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# Potential for Emergence of Foodborne Trematodiasis Transmitted by an Introduced Snail (*Melanooides 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 (*Melanooides 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 trematodiasis 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 trematodiasis 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 trematodiasis 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 trematodiasis in the United States.

The snail *Melanooides 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

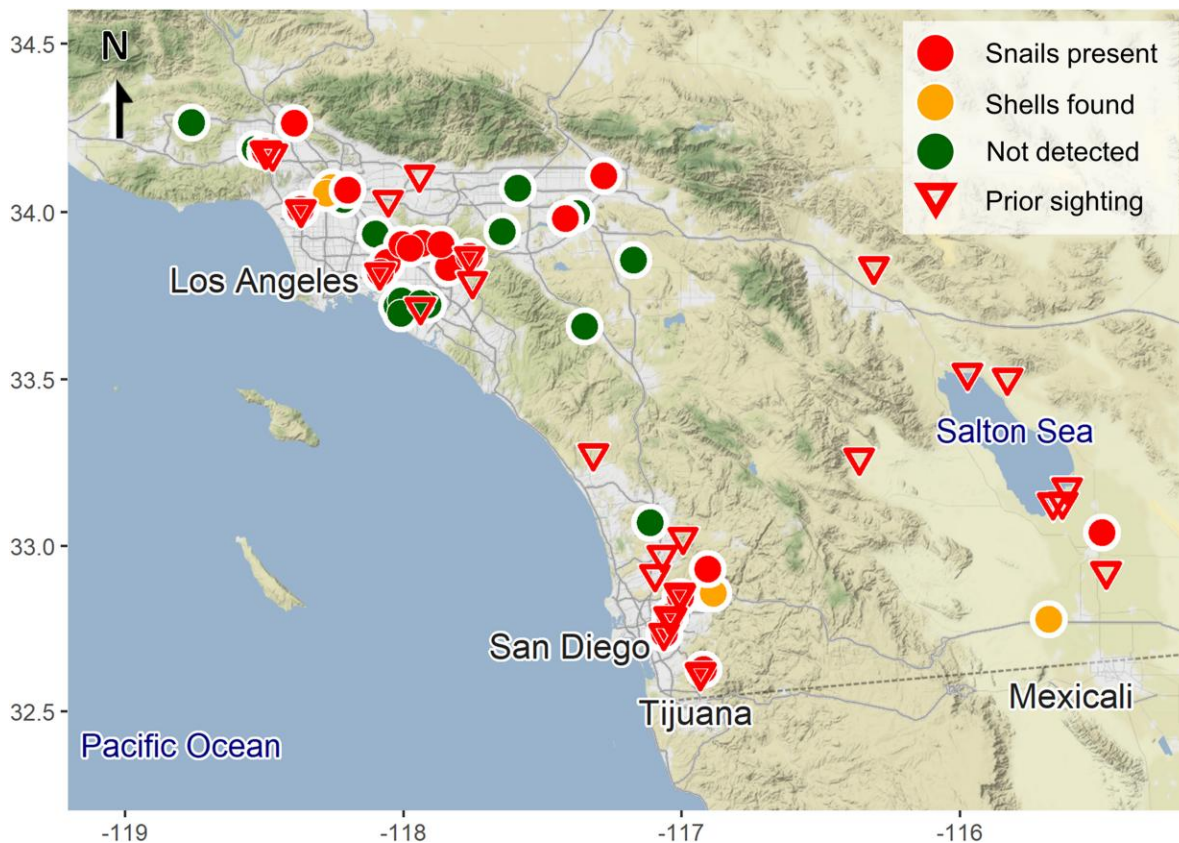
preferred habitat for *M. tuberculata* [6]. At each of the 5 sites, the team spent 20 worker minutes searching for and collecting *M. tuberculata* visually and by dragging 41-cm wide dip nets along the bottom, digging up to 2.5 cm into soft sediment. During our surveys, we also noted the presence of 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  $\geq 15$  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<sub>2</sub> 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  $\mu$ L 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- $\mu$ L reaction volume, which contained 1  $\mu$ L DNA, 2.5  $\mu$ L 10 $\times$  buffer (1.5 mM final concentration of MgCl<sub>2</sub>) (Qiagen), 0.25  $\mu$ M primers, 250  $\mu$ M dNTPs, 1 unit of Taq, and 16.3  $\mu$ L molecular water. We examined PCR products on a 1 $\times$  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 discontinuous 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 *Melanooides tuberculata*, and Whether Live Snails or Only Dead Shells Were Present**

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

<sup>a</sup>The x 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**

| County   | Locality <sup>a</sup> | n   | Overall <sup>b</sup> | Human-Infectious Species  |                                |                              | Other Species   |                 |                 |                     |
|--|-----------------------|-----|----------------------|---------------------------|--------------------------------|------------------------------|-----------------|-----------------|-----------------|---------------------|
|  |                       |     |                      | <i>Haplorchis pumilio</i> | <i>Centrocestus formosanus</i> | <i>Philophthalmus gralli</i> | 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 <sup>c</sup>   | 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]).

<sup>a</sup>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%).

<sup>b</sup>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.

<sup>c</sup>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.

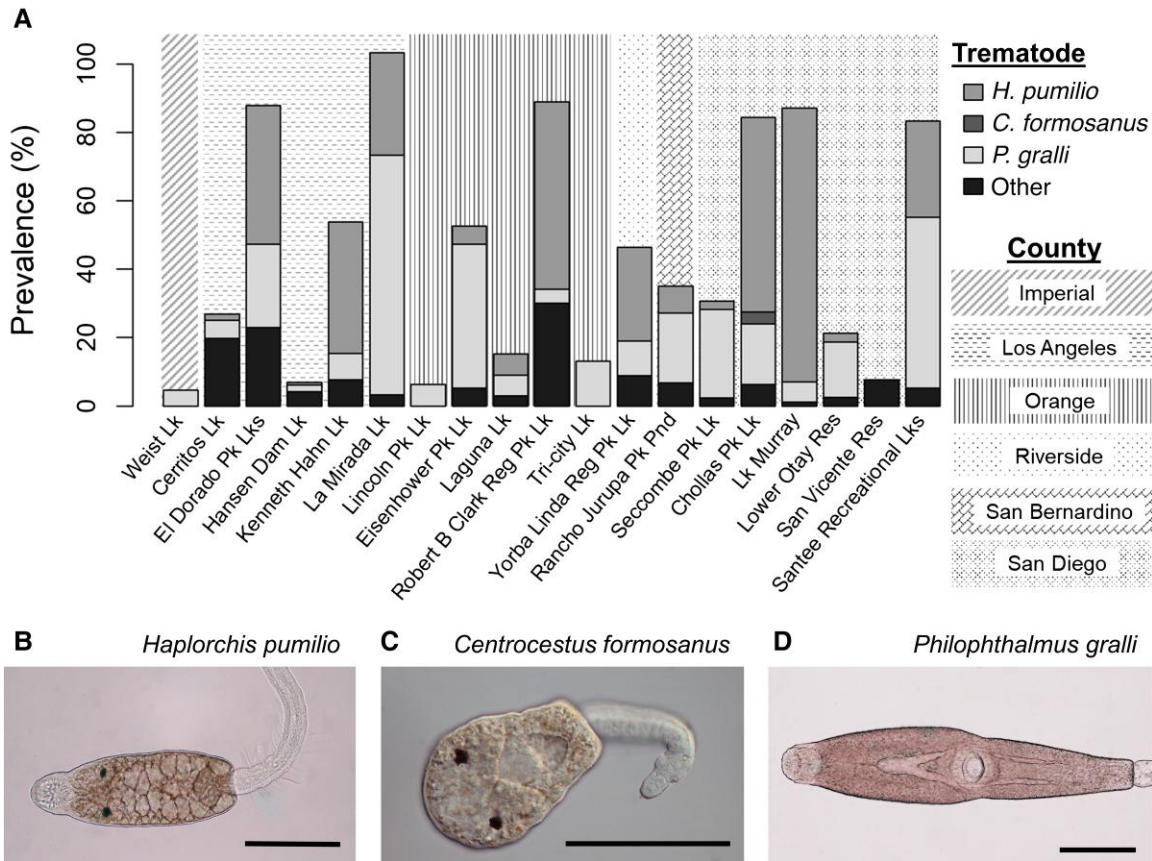
## DISCUSSION

The presence of previously unreported human-infectious parasites throughout southern California highlights the possibility for foodborne and waterborne trematodiasis 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  $\mu$ m.

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 Rencolidae, 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 *Melanooides 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 <i>M. tuberculata</i> in the Americas and perhaps not elsewhere. <sup>a,b</sup>   | Possibly    | Fishes, bivalves, or annelids             | Kidney tubules of birds                   | OK338505–OK338506     |
| Renicolid sp. 2                                       | A brownish renicolid, generally morphologically matching " <i>Cercariae Indicae</i> XIV" of Sewell [29] from India, " <i>Cercaria levantina</i> 9" of Gold and Lengy [30] from Israel, and possibly the " <i>Renicola</i> sp." reported by Pinto and Melo [31] from Brazil and the " <i>Renicolidae</i> sp." reported by Tolley-Jordan et al [16] from Florida. <sup>b</sup>  | 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 " <i>Cercaria levantina</i> 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 " <i>Lecithodendriidae</i> gen. sp." from Brazil of Lopes et al [33], which may morphologically match at least 2 species described from Asia: " <i>Cercaria levantina</i> 13" of Gold and Lengy [30] from Israel, and " <i>Cercaria</i> sp. 1" from Jordan of Ismail et al [34]. This species may also match the <i>Lecithodendriidae</i> 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 <i>Anchitrema sanguineum</i> and <i>A. longiformis</i> . <sup>c</sup> | Likely      | Insects                                   | Bats or reptiles                          | OK335156–OK335157     |
| "Armata cercaria"                                     | A potential accidental infection of <i>M. tuberculata</i> by a plagiorchoid 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  |

<sup>a</sup>Although we have consulted the majority of the vast descriptive literature concerning trematodes of *Melanooides 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.

<sup>b</sup>Renicolid sp. 1 and Renicolid sp. 2 differed by 4.9% in their 28S rDNA sequences (839 bp total).

<sup>c</sup>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 trematodiasis 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 trematodiasis 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 trematodiasis in Hawaii [39] and novel outbreaks of fishborne trematodiasis in Italy [50] further counter the notion that such trematodiasis cannot be an issue in the United States.

In short, several factors suggest the possible emergence and even ongoing presence of foodborne trematodiasis 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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## References

1. Keiser J, Utzinger J. Food-borne trematodiasis. *Clin Microbiol Rev* **2009**; 22:466–83.
2. Furst T, Keiser J, Utzinger J. Global burden of human food-borne trematodiasis: a systematic review and meta-analysis. *Lancet Infect Dis* **2012**; 12:210–21.
3. Sripa B, Kaewkes S, Intapan PM, Maleewong W, Brindley PJ. Food-borne trematodiasis in Southeast Asia: epidemiology, pathology, clinical manifestation and control. In: Zhou XN, Bergquist R, Olveda R, Utzinger J, eds. *Advances in parasitology: important helminth infections in Southeast Asia: diversity and potential for control and elimination*, pt A. Vol. 72, **2010**:305–50.
4. Fried B, Abruzzi A. Food-borne trematode infections of humans in the United States of America. *Parasitol Res* **2010**; 106:1263–80.
5. Pinto HA, Melo AL. A checklist of trematodes (Platyhelminthes) transmitted by *Melanoides tuberculata* (Mollusca: Thiaridae). *Zootaxa* **2011**; 2799:15–28.
6. CABI. Datasheet: *Melanoides tuberculata* (red-rimmed melania) [original text by B Facon and JP Pointier] invasive species compendium. Wallingford, UK: CAB International, **2020**.
7. Facon B, Pointier JP, Glaubrecht M, Poux C, Jarne P, David P. A molecular phylogeography approach to biological invasions of the new world by parthenogenetic thiarid snails. *Mol Ecol* **2003**; 12:3027–39.
8. Coelho PN, Fernandez MA, Cesar DAS, Ruocco AMC, Henry R. Updated distribution and range expansion of the gastropod invader *Melanoides tuberculata* (Muller 1774) in Brazilian waters. *Bioinvasion Rec* **2018**; 7:405–9.
9. Murray HD. The introduction and spread of thiarids in the USA. *Biologist* **1971**; 53:133–5.
10. Chalkowski K, Abigail M, Christopher AL, Sarah Z. Spread of an avian eye fluke, *Philophthalmus gralli*, through biological invasion of an intermediate host. *J Parasitol* **2021**; 107:336–48.
11. Scholz T, Aguirre-Macedo ML, Salgado-Maldonado G. Trematodes of the family Heterophyidae (Digenea) in Mexico: a review of species and new host and geographical records. *J Nat Hist* **2001**; 35:1733–72.
12. Mitchell AJ, Salmon MJ, Huffman DG, Goodwin AE, Brandt TM. Prevalence and pathogenicity of a heterophyid trematode infecting the gills of an endangered fish, the fountain darter, in two central Texas spring-fed rivers. *J Aquat Anim Health* **2000**; 12:283–9.
13. Tolley-Jordan LR, Owen JM. Habitat influences snail community structure and trematode infection levels in a spring-fed river, Texas, USA. *Hydrobiologia* **2008**; 600:29–40.
14. Nollen PM, Murray HD. *Philophthalmus gralli* - identification, growth characteristics, and treatment of an oriental eyefluke of birds introduced into continental United States. *J Parasitol* **1978**; 64:178–80.
15. Mitchell AJ, Overstreet RM, Goodwin AE, Brandt TM. Spread of an exotic fish-gill trematode. *Fisheries* **2005**; 30:11–6.
16. Tolley-Jordan L, Chadwick M, Triplett J. New records of digenetic trematodes infecting *Melanoides tuberculata* (O. F. Meuller 1774) in Florida, USA. *Bioinvasion Rec* **2022**; 11:149–64.
17. Church ML, Barrett PM, Swenson J, Kinsella JM, Tkach VV. Outbreak of *Philophthalmus gralli* in four greater

- rheas (*Rhea americana*). *Vet Ophthalmol* **2013**; 16: 65–72.
18. Pulido-Murillo EA, Furtado LFV, Melo AL, Rabelo EML, Pinto HA. Fishborne zoonotic trematodes transmitted by *Melanoides tuberculata* snails, Peru. *Emerg Infect Dis* **2018**; 24:606–8.
  19. Lopes AS, Pulido-Murillo EA, Melo AL, Pinto HA. *Haplorchis pumilio* (Trematoda: Heterophyidae) as a new fish-borne zoonotic agent transmitted by *Melanoides tuberculata* (Mollusca: Thiaridae) in Brazil: a morphological and molecular study. *Infect Genet Evol* **2020**; 85:104495.
  20. Taylor DW. Freshwater mollusks of California: a distributional checklist. *Calif Fish Game* **1981**; 67:140–63.
  21. Cohen AN, Carlton JT. Nonindigenous aquatic species in a United States estuary: a case study of the biological invasions of the San Francisco Bay and Delta. Washington. DC: United States Fish and Wildlife Service and The National Sea Grant College Program Connecticut Sea Grant, **1995**; 245.
  22. Genner MJ, Michel E, Todd JA. Resistance of an invasive gastropod to an indigenous trematode parasite in Lake Malawi. *Biol Invasions* **2008**; 10:41–9.
  23. Yanohara Y. On analysis of transmission dynamics of trematode infection 1. *Centrocestus formosanus* infection in Miyakojima, Okinawa. *Jpn J Parasitol* **1985**; 34:55–70.
  24. Lockwood AP. “Ringer” solutions and some notes on the physiological basis of their ionic composition. *Comp Biochem Physiol* **1961**; 2:241–89.
  25. Tkach VV, Littlewood DTJ, Olson PD, Kinsella JM, Swiderski Z. Molecular phylogenetic analysis of the Microphalloidea Ward 1901 (Trematoda: Digenea). *Syst Parasitol* **2003**; 56:1–15.
  26. Stecher G, Tamura K, Kumar S. Molecular evolutionary genetics analysis (MEGA) for macOS. *Mol Biol Evol* **2020**; 37: 1237–9.
  27. Newcombe RG. Two-sided confidence intervals for the single proportion: comparison of seven methods. *Stat Med* **1998**; 17:857–72.
  28. Hyndman RJ, Fan YN. Sample quantiles in statistical packages. *Am Stat* **1996**; 50:361–5.
  29. Sewell RBS. *Cercariae indicae*. *Indian J Med Res* **1922**; 10: 1–370.
  30. Gold D, Lengy J. Studies on larval stages of digenetic trematodes in aquatic mollusks of Israel; 4, on five cercariae from freshwater snail *Melanoides tuberculata* (Meuller, 1774). *Isr J Zool* **1974**; 23:143–61.
  31. Pinto HA, Melo AL. *Melanoides tuberculata* (Mollusca: Thiaridae) harboring renicolid cercariae (Trematoda: Renicolidae) in Brazil. *J Parasitol* **2012**; 98:784–7.
  32. Hechinger RF, Miura O. Two ‘new’ renicolid trematodes (Trematoda: Digenea: Renicolidae) from the California horn snail *Cerithidea californica* (Haldeman 1840) (Gastropoda: Potamididae). *Zootaxa* **2014**; 3784:559–74.
  33. Lopes AS, Pulido-Murillo EA, López-Hernández D, Melo ALD, Pinto HA. First report of *Melanoides tuberculata* (Mollusca: Thiaridae) harboring a xiphidiocercaria in Brazil: a new parasite introduced in the Americas? *Parasitol Int* **2021**; 82:102284.
  34. Ismail S, Bdair S, Issa I. Two new cercariae from *Melanoides tuberculata* (Meuller) snails in Jordan. *Helminthologia* **1988**; 25:139–45.
  35. Chai J-Y, Jung B-K. Fishborne zoonotic heterophyid infections: an update. *Food Waterborne Parasitol* **2017**; 8–9: 33–63.
  36. Scholz T, Salgado-Maldonado G. The introduction and dispersal of *Centrocestus formosanus* (Nishigori 1924) (Digenea: Heterophyidae) in Mexico: a review. *Am Midl Nat* **2000**; 143:185–200.
  37. Martin WE. The life histories of some Hawaiian heterophyid trematodes. *J Parasitol* **1958**; 44:305–18.
  38. Huston DC, Worsham MD, Huffman DG, Ostrand KG. Infection of fishes, including threatened and endangered species by the trematode parasite *Haplorchis pumilio* (Looss 1896) (Trematoda: Heterophyidae). *Bioinvasion Rec* **2014**; 3:189–94.
  39. Ching HL. Internal parasites of man in Hawaii, with special reference to heterophyid flukes. *Hawaii Med J* **1961**; 20: 442–5.
  40. Gutierrez Y, Grossniklaus HE, Annable WL. Human conjunctivitis caused by the bird parasite *Philophthalmus*. *Am J Ophthalmol* **1987**; 104:417–9.
  41. Sapp SGH, Alhabshan RN, Bishop HS, et al. Ocular trematodiasis caused by the avian eye fluke *Philophthalmus* in southern Texas. *Open Forum Infect Dis* **2019**; 6:ofz265.
  42. Greve JH, Harrison GJ. Conjunctivitis caused by eye flukes in captive-reared ostriches. *J Am Vet Med Assoc* **1980**; 177: 909–10.
  43. Diaz MT, Hernandez LE, Bashirullah AK. Experimental life cycle of *Philophthalmus gralli* (Trematoda: Philophthalmidae) in Venezuela. *Rev Biol Trop* **2002**; 50: 629–41.
  44. Kusolsuk T, Paiboon N, Pubampen S, Maipanich W, Dekumyoy P, Waikagul J. *Anchitrema sanguineum* (Digenea: Anchitremitidae) accidentally found during colonoscopy of a patient with chronic abdominal pain: a case report. *Korean J Parasitol* **2009**; 47:167–70.
  45. Chai J-Y, Jung B-K. Epidemiology of trematode infections: an update. In: Toledo R, Fried B, eds. *Digenetic trematodes*.

- 2nd ed. Cham: Springer International Publishing, 2019. 359–409.
46. Texas Department of State: Health Services. Notifiable conditions. <https://www.dshs.texas.gov/idcu/investigation/conditions/>. Accessed 16 February 2022.
  47. Florida Department of Health. Disease reporting information for health care and laboratory providers. <https://www.floridahealth.gov/diseases-and-conditions/disease-reporting-and-management/index.html>. Accessed 16 February 2022.
  48. Utah Department of Health: Bureau of Epidemiology. Utah reportable diseases. <https://epi.health.utah.gov/>. Accessed 16 February 2022.
  49. California Code of Regulations. Title 17, Section 2500: Reporting to the Local Health Authority. <https://govt.westlaw.com/SiteList>. Accessed 16 February 2022.
  50. Pozio E, Armignacco O, Ferri F, Gomez Morales MA. *Opisthorchis felineus*, an emerging infection in Italy and its implication for the European Union. *Acta Trop* 2013; 126:54–62.