Genetic Diversity of *Campomanesia adamantium* and Its Correlation with Land Use and Land Cover

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Abstract: (1) Background: *Campomanesia adamantium* is an endemic species of the Cerrado and this biome has been under threat by its constant degradation. The genetic diversity of *C. adamantium* was characterized using species-specific microsatellites in two different time periods and correlations of these parameters of genetic diversity with the land use and land cover data. (2) Methods: We used 10 microsatellite loci to analyze *C. adamantium* from five populations, collected in 2011 and 2017. Maps of land use and land cover of the collection sites in both years were generated and subsequently correlated with genetic diversity. (3) Results: No significant loss of genetic diversity was observed in the analyzed period and a positive inbreeding coefficient was observed in all populations. AMOVA and STRUCTURE showed that there is no population structure between years and populations. Significant Pearson correlations were observed in 2017 between parameters of genetic diversity and land use and land cover, with a positive correlation between expected heterozygosity and secondary vegetation, and a negative correlation between inbreeding coefficient and exposed soil. (4) Conclusions: There was no decline in genetic diversity from 2011 to 2017. The high rates of the inbreeding coefficient could lead, for the species, in the long run, to an inbreeding depression as the Cerrado fragmentation might cause a population bottleneck.

Keywords: Cerrado fragmentation; genetic variability; Guavira; microsatellite markers; temporal change

1. Introduction

The Cerrado is the second largest biome in Brazil and is considered a world biodiversity hotspot, with nearly 12,700 known plant species, of which 33.3% are endemic to the biome [1]. However, the biodiversity of Cerrado is being altered by changes to land use and land cover, which has led to a loss of some 50% of the native vegetation and compromised the genetic diversity of many species [2–4].

Among the many species of this biome, *Campomanesia adamantium* (Cambess.) O. Berg is an endemic species that is widely found in the Cerrado biome, part of the Myrtaceae family, and is commonly known as guavira, guabiroma, or gabiroma. The species *C. adamantium* presents a natural distribution in several regions of Brazil and other countries, such as Argentina and Paraguay. In 2017, the guavira (*Campomanesia* ssp.) was declared a symbol of the state of Mato Grosso do Sul.
This species is a perennial shrub or subshrub measuring 0.30 to 2 m in height that is heavily branched and usually forms thickets [3]. *C. adamantium* reproduction occurs by pollination, exhibiting self-incompatibility, that is a widespread genetic mechanism to prevent self-fertilization due to the evolutionary advantages of out-crossing [6]. Xenogamy is the predominant mode of reproduction; in addition, geitonogamy (pollination between flowers of the same plant) mode could occur too, once the effective pollination is predominantly performed by bees [5]. These factors can influence patterns of seed and pollen dispersal, which is the species’ mode of reproduction [7]. *C. adamantium* has great regional value because its fruits are commercialized and consumed fresh or used in various types of foods, such as sweets, ice cream, and beverages [8,9]. The fruits have a round shape and a color ranging from dark to light green and yellow, with a sweet and pleasant aroma. This species can resist flooding and has been used for spoiled-area reforestation projects. The species is used in traditional medicine and several studies have demonstrated its antimicrobial [10–12], antidepressant [13], and antioxidant activities [14,15], among others.

Considering the economic, medicinal, and cultural importance of this species, it is necessary to know its genetic diversity for future management and conservation programs. In order to assess the genetic diversity of plant populations, molecular markers, such as microsatellites, are commonly used, which are short-repeat tandem highly informative, codominant, multi-alleles, which are well distributed across the genome of organisms, and can be easily detected by PCR [16–18].

Studies with microsatellite markers in *C. adamantium* were conducted by Miranda et al. [19] and Crispim et al. [20] and both studies evaluated genetic diversity using markers derived from *Eucalyptus* sp., also a member of the Myrtaceae family. Recently, Crispim et al. [21] characterized a set of microsatellites specific for *C. adamantium*, and their results were different from those mentioned regarding inbreeding in native populations of the species, thus indicating that the use of species-specific microsatellite sets can provide more accurate information on genetic variability. The lack of information about the genetic diversity of *C. adamantium* using species-specific microsatellite sets as well as the relations of land use and land cover could have influenced the parameters of genetic diversity, once anthropic actions converted areas previously occupied by forest fragments into agricultural areas.

The expansion and intensification of human land use in recent decades has resulted in major changes in biodiversity [22]. The physical changes, including a reduction of habitat, from land use change can affect both gene flow and reproduction by reducing the abundance and density of plant individuals, thus resulting in a decrease in the population size and an increase in spatial isolation between populations by reducing the genetic diversity in a species [22,23].

The anthropogenic activities in Cerrado drastically converted the forest fragments in agriculture areas and these effects directly influenced the alterations in the scenario of the use and coverage of land. Therefore, these changes can form genetic variants, indicating that intense agriculture promotes a significant decrease in genetic variability and has implications for evolutionary biology, reproduction, ecology, and the conservation of this species [20]. In this context, the objectives of this study were to evaluate the genetic diversity of *C. adamantium* using species-specific microsatellites in two different time periods and the correlation of these parameters of genetic diversity with the land use and land cover data.

2. Materials and Methods

2.1. Study and Sampling Area

This study encompassed five sampling sites, four of which are located in the state of Mato Grosso do Sul, in the cities of Bonito, Dourados, Jardim, and Ponta Porã, and the fifth site, located in the Department of Amambay in Paraguay, in the Cerro Corá National Park (Figure 1). The sites Bonito, Jardim, and Cerro Corá are located in environmental reserve areas, and consequently are less affected by anthropic activity although surrounded by
farms and agricultural areas. The other sites (Dourados and Ponta Porã) are located in the vicinity of agricultural areas, where land degradation is high.

| Sample Sites | Latitude | Longitude | Location                  |
|--------------|----------|-----------|---------------------------|
| Bonito (BO)  | -21.150583 | -56.470222 | Mato Grosso do Sul, Brazil |
| Dourados (DO)| -22.137861 | -55.140056 | Mato Grosso do Sul, Brazil |
| Jardim (JA)  | -21.42875  | -56.224944 | Mato Grosso do Sul, Brazil |
| Ponta Porã (PP)| -22.712333 | -55.642111 | Mato Grosso do Sul, Brazil |
| Cerro Corá (CC)| -22.662278 | -56.031278 | Amambay, Paraguay         |

**Figure 1.** Geographic locations of sampling sites of *Campomanesia adamantium* in the years 2011 and 2017 and number of collected individuals (n) from each location in both years. Figure by Julio César Jut Solórzano 2018.

In 2017, little had changed in the sites of Bonito, Jardim, and Cerro Corá since 2011, as these areas remained preserved. On the other hand, after six years, the Ponta Porã population had almost completely disappeared, being left in a vegetation corridor of a few plants of the species. This site is surrounded by farms and is being replaced by crops. Dourados remained as a little patch of Cerrado also surrounded by farms.

Samples of *C. adamantium* leaves were collected at two time points (December 2011 and December 2017) and the number of individuals per population were sampled each year from the five sampling sites, which is shown in Figure 1. Only unique multilocus genotypes were detected across populations. In 2011, for each population, a geographical coordinate was used and from this central point the sampling occurred in a 50-m radius. So, in 2017, we located this central point and also collected within the same radius. The leaf samples were collected from the youngest plants and established at a distance of at least one meter between each thicket to avoid collecting clones from the same thicket (clones). In total, biological material was obtained from 280 individuals, of which 146 were sampled in 2011 and 134 were sampled in 2017.

### 2.2. Characterization of the Collection Sites

Land use and land cover in the collection sites were characterized by mapping to determine the degree of fragmentation of the areas occupied by the populations. The maps were constructed from high-resolution aerial images obtained from Google Earth Pro® for the periods of December 2011 and December 2017, within a radius of 1 km from the geographical coordinates located at the central point of the graph (Figure 1). The parameters assessed for land use were agriculture, building areas, water bodies, forest fragments, exposed soil, pastureland, and secondary vegetation.
The interpretation of the images was based on visual classification using the ArcGIS 10.4® software, a program used to calculate the areas and percentages of land use and land cover by each category [24].

2.3. DNA Extraction and Genotyping

The leaves (200 mg) were macerated in liquid nitrogen and DNA was extracted using the protocol adapted from Doyle and Doyle [25], using an extraction buffer containing 3% CTAB (w/v), 25 mM EDTA, 100 mM Tris-HCl (pH 8.0), 2 M NaCl, 5% PVP (w/v), and 2% betamercaptoethanol (w/v). Then, 10 species-specific microsatellite marker loci (Table S1), developed by Crispim et al. [21], were amplified.

The amplification of the markers was performed in a final volume of 10 µL containing 4.2 µL of ultrapure water (Integrated DNA Technologies, Coralville, IA, USA), 0.9 µL (10 pmol/µL) of forward and reverse primers (Thermo Fisher Scientific, Waltham, MA, USA), 3 µL of 5X FIREPol® Master MIX (Solis BioDyne, Tartu, Estonia), and 10 ng of DNA. Amplification consisted of an initial cycle at 94 °C for 6 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 60 °C for 1 min, extension at 72 °C for 1 min, and a final extension at 72 °C for 10 min using the MyCyclerTM thermal cycler (BIORAD®). The annealing temperature for all markers was set at 60 °C.

The microsatellite loci were genotyped in the fluorescence-based capillary electrophoresis equipment Fragment Analyzer (Advanced Analytical Technologies, Orangeburg, NY, USA) using the DNF-905-55 kit (1–500 bp) and 5 µL of the amplified product of each sample. Determination of fragment sizes was carried out using the software PROSize® Data Analysis version 2.0 (Advanced Analytical Technologies, Orangeburg, NY, USA).

2.4. Analysis of Genetic Diversity According to Year and Population

The genetic diversity of C. adamantium among the populations in the years 2011 and 2017 was characterized using the following parameters: number of alleles (A), allelic richness (AR), expected heterozygosity (HE), observed heterozygosity (HO), and inbreeding coefficient (FIS). The analyses were performed using the R software version 3.4.4 [26]. A was estimated in adegenet [27] package. To verify if there were any clones among the individuals, we used the “mlg” function from poppr [28]. Tests for deviation from Hardy–Weinberg equilibrium (HWE) for proportions at each population, AR, FIS, and FST were performed in diveRsity [29] package; HE and HO in PopGenKit [30] package.

2.5. Population Structure

The hierarchical analysis of molecular variance (AMOVA) was performed in terms of years and populations considering 999 permutations using poppr.

The individuals were grouped using a probabilistic approach inferred by Bayesian analysis and using the STRUCTURE software [31] and the tests were performed using the admixture model. To determine the appropriate number of inferred populations (K), STRUCTURE was run with K ranging from 2 to 10, with 200,000 burn-in periods, 500,000 iterations of Markov chain Monte Carlo (MCMC), and 10 independent repetitions for each tested K. The actual values of K were inferred from the ΔK peak value as a function of K, with the aid of the Structure Harvester software [32] based on the model proposed by Evanno et al. [33].

2.6. Correlation of Genetic Diversity with Land Use and Land Cover

PCA analysis and Pearson’s correlation (r and p-values) [34] was carried out to determine the relationship between the conditions of land use and land cover (categories in percentage of agriculture, building areas, water bodies, forest fragments, exposed soil, pastureland, and secondary vegetation) and the parameters of genetic diversity, HE, HO, and FIS. Correlations were made between each category and parameters of diversity in the two assessed years with the R software, using the Vegan [35] and Hmisc [36] packages.
3. Results

The means of the population-related genetic diversity parameters were similar in both years (2011 and 2017). $H_E$ ranged from 0.81 to 0.85 and the $F_{IS}$ showed a greater variation from 0.31 to 0.54 in 2011 (Table 1), and a similar diversity was observed in 2017, with the range of $H_E$ from 0.79 to 0.85, and that of $F_{IS}$ from 0.26 to 0.47.

Table 1. Genetic diversity for Campomanesia adamantium in the years 2011 and 2017 and for each population in each year.

| Year | N   | A   | $A_R$ (95% CI) | $H_E$ (95% CI) | $H_O$ (95% CI) | $F_{IS}$ (95% CI) | HWE |
|------|-----|-----|----------------|---------------|----------------|------------------|-----|
| 2011 | 146 | 15.5| 14.34 (13.7–14.9) | 0.86 (0.85–0.87) | 0.49 (0.46–0.52) | 0.43 (0.39–0.47) | 0   |
| 2017 | 134 | 15.3| 14.33 (13.8–14.8) | 0.87 (0.85–0.87) | 0.54 (0.49–0.56) | 0.39 (0.36–0.43) | 0   |

The population analyses showed nearly similar results in both years, with no significant loss of diversity. The $F_{IS}$ was positive for all populations, in both years, indicating the presence of inbreeding and explaining the deviations found in the Fisher’s exact test from the expected Hardy–Weinberg equilibrium proportions (Table 1).

The mean apparent outcrossing rate among individuals in the $C.\ adamantium$ populations was 0.42 in 2011 and 0.47 in 2017 (Table 2), suggesting a mixed mating system.

Table 2. Apparent outcrossing rate ($t_a$) for Campomanesia adamantium populations in the years 2011 and 2017.

| Population | 2011 | 2017 |
|------------|------|------|
| Bonito     | 0.48 | 0.58 |
| Dourados   | 0.29 | 0.45 |
| Jardim     | 0.52 | 0.54 |
| Ponta Porã | 0.42 | 0.36 |
| Cerro Corá | 0.41 | 0.41 |
| Média      | 0.42 | 0.47 |
AMOVA analysis (Table 3) did not provide any inference on population structure and revealed that the greatest variation occurred within individuals (52%) both between years and between populations. The variation between years was 1%, with a global $F_{ST}$ of 0.013 (0.0080–0.0192), and variation between populations was 3%, with a global $F_{ST}$ of 0.028 (0.0139–0.0270).

Table 3. Analysis of molecular variance (AMOVA) to evaluate the genetic structure for Campomanesia adamantium.

| Source of Variation                                      | Degree of Freedom | Sum of Squares | Mean Square | Estimated Variance | Variance Percentage |
|----------------------------------------------------------|-------------------|----------------|-------------|--------------------|---------------------|
| Among years                                              | 1                 | 22.211         | 22.211      | 0.056              | 1%                  |
| Among individuals within populations within years        | 278               | 1786.790       | 6.427       | 2.067              | 47%                 |
| Among individuals within each year                       | 280               | 642.000        | 2.293       | 2.293              | 52%                 |
| Total                                                    | 559               | 2451.002       | 4.417       |                     | 100%                |

$F_{ST} = 0.013$. $p = 0.001$

Bayesian analysis using the STRUCTURE software to assign individuals to groups showed that the most likely $K$ was 3, which is the most likely population and years structure (Figure 2). However, observing the STRUCTURE graph, it is not possible to identify three distinct genetic groups.

The $F_{ST}$ for both population and year, although it was significant, was very low. Therefore, a lack of a genetic structure for populations and years is evident.

The proportions of land use and land cover conditions are demonstrated in Figure 3. In general, over the years 2011 and 2017, there was an increase in the areas of agriculture, exposed soil, and pastureland and a decrease in forest fragments and secondary vegetation. In both years, Dourados and Ponta Porã had the highest percentages of agricultural area, and these populations together with Jardim had a decrease in the area under secondary vegetation cover.

Pearson correlation revealed a significant positive correlation between genetic diversity ($H_E$) and secondary vegetation ($p = 0.05$) and a negative correlation between the inbreeding coefficient ($F_{IS}$) and exposed soil ($p = 0.0011$) in 2017. The correlation values and their respective $p$-values are presented in Table 4.
**Figure 2.** Population genetic structure of *Campomanesia adamantium*. Δ*K* statistic of Evanno et al. [33]. Designation of the populations of *C. adamantium* in the years 2011 and 2017.

**Figure 3.** Proportions of land use and land cover categories for each sampling site for *Campomanesia adamantium* populations in the years 2011 and 2017. Figure by Julio César Jut Solórzano 2018.

**Table 4.** Spearman correlation between land use and land cover and genetic diversity estimates of the year 2017.

| Correlation                  | Pearson Statistics | \( r \) | \( p \) |
|------------------------------|--------------------|---------|-------|
| \( H_E \) and Agriculture    |                    | -0.167 | 0.788 |
| \( H_E \) and Buildings areas|                    | -0.017 | 0.978 |
| \( H_E \) and Forest fragments|                   | 0.453  | 0.444 |
| \( H_E \) and Exposed soil   |                    | -0.096 | 0.878 |
| \( H_E \) and Pastureland    |                    | -0.081 | 0.898 |
| \( H_E \) and Secondary vegetation |                | 0.874  | 0.05 * |
| \( F_IS \) and Agriculture   |                    | 0.644  | 0.24  |
| \( F_IS \) and Buildings areas|                   | -0.676 | 0.211 |
| \( F_IS \) and Forest fragments|                   | 0.173  | 0.781 |
| \( F_IS \) and Exposed soil   |                    | -0.957 | 0.011 *|
| \( F_IS \) and Pastureland   |                    | -0.183 | 0.769 |
| \( F_IS \) and Secondary vegetation |            | 0.558  | 0.328 |

\( H_E \): expected heterozygosity; \( F_IS \): inbreeding coefficient. *Significant \( p \)-value \( \leq 0.05 \).

While there was a decrease in forest fragments and secondary vegetation areas from 2011 to 2017, there was an increase in exposed soil in the same period (Figure 3). However, the increase in exposed soil areas was associated with a decrease in the inbreeding coefficient (Figure 4).
HE and Pastureland $-0.081 \ 0.898$
HE and Secondary vegetation $0.874 \ 0.05^*$
FIS and Agriculture $0.644 \ 0.24$
FIS and Buildings areas $-0.676 \ 0.211$
FIS and Forest fragments $0.173 \ 0.781$
FIS and Exposed soil $-0.957 \ 0.011^*$
FIS and Pastureland $-0.183 \ 0.769$
FIS and Secondary vegetation $0.558 \ 0.328$

$HE$: expected heterozygosity; $FIS$: inbreeding coefficient. $^*$ Significant $p$-value $\leq 0.05$.

While there was a decrease in forest fragments and secondary vegetation areas from 2011 to 2017, there was an increase in exposed soil in the same period (Figure 3). However, the increase in exposed soil areas was associated with a decrease in the inbreeding coefficient (Figure 4).

**Figure 4.** Principal component analysis (PCA) based on the genetic parameters (FIS and HE) and the land cover conditions (forest fragmentation, secondary vegetation, pastureland, and exposed soil) for the five populations (color black) of the year 2017.

**4. Discussion**

The genetic diversity of microsatellite loci showed high polymorphism, primarily through the mean number of alleles per locus (17.1) and allelic richness (16.7). This high informativity due to the number of alleles is explained by the repetition of microsatellite motifs. According to Vieira et al. [18], longer perfect repetitions lead to more frequent mutations, and new alleles are formed if DNA repair mechanisms do not correct these mutations, characterizing loci with different alleles.

Allelic richness is a measure of allelic diversity per locus and reflects the existing genetic variation. Thus, it may correlate with the adaptive potential of the species in relation to environmental changes, which indicates the evolutionary ability of the populations. Therefore, a decrease in allelic richness limits the adaptive potential, because variability depends on allelic richness [37–39]. In our results, the allelic richness was not different when compared between years and among populations, indicating that the population effective size was not affected. Miranda et al. [19] reported mean $A$, $HE$, and $H_O$ values of 6.83, 0.517, and 0.504, respectively, after analyzing the genetic diversity of *C. adamantium* by microsatellites transferred from *Eucalyptus* sp. Likewise, Crispim et al. [20] analyzed the same parameters and obtained means values of 5.49, 0.57, and 0.59, respectively. Therefore, in both studies, the values of the $A$ and $HE$ parameters were lower than those obtained in the current study regarding genetic diversity in 2011 and 2017 (Table 1) and there was no intrapopulation inbreeding [20].

Crispim et al. [21] characterized a set of 41 specific microsatellites in *C. adamantium*, out of which, 10 were used in this study, and, as expected, their results were similar to those of our study. Thus, our results indicate that specific markers provide a more accurate assessment than others of aspects related to genetic variability, possibly because the transfer of microsatellites between species may produce an inaccurate representation of genetic
Diversity ultimately causing misinterpretation of the actual situation of populations [40]. This is attributed to the fact that no inbreeding was observed in the populations in the studies by Miranda et al. [19] and Crispim et al. [20], which assessed the genetic diversity of *C. adamantium* through transferred markers.

In another study, *C. adamantium* seeds were collected from native plants, sown and transplanted into the field 11 months later, with controlled nutrient and irrigation conditions [41]. Ripe fruits were harvested within 10 to 13 months after field transplantation, giving a total period of 21 to 23 months for fruit production. Therefore, the complete reproductive cycle for the species, from germination to fruit production, is around two years, in a controlled environment [42]. There was a gap of six years between our collections, so theoretically there were two to three reproductive generations of *C. adamantium* in our study, attributed to the natural environment conditions that may have contributed to longer reproductive periods.

When the diversity of the populations in 2011 and 2017 was characterized in the five regions, there was a similarity between the genetic diversity parameters of all the regions, except those of the population of Ponta Porã, which had a lower sample number in 2017. Therefore, the genetic diversity of the population was maintained, although two plant reproductive generations had passed.

All populations had positive $F_{IS}$ and this parameter represents measures of deviations from the Hardy–Weinberg equilibrium proportions within populations. Thus, positive values indicate heterozygosity deficiency, causing inbreeding [43,44]. The observed positive $F_{IS}$ may also suggest a mixed mating system.

On analyzing the reproduction by pollination, *C. adamantium* exhibited self-incompatibility, with xenogamy being the predominant mode of reproduction; in addition, geitonogamy (pollination between flowers of the same plant), which is predominantly performed by bees, also shows effective pollination [5,8,45]. These data support the results regarding inbreeding and mode of reproduction, further confirming a mixed reproductive strategy of the species.

Activities, such as the intensification of agriculture, affect biodiversity in terms of habitat reduction as well as the presence of pollinators when exposed to agrochemicals [46]. Changes in the environment due to anthropogenic activities affect the nesting of pollinators and the distance to which they can pollinate [47], which in turn reduces gene flow [48] and leads to inbreeding.

Genetic structure described by AMOVA revealed low structuring between populations, because $F_{ST}$ values ranging from 0 to 0.05 indicate low genetic differentiation [49,50]. The population structure analyzed through Bayesian inference using the STRUCTURE software between years and among populations showed that the analysis between years also revealed low genetic structure.

The correlation analysis between the genetic diversity parameters land use and land cover data indicated environment degradation, determined by the decrease in forest fragment and secondary vegetation areas and an increase in the areas of exposed soil. Some studies have shown that habitat degradation leads to a reduction in the number and size of habitat fragments, in addition to increased isolation of these areas [51–53]. Habitat fragmentation and loss are considered the fundamental causes of a decline in biodiversity, and when it causes population decline and isolation, it puts the species survival at risk [53,54].

The highest proportion of the increase in exposed soil was observed at Jardim, characterized by the transition from pastureland areas to agricultural areas between 2011 and 2017. Similarly, there was an increase in exposed soil, agriculture, and pastureland areas, concomitant with a decrease in forest fragments and secondary vegetation for the other populations. Loss of forest fragment and secondary vegetation areas and an increase in agriculture and pasture were observed in 2017 at Ponta Porã, and there was only one vegetation corridor at the collection site along with a reduced number of *C. adamantium* individuals ($n = 15$). Therefore, population size was affected by the fragmentation and
conversion of the native habitat into agricultural area. Our results indicate that *C. adamantium* has been affected by degradation influenced by land use and land cover. The genetic diversity of the populations was reduced when compared as intact versus degraded areas in the different populations analyzed.

The *C. adamantium* species has environmental relevance, because it is used in the recovery of degraded areas [55], traditional medicine [13,15,56], and is of economic and cultural importance in the regions of Mato Grosso do Sul state [54]. Our study did not detect loss of genetic diversity in the two assessed years. The species is endemic to the biome, and therefore its genetic variability must be maintained to guarantee sustainable use and land cover of the Cerrado soil and thus ensure conservation of the species.

5. Conclusions

The six-year period was not sufficient to observe changes in the genetic diversity of *C. adamantium*. The reproductive cycle of *C. adamantium* is approximately two years; thus, the genetic diversity was not affected by habitat fragmentation in the evaluated period, even with the reduction in forest fragment and areas under secondary vegetation and the increase in areas of agriculture, pasture, and exposed soil. Significant correlations were observed in the analysis of the 2017 data between genetic diversity and land use and land cover. Considering the genetic conservation, the authors suggested that more individuals could be collected in various populations in order for *C. adamantium* to increase Ne, to ensure additive genetic variation. Therefore, these results indicate that, over a longer period of time, environment degradation by anthropic activities may alter the levels of species genetic diversity, and even reduce the size of the populations.

Supplementary Materials: The following are available online at https://www.mdpi.com/1424-2818/13/4/160/s1, Table S1: Characteristics of 10 microsatellite markers used in the genotyping of Campomanesia adamantium; Table S2: Genetic diversity for *Campomanesia adamantium* using 10 specific microsatellite loci; Table S3 Estimating global FST of Weir (1996) both using and without using the ENA correction to evaluate the effect of null alleles per locus and all loci.

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