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VECTOR COMPATIBILITY OF NEW MEXICO *GALBA* SPECIES WITH THE CANINE SCHISTOSOME *HETEROBILHARZIA AMERICANA*, INCLUDING THE FIRST REPORT OF *GALBA SCHIRAZENSIS* AS A COMPATIBLE HOST

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ABSTRACT

The indigenous North American mammalian schistosome *Heterobilharzia americana* has recently attracted attention for causing outbreaks in dogs in states outside of its southeastern U.S. distribution. Although *H. americana* has yet to be reported in New Mexico, we examined 2 New Mexico isolates of *Galba* snails to determine their susceptibility to experimental infection with an isolate of *H. americana* from Utah. One of the *Galba* isolates from the Rio Grande bosque in the Albuquerque suburb of Corrales was identified as *Galba humilis*, and like specimens of the same taxon from Utah, proved susceptible to *H. americana* (27.6% of exposed surviving snails positive). The second *Galba* isolate sourced from the northern mountains of New Mexico, which surprisingly was revealed to be *Galba schirazensis* based on cytochrome *c* oxidase 1, *16S* rRNA, and the internal transcribed spacer 2 markers, was also susceptible to *H. americana* (56.3% of exposed surviving field-derived snails and 46.4% first generation [F1] snails positive). This is the first report of the latter snail being a compatible snail host for *H. americana*. As *G. schirazensis* has a wide, albeit spotty, distribution and is considered an invasive species, it provides yet another opportunity for *H. americana* to expand its known range, potentially including the state of New Mexico as well.

Heterobilharzia americana is a North American schistosome species that infects wild and domestic mammals and can cause swimmer's itch in humans. It is particularly pathogenic in dogs and horses, and raccoons are thought to maintain the life cycle in nature. Paired male and female worms are found in the mesenteric veins, with females producing eggs that cross the intestinal wall and are excreted with the feces. Once in freshwater, the eggs hatch and the miracidia infect intermediate snail hosts within the family Lymnaeidae such as *Galba cubensis* and *Galba humilis,* with cercariae eventually emerging at night and infecting mammal definitive hosts (Lee, 1962; Malek, 1967; Rodriguez et al., 2014).

The distribution of *H. americana* has typically been considered to be the southeastern United States, but it has also been reported from Indiana (Rodriguez et al., 2014), Kansas (McKown et al., 1991), Utah (Loker et al., 2021), and California (Ehnert, 2023). Increases in raccoon abundance and distribution, the number of man-made ponds, movement of infected dogs, and acquisition of a new widespread intermediate host, *G. humilis*, all may contribute to an expansion in the range of *H. americana* (Kamler et al., 2003; Loker et al., 2021). Naturally infected snails are infrequently recovered and the compatibility of snails in the family Lymnaeidae remains largely unknown (Malek, 1967), particularly in western states such as New Mexico. Therefore, we sought to collect *Galba* snails from New Mexico to test their compatibility to *H. americana*.

The spotty nature of the distribution of *Galba* snails makes it uncertain that they can be readily collected when needed, but we did locate a population of *Galba* sp. (100 snails, 1–3 mm shell length) from a mountain stream, Rio Costilla, Valle Vidal, New Mexico (36°50′40.5456″, 105°22′29.5788″, elevation 2,682 m) in July 2021, and a second population (113 snails, 2–5 mm shell length) from a small man-made pond in the Rio Grande bosque, Liam Knight's Pond, Corrales, New Mexico (35°13′19.0128″, −106°37′26.8068″, elevation 1,529 m) in September/October 2021. Snails were brought to the Department of Biology at the University of New Mexico, cleaned and individually placed in 12-well cell culture plates with 2 ml of artificial spring water. They were held for 2 hr in the light and then 2 hr in the dark to determine if they would release (shed) cercariae, indicative of patent trematode infections. None shed cercariae. The snails were subsequently maintained in shallow pans, the bottoms of which were covered by mud including a raised central area where snails could climb above the water level. Snails were fed dried leaf lettuce and crushed shrimp pellets (see Loker et al., 2021 for details).

An isolate of *H. americana*, originally obtained from raccoon scats from Moab, Utah, was used for experimental snail exposures. The isolate had been cycling in the lab for 6 generations in mice and *G. humilis* originating from Moab, Utah (Loker et al., 2021). Animal use for this study was approved by the University of New Mexico Institutional Animal Care and Use Committee (IACUC 19-200,813-MC).

On 30 July 2021, 72 of the field-derived *Galba* sp. specimens from Rio Costilla were exposed individually in 12-well cell culture plates to 5 *H. americana* miracidia and on 18 November 2021, 33 F1 (first generation) snails descended from Rio Costilla stock, and 70 field-derived *Galba* sp. from Corrales were exposed individually to 5 *H. americana* miracidia. After 4.5 wk postexposure, the snails were isolated in 12-well cell culture plate wells in the dark for 2 hr to determine if the snails would shed *H. americana* cercariae. Shedding of this isolate of *H. americana* is known to occur during early evening hours (Loker et al., 2021). From the July 2021 exposure, 9/16 (56.3%) surviving *Galba* sp. from Rio Costilla shed *H. americana* cercariae. From the November 2021 exposure, 13/28 (46.4%) F1 lab-reared Rio Costilla *Galba* sp. shed cercariae, as did 16/58 (27.6%) of Corrales *Galba* sp. (Table I).

To determine the identity of the snail species, we used cytochrome *c* oxidase subunit 1 (*COI*) (Folmer et al., 1994), internal transcribed spacer 2 (*ITS2*) (Bargues et al., 2001), and *16S* rRNA (Palumbi, 1996) barcode markers to compare to other *Galba* species in GenBank. We sequenced the latter 2 markers as there is saturation of nucleotides for *COI* and potential mtDNA introgression (Caron et al., 2017); however, there is also a greater diversity of specimens in GenBank for *CO1* than for other mitochondrial markers. We sequenced 11 specimens of *Galba* sp. (Rio Costilla) including field-derived and experimentally exposed and shedding *H. americana* specimens, to check the possibility that the specimens obtained from this locality were of mixed lymnaeid species (Bargues et al., 2011). DNA extraction, polymerase chain reaction (PCR), and sequencing protocols were identical to those in Loker et al. (2021) for *COI*, and for *16S* and *ITS2* we followed the protocols of Alda et al. (2021). We sequenced 655 base pairs (bp) of the *COI* gene, 577 bp (Rio Costilla) and 612 bp (Corrales) of the *ITS2* region, and 425 bp of the *16S* rRNA gene. We ran maximum likelihood (ML) analysis of 592 bp of the *COI* gene to compare against 42 other *Galba* and lymnaeid species sequences in GenBank (Fig. 1). Sequences were aligned with CLUSTAL W and the best fit model of substitution was chosen using MEGA 11 (Tamura et al., 2021), which chose the General Time Reversible model (Nei and Kumar, 2000). A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories [+G, parameter = 0.4662]). Heuristic searches were utilized for ML analysis and 1,000 bootstrap replicates were run. The tree with the highest log likelihood (−3,863.73) is shown. We ran another ML analysis for the *16S* gene (tree not shown), to confirm placement of both Rio Costilla and Corrales *Galba* specimens. Sequences generated in this study were submitted to GenBank and our specimens and DNA were deposited as vouchers in the Museum of Southwestern Biology (Table I).

The specimens from Corrales were identified as *G. humilis* but, surprisingly, the *Galba* specimens from Rio Costilla fell into the *Galba schirazensis* clade based on intraclade *P*-distance values less than 1.5% for *COI*, *16S*, and *ITS2* (Vilas et al., 2005). The intraclade *P*-distance value for *COI* of our *G. humilis* ranged from 0.0% (Tennessee, KY612886) to 0.68% (Angel Fire, New Mexico, KY612852). The intraclade *P*-distance value for *G. schirazensis*

Table I. The habitat of each snail species from New Mexico (NM), their voucher numbers in the Museum of Southwestern Biology (MSB), and NCBI GenBank accession numbers. Each snail was exposed to 5 *Heterobilharzia americana* miracidia and shed 4.5 wk later. The snails that survived and shed cercariae are reported. F1 refers to the first-generation offspring derived from field-collected snails.

Sample name	Snail species	Habitat	Exposure to *H. americana*	Number of survivors shedding *H. americana*	Shedding *H. americana*	MSB numbers	GenBank accession numbers		
							COI	*16S*	*ITS2*
Gs1	*Galba schirazensis*	Costilla Creek, NM	72 (field-derived) and 33 (F1s)	13/28 (46.4%) and 9/16 (56.3%)	Yes	MSB:Host:24760	OR047454	OR047648	OR052215
Gs2	*Galba schirazensis*	Costilla Creek, NM			Yes	MSB:Host:24761	OR047455	OR047649	OR052216
Gs3	*Galba schirazensis*	Costilla Creek, NM			Yes	MSB:Host:25353	OR047456	OR047650	OR052217
Gs4	*Galba schirazensis*	Costilla Creek, NM			Yes	MSB:Host:25354	OR047457	OR047651	OR052218
Gs5	*Galba schirazensis*	Costilla Creek, NM			Yes	MSB:Host:25355	OR047458	OR047652	OR052219
Gs6	*Galba schirazensis*	Costilla Creek, NM			Not exposed—field derived	MSB:Host:25359	OR047459	OR047653	OR052220
Gs7	*Galba schirazensis*	Costilla Creek, NM			Not exposed—field derived	MSB:Host:25356	OR047460	OR047654	OR052221
Gs8	*Galba schirazensis*	Costilla Creek, NM			Not exposed—field derived	MSB:Host:25351	OR047461	OR047655	OR052222
Gs9	*Galba schirazensis*	Costilla Creek, NM			Not exposed—field derived	MSB:Host:25358	OR047462	OR047656	OR052223
Gs10	*Galba schirazensis*	Costilla Creek, NM			Not exposed—field derived	MSB:Host:25357	OR047463	OR047657	OR052224
Gs11	*Galba schirazensis*	Costilla Creek, NM			Not exposed—field derived	MSB:Host:25360	OR047464	OR047658	OR052225
Gh1	*Galba humilis*	Corrales, NM	70 (field-derived)	16/58 (27.6%)	Yes	MSB:Host:24758	OR047465	OR047659	OR052226
Gh2	*Galba humilis*	Corrales, NM			Yes	MSB:Host:24759	OR047466	OR047660	OR052227

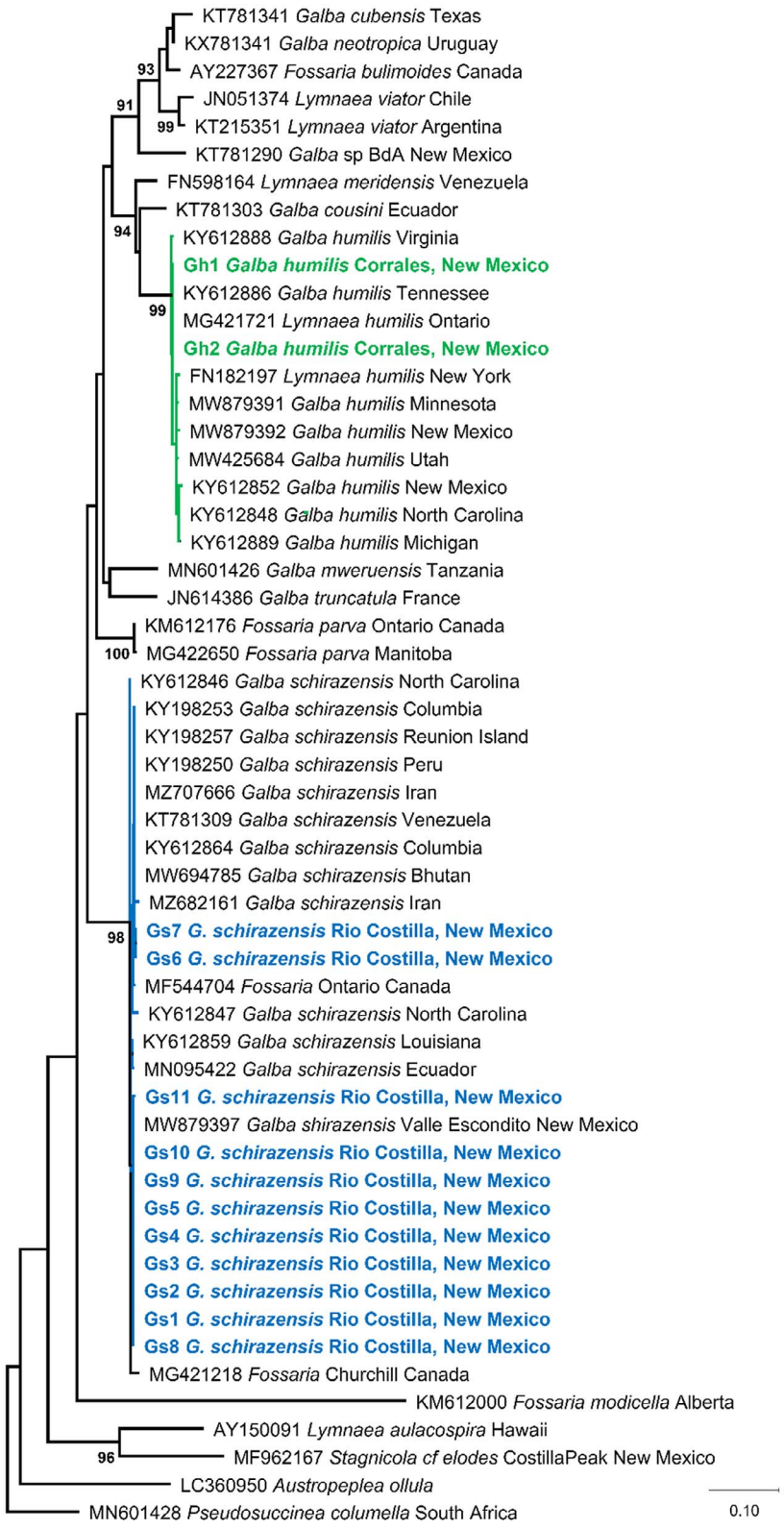

Figure 1. Phylogenetic tree based on maximum likelihood analysis of 592 positions of the cytochrome *c* subunit 1 gene from 42 lymnaeids from GenBank (accession numbers before species name) and 13 samples generated from this study. The tree is drawn with branch lengths scaled to the number of substitutions per site. There are nodes in the tree that are shown that have less than 90% support (i.e., nodes with support <90% were not collapsed). Green is the *Galba humilis* clade, and blue is the *Galba schirazensis* clade, with our samples indicated in color. See Table I for GenBank accession numbers and museum voucher numbers. Color version available online.

ranged from 0.0% (Valle Escondito, New Mexico, MW879397) to 0.49% (Iran, JF272607).

The original report of the natural, and experimental snail host for *H. americana* was *Galba cubensis* (Lee, 1962) a snail generally considered to occur in the southeast United States, the only country thus far known to harbor this parasite. *Galba cubensis* also extends into Mexico and Central America (Alda et al., 2021). Naturally occurring infections of *H. americana* from *G. humilis* from Moab

(Loker et al., 2021) have recently been reported. Lab-reared populations of *G. humilis* from Moab were also susceptible to *H. americana,* but lab-reared *Stagnicola elodes* and *Pseudosuccinea columella* were not (Loker et al., 2021). Although none of the New Mexico field-derived *Galba* snails we collected were positive for *H. americana*, both *G. humilis* from Corrales and *G. schirazensis* from Rio Costilla were experimentally susceptible. Liam Knight's Pond in Corrales, located about 1 mile from the Rio Grande, is a stocked fishing pond frequented by wildlife, likely including raccoons. Such a situation, if *H. americana* finds its way into the pond, would pose a threat to dogs and horses that live in the area and contact the pond. *Galba humilis* has also been found at Tingly Beach, Albuquerque, New Mexico, a site used for recreation purposes (Loker et al., 2021). The wide North American distribution of *G. humilis*, and its recent introduction into Japan (Saito, 2022), both areas known to harbor raccoons, could lead to even wider spread of this parasite, both in and beyond the United States.

Another factor potentially favoring the range expansion of *H. americana* is its ability to infect *G. schirazensis*. This somewhat enigmatic and invasive snail species is known from the Americas, Europe, and Asia. In the United States, it has been reported from 3 different states, New Mexico, Louisiana, and North Carolina, though its North American distribution is certainly broader given the low level of sampling, and the difficulty of differentiating it from other *Galba* species. Its origins, whether Asian (Bargues et al., 2011) or New World (Lounnas et al., 2017) remain uncertain pending more collections. It is believed to be primarily a self-fertilizing species (Lounnas et al., 2017), with a predilection to occur along the side of streams, usually out of the water (Bargues et al., 2011). We found numerous *G. schirazensis* in the water. We found them on stems of densely packed emergent sedges and in a shallow backwater of Rio Costilla. With respect to its role as a host for trematodes, *G. schirazensis* has thus far been most investigated for its possible role in transmission of *Fasciola hepatica*. Bargues et al. (2011) indicated that although *F. hepatica* rediae could be obtained in just a few experimentally exposed snails, no cercariae were produced and they considered *G. schirazensis* not to be a vector for *F. hepatica* or *Fasciola gigantica*. Caron et al. (2017) found specimens of *G. schirazensis* naturally infected with *F. hepatica* in Ecuador, recovered rediae, but no cercariae from infected snails, and concluded *G. schirazensis* was a possible vector in Ecuador as it was the only *Galba* species found in some known fascioliasis foci. Production of a few *F. hepatica* cercariae and many *Fascioloides magna* cercariae following experimental infections of a snail likely to be *G. schirazensis* was reported by Dreyfuss et al. (2015).

We know of no other studies reporting patent infections of trematodes from *G. schirazensis*, but here we report for the first time this snail is compatible with the schistosome *H. americana* following experimental exposures, with infected snails producing numerous cercariae. This will further complicate the story of *H. americana* as *G. schirazensis* is broadly distributed and invasive, as is the principal definitive host for *H. americana*, the raccoon. Establishment of *H. americana* in new locations is also likely to be facilitated by long-distance transport of infected dogs which may introduce the eggs into suitable *Galba* habitats also frequented by raccoons, which are believed to maintain the infection in nature once it is established in a new locality.

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