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Rapid Communication

First record of the trumpet snail *Melanoides tuberculata* (Müller, 1774) (Gastropoda: Thiaridae), in culture ponds for shrimp and the Pacific fat sleeper fish

César Lodeiros[1,4], Gustavo Darrigran[2], Nieves González-Henríquez[3,4], Dailos Hernández-Reyes[3], Manuel Rey-Méndez[4], Rodolfo Patricio Panta-Vélez[5], Juan José Bernal[6] and Diego Eduardo Gutiérrez Gregoric[2]

[1] *Grupo de Investigación en Biología y Cultivo de Moluscos, Departamento de Acuicultura, Pesca y Recursos Naturales Renovables, Facultad de Acuicultura y Ciencias del Mar, Universidad Técnica de Manabí, Ecuador*

[2] *División Zoología Invertebrados, Museo de La Plata, FCNyM, Universidad Nacional de La Plata. CONICET, La Plata, Argentina*

[3] *Servicio Científico Laboratorio BIOMOL, iUNAT, Universidad de Las Palmas de Gran Canaria, España*

[4] *Laboratorio de Sistemática Molecular, iARCUS, CIBUS, Campus Vida, Universidade de Santiago de Compostela, 15782-Santiago de Compostela, España*

[5] *Grupo de Investigación Biodiversidad y Ecología de Sistemas Acuáticos, Departamento de Acuicultura, Pesca y Recursos Naturales Renovables, Facultad de Acuicultura y Ciencias del Mar, Universidad Técnica de Manabí, Ecuador*

[6] *Grupo de Investigación de Nutrición y Alimentación Acuática, Departamento de Acuicultura, Pesca y Recursos Naturales Renovables, Facultad de Acuicultura y Ciencias del Mar, Universidad Técnica de Manabí, Ecuador*

Corresponding author: César Lodeiros (cesar.lodeiros@utm.edu.ec)

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Abstract

The invasive species *Melanoides tuberculata* was detected in culture ponds for shrimp (*Penaeus vannamei*) and the Pacific fat sleeper fish (*Dormitator latifrons*) in Manabí, Ecuador, through morphological and molecular identification using the COI gene. This finding confirms the presence of the Malayan snail in the Amazon-Andes ecoregion, evidencing its wide distribution in South America and its genetic relationship with populations from Iran and the United States, suggesting a recent invasion event in the continent. This is the first report of the species in culture ponds of the shrimp *P. vannamei* and fish *D. latifrons*, representing a new habitat and a potential negative factor for aquaculture activities. It is necessary to expand sampling efforts to other regions and conduct genetic analyses to identify lineages with a higher potential for transmitting parasitic infections. This information is crucial for developing early warning systems and effective management strategies. Further studies are needed to assess the potential risks of *M. tuberculata* in aquaculture systems, such as its ability to transmit parasites to farmed organisms and its possible impacts on human health.

Key words: invasive species, sanitary impact, dispersal, South America

Introduction

The trumpet snail, *Melanoides tuberculata* (Müller, 1774), is a freshwater gastropod of the family Thiaridae. Native to Africa and Asia (Pilsbry and Bequaert 1927), it is one of the most invasive freshwater species, with a wide distribution across all continents (Facon and Pointier 2022). *Melanoides tuberculata* presents characteristics that have made it a successful invasive species (Peso et al. 2010), as it stands out for presenting parthenogenesis, viviparity, high reproductive rate, great capacity to disperse through water courses and a high adaptation to new habitats, mainly on impacted environments, supporting a wide range of temperature changes (18–32 °C) for the development of stable populations (Vogler et al. 2012). It also supports long periods of desiccation, since as a strategy, adults bury themselves in the substrate and reactivate with the arrival of rains (Vega et al. 2020) and resist changes in the salinity of up to 5‰ (Albarrán Mélzer et al. 2017). On the other hand, it is a generalist-feeding species (Thomas and Tait 1984; Freitas et al. 1987).

The wide dispersal of *M. tuberculata* has been attributed mainly to the trade in ornamental aquarium plants, which is why it has been recorded in several countries (Maguire 1963; Corrêa et al. 1980; Gutiérrez Gregoric and Vogler 2010). Once released into the environment from aquariums, it expands its distribution and competes with local species (Pointier et al. 1994; Facon et al. 2003; Hernandez et al. 2003; Letelier et al. 2007). Potential future distribution studies carried out in South America (Ribas et al. 2024) mention that this species has the potential to maintain or extend its distribution, while native species such as *Biomphalaria glabrata* (Say, 1818) would present a reduction in their distribution. The first report on the presence of *M. tuberculata* on the American continent was in 1964 in Texas, United States (Murray 1964). While in South America it was cited for the first time in Venezuela in 1972, and since that date, it has been reported in Brazil, Peru, Argentina, Paraguay, Colombia, Guyana, Uruguay, Chile, and Ecuador, occupying 25 of the 52 freshwater ecoregions of this subcontinent (Darrigran et al. 2020). Of those nine countries, in Chile, it was reported only in commercial aquariums, but not in natural environments (Letelier et al. 2007).

In South America, this species has caused negative effects, having a significant environmental impact (Carranza et al. 2023), particularly on native gastropods, affecting their density or causing a displacement. There are records of affections to native populations of *B. glabrata* in Minas Gerais, of *Biomphalaria straminea* (Dunker, 1848) in Minas Gerais and in the state of Rio de Janeiro, as well as populations of *Aylacostoma tenuilabris* (Reeve, 1860) in the Tocantins River (Guimaraes et al. 2001; Giovanelli et al. 2002; Fernandez et al. 2003).

The other effects recorded in South America are sanitary, since it acts as an intermediate host of the trematode *Centrocestus formosanus* (Nishigori, 1924) (Digenea: Heterophyidae), in Venezuela (Hernández et al. 2003), Colombia (Velásquez et al. 2006), Brazil (Pinto and de Melo 2010) and Peru (Pulido-Murillo et al. 2018), which report infected humans by ingesting undercooked or raw parasitized fish, causing gastric pain and indigestion accompanied by diarrhea (Chai et al. 2013). *Melanoides tuberculata* may also act as a host for *Haplorchis pumilio* (Looss, 1896) (Digenea, Heterophyidae), a trematode that causes focal hemorrhages in the skeletal muscles of highly infected fish and may potentially affect humans by ingesting raw fish. This parasite has been detected emerging from *M. tuberculata* in Venezuela

(Díaz et al. 2008), Peru (Pulido-Murillo et al. 2018), and Brazil (Lopes et al. 2020). Finally, *Philophthalmus gralli* Mathis and Leger, 1910 (Digenea, Philophthalmidae) was also detected in Brazil in specimens of *M. tuberculata* and may affect poultry (Pinto and Melo 2010).

The shrimp culture activity in Ecuador has been in production for more than 50 years, and is one of the most important economic sectors, already exceeding 40% of exports (Arias and Torres 2019). However, the development of the cultivation of other species is necessary, depending on the diversification of aquaculture, considering multitrophic cultivation. Although the habitat of *Penaeus vannamei* (Boone, 1931) is marine-estuarine, adaptation techniques have been developed allowing the species to be cultivated in very low salinity systems, where much of the world's production comes from. With the cultivation of shrimp at low salinities, some shrimp farms have adopted its cultivation with the Pacific fat sleeper fish, *Dormitator latifrons* (Richardson, 1844), a species with great acceptance and interest in the development of its integral culture (Vega-Villasante et al. 2021). These environments constructed by man to cultivate aquatic species allow opportunities for invasive species to colonize these systems, bringing with them the possibility of a trophic imbalance and being possible hosts of risk to the health of both the cultivated organisms and humans, apart from the invasive influence on the aquatic ecosystems.

Knowing the origin of an invasive population can make it possible to establish vector management plans, in addition to being able to predict potential parasites. In this sense, the use of mitochondrial (cytochrome *c* oxidase I, COI) and ribosomal (16s rRNA) markers has had great success in understanding the patterns of origin associated with *M. tuberculata* (Van Bocxlaer et al. 2015; Duggan and Knox 2022). However, for South American populations, no COI gene sequences are available. Therefore, this work aims to report the presence of the invasive *M. tuberculata* in an environment of anthropogenic use in Ecuador, provide COI gene sequences to infer its origin or connection with other populations of the species, and warn about the potential risks associated with its presence in shrimp and fish farming at low salinity.

Materials and methods

The *M. tuberculata* samples were collected in November 2019 in the province of Manabí, Ecuador, in aquatic farming dedicated to the cultivation of the Pacific fat sleeper fish, *D. latifrons*, and white shrimp, *P. vannamei*, located at the La Isla site, Pechichal commune, San Isidro parish, Sucre Municipality (0°18′04.51″S, 80°11′33.66″W) (Figure 1).

The specimens were identified morphologically following to Facon and Pointier (2022), and total DNA was extracted from 10 specimens using the "E.Z.N.A. Mollusk DNA Kit" (Omega Bio-Tek). Partial COI gene PCR products

Figure 1. Sampling location of *Melanoides tuberculata*, Manabí province, Ecuador.

were obtained with the barcoding primer set LCO1490/HCO2198 (Folmer et al. 1994) and the modified primers jgLCO1490/jgHCO2198 (Geller et al. 2013), purified with the ExoSAP-It kit (Affimetrix) and sequenced using BigDye. v3.1 X terminator kit on ABI 3500 equipment (Thermo Fisher Scientific). The consensus sequence obtained was deposited in GenBank under the accession number PP724746.

To infer the lineage and possible origin of the *M. tuberculata* samples, a phylogenetic analysis was carried out, using as a basis the sequences available in GenBank (as of 26-12-2024) (n = 187). The total lengths of the analyzed matrices were 605 bp for *M. tuberculata*. The data were subjected to phylogenetic analysis using the maximum likelihood (ML) method. ML inference was performed using the PhyML program (Guindon and Gascuel 2003), available on the public web server Phylemon2 (http://phylemon.bioinfo.cipf.es). Statistical support for the resulting phylogenies was assessed by bootstrapping with 1000 replicates (Felsenstein 1985). All trees were edited with FigTree software.

Results and discussion

The presence of *M. tuberculata* is reported for the first time in Manabí Province, Ecuador, confirming its occurrence in the country and highlighting the broad distribution of the genus *Melanoides* across South America (Darrigran et al. 2020; Ribas et al. 2024). This region belongs to the Amazon-High Andes ecoregion, where the species had already been documented (Darrigran et al. 2020)

Our tree, based on the COI nucleotide sequence from Ecuador and representatives from GenBank (n = 184) exhibited a topology like that of Duggan and Knox (2022). As in their study, two distinct clades were recognized, and both clades had occurrences of Asian, African, and American representatives (Figure 2). Molecular analysis showed 100% similarity with populations from Iran and the United States (Figure 2). Ecuador has maintained

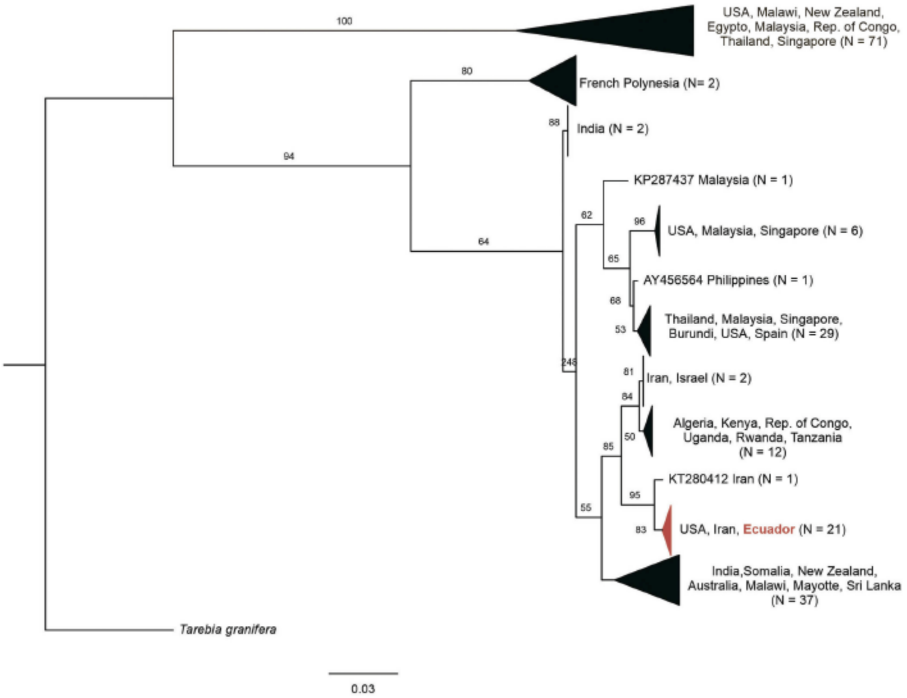

Figure 2. Phylogenetic tree of *Melanoides tuberculata*, based on the COI gene. The sequence of this study was aligned with others from NCBI. Collapsed nodes are indicated by triangles. The number of sequences per node is shown in parentheses. Nodes with support below 50% are not annotated.

an export trade relationship primarily involving bananas with Iran, and a more active one with the USA, particularly involving hydrocarbons and shrimp. This activity could have been associated with the transfer of the species to Ecuador.

Van Bocxlaer et al. (2015) proposed the existence of two globally invasive lineages of *M. tuberculata* based on the analysis of two genes (16S and COI). The first major lineage includes populations from Asia and Africa, as well as invasive populations from the Malawi-Congo region recorded in South America (Argentina and Peru, 16S gene only). The second invasive clade (Tanganyikan) includes populations from Africa, Asia, and Europe. Subsequently, Duggan and Knox (2022) incorporated COI sequences from New Zealand and other new ones available in GenBank, resulting in an analysis like that presented by Van Bocxlaer et al. (2015), with populations from the USA and New Zealand in both clades. Our phylogenetic analysis agrees with both studies, and the population from Ecuador is highly related to specimens found in Iran and the USA in the second invasive clade. This demonstrates that the sequenced specimens from Ecuador belong to a different invasion lineage than that already found in South America (Argentina and Peru), resulting from a new invasion event in the continent. This new lineage detected in South America could have new negative effects on the native fauna.

Although *M. tuberculata* had already been reported for Ecuador (Vivar et al. 1998), the findings of this study represent the first reported occurrence of this species establishing itself in an aquatic farming center, which represents a new habitat for the species and a possible negative factor for the cultivation activity. Gastropods can interfere with the feeding of cultured organisms, since the feed used for the shrimp is reduced by the consumption of snails within the ponds, either on the benthos or on the feeders, affecting the feed conversion factor. Similar cases have been reported with gastropods of the Potamididae family such as *Cerithideopsis californic*a, which inhabits muddy bottoms associated with mangroves, invading the ponds of shrimp farms and becoming a competitor for shrimp food, but also for oxygen in the ponds, producing low yields (Lodeiros and Torres 2018). The growth of these snails may also generate an imbalance in alkalinity, calcium concentration, and other important minerals in the system (Aldridge et al. 2008). Furthermore, the snails may be hosts for parasites, which would decrease the quality of the product, producing a loss of commercial value, because gastropods can be vectors of digenean parasites that can invade cultured organisms (Lodeiros and Torres 2018). In South America, cases have already been reported of fish infected with the parasitic trematodes *C. formosanus* and *H. pumilio*, which affect the muscle tissue of fish and have been associated with *M. tuberculata* as a vector (Carranza et al. 2023). On the other hand, the presence of *M. tuberculata* in the province of Manabí represents a significant risk, given its association with rice crops (Vogler et al. 2012) and the promotion of fish farming, particularly *D. latifrons* (López-Vera 2017). On the other hand, the presence of *M. tuberculata* in the province of Manabí, Ecuador, poses an additional risk due to its potential impact on rice production, one of the province's primary agricultural products (Vogler et al. 2012).

The increasing trade in aquarium plants and gastropods is significantly contributing to the spread of this invasive species (Seuffert et al. 2023). Therefore, it is crucial to assess its presence and raise awareness about its potential to invade aquaculture environments. Sampling efforts should be expanded to other regions, and genetic analyses conducted to determine whether specific lineages are more likely to transmit parasitic infections. This information would be essential for developing early warning systems and effective management strategies; however, more comprehensive studies are needed to evaluate the potential risks this species poses to aquaculture systems, particularly its ability to transmit parasitic infections to farmed fish and its potential impacts on human health.

Authors' contribution

César Lodeiros: conceptualization, data curation, formal analysis, investigation, methodology, project supervision and administration, writing – original draft, writing – review and editing. Gustavo Darrigran: conceptualization, investigation, methodology, project supervision and administration, writing – original draft, writing – review and editing. Nieves González-Henríquez: data curation, formal analysis, methodology, writing – review and editing. Dailos Hernández-Reyes: data curation, formal analysis. Manuel Rey-Méndez: data curation, formal analysis, methodology, writing – review and editing. Rodolfo Patricio Panta-Vélez: data curation, formal analysis. Juan José Bernal: data curation, formal analysis. Diego Eduardo Gutiérrez Gregoric: conceptualization, data curation, formal analysis, investigation, project supervision and administration, writing – original draft, writing – review and editing.

Authors' ORCIDs

César Lodeiros: 0000-0001-9598-2235; Gustavo Darrigran: 0000-0001-9512-8135; Nieves González-Henríquez: 0000-0003-1110-0448; Dailos Hernández-Reyes: 0000-0002-2488-5005; Manuel Rey-Méndez: 0000-0001-6102-336X; Rodolfo Patricio Panta-Vélez: 0000-0003-2969-0765; Juan José Bernal: 0000-0002-6371-2676; Diego Eduardo Gutiérrez Gregoric: 0000-0002-8001-1062.

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