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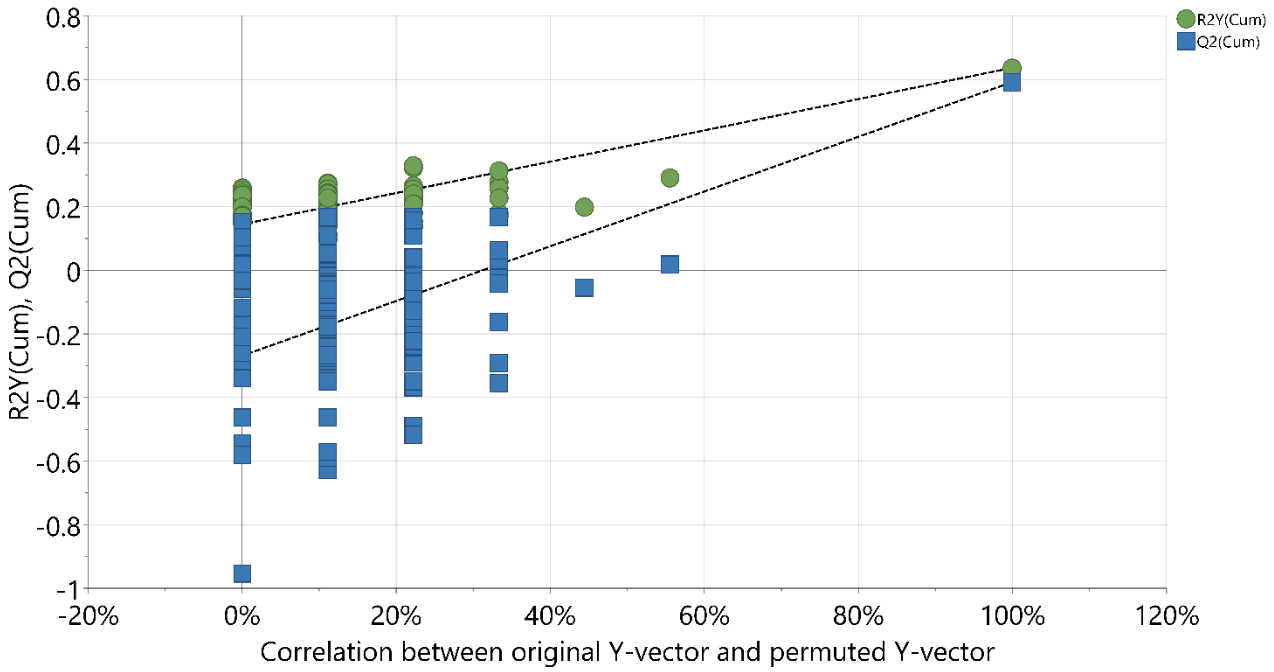

Supplementary figure 1: Permutation validation of the OPLS-DA model comparing *Holothuria cinerascens* and *Pseudocnella sykion*. Permutation test results showing intercepts at R^2=(0.0; 0.145) and Q^2=(0.0; -0.269).

Supplementary table 1: Summary of influential ^{1}H-NMR spectral regions (ppm) responsible for tissue segregation based on contribution loadings plots between *Holothuria cinerascens* and *Pseudocnella sykion*

Comparison	Species with positive association	Positively associated ppm regions (strong)	Positively associated ppm regions (lesser extent)	Negatively associated PPM regions
H. cinerascens x *P. sykion*	*H. cinerascens*	0.04, 0.92, 1.08, 1.12, 1.28, 1.32, 1.36, 1.4, 1.52, 1.56, 1.76, 1.8, 1.92, 1.96, 2.08, 2.16, 2.44, 3.2, 3.24, 3.4, 3.44, 3.48, 3.52, 3.56, 3.6, 3.64, 3.68, 3.72, 3.8, 3.84, 3.88, 3.92, 4.24, 4.56, 5.04	0.96, 1, 1.16, 1.24, 1.44, 1.48, 1.6, 1.68, 1.72, 1.84, 1.88, 2, 2.04, 2.12, 2.2, 2.28, 2.32, 2.36, 2.4, 2.56, 2.96, 3.04, 3.08, 3.76, 3.96, 4, 4.04, 4.08, 4.12, 4.16, 4.2, 4.28, 4.4, 4.52, 5.08, 5.12, 5.16, 5.2, 5.24, 5.28, 5.32, 5.4	–
H. cinerascens x *P. sykion* excluding 'HC3' and 'HC6' outliers	*H. cinerascens*	0.92, 1, 1.08, 1.12, 1.28, 1.32, 1.36, 1.4, 1.52, 1.56, 1.76, 1.8, 1.84, 1.92, 1.96, 2.08, 2.16, 2.2, 2.4, 2.44, 3.2, 3.24, 3.4, 3.44, 3.48, 3.52, 3.56, 3.64, 3.68, 3.72, 3.8, 3.84, 3.88, 3.92, 4.24	0.04, 1.16, 1.24, 1.44, 1.48, 1.6, 1.68, 1.72, 1.88, 2, 2.04, 2.12, 2.28, 2.32, 2.36, 2.56, 2.6, 2.96, 3.04, 3.08, 3.16, 3.6, 3.76, 3.96, 4, 4.04, 4.08, 4.12, 4.16, 4.2, 4.28, 4.4, 4.52, 4.56, 5.04, 5.08, 5.12, 5.16, 5.2, 5.24, 5.28, 5.32, 5.4	2.72

Supplementary table 2: Compound annotation table identifying key metabolites from the ^{1}H-NMR spectral comparison between *Holothuria cinerascens* (HC) and *Pseudocnella sykion* (PS).

Metabolite	^{1}H-NMR Chemical shifts (ppm) – HC	^{1}H-NMR Chemical shifts (ppm) – PS	Chenomx (ppm)	Human Metabolome Database	Reference Chemical Shift (ppm)	Reference	Higher in:
Acetic acid	1.91	1.90	1.9	1.91	1.91	1	HC
Alanine	1.47	1.47	1.5	1.47	1.47	2-4	HC
	3.76	3.77	3.8	3.77	3.77		
Arginine	1.66	1.64	1.6	1.53		2,4	HC
	1.72	1.71	1.7	1.59	1.68		
	1.88	1.88	1.9	1.86			
	1.92	1.91	1.9				
	3.22	3.23	3.2	3.17	1.90		
	3.78	3.76	3.8	3.77	3.23		
			6.7		3.74		
			7.2				
Ascorbic acid	3.70	3.71	3.7	3.44		5	EVEN
	3.74	3.75	3.8	3.73			
	4.05	4.02	4.0				
	4.52	4.51	4.5	4.72	4.03		
Betaine	3.25	3.25	3.3	3.25	3.25	4	HC
	3.84	3.84	3.9	3.89	3.89		
Butyric acid	0.87	0.87	0.9	0.88	0.86	6	HC
	1.51	1.51	1.5	1.54			
	2.14	2.14	2.2	2.14	2.18		
D-Glucose	3.23	3.23	3.2		3.23	4	HC
	3.38	3.38	3.4	3.39	3.40		
	3.40	3.40	3.4		3.46		
	3.45	3.45	3.5	3.52	3.52		
	3.48	3.48	3.5				
	3.52	3.52	3.5				
	3.70	3.70	3.7		3.73		
	3.72	3.72	3.7	3.72	3.82		
	3.75	3.75	3.8	3.81			
	3.82	3.82	3.8				
	3.83	3.83	3.8				
	3.88	3.88	3.9		3.88		
	4.64	4.64	4.6		4.63		
	5.21	5.21	5.2	5.21	5.22		
Dimethyl sulfone	3.16	3.16	3.1	3.14	3.14	1	EVEN
D-Xylose	3.21	3.21	3.2			7	HC
	3.31	3.31	3.3				
	3.42	3.42	3.4				
	3.48	3.48	3.5	3.52			
	3.60	3.60	3.6		3.68–3.72		
	3.61	3.61	3.6				
	3.66	3.66	3.7	3.68	4.07–4.10		
	3.69	3.69	3.7	3.68	4.08–4.11		
			3.7	3.87	4.29–4.33		
	3.88	3.88	3.9	4.00	4.43–4.46		
	4.52	4.52	4.6	4.63	4.78		
	5.19	5.19	5.2				
Ethanolamine	3.12	3.13	3.1	3.13	3.15	3,8	Even
	3.82	3.81	3.8	3.81	3.83		

Metabolite	¹H-NMR Chemical shifts (ppm) – HC	¹H-NMR Chemical shifts (ppm) – PS	Chenomx (ppm)	Human Metabolome Database	Reference Chemical Shift (ppm)	Reference	Higher in:
Galactose	3.47	3.48	3.5	3.52		[3]	HC
	3.64	3.64	3.6				
	3.69	3.69	3.7				
	3.71	3.71	3.7	3.74			
	3.73	3.73	3.7				
	3.73	3.73	3.7				
	3.76	3.76	3.8	3.82	3.81		
	3.79	3.79	3.8				
	3.84		3.8				
	3.93	3.92	3.9	3.95			
	3.98	3.98	4.0				
	4.07	4.07	4.1	4.12	4.07		
	4.57	4.57	4.6				
	5.27	5.17	5.3	5.21			
Glutamate	2.03	2.03	2.0	2.06	2.08	[4]	HC
	2.13	2.13	2.1	2.11			
	2.37	2.37	2.3	2.32			
	2.41	2.41	2.4	2.36	2.34		
	3.76	3.76	3.8	3.75	3.74		
Glutamine			2.1			[6]	HC
	2.11	2.11	2.1	2.12	2.12		
	2.14	2.14	2.4		2.44		
	2.38	2.41	2.5	2.42			
	2.40	2.46	3.8	2.46	3.70		
	3.76	3.76	6.9	3.76			
			7.6				
Glycerol	3.53	3.53	3.6	3.43		[6]	EVEN
	3.61	3.61	3.6	3.60	3.54		
	3.71	3.71	3.8	3.61	3.62		
				3.85			
Glycine	3.55	3.55	3.6	3.55	3.57	[3,4,7]	EVEN
Lysine	1.43	1.43	1.4	1.43	1.46	[2]	HC
	1.50	1.50	1.5				
	1.71	1.71	1.7	1.72	1.70		
	1.87	1.87	1.9	1.90	1.89		
	1.91	1.91	1.9	2.12			
	3.00	3.00	3.0	3.01	3.01		
	3.75	3.75	3.8	3.74	3.74		
Malonate	3.13/3.14	3.13	3.1	3.11	3.11	[3]	EVEN
Mannose	3.36	3.37	3.4	3.46	3.38	[9]	EVEN
	3.58	3.56	3.6				
	3.67	3.65	3.7	3.71			
	3.74	3.72	3.7	3.74			
		3.75	3.7				
	3.81	3.80	3.8	3.80	3.81		
	3.84	3.84	3.8	3.82	3.82		
	3.86	3.86	3.8				
	3.89	3.90	3.9				
		3.92	3.9				
		3.93	3.9	3.99			
			3.9		4.74		
	4.89	4.90	4.9		4.91		
	5.17	5.18	5.2	5.21	5.45		

Metabolite	^{1}H-NMR Chemical shifts (ppm) – HC	^{1}H-NMR Chemical shifts (ppm) – PS	Chenomx (ppm)	Human Metabolome Database	Reference Chemical Shift (ppm)	Reference	Higher in:
Methylmalonate	1.24	1.24	1.2	1.23	1.21	[5,8]	HC
	3.18	3.18	3.2	3.16			
Myo-inositol	3.27	3.26	3.3	3.27		[5]	HC
				3.54			
	3.52	3.52	3.5	3.54	3.52		
	3.60	3.61	3.6	3.61	3.60		
				3.61			
	4.04	4.06	4.1	4.05			
Proline	1.99	1.99	2.0	1.47	1.99	[2]	PS
	2.03	2.03	2.0	1.75			
	2.05	2.05	2.1	2.20	2.06		
	2.34	2.34	2.3	2.99	2.33		
	3.32	3.32	3.3	3.40	3.33		
			3.4	3.57	3.40		
	4.12	4.12	4.1		4.12		
Pyruvate	2.36	2.36	2.4	2.36	2.40	[1]	HC
Sarcosine	N/A	2.72	2.7	2.73	2.7	[10]	PS
		3.60	3.6	3.60	3.6		
				3.78			
Taurine	3.25	3.25	3.5	3.25	3.27	[3]	HC
	3.42	3.42	3.4	3.41	3.43		
Threonine	1.31	1.31	1.3	1.32	1.33	[4]	HC
	3.60	3.58	3.6	3.58	3.58		
	4.21	4.25	4.3	4.24	4.25		
Valerate	0.86	0.87	0.6	0.88		[8]	HC
	1.31	1.29	1.3	1.33	1.20		
	1.51	1.50	1.5	1.51	1.56		
	2.15	2.15	2.2	2.17			

Supplementary table 3: Comparative analysis of metabolites tentatively annotated in ESI negative mode by UPLC-QTOF-HR-MS in *Holothuria cinerascens* (HC) and *Pseudocnella sykion* (PS)

Rt	Observed m/z	Monoisotopic mass	Empirical formula (neutral)	Found in species		Compound	Reference	Mass error (%)	Mass error (ppm)
				HC	PS				
6.36	359.0743	360.08452	$C_{18}H_{16}O_8$		X	Rosmarinic acid (Levels very low)	[11] ChemSpider ID: 4474888/445104	0.000667	6.67
6.87	209.0834	210.09258	$C_8H_{18}O_4S$	X		Dibutyl sulfate or octyl sulfate or 6-methylheptyl sulfate; DBE=0	[12, 13] ChemSpider ID: 11738 Or 8547	0.000648	6.48
6.90	195.0620	196.07693	$C_7H_{16}O_4S$	X		Heptyl hydrogen sulfate	ChemSpider ID: 158845	0.003642	36.42
6.92	209.0836	210.09258	$C_8H_{18}O_4S$	X		Aliphatic organosulfate or dibutyl sulfate or octyl sulfate or 6-methylheptyl sulfate DBE=0	[12, 13] ChemSpider ID: 11738 Or 8547	0.000552	5.52
7.08	209.0838	210.09258	$C_8H_{18}O_4S$	X				0.000457	4.57
7.16	209.0844	210.09258	$C_8H_{18}O_4S$	X				0.00017	1.70
7.55	209.0842	210.09258	$C_8H_{18}O_4S$	X				0.000265	2.65
7.59	209.0840	210.09258	$C_8H_{18}O_4S$	X				0.000361	3.61
7.75	209.0839	210.09258	$C_8H_{18}O_4S$	X				0.000409	4.09
7.84	209.0838	210.09258	$C_8H_{18}O_4S$	X				0.000457	4.57
8.25	237.1141	238.12388	$C_{10}H_{22}O_4S$	X			[13]	0.000824	8.24

Rt	Observed m/z	Monoisotopic mass	Empirical formula (neutral)	Found in species HC	PS	Compound	Reference	Mass error (%)	Mass error (ppm)
						Diamyl sulfate or decyl sulfate or 8-methylnoyl hydrogen sulfate; DBE=0	ChemSpider ID: 13674908 Or 8564		
8.30	1199.5144	1200.52337	$C_{54}H_{88}O_{27}S$	X		Holothurin A1 or holothurin A4 or scabraside D; DBE=11	[14-16] ChemSpider ID: 29368578	9.55E-05	0.95
	1199.5177	1199.51915	$C_{66}H_{80}O_{19}Na$	X		Unknown; DBE=26		0.083969	-839.69
8.39	223.0990	224.10823	$C_{9}H_{20}O_{4}S$	X	X	2.6-dimethylheptyl hydrogen sulfate or 6-methyloctyl sulfate or 7-methyloctyl hydrogen sulfate; DBE=0	[13] ChemSpider ID: 23327239 or 141892 PubChem CID: 141892	0.00063	6.30
8.48	1197.5016	1198.50772	$C_{54}H_{86}O_{27}S$	X		Holothurin A (arenicolaside A) or 17-hydroxyfuscocineroside B (scabraside B) or 25-hydroxyfuscocineroside B	[14-17]	0.000142	-1.42
	1197.4994	1198.50882	$C_{70}H_{79}O_{14}Sna$	X		Unknown		0.000133	1.33
8.72	1181.5083	1182.51280	$C_{54}H_{86}O_{26}S$	X	X	Scabraside A or 24-dehydroechinoside A or 17-dehydroxyholothurin A (fuscocineroside C) or fuscocineroside B	[14-18]	0.000281	-2.81
	1181.5016	1182.50693	$C_{61}H_{82}O_{21}S$	X	X	Unknown; DBE=21		0.000211	-2.11
8.92	237.1149	238.12388	$C_{10}H_{22}O_{4}S$	X	X	Diamyl sulfate or decyl sulfate or 8-methylnoyl hydrogen sulfate; DBE=0	[13] ChemSpider ID: 13674908 Or 8564	0.000487	4.87
8.96	1199.5203	1200.52337	$C_{54}H_{88}O_{27}S$	X	X	Holothurin A1 or holothurin A4 or scabraside D; DBE=11	[14-16]	0.000396	-3.96
9.14	1197.4954	1198.50184	$C_{61}H_{82}O_{22}S$	X	X	Unknown; DBE=21		0.000116	-1.16
	1197.4998	1197.50100	$C_{70}H_{78}O_{14}Sna$	X	X	Unknown; DBE=31		0.084131	-841.31
9.30	367.2151	368.22326	$C_{17}H_{36}O_{6}S$	X	X	2-2-(tridecyloxy) ethoxy]ethyl hydrogen sulfate or C13-alkyl-2-ethoxysulfate (C13-AE2S); DBE=0	ChemSpider ID: 2289709 or PubChem CID: 3023544	9.12E-05	0.91
9.36	1181.5095	1182.51280	$C_{54}H_{86}O_{26}S$	X	X	Scabraside A or 24-dehydroechinoside A or 17-dehydroxyholothurin A (fuscocineroside C) or fuscocineroside B; DBE=12	[14-16,18]	0.000383	-3.83
	1181.5028	1181.50257	$C_{52}H_{86}O_{26}SNa$	X	X	Unknown; DBE=9		0.085393	-853.93

Rt	Observed m/z	Monoisotopic mass	Empirical formula (neutral)	Found in species		Compound	Reference	Mass error (%)	Mass error (ppm)
				HC	PS				
9.39	1181.5133	1182.52806	$C_{58}H_{86}O_{23}S$	X		Unknown		0.000587	5.87
	1181.5016	1182.50693	$C_{61}H_{82}O_{21}S$	X		Unknown; DBE=21		0.000211	-2.11
	1181.5017	1182.51040	$C_{52}H_{87}O_{26}$Sna	X		Unknown; DBE=9		7.41E-05	0.74
9.39	669.2791	670.28704	$C_{29}H_{50}O_{15}S$	X		32-([4-methylphenyl) sulfonyl]oxy)-3,6,9,12,15,18,21,24,27,30-decaoxadotriacontan-1-oic acid; DBE=5	ChemSpider ID: 123961299	1,72E-05	0.17
9.49	367.2159	368.22326	$C_{17}H_{36}O_6S$	X	X	2-[2-(tridecyloxy)ethoxy] ethyl hydrogen sulfate or C13-alkyl-2-ethoxysulfate (C13-AE2S); DBE=0	ChemSpider ID: 2289709 or PubChem CID: 3023544	0.000127	-1.27
9.67	1147.5514	1148.55897	$C_{59}H_{88}O_{20}S$	X		Unknown; DBE=16		2.22E-05	-0.22
	1147.5510	1148.55906	$C_{53}H_{89}O_{25}Na$	X		Unknown; DBE=9 **Note:** Co-elution detected		2.05E-05	0.20
9.75	1181.5153	1182.52806	$C_{58}H_{86}O_{23}S$	X		Unknown		0.000418	4.18
		1182.51040	$C_{52}H_{87}O_{26}SNa$	X		Unknown; DBE=9		0.001077	-10.77
	1181.5050	1182.51280	$C_{54}H_{86}O_{26}S$	X		Scabraside A or 24-dehydroechinoside A or 17-dehydroxyholothurin A (fuscocineroside C) or fuscocineroside B; DBE=12	[14,16,18]	2.12E+06	-0.02
9.71	251.1201	252.13953	$C_{11}H_{24}O_4S$	X		Undecyl hydrogen sulfate or 4(R),8-dimethylnonyl sulfate; DBE=0	[13] ChemSpider ID: 109828 or 34448657 PubChem CID: 24796507	0.004621	46.21
9.75	251.1299	252.13953	$C_{11}H_{24}O_4S$	X				0.000719	7.19
9.84	251.1298	252.13953	$C_{11}H_{24}O_4S$	X	X			0.000759	7.59
9.94	1295.5319	1296.53862	$C_{66}H_{88}O_{24}S$		X	Unknown; DBE=23		8.53E-05	-0.85
10.11	669.2792	670.28704	$C_{29}H_{50}O_{15}S$	X		32-([(4-methylphenyl) sulfonyl]oxy)-3,6,9,12,15,18,21,24,27,30-decaoxadotriacontan-1-oic acid; DBE=5	ChemSpider ID: 123961299	2.24E+06	0.02
10.12	251.1304	252.13953	$C_{11}H_{24}O_4S$		X	Undecyl hydrogen sulfate or (R),8-dimethylnonyl sulfate **Note:** Straight-chain or branched molecule; DBE=0	[13] ChemSpider ID: ID: 109828 or 34448657 PubChem CID: 24796507	0.00052	5.20
10.43	671.2966	672.30269	$C_{29}H_{52}O_{15}S$	X		Unknown; DBE=4		0.000258	-2.58
10.60	1177.5172	1178.52376	$C_{48}H_{90}O_{30}S$	X		Unknown		0.000107	-1.07
	1177.5054	1178.51202	$C_{62}H_{82}O_{20}S$	X	X	Unknown; DBE=22		0.000102	-1.02
	265.1478	266.15518	$C_{12}H_{26}O_4S$	X	X		[19]	0.000168	-1.68

Rt	Observed m/z	Monoisotopic mass	Empirical formula (neutral)	Found in species		Compound	Reference	Mass error (%)	Mass error (ppm)
				HC	PS				
						Lauryl sulfate/dodecyl hydrogen sulfate; DBE=0	ChemSpider ID: 8448 PubMed CID: 8778		
10.60	859.3828	860.38642	$C_{41}H_{64}O_{17}S$	X		Holothurin B (axilogoside A) or Holothurin B4 or Nobiliside B or nobiliside II (ananaside C); DBE=10	[14-18,20] ChemSpider ID: 25052543 PubChem CID: 23674754	0.000489	-4.89
	859.3779	859.37619	$C_{39}H_{64}O_{17}SNa$	X		Unknown; DBE=7		0.117611	-1176.11
10.71	711.2894	712.29775	$C_{52}H_{40}O_3$	X		Unknown; DBE=33		7.38E-05	0.74
		712.29534	$C_{50}H_{41}O_3Na$	X		Unknown; DBE=30		0.000265	-2.65
10.88	713.3018	714.31326	$C_{31}H_{54}O_{16}S$	X		35-((4-Methylphenyl) sulfonyl]oxy)-3,6,9,12,15,18,21,24,27,30,33-un-decaoxapenta-triacontan-1-oic acid; DBE=5	[20] ChemSpider ID: 123961295	0.00051	5.10
10.96	843.3841	844.39151	$C_{41}H_{64}O_{16}S$	X		Holothurin B3 or 24-dehydroechinoside B DBE=10	[14,16,17] ChemSpider ID: 76818153	4.92E-05	-0.49
11.07	655.2979	656.30778	$C_{29}H_{52}O_{14}S$	X		Unknown; DBE=3		0.000314	3.14
11.23	1127.6241	1128.63363	$C_{67}H_{93}O_{11}SNa$		X	Unknown; DBE=21		0.000151	1.51
11.25	613.2875	614.29721	$C_{27}H_{50}O_{13}S$	X		Unknown; DBE=3		0.000307	3.07
11.65	655.2977	656.30778	$C_{29}H_{52}O_{14}S$	X		Unknown; DBE=3		0.000344	3.44
11.76	655.3008	656.30778	$C_{29}H_{52}O_{14}S$	X		Unknown; DBE=3		0.000129	-1.29
11.78	619.2897	620.29613	$C_{34}H_{45}O_9Na$	X	X	Unknown; DBE=12		0.000225	-2.25
12.05	619.2881	620.29613	$C_{34}H_{45}O_9Na$	X	X	Unknown; DBE=12		3.31E-05	0.33

X, compound present; DBE, double bond equivalent
Average percentage error = 0.0061%; Average error (ppm) = 61.4815

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