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Research Article

Bases to inform a genetic line of whiteleg shrimp *Penaeus vannamei* of Mexican origin

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ABSTRACT. The whiteleg shrimp *Penaeus vannamei* is one of the most relevant aquaculture species in Latin America and globally. Among several elements, the improvement of its production depends on the larval genetic quality produced in commercial hatcheries. A strategy for achievement is setting up a long-term management plan that includes the genetic settlement of a breeding population with broad genetic variability and reduced inbreeding levels and the design of adequate management and crossbreeding schemes. The settlement of the breeding population requires a detailed characterization of the genetic composition and diversity of the breeding line(s) that are being managed. The present study evaluated the genetic composition of six wild populations from the southern and northern coasts of the Mexican Pacific (Oaxaca, Guerrero, Nayarit, and Sinaloa) and 56 breeding lots maintained in commercial hatcheries. The genetic profiles of a low-density SNP marker panel (171 and 152 loci for the wild and hatchery-reared groups, respectively) were used to estimate genetic diversity and differentiation within and among samples. The wild population presented significant genetic differences between southern and northern Pacific locations. Although these populations showed higher diversity levels than the cultivated stocks, the genetic pool of the total 56 lots was highly variable with low inbreeding levels. The genetic characteristics of the analyzed populations and cultivated stocks warrant the constitution of a Mexican-origin breeding line with future potential for selection to the environmental conditions of the northwestern region of Mexico.

Keywords: *Penaeus vannamei*; genetic diversity; genetic structure; selection, SNPs; inbreeding

INTRODUCTION

The whiteleg shrimp *Penaeus vannamei* is one of the most relevant worldwide aquaculture species, with an annual production of more than 5.8 million t, representing more than 50% of crustacean production (FAO 2022). In Mexico, this species' aquaculture has the highest production value of over 22.5 billion Mexican pesos (ca. US$ 1.1 billion) in 2022 (CONAPESCA 2023).

During 1998-2013, shrimp aquaculture in Mexico was based on a breeding line regionally known as 'Melagos' that came from an imported stock from Venezuela in 1997 (Cruz et al. 2004). In 2007, this line presented a relatively high genetic diversity but signs of accumulated inbreeding (Perez-Enriquez et al. 2009). After 2013, hatchery-reared larvae producers, moved by the necessity of reverting pathogen-associated problems [mainly white spot syndrome virus (WSSV) and acute hepatopancreatic necrosis disease (AHPND)], imported breeding lines originated from hatcheries in the USA, Central, and South America, which showed higher levels of genetic diversity than the 'Melagos' line (Perez-Enriquez et al. 2018a).

The distribution of whiteleg shrimp along the eastern tropical Pacific spans from Mexico to Peru. The

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wild population is reported to be genetically structured over a wide geographic range (Valles-Jimenez et al. 2005), but with genetic homogeneity at a regional level in Sinaloa, Mexico (Perez-Enriquez et al. 2018b) and introgression evidence from aquaculture activities (Perez-Enriquez et al. 2018c).

Genetic diversity plays a dominant role in the ability of populations to confront and adapt to environmental challenges. Thus, reducing diversity and the resulting increase in inbreeding may negatively affect biological fitness (Freeland et al. 2011). Therefore, whether or not selection strategies are being followed, management should include this element in broodstock crossbreeding programs (Gjerde 2010).

Monitoring the genetic diversity in shrimp breeding stocks is a topic widely analyzed worldwide. It is being taken into account in several commercial breeding and larvae production programs, not only in *P. vannamei* (e.g. Vela-Avitúa et al. 2013, Zhang et al. 2014, Ren et al. 2018, Knibb et al. 2020, Garcia et al. 2021, Lisboa-Silva et al. 2022, Casado et al. 2022) but in other shrimp species as well [e.g. *Penaeus japonicus* (Luan et al. 2006), *P. monodon* (Guppy et al. 2020), *Litopenaeus stylirostris* (Goyard et al. 2008), among others].

In Mexico, the aquaculture consortium Genetica Acuicola Mexicana (Genamex) is developing a long-term project on the genetic management of shrimp broodstock to, among other things, increase shrimp farmers' ability to respond to the threats of new variants of endemic pathogens and the potential emergence of new pathogens. One of the premises of this project is the integration of a genetic nucleus of Mexican origin with broad genetic diversity, from which specific lines will be developed through particular management and selection strategies.

In the present study, the breeding stocks of the consortium were genetically characterized, both from wild and aquaculture origin, to establish the basis for a Mexican genetic line that may include potential locally adapted genes.

MATERIALS AND METHODS

Sampling strategy and data management

Samples were obtained from wild and aquaculture populations. Samples from the wild were taken at six locations (n = 32 at each location) along the Mexican tropical Pacific coast in 2020-2021 by small-scale fishermen, from the localities of Cerro Grande, Oaxaca (16°14'25"N, 94°32'47"W), Laguna Tres Palos, Guerrero (16°47'39"N, 99°44'57"W), San Blas, Nayarit (21°25'58"N, 105°13'10"W), and Sinaloa from three regions: Sinaloa Sur from Teacapan (22°32'46"N, 105°44'58"W) to Los Pozos (23°00'38"N, 106°08'59"W), Sinaloa Centro from Cospita (24°05'50"N, 107°08'11"W) to Angostura (25°08'26"N, 108°20'33"W), and Sinaloa Norte from Ohuira (25°37'54"N, 108°58'38"W) to Ahome (25°42'46"N, 109°20'49"W) (Fig. 1). The leading aquaculture stocks, which were from several origins (South, Central and North America), came from 56 lots maintained within the Genamex consortium (n = 32 from each stock). Muscle samples were preserved in absolute ethanol. Subsamples were sent to the Center for Aquaculture Technologies (CAT, https://aquatech-center.com/) for DNA extraction and genoty-ping with a commercial low-density SNP panel (AQUAarray LD vannamei) with 192 SNP markers. The individual genotypes were processed with the GenAlEx program ver. 6.503 (Peakall & Smouse 2012). Individuals with more than 15% missing genotypes were discarded from the database. Departure of loci from Hardy-Weinberg Equilibrium (HWE) was calculated with GenAlEx, using an adjusted *P*-value by the sequential Bonferroni method (Rice 1989). Loci that departed from HWE or were monomorphic in all populations, with >5% of missing data, were discarded.

Genetic diversity and structure of wild populations

The genetic diversity parameters were the number of alleles per locus (Na), the effective number of alleles per locus (ne) and observed (Ho) and expected (He) heterozygosity, which were estimated for each sampling location with GenAlEx. Levels of diversity among locations were statistically compared by Student *t*-tests with *P*-values adjusted by sequential Bonferroni.

The level of genetic differentiation among the six wild locations was estimated by pairwise and global F_{ST} and with an analysis of molecular variance (AMOVA) with the Arlequin software v. 3.5 (Excoffier et al. 2005). The genetic structure was analyzed using the multivariate grouping method of discriminant analysis of principal components (DAPC) with the ADEGENET program in R (Jombart 2008), with functions found clusters and DAPC retaining 30 principal components, 3 clusters, and 2 discriminants. Also, a Bayesian analysis was run with the Structure software v. 2.3.4 (Pritchard et al. 2000) with the following parameters: '*no-admixture*' model, 20000 burnin steps, and 50000 cycles of the Markov chain, for K = 1 to 7, with five replicates each one. The most probable group number (K) was defined by the Evanno method (Evanno et al. 2005) and the average structure with the Clumpak software (Kopelman et al. 2015). To explain the genetic

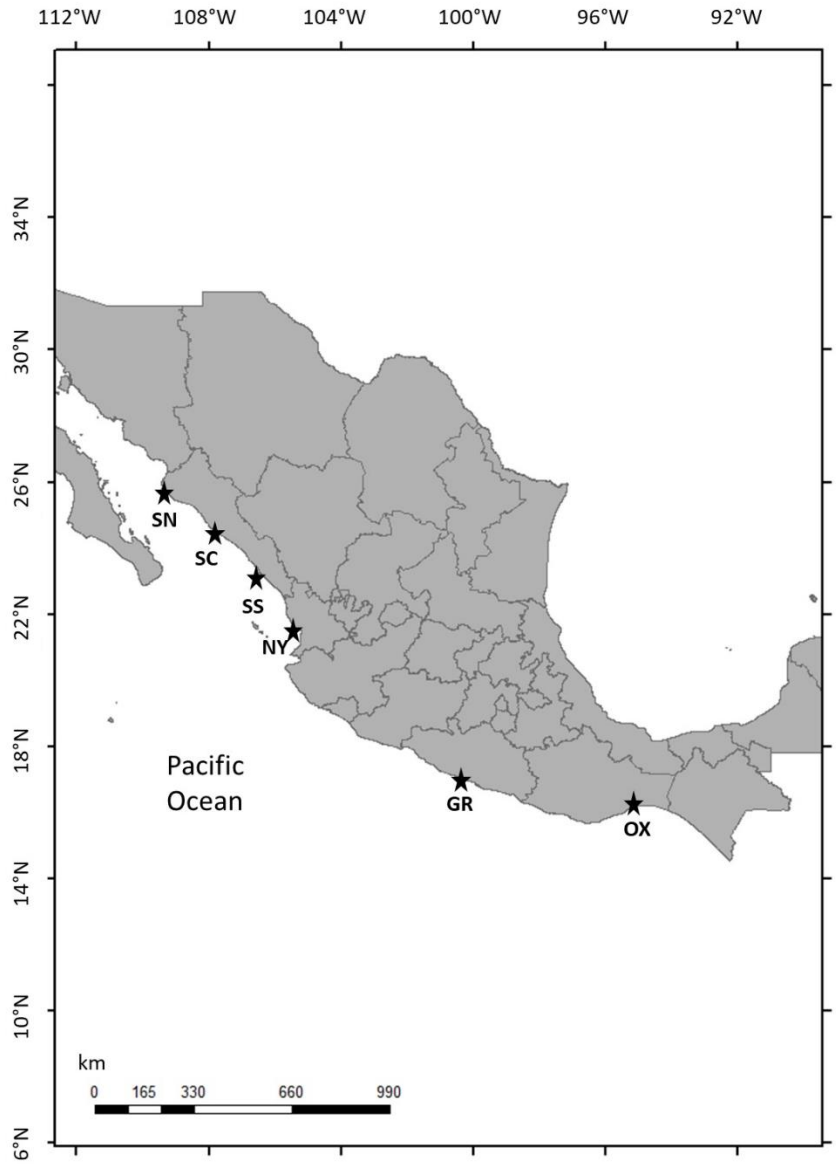

Figure 1. Sampling locations of wild populations. SN: Sinaloa Norte, SC: Sinaloa Centro, SS: Sinaloa Sur, NY: Nayarit, GR: Guerrero, OX: Oaxaca.

differentiation among wild locations as a function of the geographic distance, i.e. isolation by distance, a correlation matrix of the natural logarithm of the distance (km) *vs*. imbriding rates (*Fst*) was done with a Mantel test in Arlequin (Excoffier et al. 2005).

Genetic diversity and structure of farmed stocks

The genetic diversity was estimated as above. Farmed stocks were classified into low, medium, and high genetic diversity groups, building a matrix of arbitrary values of a low, medium, and high number of alleles per locus (≤1.6, 1.6-1.8, and >1.8, respectively) and expected heterozygosity (<0.25, 0.25-0.30, and > 0.30), respectively). Each stock's inbreeding rate (*Fis*) was estimated with the equation $Fis = 1 / (2 \times Ne)$, where Ne is the effective population size. Two methods obtained the Ne value: 1) based on the number of families represented in each lot, which was inferred by paternity tests within lots with the program Colony v. 2.0.6.9 (Wang 2009, Jones & Wang 2010); 2) based on the number of families registered during the spawning events of each lot. In both cases, because shrimp breeding occurs at a proportion of one female and one male, Ne = 2 × the number of families.

For the genetic structure analysis, the Guerrero and Sinaloa Sur locations were added to the database of the 56 cultivated stocks [generated by the companies by the mass selection approach mainly originated by introduc-

tions from South (2015), Central (2011) and North America (2013)], because they were considered representative, respectively, of the wild populations from southern and northern Mexican Pacific regions. With 58 lots, the Structure software was run using the same model as above. In this case, an analysis in phases was done so that, as a function of the most probable K, the lots were further separated as genetic groups, and with these, new genetic analyses were done.

RESULTS

Wild population diversity and genetic structure

Of the 192 SNPs in the CAT's low-density panel, 171 represented an adequate percentage of reads. The HWE analysis, considering an adjusted *P*-value by the sequential Bonferroni method ($P = 0.0003$), showed that one locus departed from equilibrium in Nayarit, Sinaloa Centro, and Sinaloa Norte in each; Guerrero, Oaxaca, and Sinaloa Sur did not show significant departures. Based on these results, the genetic diversity and structure analyses were done without discarding loci, i.e. with the 171 loci (Table S1).

High genetic diversity was observed in the number of alleles per locus (Na = 1.86-1.918) in the six locations (Fig. 2a). The adjusted values through the effective number of alleles per locus (ne) varied between 1.518-1.544 (Fig. 2a). In the case of the observed and expected heterozygosities (Ho and He, respectively), the values fluctuated from 0.297 to 0.313 (Fig. 2b). According to paired Student-t tests, there were no significant differences ($P > 0.05$) among locations in any diversity parameter (Na, ne, Ho, He). In *Fis*, the significant differences (Student-t tests, $P < 0.05$) observed between Oaxaca-Nayarit and Oaxaca-Sinaloa Centro pairs (Fig. 2c) were non-significant after the Bonferroni adjustment.

The genetic structure analysis indicated a most probable K of 2 (Evanno) or 3 (Clumpak) (Fig. S1). The bar plot indicates that the southern Pacific locations (Oaxaca and Guerrero) form a group. At the same time, those from the northern Pacific are another group, with an additional group within this corresponding to the Sinaloa Centro location (Fig. 3a). The DPAC shows a similar picture to that described above (Fig. 3b). Genetic structure was also inferred by the global AMOVA ($F_{ST} = 0.006$; $P < 0.05$), and pairwise F_{ST} (Table 1). The hierarchical AMOVA indicated significant differences between the southern and northern Pacific locations ($F_{CT} = 0.0075$; $P = 0.016$, and $F_{SC} = 0.0005$; $P = 0.38$) with a further subdivision of Sinaloa Centro in the northern Pacific, where approximately 50% of the individuals showed a distinct genetic profile (Fig. 3).

A low but significant determination coefficient ($R^2 = 0.34$; $P = 0.017$) obtained in the Mantel test suggested the rejection of the null hypothesis of panmixia. When the test was run excluding Sinaloa Centro, the Mantel test resulted in a higher and statistically significant $R^2 = 0.77$ ($P = 0.009$), indicating that isolation by distance can explain genetic differentiation among locations (Fig. 4).

Farmed stock's diversity and genetic structure

The genetic diversity of farmed stocks, which included two wild locations as references, fluctuated between 1.57-1.97 alleles per locus and 0.221-0.337 of expected heterozygosity (Fig. 5). The majority of the cultivated stocks (80%) were classified into medium- to high-diversity groups (Table 2). In addition, most stocks (87.5%) presented an inbreeding rate lower than 10% (Table 2).

The analysis with the Structure program indicated, in the first phase (56 cultivated + 2 wild = 58 stocks), a most probable K = 2, that revealed a well-defined difference of one group composed of stocks P08 and P19-P34, whose reported origin is Central America (Fig. 6a). In the second phase, which excluded the 17 stocks of the previous phase, the 39 cultivated stocks and the two wild populations showed a multimodal delta K. Still, with a K =14 as the most probable (Fig. 6b). The bar plot for K = 14 showed a high genetic heterogeneity among stocks, with a high mixture within some of them. The coincidence among the putative genetic groups with the origin records was inconsistent for all cultivated stocks (Table 3). A relevant observation was that some individuals in the cultivated stocks showed a genetic profile similar to the wild origin (e.g. P01, P09, P36, P38, P42, P44, P45, P46; Fig. 6b). The comparative analysis among the obtained groups relative to the putative origin of each stock (from the origin records at each hatchery) showed some consistent grouping [e.g. group C (Hw); group F (Nc); group O (wild)]; however, there was an apparent lack of consistency in most groups with mixtures of two or more origins within a group (Table 3).

DISCUSSION

Genetic structure of the wild population

Despite the low genetic differentiation among the studied locations, the statistical significance detected between the southern locations of the Mexican Pacific (Oaxaca and Guerrero) with those from the northern

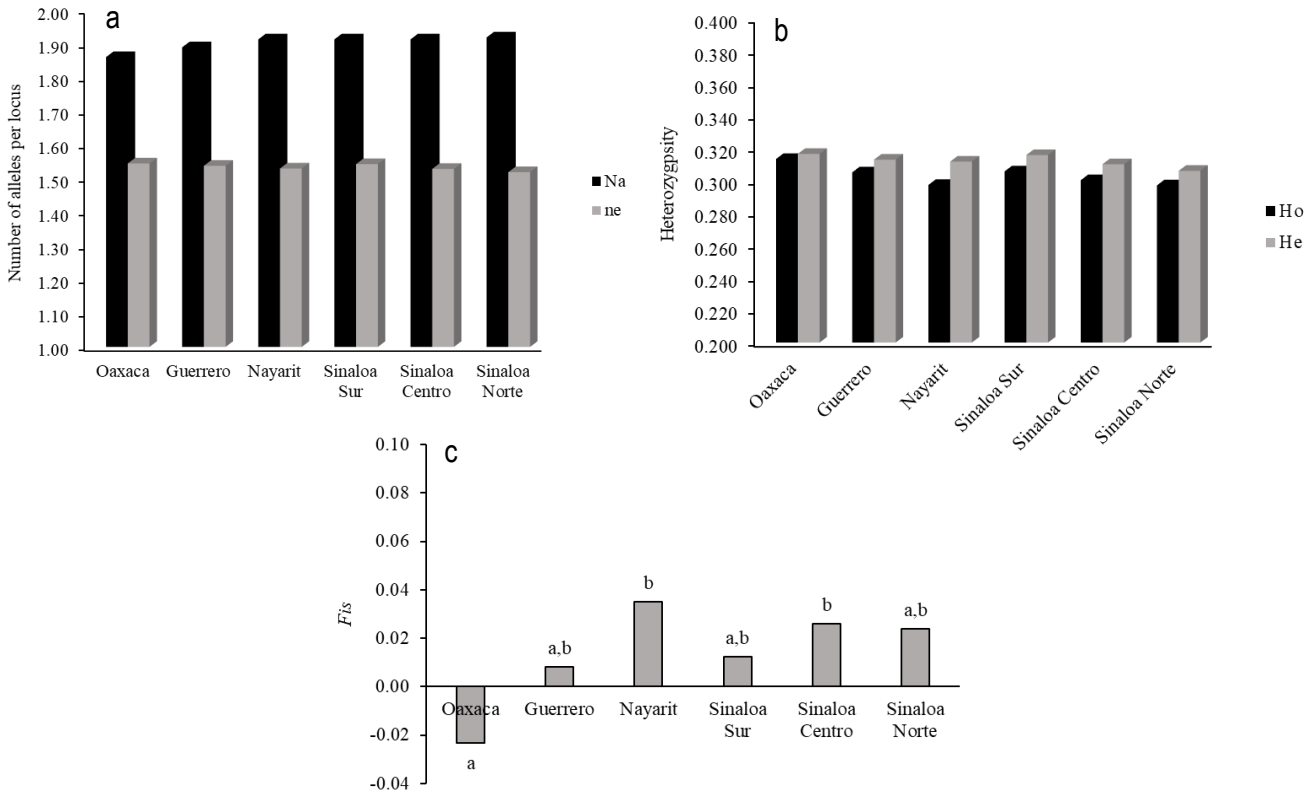

Figure 2. Genetic diversity and inbreeding in wild locations. a) Number of alleles per locus (Na) and effective number of alleles per locus (ne); b) observed (Ho) and expected (He) heterozygosity; c) inbreeding coefficient (*Fis*) (letters indicate statistical differences among groups, $P < 0.05$).

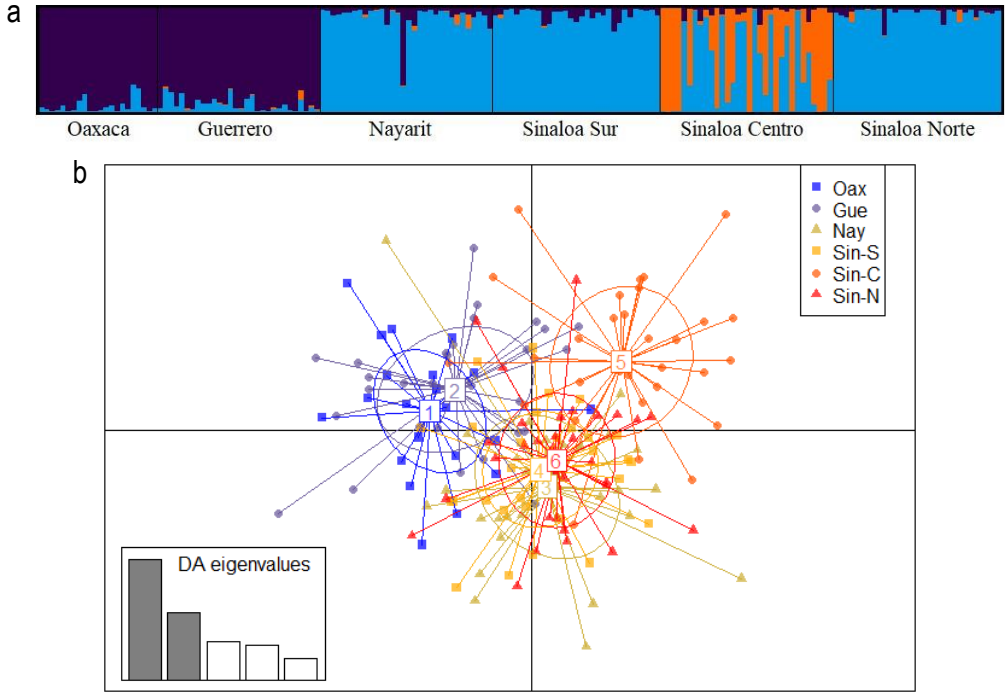

Figure 3. Genetic structure of wild locations inferred by: a) Structure, b) discriminant analysis of principal components (DAPC). 1: Oaxaca, 2: Guerrero, 3: Nayarit, 4: Sinaloa Sur, 5: Sinaloa Centro, 6. Sinaloa Norte. DA: discriminant analysis.

Table 1. Pairwise F_{ST} values among wild locations below the diagonal; *P*-values above the diagonal, with significant values in bold (significance adjusted by sequential Bonferroni $P = 0.008$).

	Oaxaca	Guerrero	Nayarit	Sinaloa Sur	Sinaloa Centro	Sinaloa Norte
Oaxaca	-	0.078	**0.006**	0.014	**0**	**0.001**
Guerrero	0.00362	-	**0.004**	0.012	**0**	**0.0001**
Nayarit	0.00716	0.00631	-	0.670	**0.002**	0.328
Sinaloa Sur	0.00596	0.0054	0.00072	-	**0.005**	0.760
Sinaloa Centro	0.01818	0.01306	0.00745	0.00633	-	**0.004**
Sinaloa Norte	0.00842	0.00895	0.00095	-0.00132	0.0063	-

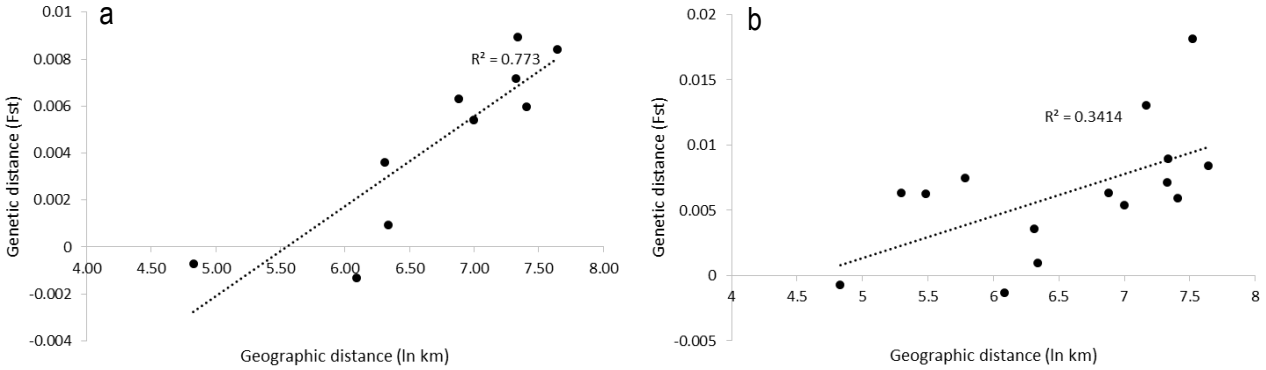

Figure 4. Isolation by distance test of wild locations based on the genetic distance vs. geographic distance in kilometers (km), standardized by natural logarithm. a) Six locations included, b) Sinaloa Centro excluded.

locations (Nayarit and Sinaloa) indicates the existence of genetic structure. This genetic structure explains the phenomenon of isolation by distance, which happens when the gene flow is inversely proportional to the geographic distance (Freeland et al. 2011). This result coincides with previous regional studies using other genetic markers such as microsatellites (Valles-Jimenez et al. 2005) and mitochondrial DNA (Valles-Jimenez et al. 2006).

The gene flow is enough to maintain the genetic homogeneity among locations in the northern Pacific region, which also coincides with Perez-Enriquez et al. (2018b). Nevertheless, the presence of individuals in Sinaloa Centro with a distinct origin suggests the possibility of introgression of individuals from shrimp farms in the region, similar to a previous study (Perez-Enriquez et al. 2018c). Unlike Perez-Enriquez et al. (2018c), who reported that in 2013, such events represented about 4%, in the present study, it might be up to 50%. Considering the potential negative impact of the modification of the genetic structure and variability caused by the release of cultivated organisms to the wild on the fitness (Utter 2003), the shrimp aquaculture sector must take pertinent management actions to avoid the scape or intentional release of larvae, juvenile or adults. The main wild shrimp species in the Gulf of California are *P. vannamei*, *P. stylirostris*, and *P. californiensis*. Their common presence in the incoming seawater channels of farms and during the harvests of *P. vannamei* semi-intensive shrimp ponds (personal experience) suggests the possibility for the introduction of *P. vannamei* wild populations to farms, where the survivor shrimps are selected by mass selection as future broodstock by companies involved in all pathogens exposed (APE) method applied after 2015 to improve pathogenic and biotoxins resistance to shrimps in successive generations.

Genetic composition and diversity of cultivated stocks

From 2011, the Mexican shrimp aquaculture industry imported genetic lines from several origins (e.g. Ecuador (2015), USA (2013), and Central America (2011) to mitigate the adverse effects of diseases (mainly WSSV and AHPND) (Perez-Enriquez et al. 2018a). According to production data, after 2013, shrimp aquaculture showed an outstanding recovery, from a minimum of 60,200 t to more than 194,000 t in 2022 (CONAPESCA 2023). It remains a pending research topic to determine in what proportion this recovery was due to the introduction of the imported

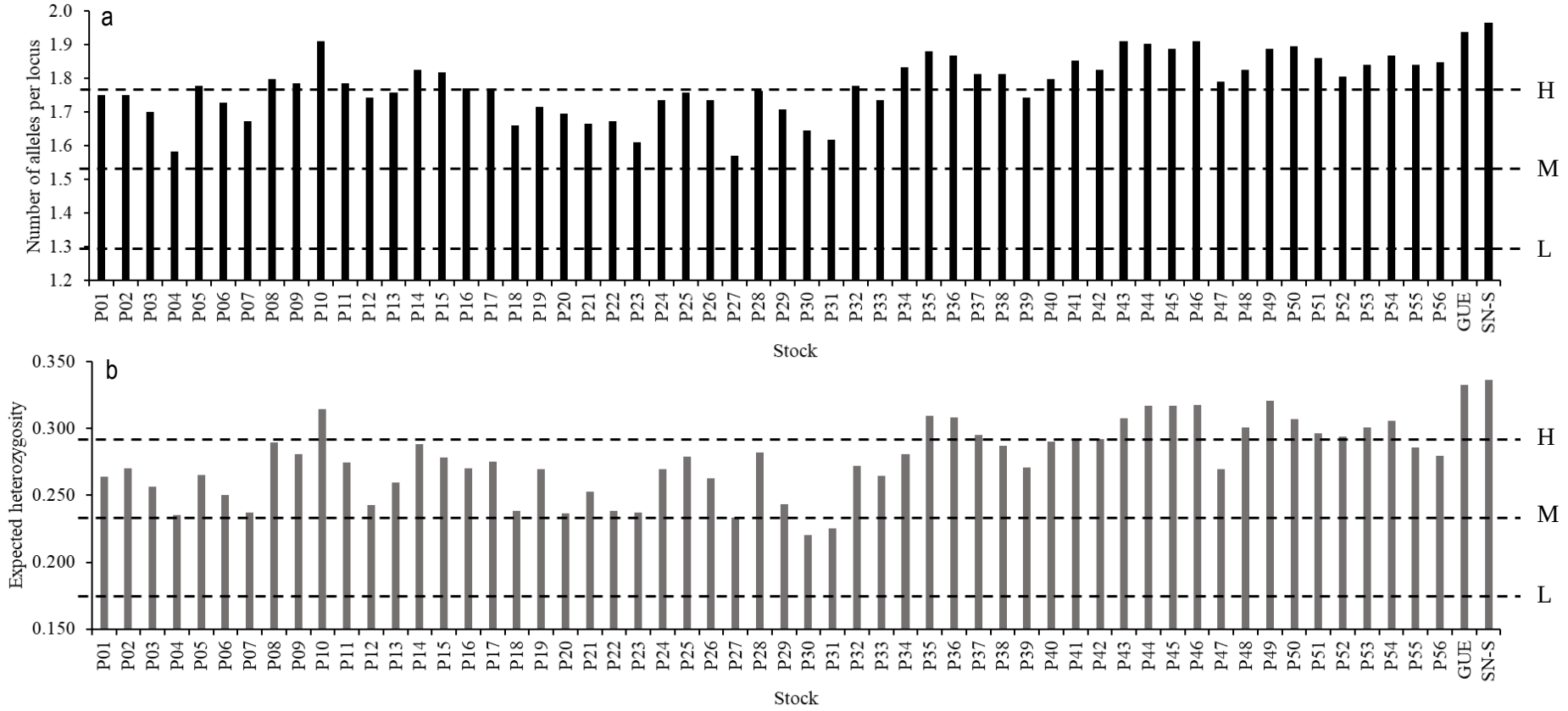

Figure 5. Genetic diversity of the 56 cultivated stocks referenced to the wild populations of Guerrero (GUE) and Sinaloa Sur (SN-S). a) Number of alleles per locus, b) Expected heterozygosity. The dotted lines represent the limits of the low (L), medium (M), and high (H) diversity categories.

Table 2. Percentages of cultivated stocks for each genetic diversity classification criteria as a function of the number of alleles per locus (Na) and expected heterozygosity (He). Inbreeding rate (F_{IS}) intervals are shown at the bottom. *For four stocks that were hybrid lines, F_{IS} = 0.0.

		He		
		Low < 0.25	Medium 0.25-0.3	High > 0.3
Na	Low < 1.6	4%	0%	0%
	Medium 1.6-1.8	16%	39%	0%
	High > 1.8	0%	20%	21%
F_{IS}		0-0.01*	0.01-0.10	> 0.10
		30.4%	57.1%	12.5%

lines, which passed by a natural selection process of shrimp cultivated in harsh environments (Lucien-Brun 2017).

Most stocks presented medium to high genetic diversity levels and low inbreeding values compared to the wild population. These indicators suggest that, on the one hand, inbreeding has not reached risky levels [if a value of 10% is taken as a reference according to Moss et al. (2007)], and, on the other hand, that there is a broadly enough genetic pool for the maintenance of the genetic diversity. In turn, this reflects that, in general, the within-lines mating strategies carried out by the commercial hatcheries owning the 56 stocks maintained by mass selection between 2011 and 2020 have been adequate. Low-risk levels have also been reported in other countries involved in *P. vannamei* aquaculture, such as Vietnam (Knibb et al. 2020), China (Ren et al. 2018), Brazil (Lisboa-Silva et al. 2022), and Cuba (Casado et al. 2022). These studies contrast with others that reported high inbreeding and loss of genetic diversity (e.g. De Freitas & Galetti 2005, Perez-Enriquez et al. 2009), suggesting there is still room for improvement in crossbreeding practices that include concepts related to the maintenance of the genetic diversity.

The genetic differentiation analysis indicates the presence of a relevant number of genetic profiles in the production stocks, from highly differentiated lines such as Nicaragua or the USA (Hawaii) to mixed lines (either intentionally or unintentionally) originating from Ecuador and the USA (Texas). The presence of these diverse genetic origins not only indicates the possibility of keeping independent lines with distinct production parameters but also of establishing new lines from an enriched gene pool. The mixing of commercial lines is a suitable management strategy for gene pool enrichment for Asian and American shrimp aquaculture (e.g. Ren et al. 2018, 2020, Knibb et al. 2020, Casado et al. 2022, Lisboa-Silva et al. 2022).

An essential element for adequate management of the cultivated lines is the follow-up and recording of all movements and transferences of the cultivated stocks within a genetic nucleus since its introduction to the hatchery. The failure of this task may result in an unwilling mixture of stocks, either during grow-out or at reproduction, and, thus, a critical loss of information about the genetic background of the lines.

Feasibility of establishing a Mexican genetic line

According to the origin records of most of the cultured stocks in this study, some individuals originated in northwest Mexico, the main productive region of farmed shrimp. Nevertheless, the genetic similarity of some cultured and wild individuals (Fig. 6) suggests the possible introduction of wild organisms into the breeding lines, as was discussed before. It is hypothesized that this non-programmed introduction may happen when farms pump water from neighbor lagoons (R. Ruiz, *com. pers.*), and survivors from grow-out ponds are selected as broodstock.

Even though introducing wild individuals is a viable alternative to enrich the gene pool of the genetic lines, there is a risk of losing the genetic gain in traits of interest that those lines might have already acquired after several generations of selection (Knibb et al. 2020). Despite this, some genetic variants in the wild populations might have been purged during selection in the actual breeding lines. However, they can perform better under adverse conditions (biological or environmental), which is relevant due to technologies that allow the analysis of genomic profiles based on thousands of markers, promoting a better knowledge of the genetic architecture of commercially important traits, especially those of polygenic nature (Palaiokostas & Houston 2018). In this regard, Strandén et al. (2019) suggested the introgression of locally adapted genes in dairy cattle to maintain production traits and overcome the challenges of climate change.

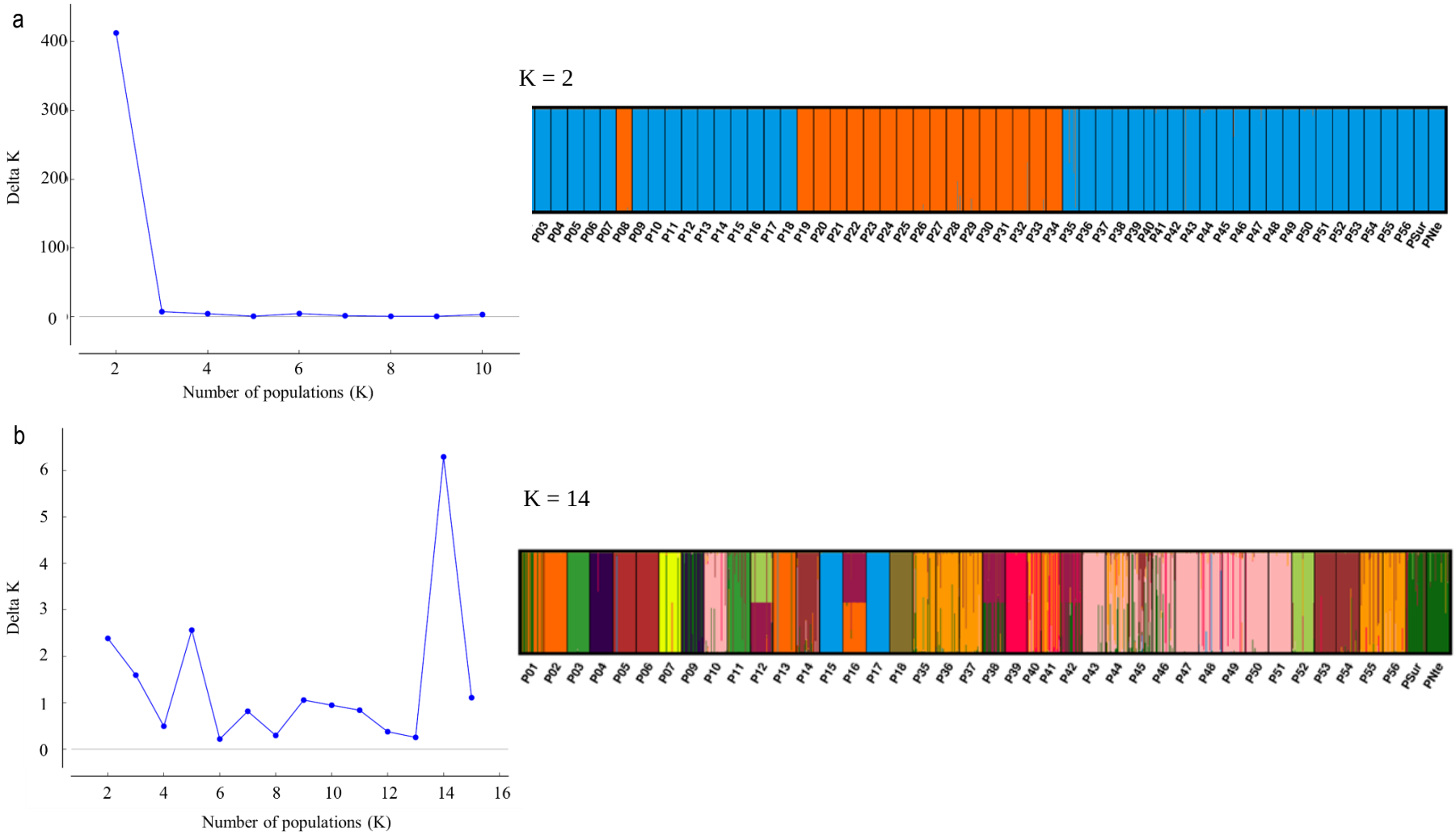

Figure 6. Delta K (statistic based on the rate of change in the log probability of data between successive K values (Evanno et al. 2005)) and bar plot graphs to determine the most probable number of groups and the grouping with the most probable K, respectively. Analysis with a) 56 cultivated and two wild stocks, and b) 39 (lots P08 and P19-P34 were excluded) and two wild stocks.

Table 3. Putative groups of cultivated lots based on the Structure analysis. The data in parenthesis correspond to the reported origins: Ec: Ecuador, Tx: Texas, Hw: Hawaii, Mx: Mexico, Nc: Nicaragua, Bu: wild from the location Buenavista, Sinaloa; PSur, and PNte: wild stocks from the southern and northern Mexican Pacific coasts, respectively; n.a.: not available. The composed origins indicate crossbreeding in the previous generation(s). Some stocks may belong to more than one putative group due to mixtures.

Putative group	Lots	Putative group	Lots
A	P01 (Hw-Tx), P02 (Tx), P13 (Tx), P16 (n.a.)	I	P12 (Ec), P16 (n.a.), P38 (Bu), P42 (Bu)
B	P03 (n.a.), P11 (Ec)	J	P14 (Ec-Tx)
C	P04 (Hw), P09 (Hw)	K	P15 (Ec), P17 (Ec-Tx)
D	P05 (Tx), P06 (Tx), P53 (Ec), P54 (Ec)	L	P18 (Ec-Tx)
E	P07 (Ec)	M	P35 (Ec), P36 (Tx), P37 (Nc), P40 (Tx), P41 (Nc), P44 (TxEc), P55 (Tx), P56 (Tx)
F	P08 (n.a.), P19-P34 (Nc)	N	P39 (Ec), P40 (Tx), P41 (Nc)
G	P10 (n.a.), P43 (Hw), P44 (TxEc), P45 (TxEc), P46 (MxEc), P47 (MxHw), P48 (MxEc), P49 (Hw-MxEc), P50 (MxEc-Hw), P51 (Hw)	O	PSur, PNte
H	P12 (Ec), P52 (Hw)		

In conclusion, the broad genetic pool maintained by mass selection in the Genamex breeding lines, including those from wild origin, may allow the development of a genetic line with locally adapted genes suitable for the environmental conditions of northwestern Mexico, from which there is potential for a long-term selective breeding program focused on the traits of interest required by the Mexican industry. This approach is justified due to the mobility of diverse pathogens from outside of the Gulf of California, such as the introductions of infectious hypodermal and hematopoietic necrosis virus (IHHNV) in 1986, WSSV in 1999(Lightner 2011), and AHPND in 2013 (Morales-Covarrubias et al. 2018), and the imminent risk of others by the dynamics of the shrimp imports from other regions. Additionally, all the farms involved in semi-intensive *P. vannamei* aquaculture have the risk of introducing pathogens from the adjacent ecosystems through the seawater interchange process (Muniesa et al. 2017). Wild *Penaeus* populations in coastal waters in the Gulf of California, adjacent to shrimp farms, where there is a potential co-evolution with pathogens, represent a risk of generating new variants of pathogens. In this sense, the wild populations represent a broad opportunity for the shrimp farms to acquire genetic diversity from wild populations naturally challenged to the same regional pathogens and their variants. From the sanitary point of view, the genetic enrichment in the actual aquaculture genetic lines with wild *P. vannamei* populations from the Gulf of California is safer than new introductions from outside regions where other pathogens have been detected. Finally, introducing new genetic diversity from the wild *P. vannamei* population through a Mexican line must consider the selection for pathogens' resistance and growth.

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SUPPLEMENTARY MATERIAL

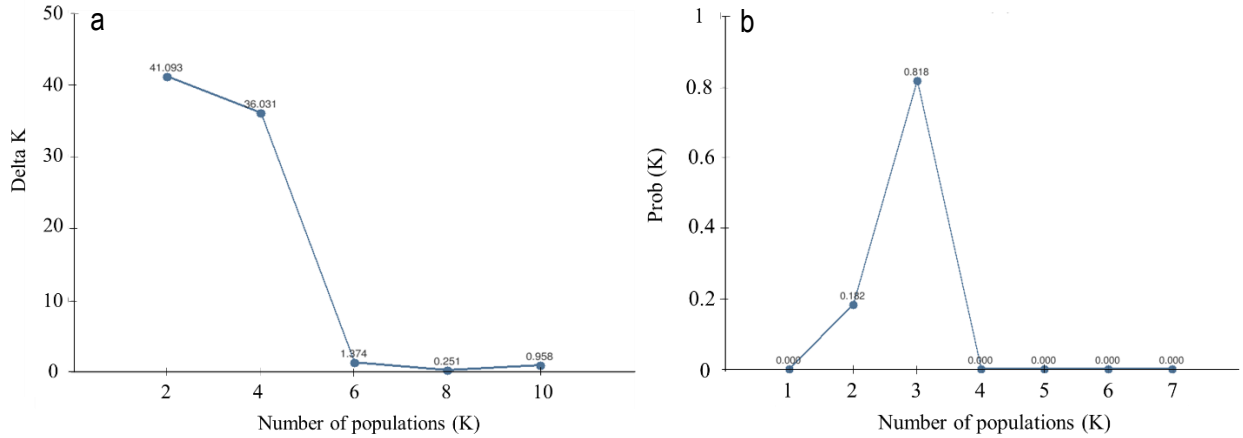

Figure S1. Most probable K in the wild population based on the a) Evanno and b) Clumpak analyses. Delta K is a statistic based on the rate of change in the log probability of data between successive K values (Evanno et al. 2005). Prob (K) is an estimate of the posterior probabilityof the data for a given K, Pr(X|K) (Pritchard et al. 2000).