CLUSTAL O(1.2.4) sequence alignment (SKK Lp Factor C sequence)

3 horseshoe crab species: Limulus polyphemus, Carcinoscorpius rotundicauda, Tachypleus tridentatus

```
Limulus             MVLASFLVSGLVLGLLAQQMHPVQSRGVDLGLCDDTRFECKCGDPGYVFNVPAKQCTYFY   60
Carcinoscorpius     MVLASFLVSGLVLGLLAQKMRPVQSKGVDLGLCDETRFECKCGDPGYVFNIPVKQCTYFY   60
Tachypleus          MVLASFLVSGLVLGILAQQMRPVQSRGVDLGLCDETRFECKCGDPGYVFNVPMKQCTYFY   60
                    **************:***:*:****:********:***************:* *******

Limulus             RWRPYCKPCDKLEAKDVCPKYKRCQECRAGLDSCVSCPPNKYGTWCSGECQCKNGGICDQ   120
Carcinoscorpius     RWRPYCKPCDDLEAKDICPKYKRCQECKAGLDSCVTCPPNKYGTWCSGECQCKNGGICDQ   120
Tachypleus          RWRPYCKPCDDLEAKDICPKYKRCQECKAGLDSCVTCPPNKYGTWCSGECQCKNGGICDQ   120
                    **********.*****:**********:*******:************************

Limulus             RTGACTCRDRYEGVHCEILQGCPLLQSDPQVQEVKNPPNDPQTIDYSCSPGFKLKGVARI   180
Carcinoscorpius     RTGACACRDRYEGVHCEILKGCPLLPSDSQVQEVRNPPDNPQTIDYSCSPGFKLKGMARI   180
Tachypleus          RTGACTCRDRYEGAHCEILKGCPLLPSDSQVQEVRNPPDNPQTIDYSCSPGFKLKGVARI   180
                    *****:*******.*****:***** ** *****:***::****************:***

Limulus             TCLPNGQWSSFPPKCIRECSMVSSLEHGKVNSPSADLIEGATLRFSCDSPYYLIGQETLT   240
Carcinoscorpius     SCLPNGQWSNFPPKCIRECAMVSSPEHGKVNALSGDMIEGATLRFSCDSPYYLIGQETLT   240
Tachypleus          SCLPNGQWSSFPPKCIRECAKVSSPEHGKVNAPSGNMIEGATLRFSCDSPYYLIGQETLT   240
                    :********.*********: *** ******: *.::**********************

Limulus             CQGNGQWSGQIPQCQKLVFCPDLDPVSHAEHQVKIGLEQKYGQFPQGTEVTYTCTGNYFL   300
Carcinoscorpius     CQGNGQWNGQIPQCKNLVFCPDLDPVNHAEHKVKIGVEQKYGQFPQGTEVTYTCSGNYFL   300
Tachypleus          CQGNGQWSGQIPQCKKLVFCPDLDPVNHAEHQVKIGVEQKYGQFPQGTEVTYTCSGNYFL   300
                    *******.******::**********.****:****:****************:*****

Limulus             MGLDTLKCNPDGSWSGTQPSCVKVADREVNCDSKAVDFLDDVGEPVRIHCPAGCSLTAGT   360
Carcinoscorpius     MGFDTLKCNPDGSWSGSQPSCVKVADREVDCDSKAVDFLDDVGEPVRIHCPAGCSLTAGT   360
Tachypleus          MGFNTLKCNPDGSWSGSQPSCVKVADREVDCDSKAVDFLDDVGEPVRIHCPAGCSLTAGT   360
                    **::************:************:*****************************
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```
Limulus              VWGTAIYHELSSVCRAAIHAGKVPNSGGAVHVVNNGPYSDFLASDLNGIKSDELKSLAQS   420
Carcinoscorpius      VWGTAIYHELSSVCRAAIHAGKLPNSGGAVHVVNNGPYSDFLGSDLNGIKSEELKSLARS   420
Tachypleus           VWGTAIYHELSSVCRAAIHAGKLPNSGGAVHVVNNGPYSDFLGSDLNGIKSEELKSLARS   420
                     **********************:******************.********:******:*

Limulus              FRFDYVSSSTAGRKSGCPDGWFEIEENCVYVTSKQRAWERAQGVCTNMAARLAVLDKDVI   480
Carcinoscorpius      FRFDYVRSSTAG-KSGCPDGWFEVDENCVYVTSKQRAWERAQGVCTNMAARLAVLDKDVI   479
Tachypleus           FRFDYVSSSTAG-RSGCPDGWFEVEENCVYVTSKQRAWERAQGVCTNMAARLAVLDKDLI   479
                     ****** ***** :*********::*********************************:*

Limulus              PSSLTETLRGKGLATTWIGLHRLDADNHFIWELMDRSSVALNDSLTFWAPGEPGNETNCV   540
Carcinoscorpius      PNSLTETLRGKGLTTTWIGLHRLDAEKPFIWELMDRSNVVLNDNLTFWASGEPGNETNCV   539
Tachypleus           PSSLTETLRGKGLTTTWIGLHRLDAEKPFVWELMDRSNVVLNDNLTFWASGEPGNETNCV   539
                     *.***********:***********:: *:*******.*.***.***** **********

Limulus              YLDIQDQLQPVWKTKSCFQPSSFVCMMDLSDKNKAKCKDPGPLENGHAKLHGQSIDGFYA   600
Carcinoscorpius      YMDIQDQLQSVWKTKSCFQPSSFACMMDLSDRNKAKCDDPGSLENGHATLHGQSIDGFYA   599
Tachypleus           YLDIRDQLQPVWKTKSCFQPSSFACMMDLSDRNKAKCDDPGPLENGHATLHGQSIDGFYA   599
                     *:**:**** ************.*******:*****.*** ******.***********

Limulus              GSSVRYSCEVLHYLSGTETVSCTSNGTWSAPKPRCIKVITCQTPPVPSYGSVDIKPPSRT   660
Carcinoscorpius      GSSIRYSCEVLHYLSGTETVTCTTNGTWSAPKPRCIKVITCQNPPVPSYGSVEIKPPSRT   659
Tachypleus           GSSIRYSCEVLHYLSGTETVTCTTNGTWSAPKPRCIKVITCQNPPVPSYGSVEIKPPSRT   659
                     ***:****************:**:******************.*********:*******

Limulus              NSISRVGSPFLRLPRLPLPLARAAGPPPKPRSAPPSTVDLSSKVKLPEGHYRVGSQAIYT   720
Carcinoscorpius      NSISRVGSPFLRLPRLPLPLARAAKPPPKPRSSQPSTVDLASKVKLPEGHYRVGSRAIYT   719
Tachypleus           NSISRVGSPFLRLPRLPLPLARAAKPPPKPRSSQPSTVDLASKVKLPEGHYRVGSRAIYT   719
                     ********************** *******: ******:**************:****

Limulus              CESRYYELLGSQGRRCDSNGKWSGRPASCIPVCGRSDSPRSPFIVNGNSTEIGQWPWQAG   780
Carcinoscorpius      CESRYYELLGSQGRRCDSNGNWSGRPASCIPVCGRSDSPRSPFIWNGNSTEIGQWPWQAG   779
Tachypleus           CESRYYELLGSQGRRCDSNGNWSGRPASCIPVCGRSDSPRSPFIWNGNSTEIGQWPWQAG   779
                     ********************:********************** **************
```

```
Limulus             ISRWLADHNMWFLQCGGALLNEKWIITAAHCVTYSATAEIIDPSQFKFYLGKYYRDDSKD   840
Carcinoscorpius     ISRWLADHNMWFLQCGGSLLNEKWIVTAAHCVTYSATAEIIDPNQFKMYLGKYYRDDSRD   839
Tachypleus          ISRWLADHNMWFLQCGGSLLNEKWIVTAAHCVTYSATAEIIDPSQFKIYLGKYYRDDSRD   839
                    *****************:*******:*****************.***:**********:*

Limulus             DDYVQVREAIEIHVNPNYDPGNLNFDIALIQLKTSVALTTRVQPICLPTDLTTRENLKEG   900
Carcinoscorpius     DDYVQVREALEIHVNPNYDPGNLNFDIALIQLKTPVTLTTRVQPICLPTDITTREHLKEG   899
Tachypleus          DDYVQVREALEIHVNPNYDPGNLNFDIALIQLKTPVTLTTRVQPICLPTDITTREHLKEG   899
                    *********:*********************** *:*************:****:****

Limulus             ALAVVTGWGLNENNTYSEMIQQAVLPVVAASTCEQGYQDSGLPLTVTENMFCAGYKQGRY   960
Carcinoscorpius     TLAVVTGWGLNENNTYSETIQQAVLPVVAASTCEEGYKEADLPLTVTENMFCAGYKKGRY   959
Tachypleus          TLAVVTGWGLNENNTYSEMIQQAVLPVVAASTCEEGYKEADLPLTVTENMFCAGYKKGRY   959
                    :***************** ***************:**::::.***************:***

Limulus             DACSGDSGGPLVFADDSRTDRRWVLEGIVSWGSPNGCGKSNQYGGFTKVNVFLSWIRQFI  1020
Carcinoscorpius     DACSGDSGGPLVFADDSRTERRWVLEGIVSWGSPSGCGKANQYGGFTKVNVFLSWIRQFI  1019
Tachypleus          DACSGDSGGPLVFADDSRTERRWVLEGIVSWGSPSGCGKANQYGGFTKVNVFLSWIRQFI  1019
                    ******************:**************.****:**********************
```

Key:

- * : Fully conserved residues.
- : : Conservation between groups of strongly similar properties (Gonnet PAM 250 score > 0.5).
- . : Conservation between groups of weakly similar properties (Gonnet PAM 250 score ≤ 0.5).
- ␣ : Non-conserved residues.

Note: I scored a residue as a non-conservative substitution in Limulus (red highlight) only if it was identified as non-conservative in this Clustal Omega alignment AND the Limulus sequence differed from BOTH of the other species for which Factor C sequence was available prior to 2013.

OVERALL non-conservative substitutions = 11/1020 = 1.1%; overall similarity = 98.9%

CLUSTAL O(1.2.4) multiple sequence alignment of Limulus polyphemus vs Tachypleus tridentatus **Factor B**

```
Limulus         MAWICVITLFALASSTLSNKVSRVGIIFPKTQNDNKQCTAKGGLKGSCKSLTDCPAVLAT   60
Tachpleus       MTWICVITLFALASATLGNKVSRVGVLFPKTRNDN-ECTARGGLKGSCKSLIDCPSVLAT   59
                *:************:**.*******::****:*** :***:********** ***:****

Limulus         LKDSFPVVCSWNGRFQPIVCCPDAAAPS-VTTTVTTIVPTKETKIPRLHIPGCGKRKVNV   119
Tachpleus       LKDSFPVVCSWNGRFQPIVCCPDAIAPPPVTTTAVTVISTKEPKLPRLHISGCGKRKVKI   119
                ********************** **  ****..*:: *** *:***** *******::

Limulus         DITTIGRSGSPILPPISTSQDLKGGRGIIAGGVEAKIGAWPWMAAVFVKNFGIGRFHCAG   179
Tachpleus       DITTVGRSGSPILPPISTPQNSTGGRGIIAGGVEAKIGAWPWMAAVFVKNFGIGRFHCAG   179
                ****:************* *: .************************************

Limulus         SIISSKYILSAAHAFLIGGRKLTPTRLAVRVGGHYVKMGQEYHVEDVIIHPDYVERENYN   239
Tachpleus       SIISNKYILSAAHAFLIGGRKLTPTRLAVRVGGHYIKRGQEYPVKDVIIHPHYVEKENYN   239
                ****.*******************************:* **** *:******.***:****

Limulus         DIAIIVLKEELNFTDLVRPICLPDPEAVTDSLKGRRVTVAGWGDLDFAGPRSQVLREVSI   299
Tachpleus       DIAIIELKEELNFTDLVNPICLPDPETVTDPLKDRIVTAAGWGDLDFSGPRSQVLREVSI   299
                ***** ***********.********:*** **.* **.********:************

Limulus         PVVPIGDCNKAYQKLNTLALKNGITKKFICAGLEEGGKDACQGDSGGPLMLVNNSSWIVT   359
Tachpleus       PVVPVDKCDQAYEKLNTPSLKNGITNNFLCAGLEEGGKDACQGDSGGPLMLVNNTRWIVV   359
                ****:..*::**:**** :******::*:************************: ***.

Limulus         GVVSFGHKCAEEGFPGVYTRVVSYLEWIAKVTNSLDQTVTN    400
Tachpleus       GVVSFGHKCAEEGYPGVYSRVASYLDWIAKVTNSLDHAVTN    400
                *************:****:**.***:**********::***
```

Key:

- * : Fully conserved residues.
- : : Conservation between groups of strongly similar properties (Gonnet PAM 250 score > 0.5).

- . : Conservation between groups of weakly similar properties (Gonnet PAM 250 score ≤ 0.5).
- : Non-conserved residues.

17/400 non-conserved residues = 4.3%; thus overall sequence similarity = 95.7%

CLUSTAL O(1.2.4) multiple sequence alignment: Pro-clotting factor, L. polyphemus vs. T. tridentatus

```
L.      MLVNNMFSLLCFPLLMSMFSCSSLGRQRRQFVFPDDEESCSNRFTNDGICKDVLNCRDLL  60
T.      MLVNNVFSLLCFPLLMSVVRCSTLSRQRRQFVFPDEEELCSNRFTEEGTCKNVLDCRILL  60
        *****:***********:. **:*.**********:** ******::* **:**:** **

L.      QKNDYNLLKESICGFEGITPKVCCPKQSIVNPITEAPPKTTTTERPPIRIPSNLPKQCGN  120
T.      QKNDYNLLKESICGFEGITPKVCCPKSSHVISSTQAPPETTTTERPPKQIPPNLPEVCGI  120
        **************************.* *   *:***:******** :** ***: **

L.      RNITTTRIIGGQEATPGAWPWMAAVYIKQGGIRSVQCGGALVTNRHVITASHCVVNSLGT  180
T.      HNTTTTRIIGGREAPIGAWPWMTAVYIKQGGIRSVQCGGALVTNRHVITASHCVVNSAGT  180
        :* ********:**  ******:********************************** **

L.      DVMRADVFSVRLGEHNLYSTNDSSDPIDFAVTSVKHHENFVLATYLNDIAILKLNDTVTF  240
T.      DVMPADVFSVRLGEHNLYSTDDDSNPIDFAVTSVKHHEHFVLATYLNDIAILTLNDTVTF  240
        *** ****************:*.*:************:************.*******

L.      THKIKPICLPYESLRYEDLAMRNPFVAGWGTTAFNGPSSAVLREVQLPIWGHEPCRQAYE  300
T.      TDRIRPICLPYRKLRYDDLAMRKPFITGWGTTAFNGPSSAVLREVQLPIWEHEACRQAYE  300
        *.:*:******..***:*****:**::********************** ** ******

L.      KDLNITNVYMCAGYADGGKDACQGDSGGPMMLPDKSGNFYLVGIVSFGKKCALPGFPGVY  360
T.      KDLNITNVYMCAGFADGGKDACQGDSGGPMMLPVKTGEFYLIGIVSFGKKCALPGFPGVY  360
        ************:****************** *:*:***:******************

L.      TKVTEFLDWIAVNMV  375
T.      TKVTEFLDWIAEHMV  375
        ********** :**
```

Key:

- * : Fully conserved residues.
- : : Conservation between groups of strongly similar properties (Gonnet PAM 250 score > 0.5).
- . : Conservation between groups of weakly similar properties (Gonnet PAM 250 score ≤ 0.5).
- : Non-conserved residues.

20/375 non-conserved residues = 5.3%; Thus, 94.7% sequence similarity between pro-clotting factor proteins from these two species