

Rapid Communication

Infection of fishes, including threatened and endangered species by the trematode parasite *Haplorchis pumilio* (Looss, 1896) (Trematoda: Heterophyidae)

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Abstract

Haplorchis pumilio (Trematoda: Heterophyidae) has become widely established around the world because of multiple introductions of its snail hosts, and because of its flexible host requirements at the second-intermediate and definitive levels. Although exotic thiarid snails introduced into North American waters have been previously reported to harbor *H. pumilio*, metacercariae of *H. pumilio* have not been reported from native fishes in the continental USA. In this study artificially exposed cyprinids to *H. pumilio* cercariae from infected snails became infected with the trematode, sometimes lethally, when exposed for only 15 minutes to high cercarial densities. Subsequent collection and examination of fountain darters *Etheostoma fonticola*, a U.S. federally endangered fish species, and the examination of archived specimens of largemouth bass *Micropterus salmoides*; the IUCN endangered *Dionda diaboli*, *Gambusia nobilis*, *Cyprinodon elegans*; and IUCN vulnerable *Etheostoma grahami* from West Texas springs resulted in new host and locality records for *H. pumilio* metacercarial infections in all species except *C. elegans*. Metacercariae were found encysted in the connective tissue of the head and at fin insertions. Conversely no integumental or visceral infections were observed, regardless of the fish species or collection locality. It is surmised that *Haplorchis pumilio* is probably present in many aquatic systems where *Melanoides tuberculata* and *Tarebia granifera* have become established, but that the metacercariae have been missed by previous investigators because of their small size and unusual anatomical location. Results from this study suggest that subsequent investigators be on the watch for these metacercariae, and that the anatomical sites typical of the worm (fin insertions, and especially the caudal peduncle) be included in routine necropsy procedures for fishes from such habitats.

Key words: exotic trematode, *Melanoides tuberculata*, *Tarebia granifera*, Texas

Introduction

Haplorchis pumilio (Looss, 1896) was first described from birds in Egypt. Various life stages of this parasite have now been reported from Africa (Sommerville 1982a), Israel (Witenberg 1929), India (Umadevi and Madhavi 2006), China and East Asia (Chen 1936; Shen 1959; Lo and Lee 1996a; Chung et al. 2011), Southeast Asia (Pearson and Ow-Yang 1982; Radomyos et al. 1998; Kay et al. 2009; Krailas et al. 2011; Chai et al. 2012), Australia (Pearson 1964), Venezuela (Díaz et al. 2008), Mexico (Scholz et al. 2001), and the USA (Tolley-Jordan and Owen 2008; snail stages only).

Adults of *Haplorchis pumilio* have been recovered from the digestive tracts of mammals,

birds, and reptiles (Sommerville 1982a; Umadevi and Madhavi 2006; Díaz et al. 2008). First intermediate hosts reported for *H. pumilio* are the thiarid snails *Melanoides tuberculata* (Müller, 1774) and *Tarebia granifera* (Lamarck, 1822) (Umadevi and Madhavi 2006; Tolley-Jordan and Owen 2008). *Haplorchis pumilio* metacercariae parasitize a broad range of second intermediate fish hosts, where they are found encysted in the soft tissue of the fin insertions, and the cartilage of the head (Sommerville 1982b; Lo and Lee 1996b). Invasion by *H. pumilio* cercariae can have severe pathological consequences for fish hosts. Cercariae penetrate through the epidermis and migrate to various parts of the body to encyst. Hemorrhaging in the skeletal muscles has been reported in

association with both the penetration and migration of cercariae (Sommerville 1982b; Umadevi and Madhavi 2006), and simultaneous penetration by a large number of cercariae can be lethal to fry and adults of multiple fish species (Sommerville 1982b; Umadevi and Madhavi 2006).

Melanoides tuberculata is thought to have been introduced to the USA through the aquarium trade sometime prior to 1950 (Murray 1971), and *Tarebia granifera* was introduced to the USA as early as 1935 (Nollen and Murray 1978). Populations of both snail species were first reported in Texas by Murray (1964), when the snails were found in the headsprings of the San Antonio River (Bexar County). Both species were later reported in the spring-fed Comal River, Comal County, Texas (Murray and Wopschall 1965). Since that time, both snail species have been reported in multiple spring-fed systems throughout Texas (Karatayev et al. 2009), and the USA (Tolley-Jordan and Chadwick 2012; Benson and Nielson 2013; USGS 2013).

Cercariae of an unidentified *Haplorchis* species were reported from exotic snails as early as 1999 in West Texas springs by McDermott (2000), and a report from Utah also included the discovery of *Haplorchis* sp cercariae from *M. tuberculata* (Harvey et al. 2005). Furthermore, *H. pumilio* has been found in snails in the Comal River in New Braunfels, Texas (Tolley-Jordan and Owen 2008), and cercariae have been collected from the water column using filtration techniques (Johnson et al. 2012; Cantu et al. 2013). It is possible that *H. pumilio* has become established in many of the aquatic systems in the USA that support reproducing populations of *M. tuberculata* and *T. granifera*.

While there are no reports of fishes from Texas infected with *H. pumilio*, it is not known how many native fishes are being exposed daily to cercariae of *H. pumilio*. Therefore, it was decided to expose a common cyprinid *Cyprinella venusta* (Girard, 1856) to *H. pumilio* cercariae in order to determine its susceptibility to *H. pumilio* cercariae, and to acquire metacercariae for comparison with wild-caught fishes. After 15 minutes in a 38 L aquarium with approximately 100 wild caught infected *M. tuberculata*, the four experimental fish were transferred to a cercariae-free aquarium for observation. On day two post-exposure, all four fish had developed large red blisters on opposite sides of the caudal peduncle, and by day 3 post-exposure, the blisters had ruptured and the fish had died (Figure 1).

Because of the mortalities and pathologies resulting from *Haplorchis pumilio* infections in fish hosts (Sommerville 1982b), five U.S. Federally Endangered Species Act-listed fish species were examined from areas where *H. pumilio* cercariae were observed in order to determine if *H. pumilio* is infecting these species. Fresh-caught specimens of fountain darters *Etheostoma fonticola* (Jordan and Gilbert, 1886), a species listed as endangered (IUCN 2011a), as well as archived specimens of largemouth bass *Micropterus salmoides* (Lacepède, 1802) and several West Texas fishes of concern: the Rio Grande darter *Etheostoma grahami* (Girard, 1859) listed as vulnerable (IUCN 2012a); the Devils River minnow *Dionda diaboli* (Hubbs and Brown, 1957) listed as endangered (IUCN 2011b), the Pecos gambusia *Gambusia nobilis* (Baird and Girard, 1853) listed as endangered (IUCN 2012b), and the Comanche Springs pupfish *Cyprinodon elegans* (Baird and Girard, 1853) that is listed as endangered (IUCN 2011c) were examined.

Methods

On 9 December 2013, five fountain darters (2.6–3.9 cm, TL) were collected from both the San Marcos (Hays County, 29°52'43.56"N, 97°55'57.36"W) and Comal Rivers (Comal County, 29°42'25.04"N, 98°07'20.48"W) ($n=10$). Fish were transported to the San Marcos Aquatic Resource Center (SMARC) in San Marcos, Texas. Fountain darters were euthanized with tricaine methane-sulfonate (FINQUEL MS-222®; Argent Chemical Laboratories Inc., Redmond, Washington), and preserved in 10% formalin (Hexion Specialty Chemicals INC. Springfield, Oregon).

Archived specimens examined from West Texas spring systems (the Devils River, Val Verde County, 29°54'04"N, 100°59'58"W; San Felipe Springs, Val Verde County, 29°22'25"N, 100°53'06"W; Phantom Lake Springs, Jeff Davis County, 30°56'06"N, 103°50'59"W; and San Solomon Springs, Reeves County, 30°56'39"N, 103°47'16"W) had been preserved and retained at the SMARC following a *Centrocestus formosanus* (Nishigori, 1924) study conducted in 2011 by McDermott et al. (in press). Five individuals each of *D. diaboli* (3.4–4.1 cm, TL), *G. nobilis* (3.6–4.8 cm, TL), *E. grahami* (3.8–4.6 cm, TL), and *Micropterus salmoides* (6.8–9.1 cm, TL) from the Devils River; five individuals each of *D. diaboli* (3.8–5.0 cm, TL), *E. grahami* (2.6–4.7 cm, TL) and *M. salmoides* (5.8–8.6 cm, TL) from San Felipe Springs; five

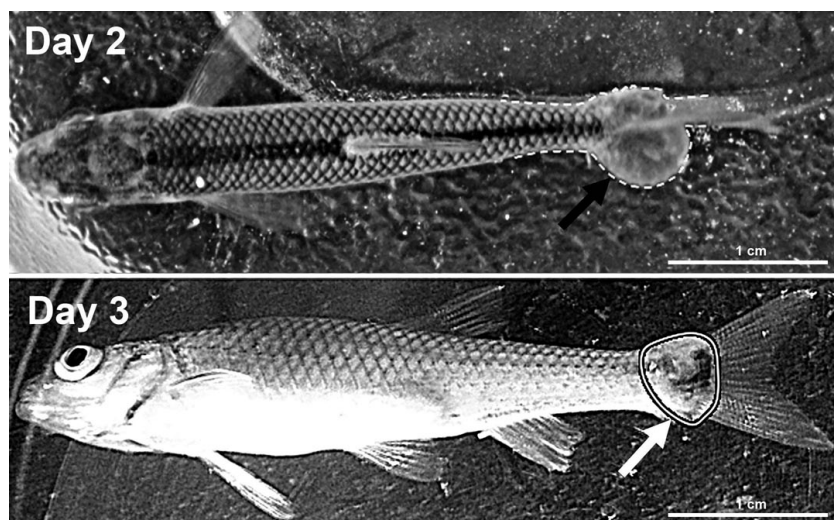


Figure 1. *Cyprinella venusta* showing blister on caudal peduncle (Day 2) that ruptured into a fatal ulcer (Day 3) following artificial exposure to a large number of *Haploorchis pumilio* cercariae for 15 minutes. Photograph by David G. Huffman.

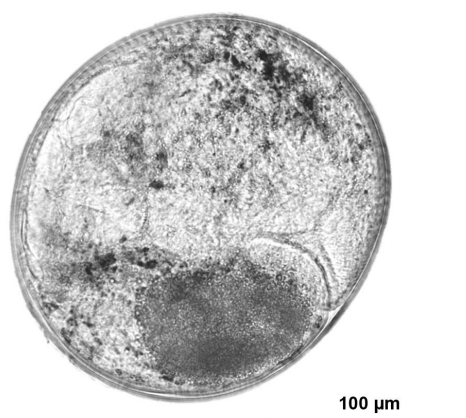


Figure 2. Encysted metacercaria of *Haplorchis pumilio* from a fountain darter. Photograph by Daniel C. Huston.

individuals each of *Cyprinodon elegans* (3.0–3.8 cm, TL) and *G. nobilis* (3.5–4.5 cm, TL) from Phantom Lake Springs; and five individuals of *C. elegans* (3.4–4.0 cm, TL) from San Solomon Springs were examined given the effect of *H. pumilio* on *Cyprinella venusta*.

Tissues around the fin insertions, skin, flesh, mouth, head, and internal organs were examined for metacercariae from fountain darters and archived specimens. All examined parts of fish were placed in petri dishes and observed with a dissecting microscope at 5–35X. The caudal peduncle of each fish was removed from the body approximately four vertebrae from the termination of the spinal column, and then divided in half sagittally. Anal,

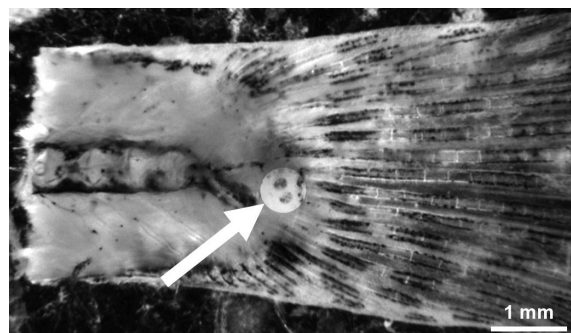


Figure 3. Lateral view of a mid-sagittal section of the caudal peduncle of a naturally infected fountain darter revealing two *Haplorchis pumilio* metacercariae (in highlighted circle at arrow). Photograph by Daniel C. Huston.

pelvic, pectoral, and dorsal fins were removed intact by cutting around the tissues of the fin insertions. In addition, internal organs, skin and muscles were removed. Finally, mandible, maxillary and isthmus tissues were removed from the head and divided into sections. Since no *H. pumilio* metacercariae had been found in the gills of the cyprinids that were artificially exposed to heavy cercarial concentrations, the gills of the additional fish in this study were not examined for *H. pumilio* infection. All of the excised tissues were then teased apart with dissecting needles, and examined for metacercariae. *Haplorchis pumilio* metacercariae were identified using a combination of descriptions provided by Scholz et al. (2001), Umadevi and Madhavi (2006), and Díaz et al. (2008) and by comparisons with metacercariae resulting from artificial infections.

Results

Haplorchis pumilio metacercariae, often with dark oval shaped excretory bladders (Figure 2), were found encysted sub-dermally in the cartilage of the head, and in tissues of the fin insertions (especially the caudal fin, Figure 3). All fountain darters from the San Marcos River, as well as those from the Comal River, were infected with *H. pumilio*. Metacercarial intensities of *H. pumilio* (min-max, mean) in the San Marcos and Comal Rivers were (3–26, 15.2) and (6–34, 15.4), respectively. In fountain darters *H. pumilio* metacercariae were found primarily in the tissue surrounding the caudal fin insertion (49%) followed by the head (26%), and the pelvic (13%), dorsal (5%), pectoral (4%) and anal (3%) fin insertions. No *H. pumilio* metacercariae were found in the skin, skeletal muscles, or internal organs of the fountain darters. *Haplorchis pumilio* infection was low in the Devils River, where one *E. grahami* and one *D. diaboli* had a single *H. pumilio* metacercaria each. No *H. pumilio* metacercariae were found in the remaining 18 fish examined from this system. At San Felipe Springs, *Dionda diaboli* had the highest *H. pumilio* metacercarial intensities (18–56, 27.6) followed by *M. salmoides* (0–20, 6.8) and *E. grahami* (0–10, 2.2). At Phantom Lake, *Gambusia nobilis* had low metacercarial intensities (0–16, 6), and no *H. pumilio* metacercariae were found in *Cyprinodon elegans* from this site, while no *H. pumilio* metacercariae were found in *C. elegans* collected from San Solomon Springs.

Discussion

Though the parthenitae of *H. pumilio* have been known to occur in Texas waters for over a decade, the unusual location of metacercarial encystment is likely the reason Texas fish have yet to be reported as infected. This is concerning because fish populations have likely hosted *H. pumilio* for many years while the implications for the health of wild fisheries and humans have not been recognized in the USA. Natural infection intensities of *Haplorchis pumilio* observed in this study were low; however, the exotic gill trematode *Centrocestus formosanus* co-occurred in all locations with *H. pumilio* (McDermott et al. in press). Accession numbers of the archival specimens matched to the data from McDermott et al. (in press) showed that approximately 50%

of the fish infected with *H. pumilio* had previously been found to have gills infected with *C. formosanus*. Episodes of mortality attributed to either *H. pumilio* or *C. formosanus* have been reported in high-density fish farms (Sommerville 1982a,b; Blazer and Gratzek 1985; Mohan et al. 1999; Ortega et al. 2009). However, there is still a paucity of data regarding the effects of these exotic trematodes on endemic fishes in natural systems. It has been suggested that *C. formosanus* can be lethal to fountain darters at metacercarial intensities above 800 in the wild (Mitchell et al. 2000), and moderate to heavy concurrent infection with *H. pumilio* could exacerbate the degenerative effects of *C. formosanus* infections, increasing the likelihood of parasite-induced morbidity, heightened predation risk, and mortality. Furthermore, infection with these parasites could intensify in periods of reduced spring discharge. Reduced spring discharge is considered to be one of the greatest threats to Texas spring endemics (Bowles and Arsuffi 1993; USFWS 1996). The combination of these exotic trematodes may present a serious threat to fountain darters, as well as the other species in this study, during drought conditions. Commercially important sport fish species such as bass and catfish could also be adversely affected by this trematode, and the location of encysted metacercariae in fish hosts provides this parasite with a more likely route of transmission to humans than *C. formosanus*, that encysts almost exclusively in the gills (Mitchell et al. 2000, 2005). *Haplorchis pumilio* has been recorded in humans in Asia (Radomyos et al. 1998; Chung et al. 2011), as humans can ingest these tiny flesh-colored metacercariae when consuming raw or lightly cooked fish (Díaz et al. 2008). Metacercariae encysted in fish can also remain viable when refrigerated for 5–8 days at 4°C (Sommerville 1982b). Currently, there is little information regarding the pathology of *H. pumilio* in humans (Kay et al. 2009), although there have been reports attributing ulcerations and gastrointestinal disturbance to heavy intensities of *Haplorchis* spp. (Chung et al. 2011).

This study is the first report of *Haplorchis pumilio* in fishes of North America north of Mexico, and lists five new spring-influenced localities in Texas for the metacercariae. This study also lists five native North American fishes as new host records for *H. pumilio*: *Dionda diaboli*, *Etheostoma fonticola*, *E. grahami*, *Gambusia nobilis*, and *Micropterus salmoides*.

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References

- Benson C, Neilson ME (2013) USGS - Nonindigenous Aquatic Species Database - *Melanoides tuberculatus*. <http://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=1037> (Accessed 31 March 2014)
- Blazer VS, Gratzek JB (1985) Cartilage proliferation in response to metacercarial infections in fish gills. *Journal of Comparative Pathology* 95: 273–280, [http://dx.doi.org/10.1016/0021-9975\(85\)90013-1](http://dx.doi.org/10.1016/0021-9975(85)90013-1)
- Bowles DE, Arsuffi TL (1993) Karst aquatic ecosystems of the Edwards Plateau regions of Central Texas, USA: a consideration of their importance, threats to their existence, and efforts for their conservation. *Aquatic Conservation: Marine and Freshwater Ecosystems* 3: 317–329, <http://dx.doi.org/10.1002/aqc.3270030406>
- Cantu V, Brandt TM, Arsuffi TL (2013) An evaluation of three sampling methods to monitor a digenetic trematode *Centrocestus formosanus* in a spring fed ecosystem. *Parasitology* 140: 814–820, <http://dx.doi.org/10.1017/S0031182013000085>
- Chai JY, De NV, Sohn WM (2012) Foodborne trematode metacercariae in fish from northern Vietnam and their adults recovered from experimental hamsters. *Korean Journal of Parasitology* 50: 317–325, <http://dx.doi.org/10.3347/kjp.2012.50.4.317>
- Chen HT (1936) A study of the Haplorchinae (Looss 1899) Poche 1926 (Trematoda: Heterophyidae). *Parasitology* 28: 40–55, <http://dx.doi.org/10.1017/S003118200002223X>
- Chung OS, Lee HJ, Kim YM, Sohn WM, Kwak SJ, Seo M (2011) First report of human infection with *Gynaecotyla squatarolae* and first Korean record of *Haplorchis pumilio* in a patient. *Parasitology International* 60: 227–229, <http://dx.doi.org/10.1016/j.parint.2010.11.003>
- Diaz MT, Hernandez LE, Bashirullah AK (2008) Studies on the life cycle of *Haplorchis pumilio* (Looss, 1896) (Trematoda: Heterophyidae) in Venezuela. *Revista Científica* 18: 35–42
- Harvey M, Wagner E, Wilson C (2005) Final Report to the Utah Reclamation Mitigation and Conservation Commission, November 2005, 23 pp
- IUCN (2011a) *Etheostoma fonticola* (Jordan and Gilbert, 1886); fountain darter. <http://www.iucnredlist.org/details/full/8114/0> (Accessed 31 March 2014)
- IUCN (2011b) *Dionda diaboli* (Hubbs and Brown, 1957); Devils River minnow. <http://www.iucnredlist.org/details/6623/0> (Accessed 31 March 2014)
- IUCN (2011c) *Cyprinodon elegans* (Baird and Girard, 1853); Comanche Springs pupfish. <http://www.iucnredlist.org/details/6150/0> (Accessed 31 March 2014)
- IUCN (2012a) *Etheostoma graham* (Girard, 1859); Rio Grande darter. <http://www.iucnredlist.org/details/8115/0> (Accessed 31 March 2014)
- IUCN (2012b) *Gambusia nobilis* (Baird and Girard, 1853); Pecos gambusia. <http://www.iucnredlist.org/details/8895/0> (Accessed 31 March 2014)
- Johnson MS, Bolick A, Alexander M, Huffman DG, Oborny E, Monroe A (2012) Fluctuations in densities of the invasive gill parasite *Centrocestus formosanus* (Trematoda: Heterophyidae) in the Comal River, Comal County, Texas, USA. *Journal of Parasitology* 98: 111–116, <http://dx.doi.org/10.1645/GE-2841.1>
- Karatayev AY, Burlakova LE, Karatayev VA, Padilla DK (2009) Introduction, distribution, spread, and impacts of exotic freshwater gastropods in Texas. *Hydrobiologia* 619: 181–194, <http://dx.doi.org/10.1007/s10750-008-9639-y>
- Kay H, Murrell KD, Hansen AK, Madsen H, Trang NT, Hung NM, Dalsgaard A (2009) Optimization of an experimental model for the recovery of adult *Haplorchis pumilio* (Heterophyidae: Digenea). *Journal of Parasitology* 95: 629–633, <http://dx.doi.org/10.1645/GE-1785.1>
- Krailas D, Namchote S, Rattanathai P (2011) Human intestinal flukes *Haplorchis taichui* and *Haplorchis pumilio* in their intermediate hosts, freshwater snails of this families Thiaridae and Pachychilidae, in southern Thailand. *Zoosystematics and Evolution* 87: 349–360, <http://dx.doi.org/10.1002/zoos.201100012>
- Lo CT, Lee KM (1996a) Pattern of emergence and the effects of temperature and light on the emergence and survival of heterophyid cercariae (*Centrocestus formosanus* and *Haplorchis pumilio*). *Journal of Parasitology* 95: 629–633
- Lo CT, Lee KM (1996b) Infectivity of the cercariae of *Centrocestus formosanus* and *Haplorchis pumilio* (Digenea: Heterophyidae) in *Cyprinus carpio*. *Zoological Studies* 35: 305–309
- McDermott KS (2000) Distribution and infection relationships of an undescribed digenetic trematode, its exotic intermediate host, and endangered fish in the springs of west Texas. Master's Thesis, Southwest Texas State University, San Marcos, 46 pp
- McDermott KS, Arsuffi TL, Brandt TM, Huston DC, Ostrand KG (in press) Exotic digenetic trematode (*Centrocestus formosanus*) distribution and occurrence, its exotic snail intermediate host (*Melanoides tuberculatus*), and fish infection rates in West Texas spring systems. *The Southwestern Naturalist*
- Mitchell AJ, Salmon MJ, Huffman DG, Goodwin AE, Brandt TM (2000) Prevalence and pathogenicity of a Heterophyid trematode infecting the gills of an endangered fish, the fountain darter, in two central Texas spring-fed rivers. *Journal of Aquatic Animal Health* 12: 283–289, [http://dx.doi.org/10.1577/1548-8667\(2000\)012<0283:PAPOAH>2.0.CO;2](http://dx.doi.org/10.1577/1548-8667(2000)012<0283:PAPOAH>2.0.CO;2)
- Mitchell AJ, Overstreet RM, Goodwin AE, Brandt TM (2005) Spread of an exotic fish-gill trematode: a far-reaching and complex problem. *Fisheries* 30: 11–16, [http://dx.doi.org/10.1577/1548-8446\(2005\)30\[11:SOAFT\]2.0.CO;2](http://dx.doi.org/10.1577/1548-8446(2005)30[11:SOAFT]2.0.CO;2)
- Mohan CV, Shanker KM, Ramesh KS (1999) Mortalities of juvenile common carp, *Cyprinus carpio* associated with larval trematode infection-a case study. *Journal of Aquaculture in the Tropics* 14: 137–142
- Murray HD (1964) *Tarebia granifera* and *Melanoides tuberculata* in Texas. *Annual Report of the American Malacological Union* 53: 15–16
- Murray HD, Woopschall LJ (1965) Ecology of *Melanoides tuberculata* (Müller) and *Tarebia granifera* (Lamarck). *Bulletin of the American Malacological Union* 32: 25–26
- Murray HD (1971) The introduction and spread of thiarids in the United States. *Biologist* 53: 133–135
- Nollen PM, Murray HD (1978) *Philophthalmus gralli*: identification, growth characteristics, and treatment of an oriental eyefluke introduced into the continental United States. *Journal of Parasitology* 64: 178–180, <http://dx.doi.org/10.2307/3279646>
- Ortega C, Fajardo R, Enriquez R (2009) Trematode *Centrocestus formosanus* infection and distribution in ornamental fishes in Mexico. *Journal of Aquatic Animal Health* 21: 18–22, <http://dx.doi.org/10.1577/H07-022.1>

- Pearson JC (1964) A revision of the subfamily *Haplorchinae* Looss 1899 (Trematoda: Heterophyidae). I. The *Haplorchis* group. *Parasitology* 54: 601–676, <http://dx.doi.org/10.1017/S003118200008269X>
- Pearson JC, Ow-Yang CK (1982) New species of *Haplorchis* from Southeast Asia, together with keys to the *Haplorchis*-group of heterophyid trematodes of the region. *Southeast Asian Journal of Tropical Medicine and Public Health* 13: 35–40
- Radomyos B, Wongsaroj T, Wilairatana P, Radomyos P, Praevanich R, Meesomboon V, Jogsuksuntikul P (1998) Opisthorchiasis and intestinal fluke infections in northern Thailand. *Southeast Asian Journal of Tropical Medicine and Public Health* 29: 123–127
- Scholz T, Aguirre-Macedo ML, Salgado-Maldonado G (2001) Trematodes of the family Heterophyidae (Digenea) in Mexico: a review of species and new host and geographical records. *Journal of Natural History* 35: 1733–1772, <http://dx.doi.org/10.1080/00222930152667087>
- Shen NX (1959) Notes on the morphology and life history of *Haplorchis pumilio* (Trematoda: Heterophyidae). *Acta Zoologica Sinica* 11: 470–481
- Sommerville C (1982a) The life history of *Haplorchis pumilio* (Looss, 1896) from cultured tilapias. *Journal of Fish Diseases* 5: 233–241, <http://dx.doi.org/10.1111/j.1365-2761.1982.tb00478.x>
- Sommerville C (1982b) The pathology of *Haplorchis pumilio* (Looss, 1896) infections in cultured tilapias. *Journal of Fish Diseases* 5: 243–250, <http://dx.doi.org/10.1111/j.1365-2761.1982.tb00479.x>
- Tolley-Jordan LR, Owen JM (2008) Habitat influences snail community structure and trematode infection levels in a spring-fed river, Texas, USA. *Hydrobiologia* 600: 29–40, <http://dx.doi.org/10.1007/s10750-007-9173-3>
- Tolley-Jordan LR, Chadwick MA (2012) *Centrocestus formosanus* Nishigori (Asian gill-trematode). In: Francis RA (ed), *A Handbook of Global Freshwater Invasive Species*. Earthscan, New York NY, USA, pp 401–419
- Umadevi K, Madhavi R (2006) The life cycle of *Haplorchis pumilio* (Trematoda: Heterophyidae) from the Indian region. *Journal of Helminthology* 80: 327–332, <http://dx.doi.org/10.1017/JOH2006359>
- United States Fish and Wildlife Service (USFWS) (1996) San Marcos/Comal Springs and associated aquatic ecosystems (revised) recovery plan. U.S. Fish and Wildlife Service, Albuquerque, New Mexico, 134 pp
- United States Geological Survey (USGS) (2013)-USGS-Nonindigenous Aquatic Species Database-*Tarebia granifera*. <http://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=1039> (Accessed 31 March 2014)
- Witenberg G (1929) Studies on the trematode family Heterophyidae. *Annals of Tropical Medicine and Parasitology* 2: 131–240