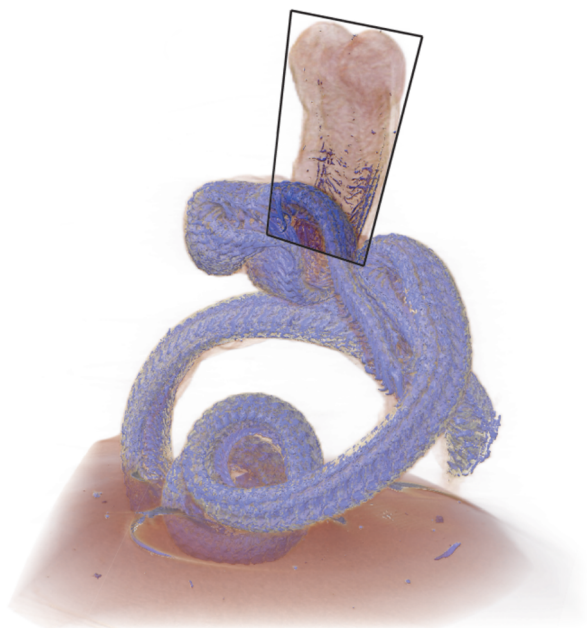
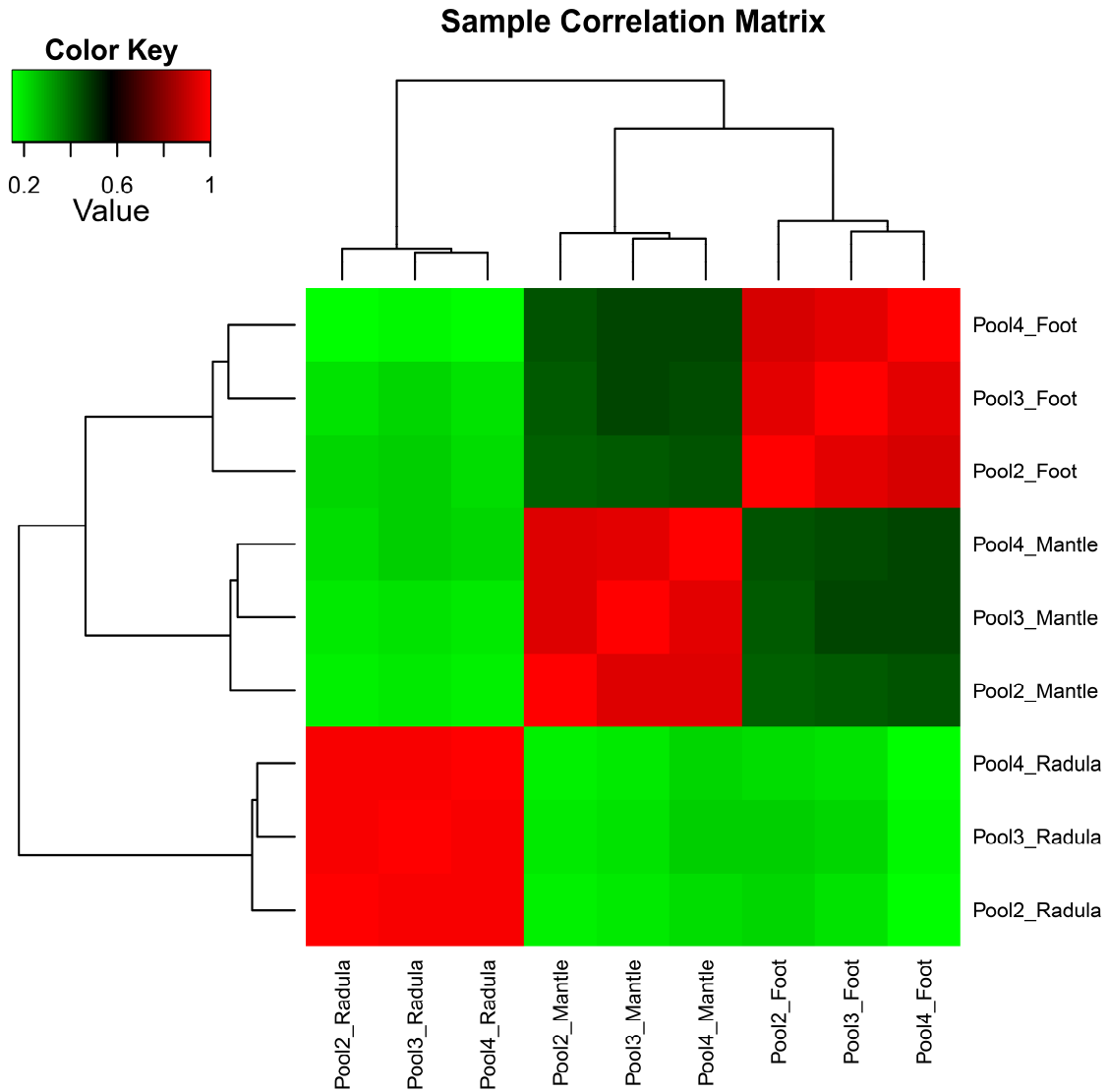


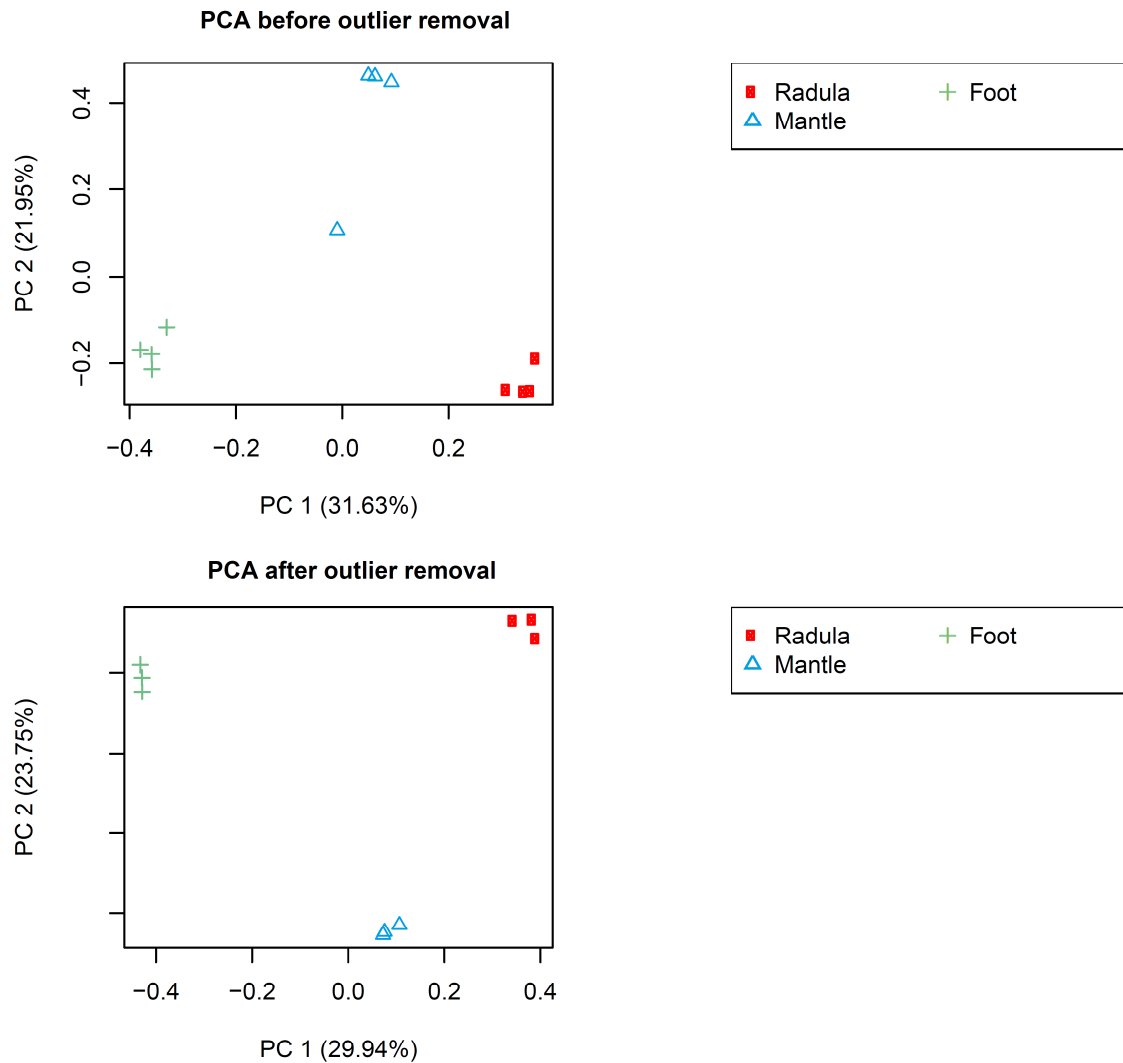
Supplement



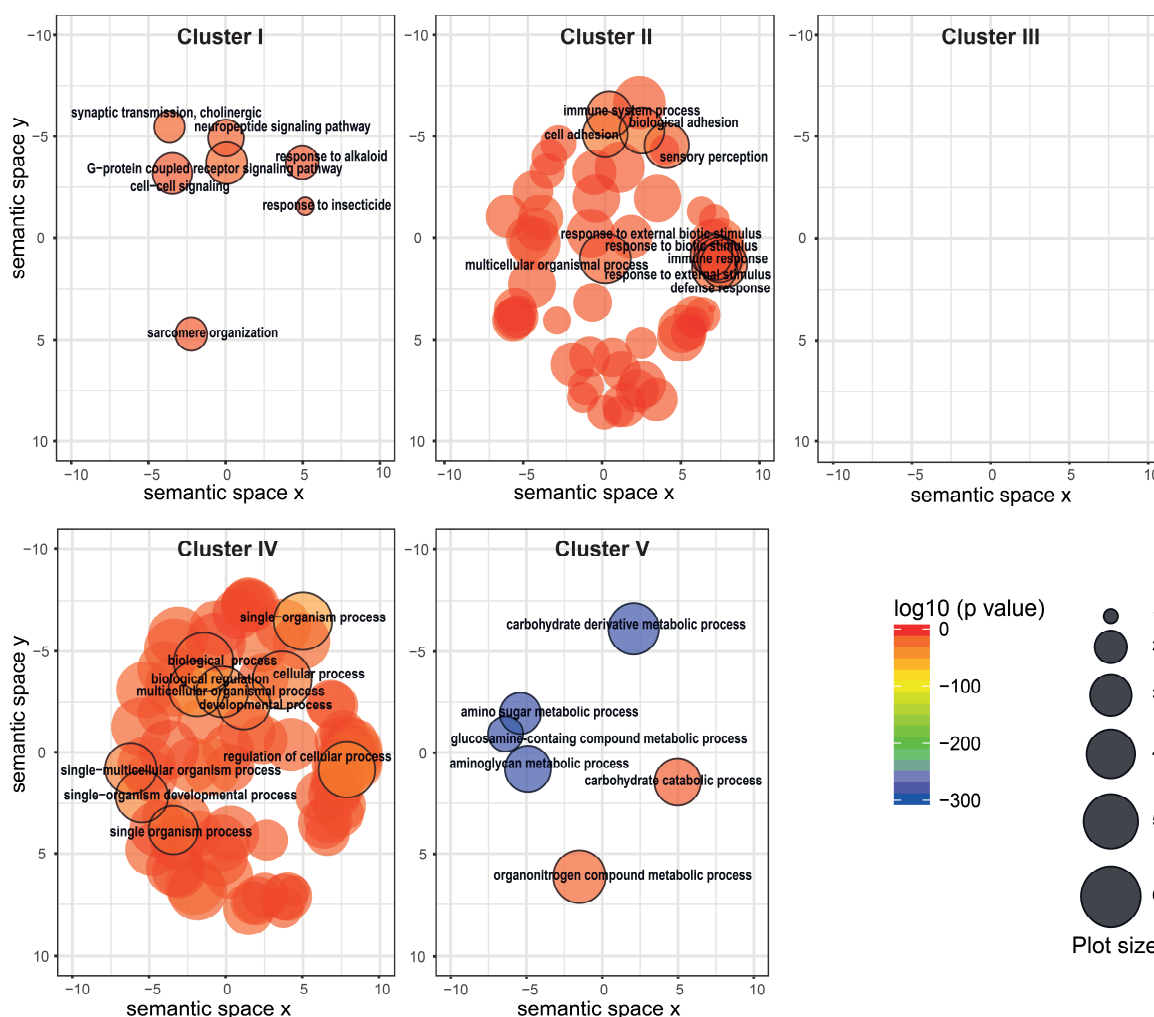
Suppl. figure 1: Micro-CT image of a dissected *Tylomelania* radula. The sampled radula forming tissue is indicated by a black box. Color differences represent differing densities and show starting tooth hardening (blue) by incorporation of minerals in the sampled region.



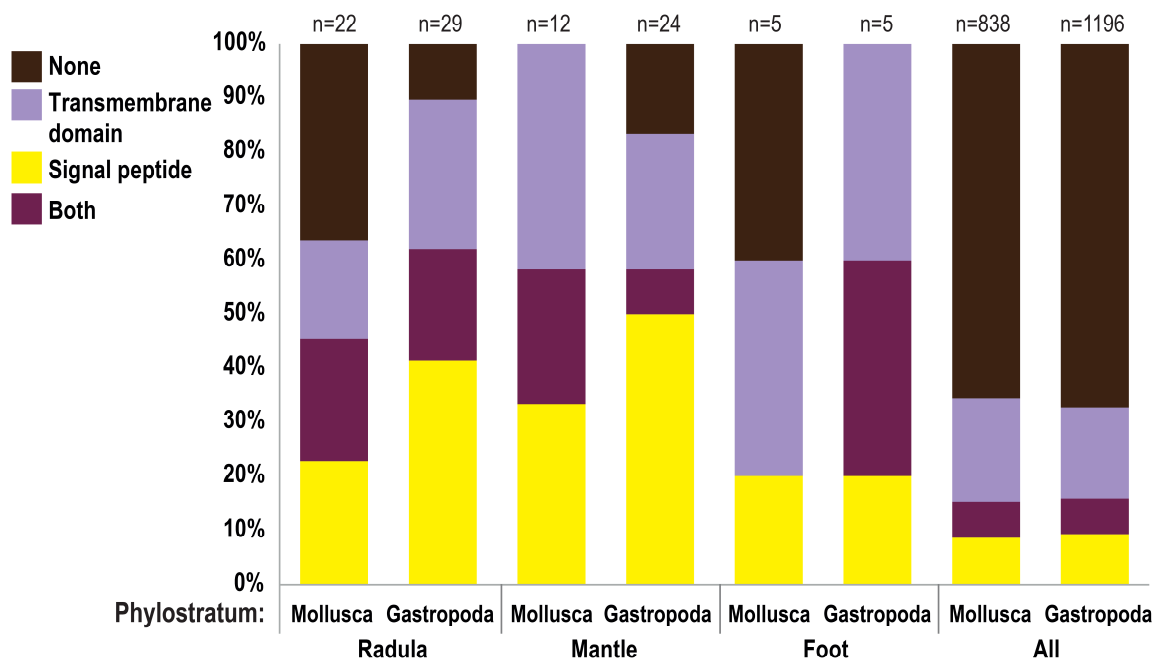
Suppl. figure 2: Heatmap of hierarchically clustered Spearman correlation matrix comparing gene expression values (\log_2 transformed CPM) across samples. Samples with more similar expression cluster together and are colored red in the heatmap.



Suppl. figure 3: Principal component analysis (PCA) of gene expression ($\log_2(\text{counts per million mapped reads})$) before (upper panel) and after (lower panel) pool1 was removed. After outlier removal, the first two principal components comprise 53.7% of the total variance in gene expression and clearly separate the three tissues, while biological replicates cluster tightly together.



Suppl. figure 4: Enriched biological processes after reduction with REVIGO (Supek et al. 2011) in each cluster I-V. More similar processes cluster together in semantic space, whereas dissimilar processes are positioned further apart. Color indicates p-values of each term and plot size indicates the frequency of the respective GO term in the Uniprot database. The ten most significantly enriched processes in each cluster are marked and labeled in the figure. Cluster III did not have any significantly enriched GO-terms.



Suppl. figure 5: Proportion of genes with signal peptides and/or transmembrane domains in genes that were predicted to have arisen either in stem-Mollusca or stem-Gastropoda, for each gene set included in the phylostratigraphic analyses.

Suppl. table 1 Read number before and after quality filtering for each sample

Sample	# mio raw pe reads	# mio quality filtered pe reads
1_Mantle	52.63	45.47
1_Radula	136.52	121.5
1_Foot	44.13	38.51
2_Mantle	37.11	32.82
2_Radula	29.14	26.23
2_Foot	41.69	36.12
3_Mantle	32.5	28.31
3_Radula	41.36	36.96
3_Foot	47.33	41.52
4_Mantle	34.33	29.69
4_Radula	27.21	24.26
4_Foot	37.29	31.92
Sum	561.24	493.31

Suppl. table 2 Enriched GO-terms in each cluster (I-V)

Cluster	GO	description	log10_p_value
Cluster_I	BP		
	GO:0007186	G-protein coupled receptor signaling pathway	-10.0682
	GO:0007271	synaptic transmission. cholinergic	-7.5887
	GO:0007218	neuropeptide signaling pathway	-7.2727
	GO:0017085	response to insecticide	-1.9269
	GO:0043279	response to alkaloid	-1.8358
	GO:0045214	sarcomere organization	-1.8156
	GO:0007267	cell-cell signaling	-1.4234
	CC		
	GO:0005576	extracellular region	-7.0356
	GO:0005578	proteinaceous extracellular matrix	-7.8087
	GO:0034702	ion channel complex	-6.0122
	GO:0045211	postsynaptic membrane	-5.3044
	GO:0005929	cilium	-4.6203
	GO:0043235	receptor complex	-4.3447
	GO:0016459	myosin complex	-3.769
	GO:1990351	transporter complex	-2.9361
	GO:0030016	myofibril	-2.293
	GO:0005581	collagen trimer	-1.467
	MF		
	GO:0005230	extracellular ligand-gated ion channel activity	-14.4207
	GO:0005509	calcium ion binding	-10.5407
	GO:0008146	sulfotransferase activity	-8.3146
	GO:0060089	molecular transducer activity	-5.3044
	GO:0051371	muscle alpha-actinin binding	-3.823
	GO:0042165	neurotransmitter binding	-3.0398
	GO:0017046	peptide hormone binding	-2.5403
	GO:0050656	3'-phosphoadenosine 5'-phosphosulfate binding	-1.9269
	GO:0004871	signal transducer activity	-1.8188
	GO:0016782	transferase activity. transferring sulfur-cont. groups	-1.7983
	GO:0042166	acetylcholine binding	-1.7963
	GO:0070405	ammonium ion binding	-1.6531
	GO:0022803	passive transmembrane transporter activity	-1.5005
Cluster_II	BP		
	GO:0007155	cell adhesion	-21.3206
	GO:0022610	biological adhesion	-21.3206
	GO:0006955	immune response	-11.2393
	GO:0009607	response to biotic stimulus	-10.4684
	GO:0009605	response to external stimulus	-9.7791
	GO:0043207	response to external biotic stimulus	-9.7231
	GO:0006952	defense response	-9.3197
	GO:0002376	immune system process	-8.1671
	GO:0007600	sensory perception	-7.4469
	GO:0032501	multicellular organismal process	-6.7082

GO:1903034	regulation of response to wounding	-6.2318
GO:0006954	inflammatory response	-5.7162
GO:0007186	G-protein coupled receptor signaling pathway	-4.9833
GO:0006835	dicarboxylic acid transport	-4.9555
GO:0072348	sulfur compound transport	-4.8082
GO:0006814	sodium ion transport	-4.4315
GO:0050808	synapse organization	-4.3361
GO:0006811	ion transport	-4.1831
GO:0051704	multi-organism process	-3.3823
GO:1990256	signal clustering	-3.3625
GO:0000281	mitotic cytokinesis	-3.2787
GO:0043154	negative regul. of cysteine-type endopept. activity involved in apopt. proc.	-2.7572
GO:0043271	negative regulation of ion transport	-2.7458
GO:0048583	regulation of response to stimulus	-2.645
GO:0032502	developmental process	-2.5793
GO:0071709	membrane assembly	-2.5033
GO:0007216	G-protein coupled glutamate receptor sign. pathway	-2.4851
GO:0034250	positive regulation of cellular amide metab. process	-2.4851
GO:0051606	detection of stimulus	-2.4851
GO:1902875	regulation of embryonic pattern specification	-2.4075
GO:0009146	purine nucleoside triphosphate catabolic process	-2.3565
GO:0030799	regulation of cyclic nucleotide metabolic process	-2.2764
GO:0040011	locomotion	-2.1869
GO:0050896	response to stimulus	-2.1664
GO:0016201	synaptic target inhibition	-2.1295
GO:0030243	cellulose metabolic process	-2.1239
GO:0042391	regulation of membrane potential	-2.1186
GO:0009215	purine deoxyribonucleoside triphosphate metab. proc.	-2.0364
GO:0043062	extracellular structure organization	-2.0016
GO:0014070	response to organic cyclic compound	-1.9938
GO:0030836	positive regulation of actin filament depolymerization	-1.8444
GO:0007610	behavior	-1.7776
GO:0010813	neuropeptide catabolic process	-1.7776
GO:0010960	magnesium ion homeostasis	-1.7571
GO:0030198	extracellular matrix organization	-1.7064
GO:0035141	medial fin morphogenesis	-1.702
GO:0016266	O-glycan processing	-1.6478
GO:2000257	regulation of protein activation cascade	-1.6199
GO:0009151	purine deoxyribonucleotide metabolic process	-1.6039
GO:0043900	regulation of multi-organism process	-1.579
GO:0031344	regulation of cell projection organization	-1.5482
GO:0051865	protein autoubiquitination	-1.5461
GO:0034248	regulation of cellular amide metabolic process	-1.5461
GO:0006928	movement of cell or subcellular component	-1.5431
GO:0045760	positive regulation of action potential	-1.484
GO:0071286	cellular response to magnesium ion	-1.4834

GO:0071694	maintenance of protein location in extracellular reg.	-1.4213
GO:0035581	sequestering of extracellular ligand from receptor	-1.4213
GO:0071554	cell wall organization or biogenesis	-1.4167
GO:0045745	pos. regul. of G-prot. coup. recept. prot. sign. pathw.	-1.37

CC

GO:0005576	extracellular region	-25.4245
GO:0044459	plasma membrane part	-19.9533
GO:0031224	intrinsic component of membrane	-12.4496
GO:0005887	integral component of plasma membrane	-11.9788
GO:0016021	integral component of membrane	-11.6228
GO:0044425	membrane part	-10.2285
GO:0009986	cell surface	-10.2231
GO:0005615	extracellular space	-9.1398
GO:0098552	side of membrane	-5.9963
GO:0030424	axon	-3.823
GO:0042995	cell projection	-3.5637
GO:0030286	dynein complex	-3.2743
GO:0016020	membrane	-2.8661
GO:0060076	excitatory synapse	-2.7969
GO:0005769	early endosome	-2.1104
GO:0097458	neuron part	-2.0352
GO:0045202	synapse	-1.6525
GO:0019898	extrinsic component of membrane	-1.6202
GO:0045121	membrane raft	-1.4297

MF

GO:0005509	calcium ion binding	-25.4245
GO:0004872	receptor activity	-11.9788
GO:0060089	molecular transducer activity	-9.4912
GO:0022804	active transmembrane transporter activity	-7.039
GO:0004871	signal transducer activity	-6.7615
GO:0030246	carbohydrate binding	-4.4611
GO:0019955	cytokine binding	-3.8561
GO:0030414	peptidase inhibitor activity	-3.7408
GO:0003777	microtubule motor activity	-3.4892
GO:0035373	chondroitin sulfate proteoglycan binding	-3.3625
GO:0005215	transporter activity	-3.0332
GO:0032554	purine deoxyribonucleotide binding	-3.0332
GO:0016793	triphosphoric monoester hydrolase activity	-3.0332
GO:0004222	metalloendopeptidase activity	-3.0332
GO:0070976	TIR domain binding	-3.0332
GO:0008832	dGTPase activity	-3.0332
GO:0003950	NAD+ ADP-ribosyltransferase activity	-2.9914
GO:0003774	motor activity	-2.8983
GO:0001640	adenylate cyclase inhib. G-prot. coup. glut. recep. act.	-2.8983
GO:0035375	zymogen binding	-2.8608
GO:0032552	deoxyribonucleotide binding	-2.6947
GO:0046872	metal ion binding	-2.4851

GO:0005201	extracellular matrix structural constituent	-2.1971
GO:0043169	cation binding	-2.1296
GO:0071813	lipoprotein particle binding	-1.922
GO:0071814	protein-lipid complex binding	-1.922
GO:0030374	ligand-depend. nucl. receptor transcript. coactiv. act.	-1.9167
GO:0005164	tumor necrosis factor receptor binding	-1.8512
GO:0045499	chemorepellent activity	-1.839
GO:0019001	guanyl nucleotide binding	-1.8384
GO:0042806	fucose binding	-1.8119
GO:0005539	glycosaminoglycan binding	-1.7954
GO:0005102	receptor binding	-1.7925
GO:0008046	axon guidance receptor activity	-1.7064
GO:0044325	ion channel binding	-1.702
GO:0008061	chitin binding	-1.6893
GO:0016817	hydrolase activity. acting on acid anhydrides	-1.6701
GO:0036458	hepatocyte growth factor binding	-1.6689
GO:0050839	cell adhesion molecule binding	-1.6555
GO:0016844	strictosidine synthase activity	-1.5329
GO:0030507	spectrin binding	-1.4979
GO:0019838	growth factor binding	-1.4906
GO:0008810	cellulase activity	-1.4669
GO:0000982	transcription factor act.. RNA polym. II core promoter proxim. reg. seq.-spec. bind.	-1.4533
GO:0000978	RNA polymerase II core promoter proximal region seq- specific DNA bind.	-1.3017

Cluster_III BP

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CC

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MF

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Cluster_IV BP

GO:0044699	single-organism process	-48.2305
GO:0008150	biological_process	-44.4164
GO:0044763	single-organism cellular process	-37.0107
GO:0065007	biological regulation	-37.0072
GO:0050794	regulation of cellular process	-34.3454
GO:0032502	developmental process	-30.157
GO:0044767	single-organism developmental process	-29.7403
GO:0032501	multicellular organismal process	-24.5637
GO:0044707	single-multicellular organism process	-24.0866
GO:0009987	cellular process	-22.2215
GO:0048583	regulation of response to stimulus	-22.0585
GO:0006040	amino sugar metabolic process	-20.2349
GO:0009966	regulation of signal transduction	-19.6438
GO:0006030	chitin metabolic process	-19.0634
GO:0006022	aminoglycan metabolic process	-18.6124

GO:0048518	positive regulation of biological process	-17.4789
GO:0048522	positive regulation of cellular process	-15.8071
GO:0051179	localization	-15.7788
GO:0048523	negative regulation of cellular process	-15.0574
GO:0065008	regulation of biological quality	-14.8033
GO:0048519	negative regulation of biological process	-13.828
GO:0007155	cell adhesion	-12.6331
GO:0022610	biological adhesion	-12.6213
GO:0005975	carbohydrate metabolic process	-11.9743
GO:0051128	regulation of cellular component organization	-10.8432
GO:0050896	response to stimulus	-10.7572
GO:0008152	metabolic process	-10.6882
GO:0071704	organic substance metabolic process	-10.6637
GO:1901135	carbohydrate derivative metabolic process	-10.1882
GO:0007610	behavior	-9.8425
GO:0032879	regulation of localization	-9.707
GO:0044708	single-organism behavior	-8.6929
GO:1901564	organonitrogen compound metabolic process	-8.6568
GO:0006793	phosphorus metabolic process	-8.3986
GO:0016192	vesicle-mediated transport	-8.3064
GO:0006486	protein glycosylation	-8.2011
GO:0030029	actin filament-based process	-8.1966
GO:0042127	regulation of cell proliferation	-8.0862
GO:0006928	movement of cell or subcellular component	-8.0381
GO:0044087	regulation of cellular component biogenesis	-7.7857
GO:0006897	endocytosis	-7.7082
GO:0051174	regulation of phosphorus metabolic process	-7.6574
GO:0070085	glycosylation	-7.59
GO:0044238	primary metabolic process	-7.5706
GO:0071840	cellular component organization or biogenesis	-7.5295
GO:0007154	cell communication	-7.4342
GO:0007167	enzyme linked receptor protein signaling pathway	-7.4342
GO:0044723	single-organism carbohydrate metabolic process	-7.3951
GO:0043170	macromolecule metabolic process	-7.3724
GO:0043401	steroid hormone mediated signaling pathway	-7.3061
GO:0042325	regulation of phosphorylation	-7.09
GO:0042221	response to chemical	-6.5571
GO:0071702	organic substance transport	-6.301
GO:0031344	regulation of cell projection organization	-6.265
GO:0040012	regulation of locomotion	-6.251
GO:0006820	anion transport	-6.1724
GO:0065009	regulation of molecular function	-5.9926
GO:0023052	signaling	-5.8967
GO:0007163	establishment or maintenance of cell polarity	-5.8445
GO:0019538	protein metabolic process	-5.8386
GO:0071577	zinc II ion transmembrane transport	-5.6952
GO:0043412	macromolecule modification	-5.6434

GO:0007015	actin filament organization	-5.6292
GO:0051049	regulation of transport	-5.464
GO:0050790	regulation of catalytic activity	-5.4342
GO:0019725	cellular homeostasis	-5.3703
GO:0006357	regul. of transcription from RNA polymerase II prom.	-5.308
GO:0044711	single-organism biosynthetic process	-5.0282
GO:0044710	single-organism metabolic process	-5.0255
GO:0044267	cellular protein metabolic process	-4.9891
GO:0007267	cell-cell signaling	-4.8669
GO:0010941	regulation of cell death	-4.5464
GO:0007043	cell-cell junction assembly	-4.5016
GO:0040011	locomotion	-4.408
GO:0046777	protein autophosphorylation	-4.3617
GO:0002682	regulation of immune system process	-4.3108
GO:0008610	lipid biosynthetic process	-3.8884
GO:0044237	cellular metabolic process	-3.7603
GO:0040007	growth	-3.7436
GO:0051246	regulation of protein metabolic process	-3.6976
GO:0034330	cell junction organization	-3.4278
GO:0006629	lipid metabolic process	-3.4154
GO:0002376	immune system process	-3.196
GO:1901566	organonitrogen compound biosynthetic process	-2.9635
GO:1901565	organonitrogen compound catabolic process	-2.706
GO:0001775	cell activation	-2.6595
GO:0006807	nitrogen compound metabolic process	-2.5858
GO:0015849	organic acid transport	-2.5749

CC

GO:0005575	cellular_component	-45.5629
GO:0044425	membrane part	-39.583
GO:0031224	intrinsic component of membrane	-39.2993
GO:0016021	integral component of membrane	-38.3021
GO:0016020	membrane	-37.4947
GO:0044464	cell part	-31.5791
GO:0044424	intracellular part	-19.5359
GO:0044459	plasma membrane part	-19.4488
GO:0044421	extracellular region part	-14.2673
GO:0043226	organelle	-13.8104
GO:0043227	membrane-bounded organelle	-13.8104
GO:0005737	cytoplasm	-12.9972
GO:0044444	cytoplasmic part	-12.896
GO:0098588	bounding membrane of organelle	-11.7511
GO:0031982	vesicle	-11.3514
GO:0031410	cytoplasmic vesicle	-11.0342
GO:0005887	integral component of plasma membrane	-10.9588
GO:0005576	extracellular region	-10.4625
GO:0044432	endoplasmic reticulum part	-10.2546
GO:0097458	neuron part	-9.7475

GO:0030054	cell junction	-9.6303
GO:0005911	cell-cell junction	-9.4747
GO:0098589	membrane region	-9.1684
GO:0042995	cell projection	-7.7677
GO:0032579	apical lamina of hyaline layer	-6.0412
GO:0065010	extracellular membrane-bounded organelle	-5.1797
GO:0043005	neuron projection	-4.8827
GO:0009986	cell surface	-4.6241
GO:0031941	filamentous actin	-4.4645
GO:0005793	endoplasmic reticulum-Golgi intermediate compartm.	-3.9722
GO:0014069	postsynaptic density	-3.5291
GO:0005938	cell cortex	-3.4248
GO:0005903	brush border	-3.3362
GO:0031673	H zone	-3.196
GO:0048471	perinuclear region of cytoplasm	-2.5795
GO:0005856	cytoskeleton	-2.55
GO:0044297	cell body	-2.2302
GO:0005829	cytosol	-1.9551
GO:0090533	cation-transporting ATPase complex	-1.7277
GO:0031974	membrane-enclosed lumen	-1.6734
GO:0036464	cytoplasmic ribonucleoprotein granule	-1.3104

MF

GO:0003674	molecular_function	-45.6695
GO:0005488	binding	-37.8713
GO:0005515	protein binding	-29.3475
GO:0043167	ion binding	-20.1619
GO:0008061	chitin binding	-17.2657
GO:0043169	cation binding	-14.8412
GO:0005509	calcium ion binding	-14.1365
GO:0003824	catalytic activity	-13.3911
GO:0097367	carbohydrate derivative binding	-12.9811
GO:0008092	cytoskeletal protein binding	-12.2158
GO:0060089	molecular transducer activity	-11.1264
GO:0004871	signal transducer activity	-9.4747
GO:0005215	transporter activity	-9.2466
GO:0043565	sequence-specific DNA binding	-8.1125
GO:0043168	anion binding	-7.9367
GO:0004872	receptor activity	-7.6189
GO:0016787	hydrolase activity	-6.9276
GO:0016773	phosphotransferase activity. alcohol group as acceptor	-6.6562
GO:0016740	transferase activity	-6.6251
GO:0005088	Ras guanyl-nucleotide exchange factor activity	-6.534
GO:0035639	purine ribonucleoside triphosphate binding	-6.4143
GO:0008194	UDP-glycosyltransferase activity	-5.9971
GO:0003677	DNA binding	-5.7518
GO:0022891	substrate-specific transmembrane transporter activity	-5.669
GO:0044877	macromolecular complex binding	-5.4819

GO:0097159	organic cyclic compound binding	-5.219
GO:0016772	transferase act.. transferr. phosphorus-cont. groups	-5.1799
GO:0001071	nucleic acid binding transcription factor activity	-5.1004
GO:0003700	transcription factor activity. seq.-specific DNA binding	-5.1004
GO:0016757	transferase activity. transferring glycosyl groups	-4.6932
GO:1901363	heterocyclic compound binding	-4.3385
GO:0016798	hydrolase activity. acting on glycosyl bonds	-4.0931
GO:0036094	small molecule binding	-4.0114
GO:0004553	hydrolase activity. hydrolyzing O-glycosyl compounds	-3.8945
GO:0030246	carbohydrate binding	-3.4497
GO:0005112	Notch binding	-3.3583
GO:1901265	nucleoside phosphate binding	-3.145
GO:0003756	protein disulfide isomerase activity	-2.9416
GO:0044212	transcription regulatory region DNA binding	-2.5412
GO:0016853	isomerase activity	-2.5281
GO:0001067	regulatory region nucleic acid binding	-2.4864
GO:0008289	lipid binding	-2.4631
GO:0005516	calmodulin binding	-2.4202
GO:0004713	protein tyrosine kinase activity	-2.3121
GO:0000146	microfilament motor activity	-2.2634
GO:0050839	cell adhesion molecule binding	-2.2036
GO:0005057	signal transducer activity. downstream of receptor	-2.0945
GO:0008237	metallopeptidase activity	-2.0812
GO:0004528	phosphodiesterase I activity	-2.0787
GO:0035091	phosphatidylinositol binding	-2.0545
GO:0030898	actin-dependent ATPase activity	-1.9854
GO:0042578	phosphoric ester hydrolase activity	-1.8678
GO:0045296	cadherin binding	-1.833
GO:0004715	non-membrane spanning prot. tyrosine kinase activity	-1.6435
GO:0004702	signal transducer. downstream of receptor. with serine/threonine kinase activity	-1.5699
GO:0008743	L-threonine 3-dehydrogenase activity	-1.5054
GO:0030742	GTP-dependent protein binding	-1.4794
GO:0030156	benzodiazepine receptor binding	-1.4404
GO:0070064	proline-rich region binding	-1.4402
GO:0003924	GTPase activity	-1.3473
GO:0008270	zinc ion binding	-1.3447
GO:0033882	choloyl-CoA hydrolase activity	-1.3276
GO:0016307	phosphatidylinositol phosphate kinase activity	-1.3095

Cluster_V BP

GO:0006040	amino sugar metabolic process	-300
GO:1901135	carbohydrate derivative metabolic process	-300
GO:1901071	glucosamine-containing compound metabolic process	-300
GO:0006022	aminoglycan metabolic process	-300
GO:1901564	organonitrogen compound metabolic process	-6.4946
GO:0016052	carbohydrate catabolic process	-3.3102

CC

GO:0005576	extracellular region	-300
MF		
GO:0008061	chitin binding	-300
GO:0004553	hydrolase activity. hydrolyzing O-glycosyl compounds	-2.7574
GO:0016798	hydrolase activity. acting on glycosyl bonds	-2.4562
GO:0097367	carbohydrate derivative binding	-2.2066

Suppl. table 3 Combination of protein families found in genes with radula-specific expression that were predicted to have originated in stem-Mollusca

Gene number	Pfam_ids	Pfams_descriptipion
12	None	None
3	PF01607.21	Chitin binding Peritrophin-A domain
2	PF00008.24; PF12661.4; PF01414.16;	EGF-like domain; Human growth factor-like EGF; Delta serrate ligand
1	PF01607.21; PF01607.21; PF01607.21; PF01607.21	Chitin binding Peritrophin-A domain; Chitin binding Peritrophin-A domain; Chitin binding Peritrophin-A domain; Chitin binding Peritrophin-A domain
1	PF14854.3	Leucine rich adaptor protein
1	PF06535.9	Repulsive guidance molecule (RGM) N-terminus
1	PF00386.18	C1q domain
1	PF15788.2	Domain of unknown function