Genetic structure among the endangered Brazilian Euterpe Edulis Mart (Arecaceae) morphotypes

Gislaine Coelho[1], Alesandro Santos[1], Ivandilson Menezes[2], Roberto Tarazi[3], Fernanda Maria Souza[4], Maria das Graças Silva[5], and F. A. Gaiotto[1]

[1]Universidade Estadual de Santa Cruz
[2]Instituto Federal Goiano
[3]BASF Discovery Breeder
[4]Universidade Estadual de Santa Cruz Departamento de Ciencias Biologicas
[5]5Centro de Pesquisa do Cacau (CEPEC), Comissão Executiva do Plano da Lavoura Cacaueira (CEPLAC)

May 5, 2020

Abstract

Euterpe edulis (Arecaceae) Mart has high ecological and economic importance providing food resources for more than 58 species of birds and 20 species of mammals, including humans. E. edulis is the second most explored non-timber product from Brazilian Atlantic Forest. Due to overexploitation and destruction of habitats, E. edulis is threatened by extinction. E. edulis populations have large morphological variations, with individuals having green, red or yellow leaf sheath. However, no study has related phenotypic distinctions between populations and their levels of genetic structure. Thus, this study aimed to evaluate the diversity and genetic structure of different E. edulis morphotypes. We sampled 250 adult individuals in eight populations with the different morphotypes. Using 14 microsatellite markers, we access genetic diversity through population genetic parameters calculated in the GenAlex program and the diveRsity package in R. We used the Wilcoxon test to verify population bottlenecks and the genetic distance of Nei and Bayesian analysis for genetic clusters. The eight populations showed low allele richness, low heterogeneity observed and high inbreeding values (f). In addition, six of the eight populations experienced genetic bottlenecks, which would partly explain the low genetic diversity in populations. Cluster analysis identified two clusters (K=2), with green morphotype genetically distinguishing from yellow and red morphotypes. Thus, we show, for the first time, a strong genetic structure among E. edulis morphotypes even for geographically close populations.

INTRODUCTION

Arecaceae is a botanical family with pantropical distribution, with about 183 genera and 2,400 species (Dransfield *et al* ., 2008). Arecaceae species are key components of forest structuring, and are also important because they provide fruits and seeds that maintain a high richness of frugivores in these environments (Galetti *et al.* , 2013; Elena *et al.* , 2014; Benchimol *et al.* , 2016). In Brazil, 37 genera and 297 species of Arecaceae are found, being approximately 46% of these endemic species (Leitman *et al* ., 2015). Studies show that this high percentage of endemism is probably related to environmental characteristics, such as soil fertility and climatic factors (Salm *et al.* , 2007; Eiserhardt *et al.* , 2011). For the Brazilian Atlantic Forest 61 Arecaceae species are found, which are in constant risk of being lost due to the exacerbated loss of their natural habitat (Leitman *et al* ., 2015; Benchimol *et al.* , 2016).

Euterpe edulis Mart (Arecaceae) has a great ecological and economic relevance (Reis *et al.* , 2000; Galetti *et al.* , 2013; Elena *et al.* , 2014; Carvalho *et al.* , 2016). In Brazil, this species occurs in forests of Galleries in the Cerrado and widely distributed in the Atlantic Forest, its main habitat of occurrence (Leitman *et al*

., 2015). *E. edulis* seeds and fruits are important food resources for about 58 bird species and 20 mammal species playing an important ecological role in maintaining the diversity of frugivores in the Atlantic Forest (Galetti *et al.* , 2013). In economic terms, the species is the second most exploited non-timber product of the Atlantic Forest Brazilian, through the extraction of its apical meristem called heart-of-palm for human consumption (Silva Matos *et al.* , 1999; Reis *et al.* , 2000; Pizo and Vieira, 2004). This illegal harvesting of heart-of-palm causes the death of individuals, which is a serious problem, since the species does not sprout or have tillering. Therefore, this is one of the causes that led the species to the list of endangered Brazilian flora (Leitman*et al* ., 2015). In addition to the negative impacts of predatory harvesting, the reduction and fragmentation of the Atlantic Forest further threatens this species (Fleury and Galetti, 2006; Carvalho*et al.* , 2015; Santos *et al.* , 2016).

E. edulis is a monoecious species with predominance of cross-pollination, performed mainly by small bees (eg *Trigona spinipes*) and seeds dispersed mainly by birds (Reis *et al.* , 2000; Gaiotto *et al.* , 2003; Galetti *et al.* , 2013). The populations of *E. edulis* may present large morphological variations, as for example, leaf sheath color and number of rachila, besides distinct demographic characteristics along their area of occurrence. Motivated by these differences, some scientists have proposed separation into two species and one variety: populations with green sheathed individuals, such as *E. edulis* ; and those with individuals having red sheaths such as *E. spiritosantensis* and; Yellow-sheathed individuals, such as *E. edulis var.clausa* (Bovi*et al* ., 1987; Reis *et al* ., 1996; Mantovani and Morellato, 2000; Silva *et al.* , 2009; Leitman *et al* ., 2015). Although there are evident morphological and demographic differences, currently, it is considered as taxonomically accepted name only *E. edulis*and the others are considered synonyms (Leitman *et al* ., 2015).

In this context, despite these peculiarities observed and discussed in the literature, there is a shortage of genetic studies that associate the phenotypic distinctions between natural populations and their levels of genetic structure. Currently, it is only known that populations of*E. edulis* in different environmental conditions are genetically different (Brancalion *et al.* , 2018; Alves-Pereira *et al.* , 2019). However, it is not known whether populations with morphological differences are also genetically different. Thus, this study had as its central objective to evaluate the genetic diversity and to verify if there is genetic differentiation between the different *E. edulis*morphotypes.

MATERIAL AND METHODS

Sampling Areas

The populations studied were chosen based on their distinct morphotypes (Figure 1 and Table 1) and geographic distances (Figure 2). Eight populations were sampled, being two in gallery forests from the*Cerrado* biome (Brazilian savanna in Distrito Federal - DF, central west of Brazil) and six from the Atlantic Forest (Bahia, northeastern of Brazil) (Figure 2).

The two populations sampled in DF are located in gallery forests, which are a type of perennial forest vegetation immersed in savanna formations, which accompany small streams and usually have nutrient-rich soils (Haridasan, 1998). These populations were sampled within protected areas, namely the Brasilia National Park (PNB) and the Roncador Ecological Reserve (IBGE), which are located 10 Km and 20 Km from Brasilia-DF, respectively.

The sampled populations in Bahia are located in the region with the largest remnants of the Atlantic Forest of northeastern Brazil (Ribeiro*et al.* , 2009), being considered a priority for conservation due to its high biodiversity (Martini *et al.* , 2007). Of these populations, four are located in protected areas and two in unprotected areas. Protected areas include: (1) The population from the Private Reserve of Natural Patrimony Serra do Teimoso (ST) located in the municipality of Jussari, which consists in a transition between the rainforest found on the tops of hills and a semideciduous forest (forest with a dry period in the year) at the base; (2) The populations of the Ecoparque de Una (EU) and the Una Biological Reserve (BR) which are located in the municipality of Una, inserted in rainforest (Thomas*et al.* , 1998); (3) The population from the Private Reserve of Natural Patrimony Estação Veracel (EV), which is located in the municipality of Eunapolis and has rainforest. The other two populations are not located within conservation

units, but they are also inserted in rainforest of the Atlantic Forest, located in particular properties which are in the municipality of Itacaré on Alto da Esperança Farm (AE), and in the municipality of Uruçuca on Boa Sorte Farm (BS).

Microsatellite Marker Analysis

Samples were collected from the root tissue of 250 randomly chosen adults and the DNA was obtained according to Doyle and Doyle (1987). Posteriorly, the genetic material was amplified with a total of 14 nuclear microsatellite fluorescent primer pairs (*EE2/ EE5/ EE23/ EE32/ EE9/EE25/ EE43/ EE45/ EE47/ EE48/ EE52/ EE54/ EE59/ EE63*) developed by Gaiotto et al (2001).

The amplification reactions were performed by PCR in a final volume of 13 μL containing 7.5 ng of genomic DNA, 1X buffer (10 mmol/L Tris-HCl, 50 mmol/L KCl and 21.5 mmol/L MgCl, pH 8.3), 0.27 μmol/L of each primer, 2.0 mmol/L $MgCl_2$, 0.25 mg/mL BSA, 0.25 mmol/L dNTP and 1U of Taq DNA polymerase in ultrapure sterile water. The PCRs were performed under the following conditions: 1 cycle at 96°C for 2 min, 34 cycles of 94°C for 1 min, the annealing of the temperature-specific primer ($T^{o}C$) for 1 minute and 72°C for 1 min. The final elongation step was performed at 72°C for 7 min. The analysis of amplicons in a denaturing gel (7 mol/L urea) with 4% polyacrylamide (Long Ranger 50% - Cambrex) was performed in a multiplex system, in a semi-automatic ABI 377 sequencer (Applied Biosystems) by using virtual filter D.

Data Analysis

To estimate the frequency of null alleles, assuming the Hardy-Weinberg equilibrium proportions and to identify possible genotyping errors, we use the MICRO-CHECKER 2.2.3 program (Oosterhout *et al.* , 2004). The populations presented low average frequency of null alleles ([?]0.08), not compromising our results. Then, we used the spreadsheet obtained of the MICRO-CHECKER with corrections of possible genotyping errors for genetic analysis, thus ensuring greater accuracy in the results.

The genetic diversity of *E. edulis* morphotypes was estimated using different population genetics parameters calculated by GenAlex 6.5 (Peakall and Smouse, 2012) and diveRsity package in the R software (Keenan *et al* ., 2013). In the GenAlex, we calculated the mean number of alleles (No); the mean number alleles effectives (Ne) and the mean private alleles (PA). Additionally, in diveRsity package, we used the divBasic function to calculate the allelic richness (AR), expected heterozygosity (H_E), observed heterozygosity (H_O) and inbreeding coefficient (f). To consider the 95% confidence interval of f , the standard deviation for H_E and H_O, and standard error for AR, we calculated 10,000 bootstraps which the divBasic function.

To verify signs of population bottlenecks, we use the Wilcoxon test in software BOTTLENECK version 1.2.02 02 (Cornuet and Luikart, 1996), testing if populations have excess heterozygosity (Piry *et al* ., 1999). The Wilcoxon test is recommended because of its power to detect population bottleneck when few molecular markers (<20 loci) are used, as is the case in our study. As microsatellites can present different mutational models, we performed the Wilcoxon test considering the Infinite Allele Model (IAM), Stepwise Mutation Model (SMM) and Two Phase Model (TPM) (Piry *et al* ., 1999). For the TPM model, the proportion of SMM in TPM = 0.000, and variance of the geometric distribution for TPM = 0.36, which generally represent the most sensitive values for microsatellite markers (Piry *et al.* , 1999).

To estimate the level of genetic structure among the populations, we calculate the observed F_{ST} values and the 95% confidence interval with 10,000 bootstraps, using the diffCalc function of the diveRsity package in software R (Keenan *et al* ., 2013) (http://www.r-project.org/). Subsequently, to assess whether geographic distance (km) influenced in F_{ST} values obtained between populations, we performed a Mantel test (Mantel, 1967) using the ecodist package (Goslee and Urban, 2007) in the software R (http://www.r-project.org/).

To evaluate the population clustering, we calculated the genetic distance from Nei in GenAlex 6.5 (Peakall and Smouse, 2012) and the Bayesian clustering in Structure program (Evanno *et al* ., 2005). Using Nei's genetic distance, we performed a heatmap analysis with the eight populations of *E. edulis* in heatmaply package (Galili*et al* ., 2017) in software R. To determine the number of groups in the heatmap, we used hierarchical clustering algorithm with the Euclidean distance and the average method, implemented in heatmaply package

(Galili *et al* ., 2017) in software R. In Bayesian model, we infer automatically the number of distinct genetic groups according to mixed model that was often correlated. An analysis of the number of populations (K) was performed for values ranging from 1 to 10 with ten independent chains, and each chain had a length of 50000 iterations followed by 100000 repetitions of the MCMC (Markov and Monte Carlo chain). The ΔK (actual number of groups) was determined on the basis of the average values of L (K) as produced for ten repetitions for each K, according to the method proposed by Evanno et al (2005), by using the Structure Harvester program (Earl and VonHoldt, 2011).

RESULTS

Genetic Diversity and population bottlenecks

The eight populations of *Euterpe edulis* showed low to moderate number of alleles and allelic richness (Mean = 7.58 and 5.48 respectively), low number of effective alleles (Mean =4.08), and all of them had private alleles (Table 1). The observed heterozygosity (H_O) was low to moderate, except for BN and RE with high values (Figure 3A). The expected heterozygosity (H_E) was moderate to high, with emphasis for AE, BN and RE (Figure 3A). The values of inbreeding coefficient (f) were high, except for BN and RE showing the lowest values, although none values are nonzero (Figure 3B).

The different mutational models (IAM, TPM and SMM) used in the Wilcoxon test for population bottlenecks showed divergent results. The IAM model detected significant excess of heterozygosity for the BS, AE, EU, ST, BN and RE populations, while the SMM and TPM models did not detect genetic bottlenecks in any of the eight *E. edulis* populations evaluated (Table 2).

Genetic Structure

The analysis of the genetic structure with F_{ST} revealed that the populations have moderate to high genetic differentiation, with the less value found for BN-RE populations (Figure 4). Although all F_{ST} values are moderate or high, they are within the confidence interval and do not differ from zero (Figure 4). The Mantel test showed that the geographical distance does not correlate significantly with the pattern of genetic differentiation found between populations (r = 0.13, p = 0.33, Figure S1).

Population clustering

The heatmap with clustering using the Nei's genetic distance, showed that the eight sampled populations form two clusters (Figure 5 and Figure S2). The populations with green morphotypes, which were sampled in the Distrito Federal (BN and RE) and southern Bahia (ST) clustered separately from the red (EU, AE and BR) and yellow (BS and EV) morphotypes sampled in southern Bahia (Figure 5).

In Bayesian clustering approaches, the eight sampled populations had the estimated number of population groups of K = 2, as the number of most likely groups (Figure 6 and Figure S3), which demonstrated that they are strongly structured (Figure 6). On average, the shared ancestry among the *E. edulis* palm was 98.8% and 99.4% in the groups marked in red and green, respectively. The red group was composed of all individuals sampled in the EU, AE, BR, BS and EV populations in southern Bahia, that have red or yellow morphotypes (Figure 1). The green group contains all the individuals sampled in the RE and BN populations in Distrito Federal and ST in southern Bahia, that have green morphotype (Figure 1).

DISCUSSION

In the present study, we recorded low genetic variability and genetic bottleneck effect in *E. edulis* populations, which also have unusual genetic structure rate for populations of this species (Gaiotto*et al.* , 2003; Santos *et al.* , 2015). In addition, the evaluated populations formed two genetic clusters, separating typical populations (green morphotype) from populations with yellow or red morphotypes that have restricted occurrence within the Brazilian Atlantic Forest. Thus, we showed, for the first time, that morphologically distinct populations of *E. edulis* have a strong genetic structure, as a possible response to local environmental factors.

Genetic diversity and population bottlenecks

In general, there is a low genetic diversity in the eight populations of*E. edulis* when considering the number and richness of alleles and the number of effective alleles compared to other populations of the species (Conte *et al.* , 2008; Carvalho *et al.* , 2015, 2017). In theory, several ecological or anthropogenic factors can negatively impact genetic diversity, such as the reduction or inefficiency of dispersers and pollinators, reduction in effective population size, causes related to habitat loss and fragmentation, or even illegal extraction of species (Young *et al.* , 1996; Chung *et al.* , 2014; Browne *et al.* , 2015; Carvalho *et al.* , 2016, 2017; Ellegren and Galtier, 2016). Even in this context, all populations had a considerable proportion of private alleles, demonstrating the importance of these areas for the conservation of this endangered species.

Considering the observed heterogeneity (H_O), six populations have low or moderate values compared to other populations of the species (Carvalho *et al.* , 2017). In addition, expected heterozygosity (H_E) values are moderate to high and always upper than H_O values in all populations, indicating an increase in the number of homozygous individuals compared to expected in the drift-mutation equilibrium in an idealized, panmictic population (so-called Wright–Fisher equilibrium). As a consequence of H_O<H_E, all populations have positive and high values of inbreeding (f), indicating non-random mating occurring, which may cause reduction of genetic diversity (Xue*et al.* , 2015). In this scenario, the genetic erosion may arise and even increase over generations, as individuals would become more related to each other as result of non-random mating within populations (Xue *et al.* , 2015). On the other hand, it is important to draw attention to the areas RE and BN, that have the highest values of H_O, H_E and the lowest of f , indicating the best genetic conservation status among the studied populations. These values are probably influenced by the occurrence of gene flow between RE and BN, which may favor the maintenance of genetic variability over time (Gaiotto *et al.* , 2003; Santos *et al.* , 2016).

The Wilcoxon test, with the Infinite Allele Mutational model (IAM), revealed that six of the eight populations studied have recently experienced genetic bottlenecks. However, the Two Phase Model (TPM) and Stepwise Mutation Model (SMM) had divergent results, not detecting genetic bottleneck in any of the evaluated populations. This result was expected to some extent because, although microsatellite markers may adhere to different mutational models (AIM, TPM, or SMM), genetic bottlenecks have been reported mainly for the AIM model (Piry *et al.* , 1999; Santos *et al.* , 2019). The genetic bottleneck or excess of H_E occurs when the population has experienced a recent reduction in effective population size, causing a reduction in the number of alleles faster than in H_E (Cornuet and Luikart, 1996; Piry *et al.* , 1999). Thus, populations that have experienced genetic bottlenecks have a H_E greater than the expected heterozygosity in drift - mutation equilibrium (H_{EQ}) (Cornuet and Luikart, 1996; Piry *et al.* , 1999). Thus, as we identified genetic bottleneck effect in most populations evaluated, at least in parts, would explain the reduction in the number and allelic richness and in the number of effective alleles reported. Moreover, even if the population did not go through a genetic bottleneck, such as BR and EV, there is evidence of genetic structure (Figure 4), which could potentially lead to non-random crossing within these areas due to isolation (Mosca *et al.* , 2014; Sexton *et al.* , 2014). Thus, we believe that both the genetic bottleneck and the degree of isolation may explain the reduction of genetic diversity in terms of number and richness of alleles, H_O<H_E, high values of*f,* and presence of private alleles in the populations.

Genetic Structure

Considering all populations, F_{ST} values were generally moderate to high, indicating a limited gene flow between populations. These F_{ST} values indicate unusual genetic divergence among *E. edulis* populations compared to other populations of the species, even when under influence of fragmentation and geographically distant (Conte *et al.* , 2008; Santos *et al.* , 2015). Thus, we believe that the differences found between the populations of this palm tree should not be related to habitat fragmentation, but to naturally occurring evolutionary events, such as local adaptation (Brancalion *et al.* , 2018). In addition, it is important to draw attention to RE and BN that have the same morphotype and are geographically close, with low to moderate F_{ST}, indicating occurrence of gene flow, as reported by Gaiotto et al (2003) for these same populations. The occurrence of gene flow between RE and BN, probably favors the highest H_O and H_E values and the lowest of f reported in present study. However, it is important to note that, although F_{ST} values were high in most

cases, all values are within the confidence interval and are not statistically different from zero.

The pattern of genetic differentiation (F_{ST}) is not explained by geographical distance as theoretically expected, in which geographically closer populations are genetically more similar (Wright, 1943; Diniz-filho *et al.* , 2013; Ramírez-Barrera *et*al.,2019). Thus, we emphasize that the pattern of genetic differentiation found between populations, is probably influenced mainly by morphological differences, as demonstrated in the cluster analysis.

Population clustering

Although F_{ST} values were moderate or high between sampled populations, clustering using Nei genetic distance and bayesian analysis identified K = 2 as the most likely number of genetic clusters. It is important to note that no F_{ST} value was statistically significant and that values greater than 0.20 allow for more accurate cluster identification and can correctly attribute about 97% of individuals to a true cluster, when using the bayesian approach (Latch *et al.* , 2006). Thus, the reported F_{ST}values probably contributed to a greater inference power of the genetic clusters.

The grouping into two clusters (Figure 5 and 6), indicates a greater genetic ancestry between populations with green morphotype that is genetically distinguished from yellow and red morphotypes. Thus, we show for the first time that it is not just a phenotypic plasticity of*E. edulis* , but there is genetic divergence between morphotypes, possibly in response to environmental or ecological divergence (Funk and Murphy, 2010). On the other hand, the grouping of the yellow morphotype with the red indicates that this morphological differentiation is probably more recent compared wich green morphotype. This would potentially explain the sharing of the gene pool between the morphotype grouped.

Although we have used microsatellite markers that are considered neutral, we believe that the genetic clusters that distinguish *E. edulis* morphotypes are a consequence of populations being shaping in different environmental or ecological conditions. This hypothesis is based on recent studies using SNPs (Single Nucleotide Polymorphism) markers, demonstrating that *E. edulis* populations inserted in different environmental and ecological conditions are genetically different (Brancalion *et al.* , 2018; Alves-Pereira *et al.* , 2019). Thus, it is believed that there is adaptation of species to different conditions and that this adaptation remains mainly locally, favoring the genetic divergence directed by the environment (Rellstab*et al.* , 2017; Sork, 2017). However, it is important to emphasize that we measured no local environmental variables and used microsatellite markers, which lead us to indicate future studies using potential markers under selection (eg: SNPs) to test our hypothesis of local adaptation with morphological and genetic differentiation.

CONCLUSION

The genetic grouping of yellow and red morphotypes indicates that these morphological variations found only in the state of Bahia and Espirito Santo in Brazil, are genetically different from the typical populations (green morphotype) of *E. edulis* found in all occurrence areas, including the Brazillian states aforementioned. Thus, we believe that unravel the relationship between phenotypic and genetic variation is an important advance in scientific knowledge for future conservation measures for this endangered species. In this sense, considering the low genetic variability reported in this study, the importance of developing conservation plans is highly recommended, particularly for populations with yellow or red morphotypes that have limited geographical occurrence.

ACKNOWLEDGEMENTS

This study was financed in part by the Coordenacao de Aperfeicoamento de Pessoal de Nivel Superior - Brasil (CAPES) - Finance Code 001. Funding was provided by Universidade Estadual de Santa Cruz (UESC) grant # 00220.1100.427 and Fundacao de Amparo a Pesquisa do Estado da Bahia (FAPESB) grant # 857/2004. Conselho de Desenvolvimento Cientifico e Tecnologico (CNPq) and Coordenacao de Aperfeicoamento de Pessoal de nivel Superior (CAPES) provided fellowships to the authors: productivity (FAG) (CNPq #306160/2017-0), postdoctoral researcher (ASS) and graduate (GMC). We thank Josiane

Amorim, Mariana Barreto, Elaini Oliveira, Marcelo Bramucci, Rodolpho Mafei, and Dario Ahnert for their field support.

COMPETING INTERESTS STATEMENT

The authors declare no conflict of interest

DATA ACCESSIBILITY

Relationship between pairwise F_{ST} and geographic distances (Figure S1), Estimated number of groups using the average method (Figure S2), Variation of the second order of the average values of maximum likelihood (Figure S3) uploaded as online Supplementary material. In addition, the raw data will be deposited at http://datadryad.org/.

AUTHOR CONTRIBUTIONS

G.M.Coelho and F.A.Gaiotto conceptualized and performed the study, contributed to writing the paper; A.S.Santos analyzed the data and wrote the paper; I.P.P.Menezes, R.Tarazi, F.M.O.Souza and M.G.C.P.C.Silva contributed to the study conception and writing the paper.

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Table 1 *E. edulis* populations sampled with their respective morphotypes, sample size, and estimation of genetic parameters.

Population	Morphotype	N	Na	Ne	PA	AR
EV	Yellow	16	5.28 (0.6)	3.04 (0.3)	1.07 (0.4)	4.41 (0.4)
BS	Yellow	40	8.36 (1.0)	4.68 (0.7)	0.57 (0.2)	5.65 (0.6)
BR	Red	40	8.28 (1.0)	3.88 (0.5)	0.64 (0.2)	5.32 (0.6)
AE	Red	40	8.93 (1.0)	4.94 (0.6)	1.35 (0.3)	5.98 (0.5)
EU	Red	33	6.64 (0.6)	3.95 (0.5)	0.86 (0.3)	4.94 (0.4)
ST	Green	20	5.50 (0.6)	3.29 (0.4)	0.78 (0.3)	4.42 (0.5)
BN	Green	28	8.36 (0.7)	4.38 (0.3)	0.71 (0.2)	6.52 (0.4)
RE	Green	33	9.28 (0.7)	4.53 (0.4)	1.00 (0.3)	6.63 (0.5)
Mean total	-	31,25	7.58	4.08	0.87	5.48

Abbreviations: N = Size samples; Na = Mean number of alleles; Ne = Mean number alleles effectives; Pa = Mean private alleles; AR = Allelic richness; ( ) estimative standard error. *EV = Private Reserve of Natural Patrimony Estação Veracel; BS = Boa Sorte Farm; BR = Una Biological Reserve; AE = Farm Alto da Esperança; EU = Ecoparque de Una; ST = Private Reserve of Natural Patrimony Serra do Teimoso; BN = Brasilia National Park; RE = Roncador Ecological Reserve.

Table 2 Results of the Wilcoxon sign-rank test.

Population	IAM	TPM	SMM
EV	0.076	0.891	0.979
BS	**0.020**	0.749	0.982
BR	0.291	0.979	0.991
AE	**0.000**	0.786	0.948
EU	**0.010**	0.786	0.987
ST	**0.039**	0.891	0.991
BN	**0.002**	0.913	0.985
RE	**0.052**	0.985	0.998

Abbreviations: IAM = Infinite Alleles Model; TPM = Two-Phase Model; SMM = Stepwise Mutation Model. Significant values are shown in bold. EV = Private Reserve of Natural Patrimony Estação Veracel; BS = Farm Boa Sorte; BR = Una Biological Reserve; AE = Farm Alto da Esperança; EU = Ecoparque de Una; ST = Private Reserve of Natural Patrimony Serra do Teimoso; BN = Brasilia National Park; RE = Roncador Ecological Reserve.

APPENDIX

Figure S1 Relationship between pairwise F_{ST} and geographic distances for the eight populations sampled of *E. edulis* , with Mantel test (r=0.134, p=0.33).

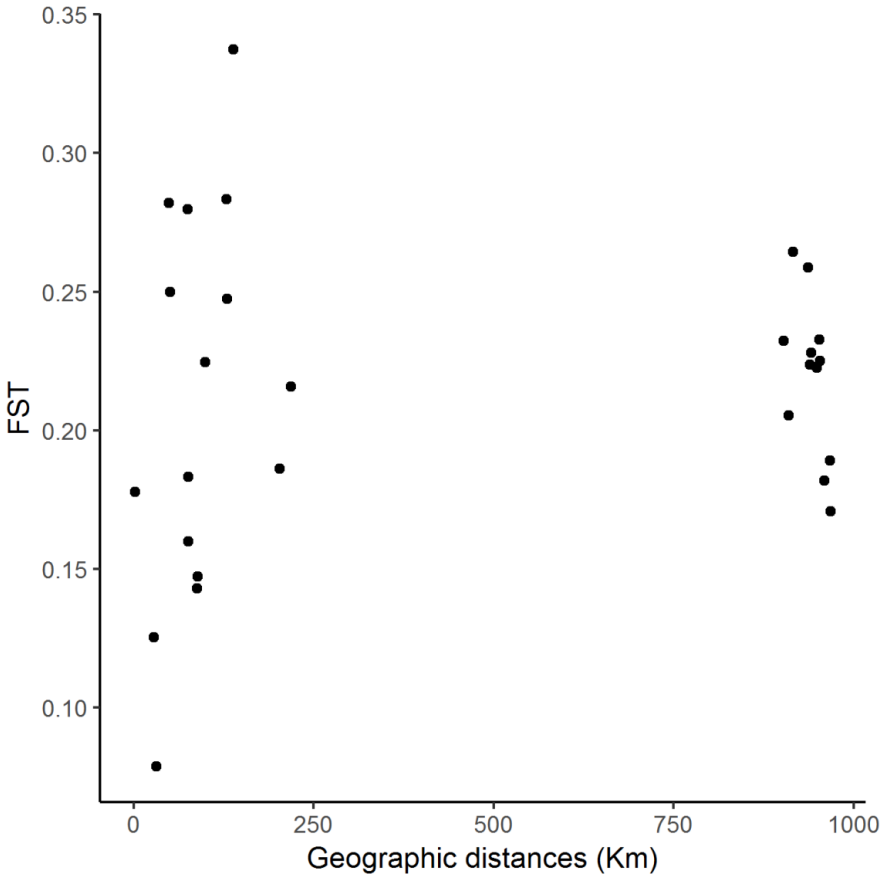

Figure S2 Estimated number of groups using the average method for the number of populations K in 250 individuals of *E. edulis*of the eight populations sampled.

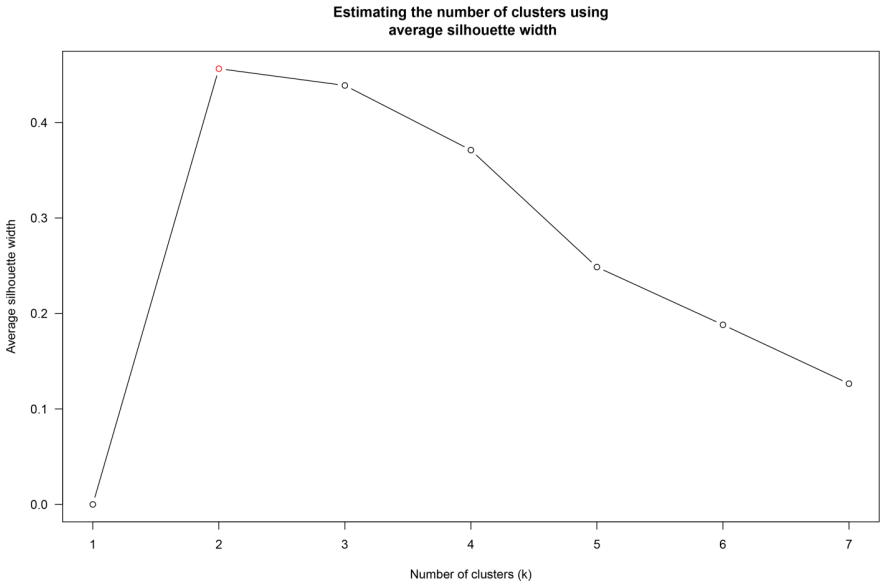

Figure S3 Variation of the second order of the average values of maximum likelihood for the number of populations K in 250 individuals of *E. edulis* of the eight populations sampled.