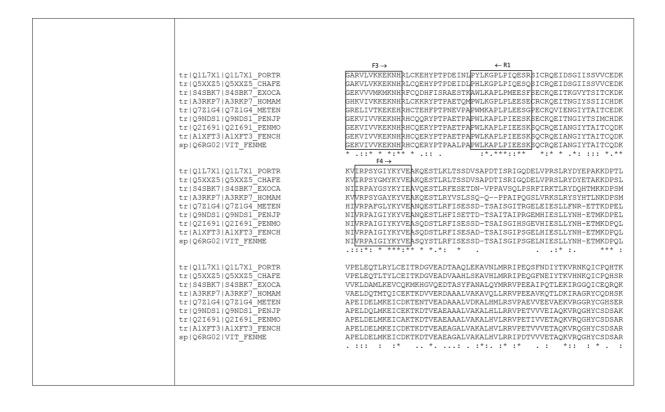
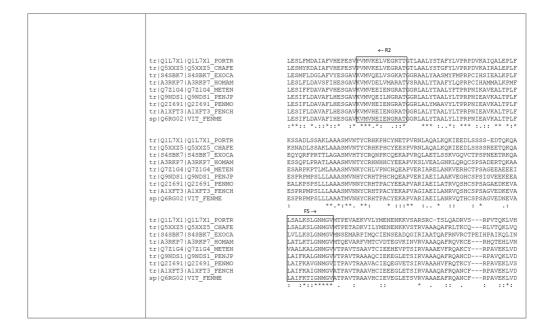
Fig. S1.

Accession nu	mbers	CLUSTAL Omega Alignment		
	tr Q1L7X1 Q1L7X1 PORTR tr Q5XXZ5 Q5XXZ5 CHAFE tr A3RKP7 A3RKP7 HOMAM tr Q721G4 Q721G4 METEN tr Q21691 Q21691 PENMO tr A1KFT3 A1KFT3 FENCH siensis tr Q1L7X1 Q1L7X1 PORTR tr Q21691 Q21691 PENMO tr A1KFT3 A1KFT3 FENCH tr Q5XXZ5 Q5XXZ5 CHAFE tr S45EK7 A3RKP7 HOMAM tr Q721G4 Q721G4 METEN tr Q9NDS1 Q9NDS1 PENJP tr Q21691 Q21691 PENMO tr A1KFT3 A1KFT3 FENCH sp Q6RG02 VIT FENME tr Q1L7X1 Q1L7X1 PORTR tr Q5XXZ5 Q5XXZ5 CHAFE tr S45EK7 A3RKP7 HOMAM tr Q71G4 Q721G4 PENDH tr Q5XXZ5 Q5XXZ5 CHAFE tr S45EK7 S45EK7 EXOCA tr A3RKP7 A3RKP7 HOMAM tr Q721G4 Q721G4 METEN tr Q5XXZ5 Q5XXZ5 CHAFE tr S45EK7 S45EK7 EXOCA tr A3RKP7 A3RKP7 HOMAM tr Q721G4 Q721G4 METEN tr Q9NDS1 Q9NDS1 PENJP tr Q91691 Q21691 PENMO tr A1KFT3 A1KFT3 FENCH	MTTHTVLLLLALTAAAVAAPYGGTTQLCSTECPLAA-AKLSFIPGKTYSYTYSGKSIVQL MTTHTV-LLLALAAAAAAPYGGTTQLCSTECPVAA-AKLSFIPGKTYSYTYTGKSQVQL -MTYSALFVFAILAAASAAPWSTTNLCSRECPVAGSPKLFYAPEKTYVYSYTGKSRINL MTTSTALLVPPLVVGAGASPMGGNTPRCSTECPVAGSPKLFYAPEKTYVYSYTGKSRINL MNSSSLVLVLALVAGGLAAPBEDEAPRCSTECPTGSPKLAYPGPKTYTYTYSGKSKVQL MTTSSLLFVLALVAGGLAAPWGADLPRCSTECPTGSPKLAYPGPKTYTYAYSGKSKVQL MTTSTLIFILAFVTGGLAAPWGADLPRCSTECPTGSPKLAYQPDKTYAPPYSGKSRVHL MTSNLLFVLAFVAGGLAAPWGADLPRCSTECPTGSPKLAYQPDKTYAPYSGKSRVHL MTTSTLLFVLAFVAGGLAAPWGADLPRCSTECPTGSPKLAYQPDKTYAPYSGKSRVHL MTTSTLLFVLAFVAGGLAAPWGADLPRCSTECPTGSPKLAYQPDKTYAYAYSGKSTVQL : : '* ** ***: '* ** ** *** ** **** ***		
	sp Q6RG02 VIT_FENME	RVQHVCAHPEDEAWAINLKKGVASAFQNSIPSLS <mark>AVSSGITVTETDV</mark> VGKCPTKYEIETE ** * **: *:: *:*: *:*: * :: * * : ***:: *		





	7
	← R3
tr Q1L7X1 Q1L7X1_PORTR	YALRPEENTEVRIAAYLAAVRCANYEDLQEIVTKISYEENTQVRGFILSHLINLQKS
tr Q5XXZ5 Q5XXZ5_CHAFE	YALRPGQNTEVRIAAYLAAVRCANYEDLQHIVTKISYEENTQVRGFILSHLINLQQS
tr S4SBK7 S4SBK7_EXOCA	VVLDPKKKTEVRIGSYLAAVKCAKYEDLKKITDKIAI AENTQVRSFILSHLQNVRES
tr A3RKP7 A3RKP7_HOMAM	IAVHPAKNTEFRIASYLMAMRCVRQKDLQMIVNNTYHEKHSKNTQVRGFILSHLLNLQDT IATHPDFETEVRIVSYLAAIKCAEMEDLEKIINKITEEKNTOVRSFVLGHLINIOES
tr Q7Z1G4 Q7Z1G4_METEN tr Q9NDS1 Q9NDS1_PENJP	IATHPOFETEVRIVSYLAAIRCAEMEDLERIINRITEERNTQVRSFVLGHLINIQES IATRPTFETEVRIASYLAAIRCAEKEHLEQIIEKISEEENTQVRGFVLGHLINIQES
tr Q21691 Q21691 PENMO	IAVRPAFGTEVRIASYLAAIRCAEMEDLEEIFEKISVEENTOVRGFILGHLLNIQES
tr A1XFT3 A1XFT3 FENCH	IAVRPAFETEVRIASYLAAVRCAEOEHLETIIEKISKEENTOVRGFVLGHLINIOES
sp Q6RG02 VIT FENME	IAVRPAFETEVRIASYLAAVRCAEOEHLETIIEKISKEENTOVRGFVLGHLINIOES
- P Z	** ** ** * * * * * * * * * * * * * * * *
tr Q1L7X1 Q1L7X1 PORTR	DAPEKQSLRYMMTNIVLPQDFEADIRKYSQNLDLSYFSESLGVGAEVESNLIYAPGSMIP
tr Q5XXZ5 Q5XXZ5_CHAFE	DAPGKQRLRYLLTNILLPQDFETDIRKYSQNLDLSYFSPSAAGLESNMIYAPGSMLP
tr S4SBK7 S4SBK7_EXOCA	TASFKGNLKNMLETIVLPSNFTKDWRKISRNVDLSYYAPTFGVGAGMESNLIYAPGSFVP
tr A3RKP7 A3RKP7_HOMAM	NTPHKDYLRYLLTNTLLPRDYKTDFRKYSRNIDMSYFAPSLGVGAGVESNIIYVPGSFVP
tr Q7Z1G4 Q7Z1G4_METEN	TCPSKEHLRYLLTDFVIPTDFERDFRRVSRNVMMGYHSAAFEMGADVESNIIYAPGAFVP
tr Q9NDS1 Q9NDS1_PENJP	TCPTKENLKYLLTNVVIPTDFEKDFRKFSRNVEMSYHAPAFGMGADLESNIIYAPGSFIP
tr Q2I691 Q2I691_PENMO	TCPTKEHLRYLLTNFVIPIDFERDFRKFSRNIDVAYHAPAFGMGAGLESNIIYAPGSFIP TCPAKENLRYLLANVVIPTDFERDFRKFSRNIDMAYHAPAFGMGAGLESNIIYAPGSFVP
tr A1XFT3 A1XFT3_FENCH sp Q6RG02 VIT FENME	TCPAKENLRYLLANV-IPTDFERDFRKFSRNIDVAYHAPAFGMGAGLESNIIYAPGSFVP
SP/QORGOZ/VII_FENME	* *: : * * * * *: * : * : * * : ***** : *
f6→	
tr Q1L7X1 Q1L7X1 PORTR	RSLGVNLTAALDGTGIPMNLGEIGARLEGLEPILADLLGPASYLKTSSYSKMFNDLVSFI
tr Q5XXZ5 Q5XXZ5 CHAFE	RSLGVNLTAAPDGTGIPINIADVGARVEGLEPIIAQLLGPASYLKTSSYSKIFNDLVSFI
tr S4SBK7 S4SBK7 EXOCA	RSVNLNLTGALGATPFNIGEIGARFEGIESIIEEMFGPESYLRKTSSRQILRDLSSNI
tr A3RKP7 A3RKP7_HOMAM	RSVDFNFTAAFEGISMNIGEVGARLEGLEPVIEEVFGPEGYLQRTSFSQILTDMTTFV
tr Q7Z1G4 Q7Z1G4_METEN	RALTMNLKADIDETHLDIGEIGARFEGVDSIIEEHFGPEGYLRKASFGKILEDITGFA
tr Q9NDS1 Q9NDS1_PENJP	RAVNLNMKAAVDETHMDLAEIGARFEGIDSIIEELFGPEGYLRKATFGKIMQDITGFA
tr Q2I691 Q2I691_PENMO	RAVNLKMKADVDEMHMDIAEIGARFEGIDSIIEELLGPQGYLHRATFGKIMEDITGFA
tr A1XFT3 A1XFT3_FENCH	RAVNMKMKADVDETHMDIAEIGARFEGVDSIIEELLGPQGYLRRATFGKIMEDITGFA
sp Q6RG02 VIT_FENME	RAVNLKMKADVDETHMDIAEIGARFVGIESIIEEPLGPQGYLRTATFGKIMEDITGFA
	*:: .:: ::::**. *:: :: :** .*:: :: *:
 1	

tr|Q1L7X1|Q1L7X1|PORTR tr|Q5XXZ5|Q5XXZ5_CHAFE tr|S4S8K7|S4S8K7|EXCCA tr|A3R8F7|A3RR97|HOMAM tr|Q7Z1G4|Q7Z1G4|METEN tr|Q9NDS1|Q9NDS1|PENJP tr|Q21G91|Q21G91|PENMO tr|A1XFT3|A1XFT3_FENCH OKNWSTIKOELEVATRERRSVDYAALESIISKLYGPHYGKFOADFFARFLGOEINYASLS QQNMSTIRQELEVAIRERRTVDDATIESIISKLYGPQYGGPQADFFARFLGGEINYASLS EETFNKINERLQGSFRQRRSIDLSQISHLFDKLYGNRH-MQKADFYARINNQEMAFGSLM EQKGNKILEKLQQDLRQKRSIDLSTLINFLGNLYSDQSRVTRADVFARFMGGEITFASLA EEKGYKVMEHLENTLRTRSIDASTIADFFNKLYGERASDVRAEVFARIMGGEVTYANIA EEKGLKVMEHIKQTLRTKRSIDSSVISDFFGKLYGEGRSHTHAEVFARIMGEITYADVA GEKGLKVMEHIKHTLRTRSIDSSVISDFFGKLYGEGRSHVHADLFARFMGHEITYADVA GEKGLKVMEHIKGTLRTRRSIDSSVIADFFSKLYGESRSHTHAELFARFMGHEITYADVA sp|Q6RG02|VIT_FENME tr|Q1L7X1|Q1L7X1 PORTR DNLODINIOHLVEASVRYLKRMLSSLKNMDLDMVKA-AOLGVDYSL-PTIOGTPLKMKLE tr |Q11.7X1 |Q11.7X1 PORTR
tr |Q5XX25 |Q5XX25 CHAFE
tr |S4SBK7 |S4SBK7 EXOCA
tr |A3RKP7 |A3RKP7 HOMAM
tr |Q721G4 |Q721G4 METEN
tr |Q9NDS1 |Q9NDS1 PENJP
tr |Q21691 |Q21691 PENMO
tr |A1KFT3 |A1KFT3 FENCH EHLQDINMHILVEASVRCPMQMLSSIKNVNLDKVKA-VQLGVDVSF-PTIQGTPLKITSE GNMKNIKMEELINNMFDSFDDMINRAAENNLDTVRA-AQLYLDYHL-PTMQGLPLKMKLE GDLKHISADEMIESFFSYFDDIPPQMKNLAHQFQHERLKLTLDYHLSPPSRAYHSKLQLN ETLKGVTADKIIETFFSLVDDSLEELKGLNLNTSKELLKLHLYYSL-PTIOGLHSRSPWK EILMGVTADKILEIFFSLVDDSLEELMGINLMTSKELLMKHLIISL-FIIGGTPSKSWM
ESLKGVTADTLIETFFSFEENSLEHMKDLNLNTART-AQLYMDYSL-PTIGGTPLKIKLA
ESLKGVTADTLIETFFSFFENSLEHMKDLNLNTART-AQLSMDYSL-PDIGGTPLRLKLA sp|Q6RG02|VIT_FENME tr|01L7X1|01L7X1 PORTR TVAVAGIKMOTNLNGL--FSGOGSSGSLFKILPSFSVETHGFIGYDAYISKSGLKMNTTV tr|Q5XXZ5|Q5XXZ5_CHAFE tr|S4SBK7|S4SBK7_EXOCA tr|A3RKP7|A3RKP7_HOMAM tr|Q7Z1G4|Q7Z1G4 METEN tr|Q9NDS1|Q9NDS1 PENJP tr|Q2I691|Q2I691 PENMO tr|A1XFT3|A1XFT3_FENCH sp|Q6RG02|VIT_FENME

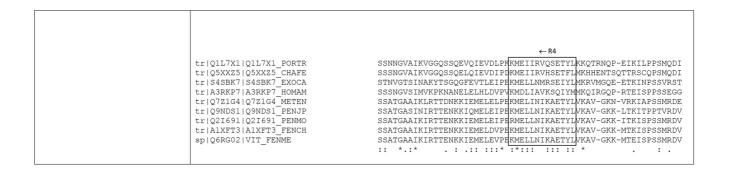


Fig. S1. Multiple alignment of VTG amino acid sequences from nine different crustacean sources. The sequences were chosen from the GenBank SwissProt database and aligned using Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/). The figure shows the alignments based upon which the CODEHOP primers have been designed. An asterisk (*) denotes identical residues; a colon (:) denotes a conserved residue substitution; a full stop (.) denotes partial conservation of the residue. The arrows above the amino acid sequences indicate the position of the sense ($F \rightarrow$) and antisense ($F \rightarrow$) primers chosen from a group of candidate primers obtained from the j-CODEHOP programme. The sequences of the sense and antisense primers, shown inside the boxes, were obtained from the Clustal Omega alignments

Clustal Omega alignment				
P.elephas	TL-PTQLRSRPLAQGPLPVEESGSVCLQEIKDGIYWSITCEDE	42		
QDE13857.1	GEKVVVTKLKNHRFCQDHYANRAETPKAWMKAPLPMEESYSECKQEITNGIYTSITCKDK	112		
AB009863.1	GHKVIVKKEKNHRLCKKRYPTPAETQMPWLKGPLPLEESECRCKQEITNGIYSSIICHDK :.***:*** . * ***.:*** *.*:	240		
P.elephas	NVIRPSYGAYKYVEANQESTT	63		
QDE13857.1	NVIKPAYGSYKYVEAHQESVLRFQSETDQIPPSVSQLPSRFIRKTLRYDQHTLKKDPSMA	172		
AB009863.1	<pre>KVVRPSYGAYKYVEAKQESTLRYVSLSSQQPPAIPQ—GSLVRKSLRYSYHTLNKDPSMV :*::*:**:******.</pre>	298		
QDE13857.1 AB009863.1	Macrobrachium potiuna (69.39% identity) Homarus americanus (67.92 % identity)			

Fig. S2. Alignment of VTG *P. elephas* amino acid sequences with homologous VTG sequences from GenBank. Identity and similarity percentages are shown at the top of each Clustal Omega alignment panel. Numbers on the left indicate the positions of the amino acids in each protein. An asterisk (*) denotes identical residues; a colon (:) denotes a conserved residue substitution; a full stop (.) denotes partial conservation of the residue.

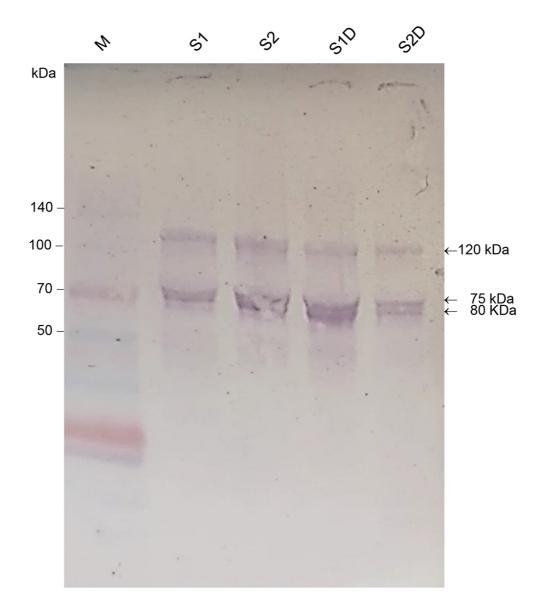


Fig. S3. Results of Western blot analysis using anti-VTG2 antibody. An aliquot of all homogenate samples with concentration of VTG120-180 ng / mL were pooled into one sample (S1). Similarly, an aliquot of all samples with a concentration of VTG 200-260 ng / mL (S2) was pooled. The same S1 and S2 samples were also digested with the subtilisin protease (S1D and S2D samples). Western Blot profiles did not highlight particular differences between the samples treated and untreated with the protease. The different concentrations of VTG detected by ELISA were also not reflected in the number of immunoreactive bands to the anti-VTG2 antibody. The arrows (←) indicate the presence of an immunoreactive band of about 120 kDa and also two very close bands between 80-75 kDa.