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Genetic diversity in natural populations of the endangered Neotropical orchid *Telipogon peruvianus*

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Abstract

Telipogon peruvianus is a highly restricted, sexually deceptive, Neotropical orchid species, endemic to the southern Peruvian Andes. It is only known from two localities, which are affected by anthropogenic disturbance. Here, we investigated whether the restricted distribution of *T. peruvianus* has led to low genetic diversity and inbreeding, thus threatening its survival. We isolated 10 novel microsatellite loci specific for *T. peruvianus* (and that also cross-amplified in related species) from two adjacent populations. We found that genetic diversity within populations was only moderately reduced, along with some evidence of inbreeding. We found low levels of genetic differentiation, suggesting connectivity by pollen/seed flow between the two populations. Effective population size was comparable to the real number of flowering individuals in the populations and we did not detect the signature of recent bottlenecks. Taken together, these results show that, despite increasing anthropogenic pressure, the two investigated populations of *T. peruvianus* still host valuable genetic diversity that should be preserved through appropriate conservation strategies.

KEYWORDS

endangered species, genetic diversity, microsatellites, Oncidiinae, tropical orchids

1 | INTRODUCTION

Both the size and number of populations in the species range are likely to have a strong impact on genetic diversity and differentiation of a rare species (Bouzat, 2010). Species with small and fragmented distributions are expected to have an elevated risk of loss of genetic variation (Loveless & Hamrick, 1984). Thus, the characterization of genetic variation and interpopulation gene flow is crucial to establish adequate management plans for endangered populations and/or species (Frankham, 2003). Genetic variation in plant species is the result of several factors, such as their reproductive biology, levels of outcrossing, life-history traits, population history, geographical range or selective pressures (Linhart & Grant, 1996). These factors also determine how genetic variation is partitioned among and within populations (Hamrick, Godt, & Sherman-Broyles, 1992). Another important factor that can be detrimental for genetic diversity in small, isolated populations is the increased likelihood of stochastic processes such as genetic drift (Byers & Waller, 1999; Ellstrand & Elam, 1993). This can either be a consequence of bottlenecks and founder effect or can be an independent process.

In animal-pollinated plant species with small and isolated populations, pollination can also be affected by a reduction in the quality and quantity of pollen and pollinator limitation (Wilcock & Neiland, 2002). These factors

together may strongly influence population genetic diversity and between-population differentiation (Rathcke & Jules, 1993; Steffan-Dewenter & Tscharntke, 1999).

Orchids are a flagship group in plant conservation (Cribb, Kell, Dixon, & Barrett, 2003) and the Neotropical orchid flora is characterized by extremely high diversity and generally restricted populations (Crain & Tremblay, 2012; Jost, 2004; Pandey, Sharma, Taylor, & Yadon, 2013; Pérez-Escobar et al., 2017; Tremblay & Ackerman, 2001). The tropical Andes is the most species-rich area in the Neotropics and the most species-rich biodiversity hotspot worldwide (Myers, Mittermeier, Mittermeier, Fonseca, & Kent, 2000; Pérez-Escobar et al., 2017). Orchids are highly characteristic plants in the Andes and epiphytic orchids can account for up to 50% of the total epiphytic flora in some Andean regions (Pérez-Escobar et al., 2017); for example, in Colombia more than 10,000 orchid species have been estimated to occur in the Andean Cordilleras (Kirby, 2016). The existing evidence indicates that Andean orchid diversity is threatened and has suffered dramatic declines in abundance, mostly due to land cover change and increased habitat fragmentation (Dodson & Gentry, 1991; Parra-Sánchez, Retana, & Armenteras, 2016). This latter is often linked to a reduction in the levels of gene flow, to an increased isolation-by-distance and to a high among-populations genetic differentiation (Muñoz, Warner, & Albertazzi, 2010; Slatkin, 1987). Therefore, quantifying the genetic variation in species with small and isolated populations is required for establishing conservation plans for the maximization of genetic diversity (Frankham, Ballow, & Briscoe, 2002; Van Dyke, 2008).

The genus *Telipogon* Kunth (Orchidaceae, Oncidiinae), which encompasses approximately 200 species (Martel & Nauray, 2013), is exclusively Neotropical and is distributed in Central America, in the Caribbean and, along the Andes, from Venezuela to Bolivia (Ackerman, 2004; Bogarín, 2012; Pridgeon, Cribb, Chase, & Rasmussen, 2009). In Peru, the genus is represented by 55 species (Collantes & Martel, 2015; Martel, Collantes, & Egoavil, 2017), with most having highly restricted distribution (Collantes & Martel, 2015; Nauray & Galán, 2008). Among these, *T. peruvianus* T. Hashim. is a highly endangered species because it has a small and fragmented distribution consisting of few populations that only occur in two localities in the basins of the Peruvian rivers Araza and Q'eros in the Cusco region (southern Peru, Martel & Nauray, 2013; Martel, Cairampoma, Stauffer, & Ayasse, 2016). Pollination in this species is exclusively mediated by insects: its flowers attract sexually excited male flies of *Eudejeania* aff. *Browni* (Tachinidae) and its pollination strategy involves the imperfect mimicry of a female fly perched on a daisy, eliciting male sexual behavior (Martel et al., 2016; Martel, Francke, & Ayasse, 2019). The negative genetic consequences of small and fragmented populations in this species may be potentially compensated for by the fact that sexually deceptive species have been shown to experience increased outcrossing, thus leading to lower levels of among-population differentiation when compared with species employing a rewarding pollination strategy (Scopece, Cozzolino, Johnson, & Schiestl, 2009; Scopece, Schiestl, & Cozzolino, 2015).

Quantifying the levels of genetic variation and gene flow between *Telipogon peruvianus* populations is of crucial importance in order to assess the extinction risk in this extremely rare Peruvian endemic species and to propose effective conservation strategies. With this aim, we developed and applied a new set of polymorphic microsatellite markers to investigate whether the highly restricted and patchy distribution severely affects the occurrence of gene flow and threatens the species survival.

2 | MATERIALS AND METHODS

2.1 | Study species and distribution

Telipogon peruvianus is an epiphytic perennial plant (Martel & Nauray, 2013). The species is self-compatible but is strictly dependent on animal vectors for pollination. This is carried out by *Eudejeania* aff. *Browni* (Tachinidae) males attracted through a specialized sexually deceptive strategy (Martel et al., 2016, 2019). *Telipogon peruvianus* blooms from June to September (with a mean flower longevity of 33 days; Martel et al., 2016) in semi-dense populations near to forest clearings or to their boundaries. As typical for the orchid family, their dust-like seeds are dispersed by wind. The species is only known from two localities: (a) near to the town of Marcapata in the Araza river basin and (b) near to the town of Quero in the Q'eros river basin, both in the Department of Cusco between 2,600 and 3,200 m a.s.l., in the basin slopes (Martel & Nauray, 2013). Plants of *T. peruvianus* can be found in the cloud forests of the southern Peruvian Andes on trees, shrubs and bamboo-like grasses, associated with mosses, and near to roads and trails in disturbed or sunny areas (Martel & Nauray, 2013). Its habitat is currently severely affected by deforestation, livestock, agriculture expansion and the inter-oceanic (Peru–Brazil) highway (Martel & Nauray, 2013; Roque & León, 2006). Hence, its conservation status has been assessed as critically endangered (Roque & León, 2006).

Plant material collection was carried out from two populations (hereafter referred to as Trucha and Haccheria)

in the cloud forest near the Araza river between 2,800 and 3,000 m a.s.l. (Marcapata district) (Figure 1A). The two populations occurred in the slopes of two contiguous mountains and are approximately 1 km apart (Figure 1A). The region is characterized by two distinct seasons: dry from May to October (austral autumn and winter) and wet from November to April (austral spring and summer). Average annual rainfall is around 765 mm and average annual temperature is 12.8°C (Climate-Data.org). In the two populations, all visible and accessible flowering plants were sampled. The size of the census population, in terms of flowering individuals, is thus expected to be comparable to the number of sampled individuals.

2.2 | Isolation of microsatellite loci

Total genomic DNA was extracted from silica-gel-dried leaf tissue using a DNeasy Plant Mini Kit (QIAGEN, Hilden, Germany) from 96 *T. peruvianus* individuals coming from the two populations (Trucha and Haccheria; see Figure 1A), and from single individuals of another 10 species of genus *Telipogon* and two species of the sister genera *Hofmeisterella* and *Trichoceros* (Neubig et al., 2012; Williams, Whitten, & Dressler, 2005). Microsatellite markers were isolated following the FIASCO protocol (Fast Isolation by AFLP of Sequences Containing repeats) with minor modifications (Zane, Bargelloni, & Patarnello, 2002). A total of 500 ng genomic DNA was completely digested with 3 units of *Mse*I (Fermentas, Vilnius, Lithuania); digested DNA was ligated to *Mse*I AFLP adaptors (5′-GACGATGAGTCCTGAG-3′ / 5′-TACTCAGGACTCAT-3′) using 1 unit of T4 DNA ligase (Fermentas, Lithuania). The digestion-ligation mixture was diluted (1:10) and directly amplified using *Mse*I adaptor-specific primers (5′- GATGAGTCCTGAGTAAN-3′) following Pinheiro et al. (2008). Approximately 1 μg of amplified DNA fragments was hybridized with 200 pmol of 5′-biotinylated (AC)15, (GA)15, (GC)15 and (AT)15 as reported in Pinheiro et al. (2008) and amplified for 30 cycles with *Mse*I adaptor-specific primers with overhang for Illumina Sequencing (Illumina, San Diego, CA).

A shotgun library, including three *T. peruvianus* individuals, was built using the Nextera DNA Sample Preparation Kit v2 (Illumina) and sequenced with a MiSeq Reagent Kit v3 2x300 bp paired-end in a MiSeq benchtop sequencer (Illumina). Raw reads were demultiplexed, adapters and indices were removed from the reads, and FASTQ files were generated using MiSeq Reporter v. 2.5.1 (Illumina). The raw sequenced reads were processed using Trimmomatic software (Bolger, Lohse, & Usadel, 2014) and paired-end reads were merged using FLASH (Magoč & Salzberg, 2011) with an overlap parameter of at least 20 nucleotides. The resulting FASTQ files were converted to the FASTA format using Fastx_toolkit software (http://hannonlab.cshl.edu/fastx_toolkit).

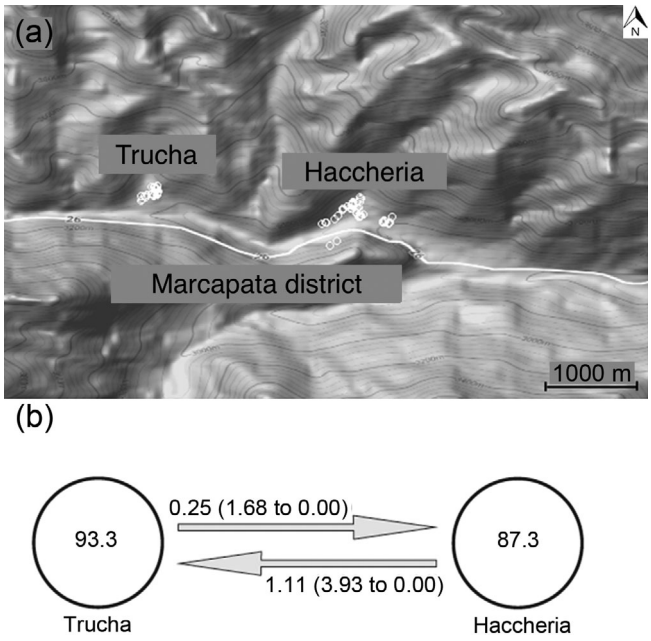

FIGURE 1 (a) Distribution map of *Telipogon peruvianus* populations near the town of Marcapata in the Araza river basin; (b) effective population size (inside circles) and number of immigrants per generation θM (below and above arrows), including 95% confidence intervals, calculated for Trucha and Haccheria populations. Arrows indicate the direction of gene flow

2.3 | Primer design and polymorphism screening of the microsatellite loci

A set of 50 potential microsatellite sequences was manually selected and primers were designed with Primer3 software (http://frodo.wi.mit.edu/primer3/). Primers with a size ranging from 18 to 24 bp and with a melting temperature (Tm) from 50 to 65°C were selected. Using the Primer-BLAST software (NCBI), selected primers were aligned with the enriched library to exclude that they match with more than one DNA region.

For each microsatellite locus, the forward primers were synthesized with a 5′-M13 tail according to Schuelke (2000), including the primer sequence and a universal M13 primer labeled with different fluorescent dyes 6-FAM, NED, JOE and HEX (Thermo Fisher Scientific-Applera, Waltham, MA). All polymerase chain reaction (PCR) amplifications were performed in an Applera 2,700 thermocycler according to PCR conditions set in Pinheiro et al. (2008). The conditions were maintained constant for all loci in order to maximize standardization.

Capillary electrophoresis of the amplified products was performed on an ABI3130 Sequencer, using LIZ (500) as internal size standard. Genotyping was conducted in the software GENEMAPPER v.3.7 (Thermo Fisher Scientific-Applera, Waltham, MA).

Out of the 50 primer pairs tested, 10 were further applied in 96 *T. peruvianus* individuals (See Table S1). We also checked the potential for cross-species amplification for the 10 loci in one sample from each of the 10 *Telipogon* species and in two species of the allied genera *Hofmeisterella* and *Trichoceros*.

2.4 | Genetic diversity estimates

Samples of the 96 individuals from the two investigated populations of *T. peruvianus* were analyzed to evaluate microsatellite polymorphism distributed among loci and populations. The microsatellite dataset was tested for genotyping errors due to stuttering, short allele dominance and null alleles using a Monte Carlo simulation of expected allele size differences with Micro-Checker (van Oosterhout, Hutchinson, Wills, & Shipley, 2004). Arlequin v. 3.5.2.2 (Excoffier & Lischer, 2010) was used to estimate the number of alleles (A), expected heterozygosity (H_E), observed (H_O) heterozygosity and deviations from Hardy–Weinberg equilibrium (HWE) for each locus.

Population-wise genetic diversity of the two populations of Trucha and Haccheria was assessed by estimating the number of alleles (A), the allelic richness (AR), the private allelic richness (PAR), and the expected (H_E) and observed (H_O) heterozygosity. The inbreeding coefficient (f) was then calculated using the software MSA v. 4.05 (Dieringer & Schlötterer, 2003) and HP-RARE v. 1.0 (Kalinowski, 2005). Significant departures from the Hardy–Weinberg equilibrium (HWE) were assessed using exact tests in Genepop v. 4.0 (Raymond & Rousset, 1995).

2.5 | Population structure and demography

Population genetic structure was assessed by estimating pairwise F_{ST} among populations using Genodive (Meirmans & Van Tienderen, 2004). We estimated the significance of the *F*-statistic using a resampling approach (with 10,000 permutations). The population differentiation estimate D_{ST} was used to verify that F_{ST} estimates were not an artifact of high intrapopulation diversity. Indeed, the Dst index was described by Jost (2008) as an unbiased estimator of genetic differentiation due to the fact that it is not affected by the high intrapopulation diversity observed in short sequence repeat (SSR) loci. To show the degree of overlap of the two populations, we performed a principal coordinate analysis (PCoA) on genetic data using GenAlEx 6.5 (Peakall & Smouse, 2012).

To detect the consequences of demography on genetic diversity, we carried out the "heterozygosity excess test" using BOTTLENECK v.1.2 (Cornuet & Luikart, 1996) as described in Cozzolino, Noce, Musacchio, and Widmer (2003). This analysis assumes that a population with a reduction in effective population size exhibits a decreasing number of alleles and levels of heterozygosity, but with a faster reduction of number of alleles than in heterozygosity (Allendorf, 1986; Maruyama & Fuerst, 1985; Watterson, 1984). Following Pinheiro et al. (2013), the occurrence of a recent bottleneck was also tested by using the *M*-ratio method (Garza & Williamson, 2001). In this test, *M* is the ratio between the number of alleles at a locus and the total range in allele sizes. Because rare alleles are lost more regularly during a population bottleneck, unless all rare alleles are at the ends of the allele size distribution, *M* will be reduced in populations having undergone a significant population size reduction. We used the software M_P_VAL (Garza & Williamson, 2001) to calculate *M*-ratios from microsatellite genotypes. Significance was evaluated using the CRITICAL_M.EXE software (http://swfsc.noaa.gov/textblock.aspx?Division=FED&id=3298). The assumption of this analysis is that bottlenecks cause an *M*-ratio value lower than the M_C threshold. We simulated M_C values modifying the parameter Theta (0.5, 2.0 and 10.0) and the proportion of single-step mutations (0.1 and 0.3); we kept the average size of non-one-step mutations (Δg) constant (at a value of 3.5). The lowest M_C values in the two investigated populations of Trucha and Haccheria were chosen to check the significance of bottlenecks (Garza & Williamson, 2001).

Following Rinaldi et al. (2019), to assess the intensity of historical gene flow we estimated the parameter Theta (as $4N_e\mu$ for biparental inherited loci, where N_e is the effective population size and μ is the mutation rate) and the number of immigrants per generation (as ThetaM, where M is the mutation-scaled effective immigration rate), using a coalescent framework in Migrate-n 3.6.4 (Beerli, 2006; Beerli & Felsenstein, 2001). In the absence of direct estimates of the mutation rate for microsatellites in orchids, the effective population size (N_e) was calculated using the mutation rate of 0.00077 as detected in other monocots (i.e., maize microsatellites, Vigouroux et al., 2002). We calculated the starting values using F_{ST} and used model averaging to assess the migration rates and Theta values. As described in Rinaldi et al. (2019), we performed the Migrate-n analyses using a static heating strategy with four short chains (temperature = 1.0, 1.5, 3.0 and 1.0×10^6) and a single long chain with 50,000 recorded steps and an increment of 50 and 20,000 steps

TABLE 1 Characteristics of microsatellite loci from *Telipogon peruvianus*, including locus name, primer sequences 5′ – 3′, repeat motif, number of alleles and allele size range

Locus	Primer sequence 5′ - 3′	Repeat motif	Number of alleles	Size range
Locus 1	F: CACGACGTTGTAAAACGACTCATGTGATGTGGTTGTGTATTGTA R:CGTGTGTTAGTTTTTTTTAGTTTGTG	$(CA)_{34}$	3	251–261
Locus 2	F: CACGACGTTGTAAAACGACTCAGAATTGAAGGAGGAGCAC R: ACACACACACATAAATTAACA	$(GT)_{19}$	3	277–281
Locus 3	F: CACGACGTTGTAAAACGACGATGAAGGACTGGACGAATG R: TCCATCTCTCTCTTTCTACTCT	$(GA)_{17}$	2	266–268
Locus 4	F: CACGACGTTGTAAAACGACATGCAAACTTATAATAACTC R: TGCGATGATGAGTCCTGAGT	$(GT)_{13}$	4	210–216
Locus 5	F: CACGACGTTGTAAAACGACAGAAGGGACATAATATGTTGC R: ATTTGTGTGTGGATCTTAACA	$(CA)_{19}$	2	168–170
Locus 6	F: CACGACGTTGTAAAACGACTCGTTCCTGATGATGAGTCCT R: ACAAGATAATATCATTTGTACCA	$(CA)_{10}$	2	135–137
Locus 7	F: CACGACGTTGTAAAACGACTGCAATATCCATATACCATTC R: ACTTTCTGTCTCTCTCTCTCTGTA	$(GA)_{22}$	12	155–177
Locus 8	F: CACGACGTTGTAAAACGACACTGTAAGTGATGCCTGCGA R: TGCAAAACACTACAGTTCCAT	$(GT)_9$ - $(GA)_{16}$	16	191–229
Locus 9	F: CACGACGTTGTAAAACGACGGGTGAGGGGGGAGGTGTGTAG R: TCAAAGAAAGTGGGCTCATGT	$(CA)_{24}$	6	218–232
Locus 10	F: CACGACGTTGTAAAACGACCATGACTCTCTATGATGAAGTCCTGA R:CTCTCTATAATGAGTCCTGAGTAA	$(AG)_{29}$	8	247–261

Note: The forward primers were synthesized with a 5′-M13 tail according to Schuelke (2000).

discarded as burn-in. The number of replicates (i.e., concurrent chains) was 10 and the stationarity of the Markov chain was calculated by evaluating the effective sample size for each parameter.

3 | RESULTS

3.1 | Amplification and polymorphism screening of the microsatellite loci

The 10 selected primer pairs showed clear amplification peaks (Table S1). Among the 10 primer pairs, seven amplified regions with the expected size range in most of the *Telipogon* species tested and four out of 10 primer pairs amplified regions with the expected size range in the two sister genera *Hofmeisterella* and *Trichoceros* (Table S2).

3.2 | Genetic diversity estimates

The 10 loci targeted in 96 *T. peruvianus* individuals showed a number of alleles per locus ranging from 2 to 16, with an average of 5.8 alleles per locus (Table 1). Analyzing the two populations independently, the 10 polymorphic loci showed different patterns. In Trucha, the observed and expected heterozygosity (H_O and H_E) ranged from 0.00 to 0.92 and from 0.09 to 0.82 (Table 2). Four loci showed a significant departure from Hardy–Weinberg equilibrium ($p < .05$). One locus was monomorphic (Locus 5). In Haccheria, the observed and expected heterozygosity (H_O and H_E) ranged from 0.02 to 0.95 and from 0.02 to 0.84 (Table 2). Eight out of 10 loci showed a significant departure from Hardy–Weinberg equilibrium ($p < .05$).

When analyzing all loci, moderate levels of genetic diversity were observed for most genetic parameters. Overall, the two populations of Trucha and Haccheria showed comparable levels of genetic diversity parameters (Table 3). The number of alleles ranged from 45 to 52, and the allelic richness ranged from 4.49 to 4.50. The expected and observed heterozygosity (H_O and H_E) per population ranged from 0.272 to 0.391 and 0.478 to 0.488, respectively. The inbreeding coefficients were high and significant in both Trucha (0.190) and Haccheria (0.437) populations. Using Micro-Checker software (van Oosterhout et al., 2004), we found no evidence for scoring error due to “stuttering” or “large allele dropout”.

TABLE 2 Number of alleles (a), observed (H_O) and expected (H_E) heterozygosity, and test for departure from Hardy–Weinberg equilibrium (HWE) of 10 short sequence repeat (SSR) loci in the two populations of *Telipogon peruvianus*

	Trucha				Haccheria			
Locus	**A**	H_O	H_E	**HWE (*p*-value)**	**A**	H_O	H_E	**HWE (*p*-value)**
Locus 1	3	0.692	0.546	.172	3	0.171	0.441	<.001
Locus 2	3	0.590	0.614	.061	3	0.264	0.509	<.001
Locus 3	2	0.500	0.382	.283	2	0.514	0.470	.455
Locus 4	4	0.428	0.426	.206	4	0.304	0.630	<.001
Locus 5	Monomorphic				2	0.015	0.015	1.000
Locus 6	2	0.086	0.085	1.000	2	0.072	0.122	<.050
Locus 7	11	0.923	0.819	<.050	10	0.953	0.756	<.001
Locus 8	10	0.181	0.794	<.001	14	0.318	0.838	<.001
Locus 9	5	0.120	0.386	<.001	5	0.057	0.525	<.001
Locus 10	4	0.000	0.337	<.001	7	0.057	0.471	<.001

TABLE 3 Characterization of genetic variability in *Telipogon peruvianus* populations

Populations/code	A	AR	PAR	H_E	H_O	f[a]
Thucha	45	4.50	0.90	0.488	0.391	0.190***
Haccheria	52	4.49	0.89	0.478	0.272	0.437***

Note: The number of alleles (A), allelic richness (AR), private allelic richness (PAR), expected (H_E) and observed (H_O) heterozygosity, and the within-population inbreeding coefficient *f* were estimated from 10 nuclear microsatellite loci for 96 individuals (26 in Trucha and 70 in Haccheria).
[a]Significant values ($p < .001$, ***) indicate departures of within-population inbreeding coefficients (*f*) from the Hardy–Weinberg equilibrium (HWE).

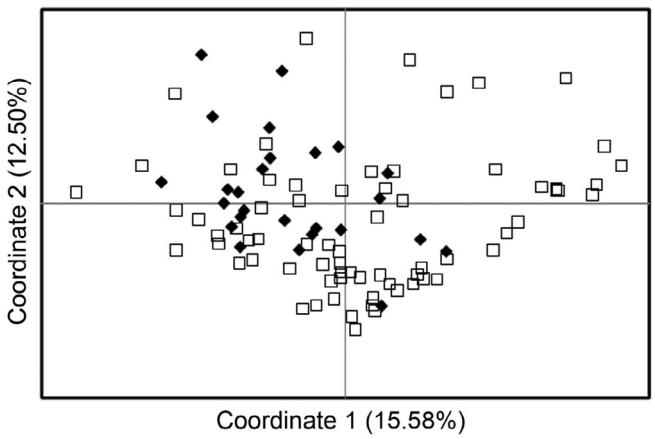

FIGURE 2 Principal coordinate analysis (PCoA) of *Telipogon peruvianus* individuals from the investigated populations of Trucha (diamonds) and Haccheria (squares), using the first two coordinates (coordinate one 15.58%, coordinate two 12.50%)

TABLE 4 Demographic estimates of *Telipogon peruvianus* populations, including the sample size (*n*), the effective population size (N_e), the lowest critical values of the *M*-ratio (M_C) simulated for each population and the *M*-ratio values (*M*)

Pop	*n*	N_e[a]	M_C	M[b]
Trucha	26	93.39 (0.00 to 259.74)	0.559	0.831
Haccheria	70	87.36 (0.00 to 238.09)	0.616	0.860

Note: The 95% confidence intervals for N_e are between parentheses.
[a]Effective population sizes were calculated using the formula $Ne = \text{theta}/4\ \mu$, where μ = mutation rate (0.00077 as found at microsatellite loci in maize; Vigouroux et al., 2002).
[b]A population is considered to have undergone a bottleneck if its *M* value falls below the lower threshold of the critical *M*-ratio (*Mc*) calculated for each population.

3.3 | Population differentiation, gene exchange and historical size reduction

Low levels of nuclear genetic differentiation among populations ($p < .001$) were found for F_{ST} (0.055) and D_{ST} (0.052). The PCoA showed a wide overlap between the two populations (Figure 2). The effective population sizes were high in populations Trucha and Haccheria (N_e = 93.3 and 87.3, respectively). Migrate-N analysis detected asymmetric migration between populations: less than one migrant per generation when Trucha acted as donor to Haccheria (0.25, maximum 1.68, minimum 0.00) and a slightly higher value when Haccheria acted as donor to Trucha (1.11, maximum 3.93, minimum 0.00; Figure 1B). The heterozygosity excess test revealed no

deviation from mutation-drift equilibrium was found for Trucha ($p = .67$) and Haccheria ($p = .94$) under the stepwise mutation model (SMM), suggesting that the two populations suffered no recent bottleneck. Bottlenecks were also not detected when using Garza & Williamson (2001) statistics, because the *M*-ratio values were much higher than the lowest critical values of *M*-ratio (M_C) simulated for each population (Table 4).

4 | DISCUSSION

Understanding the levels of genetic variation in species with small and fragmented distribution is a primary aim of conservation genetics. In this study, we analyzed two populations from one of the two only known localities of the rare endemic Peruvian orchid *T. peruvianus*. Overall, we found observed heterozygosity only slightly lower than expected within populations, whereas the inbreeding coefficient was significant in both populations. Nevertheless, a high value of N_e and absence of a bottleneck signature suggest that the increased anthropogenic pressure that is causing the depletion of the *T. peruvianus* habitat (Roque & León, 2006) has not yet compromised genetic diversity in the investigated populations.

The microsatellite markers isolated and applied in this study have been found to lead to 68.3% of cross-amplification in related species and genera, comparable with the mean value reported for monocot species (60%, Barbara et al., 2007) (Table S2). This is a highly remarkable result considering that the genus *Telipogon* encompasses many localized and highly endangered species (Nauray & Galán, 2008; Roque & León, 2006), and may also reveal the rapid diversification within the *Telipogon* alliance, as already observed in molecular phylogenetic studies (Neubig et al., 2012; Pridgeon et al., 2009; Williams et al., 2005).

The two investigated populations are characterized by a moderate heterozygosity deficiency that can be interpreted as an indication of a slightly reduced genetic variability and can be generated by stochastic processes such as genetic drift. The small population size typical of *T. peruvianus* (Martel & Nauray, 2013), which occurs in few isolated populations, might increase the likelihood of allele loss, thus accounting for the observed levels of heterozygosity. This could be eventually speeded up by the fact that in both investigated populations only a small proportion of flowers are pollinated. Low fruit set and higher fruiting failure in deceptive species compared to nectar-rewarding species have been already observed in orchids (Neiland & Wilcock, 1998; Tremblay, Ackerman, Zimmerman, & Calvo, 2005) and were recorded in one patch of *T. peruvianus* (Martel et al., 2016). Nevertheless, it should be noted that the observed levels of heterozygosity may be more locus specific rather than population specific. For instance, in the Trucha population, only three out of 10 microsatellite loci showed a significant reduction in observed heterozygosity, with one locus being in homozygosis (Table 2). Indeed, in Trucha, the expected and observed heterozygosity per population calculated from the remaining seven loci were 0.459 and 0.410, respectively. A possible occurrence of null alleles at these three loci could explain the observed heterozygotes' deficiencies. However, this is not supported by our Microchecker analysis, which showed no evidence for scoring error due to "stuttering" or "large allele dropout". Alternatively, these loci may locate in low recombinant regions of chromosomes and/or the effects of selection acting on linked sites may have led to a reduction in their microsatellite variation (Schlötterer, Vogl, & Tautz, 1997). Further analyses of the genomic regions that surround these three microsatellite loci are needed to determine whether background selection for deleterious genes or adaptive fixation is the cause of their low levels of observed heterozygosity (Begun & Aquadro, 1991; Charlesworth, Morgan, & Charlesworth, 1993).

Tropical orchids are well known for their small and naturally fragmented populations, especially in epiphyte species (Gentry & Dodson, 1987; Tremblay & Ackerman, 2001). These small and scattered populations play an important role in orchid diversification, because low levels of gene exchange coupled to genetic drift would explain the high levels of species diversity observed in the Neotropical region (Gentry & Dodson, 1987; Givnish et al., 2015; Tremblay et al., 2005). However, in our study, we found no evidence for variation in the genetic parameters caused by a finite number of parents. Also, although our analysis cannot rule out the occurrence of ancient bottlenecks, our results reveal that neither of the investigated populations suffered from recent genetic bottlenecks. In addition, the estimated effective population size (*N*e) was roughly comparable to the total number of flowering individuals. These results may suggest that the two investigated populations of this species did not undergo (yet) a dramatic fluctuation in size. The absence of detectable demographic fluctuation in a population (i.e., the absence of recent bottlenecks), may indeed allow keeping of the predicted allelic diversity, hence leading to *N*e estimates close to the observed populations size. Large *N*e buffers the extent to which random genetic drift changes allele frequencies, increases inbreeding and decreases genetic diversity. The preservation of a relatively large *N*e can be partly explained by the peculiarity of a deceptive pollination strategy that maximizes the number of reproductive individuals even

in the presence of strong pollination limitation and low reproductive success (Cozzolino & Widmer, 2005; Thakur, Rathore, Sharma, & Chawla, 2018). Accordingly, Martel et al. (2016) found that 56% of the plants in one patch in Haccheria presented at least one pollinated flower (rarely two). The low pollination success typical of sexually deceptive species can be further compensated for because *T. peruvianus* flowers are long lived (around 33 days) and a plant can hold open flowers for up to 3 months (Martel et al., 2016; Martel & Nauray, 2013), which increases the probability of plants being visited by pollinators.

Overall, within the limitation of our approach, we found very low levels of nuclear genetic differentiation ($F_{ST} = 0.055$ and $D_{ST} = 0.052$) and a number (around one) of estimated migrants per generation between Trucha and Haccheria (Figure 1B; see also PCoA in Figure 2) that is low but still relevant if compared to effective population size. As a cautionary note, the estimation of the latter may have been potentially inflated by the application of the maize microsatellite mutation rate and by the unbalanced distribution of observed heterozygosity among the 10 microsatellite loci.

Gene flow among populations can be due to pollen transfer mediated by pollinators or by seed dispersal by wind. The finding of high genetic connectivity between *T. peruvianus* populations can be explained by their proximity and by the observation that this species has sexually deceptive pollination, a strategy that has been found to promote outcross, leading to low among-population differentiation (see meta-analysis in Scopece et al., 2009). However, considering the close proximity (approximately 1 km) of the two investigated populations, the finding of a significant, although low, inbreeding coefficient, suggests that, in *T. peruvianus*, mating is mostly occurring within the investigated populations. Indeed, male pollinators, such as those pollinating *T. peruvianus*, prefer patrolling at a short range, potentially leading to strong variation in pollination success even between close patches, as demonstrated by the fact that even within the Haccheria population at least two patches (only distant by 400 m) with dramatically different levels of pollination success (56% versus 4%; Martel et al., 2016) were recorded. The striking difference in pollination success between near patches can be explained by the scarcity of male tachinid flies patrolling in one patch due to the presence of fewer nectar-rewarding plants (Martel et al., 2016) or to pollinator behavior of avoiding crossing small unconnected forest patches (e.g., small meadows associated with streams and roads, surrounded by secondary forest; Martel pers. obs.). Other studies considering epiphytic orchids as a metapopulation found that population substructure can occur even at the level of individual trees (Trapnell, Hamrick, & Nason, 2004). At this level, short-distance gene flow can occur and translate into a greater chance of inbreeding depression by mating among relatives (Murren, 2003). A low pollination success eventually associated with local assortative mating can rapidly increase genetic divergence among recently isolated populations by favoring the effect of drift and fixation of alternative alleles (Ellstrand & Elam, 1993). Thus, the effects of habitat disturbance on the foraging behavior of the specific *T. peruvianus* pollinator can potentially generate small-scale partitioning of the pollen movements and can further favor the genetic fragmentation of this rare species in a changing environment.

5 | CONCLUSIONS

Our data show that the two investigated populations of *T. peruvianus* are only undergoing a moderate reduction of genetic diversity. However, a significant inbreeding coefficient may represent the first clue for a progressive depletion of genetic parameters. These findings coupled with the observation that this species occurs in small and isolated populations suggest that an increasing of anthropogenic pressure may further expose *T. peruvianus* to a potential risk of extinction by reducing interpopulation connectivity mediated by pollinators. Therefore, immediate conservation strategies are needed in order to preserve this very rare and highly specialized species and its exclusive pollinator, including its foraging behavior.

The polymorphic genetic markers developed in this study, which are applicable also in other *Telipogon* species, may represent a very useful tool for estimating levels of genetic diversity in one of the biggest clades of the large Oncidinae subtribe that encompasses many potentially endangered tropical epiphytic species, which, like *T. peruvianus*, have small and naturally fragmented populations.

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REFERENCES

Ackerman, J. D. (2004). Notes on the Caribbean orchid Flora. *New species combinations and records. Lankesteriana*, *4*, 47–56.

Allendorf, F. W. (1986). Genetic drift and the loss of alleles versus heterozygosity. *Zoological Biology*, *5*, 181–190.

Barbara, T., Palma-Silva, C., Paggi, G. M., Bered, F., Fay, M. F., & Lexer, C. (2007). Cross-species transfer of nuclear microsatellite markers: Potential and limitations. *Molecular Ecology*, *16*, 3759–3767.

Beerli, P. (2006). Comparison of Bayesian and maximum likelihood inference of population genetic parameters. *Bioinformatics*, *22*, 341–345.

Beerli, P., & Felsenstein, J. (2001). Maximum likelihood estimation of a migration matrix and effective population sizes in n populations by using a coalescent approach. *Proceedings of the National Academy of Sciences of the U.S.A.*, *98*, 4563–4568.

Begun, D. J., & Aquadro, C. F. (1991). Molecular population genetics of the distal portion of the X chromosome in drosophila: Evidence for genetic hitchhiking of the yellow-achaete region. *Genetics*, *129*, 1147–1158.

Bogarín, D. (2012). A new *Telipogon* from Mexico close to *Telipogon standleyi* (Orchidaceae: Oncidiinae). *Lankesteriana*, *12*, 115–119.

Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics*, *30*, 2114–2120.

Bouzat, J. L. (2010). Conservation genetics of population bottlenecks: The role of chance, selection, and history. *Conservation Genetics*, *11*, 463–478.

Byers, D. L., & Waller, D. M. (1999). Do plant populations purge their genetic load? Effects of population size and mating history on inbreeding depression. *Annual Review in Ecology and Systematics*, *30*, 479–513.

Charlesworth, B., Morgan, M. T., & Charlesworth, D. (1993). The effect of deleterious mutations on neutral molecular variation. *Genetics*, *134*, 1289–1303.

Collantes, B., & Martel, C. (2015). Telipogon koechlinorum (Orchidaceae), a new species from Machu Picchu, Peru. *Brittonia*, *67*, 113–117.

Cornuet, J. M., & Luikart, G. (1996). Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics*, *144*, 2001–2014.

Cozzolino, S., Noce, M. E., Musacchio, A., & Widmer, A. (2003). Variation at a chloroplast minisatellite locus reveals the signature of habitat fragmentation and genetic bottlenecks in the rare orchid *Anacamptis palustris* (Orchidaceae). *American Journal of Botany*, *90*, 1681–1687.

Cozzolino, S., & Widmer, A. (2005). Orchid diversity: An evolutionary consequence of deception? *Trends in Ecology & Evolution*, *20*, 487–494.

Crain, B., & Tremblay, R. (2012). Update on the distribution of *Lepanthes caritensis*, a rare Puerto Rican endemic orchid. *Endangered Species Research*, *18*, 89–94.

Cribb, P. J., Kell, S. P., Dixon, K. W., & Barrett, R. L. (2003). Orchid conservation: A global perspective. In K. W. Dixon, S. P. Kell, R. L. Barrett, & P. J. Cribb (Eds.), *Orchid conservation* (pp. 1–24). Kota Kinabalu: Natural history publications.

Dieringer, D., & Schlötterer, C. (2003). Microsatellite analyzer (MSA): A platform independent analysis tool for large microsatellite data sets. *Molecular Ecology Notes*, *3*, 167–169.

Dodson, C. H., & Gentry, A. H. (1991). Biological extinction in western Ecuador. *Annals of the Missouri Botanical Garden*, *78*, 273–295.

Ellstrand, N., & Elam, D. (1993). Population genetic consequences of small population size implications for plant conservation. *Annual Review in Ecology and Systematics*, *24*, 217–242.

Excoffier, L., & Lischer, H. E. L. (2010). Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, *10*, 564–567.

Frankham, R. (2003). Genetics and conservation biology. *Comptes Rendu Biologies*, *326*, 22–29.

Frankham, R., Briscoe, D. A., & Ballou, J. D. (2002). Cambridge: Cambridge University Press.

Garza, J. C., & Williamson, E. G. (2001). Detection of reduction in population size using data from microsatellite loci. *Molecular ecology*, *10*(2), 305-318.

Gentry, A. H., & Dodson, C. H. (1987). Diversity and biogeography of neotropical vascular epiphytes. *Annals of the Missouri Botanical Garden*, *74*, 205–233.

Givnish, T. J., Spalink, D., Ames, M., Lyon, S. P., Hunter, S. J., Zuluaga, A., et al. (2015). Orchid phylogenomics and multiple drivers of their extraordinary diversification. *Proceedings of the Royal Society B: Biological Sciences*, *282*, 1553.

Hamrick, J. L., Godt, M. J. W., & Sherman-Broyles, S. L. (1992). Factors influencing levels of genetic diversity in woody plant species. *New Forest*, *6*, 95–124.

Jost, L. (2004). Explosive local radiation of the genus *Teagueia* (Orchidaceae) in the upper Pastaza watershed of Ecuador. *Lyonia*, *7*, 41–47.

Jost, L. (2008). GST and its relatives do not measure differentiation. *Molecular Ecology*, *17*, 4015–4026.

Kalinowski, S. T. (2005). HP-RARE 1.0: A computer program for performing rarefaction on measures of allelic richness. *Molecular Ecology Notes*, *5*, 187–189.

Kirby, S. H. (2016). Active tectonic and volcanic mountain building as agents of rapid environmental changes and increased orchid diversity and long-distance orchid dispersal in the tropical Americas: Opportunities and challenges. *Lankesteriana*, *16*, 243–254.

Linhart, Y. B., & Grant, M. C. (1996). Evolutionary significance of local genetic differentiation in plants. *Annual Review in Ecology and Systematics*, *27*, 237–277.

Loveless, M. D., & Hamrick, J. L. (1984). Ecological determinants of genetic structure in plant populations. *Annual Review in Ecology and Systematics*, *15*, 65–95.

Magoč, T., & Salzberg, S. L. (2011). FLASH: Fast length adjustment of short reads to improve genome assemblies. *Bioinformatics*, *27*, 2957–2963.

Martel, C., Cairampoma, L., Stauffer, F. W., & Ayasse, M. (2016). *Telipogon peruvianus* (Orchidaceae) flowers elicit pre-mating behaviour in *Eudejeania* (Tachinidae) males for pollination. *PLoS One*, *11*, e0165896.

Martel, C., Collantes, B., & Egoavil, L. (2017). *Telipogon huancavelicanus* sp. nov. (Orchidaceae) from Peru, and an

updated description of *T. deuterocuscoensis*. *Nordic Journal of Botany*, *35*, 539–545.

Martel, C., Francke, W., & Ayasse, M. (2019). The chemical and visual bases of the pollination of the Neotropical sexually deceptive orchid *Telipogon peruvianus*. *New Phytologist*, *223*, 1989–2001.

Martel, C., & Nauray, W. (2013). Notes and emended description of *Telipogon peruvianus* T.Hashim. (Orchidaceae). *Candollea*, *68*, 245–250.

Maruyama, T., & Fuerst, P. A. (1985). Population bottlenecks and non-equilibrium models in population genetics. II. Number of alleles in a small population that was formed by a recent bottleneck. *Genetics*, *111*, 675–689.

Meirmans, P. G., & Van Tienderen, P. H. (2004). GENOTYPE and GENODIVE: Two programs for the analysis of genetic diversity of asexual organisms. *Molecular Ecology Notes*, *4*, 792–794.

Muñoz, M., Warner, J., & Albertazzi, F. J. (2010). Genetic diversity analysis of the endangered slipper orchid *Phragmipedium longifolium* in Costa Rica. *Plant Systematics and Evolution*, *290*, 217–223.

Murren, C. J. (2003). Spatial and demographic population genetic structure in *Catasetum viridiflavum* across a human-disturbed habitat. *Journal of Evolutionary Biology*, *16*, 333–342.

Myers, N., Mittermeier, R. A., Mittermeier, C. G., Fonseca, G. A. B., & Kent, J. (2000). Biodiversity hotspots for conservation priorities. *Nature*, *403*, 853–858.

Nauray, W., & Galán, A. (2008). Ten new species of *Telipogon* (Orchidaceae, Oncidiinae) from southern Peru. *Anales Jardìn Botanica Madrid*, *65*, 73–95.

Neiland, M. R., & Wilcock, C. C. (1998). Fruit set, nectar reward, and rarity in the Orchidaceae. *American Journal of Botany*, *85*, 1657–1671.

Neubig, K. M., Whitten, W. M., Williams, N. H., Blanco, M. A., Endara, L., Burleigh, J. G., ... Chase, M. W. (2012). Generic recircumscriptions of Oncidiinae (Orchidaceae: Cymbidieae) based on maximum likelihood analysis of combined DNA datasets. *Botanical Journal of the Linnean Society*, *168*, 117–146.

Pandey, M., Sharma, J., Taylor, D. L., & Yadon, V. L. (2013). A narrowly endemic photosynthetic orchid is non-specific in its mycorrhizal associations. *Molecular Ecology*, *22*, 2341–2354.

Parra-Sánchez, E., Retana, J., & Armenteras, D. (2016). Edge influence on diversity of orchids in Andean cloud forests. *Forests*, *7*, 1–13.

Peakall, R., & Smouse, P. E. (2012). GenAlEx 6.5: Genetic analysis in excel. Population genetic software for teaching and research–an update. *Bioinformatics*, *28*, 2537–2539.

Pérez-Escobar, O. A., Chomicki, G., Condamine, F. L., Karremans, A. P., Bogarín, D., Matzke, N. J., ... Antonelli, A. (2017). Recent origin and rapid speciation of Neotropical orchids in the world's richest plant biodiversity hotspot. *New Phytologist*, *215*, 891–905.

Pinheiro, F., Cozzolino, S., de Barros, F., Gouveia, T. M., Suzuki, R. M., Fay, M. F., & Palma-Silva, C. (2013). Phylogeographic structure and outbreeding depression reveal early stages of reproductive isolation in the Neotropical orchid *Epidendrum denticulatum*. *Evolution*, *67*, 2024–2039.

Pinheiro, F., Santos, M. O., Barros, F., Meyer, D., Salatino, A., Souza, A. P., & Cozzolino, S. (2008). Isolation and characterization of microsatellite loci in the Brazilian orchid *Epidendrum fulgens*. *Conservation Genetics*, *9*, 1661–1663.

Pridgeon, A. M., Cribb, P., Chase, M. W., & Rasmussen, F. N. (2009). *Genera Orchidacearum Volume 5*. Epidendroideae (Part II). Oxford: Oxford University Press.

Rathcke, B. J., & Jules, E. S. (1993). Habitat fragmentation and plant-pollinator interactions. *Current Science*, *65*, 273–277.

Raymond, M., & Rousset, F. (1995). GENEPOP (version 1.2): Population genetics software for exact tests and ecumenicism. *Journal of Heredity*, *86*, 248–249.

Rinaldi, R., Cafasso, D., Strumia, S., Cristaudo, A., Sebastiani, F., & Fineschi, S. (2019). The influence of a relict distribution on genetic structure and variation in the Mediterranean tree, *Platanus orientalis*. *AoB Plants*, *11*, plz002.

Roque, J. E., & León, B. (2006). Orchidaceae endémicas del Perú. *Revista Peruana Biologia*, *13*, 759–878.

Schlötterer, C., Vogl, C., & Tautz, D. (1997). Polymorphism and locus-specific effects on polymorphism at microsatellite loci in natural *Drosophila melanogaster* populations. *Genetics*, *146*, 309–320.

Schuelke, M. (2000). An economic method for the fluorescent labelling of PCR fragments. *Nature Biotechnology*, *18*, 233–234.

Scopece, G., Cozzolino, S., Johnson, S. D., & Schiestl, F. P. (2009). Pollination efficiency and the evolution of specialized deceptive pollination systems. *American Naturalist*, *175*, 98–105.

Scopece, G., Schiestl, F. P., & Cozzolino, S. (2015). Pollen transfer efficiency and its effect on inflorescence size in deceptive pollination strategies. *Plant Biology*, *17*, 545–550.

Slatkin, M. (1987). Gene flow and the geographic structure of natural populations. *Science*, *236*, 787–792.

Steffan-Dewenter, I., & Tscharntke, T. (1999). Effects of habitat isolation on pollinator communities and seed set. *Oecologia*, *121*, 432–440.

Thakur, D., Rathore, N., Sharma, M. K., & Chawla, A. (2018). Enhanced reproductive success revealed key strategy for persistence of devastated populations in Himalayan food-deceptive orchid, *Dactylorhiza hatagirea*. *Plant Species Biology*, *33*, 191–202.

Trapnell, D. W., Hamrick, J. L., & Nason, J. D. (2004). Three-dimensional fine-scale genetic structure of the neotropical epiphytic orchid, *Laelia rubescens*. *Molecular Ecology*, *13*, 1111–1118.

Tremblay, R. L., & Ackerman, J. D. (2001). Gene flow and effective population size in *Lepanthes* (Orchidaceae): A case for genetic drift. *Biological Journal of the Linnean Society*, *72*, 47–62.

Tremblay, R. L., Ackerman, J. D., Zimmerman, J. K., & Calvo, R. N. (2005). Variation in sexual reproduction in orchids and its evolutionary consequences: A spasmodic journey to diversification. *Biological Journal of the Linnean Society*, *84*, 1–54.

van Dyke, F. (2008). *Conservation biology: Foundations, concepts, applications*. Berlin: Springer.

van Oosterhout, C., Hutchinson, W. F., Wills, D. P. M., & Shipley, P. (2004). MICRO-CHECKER: Software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Resources*, *4*, 535–538.

Vigouroux, Y., Jaqueth, J. S., Matsuoka, Y., Smith, O. S., Beavis, W. D., Smith, J. S. C., & Doebley, J. (2002). Rate and pattern of mutation at microsatellite loci in maize. *Molecular Biology and Evolution*, *19*, 1251–1260.

Watterson, G. A. (1984). Allele frequencies after a bottleneck. *Theoretical Population Biology*, *26*, 387–407.

Wilcock, C., & Neiland, M. R. M. (2002). Pollination failure in plants: Why it happens and when it matters. *Trends Plant Science*, *7*, 270–277.

Williams, N. H., Whitten, W. M., & Dressler, R. L. (2005). Molecular systematics of *Telipogon* (Orchidaceae: Oncidiinae) and its allies: Nuclear and plastid DNA sequence data. *Lankesteriana*, *5*, 163–184.

Zane, L., Bargelloni, L., & Patarnello, T. (2002). Strategies for microsatellite isolation: A review. *Molecular Ecology*, *11*, 1–16.

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