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Kathryn E. Perez

The University of Texas Rio Grande Valley

Victoria Garcia Gamboa

The University of Texas Rio Grande Valley

Caitlin M. Schneider

Romi L. Burks

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Resaca supports range expansion of invasive apple snails (*Pomacea maculata* Perry, 1810; Caenogastropoda: Ampullariidae) to the Rio Grande Valley, Texas

Kathryn E. Perez,^{1,4} Victoria Garcia Gamboa,¹ Caitlin M. Schneider,² & Romi L. Burks^{2,3}

¹University of Texas Rio Grande Valley, Department of Biology, 1201 West University Drive, Edinburg, TX, USA 78501

²Southwestern University, Environmental Studies Program, 1001 East University Avenue, Georgetown, TX, USA 78626

³Southwestern University, Department of Biology, 1001 East University Avenue, Georgetown, TX, USA 78626

⁴Corresponding author. E-mail: perezke@gmail.com

Abstract. Resacas, or oxbow lakes, form from old river channels. In the Rio Grande, resacas provide habitat for diverse wildlife, including native and non-native species. Biologists unexpectedly found pink egg masses on emergent vegetation (November 2015) and later adult apple snails (May 2016) within a resaca at a former fish hatchery in Brownsville, Texas. This report extends the non-native range of *Pomacea maculata* Perry, 1810 by 429 km southeast in Texas. Our findings imply that abandoned waterbodies, such as fish hatcheries, can act as unrecognized conduits for non-native invasive species.

Key words. New record; gastropod; non-native; irrigation network; GIS

The genus *Pomacea* Perry, 1810 (Ampullariidae) includes the largest and most diverse group of freshwater snails native to tropical and subtropical habitats in Central and South America (HAYES et al. 2015). In particular, 2 *Pomacea* species succeed globally as non-native invasive species (NNIS) (HAYES et al. 2008, HAYES et al. 2012). For example, *Pomacea maculata* Perry, 1810, a large freshwater apple snail native to South America from the Amazon south to the Rio de la Plata (HAYES et al. 2012), established NNIS populations in the United States in the early 1990s and has since spread to 8 southern states (BYERS et al. 2013, BURKS et al. 2016). Non-native apple snails consume aquatic macrophytes (BURLAKOVA et al. 2009), compete with native species (CONNER et al. 2008, POSCH et al. 2013), disrupt ecosystem services (GILIOLI et al. 2017), cause economic damages (JOSHI & SEBASTIAN 2006), and can harbour parasites that threaten wildlife (DODD et al. 2016) and human health (TEEM et al. 2013).

The greater Houston, Texas, metropolitan area (e.g., Harris, Brazoria, Fort Bend and Galveston counties; Fig. 1) has supported populations of *P. maculata* since 2000 (UNITED STATES GEOLOGICAL SURVEY 2016) in interconnected bayous, ditches and canals (BURKS et al. 2010, BURLAKOVA et al. 2010). New records appear annually in the database (UNITED STATES

GEOLOGICAL SURVEY 2016) as the highly fecund snails quickly reproduce (BARNES et al. 2008) and spread to novel habitats. However, prior to our study, no records of established apple snail populations existed from the Rio Grande Valley (RGV), the southernmost area of Texas, which lies along the northern bank of the Rio Grande River.

The RGV offers a hospitable subtropical climate for *P. maculata* and could present greater potential for reproduction and spread of this species. Resacas, otherwise known as oxbow lakes or ponds, are remnants of the Rio Grande river channel previously cut off by eroding river banks or created by sediment accumulation (ALLHANDS 1960, MORA et al. 2001). Some resacas qualify as permanent wetlands because they receive water intermittently from pumping stations. Other resacas may be subdivided and used as fish farms or fish hatcheries (ROBINSON 2010). Although these ecosystems often get overlooked by routine monitoring efforts or become abandoned once a hatchery closes, these modified resacas can still provide valuable habitat for wetland plants, aquatic invertebrates, amphibians, fish and migratory birds within the semi-arid environment of South Texas (JAHRSDOERFER & LESLIE 1988). For example, in Cameron County within the RGV (Fig. 1), a recent survey found that 17 permanent resacas served as habitat for an endemic threatened siren, a type of salamander with small forelimbs (LAFORTUNE 2015). Because they support an endangered species, legal protection prevents certain resacas from being drained. However, provision of permanent water in a semi-arid region may have the unintended consequence of also supporting NNIS, including mollusk species such as *Melanoides tuberculata* (O. F. Müller, 1774), which are known vectors of parasites that cause human infections (BENSON & NEILSON 2017) and already occur in the RGV.

Besides serving as habitat for wildlife, resacas provide additional ecosystem services as the RGV supports intensive agriculture production that relies on an intricate irrigation canal network for flood-irrigation of crops such as citrus (ENCISO et al. 2008). To feed this network, water from the Rio Grande

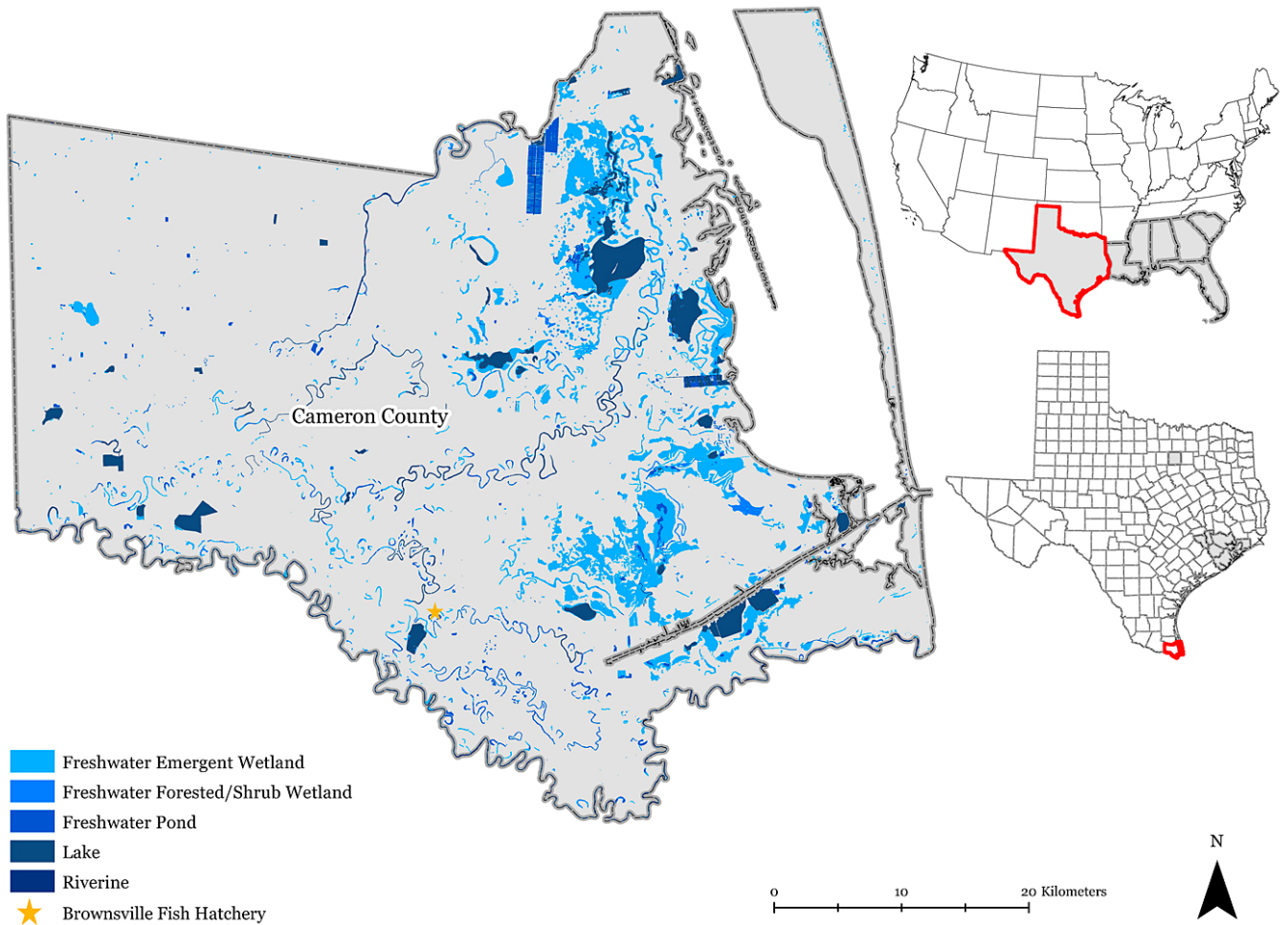


Figure 1. ArcGIS Map of the different types of wetlands and their interconnectivity within Cameron County. A red outline indicates the location of Cameron County on the inset map of Texas. A star marks the location of the Brownsville Fish Hatchery that contains the resaca that supports a reproducing population of *Pomacea maculata*. Inset maps of the U.S. and Texas indicate the previous range of *P. maculata* with gray shading. Data retrieved from USGS (2016).

River gets diverted through a network of over 3,200 km of canals and pipelines (KNIGHT 2009), some with permanent water and others that flood periodically. This hydrological network presents conditions potentially favorable to the spread of apple snails by providing more places to deposit egg clutches (BURKS et al. 2010, KYLE et al. 2011). In addition, the habitat linkages also allow snails refuge as they can move into deeper water (i.e., resacas or main irrigation canals) to avoid seasonal dry (DARBY et al. 2002) or cold conditions (NAYLOR 1996). Consequently, snails may survive unfavorable conditions and then increase their activity.

In parts of Texas, crop rotation of rice fields most likely minimizes detrimental effects of apple snails compared to those documented in Southeast Asia (BURLAKOVA et al. 2010). In Southeast Asia, herbivory by the more frequently studied *Pomacea canaliculata* (Lamarck, 1822), and likely *P. maculata* as well, annually results in billions of dollars in damage to rice (JOSHI & SEBASTIAN 2006), and causes considerable ecological damage to natural and constructed wetlands (HORGAN et al. 2014). However, the absence of a clear economic impact in Texas thus far does not imply a lack of vulnerable agricultural areas, especially those with permanent water. Although most studies focus on impacts to rice farming, apple snails may also

damage maize, sugarcane, and citrus (NAYLOR 1996), staples of RGV agriculture (ENCISO et al. 2008). Unfortunately, as *P. canaliculata* and *P. maculata* establish new populations in non-native habitats across the globe, taxonomic confusion has plagued apple snail identifications due to morphological similarities between the two *Pomacea* species and recent synonymy of *P. insularum* d'Orbigny, 1835 to *P. maculata* (HAYES et al. 2012). In this paper, we quantify initial reproductive output of a newly established population of apple snails in the RGV and genetically confirm the species identity to avoid any ambiguity.

In November 2015, researchers collected 7 snail egg clutches attached to vegetation adjacent to the resaca by clipping the stems of vegetation to prevent damaging the egg clutches (Texas Parks and Wildlife Department permit no. SPR-1014-201). The resaca occurs as part of the Lower Laguna Madre Coastal Fisheries Field Office of Texas Parks and Wildlife, formerly known as the Brownsville or Olmito Fish Hatchery, and hereafter referred to as the Brownsville Fish Hatchery (Fig. 1; 25.9856° N, -97.5311° W). Clutches occurred on concrete fixtures, emergent vegetation such as cattails, horsetails, and grasses, and plants overhanging the pond such as the Mexican Fan Palm and other trees (Fig. 2). Researchers returned to the site in May of 2016 and collected additional clutches and found



Figure 2. Images of collection site Brownsville Fish Hatchery, Brownsville, Cameron County, Texas, 20 November, 2015, collection and photo by R. Lopez and V. Garcia Gamboa. L: Overview of pond collected looking NW from bank of pond. R: Pink egg clutches on *Typha* sp.

the dry shells of approximately 20 adult snails (Fig. 3). Four of the adult shells (dry), hatchlings from all clutches examined (70% ethanol), and an unhatched egg mass (dry) were deposited at the National Museum of Natural History, Smithsonian Institution (USNM 1411272–1411287).

To examine the hydrologic landscape in which this discovery of a new apple snail population occurred, we used ArcMap (v. 10.4) to create a map of wetland polygons at a county scale (Fig. 1; Cameron County) and determined distance from the nearest population recorded in the United States Geological Survey's Nonindigenous Aquatic Species Database. Geographic Information Systems (GIS) shapefiles came from the United States Fish and Wildlife Service (USFWS) National Wetlands Inventory (U.S. FISH AND WILDLIFE SERVICE 2016).

To identify species genetically, we crushed whole hatchlings

preserved in 95% ethanol and extracted DNA using 600 μ l of Cetrimonium bromide (CTAB) extraction buffer and 30 μ l of Proteinase K, incubated overnight at 37 °C, and then followed with phenol-chloroform extraction (SAGHAI-MAROOF et al. 1984). Extracted DNA was resuspended in Tris-Ethylenediaminetetraacetic acid (TE) with RNAase and then additionally purified using the IBI Scientific PCR Clean Up Kit (IBI Scientific). We amplified the mitochondrial cytochrome oxidase I (COI) gene using universal primers (FOLMER et al. 1994) in 25 μ l reactions containing template DNA (2 μ l/reaction), reaction buffer (1 M), Taq polymerase (0.05 U/ μ l), and dNTPs (0.2 mM). Cycling parameters consisted of 1 cycle of 2 min at 92 °C, then 5 cycles of 40 sec at 92 °C, 40 sec at 40 °C, 90 sec at 72 °C; then 30 cycles of 40 sec at 92 °C, 40 sec of 50 °C, 90 sec at 72 °C, then a final extension of 72 °C for 5 min with final



Figure 3. Dry adult *Pomacea maculata* shell collected from the Brownsville Fish Hatchery in Cameron County, Texas.

incubation of 12 °C until termination of reaction. We purified successful amplicons using an IBI Scientific PCR Clean Up Kit and sent them for Sanger sequencing on an ABI3100 by Genewiz (South Plainfield, NJ).

Sequences for comparison were drawn from recent published literature (RAWLINGS et al. 2007; HAYES et al. 2009) accessible from Genbank (<http://www.ncbi.nlm.nih.gov/genbank/>). We included representative haplotypes for COI of the “*Pomacea* clade” (HAYES et al. 2009), a clade of close relatives including *P. canaliculata*, *P. maculata*, another species of *Pomacea* native in the United States (*P. paludosa* [Say, 1829]), and *P. guayanensis* (Lamarck, 1822), a member of the closely related “*Effusa* clade” (HAYES et al. 2009), as an outgroup for phylogenetic analysis. MUSCLE (EDGAR 2004) implemented at *Phylogeny.fr* (DEREEPER et al. 2008) was used to align sequences. Identical haplotypes were collapsed manually to retain a single individual consensus sequence. jModeltest (GUINDON & GASCUEL 2003, DARRIBA et al. 2012) using AIC was used for selection of an appropriate model of evolution for phylogenetic analysis. We conducted phylogenetic inference and 1000 rapid bootstrap replicates (FELSENSTEIN 1985) using the maximum likelihood algorithm carried out in RaxML v. 8.2.8 (STAMATAKIS 2006). This analysis was conducted using the Cyberinfrastructure for Phylogenetic Research Gateway (MILLER et al. 2010).

To evaluate the immediate reproductive potential of the snails, we took egg clutches collected in both November 2015 (USNM 1411284–1411287) and May 2016 to the laboratory at The University of Texas Rio Grande Valley. We allowed November clutches to hatch fully and counted hatchlings over a period of one week. The total number of eggs per clutch (EPC) included unhatched eggs and hatchlings. For the additional egg masses collected in May 2016 (USNM 1411272–1411283), we immersed entire egg masses in 1.0 M NaOH overnight to allow the entire mass to separate (BARNES et al. 2008) and included all eggs in the determination of EPC. We compared EPC counts separately to allow for seasonal variation (YOSHIDA et al. 2014). To compare with past literature, we measured maximum height of a hatchling’s shell aperture ($n = 231$), hatchling aperture height (HAH), the same measurement referred to as “operculum width” in past literature (BARNES et al. 2008: fig. 2).

According to the National Wetlands Inventory, wetlands occupied 9.27% of Cameron County (Fig. 1) in 2010 and included freshwater emergent wetlands, freshwater forested/shrub wetlands, freshwater ponds, lakes, and rivers. Within that county, the Brownsville Fish Hatchery property occurs 429.2 km from the nearest recorded population of apple snails in Brazoria county (USGS 2016). The resaca in which we found apple snails supported stands of *Typha* sp. (Fig. 2) and occupied 7.7 ha. A previous fish hatchery divided the resaca into smaller basins that each occupied approximately 0.27 ha.

Based on data from the closest gauging station, a rise in water of 1.5 m would inundate the surrounding landscape. Apple snails occurred approximately 3.26 km away from the nearest large (250.85 ha) reservoir, Resaca de la Palma (Fig. 1; visible as the large open body of water southeast of the fish hatchery) and 10.73 km away from the closest branch of the Rio Grande (Fig. 1; river forms southern boundary of map). The bleached nature of adult shells collected from the resaca

within the RGV (Figure 3) made identification of certain morphological characteristics suggestive of *P. maculata* (i.e., inside of pallial lip reddish-orange) versus *P. canaliculata* (i.e., inside of pallial lip unpigmented) (HAYES et al. 2012) difficult and examination of opercula characteristics impossible.

More so than the morphological characteristics, the highest likelihood tree (−3240.676515; Fig. 4) from our phylogenetic analysis clearly confirmed all specimens from the RGV as *P. maculata*. Sequencing and alignment resulted in 24 sequences for a 635 bp aligned portion of COI. No gaps or stop-codons occurred in this alignment. The TIM2+I+G model was selected as the most appropriate for this set of data. We found 2 different COI haplotypes, BFH1 and BFH2, that differed in 8 basepairs (out of 635). Individuals ($n = 4$) from 1 egg mass had a haplotype, BFH1 (Genbank ID KX149128; USNM 1411287), known from other U.S. introduced populations including Texas (RAWLINGS et al. 2007, MARTIN et al. 2012). The other haplotype found in the other 4 egg masses (23 individuals) sequenced, BFH2 (Genbank ID KX149127; USNM 1411285–86, 1411288–89) has been previously documented in Florida (RAWLINGS et al. 2007), Louisiana (DEATON et al. 2016), and Spain (ANDREE & LOPEZ 2013), but not previously recorded in Texas.

Six out of 7 of the clutches collected in November 2015 partially hatched, providing 231 hatchlings and many more unhatched eggs. Collectively, the 6 clutches yielded 2,479 total eggs and hatchlings with an average of 471.3 ± 187.554 EPC with a range of 48–1301 EPC (Fig. 5). Twelve clutches collected in May 2016 yielded 8,851 eggs, with an average of 804.6 ± 111.843 EPC and a range of 302–1562 EPC. Hatchling aperture height ($n = 231$) averaged 1.379 ± 0.014 mm.

In this study, we report a small population of *P. maculata* in a new drainage basin, the Rio Grande and a new geographic region, the RGV, 429 km from the nearest invasive populations in Fort Bend, Brazoria County, Texas (Fig. 1). Species identification of apple snails has historically suffered from taxonomic confusion and been difficult because of morphological similarities (HAYES et al. 2012). To avoid perpetuating the confusion that has existed, our study provides evidence from both morphological and genetic analysis that undoubtedly confirms the presence of this NNIS in the RGV.

The size of egg masses collected in November led us first to question whether these clutches could have been laid by *P. canaliculata*. These egg masses contained about 500 eggs per clutch (EPC; Fig. 5), closer to the characteristic average production of *P. canaliculata* (dashed line, values from TAMBURI & MARTIN 2011) than *P. maculata* egg masses (dotted line, values from BARNES et al. 2008). However, May clutches ($n = 12$), nearly twice as large (ca. 800 EPC) as the November samples, provided better support for an identification of *P. maculata*. BARNES et al. (2008) studied reproductive effort in female *P. maculata* in the Houston area of Texas by sampling in September, April, and June and found the lowest reproductive effort (lower number of EPC) at the beginning of the reproductive season (April, 767.7 ± 74.6). Reproductive effort then tripled by June (2247.8 ± 184.8) and then declined in September (1982.0 ± 204.2). Given the higher average temperatures in Brownsville compared to Houston, reproductive effort seems likely to continue its decline through November, resulting in

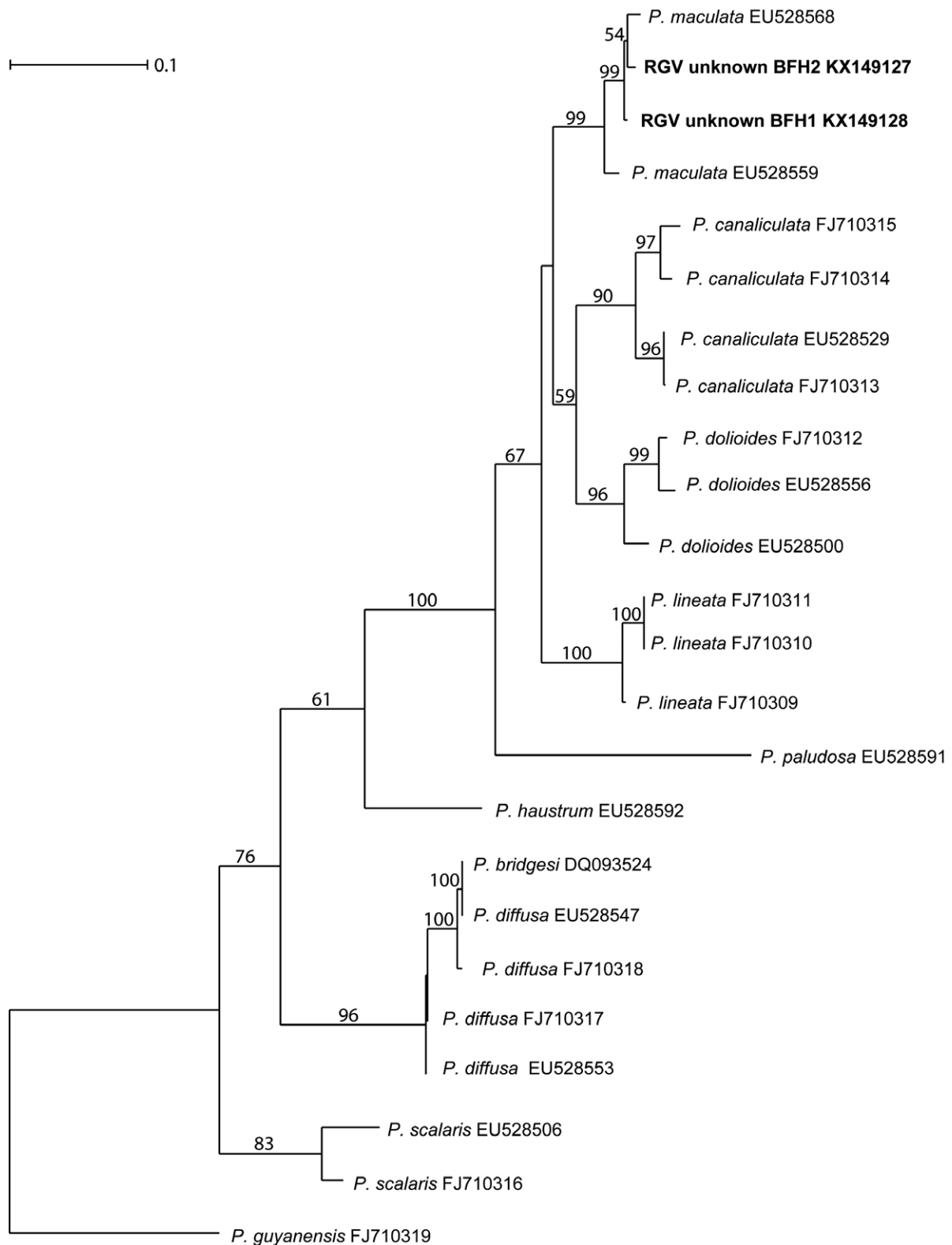


Figure 4. Maximum likelihood phylogram showing position of the Rio Grande Valley *Pomacea* haplotypes (2 haplotypes, 27 individuals sampled) in context of the *Pomacea* clade (HAYES et al. 2009). Numbers above branches are bootstrap values generated by 1000 rapid ML bootstraps in RaxML. Haplotypes from the Brownsville Fish Hatchery (BFH 1 and 2) are highlighted by bold text.

our low, almost *P. canaliculata*-level egg clutch counts (ca. 200 EPC; TAMBURI & MARTÍN 2011).

Other factors may explain the smaller than expected egg mass sizes in November. Beyond seasonal variation, the establishment of this RGV population appears very recent and clutch mass varies widely with female size (KYLE et al.

2011). Limited food availability or density may also explain the range of clutch sizes found at our RGV site. For example, TAMBURI & MARTÍN (2011) found egg mass size in *P. canaliculata* decreased as food availability decreased and TANAKA et al. (1999) found that increased density also decreased egg mass size. *Pomacea maculata* likely exhibit similar responses, and

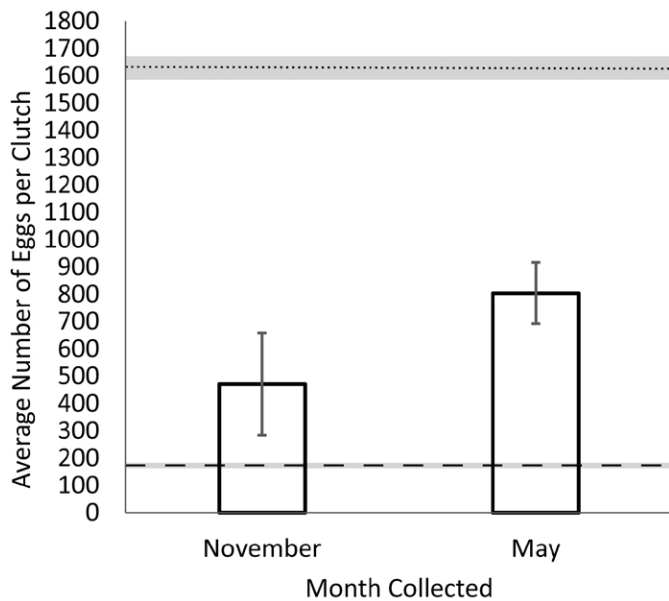


Figure 5. Average number of eggs per clutch (EPC) counted from collections in November ($n = 6$, 471 ± 187.6) and May ($n = 12$, 804.6 ± 111.8). Egg masses present in May had nearly twice as many EPC. Dotted line ($n = 67$, 1626.3 ± 121.7) shows the average EPC and gray bar shows the SEM for *P. maculata* (BARNES et al. 2008). Dashed line ($n = 318$, 186.87 ± 0.34) shows the average EPC and gray bar shows the SEM for *P. canaliculata* (TAMBURI & MARTÍN 2011).

future investigations will hopefully distinguish between these possibilities that our initial data could not. While often useful for distinguishing *P. maculata* from *P. canaliculata* (HAYES et al. 2012), we caution about application of egg clutch size in the field as the potential for variability and confusion exists when small *P. maculata* clutches approximate the expected egg clutch count of *P. canaliculata*.

As an alternative to egg clutch counts, hatchling aperture height (HAH) provides one of the most accurate comparisons between *P. canaliculata* and *P. maculata* (HAYES et al. 2012). RGV individuals exhibited an average HAH value of 1.379 ± 0.014 mm. Published data for *P. maculata* from the Houston, Texas area found a HAH of 1.14 ± 0.003 mm (BARNES et al. 2008). Measurement of a figure (HAYES et al. 2012: fig. 14) comparing 1-day old hatchlings of both species found HAH in individuals of *P. maculata* to be approximately 1 mm and 2.4 mm for *P. canaliculata* (HAYES et al. 2012). HAH (and overall size) of the RGV population's hatchlings appeared a bit larger than expected for newly hatched *P. maculata*, but still at least 1 whole mm smaller than *P. canaliculata*, thus allowing this measurement to help distinguish between the 2 species and support an identification as *P. maculata*. We hypothesize that the slight HAH differences occurred due to the rapid growth that occurs after hatching as comparable measurements occurred on Day 0 hatchlings, but the number of hours may have varied.

While morphological measures indicated an identification of *P. maculata*, we wanted the unambiguous confirmation of this new population that comes with genetic analysis. COI sequences from the RGV population formed a well-supported (99% bootstrap support) clade with other *P. maculata*, distinct from *P. canaliculata* (Fig. 4). RAWLINGS et al. (2007) reported that the U.S. apple snail invasion initially into Florida came

from sources in Brazil and Argentina. However, sampled Texas populations of *P. maculata* appeared to have invaded only from Argentina (RAWLINGS et al. 2007). Overall, low haplotype diversity in introduced populations of *Pomacea* occurs compared to native populations (HAYES et al. 2012), with most introduced populations retaining a single COI haplotype. The 2 distinct haplotypes present in Brownsville, separated by not just 1, but 8 basepairs, makes this population unexpectedly diverse. Haplotype 1 also matches populations in Argentina, Florida, Georgia, Alabama, and Texas (RAWLINGS et al. 2007, MARTIN et al. 2012). This location is the first and only place that Haplotype 2 has been documented from Texas, providing a small, yet important bit of evidence that supports a Florida or Louisiana origin of the RGV population, versus spread from other Texas populations.

Pomacea maculata has been established in the Houston, Texas area since 1989 (RAWLINGS et al. 2007), however, its movement into the RGV presents a climate potentially more favorable for the survival and population growth of this tropical/subtropical species (BYERS et al. 2013). A study of macroinvertebrate diversity (MCINTOSH 2014) in similar water bodies, including 3 resacas near (< 25 km) the Brownsville Fish Hatchery, found that water temperature ranged from 12.3 °C in winter to 34.9 °C in summer, with an average annual temperature of 25.4 °C that would provide sufficient water temperatures for year-round activity in *P. maculata*. Thus, it seems likely that this favorable climate and places of refugia could enhance the success and spread of *P. maculata* in the RGV. The presence of egg clutches in both November and May as well as adults in May suggested that the population survived winter and continued to reproduce. The question of hatchling survival still remains open. Small hatchlings would be more vulnerable to predation by crayfish (DORN & HAFSADI 2016), but the overall number of offspring potentially produced probably provides sufficient numbers to survive enhanced predation. Furthermore, the highly visible and seemingly vulnerable egg clutches are chemically defended and unpalatable to predators (GIGLIO et al. 2016). Coupled with the climate and habitat in South Texas, all these factors (e.g., ability to overwinter, hundreds of propagules and egg defense) indicate the likely persistence and spread of this species.

The persistent presence of this apple snail population in a decommissioned fish hatchery further highlights a potential reservoir for this invasive species to gain a foothold in a new geographic region. The resaca sits in a flat landscape connected hydrologically to a drainage network spanning the RGV, an area with extensive wetlands (Fig. 1). This interconnected drainage network has the potential to facilitate spread of this species throughout this geographic region. The occurrence in a resaca also poses challenges to easy eradication as the habitat supports *P. maculata*, a NNIS, along with another threatened species that warrants conservation, the Texas siren (*Siren intermedia texana* Goin, 1957) (DIXON 2013, KLINE & CARREON 2013). *Pomacea maculata* may also threaten other wildlife by transferring toxins linked to Avian Vacuolar Myelinopathy (DODD et al. 2016) or by transmitting *Angiostrongylus cantonensis* (Chen, 1935) (TEEM et al. 2013).

The RGV encompasses a single geographical region,

however, many areas outside Texas exist with similar hydro-logic landscapes covered with oxbow lakes and abandoned fish ponds. Routine monitoring of these types of ecosystems might be valuable for avoiding the potential ecosystem damage of NNIS. MARTIN et al. (2012) described the history of an apple snail introduction in Mobile, Alabama, to a similarly sized wetland. Over a 10-year period, state and local governments have attempted to control the apple snail population using chemical and biological controls, ecosystem modifications, and physical removal, yet the invasive population remains. In Florida, management effort by hand-picking has been found to be effective and cost-effective (BERNATIS & WARREN 2014) for apple snail control at the initial stages of a population that remains relatively localized. Economically, apple snails could have an impact in the RGV as current efforts seek to restore the resacas' ecosystem functions including connectivity (Resaca Restoration Project, Brownsville Public Utilities Board). The case of *P. maculata* in South Texas clearly demonstrates that abandoned waterbodies within hospitable climates may act as conduits for invasive species and thus warrant monitoring and early intervention.

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