

Supplement of Web Ecol., 18, 91–103, 2018
<https://doi.org/10.5194/we-18-91-2018-supplement>
© Author(s) 2018. This work is distributed under
the Creative Commons Attribution 4.0 License.



Supplement of

Genetic diversity and differentiation of invasive *Acacia longifolia* in Portugal

Sara Vicente et al.

Correspondence to: Helena Trindade (htrindade@fc.ul.pt)

The copyright of individual parts of the supplement might differ from the CC BY 4.0 License.

Supplement

- **Sample Identification**

Table S1: Identification of the samples from Osso da Baleia (OB) with a sample code, identification (ID) number and the geographic coordinates of the location.

Sample Code	ID N°	Geographic Coordinates
OB1	41	
OB2	42	
OB3	43	
OB4	44	
OB5	45	
OB6	46	
OB7	47	
OB8	48	
OB9	49	
OB10	50	
OB11	51	
OB12	52	
OB13	53	40°0'03.29"N
OB14	54	8°54'03.10"W
OB15	55	
OB16	56	
OB17	57	
OB18	58	
OB19	59	
OB20	60	
OB21	61	
OB22	62	
OB23	63	
OB24	64	
OB25	65	

Table S2: Identification of the samples from Pinheiro da Cruz (PC) with a sample code, identification (ID) number and the geographic coordinates of the location.

Sample Code	ID N°	Geographic Coordinates
PC1	71	
PC2	72	
PC3	73	
PC4	74	
PC5	75	
PC6	76	
PC7	77	
PC8	78	
PC9	79	
PC10	80	
PC11	81	
PC12	82	
PC13	83	38°15'01.38"N
PC14	84	8°45'07.12"W
PC15	85	
PC16	86	
PC17	87	
PC18	88	
PC19	89	
PC20	90	
PC21	91	
PC22	92	
PC23	93	
PC24	94	
PC25	95	

Table S3: Identification of samples from Vila Nova de Milfontes (VNMF) with a sample code, identification (ID) number and the geographic coordinates of the location of each individual.

Site	Sample Code	ID N°	Geographic Coordinates
	EC	0	External Control: <i>Acacia saligna</i>
MFOld	MFOld1	1	37°41'15.55"N, 8°47'31.21"W
	MFOld2	2	37°41'14.25"N, 8°47'31.91"W
	MFOld3	3	37°41'12.83"N, 8°47'32.04"W
	MFOld4	4	37°41'11.37"N, 8°47'31.83"W
	MFOld5	5	37°41'09.71"N, 8°47'31.24"W
	MFOld6	6	37°41'08.44"N, 8°47'30.38"W
	MFOld7	7	37°41'07.05"N, 8°47'29.43"W
	MFOld9	9	37°41'22.00"N, 8°47'25.92"W
	MFOld10	10	37°41'23.32"N, 8°47'24.85"W
	MFOld11	11	37°41'24.41"N, 8°47'23.95"W
	MFOld12	12	37°41'25.47"N, 8°47'23.12"W
	MFOld13	13	37°41'26.56"N, 8°47'22.20"W
	MFOld14	14	37°41'28.13"N, 8°47'21.00"W
	MFOld15	15	37°41'28.93"N, 8°47'20.12"W
	MFRec	MFRec1	16
MFRec2		17	37°40'56.39"N, 8°46'55.37"W
MFRec3		18	37°40'50.70"N, 8°46'47.68"W
MFRec4		19	37°40'49.17"N, 8°46'51.83"W
MFRec5		20	37°40'50.29"N, 8°46'38.97"W
MFRec6		21	37°40'58.22"N, 8°46'24.02"W
MFRec7		22	37°41'01.51"N, 8°46'26.62"W
MFRec8		23	37°41'04.55"N, 8°46'28.24"W
MFRec9		24	37°41'25.42"N, 8°46'24.44"W
MFRec10		25	37°41'28.35"N, 8°46'23.07"W
MFRec11		26	37°41'33.97"N, 8°46'20.10"W
MFRec12		27	37°41'32.22"N, 8°46'15.53"W
MFRec13		28	37°41'29.55"N, 8°46'03.99"W
MFRec14		29	37°41'02.08"N, 8°46'00.40"W
MFRec15		30	37°41'01.34"N, 8°45'51.66"W
MFRec16		31	37°40'57.60"N, 8°45'50.19"W
MFRec17		32	37°40'50.22"N, 8°45'48.90"W
MFRec18		33	37°40'46.58"N, 8°45'50.75"W
MFRec19		34	37°40'41.60"N, 8°45'53.25"W
MFRec20		35	37°40'32.19"N, 8°45'59.03"W
MFRec21		36	37°40'43.26"N, 8°46'15.80"W
MFRec22		37	37°41'02.28"N, 8°47'40.71"W
MFRec23		38	37°41'03.97"N, 8°47'40.28"W
MFRec24		30	37°41'05.60"N, 8°47'37.01"W
MFRec25		40	37°41'00.55"N, 8°47'41.28"W
MFCL	MFCL1	101	37°30'27.33"N, 8°27'04.48"W
	MFCL2	102	37°30'26.86"N, 8°27'02.54"W
	MFCL3	103	37°30'26.54"N, 8°27'04.00"W
	MFCL4	114	37°30'42.56"N, 8°26'24.96"W
	MFCL5	104	37°42'14.04"N, 8°46'19.78"W
	MFCL6	105	37°42'20.02"N, 8°46'22.65"W
	MFCL7	106	37°42'29.73"N, 8°46'29.84"W
	MFCL8	107	37°36'01.94"N, 8°48'55.54"W
	MFCL9	108	37°32'34.76"N, 8°47'16.25"W
	MFCL10	109	37°28'37.88"N, 8°47'45.11"W
	MFCL11	110	37°28'35.32"N, 8°44'17.50"W
	MFCL12	111	37°30'47.78"N, 8°43'35.93"W
	MFCL13	112	37°34'28.33"N, 8°44'15.55"W
	MFCL14	113	37°39'39.89"N, 8°45'28.10"W

• Inter-Simple Sequence Repeats (ISSRs) Primers

Table S4: List ISSR primers and corresponding annealing temperatures. TD - touch-down PCR cycle. ¹Primers selected for analysis of samples from Vila Nova de Milfontes (MFold and MFRec), Osso da Baleia (OB) and Pinheiro da Cruz (PC). ²Primers selected for the detailed analysis of samples from Vila Nova de Milfontes (MFold, MFRec and MFCL).

Primer	Sequence	Annealing temperature (°C)
807	(AT) ₈ T	52
808 ^{1,2}	AGAGAGAGAGAGAGAGC	52
810 ^{1,2}	GTGTGTGTGTGTGTGTCA	52
812	(GA) ₈ A	53
813 ^{1,2}	CTCTCTCTCTCTCTT	52
817 ¹	CACACACACACACAA	52
823 ²	(TC) ₈ C	54
825 ¹	(AC) ₈ T	54
826 ¹	(AC) ₈ C	58
827 ^{1,2}	(AC) ₈ G	58
834	(AG) ₈ YT	59
835	(AG) ₈ YC	59
836 ^{1,2}	(AG) ₈ YA	54
840 ^{1,2}	(GA) ₈ YT	TD 57/54
841	(GA) ₈ YC	54
846 ^{1,2}	(CA) ₈ RT	TD 57/53
849 ^{1,2}	GAGAGAGAGAGAGAGAT	52
857	(AC) ₈ YG	52
858	(TG) ₈ RT	59
862	(AGC) ₆	55
864	ATGATGATGATGATGATG	52
866	(CTC) ₆	55
868 ^{1,2}	(GAA) ₆	51
880 ¹	GGA(GAG) ₂ AGGAGA	54
892	TAG ATC TGA TAT CTG AAT TCC C	54
895	AGA GTT GGT AGC TCT TGA TC	55
899	CAT GGT GTT GGT CAT TGT TCC A	58
900	ACT TCC CCA CAG GTT AAC ACA	57
901 ^{1,2}	DHB(CG A) ₅ x	TD 57/52
903 ¹	BDB(CAC) ₅ x	TD 57/52
904 ¹	DDC(CAC) ₅	TD 57/52

• **Simple Sequence Repeats or Microsatellite (SSRs) Pairs of Primers**

Table S5: List of tested SSR primers, corresponding sequence and reference.

Primer	Sequence (5'-3')	References
BBY8P	Fw: TTGGCAAATCCGCACAGTC Rv: TGCCATCGCAACATATAGCTTC	Forrest et al. (2015)
BA1R8	Fw: GGTGCTTTTCCCCACCTTC Rv: TCTCGCTTTTCATGTGCAAG	Forrest et al. (2015)
CIDYF	Fw: CACACTTATGGGATGGGTTGC Rv: AGCTAAGGAAAGTGTACGGGAAT	Roberts et al. (2013); Forrest et al. (2015)
CPUH4	Fw: AGATGCATTGACTGAGACGG Rv: CGAATGAAGGAGATTTATGAAGAGAC	Forrest et al. (2015)
AV9GR	Fw: CCAACGACAGTGGGCAGTC Rv: CTCCGGTGTAGCAAAGGC	Forrest et al. (2015)
BVWHY	Fw: TCCTACTTCCCCAACACGC Rv: ACAAGCAGCCATTGGAAGG	Forrest et al. (2015)
APZIZ	Fw: ACACTACACTCACAACACACAC Rv: ACACGGTTTGCTTGGCTTG	Forrest et al. (2015)
AO12C	Fw: AAAACAAGAGAAGAGGACATGC Rv: TCGTAGAAACGACACGAAACG	Forrest et al. (2015)
CU0EQ	Fw: ACCACCATCTTCACCTCCAC Rv: TCCGGCGTTTCCAATAAC	Forrest et al. (2015)
ACPU7	Fw: GTTCTACGGCTAGATGGTGC Rv: TGTCATACGGCCTCACAAG	Forrest et al. (2015)
DCLOC	Fw: CAACTTGTGATTAAGTCCACGG Rv: TGTGTTGAGACTTTGTGCTG	Roberts et al. (2013)

• Gel Image

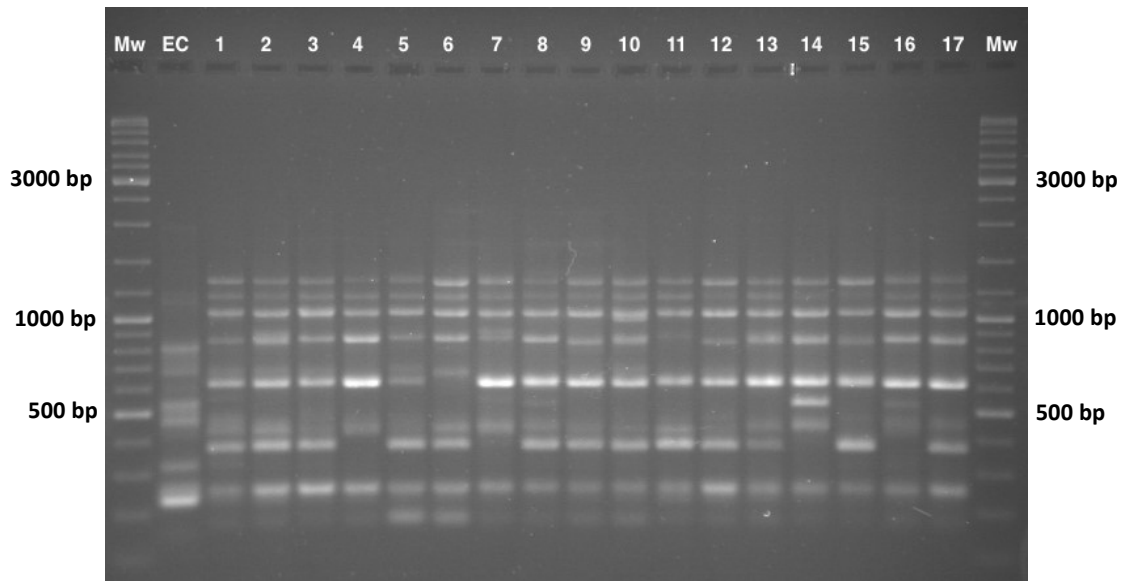


Fig. S1 Agarose gel of the amplification products obtained with ISSR primer 849. Mw – molecular weight standard, with the three most intense bands (3.000, 1.000, 500 bp) indicated; EC – external control (*A. saligna*); Lanes 1-17 – samples of *A. longifolia*

• ISSR Supplementary Results

Table S6: Total number of bands with and without the external control (EC), number and percentage of polymorphic bands (PB) for each ISSR primer for analysis of samples from Vila Nova de Milfontes (MFold, MFRec), Osso da Baleia (OB) and Pinheiro da Cruz (PC).

Primer	N° Total Bands with EC	N° Total Bands without EC	N° PB (with EC)	%PB (with EC)
808	18	16	18	100%
810	19	17	19	100%
813	24	23	23	96%
817	14	11	11	79%
825	25	24	25	100%
826	11	6	9	82%
827	17	14	17	100%
836	20	19	19	95%
840	14	14	14	100%
846	15	14	15	100%
849	13	11	12	92%
868	15	13	14	93%
880	10	10	10	100%
901	25	23	23	92%
903	20	16	18	90%
904	15	12	11	73%
TOTAL	275	243	258	94%

Table S7: Total number of bands with and without the external control (EC), number and percentage of polymorphic bands (PB) for each ISSR primer for analysis of samples from Vila Nova de Milfontes (MFold, MFRec and MFCL).

Primer	N° Total Bands With EC	N° Total Bands Without EC	N° PB (with EC)	%PB (with EC)
808	11	11	11	100%
810	17	15	17	100%
813	10	9	10	100%
823	13	13	13	100%
827	11	8	11	100%
836	11	10	11	100%
840	14	14	14	100%
846	10	10	10	100%
849	14	14	14	100%
868	9	8	9	100%
901	17	16	17	100%
TOTAL	137	128	137	100%