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### Seven ascidian (Tunicata) species from the North Eastern Mediterranean

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**Seven ascidian (Tunicata) species from the North Eastern Mediterranean****Arzu KARAHAN, Berivan TEMIZ, Esra ÖZTÜRK, Jacob DOUEK and Baruch RINKEVICH***Mediterranean Marine Science, 24 (3) 2023***Table S1.** Sampling sites' coordinates, date and salinity information.

<b>Name of sites</b>	<b>Coordinates</b>	<b>Date</b>	<b>Salinity</b>
Antalya-Kemer	36.599871°N - 30.575208°E	October- 2018	40.1
Antalya-Alanya	36.559481°N - 31.951682°E	October- 2018	38.9
Mersin-Tisan	36.157770°N - 33.683355°E	October- 2018	40.2
Mersin-Kızkalesi	36.457684°N - 34.144009°E	April-2012; October-2018	NA; 39.0
Mersin-Mezitli	36.732885°N - 34.521313°E	October- 2018	39.2
Hatay-Konack	36.360380°N - 35.820282°E	September- 2018	40.0

**Table S2.** Present study samples' details. AN: accession number.

No	Sample ID	BOLD AN	GenBank AN	Species	Collecting sites	Sampling date	Color of colony
1	L12	IMS265-20-BOLD:ABY9216	OR621356				Brown with yellow strip
2	L13	IMS271-23-BOLD:AEE3528	OR621357				Grey with creamy-yellow strips
3	L14	IMS272-23-BOLD:ABY9216	OR621354	<i>Botryllus schlosseri</i>	Konacık-Hatay	September-2018	Dark brown with grey strip
4	L16	IMS273-23-BOLD:ABY9216	OR621355				Dark brown with grey strip
5	L19	IMS274-23-BOLD:ABY9216	OR621358				Grey with cream strip
6	G3	IMS260-20-BOLD:AEEF0161	OR621352		Tisan-Mersin		Grey with brown-creamy strip
7	GK42	IMS261-20-BOLD:AEE6215	OR621350	<i>Botrylloides israeliense</i>	Kızkalesi-Mersin	October-2018	Brown with white brownish strip
8	GK28	IMS263-20-BOLD:AEE6215	OR621351		Kızkalesi-Mersin		Dark brown with creamy strip
9	C64	IMS269-22-BOLD:AEC6032	OR621353	<i>Botrylloides</i> sp.	Kemer-Antalya	October-2018	Brownish with the yellow strip
10	C6	IMS259-20-BOLD:AEE3749	OR621359		Alanya-Antalya	October-2018	Pink with white spot
11	L3	BANEM032-23-BOLD:AEE3749	OR621363		Konacık-Hatay	September-2018	Dark-brown with light brown pattern
12	L4	BANEM033-23-BOLD:AEE3749	OR621362	<i>Botryllus</i> sp. ( <i>humilis</i> )	Konacık-Hatay	September-2018	Light orange-cream
13	L6	BANEM035-23-BOLD:AEE3749	OR621361		Konacık-Hatay	September-2018	Pink
14	M2_16	BANEM052-23-BOLD:AEE3749	OR621360		Mezitli-Mersin	October-2018	Reddish-brown with pink spot
15	M2_23	IMS264-20-BOLD:ADX5608	OR621373	<i>Symplegma brakenhielmi</i>	Mezitli-Mersin	October-2018	Red
16	M18	IMS266-20-BOLD:ADC2025	OR621366				Black with grey strip
17	M7_2	IMS266-20-BOLD:ADC2025	OR621366				Black with grey strip
18	M8	IMS275-23-BOLD:ADC2025	OR621369				Black with grey strip
19	M1	IMS276-23-BOLD:ADC2025	OR621367	<i>Polyclinum constellatum</i>	Kızkalesi-Mersin	April_2012	Black with grey strip
20	M19	IMS279-23-BOLD:ADC2025	OR621370				Black with grey strip
21	M6_2	IMS277-23-BOLD:ADC2025	OR621371				Brown with cream strip
22	M9	IMS278-23-BOLD:ADC2025	OR621372				Red with creamy strip
23	M20	IMS280-23-BOLD:ADC2025	OR621368				Brown with creamy strip
24	C2	IMS281-23-BOLD:ACB6312	OR621365				
25	C3	IMS267-20-BOLD:ACB6312	OR621364	<i>Didemnum perlucidum</i>	Alanya-Antalya	October-2018	White

Table S3. NCBI Blast results.

Present study samples			NCBI BLAST result							
N	Field ID	Species Name	<u>BLAST result Scientific Name</u>	<u>Max Score</u>	<u>Total Score</u>	<u>Query Cover %</u>	<u>E value</u>	<u>Per. Ident</u>	<u>Acc. Len</u>	<u>Accession</u>
1	C6		<a href="#">Botryllus sp. hu MN-2022</a>	929	929	100	0.0	99.80%	844	<a href="#">OP221215.1</a>
2	L3		<a href="#">Botryllus sp. hu MN-2022</a>	929	929	100	0.0	99.80%	844	<a href="#">OP221215.1</a>
3	L4	<i>Botryllus humilis</i>	<a href="#">Botryllus sp. hu MN-2022</a>	922	922	100	0.0	100%	844	<a href="#">OP221215.1</a>
4	L6		<a href="#">Botryllus sp. hu MN-2022</a>	922	922	100	0.0	100%	844	<a href="#">OP221215.1</a>
5	M2_16		<a href="#">Botryllus sp. hu MN-2022</a>	893	893	100	0.0	99.39%	844	<a href="#">OP221215.1</a>
6	G3		<a href="#">Botrylloides sp. NAP1</a>	826	826	96	0.0	97.33%	530	<a href="#">MN076468.1</a>
6	G3		<a href="#">Botrylloides israeliense</a>	763	763	88	0.0	97.53%	467	<a href="#">MG009580.1</a>
7	GK42	<i>Botrylloides israeliense</i>	<a href="#">Botrylloides sp. NAP1</a>	804	804	96	0.0	96.50%	530	<a href="#">MN076468.1</a>
7	GK42		<a href="#">Botrylloides israeliense</a>	719	719	88	0.0	95.74%	467	<a href="#">MG009580.1</a>
8	GK28		<a href="#">Botrylloides sp. NAP1</a>	793	793	96	0.0	96.09%	530	<a href="#">MN076468.1</a>
8	GK28		<a href="#">Botrylloides israeliense</a>	708	708	88	0.0	95.29%	467	<a href="#">MG009580.1</a>
9	C64	<i>Botrylloides sp.</i>	<a href="#">Botrylloides sp. FLMNH 2685</a>	902	902	100	0.0	98.81%	850	<a href="#">ON053356.1</a>
10	L12		<a href="#">Botryllus schlosseri</a>	872	872	100	0.0	98.58%	867	<a href="#">MW584328.1</a>
11	L13		<a href="#">Botryllus schlosseri</a>	872	872	100	0.0	98.58%	867	<a href="#">MW584328.1</a>
12	L14	<i>Botryllus schlosseri</i>	<a href="#">Botryllus schlosseri</a>	883	883	100	0.0	98.99%	867	<a href="#">MW584328.1</a>
13	L16		<a href="#">Botryllus schlosseri</a>	878	878	100	0.0	98.78%	867	<a href="#">MW584328.1</a>
14	L19		<a href="#">Botryllus schlosseri</a>	861	861	100	0.0	98.17%	867	<a href="#">MW584328.1</a>
15	M2_23	<i>Symplegma brakenhielmi</i>	<a href="#">Symplegma sp. HAW01</a>	924	924	100	0.0	99.60%	658	<a href="#">MW278788.1</a>
15	M2_23		<a href="#">Symplegma brakenhielmi</a>	852	852	99	0.0	97.22%	553	<a href="#">MT232734.1</a>
16	M18		<a href="#">Polyclinum indicum</a>	1020	1020	100	0.0	100.00%	631	<a href="#">KX650781.1</a>
17	M7_2		<a href="#">Polyclinum indicum</a>	1018	1018	99	0.0	100.00%	631	<a href="#">KX650781.1</a>
18	M8		<a href="#">Polyclinum indicum</a>	1020	1020	100	0.0	100.00%	609	<a href="#">KX650783.1</a>
19	M1		<a href="#">Polyclinum indicum</a>	1018	1018	99	0.0	100.00%	609	<a href="#">KX650783.1</a>
20	M19	<i>Polyclinum constellatum</i>	<a href="#">Polyclinum indicum</a>	1011	1011	99	0.0	99.82%	609	<a href="#">KX650783.1</a>
20	M19		<a href="#">Polyclinum constellatum</a>	1009	1009	99	0.0	99.82%	816	<a href="#">MT873559.1</a>
21	M6_2		<a href="#">Polyclinum indicum</a>	957	957	100	0.0	98.88%	631	<a href="#">KX650781.1</a>
22	M9		<a href="#">Polyclinum indicum</a>	931	931	100	0.0	97.96%	610	<a href="#">KX650785.1</a>
23	M20		<a href="#">Polyclinum indicum</a>	909	909	98	0.0	98.09%	631	<a href="#">KX650781.1</a>
24	C2	<i>Didemnum perlucidum</i>	<a href="#">Didemnum perlucidum</a>	952	952	100	0.0	100.00%	595	<a href="#">MN184710.1</a>
25	C3		<a href="#">Didemnum perlucidum</a>	952	952	100	0.0	100.00%	595	<a href="#">MN184710.1</a>

**Table S4.** The Kimura-2 Parameter distance results of *Botryllus schlosseri* samples.

	Specimen code and name	1	2	3	4	5	6	7	8	9
1	IMS265-20 <i>Botryllus schlosseri</i> (L12)		0,00	0,00	0,00	0,00	0,01	0,01	0,01	0,01
2	IMS273-23 <i>Botryllus schlosseri</i> (L16)	0,00		0,00	0,00	0,00	0,01	0,01	0,01	0,01
3	IMS272-23 <i>Botryllus schlosseri</i> (L14)	0,00	0,00		0,00	0,00	0,00	0,01	0,01	0,01
4	IMS271-23 <i>Botryllus schlosseri</i> (L13)	0,00	0,00	0,00		0,00	0,01	0,01	0,01	0,01
5	IMS274-23 <i>Botryllus schlosseri</i> (L19)	0,01	0,01	0,01	0,01		0,01	0,01	0,01	0,01
6	MW584328.1 <i>Botryllus schlosseri</i>	0,01	0,01	0,01	0,01	0,02		0,00	0,00	0,01
7	MN064603.1 <i>Botryllus schlosseri</i>	0,02	0,01	0,01	0,02	0,02	0,00		0,00	0,01
8	HF548550.1 <i>Botryllus schlosseri</i>	0,02	0,01	0,01	0,02	0,02	0,00	0,00		0,01
9	KM587658.1 <i>Botryllus schlosseri</i>	0,02	0,02	0,02	0,02	0,03	0,02	0,01	0,01	

Above diagonal represents standard deviation. The red colors refer to the present study samples and sites, and the codes in the brackets is the sample codes.

**Table S5.** The Kimura-2 Parameter distance results of *Botrylloides israeliense*.

	Specimen code and name	1	2	3	4	5	6
1	IMS260-20 <i>Botrylloides israeliense</i> (G3)		0,01	0,01	0,01	0,01	0,01
2	MN076468.1 <i>Botrylloides</i> sp.	0,03		0,01	0,01	0,01	0,01
3	MG009580.1 <i>Botrylloides israeliense</i>	0,03	0,02		0,01	0,01	0,01
4	IMS261-20 <i>Botrylloides israeliense</i> (GK42)	0,05	0,04	0,04		0,00	0,01
5	IMS263-20 <i>Botrylloides israeliense</i> (GK28)	0,05	0,04	0,05	0,00		0,01
6	MK978805.1 <i>Botrylloides</i> sp.	0,07	0,07	0,07	0,07	0,07	

Above diagonal represents standard deviation. The red colors refer to the present study samples and sites, and the codes in the brackets is the sample codes.

**Table S6.** The Kimura-2 Parameter distance results of *Botrylloides* sp. and its possible closest relatives.

	Specimen code and name	1	2	3	4	5
1	IMS269-22 <i>Botrylloides</i> sp. (C64)		0,00	0,00	0,02	0,02
2	ON053356.1 <i>Botrylloides</i> sp.	0,01		0,00	0,02	0,02
3	ON053355.1 <i>Botrylloides</i> sp.	0,01	0,00		0,02	0,02
4	ON098241.1 <i>Botrylloides</i> sp.	0,18	0,16	0,17		0,02
5	MT873573.1 <i>Botrylloides</i> cf. <i>anceps</i>	0,17	0,16	0,16	0,14	

Above diagonal represents standard deviation. The red colors refer to the present study samples and sites, and the codes in the brackets is the sample codes.

**Table S7.** The Kimura-2 Parameter distance results of *Botryllus humilis*.

	Specimen code and name	1	2	3	4	5	6	7	8
1	IMS259-20 <i>Botryllus humilis</i> (C6)		0,00	0,00	0,00	0,00	0,00	0,00	0,00
2	BANEM032-23 <i>Botryllus humilis</i> (L3)	0,00		0,00	0,00	0,00	0,00	0,00	0,00
3	BANEM033-23 <i>Botryllus humilis</i> (L4)	0,00	0,00		0,00	0,00	0,00	0,00	0,00
4	OP221215.1 <i>Botryllus</i> _sp._ <i>hu</i>	0,00	0,00	0,00		0,00	0,00	0,00	0,00
5	OP221169.1 <i>Botryllus</i> _sp._ <i>hu</i>	0,00	0,00	0,00	0,00		0,00	0,00	0,00
6	BANEM035-23 <i>Botryllus humilis</i> (L6)	0,00	0,00	0,00	0,00	0,00		0,00	0,00
7	BANEM052-23 <i>Botryllus humilis</i> (M2_16)	0,00	0,00	0,01	0,01	0,01	0,01		0,00
8	MG009581.1 UNVERIFIED: <i>Botrylloides anceps</i>	0,01	0,01	0,01	0,01	0,01	0,01	0,01	

Above diagonal represents standard deviation. The red color refers to the present study sample and site, and the code in the brackets is the sample code.

**Table S8.** The Kimura-2 Parameter distance results of *Symplegma brakenhielmi*.

	Specimen code and name	1	2	3	4
1	IMS264-20 <i>Symplegma brakenhielmi</i> (M2_23)		0,00	0,01	0,01
2	MW278788.1 <i>Symplegma</i> _sp.	0,00		0,01	0,01
3	MT232734.1 <i>Symplegma brakenhielmi</i>	0,03	0,03		0,01
4	MW285134.1 <i>Symplegma brakenhielmi</i>	0,04	0,04	0,02	

Above diagonal represents standard deviation. The red color refers to the present study sample and site, and the code in the brackets is the sample code.

**Table S9.** The Kimura-2 Parameter distance results of *Polycylinum constellatum*.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	
1		0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,01	0,01	0,01	
2			0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,01	0,01	0,01	
3				0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,01	0,01	0,01	
4					0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,01	0,01	0,01	
5						0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,01	0,01	0,01	
6							0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,01	0,01	0,01	
7								0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,01	0,01	0,01	
8									0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,01	0,01	0,01	
9										0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,01	0,01	
10											0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,01	0,01	
11												0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,01	0,01	
12													0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,01	0,01	
13														0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,01	0,01	
14															0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,01	
15																0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	
16																	0,00	0,00	0,00	0,00	0,00	0,00	0,00	
17																		0,01	0,00	0,00	0,00	0,00	0,00	
18																			0,01	0,00	0,00	0,00	0,00	
19																				0,01	0,00	0,00	0,00	
20																					0,01	0,00	0,00	
21																						0,01	0,00	
22																							0,01	
23																								0,01

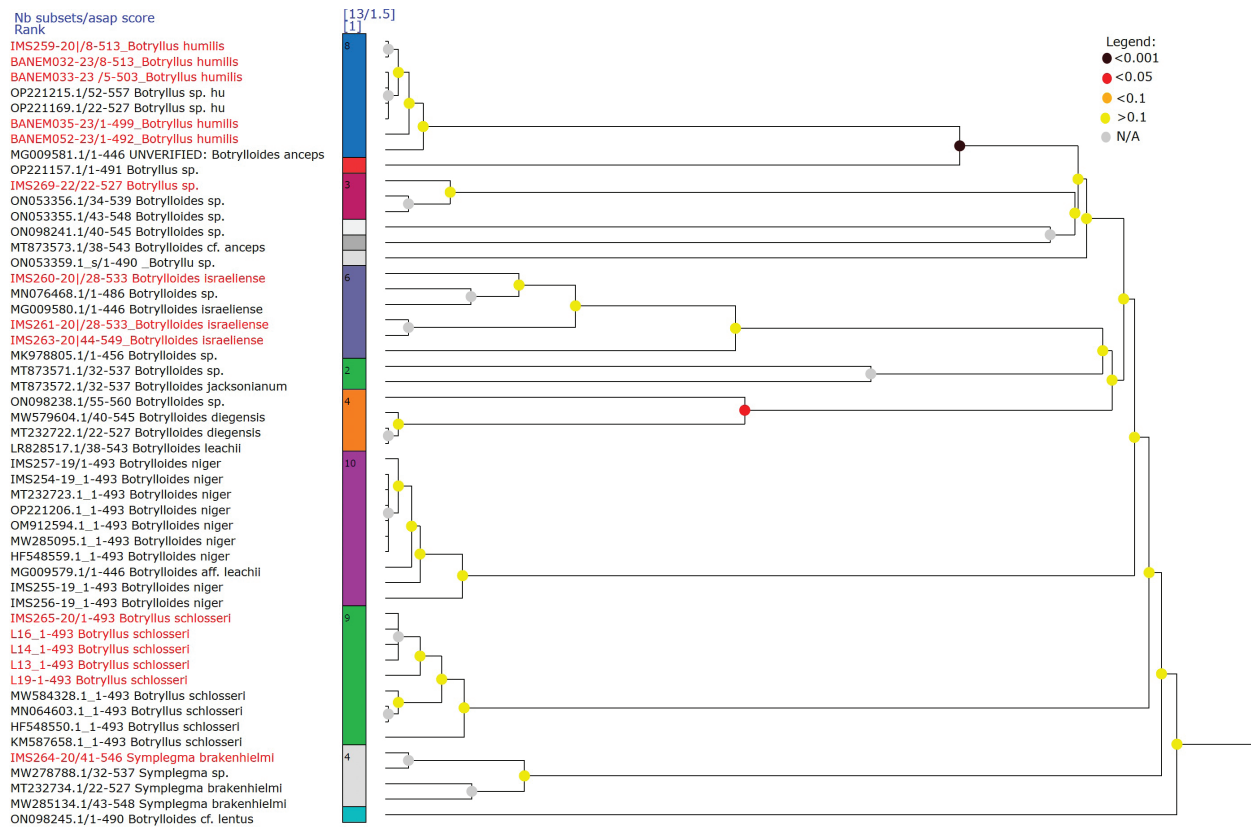
Above diagonal represents standard deviation. The red colors refer to the present study samples and sites, and the codes in the brackets is the sample codes.

**Table S10.** The Kimura-2 Parameter distance results of *Didemnum perlucidum*.

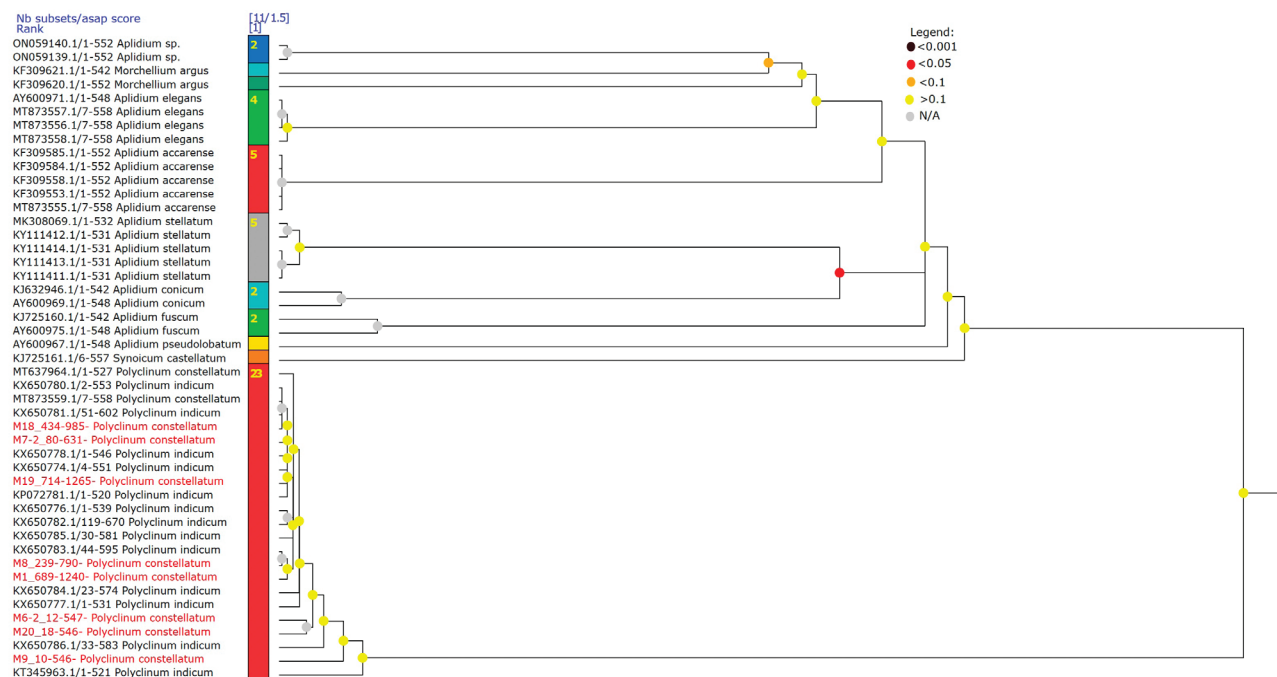
	Specimen code and name	1	2	3	4	5	6	7	8	9	10	11	12
1	MH824680 <i>Didemnum perlucidum</i>		0,00	0,00	0,01	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,01
2	KX650791 <i>Didemnum perlucidum</i>	0,01		0,00	0,01	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,01
3	KU883151 <i>Didemnum perlucidum</i>	0,01	0,00		0,01	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,01
4	KX138490 <i>Didemnum perlucidum</i>	0,02	0,02	0,02		0,01	0,01	0,01	0,01	0,01	0,01	0,01	0,01
5	KU667270 <i>Didemnum perlucidum</i>	0,00	0,01	0,01	0,02		0,00	0,00	0,00	0,00	0,00	0,00	0,01
6	KR537439 <i>Didemnum perlucidum</i>	0,00	0,01	0,01	0,02	0,00		0,00	0,00	0,00	0,00	0,00	0,01
7	JQ731735. <i>Didemnum perlucidum</i>	0,01	0,00	0,00	0,02	0,01	0,01		0,00	0,00	0,00	0,00	0,01
8	MT637962 <i>Didemnum perlucidum</i>	0,01	0,00	0,00	0,02	0,01	0,01	0,00		0,00	0,00	0,00	0,01
9	MN184710 <i>Didemnum perlucidum</i> -Australia	0,01	0,00	0,00	0,02	0,01	0,01	0,00	0,00		0,00	0,00	0,01
10	IMS281-23 <i>Didemnum perlucidum</i> (C2)	0,01	0,00	0,00	0,02	0,01	0,01	0,00	0,00	0,00		0,00	0,01
11	IMS267-20 <i>Didemnum perlucidum</i> (C3)	0,01	0,00	0,00	0,02	0,01	0,01	0,00	0,00	0,00	0,00		0,01
12	KY741541 <i>Didemnum etiolum</i>	0,06	0,06	0,06	0,08	0,06	0,06	0,06	0,06	0,06	0,06	0,06	

Above diagonal represents standard deviation. The red colors refer to the present study samples and sites, and the codes in the brackets is the sample codes.

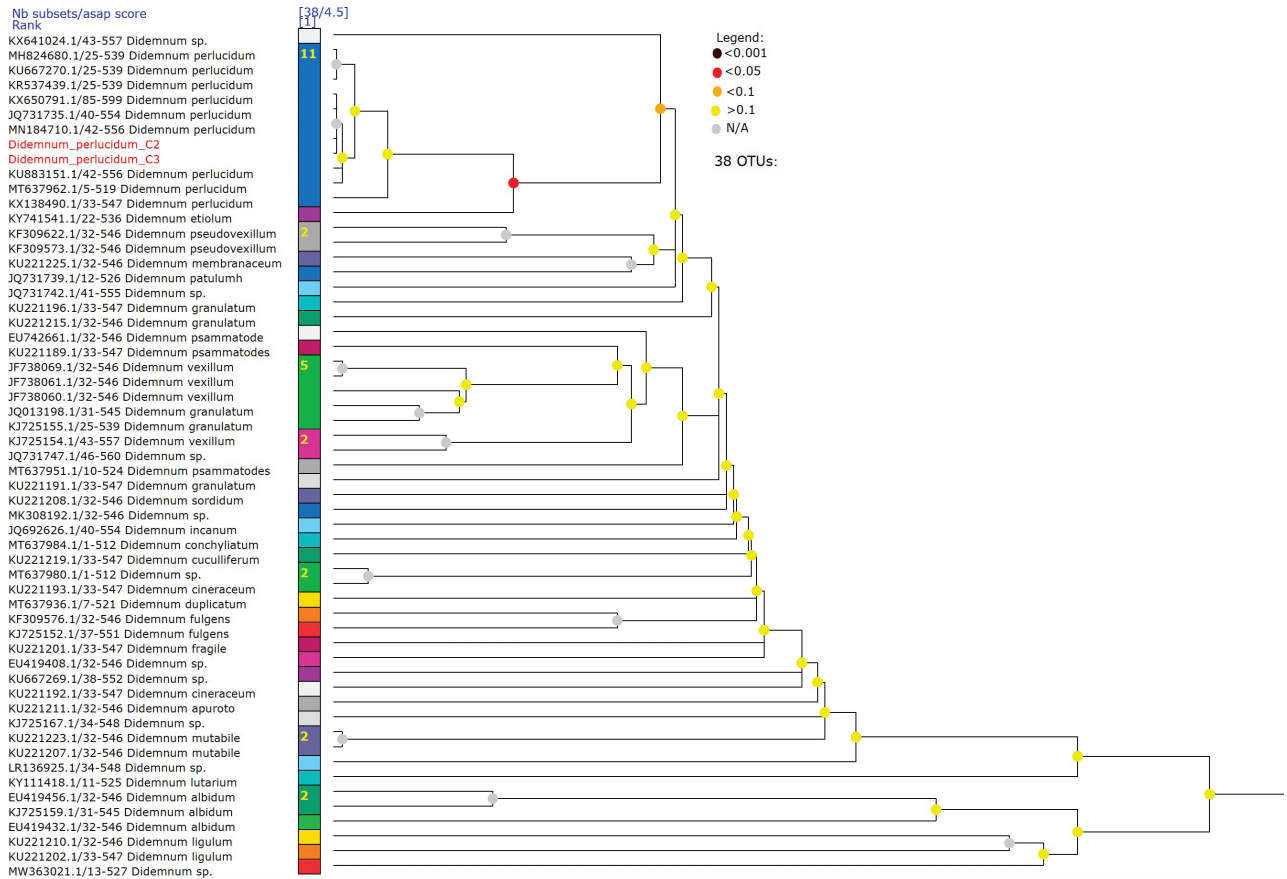




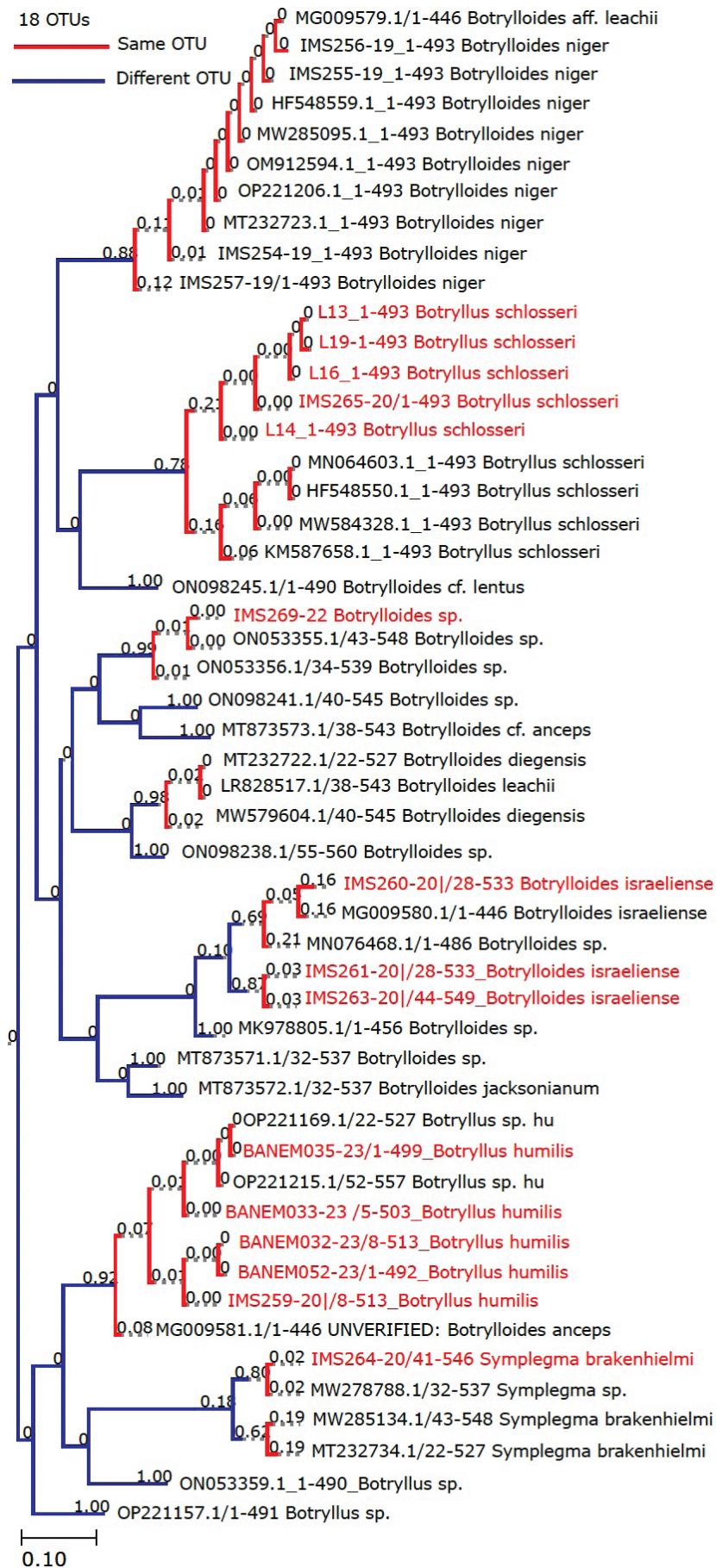
**Fig. S1:** The figure displays the ASAP scores of Styelidae specimens. Different OTUs are represented by colors on the bar, and the number inside each color bar corresponds to the assigned specimen number for that OTU. The number of subsets, assigned total OTU number, ASAP score (where lower scores indicate better partitions, Puillandre *et al.*, 2021), and the best rank column (1) are included. Samples from the present study are marked in red letters. Legend; Darker colors in the figure indicate lower probabilities, while a grey dot signifies that the probability was not computed. When a probability is very low (dark color), it suggests that the groups within the node likely correspond to different species.



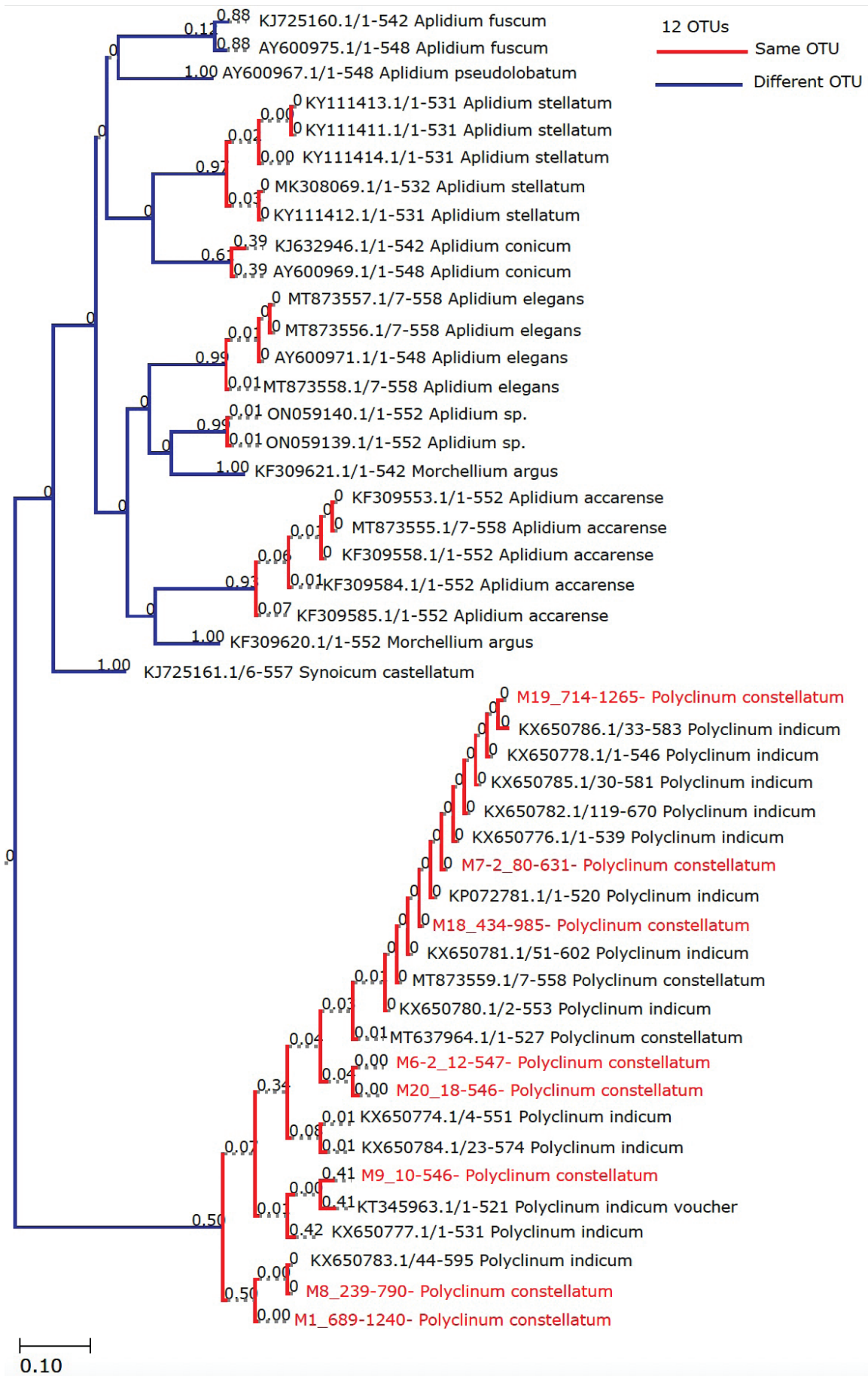
**Fig. S2:** The figure displays the ASAP scores of Polyclinidae specimens. Different OTUs are represented by colors on the bar, and the number inside each color bar corresponds to the assigned specimen number for that OTU. The number of subsets, assigned total OTU number, ASAP score (where lower scores indicate better partitions, Puillandre *et al.*, 2021), and the best rank column (1) are included. Samples from the present study are marked in red letters. Legend; Darker colors in the figure indicate lower probabilities, while a grey dot signifies that the probability was not computed. When a probability is very low (dark color), it suggests that the groups within the node likely correspond to different species.



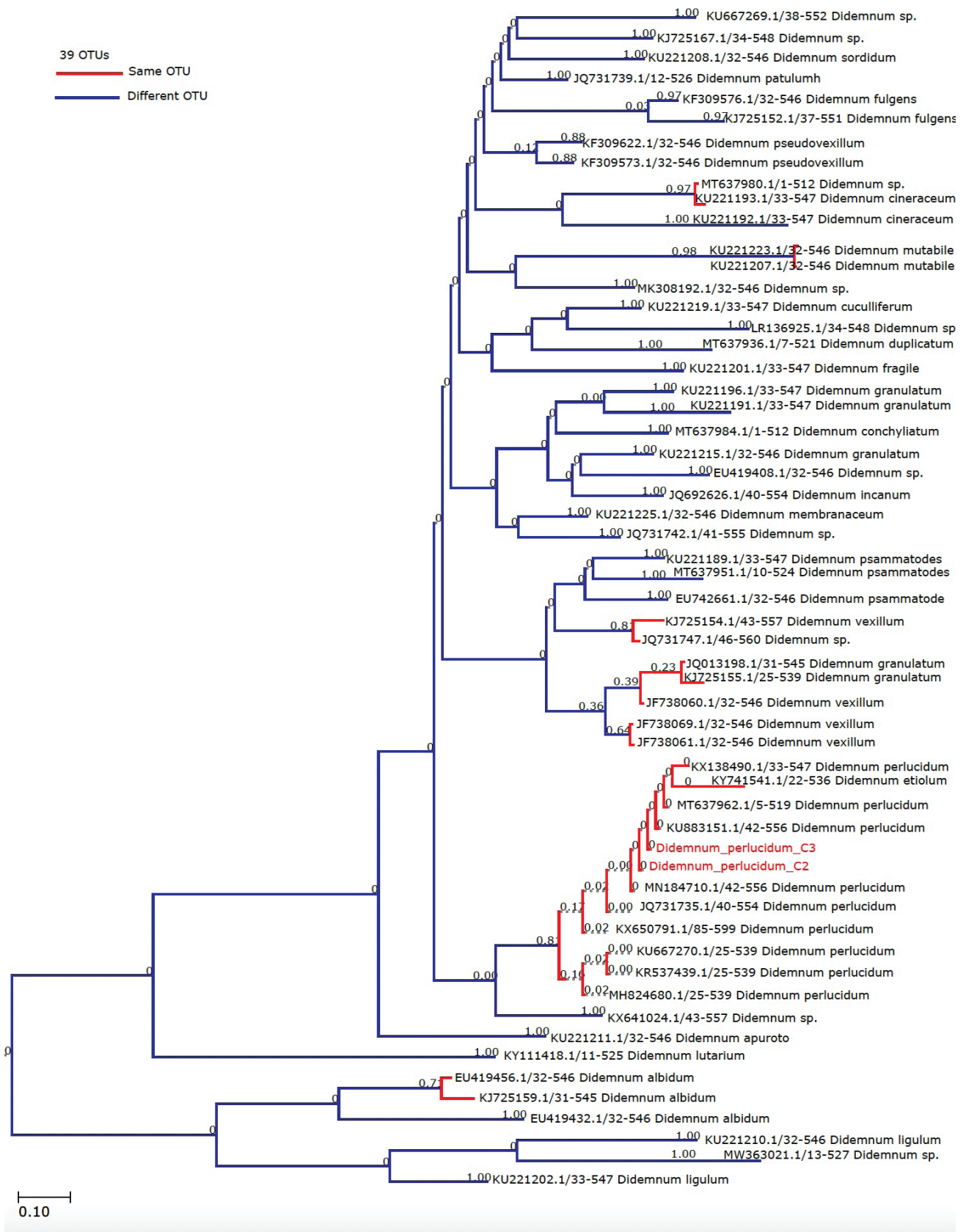
**Fig. S3:** The figure displays the ASAP scores of didemnid specimens. Different OTUs are represented by colors on the bar, and the number inside each color bar corresponds to the assigned specimen number for that OTU. The number of subsets, assigned total OTU number, ASAP score (where lower scores indicate better partitions, Puillandre *et al.*, 2021), and the best rank column (1) are included. Samples from the present study are marked in red letters. Legend; Darker colors in the figure indicate lower probabilities, while a grey dot signifies that the probability was not computed. When a probability is very low (dark color), it suggests that the groups within the node likely correspond to different species.



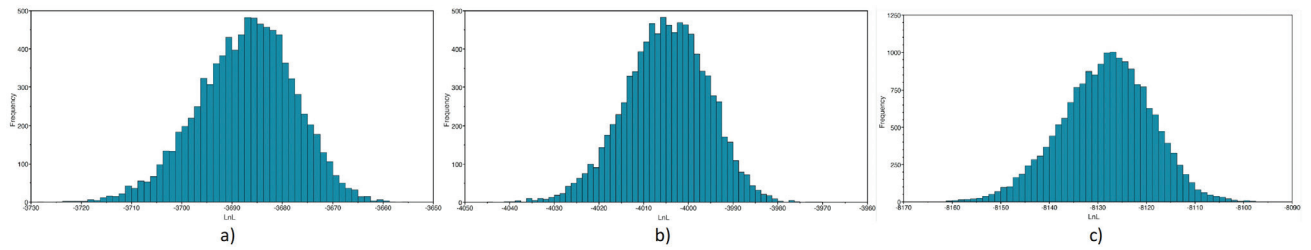
**Fig. S4:** PTP score of Styelidae. Blue lines represent different OTUs, red lines represent the same OTUs. Red letter represents present study samples.



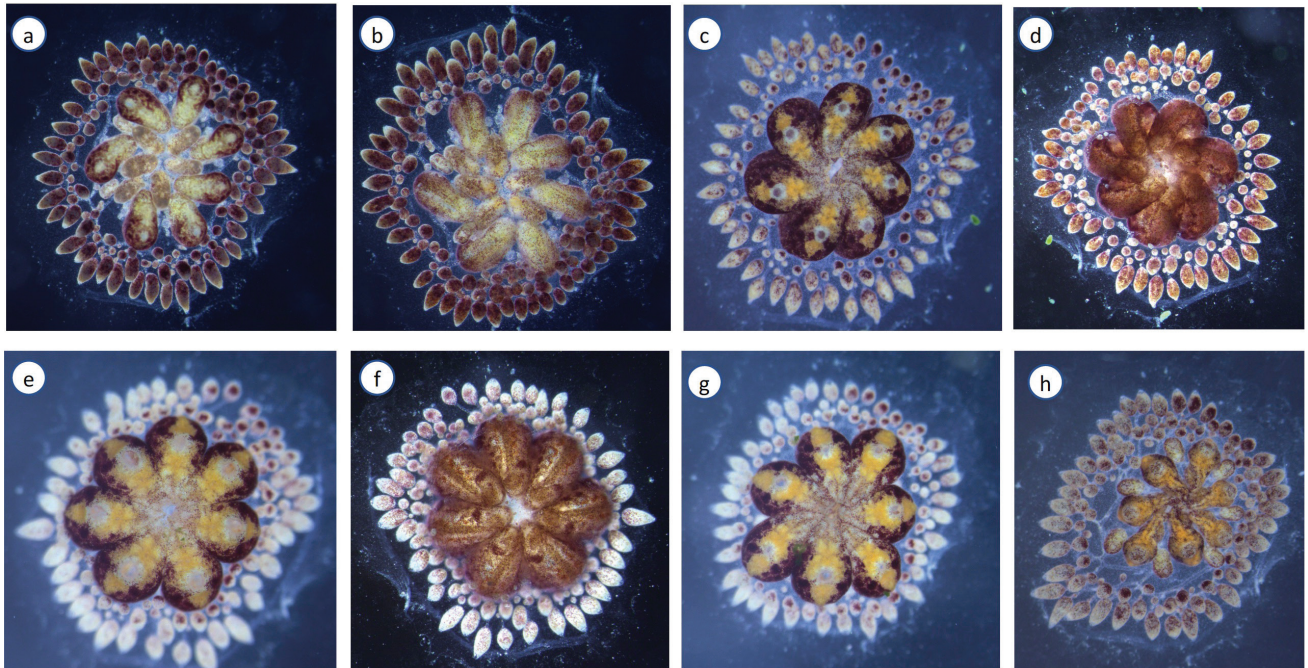
**Fig. S5:** PTP score of Polyclinidae. Blue lines represent different OTUs, red lines represent the same OTUs. Red letter represents present study samples.



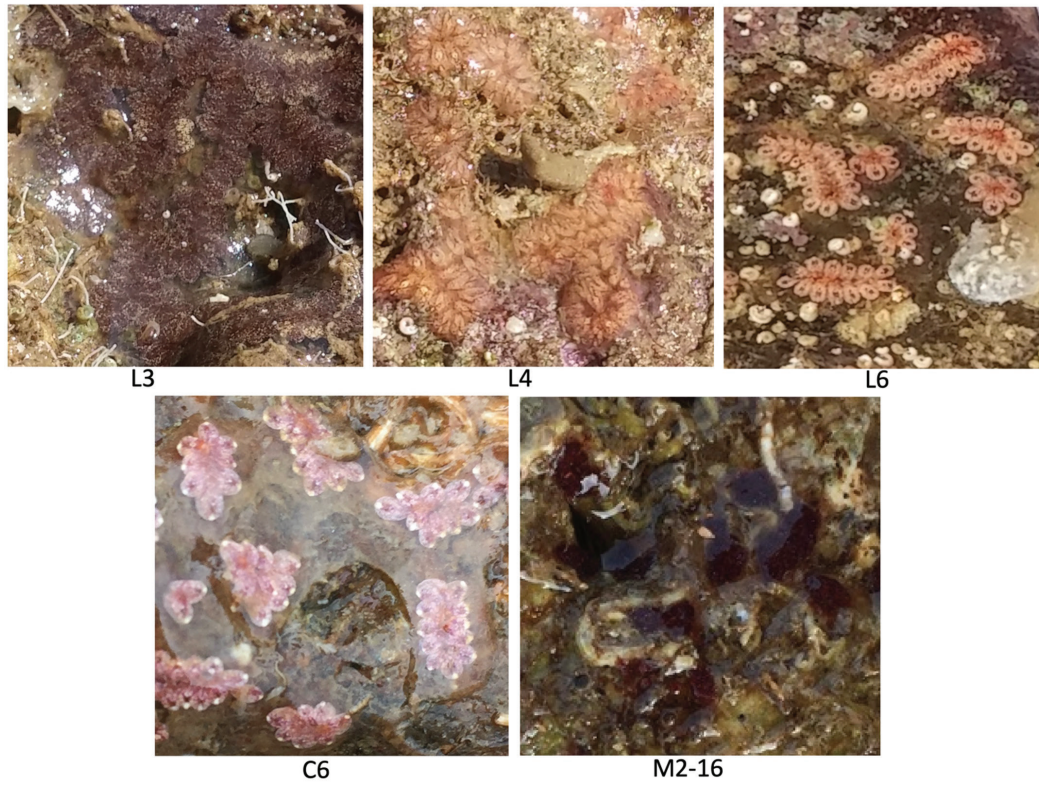
**Fig. S6:** PTP score of Didemnidae. Blue lines represent different OTUs, red lines represent the same OTUs. Red letter represents present study samples.



**Fig. S7:** LnL values of MrBayes analyses, a) Styeliadae, b) Aplousobranchia c) Didemnidae.



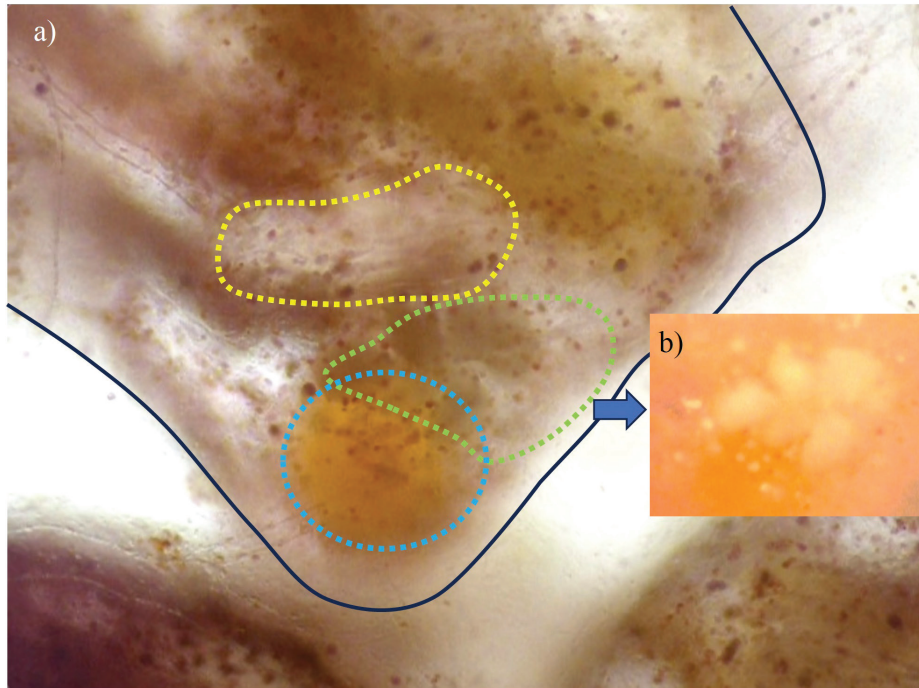
**Fig. S8:** Blastogenic cycle of *Botrylloides* sp. Dorsal and ventral view of the Blastogenic stages; a-b) stage-D (Jun 07, 2018), c-d) stage-A (Jun 08, 2018), e-f) stage-B (Jun 09, 2018), g) Dorsal-stage C (Jun 10, 2018), h) Dorsal – stage D (Jun 11, 2018).



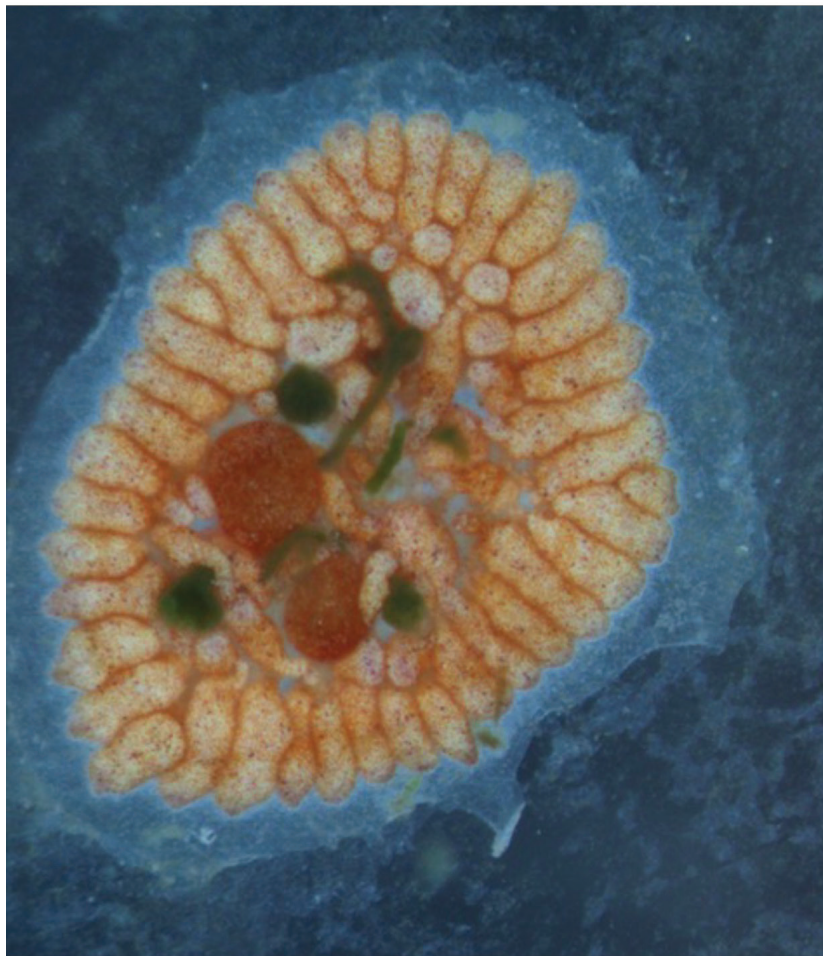
**Fig. S9:** Color morphotypes of *Botryllus humilis*, L3, L4 L6 from Hatay (Konacık), C6 Antalya (Alanya), M2\_16 Mersin (Mezitli).



**Fig. S10:** When the zooid size of *Botryllus humilis* reached up to 2 mm (the photographs were captured via a cell phone).



**Fig. S11:** Ovary and testes of *Botryllus humilis* (L6). a) yellow frame is representing heart border; green frame is testis and blue frame is ovary. b) the picture is captured by focusing the testes with only overhead light option.



**Fig. S12:** Large, condensed ampullas and irregular, small zooids of 3 years old *Botryllus humilis* colony.