Electronic Supplementary Material (ESI) for Metallomics.

Supplementary Material for

Metallomics reveal a persisting impact of cadmium on the evolution of metal-selective snail metallothioneins

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† deceased. We want to dedicate this work to our longtime cooperation partner, Silvia Atrian

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References

Table S1: PCR conditions for the neutral markers COI and 18SrDNA: a) Thermal cycles followed [1] with the annealing temperatures adjusted according to the particular primers, samples, and polymerase used. b) PCR mix for MyTaq Polymerase c) PCR mix for Rotorgene probe-PCR for samples which did not amplify with MyTaq Polymerase

a) Thermal cycles:	**step**	**temperature**	**time**	**cycles**
	Intitial step	95°C	4 min	1x
-	denaturation per cycle	95°C	30 s	38x
	annealing per cycle	50 - 65°C	45 s	
	elongation per cycle	72°C	150 s	
	final step	72°C	10 min	1x
b) PCR mix for MyTaq Polymerase	**reagent**	**volume (µl)**		
	$\mathrm{dH_2O}$	5.55		
	5x PCR Buffer	2.00		
	BSA 100µg/µl (final conc. 10µg/µl)	1.00		
	each primer (final conc. 0.2µM)	0.20		
	MyTaq polymerase Bioline	0.05		
	template DNA	1.00		
c) PCR mix for Rotorgene probe-PCR	**reagent**	**volume (µl)**		
	$\mathrm{dH_2O}$	3.6		
	2x Rotorgene Mastermix Qiagen	5.00		
	each primer (final conc. 0.2µM)	0.20		
	template DNA	1.00		

Table S2 – Lists of primers and cycling parameters applied for PCR confirmation of MT sequences

2A: Description of applied gene-specific primers used for Long Distance PCR

Species	***MT Isoform***	***Primer***	***Sequence 5'-3'***	***Tm [°C]***	***Tm_{An} [°C]***
Neritina pulligera	MT1	Fw	TCA TCG TAC TCA CCA CTC CA	58.4	57
		Rev	TGG ACA AGG GGG TAA ACG A	57.5	
	MT2	Fw	CAG TGT CGT GTG CAG AGA GA	60.5	58
		Rev	CAG TCC AGA ATG TCT TCT TCC	59.5	
Deroceras reticulatum	CdMT	Fw	GGC TTA AAC GAG GAC CTG	56.3	54
		Rev	AGC TTG ATT ATT TGT CAG GTA C	56.4	
	CdMT2	Fw	CAG TCC AGA ATG TCT TCT TCC	59.5	54.5
		Rev	CTT GAA TCT TGA GAG TGG CA	56.4	
Marisa cornuarietis	MT1	Fw	CGT GTT GTT GTG GTT GTT	51.4	49.5
		Rev	GCA CAT ACA GAC AGG AAT	51.4	
	MT2	Fw	GTC GAC AAC CGA ACC ATC CT	60.5	58.5
		Rev	GCA GAC ATC GTG TCA CTT GC	60.5	
Anentome helena	MT	Fw	TCA CTT TTC CGT GAC TTG CT	56.4	54.5
		Rev	AAA GGC TTA CAA GGC GGG G	59.5	
Aplysia californica	MT	Fw	CGT ACG TCA CCA CCA GAG AT	60.5	54
		Rev	AAT GCT CTC CGC ACG CTA	56.3	
Alinda biplicata	MT1	Fw	GTC AAG TTT ACG ATC TGC G	52.9	52
		Rev	TCA ATC CCA TCC AAC CAA C	52.9	
	MT2	Fw	AGG TCA AGT TTA CGA TCT GCG T	60.1	58
		Rev	CCT AAC CAC ACA ACT CCC GT	60.5	
	9md-MT	Fw	TCC TCG TAC CGT ATC TCA GC	60.5	58
		Rev	ACG CTC AAT GTA GTC TTA TGT TGC	62.0	
	10Md-MT	Fw	TCC TCG TAC CGT ATC TCA GC	60.5	58
		Rev	ACG CTC AAT GTA GTC TTA TGT TGC	62.0	
Patella vulgata	MT1	Fw	GAC GCA CAC AAT CAG A	48.1	47
		Rev	AGC CAT TGC ACG AGA C	50.6	
	MT2	Fw	AGT AAG ACA CCC ACA GTC AG	58.4	53
		Rev	ACG GGG ATA GAA GAC ACA A	55.0	
Lottia gigantea	MT1	Fw	CTT CTC AAA GCT TCT ACA GAA T	56.4	53
		Rev	CAA GAT TGT GTG CAT GAT GAA	55.4	
	MT2	Fw	CCG CAG AGT ACA GCA ATT CAT A	60.1	55.5
		Rev	GGT ATG TGA GTC AAC AAA GAG	57.5	

2B: Applied cycling conditions, using the Advantage 2 polymerase PCR system (see Material and Methods).

	Temp [°C]	***Time***	***Cycles***
Denaturation	95	1 min	1
Denaturation	95	30 sec	30
Annealing	See table 1	30 sec	
Extension	68	40 sec – 1 min	
Final Extension	68	5 min	1

Table S3: List of gastropod Metallothionein sequences applied for the present study. Gastropod clades and major lineages (first column) with family names (second column), species names with consecutive numbers (bold blue letter fond in square brackets) (third column), MT designations (fourth column), sequence data source and databank deposition (fifth column), PCR confirmation (sixth column), kind of metal selectivity (seventh column), reference numbers (eighth column) and consecutive sequence numbers (ninth column) are shown. MT sequences with red consecutive numbers (last column) were sequenced *de novo* and are published in the present study for the first time. All other MT sequences (last column, black consecutive numbers) are reported from previous studies (see references) or were identified from publically available databases (see material and methods). Metal selectivity features (Cd – red; Cu – blue; yellow - unspecific) of single MTs (seventh column) were either directly determined by MS methods (⚐), derived from other kinds of experimental evidence combined with sequence homology (●), or only deduced from sequence homology by alignment with related sequences of known metal-binding character (no symbol).

Clade	***Family***	***Species*** **[Species Number]**	***MT Name***	***Data source and Acc. No.***	***PCR-confirmed***	***Kind of Metal selectivity***	***References***	***Sequence No.***
Patellogastropoda	*Lottiidae*	*Lottia gigantea* **[1]**	MT1	GenBank MK770430	yes	Cd ⚐		**1**
			MT2	GenBank MK770431	yes	Cd ⚐		**2**
	Nacellidae	*Nacella polaris* **[2]**	MT	SRA; SRR2061227	no	Cd		3
	Patellidae	*Patella vulgata* **[3]**	MT1	GenBank MK443472	yes	Cd		**4**
			MT2	GenBank MK443472	yes	Cd		**5**
Vetigastropoda	*Fissurellidae*	*Megathura crenulata* **[4]**	MT	GenBank AY102647.1	-	Unspecific ⚐	[2,3]	6
	Haliotidae	*Haliotis diversicolor* **[5]**	MT	GenBank EU071824.1	-	Unspecific		7
		Haliotis discus hannai **[6]**	MT	GenBank KT895222.1	-	Unspecific		8
		Haliotis tuberculata **[7]**	MT	GenBank GEAU01019532.1	no	Unspecific		9
		Haliotis laevigata **[8]**	MT	GenBank GFTT01185788.1	no	Unspecific		10
	Tegulidae	*Tegula atra* **[9]**	MT	GenBank GFBI01039752.1	no	Unspecific		11
Neritimorpha	*Neritidae*	*Nerita peloronta* **[10]**	MT1	SRA; SRX644686	no	Unknown		12
			MT2	SRA; SRX644686	no	Unknown		13
		Neritina pulligera **[11]**	MT1	GenBank MK577683	yes	Unknown		**14**
			MT2	GenBank MK577684	yes	Unknown		**15**
	Titiscaniidae	*Titiscania limacina* **[12]**	MT	SRA; SRX644702	no	Unknown		16

Caenogastropoda	*Littorinidae*	*Littorina littorea* **[13]**	MT	GenBank AY034179.1	-	Cd ⚐	[3,4]	17
	Pomatiidae	*Pomatias elegans* **[14]**	MT1	GenBank KY636305.1	-	Cd ●	[3]	18
			MT2	GenBank KY646306.1	-	Cd ●	[3]	19
	Nassariidae	*Tritia obsoleta* **[15]**	MT	GenBank FK716482.1	no	Cd	[3]	20
	Calyptraeidae	*Bostrycapulus sp.* **[16]**	MT	SRA; SRP042651	no	Unknown		21
		Crepidula fornicata **[17]**	MT	SRA; SRP042651	no	Unknown		22
	Ampullariidae	*Pomacea bridgesii* **[18]**	MT1	GenBank KY963504.1	-	Unspecific ⚐		23
			MT2	GenBank KY963505.1	-	Unspecific ⚐		24
		Pomacea canaliculata **[19]**	MT20-III like variant X-1 like	GenBank XM_025255587.1	-	Unspecific		25
			MT20-III like variant X-2 like	GenBank XM_025255589.1	-	Unspecific		26
			MT20-III like variant X-3 like	GenBank XM_025255590.1	-	Unspecific		27
		Marisa cornuarietis **[20]**	MT1	GenBank MK577687	yes	Unspecific		**28**
			8md-MT	GenBank MK577688	yes	Unspecific		**29**
	Buccinidae	*Anentome helena* **[21]**	MT	GenBank MK577685	yes	Unspecific		**30**
Heterobranchia	*Aplysiidae*	*Aplysia californica* **[22]**	MT	GenBank MK577686	yes	Unknown		**31**
	Clionidae	*Clione limacina* **[23]**	MT	GenBank GESV01103158.1	no	Unknown		32
	Limacinidae	*Limacina retroversa* **[24]**	MT	GenBank GBXC01058817.1	no	Unknown		33

Heterobranchia ***Panpulmonata*** ***Hygrophila***	*Plakobranchidae*	*Elysia crispata* [25]	MT	GenBank KY963503	-	Unknown		**34**
		Elysia cornigera [26]	MT	GenBank GBRW01068275.1	no	Unknown		35
	Physidae	*Physella acuta* [27]	MT1	GenBank GU259686.1	-	Unspecific	5	36
			MT2	SRA; SRP042651	no	Unspecific		37
		Physella carolinae [28]	MT	SRA; SRP042651	no	Unspecific		38
		Physella hendersoni [29]	MT	SRA; SRP042651	no	Unspecific		39
		Physella gyrina [30]	MT	SRA; SRP042651	no	Unspecific		40
	Lymnaeidae	*Galba cubensis* [31]	MT	SRA; SRP042651	no	Unspecific		41
		Galba truncolata [32]	MT	SRA; SRP042651	no	Unspecific		42
		Lymnaea stagnalis [33]	MT	GenBank FX201894.1	yes	Unspecific ●		43
	Planorbidae	*Biomphalaria glabrata* [34]	MT + genomic variants	GenBank KY963495.1	-	Unspecific ⚐	5,6	44
Panpulmonata ***Stylommatophora***	*Agriolimacidae*	*Deroceras reticulatum* [35]	CdMT1	GenBank MK672823	yes	Cd		**45**
			CdMT2	eSnail database CL8321.Contig1	yes	Cd		46
	Arionidae	*Arion vulgaris* [36]	AvMT1	GenBank MF155618.1	-	Cd ⚐	7	47
			AvMT2	GenBank MF155619.1 MF155620.1	-	Cu ⚐	7	48
	Limacidae	*Lehmannia nyctelia* [37]	CdMT	eSnail database CL1241.Contig1	no	Cd		49
		Limax maximus [38]	CdMT	GenBank MH574544	-	Cd		50
Panpulmonata	*Clausiliidae*	*Alinda biplicata* [39]	CuMT1a	GenBank MK648138	yes	Cu		**51**

Stylommatophora			CuMT1b	SRX7671047	no	Cu		**52**
			CuMT2	GenBank MK639793	yes	Cu		**53**
			9md-MT	GenBank MK648139	yes	Cd		**54**
			10md-MT	GenBank MK648140	yes	Cd		**55**
	Cochlicellidae	*Cochlicella acuta* **[40]**	CdMT	eSnail database Unigene52262	no	Cd	7	56
			CuMT	eSnail database Unigene18701	no	Cu	7	57
			CdCuMT	eSnail database Unigene65576	no	Unspecific	7	58
	Helicidae	*Arianta arbustorum* **[41]**	CdMT (MTa)	Uniprot P55946	-	Cd ●		59
		Cornu aspersum **[42]**	CdMT	GenBank EF152281.1	-	Cd ●		60
			CuMT	GenBank EF178297.2	-	Cu ●		61
			CdCuMT	GenBank EF206312.1	-	Unspecific ●	8,9	62
		Cepaea hortensis **[43]**	CdMTV1	GenBank MH574545	-	Cd ●	7	63
			CdMTV2	GenBank MH574546	-	Cd ●	7	64
			CdCuMT	GenBank MH574547	-	Unspecific ●	7	65
		Cepaea nemoralis **[44]**	CdMT	GenBank GFLU01086707.1	no	Cd		66
			CuMT	GenBank GFLU01065470.1	no	Cu		67
			CdCuMT	GenBank GFLU01003885.1	no	Unspecific		68
		Helix pomatia **[45]**	CdMT	GenBank AF399740.1	-	Cd ⚐	10–12	69
			CuMT	GenBank AF399741.1	-	Cu ⚐	10–12	70
			CdCuMT1	GenBank KY420172.1	-	Unspecific ⚐		71
			CdCuMT2	GenBank KY420171.1	-	Unspecific ⚐		72
		Theba pisana **[46]**	MT	eSnail database CL8321.Contig1	no	Cu		73
	Helicoidea	*Nesiohelix samarangae* **[47]**	CdMT (MT)	GenBank EU437399.1	-	Cd		74

Table S4: Distance analysis with Identity and Similarity values (Means and Standard Deviations) of N-terminal versus C-terminal domains of Cd-selective MTs of Patellogastropoda, Caenogastropoda and Heterobranchia with the same sequences as adopted in **Fig. 3**, and for unspecific gastropod MTs**.** For N-terminal domains, calculation was performed without the N-terminal extension up to the first Cys position. For C-terminal domains, the linker region was included. Note that both similarity and identity scores are significantly higher for C-terminal domains. This supports the hypothesis of a higher degree of conservation for C-terminal domains of gastropod Cd-selective MTs. Calculations were performed using the openly accessible program package SIAS (Sequence Identity And Similarity) of the Immunomedicine group of the Universidad Complutense Madrid (http://imed.med.ucm.es/Tools/sias.html).

S4A: Cd-selective MTs

	N-terminal Domain	**C-terminal Domain**	**Difference (mean)**
Identity:			
All Cd-selective MTs	53.47% (± 20.78%)	66.17% (± 12.02%)	-12.70%
Patellogastropoda versus Littorinoidea	34.68% (± 3.07%)	66.56% (± 5.89%)	-31.88%
Patellogastropoda versus Stylommatophora	31.62% (± 2.69%)	59.10% (± 6.19%)	-27.47%
Littorinoidea versus Stylommatophora	47.49% (± 4.35%)	62.79% (± 5.97%)	-15.30%
Similarity:			
All Cd-selective MTs	58.01% (± 20.05)	71.47 (± 11.36%)	-13.46%
Patellogastropoda versus Littorinoidea	36.97% (± 3.82%)	69.31% (± 5.31%)	-32.34%
Patellogastropoda versus Stylommatophora	36.39% (± 2.99%)	63.17% (± 7.82%)	-26.78%
Littorinoidea versus Stylommatophora	54.70% (± 4.37%)	66.86% (± 7.64%)	-12.16%

S4B: Unspecific MTs

	N-terminal Domain	**C-terminal Domain**	**Difference (mean)**
Identity:			
All unspecific MTs	61.91% (± 16.11%)	59.10% (± 15.30%)	+2.80%
Vetigastropoda versus Ampullariidae	57.64% (± 5.43%)	55.86% (± 7.94%)	+1.78%
Vetigastropoda versus Hygrophila	49.36% (± 7.44%)	51.85% (± 8.33%)	-2.49%
Ampullariidae versus Hygrophila	52.75% (± 5.92%)	54.54% (± 5.63%)	-1.79%
Similarity:			
All unspecific MTs	67.087% (± 14.63%)	63.60% (± 14.60%)	+3.48%
Vetigastropoda versus Ampullariidae	62.35% (± 5.55%)	63.06% (± 7.54%)	-0.71%
Vetigastropoda versus Hygrophila	55.02% (± 7.87%)	56.14% (± 8.90%)	-1.12%
Ampullariidae versus Hygrophila	60.24% (± 7.30%)	60.03% (± 8.41%)	+0.21%

Figure S1:
Metal-binding domain organization and amino acid sequence alignment of Cu-selective (CuMTs, (framed in blue) and unspecific MT isoforms (called CdCuMTs, framed in green) from terrestrial snail and slug species of the order of Stylommatophora (clade Heterobranchia). Cys positions are underlaid in pink, conserved non-Cys positions through sequences of all species are underlaid in blue. Identical amino acid positions between pairwise aligned sequences are indicated by black stars. Domain boundaries of the N-terminal and the C-terminal domains (designated above the alignments as N and C in red letters) are symbolized by perpendicular bold red lines. The linker region between the N and the C-terminal domains are designated in black letters, their boundaries are symbolized by perpendicular dotted black lines. The gaps between the two domains indicate the lack of a second N-terminal domain (present in other gastropod MTs). MTs of species shown in red letters were sequenced for this study *de novo* and are published here for the first time. Sequences of all other species (in black letters) were identified from publically available publications or databases. Species for which metal selectivity features of respective MTs were documented experimentally by us through MS or NMR methods elsewhere are framed in blue. The bold red arrow on the right hand of the alignments points to sequence similarities between Cu-selective and unspecific MTs.

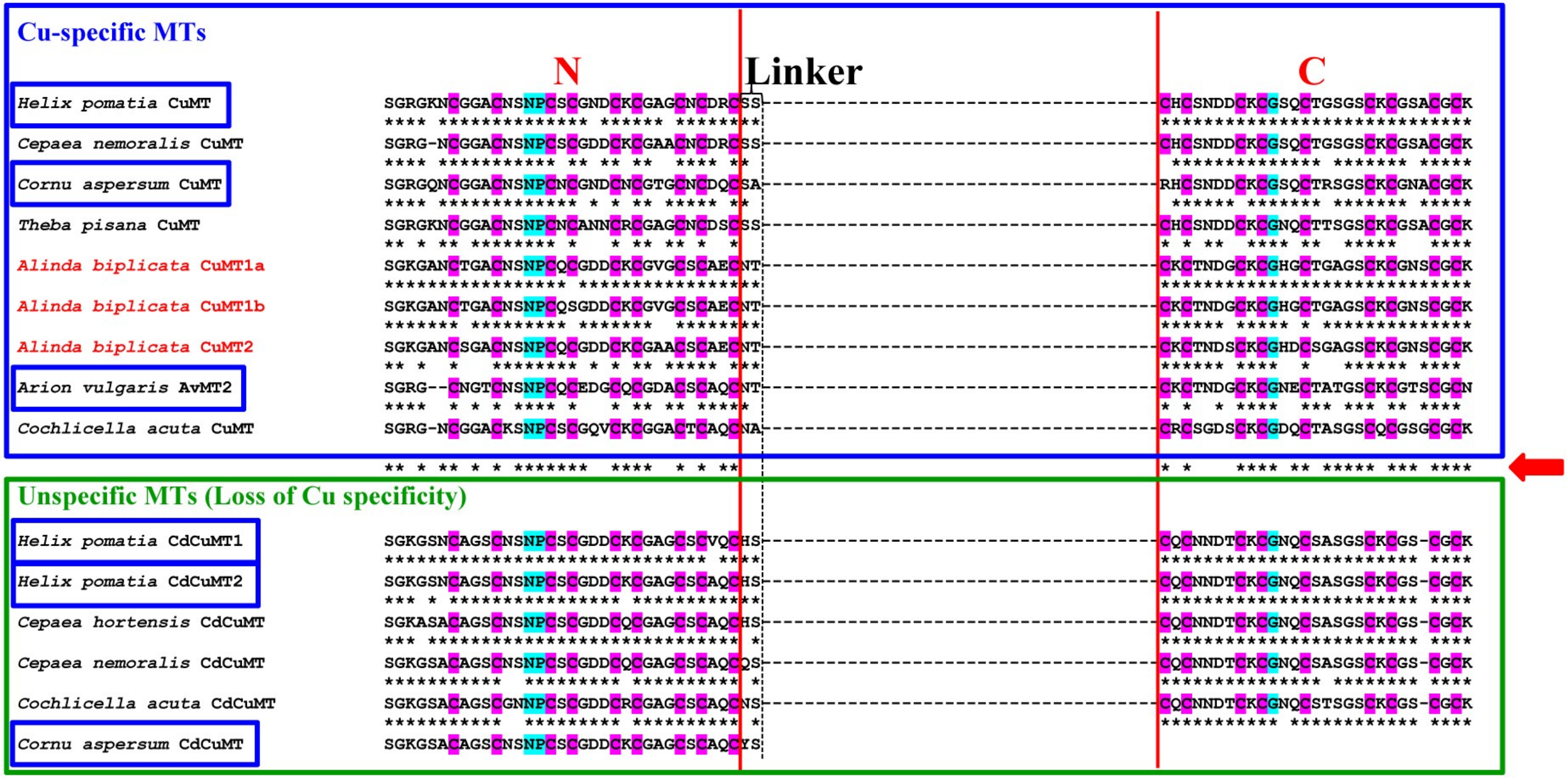


Figure S2:
Cu-selective MTs from terrestrial snails *Helix pomatia* and *Cornu aspersum* of the order of Stylommatophora (Heterobranchia). ESI-MS spectra of MT species recombinantly produced in different meal-enriched media (Cd, Zn and Cu) at the corresponding charge state (as indicated in the upper right corner) or deconvoluted. Spectra are re-drawn from data reported in [11] and [9].

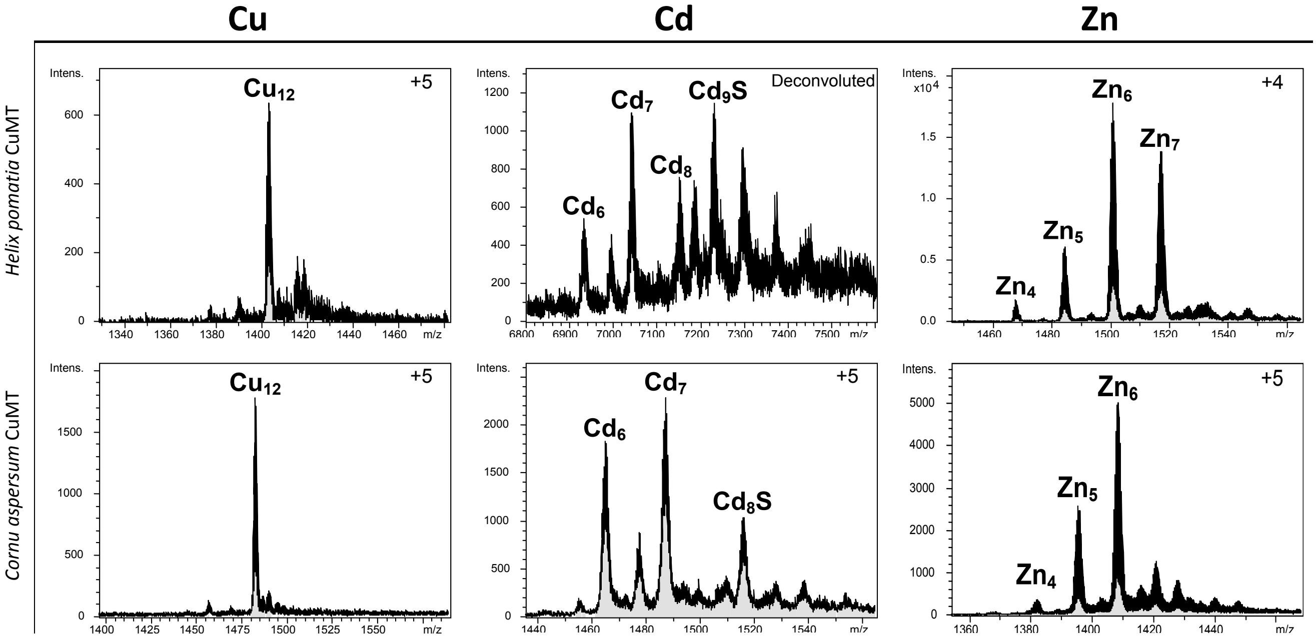
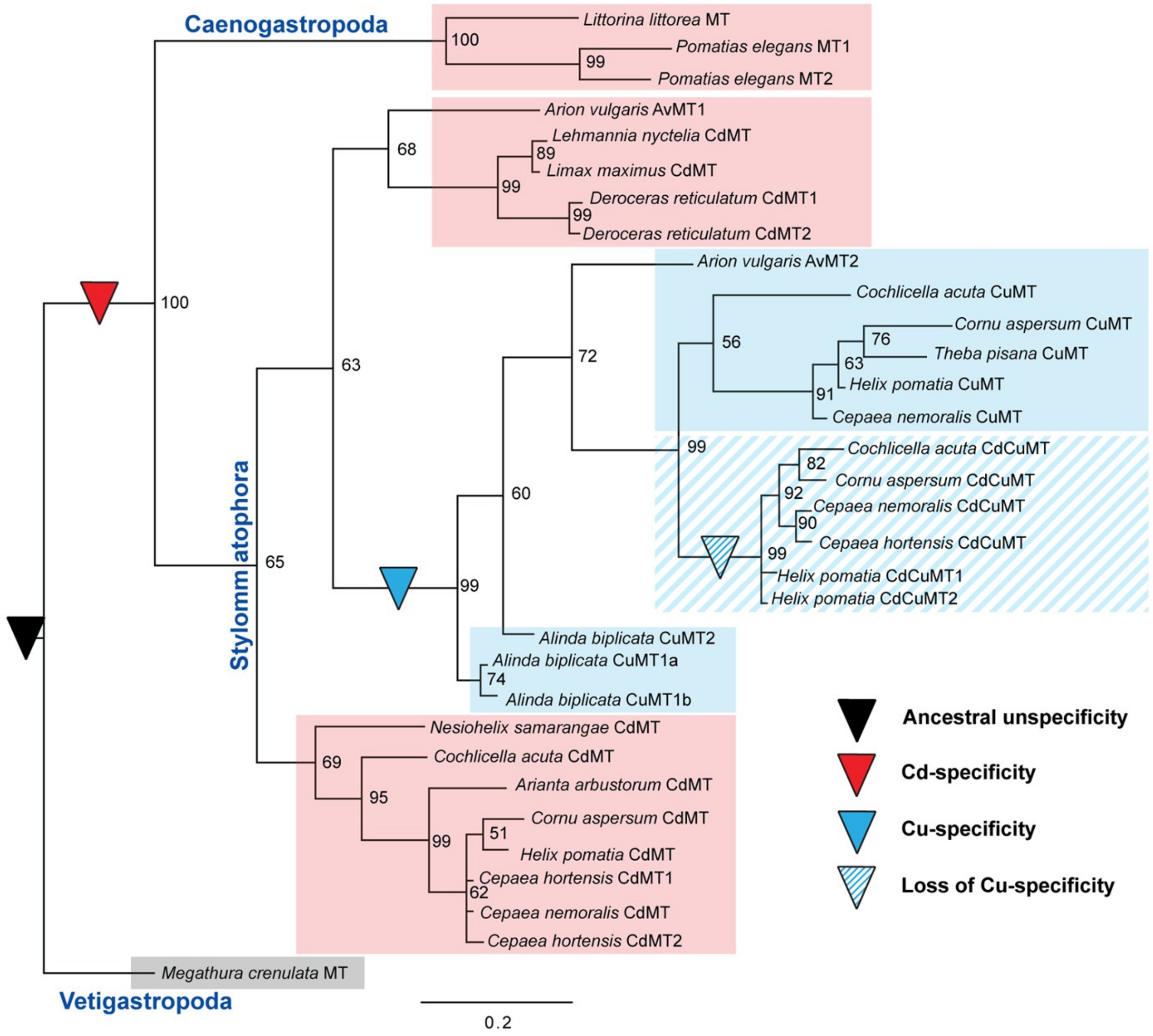


Figure S3:
Bayesian Inference tree with bootstrap support values of metal selectivity features in MTs of the clade of Caenogastropoda and in the order of Stylommatophora (a taxon of Heterobranchia with land snails and slugs), rooted against the unspecific MTs of Vetigastropoda (represented by *Megathura crenulata*, underlaid in gray). Ancient unspecificity of MTs at the root of Vetigastropoda is indicated by a black triangle. Gain of Cd selectivity in MTs at the root of divergence between Caenogastropoda and Heterobranchia (represented only by Stylommatophora) is indicated by a red triangle, species poossessing Cd-selective MTs are underlaid in pink. Gain of Cu selectivity in MTs of Stylommatophora is indicated by a blue triangle, respective species possessing Cu-selective MTs are underlaid in blue. Secondary loss of Cu selectivity in CdCuMTs of land snails and slugs is indicated by a blue hatched triangle, species with unspecific MT isoforms are highlighted within a blue hatched square. Bayesian inference calculations were made based on a manually edited MUSCLE alignment (see **alignment S5**) using the free software MrBayes (see Material and Methods)



Alignment S1:

Concatenated NEXUS alignment of the mitochondrial COI sequences (position 1-580, start=3rd codon position) and the ribosomal 18SrDNA sequences (position 581-2158; aligned using the SINA Alignment tool v. 1.2.11) of the nineteen gastropod species analysed for neutral phylogeny markers, applied for the mirrored Maximum Likelihood tree represented in **Figure 6**

Patella vulgata

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CGGCCTTAGTATATTAATTCGAGCTGAACTTGGTCAGCCAGGCGCT---CTTTTAGGCGATGACCAACTTTATAATGTTATTGTTACTGCTCACGCTTTTGTTATAATTTTCTTTTTAGTAATGCCTATAATAATTGGGGGTTTTGGTAATTGATTAG
TGCCACTTATGCTTGGGGCCCCTGATATAGCTTTTCCCCGACTTAACAACATAAGATTTTGACTTCTCCCTCCCTCTCTATTTCTTCTTTTAGCCAGAAGAGCTGTTGAAGGTGGGGTAGGTACAGGTTGAACAGTTTATCCTCCTTTATCTTCAAAT
ATTGCCCACTCAGGCCCTTCGGTAGATTTAGCTATTTTTTCTCTCCACCTAGCTGGTGCCTCTTCAATTCTGGGAGCTGCTAATTTTATTAGAACGGCCGCAAACATTCGTTGAGAAGCTATGCAACTTGAACGAATGCCTTTGTTTGTCTGATCTGT
AAAGGTAACCGCTATTTTATTACTTCTATCTTTACCTGTTTTAGCCGGGGCAATTACTATACTATTAACTGACCGTAACTTTAATACTTGTTTCTTTGACCCTAGGGTTCA-GGCT-----TGTTCCTTTCGGGGAGCGAGCCGAAACTGCGAACGGC
TCATTAG-TCAGATAAGGTTCCTTGGCGAAAA-----GCGGGTCGGTTTAATGGATAACTGTGGTAATTCTAGAGCTAATACATGC--AA-CGCACCGT-GGTC-CCCCCC-T-TTCGTTTCTCGACAAAGAGGG------GG-AAACGGC-ATTTAT
TCTCTATACCAGATCGCC-CTAGCCTGCGAAGCTACCGTC--A------AAAGTA-GC-------------GACGGGGCGAA-GCGACAAGT-GTTGAA-ATCCGAATAACTGTG--CCGATCGCGGGGTCTTTC-CGGGC-----CCCCGACGACTT
TGCCATGAAGTGTCTGTCCCATCAATTGGCGATGGTCGGCGACCTGCCTACCACGGTGTTGACGGGTAACGGGGAATCAGGGTTCGATTCCGGAGAGGGAGCCTGCGAAACGGCTACCACCTCCAAGGAGGGCAGCAGGCGCGCAACTTACCCAATCC
CGACACGGGGAGGTAGCGACGAAAAATAACGTGGCGGGGCCCTTTCGTGGTCGCCGCGGGCGGAATGAGCGCAATTTAAAAGAGTGTGCGAGGAGCTATTGGAGGGCAAGCCTGGTGCCA-GCAGCCGCGGTAATTCCAGCTCCAATAGCCTATATCG
GAGTTGCTGCGGTTAAAAAGCTCGTCGTTGGATCTCGGAGGCGGCACGGTCGGGCGCGATACGCT--GCGGGTCCGACC-------------------------GGCGAT-TTCT--C--G---------------------------GCC--GT-C-
------C-C-T--AA-CC-G-GCGCGCGGG----GCGAGGTGG--ACTTCACGG--TC--CTGCCTCGTTTTTCTCCTT-C-GCGG-GAGAGA----ACCCGCCG—CATTTTCTGTCTACCTTGAAAAAATTAGAGTGCTAAAGGCAGGCTAAAACCC
-CGA------GCCTGAATATTTCGTGCATGGAATAAGCGGGGATGCTCTTTTCCGGAGGGTGGTCCGGCCGACTTGG-----CGACGTCGCTTTCGCTGTCGGTTTCGCCGGAGA—GCTAGGATTCGAGAGGAACGGTGGGGGGCGAGCGTACCGGGC
GGGGAGAGGTGAAATTTTGTGATCCGCTCGGGACGACTCGAA-GCGAAAGCGCTGGCCCCGGACGTCTTCCTTAATCCAGAACGAAG-GTGGGTGGAGCAAAGACGATCAGATACCGTCGTAGTACCGACGGTAAACGCTGCCGACCGGGCCGCGGAC
GA-GGCTCGAGAACAGCGGTTATGGACCCGTCCGTGGCGGCTT-CGGGCAACCTGAGAGTTTACGGGTTCCGGGGGTAGTATGGTCGCAAGGCTGAAACTTGAAGGAATTGACGGAAGGGCACCACAAGGAGTGGAGCCTGCGGCTTAATTTGACTCA
ACACGGGGCAACTTACCCGGTCCGGACATCGTCAGGATTGACAGACGCTCGTGCAGTTCTTTCTCGATTCGATGGGTGGTGGTGCATGGCCGTTCGTGGCTG
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Alinda biplicata

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AGGGCTTTCTTTATTAATTCGATTAGAGTTAGGAACAAGAGGG------GTATTACTTGATGATCATTTTTTTAATGTAATTGTCACAGCGCACGCCTTCGTTATAATTTTTTTTATAGTTATACCGGTAATAATTGGGGGGTTTGGCAATTGAATAG
TACCCCTTCTCATTGCTGCACCTGATATAAGATTTCCTCGAATAAATAATATAAGCTTTTGGTTATTACCTCCGGCTTTCCTTTTATTAATTAGTAGTAGAATAGTGGAAGGGGGGGCTGGTACAGGGTGAACCGTATATCCGCCCTTAAGAGGTATT
GCCGCCCATGGTGGCGCCTCTGTTGATTTGGCAATTTTTTCTTTGCACCTTGCAGGAATATCCTCTATTTTAGGGGCTATTAATTTTATCACTACGGTATTTAATATACGTAGAAGAGGGATAAATATAGAACGAGTAAGTCTTTTTGTATGATCTAT
TTTAGTGACGGTTTTTTTATTACTACTTAGCTTACCAGTATTAGCTGGTGCGATTACTATGTTATTAACTGATCGAAATTTTAATACTTGTTTTTTTGATCCCGCAGTTCACA-CT-------GTCTC-AC----GGTGA-----AACCGCGAATGGC
TCATTAAATCAGTCGAGGTTCCTTAGATGACA------CGA-T-CC-TACTTGGATAACTGTGGCAATTCTAGAGCTAATACATGCTTAC----CA-AG--CTC-CGACC--C-TC-GC----------------------GG-AA--AGA-CGCTTT
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-AGCT-----GCCTGAATAAT-GGTGCATGGAATAATGGAATAGGA-------CC----TCGGTTCT----A-TTTT---------GTTGGTT----TTCGGAACTGGAGGT--A-----ATGATTA-ACAGGGACAAACGGGGGCATTCGTATTGCG
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Aplysia californica

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Arion vulgaris

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Biomphalaria glabrata

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Cepaea hortensis

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Anentome helena

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Cornu aspersum
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Deroceras reticulatum
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Elysia crispata
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ATCTTTCAAATGTCTGCCCTATCAAATTTCGATGGTACGTGACATGCCTACCATGTTTGTAACGGGTAACGGGGAATCAGGGTTCGATTCCGGAGAGGGAGCATGAGAAACGGCTACCACATCCAAGGAAGGCAGCAGGCGCGCAACTTACCCACTCC
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AAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGATCTC-----AGGCGCAGGCGGGCG-GTCC-GGC---------TCGC-------------------------GCCGGCTCACT--G--C----------------------------CC--GT-T-
-----GTTTCC--TG--CCC-T-ACCCGTC----TGCCGGCT-----CTCTCCCG-CGCGGTGC----TCTTCGCTGAG-C-GTCCCGGGTG------GCCG--G-------CGCGTTTACTTTGAAAAAATTAGAGTGTTCAAAGCAGGC-----CT
T-GGTC-----GCCTGAATAAT-GGTGCATGGAATAATGGAATAGGA-------CC----TCGGTTCT----A-TTTT---------GTTGGTT----TTCGGAACTGGAGGT--A-----ATGATTA-ACAGGGACAAACGGGGGCATTCGTATGGC
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GGGGAGT-T--------GATT-CGATGACTCCGCG--GGCAGCGTCCGGGAAACC—AAAGCTTTTGGGTTCCGGGGGAAGTATGGTTGCAAAGCTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGCGGCTTAATTTGACTC
AACACGGGAAAACTCACCCGGTCCGGACACTGTAAGGATTGACAGA---TTGATA-GCTCTTTCTTGATTCGGTGGGTGGTGGTGCATGGCCGTTCTTAGTTG
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Helix pomatia

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GGGTCTCTCTTTGTTAATTCGTTGAGAGTTAGGAACATCCGGC------GTATTAAGCGACGATCATTTCTATAATGTGATTGTAACTGCCCATGCATTTGTCATAATTTTTTTATGGTAATACCTATTATAATTGGTGGATTTGGAAATTGGATAG
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TCTAGTAACTGTGTTTTTACTCTTATTATCCTTACCTGTTTTAGCTGGTGCTATTACTATATTGTTGACTGATCGAAATTTTAGTACTTCTTTTTTTGATCCTGCTGTTCACA-CT-------GTCTC-AC----GGTGA-----AACCGCGAATGGC
TCATTAAATCAGTCGAGGTTCCTTAGATGACA------CGA-T-CC-TACTTGGATAACTGTGGCAATTCTAGAGCTAATACATGCTTAC----CA-AG--CTC-CGACC--C-TC-GC----------------------GG-AA--AGA-GCGCTT
TTATTAGTTC-AA--AAC-CAAT-----------CGT-CGTTGCCCT-----TCA-C-------GRGGC--GCG-GCGTTCC-----CAC-T-GGTG-A-CTCTGGATAACTTTGTGCTGATCGCATGGC----CTTAYGT-----GCC-GGCGACGC
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-----GT-TCC--TG--CCC-T-ACC-GTC----TGCCGGCT-----CTCTCCCGTGG--GTGC----TCTTCGCTGA--CGGTCCCGGGTG------GCCG--G-------CGCGTTTACTTTGAAAAAATTAGAGTGTTCAAAGCAGGC-----CT
C-GGCT-----GCCTGAATAAT-GGTGCATGGAATAATGGAATAGGA-------CC----TCGGTTCT----A-TTTT---------GTTGGTT----TTCGGAACA-GAGGT--A-----ATGATTA-ACAGGGACAAACGGGGGCATTCGTACTGC
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Limax maximus

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AGGTTTATCTTTATTAATTCGGTTAGAACTAGGGACAGCTGGT------GTTTTAATAGATGATCATTTTTTCAACGTTATTGTAACGGCTCATGCTTTTGTAATAATTTTTTTATAGTTATACCTATTATGATTGGGGGTTTTGGTAATTGAATAG
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-----GT-TCC--TG--CCC-T-ACC-GTC----TGCCGGCT-----CTCTCCCGCGG--GTGC----TCTTTGCTGAG-C-GTCCCGGGTG------GCCG--G-------CGCGTTTACTTTGAAAAAATTAGAGTGTTCAAAGCAGGC-----CT
C-GGCT-----GCCTGAATAAT-GGTGCATGGAATAATGGAATAGGA-------CC----TCGGTTCT----A-TTTT---------GTTGGTT----TTCGGAACTGGAGGT--A-----ATGATTA-ACAGGGACAAACGGGGGCATTCGTATTGC
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Littorina littorea

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TGCCTTAAGTCTACTTATTCGGGCTGAATTAGGTCAACCTGGCGCT---CTCCTGGGAGATGACCAGCTGTACAACGTTATCGTTACAGCCCACGCCTTTGTAATAATTTTCTTTCTTGTTATGCCTATAATAATTGGTGGGTTTGGAAATTGACTTG
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------A-TGT--TT--CCCATCCTACGCT----TCCCGGTTG----TTAGCCCA-TG--GTGC----TCTTCATTGAG-C-GTTTTGGGTG------GCCG--G-------AACGTTTACTTTGAAGAAATTAGAGTGTTCAAAGCAGGC------A
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Lottia gigantea

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----C-T-A---A--C-C-A-GCG--------CGCGGGGCGAGGTGGACTTCG-CG--GTCC---TGCCTCGTTTTTCT-CCTTCACGGGAGAGAACCCG--CCGCATTTTCTGTCTACCTTGAAAAAATTAGAGTGCTAAAGGCAGGC------TAA
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Marisa cornuarietis

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TGCTTTAAGAATGCTTATTCGAGCTGAACTTGGGCAACCGGGAGCC---TTGTTAGGTGATGATCAGCTTTACAATGTCATTGTTACAGCTCATGCTTTTGTAATGATTTTCTTTTTAGTTATGCCTATAATAATTGGTGGATTTGGAAATTGACTTG
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TTATTAGTTC-AA--AAC-CAGT-----------CGG-GGT-----------CTC----------------GGCCCCGTCCCT----T-T-T-GGTG-A-CTCTGGATAACTTTGTGCCGATCGCATGGC----C-TC-GA-----GCC-GGCGACGC
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C-GTTA-----GCCTGAATAAT-GGTGCATGGAATAATGGAATAGGA-------CC----TCGGTTCT----A-TTTT---------GCTGGTT----TTCGGAACACGAGGT--A-----ATGATTA-AGAGGGACAGACGGGGGCATCCGTATTGC
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Neritina pulligera

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TGCTCTTAGTTTGCTGATTCGAGCAGAGCTTGGGCAACCTGGTGCT---TTATTGGGGGATGATCAGCTTTATAATGTAATTGTGACTGCTCATGCATTTGTAATAATTTTCTTTTTGGTTATGCCAATGATAATTGGTGGGTTTGGTAACTGATTGG
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TTATTAGTTC-AA--AAC-CAAT-----------CGGGCC-----------GCA-A--------------GGCTCGTCC------GTT-T-GGTG-A-CTCTGGATAACTTTGTGCTGATCGCACGGC----C-TC-GA-----GCC-GGCGACGT
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------C-C-G---A--C-C-T-ACC--------TCCCGGTTT-------TCCCT-TG--GTGC----TCTTGACTGAG-T-GCTTCGGGTG------GCCG--G-------AACGTTTACTTTGAAAAAATTAGAGTGCTCAAAGCAGGC------G
C-T-TC-----GCCTGAATAAT-GGTGCATGGAATAATGGAATAGGA-------CC----TCGGTTCT----A-TTTT---------GTTGGTT----TTCGGAACTCGAGGT--A-----ATGATTA-AGAGGGACAGACGGGGGCATTCGTATTAC
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Physella acuta

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-----ATCTCC--TG--CCC-T-ACC-GTC----TGTCGGCT-----CTCTCCCGCGG--GTGC----TCTTCACTGAG-C-GTCCCGGGTG------GCCG--A-------CGCGTTTACTTTGAAAAAATTAGAGTGTTCAAAGCAGGC-----CT
C-G-CT-----GCCTGAATAAT-GGTGCATGGAATAATGGAATAGGA-------CC----TCGGTTCT----A-TTTT---------GTTGGTT----TTCGGAATTGGAGGT--A-----ATGATTA-ACAGGGACAAACGGGGGCATTCGTATTGC
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Pomacea bridgesii

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TTATTAGTTC-AA--AAC-CAGT-----------CGG-GGT-----------CTC----------------GGCCCCGTCCCT----T-T-T-GGTG-A-CTCTGGATAACTTTGTGCCGATCGCATGGC----C-TC-GA-----GCC-GGCGACGC
ATCTTTCAAATGTCTGCCCTATCAAATGTCGATGGTACGTGATAGGCCTACCATGTTGACTACGGGTAACGGGGAATCAGGGTTCGATTCCGGAGAGGGAGCATGAGAAACGGCTACCACATCCAAGGAAGGCAGCAGGCGCGCAACTTACCCACTCC
CAGCTCGGGGAGGTAGTGACGAAAAATAACAATACGGAACTCTTTTGAGGCT-CCGTAATTGGAATGAGTACACTTTAAACCCTTTAACGAGGATCTATTGGAGGGCAAGTCTGGTGCCA-GCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTA
AAGTTGTTGCGATTAAAAAGCTCGTAGTTGGATCTC-----AGGCATGGGCGTGCG-GTCC-GTC---------TCGCGACGGTCACTGTGCGAAGCCGGGGCGGGCGGTGCCCTTCG--C----------------------GGGGTGT-CGT-T-
CGCTTCTTTTGGCGT--CCCATCCTCCGTTTATCTCC-GGTCT---CTCCGTCCA-TG--GTGC----TCTTCGCTGAG-C-GTTATGGGCG------GCCG--G-------AACGTTTACTTTGAAGAAATTAGAGTGTTCAAAGCAGGC------T
C-GTCA-----GCCTGAATAAT-GGTGCATGGAATAATGGAATAGGA-------CC----TCGGTTCT----A-TTTT---------GCTGGTT----TTCGGAACACGAGGT--A-----ATGATTA-AGAGGGACAGACGGGGGCATCCGTATTGC
GGTGTTAGAGGTGAAATTCTTGGATCATCGCAAGACGAACAACT-GCGAAAGCATTTGCCAAGCATGTTTTCATTAGTCAAGAACGAAA-GTCAGAGGTTCGAAGACGATCAGATACCGTCGTAGTTCTGACCATAAACGATGCCAACTAGCGATCCG
CAGGTGT-T--------GCTT-CATCGACTCTGCG--GGCAGCTTCCGGGAAACC—AAAGTTTTCGGGTTCCGGGGGAAGTATGGTTGCAAAGCTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGCGGCTTAATTTGACTC
AACACGGGAAAACTCACCCGGTCCGGACACTGTAAGGATTGACAGA---TTGATA-GCTCTTTCTTGATTCGGTGGGTGGTGGTGCATGGCCGTTCTTAGTTG
```

Pomatias elegans

```
TGCTTTAAGGCTTTTAATTCGGGCTGAATTGGGGCAGCCTGGGAGT---TTGCTAGGTGATGATCAACTTTATAATGTTATTGTTACTGCTCATGCATTTGTTATAATTTTTTTTTTTGGTTATGCCAATAATGATTGGTGGTTTTGGCAATTGGTTAG
TTCCTTTAATGTTGGGGGCTCCTGATATGGCTTTTCCTCGGTTAAATAATATAAGATTTTGATTACTTCCTCCTGCGTTATTTTTATTGTTAATATCTGCGGCTGTAAAGAGGGGAGCTGGTACTGGGTGAACAGTATATCCCCCTTTGGCTGGTAAT
TTAGCACATTCGGGTGGTTCTGTTGATCTTGCTATTTTTTCGTTACATTTGGCTGGGGCATCTTCTATTTTAGGTGCTGTGAATTTCATTACTACTATTGTTAATATACAATGGTGTGGTGTTCAGTTGGAGCGGTTATCTTTGTTTGTTTGGTCGGT
TAAAATTACTGCTATTTTGCTTTTACTTTCTTTACCAGTTTTGGCTGGGGCAGTTACTATGCTTTTAACAGATCGGAATTTTAATACTACTTTTTTTGATCCTGCTGTTCACA-CC-------CTCGT-AC----GGTGA-----AACCGCGAATGGC
TCATTAAATCAGTCGAGGTTCCTTAGATGATC------CAA-A-TC-TACTTGGATAACTGTGGTAATTCTAGAGCTAATACATGC--CA----ACCAG--CTC-CGACCCTT-TC-G---------------GG------GG-AA--AGA-GCGCTT
TTATTAGTTC-AA--AAC-CAGT-----------CGGGTC------------GCA-A-------------GAC-TCGTCC------T-T-T-GGTG-A-CTCTGGATAACTTTGTGCCGATCGCATGGC----C-TC-GA-----GCC-GGCGACGC
ATCTTTCAAATGTCTGCCCTATCAAATGACGATGGTACGTGATCTGCCTACCATGTTAGCAACGGGTAGCGGGGAATCAGGGTTCGATTCCGGAGAGGGAGCATGAGAAACGGCTACCACATCCAAGGAAGGCAGCAGGCGCGCAACTTACCCACTCC
TGGCACGGGGAGGTAGTGACGAAAAATAACAATACGGAACTCTTTTGAGGCT-CCGTAATTGGAATGAGTACACTTTAAAGCCTTTAACGAGGATCTATTGGAGGGCAAGTCTGGTGCCA-GCAGCCGCGGTAATTCCAGCTCCAATAGCGTATACTA
AAGTTGTTGCGATTAAAAAGCTCGTAGTTGGATCTC-----AGGCATGGGCGCACG-GTCC--GC--------CTCGC-------------------------GGCGGT-CACT--G--T---------------------------GT--GG-T-
------TTTGT--TT--CCCATCCTACGCT----TCCCGGTTG---TTCAGCCCA-TG--GTGC----TCTTGACTGAG-C-GTTTTGGGTG------GCCG--G-------AACGTTTACTTTGAAGAAATTAGAGTGTTCAAAGCAGGC------A
C-GTC------GCCTGAATAAT-GGTGCATGGAATAATGGAATAGGA-------CC----TCGGTTCT----A-TTTT---------GCTGGTT----TTCGGAACACGAGGT--A-----ATGATTA-AGAGGGACAGACGGGGGCATCCGTATTGC
GGTGTTAGAGGTGAAATTCTTGGATCATCGCAAGACGAACAACT-GCGAAAGCATTTGCCAAGCATGTTTTCATTAGTCAAGAACGAAA-GTCAGAGGTTCGAAGACGATCAGATACCGTCGTAGTTCTGACCATAAACGATGCCAACTAGCGATTCG
CTGGTGT-T--------GCTT-CATCGACTCTGCG--GGCAGCTTCCGGGAAACC—AAAGTTTTCGGGTTCCGGGGGAAGTATGGTTGCAAAGCTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGCGGCTTAATTTGACTC
AACACGGGAAAACTCACCCGGTCCGGACACTGTAAGGATTGACAGA---TTGATA-GCTCTTTCTTGATTCGGTGGGTGGTGGTGCATGGCCGTTCTTAGTTG
```

Alignment S2:

MUSCLE alignment (manually edited) of gastropod CdMT protein sequences applied for the mirrored Maximum Likelihood tree represented in **Figure 6**.

```
>Lottia gigantea MT1
-SSEK----------------------------------------PSCCIAEYECCKTKLCCDTGPADCCKPGNKPDCCAPGKLQCKCP-G-
TCACGVGCTGVDNCKCGAGCS-CFN

>Lottia gigantea MT2
-SSEK----------------------------------------ASCCIAEYECCKTKSCCDTGPADCCKPGNKPDCCAPGKLQCKCS-G-
TCACGVGCTGVDNCKCGAGCS-CFN

>Nacella polaris MT
-SSEK----------------------------------------AACCIAEYECCKTKSCCKDGPADCCKPGNTTDCCKGKV-ACKCA-G-
SCACGAGCTGQTPCKCGAGCS-CNS

>Patella vulgata MT1
-SSQK----------------------------------------ASCCLAELECCKTKACCAKGPANCCSPGNDPNCCKS-N-ICKCN-G-
NCACGVGCTGIENCECGTGCS-CK-

>Patella vulgata MT2
-SSEK----------------------------------------AACCLAEHECCKTKSCCANGPADCCKPGKTVDCCKSQN-TCKCG-E-
SCACGAGCSGVDNCKCGSGCS-CK-

>Littorina littea MT
-SS---VF-GAGCTDVCKQTPCGCATS--GCNCTDDCKCQSC-KYGAGCT---DTCKQTPCGC---GSGCNCKEDCRCQSCST-ACKCAAG-
SCKCGKGCTGPDSCKCDRSCS-CK-

>Pomatias elegans MT1
STSGANVIYGAGCTGTCKQSPCGCKNSAAGCRCKDDCQCPACAKYGAGCT---GTCKQSPCGCKNSAAGCGCKDDCRCPACAK-SCKC--G-
TCNCGKGCTGPSNCKCDDGCS-CK-

>Pomatias elegans MT2
SSSGANAT-------------------------------------GAGCT---ETCKESPCGCKNSAAGCKCKDDCQCTTCAK-SCKCA-G-
TCNCGKGCTGPNSCKCDGGCP-CK-

>Helix pomatia CdMT
SGKGK----------------------------------------GEKCT---SACRSEPCQC---GSKCQCGEGCTCAAC-K-TCNCTSD-
GCKCGKECTGPDSCKCGSSCS-CK-

>Cornu aspersum CdMT
SGKGK----------------------------------------GEKCT---AACRNEPCQC---GSKCQCGEGCTCAAC-K-TCNCTSD-
GCKCGKACTGPDSCTCGSSCG-CK-

>Arianta arbustorum CdMT
SGKGK----------------------------------------GDLCT---AACKNEPCQC---GSKCQCGEGCACASC-K-TCNCTSD-
GCKCGEKCTGAASCKCGSSCS-CK-

>Cepaea hortensis CdMT1
SGKGK----------------------------------------GEKCT---AACRNEPCQC---GSKCQCGEGCACAAC-K-TCNCTSD-
GCKCGKECTGPDSCKCGSSCS-CK-

>Cepaea hortensis CdMT2
SGKGK----------------------------------------GEKCT---AACRNEPCQC---GSKCQCGEGCACAAC-K-TCNCTSD-
GCKCGKECTGPDSCKCGSLCS-CK-

>Cepaea nemoralis CdMT
SGKGK----------------------------------------GEKCT---AACRNEPCQC---GSKCQCGEGCACAAC-K-TCNCTSD-
GCKCGKECTGPDSCKCGSSCS-CK-

>Cochlicella acuta CdMT
SGKGK----------------------------------------AESCT---AQCQSNPCQC---GDKCQCGEGCACTSC-K-TCKCTSD-
GCKCGKECTGPASCKCGSSCS-CK-

>Nesiohelix samarangae CdMT
SGK------------------------------------------GELCT---SACKSNPCQC---GDKCQCGEGCTCSAC-K-SCHCTND-
GCNCGKECTGPTSCKCDTSCS-CK-

>Lehmannia nyctelia CdMT
SGK------------------------------------------GAKCT---GACKSEPCQC---GQNCQCGDDCSCSQC-K-TCKCSAGS
TCQCGHGCTGVESCKCGNSCS-CK-

>Limax maximus CdMT
SGK------------------------------------------GAKCT---GACKSEPCQC---GQNCQCGDDCSCSQC-K-TCKCSAGS
TCQCGHGCTGVESCKCGSSCS-CK-
```

>*Deroceras reticulatum* CdMT1
SGK--GEKCT---GDCKSEPCKC---GQNCQCGNDCTCSQC-K-TCKCSSS-
GCQCGHGCTGVESCKCGSSCT-CK-

>*Deroceras reticulatum* CdMT2
SGK--GEKCT---GDCKSEPCKC---GQNCQCGNDCTCSQC-K-TCKCSSS-
GCQCGHGCTGVESCKCGSSCT-CK-

>*Arion vulgaris* AvMT1
SGK---A-CT---GACKSEPCQC---GNNCQCGGDCDCSQC-K-TCKCTNE-
GCKCGQNCTGQATCSCEKSCS-CK-

Alignment S3:

MUSCLE alignment (manually edited) of MTs of Panpulmonata (Hygrophila + Stylommatophora + Sacoglossa) applied for the Bayesian Inference Tree represented in **Figure 7**.

```
>Tritia obsoleta MT1
--------EGTGCAGPCKQTPCGCE-SSGGCKCGGDCSCTECGR-GAGCTDACKETPCGC-ASSGGCKCTGNCSCPSCS---------------CKCGK------
-----------------------GACKCDKG-CEGPGSCKCGPG-C-----TCKKS-----------

>Littorina littorea CdMT
-----SSVFGAGCTDVCKQTPCGC--ATSGCNCTDDCKCQSC-KYGAGCTDTCKQTPCGC---GSGCNCKEDCRCQSCSTA-------------CKCAA------
-----------------------GSCKCGKG-CTGPDSCKCDRS-C-----SCK-------------

>Pomatias elegans MT1
STSGANVIYGAGCTGTCKQSPCGCKNSAAGCRCKDDCQCPACAKYGAGCTGTCKQSPCGCKNSAAGCGCKDDCRCPACAKS-------------CKCGT------
-------------------------CNCGKG-CTGPSNCKCDDG-C-----SCK-------------

>Pomatias elegans MT2
-------------------------------------SSSGANATGAGCTETCKESPCGCKNSAAGCKCKDDCQCTTCAKS-------------CKCA-------
-----------------------GTCNCGKG-CTGPNSCKCDGG-C-----PCK-------------

>Elysia crispata MT
-----------------------------------------SGK-GLICTAPCTNDPCGC---GENCQCGHACSCHSCS---------------CSCGA------
------------------------DCKCNQATCHEKSNCKCEAS-C-----SCRAK-----------

>Elysia cornigera MT
-----------------------------------------SGK-GASCPPTCRNDPCGC---GQDCQCGQDCTCSSCS---------------CLCGN------
------------------------ECKCTSASCSQGVRCRCDTL-C-----DCRPK-----------

>Helix pomatia CdMT
---------------------------------------SGKGK-GEKCTSACRSEPCQC---GSKCQCGEGCTCAACKT--------------CNCTS------
-----------------------DGCKCGKE-CTGPDSCKCGSS-C-----SCK-------------

>Cornu aspersum_CdMT
---------------------------------------SGKGK-GEKCTAACRNEPCQC---GSKCQCGEGCTCAACKT--------------CNCTS------
-----------------------DGCKCGKA-CTGPDSCTCGSS-C-----GCK-------------

>Arianta arbustorum CdMT
---------------------------------------SGKGK-GDLCTAACKNEPCQC---GSKCQCGEGCACASCKT--------------CNCTS------
-----------------------DGCKCGEK-CTGAASCKCGSS-C-----SCK-------------

>Cepaea hortensis CdMT1
---------------------------------------SGKGK-GEKCTAACRNEPCQC---GSKCQCGEGCACAACKT--------------CNCTS------
-----------------------DGCKCGKE-CTGPDSCKCGSS-C-----SCK-------------

>Cepaea hortensis CdMT2
---------------------------------------SGKGK-GEKCTAACRNEPCQC---GSKCQCGEGCACAACKT--------------CNCTS------
-----------------------DGCKCGKE-CTGPDSCKCGSL-C-----SCK-------------

>Cepaea nemoralis CdMT
---------------------------------------SGKGK-GEKCTAACRNEPCQC---GSKCQCGEGCACAACKT--------------CNCTS------
-----------------------DGCKCGKE-CTGPDSCKCGSS-C-----SCK-------------

>Cochlicella acuta CdMT
---------------------------------------SGKGK-AESCTAQCQSNPCQC---GDKCQCGEGCACTSCKT--------------CKCTS------
-----------------------DGCKCGKE-CTGPASCKCGSS-C-----SCK-------------

>Nesiohelix samarangae CdMT
-----------------------------------------SGK-GELCTSACKSNPCQC---GDKCQCGEGCTCSACKS--------------CHCTN------
-----------------------DGCNCGKE-CTGPTSCKCDTS-C-----SCK-------------

>Limax maximus CdMT
-----------------------------------------SGK-GAKCTGACKSEPCQC---GQNCQCGDDCSCSQCKT--------------CKCSA------
----------------------GSTCQCGHG-CTGVESCKCGSS-C-----SCK-------------

>Deroceras reticulatum CdMT1
-----------------------------------------SGK-GEKCTGDCKSEPCKC---GQNCQCGNDCTCSQCKT--------------CKCST------
----------------------GSGCQCGHG-CTGVESCKCGSS-C-----T---------------

>Deroceras reticulatum CdMT2
-----------------------------------------SGK-GEKCTGDCKSEPCKC---GQNCQCGNDCTCSQCKT--------------CKCSS------
-----------------------SGCQCGHG-CTGVESCKCGSS-C-----TCK-------------
```

```
>Lehmannia nyctelia CdMT
-----------------------------------------SGK-GAKCTGACKSEPCQC---GQNCQCGDDCSCSQCKT--------------CKCSA------
----------------------GSTCQCGHG-CTGVESCKCGNS-C-----SCK-------------

>Arion vulgaris AvMT1
-----------------------------------------SGK-A--CTGACKSEPCQC---GNNCQCGGDCDCSQCKT--------------CKCTN------
-----------------------EGCKCGQN-CTGQATCSCEKS-C-----SCK-------------

>Physella acuta MT1
-----------------------------------------SGK-GPNCTEACTGEQCTC---GRQLQV-------CCKT--------------CKCED------
-----------------------NACKCGEG-CTGPSTCKCESSDC-----ACK-------------

>Physella acuta MT2
-----------------------------------------SGK-GPNCTEACTGEQCTC---GDSCKCGEGCNCPSCKT--------------CKCED------
-----------------------NACKCGEG-CTGPSTCKCESSDC-----ACK-------------

>Physella carolinae MT
-----------------------------------------SGK-GPNCTEACTGEQCTC---GDSCKCGEGCNCPSCKT--------------CKCED------
-----------------------NACKCGEG-CTGPSTCKCESSDC-----ACK-------------

>Physella hendersoni MT
-----------------------------------------SGK-GPNCTEACTGEQCTC---GDSCKCGEGCNCPSCKT--------------CKCED------
-----------------------NACKCGEG-CTGPSTCKCESSDC-----ACK-------------

>Physella gyrina MT
-----------------------------------------SGK-GPNCTEACTGEQCNC---GDSCKCGEGCNCPSCKT--------------CKCED------
-----------------------NACKCGEG-CTGPSTCKCEST-C-----ACK-------------

>Galba cubensis MT
-----------------------------------------SGR-GPNCTEACRGEQCNC---GDSCKCGEGCNCPSCKT--------------CKCSA------
-----------------------EDCKCDAG-SKGTGNCKC--------------------------

>Galba truncatula MT
-----------------------------------------SGK-GPNCTEACRGEQCNC---GDSCKCGEGCNCPSCKT--------------CKCAA------
-----------------------EDCKCDAG-SKGTGNCKC--------------------------

>Lymnaea stagnalis MT
-----------------------------------------SGK-GPNCTEACTGEQCNC---GDSCKCGEGCNCPSCKT--------------CKCSA------
-----------------------EDCKCDAG-SKGTGNCKC--------------------------

>Biomphalaria glabrata MT
-----------------------------------------SGK-GPNCTEACTGEQCNC---GDSCKCGEGCNCPSCKTTKGPNCTEACTGKQ
CSCGDSCQCGEGCTCSCCKKACTKECTDTECSCGDSCKCGEG-CK-CSSCKAGK--CTKSDEGCKTEGHCAKGKCCKS

>Helix pomatia CuMT
-----------------------------------------SGR-GKNCGGACNSNPCSC---GNDCKCGAGCNCDRCSS--------------CHCSN------
-----------------------DDCKCGSQ-CTGSGSCKCGSA-C-----GCK-------------

>Cornu aspersum CuMT
-----------------------------------------SGR-GQNCGGACNSNPCNC---GNDCNCGTGCNCDQCSA--------------RHCSN------
-----------------------DDCKCGSQ-CTRSGSCKCGNA-C-----GCK-------------

>Cepaea nemoralis CuMT
-----------------------------------------SGR-G-NCGGACNSNPCSC---GDDCKCGAACNCDRCSS--------------CHCSN------
-----------------------DDCKCGSQ-CTGSGSCKCGSA-C-----GCK-------------

>Theba pisana CuMT
-----------------------------------------SGR-GKNCGGACNSNPCNC---ANNCRCGAGCNCDSCSS--------------CHCSN------
-----------------------DDCKCGNQ-CTTSGSCKCGSA-C-----GCK-------------

>Alinda biplicata CuMT1a
-----------------------------------------SGK-GANCTGACNSNPCQC---GDDCKCGVGCSCAECNT--------------CKCTN------
-----------------------DGCKCGHG-CTGAGSCKCGNS-C-----GCK-------------

>Alinda biplicata CuMT1b
-----------------------------------------SGK-GANCTGACNSNPCQS---GDDCKCGVGCSCAECNT--------------CKCTN------
-----------------------DGCKCGHG-CTGAGSCKCGNS-C-----GCK-------------

>Alinda biplicata CuMT2
-----------------------------------------SGK-GANCSGACNSNPCQC---GDDCKCGAACSCAECNT--------------CKCTN------
-----------------------DSCKCGHD-CSGAGSCKCGNS-C-----GCK-------------

>Cochlicella acuta CuMT
-----------------------------------------SGR-G-NCGGACKSNPCSC---GQVCKCGGACTCAQCNA--------------CRCSG------
-----------------------DSCKCGDQ-CTASGSCQCGSG-C-----GCK-------------
```

>*Arion vulgaris* AvMT2
---SGR-G--CNGTCNSNPCQC---EDGCQCGDACSCAQCNT--------------CKCTN------
-----------------------DGCKCGNE-CTATGSCKCGTS-C-----GCN-------------

>*Helix pomatia* CdCuMT1
---SGK-GSNCAGSCNSNPCSC---GDDCKCGAGCSCVQCHS--------------CQCNN------
-----------------------DTCKCGNQ-CSASGSCKCGS--C-----GCK-------------

>*Helix pomatia* CdCuMT2
---SGK-GSNCAGSCNSNPCSC---GDDCKCGAGCSCAQCHS--------------CQCNN------
-----------------------DTCKCGNQ-CSASGSCKCGS--C-----GCK-------------

>*Cepaea hortensis* CdCuMT
---SGK-ASACAGSCNSNPCSC---GDDCQCGAGCSCAQCHS--------------CQCNN------
-----------------------DTCKCGNQ-CSASGSCKCGS--C-----GCK-------------

>*Cepaea nemoralis* CdCuMT
---SGK-GSACAGSCNSNPCSC---GDDCQCGAGCSCAQCQS--------------CQCNN------
-----------------------DTCKCGNQ-CSASGSCKCGS--C-----GCK-------------

>*Cochlicella acuta* CdCuMT
---SGK-GSACAGSCGNNPCSC---GDDCRCGAGCSCAQCNS--------------CQCNN------
-----------------------DTCKCGNQ-CSTSGSCKCGS--C-----GCK-------------

>*Cornu aspersum* CdCuMT
---SGK-GSACAGSCNSNPCSC---GDDCKCGAGCSCAQCYS--------------CQCNN------
-----------------------DTCKCGSQ-CSTSGSCKCGS--C-----GCK-------------

Alignment S4:

MUSCLE alignment (manually edited) of MT protein sequences of Stylommatophora versus Caenogastropoda and Vetigastropoda applied for the Bayesian Inference Tree represented in **Figure S3**.

```
>Megathura crenulata MT
--SGK----------------------------------------GENCTAECKSDPCACGD---SCKCGEGCACTTCVKTEAKTTCKCG--ESCKC-EG
CKEGEACKCESGCASCK

>Arion vulgaris AvMT1
--SGK------------------------------------------ACTGACKSEPCQCGN---NCQCGGDCDCSQC------KTCKCTN-EGCKCGQN
CTGQATCSCEKSC-SCK

>Lehmannia nyctelia CdMT
--SGK----------------------------------------GAKCTGACKSEPCQCGQ---NCQCGDDCSCSQC------KTCKCSAGSTCQCGHG
CTGVESCKCGNSC-SCK

>Limax maximus CdMT
--SGK----------------------------------------GAKCTGACKSEPCQCGQ---NCQCGDDCSCSQC------KTCKCSAGSTCQCGHG
CTGVESCKCGSSC-SCK

>Deroceras reticulatum CdMT1
--SGK----------------------------------------GEKCTGDCKSEPCKCGQ---NCQCGNDCTCSQC------KTCKCSTGSGCQCGHG
CTGVESCKCGSSC-T--

>Deroceras reticulatum CdMT2
--SGK----------------------------------------GEKCTGDCKSEPCKCGQ---NCQCGNDCTCSQC------KTCKCSS-SGCQCGHG
CTGVESCKCGSSC-T--

>Nesiohelix samarangae CdMT
--SGK----------------------------------------GELCTSACKSNPCQCGD---KCQCGEGCTCSAC------KSCHCTN-DGCNCGKE
CTGPTSCKCDTSC-SCK

>Cochlicella acuta CdMT
SGKGK----------------------------------------AESCTAQCQSNPCQCGD---KCQCGEGCACTSC------KTCKCTS-DGCKCGKE
CTGPASCKCGSSC-SCK

>Arianta arbustorum CdMT
SGKGK----------------------------------------GDLCTAACKNEPCQCGS---KCQCGEGCACASC------KTCNCTS-DGCKCGEK
CTGAASCKCGSSC-SCK

>Cornu aspersum CdMT
SGKGK----------------------------------------GEKCTAACRNEPCQCGS---KCQCGEGCTCAAC------KTCNCTS-DGCKCGKA
CTGPDSCTCGSSC-GCK

>Helix pomatia CdMT
SGKGK----------------------------------------GEKCTSACRSEPCQCGS---KCQCGEGCTCAAC------KTCNCTS-DGCKCGKE
CTGPDSCKCGSSC-SCK

>Cepaea_hortensis_CdMT1
SGKGK----------------------------------------GEKCTAACRNEPCQCGS---KCQCGEGCACAAC------KTCNCTS-DGCKCGKE
CTGPDSCKCGSSC-SCK

>Cepaea nemoralis CdMT
SGKGK----------------------------------------GEKCTAACRNEPCQCGS---KCQCGEGCACAAC------KTCNCTS-DGCKCGKE
CTGPDSCKCGSSC-SCK

>Cepaea hortensis CdMT2
SGKGK----------------------------------------GEKCTAACRNEPCQCGS---KCQCGEGCACAAC------KTCNCTS-DGCKCGKE
CTGPDSCKCGSLC-SCK

>Arion vulgaris AvMT2
--SGR----------------------------------------G--CNGTCNSNPCQCED---GCQCGDACSCAQC------NTCKCTN-DGCKCGNE
CTATGSCKCGTSC-GCN

>Alinda biplicata CuMT2
--SGK----------------------------------------GANCSGACNSNPCQCGD---DCKCGAACSCAEC------NTCKCTN-DSCKCGHD
CSGAGSCKCGNSC-GCK

>Alinda biplicata CuMT1a
--SGK----------------------------------------GANCTGACNSNPCQCGD---DCKCGVGCSCAEC------NTCKCTN-DGCKCGHG
CTGAGSCKCGNSC-GCK

>Alinda biplicata CuMT1b
--SGK----------------------------------------GANCTGACNSNPCQSGD---DCKCGVGCSCAEC------NTCKCTN-DGCKCGHG
CTGAGSCKCGNSC-GCK
```

```
>Cochlicella acuta CuMT
--SGR----------------------------------------G-NCGGACKSNPCSCGQ---VCKCGGACTCAQC------NACRCSG-DSCKCGDQ
CTASGSCQCGSGC-GCK

>Cochlicella acuta CdCuMT
--SGK----------------------------------------GSACAGSCGNNPCSCGD---DCRCGAGCSCAQC------NSCQCNN-DTCKCGNQ
CSTSGSCKCGS-C-GCK

>Cornu aspersum_CdCuMT
--SGK----------------------------------------GSACAGSCNSNPCSCGD---DCKCGAGCSCAQC------YSCQCNN-DTCKCGSQ
CSTSGSCKCGS-C-GCK

>Cepaea nemoralis CdCuMT
--SGK----------------------------------------GSACAGSCNSNPCSCGD---DCQCGAGCSCAQC------QSCQCNN-DTCKCGNQ
CSASGSCKCGS-C-GCK

>Cepaea hortensis CdCuMT
--SGK----------------------------------------ASACAGSCNSNPCSCGD---DCQCGAGCSCAQC------HSCQCNN-DTCKCGNQ
CSASGSCKCGS-C-GCK

>Helix pomatia CdCuMT1
--SGK----------------------------------------GSNCAGSCNSNPCSCGD---DCKCGAGCSCVQC------HSCQCNN-DTCKCGNQ
CSASGSCKCGS-C-GCK

>Helix pomatia CdCuMT2
--SGK----------------------------------------GSNCAGSCNSNPCSCGD---DCKCGAGCSCAQC------HSCQCNN-DTCKCGNQ
CSASGSCKCGS-C-GCK

>Cornu aspersum CuMT
--SGR----------------------------------------GQNCGGACNSNPCNCGN---DCNCGTGCNCDQC------SARHCSN-DDCKCGSQ
CTRSGSCKCGNAC-GCK

>Theba pisana CuMT
--SGR----------------------------------------GKNCGGACNSNPCNCAN---NCRCGAGCNCDSC------SSCHCSN-DDCKCGNQ
CTTSGSCKCGSAC-GCK

>Helix pomatia CuMT
--SGR----------------------------------------GKNCGGACNSNPCSCGN---DCKCGAGCNCDRC------SSCHCSN-DDCKCGSQ
CTGSGSCKCGSAC-GCK

>Cepaea nemoralis CuMT
--SGR----------------------------------------G-NCGGACNSNPCSCGD---DCKCGAACNCDRC------SSCHCSN-DDCKCGSQ
CTGSGSCKCGSAC-GCK

>Littorina littorea MT
-----SSVFGAGCTDVCKQTPCGC--ATSGCNCTDDCKCQSC-KYGAGCTDTCKQTPCGCGS---GCNCKEDCRCQSCS-----TACKCAA-GSCKCGKG
CTGPDSCKCDRSC-SCK

>Pomatias elegans CdMT1
STSGANVIYGAGCTGTCKQSPCGCKNSAAGCRCKDDCQCPACAKYGAGCTGTCKQSPCGCKNSAAGCGCKDDCRCPACA-----KSCKC---GTCNCGKG
CTGPSNCKCDDGC-SCK

>Pomatias elegans CdMT2
SSSGANAT-------------------------------------GAGCTETCKESPCGCKNSAAGCKCKDDCQCTTCA-----KSCKCA--GTCNCGKG
CTGPNSCKCDGGC-PCK
```

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