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RESEARCH

Taiwan's mysterious mollusks: a deep dive into the cryptic hybridization of *Pomacea canaliculata* and *Pomacea maculata*

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Abstract

Introgressive hybridization is a pivotal force influencing genetic diversity and adaptive potential of invasive species, however to date has received less attention for even the most globally destructive species. Golden apple snails (*Pomacea* spp.), one of the world's worst invasive species, were first introduced during the 1980s into Taiwan for commerce and have since emerged as a significant threat to regional biodiversity. Two of the most destructive invasive species of *Pomacea*, *P. canaliculata* and *P. maculata*, have been reported to hybridize in its native and non-native range, the latter of which has been posited to facilitate an adaptive advantage for increasing invasiveness. Thus, our study combined mitochondrial *COI* (Cytochrome c oxidase subunit I) barcoding and nuclear *EF1-α* (elongation factor 1-alpha) gene analysis to identify putative hybridization among 254 samples collected across 14 cities/counties in Taiwan. Our investigation confirmed hybridization within the sympatric but heterogeneous distribution of *P. canaliculata* and *P. maculata* in Taiwan. The results indicated that 18.9% of the total sampled population consists of hybrids, whereas 81.1% of the samples demonstrated pure *P. canaliculata* genetics. Remarkably, no pure *P. maculata* were detected across the island, and all *P. maculata* mtDNA samples appeared to be genetically introgressed. The dearth of pure *P. maculata* populations, despite the prevalence of *canaliculata-maculata* hybrids, invites speculation about introduction mechanisms, genetic drift and maybe the nuanced influence of environmental factors on distribution dynamics, all of which required further investigation. These findings contribute significant scientific insights into the invasive dynamics of *Pomacea* in Taiwan, and the results underline findings applicable for devising targeted and effective conservation strategies in regions grappling with the challenges posed by invasive species.

Keywords Biological invasion · Introgressive hybridization · DNA barcoding · Golden Apple snails · Agricultural pests

Extended author information available on the last page of the article

Introduction

Introgressive hybridization is an important phenomenon demonstrated to increase adaptive genetic variation (Arnold 2009; Grant and Grant 2019). However, it can also impose negative consequences to wildlife, particularly when it is observed between native and invasive species or even between two invasive species (Krahulcová et al. 1996; Yamaguchi et al. 2019; Yang et al. 2020). Hybridization events may even escalate the destructive nature of invasive species and its ensuing damage to the local environment (Suehs et al. 2004; Facon et al. 2005), yet identifying such invasive hybridization events is often difficult to discern due to its cryptic nature (Banerjee et al. 2022).

Taiwan, a small island noted with an incredible array of native yet imperiled biodiversity (over 50,000 species or 2.6% of Earth's biodiversity) (Shao and Chung 2022), is also known for its continuous onslaught of invasive species (Wu et al. 2004). *Pomacea* spp. were introduced in Taiwan for commercialization during the 1980s (Cowie et al. 2006; Hayes et al. 2008), however, following its lack of success in the food market, they were subsequently released into the wild and became highly invasive with time (Wu et al. 2011). Among all the introduced species of *Pomacea*, *Pomacea canaliculata* (Lamarck,1822) is considered as one the most invasive species in the world ("top 100"; Lowe et al. 2000), causing a significant amount of economic loss in Taiwan as well as other Asian countries (specifically as an agricultural pest) (Turbelin et al. 2023). However, emphasizing its negative impact on regional biodiversity, some of the *Pomacea* spp. are also known to cryptically hybridize, making their tracking and mitigation even more difficult (Yang et al. 2020; Kannan et al. 2021). In particular, *P. canaliculata* has demonstrated overlapping morphological characteristics (shell coloration, size, thickness; Banerjee et al. 2022) with another highly invasive congener *P. maculata* Perry, 1810 [previously known as *P. insularum* (d'Orbigny, 1835)], and recently they were noted to hybridize in their native (Argentina, Brazil) and non-native ranges (China, Taiwan, Japan, USA and pacific islands) (Yang et al. 2020; Glasheen et al. 2020; Kannan et al. 2021).

Within the last few decades in Taiwan, the presence of two species, *P. canaliculata* and *P. scalaris*, were predominantly reported based on morphological characters alone (Hayes et al. 2008; Wu et al. 2011). However, the presence of a third species, *P. maculata* was identified recently based on mitochondrial *COI* and found to be co-distributed with *P. canaliculata* (Banerjee et al. 2022). While morphological distinction between *P. canaliculata and P. scalaris* is possible (Wu et al. 2011; Figure S1), however *P. maculata* were noted to be highly cryptic with *P. canaliculata*. Indeed, the presence of highly overlapping morphological characteristics further suggests the possible presence of cryptic hybrids in Taiwan (Banerjee et al. 2022). Thus, their identification and distinction based on morphological characters alone remains challenging.

Given the detrimental impacts of *Pomacea* spp. populations in Taiwan specifically, and the potential for invasive hybridization to complicate or obscure current management measures, our investigation focusses on molecular techniques to more accurately map their nation-wide distribution as well as their putative hybrids to help in developing more effective mitigation strategies. Samples were taken from 14 different counties/cities across Taiwan to identify *Pomacea* spp. The molecular identifications were done with mitochondrial *COI* barcoding (*sensu* Banerjee et al. 2024), and the interspecific hybrids were screened through combining mtDNA data with nuclear genetic (*EF1-a*: elongation factor 1-alpha)

information. Here, we anticipate that identifying the current hybridization pattern of *P. canaliculata* and *P. maculata* across Taiwan can aid us with a more impactful management strategy against invasive species.

Methods

Ethical statement

The involvement of *Pomacea* spp., and all experiments were approved by the Animal Experimentation Ethics Committee of the National Chung Cheng University, Taiwan. As targeted species are invasive and not listed under any conservation act, no special permission was needed for sampling.

Sampling

We sampled *Pomacea* spp. specimens over a period of 33 months, from April 2020 to January 2023. *Pomacea* spp. can be sampled at anytime throughout the year, but they may hibernate if food is scarce, or water is insufficient. We thus synchronized our sampling with water availability in rice fields, aiming for the rice growing season to maximize detection and capture. Our sampling campaign covered most of Taiwan, with 41 sampling locations across the country, including 14 counties/cities (Table S1). Generally, we collected 10–12 *Pomacea* spp. (field-identified up to genus based upon morphological characteristic) from each location (agricultural fields or drains). We then transported the snail samples back to the laboratory on the same day. When same-day transportation was not possible, we stored each sample in a separate sampling bag on ice until arrival back at the laboratory. In the lab, we heat-shocked the snail samples in a microwave to remove the inner tissue, and then we cleaned the tissue with double distilled water to avoid contamination and stored the tissues at 4 °C until further analysis.

Genomic DNA extraction

We selected the foot tissues of the snails for DNA extraction to avoid contamination, and DNA was extracted using Qiagen DNeasy Blood and Tissue Kit (Qiagen, Germany) following Banerjee et al. (2022). The quality and quantity were checked using nanodrop spectrophotometer (Implen, Germany) and qubit fluorometer (Thermo Fisher Scientific, USA).

Mitochondrial COI based identification

We identificatied *Pomacea* spp. using the primer sets PcanCOI (5′-TGG GGT ATG ATC AGG CC-3′), PinsCOI (5′-ATC TGC TGC TGT TGA AAG-3′), and HCO2198 (5′-TAA ACT TCA GGG TGA CAA AAA AAT CA-3′) designed by Matsukura et al. (2008) and PCR conditions were followed by Banerjee et al. (2022). Also we used the mitochondrial data generated in our previous study Banerjee et al. (2024) where we amplified the full folmer regions using the primers LCO1490 (5′-GGT CAA CAA ATC ATA AAG ATA TTG G-3′) and HCO2198 (5′ TAA ACT TCA GGG TGA CAA AAA AAT CA-3′) (Folmer et al.,

1994). The initial primers (PanCOI, PinsCOI and HCO2198) were chosen for quick multiplex PCR identification which later supported by full folmer region with LCO1490 and HCO2198 primer pairs (~680 bp). We then aligned all sequences utilizing the MUSCLE v5.1 algorithm (Edgar 2004) and subsequently edited in MEGA v11 (Tamura et al. 2021) and GENEIOUS v11.1.5 (Kearse et al. 2012).

Amplification of EF1-α for hybrid detection

We aimed to detect hybrids among the *Pomacea* spp. by amplifying a portion elongation factor 1-α (*EF1-α*) of the nuclear genome (~ 520 bp), followed by restriction endonuclease digestion. A unique restriction site for *ApaLI* present in the *EF1-α* sequence (within 520 bp) of *P. maculata* generates two fragments of 190 and 330 bp after digestion (Hayes et al. 2009; Matsukura and Wada 2017), and this particular site was absent in *P. canaliculata*, resulting in a 520 bp intact product. In the case of hybrids, all three fragments (190, 330, 520 bp) were present. This gave us a convenient and robust method for species and hybrid differentiation without the need for additional sequencing. However, we sequenced a few samples to confirm the identification.

We amplified the *EF1-α* gene using primers EF1αF (5′-TGT GAA TAA GAT GGA CAG CA-3′) and EF1αR (5′-AAT CCT AAC CTC CAA TTT TGT-3′) obtained from Hayes et al. (2009). We prepared the reaction mixture of 25 µl using 0.5 µl each of forward and reverse primer, 5 µl of 5× Fast-Run™ Taq Master Mix with Dye (Protech Technology Enterprise, Taiwan), 2 µl of DNA extract, and made a total volume of 25 µl using ultrapure water (Thermo Fisher Scientific, USA). We then carried out the PCR on the CLUBIO PCR system with a 96-well temperature gradient PCR (CB series, Scientific Biotech Corp., Taiwan), comprising the cycling parameters as follows: initial denaturation of 5 min at 94 °C followed by 36 cycles of amplification; with denaturation of 30 s at 94 °C, annealing of 30 s at 50 °C, and extension of 1 min at 72 °C, with a final extension at 72 °C for 5 min. We finally purified the PCR product using GeneSpin™ DNA Extraction/PCR Clean-up (Protech Technology Enterprise, Taiwan), following the manufacturer's protocol.

For the Restriction Fragment Length Polymorphism analysis (RFLP), we added the restriction enzyme mixed to the amplification product; 5 µl CutSmart® Buffer (New England BioLabs, USA), 1 µl *ApaLI* restriction enzyme (New England BioLabs, USA), up to a final volume of 50 µl using ultrapure water. We subsequently incubated the reaction mixture in the thermal cycler set at the following conditions: 37 °C for 30 min, then 60 °C for 15 min to deactivate the enzyme. Finally, we ran the digested product on a 2% agarose gel with a 100 bp ladder (Protech Technology Enterprise, Taiwan) to check for the presence of hybrids among all of our snail samples.

Combining mitochondrial COI and EF1-α data

We combined the mitochondrial *COI* barcodes and *EF1-α* gene data to identify the hybrids from pure species. Generally six possible combinations were noted for hybrids (Yang et al. 2020): (i) presence of both mitochondrial *COI* and *EF1-α* gene of *P. canaliculata* (pure *P. canaliculata*; CC), or (ii) both mitochondrial *COI* and *EF1-α* gene of *P. maculata* (pure *P. maculata*; MM), or (iii) mitochondrial *COI* of *P. canaliculata* and *EF1-α* gene of *P. maculata* (hybrid; CM), or (iv) mitochondrial *COI* of *P. canaliculata* and *EF1-α* gene of *P. cana-*

liculata and *P. maculata* (backcross or F2 hybrid with *P. canaliculata* haplotype; CB), or (v) mitochondrial *COI* of *P. maculata* and *EF1-α* gene of *P. canaliculata* (hybrid; MC), or (vi) mitochondrial *COI* of *P. maculata* and *EF1-α* gene of *P. canaliculata* and *P. maculata* (backcross or F2 hybrid with *P. maculata* haplotype; MB) (Fig. 1).

Results

A total of 254 *Pomacea* spp. specimens (246 *P. canaliculata* and 8 *P. maculata*) were successfully amplified using mitochondrial *COI* and *EF1-α* barcodes. Comparison of *COI* and *EF1-α* barcodes resulted in detection of a large portion of pure *P. canaliculata* (206 samples; 81.10%) that contain both the mitochondrial and nuclear barcode of *P. canaliculata* (CC type). On the other hand, the remaining 18.9% (48 samples) were found to be hybrids or demonstrated some level of genetic introgression. Among them, three kinds of genetic admixture (hybrid) classes were noted; (i) the CB type (*P. canaliculata* mitochondrial barcode with both *P. canaliculata* and *P. maculata* nuclear barcode) was the most dominant hybrid classification likely representing *P. canaliculata* backcrosses, consisting of 83.3% (40 samples) of total admixed samples; (ii) the MC type (*P. maculata* mitochondrial barcode with *P. canaliculata* nuclear barcode) were observed 12.5% (6 samples); and (iii) the MB type (*P. maculata* mitochondrial barcode with both *P. canaliculata* and *P. maculata* nuclear barcode) possibly representing *P. maculata* backcrosses were 4.2% (2 samples). Our sampling thus showed that only 16.7% (8 of 48) of the hybrids contained *P. maculata* mtDNA

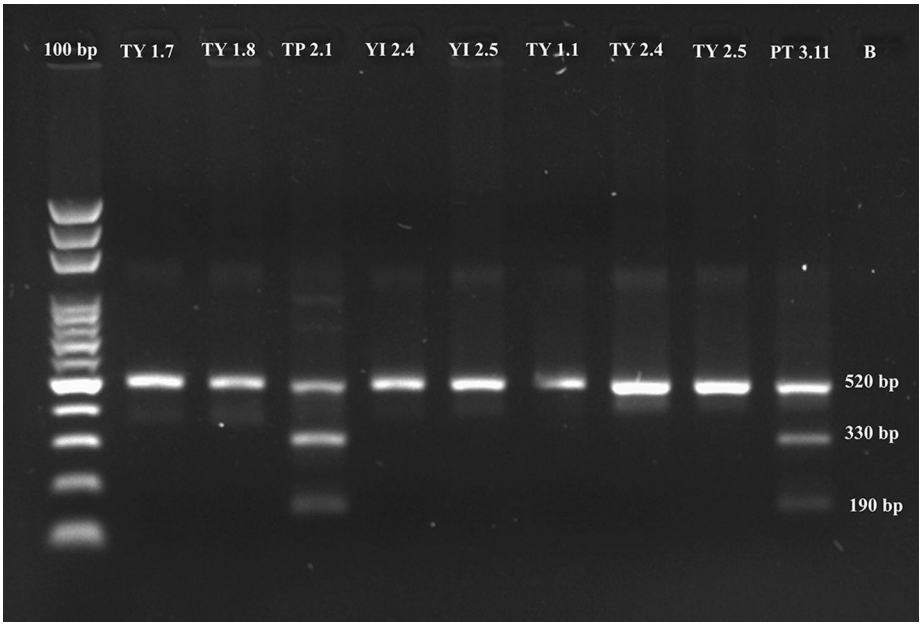

Fig. 1 Identification of hybrids among *Pomacea* spp. samples using Restriction Enzyme digestion of the nuclear *EF1-α* amplicon with *ApaLI*. *P. canaliculata* shows just one 520 bp fragment, *P. maculata* shows two fragments of 330 and 190 bp, while the hybrids exhibit all three of these fragments. Codes indicate sampling numbers, details can be found in Table S1

with varying nuclear barcode. From a nuclear DNA perspective, 42 out of the 48 hybrid individuals (87.5%) contained both *P. canaliculata* and *P. maculata EF1-α* barcodes, whereas only 6 hybrid individuals (4.2% of hybrids) contained pure *P. canaliculata EF1-α* barcodes (although with mtDNA of *P. maculata*). The complete absence of other possible genetic profiles, such as CM (*P. canaliculata* mitochondrial barcode with *P. maculata* nuclear barcode) and any pure *P. maculata* such as MM (*P. maculata* mitochondrial barcode with *P. maculata* nuclear barcode) further demonstrates genetically skewed populations across the studied region (Figs. 2 and 3). Although limited within local populations proportionately, all *P. maculata* reported from Taiwan were hybrids and consisted of either MC or MB type.

Specifically, among the cities or counties, Yilan (Total: 39%; CB: 31%, and MC: 8%), Changhua (CB: 33%), Chiayi (Total: 29%; CB: 25%, and MC: 4%) and Pingtung (Total: 27%; CB: 24%, and MB: 3%) showed highest percentage of hybrid diversity (over 25%), followed by Taipei (CB: 25%), Yunlin (CB: 23%), Kaohsiung (Total: 18%; CB: 5%, and MC: 14%), Hualien (CB: 17%), Miaoli (Total: 14%; CB: 7%, and MC: 7%), Tainan (Total: 12%; CB: 8%, and MB: 4%), Taitung (CB: 8%), and Hsinchu (CB: 7%) (Fig. 3a). However, no hybrid was reported from Taichung and Taoyuan. The rarest type, MB, was only reported from Pingtung and Tainan. The presence of MC was reported in Miaoli, Yilan, Chiayi, and Kaohsiung. The other two genetic classifications were described in almost all sampling sites (except Taichung and Taoyuan where only CC was reported).

The region specific investigation (Fig. 3b) indicate that Southern Taiwan exhibited the highest percentage of hybrids among its samples (Total: 21%; CB: 15%, MC: 4%, and MB 2%), followed by the North (Total: 18%; CB: 16%, and MC: 2%) and Middle regions (Total: 18%; CB: 16%, and MC: 2%), whereas the lowest number of hybrids were found in the East of Taiwan (CB: 12%). Importantly, the presence of three different types of hybrids (CB, MC, and MB) were observed in the South of Taiwan (Fig. 3b).

Discussion

The invasive impact of *Pomacea* is remarkable throughout Asia (Joshi 2007; Turbelin et al. 2023). Introgressive hybridization appears to be prevalent among numerous invaded countries such as mainland China, Hong Kong, Japan, South Korea, Philippines, Vietnam, and the Malaysia Peninsular (Matsukura et al. 2013; Yang et al. 2020, 2022; Kannan et al. 2021), as well as their native countries such as Brazil and Uruguay (Glasheen et al. 2020). Our study is the first to report hybridization of *P. canaliculata* and *P. maculata* in Taiwan as well. The nuclear *EF1-α* gene combined with *COI* also supports a sympatric and heterogeneous distribution of *P. canaliculata*, *P. maculata*, and their hybrids, across Taiwan (Fig. 1) (as suggested previously by Banerjee et al. 2022, 2024). Interestingly, our study showed that 18.9% of the sampled *Pomacea* individuals across Taiwan were hybrids, whereas the rest of the individuals represented pure *P. canaliculata* (81.10%) without any indication of pure *P. maculata.* While previous research has suggested the dominance of *P. canaliculata* over *P. maculata* via *COI* data alone, it is now clear that the only individuals with *P. maculata* mtDNA within Taiwan are of hybrid origin. Notably, all *P. canaliculata* in Taiwan were observed to be either pure (CC type) or introgressed with nuclear genome of *P. maculata* (CB type), however no CM type (*P. canaliculata* with pure nuclear barcode of *P. maculata*) was documented (Table S1). Furthermore, all the *P. maculata* samples were noted to be

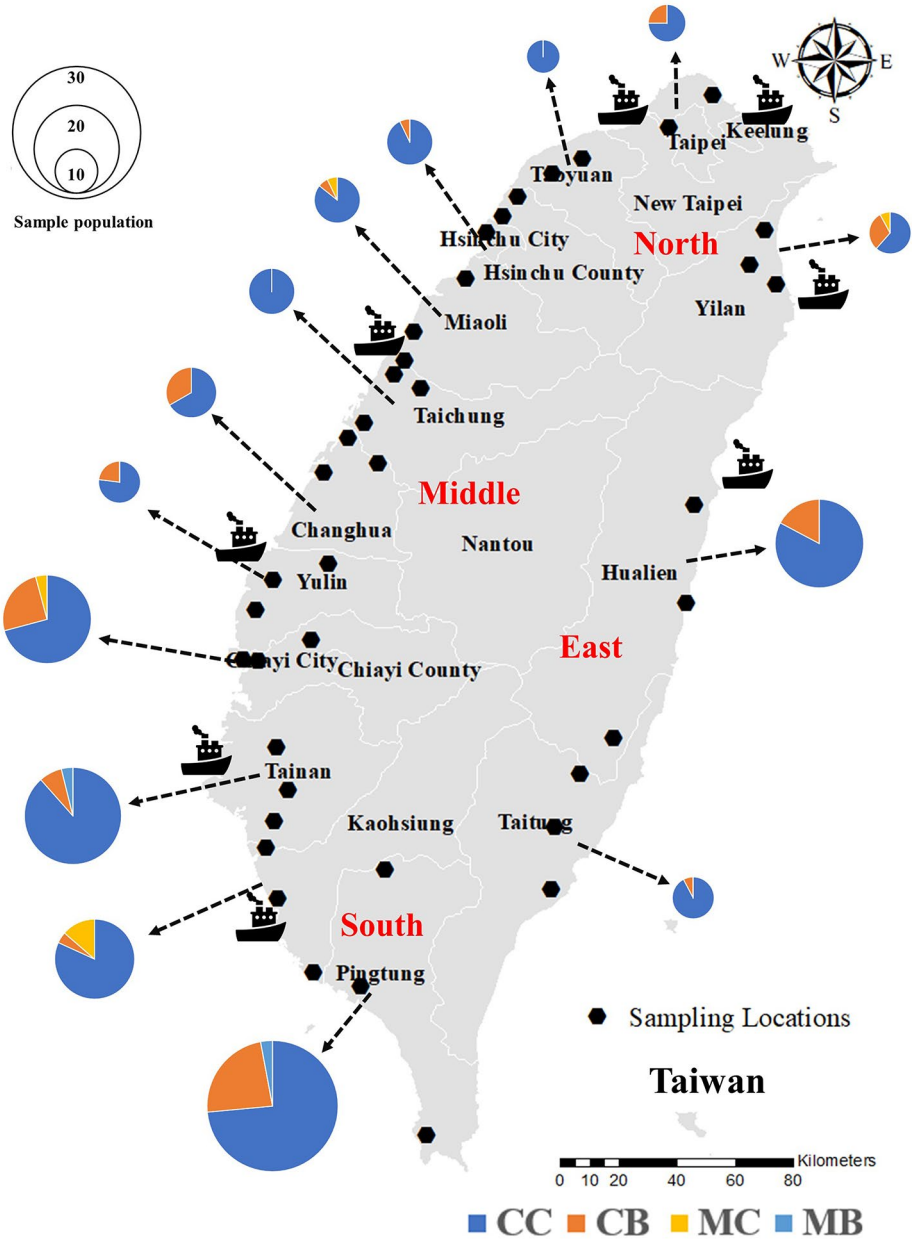

Fig. 2 Map of Taiwan showing 41 sampling locations within 14 counties/cities covering all possible land part (except mountain in the middle)

hybrids (MC, MB). A similar pattern was also reported by Yang et al. (2020) from mainland China, where the rarity of the CM type and a complete absence of the MM type (pure *P. maculata*) was reported. Yang et al. (2022) also noted a similar distribution pattern of *P. canaliculata* and *P. maculata* hybrids in Hong Kong, with only a single pure *P. maculata*

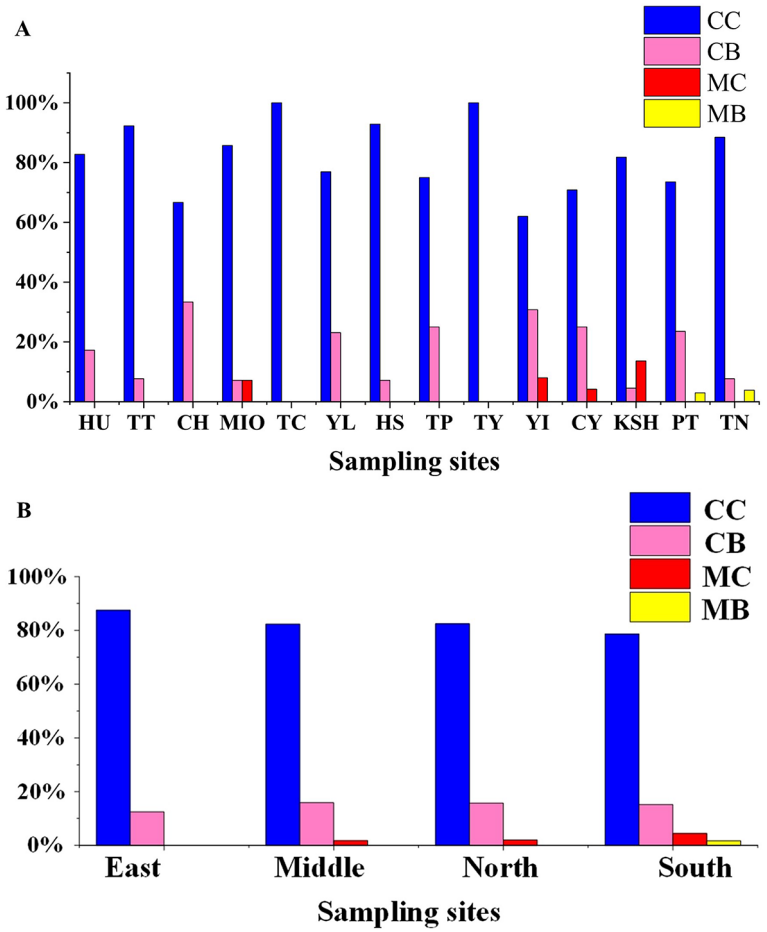

Fig. 3 The percentage of sampled *Pomacea* spp. grouped according to genetic introgression (**A**) from each of the sampled counties/cities, and (**B**) from the four regions of Taiwan: North, South, East, and Middle. In the genotype codes, the first character denotes *COI*, the second character denotes *EF1-α*. For details, please see Materials and Methods. Character keys: C- canaliculata type, M- maculata type, B- both (hybrid)

(MM) specimen reported in this location. Thus, our description of hybridization proportions for *Pomacea* spp. across Taiwan corroborate those found in other invaded Asian countries.

This putative pattern of cyto-nuclear discordance within Taiwan could signal a couple of potential scenarios: (i) stark asymmetrical introgression, or (ii) mitochondrial capture. For example, asymmtrical introgression could be a result of some type of intrinsic or extrinsic reproductive barrier preventing hybridization and gene flow between these two species. While this study did not specifically investigate the causal mechanisms into hybridization dynamics, nor their consequences, previous research by Matsukura and colleagues (Matsukura et al. 2016) were able to generate fertile hybrids between *P. canaliculata* and *P. maculata* in the laboratory, giving at least some evidence for a lack of strong intrinsic reproductive isolation. Alternatively, the cyto-nuclear discordance could be due to some type

of mitochondrial capture (e.g. Yang et al. 2020) in which the mtDNA of *P. canaliculata* is selected and incorporated over *P. maculata* during hybridization due to potential fitness advantages. Research by Yang et al. (2020) reported *P. maculata* likely prefers the nuclear genome of *P. canaliculata* to improve desiccation and cold tolerance ability, but we are unaware of any research specifically looking into mitochondrial capture in *Pomacea* spp. Since *Pomacea* spp. are dioecious (contain distinct sexes; Cowie 2002), this may also indicate a functional advantage for the maternal lineage (in this case, *P. canaliculata).* Both of the above scenarios have been observed in various species (e.g., reproductive isolation barriers, Johanneson et al. 2010, Stewart and Lougheed 2013; mitochondrial capture, Good et al. 2008; Rodriguez and Krug 2022), having evolutionary implications such as the potential to influence the adaptation and fitness of hybrid individuals in their new environment (Ellstrand and Schierenbeck 2000). Our study cannot differentiate among these scenarios nor whether either of these mechanisms occur prior to or after invasion. However, this work does elucidate complex hybridization signatures within Taiwan that warrant further investigations on those topics.

Interestingly, our study further exhibited that the percentage of hybrids discovered across Taiwan (18.9%) is distinctly lower than the percentage of hybrids in mainland China (76.45%) (Yang et al. 2020), Hong Kong (nearly 50%) (Yang et al. 2022), the Malaysian Peninsular (42.2%), as well as that of the native populations in Brazil and Uruguay (29.45%) (Glasheen et al. 2020). However, the distribution of pure *P. maculata* (MM type) is still remarkably rare within other introduced countries (China, Peninsular Malaysia, Hong Kong, Taiwan) compared to its native range (Brazil and Uruguay) (Yang et al. 2022). The dearth of pure *P. maculata* populations in their introduced zone is not consistent with the frequency of *canaliculata-maculata* hybrids, possibly suggesting historical hybridization prior to invasion. Hybrid invasive populations therefore likely act as the source for the *P. maculata* genome within Taiwan. Alternatively, *P. maculata* may be undergoing successive hybridization after introduction into Taiwan (and other Asian countries) (see also Matsukura et al. 2013), although likely to a far lesser extent. It is also possible that environmental factors may have limited and contributed to the (unequal) distribution of *P. canaliculata* and *P. maculata* as well as their hybrids (Fig. 2) (Yoshida et al. 2014; Matsukura et al. 2016). Differentiating among all of these hypotheses necessitates broad-scale, whole genomic research and comparison.

The distribution of the hybrids across Taiwan appears to follow no particular environmental or geographical boundaries, with the most common type of “CB” hybrids appearing in all sampling sites except for Taichung and Taoyuan (Middle-West to North-West; Fig. 3a). The *P. maculata-COI* hybrids, “MC” and “MB” types, were conversely more geographically restricted and commonly found in the Southern region (Fig. 3b), with a few also in the Middle-West region (Miaoli), and even the far North (Yilan). Adding more nuclear markers and broadening more sampling locales could help us to refine the current complex hybridization dynamics occurring throughout Taiwan and more effectively target mitigation strategies.

Conclusions

Our investigation combining mitochondrial and nuclear barcodes confirmed hybridization between *P. canaliculata* and *P. maculata* in Taiwan for the first time, significantly contributing to the current understanding of the invasive dynamics of these highly destructive species. The prevalence of hybrids, comprising 18.9% of the sampled population, introduces a complex dynamic to the invasive scenario within Taiwan. The absence of pure *P. maculata* in Taiwan, similar with its rarity in other introduced ranges, corroborates the dominance of *P. canaliculata* across Asia, and perhaps signals a higher prevalence of historical hybridization rather than ongoing hybridization within invaded countries. While our findings provide valuable insights into the current status of *P. canaliculata* and *P. maculata* in Taiwan, several questions remain, including whether hybridization is ongoing or a historical remanent persisting long after hybridization cessation, and whether hybridization is more prevalent prior to, or after invasion. These question remain impossible to discern with our current data because *CO1* and *EF1-α* are only single loci for mtDNA and nuDNA respectively, making it difficult to discern timing (contemporary vs. historical) and placement (native vs. invasive) of hybridization and gene flow. Additionally, understanding the potential role of environmental factors in limiting the distribution of pure *P. maculata*, and elucidating the potential role of hybridization in invasive adaptation would have broad interest to evolutionary ecologists, invasion biologists, and management practitioners alike. As we continue to unravel the intricacies of *Pomacea* invasion dynamics in Asia, it becomes crucial to monitor and manage these invasive species (e.g. Banerjee et al. 2024),and their putative hybrids (e.g. Stewart and Taylor 2020) through widespread and innovative means in order to best mitigate potential ecological consequences and further spread.

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Author contributions P.B. and C.Y.C., conceived of the idea; P.B., and N.C., performed experiments, P.B., N.C., K.A.S., and C.Y.C., prepared the first draft and revised the manuscript, figures and tables. G.D., C.W.W, P.Y.L, J.S.C, R.K.S, J.P.M., and K. H. L., gave extensive edits and revised the manuscript.

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Data availability All data obtained in this study have been deposited to GeneBank under the accession number OQ977961 - OQ978203 and OQ977020-OQ977027 and supplementary part of this paper.

Declarations

Competing interests The authors declare no competing interests.

References

Arnold ML, Martin NH (2009) Adaptation by introgression. J Biol 8(9):1–3

Banerjee P, Dey G, Maity JP, Stewart KA, Sharma RK, Chan MW, Lee K, Chen CY (2024) The unseen invaders: tracking phylogeographic dynamics and genetic diversity of cryptic Pomacea canaliculata and P. maculata (golden apple snails) across Taiwan. Ecol Evol 14(4):e11268

Banerjee P, Stewart KA, Dey G, Sharma RK, Maity JP, Chan MW, Chen CY (2022) When conventional methods fall short: identification of invasive cryptic Golden Apple snails (Pomacea canaliculata; P. maculata) using environmental DNA. Hydrobiologia 849(19):4241–4257

Cowie RH (2002) Apple snails (Ampullariidae) as agricultural pests: their biology, impacts and management. In: Barker G (ed) Molluscs as crop pests. CAB-International, Wallingford, UK, pp 145–192. https://doi.org/10.1079/9780851993201.0145

Cowie RH, Hayes KA, Thiengo SC (2006) What Are Apple Snails? Confused Taxonomy and Some Preliminary Resolution. Dalam Global Advances in Ecology and Management of Golden Apple Snails. RC Joshi and LS Sebastian (Eds), 3–23. PhilRice. Philippines

Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 32(5):1792–1797

Ellstrand NC, Schierenbeck KA (2000) Hybridization as a stimulus for the evolution of invasiveness in plants? Proc Natl Acad Sci 97(13):7043–7050

Facon B, Jarne P, Pointier JP, David P (2005) Hybridization and invasiveness in the freshwater snail Melanoides tuberculata: hybrid vigour is more important than increase in genetic variance. J Evol Biol 18(3):524–535

Folmer O, Black M, Hoeh W, Lutz W, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol Mar Biol Biotechnol 3:294–299

Glasheen PM, Burks RL, Campos SR, Hayes KA (2020) First evidence of introgressive hybridization of apple snails (Pomacea spp.) in their native range. J Molluscan Stud 86(2):96–103

Good JM, Hird S, Reid N, Demboski JR, Steppan SJ, MARTIN-NIMS TR, Sullivan J (2008) Ancient hybridization and mitochondrial capture between two species of chipmunks. Mol Ecol 17(5):1313–1327

Grant PR, Grant BR (2019) Hybridization increases population variation during adaptive radiation. Proc Natl Acad Sci 116(46):23216–23224

Hayes KA, Cowie RH, Thiengo SC (2009) A global phylogeny of apple snails: Gondwanan origin, generic relationships, and the influence of outgroup choice (Caenogastropoda: Ampullariidae). Biol J Linn Soc Lond 98(1):61–76

Hayes KA, Joshi RC, Thiengo SC, Cowie RH (2008) Out of South America: multiple origins of non-native apple snails in Asia. Divers Distrib 14(4):701–712

Johannesson K, Panova M, Kemppainen P, André C, Rolán-Alvarez E, Butlin RK (2010) Repeated evolution of reproductive isolation in a marine snail: unveiling mechanisms of speciation. Philos Trans R Soc Lond B Biol Sci 365(1547):1735–1747. https://doi.org/10.1098/rstb.2009.0256

Joshi RC (2007) Problems with the management of the golden apple snail Pomacea canaliculata: an important exotic pest of rice in Asia. Area-wide control of insect pests: from research to field implementation. Springer Netherlands, Dordrecht, pp 257–264

Kannan A, Ratnayeke S, Yow YY (2021) Molecular evidence of hybridisation in two invasive species of Pomacea (Gastropoda: Ampullariidae) in Peninsular Malaysia, vol 69. RAFFLES BULLETIN OF ZOOLOGY, pp 570–585

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Duran C (2012) Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28(12):1647–1649

Krahulcová A, Krahulec F, Kirschner J (1996) Introgressive hybridization between a native and an introduced species: Viola Lutea subsp. Sudetica versus V. Tricolor, vol 31. Folia Geobotanica, pp 219–244

Lowe S, Browne M, Boudjelas S, De Poorter M (2000) 100 of the world's worst invasive alien species a selection from the global invasive species database. Aliens 12, December 2000. The Invasive Species Specialist Group (ISSG)

Matsukura K, Izumi Y, Yoshida K, Wada T (2016) Cold tolerance of invasive freshwater snails, Pomacea canaliculata, P. maculata, and their hybrids helps explain their different distributions. Freshw Biol 61(1):80–87

Matsukura K, Okuda M, Cazzaniga NJ, Wada T (2013) Genetic exchange between two freshwater apple snails, Pomacea canaliculata and Pomacea maculata invading East and Southeast Asia. Biol Invasions 15:2039–2048

Matsukura K, Okuda M, Kubota K, Wada T (2008) Genetic divergence of the genus Pomacea (Gastropoda: Ampullariidae) distributed in Japan, and a simple molecular method to distinguish P. canaliculata and P. insularum. Appl Entomol Zool 43(4):535–540

Matsukura K, Wada T (2017) Identification of Pomacea species using molecular techniques. Biol Manage Invasive Apple Snails 3119:33–43

Rodriguez AK, Krug PJ (2022) Ecological speciation by sympatric host shifts in a clade of herbivorous sea slugs, with introgression and localized mitochondrial capture between species. Mol Phylogenet Evol 174:107523

Shao K, Chung K (2022) The National Checklist of Taiwan (Catalogue of Life in Taiwan, TaiCoL). Version 1.11. Taiwan Biodiversity Information Facility (TaiBIF). Checklist dataset https://doi.org/10.15468/auw1kd accessed via GBIF.org on 2023-12-19

Stewart KA, Lougheed SC (2013) Testing for intraspecific postzygotic isolation between cryptic lineages of Pseudacris crucifer. Ecol Evol 3:4621–4630

Stewart KA, Taylor SA (2020) Leveraging eDNA to expand the study of hybrid zones. Mol Ecol 29(15):2768–2776

Suehs CM, Affre L, Médail F (2004) Invasion dynamics of two alien Carpobrotus (Aizoaceae) taxa on a Mediterranean island: I. Genetic diversity and introgression. Heredity 92(1):31–40

Tamura K, Stecher G, Kumar S (2021) MEGA11: molecular evolutionary genetics analysis version 11. Mol Biol Evol 38(7):3022–3027

Turbelin AJ, Cuthbert RN, Essl F, Haubrock PJ, Ricciardi A, Courchamp F (2023) Biological invasions are as costly as natural hazards. Perspect Ecol Conserv 21(2):143–150

Wu JY, Wu YT, Li MC, Chiu YW, Liu MY, Liu LL (2011) Reproduction and Juvenile Growth of the Invasive Apple snails Pomacea canaliculata and P. Scalaris(Gastropoda: Ampullariidae) in Taiwan. Zoological Stud 50(1):61–68

Wu SH, Hsieh CF, Chaw SM, Rejmánek M (2004) Plant invasions in Taiwan: insights from the flora of casual and naturalized alien species. Divers Distrib 10(5–6):349–362

Yamaguchi R, Yamanaka T, Liebhold AM (2019) Consequences of hybridization during invasion on establishment success. Theoretical Ecol 12(2):197–205

Yang QQ, He C, Liu GF, Yin CL, Xu YP, Liu SW, Qiu JW, Yu XP (2020) Introgressive hybridization between two non-native apple snails in China: widespread hybridization and homogenization in egg morphology. Pest Manag Sci 76(12):4231–4239

Yang QQ, Ip JCH, Zhao XX, Li JN, Jin YJ, Yu XP, Qiu JW (2022) Molecular analyses revealed three morphologically similar species of non-native apple snails and their patterns of distribution in freshwater wetlands of Hong Kong. Divers Distrib 28(1):97–111

Yoshida K, Matsukura K, Cazzaniga NJ, 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). J Molluscan Stud 80(1):62–66

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