SUPPORTING INFORMATION

Phylogeographic and morphological analysis of *Botrylloides niger* Herdman, 1886 from the northeastern Mediterranean Sea

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Supporting Tables

Table S1. Details of sampling stations.

Region	Station	Date	Coordinates	Salinity (ppt)	pH
Antalya	Kemer	25/10/2018	36°35'-59.55"-N 30°34'-30.66"-E	40.1	7.9
	Side	24/10/2018	36°46'-00.78"-N 31°23'-07.62"-E	37.5	7.8
	Alanya	24/10/2018	36°33'-33.85"-N 31°57'-07.41"-E	39.8	8.0
	Tersane	29/10/2018	36°32'-05.14"-N 31°59'-53.89"-E	-	-
Mersin	Tisan	03/10/2018	36°09'-27.95"-N 33°40'-59.90"-E	40.2	8.3
	Kızkalesi	03/10/2018	36°27'-27.52"-N 34°08'-38.17"-E	39-40	~ 8
	Mezitli	10/4/2018	36°43'-58.76"-N 34°31'-18.53"-E	39.2	8.1
Hatay	Konacık	26/9/2018	36°21'-38.79"-N 35°49'-16.90"-E	40.0	7.9

Table S2. Details of samplings, observed haplotypes and genotypes. N: sample number. [a]: samples used for the spatial analysis, [b]: samples used for the temporal analysis, *: samples without sequences.

			Observed haplotypes			
Station	**Sampling date**	**N**	**COI**	**H3**	**18S**	**28S**
Kemer	10/2018	30[a]	H1	H01,H02,H03,H13,H14	-	-
Side	10/2018	10[a]	H1	H02,H11,H12	-	-
	10/2018	1*	-	-	-	-
Alanya	04/2012	21[a]	H1	-	-	-
	04/2012	1*	-	-	-	-
	10/2018	3[a]	H1	H09,H10	-	-
Tersane	10/2018	3[a]	H1	-	-	-
Tisan	10/2018	14[a]	H1	H01,H02,H03	-	-
Kızkalesi	04/2012	5*	-	-	-	-
	07/2014	2*	-	-	-	-
	09/2014	16*	-	-	-	-
	11/2017	18[b]	H1,H2	H02,H03,H07	H1	H1
	11/2017	3*	-	-	-	-
	07/2018	4[b]	H1	-	H1	-
	07/2018	2*	-	-	-	-
	08/2018	17[b]	H1	H02	-	-
	09/2018	26[b]	H1	H03,H08	-	-
	09/2018	3*	-	-	-	-
	10/2018	38[a,b]	H1,H2	H02,H03,H05,H06	-	-
	03/2019	1*	-	-	-	-
	04/2019	1*	-	-	-	-
Mezitli	11/2017	3[a]	H1	-	-	-
	10/2018	11[a]	H1,H3	H03,H04	-	-
Konacık	09/2018	5[a]	H1,H4	H01,H02	-	-
	TOTAL samples	**238**				
	TOTAL sequenced	**203**				
	TOTAL spatial analysis	**138**				
	TOTAL temporal analysis	**103**				

Table S3. Kızkalesi station time-series samplings. N: sample number. '0' indicates that no sample could be found during that month.

Date	N
November – 2017	18
December -2017	0
January – 2018	0
February – 2018	0
March – 2018	0
April – 2018	0
May – 2018	0
June – 2018	0
July – 2018	4
August – 2018	17
September – 2018	26
October -2018	38

Table S4. PCR programs for COI, H3, 28S rRNA and 18S rRNA genes.

PCR program	Denaturation	Annealing	Extension	Final Extension	Cycles number	Reference
DEGCOI	95ºC (1 min)	45ºC (30 sec)	72ºC (1 min)	72ºC (10 min)	35	Reem *et al*., 2018 [10]
H3	95ºC (1 min)	60ºC (45 sec)	72ºC (1 min)	72ºC (10 min)	35	Reem *et al*., 2018 [10]
28S	95ºC (1 min)	62ºC (45 sec)	72ºC (1 min)	72ºC (10 min)	35	Reem *et al*., 2018 [10]
18S	95ºC (1 min)	60ºC (45 sec)	72ºC (1 min)	72ºC (10 min)	35	Reem *et al*., 2018 [10]

Table S5. Primers used in the present study. Differences between product size and analysed size result from the trimming of low-quality sequences following alignment.

Primer name	Primer sequence	Product size	Analysed size
DEG COI F2 DEG COI R2	5-'AMWAATCATAAAGATATTRGWAC'-3 5-' AARAARGAMGTRTTRAAATTHCGATC'-3	~700 bp	519 bp
H3 F1 H3 R1	5-'ATGGCTCGTACCAAGCAGACVGC'-3 5-'ATATCCTTRGGCATRATRGTGAC'-3	~350bp	280 bp
28S C1 28S D2	5-'ACCCGCTGAATTTAAGCAT'-3 5-' TCCGTGTTTCAAGACGGG'-3	~950 bp	648 bp
18S A 18S B	5-'AACCTGGTTGATCCTGCCAGT'-3 5-'GATCCTTCTGCAGGTTCACCTAC'-3	~1750 bp	966 bp

Table S6. Accession numbers for the GenBank reference sequences. Label corresponds to the name used in Table 1.

Scientific name	Label	COI	H3	18S	28S
Botryllus schlosseri	Out-group	AY600987.1			
Botrylloides leachii		HF548553.1			
Botrylloides nigrum	BN-IL	HF548559.1			
Botrylloides leachii		HG931921.1			
Botrylloides leachii		KF309549.1			
Botrylloides leachii		KF309551.1			
Botrylloides leachii		KF309608.1			
Botrylloides leachii	BL-ES	KF309644.1			
Botrylloides nigrum		KP254541.1			
Botrylloides nigrum		KU711782.1			
Botrylloides nigrum		KU711783.1			
Botrylloides nigrum		KU711784.1			
Botrylloides nigrum		KU711785.1			
Botrylloides nigrum		KU711786.1			
Botrylloides nigrum		KU711787.1			
Botrylloides nigrum		KU711788.1			
Botrylloides nigrum		KU711789.1			
Botrylloides leachii		KY235400.1			
Botrylloides leachii		KY235401.1			
Botrylloides leachii		KY235402.1			
Botrylloides leachii		KY235403.1			
Botrylloides niger	BN-BR	LR828514.1			
Botrylloides leachii		LR828515.1			
Botrylloides leachii		LR828516.1			
Botrylloides leachii		LR828517.1			
Symplegma brakenhielmi		LS992554.1			
Botrylloides leachii	BL-IT	MG009578.1			
Botrylloides aff. leachii	BL-IL	MG009579.1			
Botrylloides aff. leachii		MG009584.1			
Botrylloides aff. leachii		MG009589.1			
Botrylloides leachii		MG009592.1			
Botrylloides aff. leachii		MG009593.1			
Botryllus schlosseri		MG009595.1			
Botrylloides leachii	BL-FR	MK978812.1			
Botrylloides diegensis	BD-FR	MN076483.1			
Botrylloides diegensis		MN175978.1			
Botrylloides diegensis		MN175979.1			
Botrylloides diegensis		MN175980.1			
Botrylloides diegensis		MN175981.1			

Scientific name	Label	COI	H3	18S	28S
Botrylloides diegensis		MN175982.1			
Botrylloides diegensis		MN175983.1			
Botrylloides diegensis		MN175984.1			
Botrylloides diegensis		MN175985.1			
Botrylloides diegensis		MN175986.1			
Botrylloides diegensis		MN175987.1			
Botrylloides diegensis		MN175988.1			
Botrylloides diegensis		MT232722.1			
Botrylloides niger		MT232723.1			
Botrylloides niger		MT232728.1			
Botrylloides niger		MT637960.1			
Botrylloides niger		MT637961.1			
Botrylloides nigrum	BN-US	MW278779.1			
Botrylloides niger		MW285094.1			
Botrylloides niger		MW285095.1			
Botrylloides diegensis		MW579604.1			
Botrylloides diegensis		MW579605.1			
Botrylloides diegensis		MW579606.1			
Botrylloides diegensis		MW579607.1			
Botrylloides diegensis		MW579608.1			
Botrylloides diegensis		MW579609.1			
Botrylloides diegensis		MW579610.1			
Botrylloides diegensis		MW579611.1			
Botrylloides diegensis		MW579612.1			
Botrylloides diegensis		MW579613.1			
Botrylloides diegensis		MW579614.1			
Botrylloides diegensis		MW579615.1			
Botrylloides diegensis		MW579616.1			
Botrylloides diegensis		MW579617.1			
Botrylloides diegensis		MW579618.1			
Botrylloides diegensis		MW579619.1			
Botrylloides diegensis		MW579620.1			
Botrylloides niger		MW817940.1			
Botrylloides diegensis		MW817941.1			
Botrylloides diegensis		MW817942.1			
Botrylloides diegensis		MW817943.1			
Botrylloides niger	BN-US	MW858360.1			
Botrylloides diegensis		MW872270.1			
Botrylloides diegensis		MW872285.1			
Botrylloides diegensis		MZ533117.1			
Botrylloides niger		OM866151.1			
Botrylloides niger		OM912589.1			

Scientific name	Label	COI	H3	18S	28S
Botrylloides niger		OM912590.1			
Botrylloides niger		OM912593.1			
Botrylloides niger		OM912594.1			
Botrylloides sp.		ON053355.1			
Botrylloides sp.		ON053356.1			
Botrylloides diegensis		ON059141.1			
Botrylloides diegensis		ON076464.1			
Botrylloides diegensis		ON076465.1			
Botrylloides diegensis		ON076466.1			
Botrylloides diegensis		ON076467.1			
Botrylloides diegensis		ON076468.1			
Botrylloides diegensis		ON076469.1			
Botrylloides cf. lentus		ON098245.1			
Botrylloides niger		OP221206.1			
Botrylloides leachii			MG009592.1		
Botryllus schlosseri			MG009595.1		
Botrylloides aff. leachii				MG009584.1	
Botrylloides aff. leachii					MG009589.1

Table S7. Life history of the 19 colonies cultured in the laboratory. Life cycle corresponds to the mean duration of the blastogenic cycle, measured as the time between two takeovers.

Colony ID	Sampling site	Sampling date	Morphotype	Haplotype	Culture start date	Life cycle (days)	Culture end date
G1	Tisan	03/10/2018	cream-violet	H1	03/10/2018	4.5	25/12/2018
G8	Tisan	03/10/2018	orange-brown	H1	03/10/2018	5	25/12/2018
G15	Tisan	03/10/2018	brown-striped	H1	03/10/2018	5	26/02/2019
Z1	Kızkalesi	03/08/2018	orange	H1	03/08/2018	6	14/03/2019
Z2	Kızkalesi	03/08/2018	orange	H1	03/08/2018	5	15/02/2019
Z4	Kızkalesi	03/08/2018	cream-violet	H1	03/08/2018	6	14/03/2019
Z12	Kızkalesi	03/08/2018	red	H1	03/08/2018	5	26/02/2019
R1	Kızkalesi	18/09/2018	orange	H1	18/09/2018	4	12/02/2019
R4	Kızkalesi	18/09/2018	brown-striped	H1	18/09/2018	6	16/05/2019
R6	Kızkalesi	18/09/2018	green-brown	H1	18/09/2018	3	22/04/2019
R8	Kızkalesi	18/09/2018	green-brown	H1	18/09/2018	5	16/05/2019
R9	Kızkalesi	18/09/2018	brown-striped	H1	18/09/2018	5	07/12/2018
R12	Kızkalesi	18/09/2018	cream-violet	H1	18/09/2018	4	16/05/2019
GK4	Kızkalesi	03/10/2018	brown-striped	H1	03/10/2018	4	14/03/2019
GK7	Kızkalesi	03/10/2018	brown-striped	H1	03/10/2018	3	01/04/2019
GK13	Kızkalesi	03/10/2018	orange-brown	H1	03/10/2018	4	07/12/2018
GK21	Kızkalesi	03/10/2018	red	H1	03/10/2018	5	16/05/2019
L15	Konacık	26/09/2018	white-brown	H4	26/09/2018	5	16/05/2019
L18	Konacık	26/09/2018	brown-striped	H1	26/09/2018	4	16/05/2019

Table S8. Pairwise comparison of the genetic differentiation (F_{st}) between the temporal populations of Kızkalesi. No statistical significance was measured for F_{st}. n.c.: Not calculated due to a lack of polymorphism.

F_{st}	11/2017	07/2018	08/2018	09/2018	11/2018
11/2017	-	0	0	0	-0.037
07/2018	-	-	n.c.	n.c.	0
08/2018	-	-	-	n.c.	0
09/2018	-	-	-	-	0
10/2018	-	-	-	-	-

Table S9. **Correspondence between sample IDs, morphotypes and the determined haplotype/genotype for COI, H3, 18S and 28S.** NA: no morphotype information. -: no haplotype/genotype information.

				Markers			
Sample index	**Group index**	**Sample ID**	**Morphotype**	**COI**	**H3**	**18S**	**28S**
1	1	C30-Kemer_OCT18	orange-brown	H1	H03	-	-
2	2	C31-Kemer_OCT18	green-brown	H1	H03	-	-
3	3	C32-Kemer_OCT18	green-brown	H1	-	-	-
4	4	C33-Kemer_OCT18	green-brown	H1	-	-	-
5	5	C34-Kemer_OCT18	orange-brown	H1	-	-	-
6	6	C35-Kemer_OCT18	green-brown	H1	H13	-	-
7	7	C38-Kemer_OCT18	green-brown	H1	H03	-	-
8	8	C39-Kemer_OCT18	white-brown	H1	-	-	-
9	9	C40-Kemer_OCT18	orange-brown	H1	H03	-	-
10	10	C41-Kemer_OCT18	orange-brown	H1	H03	-	-
11	11	C42-Kemer_OCT18	green-brown	H1	H14	-	-
12	12	C43-Kemer_OCT18	green-brown	H1	-	-	-
13	13	C44-Kemer_OCT18	black-white	H1	H02	-	-
14	14	C45-Kemer_OCT18	orange-brown	H1	-	-	-
15	15	C46-Kemer_OCT18	brown-striped	H1	-	-	-
16	16	C47-Kemer_OCT18	red	H1	H03	-	-
17	17	C48-Kemer_OCT18	green-brown	H1	H02	-	-
18	18	C49-Kemer_OCT18	cream-green	H1	H02	-	-
19	19	C50-Kemer_OCT18	black-white	H1	-	-	-
20	20	C51-Kemer_OCT18	black-white	H1	-	-	-
21	21	C52-Kemer_OCT18	red	H1	-	-	-
22	22	C54-Kemer_OCT18	white-brown	H1	-	-	-
23	23	C55-Kemer_OCT18	orange	H1	H01	-	-
24	24	C56-Kemer_OCT18	green-brown	H1	-	-	-
25	25	C58-Kemer_OCT18	green-brown	H1	H03	-	-
26	26	C59-Kemer_OCT18	brown-striped	H1	-	-	-
27	27	C60-Kemer_OCT18	brown-striped	H1	-	-	-
28	28	C61-Kemer_OCT18	brown-striped	H1	H03	-	-
29	29	C62-Kemer_OCT18	orange-brown	H1	H03	-	-
30	30	C63-Kemer_OCT18	brown-striped	H1	-	-	-
31	1	C18-Side_OCT18	green-brown	H1	-	-	-

				Markers			
Sample index	**Group index**	**Sample ID**	**Morphotype**	**COI**	**H3**	**18S**	**28S**
32	2	C19-Side_OCT18	orange-brown	H1	-	-	-
33	3	C20-Side_OCT18	orange-brown	H1	H11	-	-
34	4	C21-Side_OCT18	white-brown	-	-	-	-
35	5	C22-Side_OCT18	white-brown	H1	H02	-	-
36	6	C24-Side_OCT18	orange-brown	H1	H02	-	-
37	7	C25-Side_OCT18	red	H1	H02	-	-
38	8	C26-Side_OCT18	green-brown	H1	H02	-	-
39	9	C27-Side_OCT18	green-brown	H1	H02	-	-
40	10	C28-Side_OCT18	orange-brown	H1	H12	-	-
41	11	C29-Side_OCT18	NA	H1	-	-	-
42	1	A1_i-Alanya_APR12	orange-brown	H1	-	-	-
43	2	A3_i-Alanya_APR12	orange-brown	H1	-	-	-
44	3	A4_i-Alanya_APR12	orange-brown	H1	-	-	-
45	4	A7_i-Alanya_APR12	orange-brown	H1	-	-	-
46	5	A8_i-Alanya_APR12	orange	H1	-	-	-
47	6	A9_i-Alanya_APR12	orange-brown	H1	-	-	-
48	7	A10_i-Alanya_APR12	orange-brown	H1	-	-	-
49	8	A10_i_2-Alanya_APR12	NA	H1	-	-	-
50	9	A1-Alanya_APR12	brown-striped	H1	-	-	-
51	10	A6-Alanya_APR12	brown-striped	H1	-	-	-
52	11	A8-Alanya_APR12	white-brown	H1	-	-	-
53	12	A11-Alanya_APR12	orange-brown	H1	-	-	-
54	13	A12-Alanya_APR12	brown-striped	H1	-	-	-
55	14	A14-Alanya_APR12	brown-striped	H1	-	-	-
56	15	A15-Alanya_APR12	brown-striped	H1	-	-	-
57	16	A16-Alanya_APR12	brown-striped	H1	-	-	-
58	17	A20-Alanya_APR12	cream-violet	H1	-	-	-
59	18	A21-Alanya_APR12	brown	-	-	-	-
60	19	A23-Alanya_APR12	brown-striped	H1	-	-	-
61	20	A24-Alanya_APR12	brown	H1	-	-	-
62	21	A25-Alanya_APR12	brown-striped	H1	-	-	-
63	22	A26-Alanya_APR12	NA	H1	-	-	-
64	1	C10-Alanya_OCT18	green-brown	H1	-	-	-
65	2	C16-Alanya_OCT18	orange-brown	H1	H09	-	-
66	3	C17-Alanya_OCT18	brown-striped	H1	H10	-	-

				Markers			
Sample index	**Group index**	**Sample ID**	**Morphotype**	**COI**	**H3**	**18S**	**28S**
67	1	K1-Tersane_OCT18	green-brown	H1	-	-	-
68	2	K2-Tersane_OCT18	red	H1	-	-	-
69	3	K3-Tersane_OCT18	brown-striped	H1	-	-	-
70	1	G1-Tisan_OCT18	cream-violet	H1	-	-	-
71	2	G2-Tisan_OCT18	cream-violet	H1	H02	-	-
72	3	G4-Tisan_OCT18	cream-violet	H1	-	-	-
73	4	G5-Tisan_OCT18	green-brown	H1	-	-	-
74	5	G6-Tisan_OCT18	green-brown	H1	-	-	-
75	6	G8-Tisan_OCT18	orange-brown	H1	H03	-	-
76	7	G9-Tisan_OCT18	cream-violet	H1	-	-	-
77	8	G10-Tisan_OCT18	brown-striped	H1	-	-	-
78	9	G11-Tisan_OCT18	white-brown	H1	-	-	-
79	10	G12-Tisan_OCT18	cream-violet	H1	H01	-	-
80	11	G13-Tisan_OCT18	cream-violet	H1	-	-	-
81	12	G14-Tisan_OCT18	cream-violet	H1	-	-	-
82	13	G15-Tisan_OCT18	brown-striped	H1	-	-	-
83	14	G16-Tisan_OCT18	cream-violet	H1	-	-	-
84	1	Bides1-Kizkalesi_APR12	red-striped	-	-	-	-
85	2	Bides2-Kizkalesi_APR12	orange-brown	-	-	-	-
86	3	Bides3-Kizkalesi_APR12	brown-striped	-	-	-	-
87	4	Bides4-Kizkalesi_APR12	orange-brown	-	-	-	-
88	5	Bides5-Kizkalesi_APR12	orange-red	-	-	-	-
89	1	Bides1-Kizkalesi_JUL14	brown-striped	-	-	-	-
90	2	Bides2-Kizkalesi_JUL14	orange-brown	-	-	-	-
91	1	OB1-Kizkalesi_SEP14	orange-striped	-	-	-	-
92	2	OB2-Kizkalesi_SEP14	orange	-	-	-	-
93	3	OB3-Kizkalesi_SEP14	NA	-	-	-	-
94	4	OB4-Kizkalesi_SEP14	brown	-	-	-	-
95	5	OB5-Kizkalesi_SEP14	brown-striped	-	-	-	-
96	6	B_LUS1-Kizkalesi_SEP14	brown-striped	-	-	-	-
97	7	O1-Kizkalesi_SEP14	NA	-	-	-	-
98	8	O2-Kizkalesi_SEP14	NA	-	-	-	-
99	9	O3-Kizkalesi_SEP14	NA	-	-	-	-
100	10	O4-Kizkalesi_SEP14	orange-brown	-	-	-	-
101	11	O7-Kizkalesi_SEP14	brown	-	-	-	-

				Markers			
Sample index	**Group index**	**Sample ID**	**Morphotype**	**COI**	**H3**	**18S**	**28S**
102	12	O8-Kizkalesi_SEP14	orange-brown	-	-	-	-
103	13	O9-Kizkalesi_SEP14	NA	-	-	-	-
104	14	O10-Kizkalesi_SEP14	NA	-	-	-	-
105	15	O11-Kizkalesi_SEP14	NA	-	-	-	-
106	16	O12-Kizkalesi_SEP14	brown-striped	-	-	-	-
107	1	BN1-Kizkalesi_NOV17	brown-striped	H2	H02	H1	H1
108	2	BN2-Kizkalesi_NOV17	green-brown	H1	-	-	-
109	3	BN3-Kizkalesi_NOV17	brown-striped	H1	-	-	-
110	4	BN4-Kizkalesi_NOV17	cream-violet	H1	-	-	-
111	5	BN5-Kizkalesi_NOV17	brown-striped	H1	-	-	-
112	6	BN6-Kizkalesi_NOV17	brown-striped	H1	-	-	-
113	7	BS1-Kizkalesi_NOV17	NA	H1	-	-	-
114	8	BS2-Kizkalesi_NOV17	brown-striped	H1	H07	-	-
115	9	BS3-Kizkalesi_NOV17	green-brown	H1	-	-	-
116	10	BS4-Kizkalesi_NOV17	green-brown	H1	-	-	-
117	11	BS5-Kizkalesi_NOV17	cream-violet	H1	-	-	-
118	12	BS6-Kizkalesi_NOV17	cream-violet	H1	-	-	-
119	13	BS8-Kizkalesi_NOV17	green-brown	-	-	-	-
120	14	BV1-Kizkalesi_NOV17	orange	H1	H03	H1	H1
121	15	BV2-Kizkalesi_NOV17	orange-brown	H1	-	-	-
122	16	BV3-Kizkalesi_NOV17	orange-brown	H1	-	-	-
123	17	BV4-Kizkalesi_NOV17	orange	H1	-	-	-
124	18	BV5-Kizkalesi_NOV17	orange-brown	H1	-	-	-
125	19	BV6-Kizkalesi_NOV17	orange	-	-	-	-
126	20	BV7-Kizkalesi_NOV17	orange	-	-	-	-
127	21	BV8-Kizkalesi_NOV17	orange	H1	-	-	-
128	1	J18_BS1-Kizkalesi_JULY18	green-brown	-	-	-	-
129	2	J18_BS2-Kizkalesi_JULY18	green-brown	H1	-	H1	-
130	3	J18_BS7-Kizkalesi_JULY18	green-brown	H1	-	-	-
131	4	J18_BS8-Kizkalesi_JULY18	red	H1	-	-	-
132	5	J18_BS9-Kizkalesi_JULY18	green-brown	H1	-	-	-
133	6	J18_BS13-Kizkalesi_JULY18	red	-	-	-	-
134	1	Z1-Kizkalesi_AUG18	orange	H1	-	-	-
135	2	Z2-Kizkalesi_AUG18	orange	H1	-	-	-
136	3	Z3-Kizkalesi_AUG18	red-striped	H1	-	-	-

				Markers			
Sample index	**Group index**	**Sample ID**	**Morphotype**	**COI**	**H3**	**18S**	**28S**
137	4	Z4-Kizkalesi_AUG18	cream-violet	H1	H02	-	-
138	5	Z5-Kizkalesi_AUG18	brown-striped	H1	-	-	-
139	6	Z6-Kizkalesi_AUG18	orange-brown	H1	-	-	-
140	7	Z8-Kizkalesi_AUG18	orange-brown	H1	-	-	-
141	8	Z9-Kizkalesi_AUG18	red	H1	-	-	-
142	9	Z10-Kizkalesi_AUG18	brown	H1	-	-	-
143	10	Z11-Kizkalesi_AUG18	brown-striped	H1	-	-	-
144	11	Z12-Kizkalesi_AUG18	red	H1	-	-	-
145	12	Z14-Kizkalesi_AUG18	brown	H1	-	-	-
146	13	Z15-Kizkalesi_AUG18	brown-striped	H1	-	-	-
147	14	Z16-Kizkalesi_AUG18	green-brown	H1	-	-	-
148	15	Z19-Kizkalesi_AUG18	NA	H1	-	-	-
149	16	Z20-Kizkalesi_AUG18	brown-striped	H1	-	-	-
150	17	Z21-Kizkalesi_AUG18	brown	H1	-	-	-
151	1	R1-Kizkalesi_SEP18	orange	H1	-	-	-
152	2	R2-Kizkalesi_SEP18	red	H1	-	-	-
153	3	R4-Kizkalesi_SEP18	brown-striped	H1	H03	-	-
154	4	R5-Kizkalesi_SEP18	NA	H1	-	-	-
155	5	R6-Kizkalesi_SEP18	green-brown	H1	H08	-	-
156	6	R7-Kizkalesi_SEP18	brown-striped	H1	-	-	-
157	7	R8-Kizkalesi_SEP18	green-brown	H1	H03	-	-
158	8	R9-Kizkalesi_SEP18	brown-striped	H1	-	-	-
159	9	R10-Kizkalesi_SEP18	black-white	H1	-	-	-
160	10	R11-Kizkalesi_SEP18	green-brown	H1	-	-	-
161	11	R12-Kizkalesi_SEP18	cream-violet	H1	-	-	-
162	12	R14-Kizkalesi_SEP18	orange	H1	-	-	-
163	13	R15-Kizkalesi_SEP18	brown	H1	-	-	-
164	14	R16-Kizkalesi_SEP18	red	H1	-	-	-
165	15	R18-Kizkalesi_SEP18	red	-	-	-	-
166	16	R19-Kizkalesi_SEP18	green-brown	-	-	-	-
167	17	R21-Kizkalesi_SEP18	orange	H1	-	-	-
168	18	R25-Kizkalesi_SEP18	orange-brown	-	-	-	-
169	19	R28-Kizkalesi_SEP18	brown-striped	H1	-	-	-
170	20	R29-Kizkalesi_SEP18	red	H1	-	-	-
171	21	R32-Kizkalesi_SEP18	orange	H1	-	-	-

				Markers			
Sample index	**Group index**	**Sample ID**	**Morphotype**	**COI**	**H3**	**18S**	**28S**
172	22	R36-Kizkalesi_SEP18	orange-brown	H1	-	-	-
173	23	R37-Kizkalesi_SEP18	red	H1	-	-	-
174	24	R39-Kizkalesi_SEP18	brown-striped	H1	-	-	-
175	25	R40-Kizkalesi_SEP18	cream-violet	H1	-	-	-
176	26	R41-Kizkalesi_SEP18	orange	H1	-	-	-
177	27	R42-Kizkalesi_SEP18	green-brown	H1	-	-	-
178	28	R43-Kizkalesi_SEP18	brown-striped	H1	-	-	-
179	29	R44-Kizkalesi_SEP18	brown-striped	H1	-	-	-
180	1	GK1-Kizkalesi_OCT18	orange	H1	H03	-	-
181	2	GK2-Kizkalesi_OCT18	orange	H1	-	-	-
182	3	GK3-Kizkalesi_OCT18	brown-striped	H1	-	-	-
183	4	GK4-Kizkalesi_OCT18	brown-striped	H1	-	-	-
184	5	GK5-Kizkalesi_OCT18	brown-striped	H1	H03	-	-
185	6	GK6-Kizkalesi_OCT18	brown-striped	H1	H02	-	-
186	7	GK7-Kizkalesi_OCT18	brown-striped	H1	H05	-	-
187	8	GK8-Kizkalesi_OCT18	brown	H1	-	-	-
188	9	GK9-Kizkalesi_OCT18	orange-brown	H1	-	-	-
189	10	GK10-Kizkalesi_OCT18	brown-striped	H1	H03	-	-
190	11	GK11-Kizkalesi_OCT18	green-brown	H1	H06	-	-
191	12	GK12-Kizkalesi_OCT18	orange-brown	H1	-	-	-
192	13	GK13-Kizkalesi_OCT18	orange-brown	H1	-	-	-
193	14	GK14-Kizkalesi_OCT18	brown-striped	H1	-	-	-
194	15	GK15-Kizkalesi_OCT18	green-brown	H1	-	-	-
195	16	GK17-Kizkalesi_OCT18	orange-brown	H1	-	-	-
196	17	GK18-Kizkalesi_OCT18	orange	H1	-	-	-
197	18	GK20-Kizkalesi_OCT18	orange	H1	-	-	-
198	19	GK21-Kizkalesi_OCT18	red	H1	H02	-	-
199	20	GK22-Kizkalesi_OCT18	NA	H1	-	-	-
200	21	GK24-Kizkalesi_OCT18	brown-striped	H1	-	-	-
201	22	GK25-Kizkalesi_OCT18	orange	H1	-	-	-
202	23	GK26-Kizkalesi_OCT18	brown	H2	H03	-	-
203	24	GK27-Kizkalesi_OCT18	orange-brown	H1	-	-	-
204	25	GK29-Kizkalesi_OCT18	white-brown	H1	-	-	-
205	26	GK30-Kizkalesi_OCT18	brown	H1	-	-	-
206	27	GK31-Kizkalesi_OCT18	NA	H1	-	-	-

				Markers			
Sample index	**Group index**	**Sample ID**	**Morphotype**	**COI**	**H3**	**18S**	**28S**
207	28	GK32-Kizkalesi_OCT18	green-brown	H1	-	-	-
208	29	GK33-Kizkalesi_OCT18	orange-brown	H1	-	-	-
209	30	GK35-Kizkalesi_OCT18	NA	H1	-	-	-
210	31	GK38-Kizkalesi_OCT18	NA	H1	-	-	-
211	32	GK39-Kizkalesi_OCT18	green-brown	H1	-	-	-
212	33	GK40-Kizkalesi_OCT18	white-brown	H1	-	-	-
213	34	GK41-Kizkalesi_OCT18	orange	H1	-	-	-
214	35	GK43-Kizkalesi_OCT18	red-striped	H1	-	-	-
215	36	GK44-Kizkalesi_OCT18	NA	H1	-	-	-
216	37	GK45-Kizkalesi_OCT18	brown-striped	H1	-	-	-
217	38	GK46-Kizkalesi_OCT18	brown-striped	H1	-	-	-
218	1	KK4-Kizkalesi_MAR19	orange-brown	-	-	-	-
219	1	KK5-Kizkalesi_APR19	cream-violet	-	-	-	-
220	1	Mz_1-Mezitli_NOV17	orange-brown	H1	-	-	-
221	2	Mz_2-Mezitli_NOV17	orange-brown	H1	-	-	-
222	3	Mz_3-Mezitli_NOV17	red	H1	-	-	-
223	1	M2_12-Mezitli_OCT18	black-white	H3	-	-	-
224	2	M2_17-Mezitli_OCT18	black-white	H3	H03	-	-
225	3	M2_6-Mezitli_OCT18	black-white	H1	-	-	-
226	4	M2_7-Mezitli_OCT18	orange	H1	-	-	-
227	5	M2_10-Mezitli_OCT18	brown-striped	H1	-	-	-
228	6	M2_13-Mezitli_OCT18	orange-brown	H1	H04	-	-
229	7	M2_14-Mezitli_OCT18	brown-striped	H1	-	-	-
230	8	M2_15-Mezitli_OCT18	brown	H1	-	-	-
231	9	M2_19-Mezitli_OCT18	pink	H1	-	-	-
232	10	M2_20-Mezitli_OCT18	orange-brown	H1	-	-	-
233	11	M2_22-Mezitli_OCT18	NA	H1	-	-	-
234	1	L9-Konacik_SEP18	orange-brown	H4	-	-	-
235	2	L15-Konacik_SEP18	white-brown	H4	H01	-	-
236	3	L2-Konacik_SEP18	NA	H1	-	-	-

				Markers			
Sample index	**Group index**	**Sample ID**	**Morphotype**	**COI**	**H3**	**18S**	**28S**
237	4	L17-Konacik_SEP18	orange-brown	H1	-	-	-
238	5	L18-Konacik_SEP18	brown-striped	H1	H02	-	-

Supporting Figures

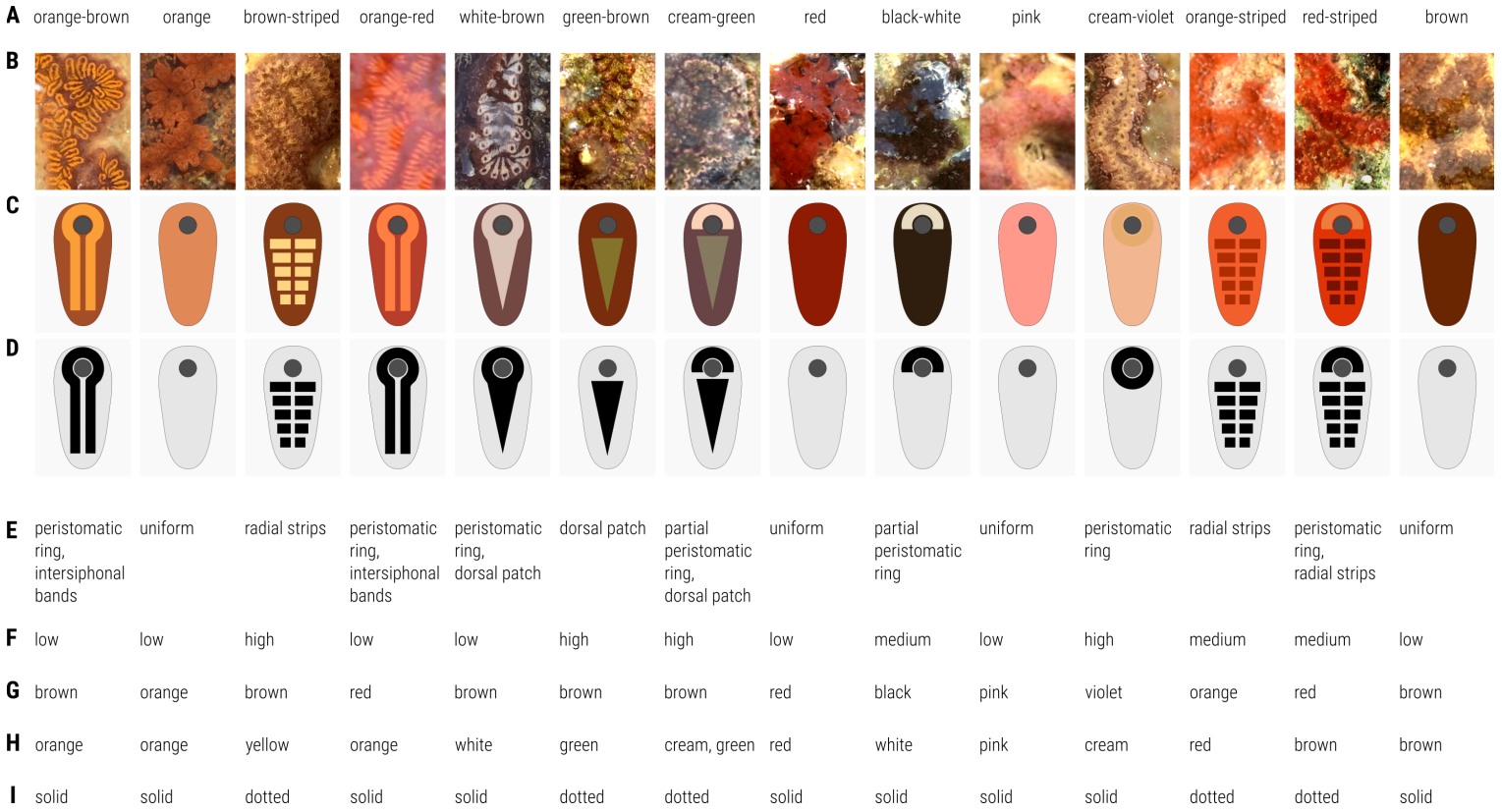

Figure S1. Description of the 14 morphotypes. A) Morphotype name, **B)** sample image, **C)** schematic representation of a typical zooid, **D)** typical pattern of the morphotype, **E)** description of the motif, **F)** variability of the motif, **G)** color of the zooid, **H)** color of the motif and **I)** type of pigmentation.

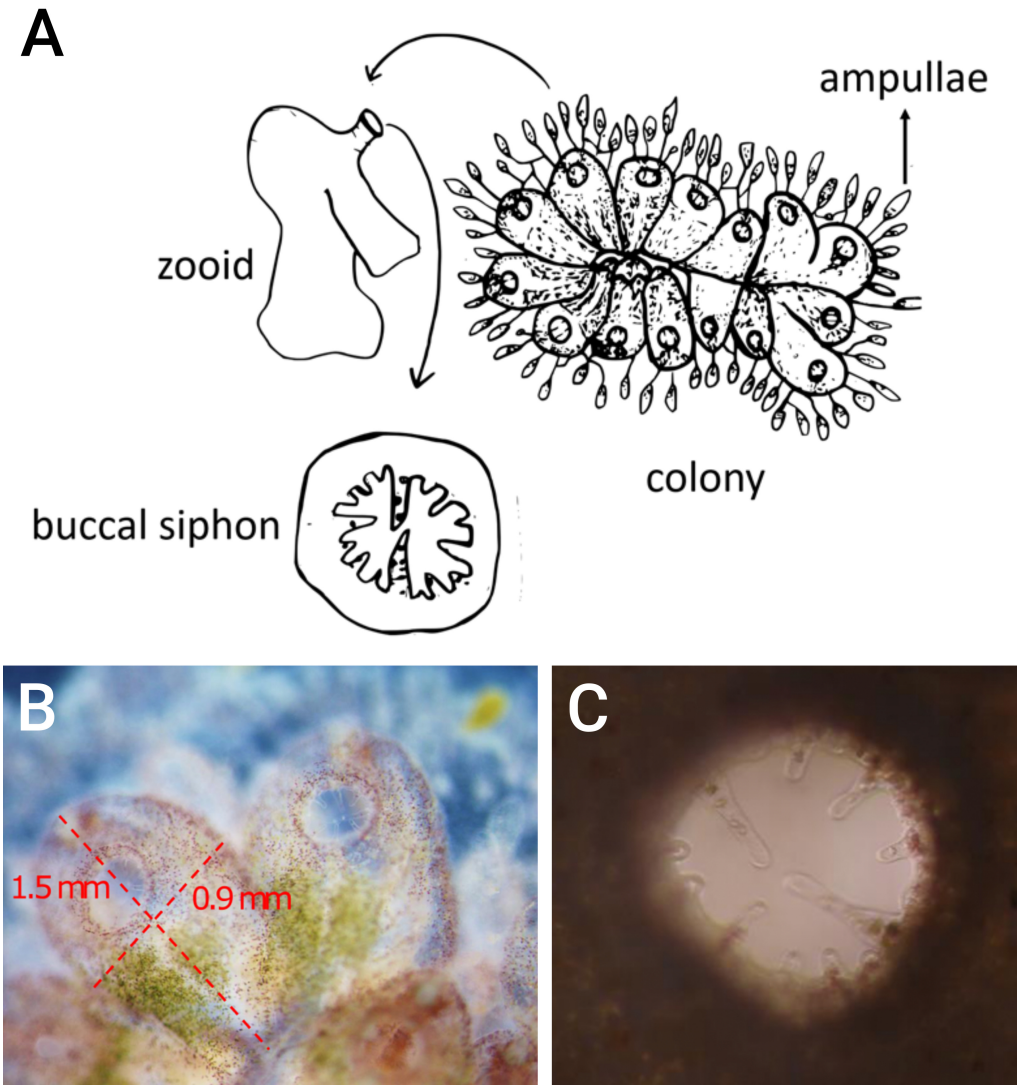

Figure S2. Morphology of *Botrylloides niger*. A) An illustration of *B. niger* colonial system, highlighting key anatomical features including colony, zooid, ampullae (vascular termini), and buccal siphon. **B**) Top view of two zooid overlaid with measurements of its size. **C**) Magnified picture of a buccal siphon showing its tentacles.

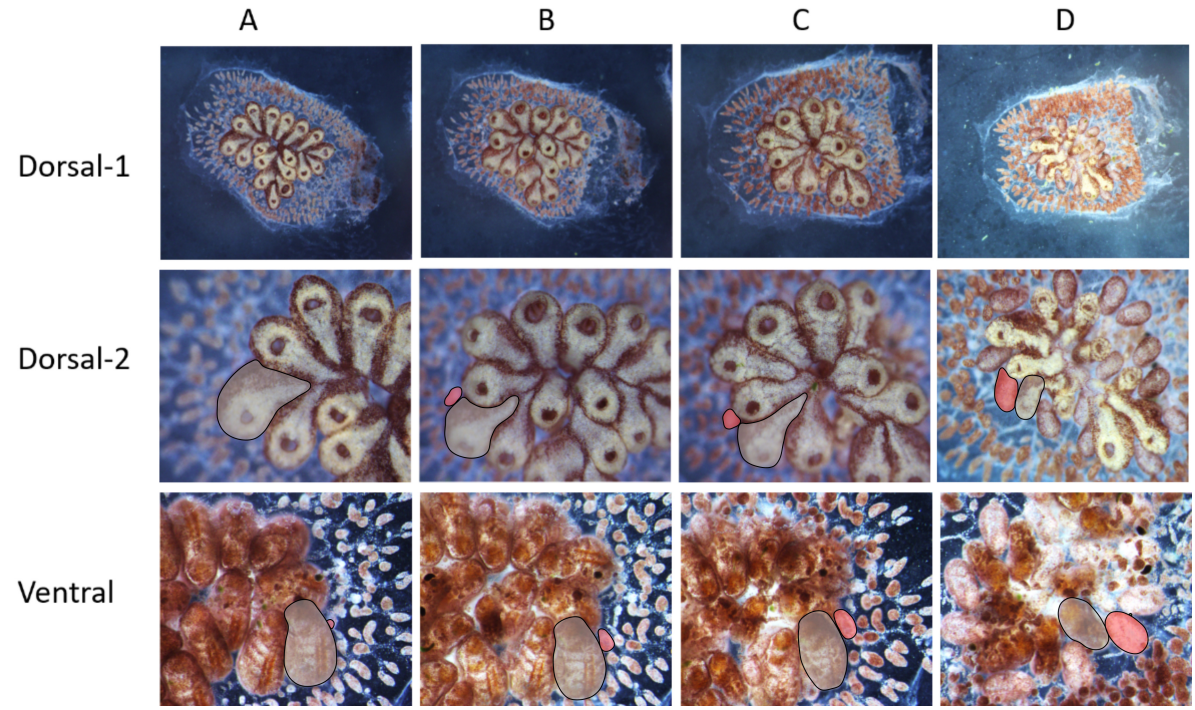

Figure S3. The blastogenic cycle of *B. niger*. The cycle was monitored on a daily basis, both from the dorsal and the ventral side of the colony. The letters above each column indicate the corresponding blastogenic stage. New parental zooids are growing new primary buds in the stage A. These new buds are increasing in size and developing secondary buds at stage B and further in stage C. The parental zooids are absorbed at stage D and the primary zooids are replacing them as the new parental zooids in the stage A of the upcoming cycle. The outline of a zooid (grey), of one of its primary bud (pink) and of one of its secondary bud (green) are depicted.

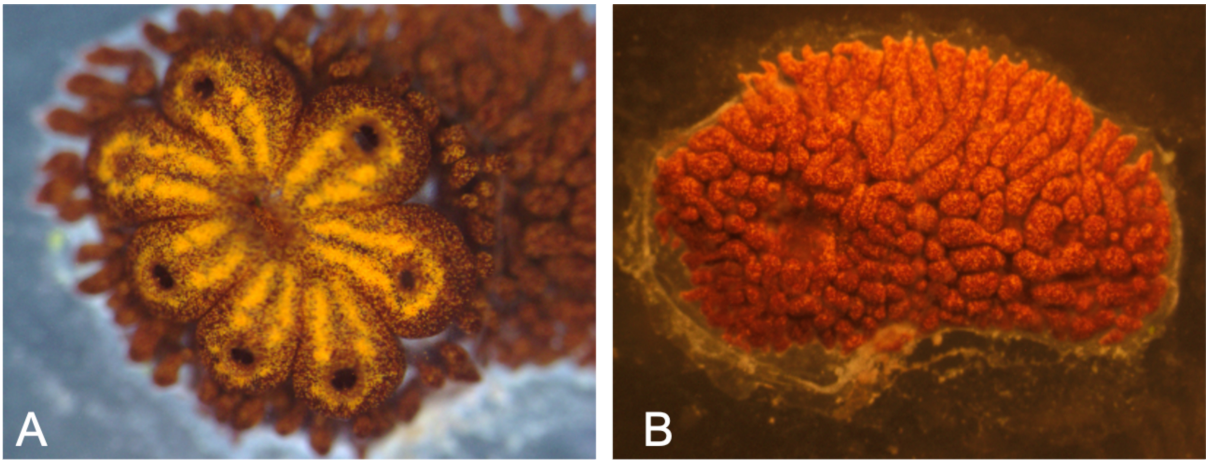

Figure S4. Hibernation of *B. niger*. A) A common *B. niger* morph during the active filter feeding phase. Seven zooids are visible with their siphons open. **B**) The same colony during hibernation. No zooid is present, only a dense mat of highly pigmented ampullae are visible inside the tunic.

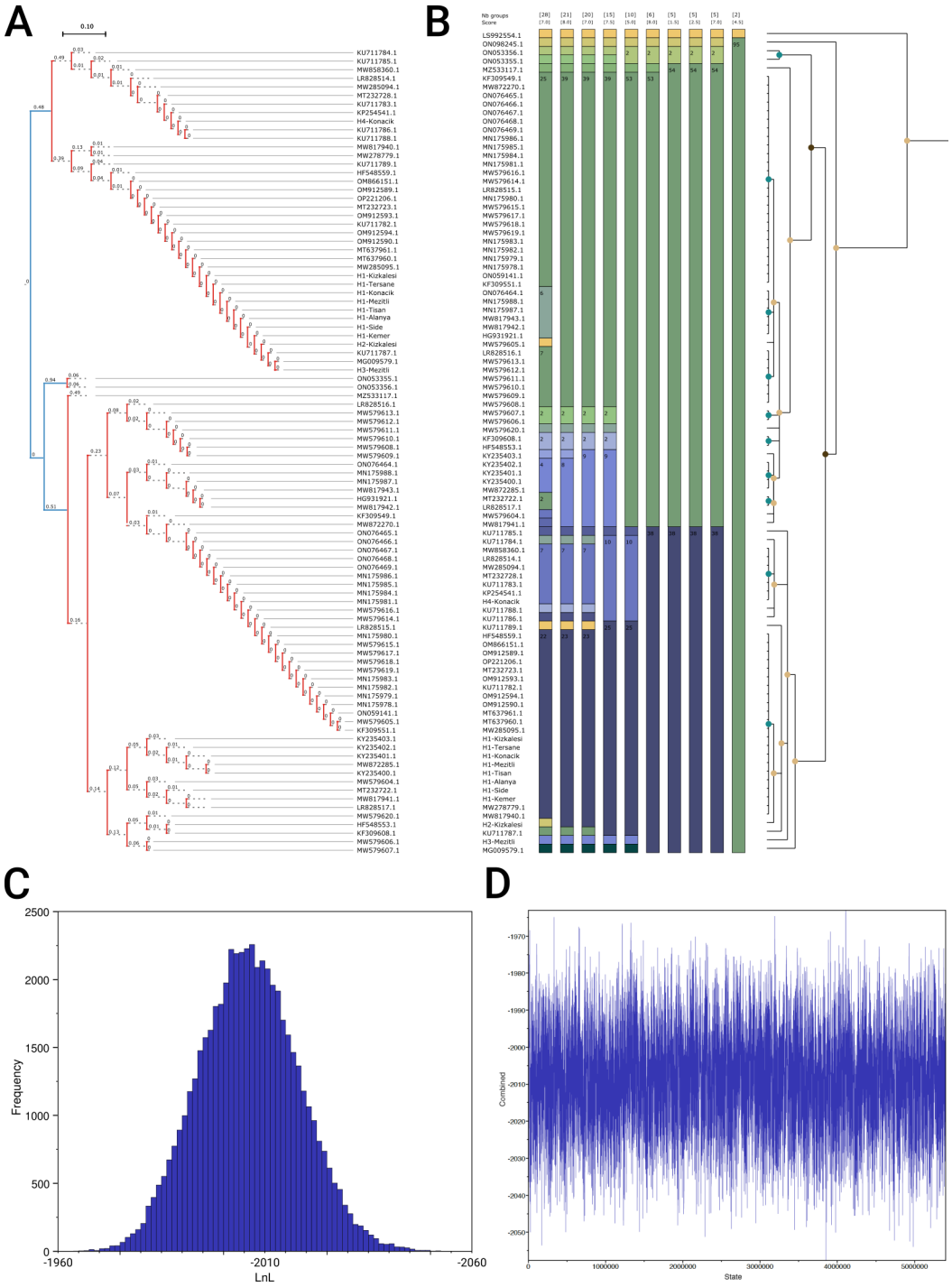

Figure S5. Delimiting Operational Taxonomic Units (OTUs) and Bayesian tracer. A) The tree-based coalescence method from the Poisson Tree Processes (PTP). Blue lines represent different OTUs, red line the same OTUs. **B)** Assemble Species by Automatic Partitioning (ASAP) score: The colors are representing different OTUs. Number inside each OTU represent the total number of samples assigned to that OTU (one is not indicated). Ten partitioning of increasing granularity (from right to left) are depicted, with their associated ASAP score (the lower the better). **C)** An example of a stationary MCMC chain displaying a Gaussian distribution of its LnL posterior probabilities, after the discarded 0.1 burn-in fraction. **D)** Traces of LnL values for two independent stationary runs combining 5400000 iterations.

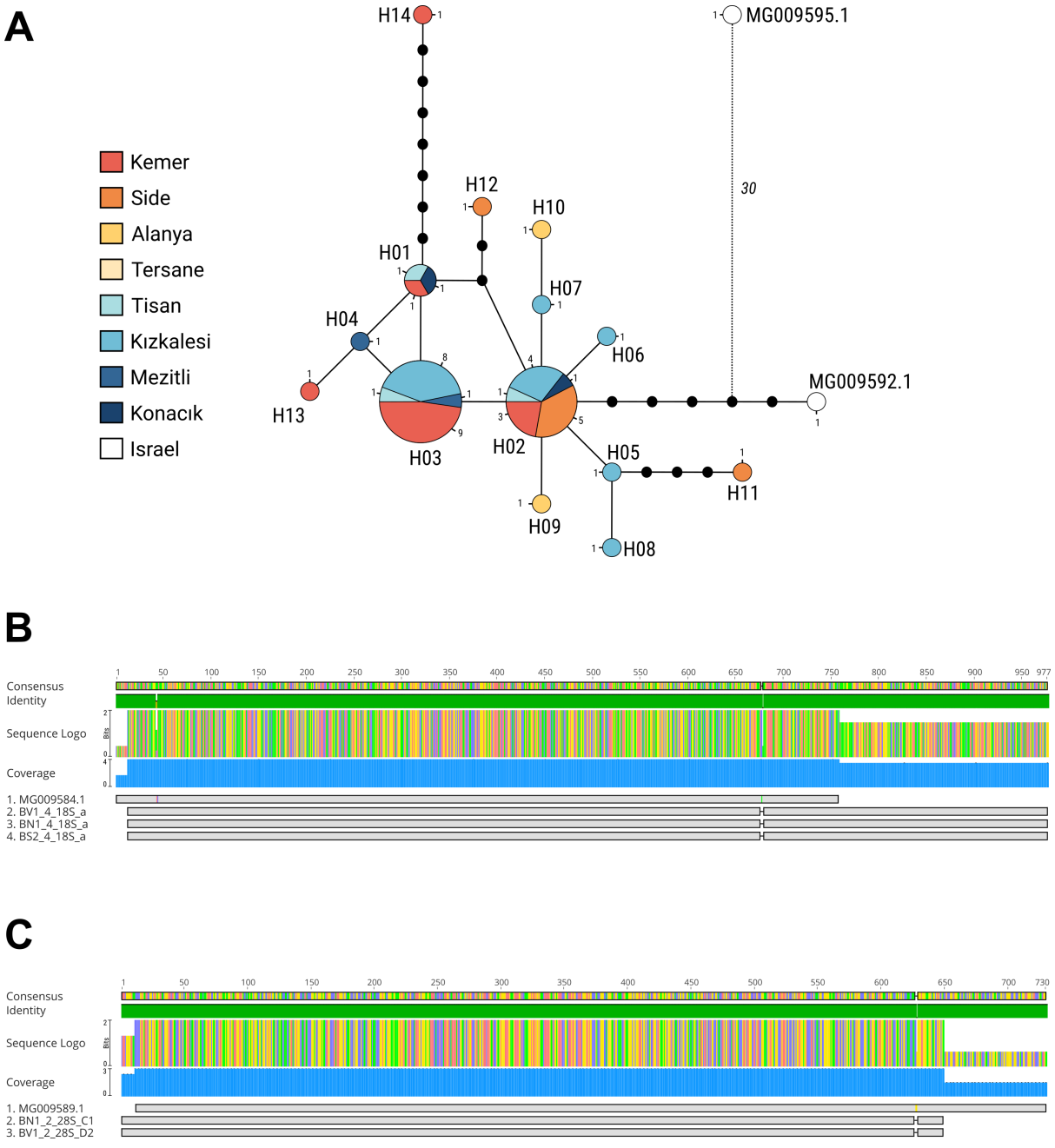

Figure S6. Nuclear gene analyses of *B. niger*. A) Median Joining Network estimation of *B. niger* based on H3 locus, overlaid with the corresponding haplotype name. Database samples: MG009592.1 - *Botrylloides leachii* - Israel; MG009595.1 - *Botryllus schlosseri* - Israel. Size differences of circles indicate frequency while colours reflect regions where the colony was sampled. Numbers represent the number of samples in each category, and each correcting edge a distance of one mutation steps between the haplotypes, with the exception of the dotted line which summarizes 30 mutations. **B**) *B. niger* 18S multiple sequences alignment profile, MG009584.1 - *Botrylloides aff. leachii* - Israel. **C**) *B. niger* 28S multiple sequences alignment profile, MG009589.1 - *Botrylloides aff. leachii* - Israel.

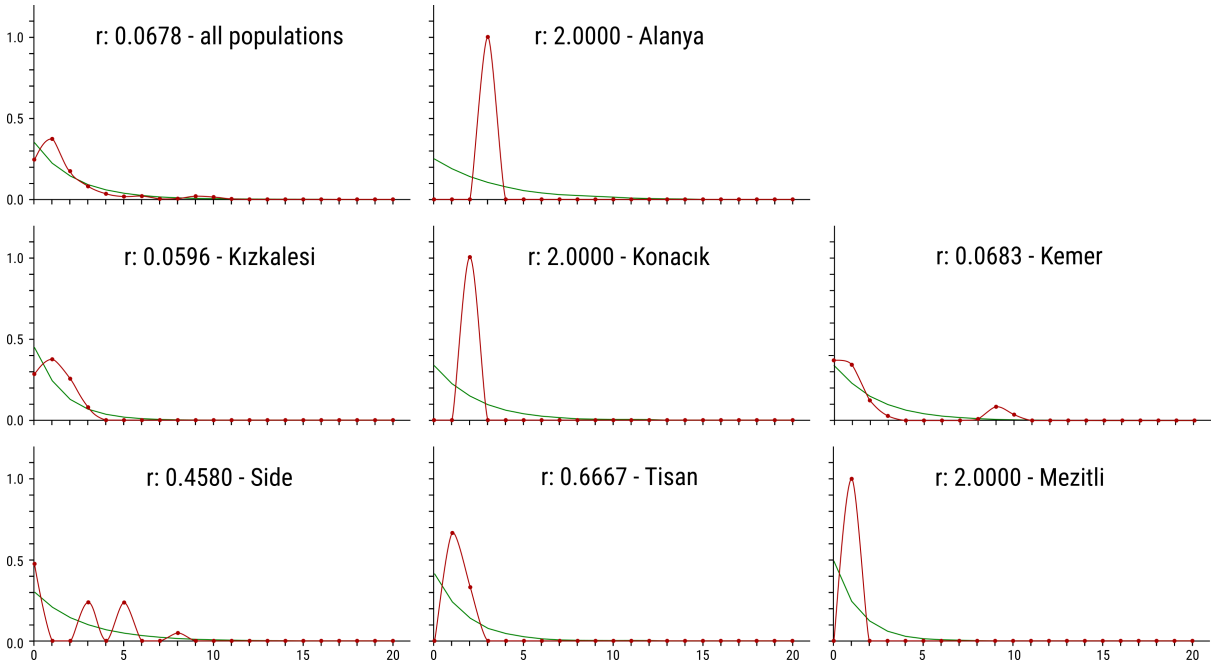

Figure S7. Population size changes analysis based on the mismatch distribution of the COI gene for Kızkalesi, Mezitli, Konacık, and all populations. Raggedness statistic values (*r*) for each analysis was supplied (*r*> 0.05). The X-axis is the number of pairwise differences while the Y-axis represents frequency. The red line indicates the observed frequency, and the green line the expected frequency for a stable population.

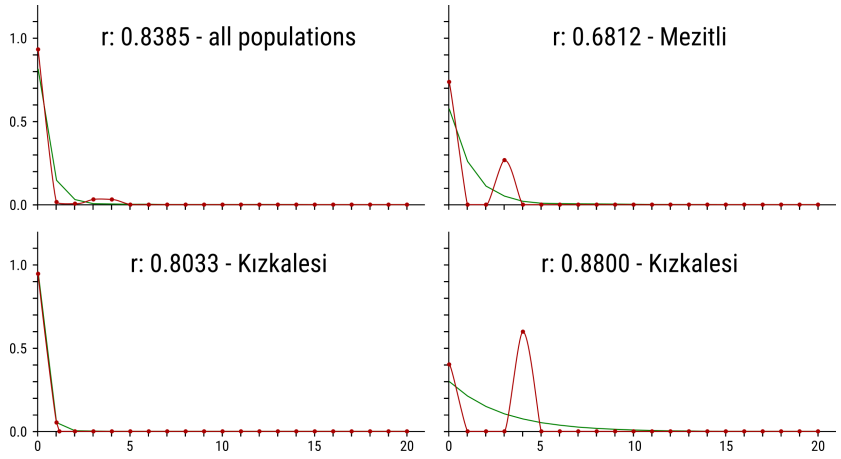

Figure S8. Population size changes of Kızkalesi time-series station based on mismatch distribution of COI. Raggedness statistic values (*r*) for each analysis was supplied (*r*> 0.05). The X-axis is the number of pairwise differences while the Y-axis represents frequency. The red line indicates the observed frequency, and the green line the expected frequency for a stable population.