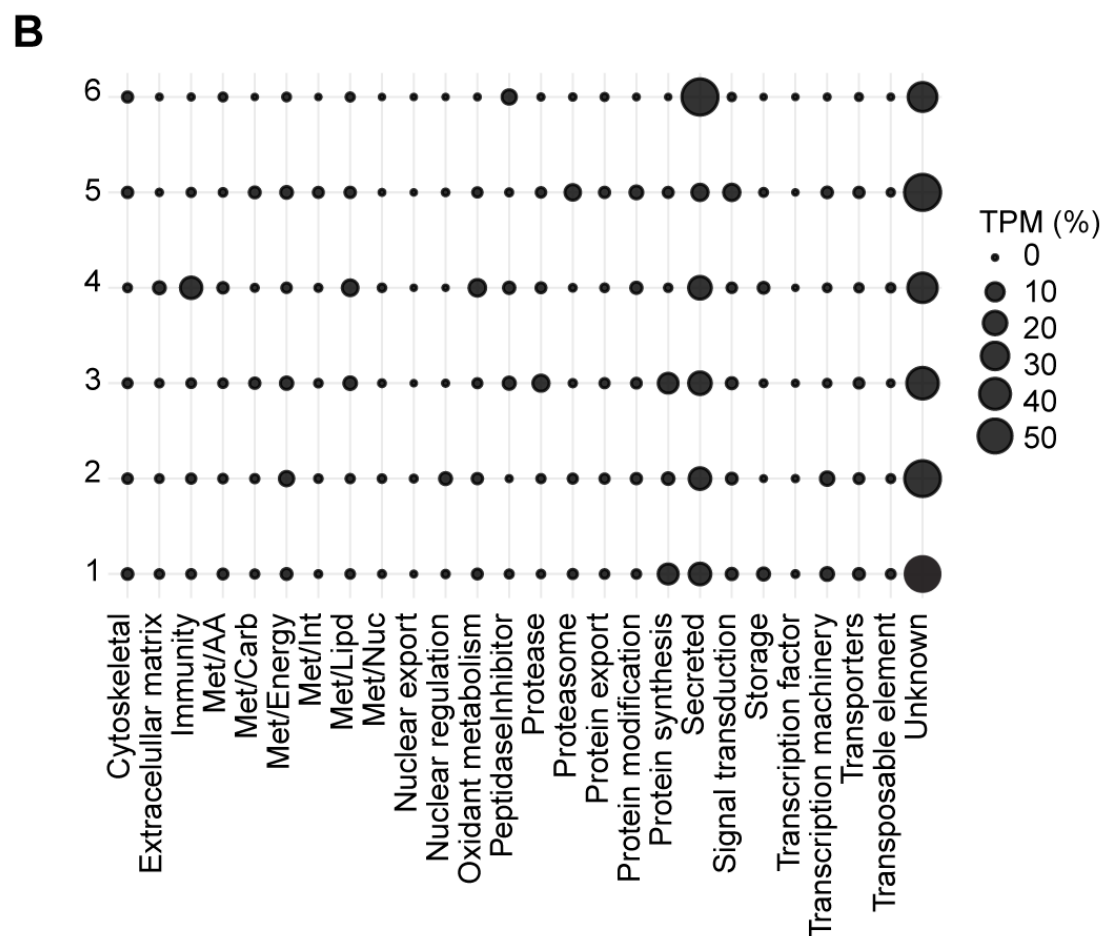
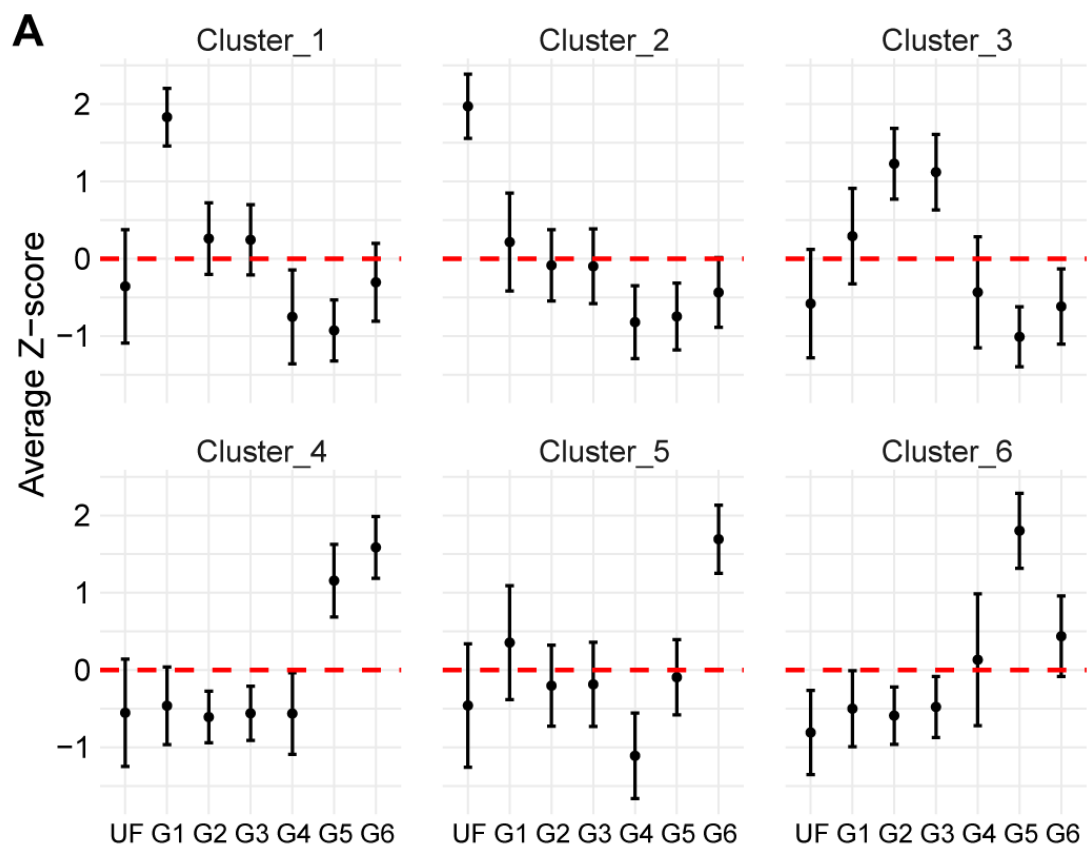


Supplementary table 1: Mapping rates of the trimmed Illumina reads to the putative CDS obtained from the *de novo* assembly of *A. americanum* midgut transcripts.

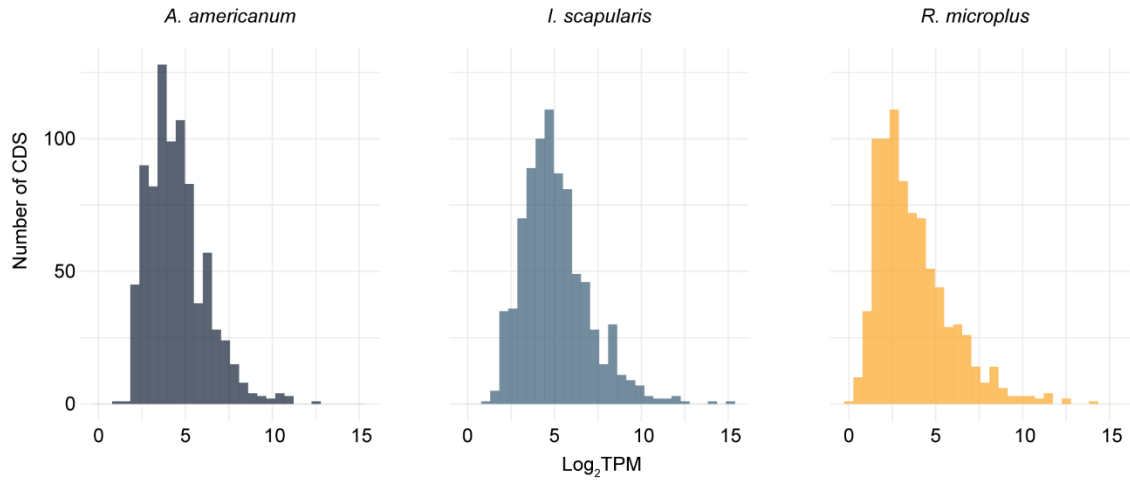
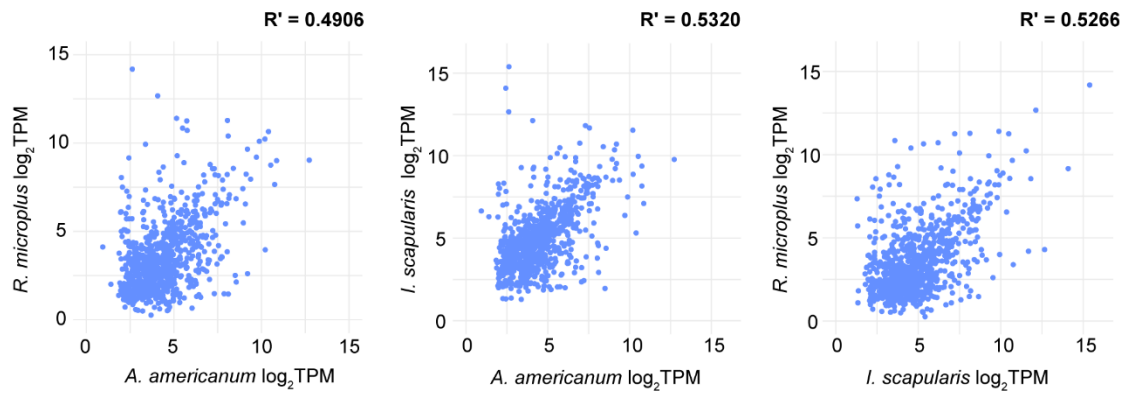
Sample	Reads not aligned	Reads aligned	Total reads	Reads aligned (%)
UF.A	28,710,730	26,258,152	54,968,882	47.76
UF.B	30,391,122	25,576,442	55,967,564	45.69
UF.C	27,004,578	23,953,610	50,958,188	47.00
G1.A	22,386,518	20,958,188	43,344,706	48.35
G1.B	23,009,394	21,885,922	44,895,316	48.74
G1.C	24,845,561	21,745,039	46,590,600	46.67
G2.A	36,856,265	33,889,303	70,745,568	47.90
G2.B	26,483,883	26,363,995	52,847,878	49.88
G2.C	27,136,423	25,931,353	53,067,776	48.86
G3.A	21,878,033	20,861,427	42,739,460	48.81
G3.B	20,460,881	19,995,267	40,456,148	49.42
G3.C	21,699,051	20,822,443	42,521,494	48.96
G4.A	19,853,516	22,085,700	41,939,216	52.66
G4.B	25,514,147	27,272,865	52,787,012	51.66
G4.C	22,419,683	26,471,151	48,890,834	54.14
G5.A	27,697,473	28,030,731	55,728,204	50.29
G5.B	24,356,043	26,071,825	50,427,868	51.70
G5.C	29,489,917	30,514,159	60,004,076	50.85
G6.A	25,198,802	25,094,764	50,293,566	49.89
G6.B	30,571,143	29,736,863	60,308,006	49.30
G6.C	23,382,954	24,569,078	47,952,032	51.23

Supplementary table 2: Benchmarking of Universal Single Copy Orthologs (BUSCO) statistics for *A. americanum* midgut at each feeding stage.

Sample	Complete (%)	Single (%)	Duplicate (%)	Fragmented (%)	Missing (%)
UF	60.6	54.9	5.7	4.9	34.5
G1	66.9	59.8	7.1	5.2	27.9
G2	64	57.5	6.5	4.9	31.1
G3	64.1	57.5	6.6	4.9	31
G4	52.1	46.4	5.7	4.1	43.8
G5	52.4	47.2	5.2	4.3	43.3
G6	59.3	53.6	5.7	4.8	35.9



Supplementary figure 1: Unsupervised clustering of transcripts identified in *A. americanum* midgut at different feeding stages. **(A)** The dots represent the average Z-score of the transcript per million (TPM) from the transcripts within each cluster and the error bars represent the standard deviation of the mean. The red dotted line marks the zero position in the y-axis. **(B)** Bubble plot representing the abundance of the transcript functional class identified in each cluster.

A**B**

Supplementary figure 2: Comparison of the shared transcripts within the midgut of *A. americanum*, *I. scapularis*, and *R. microplus* during the slow-feeding stage. **(A)** Histograms of the 823 shared transcripts among the three tick species based on their Log₂TPM values. **(B)** Scatter plot of the Log₂TPM of the 823 shared transcripts in the midgut of slow-feeding ticks. The Pearson correlation coefficient (R^2) was estimated of each pair-wise comparison.

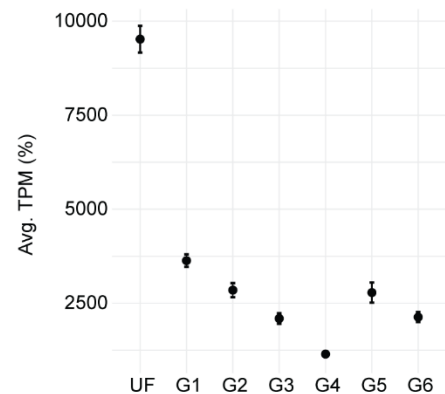
A

		*	20	*	40	*	
Amseq_4908 :	MAATQPRQNYHIDCEARINKQINMELYASYVYTS	MAYYFDRDDVALPGFH	:	50			
Dsilvarum :	MAATQPRQNYHVDCEARINKQINMELYASYVYTS	MAYYFDRDDVALPGFH	:	50			
Rsanguineu :	MAATQPRQNYHVDCEARINKQINMELYASYVYTS	MAYYFDRDDVALPGFH	:	50			
Acajennens :	MAATQPRQNYHVDCEARINKQINMELYASYVYTS	MAYYFDRDDVALPGFH	:	50			
Aparvum :	MAATQPRQNYHIDCEARINKQINMELYASYVYTS	MAYYFDRDDVALPGFH	:	50			
Aaureolatu :	MAATQPRQNYHIDCEARINKQINMELYASYVYTS	MAYYFARDDVALPGFH	:	50			
		60	*	80	*	100	
Amseq_4908 :	KFFFKCSSEEREHADKLMKYQKMRGGRVVLQPIQKPSQDEWGTGLEAMQA	:	100				
Dsilvarum :	KFFFKCSSEEREHAEKLMKYQNMGRGVVLQPIQKPAQDEWGAGLDAMQA	:	100				
Rsanguineu :	KFFFKCSSEEREHAHKLMKYQNMGRGVVLQPIQKPSQDEWGAGLDAMQA	:	100				
Acajennens :	KFFFKCSSEEREHADKLMKYQNMGRGVVLQPIQKPSQDEWGTGLEAMQA	:	100				
Aparvum :	KFFFKCSSEEREHADKFMKYQNMGRGVVLQPIQKPSQDEWGTGLEAMQA	:	100				
Aaureolatu :	KFFFKCSSEEREHADKLMKYQNMGRGVVALQPIQKPSQDEWGTGLEAMQA	:	100				
		*	120	*	140	*	
Amseq_4908 :	ALELEKAVNQSLDLHLKLATDHNDQ-----	:	126				
Dsilvarum :	ALELEKTVNQSLDLHLKLATDHNDQQLCDFLESEYLAEQVKS	IKELSDYV	:	150			
Rsanguineu :	ALELEKTVNQSLDLHLKLATDHNDQQLCDFLESEYLAEQVKAIKELSDYV	:	150				
Acajennens :	ALELEKTVNQSLDLHLKLATDHNDQQLCDFLESEYLAEQVKAIKELSDYV	:	150				
Aparvum :	ALELEKTVNQSLDLHLKLATDHNDQQLCDFLESEYLAEQVKAIKELSDYV	:	150				
Aaureolatu :	ALELEKTVNQSLDLHLKLATDHNDQQLCDFLESEYLAEQVKAIKELSDYV	:	150				
		160	*				
Amseq_4908 :	-----	:	-				
Dsilvarum :	TNLKRVGPGLGEYMFDKETLSD	:	172				
Rsanguineu :	TNLKRVGPGLGEYMFDKETLSD	:	172				
Acajennens :	TNLKRVGPGLGEYMFDKETLSD	:	172				
Aparvum :	TNLKRVGPGLGEYMFDKETLSD	:	172				
Aaureolatu :	TNLKRVGPGLGEYMFDKETLGD	:	172				

B

>Amseq_49087 putative ferritin, type-1
GGAATAAGCAGCTCCTGGAACAGCGGCCGACGGTATAAAA
GCGCGCTCCCGCCATTTCCGCCATGTTTTGGTCGATGTTA
TGCTTCAACAGTGATTGAACGAGCATCGCCAACTGTTTCA
CTCTCTCCGAACCTCTCCCAAATCCTTCTTGAATACAAGTA
CAGTTTGAGATTCCGGACGGGAACAAGACGACGAAGATGGC
CGCCACTCAGCCCCGCCAAAACCTACCACATCGATTGCGAGG
CCCGCATCAACAAGCAGATCAACATGGAACCTACGCGAGC
TACGTCTACACTTCAATGGCCTACTACTTCGACCGTGACGA
TGTGGCCCTGCCGGGATTTTCAAGTTCTTCAAGAAGTGTA
GTGAGGAGGAGCGTGAGCATGCCGATAAGCTGATGAAGTAC
CAGAAAATGCGTGAGGGGCGTGTGTGCTGCACGCCAATCCA
GAAGCCGTCGAGGACGAATGGGGCACTGGCCTGGAGGCTA
TGCAGGCCGCTCTGGAGCTGGAGAAGGCTGTCAACAGTCG
CTGCTTGACCTGCACAAGCTGGCTACTGACCACAACGATGC
TCAG

C



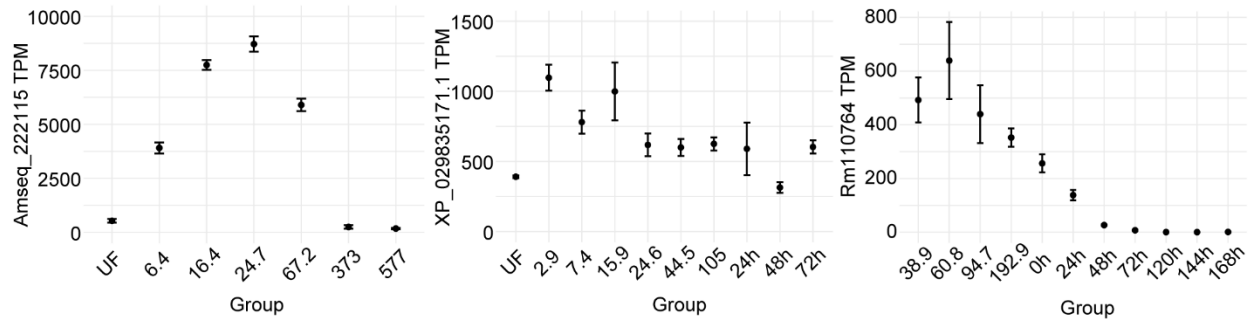
Supplementary figure 3: Primary sequence analysis of the putative type 1 ferritin

(Amseq_49087) identified in the midgut of unfed adult females *A. americanum* ticks. **(A)** Amino acid alignment of Amseq_49087 with other putative type 1 ferritins from *A. cajennenses* (JAC20482.1), *A. parvum* (JAC25210.1), *A. aureolatum* (JAT93262.1), *R. sanguineus* (AAQ54715.1) and *D. silvarum* (XP_037568710.1). **(B)** Nucleotide sequence of Amseq_49087. The iron-responsive element (IRE) is highlighted in blue and the start codon in red. **(C)** Transcriptional kinetic of Amseq_49087 in the midgut of adult females *A. americanum* ticks at different feeding stages. The dots represent the average transcript per million (TPM) in each biological condition and the error bars represent the standard deviation of the mean.

A

		*		20		*		40		*	
Amseq_2221 :	--MAR---	SVSVVAVLAVCI	-AACVASITGGWSA	EHQSSPKYKELAHYA	:	44					
Rm110764 :	MASFRIAPRGFAV	-LIVICLLGAARSVLV	GGWHRSVGDNAMFEELAHFA	:	49						
XP_0298351 :	MGVFS---	TSVVLLSLAVCG	-TLAGRSLPGGWRSRD	VYSDPAYAELAHYA	:	46					
		60		*		80		*		100	
Amseq_2221 :	VAQRTEGLENYD	TVLELTRVETQIVAGV	NYRLTFTIAGSECKIGE	IYSE	:	94					
Rm110764 :	ISRLVGDREYID	SVLELFDVKTQVVAGT	NYRIKFKVGESTCRVT	TET-YTK	:	98					
XP_0298351 :	VSSQAGDSEFYD	TVLELLELEVETQVVAG	MNYRLKFTAETACKVC	VDEYSR	:	96					
			*		120		*		140		
Amseq_2221 :	ERCPPK-ENVAKAT	CTAVVYEKPWONLRS	VTSFTCO-----	:	129						
Rm110764 :	EACVPHSRETVK	DTCTAFIYYVPWLNER	SVSSFTCEGSSTST	:	140						
XP_0298351 :	ERCLPK-VNLPKAT	CTAVVYERPWONHRE	VTSYECV-----	:	131						

B



Supplementary figure 4: Comparison of putative type-2 cystatins abundant during the slow-feeding phase of *A. americanum*, *I. scapularis* and *R. microplus* adult females. **(A)** amino acid alignment of Amseq_222115 (*A. americanum*), XP_029835171.1 (*I. scapularis*) and Rm110764 (*R. microplus*). Identical (black) and similar (gray) residues are highlighted. **(B)** Transcriptional profile of the three cystatins during the different feeding stages of adult female ticks. Data from XP_029835171.1 and Rm110764 were obtained from [20,21]. Groups represent unfed (UF), partially fed ticks grouped by their average weight (mg) or hours post-detachment (0h – 168h).

			*		20		*		40		*																																									
seqSigP -25 :	M	A	I	N	Y	C	I	L	L	T	L	F	A	V	A	F	A	Q	R	N	A	I	C	R	L	P	P	D	E	G	V	C	R	A	S	I	P	R	F	Y	F	N	P	A	E	G	K	C	S	F	:	50
Q8WPI3.1 :	--	M	K	Y	L	I	L	L	A	V	L	G	T	A	F	A	Q	R	N	G	F	C	R	L	P	A	D	E	G	I	C	K	A	L	I	P	R	F	Y	F	N	T	E	T	G	K	C	T	M	:	48	

			60		*		80		*		100																																									
seqSigP -25 :	F	I	Y	G	G	C	E	G	N	E	N	N	F	E	T	I	E	E	C	E	K	T	C	G	E	P	E	R	S	S	D	F	E	G	A	D	F	E	T	G	C	A	P	K	P	Q	R	G	F	C	:	100
Q8WPI3.1 :	F	S	Y	G	G	C	G	G	N	E	N	N	F	E	T	I	E	D	C	Q	K	A	C	G	A	P	E	R	V	S	D	F	E	G	A	D	F	K	T	G	C	E	P	A	A	D	S	G	S	C	:	98

			*		120		*		140																																				
seqSigP -25 :	K	G	F	L	D	H	W	F	F	N	V	T	S	G	E	C	E	M	F	L	Y	S	G	C	G	G	K	D	N	N	E	S	K	E	E	C	E	I	A	C	K	L	T	:	144
Q8WPI3.1 :	A	G	Q	L	E	R	W	F	Y	N	V	R	S	G	E	C	E	T	F	V	Y	G	G	C	G	N	D	N	Y	E	S	E	E	E	C	E	L	V	C	K	N	M	:	142	

Supplementary figure 5: Amino acid alignment of seqSigP-25462 from *A. americanum* and boophilin (Q8WPI3.1) from *R. microplus*. Identical (black) and similar (gray) residues are highlighted.

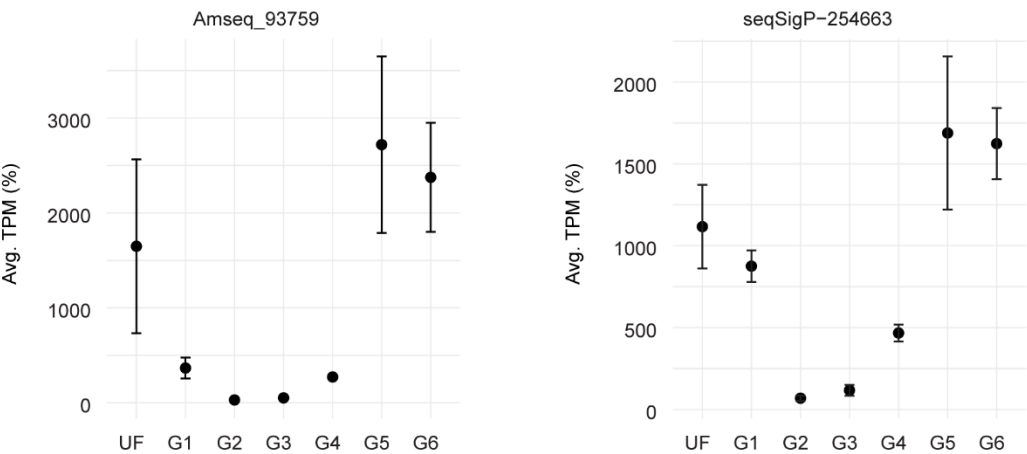
		*	20	*	40	*					
Amseq_2457 :	--MPVTPG	STAKLIAL	ISTLFYVA	-FAQSDA	QCGVPE	IIPPQ	LDPEDRIYG : 47				
P00766 :	-----	MNF-	IWL	LSYCALL	GTAFG	CGVP	AIQPVLSGLSRIVN : 36				
XP_0421439 :	-----	MKR---	FIVLS	LLLVP	TL	SKYK	GKCGKPEI	PPDTDEGDRIIG : 39			
Rm131147 :	MVISLV	VGMRDL	-ALLLT	LCSLA	LAQGN	NAKCG	APSI	PPMLDPEDRIYG : 49			
		60	*	80	*	100					
Amseq_2457 :	GSTAVP	GSWPWQ	AGIY	TNSY	QHFCG	GALIN	DYVITA	AHCVWSKLSNTVR : 97			
P00766 :	GEEAVP	GSWPWQ	VSLQ	DKTGF	HFCG	SLIN	ENWV	VTAAHCGVT	TSDVVV- : 85		
XP_0421439 :	GRTAV	GGSWPW	QVG	IY	TKY	SHFCG	GALIS	DQHVL	TAAHCVWERMATDIN : 89		
Rm131147 :	GYSAVP	GSWPWQ	AGVY	THRY	QHFCG	GALIN	DYIV	TAAHCVWSRMSTSVR : 99			
		*	120	*	140	*					
Amseq_2457 :	VHLGSH	TRLATD	DEAVY	KVEEV	CAHP	RENPT	KG--	NNRDIA	IVKLRESV : 145		
P00766 :	--AGEF	DQGS	SSEKI	QKLK	IAKV	FKNS	YNS---	LTIN	NDITLLKLSTAA : 130		
XP_0421439 :	VHLGSH	QRLAMD	STEVI	LVGV	DDVC	VT	PNERT	TGKS	AGFEDIAILKLSRRV : 139		
Rm131147 :	VHLGAY	ARRALD	NTEVI	YKVEE	VAHLN	YKPS	KR--	NSSDIA	IIKLDPV : 147		
		160	*	180	*	200					
Amseq_2457 :	NFTNTI	SPVCLP	SHKEEL	PVGS	KLYV	TGWC	AT	TRTRIL	KKS	AELKQAMTE : 195	
P00766 :	SFSQTV	SAVCLP	SASDD	FAAG	TTCV	TGWL	TRY	TNANTP	-DRL	QQASLP : 179	
XP_0421439 :	NTSATV	QFVCLP	PASGO	ELAS	GSELY	ITGW	GAT	KAKNM	KSKS	LELKQLLTT : 189	
Rm131147 :	TFTRTI	SPVCLP	SHREEL	PLGS	KYYV	TGWC	ST	TRGKF	IEKS	SRELKQAITE : 197	
		*	220	*	240	*					
Amseq_2457 :	ELRD--	EN---	CPHVN	PDVM	CASHT	EGSS	CF	GDSG	GPV	VHQR	NETWTLEF : 240
P00766 :	LLSNTN	CKKYWG	TKIKD	AMIC	CAGAS	GVSS	CM	GDSG	GPLV	CKKNG	AWTLVG : 229
XP_0421439 :	AIPTKE	CSDRD	GVPA	PE	SRE	CGTH	RS	GSSC	YGDS	GGPV	VHKANGTWTLHG : 239
Rm131147 :	ELPQNE	SA---	CSMM	PE	LL	CGTH	DY	GSSC	FGDS	GGPLV	HRKNGIWTLYG : 244
		260	*	280	*	300					
Amseq_2457 :	IVSGGP	WTCGD	ASDS	PLYFA	KVSHY	MDSF	IL	PYMD	PKTP	PKAIR	RICTIT : 290
P00766 :	IVSWG	SSTC	STST	--PG	VYAR	VTAL	VNVV	QOTL	AN--	-----	----- : 263
XP_0421439 :	IVTGG	PIVCG	SNA-D	PLFF	AKTS	SY	YVDH	FIQ	PYMD	PRTP	RRDIRKICRLT : 288
Rm131147 :	LVTSG	PYTCG	DASD	YPLIF	ARIS	HYMD	NFI	APYMD	PKTP	PRKE	IRRICTIT : 294

Supplementary figure 6: Amino acid alignment of serine peptidases from *Bos taurus* (P00766), *I. scapularis* (XP_042143948.1), *A. americanum* (Amseq_245718) and *R. microplus* (Rm131147). Identical (black) and similar (gray) residues are highlighted, while the putative catalytic residues are indicated by the red arrows.

A



B



Supplementary figure 7: (A) Amino acid alignment of *R. microplus* microplusin (GenBank: AY233212.1) and the predicted amino acid sequence of *A. americanum* microplusin-like transcripts (Amseq_93759 and seqSigP-254663). Identical (black) and similar (gray) residues are highlighted. **(B)** Transcriptional profile of Amseq_93759 and seqSigP-254663. Dots represent the average TPM at each feeding stage, while error bars represent the standard deviation of the mean.

Supplementary file 1: Windows-compatible hyperlinked Excel file containing the functional annotation of the 15,999 putative CDS identified in *A. americanum* midgut at different feeding stages.

Supplementary file 2: Excel file containing the accession numbers of the shared transcripts within the midgut of unfed and slow-feeding *I. scapularis*, *R. micrplus* and *A. americanum* adult females.