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1 **SNP markers for the genetic characterization of Mexican shrimp *Litopenaeus vannamei***
2 **broodstocks**

3

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15

16 **Abstract**

17 Selective breeding of shrimp has major potential to enhance production traits, including growth

18 and disease resistance. Genetic characterization of broodstock populations is a key element of

19 breeding programs, as it enables decisions on inbreeding restrictions, family structure, and the

20 potential use of genomic selection. Single Nucleotide Polymorphisms (SNPs) are suitable genetic

21 markers for this purpose. A set of SNPs was developed to characterize commercial breeding
22 stocks in Mexico. Individuals from local and imported lines were selected for sequencing using
23 the nextRAD technique, resulting in the identification of 2,619 SNPs. Genetic structure analysis
24 showed three to five genetic groups of Ecuadorian and Mexican origins. A subset of 1,231 SNPs
25 has potential for stock identification and management. Further, three SNPs were identified as
26 candidate sex-linked markers. The role of SNPs possibly associated with genes related to traits of
27 importance to shrimp farming, such as growth and immune response, should be further
28 investigated.

29 **Keywords:** Single Nucleotide Polymorphisms; stock identification; genetic diversity; selective
30 breeding; disease resistance

31

32 **1. Introduction**

33 Pacific whiteleg shrimp (*Litopenaeus vannamei*) is one of the most important aquaculture species
34 globally, with more than three million tonnes produced annually (FAO, 2017). Mexico is one of
35 the largest producers of whiteleg shrimp in Latin America, but the industry has been severely
36 hampered by a high incidence of infectious disease outbreaks, particularly White Spot Syndrome
37 Virus (WSSV; Esparza-Leal et al. 2012) and the bacterial Acute Hepatopancreatic Necrosis
38 Disease (AHPND; Soto-Rodriguez et al. 2015). Up until the year of peak shrimp production
39 (2009), most shrimp hatcheries relied on a breeding line called ‘Melagos’, originally imported
40 from Venezuela in 1997 (Perez-Enriquez et al., 2009), that supported an annual production
41 volume increase of ca. 9 % (CONAPESCA, 2013). While this growth performance was
42 encouraging, the line was evidently highly susceptible to WSSV, resulting in a production
43 volume decrease of more than 11 % per year in 2010 – 2012 (CONAPESCA, 2013).

44 As a strategy to help mitigate WSSV outbreaks, Mexican shrimp breeding companies, either
45 independently or through the National Association of Shrimp Larvae Producers (ANPLAC),
46 initiated the introduction of new broodstock in 2013. These stocks were mainly of Ecuadorian
47 origin as the shrimp production in this country had recovered from catastrophic outbreaks of
48 WSSV that started at the beginning of 2000 (Santana-Navarro, 2015), thought to be due to
49 genetic resistance arising from the natural selection of survivors from disease-affected ponds
50 (Lucien-Brun, 2017). Mexican official reports indicate imports from Ecuador in 2013 and 2015,
51 the USA in 2013 and 2015 [which are of Ecuadorian origin (Gervais, 2014)], Nicaragua in 2014,
52 and Colombia in 2015 [Sistema de Información Arancelaria Via Internet SIAVI
53 (<http://187.191.71.239/>); Tariff fractions 03.06.26.01 and 03.06.27.01].

54 Genetic characterization of whiteleg shrimp *Litopenaeus vannamei* breeding lines in Mexico has
55 previously been performed using microsatellite and mtDNA markers (Perez-Enriquez et al.,
56 2009; Mendoza-Cano et al., 2013; Vela-Avitúa et al., 2013). However, these evaluations were
57 focused on the ‘Melagos’ breeding line. Due to the multiple import events and subsequent
58 mixing of broodstock, the genetic structure of the currently farmed Mexican shrimp populations
59 is not well understood as no recent genetic profiling has been performed.

60 While some shrimp farming is based on the aforementioned selected broodstock lines, selective
61 breeding programs for continuous genetic improvement of shrimp are at a formative stage in
62 Mexico. A key element for these selective breeding programs is the characterization of genetic
63 diversity and composition of stocks, as it will enable decisions on the composition of base
64 populations, inbreeding restrictions, family structure, and the potential use of genomic selection
65 (FAO, 1993). The genetic characterization will also aid in stock identification for traceability
66 purposes.

67 Single Nucleotide Polymorphisms (SNPs) have been demonstrated to be suitable markers for the
68 genetic characterization of broodstock populations, parentage assignment, and genome selection
69 in several farmed aquaculture species [see review in Robledo et al. (2017)]. In *L. vannamei*,
70 Perez-Enriquez and Max-Aguilar (2016) reported a SNP-based genotyping service for parentage
71 assignment but the identity of the SNPs is not known. A commercially-available Illumina SNP-
72 chip has been created (Jones et al., 2017). However, the SNPs on the array were discovered in a
73 single-origin broodstock, and it might not represent the genetic diversity in broodstock
74 populations derived from different sources. Genotyping by sequencing technology enables
75 concurrent discovery and genotyping of genome-wide SNPs that are informative in the target
76 populations at moderate scale and cost, and has been widely applied in aquaculture species for
77 this purpose (Robledo et al. 2017).

78 The objective of this study was to assess the genetic diversity and constitution of the breeding
79 lines currently used in the Mexican shrimp aquaculture industry and to develop a set of
80 diagnostic SNP markers to identify those lines as a potential tool for stock management and
81 traceability. To do so, we carried out Nextera-tagmented reductively-amplified DNA (nextRAD)
82 sequencing in 95 individuals belonging to 21 breeding batches from 5 different hatcheries in the
83 Northwest of Mexico. Our results provide a set of SNPs for shrimp Mexican population analyses
84 and characterize current Mexican aquaculture shrimp stocks.

85

86 **2. Materials and methods**

87 *2.1. Samples*

88 A total of 95 *L. vannamei* individuals belonging to 21 breeding batches from five hatcheries
89 located in Northwestern Mexico (3 – 7 individuals per breeding line) were sampled (Table 1).
90 Those hatcheries, denoted ‘A’ – ‘E’ in the current study, represented the genetic makeup of most
91 of the shrimp currently cultivated in Mexico based on the statistics generated by ANPLAC
92 (National Larvae Producers Association, Mexico; pers. comm.). Information about the presumed
93 origin of the founders was available for two hatcheries (Table 1). A pleopod was taken from each
94 animal and kept in absolute ethanol until DNA isolation.

95 *2.2. DNA sequencing*

96 Approximately 20 mg of soft tissue were retrieved using forceps, and lysed in 500 µl of
97 proteinase-K buffer (10 mM Tris, 50 mM NaCl, 5 mM CaCl₂, 50 µg mL⁻¹ of Proteinase-K, pH
98 8.0) at 45°C overnight. The lysate was centrifuged, and the supernatant was incubated with 10 µl
99 of silica glass milk suspension (GeneClean EZ, MP Biomedicals, Santa Ana, CA) for DNA
100 binding. The suspension was centrifuged and washed twice in a washing solution (10 mM Tris,
101 pH 7.5; 80 % ethanol), and the DNA was eluted in 20 µl of Milli-Q water. DNA was quantified
102 by Qubit using the dsDNA broad range assay (Invitrogen, Palo Alto, CA), adjusted to 20 ng µl⁻¹
103 and shipped to SNPsaurus LLC (Eugene, OR) for nextRAD genotyping service. In brief,
104 nextRAD involved the construction of individual Nextera libraries (Illumina), that were
105 selectively amplified with modified multiplex Nextera primers that extend over insert regions
106 with a restrictive 9 bp sequence in their 3' ends for a systematically reduced representation of the
107 genome in the resulting libraries. Pooled libraries were sequenced in one lane of an Illumina
108 HiSeq4000 platform, using the single-end 150 bp chemistry. Reads were demultiplexed and tags
109 were removed by SNPsaurus using in-house scripts before reference construction and genotype
110 calling.

111 2.3. Data analysis

112 The number of reads per individual sample ranged from 2.2 and 3.2 million, and were deposited
113 in NCBI-SRA (BioProject PRJNA492152, BioSample accessions SAMN10094370 -
114 SAMN10094464); each sample's identifier can be associated with the corresponding SRA
115 accession using the Supplemental Table 1. A reference Fasta file of single-end 27,196 fragments
116 of 150 bp length (Ref_shrimp.fasta; Supplemental File 1) was built by clustering the reads using
117 SNPSaurus' in-house scripts. Fastq files for each individual were analyzed for quality control
118 with the program FastQC version 0.11.2
119 (<https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>). Trimmomatic version 0.36
120 (Bolger et al., 2014) was used to trim low quality leading and trailing bases (Phred < 20; 4 bases
121 window) and the Nextera adapters. Only reads longer than 32 bp post-filtering were retained.
122 Trimmed sequences were aligned to the Ref_shrimp.fasta file using the Burrows-Wheeler
123 Aligner (bwa version 0.7.8; Li and Durbin, 2009). SAMtools version 1.6 (Li et al., 2009) was
124 used to generate the bam files and remove PCR duplicates. SNP identification (alignment and
125 base quality > 20, minimum depth 10×, maximum depth 150×) was performed using Samtools
126 (mpileup command) and the bcftools software (Li et al., 2009) version 1.4 (call command). The
127 obtained vcf file was filtered using vcftools version 0.1.15 (Danecek et al., 2011) with the
128 following criteria: minor allele frequency minimum = 0.016, minimum allele counts of the minor
129 allele = 3, minimum quality = 30, maximum missing sites = 0 %, indels removed, three SNPs or
130 fewer per fragment, and to only retain loci with two alleles. A BLAST search of the flanking
131 sequence of the obtained SNPs performed against the NCBI database and SNP regions matching
132 with mitochondrial DNA, repetitive regions of penaeid shrimps [e.g. ribosomal RNA (5S, 18S,

133 28S), non-LTR-like retrotransposons, and microsatellites] or of putatively exogenous DNA
134 (bacteria) were removed.

135 Filtered vcf files were converted into Arlequin type files using PGDSpider version 2.1.1.3
136 (Lischer and Excoffier, 2012), treating each breeding stock as a separate population. Arlequin
137 version 3.5 (Excoffier et al., 2005) was used to estimate allele frequencies and genetic diversity
138 parameters (number of alleles per locus, observed and expected heterozygosity). Comparisons of
139 these parameters among broodstocks were performed using t-Student tests. Significance for
140 multiple pairwise tests was adjusted by the sequential Bonferroni correction (Rice, 1989).
141 Cavalli-Sforza genetic distance among breeding lines with 1,000 bootstrap permutations were
142 obtained to generate a Neighbor Joining tree with the software PHYLIP (Felsenstein, 1996), and
143 visualized with the Interactive Tree Of Life tool v3 (Letunic and Bork, 2016).

144 Population genetic structure was inferred by a Bayesian model-based clustering method
145 implemented by the software Structure version 2.3.4 (Pritchard et al., 2000). The model assumes
146 the presence of K genetic clusters, each characterized by a set of allele frequencies at each locus.
147 Individuals in the sample are assigned probabilistically to clusters. First, the vcf files were
148 converted to Structure type files with PGDSpider version 2.1.1.3. The running conditions for the
149 Markov chain were 10,000 burnin steps and 100,000 repetitions for K = 1 to 12. The best K
150 number of clusters was calculated by the Evanno method (Evanno et al., 2005) implemented in
151 the Structure Harvester software version 0.6.94 (Earl and von Holdt, 2012). A Discriminant
152 Analysis of Principal Component (DAPC) was also used to group broodstocks into putative
153 genetic clusters using the Adegenet R package (Jombart, 2008), using the Structure file as input.

154 *2.4. Candidate SNPs for broodstock identity and sex-linkage*

155 Individual samples were classified into the K groups obtained from the Structure analysis.
156 Pairwise F_{ST} between groups were calculated for each SNP using vcftools. Those SNPs showing
157 high levels of between-group differentiation ($F_{ST} > 0.15$; Wright, 1978) were selected as
158 candidates for group (and hence broodstock) membership identification. The candidate SNPs of
159 the 10 pairwise analyses were merged into a single list.

160 Phenotypic sex was known for samples of broodstocks E-I – E-IV. These were used to perform
161 an initial screen for putative candidate SNPs linked to sex. The 16 individuals of these stocks
162 were pooled into a male group and a female group. F_{ST} analyses were performed between the two
163 sexes for each SNP with vcftools. In this case the SNP set used for analysis was that obtained
164 from the analysis with settings of 50 bp minimum separation between sites and a maximum of
165 30% of individuals without the site. Candidate SNPs were those being heterogametic in females
166 and homogametic in males, consistent with the previously described ZW sex determining system
167 (Zhang et al., 2007; Yu et al., 2017), and with $F_{ST} > 0.4$.

168 A BLAST search of the sequences containing the candidate broodstock-identity and sex-linked
169 SNPs was performed using the current NCBI's nucleotide database, *Litopenaeus vannamei*
170 sequences Ghaffari et al. (2014), Yu et al. (2015), and Jones et al. (2017), and *Penaeus monodon*
171 sequences (Baranski et al. 2014) as reference databases. The databases of the two shrimp species
172 were downloaded from the repositories of each of the journals.

173

174 **3. Results**

175 The initial variant calling analysis resulted in 398,125 putative SNPs. After applying the filtering
176 criteria (remove indels, retain only biallelic loci, minimum MAF 0.016, SNP present in 100 % of

177 the individuals), and excluding the SNPs in repetitive regions (from BLAST) resulted in a
178 filtered set of 2,619 SNPs distributed in 1,445 fragments (loci) that were used for downstream
179 analyses.

180 The genetic diversity was significantly higher ($P < 0.025$) in the 6 lines of Hatchery B (B-I – B-
181 VI) than in the rest of the lines (Fig. 1a-d). Hatcheries C and D showed the least values for both
182 diversity indicators (Fig. 1a, c), and among these, the C-V line had the lowest values (Fig. 1b, d).

183 The Discriminant Analysis of Principal Components revealed three clearly differentiated genetic
184 groups by using two linear discriminants that explained 92% of the variance (Fig. 2). At the
185 hatchery level it can be seen that the stocks from hatcheries A and most of those from E belong
186 to the same cluster. Likewise, all stocks from B are composed of individuals of a similar genetic
187 makeup. Hatcheries C, D, and E maintain breeding lines of diverse origin.

188 The Bayesian clustering analysis showed a delta K bimodal shape (Supplemental Fig. 1). The
189 first peak found at $K = 3$, was consistent with the DAPC analysis, but the best number of clusters
190 was found at $K = 5$, indicating that beyond the three main groups (Fig. 3a), additional
191 subdivision better explains the genetic structure (Fig. 3b). Four of the five genetic clusters would
192 be composed by breeding lines from different hatcheries as shown in Table 2, highlighting
193 diverse origin of the breeding lines within hatcheries. The other group is formed exclusively by
194 all the breeding lines of Hatchery B, indicating a clear differentiation from the other hatcheries.
195 Two breeding lines of Hatchery C seem to be a mixture of two different groups (Fig. 3b): C-I of
196 C-III with C-V [groups 3 and 4, respectively (Table 2)]; and C-II of C-IV with C-V [groups 1
197 and 4, respectively (Table 2)].

198 The clustering pattern showed by the NJ tree based on Reynolds genetic distance was similar to
199 that of the Bayesian clustering, with high bootstrap values for groups 1, 3, 4, and 5 (Fig. 4), but
200 in this case C-I and C-II are clearly included in group 5, and therefore more related to C-V,
201 rather than an admixture. A membership analysis with Structure at the individual level indicated
202 that unexpected admixture within some breeding lines probably took place, such as individuals
203 D-I-1, D-III-3, D-IV-2, and E-IV-2 (Supplemental Figure 2).

204 The pairwise F_{ST} analysis according to the groups of Table 2 and excluding individuals
205 potentially coming from mixed stocks [D-I-1, D-III-3, D-IV-2, E-IV-2, C-I (1-4), C-II (1-4)],
206 resulted in 1231 SNPs in 904 loci as candidates for breeding line identification (Supplemental
207 Table 2).

208 The BLAST search of the SNP flanking sequences showed 26, 3, 151, 12, and 3 positive hits to
209 the databases of NCBI, Ghaffari et al. (2014), Yu et al. (2015), Jones et al. (2017), and Baranski
210 et al. (2014), respectively (Supplemental Table 3). Matches to genes from *Drosophila* or
211 penaeids related to immune response, fatty acids synthesis, and growth, among others functions,
212 were observed.

213 The analysis of potentially sex-linked SNPs resulted in a set of 50 loci showing $F_{ST} > 0.4$
214 between males and females, of which three loci are candidate sex-associated markers as they
215 were exclusively heterozygous in females and homozygous in males (Supplemental Table 2); a
216 linkage disequilibrium analysis indicated that the two loci are probably at the same chromosome
217 ($r > 0.7$). None matched to LG18 of Yu et al. (2017).

218 **4. Discussion**

219 This is the first report, to our knowledge, on the characterization of the genetic makeup of shrimp
220 hatchery-reared populations using SNPs as genetic markers. Previous reports have relied on
221 microsatellites (e.g. Freitas and Galetti, 2005; Perez-Enriquez et al., 2009; Vela-Avitúa et al.,
222 2013; Zhang et al., 2014; Ren et al., 2108) and mitochondrial DNA (Mendoza-Cano et al., 2013).
223 Even though these markers have been adequate to assess the genetic diversity and structure of
224 cultivated shrimp stocks, their potential to be used for other purposes, such as parentage analysis
225 and genomic selection, is limited because a putative high mutation rate (particularly of
226 microsatellites; Perez-Enriquez and Max-Aguilar, 2016), and a low genome coverage.

227 Two sets of SNP panels are currently available for *L. vannamei*. One, based on 164 SNPs
228 derived from ESTs and offered as a genotyping service (J. Stannard, CTA, pers. comm.), has
229 been used for parentage assignment in an study of heritability in growth and fatty acids content
230 (Nolasco-Alzaga et al., 2018). The second one is a commercially-available Illumina SNP-chip
231 (Jones et al., 2017), for which its usefulness to characterize breeding populations has not been
232 reported. In both cases markers are located within expressed regions, and thus neutral variation
233 might be underrepresented.

234 The SNP set developed in the present study comprises 2,619 SNPs distributed in 1,445 fragments
235 (loci), and has clearly shown differential diversities of several *L. vannamei* breeding lines
236 maintained in Mexico. Although sample sizes for each breeding stock were low, it was quite
237 clear that the genetic diversity of the imported stocks of recent years was higher than that of the
238 Mexican line (Group 4 in Table 2). Thus, it appears that the introduction of the new genetic
239 variants can be beneficial to avoid a long term accumulation of inbreeding, which could hamper
240 shrimp production traits (Moss et al., 2012).

241 Genetic differences among *L. vannamei* breeding lines were also clearly observed, and three to
242 five genetic clusters seem to be present in this sample of broodstocks used in the Mexican shrimp
243 industry. This provides a rather wide genetic background, either to inform baseline populations
244 or to test the performance of several types of between-line crosses. In China, Ren et al. (2018)
245 found enough genetic variation in 36 *L. vannamei* breeding stocks belonging to four genetic
246 clusters to inform the synthesis of a base population for future selective breeding.

247 The differentiation between the *L. vannamei* line of putatively Mexican origin with the other
248 lines is not surprising as it has founders from various origins and has been bred within-line
249 during more than 15 generations (Perez-Enriquez et al., 2009). However, the difference between
250 the *L. vannamei* Ecuadorian lines (B-I – B-VI) and those imported from Texas (lines of Group 1
251 in Table 2), which also originated from Ecuador (Gervais, 2014), is striking, but might be
252 explained by a previous mixing of WSSV-resistant animals from Ecuador and Panama (Gervais,
253 2014).

254 The acute differences among clusters found within the reduced set of 1,231 SNPs represent the
255 potential of having markers specific to *L. vannamei* breeding lines. This provides the opportunity
256 of devising a set of markers that can be used for stock traceability (in hatcheries, farms or even in
257 the market), for parentage analysis (e.g. Lapègue et al., 2014; Perez-Enriquez and Max-Aguilar,
258 2016; Bich Vo et al., 2018), and for the detection of hybrid stocks. For this purpose, individual-
259 locus assays (e.g. Taqman, KASP, integrated fluidic circuits) on a number of SNPs should be
260 tested for accuracy and repeatability in appropriate sample sizes. For the purpose of assessing
261 genetic variability, levels of inbreeding, and conformation to Hardy-Weinberg expectations, care
262 should be taken in the design of the SNP panel to avoid parameter biases.

263 Similarity searches against several published databases identified genes that may be related to
264 traits of importance for shrimp aquaculture. In penaeids several cases of up- or down-regulation
265 of genes related to the immune response during WSSV or *Vibrio parahaemolyticus* infections in
266 comparison to negative controls have been reported [e.g. Core histone protein H4 (Feng et al.,
267 2014); Argonaute-1, (Labreuche et al., 2010); Toll-4 (Ren et al., 2017); Vascular endothelial
268 growth factor 1 (Wang et al., 2015); *Penaeus monodon* AV gene (Luo et al., 2007); Fatty acid
269 synthase (Yang et al. 2011; Zuo et al., 2017)]. Large differences in the abundance of transcripts
270 of the Hypoxia inducible factor 1 alpha were detected in *L. vannamei* under normoxia and
271 hypoxia (Soñanez-Organis et al., 2009). In *P. monodon*, Magerd et al. (2013) reported the role
272 of the Thrombospondin II gene in the induction of the sperm acrosome reaction. A sequence
273 reported by Ghaffari et al. (2014) appears to be related to the ecdysis, and thus, probably with
274 growth.

275 A sex determination region in *L. vannamei* has been proposed to be in a small section of a
276 linkage group (LG18), in which a sex-associated marker has been found (Yu et al. 2017),
277 supporting a ZW/ZZ sex determination mechanism previously reported (Zhang et al., 2007). The
278 same sex determination system has been reported in *P. monodon* (Robinson et al. 2014). In our
279 study, two loci were also heterogametic in females and homogametic in males, although they did
280 not match to known sequences on LG18, nor to any public sequence in the Blast search. Further
281 analyses of these two loci with a larger sample size are needed to confirm their association with
282 the sex determination system.

283 In summary, we have developed a set of SNP markers which can effectively characterize the
284 genetic diversity and composition of broodstocks introduced to Mexico during 2013-2015. A
285 subset of these markers are potentially useful for stock identification, some of which were found

286 within genes of potential interest for the improvement of shrimp farming traits, such as immune
287 response, growth, or reproductive performance.

288

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299

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459

460 **Figures captions**

461 Fig. 1. Genetic diversity. a) and b) Number of alleles per locus per hatchery and per breeding
462 line, respectively; c) and d) Expected heterozygosity per hatchery and per breeding line,
463 respectively.

464 Fig. 2. Discriminant analysis of principal components (DAPC) for 95 genotypes of shrimp
465 breeding lines in Mexico. The axes represent the first two Linear Discriminants (LD). Each circle
466 represents a cluster and each dot represents an individual. Legends represent the breeding lines
467 of the individuals.

468 Fig. 3. Cluster memberships of breeding lines from the Bayesian genetic structure analysis. a) K
469 = 3; b) K = 5.

470 Fig. 4. Neighbor-Joining tree based on the Cavalli-Sforza genetic distance among the breeding
471 lines. Numbers along color stripes correspond to the groups of Table 3. Bootstrap percentages
472 shown in the nodes.

473 **Supplementary Figures**

474 Supplementary Fig. 1. Change rate (ΔK) for each value of K obtained from the Evanno
475 method (Evanno et al. 2005) in the Structure analysis.

476 Supplementary Fig. 2. Individual membership to genetic clusters of the 95 samples from the
477 Bayesian genetic structure analysis at K = 5.

478

Table 1. Number of individuals obtained from 21 shrimp breeding batches sampled in Mexico in 2016.

Broodstock	Batch	Sample size	Founder ID ^a
A	I	4	NA
	II	4	NA
B	I	6	EC-1
	II	6	EC-2
	III	6	EC-3
	IV	5	EC-4
	V	6	EC-5
	VI	7	EC-6
C	I	4	NA
	II	4	NA
	III	4	NA
	IV	4	NA
	V	4	NA
D	I	4	CA-1
	II	4	MX
	III	3	TX
	IV	4	CA-2
E	I	4 ^b	NA
	II	4 ^b	NA
	III	4 ^b	NA
	IV	4 ^b	NA

^a Origin of founder stocks: NA: Not available; EC-1 - EC-6: Ecuador; CA-1 and CA-2: Central America; TX: Texas; MX: Mexico

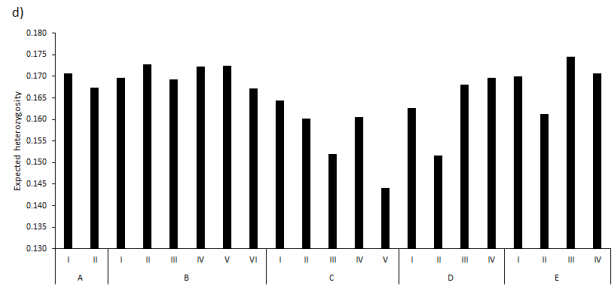
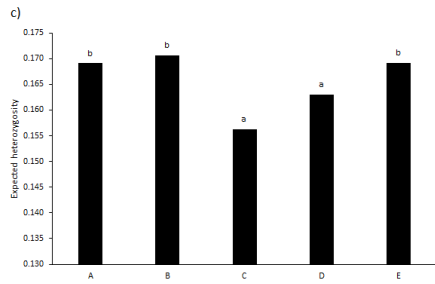
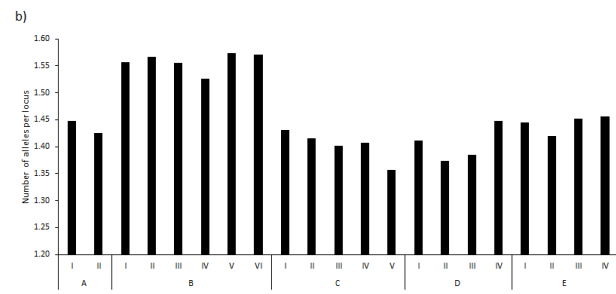
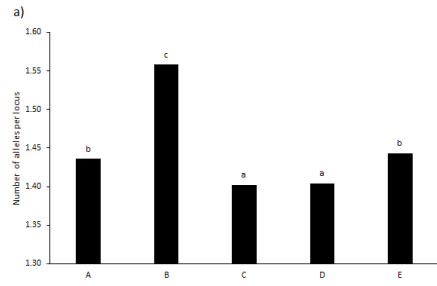
^b Sex proportion 1:1

Table 2. Proposed genetic groups based on the Bayesian clustering analysis.

Group	Member breeding lines	Putative origin ^a
1	A-I, A-II, C-IV, DIII, E-I, E-II, E-III	TX
2	B-I, B-II, B-III, B-IV, B-V, B-VI	EC
3	C-III, D-I	CA-1
4	C-V, D-II	MX
5	D-IV, E-IV	CA-2

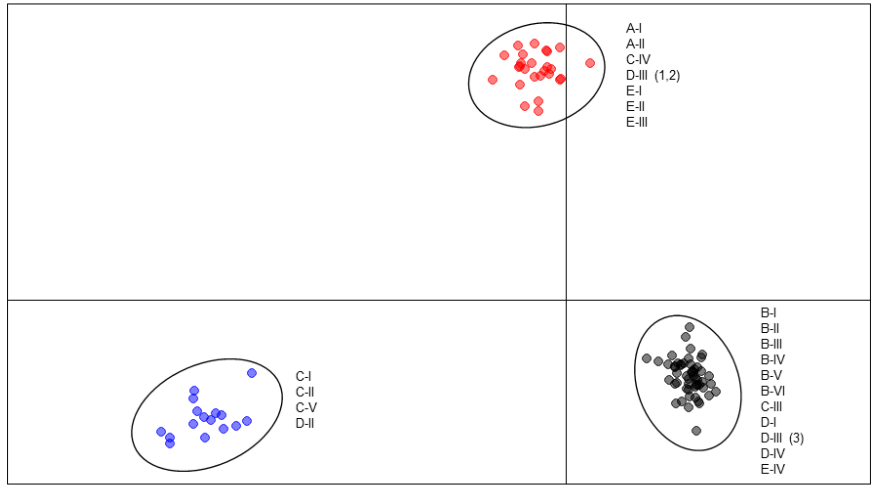
481 ^a Nomenclature according to Table 1

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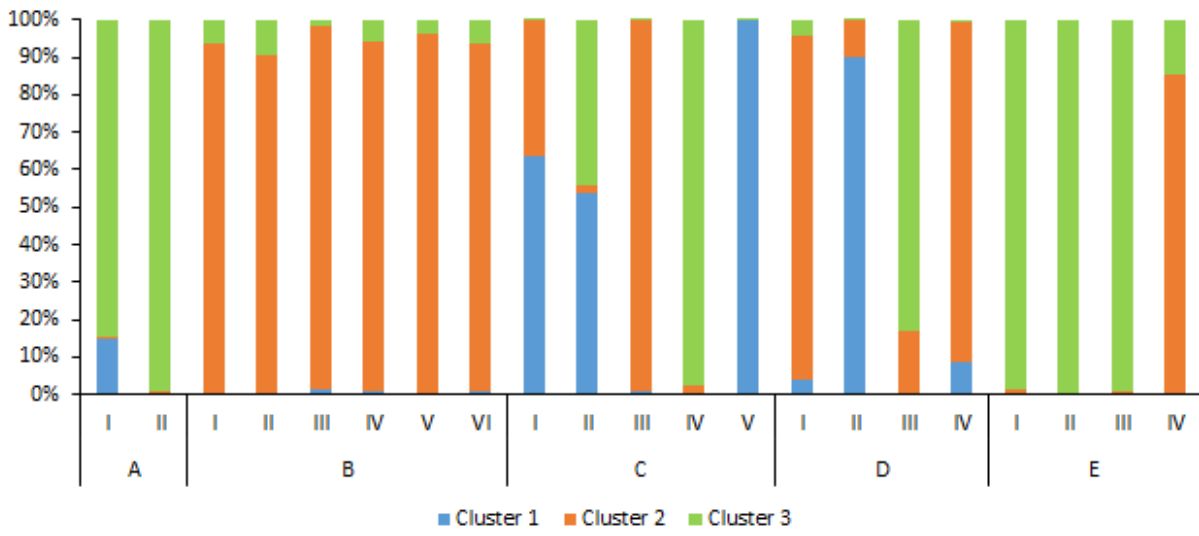
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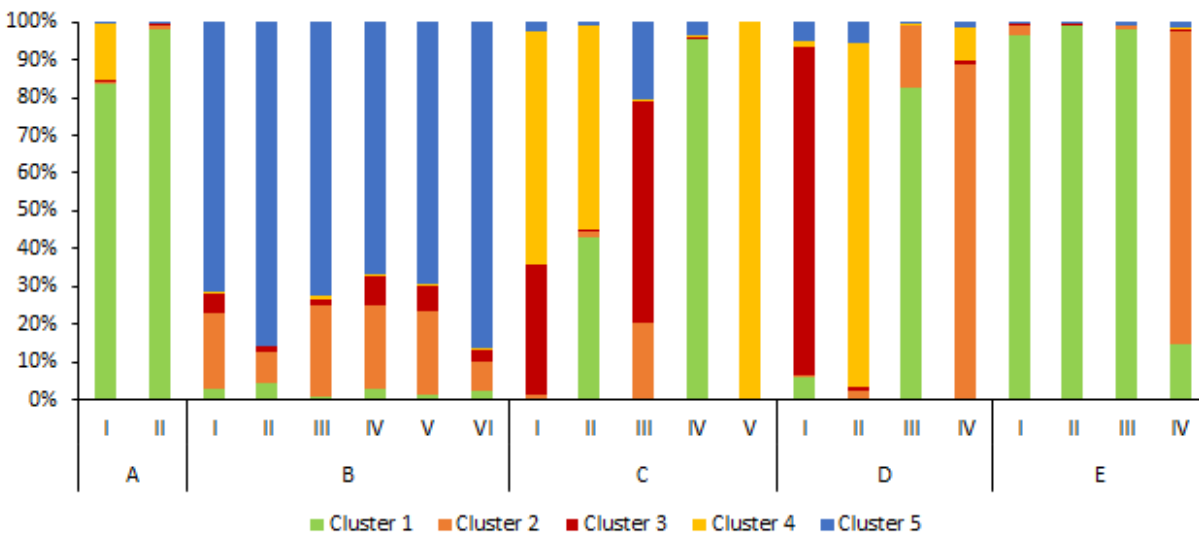
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a)

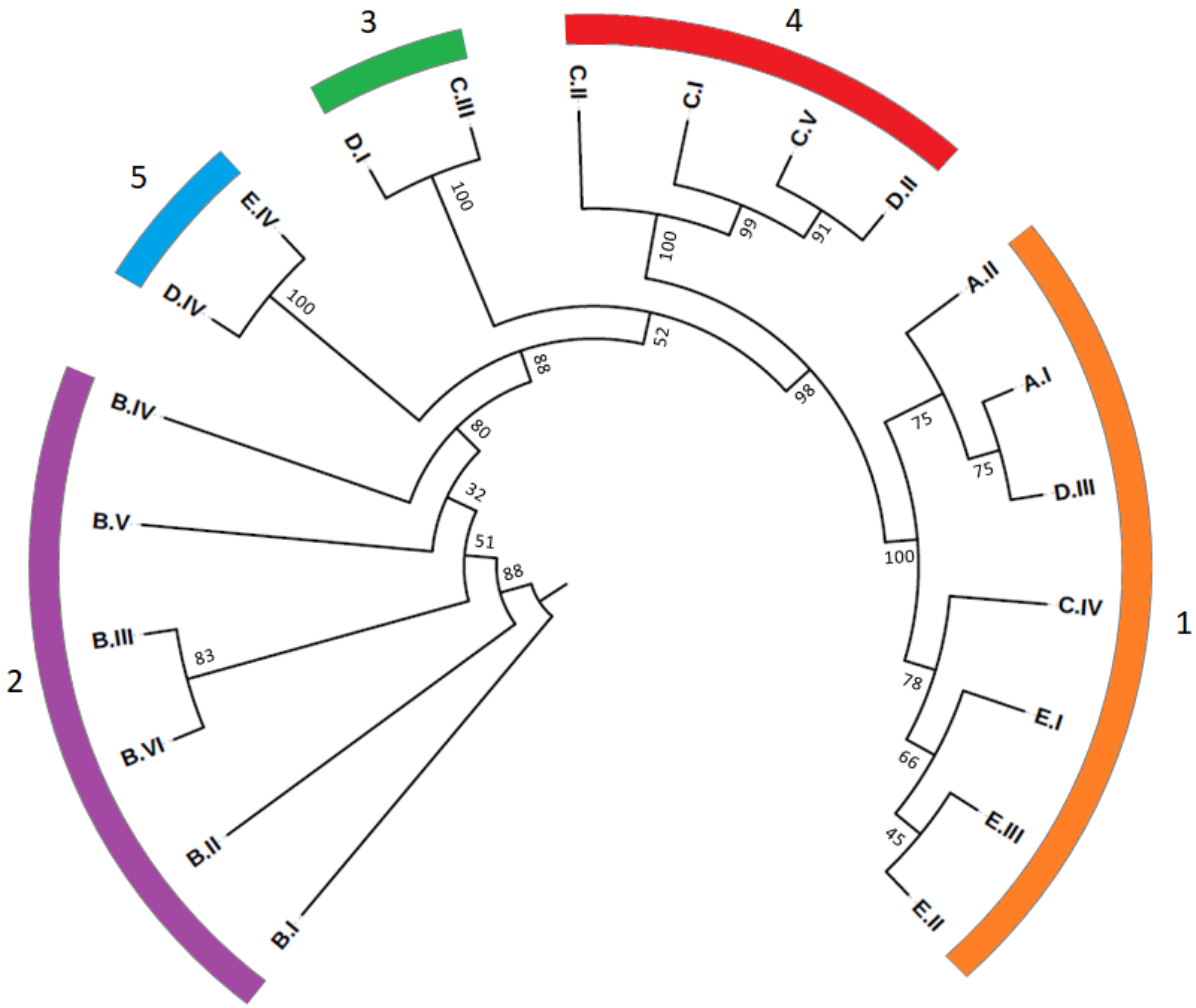


b)



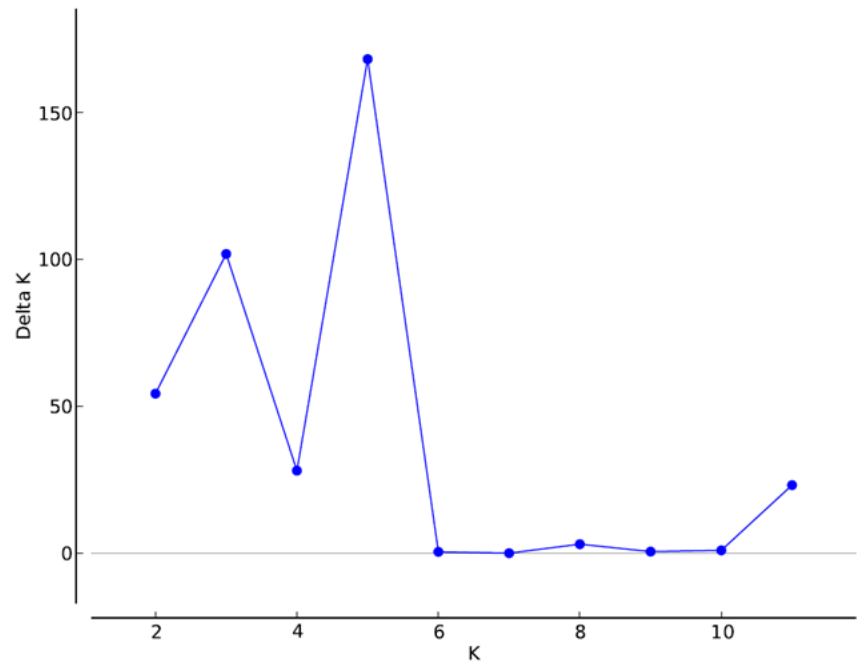
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