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Title: EXPLORING THE EFFECT OF MICROSATELLITE SIZE HOMOPLASY ON RECONSTRUCTION OF PHYLOGENETIC RELATIONSHIPS OF PICOTE SPLITFIN Zoogoneticus quitzeoensis.

Short Title: Homoplasies in Zoogoneticus microsatellites

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Abstract: This study explores the effects of microsatellite size homoplasies in the reconstruction of phylogenetic relationships and estimates of population parameters as Fixation index (FST) using as a case study a truncated microsatellite from the picote splitfin Zoogoneticus quitzeoensis. The results suggest that the use of imperfect microsatellites may have only a minor effect in phylogenetic and population studies.

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Exploring the effect of microsatellite size homoplasy on reconstruction of phylogenetic relationships of picote splitfin *Zoogoneticus quitzeoensis*

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Running title: Homoplasies in *Zoogoneticus quitzeoensis* microsatellites

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This study explores the effects of microsatellite size homoplasies in the reconstruction of phylogenetic relationships and estimates of population parameters as Fixation index (F_{ST}) using as a case study a truncated microsatellite from the picote splitfin *Zoogoneticus quitzeoensis*. The results suggest that the use of imperfect microsatellites may have only a minor effect in phylogenetic and population studies.

Key words: microsatellites, size homoplasies, phylogenetic reconstructions

A recent paper (Domínguez-Domínguez *et al.,* 2008) showed that the endangered picote splitfin *Zoogoneticus quitzeoensis* (Bean, 1898) populations can be split into two lineages that originated 3.3 My ago, as demonstrated by both mtDNA and microsatellites (microsatellite dataset from Domínguez-Domínguez *et al.,* 2007). They also reported discrepancies in mitochondrial and microsatellite data affecting the relationships of more recently diverged populations within lineage I. While mtDNA separated Moloya and Magdalena populations from others within this lineage (La Luz, Orandino, and Platanera), microsatellites relate them to the Orandino population. The discrepancy between these two genetic markers was interpreted as being caused by differences in genetic drift effects and stochastic processes affecting the genetic structure of the populations.

Despite the suggestion that size homoplasy in microsatellites (presence of alleles with equal length but different evolutionary history) may not be a significant problem in many population genetic analyses (Estoup *et al.,* 2002), the topic remains under active discussion (Ellegren, 2004).

Taking into account that the ZT1.6 microsatellite used in Domínguez-Domínguez *et al.*(2007) presents a truncated repeat structure (TG)nGG(TG)n in *Z. quitzeoensis* (Boto & Doadrio, 2003), as well as the fact that these imperfect or truncated microsatellites allow homoplasies to be detected more easily than do perfect microsatellites (Estoup *et al.,* 2002), this study explores whether discrepancies between mtDNA and microsatellite inference of phylogenetic relationships could be explained by homoplasies at this locus.

Twenty sequences were analysed from the different sized alleles present in homozygotes of the *Z. quitzeoensis* populations studied by Domínguez-Domínguez *et al.* (2007). Alleles were selected to obtain a random representation of the

populations and allele sizes and to maximise the Moloya and Magdalena population sample. Table I shows the allelic frequencies for the ZT1.6 locus as well as the sample size from the original dataset. Amplified PCR products from homozygote individuals in the Domínguez-Domínguez *et al.* (2007) dataset have been sequenced directly using the specific forward primer (Boto & Doadrio, 2003). All of the sequences matched the expected length, which was determined by the number of repeats and base pairs in the flanking region.

The results (Table II) showed that ZT1.6 alleles of the same size present a repeat structure in the Moloya/Magdalena populations different from the other populations within lineage I in Domínguez-Domínguez *et al.* (2008). This confirms that these are size-homoplasious alleles. Moreover, whereas alleles from other populations revealed a incremental variation pattern, alleles from Moloya/Magadalena, which only differed by a single repetition, showed a different repeat structure. In all sequenced alleles, the repeat flanking sequence was identical to the one present in the original clone of *Z. tequila* (Accession number AY102709).

Considering that the sequenced alleles are the most frequent alleles in the Moloya/Magdalena populations, with only two other alleles present at low frequencies, these results also suggest that analysis of phylogenetic relationships among lineage I populations could be affected by the use of this microsatellite. To explore this idea a re-analysis of the original microsatellite dataset was performed with and without the ZT1.6.microsatellite. Neighbour-joining trees using D_A distance (modified Cavalli-Sforza distance) and 5000 bootstrap replications were created using POPTREE2 software (Takezaki *et al.,* 2010). The results showed no differences in lineage I phylogenetic relationships. The same Orandino/Moloya-

Magdalena topology described by Domínguez-Domínguez *et al.* (2008) was obtained with or without the inclusion of the ZT1.6 microsatellite (Fig. 1).

The fixation indices (F_{ST}) for all population pairs were recalculated using GENEPOP (Raymond & Rousset, 1995) (version 4.0.10) both including and excluding the ZT1.6 microsatellite. The results (Table III) show that the microsatellite presence or absence has a small effect on F_{ST} values (range 0.00-0.08) in pairwise comparisons among populations of lineage I (Moloya, Magdalena, Platanera, Orandino, and La Luz). This suggests that the differences observed by Domínguez-Domínguez *et al.* (2008) when comparing mitochondrial and microsatellite markers with respect to phylogenetic relationships among lineage I populations of *Z. quitzeoensis* cannot be explained by the allele size homoplasies affecting the ZT1.6 microsatellite. Nevertheless, the possibility that the other loci could also be homoplasic cannot be ruled out.

Estoup *et al.* (2002) have proposed that molecularly accessible size homoplasy (MASH) makes up only a portion of the size homoplasy present in microsatellites. When considering the time elapsed since the divergence of *Z. quitzeoensis* lineages and homoplasy dependence on factors such as mutation rate, effective population size, and between-population divergence time, there is a high probability that the other perfect microsatellites may be homoplasic, although this homoplasy may not be molecularly accessible. The splitting in the same lineages I and II using mitochondrial DNA sequences or microsatellites (Domínguez-Domínguez *et al.,* 2008) disagrees with this interpretation, unless the similar topology obtained with these markers is simply coincidental leading to spurious results.

The authors think that the detected ZT1.6 homoplasies in the more recently diverged lineage I populations could reflect, as suggested by Domínguez-

Domínguez *et al.* (2008), a very close relatedness and a very different recent evolutionary history for those populations inhabiting water bodies that have undergone severe reductions in size as is the case with Moloya and Magdalena. In this sense, the results, which show the presence of private alleles for the ZT1.6 locus in Moloya/Magdalena, reinforce the status of these populations as an Operative Conservation Unit, as proposed by Domínguez-Domínguez *et al.* (2007) In addition, results of the current study add to the sparse bibliography with respect to the effect of homoplasies in reconstructing phylogenetic relationships.

Several studies have detected size homoplasy in microsatellites in different organisms (Primmer & Ellegren, 1998; Viard *et al.,* 1998; Angers *et al.,* 2000; Van Oppen *et al.,* 2000; Culver *et al.,* 2001; Adams *et al.,* 2004; Yokoyama *et al.,* 2004; Lia *et al.,* 2007; Barkley *et al.,* 2009; Machado *et al.,* 2010). Only a few of these studies deal directly with the effect that these homoplasies have on the reconstruction of phylogenetic relationships or on estimation of population parameters (Viard *et al.,* 1998; Angers *et al.,* 2000; Adams *et al.,* 2004; Barkley *et al.,* 2009; Machado *et al.,* 2010) and have generally reached contradictory conclusions.

Adams *et al.* (2004) found a small effect of the use of microsatellites with size homoplasy on the F_{ST} analogue R_{ST} in the tropical tree *Corythophora alta.* Angers *et al.* (2000) describe a small effect of homoplasies on the structure of the fresh water snail *Bulinus truncatus* populations. Conversely, Viard *et al.* (1998) found microsatellite size homoplasy affected the structure of *Bulinus truncatus* and *Bombus terrestris* populations. These authors also found that the use of homoplasious microsatellites may affect the reconstruction of phylogenetic relationships in *Apis mellifera*. Two recent papers (Barkley *et al.,* 2009; Machado *et*

133 *al.,* 2010) suggest however that homoplasic microsatellites have a moderate effect
134 on the reconstruction of phylogenetic relationships or population genetic studies.
135 Barkley *et al.* (2009) suggest that the use of this type of microsatellite has a slight
136 effect on the reconstruction of phylogenetic relationships within the genus *Citrus* and
137 Machado *et al.* (2010), analysing *Drosophila antonietae* populations, claim that
138 neither size homoplasy nor null alleles represent significant problems for population
139 genetic analyses, since they are compensated for by the high degree of
140 microsatellite polymorphisms.

141 The use of different methodological approaches makes comparisons difficult.
142 The present study revealed size homoplasy in a truncated microsatellite locus of *Z.*
143 *quitzeoensis*, the presence or absence of which does not seem to affect the topology
144 of phylogenetic relationships among populations of one of the two lineages
145 described for this species, and suggests a small effect of the use of microsatellites
146 exhibiting size homoplasies in phylogenetic reconstruction and F_{ST} values.

147 These results are in agreement with the observations of Adams *et al.* (2004),
148 Barkley *et al*. (2009), Machado *et al.* (2010), and with the Estoup *et al.* (2002)
149 proposal that homoplasic microsatellites are not a significant problem in many types
150 of population analyses, especially if mutation models that assume homoplasy are
151 used to calculate genetic distances, as suggested by Barkley *et al.* (2009).

152 Although that the other perfect microsatellites used in the study by Domínguez-
153 Domínguez *et al.* (2008) could also be homoplasic and the recovered phylogenetic
154 relationships spurious (even though the two main and ancient lineages are
155 recovered by both microsatellite and mitochondrial markers) the results support the
156 possibility that size homoplasy could be the product of a different evolutionary history

affecting populations such as those from Moloya and Magdalena, which inhabit water bodies that have undergone severe depletion in recent times.

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229
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231

232

Table

TABLE I. Allele frequencies for ZT1.6 microsatellite locus in *Zoogoneticus quitzeoensis* extracted from Domínguez-Domínguez *et al*. (2007) dataset

Population	Allele frequencies [a]	Number of samples
San Francisco del Rincón	**230**=0.03; **234**=0.03; **236**=0.05; **240**=0.16; **242**=0.34; **250**=0.39	19
Belisario	**232**=0.03; **236**=0.53; **240**=0.34; **244**=0.11	19
Platanera	**236**=0.21; **238**=0.08; **242**=0.71	12
Orandino	**232**=0.10; **236**=0.25; **238**=0.20; **240**=0.05; **242**=0.20; **246**=0.15; **252**=0.05	10
San Cristobal	**230**=0.04; **232**=0.08; **234**=0.04; **236**=0.21; **240**=0.54; **244**=0.08	12
Mintzita	**232**=0.06; **236**=0.12; **240**=0.71; **244**=0.12	17
La Luz	**236**=0.93; **240**=0.07	19
Moloya	**238**=0.45; **240**=0.45; **250**=0.10	10
Magdalena	**238**=0.56; **240**=0.43; **246**=0.01	7

a Allele lengths(bp) are in bold

Table

Table II. Repeat structure for zt1.6 alleles in *Zoogoneticus quitzeoensis*. Moloya and Magdalena populations are highlighted in bold

Allele length	*n*	Structure	Population
236	1	$(TG)_7GG(TG)_9$	Orandino
236	1	$(TG)_7GG(TG)_9$	La Luz
236	1	$(TG)_7GG(TG)_9$	Belisario
238	1	$(TG)_7GG(TG)_{10}$	Orandino
238	**3**	**$(TG)_2GG(TG)_{15}$**	**Moloya**
238	**2**	**$(TG)_2GG(TG)_{15}$**	**Magdalena**
240	2	$(TG)_7GG(TG)_{11}$	San Cristobal
240	1	$(TG)_7GG(TG)_{11}$	Mintzita
240	**2**	**$(TG)_9GG(TG)_9$**	**Moloya**
240	**1**	**$(TG)_9GG(TG)_9$**	**Magdalena**
242	1	$(TG)_7GG(TG)_{12}$	San Francisco
242	1	$(TG)_7GG(TG)_{12}$	Platanera
244	1	$(TG)_7GG(TG)_{13}$	Belisario
246	1	$(TG)_7GG(TG)_{14}$	Orandino
250	1	$(TG)_7GG(TG)_{16}$	San Francisco

n, number of sequenced alleles

Table

TABLE III. Estimated pairwise comparisons of F_{ST} for *Zoogoneticus quitzeoensis* populations, including (above the diagonal) or excluding (below the diagonal) ZT1.6 microsatellite

	MAG	MOL	PLA	LUZ	ORA	SFR	BEL	SCR	MIN
MAG		0.02	0,34	0.36	0.13	0.24	0.32	0.288	0.28
MOL	0.04		0.32	0.36	0.11	0.24	0.30	0.27	0.26
PLA	0.30	0.28		0.41	0.22	0.29	0.37	0.37	0.38
LUZ	0.28	0.27	0.35		0.27	0.40	0.34	0.40	0.41
ORA	0.13	0.10	0.24	0.24		0.22	0.24	0.24	0.25
SFR	0.21	0.23	0.32	0.33	0.24		0.18	0.15	0.18
BEL	0.33	0.31	0.36	0.36	0.26	0.11		0.04	0.06
SCR	0.32	0.31	0.36	0.37	0.27	0.09	0.02		0.04
MIN	0.29	0.28	0.34	0.36	0.24	0.10	0.01	0.06	

MAG, Magdalena; MOL, Moloya; PLA, Platanera; LUZ, La Luz; ORA, Orandino; SFR, San Francisco del Rincón; BEL, Belisario; SCR, San Cristobal; MIN, Mintzita.

Figure Captions

Caption Fig. 1.

Neighbour-joining tree showing the same topology in lineage I (Domínguez-Domínguez *et al*., 2008) populations, both including and excluding the ZT1.6 microsatellite from the analysis. Branch numbers show bootstrapping support with and without the presence of the ZT1.6 microsatellite.

Figure
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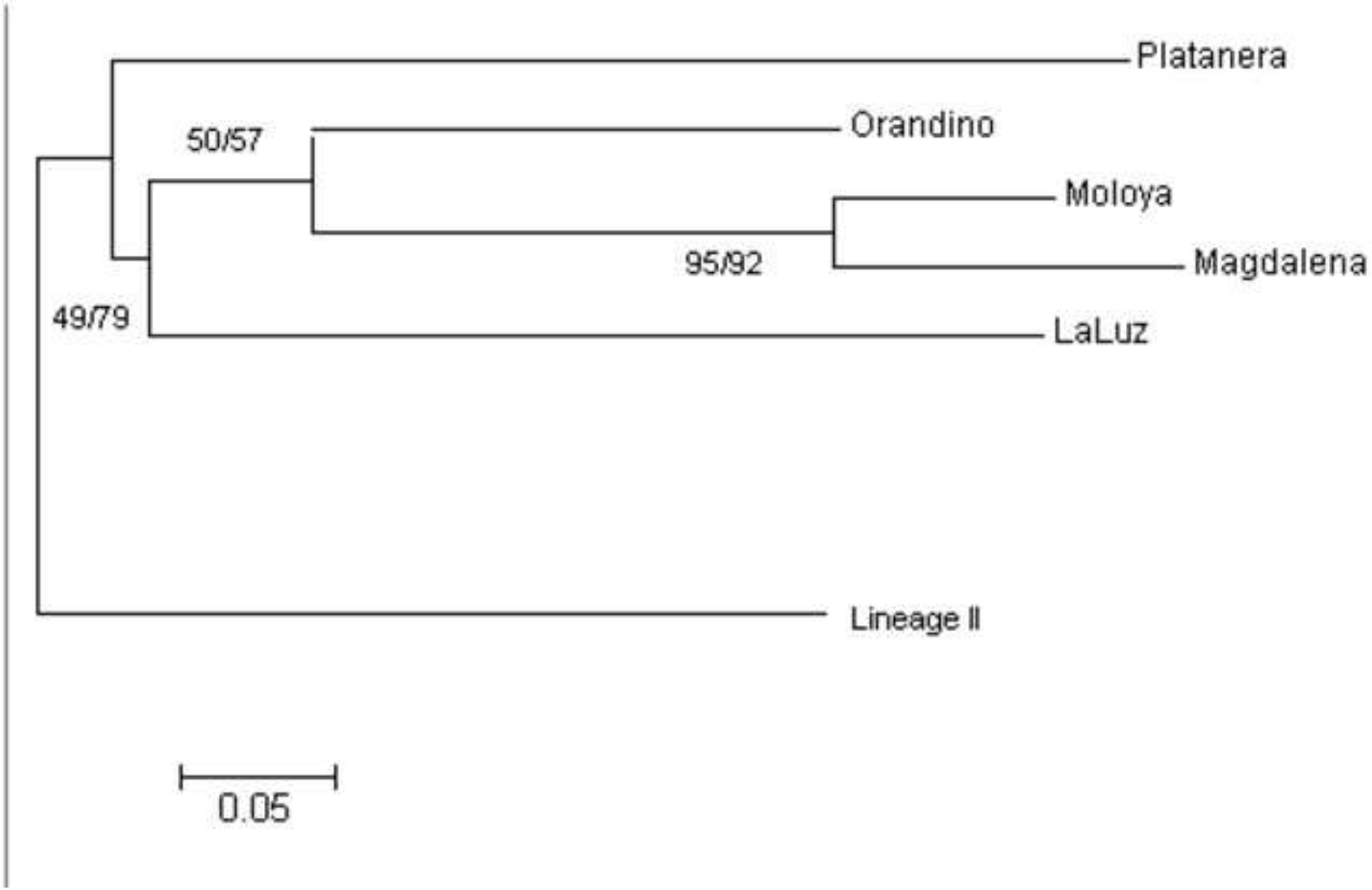

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