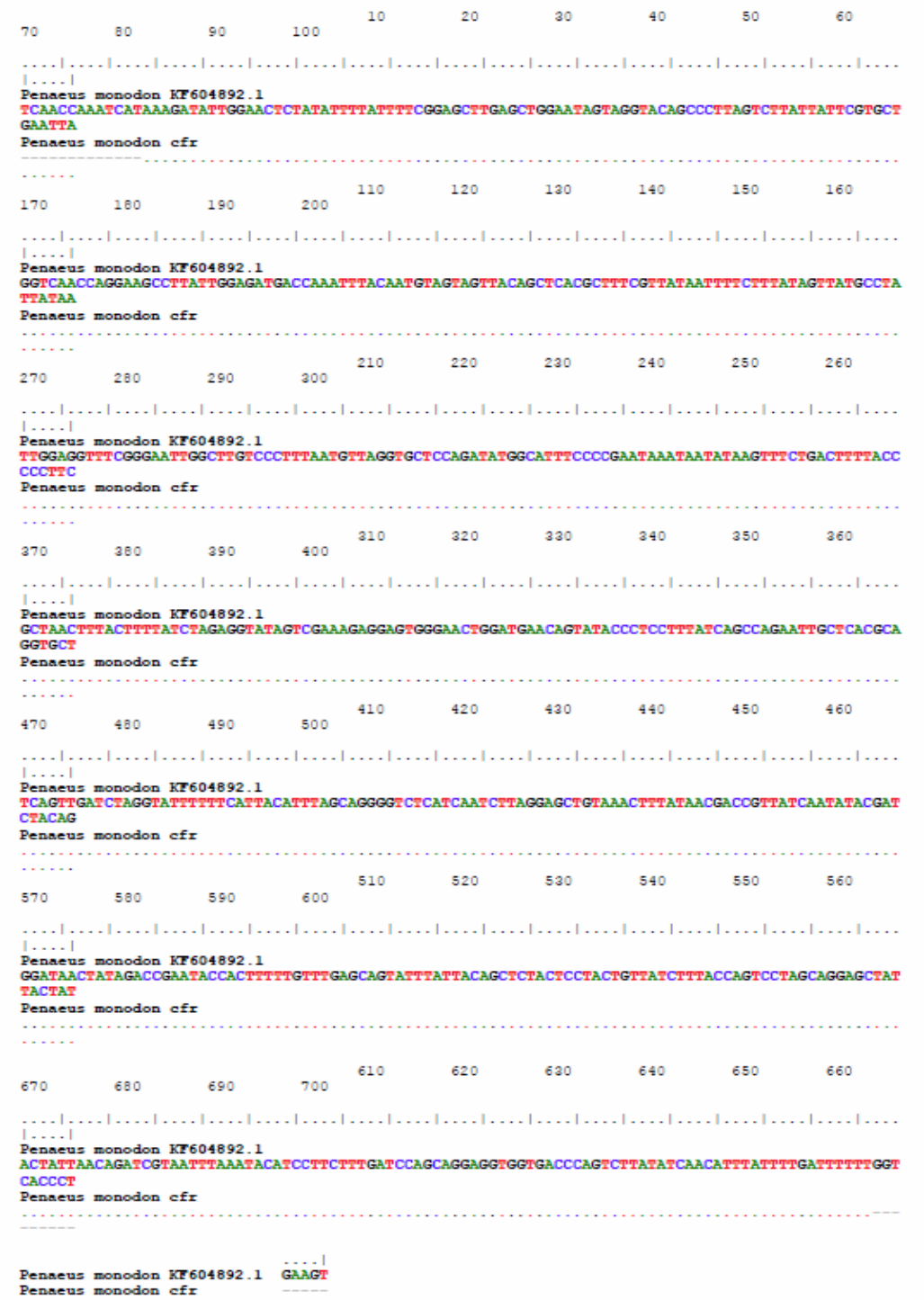

Figure 2. Alignment of the *P. monodon* mtDNA-COI region sequence (GenBank KF604892.1) and the specimen collected in Punta de las Disciplinas, Campeche, Mexico. Colored dots under the nucleotide sequence corresponding to the similarities between the two sequences.

The results from the different analyzes applied in the present study corroborated the presence of a female of *P. monodon* off the coast of Isla del Carmen, Campeche; it is the first time that morphological identification is corroborated by molecular data, relevant considering the difficulties of species identification using only external morphological characteristics. The results of the molecular analysis confirmed the morpho-

logical identification, which resulted in a 100% identity percentage to the COI sequence of *P. monodon* with coverage of 100% of the sequenced bases. Likewise, the histological analysis showed two well-developed ovaries with oocytes in advanced development, indicating that it was a female with reproductive capacity.

ACKNOWLEDGMENT

We thank the fishermen of Isla del Carmen, especially Mr. Miguel Ángel Benítez, who collected the shrimp.

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Received: 15 April 2019; Accepted: 8 September 2019

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