

## **Bacterial diversity in the freshwater sponges of Sundarban and their potential role in biomonitoring toxic element pollution**

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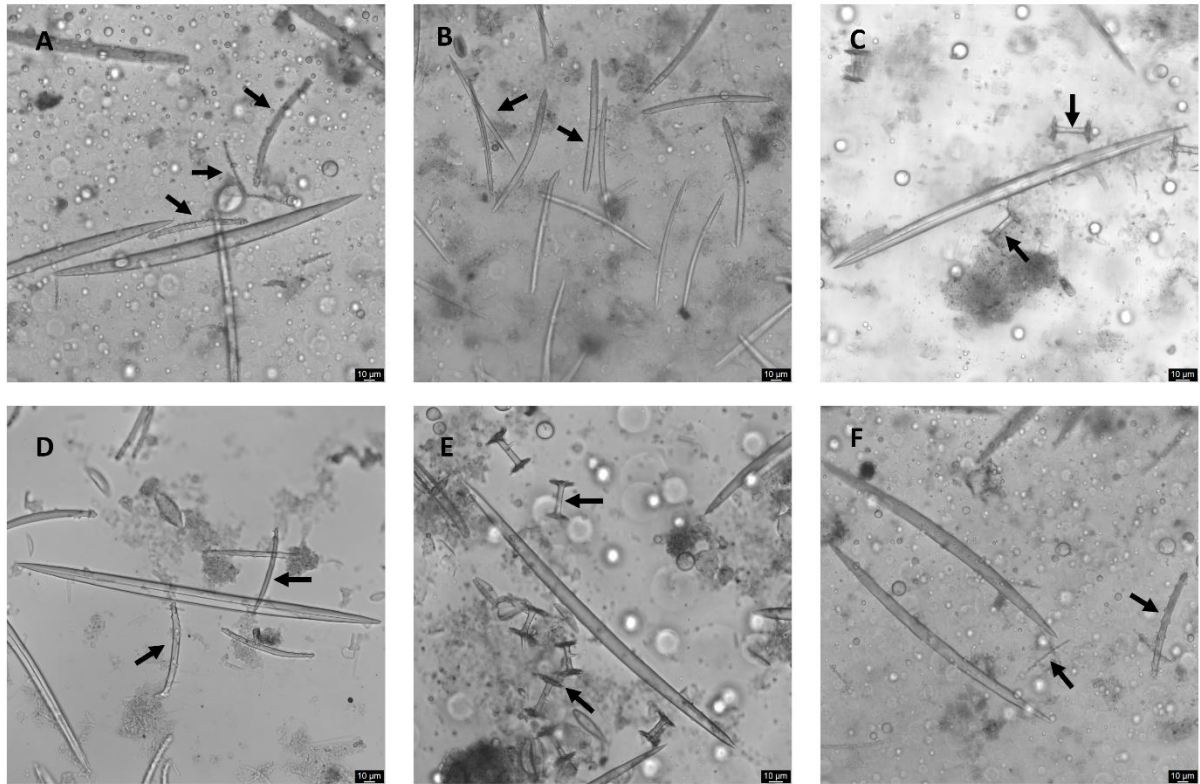
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**Table S1:** Sample IDs, sponge sample details, physicochemical parameters, and concentrations of potentially toxic elements (PTEs) measured in this study.

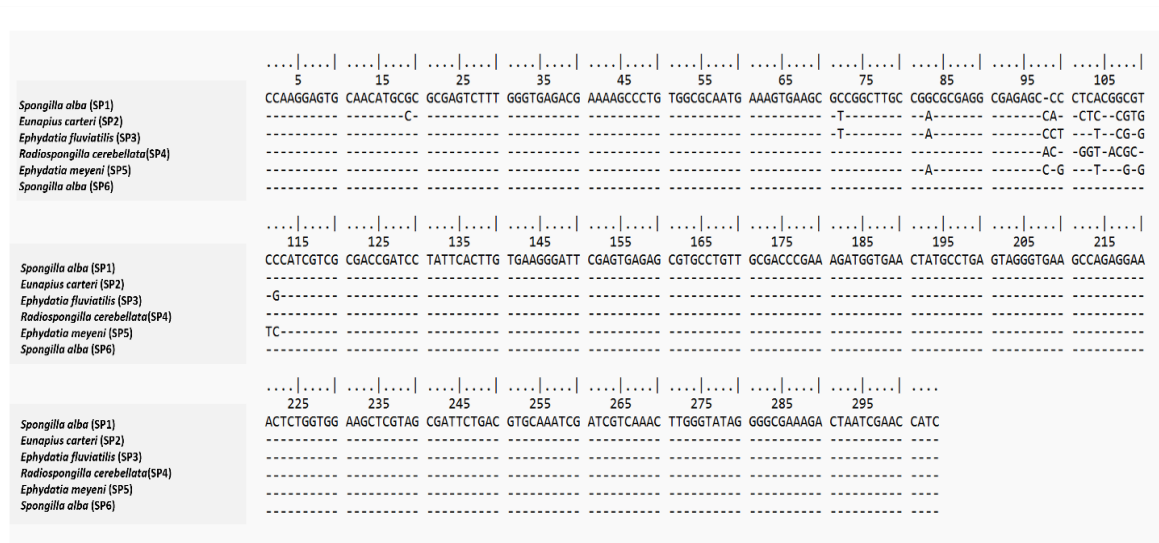
Sample ID	Group ID	Location	Sample	Physicochemical properties						Concentration of PTEs (mg/L)						
				pH	Conductivity (mS/cm)	Salinity (ppt)	TDS (mg/L)	Resistivity ( $\Omega\cdot m$ )	DO concentration (mg/L)	As	Zn	Fe	Cu	Pb	Cd	Cr
SP1.1	SP1	Sagar island	<i>Spongilla alba</i>	8.1	10.4	12.5	10.4	76.8	8.9	2.3	0.975	16.25	0.65	0	0	0.275
SP1.2	SP1	Sagar island	<i>Spongilla alba</i>	8.4	11.1	11.9	10.1	77.2	8.4	2.75	1.025	15.25	0.725	0	0	0.3
SP1.3	SP1	Sagar island	<i>Spongilla alba</i>	8	11.27	12.3	10.1	76.6	8.6	2.375	1.175	17.75	0.7	0	0	0.325
SP2.1	SP2	Sagar island	<i>Eunapius carteri</i>	7.9	13.9	16.4	12.2	81.5	7.7	3.025	1.325	14.75	0.85	0	0	0
SP2.2	SP2	Sagar island	<i>Eunapius carteri</i>	7.8	13.4	16.7	12.4	82	7.3	2.975	1.2	15.75	0.925	0	0	0
SP2.3	SP2	Sagar island	<i>Eunapius carteri</i>	8.1	13.7	16.4	12	81.8	7.3	3.9	1.15	16.5	1.025	0	0	0
SP3.1	SP3	Sagar island	<i>Ephydatia fluviatilis</i>	7.7	11.5	13.2	10.8	78.2	8.7	3.5	1.475	14.75	0.975	0	0	0
SP3.2	SP3	Sagar island	<i>Ephydatia fluviatilis</i>	8.1	11.3	13.2	10.6	77.9	8.6	4.05	1.775	14	1.15	0	0	0
SP3.3	SP3	Sagar island	<i>Ephydatia fluviatilis</i>	7.9	11.3	12.9	10.7	78	8.3	3.775	1.7	14.5	1.025	0	0	0
SP4.1	SP4	Ghoramara	<i>Radiospongilla cerebellata</i>	7.3	7.7	7	5.7	47.6	9.6	2.55	1.725	22.75	1.3	0	0	0
SP4.2	SP4	Ghoramara	<i>Radiospongilla cerebellata</i>	7.3	7.5	7.5	5.5	47.2	9.5	2.45	1.625	24	1.375	0	0	0
SP4.3	SP4	Ghoramara	<i>Radiospongilla cerebellata</i>	7.4	7.2	7.2	5.4	47.4	9.6	2.325	1.775	22.25	1.525	0	0	0
SP5.1	SP5	Ghoramara	<i>Ephydatia meyeri</i>	7.3	6.7	6.7	6.1	36.8	8.9	3.375	1.1	17.25	0.9	0	0.475	0.4
SP5.2	SP5	Ghoramara	<i>Ephydatia meyeri</i>	7.3	6.4	6.3	6.3	37	9.1	3.6	1.225	19.25	1.025	0	0.375	0.3
SP5.3	SP5	Ghoramara	<i>Ephydatia meyeri</i>	7.5	6.4	6.6	6.3	37.1	8.8	3.95	1.375	19	1.025	0	0.325	0.4
SP6.1	SP6	Ghoramara	<i>Spongilla alba</i>	7.5	6.3	7.7	6.7	42.5	9.3	4.95	0.725	14.75	0.65	0.275	0	0.225
SP6.2	SP6	Ghoramara	<i>Spongilla alba</i>	7.6	6.2	7.9	6.8	42.2	9	5.3	0.475	13.25	0.475	0.475	0	0.275
SP6.3	SP6	Ghoramara	<i>Spongilla alba</i>	7.3	6.6	7.3	6.7	42.6	9.4	5.25	0.425	14.75	0.55	0.3	0	0.4

**Table S2:** Sample IDs, water sample details, physicochemical parameters, and concentrations of potentially toxic elements (PTEs) measured in this study.

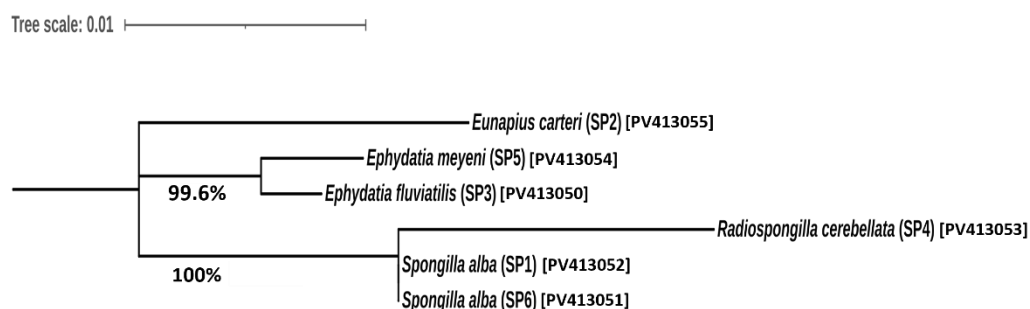
Sample id	Group id	Location	Sample	Physicochemical properties						Concentration of PTEs (mg/L)						
				pH	Conductivity (mS/cm)	Salinity (ppt)	TDS (mg/L)	Resistivity ( $\Omega\cdot\text{m}$ )	DO concentration (mg/L)	As	Zn	Fe	Cu	Pb	Cd	Cr
S1.1	S1	Sagar island	Water	8.1	10.4	12.5	10.4	76.8	8.9	0.058	0.025	0.45	0.014	0.03	0.026	0.017
S1.2	S1	Sagar island	Water	8.4	11.1	11.9	10.1	77.2	8.4	0.061	0.031	0.51	0.017	0.03	0.03	0.021
S1.3	S1	Sagar island	Water	8	11.27	12.3	10.1	76.6	8.6	0.055	0.036	0.61	0.017	0.034	0.029	0.013
S2.1	S2	Sagar island	Water	7.9	13.9	16.4	12.2	81.5	7.7	0.044	0.028	0.44	0.019	0.039	0.019	0.018
S2.2	S2	Sagar island	Water	7.8	13.4	16.7	12.4	82	7.3	0.049	0.031	0.4	0.02	0.044	0.016	0.015
S2.3	S2	Sagar island	Water	8.1	13.7	16.4	12	81.8	7.3	0.051	0.028	0.47	0.021	0.051	0.014	0.017
S3.1	S3	Sagar island	Water	7.7	11.5	13.2	10.8	78.2	8.7	0.074	0.031	0.61	0.031	0.068	0.022	0.025
S3.2	S3	Sagar island	Water	8.1	11.3	13.2	10.6	77.9	8.6	0.081	0.035	0.68	0.035	0.071	0.025	0.031
S3.3	S3	Sagar island	Water	7.9	11.3	12.9	10.7	78	8.3	0.073	0.038	0.62	0.038	0.077	0.028	0.027
S4.1	S4	Ghoramara	Water	7.3	7.7	7	5.7	47.6	9.6	0.055	0.022	0.58	0.026	0.041	0.03	0.03
S4.2	S4	Ghoramara	Water	7.3	7.5	7.5	5.5	47.2	9.5	0.058	0.023	0.61	0.019	0.043	0.025	0.032
S4.3	S4	Ghoramara	Water	7.4	7.2	7.2	5.4	47.4	9.6	0.06	0.02	0.58	0.021	0.049	0.028	0.03
S5.1	S5	Ghoramara	Water	7.3	6.7	6.7	6.1	36.8	8.9	0.039	0.016	0.34	0.011	0.024	0.019	0.011
S5.2	S5	Ghoramara	Water	7.3	6.4	6.3	6.3	37	9.1	0.037	0.015	0.33	0.01	0.029	0.02	0.014
S5.3	S5	Ghoramara	Water	7.5	6.4	6.6	6.3	37.1	8.8	0.039	0.019	0.37	0.015	0.032	0.029	0.019
S6.1	S6	Ghoramara	Water	7.5	6.3	7.7	6.7	42.5	9.3	0.045	0.032	0.51	0.019	0.035	0.029	0.017
S6.2	S6	Ghoramara	Water	7.6	6.2	7.9	6.8	42.2	9	0.049	0.033	0.55	0.025	0.036	0.031	0.02
S6.3	S6	Ghoramara	Water	7.3	6.6	7.3	6.7	42.6	9.4	0.051	0.031	0.47	0.023	0.041	0.025	0.018



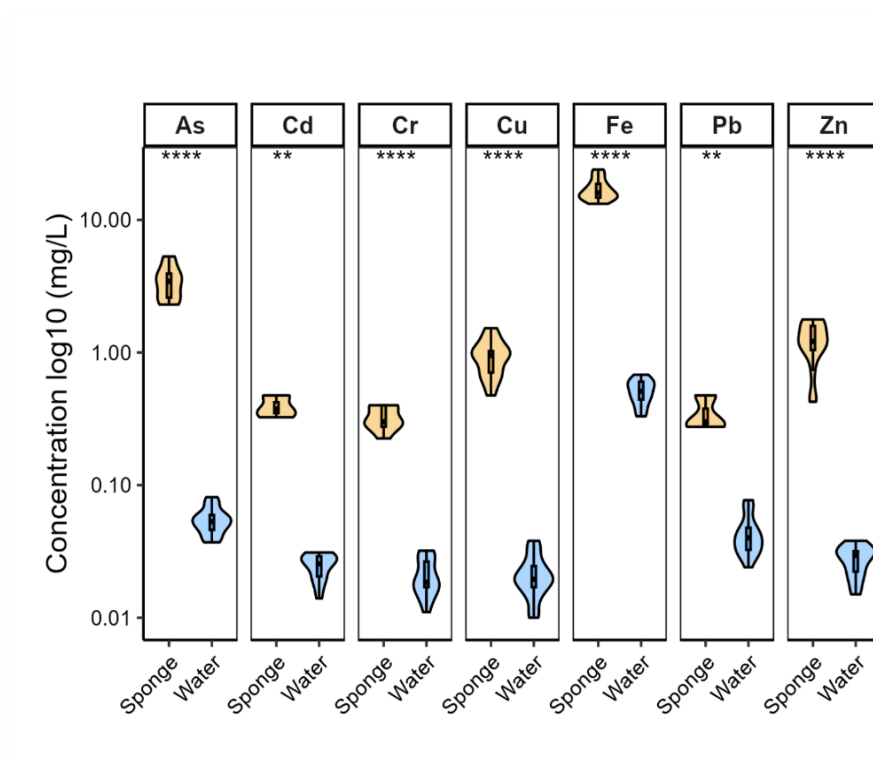
**Fig. S1:** Confocal microscopy images of sponge spicules showing megascleres and gemmoscleres. Megascleres appear as elongated spicules, while gemmoscleres are indicated by arrows. (A–F) Spicules of *Spongilla alba* (SP1), *Eunapius carteri* (SP2), *Ephydatia fluviatilis* (SP3), *Radiospongilla cerebellata* (SP4), *Ephydatia meyeri* (SP5), and *Spongilla alba* (SP6).



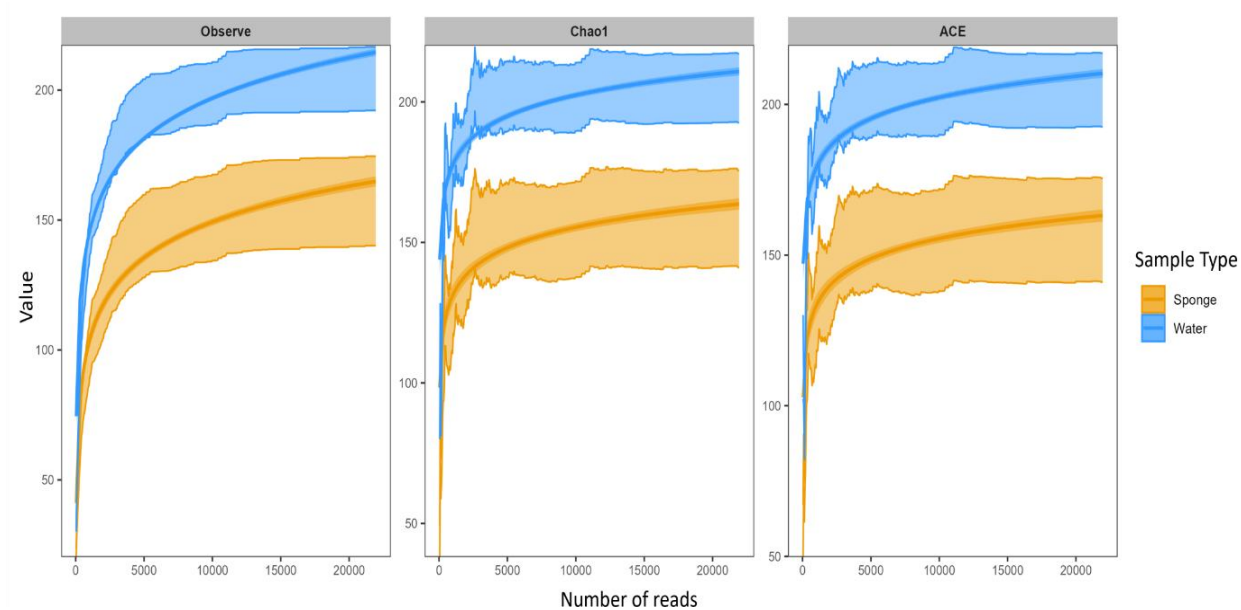
**Fig. S2:** Alignment of the 28S rDNA D3 domain from *Spongilla alba* (GenBank accession No. PV413052), *Eunapius carteri* (GenBank accession No. PV413055), *Ephydatia fluviatilis* (GenBank accession No. PV413050), *Radiospongilla cerebellata* (GenBank accession No. PV413053), *Ephydatia meyeri* (GenBank accession No. PV413054), and *Spongilla alba* (GenBank accession No. PV413051). Sequences were aligned using ClustalW. Identical sites and gaps are indicated by hyphens.



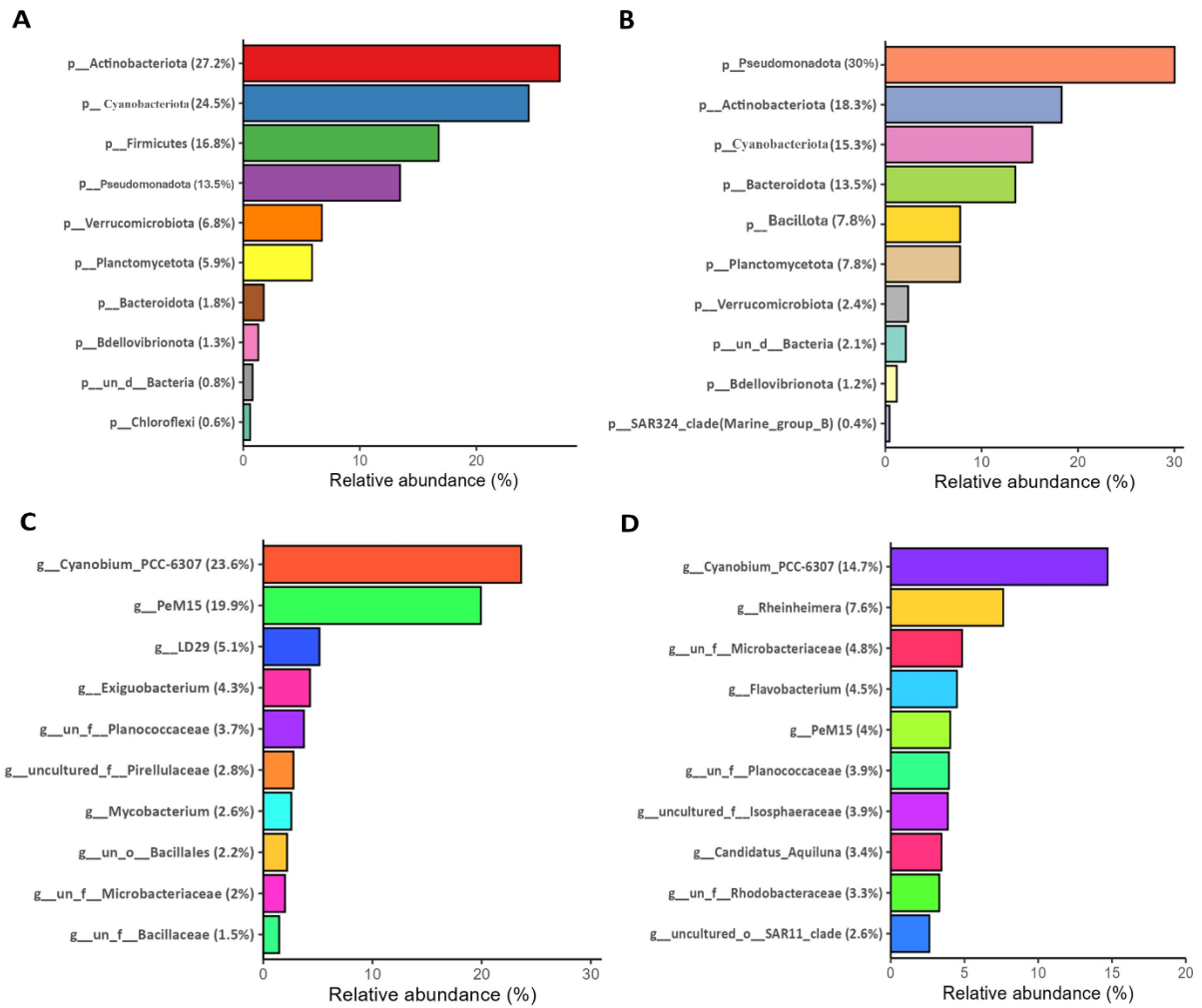
**Fig. S3:** Neighbour Joining tree based on D3 domain of 28S rDNA sequences of freshwater sponges, constructed using the Tamura 3-parameter model in MEGA. Bootstrap values (expressed as percentages based on 1000 replicates) are displayed at the nodes. The scale bar represents genetic distance.



**Fig. S4:** Concentrations of various potentially toxic elements (PTEs) measured in sponge and water samples. Significance levels are indicated as follows: \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ .

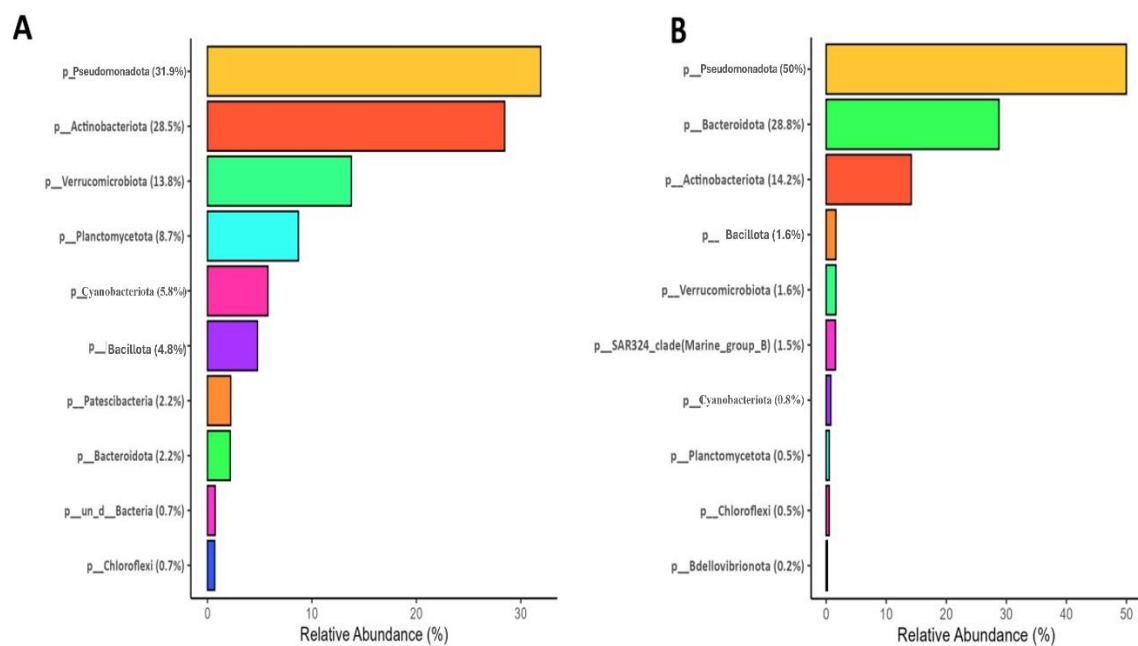


**Fig. S5:** The rarefaction curves obtained based on number of reads and the values of alpha diversity indices based on number of OTUs in sponge and water samples.

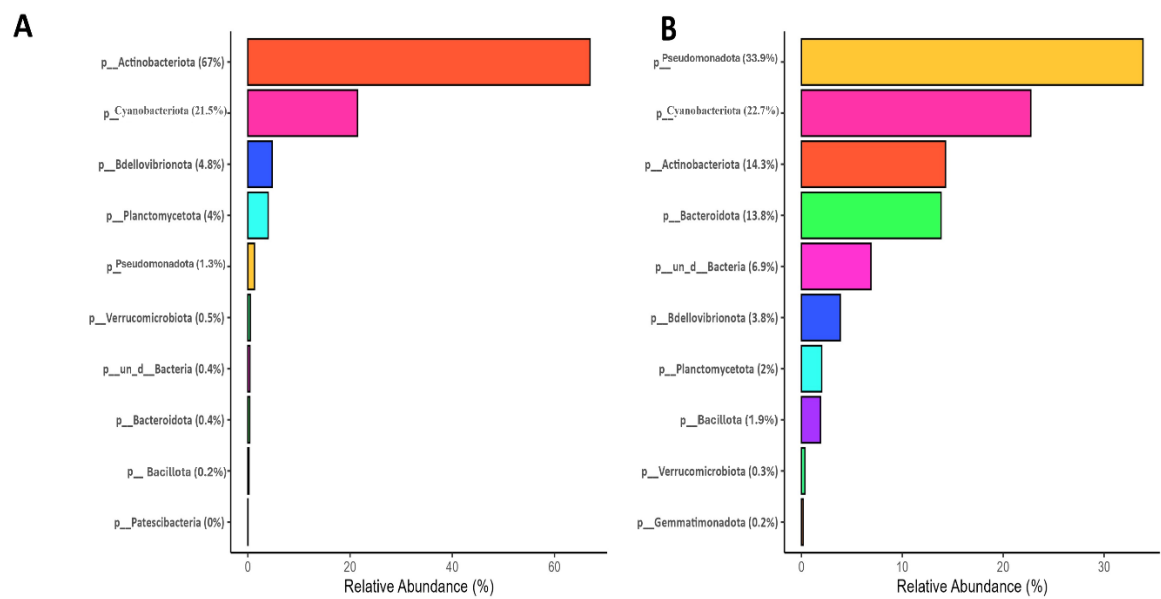


**Fig. S6:** Relative abundance of top ten phyla in sponge (A) and water samples (B), and top ten genera in sponge (C) and water (D) samples.

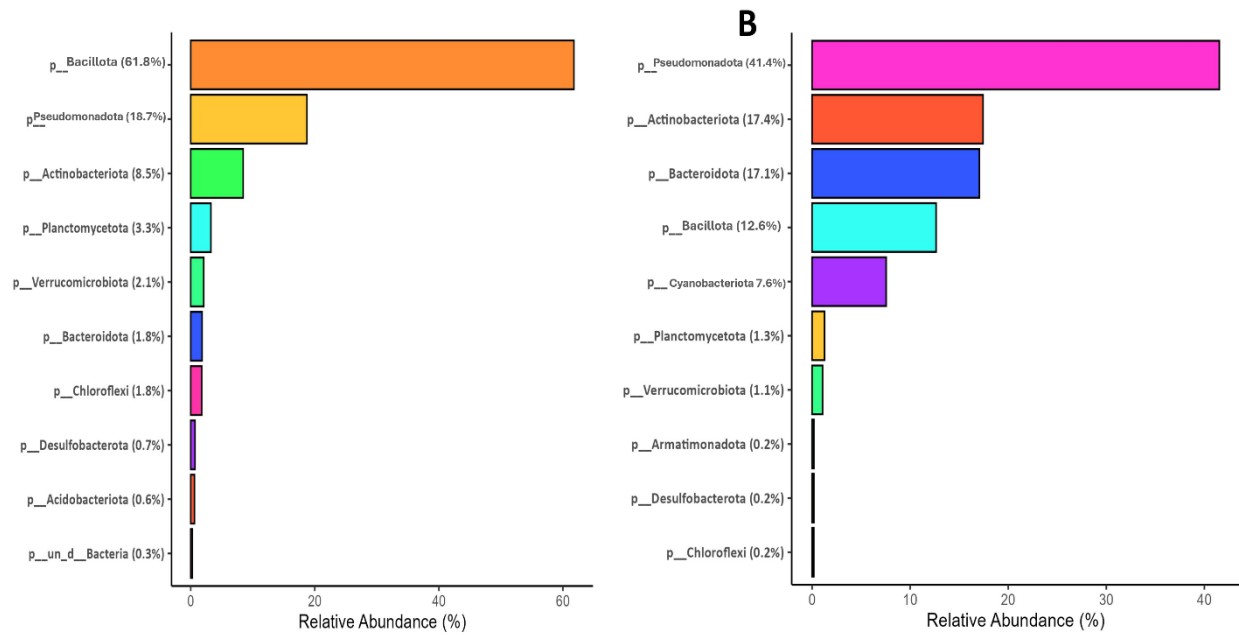




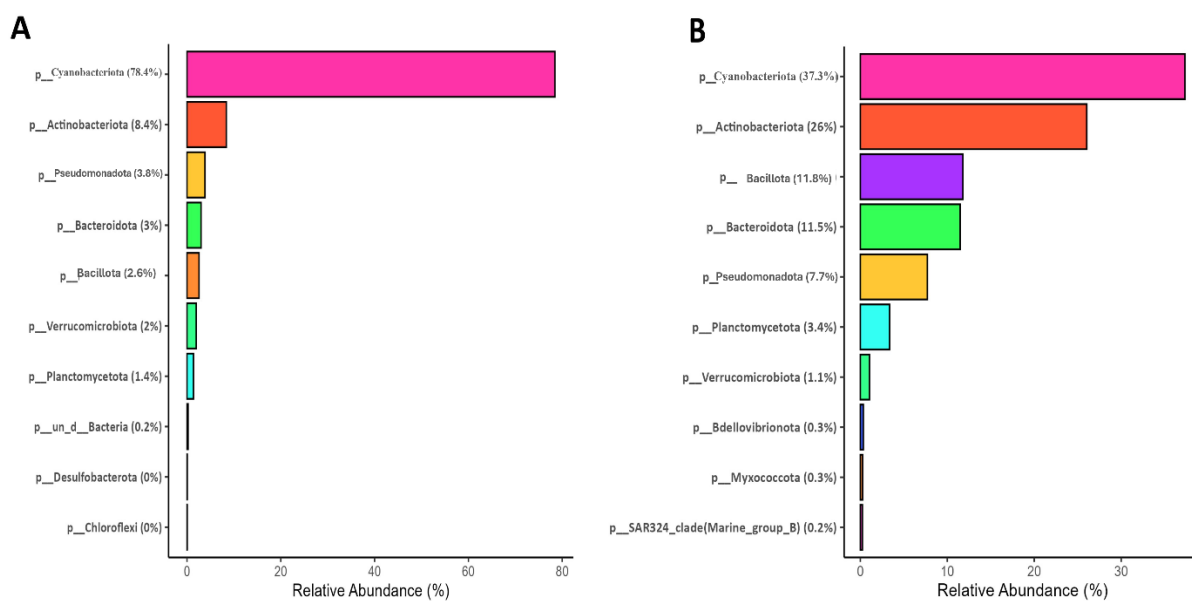
**Fig. S7:** Relative abundance of phyla in *Spongilla alba* (A) and its ambient water (B) collected from Sagar Island



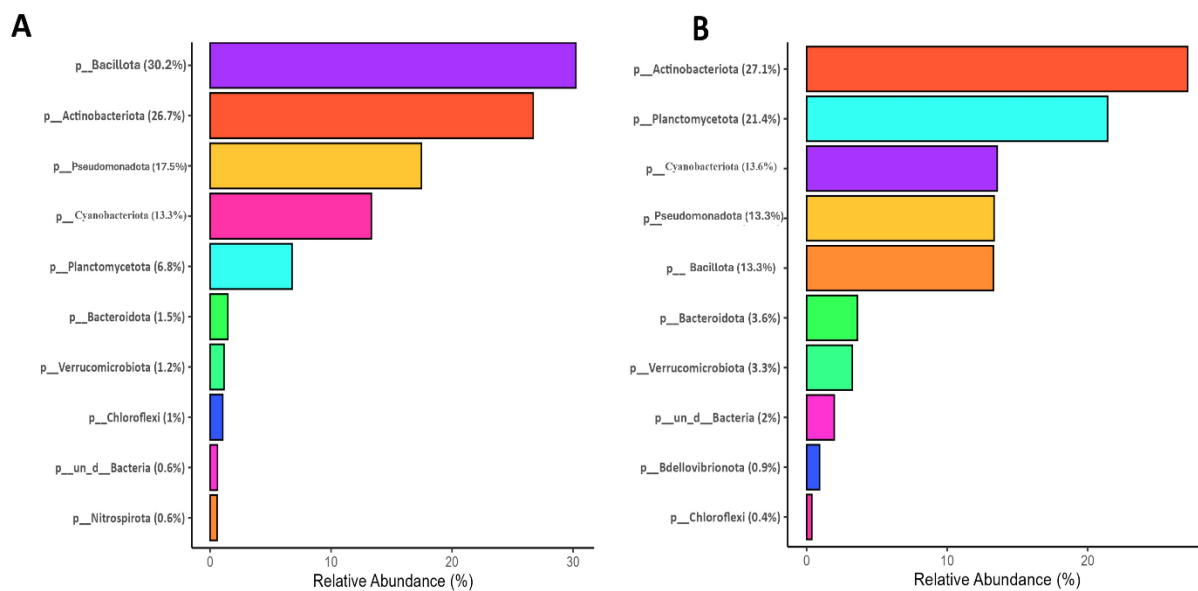
**Fig. S8:** Relative abundance of phyla in *Eunapius carteri* (A) and its ambient water (B) collected from Sagar Island



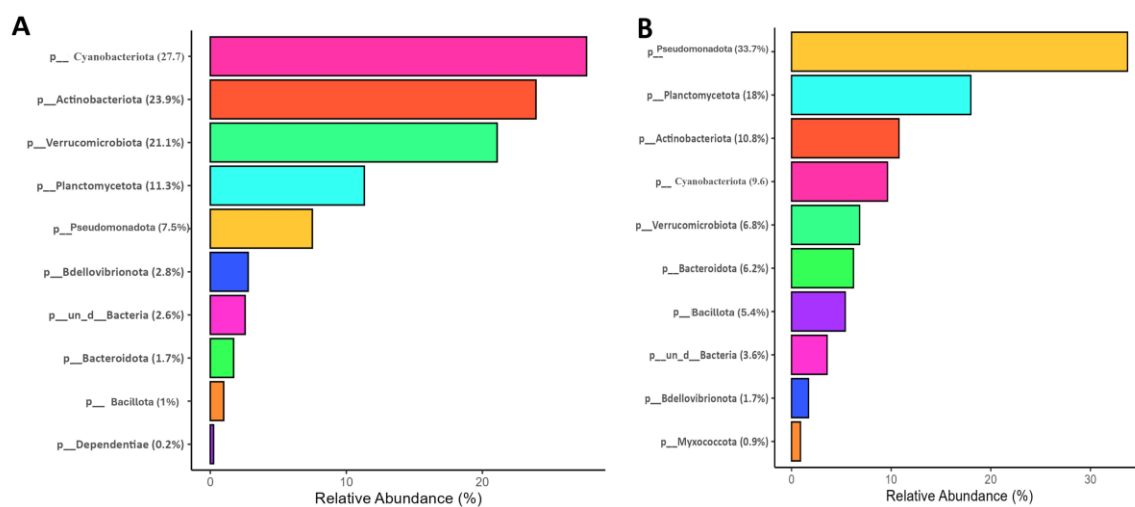
**Fig. S9:** Relative abundance of phyla in *Ephydatia fluviatilis* (A) and its ambient water (B) collected from Sagar Island



**Fig. S10:** Relative abundance of phyla in *Radiospongilla cerebellata* (A) and its ambient water (B) collected from Ghoramara



**Fig. S11:** Relative abundance of phyla in *Ephydatia meyeri* (A) and its ambient water (B) collected from Ghoramara



**Fig. S12:** Relative abundance of phyla in *Spongilla alba* (A) and its ambient water (B) collected from Ghoramara