| Primer name | Nucleotide sequence (5'→3') | Purpose |
|---|---|---|
| CXC-1F New | TGAACCCTGAGCTGAAGGCCGTGA | Real-time PCR |
| CXC-1R New | TGAAGGTCTGATGAGTTTGTCGTC | Real-time PCR |
| CXC-1R | CCTTCAGCTCAGGGTTCAAGC | Genomic PCR |
| CXC-2F New | GCTTGAACCCCGAGCTGAAAAACG | Real-time PCR |
| CXC-2R New | GTTCAGAGGTCGTATGAGGTGCTT | Real-time PCR |
| CXC-2F | CAAGCAGGACAACAGTGTCTGTGT | 3'RACE |
| CXC-2AR | GTTGCATGATTTGGATGCTGGGTAG | 5'RACE |
| CXC-1FSB | AACATATGTCTCCCAGGCCCAACTCAAAC | Southern blot |
| CXC-1RSB | CTCGAGTTATTTTGCACTGATGTGCAA | Southern blot |
| CXC1Exon1F | CAAAGTGTTTCTGCTCCTGG | Genomic PCR |
| *On*-CXC$_1$FOverEx | CATATGCAACTCAAACAAGCAGGACAACAGT | Overexpression |
| *On*-CXC$_1$ROverEx | CTCGAGTTTTGCACTGATGTGCAATTTCAA | Overexpression |
| *On*-CXC$_2$FOverEx | CATATGCAACTCAAACAAGCAGGACAACAGT | Overexpression |
| *On*-CXC$_2$ROverEx | CTCGAGCATGGCAGCTGTGGAGGGTTCCAC | Overexpression |
| β-actinrealtimeF | ACAGGATGCAGAAGGAGATCACAG | Real-time PCR |
| β-actinrealtimeR | GTACTCCTGCTTGCTGATCCACAT | Real-time PCR |
| M13F | AAAACGACGGCCAG | Nucleotide sequencing |
| M13R | AACAGCTATGACCATG | Nucleotide sequencing |
| UPM-long (0.4 µM) | CTAATACGACTCACTATAGGGCAAGCAGTGGTATCAACGCAGAGT | RACE PCR |
| UPM-short (2 µM) | CTAATAC GACTCACTATA GGGC | RACE PCR |

**Table S1.** Nakharuthai and Srisapoome (2020)

| *On*-CXC1 | Nucleotide (%) | Amino acid (%) | | *On*-CXC2 | Nucleotide (%) | Amino acid (%) | |
|---|---|---|---|---|---|---|---|
| Versus | identity | identity | similarity | Versus | identity | identity | Similarity |
| **Teleost fish** | | | | | | | |
| 1. Rock bream, *Oplegnathus fasciatus* (AB703273) | 64.5 | 49.1 | 68.1 | *O. fasciatus* | 70.7 | 57.7 | 75.4 |
| 2. Mandarin fish, *Siniperca chuatsi* (AAY79282) | 63.2 | 48.1 | 68.9 | *S. chuatsi* | 70.5 | 54.0 | 78.8 |
| 3. Atlantic halibut, *Hippoglossus hippoglossus* (ACY54778) | 52.0 | 39.3 | 51.1 | *H. hippoglossus* | 64.5 | 46.3 | 63.9 |
| 4. Common carp IL-8, *Cyprinus carpio* (ABE47600) | 44.9 | 19.1 | 34.1 | *C. carpio* | 49.4 | 21.9 | 42.6 |
| 5. Rainbow trout IL-8, *Oncorhynchus mykiss* (CAC33585) | 44.0 | 21.3 | 36.3 | *O. mykiss* | 47.3 | 23.7 | 44.4 |
| 6. Japanese flounder IL-8, *Paralichthys olivaceus* (AAL05442) | 48.4 | 25.4 | 45.9 | *P. olivaceus* | 49.6 | 29.6 | 55.0 |
| 7. Atlantic cod IL-8, *Gadus morhua* (CAD59734) | 42.2 | 21.5 | 37.0 | *G. morhua* | 47.6 | 21.4 | 46.3 |
| 8. Haddock IL-8, *Melanogrammus aeglefinus* (CAD97422) | 43.4 | 20.1 | 38.5 | *M. aeglefinus* | 48.4 | 23.9 | 48.1 |
| 9. Fugu rubripes IL-8, *Takifugu rubripes* (NP001027759) | 45.0 | 20.6 | 37.0 | *T. rubripes* | 50.3 | 24.6 | 47.2 |
| 10. Black porgy IL-8, *Acanthopagrus schlegelii* (AAY18807) | 42.1 | 18.8 | 38.5 | *A. schlegelii* | 44.1 | 24.3 | 48.1 |
| 11. European seabass IL-8, *Dicentrarchus labrax* (CAM32186) | 44.0 | 19.6 | 37.0 | *D. labrax* | 48.5 | 23.3 | 45.4 |
| 12. Bighead, *Hypophthalmichthys nobilis* (JN546631.1) | 44.8 | 22.0 | 38.5 | *H. nobilis* | 46.2 | 25.4 | 48.1 |
| 13. Channel catfish CXCL10, *Ictalurus punctatus* (AAQ01586) | 43.5 | 22.5 | 43.7 | *I. punctatus* | 46.8 | 27.0 | 55.6 |
| 14. Rainbow trout CXCL10, *Oncorhynchus mykiss* (NP001117788) | 44.3 | 22.0 | 43.7 | *O. mykiss* | 46.7 | 26.3 | 54.6 |
| 15. Zebrafish CXCL11, *Danio rerio* (XP001339307) | 42.2 | 20.6 | 40.0 | *D. rerio* | 46.0 | 24.8 | 50.0 |
| 16. Atlantic salmon CXCL10, *Salmo salar* (NP001134500) | 38.3 | 23.7 | 37.0 | *S. salar* | 42.9 | 27.7 | 44.4 |
| 17. Nile tilapia CXCL14, *Oreochromis niloticus* (XP003443444) | 43.3 | 21.4 | 37.8 | *O. niloticus* | 48.5 | 28.4 | 43.5 |
| 18. Atlantic salmon CXCL14, *Salmo salar* (NP001134297) | 44.2 | 20.0 | 36.3 | *S. salar* | 44.6 | 26.7 | 41.7 |
| 19. Rainbow trout CXCL14, *Oncorhynchus mykiss* (CCD11011) | 45.6 | 20.0 | 36.3 | *O. mykiss* | 46.5 | 26.7 | 41.7 |
| 20. Atlantic salmon CXCL13, *Salmo salar* (NP001134571) | 46.7 | 19.0 | 41.5 | *S. salar* | 47.1 | 21.1 | 44.1 |
| 21. Japanese flounder CXC, *Paralichthys olivaceus* (BAF79875) | 46.8 | 22.9 | 43.7 | *P. olivaceus* | 47.7 | 28.1 | 51.4 |
| 22. Fugu rubripes CXCL13, *Takifugu rubripes* (NP001233223) | 41.4 | 21.3 | 39.3 | *T. rubripes* | 49.4 | 23.9 | 48.1 |
| 23. Zebrafish CXCL12, *Danio rerio* (NP840092) | 44.0 | 17.1 | 38.5 | *D. rerio* | 46.4 | 20.4 | 49.1 |
| 24. Large yellow croaker CXCL12, *Larimichthys crocea* (ACF35274) | 43.8 | 13.9 | 35.6 | *L. crocea* | 46.4 | 17.3 | 44.4 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Higher vertebrates** | | | | | | | |
| 25. African clawed frog CXCL14, *Xenopus laevis* (NP001086198) | 45.8 | 20.0 | 41.5 | *X. laevis* | 47.8 | 21.2 | 44.4 |
| 26. African clawed frog CXCL12, *Xenopus laevis* (CAC82196) | 43.5 | 18.7 | 47.7 | *X. laevis* | 47.7 | 21.4 | 51.9 |
| 27. Cattle IL-8, *Bos taurus* (NP_776350) | 46.6 | 17.9 | 38.5 | *B. taurus* | 47.0 | 22.1 | 49.1 |
| 28. Sheep IL-8, *Ovis aries* (NP_001009401) | 47.6 | 17.1 | 38.5 | *O. aries* | 46.7 | 21.2 | 49.1 |
| 29. Pig IL-8, *Sus scrofa* (NP999032) | 47.9 | 17.1 | 37.8 | *S. scrofa* | 47.5 | 21.2 | 48.1 |
| 30. Dog IL-8, *Canis lupus familiaris* (NP001003200) | 47.2 | 17.9 | 38.5 | *C. lupus* | 48.7 | 21.2 | 49.1 |
| 31. Domestic cat IL-8, *Felis catus* (NP001009281) | 46.5 | 17.9 | 37.0 | *F. catus* | 45.8 | 21.2 | 46.3 |
| 32. Horse IL-8, *Equus caballus* (NP001077420) | 46.5 | 19.3 | 34.8 | *E. caballus* | 46.6 | 23.0 | 44.4 |
| 33. Human IL-8, *Homo sapiens* (NP000575) | 44.3 | 16.4 | 36.3 | *H. sapiens* | 46.3 | 20.4 | 46.3 |
| 34. Rhesus monkey IL-8, *Macaca mulatta* (NP001028137) | 45.7 | 17.9 | 37.0 | *M. mulatta* | 43.9 | 21.2 | 47.2 |
| 35. Chicken IL-8, *Gallus gallus* (NP990829) | 43.9 | 23.0 | 39.3 | *G. gallus* | 48.1 | 27.7 | 49.1 |
| 36. House mouse CXCL1, *Mus musculus* (NP032202) | 43.4 | 18.6 | 36.3 | *M. musculus* | 45.7 | 21.2 | 45.4 |
| 37. Human CXCL3, *Homo sapiens* (NP002081) | 42.4 | 18.4 | 39.3 | *H. sapiens* | 46.2 | 20.8 | 50.0 |
| 38. Human CXCL11, *Homo sapiens* (NP005400) | 40.6 | 23.3 | 45.2 | *H. sapiens* | 44.7 | 26.3 | 51.9 |
| 39. Horse CXCL10, *Equus caballus* (NP001108412) | 47.7 | 23.0 | 43.7 | *E. caballus* | 48.9 | 27.7 | 54.6 |
| 40. Pig CXCL10, *Sus scrofa* (ABD18444) | 46.1 | 21.2 | 42.2 | *S. scrofa* | 47.1 | 25.5 | 52.8 |
| 41. Rhesus monkey CXCL13, *Macaca mulatta* (NP001028052) | 44.3 | 17.3 | 40.7 | *M. mulatta* | 49.9 | 21.2 | 47.7 |
| 42. HumanCXCL13, *Homo sapiens* (NP006410) | 44.6 | 17.3 | 40.7 | *H. sapiens* | 46.7 | 20.4 | 47.7 |

**Table S2.** Nakharuthai and Srisapoome (2020)

| | | |
|---|---|---|
| nt1 | CGAATCCTCACAGCACTCCTGTCAACAATCAGTCTTTAATTGTTGCTAGTCTTTCTCTGG | nt60 |
| nt61 | CTGCATTATTATCTTTGCTGCACGTTTTCAGCACCGGTGTCATCTTGAAAACAACA**ATG**T | nt120 |
| aa1 | **M** S | aa2 |
| nt121 | CCACCCTCATCAAAGTGTTTCTGCTCCTGGCTGTCATGGTCTGCATCTCCCAGGCCCAAC | nt180 |
| aa3 | T L I K V F L L L A V M V C I S Q A Q L | aa22 |
| nt181 | TCAAACAAGCAGGACAACAGTGTCTGTGTCACCGTGTCAGGAATCGTCTCGGCATGAAGT | nt240 |
| aa23 | K Q A G Q Q C L C H R V R N R L G M K S | aa42 |
| nt241 | CTGAAATAAAGGACGTACAGATCTACCCAGCAACCAAATTCTGCAACAAAGTGGAGATTG | nt300 |
| aa43 | E I K D V Q I Y P A T K F C N K V E I V | aa62 |
| nt301 | TCGTTACCTTGAACAGTGGTCTCCGCTATTGCTTGAACCCTGAGCTGAAGGCCGTGAAAA | nt360 |
| aa63 | V T L N S G L R Y C L N P E L K A V K R | aa82 |
| nt361 | GACTCGTGACTAACATCATGGATAAAAAGCAGAAGACCACTTCCTCCCCAGTGGAACCCT | nt420 |
| aa83 | L V T N I M D K K Q K T T S S P V E P S | aa102 |
| nt421 | CCACAGCTGCCAATATAATTTTGACGACAAACTCATCAGACCTTCATATCATTTGTAGTA | nt480 |
| aa103 | T A A N I I L T T N S S D L H I I C S I | aa122 |
| nt481 | TTTTTAAAGAATATTGCTTGAAATTGCACATCAGTGCAAAA**TAA**TCAGCATTACTAAAAT | nt540 |
| aa123 | F K E Y C L K L H I S A K * | aa135 |
| nt541 | ATGTCTCAGGGAAATGGAAAATATTCAAGAATTTTCCACTTGAATTAGTCCTTTAACATA | nt600 |
| nt601 | ATGAAACACAGCAGCAACATTTAGCACATGCAAACAGCACAGCACAGCCAGTCACACACA | nt660 |
| nt661 | GTCCTGCTATACATAACAAGAGGTAGAGGCTAAAGAAGACAAAATCAAACAAACAAACAA | nt720 |
| nt721 | GACAGTTGATTTTAAGCAGGTTTCAGTTGCTGAAAAAAATGATATTCTAGAAATATGAAG | nt780 |
| nt781 | GTATGACACCAAAGGATGGTTTCCCTCTTGGAGCATGAATGTTCCCTCTAATTCTGAATT | nt840 |
| nt841 | TCATTAATTTGACCCCTGAGATGATTTTCATTTATTCTTTAGTCCTGCTCTAGTTATACT | nt900 |
| nt901 | TATTTATACTTTTTACTTATATATTTAATTAGTCCCTTCAGGATTTTACTCTCTGTATTC | nt960 |
| nt961 | CACTTTGTCCTCCTCCCATGTTAAGTTTAATTCATGTGTAAGCATTTCCCTGCTGCAGTT | nt1020 |
| nt1021 | GTTGTTATTCATGTCCCACTTTAGTTTTGTAATAATTTGTCTTCTGTGTTTCATGTTTAA | nt1080 |
| nt1081 | TTTTACTTCACGTCCTAGCTCATTGCCATTGTCTTCAACTGTGTGATTACCTTGTGTCTG | nt1140 |
| nt1141 | TTTATCCTCCTGTTTTCAATCTCCTGACATGTGTGTATAAATAGTCCTGTCTATGATCCA | nt1200 |
| nt1201 | TTTGGCAAAATAAATCCATTTGGTATCCTTGTTGGTCTTCAGACAACAATTCTGACGGCT | nt1260 |
| nt1261 | TAAGAACCAAATGGGACAGACAAAAAAAATAAAAATAAATAAATAAATAAATAAATAGT | nt1320 |
| nt1321 | CCTGTCAAAAAAAAAAAAAAAAAAAA | nt1346 |

**Figure S1.** Nakharuthai and Srisapoome (2020)

```
nt1     ACATGGGATCTTTGCTGCACGTTTTCAGCACCGGTGTCATCTTGAAACCAACAATGTCCA   nt60
aa1                                                          M  S  T   aa3
nt61    CCCTCATCAAAGTGTTTCTGCTCCTGGCTGTCATGGTCTGCATCTCCCAGGCCCAACTCA   nt120
aa4      L  I  K  V  F  L  L  L  A  V  M  V  C  I  S  Q  A  Q  L  K    aa23
nt121   AACAAGCAGGACAACAGTGTCTGTGTCACCGTGTCAGGAATCGTCTCGGCATGAAGTCTG   nt180
aa24     Q  A  G  Q  Q  C  L  C  H  R  V  R  N  R  L  G  M  K  S  E    aa43
nt181   AAATAAAGGACGTACAGATCTACCCAGCAACCAAATCATGCAACAAAGTGGAGATTCTCG   nt240
aa44     I  K  D  V  Q  I  Y  P  A  T  K  S  C  N  K  V  E  I  L  V    aa63
nt241   TTACCTTCAACAGTGGTCTCCGCTATTGCTTGAACCCCGAGCTGAAAAACGTGAAAAGAC   nt300
aa64     T  F  N  S  G  L  R  Y  C  L  N  P  E  L  K  N  V  K  R  L    aa83
nt301   TCGTGACTAACATCATGGATAAAAAGCAGAAGACCACTTCCTCCCCAGTGGAACCCTCCA   nt360
aa84     V  T  N  I  M  D  K  K  Q  K  T  T  S  S  P  V  E  P  S  T    aa103
nt361   CAGCTGCCATGTGATTGTCTTTTAAGCTGCCTGAAAGAAAAAGCACCTCATACGACCTCT   nt420
aa104    A  A  M  *                                                    aa106
nt421   GAACCCTCAACAAAGTCACTTTGACAAAAACAAAAAAAACAAACATGGACATGAAATGAT   nt480
nt481   TGTAGTTTTTTTATTTAATTTTTTTATACATGCTTTTATTTTATCTCCACTCTTTTGTA    nt540
nt541   TCCTGTTTTTATTCTACTGTGTTTTTGTAAATAGACGAATGCCTCTTTCTATCTTTTGAT   nt600
nt601   ATTTTTTTAATAAAATTTCTAACTCTAAAAAAAAAAAAAAAAAAAAAAAAAAA          nt653
```

**Figure S2.** Nakharuthai and Srisapoome (2020)

```
On-CXC1       MSTLIKVFLLLAVMVCISQAQLKQAGQQCLCHRVRNRLGMKSEIKDVQIYPATKFCNKVEIVVTLNSGLRYCLN
On-CXC2       MSTLIKVFLLLAVMVCISQAQLKQAGQQCLCHRVRNRLGMKSEIKDVQIYPATKSCNKVEILVTFNSGLRYCLN
              ****************************************************** ******:**:*********

On-CXC1       PELKAVKRLVTNIMDKKQKTTSSPVEPSTAANIILTTNSSDLHIICSIFKEYCLKLHISAK
On-CXC2       PELKNVKRLVTNIMDKKQKTTSSPVEPSTAAM-----------------------------
              **** **************************
```

| *On*-CXC1 | Nucleotide | Amino acid | |
|---|---|---|---|
| VS | Identity (%) | Identity (%) | Similarity (%) |
| *On*-CXC2 | 76.0 | 73.7 | 76.3 |

**Figure S3.** Nakharuthai and Srisapoome (2020)

**Figure S4.** Nakharuthai and Srisapoome (2020)

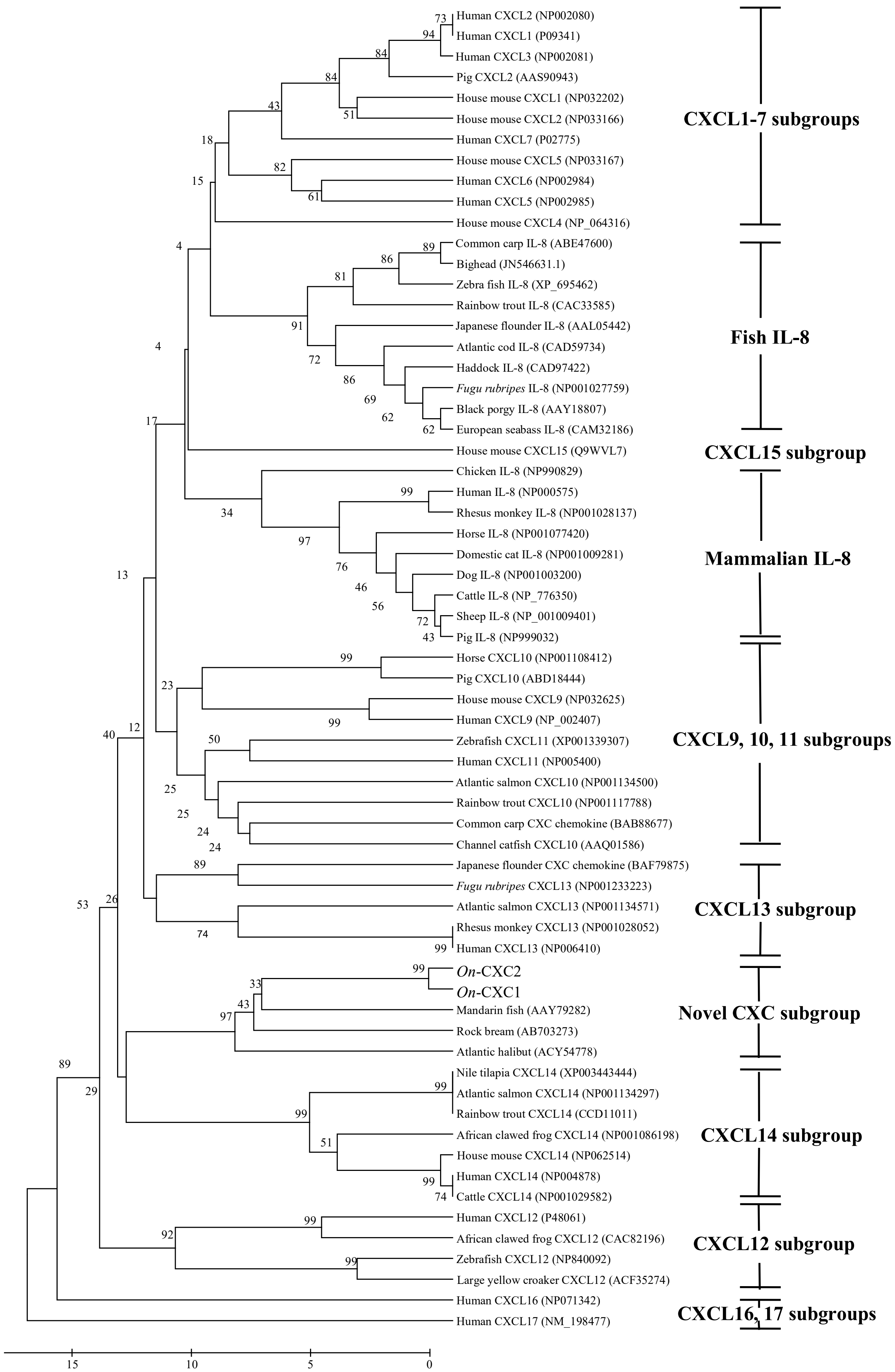


**Figure S5.** Nakharuthai and Srisapoome (2020)

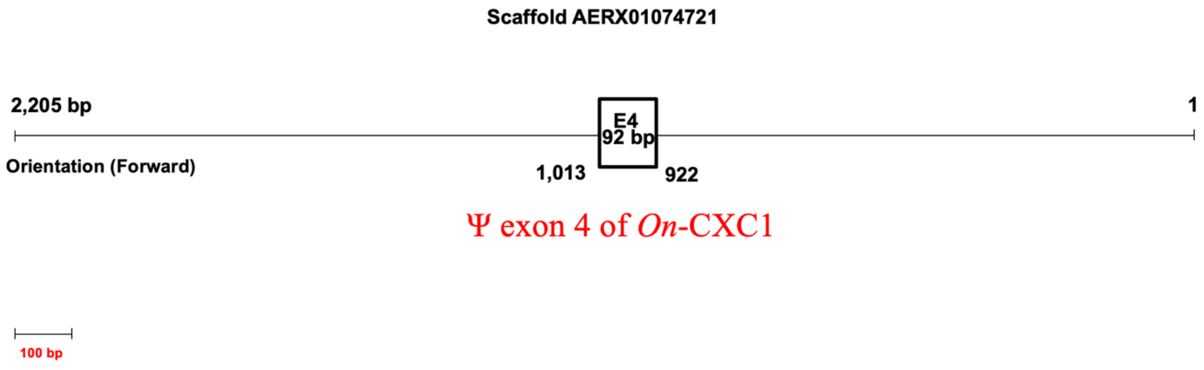


**Figure S6A**. Nakharuthai and Srisapoome (2020)

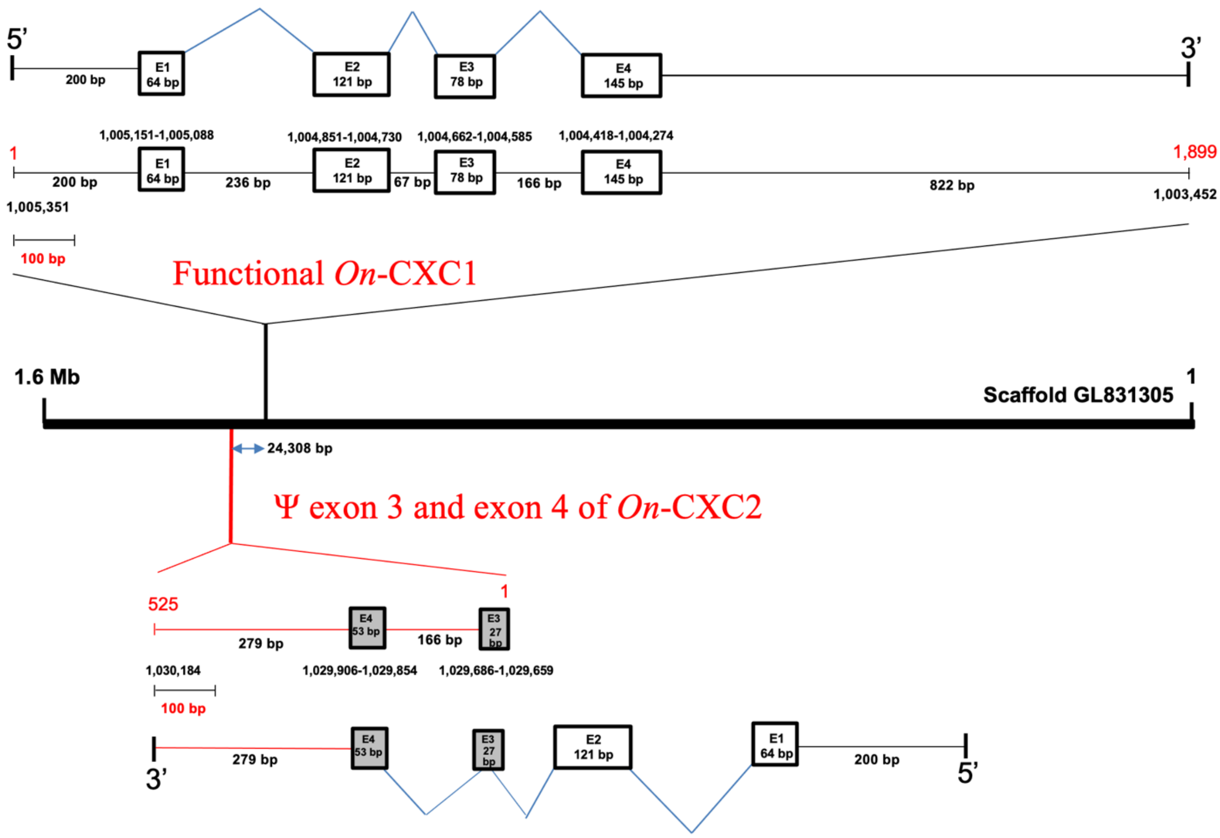


**Figure S6B**. Nakharuthai and Srisapoome (2020)

**Supplementary Materials**

**Table S1.** Oligonucleotide primers used in this study

**Table S2.** Homological analyses of nucleotide and amino acid sequences of *On*-CXC1 and *On*-CXC2 against CXC chemokines of other vertebrates.

**Figure S1.** Nucleotide and deduced amino acid sequences of the Nile tilapia CXC chemokine (*On*-CXC1) cDNA represented by the upper and lower lines, respectively. The start (ATG) and stop (TAG) codons are in bold. The signal peptide cleavage sites are underlined. Four cysteine residues are circled. Polyadenylation sites (ACTAAA, TATAAA and AATAAA) are underlined with a double line, and the 4 instability motifs (ATTTA) are underlined. The shaded letters indicate the $(GAA)_{12}$ microsatellite sequences.

**Figure S2.** Nucleotide and deduced amino acid sequences of the Nile tilapia CXC chemokine (*On*-CXC2) cDNA represented by the upper and lower lines, respectively. The start (ATG) and stop (TAG) codons are in bold. The signal peptide cleavage sites are underlined. Four cysteine residues are circled. Polyadenylation sites (AAAAAG, AAAACA, AATAGA and AATAAA) are underlined with a double line, and two instability motifs (ATTTA and ATTTTA) are underlined. The shaded letters indicate the $(GAA)_5$ microsatellite sequences.

**Figure S3.** Sequence alignment between *On*-CXC1 and *On*-CXC2. The four conserved cysteines (C) residues are boxed. The comparisons of the nucleotide and amino acid sequences of *On*-CXC1 and *On*-CXC2 are shown in the table.

**Figure S4.** Multiple sequence alignment of *On*-CXC1and *On*-CXC2 with other known CXC chemokines from various species. The four conserved cysteine residues are boxed and marked with asterisks. Gaps in the alignment are represented with dashes (-), and the ELR motif is shaded.

**Figure S5.** Phylogenetic tree showing the relationships between the Nile tilapia CXC chemokine (*On*-CXC1, *On*-CXC2) amino acid sequences and other known CXC chemokines from various species. The numbers at the relevant branches refer to bootstrap values (1,000 replicates).

**Figure S6.** Genomic structure and organization comparison of the Nile tilapia CXC chemokine genes with other known CXC chemokines of other vertebrates. (A) Scaffold AERX01074721 and (B) scaffold GL831305. The four exons are indicated by rectangles and the three introns by lines. The sizes of exons are indicated in the rectangles.