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Potential for Emergence of Foodborne Trematodiases Transmitted by an Introduced Snail (*Melanoides tuberculata*) in California and Elsewhere in the United States

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We document that 3 human-infectious trematodes and their introduced first intermediate host snail (*Melanoides tuberculata*) are widespread throughout southern California. We surveyed 41 fishing localities, 19 of which harbored snails infected with zoonotic trematodes. Two of the parasites, *Haplorchis pumilio* and *Centrocestus formosanus*, are fishborne intestinal trematodes recognized as being important human pathogens in other areas of the world; the third, *Philophthalmus gralli*, can infect the human eye. An additional 5 species detected infecting *M. tuberculata* are likely of little direct threat to people; however, they may be recently introduced to the Americas, highlighting the risk that additional pathogenic trematodes transmitted by the snail in its native range could be introduced to the United States. The current, possible human-infection risk in California clarifies the need to consider the introduced snail and its parasites from a public health perspective anywhere in the United States the snail has been introduced.

Keywords. conjunctivitis; foodborne diseases; gastroenteritis; helminths; heterophyidae; invasive species; neglected diseases; Trematoda; waterborne diseases; zoonoses.

Lay summary. We report that 3 human-infecting trematodes and their introduced intermediate host snail are widespread in southern California freshwater fishing localities. Eating undercooked or underfrozen fish is the way people get infected by 2 of the parasite species, which are recognized as important human pathogens in other areas of the world. We also found 5 non–human-infectious trematodes carried by the snail that may be cointroduced, highlighting the possibility that other dangerous pathogens transmitted by the snail where it is native could arrive later or already be present in the United States. The common presence of the human-infecting fishborne trematodes at fishing localities, the widespread popularity of eating uncooked fish (eg, as sashimi, sushi, poke, or ceviche), and the potential for additional human-infecting trematodes to also be introduced, all justify consideration of the introduced snail and its parasites from a public health perspective in California and other areas in the United States where the parasites or the host snail have already been reported.

Foodborne trematodiases are among the most important neglected infectious diseases of the world, with around a billion people estimated to be at risk [1, 2]. These diseases can involve a wide range of pathologies, including abdominal pain, chronic cough, hepatomegaly, bile-duct cancer, and brain hemorrhage [1, 3]. Foodborne trematodiases result from infection by trematode flatworms that transmit to people who eat second intermediate host organisms carrying infectious stages (metacercariae). These infectious stages, in turn, originate from larval stages using first intermediate host snails. Foodborne trematodiases have not historically been a major public health concern in the United States, likely given a general lack of snails known to transmit injurious trematodes and the general rarity of reported infections originating locally [4]. However, the introduction of one such snail in recent decades opens the door for the possible emergence of foodborne trematodiases in the United States.

The snail *Melanoides tuberculata* serves as first intermediate host for at least 11 human-infectious trematode species [5]. Native to Asia and Africa, the snail is now established worldwide; it was likely introduced via the aquarium trade to the Americas in the 1950s and has since expanded from the United States to southern Brazil [6–10]. At least 3 human-pathogenic, zoonotic trematodes have been cointroduced with the snail in the Americas [5 and references therein, 11]. Reports from the continental United States include all 3 species in Texas [12–14] and Florida [15, 16], 1 species in Arizona [17], and another in Utah [15]. However, *M. tuberculata* snails are much more widespread throughout the United States [10]

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than the few places the trematodes have been reported. Because these trematodes are dispersed by birds, which serve as the trematodes' typical final hosts, there is a high probability that the parasites will also be much more widespread in the United States than currently recognized. Despite this possibility, and in contrast to elsewhere in the Americas (particularly South America [18, 19]), there appears to have been no substantial consideration from a public health perspective in the United States of the introduction of *M. tuberculata* and its suite of trematodes (but see [9] and [10]).

During exploratory surveys in September 2019 and February 2020, we collected 22 *M. tuberculata* snails at a San Diego fishing locality (Lake Murray). Every snail was infected with either *Haplorchis pumilio* or *Philophthalmus gralli*, which are both zoonotic human pathogens (see below). The finding was surprising; at that time, not even the snail was widely appreciated as being common in California. For instance, the snail was not included in the California Department of Fish and Wildlife's list of invasive invertebrates (https://wildlife.ca.gov/Conservation/Invasives/Species) and we could only find 2 reports of the snail's presence at single localities in California [20, 21]. However, research-grade citizen-science observations on https://www.iNaturalist.org revealed that *M. tuberculata* was widespread throughout the region (Figure 1). Because we suspected that the snail's human-infectious trematodes would also be widespread, as they can widely disperse in their final host birds, we conducted a broader sampling of the snails and their trematodes in southern California.

Here, we document that the snail, its 3 human-infectious trematodes known from elsewhere in the Americas, and several other trematodes potentially introduced to the Americas are all established in areas of possibly high exposure risk throughout southern California, one of the most populous metropolitan areas in the United States.

METHODS

Because the most medically important trematodes transmitted by *M. tuberculata* elsewhere in the Americas are fishborne, we focused our surveys on lakes listed as fishing localities by the California Department of Fish and Wildlife (https://apps.wildlife.ca.gov/fishing). We excluded lakes that occurred above 305 m (1000 feet), as all research-grade iNaturalist observations in southern California were below that elevation (consistent with the tropical to warm-temperate climate preferences of the snail [6]). This left us with a list of 55 suitable freshwater fishing localities to survey.

From spring to fall 2021, we surveyed 41 of the 55 localities for *M. tuberculata* (Figure 1 and Table 1). For each locality, a team of 1 to 3 people visited 5 spatially interspersed sites consisting of approximately 200-m stretches of shoreline from the water edge to 1.3 m depth; such shallow waters are the typical preferred habitat for *M. tuberculata* [6]. At each of the 5 sites, the team spent 20 worker minutes searching for and collecting *M. tuberculata* visually and by dragging 41-cm wide dip nets along the bottom, digging up to 2.5 cm into soft sediment. During our surveys, we also noted the presence of dead *M. tuberculata* shells with unworn periostracum covering their shells; such fresh-dead shells indicate that a site had recently harbored live snails or currently harbored live snails that went undetected by our survey.

We collected live snails at sites where we detected *M. tuberculata*, emphasizing snails ≥ 15 mm shell length given the general increase of infection probability with snail size/age [22, 23], and had a target of 300 snails per locality. In total, we collected 3164 snails. We kept snails alive until dissection in small plastic laboratory terraria, using filtered tap water treated with a dechlorinator (Top Fin Water Conditioner, lot number 61724), air stones, and ad libitum feeding on boiled romaine lettuce. *M. tuberculata* snails housed in this way suffered no major mortality and could be maintained for at least 4 months with no obvious loss of health (unpublished data).

We identified trematode infections by dissecting snails, typically within 14 days of collection (up to a maximum of 41 days). We first gently cracked their shells with a hammer and, under a dissecting microscope, teased apart the tissues in *Helix pomatia* saline (NaCl 97.5 mM, KCl 2.0 mM, $CaCl_2$ 1.0 mM) [24]. Using microscopical examination, we compared worm morphology to literature descriptions (see references cited in Pinto and Melo [5]). For each locality, we collected voucher specimens in 95% ethanol: up to 5 whole, deshelled individuals infected with each species of trematode, and at least 5 uninfected snails.

We genetically confirmed morphological identifications where possible by sequencing the 28S ribosomal DNA (rDNA) locus. We extracted genomic DNA from 1 to 5 cercariae per infected snail in 1 μL of a 2 mg/mL proteinase K solution (Qiagen), amplified 28S rDNA using existing primers (dig12 and 1500R [25]) and polymerase chain reaction (PCR) protocols slightly modified from Tkach et al [25] to use a 25-μL reaction volume, which contained 1 μL DNA, 2.5 μL 10× buffer (1.5 mM final concentration of $MgCl_2$) (Qiagen), 0.25 μM primers, 250 μM dNTPs, 1 unit of Taq, and 16.3 μL molecular water. We examined PCR products on a 1× Tris/borate/ethylenediaminetetraacetic acid (TBE) agarose gel and shipped successful products to Eton Biosciences (San Diego, CA) for Sanger sequencing. We used MEGA X [26] to trim the trace files by eye prior to a discontiguous megaBLAST comparing each sequence to all 28S Platyhelminthes sequences present in GenBank (http://www.ncbi.nlm.nih.gov) as of 26 September 2022. We archived newly generated sequences in GenBank.

We report prevalence of infection as the percentage of snails infected by trematode first intermediate host stages. Overall prevalence considers simultaneous multispecies infections in

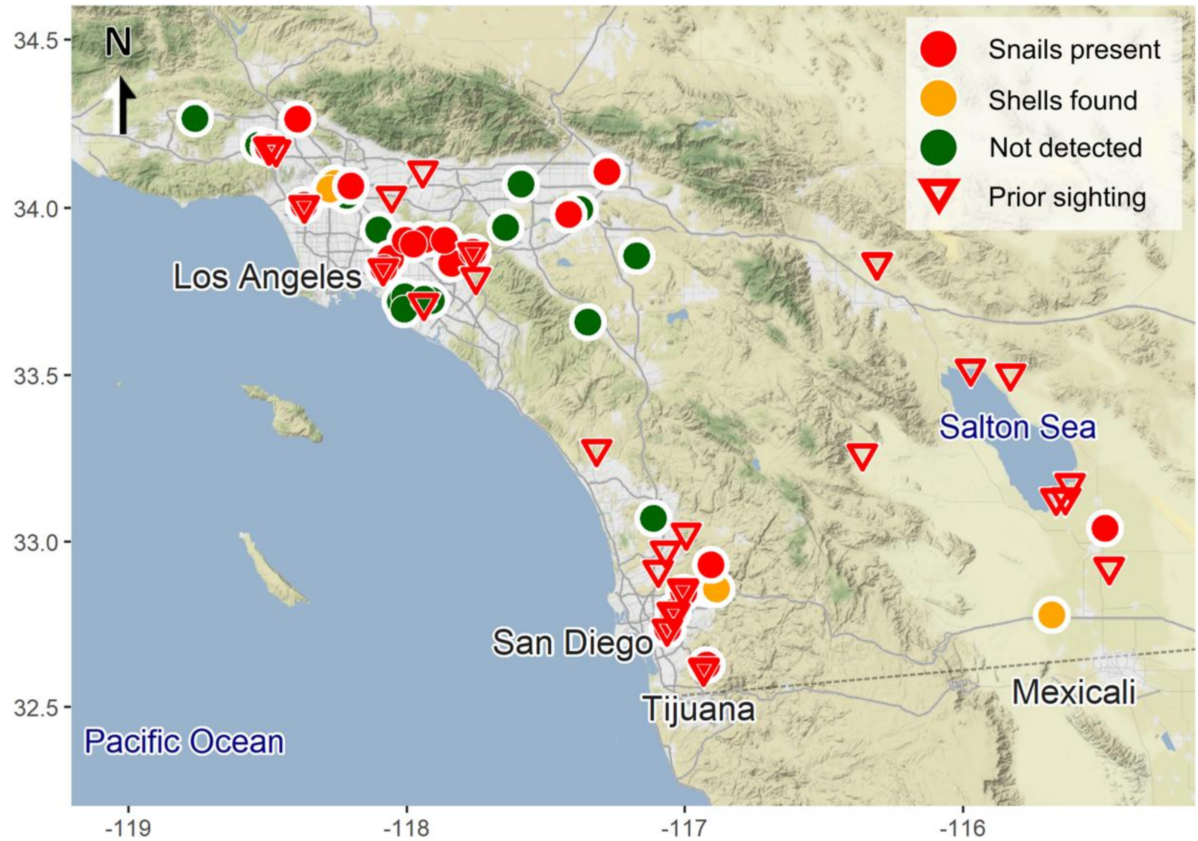

Figure 1. The introduced snail, *Melanoides tuberculata,* is widespread throughout southern California. The 25 sites with research-grade *M. tuberculata* observations from iNaturalist.org (red inverted triangles) are well-interspersed among the 41 fishing localities surveyed during this study (circles). Circles of different color (see legend) mark the 20 localities where we detected live snails, the 5 localities where we observed only fresh dead shells, and the 16 localities where we did not detect snails. Map tiles by Stamen Design, under CC BY 3.0. Data by OpenStreetMap, under ODbL.

the same snail as 1 infected snail. We calculated 95% confidence intervals for prevalence using the Wilson score interval [27]. Among-locality prevalences were calculated as unweighted means, with interquartile ranges calculated using method 7 of Hyndman and Fan [28]. We did not include 2 snails that we facultatively collected and examined from Lake Miramar in these calculations, but, for completeness, do report those infections as a footnote.

RESULTS

We detected live *M. tuberculata* snails at 20 of the 41 surveyed fishing localities (Figure 1 and Table 1). At 5 localities lacking live snails, we observed relatively unworn, dead *M. tuberculata* shells (Figure 1 and Table 1), indicating that those localities had recently supported live *M. tuberculata* or had live snails that went undetected by our survey. Hence, at least 25 of the 41 localities (61%) supported or had recently supported live *M. tuberculata* populations.

Trematode first intermediate host infections occurred at all but 1 of the localities with live *M. tuberculata* snails. Among the 20 localities with live snails, overall infection prevalence averaged 39% (interquartile range, 12%–59%; Table 2).

Two trematode species morphologically and genetically matched the zoonotic fishborne trematodes *H. pumilio* and *Centrocestus formosanus*. Our four 28S rDNA sequences for *H. pumilio* (GenBank, OK335796–OK335799) were 734, 702, 521, and 613 bp long and provided a 98.9%–100.0% match to the 10 *H. pumilio* sequences already in GenBank (HM004173, HM004186, HM004191, KY369155, KY369156, KY369157, KX815125, MN745941, MG738252, MT840091). Our 2 *C. formosanus* sequences (GenBank, OK335803 and OK335804) were 728 and 438 bp long and provided a 99.8%–100% match to the 13 *C. formosanus* sequences previously deposited in GenBank (HQ874609, KY075663, KY075664, KY075665, KY351633, KY369153, KY369154, MG738251, MK876840, MK876841, MK876842, MK876844, MZ570131).

H. pumilio was the most common trematode (Figure 2 and Table 2), composing 48% of all trematode infections, having an overall prevalence of 19% and occurring at 15 of the 20 sites with live snails. In contrast, *C. formosanus* was quite rare, being detected at only 1 locality and at low prevalence (3%; Figure 2 and Table 2).

The other encountered human-pathogenic trematode morphologically and genetically matched *P. gralli*. Our 3 *P. gralli*

Table 1. The 41 Southern California Fishing Localities Surveyed for the Introduced Trematode First Intermediate Host Snail *Melanoides tuberculata*, and Whether Live Snails or Only Dead Shells Were Present

County	Locality	Latitude	Longitude	Live Snails[a]	Dead Shells Only[a]
Imperial	Sunbeam Lake	32.780	−115.680		×
	Weist Lake	33.042	−115.490	×	
Los Angeles	Cerritos Lake	33.852	−118.060	×	
	Downey Wilderness Park Lake	33.936	−118.101		
	Echo Park Lake	34.073	−118.260		×
	El Dorado Park Lakes	33.821	−118.085	×	
	Hansen Dam Lake	34.266	−118.393	×	
	Hollenbeck Park Lake	34.040	−118.218		
	Kenneth Hahn Lake	34.009	−118.371	×	
	La Mirada Lake	33.904	−118.007	×	
	Lake Balboa	34.182	−118.497	×	
	Lincoln Park Lake	34.066	−118.203	×	
	MacArthur Park Lake	34.058	−118.278		×
	Reseda Park Lake	34.189	−118.534		
Orange	Carr Park Lake	33.722	−118.023		
	Centennial Lake	33.726	−117.912		
	Eisenhower Park Lake	33.836	−117.838	×	
	Greer Park Lake	33.737	−118.009		
	Huntington Park Lake	33.698	−118.011		
	Laguna Lake	33.908	−117.936	×	
	Mile Square Park Lake	33.729	−117.939		
	Robert B Clark Regional Park Lake	33.894	−117.978	×	
	Tri-city Lake	33.904	−117.866	×	
	Yorba Linda Regional Park Lake	33.869	−117.765	×	
Riverside	Lake Elsinore	33.659	−117.350		
	Lake Evans	33.996	−117.380		
	Perris Lake	33.857	−117.173		
	Rancho Jurupa Park Pond	33.981	−117.419	×	
San Bernardino	Cucamonga Guasti Park Lake	34.073	−117.590		
	Prado Park Lake	33.943	−117.647		
	Seccombe Park Lake	34.109	−117.282	×	
San Diego	Chollas Lake Park	32.737	−117.063	×	
	El Capitan Reservoir	32.737	−117.063		×
	Lake Hodges	33.071	−117.114		
	Lake Jennings	32.858	−116.888		×
	Lake Murray	32.787	−117.043	×	
	Lindo Lake	32.859	−116.917		
	Lower Otay Reservoir	32.627	−116.923	×	
	San Vicente Reservoir	32.930	−116.906	×	
	Santee Recreational Lakes	32.846	−117.005	×	
Ventura	Rancho Simi Park Lake	34.267	−118.764		

[a]The × symbols indicate the detection of snails or shells while blank cells reflect that none were detected.

sequences (GenBank, OK335800–OK335802) were 290, 230, and 585 bp long and were a 99.6%–100.0% match to the 3 confirmed *P. gralli* sequences previously deposited (MZ088139, JQ246434, JQ627832). This species was the second most common trematode encountered (17% overall prevalence) and was the most widespread, occurring at 18 of 20 localities harboring live *M. tuberculata* (Figure 2 and Table 2).

We encountered 5 additional trematode species that are likely not of direct public health relevance given their taxonomic affinities and probable life cycles (Figure 2, Table 2, Table 3, and "Discussion"). Three of these species morphologically matched trematodes described from *M. tuberculata* from the Eastern Hemisphere, and 2 of these 3 have not previously been reported from the Americas (Table 3). A fourth trematode also appears to have never been reported infecting *M. tuberculata* in the Americas, and we could find no record of it from Asia or Africa (see qualification in Table 3). The fifth additional trematode, observed only once in a double infection and which appeared to be poorly developing, may represent an accidental spillover infection by a species typically infecting another local snail (Table 3).

Table 2. Prevalences of Human-Infectious and Other Trematode Species and Sample Sizes of Their Introduced First Intermediate Host Snail *Melanoides tuberculata* From 20 Fishing Localities in Southern California, USA

				Human-Infectious Species			Other Species			
County	Locality[a]	n	Overall[b]	*Haplorchis pumilio*	*Centrocestus formosanus*	*Philophthalmus gralli*	Renicolid sp. 1	Renicolid sp. 2	Renicolid sp. 3	Lecithodendriid sp.
Imperial	Weist	109	5 (2–10)	0 (0–3)	0 (0–3)	5 (2–10)	0 (0–3)	0 (0–3)	0 (0–3)	0 (0–3)
Los Angeles	Cerritos	360	23 (18–27)	2 (1–4)	0 (0–1)	5 (3–8)	19 (15–24)	0 (0–1)	0 (0–1)	1 (0–2)
	El Dorado	74	50 (39–61)	41 (30–52)	0 (0–5)	24 (16–35)	20 (13–31)	0 (0–5)	0 (0–5)	3 (1–9)
	Hansen Dam	146	7 (4–12)	1 (0–4)	0 (0–3)	2 (1–6)	0 (0–3)	0 (0–3)	0 (0–3)	4 (2–9)
	K. Hahn	13	54 (29–77)	38 (18–64)	0 (0–23)	8 (0–33)	0 (0–23)	0 (0–23)	0 (0–23)	8 (0–33)
	La Mirada	30	97 (83–100)	30 (17–48)	0 (0–11)	70 (52–83)	3 (0–17)	0 (0–11)	0 (0–11)	0 (0–11)
	Balboa[c]	556	0 (0–1)	0 (0–1)	0 (0–1)	0 (0–1)	0 (0–1)	0 (0–1)	0 (0–1)	0 (0–1)
	Lincoln Park	63	6 (2–15)	0 (0–6)	0 (0–6)	6 (2–15)	0 (0–6)	0 (0–6)	0 (0–6)	0 (0–6)
	Robert B Clark	296	73 (67–77)	55 (49–60)	0 (0–1)	4 (2–7)	29 (24–34)	0 (0–1)	1 (0–3)	0 (0–1)
Orange	Eisenhower	19	53 (32–73)	5 (0–25)	0 (0–17)	42 (23–64)	0 (0–17)	0 (0–17)	0 (0–17)	5 (0–25)
	Laguna	66	15 (8–26)	6 (2–15)	0 (0–6)	6 (2–15)	3 (1–10)	0 (0–6)	0 (0–6)	0 (0–6)
	Tri-city	38	13 (6–27)	0 (0–9)	0 (0–9)	13 (6–27)	0 (0–9)	0 (0–9)	0 (0–9)	0 (0–9)
	Yorba Linda	190	45 (38–52)	27 (22–34)	0 (0–2)	10 (6–15)	1 (0–3)	1 (0–4)	3 (1–6)	4 (2–8)
Riverside	Rancho Jurupa	294	32 (27–37)	8 (5–11)	0 (0–1)	20 (16–25)	0 (0–2)	0 (0–1)	0 (0–2)	6 (4–9)
San Bernardino	Seccombe	124	31 (23–39)	2 (1–7)	0 (0–3)	26 (19–34)	0 (0–3)	0 (0–3)	0 (0–3)	2 (1–7)
San Diego	Chollas	288	79 (74–83)	57 (51–63)	3 (2–6)	18 (14–23)	5 (3–8)	1 (0–2)	0 (0–1)	0 (0–2)
	Murray	85	87 (78–93)	80 (70–87)	0 (0–4)	6 (3–13)	0 (0–4)	0 (0–4)	0 (0–4)	1 (0–6)
	Lower Otay	80	19 (12–29)	3 (1–9)	0 (0–5)	16 (10–26)	0 (0–5)	0 (0–5)	0 (0–5)	3 (1–9)
	San Vicente	235	8 (5–12)	0 (0–2)	0 (0–2)	0 (0–2)	0 (0–2)	0 (0–2)	4 (2–7)	4 (2–7)
	Santee	96	81 (72–88)	28 (20–38)	0 (0–4)	50 (40–60)	4 (2–10)	0 (0–4)	1 (0–6)	0 (0–4)
Among-locality mean prevalence, %, and interquartile range			39 (12–59)	19 (1–32)	0.2 (0–0)	17 (5–21)	4 (0–4)	0.1 (0–0)	0.4 (0–0.1)	2 (0–4)

Data are % infected (95% confidence interval [CI]).

[a]See Table 1 for full locality names. Furthermore, we facultatively collected 2 snails from Lake Miramar (San Diego County), not shown here, each infected with *P. gralli* (100% prevalence; 95% CI, 34%–100%).

[b]Overall infection prevalence also includes (1) a renicolid infection that was too young to identify (likely renicolid sp. 1 or sp. 2) at Yorba Linda Regional Park Lake, and (2) a single unidentified trematode producing "Armata" cercaria at Chollas Lake Park.

[c]All snails at Balboa were consistent with *M. tuberculata* but were morphologically distinct from other observed snails. These were likely a distinct clone, as observed in other *M. tuberculata* invasions worldwide [7]. Infection susceptibility can vary among clones [22], which likely explains the lack of observed infections at this locality.

DISCUSSION

The presence of previously unreported human-infectious parasites throughout southern California highlights the possibility for foodborne and waterborne trematodiases to emerge in California and, indeed, elsewhere in the United States where the snail has been introduced.

We detected 2 fishborne zoonotic trematodes, *H. pumilio* and *C. formosanus*. Both of these are known to infect humans where *M. tuberculata* is endemic, particularly Southeast Asia (reviewed in Chai and Jung [35]). *H. pumilio* was the single most common trematode that we found and is specifically recognized as a pathogen of global importance [35]. Both trematodes are introduced elsewhere in the Americas, including Peru [18], Brazil [19], Mexico [36], and other US states [12, 13, 15, 16, 37]. In the United States, they have previously garnered attention largely given their impacts of the metacercaria stages on endangered or commercially important second intermediate host fish in Texas and Florida [12, 15, 38]. These introduced trematodes may have additional veterinary and wildlife disease importance, as the adults can also infect cats, dogs, and wild animals [16, 35].

Concerning human infections, adult stages of these trematodes, like other members of the trematode family Heterophyidae, can cause pathologies ranging from mild discomfort to death (see [1, 3, 35] and references therein). People are infected primarily by eating raw, undercooked, or pickled fish carrying the infectious stages (metacercariae). Because our study revealed that these parasites are present in fishing localities, because their metacercariae can infect a wide range of fishes eaten by people [35] and have been reported from a wide range of fishes in the Americas [11, 15], and because uncooked fish are commonly eaten in California, including as ceviche, poke, sashimi, and sushi (unpublished observations), there is a real possibility for *H. pumilio* and *C. formosanus* to cause foodborne trematodiasis in California. Furthermore, this basic epidemiological logic, along with Ching's [39] documentation of fishborne heterophyid infections in residents of Hilo and Honolulu (Hawaii) as recently as the 1950s, underscores the possibility for these species to infect people elsewhere in the United States where *M. tuberculata* occurs.

P. gralli is also human infectious, transmitting to humans via ingestion of aquatic animals or plants harboring metacercariae

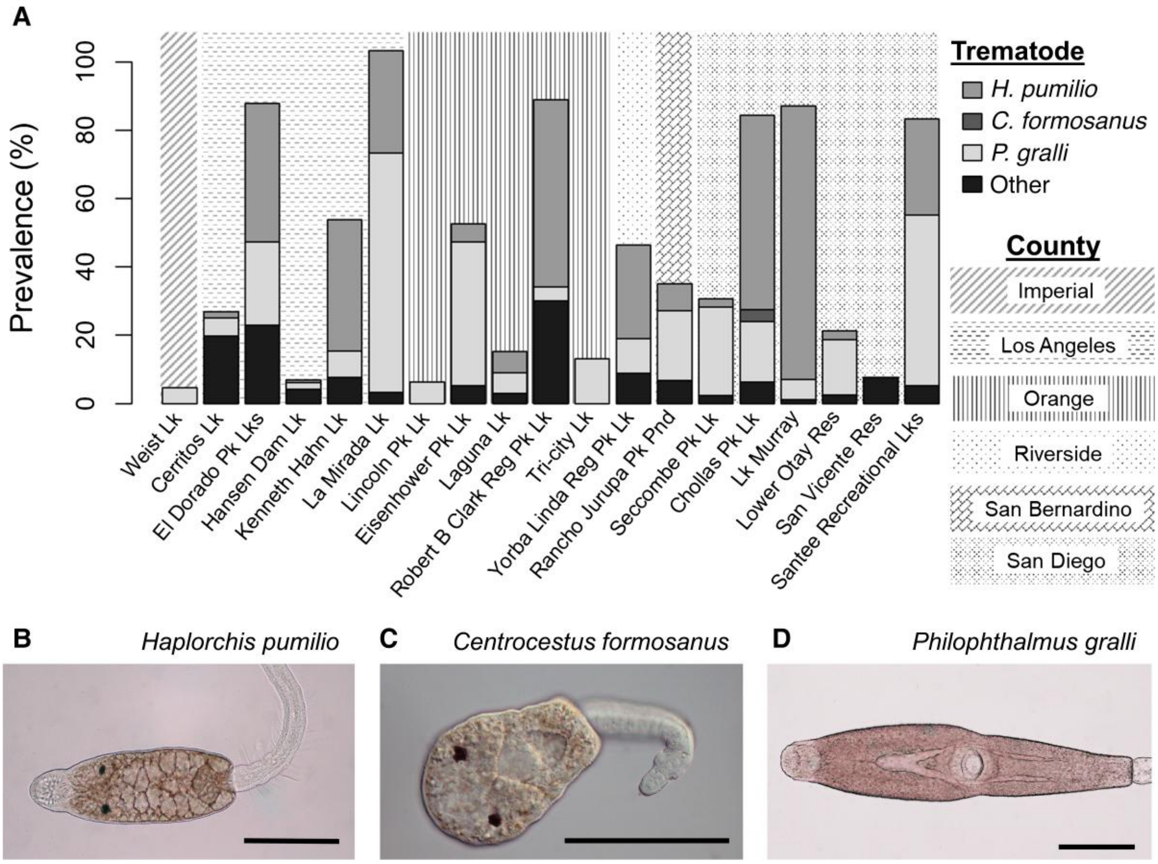

Figure 2. Human-infectious trematodes are present in the *Melanoides tuberculata* intermediate host in every surveyed Southern California county. *A*, Observed parasite prevalences at 19 localities at which live *M. tuberculata* occurred. Stacked bar height is sometimes greater than the overall prevalence of infection of Table 2 given the presence of double-species infections. Non-human-infectious trematodes are pooled into a single other category. Survey localities are arranged alphabetically within county (background shading). Two localities are excluded: Lake Balboa, in which we observed a possibly unique and completely infection-resistant clone of *M. tuberculata* (see Table 2 footnote), and Lake Miramar, from which we facultatively collected only 2 snails (each of which were infected with *Philophthalmus gralli*). *B*, Cercaria of *Haplorchis pumilio*. *C*, Cercaria of *Centrocestus formosanus*. *D*, Cercaria of *P. gralli*. All cercariae were photographed live, heat-stunned, and without staining. Scale bars = 100 μm.

or by direct contact with swimming infectious cercariae. *Philophthalmus* species can infect the eyes of humans and cause conjunctivitis [40]. A recent case in Texas [41] could have very well originated from *M. tuberculata*. *P. gralli* can also cause substantial problems in captive birds [17, 42] and therefore is of veterinary and possibly wildlife disease importance. Although *P. gralli* may be of less human health concern than the above 2 species, it was the second most common trematode in our surveys and appears quite broadly distributed elsewhere in the Americas, having been reported from *M. tuberculata* in Arizona [17], Florida [42], Texas [14], and farther south in the Americas [10, 43].

The 5 additional trematode species that we encountered infecting *M. tuberculata* probably rarely or never infect people given their taxonomic affinities and probable life cycles. Three of the species belong to the family Renicolidae, species of which infect the kidney tubules and ureters of bird final hosts. These trematodes do not infect mammals, including humans. The fourth additional species likely infects bats as final hosts and therefore could possibly infect humans. However, transmission probably requires eating raw insects. Eating raw insects likely does not happen frequently enough in the United States for this parasite to be of major concern for people, despite a report of human infection in Thailand by *Anchitrema sanguineum* [44], which is closely related or identical to this fourth trematode. The transmission and host use of the fifth species is unresolved, but it may not typically infect *M. tuberculata*. Therefore, although of possible veterinary and wildlife health importance, these 5 additional trematodes infecting *M. tuberculata* are probably of little direct public health concern.

Despite likely being incapable of infecting people, the above 5 additional trematodes do highlight a different public health concern. Because those trematodes are also possibly or likely introduced to the Americas, their presence emphasizes the risk that other pathogenic foodborne trematodes transmitted by *M. tuberculata* in its native range could be, or already have been, introduced to the United States. Such trematodes include human liver flukes, such as *Opisthorchis viverrini* and possibly

Table 3. Details on Additional Trematode Species of Unlikely Public Health Importance Encountered Infecting Introduced *Melanoides tuberculata* Snails in Southern California

Species	Descriptive and Taxonomic Notes	Introduced?	Likely 2nd Intermediate Hosts	Likely Final Host Use	GenBank Accession No.
Renicolid sp. 1	A large, white renicolid, often with yellow pigment between the oral and ventral suckers, which has not previously been reported from *M. tuberculata* in the Americas and perhaps not elsewhere.[a,b]	Possibly	Fishes, bivalves, or annelids	Kidney tubules of birds	OK338505–OK338506
Renicolid sp. 2	A brownish renicolid, generally morphologically matching "*Cercariae Indicae* XIV" of Sewell [29] from India, "*Cercaria levantina* 9" of Gold and Lengy [30] from Israel, and possibly the "*Renicola* sp." reported by Pinto and Melo [31] from Brazil and the "Renicolidae sp." reported by Tolley-Jordan et al [16] from Florida.[b]	Likely	Fishes, bivalves, or annelids	Kidney tubules of birds	OK338507–OK338508
Renicolid sp. 3	A xiphidiocercaria, fitting the morphological diagnosis of renicolid cercariae [32], that morphologically and behaviorally matches "*Cercaria levantina* 12" of Gold and Lengy [30] from Israel.	Likely	Fishes, bivalves, or annelids	Kidney tubules of birds	No sequence obtained
"Lecithodendriidae gen. sp." sensu Lopes et al (2021)	A xiphidiocercaria that morphologically and genetically (99.8%) matches "Lecithodendriidae gen. sp." from Brazil of Lopes et al [33], which may morphologically match at least 2 species described from Asia: "*Cercaria levantina* 13" of Gold and Lengy [30] from Israel, and "*Cercaria* sp. 1" from Jordan of Ismail et al [34]. This species may also match the Lecithodendriidae of Tolley-Jordan et al [16] from Florida. We also found that this species genetically matched (100%) 2 GenBank records (MW683332, MW683334) from adult trematodes reported as *Architrema sanguineum* and *A. longiformis*.[c]	Likely	Insects	Bats or reptiles	OK335156–OK335157
"Armata cercaria"	A potential accidental infection of *M. tuberculata* by a plagiorchioid trematode that usually infects another snail species; it was encountered 1 time in a double infection and appeared to be poorly developing.	Unknown	Molluscs, arthropods, fish, or amphibians	Fish, amphibian, reptile, bird, or mammal	No sequence obtained

[a]Although we have consulted the majority of the vast descriptive literature concerning trematodes of *Melanoides tuberculata*, we could not access every reference (see the extensive citation list in Pinto and Melo's checklist [5]). There is therefore a chance that "Renicolid sp. 1" has been morphologically described in Asia or Africa.

[b]Renicolid sp. 1 and Renicolid sp. 2 differed by 4.9% in their 28S rDNA sequences (839 bp total).

[c]These GenBank *Anchitrema* spp. records had not been peer reviewed at the time of writing. In addition, cercariae from *Anchitrema* spp. have not been previously described. If the GenBank species IDs do belong to an *Anchitrema* species, our findings would reveal the first intermediate host stages for an *Anchitrema* species and that *Anchitrema* truly belongs in the Lecithodendriidae, from which it was removed decades ago.

Clonorchis sinensis, which can cause cholangiocarcinoma [3, 45]. Adult stages of these parasites have been continually imported into the United States in human immigrants [4], making the introduction and establishment of these trematodes a very real possibility.

Despite existing knowledge that *M. tuberculata* and 3 of its human-infectious trematodes are already introduced in the United States [12–16], the possible public health implications have not elicited substantial attention. This lack of attention is particularly striking when considering the 2 introduced fishborne trematodes, *C. formosanus* and *H. pumilio*. Although we are currently obtaining confirmation, given the trematodes' known biology and ecology (see above), we should expect that their infectious stages will be present in fishes caught for food in the United States wherever those fish co-occur with infected snails.

Given the logical expectation that *C. formosanus* and *H. pumilio* will infect fishes that people eat, what explains the prior lack of public-health consideration in the United States, which stands in contrast to the recognition of possible impacts to human health in Peru [18] and Brazil [19]? A possible reason may have been a perception that Americans typically cook their fish, which would kill the infectious stages and minimize human infection risk. However, that idea overlooks the widespread popularity of eating uncooked fish in the United States, including as sushi, sashimi, ceviche, and poke (personal observations). It also appears unreasonable to dismiss a possible public health concern given an apparent lack of widespread cases of infections by these trematodes in the peer reviewed literature or in the news. Given the historical rarity of home-grown foodborne trematodiases in the continental United States [4], we suspect that clinicians and the public are generally not attuned to the possibility and that such cases may frequently go undiagnosed. Furthermore, even if diagnosed by a clinician or laboratory, such cases may go unreported to local or state health authorities. This possibility is enhanced by foodborne trematodiases not being explicitly listed as reportable diseases in the 4 states (Texas, Florida, Utah, and California) where the fishborne parasites carried by *M. tuberculata* have been reported [46–49]. The relatively recent occurrence of local fishborne trematodiases in Hawaii [39] and novel outbreaks of fishborne trematodiases in Italy [50] further counter the notion that such trematodiases cannot be an issue in the United States.

In short, several factors suggest the possible emergence and even ongoing presence of foodborne trematodiases in California and possibly other areas in the United States: (1) the introduced *M. tuberculata* snail is widespread, including at localities where people catch fish for eating; (2) the snail carries at least 3 of its human-pathogenic trematodes, including 2 that use a wide range of fishes to transmit to people and are recognized as being of public health importance elsewhere in the world; and (3) the snail also carries several other potentially introduced trematodes, highlighting the possibility that additional human-pathogenic trematodes are already introduced or may be introduced in the future. Taken together, these factors clearly call for additional consideration of *M. tuberculata* and its trematodes from a public health perspective in California and wherever else the snail is present in the United States.

Notes

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