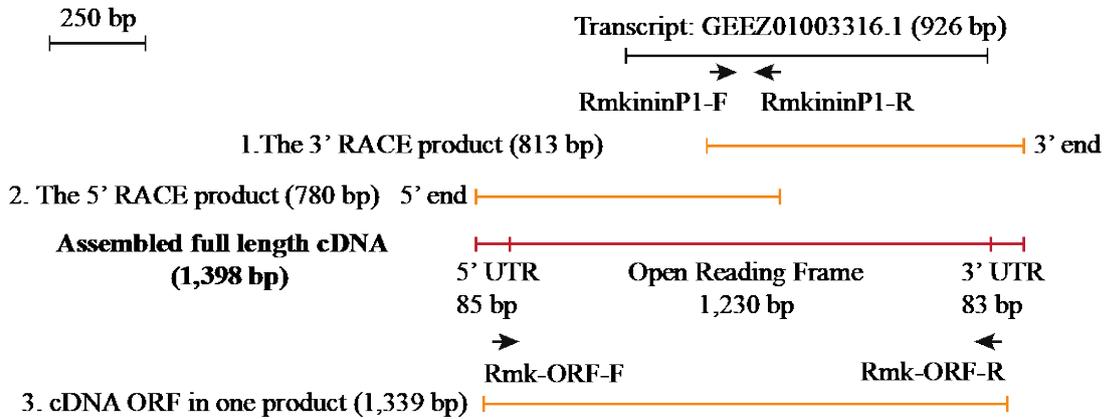
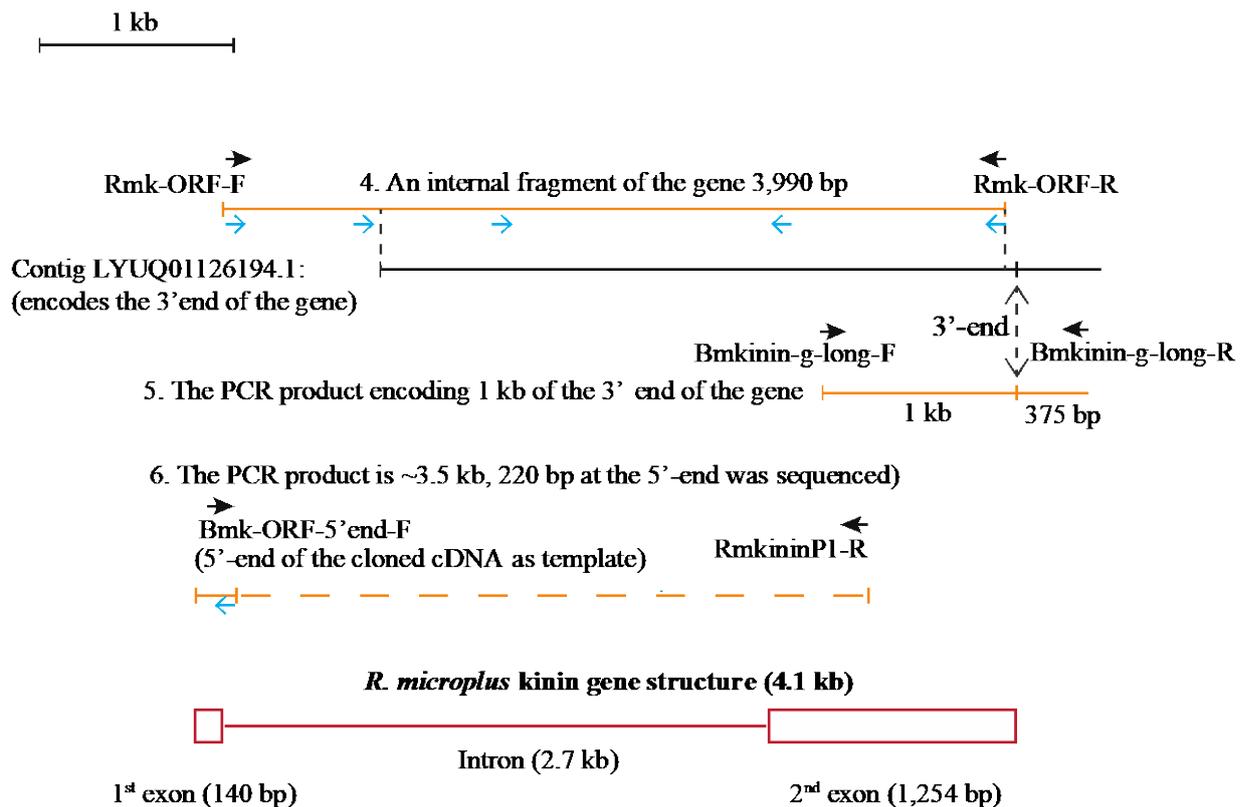


**Title: The cattle fever tick, *Rhipicephalus microplus*, as a model for forward pharmacology to elucidate kinin GPCR function in the Acari**

**A. The putative full-length kinin precursor cDNA**



**B. Amplification of the kinin gene from gDNA**



**Supplementary Figure 1. The workflow for cloning the putative kinin precursor cDNA from *Rhipicephalus microplus* and verification of the gene structure by sequencing genomic PCR products.**

In this figure, within the panels A and B, all lines representing sequences are aligned vertically to the corresponding sequence location. Notice a different scale in bp on the left of each panel.

The solid black lines represent either transcriptome (A) or genomic (B) sequences identified through local NCBI BLAST using the putative kinin precursor (amino acid sequence) from *Ixodes scapularis* as query. The black arrows denote the direction and relative location of the primers on the template above the primers. The solid orange lines depict the sequenced PCR products obtained in this study. Red lines and boxes represent the deduced full-length cDNA (A) or the kinin gene (B) obtained by assembling the sequenced PCR products.

The blue arrows denote the position of primers used for sequencing. The dashed orange line depicts the region of a PCR product which was not sequenced (sequencing was not necessary because the overlapping sequence of ~200 bp at the 5' end was enough to align this product with the PCR product in reaction 4 above). **(A) The putative full-length kinin precursor cDNA from *R. microplus*:** Cloning of 5'- and 3'- RACE PCR products. 1-2). A transcript predicted to encode a partial sequence of the putative kinin precursor was used to design a pair of gene-specific primers for the 3'- and 5'- RACE PCRs. The sequences of these 3'- and 5'- RACE PCR products (1-2) were aligned with DNASTAR (Lasergene, Madison, WI) to assemble the full-length cDNA. (3). A pair of primers was designed outside of the open reading frame (ORF) and were used to amplify the cDNA ORF in one product. **(B) Amplification of the kinin gene from genomic DNA.** (4). First, we amplified an internal fragment of the gene with a pair of gene-specific primers. (5). Secondly, the 3'-end of the kinin gene was amplified with a gene-specific forward primer and a reverse primer located outside of the ORF 3'-end. The sequence of this latter reverse primer was obtained from a genomic contig encoding the 3'-end fragment of the kinin gene (black line). (6). The 5'-end of the kinin gene was amplified using a forward primer which binds to the 5'-end of the cDNA, and with a reverse primer within the ORF. The DNA sequences from the PCR products above (4-6) were aligned with DNASTAR to obtain the sequence of the kinin gene shown in red: boxes represent exons, and the solid line represents the intronic region. The exons were identified by aligning the cDNA sequence with the gene sequence.

5' ACATGGGGaGCAACAGGCGCTGCTAGAGGCCCATCGACGGCAGCGGAAGCGTTGGCATCGCCATCGTGTCCAGCACAG 80  
5' CAGCCATGGGAACGGTACTGCACCGTCCGGGGGTGCTGGTGTACTTCCACCGTCCGGGAATCACTTGTGTGCTTCTT 160  
M G T V L H R R R G A A G A N F T V G E S L V L L L  
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25

5' GTCCTGGGGGTCAATGGCTTCCATTAGACGCCCTGGGCTCAGCAGTCTGGGCGAGCGACGAGCCCCGCTCCCTACTTTT 240  
V L G V I A F P L D A W A Q H V V G S D E A R S L L S  
26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52

5' AAGAGGTGGTGACACGTAATCCGCTGGAACATCTCGCCCGCCGCGCTGCAGCACATGCGTTCGAAAGCTTCAAGCGCC 320  
R G G D T L I R W N I S P A A L Q H M R S E S F K R  
53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78

5' AGTTCAGTCCATGGGGCGCAAGCGGAACGCCCGCATTCGAATCCCTGTGACGCAGCCGGCAATCAGGAGAGCCAC 400  
Q F S P W G G K R N A A A F E S L L D A A G N Q E S H  
79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100 101 102 103 104 105

5' CGGCACCGTCTGGCAGCGGATGCCTCGTACAAGTCCGTTTGCACCCGTGGACATCGCAGTGAGGGCCGCCGATTT 480  
R H R L A A S A D A S Y K V R R L H P V D I A V A D L  
106 107 108 109 110 111 112 113 114 115 116 117 118 119 120 121 122 123 124 125 126 127 128 129 130 131 132

5' GTTCAGTCCATGGGGCGGCAAAAGAACGGACGACAAGAAGGACAAAGACCAGACGTTCAACCCTTGGGGAGGCAAGAGGG 560  
F S P W G G K R T D D K K D K D Q T F N P W G G K R  
133 134 135 136 137 138 139 140 141 142 143 144 145 146 147 148 149 150 151 152 153 154 155 156 157 158

5' CAGGAGACCACTTCGGTTCCCTGGGGAGGCAAGAGGGACAGTTCAGCGCGTGGGGAGGCAAGCGGCAGCAGGACGCAAG 640  
A G D H F G S W G G K R D T F S A W G G K R Q Q D S K  
159 160 161 162 163 164 165 166 167 168 169 170 171 172 173 174 175 176 177 178 179 180 181 182 183 184 185

5' AACGCCTTCAGTCCGTGGGGAGGYAAGAGAGCCGTGAGAAGCCGACGGCCAGAAACGACGCCGCTAGGGCCAAGCAGGA 720  
N A F S P W G G K R A V R S P T A R N D A A R A K Q E  
186 187 188 189 190 191 192 193 194 195 196 197 198 199 200 201 202 203 204 205 206 207 208 209 210 211 212

5' GGACGGAGAGGAGGACGAAGAGCGGAGCTTCGCACCTTGGGGAGGAAAGAGGGGAACCGGGGAAGACCAGGCGTTTCTC 800  
D G E E D E E R S F A P W G G K R G T G E D Q A F S  
213 214 215 216 217 218 219 220 221 222 223 224 225 226 227 228 229 230 231 232 233 234 235 236 237 238

5' CATGGGGAGGAAAAAGGGGCGACGACGGGATACGTTTTCACGCCCTGGGGAGGCAAGCGCGATGACCGCTTCAATCCA 880  
P W G G K R G D D G D T S F T P W G G K R D D R F N P  
239 240 241 242 243 244 245 246 247 248 249 250 251 252 253 254 255 256 257 258 259 260 261 262 263 264 265

5' TGGGGAGGCAAGCGAGAAGGACCCTTCAGCCCTGGGGAGGTAAGAGGGATGGCTCGAACAAGGAAGGATTCTTCAACC 960  
W G G K R E G P F S P W G G K R D G S N K E G F F N P  
266 267 268 269 270 271 272 273 274 275 276 277 278 279 280 281 282 283 284 285 286 287 288 289 290 291 292

5' TTGGGGAGGCAAGCGCGGAGCAGACGACCCGTTCAACCCTTGGGGAGGCAAGAGGCAGGATTCTTCAATCCTTGGGGAG 1040  
W G G K R G A D D P F N P W G G K R Q D S F N P W G  
293 294 295 296 297 298 299 300 301 302 303 304 305 306 307 308 309 310 311 312 313 314 315 316 317 318

5' GCAAGAAGGAAGATGGAGTCTTCAGGCCCTGGGGTGGAAAGAAGGAAGACAACGTC TTTAGGCCCTGGGGAGGCAAAAAG 1120  
G K K E D G V F R P W G G K K E D N V F R P W G G K K  
319 320 321 322 323 324 325 326 327 328 329 330 331 332 333 334 335 336 337 338 339 340 341 342 343 344 345

5' GAGGGCAACGTGTTGGGCCCTGGGGAGGCAAAAAGAGGACGCGACACCGAGCCTTTCGTTGAGTAGGGCATTAAACTA 1200  
E G N V F G P W G G K R E D A T P S L S V S R A L N Y  
346 347 348 349 350 351 352 353 354 355 356 357 358 359 360 361 362 363 364 365 366 367 368 369 370 371 372

5' CGGTGGTCCAGCAAGCCATGACGTGGATGCAGGGTCTTCCGCAAGAAGCGAGATTCTTCTGCGAGCGAACAAAAGGGTA 1280  
G G P A S H D V D A G S L R K K R D S S A S E Q K G  
373 374 375 376 377 378 379 380 381 382 383 384 385 386 387 388 389 390 391 392 393 394 395 396 397 398

5' CGACGTCGAACGGTGAGAAAAGTGGGAGGACGTAACCACTGACCGAGTCACTGTAGCGAAGCGCCCGTTCGAAAAACAT 1360  
T T S N G E K S G R T  
399 400 401 402 403 404 405 406 407 408 409 410

5' CTCATCGCACGTCCCAAAAAAAAAAAAAAAAAAAAAA 1398

**Supplementary Figure 2. Cloned cDNA sequence and predicted amino acid sequence of putative kinin precursor from *Rhipicephalus (Boophilus) microplus*.**

The full-length cDNA sequence is 1,398 bp, with 1,227 bp ORF that encodes a 409 amino acid residue protein. The predicted enzyme cleavage sites are boxed. Seventeen kinin sequences were predicted. See Fig. 3.

Majority	----	LRXR-AXX-----	XXXLX-----	MSXXXLXQXVXXSDGDXXXSRXGDXXIRWNISPATLQHMRS--	ESFKR	
		10	20	30	40	50
O. turicata		--MAPAARSSQT-----	FCTLRYLILSISLSSAYASVEESDGRDDSDFEDMIRWNISPDTLQRLRARREAIAR			69
H. dromedarii		-----	-----	-----	-----	MRS--
D. variabilis		-----	-----	-----	-----	MRS--
A. sculptum		MG--LRRRAAG-----	ETLLRLRVLVSLSLDSVAQHMVGSDEARSLLSRGGDTLIRWNISPATLQHMRS--			ESFKR
R. sanguineus		MGTVLRRRGAAGANFAVGETLMLQLLVVIAFSSDAWAQHVVGSDARSLLSRGGDTLIRWNISPAALQHMRS--				ENFKR
R. microplus		MGTVLRHRRGAAGANFTVGESLVLVLLVGVIAFPLDAWAQHVVGSDARSLLSRGGDTLIRWNISPAALQHMRS--				ESFKR
Ix. ricinus		--MELRGHDAQELR-----	FLWTLTATTFLLMSMGLCQDVDSGSGDLGRSSRVGESFIRWNISPATLQHMRS--			ESFKR
Ix. scapularis-mRNA		--MELRGHDAQELR-----	FLWTLTATTFLLMSMGLCQDVDSGSGDLGRSSRVGESFIRWNISPATLQHMRS--			ESFKR
Ix. scapularis-gDNA		-----	MSMGLCQDVDSGSGDLGRSSRVGESFIRWNISPATLQHMRS--			ESFKR
Majority		QFSP-WGGKRN-AA	XDSLXAGXQXSRHLA	ADASYKVRRLHXGD-QPRD-DSFNPWGGKRXDXK-----		
		90	100	110	120	130
O. turicata		PFVPRWGNQIQG---	DAAVGKPYGN-----	YRDVWIYGK---KD-----		102
H. dromedarii		QFSP-WGGKRNAALESLLD	SPGNQESRRHLLAADASYKVRRLHPA--	GVRDADSFNPWGGKRTDDKKDKDQTFNPWGGK		85
D. variabilis		QFSP-WGGKRNAAFESLLD	SPGQQSHRHLAADASYKVRRLHHTG-SPRNADSF	-----P-----		64
A. sculptum		QFSP-WGGKRN-AAFDYLL	DAPGGD--H-QVADAASSYKVRVHHTP-SLRN-	-----		116
R. sanguineus		QFSP-WGGKRNAAFESLLD	SAGNQESRRHLLAADASYKVRRLHPASIGVRDADSF	SPWGGKRTDDKKDKDQTFNPWGGK		157
R. microplus		QFSP-WGGKRNAAFESLLD	AAGNQESRRHLLAADASYKVRRLHPVDIAVRAADL	FSPWGGKRTDDKKDKDQTFNPWGGK		157
Ix. ricinus		QFSP-WGGKRG--VLDQAL	PTAHLRSGPLYLYKALHSPGAMGERRGDKQDD-ET	FNPWGGKRENDK-----		133
Ix. scapularis-mRNA		QFSP-WGGKRG--VLDQAL	PTAHLRSGPLYLYKALHSPRAMGERRGDKQDD-EA	FNPWGGKRENDK-----		133
Ix. scapularis-gDNA		QFSP-WGGKRG--VLDQAL	PTAHLRSGPLYLYKALHSPRAMGERRGDKQPED-EA	FNPWGGKRENDK-----		110
Majority		-----	-----	-----	-----	DXERSFAPWGGKRG----
		170	180	190	200	210
O. turicata		-----	-----	-----	-----	AHFNWGGKRDGTHT
H. dromedarii		R-----	-----	-----	-----	DSVRVKQEDGEDEERSFAPWGGKRG----
D. variabilis		-----	-----	-----	-----	ERSFAPWGGKRG----
A. sculptum		-----	-----	-----	-----	VFAPWGGKRG----
R. sanguineus		RAAAGDRFGSWGKRETF	NAWGGKRQDD-KNSFSPWGGKRAVRSPTARNEDAAR	QDDGEEDEERSFAPWGGKRG----		232
R. microplus		R--AGDHFGSWGKRDTF	SAWGGKRQDDSKNAFSPWGGKRAVRSPTARN-DAARA	KQEDGEDEERSFAPWGGKRG----		230
Ix. ricinus		-----	-----	-----	-----	DKELSFNPWGGKRGTFSS
Ix. scapularis-mRNA		-----	-----	-----	-----	DKELSFNPWGGKRGTFSS
Ix. scapularis-gDNA		-----	-----	-----	-----	DKELSFNPWGGKRGTFSS
Majority		-XGEDXAFS-----	-----	-----	-----	PWGGKRXDDGD---TXFXPWGGKREDR---FNPWGGKRE
		250	260	270	280	290
O. turicata		RSGLDDSSG-----	-----	-----	-----	SWSEKK---D-----NFPWGGKKEAS---FNPWGGKRD
H. dromedarii		-AGEDQAFS-----	-----	-----	-----	PWGGKRDGD---TSFAPWGGKREDR---FNPWGGKRE
D. variabilis		-AAEDQAFS-----	-----	-----	-----	PWGGKRDSEED---TSFAPLGGKREDR---FNPWGGKRE
A. sculptum		-AAEDRAFS-----	-----	-----	-----	PWGGKRGDDGG---ETFAPWGGKREDR---FNPWGGKRE
R. sanguineus		-TGEDQAFS-----	-----	-----	-----	PWGGKRGDDGD---TSFTPWGGKREDR---FNPWGGKRE
R. microplus		-TGEDQAFS-----	-----	-----	-----	PWGGKRGDDGD---TSFTPWGGKRDDR---FNPWGGKRE
Ix. ricinus		WGGKRDFTG-----	-----	-----	-----	PWGGKRDFTGAWGGKRDFTGPWGGKRDQKESGFNPWGGKRE
Ix. scapularis-mRNA		WGGKRDFTG-----	-----	-----	-----	PWGGKRDFTGAWGGKRDFTGPWGGKRDQKESGFNPWGGKRE
Ix. scapularis-gDNA		WGGKRDFTGSWGKRDFTG	PWGGKRDFTGPWGGKRDFTGPWGGKRDFTG	PWGGKRDFTGPWGGKRDFTG	PWGGKRDFTG	PWGGKRDQKESGFNPWGGKRE
Majority		GPFPWGGKRE-----	GSNKEGFFNPWGGKRGADDS-FNPWGGKRE-----	EDSFNPWGGKXX--XDTAFXPWGGK		
		330	340	350	360	370
O. turicata		N-FNPWGGKRD-----	NFPWGGK--DSSI	FNPWGGKRS	DGSKDEL	SFNPWGGKRDQGVGTIFGPWGGK
H. dromedarii		GPFPWGGKRD-----	GSNKEGFFNPWGGKRGADDS-FNPWGGKRE-----	ADDSFNPWGGK--QD-SFNPWGGK		215
D. variabilis		GPFPWGGKRE-----	GSNKEGFFNPWGGKRGADDS-FNPWGGKRE-----	QDSFNPWGGK--ESGVFRPWGGK		178
A. sculptum		GPFPWGGKRE-----	GSNKEGFFNPWGGKRGADDS-FNPWGGKRE-----	ADDAFNPWGGKGG--ADAAFPWGGK		232
R. sanguineus		GPFPWGGKRD-----	GSNKEGFFNPWGGKRGADDS-FNPWGGKRE-----	QDSFNPWGGK--EDGVFRPWGGK		334
R. microplus		GPFPWGGKRD-----	GSNKEGFFNPWGGKRGADDP-FNPWGGKRE-----	QDSFNPWGGK--EDGVFRPWGGK		332
Ix. ricinus		DPFPWGGKKEKDN	AFSPWGGKREQNFNPWGGKKT	TKDSTFSPWGGKRE-----	EGFPNPWGGKGD--SDTAFAPWGGK	273
Ix. scapularis-mRNA		DPFPWGGKKEKDN	AFSPWGGKREQNFNPWGGKKT	TKDSTFSPWGGKRE-----	EGFPNPWGGKGD--SDTAFAPWGGK	263
Ix. scapularis-gDNA		DPFPWGGKKEKDN	AFSPWGGKRDQNFNPWGGKKT	TKDSTFSPWGGKRE-----	EGFPNPWGGKGD--SDTAFAPWGGK	280

Majority	RED-VFNPWGGKRD---DXVFSWGGKRED-----FGXXA---DXLEDAXSRKKRDSXSEHKGTTSXG	
	410      420      430      440      450      460      470      480	
O. turicata	REN-LFNPWGGKREKS--EMTFNPWGGKKSE-----LAFSPWM-----SASSRIKRDSTINTDMFIQHPK	274
H. dromedarii	REDGVFRPWGGKKD---DNVFRPWGGKKED-----NV--FGPWG---GKREDAGSLRKKRDSSTSEHKVTTSHG	276
D. variabilis	KEDKVFPGWGGKRD---DDVFGPWGGKREQVSSSSSHSAGRGFPFGGAT---EHGVDAESLRKKRDSMSEHQVTTSYS	251
A. sculptum	RQD-SFNPWGGKRE---DVFVFRPWGGKKED---N-----VFRPWG---GKKEDN-VFRPWGGKRQGD--GFKPWG	289
R. sanguineus	KEDNVFRPWGGKKE---DNVFAPWGGKREDALPS--LYVGRGFNYGGTA---SHGVDAGSLRKKRDSSTSEHKGTTSYG	405
R. microplus	KEDNVFRPWGGKKE---GNVFGPWGGKREDATPS--LSVSRALNYGGPA---SHDVDAGSLRKKRDSASEQKGTTSNG	403
Ix. ricinus	RDN-NFNPWGGKRDNGNKDSSFSPWGGKRES-----FGVQASDPDSLEDHSPSRNKRDSRRVPTKNSAR	338
Ix. scapularis-mRNA	RDN-NFNPWGGKRDNGNKDSSFSPWGGKRES-----FGVQALDPDSLEDHSPSRNKRDSRRVPTKNSAQ	328
Ix. scapularis-gDNA	RDN-NFNPWGGKRDNGNKDSSFSPWGGKRES-----FGVQALDPDSLEDHSPSRNKR	332
Majority	GKSGST---	
	490	
O. turicata	VQKASPRPL	283
H. dromedarii	GKSASA	282
D. variabilis	GKSGGT	257
A. sculptum	GKREEDT	296
R. sanguineus	GKSGST	411
R. microplus	EKSGRT	409
Ix. ricinus	SAISSVAKTF	348
Ix. scapularis-mRNA	STIRSVAKTF	338
Ix. scapularis-gDNA		332

**Supplementary Figure 3. Multiple amino acid sequence alignment of the deduced kinin precursors from eight tick species using Clustal W method by MegAlign (Lasergene, Madison, WI).**

There are two sequences from *Ixodes scapularis*, the first one was deduced from three transcriptome sequences (GBBN01023680.1, GGIX01408871.1, GBBN01027580.1), and the second was deduced from a genome scaffold (DS680282) (Gulia-Nuss and Caffrey, 2016). The amino acid sequence from the latter lacks 23 residues at the N- terminus and also 23 residues at the C-terminus. The sequence deduced from the transcriptome (*Ix. scapularis*-mRNA) misses some internal amino acids within positions 250-293.

Gulia-Nuss, M., Nuss, A.B., Meyer, J.M., Sonenshine, D.E., Roe, R.M., Waterhouse, R.M., Sattelle, D.B., de la Fuente, J., Ribeiro, J.M., Megy, K., Thimmapuram, J., Miller, J.R., Walenz, B.P., Koren, S., Hostetler, J.B., Thiagarajan, M., Joardar, V.S., Hannick, L.I., Bidwell, S., Hammond, M.P., Young, S., Zeng, Q., Abrudan, J.L., Almeida, F.C., Ayllon, N., Bhide, K., Bissinger, B.W., Bonzon-Kulichenko, E., Buckingham, S.D., and Caffrey, D.R., Caimano, M.J., Croset, V., Driscoll, T., Gilbert, D., Gillespie, J.J., Giraldo-Calderon, G.I., Grabowski, J.M., Jiang, D., Khalil, S.M., Kim, D., Kocan, K. M., Koci, J., Kuhn, R.J., Kurtti, T.J., Lees, K., Lang, E.G., Kennedy, R.C., Kwon, H., Perera, R., Qi, Y., Radolf, J.D., Sakamoto, J.M., Sanchez-Gracia, A., Severo, M.S., Silverman, N., Simo, L., Tojo, M., Tornador, C., Van Zee, J.P., Vazquez, J., Vieira, F.G., Villar, M., Wespiser, A.R., Yang, Y., Zhu, J., Arensburger, P., Pietrantonio, P.V., Barker, S.C., Shao, R., Zdobnov, E.M., Hauser, F., Grimmelikhuijzen, C.J., Park, Y., Rozas, J., Benton, R., Pedra, J.H., Nelson, D.R., Unger, M.F., Tubio, J.M., Tu, Z., Robertson, H.M., Shumway, M., Sutton, G., Wortman, J.R., Lawson, D., Wikel, S.K., Nene, V.M., Fraser, C.M., Collins, F.H., Birren, B., Nelson, K.E., Caler, E., Hill, C.A. (2016). Genomic insights into the *Ixodes scapularis* tick vector of Lyme disease. *Nat. Commun.* 7, 10507.



