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Evidence for an additional population of the cave-dwelling squat lobster *Munidopsis polymorpha* in Lanzarote (Canary Islands): implications for management and conservation

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SUPPLEMENTARY ANALYSES

Non-parametric visualization of the genetic structure

We conducted exploratory multivariate analyses. We first generated an allele-frequency table for each individual genotype using tab with mean imputation of missing values. We then performed a Principal Component Analysis (PCA) using the function 'prcomp' included in the R package 'stats', to calculate and visualize the two major axes of genetic variation without assumptions about population structure. Additionally, we performed a Discriminant Analysis of Principal Components (DAPC) using the function 'dapc' included in the R package 'adegenet' (Jombart & Ahmed, 2011). We retained up to 10 principal components (or n–1, whichever was smaller) and a maximum of three discriminant functions. Both PCA and DAPC were run on the complete dataset of eight loci and a filtered dataset of five loci with low null-allele estimates (see below). Results were visualized using ggplot2 (Wickham, 2016). Because sample sizes were small and uneven, we interpret PCA/DAPC patterns qualitatively; these results are provided in Supplementary Fig. S1.

Evaluation of the impact of null alleles

We estimated null-allele frequencies per locus using the function 'null.all' included in the R package 'adegenet' (Jombart & Ahmed, 2011), and inspected their correspondence with HWE deviations, using the function 'hw.test' in the R package 'pegas' version 1.3 (Paradis, 2010). Three loci (Mp-1, Mp-3, Mp-6) showed both elevated null-allele frequency (>0.10) and HWE departures consistent with heterozygote deficits in at least some sites, whereas the remaining loci behaved well. Because removing such loci can both reduce artefacts and discard real variation (Cabezas et al., 2012), we retained the full eight-locus data set for the main analyses but also constructed a filtered data set including only loci with low null-allele frequencies and no consistent HWE problems (Mp-2, Mp-4, Mp-5, Mp-7, Mp-8). We repeated all diversity and differentiation analyses on this reduced marker set as a sensitivity analysis. Full quality control results and the filtered-loci summaries are reported in Supplementary Tabs. S3–S5 and Fig. S2.

The locus-level null-allele screen was consistent with the HWE tests: Mp-1, Mp-3, and Mp-6 showed the highest inferred null-allele frequencies and the clearest tendency towards heterozygote deficits at some sites, whereas Mp-2, Mp-4, Mp-5, Mp-7, and Mp-8 showed low null-allele estimates and behaved well across sites (Supplementary Table S3-S4). Repeating all diversity and differentiation analyses on this filtered five-locus data set produced results that were qualitatively identical to those obtained with all loci (Supplementary Tab. S5, Fig. S2): Charcos de Luis retained the highest allelic richness and the largest proportion of private alleles, and pairwise FST values continued to support a clear differentiation between Charcos de Luis and the lava-tube sites, with FST confidence intervals overlapping the estimates from the full data set.

We also explored STRUCTURE v2.3.4, but due to extremely small sample sizes ($n < 10$ per group), allele-frequency estimates were unstable across runs, and no reproducible clustering was obtained; therefore, these results are not discussed further.

References

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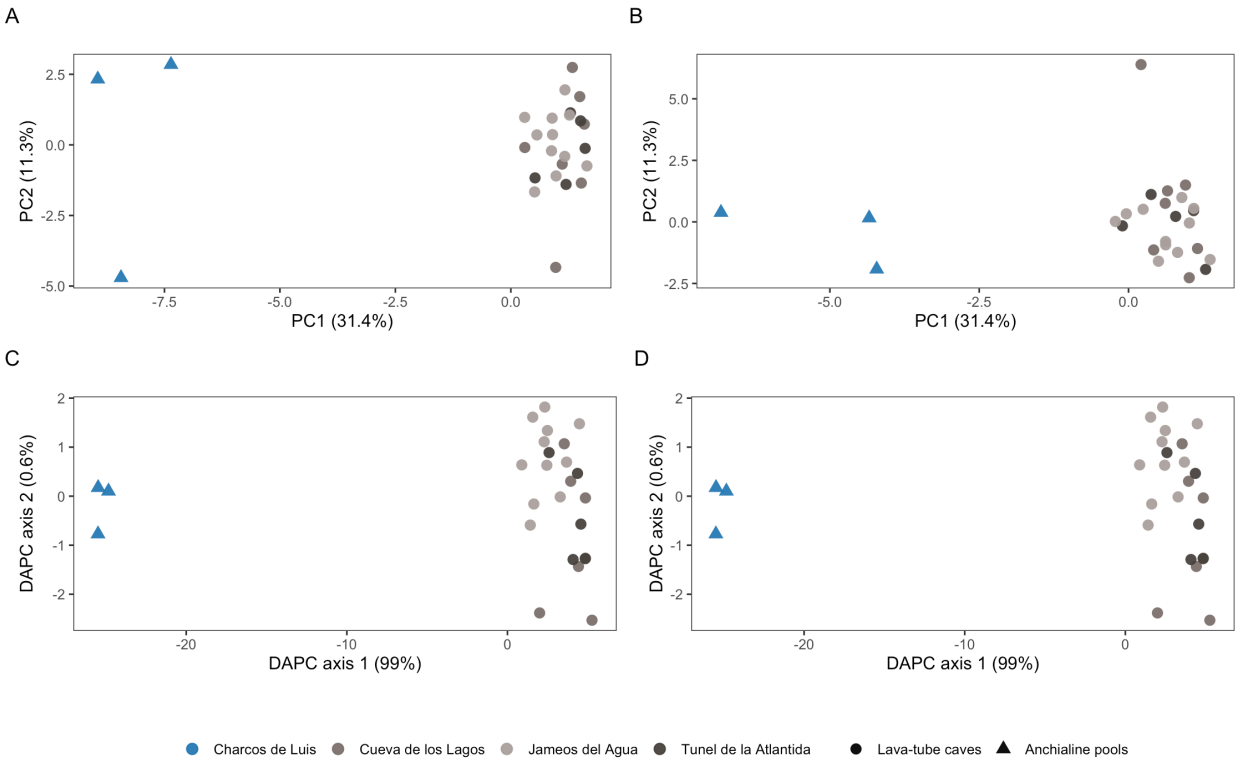

Fig. S1. Exploratory multivariate analyses of microsatellite genotypes in *Munidopsis polymorpha*. **A.** Principal Component Analyses (PCA) based on the full eight-locus dataset and **B.** PCA based on the five-locus filtered dataset excluding loci with elevated null-allele frequencies. **C.** Discriminant Analysis of Principal Components (DAPC) for the full dataset. **D.** DAPC based on the filtered 5-locus dataset. Points represent individual multilocus genotypes coloured by sampling locality (Cueva de los Lagos, Jameos del Agua and Túnel de la Atlántida within La Corona lava tube, and Charcos de Luis) and shaped by habitat type (lava tube, anchialine pools).

Tab. S1. Summary of microsatellite genotypes for *Munidopsis polymorpha*. Each row corresponds to a genotyped individual (MP.L01–MP.L26); columns are the microsatellite loci. Values are fragment lengths (in base pairs). Two values are shown per locus because these are diploid nuclear markers; different lengths indicate different alleles. Private alleles are highlighted. Individuals are grouped by sampling locality. "NP" indicates that the genotype was not produced.

Locality	ID	Mp-8		Mp-1		Mp-4		Mp-5		Mp-2		Mp-3		Mp-6		Mp-7	
Cueva de los Lagos (N=7)	MP.L01	205	205	367	367	157	157	311	311	266	269	311	311	449	449	227	227
	MP.L02	201	205	367	367	177	177	311	318	266	269	311	311	449	449	227	225
	MP.L03	201	205	367	367	177	177	311	314	266	265	311	311	449	449	221	221
	MP.L04	201	205	368	377	157	177	311	314	266	269	311	311	449	449	227	225
	MP.L05	205	205	**395**	**395**	155	155	311	314	266	265	311	311	449	449	225	225
	MP.L06	205	201	367	367	177	177	311	311	266	269	311	311	449	449	221	221
	MP.L07	205	205	367	367	157	177	311	311	266	265	311	311	449	449	221	225
Túnel de la Atlántida (N=5)	MP.L08	201	205	367	367	157	177	311	314	266	265	311	311	449	449	221	221
	MP.L09	205	201	367	367	157	177	311	311	266	269	311	311	449	449	221	221
	MP.L10	205	201	367	367	177	177	311	311	266	269	311	311	449	449	221	225
	MP.L14	205	**209**	367	367	177	177	311	311	266	265	311	314	449	449	221	227

	MP.L15	205	205	367	367	152	172	314	314	265	265	310	310	NP	NP	321	**323**
Jameos del Agua (N=11)	MP.L16	205	221	367	367	152	172	314	314	269	269	310	310	249	249	321	327
	MP.L17	205	205	367	367	172	172	314	314	265	265	310	310	NP	NP	321	325
	MP.L18	NP	NP	NP	NP	152	172	NP	NP	269	269	310	310	NP	NP	NP	NP
	MP.L19	205	205	367	367	172	172	314	314	269	269	310	310	NP	NP	321	321
	MP.L20	NP	NP	NP	NP	152	152	NP	NP	265	265	310	310	NP	NP	NP	NP
	MP.L21	205	205	367	367	152	152	314	314	265	269	310	310	249	249	321	321
	MP.L22	205	205	367	367	152	172	314	314	265	269	310	310	NP	NP	NP	NP
	MP.L23	205	205	367	367	172	172	314	314	265	269	310	310	NP	NP	321	321
	MP.L24	205	205	367	367	152	172	314	314	**257**	269	310	310	NP	NP	NP	NP
	MP.L25	205	221	NP	NP	172	172	314	314	265	265	310	310	NP	NP	NP	NP
	MP.L26	205	205	367	367	152	172	314	314	265	265	310	310	NP	NP	NP	NP
Charcos de Luis (N=3)	MP.L11	**217**	**217**	367	387	172	**204**	NP	NP	NP	NP	**294**	310	**2411**	**2411**	**3099**	**3099**

MP.L12	205	**217**	387	387	172	**212**	NP	NP	269	269	**2822**	**3022**	**2441**	**2441**	**3099**	**3099**
MP.L13	205	**217**	387	387	**212**	**212**	NP	NP	265	265	**2945**	**3022**	**2445**	249	**3099**	327

Tab. S2. Summary of the primers used for the amplification of the eight microsatellites used in the study of *Munidopsis polymorpha*. The sequences of the forward and reverse primers, the microsatellite sequence, and the average amplified fragment size are indicated.

Locus	Primer	Repeat motif	Size cloned allele
Mp-1	F:HEX-TTCCACAATGAGCACTGGAC	(CTAT)16	350
	R:GCATATGTGGAGCCTGGATT	(CTAT)16	350
Mp-2	F:6-FAM- GGAGAGGGAGTTATCGAGAGG	(CTAT)15TTAT(CTAT)6	249
	R:GGGGAGTCTGGAATTAAATGG	(CTAT)15TTAT(CTAT)6	249
Mp-3	F:NED-CGTCTTTGGCTGCGACTAA	(TATC)15	293
	R:ACGGCGTCAGGCAATAAATA	(TATC)15	293
Mp-4	F:6-FAM-TGACCAAACAAATATTCCTTAGTAGG	(GATA)13	154
	R:CTCATGTGGGCCTCTGAATA	(GATA)13	154
Mp-5	F:HEX-TCGTGCTTCTCTTTCAATGC	(TATC)34	296
	R:ACAATGGGAATGAGGGGAAG	(TATC)34	296
Mp-6	F:AACCTCTCCTTGCCTTCCTT	(TTTG)9	236
	R:TCGTTGGCAGAGGTAACAAAC	(TTTG)9	236
Mp-7	F:6-FAM- CTCCAGGCACAGATACTGACAC	(TG)17	301
	R:GGTGATGACTGAACACAAGTCC	(TG)17	301
Mp-8	F:AGCATCAATTCTGCCCTTTC	(TCCA)4(TCTA)9(TC)2(TATC)10	189
	R:GATCACCCCACTTGAAGGAA	(TCCA)4(TCTA)9(TC)2(TATC)10	189

Tab. S3. Genotyping quality control by site x locus. Columns report, for each locus within each site: N sampled (individuals sampled at that site), N genotyped (non-missing diploid genotypes), Call rate (% = N genotyped / N sampled), HWE p (Hardy–Weinberg exact test; 10,000 Monte-Carlo replicates), HWE category (whether the HWE test was performed and, if not, the reason), and LD pairs (BH<0.05) (within-site locus pairs significant for linkage disequilibrium after Benjamini–Hochberg correction; "none" indicates no significant pairs for that locus). "HWE tested" = test run with ≥5 genotypes and polymorphism; "HWE n<5" = fewer than 5 usable genotypes (unassessable); "HWE sparse table" = extremely sparse genotype contingency (test not computed; treated as unassessable); A dash "–" under HWE p indicates unassessed. Site name abbreviations: Lagos = Cueva de los Lagos; Túnel = Túnel de la Atlántida; Jameos = Jameos del Agua; Luis = Charcos de Luis.

Locus	Locality	N sampled	N genotyped	Call Rate	HWE p	HWE category	LD pairs (BH<0.05)
Mp-1	Lagos	7	7	100	-	HWE sparse table	none
Mp-2	Lagos	7	7	100	1	HWE tested	none
Mp-3	Lagos	7	7	100	-	HWE n<5	none
Mp-4	Lagos	7	7	100	1	HWE tested	none
Mp-5	Lagos	7	7	100	1	HWE tested	none
Mp-6	Lagos	7	7	100	-	HWE n<5	none
Mp-7	Lagos	7	7	100	-	HWE sparse table	none
Mp-8	Lagos	7	7	100	-	HWE sparse table	none
Mp-1	Túnel	5	5	100	-	HWE n<5	none
Mp-2	Túnel	5	5	100	1	HWE tested	none
Mp-3	Túnel	5	5	100	1	HWE tested	none
Mp-4	Túnel	5	5	100	1	HWE tested	none
Mp-5	Túnel	5	5	100	-	HWE n<5	none
Mp-6	Túnel	5	4	80	-	HWE n<5	none

Mp-7	Túnel	5	5	100	-	HWE sparse table	none
Mp-8	Túnel	5	5	100	-	HWE sparse table	none
Mp-1	Jameos	11	8	72.7	-	HWE n<5	none
Mp-2	Jameos	11	11	100	-	HWE sparse table	none
Mp-3	Jameos	11	11	100	-	HWE n<5	none
Mp-4	Jameos	11	11	100	1	HWE tested	none
Mp-5	Jameos	11	9	81.8	-	HWE n<5	none
Mp-6	Jameos	11	2	18.2	-	HWE n<5	none
Mp-7	Jameos	11	5	45.5	-	HWE sparse table	none
Mp-8	Jameos	11	9	81.8	1	HWE tested	none
Mp-1	Luis	3	3	100	-	HWE n<5	none
Mp-2	Luis	3	2	66.7	-	HWE n<5	none
Mp-3	Luis	3	3	100	-	HWE n<5	none
Mp-4	Luis	3	3	100	-	HWE n<5	none
Mp-5	Luis	3	0	0	-	HWE n<5	none
Mp-6	Luis	3	3	100	-	HWE n<5	none
Mp-7	Luis	3	3	100	-	HWE n<5	none
Mp-8	Luis	3	3	100	-	HWE n<5	none

Tab. S4. Site-level genetic summary, comparing the full 8-loci dataset with the filtered 5-loci dataset. N, individuals genotyped; AR, allelic richness rarefied to the smallest site sample size (n = 3); Ho/He, observed/expected heterozygosity (means across loci); FIS, inbreeding coefficient (Weir & Cockerham); Private alleles, alleles unique to a site across all loci; Call Rate mean, mean % of individuals successfully genotyped per locus; HWE tested, number of locus × site combinations with ≥5 genotypes that were tested for Hardy–Weinberg equilibrium; LD sig pairs n, number of within-site locus pairs significant for linkage disequilibrium after Benjamini–Hochberg correction ($q < 0.05$); FST *vs* Charcos (95% CI), Weir–Cockerham *F*ST between each lava tube site and Charcos de Luis ("–" for Charcos). Many HWE/LD contrasts were unassessable due to small sample sizes.

Metric	**Dataset**	**Lagos**	**Túnel**	**Jameos**	**Luis**
N	-	7	5	11	3
AR	Full	1.58	1.57	1.39	2.2
	Filtered	1.81	1.83	1.63	2.05
Ho/He	Full	0.32/0.30	0.33/0.27	0.18/0.21	0.48/0.63
	Filtered	0.46/0.42	0.48/0.39	0.29/0.33	0.42/0.65
FIS	Full	-0.07	-0.18	0.09	0.15
	Filtered	-0.08	-0.22	0.09	0.19
Private alleles	Full	2	3	1	9
	Filtered	1	2	1	4
Call rate mean	Full	100	97.5	75	83.3
	Filtered	100	100	81.8	73.3
HWE tests	Full	3	3	2	0
	Filtered	3	2	2	0
LD sig. pairs	Full	0	0	0	0
	Filtered	0	0	0	0
FST *vs* Charcos	Full	0.46	0.47	0.52	-
	Filtered	0.3	0.32	0.37	-