Unravelling the global invasion routes of a worldwide invader, the red swamp crayfish (Procambarus clarkii)

Francisco Oficialdegui, Miguel Clavero, Marta Sánchez, Andy J Green, Luz Boyero, Thomas Michot, Kristie Klose, Tadashi Kawai, Christophe Lejeusne

i. Title Page

Article Title

Unravelling the global invasion routes of a worldwide invader, the red swamp crayfish (*Procambarus clarkii*)

The full names of the authors

Oficialdegui, Francisco J.[1,*], Clavero, Miguel[2], Sánchez, Marta I.[1,3], Green, Andy J.[1], Boyero, Luz[4,5], Michot, Thomas C.[6], Klose, Kristie[7], Kawai, Tadashi[8], Lejeusne, Christophe[9,10].

* oficialdegui@ebd.csic.es Francisco Javier Oficialdegui. Department of Wetland Ecology. Estación Biológica de Doñana (EBD-CSIC). C/Américo Vespucio 26. Isla de la Cartuja. 41092. Seville (Spain). Tel. (+34) 954 466 700; ext 1433. ORCID: 0000-0001-6223-736X

The author's institutional affiliations where the work was carried out, with a footnote for the author's present address if different from where the work was carried out

1. Department of Wetland Ecology. Estación Biológica de Doñana (EBD-CSIC). Seville (Spain).
2. Department of Conservation Biology. Estación Biológica de Doñana (EBD-CSIC). Seville (Spain).
3. Instituto Universitario de Investigación Marina (INMAR) Campus de Excelencia Internacional/Global del Mar (CEI·MAR) Universidad de Cádiz. Puerto Real, Cádiz (Spain).
4. Department of Plant Biology and Ecology. Faculty of Science and Technology. University of the Basque Country (UPV/EHU). Leioa, (Spain).
5. IKERBASQUE. Bilbao. (Spain).
6. Institute for Coastal and Water Resources, University of Louisiana, Lafayette, LA, (U.S.A.).
7. Marine Science Institute. University of California, Santa Barbara, CA, (U.S.A.).
8. Wakkanai Fisheries Institute, 4-5-15 Suehiro, Wakkanai, 097-0001 Hokkaido, (Japan).
9. Sorbonne Université. CNRS. Station Biologique de Roscoff. UMR 7144 AD2M. Roscoff (France).
10. Institut Méditerranéen de Biodiversité et d'Ecologie Marine et Continentale (IMBE), UMR 7263. Aix Marseille Université. CNRS. IRD. Avignon Université. Station Marine d'Endoume. Marseille (France).

Keywords

Admixture, invasion hubs, invasion process, mitochondrial DNA, propagule pressure,

ii. Summary

1. Understanding how introduced species succeed and become widely distributed within non-native areas is critical to reduce the threats posed by them. Our goal was to reconstruct the main invasion routes and invasion dynamics of a global freshwater invader, the red swamp crayfish, *Procambarus clarkii*, through the analysis of its genetic variability in both native and invasive ranges.

2. We inferred invasion routes and population structure from the analysis of a fragment (608bp) of the mitochondrial marker COI from 1,062 individuals of *P. clarkii* in addition to 354 GenBank sequences, for a total of 122 populations (22 natives and 100 invaded). Genetic structure was assessed using analysis of molecular variance and non-metric multidimensional scaling analyses. We analysed haplotype frequencies for the genetic variability in each locality and region. The haplotype network was depicted by using PopART software.
3. A high haplotype diversity was found in the native range (Hd: 0.90), but also in some non-native areas, such as western United States (Hd: 0.80), areas of Mexico (Hd: 0.78) and some hotspots in Europe (e.g., southern Spain or Italy), suggesting a complex pattern of multiple introductions. We grouped all localities in five differentiated groups according to biogeographic origin: the native area, West Americas, East United States, Asia and Europe. Additionally, the identification of 15 haplotypes shared between at least two localities, the phylogenetic network estimation and indices of genetic differentiation among localities allowed us to identify a large genetic admixture in the native range; the two independent invasion routes (i.e. westwards and eastwards) in US from the native range (Louisiana and Texas) with translocations within each area; a stepping-stone introduction from US to Japan (involving few individuals) themselves introduced to China afterwards; the entry of *P. clarkii* from Louisiana (US) into southern Spain and their multiple secondary introductions over Europe as well as other possible introductions in central Europe.
4. Our study emphasizes the need for unravelling the global invasion routes and the demographic processes underlying the introduction of exotic species (i.e., admixture, bridgehead invasion effect and propagule pressure) to control the spread of invasive species. Our findings highlight the value of genetic analyses to identify the geographic origin of source populations as well as the variability of invaded areas in order to reconstruct invasion dynamics and facilitate management of invasive species (e.g. through environmental DNA monitoring).

iii. Main Text

Introduction

Humans have transported species across biogeographical barriers and introduced them to new territories for millennia (Forcina et al., 2015), but large-distance movements of species have increased exponentially in recent decades (Hulme, 2009; Lenda et al., 2014) driving to an homogenization of biotas that has involved the break-down of long-established biogeographical barriers (Capinha et al., 2015). Those species that are transported by humans, released into new environments, able to survive, establish self-sustained populations, thrive, become abundant and spread geographically, are considered invasive species (Jeschke et al., 2014). Biological invasions today are perceived as major components of global change, with severe negative environmental (Simberloff et al., 2013; Blackburn et al., 2014; Jeschke et al., 2014) and socio-economic impacts (Vilà et al., 2010). To manage invasive species, it is of vital importance to identify invasion

routes (De Kort et al., 2016). However, most knowledge about the transport routes of invasive species is based on historical and observational data, which are usually scarce, confusing and sometimes inaccurate (Roman, 2006; Haydar, 2012). Population genetic studies provide valuable tools to identify areas of geographic origin of introductions, to detect single versus multiple introductions, and to describe expansion patterns (Lejeusne et al., 2014; Cristescu, 2015; Blakeslee et al., 2017; O'Hanlon et al., 2018; Fang et al., 2018), though caution must be used when interpreting demographic history over such short timescales (Fitzpatrick et al., 2012). Such information can be useful for the management of invasive species and for the prevention of future introductions (Estoup & Guillemaud, 2010), and phylogeographic studies have been proposed as an integral tool of biodiversity conservation planning (van de Crommenacker et al., 2015).

Many invasive species have high economic value, which often results in their deliberate introduction by humans into non-native areas where they can spread rapidly due to secondary introductions (see Audzijonyte et al., 2017; Cao et al., 2017; Huang et al., 2017). Unlike accidental introductions that are facilitated by humans, species that are deliberately introduced may have a higher chance of success because humans take action to ensure such success (Pyšek et al., 2011). For example, deliberate introductions often involve a high propagule pressure (i.e. number of introduction events and/or size of propagules; Lockwood et al., 2005; Simberloff, 2009), the genetic admixture of introduced populations (i.e. the mixing of populations from genetically distinct source populations; Dlugosch & Parker, 2008; Rius & Darling, 2014; Hufbauer, 2017), and the subsequent invasive bridgehead effect (i.e. a particularly successful invasive population serves as a source for new introductions, Lombaert et al., 2010; Estoup & Guillemaud, 2010).

Due to the high intensity of human disturbances and the high connectivity among inland water systems, freshwater environments are especially susceptible to invasions (Strayer, 2010; Havel et al., 2015; Tricarico et al., 2016). Also, many freshwater species can be harvested from the wild and/or cultivated in farms for commercial purposes, providing a high socioeconomic value (e.g., aquaculture; FAO, 2011). Among these, several invertebrates are valued either for human consumption or as food for other cultured animals (e.g. shrimp or prawn for fishes, crayfish for bullfrogs, etc.), which provides a high economic return (Resh & Rosenberg, 2015). Freshwater crayfish are favoured for farming since they do not have a larval phase and are polytrophic, they are relatively easy to rear compared with other cultured crustaceans (Holdich, 1993), and their consumption has a long-lasting tradition in many regions worldwide (Gherardi, 2011). The red swamp crayfish, *Procambarus clarkii* (Girard 1852), native to southern USA and northern Mexico, has been successfully introduced into all continents except Australia and Antarctica (Loureiro et al., 2015) mainly due to its economic value (Hobbs et al., 1989). Owing to its biological and ecological characteristics, this crayfish is considered one of the worst invasive species worldwide, causing serious damage to biodiversity (e.g., other crayfish species, fish, amphibians, macroinvertebrates and macrophytes) and to human infrastructure and ecosystem services (e.g., irrigation canals, water quality, rice crops, etc.) (Geiger et al., 2005;

Twardochleb et al., 2013). *Procambarus clarkii* is one of the most economically valuable aquatic species to be farmed (Huner, 2002; Souty-Grosset et al., 2016), generating tens of billions of US dollars (USD) per year in the world (http://www.fao.org/fishery/culturedspecies/Procambarus_clarkii/en#tcNA0064).

The first introductions of *P. clarkii* out of its native area took place in the early 20th century, when it was taken to the Hawaiian Islands (1923), the Pacific drainages of USA (1924), Japan (1927) and China (1929), with different motivations including aquaculture, fishing activities and food for cultured American bullfrogs *Lithobates catesbeianus* (Holmes, 1924; Penn, 1954; Brasher et al., 2006). *Procambarus clarkii* was often able to spread rapidly, occupying the rivers and lakes of non-native areas (e.g. Riegel, 1959, for California; Yue et al., 2010, for China). In the mid-1960s, a batch of crayfish was sent to Uganda from Louisiana, then translocated to Kenya, and later to other African countries (Huner, 1977; Lowery & Mendes, 1977). Concurrently, it artificially spread out of its native area in Mexico, and then to Costa Rica, Puerto Rico, Venezuela, and the Dominican Republic in the 1970s (Huner, 1977), eventually reaching Brazil in the mid-1980s (Huner, 1986). In Europe, it was deliberately and legally introduced into Spain (Badajoz and Seville in 1973 and 1974, respectively) from Louisiana (Habsburgo-Lorena, 1978; 1986). In only 45 years, *P. clarkii* has colonized many countries in Europe, being widely established in Spain, Portugal, France, Italy, Belgium, Netherlands, Germany and the United Kingdom (see Kouba et al., 2014 for the entire European distribution of this species).

To date, most genetic studies of *P. clarkii* have focused on genetic variability at a regional scale (e.g., Barbaresi et al., 2007; Torres & Álvarez, 2012; Quan et al., 2014; Yi et al., 2018; Almerão et al., 2018). Of these, very few studies have attempted to unveil the invasion routes, and when performed, they did so only at a regional scale. Hence, almost nothing is known about the population genetics and invasion routes of *P. clarkii* at a global scale. The general objective of this study is to provide a comprehensive overview of the global invasion history of *P. clarkii*. We included not only most of the non-native range of this species in the Northern Hemisphere, but also an exhaustive sampling of its native area in order to confirm the invasion sources and routes previously in the literature and detect previously unreported ones. Hence, our specific objectives were: 1) to describe the invasion dynamics of *P. clarkii* at continental and global scales, identifying the main invasion routes; and 2) to examine the genetic variability and population structure of *P. clarkii* in the native area and across the non-native range, with special focus on Europe, to reveal potentially unreported introductions not cited in the literature.

Methods

Sampling

We collected 1,062 specimens of *P. clarkii* from 72 localities: 15 native (States of Louisiana and Texas, USA) and 57 non-native localities distributed within the Northern Hemisphere (i.e., western US, eastern US, Europe and Japan) (Table 1 and Fig. 1). Crayfish were individually preserved in 96% ethanol. Average sample size per locality was 14.7 ± 6.6 individuals (mean ± SE; range 2-21) (Table 1). We included in our dataset the information for 354 additional individuals from 7 native and 43 non-native localities that we obtained from data already published in previous studies (Genbank Accession numbers: AY701195; JF438001- JF438004; JN000898- JN000908; JX120103- JX120108) available from Taylor & Knouft (2006), Filipová et al. (2011), Torres & Álvarez (2012) and Li et al. (2012), respectively. Thus, a total of 1,416 individuals from 22 native and 100 introduced localities (Fig. 1a; Table 1) were used for this study. The sequences recently published by Almerão et al. (2018) were not added into our global analyses of this study because our sequences were larger than theirs. Even so, we compared their results in a subsequent analysis (see Supplementary Material, Table S1 for synonymous haplotypes).

DNA extraction and sequencing

Genomic DNA was extracted from muscle tissue (gill tissue at LEN, FOR and PER localities; see Table 1 for more details) using a modified DNA salt-extraction protocol (composition: NaCl 25 mM, Tris 12.5 mM (pH 8.0), EDTA 12.5 mM (pH 8.0), 31.5 µL SDS 10%) and proteinase K (Aljanabi & Marinez, 1997). Logistical support was provided by the Laboratorio de Ecología Molecular, Estación Biológica de Doñana, CSIC (LEM-EBD). A fragment of the mitochondrial gene coding for the cytochrome *c* oxidase subunit I (COI) gene was amplified using the primers LCO1490 and HCO2198 (Folmer et al., 1994). Amplifications were carried out in a 20 µl reaction volume, with 1-5 µl of genomic DNA, 2 µl of 10x buffer, 0.8 µl of $MgCl_2$ (50 mM), 0.16 µl dNTP (100 mM), 0.5 µl primer LCO 1490, 0.5 µl primer HCO 2198 and 0.12 µl TAQ polymerase (Bioline). Polymerase chain reaction (PCR) consisted of an initial denaturation step at 94ºC for 5 min, followed by 30 amplification cycles (94ºC for 1 min, 47ºC for 1 min and 72ºC for 1 min) and a final elongation step at 72ºC for 5 min. Sequencing was performed by Macrogen Europe Company.

Genetic analyses

Sequences were edited using the software Sequencher™ v4.9 (Gene Codes Corp., © 1991–2009, Ann Arbor, MI 48108). Nucleotide sequences were aligned using the algorithm CLUSTAL W implemented in BioEdit (Hall, 1999). No insertions nor deletions (indels) were found. A hierarchical series of tests based on the

Bayesian Information Criterion (BIC) was applied to identify the most appropriate nucleotide substitution model among 88 models tested, as implemented in jModelTest 2 (Darriba et al., 2012). We used the nested model Tamura & Nei (1993) with 133 parameters, 1382.91 –lnL for onwards analyses. DnaSP 6.0 software was used to calculate the number of polymorphic sites (S), haplotype diversity (Hd), nucleotide diversity (π), and total number of synonymous and non-synonymous mutations, for which nucleotide sequences were translated into amino acid sequences using the *Drosophila* mitochondrial genetic code (Rozas et al., 2017). The haplotype network was inferred by the TCS method (Clement et al., 2000) implemented in PopART software (Leigh & Bryant, 2015).

Because of a smaller sampling size (1 or 2 individuals), ILL, LAf, FR1, FR2, FR3 and VAL localities were excluded from downstream analyses. Pairwise ϕ_{ST} (*Phi*$_{ST}$) and hierarchical analysis of molecular variance (AMOVA) were calculated using Arlequin 3.5 (Tamura & Nei, 1993; Excoffier & Lischer, 2010). To examine the genetic differentiation between any two of the populations, ϕ_{ST} calculations were calculated assuming gamma-distributed substitution rates using the Tamura and Nei model (Tamura & Nei, 1993) to compute a distance matrix and 10,000 bootstrap pseudo-replicates were used to estimate the standard error. The *p-values* were corrected for multiple comparisons using the false discovery rate (FDR) control according to the Benjamin and Hochberg (BH) correction method (Benjamin & Hochberg, 1995). To ascertain the genetic structure of populations, AMOVAs were performed based on 10,000 random permutations. Due to the large native range of *P. clarkii*, we classified native localities into five groups according to natural (e.g. river catchments) and administrative (e.g. country or state frontiers) boundaries: Mexico, Texas, Louisiana east (east of the Atchafalaya River), Louisiana west (west of the Atchafalaya River), and Mississippi River upstream (upstream starting at Monroe, Memphis and north to Illinois). However, for all datasets, two different *a priori* hypotheses were tested: (a) native versus introduced localities, and (b) population grouping according to biogeographical distribution of this species into 5 zones: (1) native area, (2) West Americas which included all samples from the USA west of Texas, including California, Oregon and Washington State, plus all samples from Hawaii and invaded Central America, (3) East United States (from Louisiana to the Atlantic Ocean and Chicago), (4) Asia, and (5) Europe. Another *a priori* hypothesis was analysed exclusively for European populations to test whether there were one (i.e., the whole of Europe) or two genetic clusters within Europe (i.e., southern and northern areas of *P. clarkii* distribution). A dissimilarity matrix of Jost's D_{est} distances was also calculated with 10,000 replicates using SPADE (Jost, 2008; Chao & Shen, 2012). Based on ϕ_{ST} and D_{est} estimates, two non-metric multidimensional scaling (NMDS) analyses were used to graphically represent the differentiation among localities and their respective zones (described above) using the *vegan* package in R (Oksanen, 2013).

Results

Among the 1,416 specimens of *P. clarkii* analysed, we obtained a matrix of 608 base pairs (bp) of the cytochrome *c* oxidase subunit I (COI) with 54 polymorphic sites, yielding 65 haplotypes. Sequences of all haplotypes were submitted to GenBank and assigned Accession Numbers: MK026671 - MK026735. Most of the nucleotide substitutions were synonymous, but four non-synonymous changes were identified (2 substitutions at the 1st position corresponding to a change from an Isoleucine to a Valine, and from a Methionine to a Valine, respectively; and 2 substitutions at the 2nd position corresponding to a change from a Valine to an Alanine, and from a Threonine to a Methionine, respectively). Of these four non-synonymous changes, one singleton was found in the ALB locality (Spain) and three parsimony sites were located at SMA and COM localities in Texas (US) and in the DU locality in the invaded area of Mexico (see abbreviations in Table 1 and marked in haplotype network in Fig. 2).

The overall haplotype diversity (Hd) and nucleotide diversity (π) were 0.76 and 0.0040, respectively. The native area showed the highest haplotype and nucleotide diversity (0.90 and 0.0055, respectively), with the highest figures being found in WOO, DES, MON and PIE localities (Table 1). For invaded areas, haplotype and nucleotide diversities varied considerably between regions: Hd: 0.80 and π: 0.0048 in non-native US, Hd: 0.78 and π: 0.0056 in non-native Mexico, Hd: 0.46 and π: 0.0023 in Asia and Hd: 0.58 and π: 0.0022 in Europe, respectively (for localities see Table 1). Of the entire dataset, a total of 15 haplotypes were shared between at least two sampling localities (Fig. 1), of which Hap_04 was present at high frequency almost worldwide, irrespective of the native (up to 13 localities) or non-native (up to 76 localities) status of the populations. Other haplotypes were shared between continents, including coincidences between US and Europe (Hap_01, Hap_03, Hap_05, Hap_09 and Hap_29) and North America and Asia (Hap_02 found in invaded localities in Mexico, California, Japan and China, and Hap_40 shared between the native area and Japan). Conversely, 50 haplotypes were restricted to one locality (i.e., private haplotypes), 29 of them found in the native area and 21 being exclusive to one of the invaded localities (see Supplementary Material, Table S2).

The statistical parsimony haplotype network showed a star-like structuring centered around the Hap_04, which appeared in almost half of sampled crayfish (618 specimens) geographically widely distributed over all zones (13 native and 62 invaded localities) (Fig. 2 and see Supplementary Material, Table S2). Moreover, there were other smaller star-like structuring around three haplotypes (Hap_01, Hap_20 and Hao_09). Hap_01 was mainly distributed in the US, both in its native (9 localities in Louisiana) and non-native range (TOP and PIN in western US; and PER, LEN, FOR and CHI in Atlantic area), but also was found in two Spanish localities (ECO and GIJ). Hap_20 was found in Louisiana and across the western US. In addition, it closely joined (1 mutation) to Hap_28 which is widely found in Asia. Hap_09 was broadly distributed among the native localities in Louisiana, as well as in three invaded North American localities, and two Spanish and one French locality (AR4, CHO and FR1, respectively). In addition, this central haplotype was connected by only one mutation with Hap_40 which was present in Japan. A thorough analysis of the haplotype network in the native range (see Supplementary Material, Fig. S1) showed no clear

genetic structure except for Texas localities (Hap_15-19 and Hap_48-50), Mexico localities (Hap_61-62) and the Hap_20 which was mainly found in southeastern Louisiana. Additionally, Hap_04, Hap_01 and Hap_09 were widely distributed over all localities in the native range as well as many localities grouped evolutionarily differentiated haplotypes (e.g., MON, NAT or LO localities), indicating a large genetic admixture in Louisiana.

Due to the large native area of *P. clarkii*, we tested whether the native area clustered into 5 groups (Mexico, Texas, Mississippi River upstream, Louisiana east, and Louisiana west). As haplotype network showed, AMOVA also revealed a slight genetic structure within the native area, where a small fraction of the total variance was due to between-group variance (29.0%); nevertheless, most of the variance was explained by variation within populations (61.6%) (Table 2). This may be due to the high proportion of private haplotypes (Fig. 1b and Fig. 1c). For the whole dataset, native and invaded areas worldwide were not clustered into two different genetic groups because only 14.3 % of total variation was due to differences between groups (Table 3), indicating the high variability of *P. clarkii* in the invaded range. However, after classifying the whole set of localities according to their biogeographical ranges (see Methods), 39.6% of total variation was still explained by differences within localities, but 36.0% of total variance was due to differences among these 5 established zones (Table 3). This result seems to indicate a slight genetic structure among these zones. In Europe, a moderate genetic structure was detected between the northern and southern distribution areas of *P. clarkii* (Fig. 1g), where Hap_04 was predominant in South Europe and Hap_11 in Central Europe. AMOVA analyses showed that 40.7 % of explained variance was due to differences between both genetic clusters (Table 4).

In a NMDS plot based on D_{est} distances (Fig. 3), most localities remained within the 95% confidence intervals (CI) of the native area group, except some localities from Japan, China and western North America, in which Hap_28 was present at high frequency. This is due to the fact that we did not find this haplotype in the native area, despite the exhaustive sampling done there. All localities from eastern US were closely grouped within the range of the native area. However, the localities from western North America were more different from each other, for instance PIN was closer to European localities, whereas VEN and CH were more similar to Asian locations. In addition, the other locality from western North America not only had a clear proximity to each other but also to localities from within the native area (COM, SMA, COC and NL), indicating a similarity among them. The result of AMOVA analysis for European populations, where two genetic clusters were found between localities from the northern and southern European distributions, was also reinforced by NMDS plot. LON, HOL, ECA, BIO and BRI localities (depicted by a green triangle in the NMDS plot) were situated all together outside the 95% CIs of the European group, having greater proximity to Texas and Mexico localities from the native area and those from western America than to south European localities.

Discussion

The high haplotype diversity of *P. clarkii* found in some invaded localities suggests that its global invasion, driven mostly by human-mediated introductions, may have involved admixture in the native range, an invasive bridgehead effect, and high propagule pressure. However, we also detected low levels of genetic diversity in some non-native areas (e.g. Asia), attributable to potential bottlenecks or founder effects. Our results allow the identification of the likely geographic origin and main routes of invasion, helping us to understand how the invasion has happened over a long time scale (Fig. 4).

The native range of the red swamp crayfish

Admixture has been proposed to be a causal mechanism triggering the invasiveness of some introduced species (Kolbe et al., 2007; van Boheemen et al, 2017; Fischer et al, 2017; Wagner et al., 2017; but see also, Rius & Darling, 2014) by enhancing genetic variability, thus improving population growth, decreasing the risk of extinction, and favouring adaptation to novel environments. In the present study, we found the highest haplotype diversity in the native area. The vast majority of haplotypes found in invaded areas also appeared in Louisiana but not in other native populations of US or Mexico. This pattern is arguably related to the commercial exploitation of this species in Louisiana, where *P. clarkii* has been reared, harvested and sold globally by food-industry companies for a long time (Gary, 1975; Alford et al., 2017). Although some genetic clusters seem to be differentiated between east and west Louisiana (Hap_01, Hap_04 and Hap_20 predominated in Louisiana east while Hap_03 and Hap_08 predominated in Louisiana west), Texas and Mexico localities, most of the genetic variation in those areas occurred within localities. The lack of a clear genetic structure in the native area might imply a pattern of admixture owing to farming activities in Louisiana, in which crayfish are often exchanged and translocated from wild to captive populations (Huner, 2002). Similar genetic patterns of native admixed populations have been identified in other species related to aquaculture such as the topmouth gudgeon, *Pseudorasbora parva*, when they were translocated together with Chinese carp species (Hardouin et al., 2018). The exchanges found in the Louisiana populations do not seem to have occurred in Texas, since eight private haplotypes were found in two populations and almost all haplotypes were grouped together (see Hap_15-19 and Hap_48-50). Admixed source populations, like for *P. clarkii* in Louisiana, can lead to high genetic variability in invasive populations, thus allowing the invasive species to face novel environments and to thereby increase invasion success in the introduced range. But this assumption should be interpreted with caution since some species are able to show high invasiveness despite low genetic variability, such as *Procambarus virginalis*, a potentially highly invasive parthenogenetic crayfish that is able to establish wild populations from a single released individual (Feria & Faulkes, 2011).

The invasion dynamics of the red swamp crayfish

According to the literature, the first invasion of *P. clarkii* took place in Hawaiian streams in 1923 (Brasher et al., 2006) and in California in 1924 (Holmes, 1924). However, the geographical origins of both invasions remain unclear. In 1934, another event of introduction occurred in the island of Oahu, Hawaii, from Santa Barbara, California, from which subsequently *P. clarkii* apparently spread over the rest of the Hawaii archipelago (Penn, 1954). The Hap_27 found in each of these US states (California and Hawaii) may confirm this second introduction event of *P. clarkii* from California, but this result should be treated with caution since only four crayfish were sampled from Hawaii. In the continental USA, the California introduction was followed by later introductions to Oregon in the early 1980s (Larson & Olden, 2011) and to Washington State in the 2000s (Mueller, 2001). Theoretically, we might expect higher genetic variability in California, with a decrease of variability from the place of first introduction (California) northwards (Washington, Oregon) due to secondary bottlenecks and/or founder effects. Our results seem to indicate a more complex pattern of invasion (Fig. 4), in which shared haplotypes between populations in the western US confirm the connectivity among localities (Hap_01, Hap_09, Hap_20 or Hap_27). The development of the crayfish industry in California, where *P. clarkii* has been cultured and traded for many years, seems to have contributed to the dispersal of the crayfish along the West Coast of the US (Comeaux, 1978; Mueller, 2001). Moreover, this scenario might have been favoured by the large number of biological supply companies in this area, and also by the use of live animals for classroom observations, some of which were given to students after school-years to take home and were probably released in the wild later on (Larson & Olden, 2008).

Regarding the origin of California populations, we were not able to identify the precise geographic origin of this invasion because though low ϕ_{ST} values were found among California and native localities, and these native localities were not close to each other (see Supplementary Material, Table S3). The haplotypes found in Topanga Creek (TOP) suggest that the origin of this invasion came from southeastern Louisiana, but the presence of the Hap_08 (mainly distributed in western Louisiana; and considerably distinct from ancestral haplotype), would contradict this idea. For the other two California localities (VEN and SYZ), we were also unable to unveil their origins accurately because their haplotypes were not shared with the native area; however, their private haplotypes were more evolutionarily related to Hap_04 and Hap_20, which would indicate again a possible origin from southeastern Louisiana. Given that crayfish populations in Topanga Creek were recently established (around 2001, Garcia et al., 2015), this population could come from previous established populations in California, having undergone a possible bottleneck. If so, we could be underestimating the haplotype diversity in the area and more haplotypes would be present in California. This latter surmise is reinforced by the fact that *P. clarkii* has long been considered a pest in southern California (Riegel, 1959), the higher haplotype diversity in population WAV (Oregon) and the presence of Hap_04 at a high frequency only in PIN (Washington State). This suggests that (1) more genetic variability is to be expected in California, acting as an admixed or bridgehead zone because of its anteriority in

introduction and the numerous biological supply companies which can move live crayfish, or (2) other distinct introduction events may have occurred in northern states from the native area, which seem unlikely given the great demand for the crayfish industry in California (Comeaux, 1978) and since the northwest US is the native range of another commercially and culturally important crayfish species (i.e., signal crayfish, *Pacifastacus leniusculus*) (Holdich, 1993).

Localities from the eastern US showed a different haplotype frequency (Hap_01 was the most common haplotype) from those of the western part of the country, suggesting another independent route of invasion to North Carolina and north of Illinois with subsequent secondary events (Fig. 4). This pattern is congruent with the native area located in the middle of the US, making it easier to move crayfish in two independent directions than from coast to coast, as well as the presence of one of the biggest biological supply companies in North Carolina (US) which was supplying most of the eastern US invaded areas. Although the eastern US is a suitable area for *P. clarkii* (Larson & Olden, 2011), the low haplotype diversity found in eastern localities of the US (from Hd = 0.20 in LEN to Hd = 0.50 in FOR) suggests a low propagule pressure from the native area or from shipments of the biological supply company in North Carolina.

In Asia, according to the literature (Penn, 1954), 100 specimens of *P. clarkii* were carried from New Orleans to Japan in 1927, of which only 20 specimens arrived alive to a pond near Tokyo (Penn, 1954; Kawai & Kobayashi, 2005); two years later, *P. clarkii* from Japan were translocated to Nanjing, in China (Li et al., 2007). This historical report perfectly matches the genetic pattern (i.e., founder effect and strong bottleneck) found in Japanese and, overall, Chinese populations of *P. clarkii* (Yue et al., 2010; Li et al., 2012; Zhu et al., 2013, this study), in which a smaller batch was introduced to Japan and subsequent invasions came from the Japan population with few founders (Fig. 4). The lack of ectoparasites of the order Branchiobdellida is often attributed to long shipments in poor conditions (Gelder & Williams, 2015; Clavero et al., 2016). Kawai & Kobayashi (2005) found no Branchiobdellida on Japanese specimens of *P. clarkii*, a pattern that could support the hypothesis that all specimens of *P. clarkii* in Japan (and thus, in China) descend from the initial introduction at the end of 1920s. Our results show low haplotype diversity in Japanese and Chinese populations (Hd = 0.48 and 0.35, respectively) with only four haplotypes appearing in the extensive area sampled, only two of them at high frequency (Hap_04 and Hap_28), as similarly found by Li et al. (2012). Apart from Asian populations, Hap_28 was only found in few individuals of the CH population (Mexico) and VEN (California) but not in the native area. Surprisingly, our genetic results seem to contradict previous literature because neither Hap_28 nor Hap_40 appeared in localities sampled around the native locations of New Orleans, Louisiana. The finding of Hap_28 in California and the similar date of both introductions (i.e., California in 1924 and Japan in 1927) suggests that a route of invasion from California to Japan is more plausible (Fig. 4). Additionally, as Hap_28 was a rare haplotype in our sampling (only 5 of the 988 non-Asian individuals carrying this haplotype), another old introduction into Asia seems unlikely because a different

haplotype frequency would be expected. This strong genetic bottleneck did not prevent *P. clarkii* from invading successfully (Estoup et al., 2016) and becoming a pest across Japanese and Chinese territories (Penn, 1954; Kawai, 2017). A similar pattern has been recorded for the parthenogenetic crayfish, *P. virginalis*, in other areas (Feria & Faulkes, 2011). Finally, the presence of the Hap_40 in TOK (Japan) and NAT (northwest Louisiana) led to two possible hypotheses: (1) this haplotype was present in the initial translocated batch but has been lost in subsequent secondary invasions by genetic drift or bottleneck, or (2) one new introduction event has recently occurred from the native area but has not been spread yet (nor been reported). Of both hypotheses, the first one seems more plausible, but we are not able to resolve them.

Of all invaded areas, the European invasion by *P. clarkii* has perhaps been the best reported, with the first two events of introduction from Louisiana to Spain (Halsburgo-Lorena, 1978) and later into other European countries (i.e., in Spain Gutiérrez-Yurrita, et al., 1999; in France Changeux, 2003 and Laurent, 1997; in Italy Gherardi et al., 1999) (Fig. 4). The invasion routes through European countries and connectivity between European populations are poorly understood, possibly because they are due to multiple and uncontrolled deliberate introductions by private citizens (Clavero, 2016; this likely also occurred with signal crayfish, *P. leniusculus*, see Petrusek et al., 2017). In European populations, we found a moderately high overall haplotype diversity (Hd = 0.58; i.e. lower than for invasive US populations, but higher than in Asia). The European invasion has probably not been based on as many introduction events as invasive American populations (e.g., California) given the differences in proximity to the native area (a possible cause of the higher haplotype diversity found on the American continent); however, the large number of *P. clarkii* imported to Spain (100 kg, around 6,500 crayfish) probably also included high genetic variability from the native area compared to the Asian introduction. According to our results, a clear decrease in haplotype diversity was found from the initial sites of introduction (Hd = 0.66 in rice fields near Seville, and Hd = 0.72 in Doñana National Park) northwards, excepted for TOS in Italy (Hd = 0.72), which could be explained by intensive farming activities on Lake Massaciuccoli (Gherardi et al., 1999) or a second introduction.

The most surprising result was the finding of two independent genetic groups in Europe. The Hap_04 was widely distributed over the Iberian Peninsula, South France and Italy, while the Hap_11 predominated in Northern France and Italy, Belgium, the Netherlands and United Kingdom, but was not found in the Iberian Peninsula. Two possible scenarios could explain this result: (1) we did not capture all haplotypes from the first introduction in southern Europe, and northern populations have undergone a strong bottleneck; or (2) another unreported introduction from outside Europe has occurred, independently from those reported from southern Spain (Fig. 4). The first scenario is unlikely, due to the extensive sampling effort on both the Iberian Peninsula and the native area. In such a scenario, the Hap_11 should have appeared in the Iberian Peninsula because of other high frequencies in North Europe. Moreover, Almerão et al. (2018) found nine

haplotypes in Central France, four of which seem to match with our database but not Hap_11. On the other hand, unreported introductions of *P. clarkii* could be a consequence of the sales in pet shops which are common and one of the primary introduction pathways in Central Europe (Chucholl, 2015; Faulkes, 2015). These results, however, also support previous historical reports (Laurent, 1990; Holdich, 2002) suggesting how live *P. clarkii* may have been brought from Kenya to Europe in the 1970s. Both hypotheses could explain the presence of this haplotype across the northern European range of *P. clarkii*. To clarify our results, samples from pet shops or African samples of *P. clarkii* should be obtained in order to resolve the likely second invasion route. The second scenario therefore seems the most plausible (as Barbaresi et al., 2007, also suggested), with a plausible introduction from Kenya to Central Europe.

National and international translocations have occurred within Europe (Fig. 4). On the one hand, we found Hap_04 and Hap_05 to be highly frequent all over the Iberian Peninsula to South France, which perfectly matches with the literature signaling where live specimens having been translocated from South Spain (Laurent, 1997). On the other hand, the presence of the Hap_06 at higher frequencies in southern Portugal and dated reports of introduction events across Portugal seem to confirm the spread of *P. clarkii* from south (near the first introduction site in 1973; Cruz & Rebelo, 2007) to north Portugal (Gutiérrez-Yurrita et al., 1999). In addition, Hap_06 was also found in MAD (Spain) and LAZ (Italy), suggesting a connection among these invaded areas as well as POR in Portugal and LEZ in the Ebro Basin, Northern Spain (Fig. 4). Another possible connection was between TOS in Italy and southern Spain, with most haplotypes shared, suggesting another possible invasion route. Continuous exchanges and secondary translocations of *P. clarkii* through invaded areas have produced a very complex invasion process, which could accelerate the invasiveness of this kind of species (Wagner et al., 2017).

Our results provide a clear example of how different features of introduction events and invasion processes (e.g. genetic admixture, propagule pressure or secondary introductions) can generate contrasting genetic diversity patterns across non-native populations of a global invader (Roman & Darling, 2007). For example, Asian populations of *P. clarkii* underwent a strong bottleneck as a consequence of the introduction of few individuals in a single introduction event, which arguably originated from an already introduced population (probably in western US) that might have already gone through previous bottlenecks. Genetic diversity was notably higher in the *P. clarkii* populations in western US, probably due to the existence of numerous introduction events (e.g. facilitated by vicinity to the native range and the development of biological supply companies), involving large batches of individuals with high genetic admixture. The European case is apparently intermediate, with numerous individuals imported from an admixed native range to SW Spain, from which the species expanded across the continent through multiple secondary introductions, involving a clear loss in haplotype diversity. However, higher genetic variabilities were found in European (Petrusek

et al., 2017) and Japanese populations of *P. leniusculus* (Usio et al., 2016) in comparison to that reported by us for *P. clarkii*, arguably due to the combination of several introduction events involving large batches of individuals and coming from a variety of origins in the US, including native and non-native populations. A striking pattern deriving from our results is that the invasiveness of *P. clarkii* does not seem to depend, at least in the short- and mid-term, on the genetic diversity of introduced populations. Although genetic diversity can fuel invasiveness by allowing the efficient adaptation of introduced populations to spatial and temporal variability in the recipient ecosystems, the relationship between those two features is obscure (Estoup et al., 2016). There is growing evidence that the loss of genetic diversity in introduced populations can be compensated through epigenetic processes (Estoup et al., 2016). The most extreme example of high invasiveness with low genetic variability is the clonal species *P. virginalis* (Feria & Faulkes, 2011), which is able to thrive in a wide variability of environmental conditions (Andriantsoa et al., 2019).

Apart from informing about invasion routes, our results might also be relevant for new approaches for the detection and surveillance of invasive species. Environmental DNA (eDNA) is a rapidly emerging monitoring tool for freshwater invasive species based on the persistence of DNA fragments in the environment (Ficetola et al., 2008; Mauvisseau et al., 2018). Large-scale phylogeographic studies provide accurate datasets for improving invasive species detection protocols based on eDNA (Ficetola et al., 2008; Larson et al., 2017). Admixture in both native and invasive ranges, as well as the bridgehead invasive effect, has led to large intraspecific genetic variability within and among invaded areas, which may reduce the efficacy of eDNA protocols (Wilcox et al., 2015). In fact, the spatial gradients in genetic variability and the presence of different genetic clusters in Europe reported here, probably led to the failure of eDNA probes in detecting French populations of *P. clarkii* (Tréguier et al., 2014; Mauvisseau et al., 2018), which had worked well with the less variable Chinese populations (Cai et al., 2017). Our study may thus be useful for the development of better site-specific eDNA-based protocols to detect *P. clarkii* (Manfrin et al., 2019).

Conclusions

Our results illustrate extensive admixture of *P. clarkii* in its native area, report two independent invasion routes in the US (i.e., westwards and eastwards), and support the historical reports of a single introduction event into Asia involving few individuals. They also suggest that Europe may have received *P. clarkii* through more introduction routes than the frequently reported imports into Spain. To find other likely introduction routes, more effort should be put on sampling in previously unstudied sites (e.g., Texas, pet shops, biological supply trade and/or Southern Hemisphere countries where other introduced populations might act as sources of invasion, for example, African or South American populations).

We have traced the complex scheme of invasion of *P. clarkii* (Fig. 4), with a key role for human-mediated dispersal. The economic value of *P. clarkii* and the ease with which it is transported have favoured the spread of the species worldwide (largely for aquaculture, the aquarium trade and other forms of human exploitation as food) as the consequence of multiple subsequent introduction events. Genetic admixture, invasive bridgehead effects, extensive genetic variation in the native area and high propagule pressure are apparent drivers of genetic variability across its broad geographic distribution. Such extensive genetic variability in invaded areas should be taken into account to improve management measures based on mtDNA for environmental detection of this invasive species. Overall, invasive species, and invasive crayfish in particular, continue to be artificially introduced into more countries through the aquarium trade (e.g. fish species, Strecker et al., 2011; crayfish species, Patoka et al., 2014 and particularly *P. virginalis*, Faulkes 2015). The example of the successful worldwide invasion of *P. clarkii* highlights the high spread potential of intentionally introduced freshwater species, especially those species also involved in aquaculture (Naylor et al., 2001). Once a species has been introduced in a new territory, management strategies aimed at reducing the spread and impacts of invasive species should focus on avoiding secondary introductions and would benefit from the early detection of potential invasion hubs.

iv. Acknowledgements

We thank R. López, M. A. Bravo, and Á. Vidal for field and lab assistance, as well as the many colleagues who kindly provided samples from Spain [J. Molero, J. L. Grau, E. Carnerero, A. Pradilla (CCEDCV - EL PALMAR), J. Rucio & M. Alcántara (Aragón Government), L.M. Álvarez (Asturias Government), Parque Martioda & J. Carreras (Alava Provincial Council), J. L. Moreno, N. Franch (Parc Natural del Delta de l'Ebre), Centro de Acuicultura Vegas del Guadiana (Extremadura Government), L. Valls, F. J. Galindo (Andalusia Government), F. Unzu & L. Lopo (La Rioja Government), J. Bosch, Presi, Kike, F. J. Oliva, I. Vedia, A. Mestre and F. Mesquita], Europe: [F. Ribeiro, A. F. Filipe, I. Roessink, D. Ghia, E. Tricarico, R. Cammaerts, P. Fossat, D. Nicolas, E. Petit, O. Adrien and J. Cucherousset] and the United States [R. Fagundo & B. Williams (North Carolina Museum of Natural Sciences), T. García, J. Olden & L. Kuehne, E. R. Larson, C. Gabor and R. Keller]. We also thank both Laboratorio de Ecología Molecular (LEM-EBD) and Laboratorio de SIG y Teledetección (LAST-EBD) at Estación Biológica de Doñana (CSIC) for providing logistical support. In addition, we thank Eric R. Larson and another anonymous reviewer who improved the final version of this manuscript. F.J.O. was supported by an Andalusia Government grant. This study was funded by the Andalusia Government (RNM-936) and the bilateral CNRS-CSIC PICS programs (PICS07360 and PIC2015FR4).

Conflicts of Interest

The authors declare no conflict of interest.

v. References

Alford, A. B., Kaminski, R. M., Grado, S. C., D'Abramo, L. R., & Avery, J. L. (2017). Harvest of crayfish as an ecosystem service of wetlands compared to production systems with planted forage. *Aquaculture Economics & Management*, 21, 295-313.

Aljanabi, S. M., & Marinez, I. (1997). Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. *Nucleic Acids Research*, 25, 4692–4693.

Almerão, M. P., Delaunay, C., Coignet, A., Peiró, D. F., Pinet, F., & Souty-Grosset, C. (2018). Genetic diversity of the invasive crayfish *Procambarus clarkii* in France. *Limnologica*, 69, 135-141.

Andriantsoa, R., Tönges, S., Panteleit, J., Theissinger, K., Carneiro, V. C., Rasamy, J., & Lyko, F. (2019). Ecological plasticity and commercial impact of invasive marbled crayfish populations in Madagascar. *BMC Ecology*, 19 (1), 8.

Audzijonyte, A., Baltrūnaitė, L., Väinölä, R., & Arbačiauskas, K. (2017). Human-mediated lineage admixture in an expanding Ponto-Caspian crustacean species *Paramysis lacustris* created a novel genetic stock that now occupies European waters. *Biological Invasions*, 19 (8), 2443-2457.

Barbaresi, S., Gherardi, F., Mengoni, A., & Souty-Grosset, C. (2007). Genetics and invasion biology in fresh waters: a pilot study of *Procambarus clarkii* in Europe, pp. 381-400. In, F. Gherardi (ed.), Biological Invaders in Inland Waters: Profiles, Distribution, and Threats. Springer, Dordrecht. The Netherlands.

Blackburn, T. M., Essl, F., Evans, T., Hulme, P. E., Jeschke, J. M., Kühn, I., ... & Pergl, J. (2014). A unified classification of alien species based on the magnitude of their environmental impacts. *PLoS biology*, 12(5), e1001850.

Blakeslee, A. M. H., Kamakukra, Y., Onufrey, J., Makino, W., Urabe, J., Park, S., … Miura, O. (2017). Reconstructing the Invasion History of the Asian shorecrab, *Hemigrapsus sanguineus* (De Haan 1835) in the Western Atlantic. *Marine Biology*, 164:47.

Brasher, A. M., Luton, C. D., Goodbred, S. L., & Wolff, R. H. (2006). Invasion patterns along elevation and urbanization gradients in Hawaiian streams. *Transactions of the American Fisheries Society*, 135(4), 1109-1129.

Cai, W., Ma, Z., Yang, C., Wang, L., Wang, W., Zhao, G., Geng, Y. & Douglas, W. Y. (2017). Using eDNA to detect the distribution and density of invasive crayfish in the Honghe-Hani rice terrace World Heritage site. *PloS one*, 12(5), p.e0177724.

551 Cao, L. J., Wang, Z. H., Gong, Y. J., Zhu, L., Hoffmann, A. A., & Wei, S. J. (2017). Low genetic diversity but
552 strong population structure reflects multiple introductions of western flower thrips (Thysanoptera:
553 Thripidae) into China followed by human-mediated spread. *Evolutionary Applications*, 10(4), 391-401.

554 Capinha, C., Essl, F., Seebens, H., Moser, D., & Pereira, H. M. (2015). The dispersal of alien species redefines
555 biogeography in the Anthropocene. *Science*, 348 (6240), 1248-1251.

556 Changeux, T. (2003). Evolution de la répartition des écrevisses en France métropolitaine selon les enquêtes
557 nationales menées par le Conseil Supérieur de la Pêche de 1977 à 2001. *Bulletin Français de la Pêche et de la*
558 *Pisciculture*, 370-371, 15-41.

559 Chao, A., & Shen, T. J. (2012). Program SPADE (Species Prediction And Diversity Estimation). Program and
560 User's Guide published at <http://chao.stat.nthu.edu.tw>.

561 Chucholl, C. (2015). Marbled crayfish gaining ground in Europe: the role of the pet trade as invasion
562 pathway. In Kawai T, Faulkes Z and Scholtz G (eds). *Freshwater Crayfish: a global overview*. Boca Raton
563 (FL), CRC Press, p. 83-114.

564 Clavero, M. (2016). Species substitutions driven by anthropogenic positive feedbacks: Spanish crayfish
565 species as a case study. *Biological Conservation*, 193, 80-85.

566 Clavero, M., Nores, C., Kubersky-Piredda, S., & Centeno-Cuadros, A. (2016). Interdisciplinarity to
567 reconstruct historical introductions: solving the status of cryptogenic crayfish. *Biological reviews*, 91 (4),
568 1036-1049.

569 Clement, M., Posada, D., & Crandall, K.A. (2000). TCS: a computer program to estimate gene genealogies.
570 *Molecular Ecology*, 9, 1657–1659.

571 Comeaux, M. C. (1978). The crawfish industry of California and the Northwest. *The California Geographer*, 18,
572 121-135.

573 Cristescu, M. E. (2015). Genetic reconstructions of invasion history. *Molecular ecology*, 24 (9), 2212-2225.

574 Cruz, M. J. & Rebelo, R. (2007). Colonization of freshwater habitats by an introduced crayfish, *Procambarus*
575 *clarkii*, in Southwest Iberian Peninsula. *Hydrobiologia*, 575, 191–201.

576 Darriba, D., Taboada, G. L., Doallo, R., & Posada, D. (2012). jModelTest 2: more models, new heuristics and
577 parallel computing. *Nature methods*, 9 (8), 772.

578 Dlugosch, K. M., & Parker, I. M. (2008). Founding events in species invasions: genetic variation, adaptive
579 evolution, and the role of multiple introductions. *Molecular Ecology*, 17, 431-449.

580 Estoup, A., & Guillemaud, T. (2010). Reconstructing routes of invasion using genetic data: why, how and so
581 what? *Molecular Ecology*, 19, 4113-4130.

582 Estoup, A., Ravigné, V., Hufbauer, R., Vitalis, R., Gautier, M., & Facon, B. (2016). Is there a genetic paradox
583 of biological invasion?. *Annual Review of Ecology, Evolution, and Systematics*, 47, 51-72.

584 Excoffier, L., Laval, G., & Schneider, S. (2005). Arlequin ver. 3.0: An integrated software package for
585 population genetics data analysis. *Evolutionary Bioinformatics Online*, 1, 47-50.

586 Fang, B., Merilä, J., Ribeiro, F., Alexandre, C. M., & Momigliano, P. (2018). Worldwide phylogeny of three-
587 spined sticklebacks. *Molecular Phylogenetics and Evolution*, 127, 613-625.

588 FAO (Food and Agriculture Organization of the United Nations) (2011). The state of world fisheries and
589 aquaculture 2009. FAO, Rome.

590 Faulkes, Z. (2015). A bomb set to drop: parthenogenetic Marmokrebs for sale in Ireland, a European location
591 without non-indigenous crayfish. *Management of Biological Invasions*, 6 (1), 111–114.

592 Feria, T. P., & Faulkes, Z. (2011). Forecasting the distribution of Marmorkrebs, a parthenogenetic crayfish
593 with high invasive potential, in Madagascar, Europe, and North America. *Aquatic Invasions*, 6, 55-67.

594 Ficetola, G. F., Miaud, C., Pompanon, F., & Taberlet, P. (2008). Species detection using environmental DNA
595 from water samples. *Biology letters*, 4, 423-425.

596 Filipová, L., Grandjean, F., Chucholl, C., Soes, D. M., & Petrusek, A. (2011). Identification of exotic North
597 American crayfish in Europe by DNA barcoding. *Knowledge and Management of Aquatic Ecosystems*, (401),
598 11.

599 Fischer, M. L., Salgado, I., Beninde, J., Klein, R., Frantz, A. C., Heddergott, M., … Hochkirch, A. (2017).
600 Multiple founder effects are followed by range expansion and admixture during the invasion process of
601 the raccoon (*Procyon lotor*) in Europe. *Diversity and Distributions*, 23 (4), 409-420.

602 Fitzpatrick, B. M., Fordyce, J. A., Niemiller, M. L., & Reynolds, R. G. (2012). What can DNA tell us about
603 biological invasions?. *Biological Invasions*, 14, 245-253.

604 Folmer, O. M., Black, M., Hoeh, R., Lutz, R., & Vrijehoek, R. (1994). DNA primers for amplification of
605 mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine*
606 *Biology and Biotechnology*, 5, 304–313.

607 Forcina, G., Guerrini, M., van Grouw, H., Gupta, B. K., Panayides, P., Hadjigerou, P., … Barbanera, F. (2015).
608 Impacts of biological globalization in the Mediterranean: Unveiling the deep history of human-mediated
609 gamebird dispersal. *Proceedings of the National Academy of Sciences*, 112 (11), 3296-3301.

610 Garcia, C., Montgomery, E., Krug, J., & Dagit, R. (2015). Removal efforts and ecosystem effects of invasive
611 red swamp crayfish (*Procambarus clarkii*) in Topanga Creek, California. *Bulletin of the Southern California*
612 *Academy of Sciences*, 114 (1), 12-21.

613 Gary, D. L. (1975). The geography of commercial crayfish ponds in south Louisiana. *Freshwater Crayfish*, 2,
614 117-124.

615 Geiger, W., Alcorlo, P., Baltanás, A., & Montes, C. (2005). Impact of an introduced Crustacean on the trophic
616 webs of Mediterranean wetlands. *Biological Invasions*, 7, 49-73.

617 Gelder, S. R. & Williams, B. W. (2015). Clitellata: Branchiobdellida. In: *Ecology and General Biology: Thorp and*
618 *Covich's Freshwater Invertebrates* (eds Thorp J, Rogers DC). Academic Press, New York, pp. 551–563.

619 Gherardi, F., Baldaccini, G. N., Barbaresi, S., Ercolini, P., De Luise, G., Mazzoni, D., & Mori, M. (1999). "The
620 situation in Italy". In Crayfish in Europe as alien species. How to make the best of a bad situation?, Edited
621 by: Gherardi, F and Holdich, DM. 107–128. Rotterdam: A.A. Balkema.

622 Gherardi, F. (2011). Towards a sustainable human use of freshwater crayfish (Crustacea, Decapoda,
623 Astacidea). *Knowledge and Management of Aquatic Ecosystems*, (401), 02.

624 Gutiérrez-Yurrita, P. J., Martinez, J. M., Ilhéu, M., Bravo-Utrera, M. A., Bernardo, J. M., & Montes, C. (1999).
625 The status of crayfish populations in Spain and Portugal. *Crustacean Issues*, 11, 161-192.

626 Habsburgo-Lorena, A. S. (1978). Present situation of exotic species of crayfish introduced into Spanish
627 continental waters. *Freshwater Crayfish*, 4, 175–184.

628 Habsburgo-Lorena, A. S. (1986). The status of the *Procambarus clarkii* population in Spain. *Freshwater Crayfish*,
629 6, 131–136.

630 Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for
631 Windows 95/98/NT. In *Nucleic Acids Symposium Series* (Vol. 41, No. 41, pp. 95-98). [London]: Information
632 Retrieval Ltd., c1979-c2000.

633 Hardouin, E. A., Andreou, D., Zhao, Y., Chevret, P., Fletcher, D. H., Britton, J. R., & Gozlan, R. E. (2018).
634 Reconciling the biogeography of an invader through recent and historic genetic patterns: the case of
635 topmouth gudgeon *Pseudorasbora parva*. *Biological Invasions*, 20, 2157–2171.

636 Havel J. E., Kovalenko K. E., Thomaz S. M., Amalfitano S., & Kats L. B. (2015). Aquatic invasive species:
637 challenges for the future. *Hydrobiologia*, 740, 147–170.

638 Haydar, D. (2012). What is natural? The scale of cryptogenesis in the North Atlantic Ocean. *Diversity and*
639 *Distributions*, 18 (2), 101-110.

640 Hobbs, H., Jass, J., & Huner, J. A. (1989). A review of global crayfish introductions with particular emphasis
641 on two North American species. *Crustaceana*, 56, 299-316.

642 Holdich, D. M. (1993). A review of astaciculture: freshwater crayfish farming. *Aquatic Living Resources*, 6 (4),
643 307-317.

644 Holdich, D. M. (2002). Distribution of crayfish in Europe and some adjoining countries. *Bulletin Français de la*
645 *Pêche et de la Pisciculture*, 367, 611-650.

646 Holmes, S. J. (1924). The genus *Cambarus* in California. *Science*, 60 (1555), 358-359.

647 Huang, J., Tang, S., Cai, F., Lin, Y., & Wu, Z. (2017). Microsatellite evidence of dispersal mechanism of red
648 swamp crayfish (*Procambarus clarkii*) in the Pearl River basin and implications for its management.
649 *Scientific Reports*, 7 (1), 8272.

650 Hufbauer, R. A. (2017). Admixture is a driver rather than a passenger in experimental invasions. *Journal of*
651 *Animal Ecology*, 86 (1), 4-6.

652 Hulme, P. E. (2009). Trade, transport and trouble: managing invasive species pathways in an era of
653 globalization. *Journal of Applied Ecology*, 46 (1), 10-18.

654 Huner J. V. (1977). Introductions of the Louisiana Red Swamp Crayfish, *Procambarus clarkii* (Girard); an
655 update. *Freshwater Crayfish*, 3, 193-202.

656 Huner, J. V. (1986). Distribution of the red swamp crawfish. *Crawfish Tales*, 5 (3), 16-18.

657 Huner J. V. (2002). *Procambarus*. In: Holdich D. (ed.), Biology of Freshwater Crayfish. Blackwell Science Ltd.,
658 Oxford, 541–584.

659 Jeschke, J. M., Bacher, S., Blackburn, T. M., Dick, J. T., Essl, F., Evans, T., … Kumschick, S. (2014). Defining
660 the impact of non-native species. *Conservation Biology*, 28 (5), 1188-1194.

661 Jost, L. (2008). GST and its relatives do not measure differentiation. *Molecular Ecology*, 17, 4015-4026.

662 Kawai, T., & Kobayashi, Y. (2005). Origin and current distribution of the alien crayfish *Procambarus clarkii*
663 (Girard, 1852) in Japan. *Crustaceana*, 78 (9), 1143–1149.

664 Kawai, T. (2017). A Review of the Spread of *Procambarus clarkii* across Japan and its Morphological
665 Observations. *Freshwater Crayfish*, 23 (1), 41-53.

666 Kolbe, J. J., Glor, R. E., Schettino, L. R., Lara, A. C., Larson, A., & Losos, J. B. (2007). Multiple sources,
667 admixture, and genetic variation in introduced Anolis lizard populations. *Conservation Biology*, 21(6),
668 1612-1625.

669 De Kort, H., Mergeay, J., Jacquemyn, H., & Honnay, O. (2016). Transatlantic invasion routes and adaptive
670 potential in North American populations of the invasive glossy buckthorn, *Frangula alnus*. *Annals of*
671 *botany*, 118(6), 1089-1099.

672 Kouba, A., Petrusek, A., & Kozák, P. (2014). Continental-wide distribution of crayfish species in Europe:
673 update and maps. *Knowledge and Management of Aquatic Ecosystems*, 413, 05.
674 Larson, E. R., & Olden, J. D. (2008). Do schools and golf courses represent emerging pathways for crayfish
675 invasions. *Aquatic Invasions*, 3, 465-468.
676 Larson, E. R., & Olden, J. D. (2011). The state of crayfish in the Pacific Northwest. *Fisheries*, 36, 60-73.
677 Larson, E. R., Renshaw, M. A., Gantz, C. A., Umek, J., Chandra, S., Lodge, D. M. & Egan, S. P. (2017).
678 Environmental DNA (eDNA) detects the invasive crayfishes *Orconectes rusticus* and *Pacifastacus*
679 *leniusculus* in large lakes of North America. *Hydrobiologia*, 800, 173-185.
680 Laurent, P. J. (1990). Point sur les risques engendrés par l'introduction intempestive de *Procambarus clarkii*,
681 l'Ecrevisse rouge des marais de Louisiane. *Courrier de la Cellule Environnement INRA*, 11 (11), 7-10.
682 Laurent, P. J. (1997). Introductions d'écrevisses en France et dans le monde, historique et conséquences.
683 *Bulletin Français de la Pêche et de la Pisciculture*, (344-345), 345-356.

684 Leigh, J. W., & Bryant, D. (2015). POPART: full-feature software for haplotype network construction. *Methods*
685 *in Ecology and Evolution*, 6, 1110-1116.

686 Lenda, M., Skórka, P., Knops, J. M. H., Moron, D., Sutherland, W. J., Kuszewska, K., & Woyciechowski, M.
687 (2014). Effect of the Internet Commerce on Dispersal Modes of Invasive Alien Species. *PLoS One*, 9 (6):
688 e99786.

689 Lejeusne, C., Saunier, A., Petit, N., Béguer, M., Otani, M., Carlton, J. T., … Green, A. J. (2014). High genetic
690 diversity and absence of founder effects in a worldwide aquatic invader. *Scientific Reports*, 4, 5808.

691 Li, J. L., Dong, Z. G., Li, Y. S., & Wang, C. H. (2007). Invasive aquatic species in China. Shanghai: Shanghai
692 Science and Technology Publisher.

693 Li, Y. H, Guo, X. W., Cao, X. J., Deng, W., Luo, W., & Wang, W. M. (2012). Population genetic structure and
694 post-establishment dispersal patterns of the red swamp crayfish *Procambarus clarkii* in China. *PLoS One*, 7
695 (7): e40652.

696 Lockwood, J. L., Cassey, P., & Blackburn, T. M. (2005). The role of propagule pressure in explaining species
697 invasions. *Trends in Ecology & Evolution*, 20 (5), 223-228.

698 Lombaert, E., Guillemaud, T., Cornuet, J. M., Malausa, T., Facon, B., & Estoup, A. (2010). Bridgehead effect in
699 the worldwide invasion of the biocontrol harlequin ladybird. *PloS one*, 5 (3), e9743.

700 Loureiro, T. G., Anastácio, P. M. S. G., Araujo, P. B., Souty-Grosset, C., & Almerão, M. P. (2015). Red swamp
701 crayfish: biology, ecology and invasion-an overview. *Nauplius*, 23 (1), 1-19.

702 Lowery, R. S., & Mendes, A. J. (1977). The biology of *Procambarus clarkii* in Lake Naivasha, Kenya; with a note
703 on its distribution. *Freshwater Crayfish*, 3, 203-210.

704 Manfrin, C., Souty-Grosset, C., Anastácio, P., Reynolds, J., & Giulianini, P. (2019). Detection and Control of
705 Invasive Freshwater Crayfish: From Traditional to Innovative Methods. *Diversity*, 11 (1), 5.

706 Mauvisseau, Q., Coignet, A., Delaunay, C., Pinet, F., Bouchon, D., & Souty-Grosset, C. (2018). Environmental
707 DNA as an efficient tool for detecting invasive crayfishes in freshwater ponds. *Hydrobiologia*, 805(1), 163-
708 175.

709 Mueller, K. W. (2001). First record of the red swamp crayfish, *Procambarus clarkii* (Girard, 1852) (Decapoda,
710 Cambaridae), from Washington state, USA. *Crustaceana*, 74 (9), 1003-1007.

711 Naylor, R. L., Williams, S. L., & Strongm D. R. (2001). Aquaculture – a gateway for exotic species. *Science*,
712 294, 1655–56.

713 O'Hanlon, S. J., Rieux, A., Farrer, R. A., Rosa, G. M., Waldman, B., Bataille, A., ... Martin, M. D. (2018). Recent
714 Asian origin of chytrid fungi causing global amphibian declines. *Science*, 360(6389), 621-627.

715 Oksanen, J. (2013). Vegan: Community Ecology Package v. R package version 2.0-7

716 Patoka, J., Kalous, L., & Kopecký, O. (2014). Risk assessment of the crayfish pet trade based on data from the
717 Czech Republic. *Biological Invasions*, 16, 2489-2494.

718 Penn, G. H. Jr. (1954). Introduction of American crawfishes into foreign lands. *Ecology*, 35, 296.

719 Petrusek, A., Filipová, L., Kozubíková-Balcarová, E., & Grandjean, F. (2017). High genetic variation of
720 invasive signal crayfish in Europe reflects multiple introductions and secondary
721 translocations. *Freshwater Science*, *36*, 838-850.

722 Pyšek, P., Jarošík, V., & Pergl, J. (2011). Alien plants introduced by different pathways differ in invasion
723 success: unintentional introductions as a threat to natural areas. *PLoS One*, 6(9), e24890.

724 Quan, A. S., Pease, K. M., Breinholt, J. W., & Wayne, R. K. (2014). Origins of the invasive red swamp crayfish
725 (*Procambarus clarkii*) in the Santa Monica Mountains. *Aquatic Invasions*, 9, 211-219.

726 Resh, V. H., & Rosenberg, D. M. (2015). Economic Aspects of Freshwater Invertebrates. *In Thorp and Covich's*
727 *Freshwater Invertebrates (Fourth Edition)* (pp. 93-109).

728 Riegel, J. A. (1959). The systematics and distribution of crayfishes in California. *California Fish and Game*, 45,
729 29-50.

730 Rius, M., & Darling, J. A. (2014). How important is intraspecific genetic admixture to the success of
731 colonising populations?. *Trends in Ecology & Evolution*, 29, 233-242.

732 Roman, J. (2006). Diluting the founder effect: cryptic invasions expand a marine invader's range. *Proceedings*
733 *of the Royal Society of London B: Biological Sciences*, 273, 2453-2459.

734 Roman, J., & Darling, J. A. (2007). Paradox lost: genetic diversity and the success of aquatic invasions. *Trends*
735 *in ecology & evolution*, 22 (9), 454-464.

736 Rozas, J., Ferrer-Mata, A., Sánchez-DelBarrio, J. C., Guirao-Rico, S., Librado, P., … Sánchez-Gracia, A. (2017).
737 DnaSP 6: DNA sequence polymorphism analysis of large data sets. *Molecular biology and evolution*, 34,
738 3299-3302.

739 Simberloff, D. (2009). The role of propagule pressure in biological invasions. *Annual Review of Ecology,*
740 *Evolution, and Systematics*, 40, 81-102.

741 Simberloff, D., Martin, J. L., Genovesi, P., Maris, V., Wardle, D. A., Aronson, J., ... Pyšek, P. (2013). Impacts of
742 biological invasions: what's what and the way forward. *Trends in Ecology and Evolution*, 28, 58-66.

743 Souty-Grosset, C., Anastácio, P. M., Aquiloni, L., Banha, F., Choquer, J., Chucholl, C., & Tricarico, E. (2016).
744 The red swamp crayfish *Procambarus clarkii* in Europe: impacts on aquatic ecosystems and human well-
745 being. *Limnologica*, 58, 78-93.

746 Strayer, D. L. (2010). Alien species in fresh waters: ecological effects, interactions with other stressors, and
747 prospects for the future. *Freshwater Biology*, 55, 152–174.

748 Strecker, A. L., Campbell, P. M., & Olden, J. D. (2011). The aquarium trade as an invasion pathway in the
749 Pacific Northwest. *Fisheries*, 36, 74-85.

750 Tamura, K., & Nei, M. (1993). Estimation of the number of nucleotide substitutions in the control region of
751 mitochondrial DNA in humans and chimpanzees. *Molecular Biological Evolution*, 10, 512-526.

752 Taylor, C. A., & Knouft, J. H. (2006). Historical influences on genital morphology among sympatric species:
753 gonopod evolution and reproductive isolation in the crayfish genus Orconectes (Cambaridae). *Biological*
754 *Journal of the Linnean Society*, 89, 1-12.

755 Torres, E., & Álvarez, F. (2012). Genetic variation in native and introduced populations of the red swamp
756 crayfish *Procambarus clarkii* (Girard, 1852) (Crustacea, Decapoda, Cambaridae) in Mexico and Costa Rica.
757 *Aquatic Invasions*, 7, 235-241.

Tréguier, A., Paillisson, J. M., Dejean, T., Valentini, A., Schlaepfer, M. A., & Roussel, J. M. (2014). Environmental DNA surveillance for invertebrate species: advantages and technical limitations to detect invasive crayfish *Procambarus clarkii* in freshwater ponds. *Journal of Applied Ecology*, 51 (4), 871-879.

Tricarico, E., Junqueira, A. O., & Dudgeon, D. (2016). Alien species in aquatic environments: a selective comparison of coastal and inland waters in tropical and temperate latitudes. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 26, 872-891.

Twardochleb, L. A., Olden, J. D. & Larson, E. R. (2013). A global meta-analysis of the ecological impacts of nonnative crayfish. *Freshwater Science*, 32, 1367-1382.

van Boheemen, L. A., Lombaert, E., Nurkowski, K. A., Gauffre, B., Rieseberg, L. H., & Hodgins, K. A. (2017). Multiple introductions, admixture and bridgehead invasion characterize the introduction history of *Ambrosia artemisiifolia* in Europe and Australia. *Molecular ecology*, 26, 5421-5434.

van de Crommenacker, J., Bourgeois, Y. X. C., Warren, B. H., Jackson, H., Fleischer-Dogley, F., Groombridge, J., & Bunbury, N. (2015). Using molecular tools to guide management of invasive alien species: assessing the genetic impact of a recently introduced island bird population. *Diversity and Distributions*, 21, 1414-1427.

Vilà, M., Basnou, C., Pyšek, P., Josefsson, M., Genovesi, P., Gollasch, S., … Hulme, P. E. (2010). How well do we understand the impacts of alien species on ecosystem services? A pan-European, cross-taxa assessment. *Frontiers in Ecology and the Environment*, 8, 135-144.

Wagner, N. K., Ochocki, B. M., Crawford, K. M., Compagnoni, A., & Miller, T. E. (2017). Genetic mixture of multiple source populations accelerates invasive range expansion. *Journal of Animal Ecology*, 86, 21-34.

Yi, S., Li, Y., Shi, L., Zhang, L., Li, Q., & Chen, J. (2018). Characterization of Population Genetic Structure of red swamp crayfish, *Procambarus clarkii*, in China. *Scientific Reports*, 8, 5586.

Yue, G. H., Li, J., Bai, Z., Wang, C. M., & Feng, F. (2010). Genetic diversity and population structure of the invasive alien red swamp crayfish. *Biological Invasion*, 12, 2697–2706.

Zhu, B. F., Huang, Y., Dai, Y. G., Bi, C. W., & Hu, C. Y. (2013). Genetic diversity among red swamp crayfish (*Procambarus clarkii*) populations in the middle and lower reaches of the Yangtze River based on AFLP markers. *Genetic Molecular Research*, 12, 791-800.

vi. Tables

Table 1. Genetic diversity parameters based on the COI gene for each *Procambarus clarkii* locality. Note that localities are grouped in biogeographical zones (see Methods). Sequences retrieved from GenBank are

788 shown in italics (see references in Materials and Methods section). *: 0.05 > p > 0.01; **: 0.01 > p > 0.001; ***: p
789 < 0.001

Country	Locality	Code	Lon	Lat	N	*h*	Hd	π	R2	Tajima	Fs
NATIVE RANGE					179	39	0.902	0.00549			
East Louisiana											
	Poison	LA2	30.220	-91.614	20	5	0.795	0.00349	0.172	0.810	0.854
	Haha Bay	LA4	30.147	-91.628	20	8	0.775	0.00395	0.104	-0.519	-1.573
	Baton Rouge	BAT	30.370	-91.189	4	3	0.833	0.00302	0.265	1.090	0.006
	Morgan City	MOR	29.767	-91.127	10	6	0.867	0.00428	0.157	0.215	-1.164
	Pierre Part	PIE	29.950	-91.283	10	8	0.956	0.00450	0.135	-0.144	-3.882**
	Des Allemands	DES	29.798	-90.505	4	4	1.000	0.00630	0.114*	0.039	-0.884
	Jean Lafitte	JEA	29.732	-90.075	9	4	0.694	0.00265	0.172	-0.526	-0.061
	New Orleans	*LAf*	*29.950*	*-90.083*	*1*	*1*					
West Louisiana											
	Abbeville	ABB	29.911	-92.200	4	3	0.833	0.00247	0.276	-0.754	-0.288
	Alexandria	ALE	31.097	-92.493	4	3	0.833	0.00877	0.327	-0.222	1.606
	Woodworth	WOO	31.186	-92.467	4	4	1.000	0.00356	0.223	-0.065	-1.741*
	Natchitoches	NAT	31.740	-93.077	17	6	0.765	0.00816	0.199	1.495	2.133
	Kaplan	*LAt*	*29.991*	*-92.260*	*5*	*2*	*0.600*	*0.00197*	0.300	1.459	1.688
	Calcasieu L.	*LO*	*29.870*	*-93.260*	*10*	*4*	*0.533*	*0.00643*	0.143	-0.352	2.256
Upstream Mississippi River											
	Monroe	MON	32.497	-91.669	5	4	0.900	0.00724	0.218	0.132	0.286
	Memphis	MEM	35.366	-90.033	5	2	0.400	0.00066	0.400	-0.817	0.090
	Horseshoe	*ILL*	*37.138*	*-89.343*	*1*	*1*					
Texas											
	Comal	COM	29.711	-98.134	18	6	0.686	0.00170	0.103	-0.917	-2.350*
	San Marcos	SMA	29.882	-97.934	14	3	0.560	0.00103	0.157	-0.011	-0.072
Mexico											
	Sabinas Hidalgo	*NL*	*26.483*	*-100.221*	*4*	*1*					
	Río Jiménez	*CON*	*29.154*	*-100.764*	*5*	*2*	*0.400*	*0.00066*	0.400	-0.817	0.090
	Río Sabinas	*COC*	*27.969*	*-101.582*	*5*	*1*					
WEST AMERICAS											

Non-native US											
	Santa Ynez	SYZ	34.557	-119.881	10	3	0.378	0.00190	0.187	-1.388″	0.762
	Topanga	TOP	34.064	-118.587	20	5	0.679	0.00859	0.194	1.541	4.185
	Ventura	VEN	34.345	-119.299	4	2	0.667	0.00439	0.333	2.080	2.719
	Pine	PIN	47.587	-122.044	21	3	0.581	0.00183	0.186	0.883	1.537
	Waverly	WAV	44.640	-123.069	20	7	0.832	0.00501	0.121	-0.356	0.057
	Waiau	HW	19.713	-155.149	3	1					
Non-native Mexico					13	4	0.782	0.00557			
	Teopisca	*CHIS*	*16.554*	*-92.476*	*5*	*1*					
	El Arenal	*DU*	*24.043*	*-104.428*	*5*	*2*	*0.600*	*0.00493*	0.300	1.686	3.526
	Las Varas	*CHt*	*29.797*	*-106.693*	*3*	*1*					
Costa Rica	*Cachí Dam*	*CR*	*9.825*	*-83.821*	*4*	*2*	*0.500*	*0.00082*	0.433	-0.612	0.172
EAST USA											
	Pee Dee	FOR	36.150	-80.291	4	2	0.500	0.00082	0.433	-0.612	0.172
	Pamplico	LEN	35.244	-77.559	10	2	0.200	0.00033	0.300	-1.112	-0.339
	Albemarle	PER	36.268	-76.378	21	3	0.338	0.00100	0.111	-0.707	0.204
	North Shore	CHI	42.032	-87.710	20	5	0.442	0.00113	0.105	-1.888*	-2.091*
EUROPE											
Spain					355	13	0.469	0.00239			
	Balboa	EXT	38.883	-6.871	20	2	0.395	0.00065	0.197	0.723	0.976
	Manecorro	MAN	37.124	-6.489	20	5	0.716	0.00443	0.143	0.214	1.571
	Cantaritas	AR4	37.046	-6.213	20	5	0.663	0.00447	0.140	0.243	1.596
	Colomera	GRA	37.384	-3.719	20	3	0.468	0.00300	0.154	0.255	2.904
	Hueznar	HUE	37.933	-5.697	20	2	0.100	0.00066	0.218	-1.868*	0.998
	Arreo	ALA	42.778	-2.991	20	2	0.479	0.00315	0.239	2.024	5.159
	Elorz	EL	42.798	-1.667	19	4	0.585	0.00275	0.105	-0.921	1.200
	Expo	EXP	41.671	-0.909	13	3	0.615	0.00405	0.200	1.009	3.086
	Gijón	GIJ	43.536	-5.640	15	3	0.257	0.00085	0.121	-1.317	-0.379
	Jiloca	JIL	40.544	-1.293	15	2	0.419	0.00207	0.210	1.078	3.248
	Leza	LEZ	42.441	-2.311	20	2	0.268	0.00177	0.134	-0.138	3.143
	Almenara	ALM	39.761	-0.183	20	1					

	Brugent	BRU	42.006	2.607	20	2	0.337	0.00222	0.168	0.565	3.843
	Ecomuseu	ECO	40.724	0.722	20	3	0.195	0.00099	0.159	-2.056**	0.136
	Alpedrete	MAD	40.667	-4.016	20	2	0.442	0.00073	0.221	1.026	1.169
	Júcar	ALB	39.148	-1.809	11	3	0.618	0.00114	0.192	0.036	-0.113
	Mundo	MUN	38.458	-1.761	20	1					
	Sa Pobla	SAP	39.791	3.063	5	2	0.400	0.00263	0.400	-1.094″	2.202
	Soller	SOL	39.787	2.794	5	3	0.700	0.00428	0.205	0.562	1.090
	Carucedo	CAR	42.488	-6.784	5	2	0.400	0.00132	0.400	-0.973	1.040
	Chozas	CHO	42.518	-5.714	4	2	0.500	0.00247	0.433	-0.754	1.716
	Valparaiso	VAL	41.995	-6.288	2	2	1.000	0.01151	0.500	0.000	1.946
	Pisuerga	VLB	41.801	-4.588	21	3	0.343	0.00172	0.105	-0.742	1.384
Portugal					114	4	0.399	0.00118			
	Aboboda	ABO	38.736	-9.319	15	2	0.343	0.00169	0.171	0.342	2.710
	Lousal	LOU	38.027	-8.431	20	2	0.479	0.00079	0.239	1.262	1.311
	Alpiarça	POR	39.245	-8.594	20	4	0.642	0.00291	0.123	-0.727	1.429
	R. de Monsaraz	REG	38.478	-7.522	20	2	0.505	0.00083	0.253	1.430	1.409
	Requeixo	REQ	40.592	-8.526	20	2	0.100	0.00016	0.218	-1.164	-0.879″
	Vila-Rica	VILA	41.229	-7.096	19	1					
France					84	5	0.561	0.00218			
	Marais Bruges	BOR	44.903	0.596	20	1					
	Briere	BRI	47.343	-2.246	20	1					
	Tour du Valat	CAM	43.508	4.668	20	2	0.526	0.00346	0.263	2.511	5.567
	Lamartine	TOU	43.506	1.341	21	2	0.095	0.00016	0.213	-1.164	-0.919″
	Rochechevreux	*FR1*	*45.467*	*1.217*	*1*	*1*					
	Rochechevreux	*FR2*	*46.467*	*1.217*	*1*	*1*					
	Givrezac	*FR3*	*45.403*	*0.216*	*1*	*1*					
Italy					60	6	0.731	0.00266			
	Bernate	BER	45.485	8.795	20	2	0.479	0.00079	0.239	1.262	1.311
	Monterotondo	LAZ	42.052	12.547	20	2	0.505	0.00083	0.253	1.430	1.409
	Fucecchio	TOS	43.810	10.794	20	5	0.716	0.00448	0.150	0.257	1.608

Holland	Hardinxveld-Giess	HOL	51.817	4.836	20	1					
Belgium					19	2	0.409	0.00067			
	Bioul	BIO	50.339	4.809	12	1					
	Ecaussinnes	ECA	50.576	4.139	7	2	0.476	0.00078	0.238	0.559	0.589
United Kingdom	Hampstead-Heath	LON	51.561	-0.162	20	1					
ASIA											
Japan					122	4	0.476	0.00237			
	Wakamatsu	FUK	33.911	130.782	20	1					
	Hourai	HOK	42.939	143.224	20	1					
	Waga	IWA	39.436	140.776	9	2	0.556	0.00274	0.278	1.948	3.276
	Ohfuna	KAN	35.353	139.529	20	2	0.521	0.00257	0.261	2.266	4.362
	Rakusho	OKA	34.714	133.933	20	3	0.279	0.00106	0.108	-0.626	0.286
	Ohtsu	SHI	35.013	135.865	10	2	0.533	0.00263	0.267	1.831	3.338
	Kanda	TOK	35.685	139.774	13	3	0.410	0.00240	0.164	-1.335″	1.625
	Saitama	*SA*	*35.850*	*139.650*	*10*	*2*	*0.533*	*0.00263*	0.267	1.831	3.338
China					293	2	0.350	0.00173			
	Shanghai	*SH*	*31.030*	*121.230*	*8*	*2*	*0.571*	*0.00282*	0.286	1.982	3.149
	Jiaxing	*JX*	*30.750*	*120.770*	*10*	*2*	*0.356*	*0.00175*	0.178	0.021	2.334
	Binhu. Wuxi	*WXB*	*31.520*	*120.280*	*7*	*2*	*0.571*	*0.00282*	0.286	1.811	2.920
	Nantong	*NT*	*32.020*	*120.870*	*7*	*2*	*0.286*	*0.00141*	0.350	-1.358″	1.514
	Xiaba village	*XB*	*32.200*	*118.870*	*8*	*2*	*0.571*	*0.00282*	0.286	1.981	3.149
	Wuxi	*WX*	*31.570*	*120.300*	*8*	*2*	*0.536*	*0.00264*	0.268	1.601	2.988
	Wangjiang	*WJ*	*30.120*	*116.700*	*8*	*1*					
	Maanshan	*MAS*	*31.550*	*118.500*	*10*	*2*	*0.356*	*0.00175*	0.178	0.021	2.338
	Chaohu	*CH*	*31.620*	*117.870*	*8*	*2*	*0.429*	*0.00211*	0.214	0.458	2.469
	Hefei	*HF*	*31.820*	*117.230*	*7*	*2*	*0.286*	*0.00141*	0.350	-1.358″	1.514
	Dingyuan	*DY*	*32.280*	*117.830*	*10*	*2*	*0.556*	*0.00274*	0.278	2.057	3.451
	Nanbei Port	*NBP*	*29.720*	*116.170*	*8*	*2*	*0.250*	*0.00123*	0.331	-1.448″	1.415
	Zhongxian	*ZX*	*30.280*	*108.030*	*10*	*2*	*0.533*	*0.00263*	0.267	1.831	3.338

Jianyang	*JY*	*30.380*	*104.550*	*10*	*2*	*0.467*	*0.00230*	0.233	1.152	2.985
Chongqing	*CQS*	*29.550*	*106.530*	*6*	*1*					
Ningbo	*NB*	*29.880*	*121.550*	*7*	*1*					
Xuyi-culture	*XYC*	*33.000*	*118.500*	*7*	*1*					
Xuyi-wild	*XYW*	*33.030*	*118.420*	*10*	*1*					
Xiaguan district	*XG*	*32.080*	*118.750*	*10*	*1*					
Baguazhou township	*BGT*	*32.170*	*118.820*	*8*	*1*					
Guangfengwei	*CJR*	*30.120*	*116.870*	*8*	*1*					
Sanli township	*SLT*	*29.750*	*116.220*	*8*	*1*					
Poyang lake	*PYL*	*28.870*	*116.430*	*10*	*1*					
Youlan. Nanchang	*NCYL*	*28.520*	*116.120*	*6*	*1*					
Nanhu lake	*NHL*	*30.020*	*114.030*	*8*	*1*					
Yuni Lake	*YNL*	*30.000*	*112.200*	*7*	*1*					
Xiantao	*XT*	*30.300*	*113.400*	*8*	*1*					
Qianjiang	*QJ*	*30.400*	*112.600*	*10*	*1*					
Liangzi lake	*LZL*	*30.000*	*114.000*	*10*	*1*					
Honghu lake	*HLL*	*29.700*	*113.400*	*8*	*1*					
Changhu lake	*CHL*	*30.300*	*112.100*	*6*	*1*					
Yuanjiang	*YJ*	*28.850*	*112.370*	*10*	*1*					
Ningxiang	*NX*	*28.280*	*112.550*	*8*	*1*					
Dongting lake	*DTL*	*29.300*	*113.020*	*10*	*1*					
Dongting Lakeside	*DTLs*	*29.350*	*113.130*	*9*	*1*					

Number of sequences (N), number of haplotypes (*h*), haplotype diversity (Hd), nucleotide diversity (π).

797 **Table 2.** Analysis of Molecular Variance (AMOVA) within the native area of *Procambarus clarkii*, giving corresponding
798 values for F_{CT} (difference among groups), F_{SC} (differences among localities within groups), and F_{ST} (differences among all
799 localities). Five groups were considered: the native localities in Mexico, Texas, east Louisiana, west Louisiana and
800 upstream Mississippi River.

Source of Variation	d.f.	Sum of squares	Variance components	Percentage of variation
México - Texas – E. Louisiana – W. Louisiana – Upstream Mississippi River				
Among groups	4	77.228	0.53574	29.04 (F_{CT} = 0.290, p = 0.000)
Among localities within groups	15	39.135	0.17294	9.38 (F_{SC} = 0.132, p = 0.002)
Within localities	157	178.350	1.13598	61.58 (F_{ST} = 0.384, p = 0.000)
Total	176	294.712	1.84466	

801

802 **Table 3.** Analysis of Molecular Variance (AMOVA) among the native and introduced localities of *Procambarus clarkii*
803 worldwide and among the five zones (native range, west Americas, east USA, Europe and Asia), listing the
804 corresponding values for F_{CT} (difference among groups), F_{SC} (differences among localities within groups), and F_{ST}
805 (differences among all localities)

Source of Variation	d.f.	Sum of squares	Variance components	Percentage of variation
Native Area – Invaded Area				
Among groups	1	69.842	0.19760	14.35 (F_{CT} = 0.143, p = 0.000)
Among localities within groups	114	932.781	0.63021	45.76 (F_{SC} = 0.534, p = 0.000)
Within localities	1293	710.207	0.54927	39.89 (F_{ST} = 0.601, p = 0.000)
Total	1408	1712.829	1.37708	
Native Area – West Americas – East USA – Europe – Asia				
Among groups	4	491.791	0.49942	36.04 (F_{CT} = 0.360, p = 0.000)
Among localities within groups	111	510.831	0.33716	24.33 (F_{SC} = 0.380, p = 0.000)
Within localities	1293	710.207	0.54927	39.63 (F_{ST} = 0.604, p = 0.000)
Total	1408	1712.829	1.38585	

Table 4. Analysis of Molecular Variance (AMOVA) within Europe between northern and southern distribution of *Procambarus clarkii*, listing the corresponding values for F_{CT} (difference among groups), F_{SC} (differences among localities within groups), and F_{ST} (differences among all localities).

Source of Variation	d.f.	Sum of squares	Variance components	Percentage of variation
North (UK, HOL, BIO, ECA, BRI) – **South European distribution** (rest of European localities)				
Among groups	1	59.646	0.40773	40.74 (F_{CT} = 0.407, p = 0.000)
Among localities within groups	37	101.955	0.13481	13.47 (F_{SC} = 0.227, p = 0.000)
Within localities	628	287.734	0.45817	45.78 (F_{ST} = 0.542, p = 0.000)
Total	666	449.334	1.00071	

vii. Figure captions

Figure 1. Haplotype frequencies of *Procambarus clarkii* in the 122 localities distributed worldwide. The size of pie charts is proportional to the sample size. Haplotypes restricted to one sampling locality (i.e., private haplotypes) are coloured in white within pie charts, while haplotypes shared between localities are shaded using colours. Black spots show each one of the 122 localities used, and dark grey areas represent the native range of *Procambarus clarkii*. a) Global map; b) United States and Mexico; c) Hawaiian Islands; d) Costa Rica; e) close-up of Louisiana (US) within its native range; f) East Asia (China and Japan) and; g) Europe.

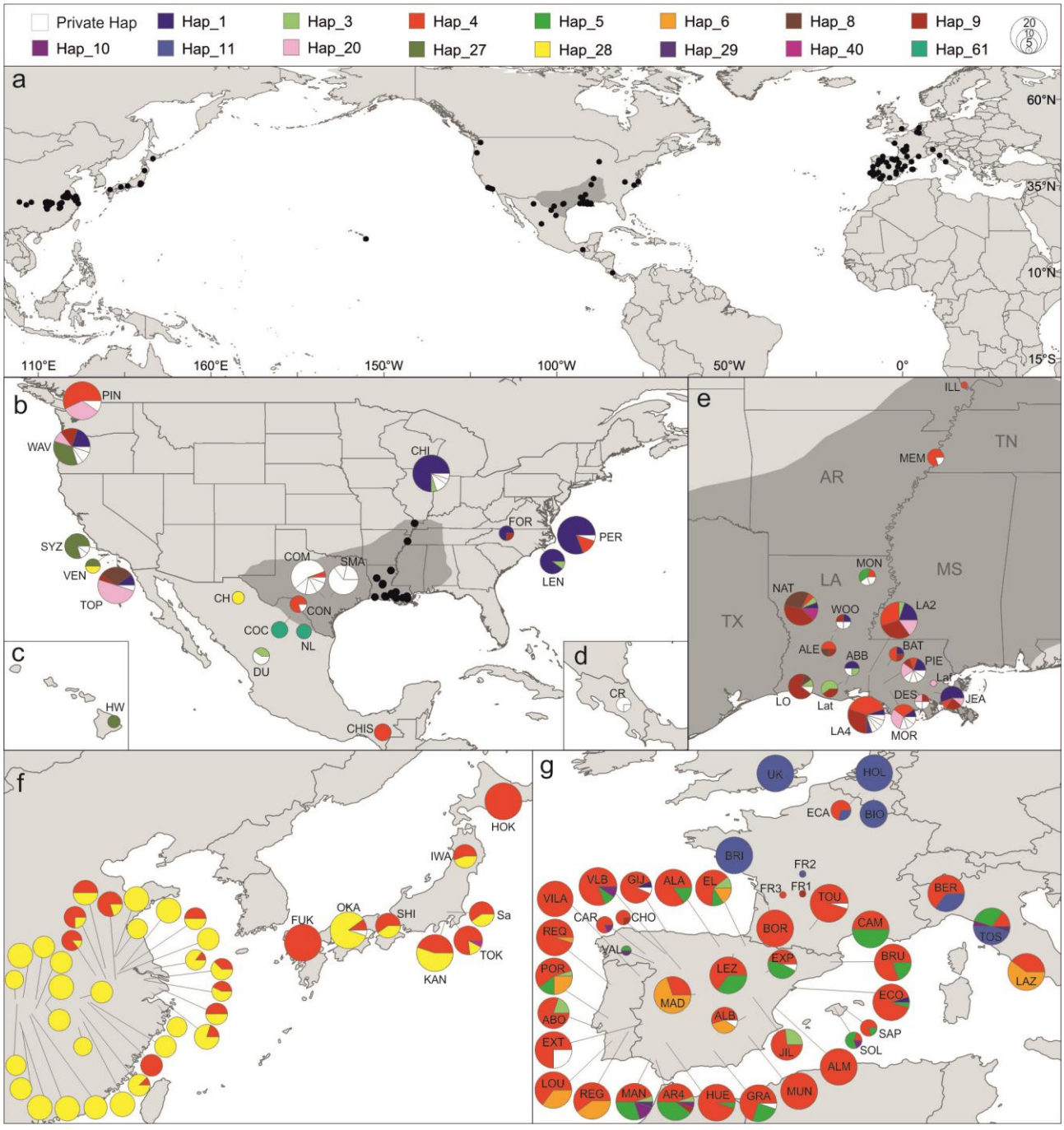

Figure 2. Haplotype network (statistical parsimony-based) for cytochrome c oxidase subunit I (COI) sequences of the red swamp crayfish, *Procambarus clarkii*. Each circle represents one haplotype and its size is proportional to the haplotype frequency. Within the network, each line between haplotypes represents a mutational change and small black dots show unsampled haplotypes inferred from the data. Localities from the same geographical region share the same colour. Haplotypes with non-synonymous changes are indicated by *.

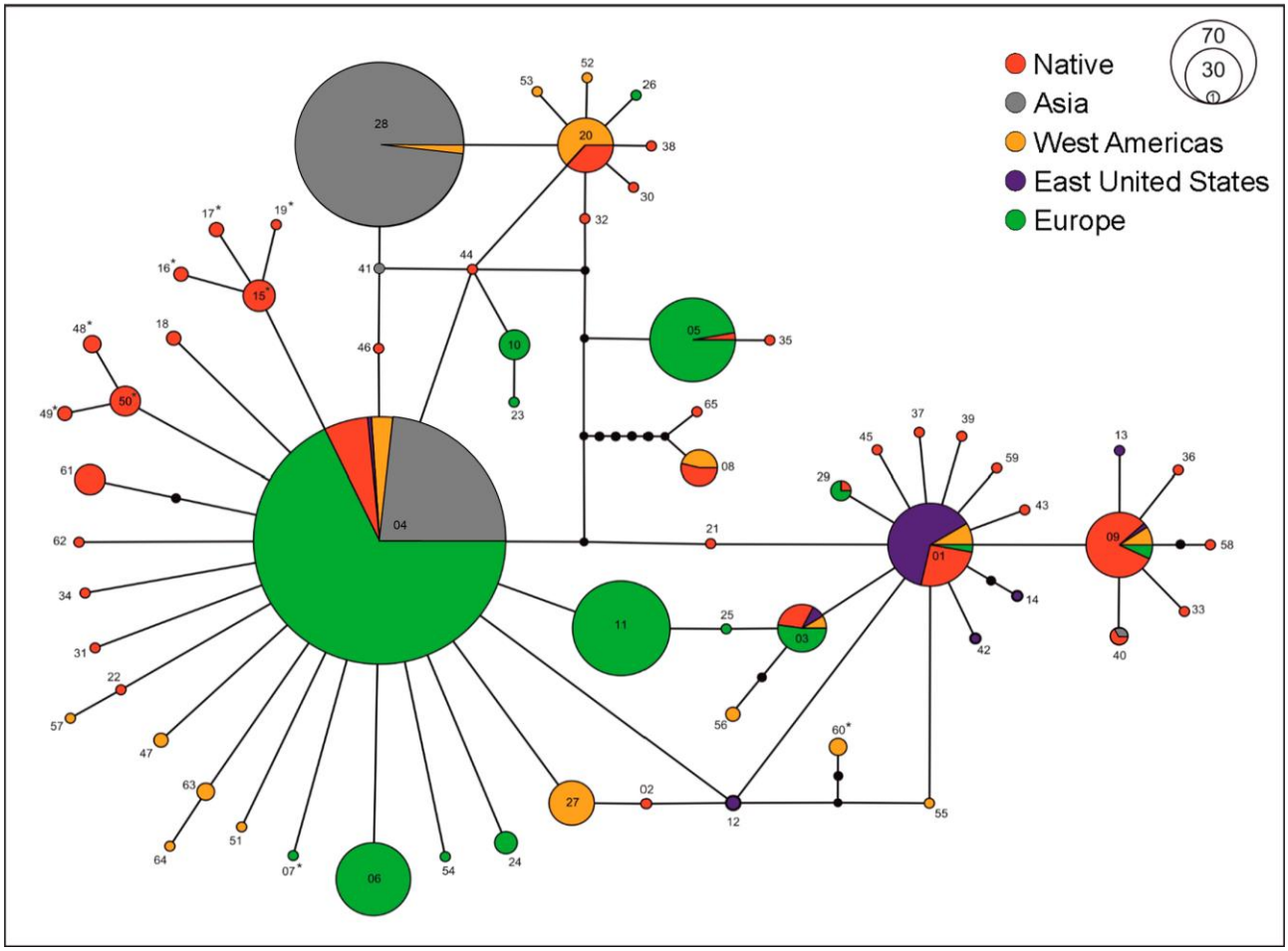

Figure 3. NMDS analysis on D_{est} Jost distances. The graph depicts the pairwise dissimilarity between localities in a low-dimensional space where each point represents one population, ellipses depict established groups and dashed ellipses their 95% confidence intervals (CI). For a better interpretation, a green triangle indicates overlapping Central European localities (BIO, LON, BRI and HOL), a black "X" indicates the Mexican and Chinese overlapping localities (CHt, NB, XYc, XYw, XG, BGt, CJr, SLt, PYL, NCyL, NHL, YNL, XT, QJ, LZL, HLL, CHL, YJ, NX, DTL, DTLs) and a black star indicates the European, Asian and Mexican overlapping localities (BOR, VILA, MUN, ALM, WJ, CQs, HOK, FUK, CHIS).

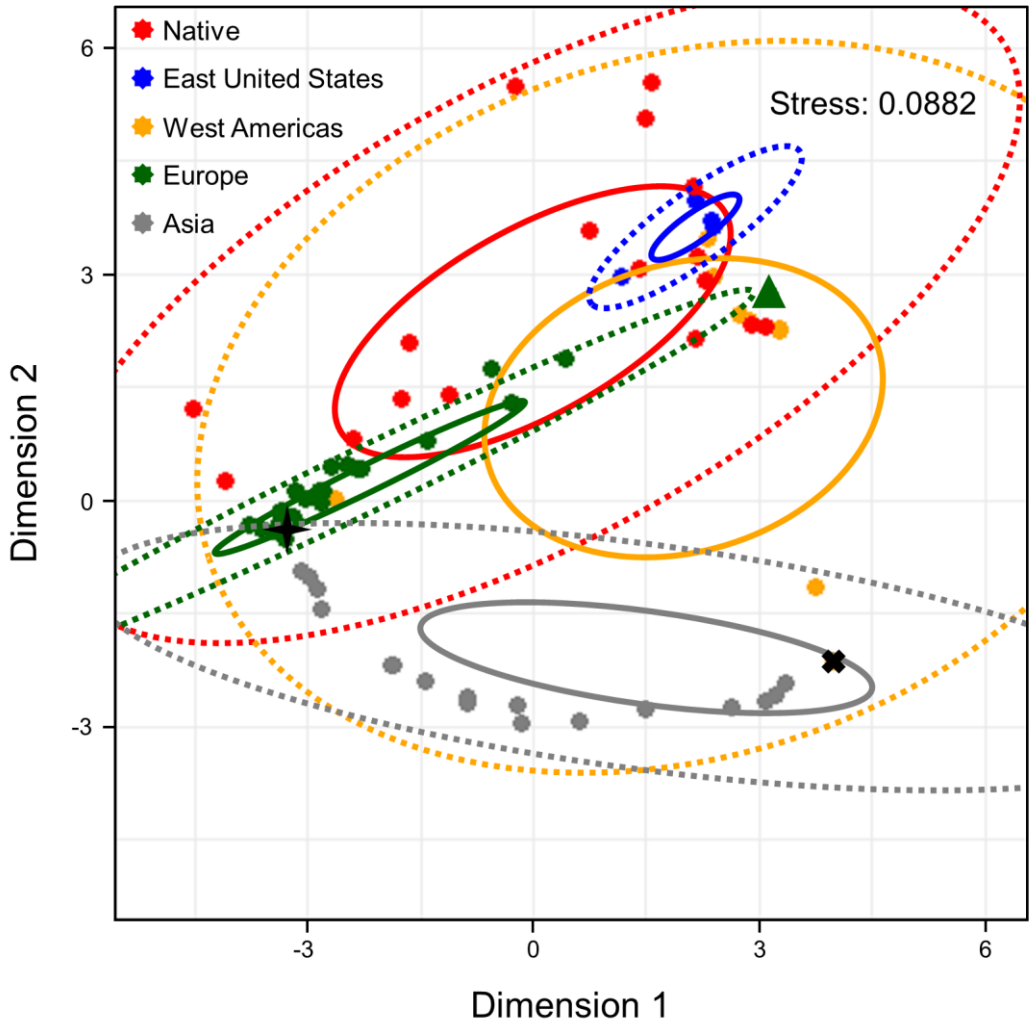

Figure 4. The global invasion routes of the red swamp crayfish, *Procambarus clarkii*, native from southern US and northeastern Mexico, based on mtDNA (present study) and reports from the literature. Main and secondary introduction routes are confirmed, described and hypothesized. Relevant invasion hubs, which usually act as recipients and sources of new invasions, are shown as red circles: Louisiana (in the native range), California, Kenya and Spain.