Supplemental Information for:

Host preferences of coexisting Perkinsea parasitoids during coastal dinoflagellate blooms

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Evaluation of blank and mock samples

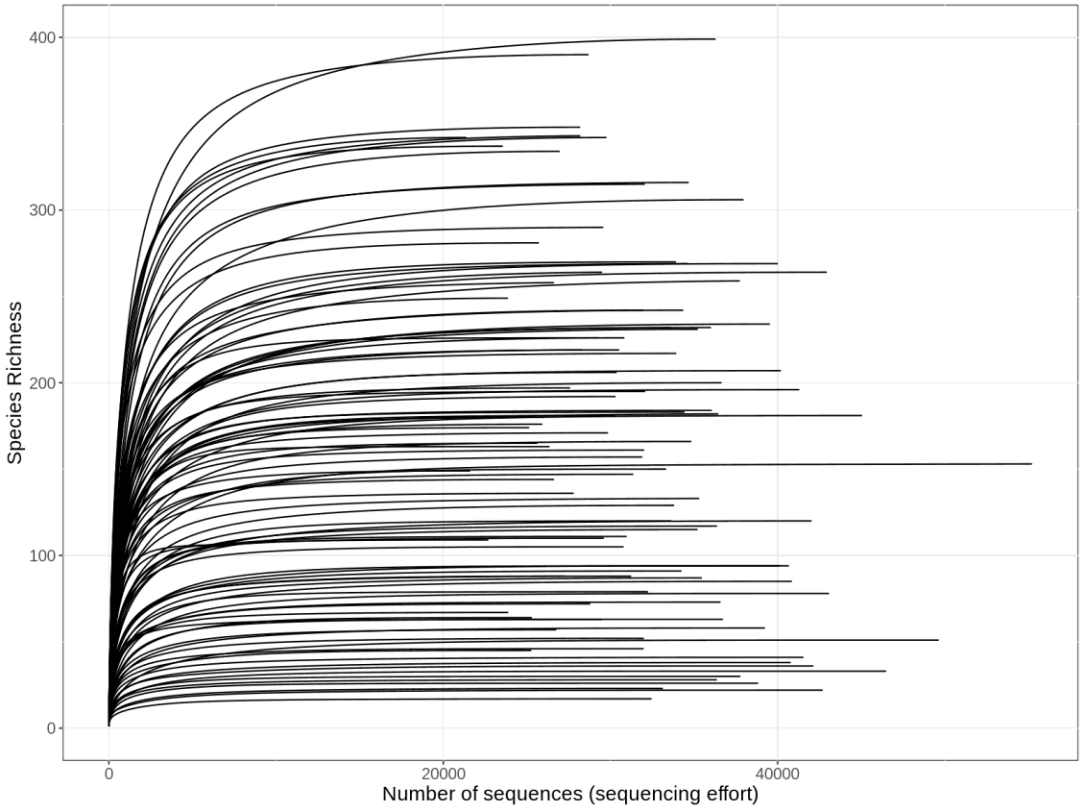
Negative (blank) and positive (mock) controls were processed during metabarcoding sequencing as reference standards. One blank sample containing autoclaved MilliQ water and one mock sample containing dinoflagellate and parasitoid cultured cells at known abundances were processed by serial filtration, yielding one small (0.8–10 µm) and one large (10–200 µm) fraction for each sample. Two samples of autoclaved sediments obtained from different locations containing the mock community were also processed.

All samples analyzed showed richness saturation (Suppl. Fig. 1), indicating that the sequencing effort was sufficient to obtain a robust characterization of the microeukaryotic community. An MDS analysis was then performed with the community information to evaluate the similarity between locations, fractions (small, large and sediment) and sample replicates (Suppl. Fig. 2). Blank and mock samples were also included in the analysis to use them as negative and positive controls, respectively. All samples corresponding to the same location generally clustered together, even though the similarity between small and large seawater fractions was higher than for sediment ones. All sample replicates were in agreement, showing high similarity.

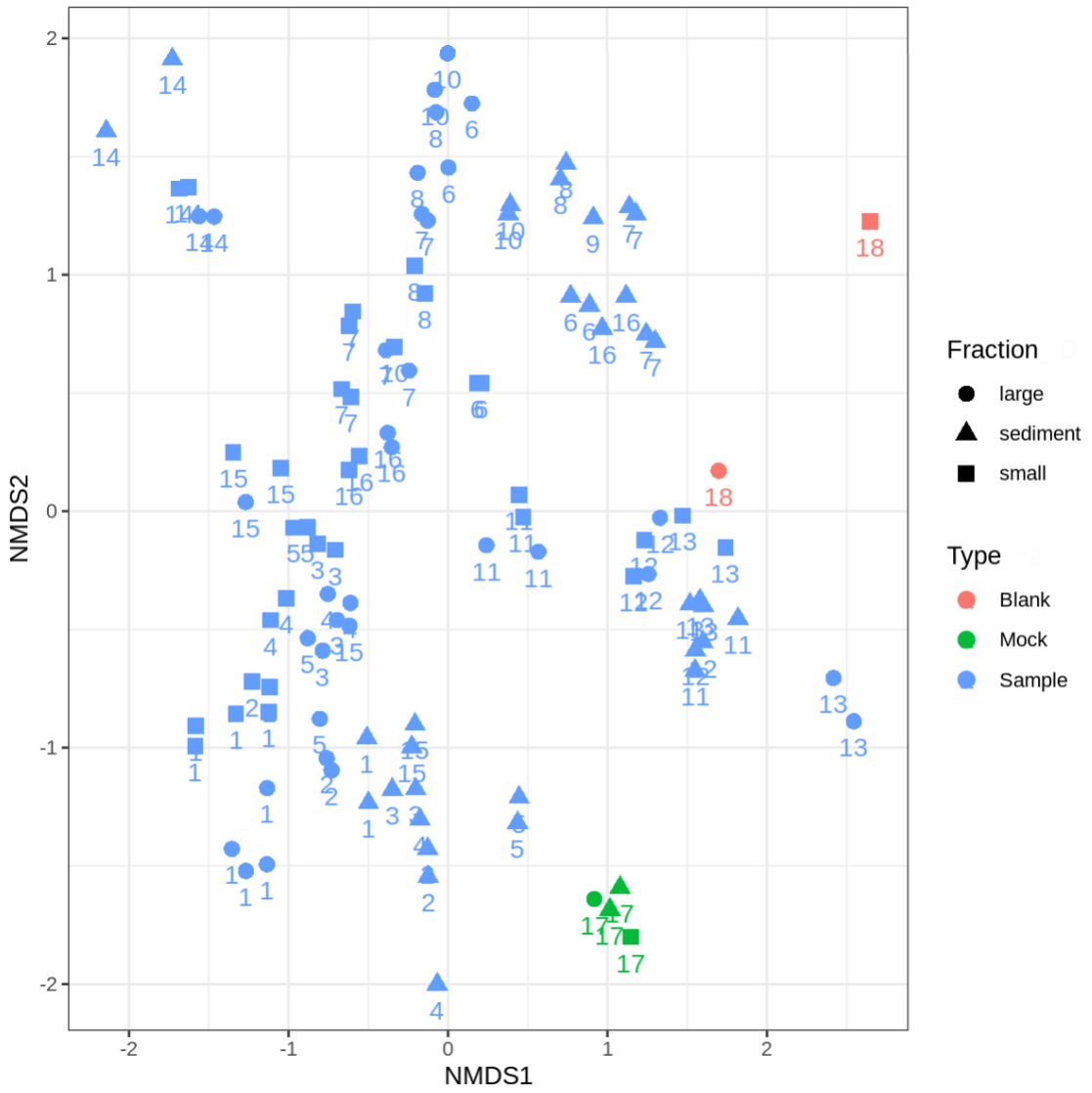
The results from the two fractions of blank sample differed (Suppl. Fig. 2). The small fraction contained only a few ASVs with a low number of reads, and its community composition was not related to that of the study samples. By contrast, the community composition of the large fraction of the blank sample was similar to that of other environmental samples. A detailed analysis of the data showed that the latter fraction included some of the same ASVs present in the previously processed sample, suggestive of cross-contamination during the DNA extraction process. The possibility of other cross-contaminations between environmental samples was evaluated, but all of the fraction replicates for each sample were of high similarity, and the

different fractions for each water sample generally clustered together. We thus concluded that the cross-contamination was limited to the blank sample.

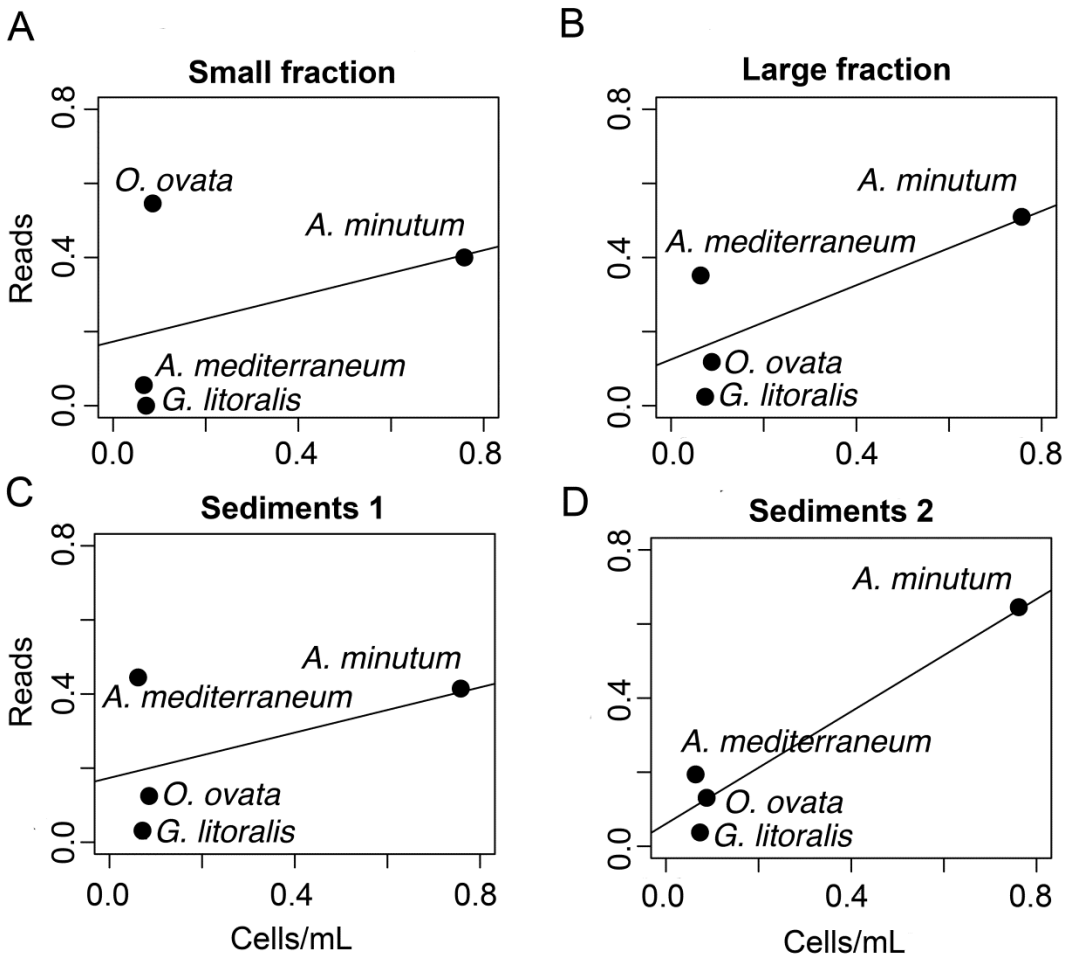
The four samples corresponding to the mock community, one for the small and one for the large fraction, and two for sediments, showed high similarity (Suppl. Fig. 2), in agreement with the fact that all of them contained exactly the same dinoflagellate and parasite community. All reads obtained in the mock community samples, which included several target organisms from this study, belonged to those species added from cultures. Therefore, our metabarcoding method allowed the recovery, amplification and sequencing of target Dinophyceae and Perkinsea. A possible relationship between the number of reads obtained for each organism and the number of cells mL^{-1} added at each sample was evaluated. Firstly, all values were normalized based on the contribution of each species to the total. Next, the relationship between the two variables was determined using a linear regression for each sample (small fraction, large fraction, sediment 1 and sediment 2). No relationship was found ($p > 0.5$) when the values for all eight organisms were processed together, i.e., 4 cultured dinoflagellates species and the hosts present in the parasitoid cultures and the 4 cultured parasitoids (data not shown). The two groups of organisms were then processed independently and the contribution of each species with respect to the total of its group was calculated. For the group of dinoflagellates (Suppl. Fig. 3), the only correlations were found between the number of reads and the cells mL^{-1} of each species in the sediment 2 sample ($p < 0.05$ and $R^2 = 0.94$; Suppl. Fig. 2D). But even in this case, the relation was dominated by the presence of *A. minutum*, which had a strong contribution to cell abundances and to the number of reads. By contrast, the correlations between sequencing reads and cellular abundances of parasitoids (Suppl. Fig. 4) were significant for all samples ($p < 0.05$). The values were lower in the water samples, ($R^2 = 0.84$ in the small fraction and $R^2 = 0.81$ in the large fraction; Suppl. Fig. 4A, B) than in the sediment samples ($R^2 = 0.99$ in sediment 1 and $R^2 = 0.98$ in sediment 2; Suppl. Fig. 4C, D). Even though metabarcoding is considered as a semi-quantitative method, the results obtained for parasitoid suggest that a good relationship between the number of reads and their cellular abundances can be established in results obtained for environmental samples.



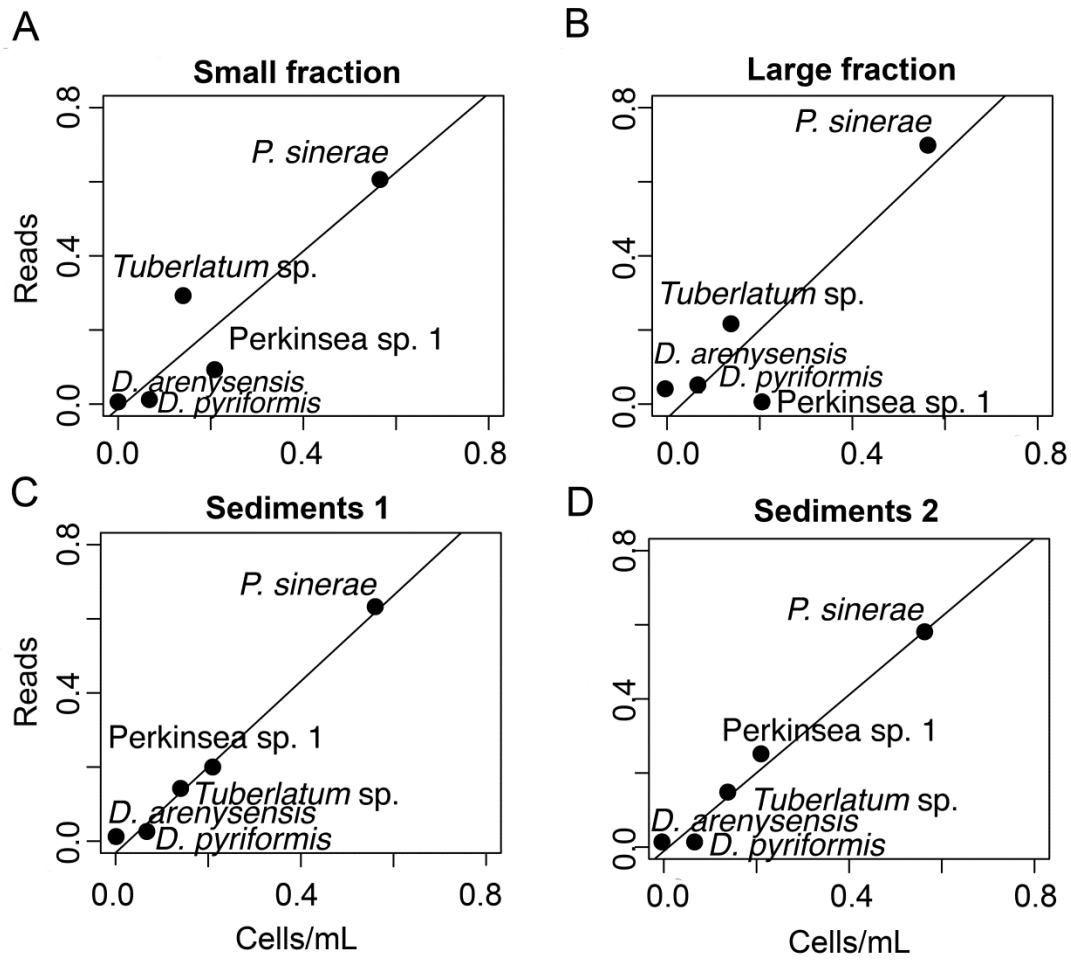
Supplementary Figure 1: Rarefaction curves of all the samples, showing the richness saturation. The horizontal axis indicates the number of sequences (sequencing effort) and the vertical axis the species richness (number of ASVs).



Supplementary Figure 2. Non-metric multidimensional scaling (NMDS) ordination plot, based on Bray-Curtis distances between all the samples including the mocks and blanks. The type of sample is indicated by the different colours (blanks in red, mock in green and samples in blue) and the fraction by the different shapes (small in squares, large in circles and sediments in triangles). The numbers represent the sampling, as described in Table 1.



Supplementary Figure 3: Scatterplot of the relation between the normalized contribution of reads (y axis) and cells mL^{-1} (x axis) of each dinoflagellate culture to the total. A) Small fraction (0.8–10 µm). B) Large fraction (10–200 µm). C) Sediment sample 1. D) Sediment sample 2.



Supplementary Figure 4: Scatterplot of the relation between the normalized contribution of reads (y axis) and cells mL^{-1} (x axis) of each parasitoid culture to the total. A) Small fraction (0.8–10 µm). B) Large fraction (10–200 µm). C) Sediment sample 1. D) Sediment sample 2.

Supplementary Table 1: Relation of metabarcoding samples, including the total number of eukaryotic reads and ASVs obtained, and those corresponding to Dinophyceae and Perkinsea, including the community percentage (%) they represent.

Sample				Eukaryotes		Dinophyceae			Perkinsea		
Code	Location	Replicate	Fraction	Reads	ASVs	Reads	%	ASVs	Reads	%	ASVs
CP001	El Masnou	R1	small	27801	136	9288	33.4	13	0	0	0
CP002	El Masnou	R2	small	31364	147	9920	31.6	13	0	0	0
CP003	El Masnou	R1	small	36732	63	32712	89.1	3	0	0	0
CP004	El Masnou	R2	small	40850	85	36066	88.3	4	0	0	0
CP005	Vilanova	R1	small	43078	78	3337	7.7	12	0	0	0
CP006	Vilanova	R2	small	49637	51	5162	10.4	13	0	0	0
CP007	Llavaneres	R1	small	30241	181	12120	40.1	52	0	0	0
CP008	Llavaneres	R2	small	33917	270	16632	49	49	0	0	0
CP009	Sant Pol	R1	small	33812	129	17270	51.1	23	95	0.28	3
CP010	Sant Pol	R2	small	33632	120	19240	57.2	24	75	0.22	3
CP011	Sitges	R1	small	27590	197	2485	9	14	50	0.18	1
CP012	Sitges	R2	small	30528	219	2072	6.8	15	0	0	0
CP013	Barcelona	R1	small	25724	281	5714	22.2	36	29	0.11	1
CP014	Barcelona	R2	small	29574	290	7017	23.7	36	19	0.06	1
CP015	Cambrils	R1	small	22696	109	9936	43.8	21	0	0	0
CP016	Cambrils	R2	small	36444	182	15140	41.5	32	0	0	0
CP017	Muga	R1	small	26357	163	13334	50.6	25	0	0	0
CP018	Muga	R2	small	25141	174	13292	52.9	24	0	0	0
CP019	Estartit	R1	small	29859	171	7620	25.5	20	0	0	0
CP020	Estartit	R2	small	26623	144	5680	21.3	21	0	0	0
CP021	Fra Ramon	R1	small	25265	45	4740	18.8	5	0	0	0
CP022	Fra Ramon	R2	small	40785	38	12325	30.2	10	0	0	0
CP023	Aiguafreda	R1	small	23891	67	18296	76.6	16	0	0	0
CP024	Aiguafreda	R2	small	35465	87	27256	76.9	23	0	0	0
CP025	L'Arenal	R1	small	32082	195	15394	48	23	0	0	0
CP026	L'Arenal	R2	small	34434	183	19228	55.8	25	0	0	0
CP027	L'Ametlla	R1	small	36065	184	12982	36	32	0	0	0
CP028	L'Ametlla	R2	small	31907	157	14543	45.6	31	2	0.01	1
CP029	Pals	R1	small	25295	64	23693	93.7	18	8	0.03	1
CP030	Pals	R2	small								
CP031	L'Arenal	R1	small	29284	226	6584	22.5	26	47	0.16	3
CP032	L'Arenal	R2	small	34614	269	12021	34.7	32	15	0.04	2
CP033	Cubelles	R1	small	34249	91	30233	88.3	26	0	0	0
CP034	Cubelles	R2	small	42037	120	36448	86.7	34	0	0	0

CP037	El Masnou	R1	large	39238	58	37099	94.5	13	0	0	0
CP038	El Masnou	R2	large	46493	33	45974	98.9	12	0	0	0
CP039	El Masnou	R1	large	36367	28	36118	99.3	12	0	0	0
CP040	El Masnou	R2	large	42696	22	42554	99.7	8	0	0	0
CP041	Vilanova	R1	large	29488	63	13071	44.3	26	0	0	0
CP042	Vilanova	R2	large	36585	73	12200	33.3	29	0	0	0
CP043	Llavaneres	R1	large	55217	153	46776	84.7	60	27	0.05	2
CP044	Llavaneres	R2	large	32018	161	24905	77.8	63	49	0.15	1
CP045	Sant Pol	R1	large	31988	46	27716	86.6	28	250	0.78	2
CP046	Sant Pol	R2	large	31998	52	27257	85.2	29	169	0.53	3
CP047	Sitges	R1	large	40195	207	3486	8.7	25	66	0.16	2
CP048	Sitges	R2	large	45045	181	11566	25.7	31	60	0.13	3
CP049	Barcelona	R1	large	30307	192	16743	55.2	30	14	0.05	1
CP050	Barcelona	R2	large	42946	264	23921	55.7	32	33	0.08	1
CP051	Cambrils	R1	large	40674	94	31948	78.5	26	0	0	0
CP052	Cambrils	R2	large	40109	94	35492	88.5	34	0	0	0
CP053	Muga	R1	large	27287	88	22292	81.7	23	0	0	0
CP054	Muga	R2	large	29606	110	22300	75.3	28	0	0	0
CP055	Estartit	R1	large	33333	150	25567	76.7	35	4	0.01	1
CP056	Estartit	R2	large	30974	111	24106	77.8	37	0	0	0
CP057	Fra Ramon	R1	large	28794	72	14921	51.8	12	0	0	0
CP058	Fra Ramon	R2	large	32247	79	20157	62.5	16	0	0	0
CP059	Aiguafreda	R1	large	37774	30	37401	99	18	0	0	0
CP060	Aiguafreda	R2	large	41546	41	39668	95.5	23	0	0	0
CP061	L’Arenal	R1	large	30791	105	11080	36	38	0	0	0
CP062	L’Arenal	R2	large	35312	133	16922	47.9	64	0	0	0
CP063	L’Ametlla	R1	large	26766	57	26096	97.5	26	0	0	0
CP064	L’Ametlla	R2	large	31234	88	27447	87.9	35	0	0	0
CP065	Pals	R1	large	32463	17	32406	99.8	10	0	0	0
CP066	Pals	R2	large	33131	23	33044	99.7	16	0	0	0
CP067	L’Arenal	R1	large	36384	117	31888	87.6	46	0	0	0
CP068	L’Arenal	R2	large	35210	115	32125	91.2	45	16	0.05	1
CP069	Cubelles	R1	large	42138	36	35354	83.9	16	20	0.05	1
CP070	Cubelles	R2	large	38837	26	30101	77.5	11	11	0.03	1
CP073	El Masnou	R1	sediment	34838	166	23643	67.9	21	0	0	0
CP074	El Masnou	R1	sediment	41299	196	18173	44	38	0	0	0
CP075	Vilanova	R1	sediment	23875	249	8343	34.9	49	0	0	0
CP076	Vilanova	R2	sediment	26618	258	8483	31.9	52	0	0	0
CP077	Llavaneres	R1	sediment	33613	232	5669	16.9	14	5	0.01	1
CP078	Llavaneres	R2	sediment	36014	232	6858	19	16	0	0	0

CP079	Sant Pol	R1	sediment	25924	176	17268	66.6	35	191	0.74	4
CP080	Sant Pol	R2	sediment	35220	231	22472	63.8	41	462	1.31	4
CP081	Sitges	R1	sediment	34355	242	3909	11.4	17	0	0	0
CP082	Sitges	R2	sediment	33938	217	5975	17.6	13	0	0	0
CP083	Barcelona	R1	sediment	29763	342	4683	15.7	24	20	0.07	2
CP084	Barcelona	R2	sediment	36291	399	6745	18.6	27	36	0.1	2
CP085	Cambrils	R1	sediment	37739	259	13945	37	29	0	0	0
CP086	Cambrils	R2	sediment	36645	200	18102	49.4	25	2	0.01	1
CP087	Estartit	R1	sediment	29503	264	3401	11.5	28	154	0.52	1
CP088	Estartit	R2	sediment	6158	106	91	1.5	4	22	0.36	1
CP089	Muga	R1	sediment	28190	348	3697	13.1	42	51	0.18	5
CP090	Muga	R2	sediment	28692	390	3530	12.3	35	37	0.13	3
CP091	Fra Ramon	R1	sediment	25627	165	346	1.4	4	32	0.12	1
CP092	Fra Ramon	R2	sediment	21613	149	134	0.6	6	52	0.24	1
CP093	Aiguafreda	R1	sediment	34679	316	10568	30.5	29	0	0	0
CP094	Aiguafreda	R2	sediment	32049	315	11362	35.5	24	0	0	0
CP095	L’Arenal	R1	sediment	28191	343	2279	8.1	30	3	0.01	1
CP096	L’Arenal	R2	sediment	26968	334	2036	7.5	28	8	0.03	1
CP097	L’Ametlla	R1	sediment	21385	342	2458	11.5	16	0	0	0
CP098	L’Ametlla	R2	sediment	23562	337	2044	8.7	15	0	0	0
CP099	Pals	R1	sediment	40005	269	11307	28.3	23	0	0	0
CP100	Pals	R2	sediment	39537	234	13226	33.5	26	40	0.1	2
CP101	L’Arenal	R1	sediment	30373	206	3981	13.1	21	0	0	0
CP102	L’Arenal	R2	sediment	26034	180	2369	9.1	16	0	0	0
CP103	Cubelles	R1	sediment	31978	242	3615	11.3	18	9996	31.26	4
CP104	Cubelles	R2	sediment	28332	219	3016	10.6	16	8076	28.5	3
CP105	Sa Riera	R1	sediment	37967	306	24791	65.3	35	89	0.23	1
CP106	Cubelles	R1	sediment	30836	226	3368	10.9	16	8953	29.03	3

Supplementary Table 2: List of Perkinsea parasitoid cultures established, including details of its isolation source, molecular rDNA sequences obtained, and dinoflagellate hosts used for maintaining the cultures.

Species	Location	Date	18S rDNA	Host	Strain	Origin	Year of isolation
Parvilucifera sinerae	Barcelona	28-mar-19	MT606014	*Alexandrium minutum*	Arenys	Arenys (Catalan coast)	2018
Parvilucifera sinerae	Cambrils	15-may-19	MT606015	*Alexandrium minutum*	Arenys	Arenys (Catalan coast)	2018
Parvilucifera sinerae	Pals	4-jul-19	MT606016	*Gymnodinium litoralis*	UNISS1	Sardinia (Mediterranean Sea)	2010
Dinovorax pyriformis	Llavaneres	25-jul-18	MT606012	*Ostreopsis* sp.	OOPM18	Llavaneres (Catalan coast)	2018
Dinovorax pyriformis	Sitges	7-ago-18	MT606013	*Ostreopsis* sp.	OOPM18	Llavaneres (Catalan coast)	2018
Dinovorax pyriformis	Cubelles	27-ago-19	MT606011	*Ostreopsis* sp.	OOPM18	Llavaneres (Catalan coast)	2018
Tuberlatum sp.	Sant Pol	25-jul-18	MT606017	*Alexandrium taylorii*	VGO703	Alfacs (Catalan coast)	2003
Perkinsea sp. 1	Sant Pol	25-jul-18	MN721815	*Alexandrium minutum*	Arenys	Arenys (Catalan coast)	2018
				Alexandrium taylorii	VGO703	Alfacs (Catalan coast)	2003
				Alexandrium minutum	Arenys	Arenys (Catalan coast)	2018