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Detection of Trematodes from the Host Exotic Aquatic Snail *Melanoides tuberculata* in an Urban Stormwater System

Jason M. Post [1], Rachael J. Reasch [2] and Emily S. Bailey [3,*]

[1] Geography and Geospatial Technology Program, Tohono O'odham Community College, Sells, AZ 85634, USA
[2] Pima County Health Department, Tucson, AZ 85714, USA
[3] Department of Public Health, College of Pharmacy and Health Sciences, Campbell University, Buies Creek, NC 27506, USA
* Correspondence: ebailey@campbell.edu

Simple Summary: Exotic freshwater snails have been detected with increasing frequency in a number of water bodies in North America. As these snails are a well-known host for trematodes that cause both human and animal disease, the detection of pathogenic trematodes is of public health concern. Here we describe the detection of trematodes in a body of water used by humans and animals in a neighborhood of Los Angeles.

Abstract: The red-rimmed melania or Malaysian Trumpet Snail, *Melanoides tuberculata*, is a common exotic freshwater snail that has been invading an increasing number of water bodies in North America. As a well-known host for trematodes causing human and animal diseases, the pattern of invasion and parasitic infection for this species is of great concern. Snail specimens were collected from an urban stream in Los Angeles that drains into a fragile, protected wetland ecosystem. Molecular analysis identified four trematode species: *Haplorchis pumilio*, *Fasciola jacksoni*, *Parorchis sp. TH-2019*, and an unclassified trematode species, *Trematoda distomecercaria WN-2016*. *H. pumilio* is responsible for haplorchiasis infections, previously considered endemic to Asia. *F. jacksoni* infection is a significant cause of mortality in Asian elephants. This study represents the first occurrence of *F. jacksoni* in North America and a novel occurrence of that trematode in association with *M. tuberculata*. This study also represents new occurrences of *Parorchis sp. TH-2019* and an unclassified trematode species, *Trematoda distomecercaria WN-2016*, within California and North America. *Parorchis sp. TH-2019* has previously only been documented in a marine whelk. This identification of these trematodes in association with *M. tuberculata* further exemplifies the need for ongoing monitoring and detection, especially considering the significance of *H. pumilio* and haplorchiasis to public health.

Keywords: zoonotic disease; zoonoses and public health; invasive species; snail; trematode

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1. Introduction

The red-rimmed melania or Malaysian Trumpet Snail, *Melanoides tuberculata* (Müller, 1774), is a benthic freshwater thiarid snail native to Africa and Asia [1]. This range extends from Africa eastward to the East Indies [2] and includes southern Asia, Madagascar, northern Australia [3,4], the Mediterranean, and the Pacific Islands [5]. This species has been recorded in many parts of Europe, including Spain, Malta, Austria, the Netherlands, Hungary, and Germany [6]. It is well established in the Americas and has been documented from Florida southward to Argentina and Paraguay [7].

M. tuberculata were first observed within the United States in the 1960s with initial observations in Texas and Florida [8]. The literature suggests that this species was initially introduced through the ornamental plant and aquarium trades [9]. Freshwater snails are some of the most-kept freshwater organisms because of their ability to control algae and waste in aquaria [10,11]. Passive distribution through flood events and artificial drainage

infrastructure further contributes to its spread and establishment, especially in urban areas [1,7,12,13]. *M. tuberculata* can resist desiccation during transport or drought for multiple days at a time [14] and has demonstrated an ability to outcompete native snails [15].

Temperature is a limiting factor for the spread and success of *M. tuberculata* [16,17]. When the climate is not naturally suitable, *M. tuberculata* lives in thermally altered waterways [6], such as springs, polluted canals, and channels [4,6], and has been found in the heat exchanger of a power plant station in Poland [6]. These warmer conditions allow the snails to thrive while the temperatures are cold in the winter. Urban storm drains have the potential to house large colonies of *M. tuberculata.* They provide optimal ecosystems for snail growth as conditions yield substantial food, protection, and shelter from freezing temperatures [1,13].

A major concern with the introduction of exotic freshwater aquatic snails such as *M. tuberculata* is their status as intermediate hosts for parasites causing both human and animal diseases [13,18]. *M. tuberculata* is an intermediate host of several species of trematodes, such as eye flukes (*Philophthalmus* sp., *Philophthalmus gralli*, *Philophthalmus anatinus*, and *Philophthalmus lucknowensis*) and the first intermediate host of *Centrocestus* sp., *Haplorchis* sp. [1,19,20]. In Central Texas, *M. tuberculata* has been found to host *Centrocestus formosus*, which causes fish gill infection, and *Philophthalmus gralli*, which infects waterfowl [12]. The diversity of pathogenic trematodes associated with *M. tuberculata* presents a concern for public health because of their potential to cause diseases in humans. Infections such as schistosomiasis, paragonimiasis, clonorchiasis, opisthorchiasis, echniostomiatidiasis, and fasciolopsiasis are caused by trematodes hosted by *M. tuberculata* [13,21,22]. Exotic occurrences of *M. tuberculata* have been linked to the introduction of trematode parasites that impact federally protected species, including fish and birds [12]. *M. tuberculata* invasions contributed to the introduction of gill-infesting trematodes into eleven states in Mexico. This led to an outbreak of *Centrocestus formosus* in almost 40 species of fish [23]. Additionally, similar invasions occurred in China, where native waterfowl were infected with *Philophthalmus gralli*, also caused by the invasion of *M. tuberculata* [24]. Recent studies on *M. tuberculata*, such as Tolley-Jordan et al. (2022), represent new records of trematodes in North America [25].

In California, the snail was first observed in Riverside County in 1972 in a drainage ditch near the Salton Sea [26]. Since then, an increasing number of observations of *M. tuberculata* have occurred throughout much of southern and central California [27]. Both the biotic and abiotic processes contributing to the spread, establishment, and success of *M. tuberculata* in California are not well studied. In addition, little is known about trematode infection rates of *M. tuberculata* in California waters, including the incidence and prevalence of associated infections. In 2021, *M. tuberculata* was observed in a reservoir that serves as part of the local municipal water supply near Westlake Village, CA, just north of Los Angeles [28].

This study applies established methods of trematode detection [29] to identify the trematodes associated with exotic *M. tuberculata* in an urban creek. Current literature not only suggests that occurrences of *M. tuberculata* are increasing, but associated trematodes identify gaps in how the exotic snail is viewed by natural resource managers and public health professionals. Building on these current trends, this study provides another example of how exotic *M. tuberculata* poses as a reservoir for potentially harmful trematodes. While the trematodes associated with *M. tuberculata* in native waters have been substantially studied [18], patterns of infection and associated trematodes in exotic waters remain comparatively understudied. A novel application to a recently discovered exotic population of *M. tuberculata* represents an early yet modest contribution to the patterns of trematode occurrence in California urban waters. Further, this study provides the basis for management and public health considerations in Los Angeles and the greater Southern California region.

2. Materials and Methods

2.1. Sample Collection

Study personnel from Tohono O'odham Community College and Texas Tech Health Sciences Center traveled to the Playa Vista neighborhood of Los Angeles to collect samples of the exotic thiarid snail *Melanoides tuberculata*. Samples were collected from Bluff Creek (Figure 1), a small urban creek designed to convey stormwater from park fountains to the Pacific Ocean via Ballona Creek and Ballona Freshwater Marsh (Los Angeles Region, California Regional Water Resources Control Board 2009, City of Los Angeles 2009). Bluff Creek is directly fed by the Los Angeles municipal storm drain system. Samples were collected on 25 September 2021, from the northernmost storm drain outfall pipe discharging into Bluff Creek (Figure 2). Tongs were used to carefully remove organisms affixed to concrete or vegetation. A total of fifty-six snails were collected and placed into 50 mL sterile containers with UV-sterilized deionized water. Samples were flash frozen and transferred on dry ice to the laboratory for processing. Sample processing and molecular assays were performed at the Texas Tech University Health Sciences Center in Abilene, Texas.

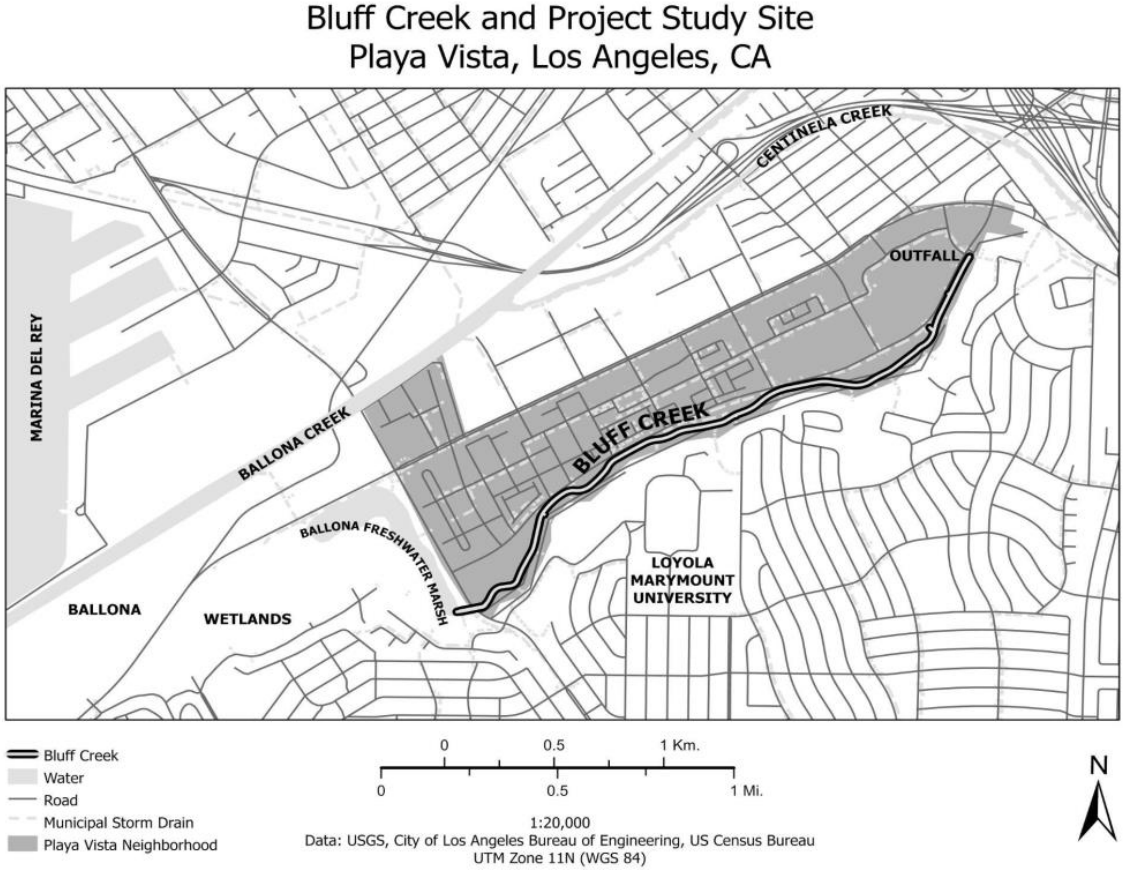

Figure 1. Map of Bluff Creek and the Environs, Playa Vista, Los Angeles, CA.

2.2. Laboratory Testing

We adapted previously published techniques for molecular analysis of trematodes, cestodes, and nematodes [29]. Briefly, trematode, cestode, and nematode DNA was extracted from environmental samples using the Qiagen DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) and was then assessed with a conventional (28S rRNA gene) PCR assay using the Invitrogen Platinum Taq DNA Polymerase Kit (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Partial genome sequencing was performed by Genewiz (Genewiz, South Plainfield, NJ, USA). Sequences were then compared to the NCBI sequence data using the BLAST application of BioEdit 7.1.9 (Ibis Biosciences, Carlsbad, CA, USA).

Trematode cercariae emitted from captive and collected *M. tuberculata* into water have been previously used to identify and detect species present within the snails [30]. The deionized water that contained *M. tuberculata* specimens during sample collection was therefore included in the PCR assay.

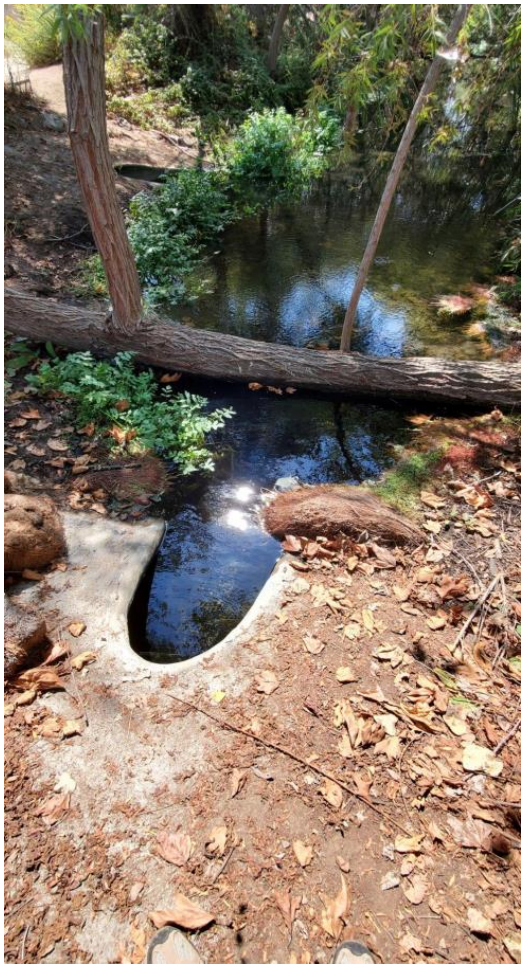

Figure 2. Storm drain outfall pipe feeding Bluff Creek, Los Angeles, CA (September 2021).

3. Results

Four species of trematodes (Table 1) were detected. Sequences with a matching identity of 80% or higher were considered successful matches based on previous methods evaluating partial sequences [29]. Two species, *H. pumilio* and *F. jacksoni*, were detected within specimens of *M. tuberculata*, while the unclassified *Trematoda distomecercaria WN-2016* and *Parorchis sp. TH-2019* were identified only in frozen deionized water samples containing *M. tuberculata*. This is highly suggestive that these were emitted from *M. tuberculata* and may have represented a different life stage (egg, miracidiae, and cercariae) than *H. pumilio* or *F. jacksoni*. The deionized water used to transport *M. tuberculata* samples was sterile prior to collection and flash freezing with dry ice. Since *M. tuberculata* is an intermediate host for trematodes, *H. pumilio* and *F. jacksoni* most likely formed sporocysts within the snail. Since samples were flash frozen with dry ice within one hour of collection, there was limited opportunity for cercariae emission. This suggests that the unclassified *Trematoda distomecercaria WN-2016* and *Parorchis sp. TH-2019* may have only recently infected or unsuccessfully infected *M. tuberculata*. It is also possible that they were located superficially on the shell of the snail at the time of collection. Since *Parorchis sp. TH-2019* has only been previously associated with a marine snail [31], its life history, infection rates, and success in freshwater snail hosts remain unknown.

Table 1. Trematodes detected in *M. tuberculata* samples.

BLAST * Result	% Identity	NCBI⁺ Accession Number	*M. tuberculata* Specimens	Emitted From *M. tubeculata* Samples (into DI Water)	Previously Found in *M. tuberculata*
Fasciola jacksoni	83.70%	MN970006.1	X		
Haplorchis pumilio	100%	MG738252.1	X		X [18]
Haplorchis pumilio	98.11%	MN745941.1	X		X [18]
Parorchis sp. TH-2019	100%	LC438643.1	X	X	
Unclassified *Trematoda sp. distomecercaria WN-2016*	100%	KU820969.1	X	X	

* BLAST is the Basic Logical Alignment Search Tool. ⁺NCBI is the National Center for Biotechnology Information

Two different records of *Haplorchis pumilio* were identified. This is most likely the first documented occurrence in California waters. The discovery of *Fasciola jacksoni* is not only the first in California, but also in North America. *H. pumilio* has been found to be hosted by *M. tuberculata* in previous studies [18], but this is also the first documented association of *F. jacksoni*, *Parorchis sp. TH-2019*, and unclassified *Trematoda distomecercaria WN-2016* with *M. tuberculata* snails. The detection of *Parorchis sp. TH-2019* and unclassified *Trematoda distomecercaria WN-2016* also represent new occurrences for the United States and North America in general.

4. Discussion

The findings of this study are consistent with current trends of increasing new occurrences of *M. tuberculata* associated with novel detections of associated trematodes previously not found in North America [25]. Therefore, *M. tuberculata* is an important reservoir and vector for the introduction of exotic trematodes into the waters of the United States. Monitoring and surveillance efforts are needed to track the spread of these trematodes, especially those of medical and veterinary significance [21]. With the introduction of exotic trematodes, the potential for the spread of related infections exists. In many cases, such as with *F. jacksoni*, human infection is considered possible upon consumption of contaminated food or water but has not been documented [32]. The unknown human pathogenic effect of many exotic trematodes is in and of itself a public health concern.

Animals contribute to the rapid spread of exotic snails, such as *M. tubercualta*, once introduced to aquatic environments. Birds are capable of carrying them on their feet or feathers [33,34]. Mammals may also transport snails on their hooves or feet or by depositing mud on their bodies [21]. *Parorchis sp. TH-2019* has only previously been isolated from *Rapana venosa*, a marine whelk, in a tidal basin (Sihwa Lake) of Gyeonggi Province, South Korea [31]. Since the study site, Bluff Creek, is less than three kilometers from the nearest saltwater source (Ballona Creek, Ballona Wetlands, and Santa Monica Bay), the introduction of this marine trematode into Bluff Creek is understandable.

The nearby Ballona Wetlands constitute an important habitat and wildlife corridor within the city and are the largest remaining coastal wetlands in Los Angeles County [35,36]. Trematode infections spread by *M. tuberculata* have been shown to infect ecologically vulnerable and protected species of fish and birds [12]. This presents a major ecological concern since the Ballona Wetlands provide critical habitat for numerous native plants, birds, fish, and mammals isolated by urbanization [35].

Scientific or other equipment, including boots, may also inadvertently transport the snails and associated trematodes [37]. Public parks and hiking trails directly link Bluff Creek with the Ballona Wetlands and the nearby Pacific Ocean. The recreation corridor surrounding Bluff Creek may further contribute to the spread of *M. tuberculata* and the associated trematodes.

The unclassified *Trematoda distomecercaria WN-2016* detected here may reflect a new species. It has only previously been found in freshwater snails in the Chiang Mai Province of

Thailand [38]. Like *Parorchis sp. TH-2019*, its significance as a human or veterinary pathogen remains unknown. Neither of these has been previously identified from *M. tuberculata*.

F. jacksoni has not been previously found in association with *M. tuberculata* or in North American waters. The major definitive host is the Asian elephant (*Elephas maximus*), and *F. jacksoni* has been found only in India, Sri Lanka, Nepal, Myanmar, and Malaysia [39,40]. While it is not known what effect *F. jacksoni* will have on humans or wildlife in North America, this trematode has led to significant mortality and the decline of Asian elephants [39,40]. The intermediate host for this species is unknown, identifying a need for further research to explore how *F. jacksoni* spreads. Due to the infection of large mammals such as elephants, human infection is hypothesized but has not been confirmed [32].

H. pumilio has been found to infect humans [41,42]. *H. pumilio* also infects domestic cats and dogs [43]. Foodborne haplochoriasis has caused serious pathological issues in human hosts [42,44–46]. Haplorchiasis has been documented in Southeast Asia [41] and Venezuela [30]. Diaz et al., importantly, linked the human haplochoriasis cases to *H. pumilio* spread by *M. tuberculata* [30]. A *H. pumilio* infection typically results from consuming raw or undercooked fish. Popular raw food habits may increase infection [47].

Culturally sensitive health education, prevention, and intervention programs are needed to directly address raw food consumption and reduce rates of trematode infection [22]. Los Angeles County is facing an unprecedented number of people experiencing homelessness, with estimates exceeding 69,000 unhoused residents [48]. An estimated 140 people experiencing homelessness have created camps (Figure 3) in the area immediately surrounding Bluff Creek and the Ballona Freshwater Marsh [49]. The presence of detected trematodes raises concerns for the vulnerable population of homeless residents who use public spaces to set up shelters, as there is no other water supply in the area. Rates of contact with the water and consumption of local fish need to be further evaluated to more accurately understand the risk posed by trematode infections.

Figure 3. Homeless camp adjacent to Ballona Freshwater Marsh, Los Angeles, CA (November 2021).

Ismail and Arif (1993) recorded the incidence of parasitic trematodes in *M. tuberculata* to be as high as 92% [50]. These snails are of medical significance because of their ability to be an intermediate host and transmission vector for parasitic trematodes that can affect humans, wildlife, pets, and livestock [1,13,18,51,52]. *M. tuberculata* is spreading throughout the waters of the U.S. and is able to successfully colonize both urban and suburban waters, further contributing to a public health concern [13,25].

The major public health threat associated with *H. pumilio* and many other trematode infections facilitated by *M. tuberculata* as an intermediate host centers around foodborne illness, as the consumption of raw or undercooked fish containing the parasites is a common infection pathway [22,41,47]. Precautions must be taken to limit the spread of exotic aquatic freshwater snails and resulting trematode infections. Madsen and Frandsen [21] recommended a global collaborative approach, suggesting that the World Health Organization (WHO) and Food and Agriculture Organization (FAO) identify prevention strategies specifically targeting the intermediate snail host species. Further, these authors recommend more stringent inspections on a global scale to help limit the introduction and spread of these snails [21]. Both public education and regulation are also needed to help decrease the negative outcome of the future establishment and spread of exotic species [12].

This study represents a modest contribution to the body of knowledge on trematodes spread through *M. tuberculata* invasions in urban waters. The initial detection of trematodes associated with *M. tuberculata* in an urban storm drain system identifies a need for further research on the actual risks to public health posed by these parasites. Further, a need for ongoing monitoring in surrounding urban waters is identified, and future work should include an expanded set of PCR primers to identify other potentially pathogenic trematodes that may be present in the *M. tuberculata* samples. In addition, as work in geographic information systems (GIS) and mapping becomes more readily available for use in studies on zoonotic disease, the evaluation of location and the spread of pathogens will be increasingly important and should be considered in future work.

5. Conclusions

Findings presented here are consistent with a trend in the literature, where expanding invasions and established populations of exotic *M. tuberculata* are associated with increasing novel introductions of associated trematodes. Furthermore, this study documents the first occurrences in North America of *F. jacksoni*, *Parorchis sp. TH-2019*, and unclassified *Trematoda distomecercaria WN-2016*. This detection of *Parorchis sp. TH-2019* suggests that in close proximity to coastal wetlands, trematodes previously considered to be marine may invade freshwater ecosystems. This process may be easily facilitated by birds and human foot traffic along hiking trails. This also represents the first documented association with *M. tuberculata*. In exotic populations, *M. tuberculata* may serve as an opportunistic intermediate host for marine trematodes. *M. tuberculata* should be carefully monitored due to its ability to host a diversity of trematodes. Over 37 species of trematodes, representing 25 genera and 17 families, have been associated with *M. tuberculata* [18]. Recent studies show that these numbers continue to grow [25].

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Institutional Review Board Statement: In a determination by permitting staff biologists at the California Department of Fish and Wildlife, Bluff Creek, Playa Vista Park fountains, and the urban stormwater system are not considered waters of the State of California, thus no scientific collection permit was required. A CADFW chain of custody form (CA DFW 1379c) accompanied samples throughout transit to ensure no accidental reintroduction or escape occurred in California waterways.

Data Availability Statement: The data included in this study are available from the corresponding author upon reasonable request.

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