

Supplementary Material for Immune-metabolic implication of hepcidin genes on gilthead seabream (*Sparus aurata*) iron homeostasis, storage, and regulation

Supplementary Tables

Supplementary Table 1. Hepcidin protein sequences of Eupercaria fish.

UniProt Code	Order	Family	Species
A0A0A7DK26	Chaetodontiformes	Leiognathidae	<i>Leiognathus equula</i>
A0A0F8BM78	Perciformes	Sciaenidae	<i>Larimichthys crocea</i>
A0A0G2QNF5	Perciformes	Sciaenidae	<i>Larimichthys crocea</i>
A0A0G2QNF6	Perciformes	Sciaenidae	<i>Larimichthys crocea</i>
A0A0G2QNF7	Perciformes	Sciaenidae	<i>Larimichthys crocea</i>
A0A0G2QNG3	Perciformes	Sciaenidae	<i>Larimichthys crocea</i>
A0A0G2QNG8	Perciformes	Sciaenidae	<i>Larimichthys crocea</i>
A0A0G2SKE2	Perciformes	Moronidae	<i>Dicentrarchus labrax</i>
A0A0G2SKP8	Perciformes	Moronidae	<i>Dicentrarchus labrax</i>
A0A0G2SKR4	Perciformes	Moronidae	<i>Dicentrarchus labrax</i>
A0A0G2SL28	Perciformes	Moronidae	<i>Dicentrarchus labrax</i>
A0A142LQX1	Perciformes	Scatophagidae	<i>Scatophagus argus</i>
A0A142LQX2	Perciformes	Scatophagidae	<i>Scatophagus argus</i>
A0A221S5V4	Perciformes	Cottidae	<i>Trachidermus fasciatus</i>
A0A286RS38	Perciformes	Sciaenidae	<i>Argyrosomus regius</i>

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A0A3B5JZ21	Tetraodontiformes	Tetraodontidae	<i>Takifugu rubripes</i>
A0A3B5K9Z8	Tetraodontiformes	Tetraodontidae	<i>Takifugu rubripes</i>
A0A3B5KJ12	Tetraodontiformes	Tetraodontidae	<i>Takifugu rubripes</i>
A0A3Q3EDR8	Labriformes	Labridae	<i>Labrus bergylta</i>
A0A3Q3EER6	Labriformes	Labridae	<i>Labrus bergylta</i>
A0A3Q3EF34	Labriformes	Labridae	<i>Labrus bergylta</i>
A0A3Q3EFV6	Labriformes	Labridae	<i>Labrus bergylta</i>
A0A3Q3LLD2	Labriformes	Labridae	<i>Labrus bergylta</i>
A0A3Q3WXJ3	Tetraodontiformes	Molidae	<i>Mola mola</i>
A0A3Q3XE98	Tetraodontiformes	Molidae	<i>Mola mola</i>
A0A3Q4BQH2	Tetraodontiformes	Molidae	<i>Mola mola</i>
A0A484D7H1	Perciformes	Percidae	<i>Perca flavescens</i>
A0A484D831	Perciformes	Percidae	<i>Perca flavescens</i>
A0A4U5V1Z2	Perciformes	Sciaenidae	<i>Collichthys lucidus</i>
A0A4U5V3A2	Perciformes	Sciaenidae	<i>Collichthys lucidus</i>
A0A4U5V3F1	Perciformes	Sciaenidae	<i>Collichthys lucidus</i>
A0A4U5V583	Perciformes	Sciaenidae	<i>Collichthys lucidus</i>
A0A4V6XYT9	Perciformes	Sciaenidae	<i>Collichthys lucidus</i>
A0A4Z2EKA7	Perciformes	Liparidae	<i>Liparis tanakae</i>
A0A516UX08	Perciformes	Serranidae	<i>Centropristis striata</i>
A0A5C1K3A1	Perciformes	Sebastidae	<i>Sebastiscus marmoratus</i>
A0A5C6MEA9	Tetraodontiformes	Tetraodontidae	<i>Takifugu flavidus</i>

A0A5C6MEB4	Tetraodontiformes	Tetraodontidae	<i>Takifugu flavidus</i>
A0A5C6MFY1	Tetraodontiformes	Tetraodontidae	<i>Takifugu flavidus</i>
A0A5C6PIG2	Tetraodontiformes	Tetraodontidae	<i>Takifugu flavidus</i>
A0A5C6PJI5	Tetraodontiformes	Tetraodontidae	<i>Takifugu flavidus</i>
A0A5C6PL24	Tetraodontiformes	Tetraodontidae	<i>Takifugu flavidus</i>
A0A5C6PL34	Tetraodontiformes	Tetraodontidae	<i>Takifugu flavidus</i>
A0A5J5DEQ9	Perciformes	Percidae	<i>Etheostoma spectabile</i>
A0A5P9K6W7	Tetraodontiformes	Tetraodontidae	<i>Takifugu pardalis</i>
A0A671WFP8	Spariformes	Sparidae	<i>Sparus aurata</i>
A0A671WFQ7	Spariformes	Sparidae	<i>Sparus aurata</i>
A0A671WFR7	Spariformes	Sparidae	<i>Sparus aurata</i>
A0A671WFU3	Spariformes	Sparidae	<i>Sparus aurata</i>
A0A671WFO4	Spariformes	Sparidae	<i>Sparus aurata</i>
A0A671WFX4	Spariformes	Sparidae	<i>Sparus aurata</i>
A0A671WGL1	Spariformes	Sparidae	<i>Sparus aurata</i>
A0A671WGN1	Spariformes	Sparidae	<i>Sparus aurata</i>
A0A671WGP5	Spariformes	Sparidae	<i>Sparus aurata</i>
A0A671WGQ6	Spariformes	Sparidae	<i>Sparus aurata</i>
A0A671WIH8	Spariformes	Sparidae	<i>Sparus aurata</i>
A0A671WIJ8	Spariformes	Sparidae	<i>Sparus aurata</i>
A0A671WIK3	Spariformes	Sparidae	<i>Sparus aurata</i>
A0A671WIM2	Spariformes	Sparidae	<i>Sparus aurata</i>

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A0A671WIN7	Spariformes	Sparidae	<i>Sparus aurata</i>
A0A671WIP4	Spariformes	Sparidae	<i>Sparus aurata</i>
A0A671WMC6	Spariformes	Sparidae	<i>Sparus aurata</i>
A0A671WME1	Spariformes	Sparidae	<i>Sparus aurata</i>
A0A671WMH5	Spariformes	Sparidae	<i>Sparus aurata</i>
A0A671WMJ0	Spariformes	Sparidae	<i>Sparus aurata</i>
A0A671Y2Z0	Spariformes	Sparidae	<i>Sparus aurata</i>
A0A674MRJ1	Tetraodontiformes	Tetraodontidae	<i>Takifugu rubripes</i>
A0A674MZ52	Tetraodontiformes	Tetraodontidae	<i>Takifugu rubripes</i>
A0A674N256	Tetraodontiformes	Tetraodontidae	<i>Takifugu rubripes</i>
A0A674N5U9	Tetraodontiformes	Tetraodontidae	<i>Takifugu rubripes</i>
A0A674NVV8	Tetraodontiformes	Tetraodontidae	<i>Takifugu rubripes</i>
A0A674P4K6	Tetraodontiformes	Tetraodontidae	<i>Takifugu rubripes</i>
A0A674PE74	Tetraodontiformes	Tetraodontidae	<i>Takifugu rubripes</i>
A0A674PS50	Tetraodontiformes	Tetraodontidae	<i>Takifugu rubripes</i>
A0A6A5FGJ8	Perciformes	Percidae	<i>Perca fluviatilis</i>
A0A6A5FI96	Perciformes	Percidae	<i>Perca fluviatilis</i>
A0A6I9NDV1	Perciformes	Nototheniidae	<i>Notothenia coriiceps</i>
A0A6I9P6Q3	Perciformes	Nototheniidae	<i>Notothenia coriiceps</i>
A0A6I9PTK1	Perciformes	Nototheniidae	<i>Notothenia coriiceps</i>
A0A6I9Q0K9	Perciformes	Nototheniidae	<i>Notothenia coriiceps</i>
A0A6J2R947	Perciformes	Bovichtidae	<i>Cottoperca gobio</i>

A0A6J2RAN9	Perciformes	Bovichtidae	<i>Cottoperca gobio</i>
A0A6J2RBX1	Perciformes	Bovichtidae	<i>Cottoperca gobio</i>
A0A6J2RCZ0	Perciformes	Bovichtidae	<i>Cottoperca gobio</i>
A0A6J2RDB7	Perciformes	Bovichtidae	<i>Cottoperca gobio</i>
A0A6J2RE69	Perciformes	Bovichtidae	<i>Cottoperca gobio</i>
A0A6M5XI51	Perciformes	Platycephalidae	<i>Sorsogona tuberculata</i>
A0A6M5XPK9	Gerreiformes	Gerreidae	<i>Gerres filamentosus</i>
A0A6P8W9A8	Perciformes	Bathydraconidae	<i>Gymnodraco acuticeps</i>
A0A7J5XQN3	Perciformes	Nototheniidae	<i>Dissostichus mawsoni</i>
A0A7S6P375	Perciformes	Serranidae	<i>Epinephelus malabaricus</i>
A0A8C2ZMY7	Perciformes	Cyclopteridae	<i>Cyclopterus lumpus</i>
A0A8C2ZMY8	Perciformes	Cyclopteridae	<i>Cyclopterus lumpus</i>
A0A8C2ZPP5	Perciformes	Cyclopteridae	<i>Cyclopterus lumpus</i>
A0A8C3G2S7	Perciformes	Cyclopteridae	<i>Cyclopterus lumpus</i>
A0A8C3G3Y6	Perciformes	Cyclopteridae	<i>Cyclopterus lumpus</i>
A0A8C4DSN4	Perciformes	Moronidae	<i>Dicentrarchus labrax</i>
A0A8C4DV20	Perciformes	Moronidae	<i>Dicentrarchus labrax</i>
A0A8C4DVL0	Perciformes	Moronidae	<i>Dicentrarchus labrax</i>
A0A8C4F477	Perciformes	Moronidae	<i>Dicentrarchus labrax</i>
A0A8C4GHW7	Perciformes	Moronidae	<i>Dicentrarchus labrax</i>
A0A8C4GWR5	Perciformes	Moronidae	<i>Dicentrarchus labrax</i>
A0A8C9XFH5	Perciformes	Percidae	<i>Sander lucioperca</i>

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A0A8C9XX62	Perciformes	Percidae	<i>Sander lucioperca</i>
A0A8C9Y0E8	Perciformes	Percidae	<i>Sander lucioperca</i>
A0A8C9Y0H5	Perciformes	Percidae	<i>Sander lucioperca</i>
A0A8C9Y1V9	Perciformes	Percidae	<i>Sander lucioperca</i>
A0A8C9YA57	Perciformes	Percidae	<i>Sander lucioperca</i>
A0A8D0CQR4	Perciformes	Percidae	<i>Sander lucioperca</i>
A0A8D0CUV5	Perciformes	Percidae	<i>Sander lucioperca</i>
A0A8D0CUV7	Perciformes	Percidae	<i>Sander lucioperca</i>
A1Z0M0	Perciformes	Sciaenidae	<i>Larimichthys crocea</i>
A5Z0S6	Perciformes	Percidae	<i>Perca fluviatilis</i>
B2RFG6	Spariformes	Sparidae	<i>Sparus aurata</i>
B2X7F5	Spariformes	Sparidae	<i>Sparus aurata</i>
B2X7F7	Spariformes	Sparidae	<i>Sparus aurata</i>
B3FKC0	Perciformes	Eleginopidae	<i>Eleginops maclovinus</i>
B3FKC3	Perciformes	Nototheniidae	<i>Notothenia angustata</i>
B3FKC4	Perciformes	Eleginopidae	<i>Eleginops maclovinus</i>
B3FKC5	Perciformes	Nototheniidae	<i>Notothenia angustata</i>
B3FKC6	Perciformes	Channichthyidae	<i>Chaenocephalus aceratus</i>
B3FKC7	Perciformes	Nototheniidae	<i>Dissostichus mawsoni</i>
B3FKC8	Perciformes	Nototheniidae	<i>Dissostichus mawsoni</i>
B3FKC9	Perciformes	Nototheniidae	<i>Notothenia angustata</i>
B3FKD0	Perciformes	Nototheniidae	<i>Notothenia angustata</i>

B3FKD1	Perciformes	Nototheniidae	<i>Dissostichus mawsoni</i>
B3FKD2	Perciformes	Bathydraconidae	<i>Gymnodraco acuticeps</i>
B3FKD5	Perciformes	Nototheniidae	<i>Dissostichus mawsoni</i>
B3FKD6	Perciformes	Nototheniidae	<i>Dissostichus mawsoni</i>
B3FKD7	Perciformes	Nototheniidae	<i>Notothenia angustata</i>
B3FKD8	Perciformes	Artedidraconidae	<i>Pogonophryne scotti</i>
B3FKD9	Perciformes	Nototheniidae	<i>Notothenia angustata</i>
B3FKE0	Perciformes	Zoarcidae	<i>Lycodichthys dearborni</i>
B3FKE1	Perciformes	Zoarcidae	<i>Lycodichthys dearborni</i>
B5A4A5	Centrarchiformes	Oplegnathidae	<i>Oplegnathus fasciatus</i>
B5A4A6	Centrarchiformes	Oplegnathidae	<i>Oplegnathus fasciatus</i>
B5A4A7	Centrarchiformes	Oplegnathidae	<i>Oplegnathus fasciatus</i>
B6ZJU9	Spariformes	Sparidae	<i>Pagrus auriga</i>
B6ZJV0	Spariformes	Sparidae	<i>Pagrus auriga</i>
B6ZJV1	Spariformes	Sparidae	<i>Pagrus auriga</i>
B6ZJV2	Spariformes	Sparidae	<i>Pagrus auriga</i>
B7UE76	Pempheriformes	Lateolabracidae	<i>Lateolabrax japonicus</i>
B8QGA6	Centrarchiformes	Centrarchidae	<i>Micropterus salmoides</i>
B8QGA7	Centrarchiformes	Centrarchidae	<i>Micropterus salmoides</i>
B8QGA8	Centrarchiformes	Centrarchidae	<i>Micropterus dolomieu</i>
B8QGA9	Centrarchiformes	Centrarchidae	<i>Micropterus dolomieu</i>
C1IDZ8	Perciformes	Sebastidae	<i>Sebastes schlegelii</i>

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D5FZW1	Centrarchiformes	Sinipercidae	<i>Siniperca chuatsi</i>
E2IFI1	Perciformes	Serranidae	<i>Epinephelus coioides</i>
F1CNA5	Perciformes	Serranidae	<i>Epinephelus fuscoguttatus</i>
F1JYM8	Perciformes	Serranidae	<i>Epinephelus moara</i>
F1JYM9	Perciformes	Serranidae	<i>Epinephelus moara</i>
F1JYN0	Perciformes	Serranidae	<i>Epinephelus moara</i>
F1JYN1	Perciformes	Serranidae	<i>Epinephelus moara</i>
F1JYN2	Perciformes	Serranidae	<i>Epinephelus moara</i>
G0Z5J6	Perciformes	Sciaenidae	<i>Miichthys miiuy</i>
G0Z5J7	Perciformes	Sciaenidae	<i>Miichthys miiuy</i>
G0Z5J8	Perciformes	Sciaenidae	<i>Miichthys miiuy</i>
G3EWI3	Centrarchiformes	Oplegnathidae	<i>Oplegnathus fasciatus</i>
G3EWI4	Centrarchiformes	Oplegnathidae	<i>Oplegnathus fasciatus</i>
G3EWI5	Centrarchiformes	Oplegnathidae	<i>Oplegnathus fasciatus</i>
G3NPA3	Perciformes	Gasterosteidae	<i>Gasterosteus aculeatus</i>
G3NPA5	Perciformes	Gasterosteidae	<i>Gasterosteus aculeatus</i>
G3PFW0	Perciformes	Gasterosteidae	<i>Gasterosteus aculeatus</i>
G5DD15	Perciformes	Serranidae	<i>Epinephelus malabaricus</i>
G8D464	Perciformes	Serranidae	<i>Alphestes immaculatus</i>
H2RK16	Tetraodontiformes	Tetraodontidae	<i>Takifugu rubripes</i>
H3BX45	Tetraodontiformes	Tetraodontidae	<i>Tetraodon nigroviridis</i>
H3DDC3	Tetraodontiformes	Tetraodontidae	<i>Tetraodon nigroviridis</i>

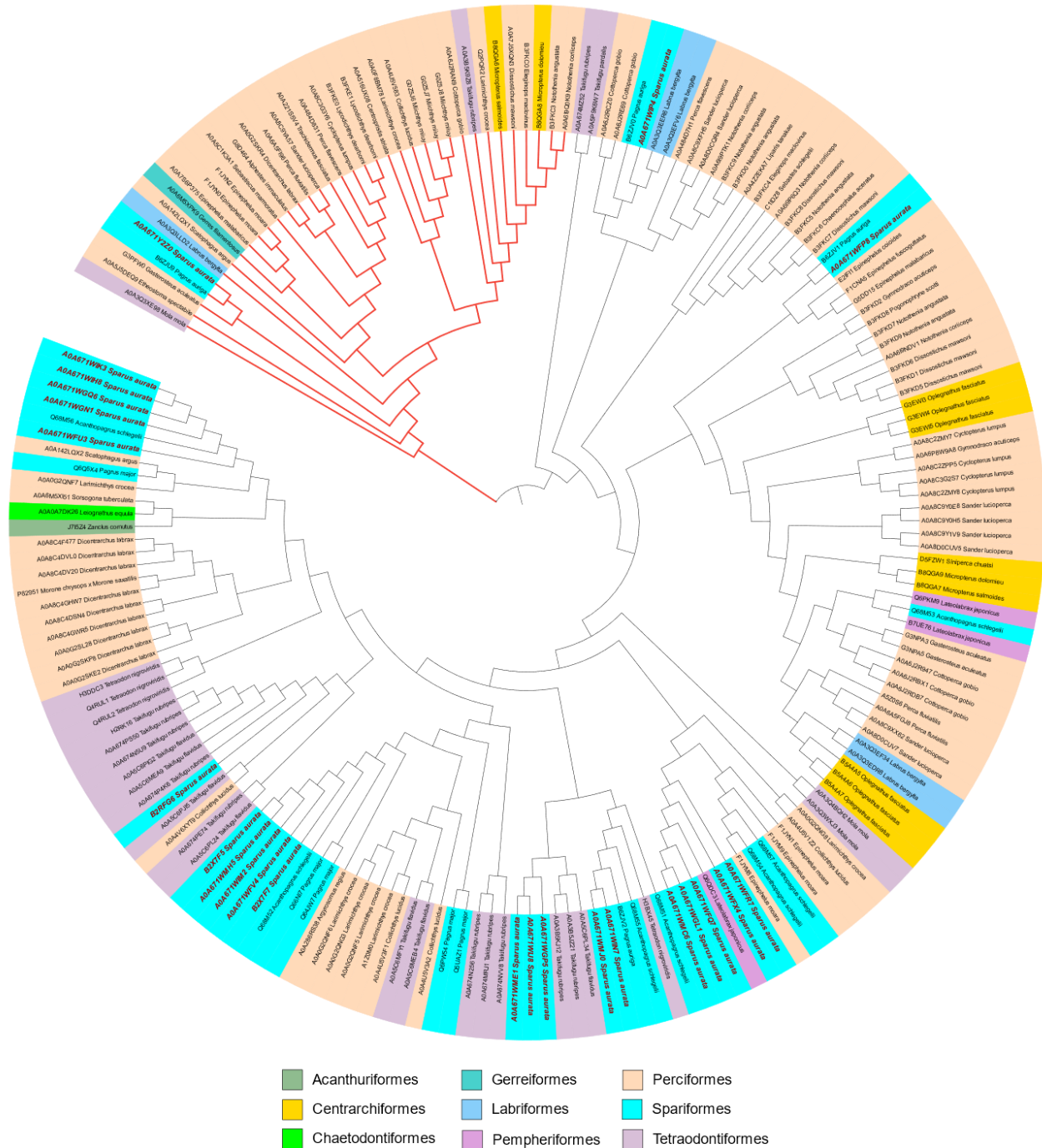
J7I5Z4	Acanthuriformes	Zanclidae	<i>Zanclus cornutus</i>
P82951	Perciformes	Moronidae	<i>Morone chrysops</i> x <i>Morone saxatilis</i>
Q2PQR2	Perciformes	Sciaenidae	<i>Larimichthys crocea</i>
Q4RUL1	Tetraodontiformes	Tetraodontidae	<i>Tetraodon nigroviridis</i>
Q4RUL2	Tetraodontiformes	Tetraodontidae	<i>Tetraodon nigroviridis</i>
Q5UAZ1	Spariformes	Sparidae	<i>Pagrus major</i>
Q64JW7	Spariformes	Sparidae	<i>Pagrus major</i>
Q66NI7	Spariformes	Sparidae	<i>Pagrus major</i>
Q68M51	Spariformes	Sparidae	<i>Acanthopagrus schlegelii</i>
Q68M52	Spariformes	Sparidae	<i>Acanthopagrus schlegelii</i>
Q68M53	Spariformes	Sparidae	<i>Acanthopagrus schlegelii</i>
Q68M54	Spariformes	Sparidae	<i>Acanthopagrus schlegelii</i>
Q68M55	Spariformes	Sparidae	<i>Acanthopagrus schlegelii</i>
Q68M56	Spariformes	Sparidae	<i>Acanthopagrus schlegelii</i>
Q68M57	Spariformes	Sparidae	<i>Acanthopagrus schlegelii</i>
Q6PKM9	Pempheriformes	Lateolabracidae	<i>Lateolabrax japonicus</i>
Q6PW54	Spariformes	Sparidae	<i>Pagrus major</i>
Q6Q5X4	Spariformes	Sparidae	<i>Pagrus major</i>
Q6QDC3	Pempheriformes	Lateolabracidae	<i>Lateolabrax japonicus</i>

Supplementary Table 2. Properties of mature sea bream hepcidins.

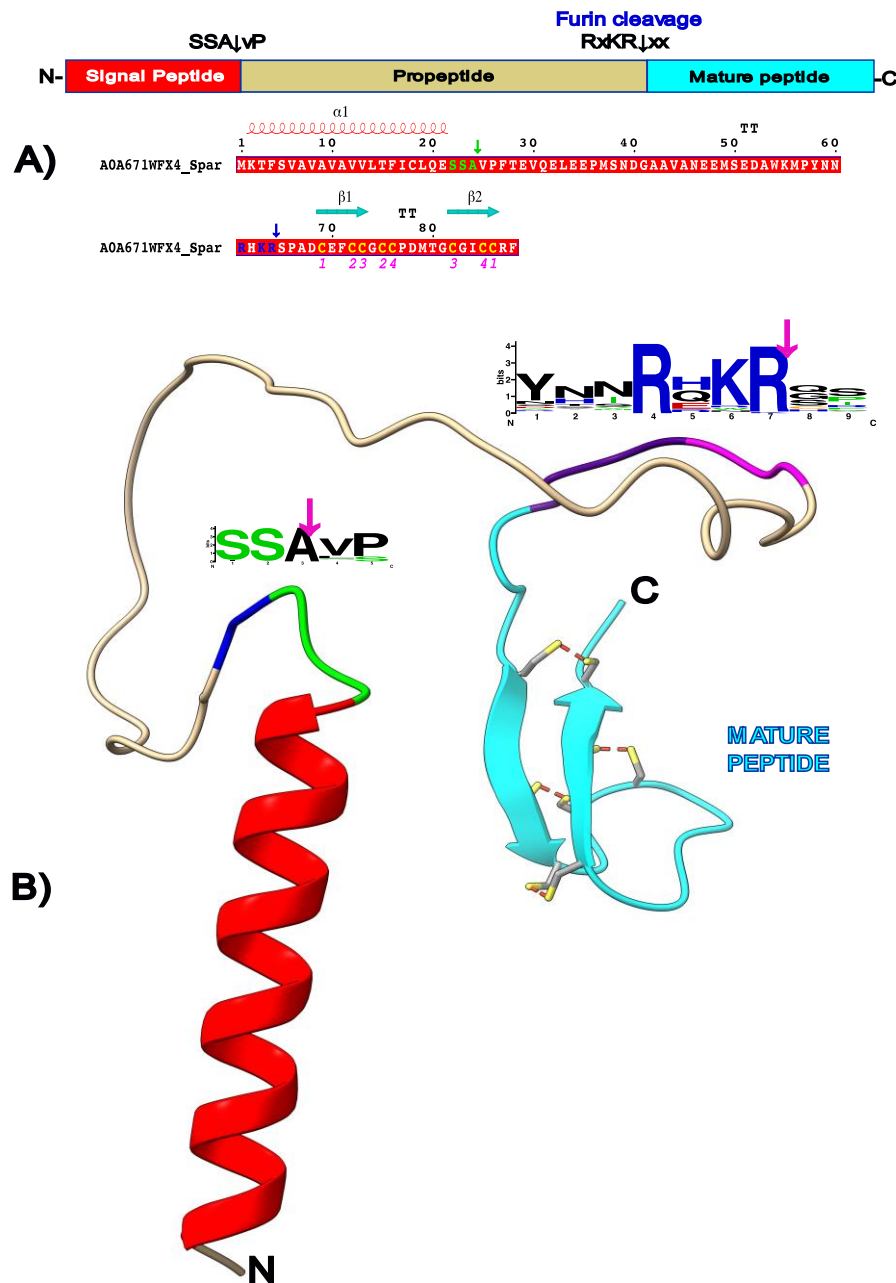
UniProt Code	Mature peptide	pI	Net charge pH 7.0	Estimated Water solubility
B2X7F7	HWKCRFCCRCCPRMRGCGLCRRF	12.7	6.1	Good
A0A671WMH5	CRFCCRCCPRMRGCGLCRRF	12.7	5.0	Good
B2RFG6	RRCKFCCGCCPGMRVCGVCCRF	12.6	5.0	Good
A0A671Y2Z0*	QSHISMCYYCCNCCRANKGCGYCCKF	10.2	3.1	Poor
A0A671WFM3	SPAGCKFCCGCCPNMRGCGLCRRF	12.1	3.0	Poor
A0A671WGM6	SPAGCKFCCGCCPNMRGCGVCCRF	12.1	3.0	Poor
A0A671WFP8	QKPQSGLIRCSYCCDCCALGVCGMCCKRGLLYEH	9.9	2.1	Poor
A0A671WIN7	CRFCCGCCPNMIGCGTCCKF	11.5	2.0	Poor
A0A671WFR7	CRFCCGCCPDTTGCNVCCRF	11.2	1.0	Poor
A0A671WIP4	SLSGCIFCCGCCPIMTRCGICCYQWV	9.6	1.0	Poor
A0A671WIJ8	CRFCCDCCPPDMSGCGTCCKF	7.1	0.0	Poor
A0A671WGL1	SPADCRFCCGCCPDMTGCGVCCTF	3.7	-1.0	Poor
A0A671WFX4	SPADCEFCGCCPDMTGCGICCRF	3.5	-2.0	Poor

*A0A671Y2Z0, hepcidin protein of type I.

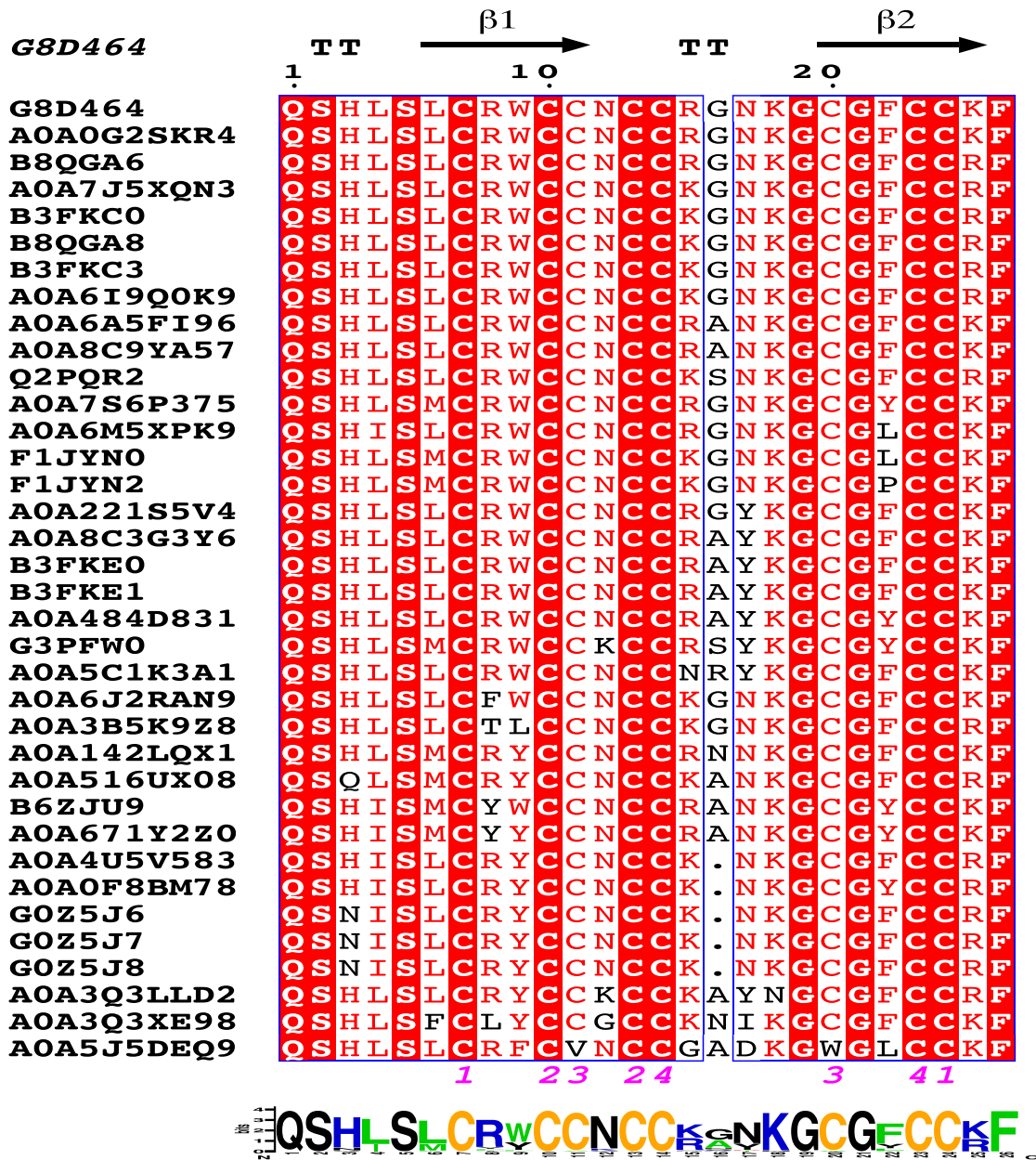
Supplementary Figures



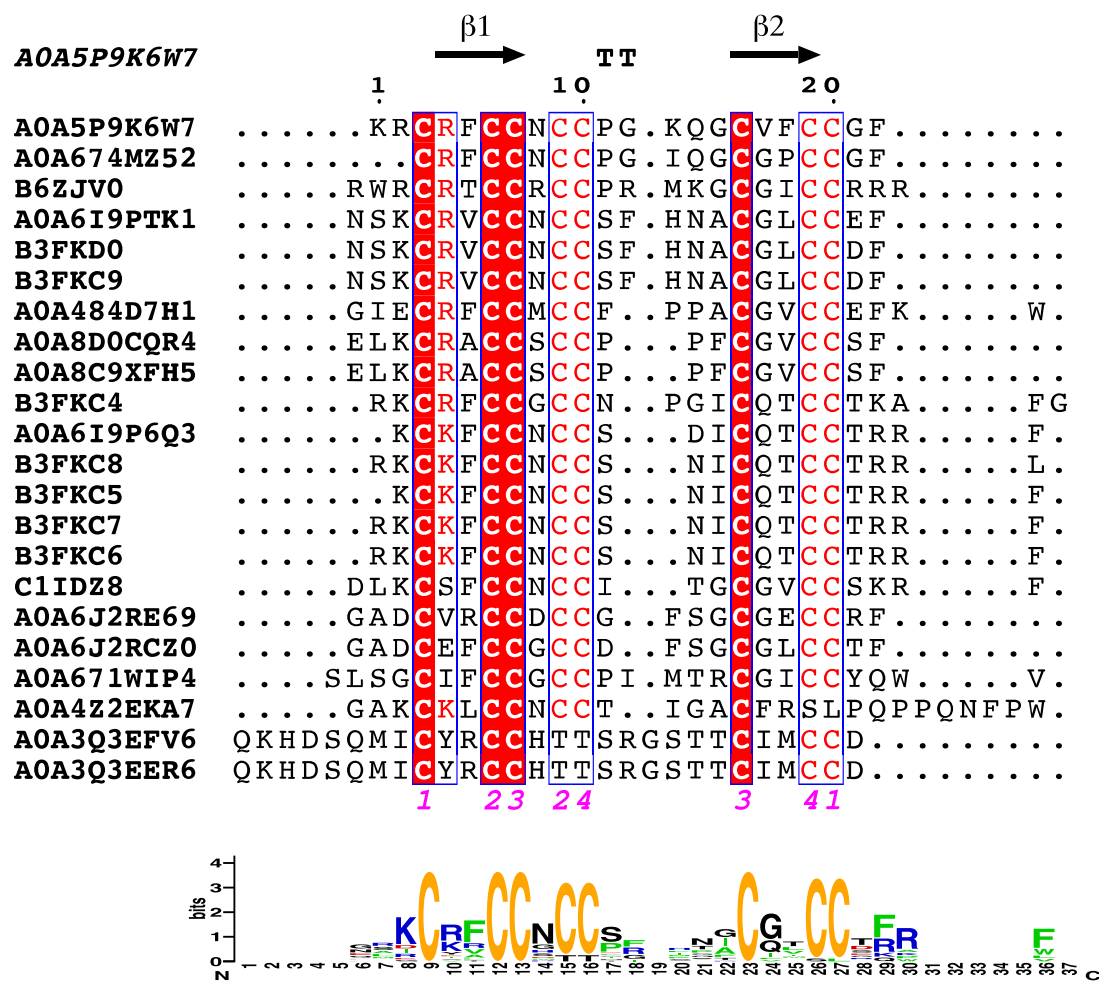
Supplementary Figure 1. Phylogenetic analysis of mature hepcidins in the different Eupercaria orders. The Neighbour-Joining (NJ) tree was obtained from 1000 replicates as described in Materials and Methods. The codes of the hepcidins are summarized in Supplementary Table S1.



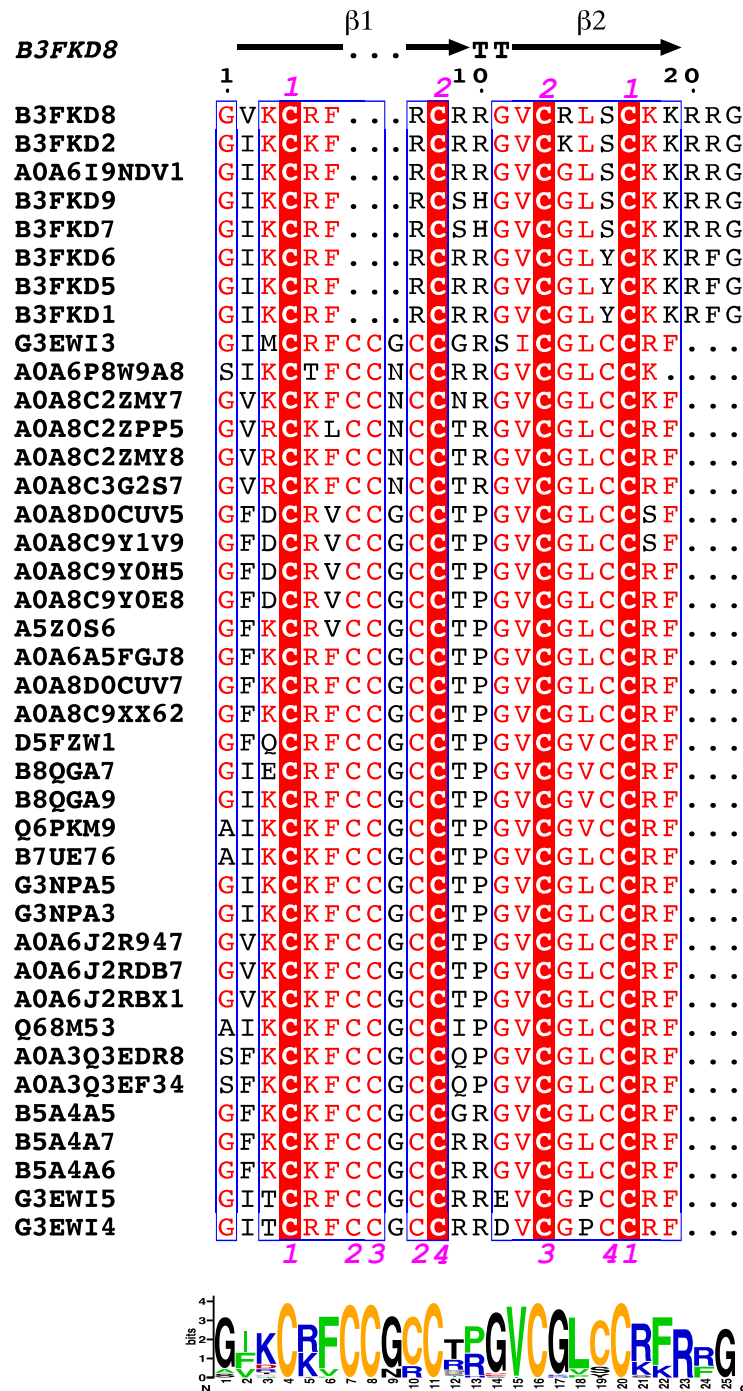
Supplementary Figure 2. Domain architecture of the hepcidin-like antimicrobial peptide (AMP) domains of gilthead seabream. A) Signal peptide domains are indicated in red, propeptide in brown and mature peptide in cyan. This structure is based on the hepcidin sequence corresponding to the UniProt sequence A0A671WFX4, and disulfide bridges are indicated by pairs of numbers below the sequence. B) Structure of the hepcidin corresponding to the A0A671WFX4 sequence, showing the three regions described above and the consensus sequences within Eupercaria of both the signal peptide cleavage site and the furin cleavage site, that releases the mature peptide.



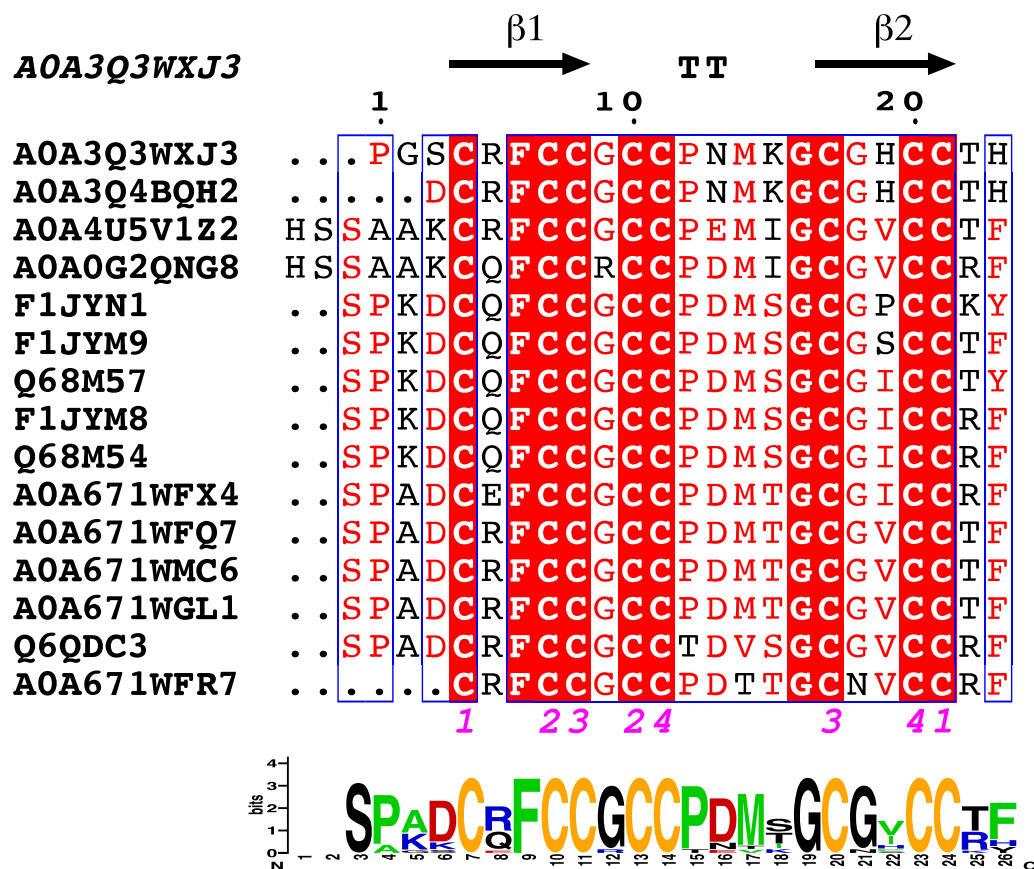
Supplementary Figure 3. Sequence alignment and logo of the sequences corresponding to clade 1. The UniProt sequences correspond to those shown in Figure 6 and Supplementary Table S1.



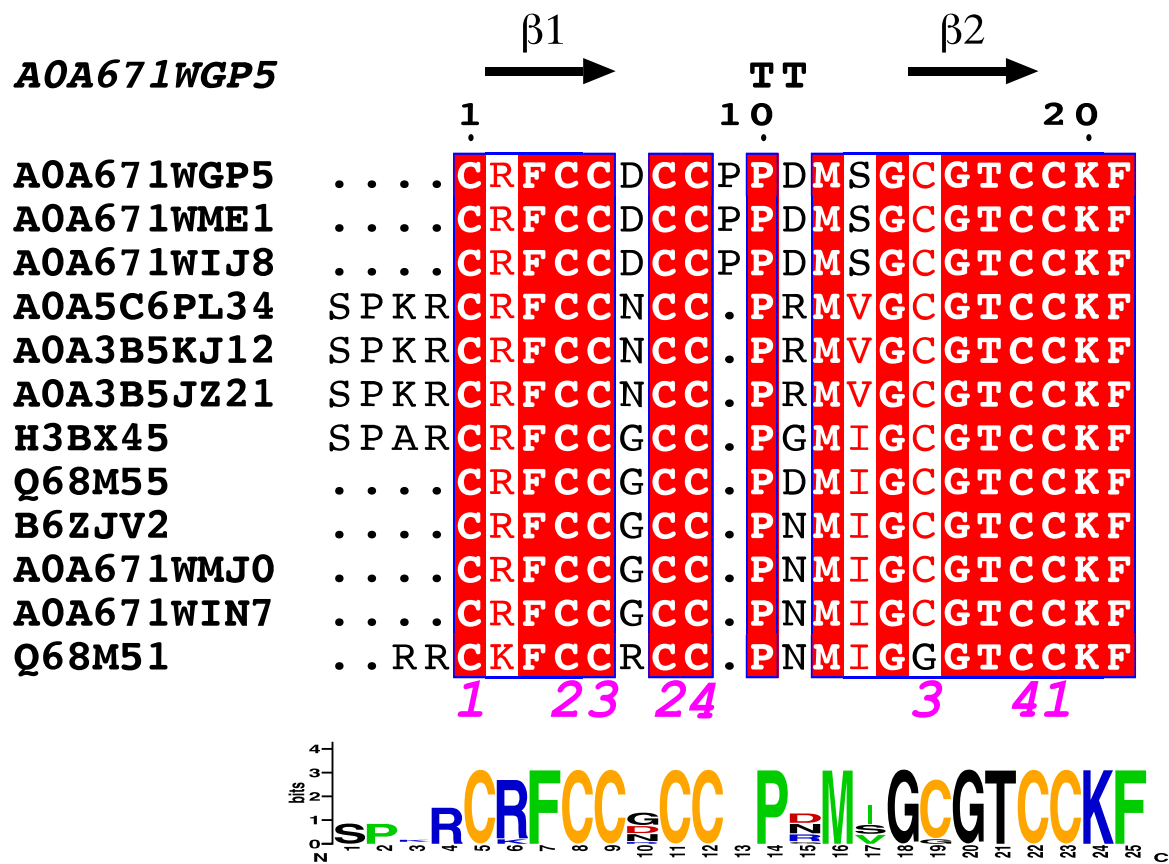
Supplementary Figure 4. Sequence alignment and logo of the sequences corresponding to clade 2.1. The UniProt sequences correspond to those shown in Figure 6 and Supplementary Table S1.



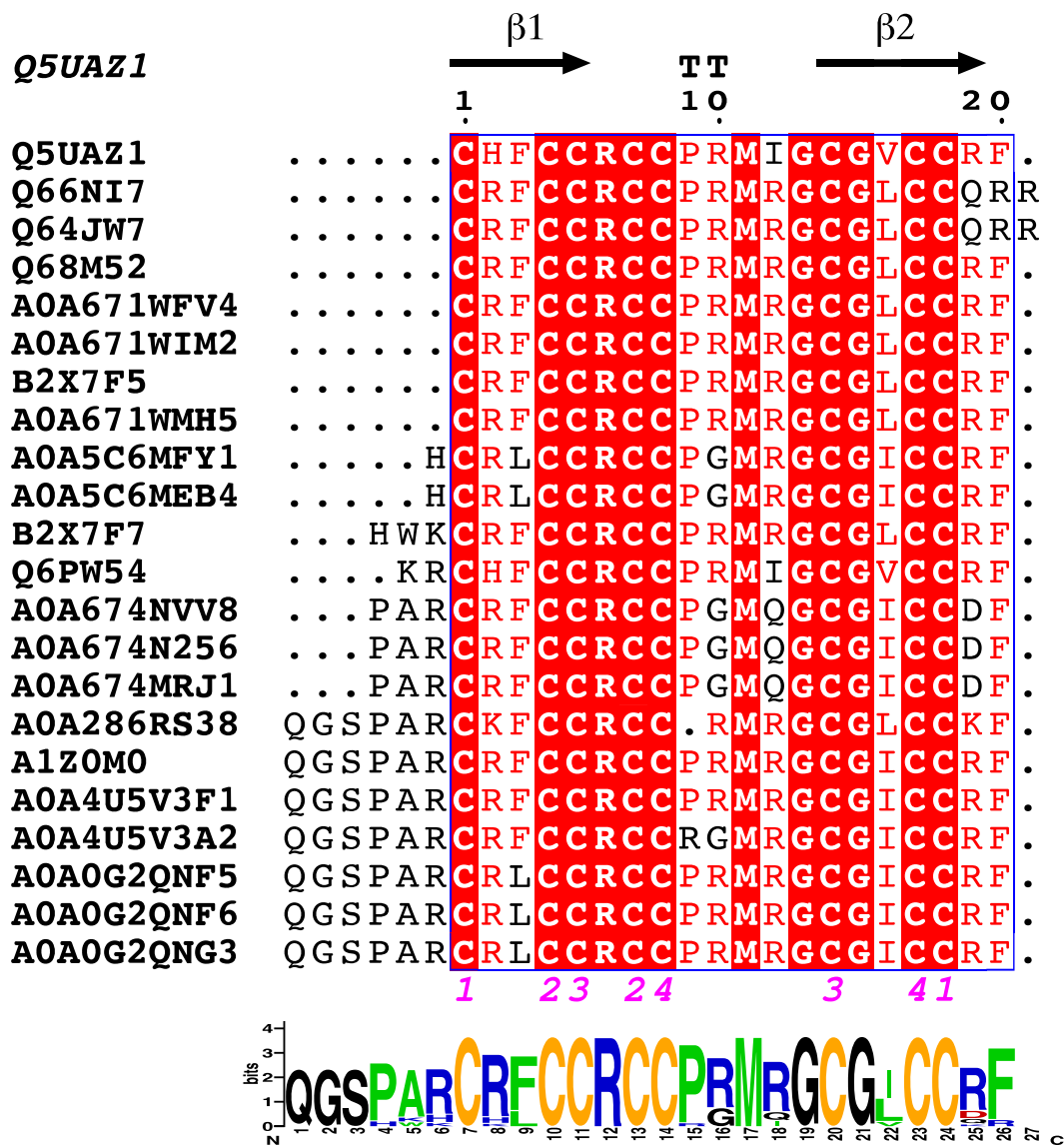
Supplementary Figure 6. Sequence alignment and logo of the sequences corresponding to clade 2.3. The UniProt sequences correspond to those shown in Figure 6 and Supplementary Table S1.



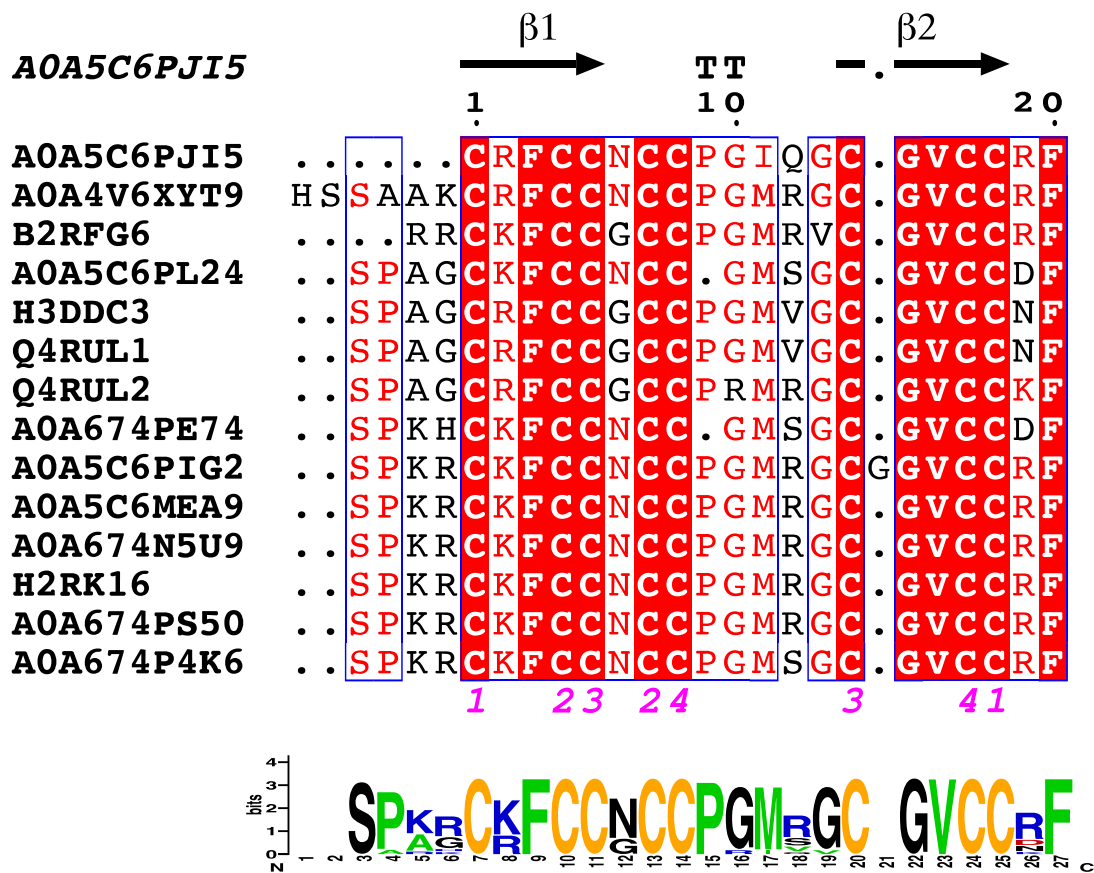
Supplementary Figure 7. Sequence alignment and logo of the sequences corresponding to clade 2.4. The UniProt sequences correspond to those shown in Figure 6 and Supplementary Table S1.



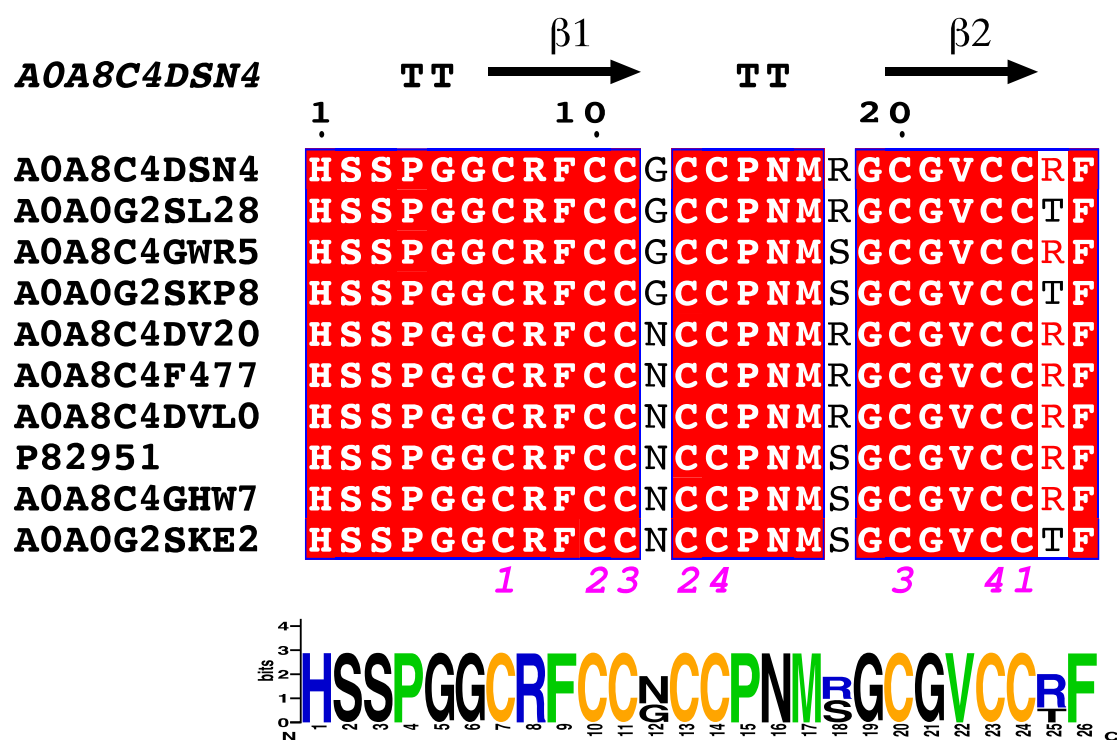
Supplementary Figure 8. Sequence alignment and logo of the sequences corresponding to clade 2.5. The UniProt sequences correspond to those shown in Figure 6 and Supplementary Table S1.



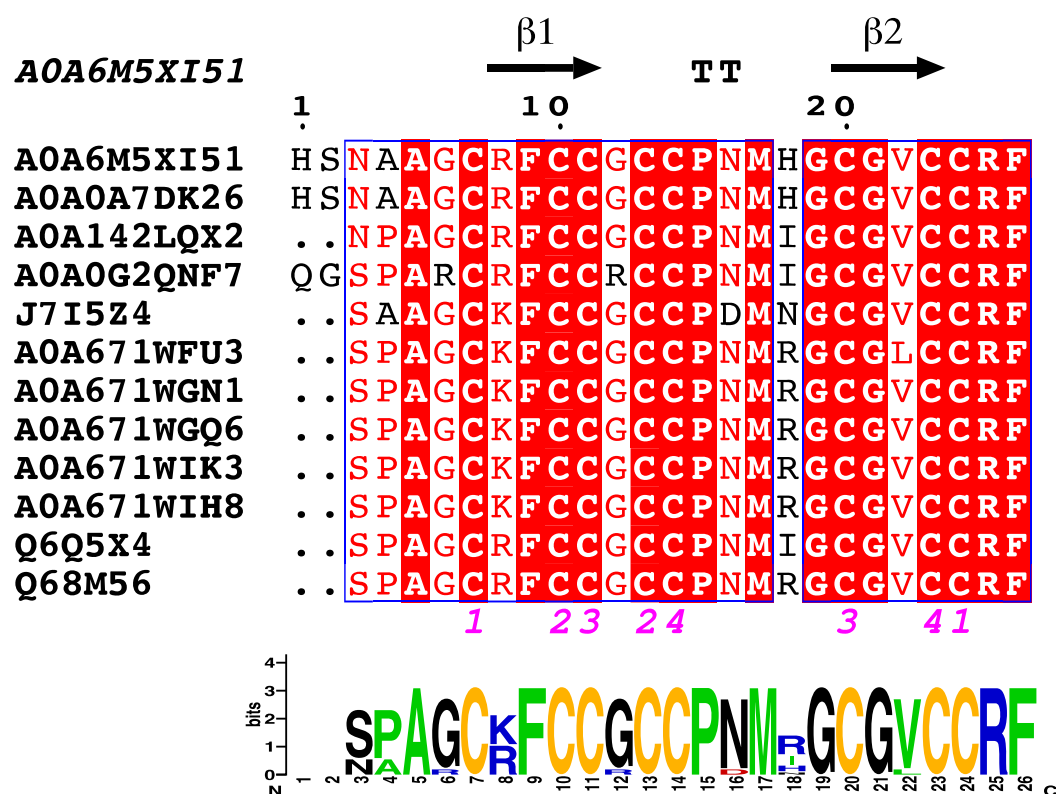
Supplementary Figure 9. Sequence alignment and logo of the sequences corresponding to clade 2.6. The UniProt sequences correspond to those shown in Figure 6 and Supplementary Table S1.



Supplementary Figure 10. Sequence alignment and logo of the sequences corresponding to clade 2.7. The UniProt sequences correspond to those shown in Figure 6 and Supplementary Table S1.



Supplementary Figure 11. Sequence alignment and logo of the sequences corresponding to clade 2.8. The UniProt sequences correspond to those shown in Figure 6 and Supplementary Table S1.



Supplementary Figure 12. Sequence alignment and logo of the sequences corresponding to clade 2.9. The UniProt sequences correspond to those shown in Figure 6 and Supplementary Table S1.