Freshwater snails (Mollusca: Gastropoda) from the Commonwealth of Dominica with a discussion of their roles in the transmission of parasites

Will K. Reeves¹, Robert T. Dillon, Jr.², and Gregory A. Dasch³

¹ USDA Agricultural Research Service, Arthropod-Borne Diseases Research Laboratory, Agriculture Building, Room 5031, Department 3354, 1000 E University Avenue, Laramie, Wyoming 82071-2000, U.S.A., wreeves@alumni.clemson.edu

² Department of Biology, College of Charleston, Charleston, South Carolina 29424, U.S.A., dillonr@cofc.edu

³ Centers for Disease Control and Prevention, 1600 Clifton Road NE, MS G-13, Atlanta, Georgia 30333, U.S.A.

Abstract: We collected six species of freshwater snails from Dominica, including *Biomphalaria kuhniana* (Clessin, 1883), *Gundlachia radiata* (Guilding, 1828), *Helisoma* (=*Planorbella*) trivolvis (Say, 1817), *Melanoides tuberculata* (Müller, 1774), *Neritina punctulata* Lamarck, 1816, and *Physa marmorata* Guilding, 1828. Our collections indicate that un-reported species such as *G. radiata* and *H. trivolvis* are established on Dominica, West Indies. We tested a limited number of *M. tuberculata* for rickettsial pathogens, *Neorickettsia* spp., but did not identify this agent. Three species of snails previously reported from Dominica, *Biomphalaria glabrata* (Say, 1818), *Biomphalaria straminea* (Dunker, 1848), and *Thiara granifera* (Lamarck, 1822), were not collected. Our data suggest that *B. glabrata* has not re-emerged as a prominent component of the freshwater snail fauna since it disappeared or was locally eradicated. In addition, previous reports of *B. straminea* were probably misidentifications of *B. kuhniana*, and some abnormally large specimens of *M. tuberculata* from Freshwater Lake could be misidentified as *T. granifera*. Our sampling was not adequate to demonstrate that *T. granifera* was absent from Dominica. We determined that *B. kuhniana* was not eradicated by previous molluscan control regimes. Additional studies on the relationships of freshwater snails in Dominica to helminths of animals and humans are needed to understand the public and veterinary health significance of these snails.

Key words: Biomphalaria, Gundlachia, Helisoma, Physa, West Indies

The Commonwealth of Dominica is a small (790 km^2) mountainous island nation in the West Indies that receives over 900 cm of rain per year (Grell 1976). The freshwater snail fauna of Dominica has been studied in regard to its significance in the transmission of schistosomiasis (e.g., Noblet and Damian 1991), but the snail fauna has been largely ignored in other regards. Freshwater snails are the primary intermediate hosts for most trematodes, some nematodes, and some rickettsial pathogens (Neorickettsia spp.). There have been no reports of autochthonous schistosomiasis on Dominica, but visitors and immigrants harboring the worm have been documented (Grell 1976, Prentice 1980, Grell et al. 1981, Noblet and Damian 1991, Adedayo and Nasiiro 2004). There remains a potential for transmission and establishment of schistosomiasis as long as susceptible populations of Biomphalaria Preston, 1910 are established on the island. Biomphalaria glabrata (Say, 1818) was reported on Dominica (Prentice 1980) but more recent surveys (Noblet and Damian 1991) indicate that this snail was replaced by Biomphalaria straminea (Dunker, 1848). In addition, two molluscan intermediate hosts of the trematode Paragonimus westermani were introduced on Dominica (Noblet and Damian 1991). The potential to establish this trematode is relatively small because it is not established on neighboring islands. Prosobranch molluscs are the intermediate hosts for trematodes that transmit Neorickettsia spp. to humans and

domestic animals throughout the Americas (Pusterla *et al.* 2000, Headley *et al.* 2004). *Neorickettsia* spp. have not been reported from Dominica. We conducted a survey of freshwater ponds, lakes, and rivers to determine the distribution of freshwater snails on Dominica. We tested selected *Melanoides tuberculata* (Müller, 1774) for the presence of *Neorickettsia* by PCR amplification of the 16S rRNA gene of *Neorickettsia*.

MATERIALS AND METHODS

Snails were collected (sites listed in Table 1) by removing them from vegetation and mud using nets and sieves or by snorkeling in streams and removing them from rocks. All specimens were preserved in 99% ethanol, which was changed completely after 24 hours.

All snails were identified with morphological characters. DNA was extracted from individual specimens in the genus *Biomphalaria* and 9 *Melanoides tuberculata* from each collection locality. The DNA extraction, PCR, PCR cleanup, and sequencing followed the techniques described by Reeves *et al.* (2006) with the following modifications. We extracted DNA from individual snails and amplified the internal transcribed spacer 2 (ITS-2) and a portion of the 28S rRNA gene from each specimen of *Biomphalaria*, using the primers de-

| Collection site | Habitat type | Collection date | Species collected |
|---|-------------------------|-----------------|--|
| Springfield Estate, Parish of Sainte Paul | Pond and outflow stream | 12-17 May 2005 | Biomphalaria kuhniana Melanoides tuberculata Neritinia punctulata Physa marmorata |
| Roseau Botanic Garden, Roseau, Parish of Sainte George | Artificial pond | 13 May 2005 | B. kuhniana Helisoma trivolvis M. tuberculata P. marmorata |
| Roseau, open sewer drains, Parish of Sainte George | Standing water | 13 May 2005 | B. kuhniana P. marmorata |
| Roseau River, Roseau, Parish of Sainte George | River | 13 May 2005 | N. punctulata |
| Clark Hall River, Parish of Saint Paul | River | 14 May 2005 | Gundlachia radiata M. tuberculata N. punctulata |
| Freshwater Lake, Parish of Saint George | Lake | 15 May 2005 | B. kuhniana M. tuberculata P. marmorata |
| Miranda's Pond, Parish of Saint George | Pond | 16 May 2005 | B. kuhniana N. punctulata |
| Cochran, unnamed pond, Parish of Sainte Paul | Pond | 16 May 2005 | G. radiata |
| Middleham Falls, Parish of Sainte Paul | River | 16 May 2005 | N. punctulata |

Table 1. Collection data and snail species identified from sites in Dominica, West Indies in 2005.

scribed by Caldeira *et al.* (2004). DNA extracts from *M. tuberculata* were screened for DNA of *Neorickettsia* by PCR using the EHR16SD and EHR16SR PCR primers to amplify the 16S rRNA gene of *Neorickettsia* as described by Inokuma *et al.* (2000). Positive controls with DNA from *Helisoma trivolvis* (Say, 1817) or *Wolbachia* sp. and a negative control with distilled water were used. We used positive controls that could be amplified by PCR but represented organisms other than those examined in our study.

Voucher specimens of snails were deposited in the Academy of Natural Sciences of Philadelphia. DNA sequences for the ITS-2 and a portion of the 28S rRNA gene of *Biomphalaria kuhniana* (Clessin, 1883) (GenBank Accession #DQ111952) were deposited in GenBank.

RESULTS AND DISCUSSION

We did not collect *Biomphalaria glabrata* or *Biomphalaria straminea* but did collect *Biomphalaria kuhniana* from isolated ponds and Freshwater Lake (Table 1). The DNA sequences for the ITS-2 and a portion of the 28S rRNA gene from our specimens were 100% identical to those of *B. kuhniana* (GenBank #s AY030380, AY030378, AY030379) from Columbia, Dominica, and Venezuela. *Biomphalaria kuhni*

ana was described from a "Chinese well" in Panama and is morphologically similar to B. straminea (Paraense 2003). The two species can be separated with molecular and morphological characters. Biomphalaria kuhniana is not a competent intermediate host for Schistosoma mansoni (Paraense 2003), which could explain why schistosomiasis has not become established on Dominica even though Biomphalaria and occasional transient human infections with S. mansoni have been reported in Dominica. Noblet and Damian (1991) reported populations of B. straminea in artificial ponds on the island but not from Freshwater Lake. However, we suggest that the previous reports of B. straminea were misidentifications of B. kuhniana, which was not included in the key by Malek (1985), used to diagnose snails in the previous surveys. In addition we collected B. kuhniana in Freshwater Lake, which is a new locality for this snail. DeJong et al. (2001) had reported a population of B. kuhniana from Roseau, Dominica. Biomphalaria kuhniana is not known to serve as the intermediate host of trematodes parasitic to humans or domestic animals; however, little research has focused on the possibility that this snail is a host to helminths other than S. mansoni. Other species of Biomphalaria are intermediate hosts to echinostomatid trematodes and nematodes in the genus Angiostrongylus (Malek 1980).

We collected ancylid limpets in both streams and ponds.

All limpets were morphologically identified as *Gundlachia radiata* (Guilding, 1828), which has not been previously reported from Dominica, but is known from neighboring islands (Starmuhler 1984, Malek 1986). *Gundlachia radiata* is not considered an intermediate host to trematodes of medical significance. It does harbor anisakid nematodes, which are parasitic to fish and some fish-eating mammals (Thiengo *et al.* 2000). Ancylidae are small and often go unnoticed by collectors. These limpets might play important roles in the natural cycles of helminths in Dominica but are currently unstudied.

Helisoma trivolvis (Say, 1817), a planorbid snail that could be mistaken for Biomphalaria by untrained collectors, was collected in the metal ponds at the Roseau Botanic Garden. Helisoma trivolvis is established in the Dominican Republic and possibly Haiti and Cuba (Ayvazian and Mallett 1986, Paraense 2003). A congeneric species, Helisoma duryi (Wetherby, 1879) is also established in the Caribbean (e.g., Pointier 2001). Helisoma trivolvis is naturally resistant to infection by Schistosoma mansoni (Ayvazian and Mallett 1986), but this snail is an intermediate host to clinostomatid, cyclocoeliid, echinostomatid, and strigeid trematodes and is used as a laboratory host to nematodes in the genus Angiostrongylus (Malek 1980, Ponder and Fried 2004). Humans and domestic animals can be infected by some of these worms, including Alaria canis, which can cause fatal infections in humans (Malek 1980). As with other zoonotic trematodes, infections of humans are accidental and usually involve eating uncooked meat harboring metacercariae.

We did not collect Thiara granifera (Lamarck, 1822), but the specimens of Melanoides tuberculata from Freshwater Lake were abnormally large and were initially misidentified as T. granifera. Melanoides tuberculata was collected in both streams and ponds. We did not amplify DNA from Neorickettsia spp. in any of our collections of M. tuberculata. This exotic snail was possibly introduced to Dominica around 1975 (Pointier and McCullough 1989). Melanoides tuberculata is a potential biological control agent for Biomphalaria spp. because the two snails appear to compete, and M. tuberculata might exclude Biomphalaria spp. in some habitats (Pointier and McCullough 1989). Our data indicate that exclusion does not occur in Dominica (Table 1). Melanoides tuberculata is not a suitable host for Schistosoma mansoni but is an intermediate host to Paragonimus westermani, a lung fluke. There is a possibility that *P. westermani* or other Paragonimus spp. will become established on Dominica, because both M. tuberculata and the freshwater-crab, secondintermediate hosts of P. westermani, are present (Noblet and Damian 1991). Carnivorous mammals are natural hosts for this fluke so zoonotic cycles of the parasite could become established without human infections. *Melanoides tuberculata* can serve as the intermediate host to other trematodes that occasionally infect humans, including eye flukes (*Philophthalmus* spp.) of birds (Dimitrov *et al.* 2000, Lamothe-Argumendo *et al.* 2003). *Melanoides tuberculata* is also an intermediate host to *Heterophyes heterophyes*, a fluke of fish-eating mammals and birds (Malek 1980). *Heterophyes heterophyes* can infect humans.

Physa marmorata Guilding, 1828 was collected from ponds and water tanks with freshwater plants. Physid snails are often overlooked as intermediate hosts of helminths, but P. marmorata is the intermediate host for the echinostomatid trematodes, Echinostoma luisrevi and Echinostoma paraensei (Maldonado et al. 2001, 2003). Physa spp. are intermediate hosts for diplostomatid, echinostomatid, and strigeid trematodes and are laboratory hosts for nematodes in the genus Angiostrongylus (Malek 1980). In addition, Physa spp. serve as hosts to nematomorph worms and the oligochaete Chaetogaster sp., which are parasites of invertebrates (Gamble and Fried 1976, Hanelt et al. 2001). The public health or veterinary significance of populations of P. marmorata on Dominica are unknown, but further studies could prove this snail a host to helminths of economic significance. Noblet and Damian (1991) reported collecting Physa cubensis Pfeiffer, 1839 in Dominica. Physa cubensis is a junior synonym of Physa acuta Draparnaud, 1805 (Paraense and Pointier 2003). However, we collected P. marmorata in the same habitats and localities that Noblet and Damian (1991) reported P. acuta. These older reports of P. acuta thus probably represent misidentifications of P. marmorata.

The neritid snail *Neritina punctulata* Lamarck, 1816 was collected from both streams and fish ponds. *Neritina punctulata* occurs in streams throughout Dominica (Starmuhlner 1984) and breeds in streams with eggs attached to boulders. *Neritina punctulata* is possibly the largest freshwater snail on Dominica and is not known to harbor parasites of humans or domestic animals. However, this snail is eaten by humans on Dominica. As long as the snails are adequately cooked, they would pose no threat even if they were intermediate hosts to helminths or other infectious agents. A detailed study of the potential helminths or other infectious agents pathogenic to humans in *N. punctulata* will thus have public health implications.

Exotic snails continue to be introduced into Dominica. There is at least one tropical fish store on the island, and tropical ampullarids, physids, and planorbids are sold by the tropical fish industry. Freshwater plants such as water lettuce (*Pistia stratiotes*) are transported from one Caribbean Island to another by travelers and are used as ornamental plants in local water gardens or fishponds. Snails such as *Biomphalaria* spp. can be transported on these plants.

ACKNOWLEDGMENTS

We thank J. Andre for issuing collection and research permits, P. H. Adler, R. W. Blob, J. A. Korecki, B. A. Powell, P. D. McMillan, A. G. Wheeler, Jr., and N. G. L. Osler for logistical and collecting assistance in Dominica, and the American Society for Microbiology for partially supporting this research. The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the funding agency.

LITERATURE CITED

- Adedayo, O. and R. Nasiiro 2004. Intestinal Parasitoses. *Journal of the National Medical Association* **96**: 93-96.
- Ayvazian, S. G. and J. C. Mallett. 1986. Resistance of *Helisoma tri*volvis from the Dominican Republic to infection by the trematode *Schistosoma mansoni*. Journal of Parasitology **72**: 785-786.
- Caldeira, R. L., L. K. Jannotti-Passos, P. M. Lira, and O. S. Carvalho. 2004. Diagnostic of *Biomphalaria* snails and *Schistosoma mansoni*: DNA obtained from traces of shell organic material. *Memorias do Instituto Oswaldo Cruz* 99: 499-502.
- DeJong, R. J., J. A. T. Morgan, W. L. Paraense, J. Pointier, M. Amarista, P. F. K. Ayeh-Kumi, A. Babiker, C. S. Barbosa, P. Bermond, A. P. Canese, C. Pereira de Souza, C. Dominguez, S. File, A. Gutierrez, R. N. Incani, T. Kawano, F. Kazibew, J. Kpikpi, N. J. S. Lwambo, L. E. Velasquez, M. Yong, C. M. Adema, B. V. Hofkin, G. M. Mkoji, and E. S. Loker. 2001. Evolutionary relationships and biogeography of *Biomphalaria* (Gastropoda: Planorbidae) with implications regarding its role as host of the human blood fluke, *Schistosoma mansoni. Molecular Biology and Evolution* 18: 2225-2239.
- Dimitrov, V., I. Kanev, M. Panaiotova, V. Radev, and D. Gold. 2000. Argentophilic structures of the miracidia and cercariae of *Philophthalmus distomatosa* n. comb. from Israel. *Journal of Parasitology* 86: 1239-1243.
- Gamble, H. P. and B. Fried. 1976. Experimental evidence for parasitism in the relationship between *Chaetogaster limnaei limnaei* (Oligochaeta) and *Physa acuta* (Gastropoda). *The Veliger* 18: 393-395.
- Grell, G. A. C. 1976. Medical disorders in a small Caribbean island. Annals of Tropical Medicine and Parasitology **70**: 1-10.
- Grell, G. A. C., P. Desai, E. Watty, R. Muller, and G. R. Serjeant. 1981. A survey of parasites in primary school children in Dominica, West Indies. *Annals of Tropical Paediatrics* 1: 155-160.
- Hanelt, B., L. E. Grother, and J. Janovy. 2001. Physid snails as sentinels of freshwater nematomorphs. *Journal of Parasitology* 87: 1049-1053.
- Headley, S. A., O. Vidotto, D. Scorpio, J. S. Dumler, and J. Mankowski. 2004. Suspected cases of *Neorickettisa*-like organisms in Brazilian dogs. *Annals of the New York Academy of Science* 1026: 79-83.
- Inokuma, H., D. Raoult, and P. Brouqu. 2000. Epidemiological survey of *Anaplasma platys* and *Ehrlichia canis* using ticks

collected from dogs in Japan. *Veterinary Parasitology* **38**: 4219-4221.

- Lamothe-Argumedo, R., S. P. Diaz-Camacho, and Y. Nawa. 2003. The first human case in Mexico of conjunctivitis caused by the avian parasite, *Philophthalmus lacrimosus. Journal of Parasitol*ogy 89: 1983-1985.
- Maldonado, A., Jr., G. O. Vieira, J. S. Garcia, L. Rey, and R. M. Lanfredi. 2001. Biological aspects of a new isolate of *Echinostoma paraensei* (Trematoda: Echinostomatidae): Susceptibility of sympatric snails and the natural vertebrate host. *Parasitology Research* 87: 853-859.
- Maldonado, A., Jr., G. O. Vieira, and R. M. Lanfredi. 2003. Echinostoma luisreyi n. sp. (Platyhelminths: Digenea) by light and scanning electron microscopy. Journal of Parasitology 89: 800-808.
- Malek, E. A. 1980. *Snail-Transmitted Parasitic Diseases*. Vol. II. CRC Press, Boca Raton, Florida.
- Malek, E. A. 1985. Snail hosts of schistosomiasis and other snailtransmitted diseases in tropical America: A manual. Scientific Publication no. 478, Pan American Health Organization, World Health Organization, Washington D.C., U.S.A., 325 pp.
- Malek, E. A. 1986. Freshwater and terrestrial snails of Saint Lucia, West Indies. *The Nautilus* **100**: 143-147.
- Noblet, G. P. and R. T. Damian. 1991. On the status of *Biomphalaria* and *Thiara* snails and the threat of schistosomiasis and paragonimiasis on Dominica, West Indies. *Journal of Medical and Applied Malacology* **3**: 1-6.
- Paraense, W. L. 2003. A bird's eye survey of Central American planorbid molluscs. *Memorias do Instituto Oswaldo Cruz* 98: 51-67.
- Paraense, W. L. and J. Pointier. 2003. Physa acuta Draparnaud, 1805 (Gastropoda: Physidae): A study of topotypic specimens. Memorias do Instituto Oswaldo Cruz 98: 513-517.
- Pointier, J. 2001. Invading freshwater snails and biological control in Martinique Island, French West Indies. *Memorias do Instituto Oswaldo Cruz* 96: 67-74.
- Pointier, J. and F. McCullough. 1989. Biological control of the snail hosts of *Schistosoma mansoni* in the Caribbean area using *Thiara* spp. *Acta Tropica* 46: 147-155.
- Ponder, E. L. and B. Fried. 2004. Effects of snail size and diet on encystment of *Echinostoma caproni* cercariae in juvenile *Helisoma trivolvis* (Colorado strain) and observations on the survival of infected snails. *Journal of Parasitology* **90**: 422-424.
- Prentice, M. A. 1980. Schistosomiasis and its intermediate hosts in the Lesser Antillean Islands of the Caribbean. *Bulletin of the Pan American Health Organization* 14: 258-268.
- Pusterla, N., E. Johnson, J. Chae, J. B. Pusterla, E. DeRock, and J. E. Madigan. 2000. Infection rate of *Ehrlichia risticii*, the agent of Potomac horse fever, in freshwater stream snails (*Juga yrekaensis*) from northern California. *Veterinary Parasitology* 92: 151-156.
- Reeves, W. K., L. A. Durden, and G. A. Dasch. 2006. A spotted fever

group *Rickettsia* from an exotic tick species, *Amblyomma exornatum* (Acari: Ixodidae), in a reptile breeding facility in the United States. *Journal of Medical Entomology* **43**: 1099-1101.

- Starmuhlner, F. 1984. Occurrence, longitudinal distribution and geographical range of the fresh- and brackish water mollusks of the Lesser Antillean Islands (Guadeloupe, Dominica and Martinique). *Sossiana* **12**: 83-102.
- Thiengo, S. C., S. B. Santos, J. J. Vicente, and R. M. Pinto. 2000. Occurrence of *Contracaecum* sp. larvae (Nematoda, Anisakidae) in *Gundlachia radiata* (Guilding, 1828) (Mollusca, Gastropoda, Ancylidae) in Brazil. *Journal of Invertebrate Pathology* 75: 178-179.

Accepted: 5 March 2007; final corrections received: 2 November 2007