

WEAK POPULATION SPATIAL GENETIC STRUCTURE AND LOW INFRASPECIFIC SPECIFICITY FOR FUNGAL PARTNERS IN THE RARE MYCOHETEROTROPHIC ORCHID *EPIPOGIUM APHYLLUM*

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Table S1: Details of sampling locations and effort;

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Table S1. Details of sampling locations and sampling effort of the 27 populations of *E. aphyllum*; N_{ITS} - population sample sizes for ribosomal DNA internal transcribed spacer diversity; N_{DNA} - population sample sizes for cpDNA diversity; N_{HAP} – number of haplotypes after correction for clones; N_{SSR} – population sample size for nuclear microsatellite diversity; MLG - number of multilocus genotypes observed in population after correction for clones; eMLG – number of expected MLG at the smallest sample size ≥ 10 based on rarefaction; *R* – genotypic richness; *R_t* – genotypic richness in population ≥ 10 based on rarefaction; *na* – not assessed. In bold, populations ≥ 4 MLG for which population genetic analyses have been conducted.

Location in range	Region	Code	Location of population	Lat/long (N/E)	N _{ITS}	N _{DNA}	N _{HAP}	N _{SSR}	MLG (eMLG)	<i>R</i> (<i>R_t</i>)
WESTERN	Pyrenees	TVF	Thuès-entre-Valls	42.515/2.252	-	3	3	4	4	1.000
		CXF	Cazaux	43.052/1.509	-	2	1	2	1	<i>na</i>
		OOF	Oô	42.796/0.505	-	2	1	2	1	<i>na</i>
	Massif Central	SCF	Saint-Clément	44.924/2.661	3	1	1	5	3	0.500
		BGF	Bois de Grandval	45.032/2.769	-	7	3	7	3	0.333
		MOF	Malbo	45.000/2.734	2	21	5	26	5 (3.09)	0.154 (0.084)
		SSF	Saint-Paul-de-Salers	45.140/2.517	-	1	1	1	1	<i>na</i>
		PSF	Ponteils	44.891/3.958	1	-	-	2	2	<i>na</i>
		PEF	Picherande	45.463/2.769	1	3	1	3	1	<i>na</i>
SOUTHERN	Alps	BNF	Boscodon	44.494/6.453	-	4	4	4	4	1.000
		BRF	Brizon	46.036/6.440	-	-	-	2	1	<i>na</i>
		LVF	Lans en Vercors	45.129/5.588	1	1	1	2	2	<i>na</i>
		VLF	Villard-de-Lans	45.069/5.550	-	1	1	1	1	<i>na</i>
		VUS	Val d’Uina	46.608/7.121	6	6	6	6	6	1.000
	Carpathians	OPP	Ojcowski National Park	50.185/19.817	11	25	11	32	11 (11.00)	0.322 (0.322)
		TMP	Tatra Mountains	49.255/19.897	5	26	8	35	9 (8.49)	0.235 (0.220)
NORTHERN	Baltic region	WEP	Wejherowo	54.624/18.212	1	32	1	80	1 (1)	0.000 (0.000)
		KRP	Kozi Rynek	53.799/23.227	1	1	1	1	1	<i>na</i>
		SAN	Snåsa	64.200/12.200	3	2	2	4	3	0.666
		RKS!	Ragunda Kommun	63.100/16.300	1	1	1	1	1	<i>na</i>
		HIE	Hiiumaa Island	58.933/24.241	1	1	1	1	1	<i>na</i>
		JAE	Jouga	59.176/27.465	1	1	1	1	1	<i>na</i>
		KEE	Kose	59.329/27.499	1	1	1	1	1	<i>na</i>
		JEE	Jänioe	59.264/25.518	1	2	2	7	5	0.666
		LDR	Luzhsky District	58.983/29.688	-	4	4	4	4	1.000
		VRR	Vologda Region	59.693/37.911	2	3	1	4	1	0.250
EAST ERN	Asia	ZDR	Zeysky District	54.085/126.874	2	7	7	10	8 (8.00)	0.778 (0.778)
TOTAL					44	158	69	248	82	(0.265)

! – sample from Jodrell Kew DNA bank (accession no. 19248)

Table S2. Microsatellite loci for *E. aphyllum*, with repeated motif, primer sequences (5'-3' sequence), amplicon size range, and PCR annealing temperatures (T_m).

Marker name	Motif	Forward primer	Reverse primer	Size range (bp)	No of alleles	T_m
Epi_8	(AAG) ₅	AACCCTTTAAGCCAGTGGGA	TCAACCCGTCTTGTTTCAGGT	207-213	3	60
Epi_13	(AG) ₁₃	TTCGATCGATCACCAACCTAA	CCTCTTGAAGGCTTTGAAGGTT	214-244	8	64
Epi_17	(AG) ₉	GAATGTCTTCCCATTCGACG	AGTCGTGCATGTTTGACCCT	177-191	6	63
Epi_21	(AT) ₈	ACGATGCACTTGAGACCTGA	CGAGGGTTATTAAGGCTTGATTATT	185-191	4	58
Epi_22	(AAG) ₉	AGTATGGCGCTTTCGCTTC	CTCCGGAGCAGGATGAAATA	139-163	8	60
Epi_26	(TC) ₇ C(TC) ₂	TGTTGAAGAACCTGTGGACTAAA	TATCCCATTGTTTCGGACCAG	313-317	3	58
Epi_45	(AG) ₁₁	CCGATCAACGAATGGACAA	TGGTCTTCATCTCTACAGTCT	183-211	13	60
Epi_49 ^a	(AT) ₁₁	AGGGAGGCGCATATCAATTA	GCCCACGTATGAGGGTAAAA	189-205	9	60
Epi_51	(AG) ₁₄	GAAGACTTTGACTGTTTCGTTCC	ACACCTGATTGCTCCCAGTT	(218)226-238	7	60
Epi_53 ^a	(AT) ₁₃	CAAATGGAAGAGCCAGGTGA	TCCCTAACAAGCTTTCCCAG	253-273	10	60
Epi_55 ^a	(AG) ₁₁	TGTTCTCCCTCTATCTCCCG	AGGGCTCTGAGAAACCCTTG	188-210	10	60
Epi_60 ^b	(AT) ₁₂	ATTGAGCAAAGCAGATGGCT	CAATGCCCGATCAGTTAAGC	146-152	4	58
Epi_61	(AAT) ₁₁	ATTAACGGGTGAGGTGATCG	CCAGCAAGAAAGGAGTGTCC	205-214	4	60

^a discarded because of evidence of null alleles.

^b discarded due to missing data

Table S3. Plastid loci for *E. aphyllum*, with primer names and sequences.

	Locus	Foreward primer	Reverse primer
1	<i>accd-trnE</i> (UUC)	<i>accd-5447F:</i> AATTCTTCATTTTCTTCAGTACTACCA	<i>trnE-5989R:</i> ACTGGGTTGAGCTGGATTG
2	<i>rps4</i>	<i>rps4-1F:</i> CGAAGTTTGTCCGCTCTACC	<i>rps4-1R</i> ATTCGACAACCAATAGCTCA
3	<i>rps12-trnL</i> (GCA)	<i>rps12-1F:</i> AGAGTGTATGTGCGATTGTTT	<i>trnL-1R:</i> CATCTGGATTGAACCAGAGTAT

Table S4. PCR conditions for plastid loci amplification.

	Locus	Initial de-naturation	Denaturation	Annealing	Extension	Final Extension
1	<i>accd-trnE</i> (UUC)	96 °C for 5 min	95°C for 30 s	58.5 °C for 30 s	72 °C for 120 s	72 °C for 10 min
			30 cycles			
2	<i>rps4</i>	96 °C for 5 min	95°C for 30 s	58.5 °C for 30 s	72 °C for 120 s	72 °C for 10 min
			30 cycles			
3	<i>rps12-trnL</i> (GCA)	96 °C for 5 min	95°C for 30 s	60 °C for 30 s	72 °C for 45 s	72 °C for 7 min
			Touch town -1 °C/cycle, 10 cycles			
			95°C for 30 s	50 °C for 30 s	72 °C for 45 s	
			25 cycles			

Table S5. Description of the 22 plastid DNA haplotypes identified in *E. aphyllum*. Position of mutations in the base pairs (†) of the aligned *rps4*, *accd-trnE*(UUC) and *rps12-trnL*(GCA) matrix respectively. Condition found in the majority of samples is marked as “0”. Values 4-8 in a position 206 of *rps12-trnL* alignment constitute number of repeats (n) of microsatellite motif (TA)_n.

locus	<i>rps4</i>	<i>accd-trnE</i> (UUC)											<i>rps12-trnL</i> (GCA)																	
Haplotype	21†	138	152	155	319	341	343	350	353	380	462	473	75	87	104	122	192	202	206	247	297	303	314	426	445	483	491	494	504	537
H-1	1	0	1	0	0	0	0	1	0	0	1	0	0	0	0	1	0	0	7	0	0	0	0	1	0	0	0	0	0	0
H-2	0	0	1	0	0	0	0	1	0	0	1	0	0	0	1	1	0	0	7	0	0	0	1	1	0	0	0	0	0	0
H-3	0	0	1	0	0	0	0	1	0	0	1	0	0	0	0	1	0	0	7	0	0	0	1	1	0	0	0	0	0	0
H-4	0	0	1	0	1	0	0	1	0	0	1	0	0	0	0	1	0	0	7	0	0	0	1	1	0	0	0	0	0	0
H-5	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0
H-6	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	6	0	0	0	0	0	0	0	0	0	0	0
H-7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7	0	0	0	0	0	0	0	0	0	0	0
H-8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7	0	0	0	0	0	0	0	1	0	0	1
H-9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	0	0	0	0	0	0	0	1	0	0	0
H-10	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	7	0	0	0	0	0	1	1	0	0	0	0
H-11	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	7	0	0	0	0	0	0	0	1	0	0	0
H-12	0	0	0	1	0	0	0	0	0	0	0	1	1	0	0	0	0	1	4	0	0	0	0	0	0	0	0	0	0	0
H-13	0	0	0	1	0	0	0	0	0	0	0	1	1	1	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0
H-14	0	0	0	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	6	0	0	1	0	0	0	0	0	0	0	0
H-15	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	6	0	0	1	0	0	0	0	0	0	0	0
H-16	0	0	0	2	0	0	0	0	0	0	0	0	1	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0
H-17	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	1
H-18	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0
H-19	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	5	1	0	0	0	0	0	0	0	0	0	0
H-20	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	1	0	0
H-21	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	5	0	1	0	0	0	0	0	0	1	0	0
H-22	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	1	1	0	0

Table S6. Pairwise F_{ST} for nine populations of *E. aphyllum* based on nuclear SSR (lower diagonal) and plastid DNA (upper diagonal). Bolded values are statistically significant with $P < 0.05$

	TVF	MOF	BNF	VUS	OPP	TMP	JEE	LDR	ZDR
TVF		0.205	0.250	0.143	0.306	0.250	0.000	1.000	0.344
MOF	0.121		0.006	-0.002	0.126	0.076	0.080	0.558	0.092
BNF	0.168	0.052		-0.051	0.043	0.020	0.111	0.500	0.000
VUS	0.290	0.106	0.055		0.074	0.002	0.023	0.456	0.032
OPP	0.132	0.086	0.064	0.095		0.004	0.233	0.282	0.097
TMP	0.166	0.109	0.079	0.113	0.100		0.157	0.184	0.073
JEE	0.288	0.169	0.026	0.089	0.123	0.121		1.000	0.256
LDR	0.184	0.118	0.102	0.119	0.106	0.042	0.066		0.397
ZDR	0.217	0.077	0.006	0.055	0.099	0.058	0.090	0.093	

Table S7. Pairwise F_{ST} on a regional level *E. aphyllum* diversity based on nuclear SSR (lower diagonal) and plastid DNA (upper diagonal). Bolded values are statistically significant with $P < 0.05$.

	Pyrenees	Massif Central	Alps	Carpathians	North Europe	East
Pyrenees		0.179	0.189	0.275	0.238	0.438
Massif Central	0.033		-0.004	0.064	0.057	0.128
Alps	0.110	0.060		0.024	0.076	0.048
Carpathians	0.058	0.050	0.035		0.075	0.062
North Europe	0.070	0.049	0.024	0.013		0.188
Asia	0.151	0.090	0.027	0.056	0.044	

Table S8. Identification of symbiotic fungal species of *E. aphyllum* found in present study. For the species of *Inocybe* their affiliation to the alignment groups (AG) according to Ryberg *et al.* (2009) is given. WEP- Wejherowo, TMP- Tatra Mountains, KRP - Kozi Rynek, OPP - Ojcowski National Park.

alignment group	OTU	Population	Sample name	GenBank accession No	The most similar sequence in UNITE (UDB) or GenBank : species. (accession No)	E value	Maximum identity (%)
AG-2	OTU3	WEP*	WEP_Ea202	KX867466	<i>Inocybe glabrescens</i> Velen. (HQ604513)	0.0	98
	OTU8	WEP	WEP_Ea482 WEP_Ea93	KX867483 KX867484	<i>Inocybe nitidiuscula</i> (Britzelm.) Lapl. (AM882913)	0.0	99
		KRP	KRP_Ea1	OL461961			
	OTU9	WEP	WEP_Ea507	KX867485	<i>Inocybe flocculosa</i> (Berk.) Sacc.(AM882992)	0.0	99
	OTU15	TMP	TMP_Ea17-2	OL461963	<i>Inocybe sindonia</i> (Fr.) P. Karst. (HQ604384)	0.0	99
AG-3	OTU14	TMP	TMP_Ea17-4 TMP_Ea17-5	OL461964 OL461965	<i>Inocybe cervicolor</i> (Pers.) Quél. (UDB032065)	0.0	98
AG-5	OTU1	WEP	WEP_Ea125	KX867464	<i>Inocybe lilacina</i> (Peck) Kauffman (JF908236)	0.0	100
	OTU5	WEP	WEP_Ea256	KX867468	<i>Inocybe posterula</i> (Britzelm.) Sacc. (JF908223)	0.0	99

AG-7	OTU13	WEP	WEP_Ea429	KX867502	<i>Inocybe subbrunnea</i> Kühner (KJ399945)	0.0	99
			WEP_Ea460	KX867503			
		TMP	TMP_Ea17-1	OL461962			
	OTU11	WEP	WEP_Ea234	KX867492	<i>Inocybe cf. castanea</i> Velenovsky (HQ604374)	0.0	99
AG-8	OTU7	WEP	WEP_Ea34	KX867482	<i>Inocybe griseolilacina</i> J.E. Lange (AM882728)	0.0	99
	OTU4	WEP	WEP_Ea218	KX867467	<i>Inocybe subnudipes</i> Kühner (UDB015353)	0.0	99
AG-13	OTU2	WEP	WEP_Ea194-P2	KX867465	<i>Inocybe cf. mixtilis</i> (Britzelm.) Sacc. (AM882836)	0.0	99
	OTU10	WEP	WEP_Ea115, WEP_Ea154, WEP_Ea162, WEP_Ea226, WEP_Ea233, WEP_Ea346, WEP_Ea192, WEP_Ea406	KX867486 to KX867494	<i>Inocyte mixtilis</i> (Britzelm.) Sacc (HQ604488)	0.0	100
AG-16	OTU6	WEP	WEP_Ea277, WEP_Ea279, WEP_Ea214, WEP_Ea264, WEP_Ea300, WEP_Ea342, WEP_Ea453, WEP_Ea457, WEP_Ea87, WEP_EaAlb, WEP_Ea194-P1, WEP_Ea501	KX867469 to KX867481	<i>Inocybe terrigena</i> Fr. Kühner (AM882864)	0.0	99
		TMP	TMP_Ea14-2	KX940905			

	OTU12	WEP	WEP_Ea441, WEP_Ea182, WEP_Ea119, WEP_Ea171, WEP_Ea380, WEP_Ea287, WEP_Ea297, WEP_EaRos	KX867495 to KX867494	<i>Inocybe leucoblema</i> Kühner (HM209789)	0.0	99
	OTU16	OPP	OPP_Ea17-2, OPP_Ea17-3, OPP_Ea17-4, OPP_Ea17-5,	OL461967 to OL461970	<i>Hebeloma sinapizans</i> (Paulet) Gillet (HF678206)	0.0	99
	OTU17	TMP	TMP_Ea17-3	OL461966	<i>Hebeloma incarnatulum</i> A. H. Sm. (AF430291)	0.0	99
	OUT 18	OPP	OPP_Ea17-4T	OL461971	<i>Tomentellopsis</i> sp (AJ893355)	0.0	94

*40 specimens of *E. aphyllum* from WEP represent the same multilocus genotype.