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BRIEF NOTE

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A new multiplexed microsatellite tool for metapopulation studies in the overexploited endemic limpet *Patella aspera* (Röding, 1798)

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Background: Patellid limpets are ecologically important keystone grazers having a long history of overexploitation in the Macaronesian Archipelagos (NE Atlantic islands), where some species, such as *Patella aspera*, are under serious risk.[1,2] *Patella aspera* is a protandric sequential hermaphrodite species with external fertilization, in which individuals start off as males but may undergo a sex reversal with age.[3] Hence, exploitation tends to focus on the larger females in the population as larger limpets (predominantly females) are selectively removed. Despite conservation legislation in Canaries, Madeira and Azores, limpets are under severe pressure and few individuals survive long enough to become females, a phenomenon that severely restricts the effective population size.[4] New conservation actions for the protection and sustainable use of limpets in Macaronesian Archipelagos are urgently needed and should be based on a multidisciplinary framework based on knowledge of the population dynamics and connectivity of this species.

Samples, genetic analysis and results: A total of 309 microsatellite loci were isolated from the *P. aspera* genome using a 454 sequencing platform, and three multiplex sets comprising 17 loci for rapid population genetic analyses were developed in this species (Appendix S1; Table S1). Samples from three populations of *P. aspera* ($n = 127$) from the Macaronesian islands (two from the archipelago of Azores and one from the Canaries) were fully genotyped using capillary electrophoresis. Genetic diversity and population differentiation estimates were assessed (Appendix S1). All loci were polymorphic, and a large deficit in heterozygosity was commonplace across markers and samples (Table 1; Table S2). Such a deficit probably arose as a consequence of complex population processes resulting from overexploitation as well as from the usual null allele impact in molluscan microsatellites. Nonetheless, no proper inferences on the causality of this phenomenon can be made until assessed by large-scale population genotyping studies.

Pairwise comparisons of diversity among the three populations analyzed showed a significant F_{ST} distance in 12 of the 17 markers analyzed (Table S2), averaging $F_{ST} = 0.047$ ($P < 0.001$). Noteworthy, hierarchical AMOVAs showed that such interpopulation genetic distance was caused by the divergence between each of the Azorean samples and the Canarian sample ($F_{ST} = 0.074$, $P < 0.001$), but not between the Azorean samples ($F_{ST} = 0.017$, $P > 0.01$) (Table S3).

Comments: Despite the low number of samples analyzed, present data suggest that the divergence found between archipelagos amounts to that expected between allopatric species rather than among conspecific populations. Next steps include the study of the genetic population structure of *P. aspera* throughout its distribution range so that future efforts can focus on identifying scales of connectivity and hence stock integrity across the Macaronesian Archipelagos. These novel tools can be useful to inform management of fisheries and preserve endemic limpet stocks that have been severely depleted in Macaronesia.

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Table 1 Mean genetic diversity estimates based on 17 microsatellite loci for three *Patella aspera* populations from the Macaronesian islands of S. Miguel (Azores, SMI_1 and SMI_2) and Gran Canaria (Canaries, CAN).

	n	N_A (±SE)	H_O (±SE)	H_E (±SE)	F_{IS} (±SE)
SMI_1	48	10.1 (1.5)	0.393 (0.050)	0.718 (0.042)	0.463* (0.056)
SMI_2	49	10.5 (1.4)	0.381 (0.051)	0.732 (0.039)	0.497* (0.062)
CAN	30	8.2 (0.9)	0.278 (0.061)	0.669 (0.052)	0.607* (0.078)
All	127	9.6 (0.7)	0.351 (0.032)	0.707 (0.026)	0.514* (0.057)

n, number of individuals; N_A, number of alleles per sample; H_O, observed heterozygosity; H_E, unbiased expected heterozygosity; F_{IS}, inbreeding coefficient; SE, standard error.
*Significant departure from Hardy–Weinberg equilibrium after sequential Bonferroni correction.

BDP/63040/2009 and SFRH/BPD/69232/2010 respectively.

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Supporting information

Additional supporting information may be found in the online version of this article.

Appendix S1 Materials and methods.

Table S1 Characteristics of 17 microsatellite loci developed for *Patella aspera* and co-amplified in three multiplex reactions (PasMix I, II, III).

Table S2 Gene diversity of 17 microsatellite loci in three *Patella aspera* samples from the Macaronesian islands of S. Miguel (Archipelago of Azores, SMI_1, $n = 48$ and SMI_2, $n = 49$) and Gran Canaria (Archipelago of Canaries, $n = 30$).

Table S3 Hierarchical AMOVA (weighted average over loci) and *F*-statistics for *P. aspera* samples, with variance partitioned across samples and archipelagos.