Niche conservatism and invasion potential of *Pomacea canaliculata* and *Pomacea maculata* in two invaded regions

Zhong Qin

South China Agricultural University

Jia-En Zhang (✉ jeanzh@scau.edu.cn)

South China Agricultural University

Benliang Zhao

South China Agricultural University

Zhaoji Shi

South China Agricultural University

Zeheng Xiao

South China Agricultural University

Qinlin Jing

South China Agricultural University

Research Article

Keywords: Niche conservatism, Ecological niche models, Niche dynamics, *Pomacea canaliculata*, *Pomacea maculata*, Invasive species

Posted Date: May 27th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-209740/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

The most noxious apple snails (*Pomacea canaliculata* and *P. maculata*) native to South America, currently has two distinct invaded ranges in China and the United States. Whether the environmental niches of the two closely related species have changed or remained stable (niche conservatism hypothesis) during the invasion process has become an important issue in forecasting their potential geographic distributions. For each *Pomacea* snail, two ecological niche models (ENMs, employing BIOMOD2) were generated based on bioclimatic variables and occurrence records in: (1) the native range; (2) the different invasive range. Conservation of ecological niche between the native and invasive snail populations were then tested by principal component and niche dynamics analysis. According to all models, precipitation contributed most to distribution of *P. maculata*, whereas low temperature was another most influential factor for spread of *P. canaliculata*. Niche conservatism were indicated by niche similarity tests and high niche stability for both *Pomacea* snails during their invasions in two regions. Niche expansions of *P. canaliculata* were relatively larger than unfilling values, whereas niche expansions of *P. maculata* were lower than unfillings. High niche unfilling for *P. maculata* in the United States revealed a great potential for further expansion in this region. We discuss the possible roles of physiological tolerances, genetic variation, residence time and hybridization in shaping niche changes for *Pomacea* snails during their invasion processes. Findings of this work can improve the understanding of potential mechanisms for niche differentiation and provide a theoretical basis for forecasting the invasion potential of *Pomacea* snails.

Introduction

The snail genus *Pomacea* contains a successful group of aquatic invaders that have caused detrimental agricultural and environmental impacts and substantial economic losses in many countries of the world. Among the most troublesome and much concerned are the channeled apple snail *Pomacea canaliculata* (Lamarck, 1819) and the channeled apple snail and the island apple snail *Pomacea maculata* (Perry, 1810) [formerly *P. insularum* (d'Orbigny, 1835)] (Ampullariidae). The two apple snails are native to freshwater wetlands of South America, but have been widely introduced into North America, Europe, east and southeast Asia and the Pacific Islands after around 1980 (Hayes et al. 2008; Lopez Robles et al. 2010). The wide host range and voracious appetite (Lach et al. 2000), large fecundity (Naylor 1996) and highly adaptable to various harsh environmental conditions (Halwart 1994; Oya 1987), has help the *Pomacea* snails colonize varieties of agricultural habitats and wetlands successfully.

The rapid spread of invasive *Pomacea* snails across the continents and the resulting threats and damages have prompted the task of improved understanding of potential geographic distributions for effective control strategies and measures. Ecological niche models (ENMs), a powerful methodological tool to predict the potential invasive ranges of species based on observed occurrence records and associated abiotic environmental variables ((Guisan and Thuiller 2005; Rödder and Lötters 2010) has been intensively used in modeling species invasions under the assumption that species retain their niche properties during invasion (i.e. niche conservatism). Previous field surveys and reports have identified the

colonization areas of *Pomacea* snails and provided some insights in identification of regions with a high invasion potential using niche-based models (Byers et al. 2013; Lei et al. 2017). More specific information (e.g. the degree to which the snail is at equilibrium with current environmental conditions) is urgently needed to refine the snail's predicted conceivable distribution.

Introduced species have a higher probability of successfully establishing viable populations in areas with a climate that is similar to the native region (Di Febbraro et al. 2013). However, it is also possible that a niche shifts during biological invasion events (Guisan et al. 2014), mainly because of nonanalogous climate conditions between native and invaded areas (Ribas et al. 2018). Studies on plants (Petitpierre et al. 2012), birds (Strubbe et al. 2013), insects (Petersen 2013) and vertebrates (freshwater fish, mammals, birds and amphibians) (Strubbe et al. 2015) have reported many cases of climatic mismatches during invasion. Such climatic mismatches did not coincide with the fundamental assumption of ENMs and make it difficult to identify the potential invasive areas accurately. Thus, knowledge of niche conservatism between native and invaded areas are very important in forecasting the spread of invasive species. A recently developed framework (COUE scheme: centroid shift, overlap, unfilling, and expansion; (Petitpierre et al. 2012) in ENMs provided the technical development for analyses of niche dynamics and niche comparisons. These analyses have been widely used to test niche changes and to explore the consequences of invasive potential (Hill et al. 2017; Torres et al. 2018). However, few studies have addressed the niche status of *Pomacea* species during their invasion process and project their potential range.

Pomacea canaliculata and *P. maculata* exhibit a high degree of morphological similarity, yet can be genetically differentiated using mitochondrial DNA (COI) analysis and shell, anatomy and egg morphology (Hayes et al. 2008). Molecular phylogenetic data shown that the two species were initially intentionally introduced to south and southeast Asia as an aquaculture food source and more recently into mainland US and Hawaii through the aquarium pet trade and for biological aquatic weed control (Brito and Joshi 2016). In US, the earliest report of an established population of *P. canaliculata* in California was 1997 in Lake Miramar, north of San Diego (Cerutti 1998), whereas the earliest genetically confirmed specimen of *P. insularum* was collected in 2002 in Lake Munson (FLMNH 298514). Currently *P. canaliculata* was restricted to Arizona and California, whereas *P. insularum* occurred in Texas, Georgia, and Florida. *Pomacea canaliculata* was presumed to be the only non-native apple snail species in China, until molecular phylogenetic data was successfully used to separate it from *P. maculata* population around 2007 (Rawlings et al. 2007). *Pomacea canaliculata* was intentionally introduced to Zhongshan, Guangdong province, China, in the early 1980s, and its range has extended from the Pearl River valley northwards to the Yangtze River basin, covering a broad range of thermal and seasonal habitats in 17 southern provinces (Yang et al. 2018). Of the areas being invaded by *P. canaliculata*, 8 provinces (Sichuan, Chongqin, Zhejiang, Yunan, Jiangxi, Guangdong and Guangxi) found the establishment of *P. maculata* in recent investigations and genetic studies. Records of the two *Pomacea* snails across the two separated continents provide the opportunity to examine niche conservatism with respect to environmental variations and quantify the extent of niche differentiation in two closely related species.

We examine whether or not niches of *P. canaliculata* and *P. maculata* are conserved during their invasion across the two regions (i.e. China and US) using ecological niche models (ENMs), with the hypothesis that both snails would respond similarly to environmental variables in invasive regions, and there will be conservation of niches between the native and introduced ranges. The aim of this study was to (1) investigate how environmental variables explain distributions of the two *Pomacea* snails in two regions; (2) assess the extent of niche differences and to calculate niche metrics for comparing niches of the two *Pomacea* snails.

Materials And Methods

Species data

Occurrence records of *P. canaliculata* and *P. maculata* in native range of South America were derived from the Global Biodiversity Information Facility Data Portal (GBIF; www.gbif.org) and CABI Invasive Species Compendium (ISC; <https://ckan.cabi.org>) (Fig. 1). For US, records of the two *Pomacea* species were compiled from USGS Nonindigenous Aquatic Species database (USGS-NAS 2020). Distribution data of the two *Pomacea* species in China sourced from: (1) published records in journals, books and reports (Lv et al. 2013; Song 2010; Yang et al. 2018); (2) molecular phylogenetic identification of specimens observed during field survey (mostly opportunistic sampling) by the authors, and (3) results of haplotype diversity analysis on specimens from different geographical origins in south China (provided by China Jiliang University). Because *P. maculata* has a synonymous name *P. insularum*, we queried each name in species distribution sources to assemble native and invasive occurrence data.

Specimens with uncertain location or non-reliable identification were discarded. For *Pomacea* occurrences in South America and US, specimens collected and genetically verified from the year 2007 to the present were used for niche analysis. For *Pomacea* occurrence of China, specimens collected after 2010 were used to be in approximate concordance with the clarification of the genetic distinctiveness of the two congeneric Ampullariidae snail species. Records of *Pomacea* species were adjusted to the raster of the climatic variables with a spatial resolution of 2.5 arcmin. To minimize spatial autocorrelation, one records were randomly selected within each grid cell. The resulting datasets of *P. canaliculata* contained 75 unique occurrence localities in native, 35 and 66 locations for invasive ranges of China and US. Datasets of *P. maculata* contained 41 unique occurrence localities in native, 485 and 20 locations for the two invasive ranges respectively (see Fig. 6 and Fig. 7).

Environmental variable

Six out of 19 available bioclimatic variables were derived from Worldclim database (www.worldclim.org; Version 2.0) for the 1970–2000 period at 2.5 arcmin spatial resolution (c. 5 km * 5 km). The subsets comprised of two temperature variables including maximum temperature of the warmest month (bio5) and minimum temperature in the coldest month (bio6), three precipitation variables including annual precipitation (bio12), precipitation of the driest quarter (bio17), and precipitation of the warmest quarter (bio18) as well as geographic elevation. These bioclimatic variables represent annual trends, seasonality

and extremes that are appropriate to explain species survival (Hijmans et al. 2005) and were thus selected based on their known or probable ecological relevance to the snail. Multicollinearity among the six candidate variables and possible overfitting were assessed based on Pearson correlation and variance inflation factor statistics (VIF) using *usdm* package in R (Naimi et al. 2014). No sign of strong multi-collinearity existed among the six candidate variables (Spearman rank correlation < 0.7 and VIF < 10 (Dormann et al. 2013). This set of bioclimatic variables has been considered as the most influential in predicting distribution of *P. maculata* in the southeastern US (Byers et al. 2013).

Geographic backgrounds

The background environments for climate niche comparison should only include areas that have been accessible to the species (Barve et al. 2011). To delimit the geographic background for niche comparison of the two *Pomacea* snails, a gridded Köppen-Geiger climate classification for the period 1951–2000 at a resolution of 30 sec derived from the CliMond database (Kriticos et al. 2012); www.climond.org), was used for sub-setting the available environment for the species within each range. The Köppen-Geiger system was selected because it classifies climate into 5 main classes and 30 sub-types based on threshold values and seasonality of monthly air temperature and precipitation, and has been widely used to define the background based on species distribution records. The distribution maps for both *Pomacea* snails spatially intersected with the Köppen-Geiger climate zones at 2.5 arcmin resolution, respectively. The resulting Köppen-Geiger polygons containing one or more species records were included in the background of each range.

In South America, the geographic background of the two *Pomacea* snails was delimited by considering their demographic history, field survey collections and molecular phylogenetic data. The range of *P. canaliculata* is restricted to the Lower Paraná, Uruguay, and La Plata basins, while *P. maculata* has a much larger range, occurring throughout much of western Brazil, from the border of Paraguay in the south to the Amazon Basin in the north (Hayes et al. 2012). A 10-km buffer zone for the minimal convex hull covering all occurrence locations were drawn to define the background for each *Pomacea* snail. For invasive China and US ranges, the Köppen-Geiger polygons where the species occurred were considered to be potentially suitable for the species. Pseudo-absences were then extract randomly from the native and invasive backgrounds as described below.

Testing niche conservatism

An analytical framework developed by Broennimann et al. (2012) was employed to assess the environmental niche variation of the two *Pomacea* snail between their native range and the introduced ranges (China, US) separately. This method creates a global environmental space that covers all the environmental conditions where the species occurs, including both native and invaded areas, and generates occupancy values based on comparisons of the species occurrence data with the global environmental space (Broennimann et al. 2012). For each *Pomacea* snail, principal component analysis (PCA) using the selected six environmental variables, combined with the species occurrence data and the species pseudo-absence data were performed. The first two axes of PCA were used to create an

environmental grid of 100×100 cells (global environmental space), where species occupancy was allocated (Broennimann et al. 2012). Species' densities of occurrence within each grid cell were calculated using a kernel function to generate smooth distribution for native and invasive datasets, respectively. The niche metrics accounting for niche overlap, niche similarity, niche equivalency was compared between native and non-native ranges for the two *Pomacea* snails, respectively. To complete this, the Schoener's D metrics, an index ranging from 0 (no overlap) to 1 (complete overlap) were used to measure niche overlap (Schoener 1970). Niche equivalency and niche similarity test were performed based on the 95% confidence interval to test the null hypothesis of the similar and equivalent niches for both *Pomacea* snails in their native and introduced regions, respectively (Warren et al. 2008). The significance of similarity and equivalency tests was assessed by 1,000 permutations. Three niche metrics namely: niche stability (proportion of the native niche observed in the exotic niche), unfilling (proportion of the native niche not occupied in the exotic niche) and expansion (new environmental requirements observed in the exotic niche), were calculated to quantify the niche dynamics of *Pomacea* species (Petitpierre et al. 2012). A proportion of 95% of the intersection area between the native and invaded gridded environmental space were used to control for possible environmental outliers. Additionally, the niche metrics were also calculated for comparisons between species in the same geographical region. To complete this, the proportion of environmental space occupied by *P. canaliculata* or *P. maculata* solely as well as by both species were quantified using the similar method mentioned above. All niche comparisons were performed with the ecospat package (Di Cola et al. 2017) in R environment (R Development Core Team).

Ecological niche modeling

Potential distribution of *Pomacea* snails in their native and invasive ranges were predicted using the same climatic variables, occurrence localities as described in niche conservatism tests. Two different models, based on *Pomacea* species occurrences of the native range and invaded ranges only, were built for each species separately. The resulting models were projected onto the study invasive range to test their performances.

The BIOMOD2 modelling framework implemented in R 4.0.2 (R Development Core Team 2012) was employed to project the potential distribution of *P. canaliculata* or *P. maculata*. An ensemble of three modeling techniques were performed: generalized boosted models (GBM), artificial neural networks (ANN) and random forests (RF). The mean of probabilities of the three selected algorithms were used for ensemble building, which has been proved to supply more robust predictions than other ensemble building techniques (Marmion et al. 2009). The equal number of pseudo-absences were generated by randomly sampling from the grid cells of each study range (Barbet-Massin et al. 2012). Default settings for three modeling techniques were used, except that 3000 trees were used as fitting basis for GBMs and 500 trees were built for RF. For each *Pomacea* snail, 70% of occurrence records were randomly selected for calibration and 30% reserved for evaluation by cross-validation. Ten replicate runs for each model. To compare the magnitude of influence of predictors in the models, variable importance values were derived using the function provided in BIOMOD2. Performance of each modeling technique were evaluated by the

true skill statistic (TSS). Only the best fitted model runs above critical TSS values (> 0.4) and AUC (> 0.7) were implemented in the final ensemble model run. To constrain model uncertainty, consensus prediction models were obtained using a TSS weighted average method to account for the predictive power of each algorithm. Binary suitable/non-suitable map for *Pomacea* snails were created using the threshold that maximizes the true skill statistic (TSS), which was known to improve the accuracy of prediction (Jiménez-Valverde and Lobo 2007).

To examine analogy of the presumed introduction locations with native range, the multivariate environmental similarity surface (MESS) analysis was performed for each *Pomacea* snails separately. In MESS analysis, the environment of grid cells occupied by the snail in a specific invasive range was compared with that of the native range, with respect to the set of selected environmental variables (Elith et al. 2010). The grid cells having positive value indicate similar environment between two ranges whereas grid cells with the dissimilar environment for at least one variable received negative values (Broennimann et al. 2014). MESS analysis was conducted in R with the ecospat package (Di Cola et al. 2017). MESS output values (climatic similarity) were reported at the presence records in the range where the model was projected.

Results

Niche dynamics and comparisons

The invasive China niche centroid of *Pomacea* snails shifted towards areas with higher annual precipitation (bio12) and elevation, and lower minimum temperature in the coldest month (bio6) (Fig. 2 (b); (Fig. 3 (b); Fig. 4). The invasive US niche centroid of *P. canaliculata* showed a general broader range of precipitation conditions (i.e, bio12 and bio18) than the native niche (Fig. 2 (d); Fig. 5 (b-c)), whereas the niche centroid of *P. maculata* moved towards areas with narrower variability in precipitation conditions and a higher maximum temperature of the warmest month (bio5) (Fig. 3 (d); Fig. 5 (d-f)).

The first two axes of the PCA used to compare the environmental niches of the *Pomacea* snails between the native and invaded China explained proportions of variance (*P. canaliculata*: 80.65%; *P. maculata*: 81.73%). The two first axes of PCA-env explained slightly lower proportions (*P. canaliculata*: 72.59%; *P. maculata*: 78.32%) of variance across the occupied native and invaded US environments. For native-China ranges, all the environmental predictors load strongly in the PC1 axis (around 62.0% for the two *Pomacea* snails), with both temperature (loading values from -0.77 to -0.85) and precipitation (loading values from -0.67 to -0.89) variables being negatively correlated and elevation positively correlated (loading values around 0.80) (Fig. 2 (c); Fig. 3 (c)). Annual precipitation (bio12) contributed most to the PC1 axis, followed by minimum temperature in the coldest month (bio6). The PC1 axis explained maximum environmental variation (53.7%-62.7%) for native-US ranges, with both temperature (loading values from -0.59 to -0.84) and precipitation (loading values from -0.81 to -0.92) variables being negatively correlated and elevation positively correlated (loading values around 0.62) (Fig. 2 (e); Fig. 3 (e)). Maximum temperature of the warmest month (bio5) positively contributed most to the PC2 axis,

while bio12 contributed most negatively to the PC1 axis, followed by precipitation of the warmest quarter (bio18).

Niche change indices of *P. canaliculata* and *P. maculata* revealed moderate and low degree of niche expansions in invasive ranges respectively, although larger niche stabilities were detected for *P. maculata* (Table 1). Niche unfilling were no more than 0.1 for *P. maculata*. In US range, *P. canaliculata* occupied the largest area in the PCA space which signified the wide range of environments experienced by the species in this region. The exotic US niche for *P. maculata* was unique because of highest degree of stable and unfilled niches, and was concurrent with the lowest Schoener's *D* value.

Table 1
Niche change metrics of *Pomacea canaliculata* and *P. maculata* based on the analogous (common) environmental spaces between the native region (Europe) and the two introduced regions (China and the United States).

Species	<i>P. canaliculata</i>		<i>P. maculata</i>	
Region	Native-China	Native-US	Native-China	Native-US
Overlap (O)	0.4358	0.2833	0.4885	0.1778
Equivalence (<i>p</i> value)	0.9802	1.0000	0.7326	1.0000
Similarity (<i>p</i> value)	0.0990	0.1782	0.0594	0.2178
Expansion (E)	0.2366	0.3918	0.0713	0.0262
Stability (S)	0.7634	0.6082	0.9287	0.9738
Unfilled (U)	0.0349	0.0973	0.2932	0.6621

For each *Pomacea* snail, the niche comparisons showed modest overlaps (Schoener's *D* values around 0.4) among environmental conditions occupied in native-China ranges, and low overlaps (Schoener's *D* values of 0.28 and 0.18 for *P. canaliculata* and *P. maculata*) in native-US ranges (Table 1). The hypothesis of niche equivalency was confirmed for both *Pomacea* snails and invasive regions, given that the observed overlaps between invaded and native ranges were significantly higher than 95% of simulated overlaps (Table 1). All the observed niche overlap between the native and invasive niches were significantly higher than the random niche overlap ($p \leq 0.05$), indicating equivalency of the environmental niches.

Among the six environmental candidates, the proportion of niche expansion of *Pomacea* snails in invasive China range was highest for bio18 (*P. canaliculata*: 0.2850; *P. maculata*: 0.1687), followed by elevation (*P. canaliculata*: 0.2235; *P. maculata*: 0.1038) (Table 2). Full niche stability of both *Pomacea* snails were detected along precipitation of the driest quarter (bio17). Niche dynamics in invasive US displayed different species-specific pattern. For *P. canaliculata*, the proportion of niche expansion was highest for bio18 (0.3388) and bio12 (0.1718), with a considerable proportion of niche unfilling for elevation (0.0679). For *P. maculata*, varying degree of niche unfillings were observed for each environmental variable, with peak proportions along bio6 (0.5641) and bio12 (0.2152) (Table 2).

Table 2

Niche dynamics of *Pomacea canaliculata* and *P. maculata* along the environmental variables.

Region	Niche dynamics	bio5	bio6	bio12	bio17	bio18	elevation
Native-China ^{sp1}	Expansion (E)	0.0152	0.0200	0.0351	0.0000	0.2850	0.2235
	Stability (S)	0.9848	0.9800	0.9649	1.0000	0.7150	0.7765
	Unfilled (U)	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Native-US ^{sp1}	Expansion (E)	0.0129	0.0129	0.1718	0.0000	0.3388	0.0000
	Stability (S)	0.9871	0.9871	0.8282	1.0000	0.6612	1.0000
	Unfilled (U)	0.0043	0.0000	0.0000	0.0000	0.0000	0.0679
Native-China ^{sp2}	Expansion (E)	0.0000	0.0000	0.0000	0.0000	0.1687	0.1038
	Stability (S)	1.0000	1.0000	1.0000	1.0000	0.8313	0.8962
	Unfilled (U)	0.0044	0.0615	0.0009	0.0000	0.0041	0.0000
Native-US ^{sp2}	Expansion (E)	0.0000	0.0000	0.0000	0.0000	0.0005	0.0000
	Stability (S)	1.0000	1.0000	1.0000	1.0000	0.9995	1.0000
	Unfilled (U)	0.5641	0.0034	0.2152	0.0062	0.0080	0.1011
Note: (1) The initial set of six candidate variables were selected by assessment of their ecological relevance to <i>Pomacea</i> snails and collinearity tests of VIF. Variables definition: bio5: maximum temperature of the warmest month (°C * 10); bio6: minimum temperature of coldest month (°C * 10); bio12: annual precipitation (mm); bio17: precipitation of driest quarter (mm); bio18: precipitation of warmest quarter (mm).							
(2) Different upper letters indicate the snail species. "sp1" represents <i>Pomacea canaliculata</i> , whereas "sp2" represents <i>P. maculata</i> . The highest values of niche expansion and unfilled were set in bold.							

Predicted distributions

The ensemble modeling of the current distribution of *Pomacea* snails obtained high evaluation scores (TSS values of all the ENMs were great than 0.85). Models computed from the native or invasive ranges showed high predictive performance generally. The native model identified environmental suitabilities (on average, ≥ 0.274 for *P. canaliculata* and ≥ 0.473 for *P. maculata*) in southeast China and included habitats being invaded during the early phase of invasions (Fig. 6 (a); Fig. 6 (c)). In US, projections from the native model yielded environmental suitability (on average, ≥ 0.283 for *P. canaliculata* and ≥ 0.494 for *P. maculata*) in southeastern US and an area that extending from north Louisiana to central portions of Missouri to south Illinois and adjacent regions (Fig. 7 (a); Fig. 7 (c)).

Both the native and China-range models for *P. maculata* overpredicted the extent occupied in southeastern US. The species did not fully occupy all the environmental space that is occupied in the native range. Occurrences of *Pomacea* snails in invasive China range were better predicted with the

invasive US models. Environmental suitabilities for *P. canaliculata* were identified in south China and patches in southeast (Fig. 6 (b)). Parts of south China, portions in southeast coastal areas, contiguous area of western Sichan and Chongqing were predicted to be suitable for *P. maculata* (Fig. 6 (d)). Similarly, presences of *P. canaliculata* or *P. maculata* in invasive US range was better predicted using the models calibrated on invasive China range (Fig. 7 (b); Fig. 7 (d)). Moreover, the niche overlaps between the two invasive ranges were higher than niche overlaps between the native-invasive US ranges. These niche overlaps were more equivalent than random.

The MESS analysis for *P. canaliculata* identified non-analog small patches scattered at the margin of eastern Taiwan, parts of southwest regions in China (Fig. 8 (a-c)). The margin of western US and in scattered habitats of central US were non-analog to the native range (Fig. 8 (d-f)). For *P. maculata*, the non-analog environments detected in China were almost the same as those found for *P. canaliculata* except that southwest regions in China displayed analog to the native range (Fig. 9 (a-c)). Regions at southwest margins of US had non-analog environments to the native range (Fig. 9 (d-f)).

Table 3 Performance of the ensemble models for *Pomacea canaliculata* and *P. maculata* based on TSS values (TSS: True kill statistic).

Note *: models based on *Pomacea* species occurrences from the native range or invaded ranges only, were projected to the target invasive range to test their performances.

Species	Models *	Testing data	Cut off	Sensitivity	Specificity
<i>P. canaliculata</i>	Native-China	0.862	332	97.059	89.189
	US-China	0.996	679	99.93	99.93
	Native-US	0.836	393	94.118	89.527
	China-US	0.940	489	98.333	95.703
<i>P. maculata</i>	Native-China	0.938	473	99.92	93.750
	US-China	0.974	322	99.79	97.612
	Native-US	0.892	401	97.368	91.875
	China-US	0.987	613	99.90	98.684

Discussion

Environmental factors affecting the potential distribution

Regional distribution of *P. canaliculata* and *P. maculata* are currently spanning a wide range of environments between the native South America and invasive ranges (Fig. 1). Difference in local adaptation of *Pomacea* populations across the ranges may connect with the environmental variables, which contributed the most to regional ENMs. When native *Pomacea* populations spread to invasive China, annual precipitation (bio12) and minimum temperature in the coldest month (bio6) were the most influential factors, which revealed the necessity of moisture and thermal environments for *Pomacea* species to establish permanently. These findings aligned with numerous biological studies and modeling work on *Pomacea* snails (Lv et al. 2006; Yoshida et al. 2016). In invasive US, annual precipitation (bio12) followed by precipitation of the warmest quarter (bio18) contributed the most, indicating the preference of *Pomacea* species to moisture environments. Besides the two variables, maximum temperature of the warmest month (bio5) contributed a lot to model accuracy, suggesting the hottest weather conditions could be a determining factor for *Pomacea* species. Extreme temperature conditions in invasive ranges therefore, highlighted the constraints of thermal tolerance on establishment of *Pomacea* species. *Pomacea canaliculata* is generally considered to high adaptability and variability in thermal tolerance which allow them to colonize areas with high peak temperatures (e.g. in the summer in Hawaii, Hainan island) as well as cold winter conditions (e.g. in Ibaraki of Japan) (Wada and Matsukura 2007). This species had acquired sufficient cold tolerance to colonize East Asia before they were introduced (Wada and Matsukura 2011; Yoshida et al. 2014). In east and south-east Asia, the apple snails have invaded a wide range from tropical to temperate regions after several introductions (Hayes et al. 2008), northern expansion of this species were determined by low temperature during winter (Ito 2002; Syobu et al. 2001). Compared with *P. canaliculata*, *P. maculata* has poorer tolerance of both cold and desiccation (Yang et al. 2020), which was reflected in its distribution in southern, western coastal regions and Florida in US. These findings on difference in ecophysiological tolerances of the two *Pomacea* snails, has implications for understanding differential spread potentials in the invasive range.

Patterns of niche dynamics and possible explanations

Pomacea maculata has a much larger native range than *P. canaliculata* (Hayes et al. 2012). Comparisons of environmental variables in *P. canaliculata* and *P. maculata* native occurrences showed statistically significant differences for half of 6 variables used in this study (Table 2). Elevation values for native occurrences of the two *Pomacea* snails revealed no significant difference between species elevation profiles. Instead, both species were reported to inhabit in low elevation fresh water bodies, wetland areas and paddy field in their native ranges (Hayes et al. 2015). This indicated that the two *Pomacea* snails occur under similar environmental conditions despite the differences in native range sizes.

During invasion in US and China regions, both *P. canaliculata* and *P. maculata* retain signatures of their native environmental niche, but also indicate changes in environmental space following introduction. The two species showed niche conservatism (niche stability) in two invasive regions as indicated by the higher stability values of 0.76 and 0.97, respectively and lower unfilling and expansion values (Table 1). Niche expansions of *P. canaliculata* were relatively larger (0.2366 and 0.3918 in invasive China and US) compared with unfilling (no more than 0.1 for two invasive ranges). Contrastly, niche expansions of *P.*

maculata (no more than 0.1) were relatively rare compared with unfilling (0.2932 and 0.6621 in invasive China and US). Differences in niche dynamics of the two *Pomacea* snails can be ascribed to physiological tolerances, genetic variation, residence time and dispersal limitations.

In invasive China, populations of *P. canaliculata* was introduced more than once from multiple locations in Argentina, while just a single lineage of *P. maculata* from Brazil was introduced and established in China (Yang et al. 2018). *Pomacea maculata* populations of China had a much lower haplotype diversity than that in their native countries Argentina and Brazil (Hayes et al. 2008). Currently *P. canaliculata* distributed more widely than *P. maculata*. The multiple introduction events of *P. canaliculata* enhanced genetic diversity, allowing the species to occupy an environmental space that coincides with the native niche (Ahmad et al. 2019), and also creating the opportunity for niche expansion when individual snail face novel environments. The resulting effects may explain why *P. canaliculata* have colonized a larger portion of the suitable environments than *P. maculata*, despite similar introduction dates in this region.

Our findings of a high proportion of niche stability support niche conservatism between US invasive *P. maculata* populations and the native populations, but also indicate that currently *P. maculata* does not occupy all the suitable areas available in US. Furthermore, hybridization of *P. canaliculata* and *P. maculata* was unlikely to have occurred because of the low genetic variation found in *Pomacea* snails from Louisiana and Texas (Mueck 2017). *Pomacea maculata* populations having large niche unfilling may attribute to the short time since introduction in this region (nor more than 40 years). Niche unfilling seems to be larger for species introduced recently and into a small number of locations, compared to those with ancient colonization history and introduced in several points in space (Strubbe et al. 2015). In US, niche unfilling was larger for *P. maculata* introduced recently and into a small number of locations, when compared with *P. canaliculata* being introduced a few years earlier. Despite the short colonization span, increasing human mediated importation, acquisition and transportation makes *P. maculata* especially capable of invading new suitable habitats and lead to explosive population growth. The finding was consistent with studies on species with different invasion histories (Cunze et al. 2018; Sales et al. 2016). Given the high level of niche unfilling in US, the invasive potential of *P. maculata* in this region might be extremely high. Therefore, effective population control in areas already invaded is needed to prevent further expansion of *P. maculata* into adjacent southeastern areas.

Both *P. canaliculata* and *P. maculata* can occupy new environments in the invaded ranges because of preadaptation to those environments in their native range (Rawlings et al. 2007). For instance, the snails can survive adverse conditions by retreating into their shell and closing it firmly with the operculum (Horgan et al. 2014). The presence of adaptive capacity could lead to a realized niche expansion in the invaded range. However, challenges remain to differentiate between an evolutionary change, phenotypic plasticity and ecological responses (Moran and Alexander 2014). In Asia, *P. canaliculata* has been reported to survive months of drought by digging deep into the mud and closing their operculum, surfacing again after renewed flooding. In the same way snails hibernate in winter at the northern border of their distribution (Oya 1987). Such burrowing behavior was not evidenced in *P. maculata*. but this species has the capacity to move over land and sustain an aestivated state for over ten months, which

may lead to an increase in geographic range and viability (Mueck 2017). More detailed comparative studies of the two *Pomacea* snails are expected to offer new insights into the ecological or evolutionary processes that allow species to colonize new environments.

Potential causes of niche changes

Hybridization or other genetic changes in populations may allow for greater changes in niche space, which in turn may lead to increased invasiveness and more rapid range shifts or better adaptation (Thornton and Murray 2014). In South America, *P. canaliculata* and *P. maculata* occur sympatrically in the Paraná and Uruguay river basins of Argentina and Uruguay, a small region in relation to the full geographic distribution of both *Pomacea* snails (Hayes et al. 2012). It is conceivable that hybridization may impact the niche expansion of *Pomacea* snails. Recent studies documented the existence of substantial introgressive hybridization between invasive *P. canaliculata* and *P. maculata* populations (Yang et al. 2020), though the two species were believed to have significant genetic differentiation and limited gene flow among different countries. Genetic exchange and possible ongoing hybridization between *P. canaliculata* and *P. maculata* populations, were supported by the sympatrical occurrence and mating in the field (e.g., paddy field), both in native areas (Argentina) and invasive Asia countries (e.g. Japan and China) (Matsukura et al. 2008). Although additional evidence from other ranges are few for the present, the existence of hybridization between the two *Pomacea* snails have been associated with rapid growth, stronger fitness, and changes in morphological features of their egg clutches (Yang et al. 2020). Hybridization or other genetic changes in apple snails may impact species range dynamics and contribute to range expansion. The potential influence of hybridization on patterns of niche differentiation was not investigated in this study. However, we suggest that a more comprehensive genetic analysis of the two *Pomacea* populations in regions (e.g. south China, US) to investigate any evidence of hybridization. To explore the niche differentiation of the species by hybridization during the invasion process, development of ecological niche models that include dispersal limitations and biotic interactions should be considered in future.

Uncertainties and challenges

We compare the realized environmental niches of two *Pomacea* snails in their native (East Asia) and invasive (US and China) ranges respectively. Niche conservatism was indicated by niche similarity tests and high niche stability, with the results showing that the native and invaded niches of *Pomacea* snails were more equivalent than random and were more similar than expected by chance. The findings were compatible with the reports on a variety of introduced non-native species, which have emphasized the prevalence of and important role played by niche conservatism during different stages of biological invasions (Broennimann et al. 2014; Petitpierre et al. 2012). However, a recent study on 22 invasive freshwater invertebrates in New Zealand indicated that 90% of these species (including *P. canaliculata*) showed a significant niche change (Torres et al. 2018). Niche conservatism of *P. canaliculata* and *P. maculata* populations in our study seems to be special cases since niche shifts are more common for freshwater invertebrates than they are for other organisms (e.g. Parravicini et al. 2015). Such a difference would reflect distinctive selection of variables for defining the species niche, sample size, study

regions, ect. The selection of the environmental predictors for niche modeling is a source of uncertainty in model predictions. We based the niche analysis on the several environmental factors only for this study. Additional variables (e.g., land cover, soil type, and pH) having effects on snail growth and reproduction, may allow the precise characterization of aquatic species' environmental niche but not available in the bioclim dataset (Broennimann et al. 2007). Human or flood regime-mediated dispersal and altered biotic processes (e.g., competition, predation) are effective in shaping freshwater species distributions (Gallardo et al. 2015; Loo et al. 2007). Ignoring these factors may potentially lead to substantial uncertainties in assessing niche conservatism, because these factors typically become important at the regional or local scale. Therefore, more intensive research efforts are needed to combine the biotic (e.g, ecophysiological tolerances, behavior flexibility, adaptive genetic variation, competition) and abiotic factors (e.g, land cover, habitat type) in the distribution models for *Pomacea* snails to have a more refined understanding of successful establishment and niche dynamics during the invasion process.

Targeting geographical background specifically involved in the invasion process can help capturing detailed niche dynamics in invaded ranges. Here we refined geographical backgrounds to two invasive ranges (China and US) when assessing the niche stasis of the two South America introduced *Pomacea* snail populations. Because lack of reliable identified species of apple snail information, patterns of environmental niche dynamics across other regions were not investigated in this study. Further work in this regard would be (i) acquire more detailed genetic data sampled across native and invasive distributions; (ii) assess whether the *Pomacea* snail will be able to occupy additional ecological niches beyond its native range and expand its current geographic distribution globally.

Declarations

Acknowledgement

This work was supported by the National Natural Science Foundation of China (31870525, 41871034, U1131006), Science and Technology Planning Project of Guangdong Province of China (2019B030301007), Guangdong Modern Agricultural Technology Innovation Team Construction Project (2019KJ134, 2019KJ105). We thank anonymous reviewers for providing helpful comments on earlier versions of this manuscript.

Author contribution statement

Zhong Qin: Data analysis, devised and drafted the manuscript. Jia-En Zhang: Conceptualization, review and editing. Benliang Zhao and Zeheng Xiao: Data collection and filtering. Zhaoji Shi: Map creation and interpretation.

Data accessibility

Datasets in the article can be accessed at Dryad Digital Repository with doi:10.5061/dryad.2fqz612n7

References

1. Ahmad R, Khuroo AA, Charles B et al (2019) Global distribution modelling, invasion risk assessment and niche dynamics of *Leucanthemum vulgare* (Ox-eye Daisy) under climate change. *Sci Rep-Uk* 9:11395
2. Barbet-Massin M, Jiguet F, Albert CH, Thuiller W (2012) Selecting pseudo-absences for species distribution models: how, where and how many? *Methods Ecol Evol* 3:327–338
3. Barve N, Barve V, Jiménez-Valverde A et al (2011) The crucial role of the accessible area in ecological niche modeling and species distribution modeling. *Ecol Model* 222:1810–1819
4. Brito F, Joshi R (2016) The golden apple snail *Pomacea canaliculata*: A review on invasion, dispersion and control. *Outlooks Pest Manage* 27:157–163
5. Broennimann O, Fitzpatrick MC, Pearman PB et al (2012) Measuring ecological niche overlap from occurrence and spatial environmental data. *Global Ecol Biogeogr* 21:481–497
6. Broennimann O, Mráz P, Petitpierre B, Guisan A, Müller-Schärer H (2014) Contrasting spatio-temporal climatic niche dynamics during the eastern and western invasions of spotted knapweed in North America. *J Biogeogr* 41:1126–1136
7. Broennimann O, Treier UA, Müller-Schärer H et al (2007) Evidence of climatic niche shift during biological invasion. *Ecol Lett* 10:701–709
8. Byers JE, McDowell WG, Dodd SR et al (2013) Climate and pH predict the potential range of the invasive apple snail (*Pomacea insularum*) in the southeastern United States. *Plos One* 8:e56812
9. Cerutti R (1998) An infestation of *Pomacea canaliculata* (Lamarck, 1804) in Lake Miramar, San Diego, California. *Festivus* 30:25–27, 29
10. Cunze S, Kochmann J, Koch LK, Klimpel S (2018) Niche conservatism of *Aedes albopictus* and *Aedes aegypti* - two mosquito species with different invasion histories. *Sci Rep-Uk* 8:7733
11. Di Cola V, Broennimann O, Petitpierre B et al (2017) ecospat: an R package to support spatial analyses and modeling of species niches and distributions. *Ecography* 40:774–787
12. Di Febbraro M, Lurz P, Genovesi P et al (2013) The use of climatic niches in screening procedures for introduced species to evaluate risk of spread: A Case with the American Eastern Grey Squirrel. *Plos One* 8:e66559
13. Dormann CF, Elith J, Bacher S et al (2013) Collinearity: a review of methods to deal with it and a simulation study evaluating their performance. *Ecography* 36:027–046
14. Elith J, Kearney M, Phillips S (2010) The art of modelling range-shifting species. *Methods Ecol Evol* 1:330–342
15. Gallardo B, Zieritz A, Aldridge D (2015) The importance of the human footprint in shaping the global distribution of terrestrial, freshwater and marine invaders. *Plos One* 10:e0125801
16. Guisan A, Petitpierre B, Broennimann O, Daehler C, Kueffer C (2014) Unifying niche shift studies: insights from biological invasions. *Trends Ecol Evol* 29:260–269

17. Guisan A, Thuiller W (2005) Predicting species distribution: offering more than simple habitat models. *Ecol Lett* 8:993–1009
18. Halwart M (1994) The golden apple snail *Pomacea canaliculata* in Asian rice farming systems: present impact and future threat. *Int J Pest Manage* 40:199–206
19. Hayes KA, Burks RL, Castro-Vazquez A et al (2015) Insights from an integrated view of the biology of apple snails (Caenogastropoda: Ampullariidae). *Malacologia* 58:245–303
20. Hayes KA, Cowie RH, Thiengo SC, Strong EE (2012) Comparing apples with apples: clarifying the identities of two highly invasive Neotropical Ampullariidae (Caenogastropoda). *Zool J Linn Soc-Lond* 166:723–753
21. Hayes KA, Joshi RC, Thiengo SC, Cowie RH (2008) Out of South America: multiple origins of non-native apple snails in Asia. *Divers Distrib* 14:701–712
22. Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A (2005) Very high resolution interpolated climate surfaces for global land areas. *Int J Climatol* 25:1965–1978
23. Hill MP, Gallardo B, Terblanche JS (2017) A global assessment of climatic niche shifts and human influence in insect invasions. *Global Ecol Biogeogr* 26:679–689
24. Horgan FG, Stuart AM, Kudavidanage EP (2014) Impact of invasive apple snails on the functioning and services of natural and managed wetlands. *Acta Oecol* 54:90–100
25. Ito K (2002) Environmental factors influencing overwintering success of the golden apple snail, *Pomacea canaliculata* (Gastropoda: Ampullariidae), in the northernmost population of Japan. *Appl Entomol Zool* 37:655–661
26. Jiménez-Valverde A, Lobo JM (2007) Threshold criteria for conversion of probability of species presence to either or presence-absence. *Acta Oecol* 31:361–369
27. Kriticos DJ, Webber BL, Leriche A et al (2012) CliMond: global high-resolution historical and future scenario climate surfaces for bioclimatic modelling. *Methods Ecol Evol* 3:53–64
28. Lach L, Britton DK, Rundell RJ, Cowie RH (2000) Food preference and reproductive plasticity in an invasive freshwater snail. *Biol Invasions* 2:279–288
29. Lei J, Chen L, Li H (2017) Using ensemble forecasting to examine how climate change promotes worldwide invasion of the golden apple snail (*Pomacea canaliculata*). *Environ Monit Assess* 189:404
30. Loo SE, Keller RP, Leung B (2007) Freshwater invasions: using historical data to analyse spread. *Divers Distrib* 13:23–32
31. Lopez Robles MA, Altaba C, Andree K, Lopez Robles V (2010) First invasion of the Apple snail *Pomacea insularum* in Europe. *Tentacle* 18:26–28
32. Lv S, Zhang Y, Liu HX et al (2013) Phylogenetic evidence for multiple and secondary introductions of invasive snails: *Pomacea* species in the People's Republic of China. *Divers Distrib* 19:147–156
33. Lv S, Zhou X, Zhang Y et al (2006) The effect of temperature on the development of *Angiostrongylus cantonensis* (Chen 1935) in *Pomacea canaliculata* (Lamarck 1822). *Parasitol Res* 99:583–587

34. Marmion M, Parviainen M, Luoto M, Heikkinen RK, Thuiller W (2009) Evaluation of consensus methods in predictive species distribution modelling. *Divers Distrib* 15:59–69
35. Moran EV, Alexander JM (2014) Evolutionary responses to global change: lessons from invasive species. *Ecol Lett* 17:637–649
36. Mueck K (2017) Physiology of the Invasive Apple Snail. In: *Pomacea maculata* (Perry, 1810), in Louisiana. University of Louisiana at Lafayette, Louisiana
37. Naimi B, Hamm NAS, Groen TA, Skidmore AK, Toxopeus AG (2014) Where is positional uncertainty a problem for species distribution modelling? *Ecography* 37:191–203
38. Naylor R (1996) Invasions in agriculture: assessing the cost of the golden apple snail in Asia. *Ambio* 25:443–448
39. Oya S (1987) Overwintering of the apple snail, *Pomacea canaliculata* (Lamarck) in north Kyushu. *Jpn J Appl Entomol Z* 31:206–212
40. Parravicini V, Azzurro E, Kulbicki M, Belmaker J (2015) Niche shift can impair the ability to predict invasion risk in the marine realm: an illustration using Mediterranean fish invaders. *Ecol Lett* 18:246–253
41. Petersen MJ (2013) Evidence of a climatic niche shift following North American introductions of two crane flies (Diptera; genus *Tipula*). *Biol Invasions* 15:885–897
42. Petitpierre B, Kueffer C, Broennimann O et al (2012) Climatic niche shifts are rare among terrestrial plant invaders. *Science* 335:1344–1348
43. Rawlings TA, Hayes KA, Cowie RH, Collins TM (2007) The identity, distribution, and impacts of non-native apple snails in the continental United States. *Bmc Evol Biol* 7:97
44. Ribas LGDS, de Cássia-Silva C, Petsch DK, Silveira MJ, Lima-Ribeiro MS (2018) The potential invasiveness of an aquatic macrophyte reflects founder effects from native niche. *Biol Invasions* 20:3347–3355
45. Rödder D, Lötters S (2010) Explanative power of variables used in species distribution modelling: An issue of general model transferability or niche shift in the invasive Greenhouse frog (*Eleutherodactylus planirostris*). *Die Naturwiss* 97:781–796
46. Sales LP, Ribeiro BR, Hayward MW et al (2016) Niche conservatism and the invasive potential of the wild boar. *J Animal Ecol* 86:1214–1223
47. Song HM (2010) Sequencing cytochrome oxidase subunit I of mitochondrial DNA and the taxonomic status of apple snails. *Chin J Zool* 45:1–7
48. Strubbe D, Beauchard O, Matthysen E (2015) Niche conservatism among non-native vertebrates in Europe and North America. *Ecography* 38:321–329
49. Strubbe D, Broennimann O, Chiron F, Matthysen E (2013) Niche conservatism in non-native birds in Europe: niche unfilling rather than niche expansion. *Global Ecol Biogeogr* 22:962–970
50. Syobu S, Mikuriya H, Yamaguchi J et al (2001) Estimating the overwintering mortality of the apple snail, *Pomacea canaliculata* (Lamarck)(Gastropoda: Ampullariidae) in a paddy field of Southern

- Japan using temperature data. Jpn J Appl Entomol Z 45:203–207
51. Thornton DH, Murray DL (2014) Influence of hybridization on niche shifts in expanding coyote populations. Divers Distrib 20:1355–1364
 52. Torres U, Godsoe W, Buckley HL et al (2018) Using niche conservatism information to prioritize hotspots of invasion by non-native freshwater invertebrates in New Zealand. Divers Distrib 24:1802–1815
 53. Wada T, Matsukura K (2007) Seasonal changes in cold hardiness of the invasive freshwater apple snail, *Pomacea canaliculata* (Lamarck) (Gastropoda: Ampullariidae). Malacologia 49:383–392
 54. Wada T, Matsukura K (2011) Linkage of cold hardiness with desiccation tolerance in the invasive freshwater apple snail, *Pomacea canaliculata* (Caenogastropoda: Ampullariidae). J Mollus Stud 77:149–153
 55. Warren DL, Glor RE, Turelli M (2008) Environmental niche equivalency versus conservatism: quantitative approaches to niche evolution. Evolution 62:2868–2883
 56. Yang Q, He C, Liu G et al (2020) Introgressive hybridization between two non-native apple snails in China: widespread hybridization and homogenization in egg morphology. Pest Manag Sci 12:4231–4239
 57. Yang QQ, Liu SW, He C, Yu XP (2018) Distribution and the origin of invasive apple snails, *Pomacea canaliculata* and *P. maculata* (Gastropoda: Ampullariidae) in China. Sci Rep-Uk 8:1185
 58. 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 Mollus Stud 80: 62–66
 59. Yoshida K, Yusa Y, Yamanishi Y, Matsukura K, Wada T (2016) Survival, growth and reproduction of the invasive apple snail *Pomacea canaliculata* in an irrigation canal in southern Japan. J Mollus Stud 82:600–602

Figures

Figure 1

Occurrence records of *Pomacea canaliculata* (red filled circles) and *P. maculata* (black filled square) collected in native South America range. Corresponding convex hulls represent the buffered minimum convex polygon for occurrence locations.

(a) Native-sp1

(b) Native-China

(c) correlation circle

(d) Native-US

(e) correlation circle

Figure 2

Environmental niche changes of *P. canaliculata* based on principal component analysis in two introduced regions. The small fig (a) represents niche patterns of native snail population. Patterns of niche changes in China and the United States are described in fig (b) and fig (d). The first two axes of each PCA represent the density of species occurrences and the environmental space. Solid and dashed lines indicate 100% and 90% of all the available environments. The blue color represents the niche overlap

between native and introduced region. The green and red color represent niche unfilling, niche expansion environments. The red arrows show the change in the niche centroid between native and introduced range. Fig(c) and fig (e) describe the impacts of environmental variables to niche changes in China and the United States, respectively. The correlation circles represent the variable importance along the first two principle axes.

Figure 3

Predicted distribution of *Pomacea* snails in invaded China using the ensemble niche modeling approaches. Models calibrated in either the native South America range (*P. canaliculata*: fig (a); *P. maculata*: fig (c)) or invasive US region (*P. canaliculata*: fig (b); *P. maculata*: fig (d)) and projected onto invasive China region. Grey shading on the binary maps indicates areas with suitable environmental

requirements for Pomacea snails. Known localities of *P. canaliculata* (blue filled square) and *P. maculata* (red filled circles) are displayed in corresponding small figures. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [suplFigures.docx](#)