



# Fluctuating Asymmetry in Broodstocks of *Litopenaeus Vannamei* in Different Cultured Generations

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## Abstract

**Background:** Captive stocks of the marine shrimp *Litopenaeus vannamei* were formed in Brazil by inputs of wild and cultured individuals of different origins in the Pacific, with subsequent intense exchange of samples between national shrimp farms. However, a number of pure and isolated founding broodstocks of different origins were maintained as closed lineages for several generations. These lines provide a particularly useful model for detecting genetic-based phenotypic effects, caused by a potential endogamy. When analyzed under standardized environmental conditions, indices of Fluctuating Asymmetry (FA) reflect ontogenetic instabilities derived from the genetic effects exhibited by a particular broodstock. Here we assess the degree of FA in two broodstocks of *Litopenaeus vannamei* from different geographic origins and cultured generations.

**Findings:** Segments of locomotor, pereopod and pleopod appendages were particularly useful in assessing FA among broodstocks. A high level of FA was identified in both stocks, more marked in the broodstock with the largest number of cultured generations, in relation to wild populations of species.

**Conclusions:** The data reveal that even a few cultured generations without crossbreeding monitoring can have a negative impact on the genetic patterns of commercial broodstock, demonstrating the validity of FA analysis in inferring endogamy in these captive stocks.

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**Keywords:** Morphology variation; Developmental instability; Shrimp farming; Penaeidae; Fluctuating asymmetry.

## Introduction

Marine shrimp farming in Brazil emerged in the 1970s, with a better understanding of the reproduction cycle and the large-scale post-larvae production of native species. The onset of this activity occurred in the northeast of the country with the native species *Farfantepenaeus brasiliensis* and the exotic species

*Penaeus japonicus* [1,2]. In the 1980s, the shrimp industry expanded primarily due to the introduction of *Litopenaeus vannamei*, from the east coast of the Indo-Pacific, which shows an ability to adapt to a wide range of aquaculture conditions and high productivity.



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Currently, a little variable genetic profile of cultured stocks in Brazil already shows signs of inbreeding in some cases [3,4]. In fact, the high selection pressure during the past 30 years of breeding has promoted a considerable impact on its genome [5]. As a consequence, diminished genetic variability has well-known effects on productivity, including increased deformities, reduced fecundity and disease resistance, among others [6-8].

During the growth of an organism there are mechanisms in place to ensure adherence to a pre-established developmental path. Under these conditions, the embryogenic development is considered to be channeled and the effect on the genome is expressed by stable growth. On the other hand, despite the ability of the organism to control symmetric growth, random errors can occur in this process, resulting in unstable development. Genic complexes can act as determinants of this channeling [9]. Although the genetic bases of channeling and stable development are not fully understood, they have been attributed to the interaction between genic balance, co-adapted genic complexes and genome heterozygosity [10,11].

In addition to environmental factors, such as temperature, dissolved oxygen, pollution and food scarcity, which may lead to unstable development [12], others that interfere in genic balance (endogamy, hybridization, mutations and periods of intense directional selection) can increase the likelihood of genetic errors, affecting the normal development of organisms. Stable development can be measured by different approaches, such as by comparing the right and left structures of organisms with bilateral symmetry. Subtle deviations in bilateral symmetry (fluctuating asymmetry) have been shown to be valuable bio-indicators. Fluctuating Asymmetry (FA) is considered the most sensitive measure of stable development [13,14] and can be characterized as expected small variations in symmetrical development, randomly produced and resulting from the inability of the individual to establish similar development on both sides of the body. Because of its low bench cost and wide range of applications in biological systems, the FA analysis may represent a practical inexpensive routine to indirectly determine the genetic quality of shrimp broodstock. Despite this, data on FA expression in penaeid shrimp are still scarce [15], primarily with respect to its applicability in the genetic and environmental monitoring of economically viable broodstocks.

In this study, analyses of FA variations were carried out in *L. vannamei* individuals from captive stocks of different origins and generations, aiming of establishing parameters for the use of FA and estimating the effects of endogamy on ontogenetic development of this important aquaculture shrimp species.

## Material and methods

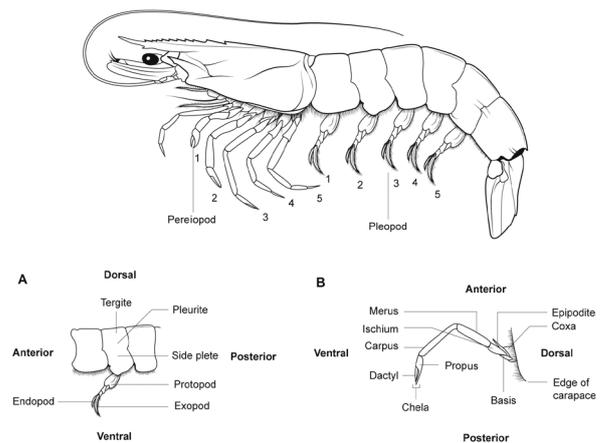
### Broodstock characterization

For analyses of FA we used samples containing 30 (15 males and 15 females) white shrimp (*Litopenaeus vannamei*) aged thirteen months, under standardized culture conditions, from two closed broodstock lineages obtained from a commercial shrimp farm in Rio Grande do Norte state, Northeast Brazil. A lineage, denominated Ecuador broodstock hereafter, was originally founded by wild post-larvae imported from Ecuador and kept in a closed breeding system and studied here in its 4<sup>th</sup> captive generation (F<sub>4</sub>). The second closed lineage, named Venezu-

ela hereafter, was founded by cultured animals, imported from a commercial farm from Venezuela and studied here in its 20<sup>th</sup> captive generation (F<sub>20</sub>). Of note, the Venezuela broodstock, in the generation under analysis, showed good growth performance, but were particularly subject to viral infections and reduced reproductive capability.

### Character measurement

Ten pairs of locomotor appendages (Pleopod (PL) and Pereiopod (PR)) of each animal (Figure 1) were carefully dissected, removed, arranged on a glass slide under a stereoscopic microscopy and individually measured with a precision caliper (0.005 mm resolution). Measures of the segments and total length were obtained from each right and left appendix (Table 1). Each measure obtained was repeated more than once in order to significantly minimize possible errors.



**Figure 1:** Schematic view of appendage pairs analyzed in *Litopenaeus vannamei*. a) pleopods and b) pereiopods.

Morphologically, pleopods consist of a basal part, namely the protopod with coxa and basis, the former reduced and apparently absent. These appendages terminate in two foliaceous branches, the endopod and exopod. Total Pleopod Length (TPL) is given by the sum of the basis and the exopod. The measures in pleopods were taken along the central axis of each segment. Similarly, Pereiopods (PR) are morphologically composed of the protopod, formed by two segments, the coxa and basis; and the exopod or external branch, rudimentary on all legs, with the endopod attached to the basis. The endopod has five segments, progressively more distal, ischium, merus, carpus, propus and dactyle. The chelae are located at the extremities of the 1<sup>st</sup> and 3<sup>rd</sup> pairs of pereiopods and the dactyles on the 4<sup>th</sup> and 5<sup>th</sup> pairs, both not measured. There is no clear separation between the propus and the carpus in the first three pairs; thus the three visible segments were measured. Four measures were taken in the other pairs (ischium, merus, carpus, propus). In pereiopods the measures were obtained along the outer surface of the appendages, from the intersection between the exopod and endopod. Total Pereiopod Length (TPL) was considered the sum of the endopod segments, except the dactyles.

### Index and estimates of fluctuating asymmetry

The bilateral asymmetry of a given characteristic is defined as the absolute value of the difference between the Right and Left Side (R-L), or as the ratio between the measures of each

side (R/L) of an individual. Given that FA can be dependent on the size of the structure, it is recommended that the values (R-L) be related to the mean values of the size of the characteristic (R+L)/2, and correlation analyses established to test this dependency [16]. If the values of the difference between the right and left sides are correlated with the mean values of the size of the structure, then FA will be based on the ratio between (R-L) and (R+L)/2 and considered relative FA. Otherwise, only the absolute values of the differences between R and L are used.

The FA value of each structure studied herein was calculated for each individual (FA<sub>j</sub>) as

$$(FA_j) = \left\{ \frac{(R-L)}{[(R+L)/2]} \right\},$$

Where, R= mean of right side measures, L= mean of left side measures.

Fluctuating Asymmetry for Each Broodstock (FA<sub>b</sub>) was estimated as the mean of the module of FA<sub>j</sub>:

$$(FA_b) = S | FA_{ij} / n$$

Where, n= sample size.

### Statistical analyses

The degree of dependency of asymmetry in relation to the size of the structure was tested using Spearman's correlation coefficients. Asymmetry values (R-L) were correlated to the mean values of the size of the characteristic (R+L)/2 [16]. To analyze the occurrence of fluctuating asymmetry (FA) in each structure, normal distribution pattern was assessed using the Kolmogorov-Smirnov test [17]. Deviation from normality was established by the significant distance from Kolmogorov-Smirnov (KS-d), where rejection of the H<sub>0</sub> hypothesis leads to not assuming normal distribution for the frequency of FA values. Next, it was determined if mean asymmetry values deviated from zero by applying the student's t-test, when distribution was considered normal, and the Wilcoxon test when it was not [18]. Analyses of the deviation from the mean zero and the normality of frequency distributions of asymmetry values were separately performed in each broodstock (n=30 individuals) and in the total sample (n=60 individuals).

Comparison of the mean FA values obtained in pleopods and pereopods between the two broodstocks was conducted using the student's t-test. In this comparison, FA values used in module to assess the magnitude of the asymmetric value. For all analyzed characters, the effect of sex and broodstock origin, as well as the interaction between these variables were tested by applying analysis of variance of two factors [16,19]. Furthermore, the Tukey test was used for different samples to detect the difference between them, when there was significance in some source of variation in the analyses of variance [18]. All the tests were carried out at a significance level of 5%.

## Results

### Dependency of asymmetry on mean structure size

It is often relevant to compare asymmetry with the size of the characteristic to determine whether or not there is dependency between the two. In the Ecuador broodstock, 26.8% of the structures measured (PL13, PL22, PL32, PL33, PL43, PLT4, PL53, PLT5, PR22, PR54 and PRT5) demonstrated a significant correlation between asymmetry and size of the characteris-

tic (Table 1). On the other hand, in the Venezuela broodstock, 14.6% of the structures measured, represented by PL13, PL43, PL53, PR33, PRT4 and PR51 characters, showed significant correlation coefficients.

**Table 1:** Pleopod (PL) and Pereiopod (PR) segments used to estimate FA in *Litopenaeus vannamei* broodstock. The first number indicates the appendage and the second the segment (in pleopods, 1-basis; 2-endopod; 3-exopod; in pereopods, 1-ischium; 2-merus; 3-carpus; 4-propus).

Pleopods	Pereopods
PL11, PL13, PLT1	PR11, PR12, PR13, PRT1
PL21, PL22, PL23, PLT2	PR21, PR22, PR23, PRT2
PL31, PL32, PL33, PLT3	PR31, PR32, PR33, PRT3
PL41, PL42, PL43, PLT4	PR41, PR42, PR43, PR44, PRT4
PL51, PL52, PL53, PLT5	PR51, PR52, PR53, PR54, PRT5

### Levels of Fluctuating Asymmetry (FA)

The presence of FA was evidenced when the distribution of asymmetry values (R-L) obeyed the normal distribution pattern and the mean of this distribution was significantly equal to zero. Normality of FA frequency distribution, calculated for each individual, was conducted considering the total sample (n=60) and broodstocks (N=30) separately, where N corresponds to sample size. Only 50% of the frequency distribution pattern in the broodstocks was normal (Table 2). In the Ecuador broodstock, 80.5% of the body structures measured showed normal distribution, except PL13, PLT1, PL22, PL32, PL33, PLT3, PL43 and PRT5, while in the Venezuela broodstock, this percentage was 70%, except PL23, PL43, PLT4, PR33, PRT3, PR42, PR43, PR44 and PRT4 (Table 3).

For the characters that exhibited normal distribution in individual FA values, the student's t-test was applied to assess the deviation of the mean FA value in relation to the mean of zero, considering the total sample (n=60) and broodstocks separately (n=30). A similar number of characters measured for both broodstocks displayed mean individual FA values of zero, 36 and 38 characters in the Ecuador and Venezuela broodstocks, corresponding to 87.8% and 92.7%, respectively. Eighteen characters expressed fluctuating asymmetry in the Ecuador broodstock and 29 in the Venezuela broodstock, corresponding to 43.9% and 70.7%, respectively, of the characters measured (Table 3).

### Effect of sex and broodstock on the expression of fluctuating asymmetry

Analysis of variance revealed the significant effect of the broodstock (Table 3) for the endopod of the 4<sup>th</sup> Pleopod (PL42), the basis of the 5<sup>th</sup> Pleopod (PL51), the merus of the 1<sup>st</sup> Pereiopod (PR12) and the ischium of the 4<sup>th</sup> Pereiopod (PR41). The mean FA values of these characters were significantly higher for the Venezuela broodstock when compared to the Ecuador broodstock.

FA values varied independently either between genders and broodstocks for most of the structures measured. However, the effect of sex (Table 3) was significant for the exopod of the 3<sup>rd</sup> pleopod (PL33) and the total length of the 5<sup>th</sup> Pleopod (PLT5). Mean FA was higher in females than in males for the aforementioned characters.

**Table 2:** Deviation from normal distribution (KS-d) and t-test in Pleopod (PL) and pereopod (PR) measures and detection of Fluctuating Asymmetry (FA) in Ecuador and Venezuela broodstocks. WT-Wilcoxon Test; (+) Normal distribution and mean distribution of zero; (-) Non-normal distribution and mean different from zero. Significant values are marked in bold ( $p < 0.05$ ).

Segment	Ecuador				FA	Venezuela				FA	FA <sub>tot</sub>
	Normality		Mean = 0			Normality		Mean = 0			
	KS-d	p	T	p		KS-d	p	T	p		
PL11	0.090	>0.100	0.740	0.456	+	0.140	>0.100	0.622	0.539	+	+
PL13	0.267	<0.050	WT	0.673	-	0.110	>0.100	0.484	0.632	+	-
PLT1	0.308	<0.010	WT	0.734	-	0.140	>0.100	0.999	0.326	+	-
PL21	0.170	>0.100	0.991	0.330	+	0.060	>0.100	0.047	0.963	+	+
PL22	0.327	<0.010	WT	0.264	-	0.090	>0.100	2.105	0.044	-	-
PL23	0.160	>0.100	1.229	0.229	+	0.246	<0.050	WT	0.629	-	+
PLT2	0.150	>0.100	0.838	0.409	+	0.150	>0.100	0.676	0.504	+	+
PL31	0.100	>0.100	2.635	<b>0.013</b>	-	0.150	>0.100	0.899	0.376	+	-
PL32	0.393	<0.010	WT	0.679	-	0.110	>0.100	0.674	0.506	+	-
PL33	0.300	<0.010	WT	0.344	-	0.180	>0.100	0.127	0.900	+	-
PLT3	0.257	<0.05	WT	0.210	-	0.160	>0.100	0.464	0.647	+	-
PL41	0.130	>0.100	0.588	0.561	+	0.160	>0.100	1.932	0.063	+	+
PL42	0.220	>0.100	0.199	0.844	+	0.120	>0.100	0.071	0.944	+	+
PL43	0.328	<0.010	WT	0.837	+	0.268	<0.050	WT	0.766	-	+
PLT4	0.220	>0.100	0.920	0.365	-	0.260	<0.050	WT	0.820	-	-
PL51	0.120	>0.100	1.182	0.247	+	0.080	>0.100	0.099	0.922	+	+
PL52	0.464	<0.010	WT	0.136	+	0.110	>0.100	1.191	0.243	+	+
PL53	0.210	>0.100	0.017	0.986	-	0.200	>0.100	2.330	<b>0.027</b>	-	-
PLT5	0.190	>0.100	0.525	0.604	-	0.240	0.070	1.973	0.058	+	-
PR11	0.120	>0.100	1.012	0.320	+	0.110	>0.100	1.038	0.308	+	+
PR12	0.160	>0.100	0.708	0.485	-	0.220	>0.100	0.715	0.480	+	-
PR13	0.160	>0.100	1.232	0.228	-	0.130	>0.100	0.363	0.719	+	-
PRT1	0.200	>0.100	0.279	0.782	+	0.170	>0.100	0.743	0.463	+	+
PR21	0.170	>0.100	0.099	0.922	+	0.140	>0.100	1.552	0.132	+	+
PR22	0.100	>0.100	2.122	<b>0.043</b>	+	0.180	>0.100	0.017	0.987	+	+
PR23	0.150	>0.100	3.241	<b>0.003</b>	+	0.240	0.067	0.061	0.952	+	+
PRT2	0.080	>0.100	1.862	0.073	-	0.210	>0.100	1.342	0.190	+	-
PR31	0.220	>0.100	1.123	0.271	+	0.130	>0.100	0.785	0.439	+	+
PR32	0.140	>0.100	2.853	<b>0.008</b>	-	0.200	>0.100	1.238	0.226	+	-
PR33	0.210	>0.100	0.286	0.777	-	0.286	<0.050	WT	0.886	-	-
PRT3	0.170	>0.100	0.665	0.511	-	0.288	<0.050	WT	0.847	-	-
PR41	0.170	>0.100	0.213	0.833	+	0.100	>0.100	0.341	0.736	+	+
PR42	0.200	>0.100	2.458	<b>0.020</b>	-	0.251	<0.050	WT	0.464	-	-
PR43	0.120	>0.100	0.458	0.683	-	0.342	<0.010	WT	0.124	-	-
PR44	0.220	>0.100	0.895	0.378	-	0.362	<0.010	WT	0.254	-	-
PRT4	0.180	>0.100	0.459	0.650	-	0.391	<0.010	WT	0.624	-	-

PR51	0.140	>0.100	0.748	0.461	+	0.100	>0.100	0.048	0.962	+	+
PR52	0.210	>0.100	0.324	0.748	+	0.070	>0.100	0.374	0.712	+	+
PR53	0.240	0.058	0.748	0.461	-	0.200	>0.100	1.343	0.190	+	-
PR54	0.210	>0.100	0.461	0.648	-	0.080	>0.100	1.292	0.207	+	-
PRT5	0.347	<0.010	WT	0.109	-	0.080	>0.100	2.122	0.043	-	-

**Table 3:** Analysis of variance of the effects of Sex (S) and Broodstock (B) in Pleopods (PL) and Pereiopods (PR) of *L. vannamei*. Significant values showed in bold ( $p < 0.05$ ).

Segment	Effect	F	p
PL22	S	2.584	0.114
	B	2.211	0.143
	Interaction	4.493	<b>0.038</b>
PL23	S	1.160	0.286
	B	0.014	0.907
	Interaction	4.212	<b>0.045</b>
PL33	S	4.641	<b>0.036</b>
	B	1.546	0.219
	Interaction	1.193	0.279
PL42	S	2.222	0.141
	B	6.110	<b>0.017</b>
	Interaction	0.593	0.444
PL51	S	2.857	0.097
	B	9.128	<b>0.004</b>
	Interaction	0.240	0.626
PLT5	S	4.342	<b>0.042</b>
	B	0.000	0.999
	Interaction	0.404	0.527
PR12	S	2.018	0.161
	B	4.585	<b>0.037</b>
	Interaction	4.185	<b>0.046</b>
PR31	S	0.020	0.887
	B	0.291	0.591
	Interaction	6.138	<b>0.016</b>
PR41	S	0.588	0.446
	B	7.157	<b>0.010</b>
	Interaction	0.358	0.552
PR51	S	0.920	0.342
	B	0.325	0.571
	Interaction	6.289	<b>0.015</b>
PR53	S	0.147	0.703
	B	0.087	0.769
	Interaction	4.351	<b>0.042</b>

Significant interaction between sex and broodstock was observed (Table 3), primarily in relation to the endopod and exopod of the 2<sup>nd</sup> Pleopod (PL22, PL23), merus of the 1<sup>st</sup> Pereiopod (PR12), ischium of the 3<sup>rd</sup> Pereiopod (PR31) and ischium and carpus of the 5<sup>th</sup> Pereiopod (PR51, PR53). The mean FA of females from the Venezuela broodstock ( $F_{20}$ ) was higher than that of the Ecuador broodstock with respect to PL23, PR12, PR51 and PR53.

### Discussion

A number of genetic factors, such as endogamy, hybridization and mutation, can promote severe instability in the ontogenetic development. Among these conditions, reduced genetic variability due to endogamy favors the expression of harmful recessive alleles, which, when expressed in recessive homozygotes, tend to interfere in the stability of phenotypic development [20,21]. Although these variations can be perceived by determining fluctuating asymmetry, few data using this methodology are available for marine shrimp [15,22].

The bilateral asymmetry patterns obtained in wild populations submitted to natural selection indicate that a certain degree of FA seems to be a tolerable and normal condition in penaeids. Indeed, analysis of FA in a natural population of *Litopenaeus schmitti* from the Western Atlantic, submitted to normal heterozygous conditions and under the effect of natural selection, revealed the occurrence of a low level of FA, detected in 27.7% of pereiopod segments [15]. Even though the mean FA values for native populations of *L. vannamei* were not available, because of their phylogenetic proximity, the FA index identified under natural conditions in *L. schmitti* result in reasonable estimations for native populations of other congeneric species, such as *L. vannamei*.

In contrast to the genetic patterns of wild populations of *Litopenaeus* species, that reveal greater genetic diversity [23], captive stocks of *L. vannamei*, on the other hand, are often more subject to genetic erosion and endogamy [3,4,6,7,24]. This condition favors the occurrence of fluctuating asymmetries, due to the increase of homozygosity and the expression of harmful alleles [22,25].

Detailed analyses of FA showed a different expression between the two broodstocks. Considering that Ecuador ( $F_4$ ) and Venezuela ( $F_{20}$ ) broodstocks were submitted to the same culture conditions, the divergences observed in FA can be attributed specifically to the variation caused by the number of broodstock generations in captivity [7].

Analyzing the loss of genetic variability using dominant DNA markers, in five successive generations of *L. vannamei* under endogamic crossbreeding ( $F_5$ - $F_9$ ), identified a progressive reduction in polymorphic loci, associated with a marked increase in the mean genetic similarity in each generation. A number of loci in generation  $F_9$  were restricted to less than 8% of broodstock individuals. Thus, since the formation of both broodstocks is

based on few breeders, selected by phenotypic characteristics, it can be inferred that they also exhibit growing genetic similarity between the generations of the culture. Furthermore, the Venezuela broodstock was previously studied using multilocus analysis when it was in generation F<sub>17</sub> [6], showing a high level of genetic similarity when compared to the other broodstock in generation F<sub>8</sub>.

A number of studies have demonstrated the relationship between heterozygosity, stable ontogenetic development and adaptive characteristics. In the freshwater shrimp *Eulimnadia texana*, genetic diversity, body size and fecundity were significantly reduced in more endogamic individuals [26]. Similarly, in fish, endogamy was associated with a lower fertility and growth rate and increased mortality [24,27,28].

It has been suggested that the association between increased developmental instability, expressed by bilateral asymmetry and endogamy is a common response to different phylogenetic groups. In marsupials, genetic analysis using microsatellites in an insular population showed low levels of genetic variation, indicating reduced fecundity in females and a rise in the FA levels. Endogamy in these populations was associated with high rates of extinction due to alterations that led to the reduction in the adaptive value of individuals [29]. In mammals, endogamic generations of *Rattus villosissimus* exhibited a tendency for larger and shorter skulls, in addition to an increase in FA in a number of skeletal characters [30]. In lines of male *Gazella cuvieri*, analysis of oro-aboral diameter of the horns indicated that FA was positively related to endogamy, suggesting that bilateral asymmetry can be used as an indicator of genetic stress [31]. In humans, the jaws of inbred populations expressed significantly more FA [32].

The expression of fluctuating asymmetry may depend on the amount of stress, which the organism is exposed during development [33], and the heterosis level of individuals [14]. Although some studies on insects showed a relationship between endogamy and FA, which is generally sensitive in detecting developmental stressors, sample sizes and genetic patterns of populations may interfere in the FA measurement. While endogamic lines of *D. melanogaster* were as asymmetric as the non-endogamics [34,35], other organisms exhibited an increase [36] or decrease [37] in asymmetry, when meristic and metric characteristics were measured across endogamic generations. Recent analysis using geometric morphometry identified sexual dimorphism in the body patterns of *L. vannamei* [38]. Indeed, sexual dimorphism is a dominant condition among crustacean species and if the locomotor appendages are involved, these structures may exhibit a different expression of FA between genders.

The data presented herein demonstrated that the females show higher average asymmetry than males. FA was more marked in females in the third and fifth pleopod, whose primary function is swimming. In males, the first two pairs participate in reproduction. The first pair forms the copulator organ and the second one assists the copulation. A higher susceptibility for bilateral asymmetry of these appendages in females than males could be reflecting a sex-biased adaptive value reduction in this shrimp. In fact, the interaction between sex and broodstock analysis identified a significant interaction, suggesting that both variables contributed to the FA variation. The first and fifth pereopod and the second pleopod were more asymmetric in females from the Venezuela broodstock than those from Ecuador. In contrast, males showed higher mean FA in the Ec-

cuador broodstock. Given that the first three pairs of pereopods showed chelae and are used for handling prey and food as well as for defense [39], these data can demonstrate that adaptively important characters were impacted by higher levels of asymmetry.

The data obtained from *L. vannamei* indicated for the first time that the larger the number of generations in captivity, inferring a higher degree of endogamy, the higher will be the FA levels. Considering that the appendages with the highest FA levels were functionally associated with reproduction (especially in females) and feeding, our findings could be very useful for aquaculture broodstocks management. Moreover, being a low-cost and efficient methodology, FA could be view as a very feasible tool for monitoring genetic background, mainly when closed lineages are maintained across several generations.

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