



First evidence of introgressive hybridization of apple snails (*Pomacea* spp.) in their native range

Paul M. Glasheen¹, Romi L. Burks¹, Sofia R. Campos¹ and Kenneth A. Hayes^{2,3}

¹*Department of Biology, Southwestern University, 1011 East University Avenue, Georgetown, TX 78626, USA;*

²*Bernice Pauahi Bishop Museum, 1525 Bernice St., Honolulu, HI 96817, USA; and*

³*National Museum of Natural History, Smithsonian Institution, Washington, DC 20560, USA*

Correspondence: K.A. Hayes; e-mail: hayes.ken@gmail.com

(Received 26 February 2019; editorial decision 13 June 2019)

ABSTRACT

Genetic variation facilitates both natural range expansions and anthropogenic invasions. Contrary to expectations, hybridization does not always impact negatively on biodiversity. Increasing evidence indicates advantageous roles for introgressive hybridization in maintaining standing genetic variation. Hypothesizing that hybridization may contribute to the evolutionary and invasive success of a diverse group of freshwater snails (Ampullariidae, commonly known as apple snails), we estimated the frequency of hybridization between two globally invasive species of *Pomacea*, *Pomacea canaliculata* (Lamarck, 1822) and *P. maculata* Perry, 1810, in their native range. While previous work in Asia has uncovered the occurrence of extensive hybridization, we provide the first phylogenetic evidence of a high degree of hybridization (30%) between these species in Uruguay and Brazil. Hybrids carried both heterozygous and homozygous combinations of elongation factor 1- α (EF1 α) nuclear alleles in both mating directions, indicating that hybridization has occurred over multiple generations and likely preceded introductions outside the native range. Among the five sites in Brazil previously documented as containing only *P. maculata*, one far northern population (Careiro Castanho), which is thousands of kilometres from the northern range of *P. canaliculata*, unexpectedly contained hybrids. This may be the result of human-facilitated introductions. Together with recent work from Asia, our investigations in the native range of apple snails support a reframing of historical perspectives of hybridization as a driver of extinction and diversity loss towards a modern paradigm where hybridization may promote diversification and contribute to the survival of evolutionary lineages such as molluscs.

INTRODUCTION

While gastropods remain understudied relative to other organisms (Régnier *et al.*, 2015) and ignored even in reviews of hybridization (Mallet, 2005), they present novel opportunities to better understand evolutionary and taxonomic complexities associated with hybridization. Gastropods often exhibit a wide range of morphological variation that makes delineating species and establishing stable taxonomy problematic (Hayes *et al.*, 2012). At one end of the range, a single species can exhibit extreme intraspecific morphological variation, as occurs in the marine snail *Littorina saxatilis* (Teshima *et al.*, 2003). Such variation creates confusion in identification, leading in this case to over a hundred synonymies for this species and the moniker as the champion of taxonomic redundancies (Cressey, 2015). At the other end of the spectrum are ‘invisible’ or cryptic species that are often difficult to distinguish solely by external morphology (Yang *et al.*, 2018).

Morphologically similar species may appear the same to a non-expert, and when relevant taxonomic expertise is not available, may often require genetic analysis to distinguish them from other species. These opposing challenges of overestimating *vs* underestimating species diversity reinforce the need to move towards integrative taxonomy (Dayrat, 2005). Integrative taxonomy involves species identifications based on multiple, complementary perspectives including, but not limited to, comparative morphology, phylogenetics and ecology (Dayrat, 2005; Hayes *et al.*, 2012). Reliance on integrative taxonomy increases the likelihood of consistent and accurate species delineation across observers, and this, in turn, may lead to the discovery of more hybrids and the detection of previously unidentified novel species in natural systems.

While introgressive hybridization has long been considered important in the evolution of plants (Roberts, 1929; Abbott, 1992; Rieseberg & Carney, 1998), theorists have also begun to recognize it as a potentially important source of novel genetic variation

in the evolutionary success of animals (Dowling & DeMarais, 1993; Grant & Grant, 1998; Pennisi, 2016, 2018; Kagawa & Takimoto, 2018). If hybridization creates advantageous novel allele combinations, then selective advantages emerge for species that demonstrate a propensity for hybridization, with such species avoiding a loss of fitness in the process. The immediate benefit of novel allele combinations following hybridization has been observed previously in freshwater snails (Facon *et al.*, 2005). Additionally, hybridization provides a novel source of standing genetic variation that aids in avoiding extinction (Rhymer & Simberloff, 1996), contributes to adaptive radiation (Arnold & Kunte, 2017) and confers other potential benefits on widely distributed taxa (Harrison & Larson, 2014). Hybrids also create new ecological niches or occupy existing ones and thus have different impacts on their environments. For example, hybrids may exhibit hybrid vigour, possessing greater tolerances to physical or environmental stresses than either parent species (Matsukura *et al.*, 2015). Furthermore, the ability to hybridize may facilitate biological invasions, allowing initial colonizers to expand their ranges quickly and subsequently to dominate ecosystems (Lucek *et al.*, 2010; Lack *et al.*, 2012; Hall, 2016).

Classically, speciation results in distinct lineages that are reproductively and genetically isolated. However, shared alleles may persist in these lineages because of incomplete lineage sorting between recently diverged species pairs. This may potentially blur the lines of identification when individuals are mistaken for hybrids or may mask differences between distinct species (Mallet, 2005; Toews & Brelsford, 2012; Harrison & Larson, 2014). Furthermore, shared ancestral polymorphisms may be confused for introgressive or hybrid alleles. Incomplete lineage sorting fails to leave a predictable biogeographical pattern in isolated and overlapping populations (Toews & Brelsford, 2012). Hybridization, in contrast, results in biogeographic inconsistencies and discordance between mitochondrial and nuclear DNA (Toews & Brelsford, 2012).

Hybridization may occur in the native range long before a species undergoes an anthropogenic range expansion. Thus, hybrids may inherit different advantageous alleles from either parent and consequently may increase their fitness. Conversely, deleterious recessive alleles, which have accumulated and become fixed in either parent population, can be masked after a hybridization event by introgressive dominant alleles that purge the impacts of ‘mutation loading’ (Coutellec & Caquet, 2011). Regardless of the mechanism, hybrid vigour or heterosis often results in increased fitness of hybrids relative to parental populations (Coutellec & Caquet, 2011). Even rare hybridization events that involve only a small proportion of individuals in a population may dramatically alter the genetic diversity of the population as a whole (Mallet, 2005).

Gastropods, such as apple snails, represent excellent models (Hayes *et al.*, 2009a, 2015) for understanding evolutionary processes, including range expansion and invasion success. In the tropics and subtropics, molluscs account for a substantial portion of freshwater biodiversity (Strong *et al.*, 2008). The diverse genus *Pomacea* contains several globally invasive species, notably *P. canaliculata* (Lamarck, 1822) and *P. maculata* Perry 1810, both of which are classic examples of invasive success and threaten ecological systems and public health on a global scale (Hayes, Cowie & Thiengo, 2009b; Hayes *et al.*, 2012, 2015; Horgan, Stuart & Kudavidanage, 2014). In addition, the genus *Pomacea* contains several morphologically cryptic species (Hayes *et al.*, 2012). Consequently, apple snails serve as good study systems for understanding the complex processes driving diversification and speciation. While the lower vagility of snails may translate to lower range expansion potential (Angert *et al.*, 2011), anthropogenic dispersal paired with adaptive traits often increases the pace of invasion (Capinha *et al.*, 2015).

Some species of *Pomacea* possess traits traditionally identified as facilitating invasiveness; these traits include a generalist herbivorous

diet (Saveanu & Martín, 2013; Burks *et al.*, 2017), tolerance to drying (Hayes *et al.*, 2009a; Glasheen *et al.*, 2017; Mueck *et al.*, 2018) and reproductive strategies, such as high fecundity (Barnes *et al.*, 2008; Tamburi & Martín, 2011), terrestrial oviposition (Burks, Kyle, & Trawick, 2010) and possibly introgressive hybridization (Matsukura *et al.*, 2013). Interestingly, non-native *P. canaliculata* and *P. maculata* populations in Japan readily hybridize (Matsukura *et al.*, 2013, 2015), indicating a possible role for hybridization during invasion. Such populations occur at range extremes and are likely to be subjected to repeated cycles of selective pressure that mimic founder effects (Carson & Templeton, 1984). This pressure, in turn, would favour fluidity in breeding stringency, thereby facilitating true introgression (Toews & Brelsford, 2012), long-term diversification (Pennisi, 2018) and dispersal (Pfenning, Kelly & Pierce, 2016). Though the occurrence of hybridization has been observed in the invasive ranges of apple snails (Matsukura *et al.*, 2013, 2015; Yang *et al.*, 2018), it remains unconfirmed in the native ranges of these species. To begin to understand the role hybridization may have played in facilitating invasion success and diversification, we investigated whether hybridization occurs in the native range of species and if so at what frequency. To address this, we collected samples from the native ranges of *P. canaliculata* and *P. maculata* in Uruguay and Brazil. We used these samples to identify hybrids and characterize hybridization frequency, as well as to rule out incomplete lineage sorting as an alternative explanation for previously observed genetic admixture.

MATERIAL AND METHODS

Sample collection

Apple snails, which were initially identified morphologically as *Pomacea canaliculata* and *P. maculata*, were collected from geographically diverse freshwater sources in Uruguay (Fig. 1). Sampling was carried out from December to January in five consecutive years (2011–2015). All specimens were killed by drowning in deoxygenated water (Fukuda, Haga & Tataru, 2008) and fixed in 95–100% ethanol. At the time of fixation, a small piece of foot tissue was removed and preserved in 95–100% ethanol for subsequent DNA extraction. Samples from additional populations of *P. maculata* from Brazil (Fig. 1) were collected as part of previous studies (Hayes, 2009; Hayes *et al.*, 2009b, 2012), and sequences from these samples provided representatives of pure or non-overlapping populations for this species. From field collections across Brazil and Uruguay (Table 1), we selected 146 snails to analyse for hybridization. These samples came from eight sites: two populations containing both *P. canaliculata* and *P. maculata* (i.e. overlapping: Parque Rodó Montevideo and Punta Gorda), one ‘pure’ *P. canaliculata* population occurring in isolation (Magallanes) and five other sites in Brazil, which had been previously identified as containing only *P. maculata* (Table 1, Fig. 1). All specimens used in this study are currently housed in the collections of the Bishop Museum, Honolulu, USA, the Instituto Oswaldo Cruz, Rio de Janeiro, Brazil, or Southwestern University, Georgetown, Texas, USA. All specimens will be vouchered at the museums associated with their country of origin following the completion of ongoing research beyond the present study.

DNA extraction and loci amplification

Total genomic DNA (gDNA) was extracted from *c.* 5 mg of foot tissue using the NucleoSpin® extraction kit (Macherey-Nagel, Pennsylvania, USA) following the manufacturer’s instructions with a final elution volume of 150 µL. A 658-bp fragment of the mitochondrial cytochrome *c* oxidase subunit I (COI) gene was amplified using primers LCO1490 and HCO2198 (Folmer *et al.*, 1994). To evaluate hybridization, a 460-bp fragment of the nuclear

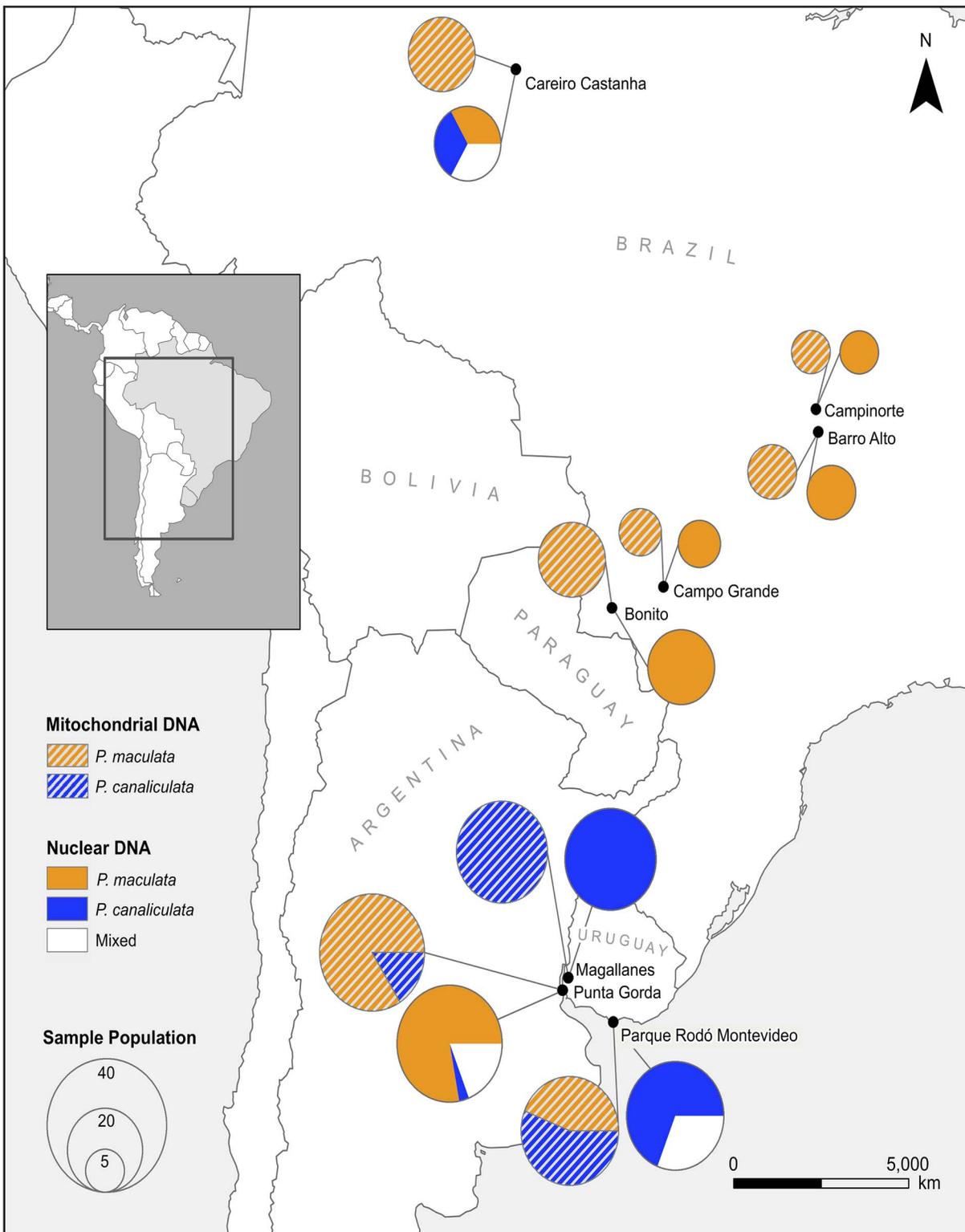


Figure 1. Map showing the sites sampled for *Pomacea maculata* and *P. canaliculata* in Uruguay and Brazil, with inset of South America. Colours and patterns show the varying degrees of concordant and discordant mitochondrial (COI) and nuclear haplotypes (EF1 α). Blue indicates *P. canaliculata* and orange represents *P. maculata*. Hatched pattern denotes COI haplotypes and solid colours show EF1 α identity. White represents the percentage of snails at a site with one EF1 α allele from *P. canaliculata* and one EF1 α from *P. maculata* (mixed). Population names correspond to those in Table 1.

elongation factor 1- α (EF1 α) was also amplified using primers from Hayes *et al.* (2009b). Amplifications for both loci were carried out in 25 μ L reactions containing 1 U MangoTaqTM (Bioline, USA), 1 \times

MangoTaqTM Buffer, 2.5 mM MgCl₂, 200 μ M dNTPs, 0.16 μ M of each primer and 1 μ L gDNA (25–50 ng); cycling parameters followed Hayes *et al.* (2009b).

Table 1. Sample populations and genotypes.

Site	Region	Coordinates	Individuals collected	COI <i>P. can</i>	COI <i>P. mac</i>	Hybrid total	EF1 α heterozygous	EF1 α homozygous
Parque Rodó Montevideo	Uruguay (SE)	34°54.81'0"S, 56°10.2'0"W	32	18	14	19	10	9
Punta Gorda	Uruguay (SW)	33°54'33.5"S, 58°25'03.2"W	37	6	31	14	7	7
Magallanes	Uruguay (SW)	33°30'05.4"S, 58°10'38.8"W	28	28	0	0	0	0
Bonito	Brazil (SW)	21°15'53.0"S, 56°33'34.4"W	15	0	15	0	0	0
Campo Grande	Brazil (SW)	20°30'7.63"S, 54°36'59.05"W	6	0	6	0	0	0
Barro Alto	Brazil (Central)	15°3'57.89"S, 48°58'31.48"W	8	0	8	0	0	0
Campinorte	Brazil (Central)	14°19'48.29"S, 49°7'51.96"W	5	0	5	0	0	0
Careiro Castanho	Brazil (NW)	03°49'23.0"S, 60°21'39.0"W	15	5	10	10	5	5
		Total	146	57	89	43	22	21

Site information and species identities, as determined by phylogenetic analysis. For hybrids, heterozygous denotes an individual with an EF1 α haplotype from both species' lineages. Homozygous hybrids carried two EF1 α haplotypes that were discordant with their COI species haplotype. *Pomacea canaliculata* and *P. maculata* have been abbreviated as '*P. can*' and '*P. mac*', respectively.

Cloning

When direct sequencing of amplified EF1 α failed because of the presence of multiple divergent alleles within an individual, we used TOPO TA Cloning kits (Invitrogen, USA) to isolate individual alleles prior to sequencing. Cloning followed the manufacturer's instructions, and we initially sequenced 9–14 positive clones from each of 13 snails. We used rarefaction analysis to determine the number of sequences necessary to capture allelic diversity. This indicated that sequence data from five clones would sufficiently capture EF1 α haplotype diversity for each individual (average = 5.2, $E = 1.9$, variance = 0.06, 95% CI = 1.31–2.00). As such, we sequenced at least five EF1 α clones in both directions from all remaining individuals.

Sequencing

Prior to sequencing at the Institute for Cellular and Molecular Biology, University of Texas, Austin, USA, all amplified products were checked for specificity and quality via gel electrophoresis. Cycle sequencing was performed with ABI BigDye terminator kits (Perkin-Elmer Applied Biosystems, Foster City, CA), and sequences were electrophoresed and analyzed on an ABI 3730xl (Perkin-Elmer Applied Biosystems) machine. Electropherograms were checked for errors and ambiguous calls before assembling contiguous sequences in Geneious v. 11.0.2 (Kearse et al., 2012). All sequences were checked against publicly available databases using BLAST (Johnson et al., 2008) to verify locus and taxon identities prior to phylogenetic analysis. All unique sequences generated from this study are available via GenBank (COI accession numbers MK992470–992488; EF1 α accession numbers MN590311–MN590410).

Phylogenetic analyses

To evaluate introgressive hybridization, we compared biparentally inherited nuclear lineages of EF1 α to uniparentally inherited mitochondrial lineages in a phylogenetic framework. We used the COI sequences from additional *Pomacea* spp. (Hayes, 2009; Hayes et al., 2009b), along with sequences from all unique haplotypes sequenced in this study, in a maximum-likelihood (ML) phylogenetic framework to cluster species. This was compared with clustering patterns of EF1 α to reveal possible hybrids. All sequences were aligned using the MUSCLE plugin v. 3.6 (Edgar, 2004) in Geneious prior to selection of the best fit nucleotide substitution model using the corrected Akaike's information criterion (AICc) in jModelTest v. 2.1.7 (Durriba et al., 2012). For COI haplotypes, we used the masking function in Geneious to create separate alignments to compare the phylogenetic signal between datasets including and excluding the third codon position. Phylogenetic analyses were carried out in PhyML v. 3.0 (Guindon et al., 2010) with the parameters from the best fit model (GTR + G and TPM3 + G for COI and EF1 α , respectively) using 1000 bootstrap replicates to evaluate topological stability (Felsenstein, 1985). For both phylogenetic analyses, we included *P. paludosa* as the outgroup (GenBank acc. nos.: COI, EU528590.1 and EU528591.1; EF1 α , FJ710347.1 and FJ710348.1). We identified hybrids as those in which EF1 α haplotypes were incongruent with assigned COI identity.

RESULTS

In total, 43 of the 146 samples analysed (30%) showed evidence of hybridization (Table 1). The COI phylogenies recovered all taxa as monophyletic consistent with their morphological identifications (Fig. 2). In contrast, the EF1 α -based phylogenies revealed

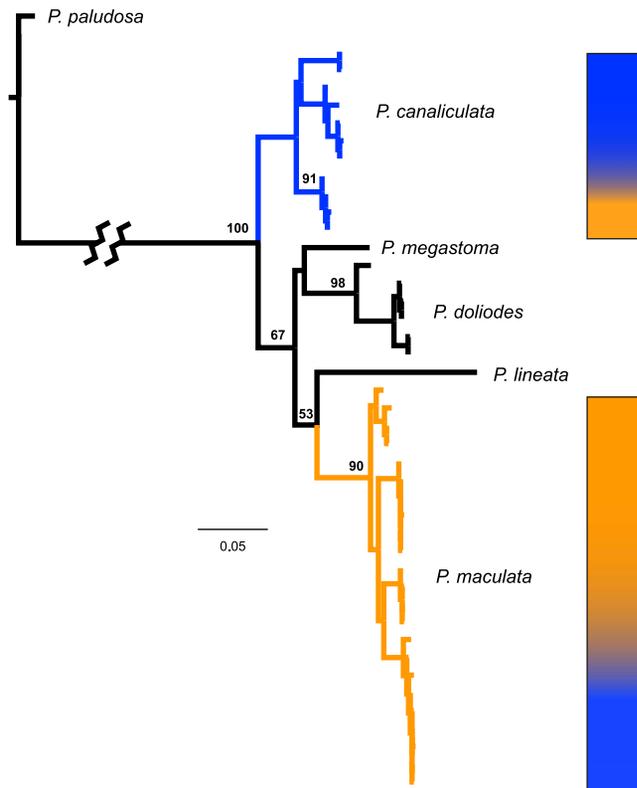


Figure 2. ML phylogeny of selected *Pomacea* species based on COI sequence data (reconstructed using the GTR + G substitution model and 1,000 bootstrap replicates). Only bootstrap support values > 50% are shown. Branches are coloured blue for *P. canaliculata* lineages and orange for *P. maculata*. Coloured boxes illustrate the percentage of concordant EF1 α alleles (same colour as the branches of the associated lineage, e.g. both are blue) vs discordant alleles (different colours to the branches of the associated lineage e.g. blue vs orange) relative to COI (11% and 36% for *P. canaliculata* and *P. maculata*, respectively). Scale bar indicates substitutions per site.

a directional pattern of hybridization with 74% (32) of hybrids carrying a *Pomacea maculata* COI haplotype, while only 26% (11) of hybrids had a *P. canaliculata* COI haplotype (Fig. 2). Most hybrids (77%) occurred at Uruguayan sites containing both *P. canaliculata* and *P. maculata* (Fig. 1); at these sites, we identified 33 of 69 (48%) snails as hybrids. The remaining 23% (10) of hybrids occurred at a site in northern Brazil (Careiro Castanho). At this site, all snails collected carried COI *P. maculata* haplotypes, but 10 of the 15 collected individuals were hybrid (Table 1, Fig. 1); EF1 α haplotypes occurred in equal proportions of homozygous *P. maculata* (i.e. non-hybrid), homozygous *P. canaliculata* (i.e. hybrid backcrosses) and mixed *P. canaliculata* and *P. maculata*.

In contrast to the even distribution of EF1 α alleles at the Brazilian site, both of the Uruguayan sites showed skewed distributions with smaller proportions of mixed haplotypes and a predominance of either *P. maculata* (Punta Gorda) or *P. canaliculata* (Parque Rodó Montevideo). Curiously, we did not find any homozygous EF1 α alleles for *P. maculata* at Parque Rodó Montevideo, although we identified 14 snails as *P. maculata* based on the COI data. As expected, no samples from our site of isolated *P. canaliculata* contained hybrids (Fig. 1). While 51% (22) of the hybrids carried one EF1 α haplotype that clustered with the *P. maculata* clade and one EF1 α haplotype that clustered with the *P. canaliculata* clade (referred to as ‘mixed’ or heterozygous), the remaining 49% (21) of hybrids were homozygous, being characterized by EF1 α haplotypes that clustered with clades that were incongruent with their respective COI haplotypes (Table 1, Figs. 1, 2).

DISCUSSION

Our results demonstrate, for the first time, a clear pattern of introgressive hybridization between *Pomacea canaliculata* and *P. maculata* in their native range. The presence of hybrids with both EF1 α haplotypes that cluster in clades incongruent with the respective COI haplotypes further supports the interpretation of hybrid backcrosses or multiple hybrid generations. Though *P. canaliculata* and *P. maculata* may have diverged as much as 25 Myr (Hayes *et al.*, 2009a, 2009b, 2015), we observed a hybridization rate of 30%. Consequently, we suggest that hybridization in these snails has not emerged as a novel phenomenon in the invaded range, but more likely constitutes an ongoing process with a long evolutionary history (Pennisi, 2018). We further speculate that the prevalence of hybridization in the native range may have facilitated invasion success of these *Pomacea* species in non-native habitats.

Both populations where the two species overlapped contained hybrids. Given that we did not observe similar patterns in five of six non-overlapping populations (Toews & Brelsford, 2012), our data support the hypothesis of introgressive hybridization instead of the alternative explanation of incomplete lineage sorting (Matsukura *et al.*, 2013). Except for a single population near Manaus, Brazil, all EF1 α sequences from single-species populations clustered with the corresponding COI sequence. People often fish using apple snails of unknown identity as bait (Hayes, personal observation), and in this way *P. canaliculata* may be transported far outside its native range. Thus, ongoing anthropogenic dispersal (Capinha *et al.*, 2015) may be a further contributory factor to the spread of this species, providing one plausible explanation for the unexpected hybrids we found at the site in Careiro Castanho, Brazil. This is further supported by the clustering of the COI haplotype from these individuals with geographically distant Argentinean and Uruguayan haplotypes instead of geographically proximate Brazilian haplotypes, a pattern suggestive of a recent introduction. Connectivity between drainage basins also does not explain the patterns we observed in Brazil. The Careiro Castanho mixed site occurs in the Amazonas drainage basin, whereas two of the ‘pure’ *P. maculata* populations (Barro Alto and Campinorte) are located in the basin of the Tocantins River. The two other ‘pure’ *P. maculata* sites in Brazil (Campo Grande and Bonito) occur in the basin of the Paraná River, a tributary of the Río de la Plata. Both the mixed site at Punta Gorda and the ‘pure’ *P. canaliculata* site at Magallanes occur within the basin of the Uruguay River, another tributary of the larger Río de la Plata.

Several previous genetic studies provide diversity and distribution information on the source of non-native populations in Asia and elsewhere (Rawlings *et al.*, 2007; Hayes *et al.*, 2008; Matsukura *et al.*, 2013; Yang *et al.*, 2018). Discovery of hybrids in the native range may provide further insight on other studies. Matsukura *et al.* (2013) was the first to demonstrate that EF1 α sequences of both species occurred in both COI species clades (their ‘clade C’ and ‘clade M’ represent *P. canaliculata* and *P. maculata*, respectively), raising the possibility of either incomplete lineage sorting or genetic exchange between the two species. Matsukura *et al.* (2015) crossed strains to produce F1 hybrids in the laboratory and demonstrated ongoing hybridization as a mechanism to explain the patterns observed in the wild. However, without samples from overlapping and non-overlapping populations in the native range, evidence of genetic exchange in Japan alone failed to reveal whether hybridization was happening prior to their introduction, or was the result of incomplete lineage sorting. By examining sequences from sympatric and allopatric populations of both species found in Uruguay or Brazil, our study is the first to provide data that rules out incomplete lineage sorting.

Supporting the hypothesis that hybridization likely occurs between more than just these two species of apple snails, Yang *et al.* (2018) found multiple populations of an additional undescribed species of *Pomacea* with EF1 α alleles that were identified as *P.*

maculata, *P. canaliculata* or both. Nine sites contained only snails with 'hybrids' (defined as those with discordant EF1 α alleles), six sites with hybrids and *P. canaliculata*, and three sites with hybrids and *P. maculata*. The fact that the undescribed species did not co-occur with either *P. canaliculata* or *P. maculata* further supports the view that hybridization likely occurred prior to introduction, possibly in the native range (Yang *et al.*, 2018). However, multiple introductions throughout Asia (Hayes *et al.*, 2008) may have moved hybrids more than once, making it difficult to determine the exact origin of a given hybrid population.

The complex ecology and reproductive flexibility of apple snails exemplify many issues of invasion biology and hybrid identification (Hayes *et al.*, 2015; Burks *et al.*, 2017). The two native South American ampullariids *P. canaliculata* and *P. maculata* are highly successful invasive species in the USA, East Asia and Europe (Hayes *et al.*, 2008; Oscoz, Tomds, & Duron, 2010; Burks *et al.*, 2017). These two species have received increased attention in the literature in recent decades largely because of their impacts on agricultural crops in Asia (Horgan *et al.*, 2014; Hayes *et al.*, 2015). An evolutionary history that includes introgressive hybridization may help explain how *Pomacea* species have overcome the challenges of dramatic population fluctuations and passive dispersal, which can result in extreme population bottlenecks and decrease population viability (Carson & Templeton, 1984). The processes in that populations in their native range are subjected to as a result of natural disruptions, such as flooding and drought (e.g. loss of genetic variation, inbreeding), mirror those in newly invasive populations (Angert *et al.*, 2011). In both cases, spatial and environmental overlap of the two species facilitates the potential for hybridization to recover genetic variation (Pennisi, 2016).

The high proportion of hybrid individuals observed across the part of the native range studied by us indicates that 'beneficial' diversification via hybridization and subsequent shedding of deleterious alleles may have outweighed possible decreases in fitness (Pfenning *et al.*, 2016). For example, distinct overwintering temperature tolerances exist for invasive *P. maculata*, *P. canaliculata* and their hybrids (Matsukura *et al.*, 2015). The observed resilience of *Pomacea* species to environmental stressors (Matsukura *et al.*, 2015; Glasheen *et al.*, 2017; Mueck *et al.*, 2018) also supports the hypothesis that species that frequently hybridize benefit from a diverse suite of introgressed and conserved adaptive traits (Capinha *et al.*, 2015; Kagawa & Takimoto, 2018). Consistent with this theory, both non-hybrid *P. maculata* and *P. canaliculata* possess many of the qualities associated with range expansions and invasiveness (Yoshida *et al.*, 2014; Burks *et al.*, 2017; Glasheen *et al.*, 2017). Hybridization can promote overall retention of adaptive introgressed alleles, while simultaneously purging maladaptive allele combinations (Rhymer & Simberloff, 1996), a benefit that has been observed following hybridization among invasive freshwater snails belonging to the family Thiariidae (Facon *et al.*, 2005). Consequently, it seems reasonable to infer that in ampullariids hybridization may have helped to drive diversification and adaptation and may possibly have facilitated invasion outside the native range. However, additional studies of pure and hybrid lines are necessary to more fully understand the role of hybridization in the evolution of ampullariid snails.

Diverse gastropod species such as apple snails are also of interest to researchers working on the non-allopatric speciation. Often called ecological speciation (*sensu* Rolán-Alvarez *et al.*, 2004), non-allopatric speciation reflects a scenario where two distinct genetic lineages develop, despite the fact that the source population has not been separated by a vicariance event. In such cases, the line between 'true' hybridization and simple introgression or gene flow between isolated populations becomes blurred (Johannesson *et al.*, 2010). Our results, however, clearly separate incomplete lineage sorting from introgressive hybridization (Toews & Brelsford, 2012). Together with recent work from Asia, our work on apple snails in their native range supports a shift from historical perceptions of hybridization as a driver of extinction and diversity

loss (Capinha *et al.*, 2015; Kagawa & Takimoto, 2018) towards a paradigm where hybridization is a driver of diversification. Hybridization can thus play a vital role in the survival of phylogenetically diverse evolutionary lineages, such as the Mollusca (Régnier *et al.*, 2015).

ACKNOWLEDGEMENTS

We thank Dr Mariana Meerhoff, Fabrizio Scarabino, Cristhian Clavijo, Clementina Calvo, Averi Segrest, Kevin Scriber and Zahra Mansur for their assistance with snail collections in Uruguay. Dr Norine Yeung at the University of Hawaii and Carissa Bishop from Southwestern University provided invaluable laboratory support with genetic analysis. We also thank Dr Silvana Thiengo (Fundação Oswaldo Cruz, Rio de Janeiro, Fiocruz) and Dr Robert Cowie (University of Hawaii at Manoa) for their help in facilitating and participating in Brazilian field collections. In addition, we thank Simone Yoxall for assisting with GIS and the assembly of Fig. 1. Funding was provided by the Keck Foundation, Mellon Foundation, Sam Taylor Foundation, Howard Hughes Medical Institute, the National Science Foundation's (NSF) Office of International Science and Engineering (grant nos. OISE-1130297, OISE-1130694 and OISE-1502853), the NSF's Division of Environmental Biology (DEB-1120906) and Howard University (to K.A.H.). This is contribution no. 2109-002 of the Bishop Museums Hawaii Biological Survey.

REFERENCES

- ABBOTT, R.J. 1992. Plant invasions, interspecific hybridization and the evolution of new plant taxa. *Trends in Ecology and Evolution*, **7**: 401–405.
- ANGERT, A.L., CROZIER, L.G., RISSLER, L.J., GILMAN, S.E., TWEKSBURY, J.J. & CHUNCO, A.J. 2011. Do species' traits predict recent shifts at expanding range edges? *Ecology Letters*, **14**: 677–689.
- ARNOLD, M.L. & KUNTE, K. 2017. Adaptive genetic exchange: a tangled history of admixture and evolutionary innovation. *Trends in Ecology and Evolution*, **32**: 601–611.
- BARNES, M.A., FORDHAM, R.K., BURKS, R.L. & HAND, J.J. 2008. Fecundity of the exotic applesnail *Pomacea insularum*. *Journal of the North American Benthological Society*, **27**: 738–745.
- BURKS, R.L., KYLE, C.H. & TRAWICK, M.K. 2010. Pink eggs and snails: field oviposition patterns indicate shallow aquatic systems susceptible to invasion by *Pomacea insularum*. *Hydrobiologia*, **646**: 243–251.
- BURKS, R.L., BERNATIS, J., BYERS, J.E., CARTER, C., MARTIN, C.W., MCDOWELL, W.G. & VAN DYKE, J. 2017. Identity, reproductive potential, distribution, ecology and management of invasive *Pomacea maculata* in the southern United States. In: *Biology and management of invasive apple snails* (R.H. Cowie, R.C. Joshi & L.C. Sebastian, eds), pp. 293–344. Philippines Rice Institute, Philippines.
- CAPINHA, C., ESSL, F., SEEBANS, H., MOSER, D. & PEREIRA, H.M. 2015. The dispersal of alien species redefines biogeography in the Anthropocene. *Science*, **348**: 1248–1251.
- CARSON, H.L. & TEMPLETON, A.R. 1984. Genetic revolutions in relation to speciation phenomena: the founding of new populations. *Annual Review of Ecology and Systematics*, **15**: 97–131.
- COUTELLE, M.A. & CAQUET, T. 2011. Heterosis and inbreeding depression in bottlenecked populations: a test in the hermaphroditic freshwater snail *Lymnaea stagnalis*. *Journal of Evolutionary Biology*, **24**: 2248–2257.
- CRESSEY, D. 2015. Dolphins, diatoms and sea dragons join census of all known marine life. *Nature News*. www.nature.com/news/.
- DARRIBA, D., TABOADA, F.L., DOALLO, R. & POSADA, D. 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods*, **9**: 772.
- DAYRAT, B. 2005. Integrative taxonomy. *Biological Journal of the Linnean Society*, **85**: 407–415.
- DOWLING, T.E. & DEMARAIS, B.D. 1993. Evolutionary significance of introgressive hybridization in cyprinid fishes. *Nature*, **362**: 444–446.

- EDGAR, R.C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, **32**: 1792–1797.
- FACON, B., JARNE, P., POINTIER, J.P. & DAVID, P. 2005. Hybridization and invasiveness in the freshwater snail *Melanooides tuberculata*: hybrid vigour is more important than increase in genetic variance. *Journal of Evolutionary Biology*, **18**: 524–535.
- FELSENSTEIN, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*, **39**: 783–791.
- FOLMER, O., BLACK, M., HOEH, W., LUTZ, R. & VRIJENHOEK, R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology & Biotechnology*, **3**: 294–299.
- FUKUDA, H., HAGA, T. & TATARA, Y. 2008. Niku-nuku: a useful method for anatomical and DNA studies on shell-bearing molluscs. *Zoosymposia*, **1**: 5–38.
- GLASHEEN, P.M., CALVO, C., MEERHOFF, M., HAYES, K.A. & BURKS, R.L. 2017. Survival, recovery, and reproduction of apple snails (*Pomacea* spp.) following exposure to drought conditions. *Freshwater Science*, **36**: 316–324.
- GRANT, P.R. & GRANT, B.R. 1998. Speciation and hybridization of birds on islands. In: *Evolution on islands* (P.R. Grant, ed.), pp. 142–162. Oxford University Press, Oxford.
- GUINDON, S., DUFAYARD, J.F., LEFORT, V., ASINIMOVA, M., HORKIJK, W. & GASCUEL, O. 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Systematic Biology*, **59**: 307–321.
- HALL, R.J. 2016. Hybridization helps colonizers become conquerors. *Proceedings of the National Academy of Sciences of the USA*, **113**: 9963–9964.
- HARRISON, R.G. & LARSON, E.L. 2014. Hybridization, introgression, and the nature of species boundaries. *Journal of Heredity*, **105**: 795–809.
- HAYES, K.A., JOSHI, R.C., THIENGO, S.C. & COWIE, R.H. 2008. Out of South America: multiple origins of non-native apple snails in Asia. *Diversity and Distributions*, **14**: 701–712.
- HAYES, K.A. 2009. Evolution, molecular systematics and invasion biology of Ampullariidae. PhD thesis. University of Hawaii. Honolulu, HI.
- HAYES, K.A., COWIE, R.H., JØREGENSEN, A., SCHULTHEISS, R., ALBRECHT, C. & THIENGO, S.C. 2009a. Molluscan models in evolutionary biology: apple snails (Gastropoda: Ampullariidae) as a system for addressing fundamental questions. *American Malacological Bulletin*, **27**: 47–58.
- HAYES, K.A., COWIE, R.H. & THIENGO, S.C. 2009b. A global phylogeny of apple snails: Gondwanan origin, generic relationships, and the influence of outgroup choice (Caenogastropoda: Ampullariidae). *Zoological Journal of the Linnean Society*, **98**: 61–76.
- HAYES, K.A., COWIE, R.H., THIENGO, S.C. & STRONG, E.E. 2012. Comparing apples with apples: clarifying the identities of two highly invasive Neotropical Ampullariidae (Caenogastropoda). *Zoological Journal of the Linnean Society*, **166**: 723–753.
- HAYES, K.A., BURKS, R.L., CASTRO-VAZQUEZ, A., DARBY, P.C., HERAS, H., MARTÍN, P.R., QIU, J.-W., THIENGO, S.C., VEGA, I.A., WADA, T., YUSA, Y., BURELA, S., CADIerno, M.P., CUETO, J.A., DELLAGNOLA, F.A., DREON, M.S., FRASSA, M.V., GIRAUD-BILLOU, M., GODOY, M.S., ITUARTE, S., KOCH, E., MATSUKURA, K., PASQUEVICH, M.Y., RODRIGUEZ, C., SAVEANU, L., SEUFFERT, M.E., STRONG, E.E., SUN, J., TAMBURI, N.E., TIECHER, M.J., TURNER, R.L., VALENTINE-DARBY, P.L. & COWIE, R.H. 2015. Insights from an integrated view of the biology of apple snails (Caenogastropoda: Ampullariidae). *Malacologia*, **58**: 245–302.
- HORGAN, F.G., STUART, A.M. & KUDAVIDANAGE, E.P. 2014. Impact of invasive apple snails on the functioning and services of natural and managed wetlands. *Acta Oecologica*, **54**: 90–100.
- JOHANNESON, K., PANOVA, M., KEMPPAINEN, P., ANDRÉ, C., ROLÁN-ALVAREZ, E. & BUTLIN, R.K. 2010. Repeated evolution of reproductive isolation in a marine snail: unveiling mechanisms of speciation. *Philosophical Transactions of the Royal Society of London Series B*, **365**: 1735–1747.
- JOHNSON, M., ZARETSKAYA, I., RAYTSELIS, Y., MEREZHUK, Y., MCGINNIS, S. & MADDEN, T.L. 2008. NCBI BLAST: a better web interface. *Nucleic Acids Research*, **36**: W5–W9.
- KAGAWA, K. & TAKIMOTO, G. 2018. Hybridization can promote adaptive radiation by means of transgressive segregation. *Ecology Letters*, **21**: 264–274.
- KEARSE, M., MOIR, R., WILSON, A., STONES-HAVAS, S., CHEUNG, M., STURROCK, S., BUXTON, S., COOPER, A., MARKOWITZ, S., DURAN, C., THEIRER, T., ASHTON, B., MEINTJES, P. & DRUMMOND, A. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, **28**: 1647–1649.
- LACK, J.B., GREENE, D.U., CONROY, C.J., HAMILTON, M.J., BRAUN, J.K., MARES, M.A. & VAN DEN BUSSCHE, R.A. 2012. Invasion facilitates hybridization with introgression in the *Rattus rattus* species complex. *Molecular Ecology*, **21**: 3545–3561.
- LUCEK, K.A.Y., ROY, D., BEZAULT, E., SIVASUNDAR, A. & SEEHAUSEN, O.L.E. 2010. Hybridization between distant lineages increases adaptive variation during a biological invasion: stickleback in Switzerland. *Molecular Ecology*, **19**: 3995–4011.
- MALLET, J. 2005. Hybridization as an invasion of the genome. *Trends in Ecology & Evolution*, **20**: 229–237.
- MATSUKURA, K., OKUDA, M., CAZZANIGA, N.J. & WADA, T. 2013. Genetic exchange between two freshwater apple snails, *Pomacea canaliculata* and *Pomacea maculata* invading East and Southeast Asia. *Biological Invasions*, **15**: 2039–2048.
- MATSUKURA, K., IZUMI, Y., YOSHIDA, K. & WADA, T. 2015. Cold tolerance of invasive freshwater snails, *Pomacea canaliculata*, *P. maculata*, and their hybrids helps explain their different distributions. *Freshwater Biology*, **61**: 80–87.
- MUECK, K., DEATON, L.E., LEE, A. & GUILBEAUX, T. 2018. Physiology of the apple snail *Pomacea maculata*: aestivation and overland dispersal. *Biological Bulletin*, **235**: 43–51.
- OSCOZ, J., TOMDS, P. & DURON, C. 2010. Review and new records of non-indigenous freshwater invertebrates in the Ebro River basin (Northeast Spain). *Aquatic Invasions*, **5**: 263–284.
- PENNISI, E. 2016. Shaking up the tree of life. *Science*, **354**: 817–821.
- PENNISI, E. 2018. Ancient hookups between different species may explain Lake Victoria's stunning diversity of fish. *Science News*, www.sciencemag.org/news/
- PFENNING, K.S., KELLY, A.L. & PIERCE, A.A. 2016. Hybridization as a facilitator of species range expansion. *Proceedings of the Royal Society of London Series B*, **283**: 20161329.
- RAWLINGS, T.A., HAYES, K.A., COWIE, R.H. & COLLINS, T.M. 2007. The identity, distribution, and impacts of non-native apple snails in the continental United States. *BMC Evolutionary Biology*, **7**: 97.
- RÉGNIER, C., ACHAZ, G., LAMBERT, A., COWIE, R.H., BOUCHET, P. & FONTAINE, B. 2015. Mass extinction in poorly known taxa. *Proceedings of the National Academy of Sciences of the USA*, **112**: 7761–7766.
- RHYMER, J.M. & SIMBERLOFF, D. 1996. Extinction by hybridization and introgression. *Annual Review of Ecology & Systematics*, **27**: 83–109.
- RIESEBERG, L. & CARNEY, S. 1998. Plant hybridization. *New Phytologist*, **140**: 599–624.
- ROBERTS, H.F. 1929. *Plant hybridization before Mendel*. Oxford University Press, Oxford.
- ROLÁN-ALVAREZ, E., CARBALLO, M., GALINDO, J., MORAN, P., FERNANDEZ, B., CABALLERO, A., CRUZ, E., BOULDING, E.G. & JOHANNESON, K. 2004. Nonallopatric and parallel origin of local reproductive barriers between two snail ecotypes. *Molecular Ecology*, **13**: 3415–3424.
- SAVEANU, L. & MARTÍN, P.R. 2013. Pedal surface collecting as an alternative feeding mechanism of the invasive apple snail *Pomacea canaliculata* (Caenogastropoda: Ampullariidae). *Journal of Molluscan Studies*, **79**: 11–18.
- STRONG, E.E., GARGOMINY, O., PONDER, W.F. & BOUCHET, P. 2008. Global diversity of gastropods (Gastropoda; Mollusca) in freshwater. *Hydrobiologia*, **595**: 149–166.

- TAMBURI, N.E. & MARTÍN, P.R. 2011. Effects of food availability on reproductive output, offspring quality and reproductive efficiency in the apple snail *Pomacea canaliculata*. *Biological Invasions*, **13**: 2351–2360.
- TESHIMA, H., DAVISON, A., KUWAHARA, Y., YOKOYAMA, J., CHIBA, S., FUKUDA, T., OGIMURA, H. & KAWATA, M. 2003. The evolution of extreme shell shape variation in the land snail *Ainohelix editha*: a phylogeny and hybrid zone analysis. *Molecular Ecology*, **12**: 1869–1878.
- TOEWS, D.PL. & BRELSFORD, A. 2012. The biogeography of mitochondrial and nuclear discordance in animals. *Molecular Ecology*, **16**: 3907–3930.
- YANG, Q., LIU, S., HE, C., COWIE, R.H., YU, X. & HAYES, K.A. 2018. Invisible apple snail invasions: importance of continued vigilance and rigorous taxonomic assessments. *Pest Management Science*, **75**: 1277–1286.
- YOSHIDA, K., MATSUKURA, K., CAZZANIGA, N.J. & WADA, T. 2014. Tolerance to low temperature and desiccation in two invasive apple snails, *Pomacea canaliculata* and *P. maculata* (Caenogastropoda: Ampullariidae), collected in their original distribution area (northern and central Argentina). *Journal of Molluscan Studies*, **80**: 62–66.