Latin American Journal of Aquatic Research, 52(4): 595-604, 2024
DOI: 10.3856/vol52-issue4-fulltext-3182

Research Article

Populational differences in freshwater prawn *Macrobrachium tenellum* Smith (Decapoda: Palaemonidae) from three river basins of Oaxaca, Mexico, determined with microsatellite markers

Itandehui Gutiérrez-Méndez[1], Héctor Rodríguez-Magadan[2], Nathali Martínez-Salazar[3] Marcelo García-Guerrero[1] & Rodolfo de los Santos-Romero[3]

[1]Laboratorio de Acuacultura Experimental, Instituto Politécnico Nacional CIIDIR Unidad Oaxaca, Santa Cruz Xoxocotlán, Oaxaca, México

[2]Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma Benito Juárez de Oaxaca, México

[3]Campus Instituto Tecnológico del Valle de Oaxaca, Tecnológico Nacional de México Santa Cruz Xoxocotlán, Oaxaca, México

Corresponding author: Rodolfo de los Santos-Romero (rodolfo.sr@voaxaca.tecnm.mx)

ABSTRACT. A study determining microsatellite markers was conducted to compare similarities among three different populations of *Macrobrachium tenellum* prawns from three different hydrological basins in Oaxaca, Mexico. In total, nine different markers in the population were used, of which six coded for the three populations and three did not. Although different, the three populations are closely genetically related, with an average allele richness from 6.845 to 16.754 and a heterozygosity of 0.166 to 0.264. However, the relationship could be much closer, particularly between them. The possible applications and scope of this knowledge are commented on and discussed.

Keywords: *Macrobrachium tenellum*; genetic conservation; molecular marker; genetic diversity; genetic variability; molecular tools; freshwater prawns

INTRODUCTION

Research in tropical coastal ecosystems has demonstrated that freshwater Decapoda is important for the balance of aquatic communities (March et al. 1998). Among those, the genus of freshwater prawns *Macrobrachium* Bate, 1868 plays a key role in coastal freshwater ecosystems equilibrium. These prawns are distributed mainly in the tropics and several subtropical areas (Murphy & Austin 2005), where some dominant ecological groups are amphidromous species, spending most of their life cycle in freshwater (Bauer 2011). Research on this species is important since migratory freshwater Decapoda are often important biotic components of tropical stream communities (March et al. 1998). Their migration is an important functional link between stream headwaters, downstreams, and estuaries (March et al. 1998, Bauer 2013). Nowadays, human activities are significantly altering the natural process that allows the establishment of species with wide distribution, such as five species of *Macrobrachium* that live in rivers, estuaries, and lagoons on the Mexican Pacific slope: *M. americanum* Bate, 1868, *M. digueti* Bouvier, 1895, *M. hobbsi* Villalobos-Hiriart & Nates-Rodríguez 1990, *M. occidentale* Holthuis, 1950, and *M. tenellum* Smith, 1871; García-Velazco et al. (2018, 2021). Because of overfishing, pollution, and habitat loss, wild populations of those species are decreasing every year, facing problems with their conservation (García-Guerrero et al. 2013). Every species confronts a different situation, but since its relative abundance and easy catching, this problem is

Associate Editor: Fernando Vega

very evident with *M. tenellum*. This prawn lives in river streams, estuaries, and coastal lagoons (Murphy & Austin 2005). Wild populations of this species can be found from the North Pacific coast of Mexico to Peru coasts in South America. To manage its exploitation effectively and to mitigate the potentially adverse effects of overexploitation and pollution, a better understanding of all its ecological cues is required.

Understanding populational issues of amphidromous prawns is important in tropical areas where human intervention is increasingly disrupting the natural flow of rivers to estuaries. The demand for fresh water in these regions has resulted in the widespread construction of dams and water intakes, interfering with the natural need of such populations to move or migrate into a large area. Today, many of its biological traits, some key for its conservation such as population structure, reproduction issues, distribution, and phylogenetical variations among different wild populations, are not well known (García-Guerrero et al. 2013). Assessments of the distribution of genetic diversity in the wild are required to develop conservation strategies (March et al. 1998, González 2003, Manel et al. 2005). If any animal's live resources are extensively exploited or its natural habitats are damaged by human activity, developing and proposing strategies for their sustainable management is required. Analyzing its current populational status with genetic criteria is useful (Mohanty et al. 2016). One way to do this is by using molecular genetic markers. The most common are allozymes, mitochondrial DNA (mtDNA), single nucleotide polymorphisms (SNP), and microsatellites (Liu & Cordes 2004, Charoentawee et al. 2006, Divu et al. 2008, Mohanty et al. 2016, Dehmord et al. 2023). From those, microsatellites are simple repeating sequences of 1-6 nucleotides in different parts of the genetic material of DNA, and with them, we can track genetic variations among closely related populations (Dehmord et al. 2023). They are co-dominant markers due to their occurrence at a high degree of polymorphism and simple Mendelian inheritance, as well as because their genotyping can be automated (González 2003). Because of this, they are useful for defining a single multilocus genotype. They are also of particular research interest where a very fine resolution scale is required, and other types of markers could have limitations, such as the analysis of paternity, kinship, assignment of individuals to a particular strain, or mating planning (Yu et al. 2019). Among those options, ADN microsatellites are the most appropriate tools for studies of genetic structure and biological conservation (Jarne & Lagoda 1996) because their application as genetic markers allows a rapid progress in kinship assignments, genetic variability assessments, consanguinity determination and identification of commercially important species with potential for aquaculture (Liu & Cordes 2004, Arif et al. 2011).

Molecular markers are a useful tool for genetic diversity studies, allowing the identification of populations with specific genetic characteristics (Qiao et al. 2013). In this sense, micro-satellites can help detect relationships and genetic differences between populations of the same species. It is useful to determine if only one or several populations are moving in a certain area, how they move, or if there is a genetic exchange between them. Due to their high polymorphism and co-dominant inheritance, microsatellites have assumed an increasingly important role as markers in population genetics (Song & Kim 2011, Qiao et al. 2013). Most research with microsatellites on *Macrobrachium* prawns has been done with Asian species of fishing and aquaculture importance (Song & Kim 2011, Qiao et al. 2013). For example, in *M. nipponense*, Feng & Li (2008) found 12 polymorphic microsatellites from China without cross-species amplification. There is also some research on species from South America, which offers the possibility of conducting comparative studies of gene flow between populations of the same species. With this, it can be inferred that they are related. Approximately 567 microsatellite loci of *Macrobrachium* prawns are integrated into GenBank, but this includes information on only five species: *M. nipponense* (294), *M. rosenbergii* (209), *M. carcinus* (8), *M. hainanense* (6), *M. malcolmsonii* (5).

Some molecular genetic tools have been previously used to study the populations of the different species of this genus distributed on the Mexican Pacific coast. Still, this research focuses primarily on detecting synonymy or the validity of morphological descriptions using genetic tools other than microsatellites (García-Velazco et al. 2017, 2018, 2021). None of this previous research includes *M. tenellum*. Specific microsatellite loci information for most species of the genus in the region is unknown and required as a tool for conservation strategies. For *M. tenellum*, there is not a single panel that helps to describe the amount of genetic variability in wild populations, and this is important for species with a very wide distribution area, such as *M. tenellum*. The cross-amplification technique could be very useful in comparing populations of close locations. This strategy involves amplifying the DNA of a group of specimens of the same species using markers designed for other species, preferably from the

same genus (Barbará et al. 2007). For species such as *M. rosenbergii* and *M. nipponense*, several genetic studies have focused on evaluating the genetic diversity in populations of such species in aquaculture farms or the wild (Mohanty et al. 2016, Song et al. 2016, Atin et al. 2017). In America, wild populations of *M. amazonicum* have been examined. From such research, it has been suggested that genetic variability exists among different populations due to some degree of isolation between them (Vergamini et al. 2011). Examples with other crustaceans, such as *Palaemon paucidens*, suggest that elevated levels of genetic diversity will be shown in certain areas. However, in some other areas, this species has low genetic variability due to habitat differences and isolation due to biogeographic barriers (Song et al. 2016). With this previous evidence, it would be possible to provide recommendations for breeding or conservation programs to maintain high levels of genetic variation in culture by identifying genetically diverse specimens for reproduction (Feng & Li 2008). In addition to being useful for sustainable management and fisheries of this species, this information would also be useful as a provider of the *M. tenellum* germplasm, an important live fishery and ecological resource in its native area.

This research aims to track genetic differences that can cause variations among freshwater prawn *M. tenellum* specimens from three different river basins using microsatellite markers methods of cross-amplification.

MATERIALS AND METHODS

Sampling specimens

M. tenellum prawns were randomly gathered from three different hydrological basins all in Oaxaca, Mexico: Tonameca (15°41'N, 96°37'W), Manialtepec (15°55'N, 97°14'W) and Colotepec (15°48'N, 97°01'W) during the summer of 2021. The sampling sites were selected because previous visits showed that it was relatively easy to carry out sampling with a similar procedure. These three basins were selected for the research because a wide distribution of the species under study had previously been observed. One hundred five specimens were collected (an average of 35 from each basin) as a minimum number in the population without generating impact. Prawns were transported alive in separate containers to the Laboratorio Experimental de Acuicultura CIIDIR Oaxaca, where they were killed by thermal shock. Immediately, a tissue sample was removed from each of them and frozen at -20°C for subsequent DNA extraction.

Genomic DNA extraction

The genomic ADN was isolated using the procedure established by the producer of the genomic DNA purification kit (PureLink® Genomic DNA-Invitrogen), using 30 mg of fresh abdominal tissue per specimen. The quality of the extracted DNA was evaluated with agarose gel (0.8%) (Ultrapure® Agarosa, Invitrogen) electrophoresis at 100 for 30 min. The concentration and purity of DNA were measured by spectrophotometric analysis (Nanodrop 2000: Thermo Scientific) with a 260/280 absorbance ratio.

PCR amplifications were done with 25 µL reaction mixtures containing 2 µL of template DNA, 2 µL of each forward and reverse primer, 8.5 µL of sterile dH20, and 12.5 µL Dream Taq Green PCR Master Mix (2x) (Thermo Scientific). Since there is no previous register of isolated microsatellites for *M. tenellum*, the samples were genotyped with nine dinucleotide microsatellite loci (Y7, Y16, Y42, W15, W24, Mr7-133, Mr 4-8, Mr4-85 and Mr7-88) all isolated from *M. rosenbergii* using cross-amplification method (Table 1) (Bhat et al. 2009, Dai et al. 2012, Yu et al. 2019).

The PCR profile was programmed for 5 min for initial denaturation at 95°C, followed by 35 cycles of denaturation at 95°C for 30 s and an additional 30 s at the specific annealing temperature (48-60°C) for each primer pair, extension at 72°C for 5 min. The PCR products were assessed in polyacrylamide gel at 100V for 75 min. The gels were visualized in an ultraviolet light transilluminator. Allele scoring and sizing were made using the molecular weight ladder HyperLadder 100 pb (Bioline) and using an electrophoresis image analysis software called GelAnalyzer [GelAnalyzer 19.1 (www.gelanalyzer.com) by Ph.D. Istvan Lazar Jr., and Ph.D. Istvan Lazar Sr.].

Genetic data analyses

Genetic diversity within each one of the three populations analyzed, including the total number of alleles per locus (A), allelic richness (A_r), observed heterozygosity (H_o), and expected heterozygosity (H_e), were calculated using GenAlEx 6.51b2 for each locus and population (Peakall & Smouse 2012), and was also used to estimate F statistics (F_{it}, F_{is} and F_{st}) (Weir & Cockerham 1984) for each locus and the pairwise Fst between populations. The *P*-values for Hardy-Weinberg (HW) expectations tests were calculated using a Markov chain randomization method using Arlequin 3.5.1.2 computer program (Excoffier & Lischer 2010). Nei's genetic distance (D_A) (Nei 1972) between populations was calculated by POPGEN version 3.2. The neighbor-joining (NJ) tree was gene-

rated using Molecular Evolutionary Genetics Analysis (MEGA) software version 11.0 (Tamura et al. 2021) based on D_A to reveal the genetic relationships among the three populations. A population structure test for the three different sampled basins was carried out using the software Structure 2.3.4 (Pritchard et al. 2000), which assessed the sample's genomic clustering (K) to obtain a representative value of K for data modeling. With this purpose, the twelve independent runs length was set to 100,000 burn-ins followed by 100,000 iterations.

RESULTS

Nine polymorphic microsatellite loci were detected (Table 1). From those, six (Y7, Y16, Y42, Mr7-133, Mr4-85, and Mr7-88) were amplified for *M. tenellum* in all sampled populations, and three monomorphic loci did not amplify for all populations. W15 amplified with prawns from Tonameca, W24 amplified for Colotepec and Manialtepec, and Mr4-8 only for those of Tonameca and Manialtepec. Those last were excluded from the intrapopulation analysis. The three populations had high polymorphism (85.19 ± 7.41%), with 145 alleles detected. The total alleles registered by locus ranged from six in microsatellite Mr7-88 to 50 in Y7 (Table 2).

The three prawn populations exhibited relatively high genetic variation with an average of A ranging from 8.889 (Manialtepec) to 22.556 (Tonameca) and an average A_r of 6.845 (Manialtepec) to 16.754 (Tonameca). The H_o of 0.166 (Manialtepec) to 0.264 (Tonameca) and the H_e of 0.680 (Manialtepec) to 0.908 (Tonameca) had higher values in the loci for the Tonameca and Manialtepec places. The mean H_o of all populations at all nine loci were lower than expected. Significant deviations from HW expectations were observed at all loci except for Y7 loci from Tonameca. Genetic differentiation (F_{st}) results are presented between the three populations for each locus (Table 2). In the Table, the mean value of F_{st} was 0.746 ± 0.058, denoting high genetic differentiation. The pairwise F_{st} coefficients between the three populations are shown in Table 3 below the diagonal. The F_{st} values go from 0.147 (between Tonameca and Colotepec) to 0.259 (between Manialtepec and Colotepec). The Tonameca and Colotepec samples had low genetic differentiation compared to the high differentiation from Manialtepec and Colotepec. The F_{st} of each population pair was statistically significant ($P < 0.05$).

According to the D_A estimator, the populations of Tonameca and Manialtepec had the highest genetic distance values. Table 3 shows the high D_A values for the prawn samples from Tonameca and Manialtepec (2.289), while the samples of Colotepec and Manialtepec had the lowest values (2.084). The genetic relationship between populations is shown (Fig. 1). The samples from Manialtepec and Colotepec converged, and those from Tonameca were separated.

Analysis of molecular variance (AMOVA) indicated that 4.703% of the total variance was between populations. In comparison, 65.796% of the total variation and 29.502% of the variation corresponded to differences between and within populations, respectively (Table 4).

DISCUSSION

Molecular tools for analyzing wild populations of aquatic species aim to understand how such populations disperse or migrate, which helps assess natural gene flow (Manel et al. 2005). Because of its amphidromy, these tools on *Macrobrachium* prawns are even more useful in explaining this specialized way of dispersing and spreading between different aquatic ecosystems, something unlikely for marine shrimps. In aquaculture, molecular tools help identify genetic diversity variations for selection purposes (Qiao et al. 2013).

Macrobrachium prawns are widely distributed in coastal areas, with a high diversity of haplotypes but without evidence of a significant geographic structure that can assess its adaptive potential to overcome changes and environmental pressure (Valle et al. 2015). There is little previous research on the genus. Still, such research suggests subtle genetic structures at small and large geographic scales (Qiao et al. 2013), which is particularly useful while analyzing genetic exchange between populations in which its natural dispersal process is disrupted by human intervention, as in the present research. The greater variability of microsatellites increases the probability of finding out if a sample from a population has a unique genotype and is useful for identifying and monitoring how populations move or migrate (Wenner et al. 2005). Dambach et al. (2013), in their research with the caridean shrimp *Nematocarcinus lanceopes*, mention that the use of several polymorphic microsatellite loci allows for determining the genetic structure of populations at a small geographic scale, helping to verify if there is a differentiation between populations, with a high certainty in the genetic structure if we rely only on a single mitochondrial marker. It is considered that the use of a large size of microsatellite markers allows monitoring of the effects of rapid change in the environment on the population genetic structure of

Table 1. List of microsatellite loci amplified previously for populations of *Macrobrachium* prawns.

Locus name	Primer (5´-3´)	Annealing temperature (ºC)	Allele range (bp)	Reference
Y7	F: ATGCCTGGAAAGAATGAG R: TTGTCTGAGCCTGAAACC	51	298-347	Yu et al. (2019)
Y16	F: ATTCGGTATCAGCTCTGC R: AGGTCATCACCCTTTCCA	57	189-216	Yu et al. (2019)
Y42	F: GGGCTCAAGAACGCTATG R: GACCCAATTACTGCTCAAAGG	48	152	Dai et al. (2012)
W15	F: TATTACGATTCCGTGGCACA R: ATATTCTTTGTAGCGGCTGG	54		Dai et al. (2012)
W24	F: AGGATTTCTGCGAGGTCTTG R: CGTGTTGTTCTTCATAGGCTTC	54	171-213	Dai et al. (2012)
Mr7-133	F: TGCCTGGAAAGAATGACC R: ACCTCGAACTGTCGCAACTT	56	242	Bhat et al. (2009)
Mr4-8	F: TCAGTGCTGGGGTGTGAA R: TGTCCTAGGATGAGGAAAGCA	56	219	Bhat et al. (2009)
Mr4-85	F: CCGGAAATTACGCCAAAATA R: GAATACGCGGCTTGAAGAG	54	183	Bhat et al. (2009)
Mr7-88	F: TATTACGATTCCGTGGCACA R: ATATTCTTTGTAGCGGCTGG	55	249	Bhat et al. (2009)

this species (Li et al. 2021). In this sense, changes in genetic diversity experienced by populations due to geographical isolation, domestication through cultivation, or hybridizations generated in the wild can be studied with microsatellite-type markers. Such is the case of that reported by Wahidah et al. (2023). In such research and using six haplotypes, it was possible to estimate in populations of *M. rosenbergii* in Indonesia how geographical isolation and domestication might cause genetic diversity differences among populations.

When there are differences in the AMOVA, the populations may have genetic variations due to non-random factors, causing data deviation (Briñez et al. 2011). According to Caraballo et al. (2010), this suggests that non-random factors produced a deviation. For *M. tenellum*, this might be a natural phenomenon. If a population is in HW equilibrium for a gene, it may not change, and the allele frequencies will remain the same along generations. On the other hand, the HW balance can also occur if a particular habitat is isolated from the sea, avoiding gene flow. These low values of genetic balance would mean negative effects due to the low variability. Thus, it would reduce biological efficacy and adaptive potential to face environmental changes. In such cases, there would be serious genetic risks for the persistence of the species (Valle et al. 2015, Dehmord et al. 2023).

On the other hand, when the D_A between the populations is reduced or negative, a severe deficiency of heterozygotes might occur, causing an imbalance in the HW parameter and, if there are high values of F_{is} (inbreeding coefficient within populations) in all loci, this could be a consequence of consanguinity (Rivera-García & Grijalva-Chon 2006). In the present research, the genetic deviations between different populations are small when the heterozygosity and F_{is} values are low. If this occurs, the attachment indices (F_{is}) suggest a certain degree of variation at inbreeding levels. To complement the genetic fixation, the F_{st} (coefficient of genetic differentiation between populations) indicates a high coincidence in the allelic frequencies observed in the different subpopulations. Finally, the F_{it} complements the information from the individual analysis of the lines. Thus, this suggests a significant deficit of heterozygotes in the sampled population. This research's F_{st}, F_{is}, and F_{it} values (total inbreeding coefficient) suggest low genetic diversity, expected heterozygosity, and frequency. The allele of the three populations may be genetically close, perhaps due to the spreading and speciation mechanisms occurring in the area. Dehmord et al. (2023), indicate that the number of alleles in wild populations is related to the increase in consanguinity among them, as occurs in aquaculture practices. In such cases, the allele frequency is significantly reduced after several generations. It is considered that increasing alleles could increase homozygosity and decrease heterozygosity in populations. Valle et al. (2015), state that low levels of

Table 2. Genetic variability at nine microsatellite loci in three populations of *Macrobrachium tenellum,* including N: sample size, A: total number of alleles, A_r: allelic richness, H_o: observed heterozygosity, H_e: expected heterozygosity, F: fixation index, and HW: *P*-value for test of Hardy-Weinberg expectations. *Monomorphic alleles.

Population (N)	Locus									Average
	Y7	Y16	Y42	W15	Mr7-133	Mr7-88	Mr4-85	W24	Mr4-8	across loci
Tonameca (35)										
A	50	7	13	22	19	24	39	19	10	22.55 ± 13.09
A_r	40.833	4.531	8.010	18.750	13.563	19.505	26.575	13.091	5.927	16.754 ± 9.78
I	3.815	1.697	2.303	3.014	2.747	3.078	3.484	2.722	1.992	2.761 ± 0.49
H_o	1.000	0.000	0.000	0.000	0.500	0.031	0.765	0.083	0.000	0.264 ± 0.29
H_e	0.976	0.779	0.875	0.947	0.926	0.949	0.962	0.924	0.831	0.908 ± 0.03
F	-0.011	1.000	1.000	1.000	0.473	0.968	0.220	0.912	1.000	
HW	0.250	0.000	0.000	0.000	0.000	0.000	0.001	0.000	0.000	
Manialtepec (18)										
A	10	16	9	0*	14	6	15	0*	10	8.889 ± 5.61
A_r	7.364	12.250	8.000	0.000	10.376	4.500	11.538	0.000	7.579	6.845 ± 4.28
I	2.139	2.622	2.138	0.000	2.490	1.633	2.569	0.000	2.138	1.748 ± 0.98
H_o	0.000	0.429	0.000	0.000	0.619	0.000	0.200	0.000	0.250	0.166 ± 0.22
H_e	0.864	0.918	0.875	0.000	0.904	0.778	0.913	0.000	0.868	0.680 ± 0.37
F	1.000	0.552	1.000		0.337	1.000	0.794		0.733	
HW	0.000	0.000	0.000		0.000	0.000	0.000		0.000	
Colotepec (25)										
A	25	21	24	21	18	19	23	0*	0*	16.778 ± 9.21
A_r	19.525	16.216	19.048	14.582	14.297	16.030	20.167	0.000	0.000	13.318 ± 7.39
I	3.091	2.916	3.060	2.850	2.770	2.872	3.067	0.000	0.000	2.292 ± 1.23
H_o	0.333	0.033	0.500	0.458	0.087	0.000	0.182	0.000	0.000	0.177 ± 0.19
H_e	0.949	0.938	0.948	0.931	0.930	0.938	0.950	0.000	0.000	0.732 ± 0.39
F	0.661	0.966	0.492	0.524	0.910	1.000	0.817			
HW	0.000	0.000	0.000	0.000	0.000	0.000	0.000			
All populations										
A	28	15	15	14	17	16	26	6	7	16.074 ± 2.21
A_r	22.574	10.999	11.686	11.111	12.746	13.345	19.427	4.364	4.502	12.306 ± 1.76
I	3.015	2.412	2.501	1.996	2.669	2.528	3.040	0.907	1.377	2.267 ± 0.27
H_o	0.444	0.154	0.167	0.153	0.402	0.010	0.382	0.028	0.083	0.203 ± 0.05
H_e	0.929	0.879	0.899	0.626	0.920	0.888	0.942	0.308	0.566	0.773 ± 0.06
F	0.541	0.833	0.824	0.074	0.561	0.989	0.598	0.910		0.746 ± 0.06
HW	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001			

Table 3. Matrix of pairwise F_{st} values (below diagonal) and genetic distances (DA: above diagonal) between three populations of *Macrobrachium tenellum*.

	Tonameca	Manialtepec	Colotepec
Tonameca		2.289	2.230
Manialtepec	0.159		2.084
Colotepec	0.147	0.259	

genetic differentiation are observed more often in populations exposed to genetic bottlenecks, especially those maintained in captivity or in closed environments where access to wild re-stocking is impossible.

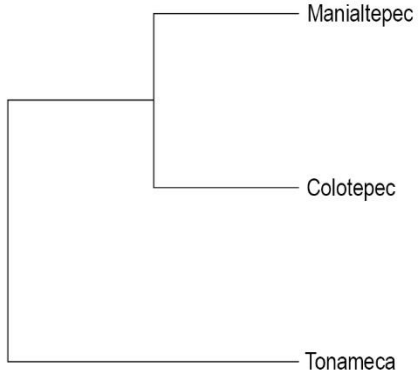

Figure 1. Representation of Neighbor-joining Nei's (1972) standard genetic distance among the three populations of *Macrobrachium tenellum* based 1000 replicates.

Table 4. Analysis of molecular variance (AMOVA) results for three populations of *Macrobrachium tenellum*.

Source of variation	Sum of squares	Variance components	Percentage variation	Fixation index	Average over all loci
Among populations	22.249	0.138	4.703	F_{is}	0.690
Among individuals within populations	326.869	1.930	65.796	F_{st}	0.047
Within individuals	64.000	0.865	29.502	F_{it}	0.705
Total	413.118	2.933	100		

In agreement with Li et al. (2021), Azuma et al. (2022) and Makombu et al. (2022), the use of molecular markers in wild populations of prawns helps to deduct the nucleotide diversity, the heterozygosity (H_{et}) of the population and the F_{st}. It can provide clues to verify the effects of hybridization, which are valuable when proposing genetic improvement strategies in those species with potential for aquaculture. However, in most previous research, the genetic diversity of the populations is addressed by comparing the indexes indicating the polymorphisms (HW, F_{is}, F_{st}) of the markers. The information they produce is statistically significant in such a way that it can be inferred if there is sufficient genetic flow in the populations, where it is possible to show that they are different and, therefore, have good probabilities for reproductive selection processes. Such is the case of the populations of present research in where prawns from the Tonameca River display greater genetic distance and average heterozygosity concerning the populations of the Manialtepec River and Colotepec River (Fig. 1). In agreement with this figure, the assumption that geographical distance is an important factor for the genetic differences of the populations is validated (Dambach et al. 2013, Wang et al. 2024) and therefore, is a viable population for management.

Molecular microsatellite markers are very useful for finding differences or similarities between two or more populations of the same species in areas in which it is not clear if there is a genetic exchange between them due to any natural or human-made barriers, as occurs with *Macrobrachium* prawns. At the same time, they move from one basin to another. In such cases, prawns from Colotepec and Manialtepec may share close genetic similarities, while those from Tonameca are somewhat distant from both. The proximity of the Colotepec and Manialtepec basins and the genetic similarities between the samples in the present research suggest a gene flow between them in recent times. One or more tributaries or passages may connect both basins, providing a way of interchange.

Among the previous research analyzing microsatellites in *Macrobrachium* prawns, Tan et al. (2005) report 19 different microsatellite regions in *M. rosenbergii*. They state that these are part of critical regions and have had mutations. A similar situation could occur with *M. tenellum* if larger microsatellite regions are identified. The high similarity between the sequences found or homologous sequences could suggest a possible evolutionary similarity between lineages, but this has still to be determined for *M. tenellum*. On the other hand, Song & Kim (2011), characterized 15 polymorphic microsatellite loci of *M. nipponense*. They also analyzed the cross-species amplification potential of their 15 primer sets and included the 12 sets reported by Feng & Li (2008), using seven related taxa. Authors state that null alleles may exist at various loci, as suggested by the general excess of homozygotes for most allele size classes. In this case, the excess homozygosity can be partly attributed to the fact that the specimens used for genotyping were obtained from distinct locations, as in *M. tenellum* in the present research. In agreement with present results, Wang et al. (2024), indicate that there is a positive correlation between genetic distance and geographical distance. In such research, genetic differences in populations of *M. nipponense* commonly occur if geographic distances are long, suggesting isolation might cause genetic distances, although they cannot be evident quickly. In agreement with the author, generations between 40 and 60 must display this effect. So, this may apply to species from the same basin but different habitats.

On the other hand, Qiao et al. (2013), determined if *M. nipponense* prawns from various stocks could be genetically structured across their latitudinal distribution interval due to geographic heterogeneity. They sampled seven different wild populations, all in or near the main current of the same river. They found 20 different microsatellite markers, suggesting high genetic diversity in the sampled river compared to other rivers previously studied, where the mean H_{et} was lower. According to the authors, the deficiency of H_{et} is a risk in the conservation of germplasm because it can cause a loss of genetic diversity over time due to a greater inbreeding depression. Likewise, the level of H_{et} is a

good index of genetic variability since it can be tested with any species regardless of its environment or genetic structure. The authors concluded that the *M. nipponense* population from the sampled river is in better condition, with considerable genetic diversity within individuals. The authors also compare their results with previous data from other lakes or estuaries, finding that population differentiation, inferred from microsatellite analysis, was greater in rivers than lakes, which the better dispersal capacity in such environments could explain. If the number of identified molecular markers is large and polymorphism is high in any population, this suggests a broad genetic pool, possibly suitable for breeding programs.

Studies with molecular markers can allow us to recreate maps of original migrations and distribution areas, which contributes to a better understanding of the species in ecological terms. It is important to consider that the genetic variations in the three basins included in this research may be caused by alterations that such environments have due to natural or human causes. Overfishing, for example, since this might produce a populational imbalance. The degree of genetic diversity is also related to the intrinsic features of the species. Liu et al. (2020), analyzing several populations of *Procambarus clarkii*, determined that the observed genetic diversity largely results from its distribution and that species with a wider distribution might have a higher level of genetic variability than those with a narrower distribution that will cause a correlation between the genetic diversity of the population and determine how such population is distributed along a particular area or can colonize different habitats. In agreement with this, genetic distances in present research can cause variations due to the amphidromous attribute that *M. tenellum* possesses. Such attributes allow this species to spread over a wide area composed of different habitats. These assumptions should be considered while proposing development and conservation strategies, such as re-stocking programs, fisheries management, or aquaculture practices.

Credit author contribution

I. Gutiérrez-Méndez: conceptualization, validation, methodology, formal analysis, writing-original draft; H. Rodríguez-Magadan: methodology, formal analysis, validation; N. Martínez-Salazar: methodology, review and editing; M. García-Guerrero: conceptualization, funding acquisition, project administration, supervision, review, and editing; R. de los Santos-Romero: conceptualization, funding acquisition, formal analysis, validation, review, and editing. All authors have read and accepted the published version of the manuscript.

Conflict of interest

The authors declare no potential conflict of interest in this manuscript.

ACKNOWLEDGMENTS

ISGM thanks CONAHCYT for the scholarship. MGG thanks funding from SNII-CONAHCYT, EDI-IPN, and COFAA-IPN. RdelosSR thanks TecNM and Instituto Tecnológico del Valle de Oaxaca for their support.

REFERENCES

Arif, I.A., Khan, H.A., Bahkali, A.H., Al Homaidan, A.A., Al Farhan, A.H., Al Sadoon, M., et al. 2011. DNA marker technology for wildlife conservation. Saudi Journal of Biological Sciences, 18: 219-225. doi: 10.1016/j.sjbs.2011.03.002

Atin, K.H., Christianus, A., Fatin, N., Lutas, A.C., Shabanimofrad, M. & Subha, B. 2017 Genetic diversity analysis in Malaysian giant prawns using expressed sequence tag microsatellite markers for stock improvement program. Genetics and Molecular Research, 16: 1-10. doi: 10.4238/gmr16035685

Azuma, N., Kuroda, M., Katogi, Y. & Chiba, S. 2022. Genetic differentiation in *Palaemon septemtrionalis* between populations of two rivers examined by newly developed short tandem repeat markers. Crustacean Research, 51: 123-127. doi: 10.18353/crustacea.51.0_123

Barbará, T., Palma-Silva, C., Paggi, G.M., Bered, F., Fay, M.F. & Lexer, C. 2007. Cross-species transfer of nuclear microsatellite markers: potential and limitations. Molecular Ecology, 16: 3759-3767. doi: 10.1111/j.1365-294X.2007.03439.x

Bauer, R.T. 2011. Amphydromy and migrations of freshwater shrimps. I. Costs, benefits, evolutionary origins, and an unusual case of amphydromy. In: Asakura, A. (Ed.). New frontiers in crustacean biology. Brill, Leiden, pp. 145-156.

Bauer, R.T. 2013. Amphidromy in shrimps: a life cycle between rivers and the sea. Latin American Journal of Aquatic Research. 41: 633-650. doi: 103856/vol41-issue4-fulltext-2

Bhat, S., Patel, A., Das, P., Meher, P.K., Pillai, B.R. & Jayasankar, P. 2009. Isolation and characterization of

microsatellite loci in giant freshwater prawn *Macrobrachium rosenbergii*. Conservation Genetics, 10: 1473-1475. doi: 10.1007/s10592-008-9763-2

Briñez, R.B., Caraballo, O.X. & Salazar, V.M. 2011. Diversidad genética en seis poblaciones de tilapia roja, usando microsatelites como marcadores genéticos. Revista MVZ Córdoba, 16: 2491-2498.

Caraballo, D.A., Bellucio, P.M. & Rossi, M.S. 2010. The library model for satellite DNA evolution: a case study with the rodents of the genus *Ctenomys* (Octodontidae) from the Iberá marsh, Argentina. Genetica, 138: 1201-1210. doi: 10.1007/s10709-010-9516-2

Charoentawee, K., Supawadee, P. & Uthairat, N. 2006. Isolation and characterization of microsatellites in giant freshwater prawn *Macrobrachium rosenbergii*. Molecular Ecology Notes, 6: 823-825. doi: 10.1111/j.1471-8286.2006.01358.x

Dai, X.L., Deng, P.P., Shi, Y.H., He, A.Y., Jiang, F. & Ding, F. 2012. A preliminary study on re-developing of *Macrobrachium rosenbergii* microsatellite markers and influence factors. Biotechnology Bulletin, 10: 141-149.

Dambach, J., Raupach, M.J. & Mayer, C. 2013. Isolation and characterization of nine polymorphic microsatellite markers for the deep-sea shrimp *Nematocarcinus lanceopes* (Crustacea: Decapoda: Caridea). BMC Research Notes, 6: 75. doi: 10.1186/1756-0500-6-75

Dehmord, L.M., Nazari, M.A., Chermahini, M.A. & Pazir, K.M. 2023. Application of microsatellite DNA method in determining the genetic diversity of farmed broodstocks of *Penaeus vannamei* (Boone, 1931) in hatchery centers of Bushehr Province. Latin American Journal of Aquatic Research, 51: 343-350. doi: 10.3856/vol51-issue3-fulltext-3001

Divu, D., Khushiramani, R., Malathi, S., Karunasagar, I. & Karunasagar, I. 2008. Isolation, characterization and evaluation of microsatellite DNA markers in giant freshwater prawn *Macrobrachium rosenbergii*, from South India. Aquaculture, 284: 281-284. doi: 10.1016/j.Aquaculture.2008.07.023

Excoffier, L. & Lischer, H. 2010. Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources, 10: 564-567. doi: 10.1111/j.1755-0998.2010.02847.x

Feng, J.B. & Li, J.L. 2008. Twelve polymorphic microsatellites in Oriental river prawn, *Macrobrachium nipponense*. Molecular Ecology Resources, 8: 986-988. doi: 10.1111/j.1755-0998.2008.02129.x

García-Guerrero, M., Becerril-Morales, Vega-Villasante, F. & Espinosa-Chuarand, L. 2013. Los langostinos del género *Macrobrachium* con importancia económica y pesquera en América Latina: conocimiento actual, rol ecológico y conservación. Latin American Journal of Aquatic Research, 41: 651-675. doi: 10.3856/vol41-issue4-fulltext-3

García-Velazco, H., Maeda-Martínez, A.M., Obregón-Barboza, H. & Murugan, G. 2018. The systematics of the amphidromous shrimp *Macrobrachium hobbsi* Nates & Villalobos, 1990 (Decapoda: Caridea: Palaemonidae) from the Mexican Pacific slope. Journal of Crustacean Biology, 38: 586-599. doi: 10.1093/jcbiol/ruy054

García-Velazco, H., Maeda-Martínez, A.M., Obregón-Barboza, H., Campos-Torres, O. & Murugan, G. 2017. The systematics of the Mexican populations of *Macrobrachium digueti* (Bouvier, 1895) (Decapoda: Caridea: Palaemonidae). Journal of Crustacean Biology, 37: 168-186. doi: 10.1093/jcbiol/rux008

García-Velazco, H., Maeda-Martínez, A.M., Obregón-Barboza, H., Ruiz-Campos, G., Rodríguez-Almaraz, G. & Murugan, G. 2021. The systematics of the amphidromous shrimp *Macrobrachium americanum* Spence Bate, 1868 (Decapoda: Caridea: Palaemonidae) from the Mexican Pacific slope. Journal of Crustacean Biology, 4: 1. doi: 10.1093/jcbiol/ruaa098

González, E. 2003. Microsatelites, sus aplicaciones en la conservación de la biodiversidad. Graellsia, 59: 377-388. doi: 10.3989/graellsia.2003.v59.i2-3.253

Jarne, P. & Lagoda, P. 1996. Microsatellites, from molecules to populations and back. Trends in Ecology & Evolution, 11: 424-429. doi: 10.1016/0169-5347(96)10049-5

Li, Y., Fan, W., Huang, Y., Huang, Y., Du, X., Liu, Z., et al. 2021. Comparison of morphology and genetic diversity between broodstock and hybrid offspring of oriental river prawn, *Macrobrachium nipponense* based on morphological analysis and snp markers. Animal Genetics, 52: 461-471. doi: 10.1111/age.13081

Liu, Z. & Cordes, J.F. 2004. DNA marker technologies and their applications in aquaculture genetics. Aquaculture, 238: 137. doi: 10.1016/j.aquaculture.2004.05.027

Liu, F., Qu, Y.K., Geng, C., Wang, A.M., Zhang, J.H., Li, J.F., et al. 2020. Analysis of the population structure and genetic diversity of the red swamp crayfish (*Procambarus clarkii*) in China using SSR markers. Electronic Journal of Biotechnology, 47: 59-71. doi: 10.1016/j.ejbt.2020.06.007

Makombu, J.G., Cheruiyot, E.K., Stomeo, F., Thuo, D.N., Oben, P.M., Oben, B.O., et al. 2022. Species-informative SNP markers for characterizing freshwater prawns of genus *Macrobrachium* in Cameroon. Plos One, 17: e0263540.

Manel, S., Gaggiotti, O.E. & Waples, R.S. 2005. Assignment methods: matching biological questions with appropriate techniques. Trends in Ecology and Evolution, 20: 136-142. doi: 10.1016/j.tree.2004.12.004

March, J., Benstead, P., Pringle, M. & Scatena, F.N. 1998. Migratory drift of larval freshwater shrimps in two tropical streams, Puerto Rico. Freshwater Biology, 40: 261-273. doi: 10.1046/j.1365-2427.1998.00352.x

Mohanty, P., Lakshman, S., Bindu, R.P., Pallipuram, J. & Paramananda, D. 2016. Genetic divergence in Indian populations of *M. rosenbergii* using microsatellite markers. Aquaculture Research, 47: 472-481. doi: 10.1111/are.12508

Murphy, N.P. & Austin, C.M. 2005. Phylogenetic relationships of the globally distributed freshwater prawn genus *Macrobrachium* (Crustacea: Decapoda: Palaemonidae): biogeography, taxonomy and the convergent evolution of abbreviated larval development. Zoologica Scripta, 34: 187-197. doi: 10.1111/j.1463-6409.2005.00185.x

Nei, M. 1972. Genetic distance between populations. American Naturalist, 106: 283-292. doi: 10.1086/282771

Peakall, R. & Smouse, P.E. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research update. Bioinformatics, 28: 2537-2539. doi: 10.1093/bioinformatics/bts460

Pritchard, J., Stephens, M. & Donnelly, P. 2000. Inference of population structure using multilocus genotype data. Genetics, 155: 945-959. doi: 10.1093/genetics/155.2.945

Qiao, H., Lv, D., Jiang, S.F., Sun, S.M., Gong, Y.S., Xiong, Y.W., et al. 2013. Genetic diversity analysis of oriental river prawn, *Macrobrachium nipponense*, in Yellow River using microsatellite marker. Genetics and Molecular Research, 12: 5694-5703. doi: 10.4238/2013. November.18.18

Rivera-García, M. & Grijalva-Chon, J.M. 2006. Variabilidad y diferenciación genética en camarón blanco *Penaeus* (*Litopenaeus*) *vannamei* de bajo y alto crecimiento. Ciencias Marinas, 32: 1-11.

Song, K.H. & Kim, W. 2011. Isolation and characterization of polymorphic microsatellites from the oriental river prawn *Macrobrachium nipponense* (Caridea: Palaemonidae). Journal of Crustacean Biology, 31: 138-141. doi: 10.1651/09-3250.1

Song, K.H., Jung, J. & Kim, W. 2016. Genetic variation in a freshwater prawn species, *Palaemon paucidens*, in South Korea. Biochemical Systematics and Ecology, 65: 23-32. doi: 10.1016/j.bse.2016.01.001

Tamura, K., Stecher, G. & Kumar, S. 2021. MEGA 11: Molecular Evolutionary Genetics Analysis Version 11. Molecular Biology and Evolution, 38: 3022-3027. doi: 10.1093/molbev/msab120

Tan, S., Bhassu, S., Hassan, R., Yusoff, K., Jamari, Z., Siraj, S. & Guan, T. 2005. Single Locus microsatellite development for the malaysian giant freshwater prawns, *Macrobrachium rosenbergii*. Pertanika Journal of Tropical Agricultural Science, 28. 87-94.

Valle, A.G., Fresneda-Rodríguez, A., Chasqui, L. & Caballero, S. 2015. Diversidad genética del langostino blanco *Litopenaeus schmitti* en el Caribe colombiano. Boletín de Investigaciones Marinas y Costeras, 44: 237-251.

Vergamini, F.G., Pileggi, L.G. & Mantelatto, F.L. 2011. Genetic variability of the Amazon River prawn *Macrorachium amazonicum* (Decapoda, Caridea, Palaemonidae). Contributions to Zoology, 80: 67-83. doi: 10.1163/18759866-08001003

Wahidah, S.B., Omar, A., Trijuno, D.D., Nugroho, E. & Khatimah, A.K. 2023. Genetic variation of giant freshwater prawns *Macrobrachium rosenbergii* wild population of south Sulawesi, Indonesia. Biodiversitas, 24: 3081-3090. doi: 10.13057/biodiv/d240564

Wang, D., Hu, L., Zhang, F., Zheng, F., Gong, M., Xiong, F., et al. 2024. Genetic structure of *Macrobrachium nipponense*, an important farmed freshwater shrimp in China, in the Three Gorges Reservoir. Aquatic Living Resources, 37: 5. doi: 10.1051/alr/2024003

Weir, B.S. & Cockerham, C.C. 1984. Estimating F-statistics for the analysis of population structure. Evolution, 38: 1358-1370. doi: 10.2307/2408641

Wenner, E.L., Knott, D.M., Barans, C.A., Wilde, S., Blanton, O. & Amft, J. 2005. Key factors influencing transport of white shrimp (*Litopenaeus setiferus*) post-larvae into the Ossabaw Sound system, Georgia, USA. Fisheries Oceanography, 14: 175-194. doi: 10.1111/j.1365-2419.2005.00328.x

Yu, L., Zhu, X., Liang J., Fan, J. & Chen, C. 2019. Analysis of genetic structure of wild and cultured giant freshwater prawn (*Macrobrachium rosenbergii*) using newly developed microsatellite. Frontiers in Marine Science, 6: 1-9. doi: 10.3389/fmars.2019.00323

Received: November 22, 2023; Accepted: June 7, 2024