

3. Results

3.1. Sequence analysis of *C. macrocephalus* C3 cDNA

The partial cDNA sequence for *C. macrocephalus* C3 was obtained by RACE-PCR and consisted of 4140 bp, comprising an incomplete open reading frame of 3933 bp and a 3' UTR of 233 bp containing the poly A tail (Fig 1). The 3' UTR contained a consensus polyadenylation signal (AATAAA) 18 bp upstream from the poly A tail. The incomplete open reading frame of *C. macrocephalus* C3 sequence encoded a part of protein consisting of 1311 amino acid residues. Three possible N-glycosylation sites were observed at amino acid position 507, 599 and 978 (Fig. 1).

The deduced 1311 amino acid sequence of the partial *C. macrocephalus* C3 was compared with the corresponding region of other known C3 sequences. As shown in Table 2, the partial *C. macrocephalus* C3 had greater similarity to the corresponding part of C3s from teleost fish (52-64% identity; 70-79% similarity) than to those of mammals (41-45% identity; 62-64% similarity). Fig. 2 shows amino acid sequence alignment of the partial *C. macrocephalus* C3 with the corresponding part of other known C3s using CLUSTAL X program, indicating the conserved amino acids and several functionally important sites. The alignment revealed that this part of *C. macrocephalus* C3 contained the partial β chain located at the NH₂-terminal end and the complete α chain located at the COOH-terminal. The potential β - α processing signal of *C. macrocephalus* C3 (RKRR motif) was found to align perfectly with the homologous position of other C3s. The cysteine residues related with disulfide bond between the β - α chains in human were conserved in *C. macrocephalus* C3. Likewise, *C. macrocephalus* C3 possessed the factor I and C3 convertase cleavage sites (Arg-Ser) as found in human, with the exception of the second factor I cleavage site (Arg-Thr). Moreover, the thiolester site (GCGEQ) of *C. macrocephalus* was completely identical to those of other C3s. The key amino acids for other complement proteins, such as factor B, and factor H as well as properdin binding were also conserved in *C. macrocephalus* C3.

Table 2 Amino acid comparison between *C. macrocephalus* C3 and other C3s.

Species	GenBank	% identity/
	Accession no.	similarity
Common carp, <i>Cyprinus carpio</i>	AB016210	64/79
Zebrafish, <i>Danio rerio</i>	XM002660575	61/78
Rainbow trout, <i>Oncorhynchus mykiss</i>	L24433	58/74
Japanese flounder, <i>Paralichthys olivaceus</i>	AB021653	54/71
Spotted wolffish, <i>Anarhichas minor</i>	AJ309570	53/69
Japanese medaka, <i>Oryzias latipes</i>	AB025576	52/70
Norway rat, <i>Rattus norvegicus</i>	NM016994	45/64
Cattle, <i>Bos taurus</i>	AM086793	45/63
Human, <i>Homo sapiens</i>	AY513239	41/62

Human C3	--RSGLPIPTVSPYQIHFTKTPKFKPGMPFDMVFTNPDGSPAYR-VFVAVQGEOT-----VQSLT	Human C3	-----SLVVKSGQ--SEDRQVFGQQTMLKIEGDHGAARVVLAVDQGVFLNNKNNLTQSKIMDVVEKADIGCTPGSGKD
Mouse C3	--ERSGPIPTVSPYQIHFTKTPKFKPAMPFDLMVFTNPDGSPASK-VLVVVGQSN-----AKALT	Mouse C3	SCVG-----TLVVGSDP--RDNHGAARVGLVAVDQGVFLNNKNNLTQSKIMDVVEKADIGCTPGSGKD
Rat C3	--ERSGPIPTVSPYQIHFTKTPKFKPAMPFDLMVFTNPDGSPARR-VFVVTQGSQ-----AQALT	Rat C3	SCVG-----TLVVGSDP--KQDKHRRPGQQTMLKIEGDHGAARVGLVAVDQGVFLNNKNNLTQSKIMDVVEKADIGCTPGSGKD
Fig C3	--ERSGPIPTVSPYQIHFTKTPKFKPAMPFDLMVFTNPDGSPARR-IPVVTQDFK-----VRSLT	Fig C3	SCVG-----TLVVGKGG--KQDKHRRPGQQTMLKIEGDHGAARVGLVAVDQGVFLNNKNNLTQSKIMDVVEKADIGCTPGSGKD
Cattle C3	--ERSGPIPTVSPYQIHFTKTPKFKPAMPFDLMVFTNPDGSPARR-IPVVTQDFK-----VQSLT	Cattle C3	SCMG-----TLVVGKGG--KQDKHRRPGQQTMLKIEGDHGAARVGLVAVDQGVFLNNKNNLTQSKIMDVVEKADIGCTPGSGKD
Chicken C3	--ERSGPIPTVSPYQIHFTKTPKFKPAMPFDLMVFTNPDGSPARR-IPVVTQDFK-----VQSLT	Chicken C3	TCMG-----TLVVGKGG--KQDKHRRPGQQTMLKIEGDHGAARVGLVAVDQGVFLNNKNNLTQSKIMDVVEKADIGCTPGSGKD
Cobra C3	--EQGGHIVTSPYQIHFTKTPKFKPAMPFDLMVFTNPDGSPARR-IPVVTQDFK-----VQSLT	Cobra C3	TCMG-----TLVVGKGG--KQDKHRRPGQQTMLKIEGDHGAARVGLVAVDQGVFLNNKNNLTQSKIMDVVEKADIGCTPGSGKD
Xenopus C3	--EIEGIVTSPYQIHFTKTPKFKPAMPFDLMVFTNPDGSPARR-IPVVTQDFK-----VQSLT	Xenopus C3	TCMG-----TLVVGKGG--KQDKHRRPGQQTMLKIEGDHGAARVGLVAVDQGVFLNNKNNLTQSKIMDVVEKADIGCTPGSGKD
Trout C3-1	--EKRGIVTSPYQIHFTKTPKFKPAMPFDLMVFTNPDGSPARR-IPVVTQDFK-----VQSLT	Trout C3-1	TCMG-----TLVVGKGG--KQDKHRRPGQQTMLKIEGDHGAARVGLVAVDQGVFLNNKNNLTQSKIMDVVEKADIGCTPGSGKD
Trout C3-2	--EKRGIVTSPYQIHFTKTPKFKPAMPFDLMVFTNPDGSPARR-IPVVTQDFK-----VQSLT	Trout C3-2	TCMG-----TLVVGKGG--KQDKHRRPGQQTMLKIEGDHGAARVGLVAVDQGVFLNNKNNLTQSKIMDVVEKADIGCTPGSGKD
Trout C3-3	--EKRGIVTSPYQIHFTKTPKFKPAMPFDLMVFTNPDGSPARR-IPVVTQDFK-----VQSLT	Trout C3-3	TCMG-----TLVVGKGG--KQDKHRRPGQQTMLKIEGDHGAARVGLVAVDQGVFLNNKNNLTQSKIMDVVEKADIGCTPGSGKD
Trout C3-4	--EKRGIVTSPYQIHFTKTPKFKPAMPFDLMVFTNPDGSPARR-IPVVTQDFK-----VQSLT	Trout C3-4	TCMG-----TLVVGKGG--KQDKHRRPGQQTMLKIEGDHGAARVGLVAVDQGVFLNNKNNLTQSKIMDVVEKADIGCTPGSGKD
Flounder C3	--ELRGIVTSPYQIHFTKTPKFKPAMPFDLMVFTNPDGSPARR-IPVVTQDFK-----VQSLT	Flounder C3	TCMG-----TLVVGKGG--KQDKHRRPGQQTMLKIEGDHGAARVGLVAVDQGVFLNNKNNLTQSKIMDVVEKADIGCTPGSGKD
Wolfish C3	--ELRGIVTSPYQIHFTKTPKFKPAMPFDLMVFTNPDGSPARR-IPVVTQDFK-----VQSLT	Wolfish C3	TCMG-----TLVVGKGG--KQDKHRRPGQQTMLKIEGDHGAARVGLVAVDQGVFLNNKNNLTQSKIMDVVEKADIGCTPGSGKD
Medaka C3-1	--ELRGIVTSPYQIHFTKTPKFKPAMPFDLMVFTNPDGSPARR-IPVVTQDFK-----VQSLT	Medaka C3-1	TCMG-----TLVVGKGG--KQDKHRRPGQQTMLKIEGDHGAARVGLVAVDQGVFLNNKNNLTQSKIMDVVEKADIGCTPGSGKD
Medaka C3-2	--ELRGIVTSPYQIHFTKTPKFKPAMPFDLMVFTNPDGSPARR-IPVVTQDFK-----VQSLT	Medaka C3-2	TCMG-----TLVVGKGG--KQDKHRRPGQQTMLKIEGDHGAARVGLVAVDQGVFLNNKNNLTQSKIMDVVEKADIGCTPGSGKD
Carp C3-81	--ELRGIVTSPYQIHFTKTPKFKPAMPFDLMVFTNPDGSPARR-IPVVTQDFK-----VQSLT	Carp C3-81	TCMG-----TLVVGKGG--KQDKHRRPGQQTMLKIEGDHGAARVGLVAVDQGVFLNNKNNLTQSKIMDVVEKADIGCTPGSGKD
Carp C3-82	--ELRGIVTSPYQIHFTKTPKFKPAMPFDLMVFTNPDGSPARR-IPVVTQDFK-----VQSLT	Carp C3-82	TCMG-----TLVVGKGG--KQDKHRRPGQQTMLKIEGDHGAARVGLVAVDQGVFLNNKNNLTQSKIMDVVEKADIGCTPGSGKD
Carp C3-8	--ELRGIVTSPYQIHFTKTPKFKPAMPFDLMVFTNPDGSPARR-IPVVTQDFK-----VQSLT	Carp C3-8	TCMG-----TLVVGKGG--KQDKHRRPGQQTMLKIEGDHGAARVGLVAVDQGVFLNNKNNLTQSKIMDVVEKADIGCTPGSGKD
Zebrafish C3	--ELRGIVTSPYQIHFTKTPKFKPAMPFDLMVFTNPDGSPARR-IPVVTQDFK-----VQSLT	Zebrafish C3	TCMG-----TLVVGKGG--KQDKHRRPGQQTMLKIEGDHGAARVGLVAVDQGVFLNNKNNLTQSKIMDVVEKADIGCTPGSGKD
Catfish C3	--ELRGIVTSPYQIHFTKTPKFKPAMPFDLMVFTNPDGSPARR-IPVVTQDFK-----VQSLT	Catfish C3	TCMG-----TLVVGKGG--KQDKHRRPGQQTMLKIEGDHGAARVGLVAVDQGVFLNNKNNLTQSKIMDVVEKADIGCTPGSGKD
Lamprey C3	--ELRGIVTSPYQIHFTKTPKFKPAMPFDLMVFTNPDGSPARR-IPVVTQDFK-----VQSLT	Lamprey C3	TCMG-----TLVVGKGG--KQDKHRRPGQQTMLKIEGDHGAARVGLVAVDQGVFLNNKNNLTQSKIMDVVEKADIGCTPGSGKD
Hagfish C3	--ELRGIVTSPYQIHFTKTPKFKPAMPFDLMVFTNPDGSPARR-IPVVTQDFK-----VQSLT	Hagfish C3	TCMG-----TLVVGKGG--KQDKHRRPGQQTMLKIEGDHGAARVGLVAVDQGVFLNNKNNLTQSKIMDVVEKADIGCTPGSGKD
Amphioxus C3	--ELRGIVTSPYQIHFTKTPKFKPAMPFDLMVFTNPDGSPARR-IPVVTQDFK-----VQSLT	Amphioxus C3	TCMG-----TLVVGKGG--KQDKHRRPGQQTMLKIEGDHGAARVGLVAVDQGVFLNNKNNLTQSKIMDVVEKADIGCTPGSGKD
Sea urchin C3	--ELRGIVTSPYQIHFTKTPKFKPAMPFDLMVFTNPDGSPARR-IPVVTQDFK-----VQSLT	Sea urchin C3	TCMG-----TLVVGKGG--KQDKHRRPGQQTMLKIEGDHGAARVGLVAVDQGVFLNNKNNLTQSKIMDVVEKADIGCTPGSGKD

Fig. 2 Alignment of C3 amino acid sequences between *C. macrocephalus* and other known sequences. The 1311 amino acid sequence of *C. macrocephalus* C3 (Catfish C3, Genbank accession no. AB636135) was aligned with the corresponding part of *Homo sapiens* C3 (Human C3, Genbank accession no. K02765), *Mus musculus* C3 (Mouse C3, Genbank accession no. NM009778), *Rattus norvegicus* C3 (Rat C3, Genbank accession no. NM016994), *Bos taurus* C3 (Cattle C3, Genbank accession no. NM001040469), *Gallus gallus* (Chicken C3, Genbank accession no. NM205405), *Naja naja* (Cobra C3, Genbank accession no. Q0331), *Xenopus tropicalis* (Xenopus C3, Genbank accession no. BC168633), *Uncarhynchus mykiss* (Trout C3-1, Genbank accession no. P98093), *O. mykiss* (Trout C3-3, Genbank accession no. U 61753), *O. mykiss* (Trout C3-4, Genbank accession no. AF271080), *Paralichthys olivaceus* C3 (Flounder C3, Genbank accession no. AB021653), *Anarhichus minor* C3 (Wolfish C3, Genbank accession no. AJ309570), *Oryzias latipes* C3-1 (Medaka C3-1, Genbank accession no. NM_001105082), *O. latipes* C3-2 (Medaka C3-2, Genbank accession no. NM_001105083), *Cyprinus carpio* C3-H1 (Carp C3-H1, Genbank accession no. AB016210), *C. carpio* C3-H2 (Carp C3-H2, Genbank accession no. AB016212), *C. carpio* C3-S (Carp C3-S, Genbank accession no. AB016213), *Danio rerio* C3 (Zebrafish C3, Genbank accession no. XM002660575), *Lethenteron japonicum* C3 (Lamprey C3, Genbank accession no. AY359861), *Eutretus burger* (Hagfish C3, Genbank accession no. P98094), *Branchiostoma belcheri* (Amphioxus C3, Genbank accession no. AB050668), and *Strongylocentrotus purpuratus* (Sea urchin C3, Genbank accession no. NM214521) using the CLUSTAL X program. Conservation of amino acid identity is shown with an asterisk '*' whereas ':' and '.' indicate high and low levels of amino acid similarity, respectively.

Human C3	VVPEGIRNNNTVAVRTLDEPRLGGVGQKEDI PPAADS	DQVPTDSETRILLGLG-TPVAQ
Mouse C3	VVPEGMRIKNTVAHTLDEPRLGGVGQKVDVPPAADS	DQVPTDSETRILLGLG-TPVAQ
Rat C3	VVPEGMRIKNTVAVRTLDEPRLGGVGQKQREVD PPAADS	DQVPTDSETRILLGLG-TPVAQ
Fish C3	VVPEGRVRKVTVTRTLDEPHKGQGVQREIE PPAADS	DQVEDTSETRILLGLG-TPVAQ
Fish C3	VVPEGRVQKNTVAVRTLDEPHGGVGQREIE PPAADS	DQVPTDSETRILLGLG-TPVAQ
Chicken C3	VVPEGRRLKTKVIVLQVQKQVQKQVLS	DQVPTDSETRILLGLG-TPVAQ
Cobra C3	VVEGER-NKVTIEELDPSSVGGQVQELTVIANKL	DQVPTDEVTETRSVLG-TPVAQ
Xenopus C3	VVPEGMRIAQQVLTIVLEPQVKGKQVQEEIKALNPK	NVVFRTDITITLGLG-TPISQ
Trout C3-1	VVAEGVLV-KETNVLNPKVHG-GEQTSVSGVPR	NQVNSDADTLTSLVAGECTSVQ
Trout C3-2	VVPEGVTLRLQVNLKVLDPKHHG-GTDTVPVRS-GPLA	NGQVNTPARTHSITVSG-EDLAS
Trout C3-3	VVAEGVTLVRLQVNLKVLDPKHHG-GTDTVPVRS-GALT	DQVNTLTAHSITVSG-QGLAR
Flounder C3	VVPEGLVIL-QPQITLDPKHHG-GTDTVPVRS-GPLA	DWVPTDSETRILLGLG-TPVAQ
Wolfish C3	VVPEGVGVQ-SFQITLDPSTQVAGKQKQETLVSGIKPK	DLVQDTPQTSIQISVTRGVQVSG
Medaka C3-1	VVPGVLGV-IAESTLDPKHHGKNGQVQLNSINPES	NFI PESTPTSIQISVTRGVQVSG
Medaka C3-2	VVPEGLVL-TAKTVTLDPKHHGKNGQVQLNSINPES	NMI PESTPTSIQISVTRGVQVSG
Carp C3-B1	VVSGVGLVLRKNNKVELNPKVT--GEKFLVSKDIPV	DRVGTVPANTHSITVSG-ELIAQ
Carp C3-B2	VVSGVGLTVRRNVELNPKVT--GEKFLVSKDIPV	NKRVPTVPANTHSITVSG-EEITQ
Carp C3-B3	VVSGVGLTVRRNVELNPKVT--GEKFLVSKDIPV	DRVGTVPANTHSITVSG-EEISQ
Carp C3-B4	VVSGVGLVPLGRNVELNPKHHK--GEKFLVSKDIPV	DRVGTVPANTHSITVSG-EEISQ
Zebrafish C3	VVAEGVGLPLGRNVELNPKVKN--GDSPIVLRADIPV	DRVGTVPANTHSITVSG-EEISQ
Catfish C3	VVSGVGLTLEPQGNLNPSPRS-PGGVGVNKLKSDIPN	DQVNSPASTYLSITVSG-EEVQV
Lamprey C3	VVAEGVDRTIRSSRSVHVEERE--TFTEIHEISP	DGVNSDADTLTSLVSG-DELAE
Anguill C3	VVSGVGLVEHMSRSVQPRRHGG--QQVIVDNEPTP	NVVFTEMSAFSLAQSG-NLVAE
Aquaticox C3	TVVEGVGVRLVRS IFVDP-GRKARDKRRHGEVALPTEHVD	PDNGGLQVVDVRLPPTETEGSGEQVCAV IGM-DIMQ
Human C3		

Human C3 MTEADVAERLKHILITPVGCGGCGNIMGHTPTVIAVHYLDSTQWEKFG-LEKRGALIELIKKGYTQQLAFKQPSSSAFAA
 Mouse C3 MAEDVDVGRGLKHILITPVGCGGCGNIMGHTPTVIAVHYLDSTQWEKFG-LEKRGALIELIKKGYTQQLAFKQPSSSAFAA
 Rat C3 MAEDVDVGRGLKHILITPVGCGGCGNIMGHTPTVIAVHYLDSTQWEKFG-LEKRGALIELIKKGYTQQLAFKQPSSSAFAA
 Pig C3 MTEADIDGRLKHILITPVGCGGCGNIMGHTPTVIAVHYLDSTQWEKFG-LEKRGALIELIKKGYTQQLAFKQPSSSAFAA
 Cattle C3 MVEDAIDGERLKHILITPVGCGGCGNIMGHTPTVIAVHYLDSTQWEKFG-LEKRGESLILPKGYTQQLAFKQSSAAFA
 Chicken C3 LVEKATDGLNLHLITPVGCGGCGNIMGHTPTVIAVHYLDSTQWEFTG-INRRTAEILIKKGYTQQLAYKRGESFAA
 Xenopus C3 LTESIDGSLKHLILITPVGCGGCGNIMHTPSVATITLDATGQWENLG-VDREATAIKGVTGVAQMYKADHSYAA
 Trout C3-1 LVEQVDSGRLKHILITPVGCGGCGNIMHTPLVPIATLYLDKTNQWEAVG-FQKRDELQIHKIGYTNAGYKRPKSSYAA
 Trout C3-2 LVEQVDSGSLGLLQIPVGCGGCGNIMHTPLVPIATLYLDKTNQWEAVG-FQKRDELQIHKIGYTNAGYKRPKSSYAA
 Trout C3-3 TIEAASISGSLGLLQIPVGCGGCGNIMHTPLSPHITWRPTNVRKGLG-LERRNQASIGYQEGELAYKPKSSYAA
 Trout C3-4 TIEAASISGSLGLLQIPVGCGGCGNIMHTPLVPIATLYLDKTNQWEAVG-FQKRDELQIHKIGYTNAGYKRPKSSYAA
 Flounder C3 LVENAISGSMGTILVTPVGCGGCGNIMHTPLVPIATLYLDKTNQWEAVG-FQKRDELQIHKIGYTNAGYKRPKSSYAA
 Wolfish C3 LVENAISGTSLSLQIPVGCGGCGNIMHTPLVPIATLYLDKTNQWEAVG-FQKRDELQIHKIGYTNAGYKRPKSSYAA
 Modaka C3-1 LVENAISGSMGTILVTPVGCGGCGNIMHTPLVPIATLYLDKTNQWEAVG-FQKRDELQIHKIGYTNAGYKRPKSSYAA
 Modaka C3-2 LVENAISGSLGTILQIPVGCGGCGNIMHTPLVPIATLYLDKTNQWEAVG-FQKRDELQIHKIGYTNAGYKRPKSSYAA
 Carp C3-1 TVEQASISGFMRLIVPVGCGGCGNIMHTPLVPIATLYLDKTNQWEAVG-FQKRDELQIHKIGYTNAGYKRPKSSYAA
 Carp C3-2 TVEQASISGFMRLIVPVGCGGCGNIMHTPLVPIATLYLDKTNQWEAVG-FQKRDELQIHKIGYTNAGYKRPKSSYAA
 Carp C3-3 TVEQASISGFMRLIVPVGCGGCGNIMHTPLVPIATLYLDKTNQWEAVG-FQKRDELQIHKIGYTNAGYKRPKSSYAA
 Zebrafish C3 TVEQASISGFMRLIVPVGCGGCGNIMHTPLVPIATLYLDKTNQWEAVG-FQKRDELQIHKIGYTNAGYKRPKSSYAA
 Catfish C3 TVEQASISGFMRLIVPVGCGGCGNIMHTPLVPIATLYLDKTNQWEAVG-FQKRDELQIHKIGYTNAGYKRPKSSYAA
 Lamprey C3 THYNCLDKAISINLIGITPVGCGGCGNIIQAAPTLLTLYLSDQWEKIEG-LHRGEEAIFLQKQSYRELSYKADHSYAA
 Hagfish C3 TIGNTLKGSINLNLRLPVGCGGCGNIMHTPTVIVARYLSDNMGDGLPQKSSPFDITVSGFASQILTRPKDYSYAA
 Amphioxus C3 TITTTTGGG-LOTLLRLTTCGCGGCGNIIKARYNTLYSLHCTDQTKTNG--EKAQVIFQGYNQLSHRAPGSCFVS
 Human C3-1 TITTTTGGG-LOTLLRLTTCGCGGCGNIIKARYNTLYSLHCTDQTKTNG--EKAQVIFQGYNQLSHRAPGSCFVS

Human C3
Mouse C3
Rat C3
Pig C3
Catfish C3
Chicken C3
Cobra C3
Xenopus C3
TROUT C3-1
TROUT C3-2
TROUT C3-4
Flounder C3
Wolfish C3
WASH-3
Medaka C3-1
Medaka C3-2
Carp C3-B1
Carp C3-B2
Carp C3-H1
Zebrafish C3
Catfish C3
Lamprey C3
Bagfish C3
Amphioxus C3
Sea urchin C3

EVNR--FAPSTWLTAVYVKVFLAANLI-AIDSPQVLGCAVWLILEKQKPDGVQEDPAVIBQMIGL-LRNHNKMDMAI
FNNR--PSTWLTAVYVKVFLAANLI-AIDSPQVLGCAVWLILEKQKPDGVQEDPQVHQEMIGL-FRNAKAEADVL
FQNR--LSSTLTAVYVKVFANANLI-AIDSPQVLGCAVWLILEKQKPDGVQEDPQVFNQVHWIKEMIGL-KQTEKEDVSL
FYNR--PSTWLTAVYVKVFLAANLI-AIDSKLQETVWLILEKQKPDGVQEDPQVQVHWIKEMIGL-FRDTREKDVSL
FTTR--PSTWLTAVYVKVFAANAMV-DIEPVCVGAIKWLILEKQKPDGVQEDPAVPIHKEMVGG-VYQ-APSPVSL
FVNR--ASSSWLTAVYVKVFAANAMVDVIEICGGVWLILNRQQPDGVFNKAAPVPIHGEKLHG-TKG-APPEASL
WKDR--FSTWLTGVYKVGNAQVEF-DIEANVLGCAIKWLILEKQKPDGVFNAPYAVIHGEVGTITGAAEADSL
WGRN--QSTWLTAVYVKVFAMSTLI-SVQENLVCTAVKWLILQTPQDGFNEFAPIHAETGN--VRGSDDSAM
WYNR--PSTWLTAVYVKVFAANLI-VIEISVCAVWLHKRRQNPDETEKTLKLLTHQWGL-VLGDVDDSL
FLYE--RSGWLTAVYVKVFAANLI-VIEISVCAVWLHKRRQNPDETEKTLKLLTHQWGL-VLGDVDDSL
FASH--GSSSWLTAVYKVFAMANSVL-AVQNNDICAVKWLILHAQQPDGVKVFVGHAEHIGL-VKGTDSBCLH
WACR--PSAVALTAVYKVFAMANSVL-AVQNNDICEAKYLLHAQQPDGVFKDPAVPIHGEHTEG--CSRARFPCSL
WADH--GSSSWLTAVYVKSHANLVL-AIKHEIKDAVKFLILRAQQPDGFTTGKMYHIGEMIGL-VRGSDSDAM
YPRS--PSSSLTAVYVKVFSHANLVL-AIRNHKICDAVKFLILRAQQPDGFTTEVGRVSAVYMGDQ-VQGYSDSDAM
WTHR--PSTWLTAVYKVFAMANDA-AIEENVLCAKWLILHKKQDGLPGSFKEESAIVHGEVGD-VRKGDDADSL
WTHR--PSTWLTAVYKVFAMANDA-TIEBEKVLCAEWLILHKRTQDGSFKEESAIVHGEVGD-VQGNDDADSL
WIDR--PSTWLTAVYKVFAMANSVL-TIENVCASLAKWLILHKKQDGLPGSFKEESAIVSGMGVGD-VQGNDDADSL
WIDR--PSTWLTAVYKVFAMANSVL-TIENVCASLAKWLILHKKQDGLPGSFKEESAIVSGMGVGD-VQGNDDADSL
WIDR--PGSTWLTAVYKVFALANLI-SVDENVLCAVWLILHAQQPDGVFNKAAPVPIHGEKLHG-VQTEKEDVSL
WTHR--PSTWLTAVYVKVYSLAKRVL-IDNQLCEGVPEWIKKRNQSDGSVREDEPQVIRHMQQGS-VGGETHGVSH
FLNR--ASSTWLTAFVAKVYSQARQLV-FIPVSIKGSVPEWIKKRNQSDGSVREDEPQVIRHMQQGS-VGGETHGVSH
WGNNRYRCPSTWLTAFVYKQAKRVL-SIDAEAVKWLILHKKQDGLPGSFKEESAIVKHHREHTEG--VQGDAM
WTHR--PGSTWLTAVYKVFYSQANR-VLEGHVGSINWILNDGQLSGRQAFQSQVQVHQEMIGL-VKGETH

13

Human C3	VTVYHAKNQLGLT-CNKFDLKVTLKPAETAKR	
Mouse C3	VAVYHAKLSKVLT-CNKFDLRVLSIRAPETAATK	
Rat C3	VTVYHAKVVGKAT-CNKFDLRVTLKPAETAATK	
Fig C3	VTVYHMGKAGKGT-CNKFDLKVLSIHAEPEVKK	
Cattle C3	VTVYHAKLHGKVS-CNKFDLRVLSIRAPETAATK	
Chicken C3	VTVYHAKVPEKMGKCNFKDLRSVEDVAG-RE	
Cobra C3	VTVYNAQLREDAMCNKPHLDVSVENHNLKNG	
Xenopus C3	VTVYHALLTEKPEKCSNFELSVMKV-EELIARR	
Trout C3-1	VSLYYALPEEKSDCSDFKLSVTLTKMDKTSHE	
Trout C3-2	VSLYYATPEKMGKCNKFNVLVDVLIKQAVSLN	
Trout C3-3	VSLYYARPEKMGKCNKFNVLVDVLIPTQVSLIK	
Flounder C3	VSLYYALPQKKECDCKGKSNVSLLEIF-ELADL	
Wolfish C3	VSLYYAHPKKEKSDCKGFDMTVQLLPAEKMDQ	
Medaka C3-1	VSLYYALPEEKESDCKGFQDVSQVLLPKNIGT	
Medaka C3-2	VSLYYALPEEKESDCKGKFNDSVQLLFDNIGL	
Carp C3-01	LTLYYARFPEKKS DCTTFDITLVQKEDNE-ANQ	
Carp C3-02	LTLYYARFPEKKS DCTTFENLITVQKEDNE-VNK	
Carp C3-03	LTLYYARFVKNKSDCTLFDITLVQKEDNKSGE	
Zebrafish C3	LTLYYARFPEKKS DCTLFDLSVTKIDKDE-SK	
Catfish C3	YTLKYARFPEKKS DTLFDLSVQKREVSNSA	
Lamprey C3	HSVFNKVAPESSKS-CSTDLKVTNTEANDGERPQGLRGWDFGK	
Hagfish C3	VKNKRNKVTIKK-CXGFSLEITNLDNGQVFE	ARRKR
Amphioxus C3	EVNVTNTHQNRCELEFENLITLVQKEDNE	
Sea urchin C3	ELAYTEKTSRDKTCEPEFENLITLVQKEDNE	

	Protein binding site
Human C3	-----PQDAKNTMILIEICTRYR-----SDQDQMSILDSIMSGTGFAPDPTDLQKLANGVD--RYISKYIKDLKAFSDRNTLI
Mouse C3	-----PEAKNTMILIEICTRYL-----SDVDATSLIDISIMSGTGFAPDPTDLQLASGVD--RYISKYKMKNAKSNKNTLI
Human C3	-----PQDAKNSMIDLEICTRYL-----SDVDATSLIDISIMSGTGFAPDPTDLQLASGVD--RYISKYKMKNAKSNKNTLI
Pig C3	-----PQDAKNSMIDLEICTRYL-----SDVDATSLIDISIMSGTGFAPDPTDLQLASGVD--RYISKYKMKNAKSNKNTLI
Cattle C3	-----PQDAKNSMIDLEICTRYL-----SDVDATSLIDISIMSGTGFAPDPTDLQLASGVD--RYISKYKMKNAKSNKNTLI
Chicken C3	-----VEGVIRSVKVICTIRFL-----PDVDATSLIDISIMSGTGFAPDPTDLQLASGVD--RYISKYKMKNR--SNKNTLI
Cobra C3	-----AKGGAALKRLKICITRYL-----PEVDSTMTIDISIMSLTGFPDPAEDLRKANGVD--RYISKYKFNKDLNRSNRLI
Xenopus C3	-----PEGARSTVTEACARYL-----SKDDATSLIDISIMSGTGFAPDPTDLQLASGVD--RYISKYKFNKNGKQCTVV
Trout C3-1	-----DAKESFMTLIEVLKYK-----SERDATSLIDIGLGLTGTVDPDQLQLSGRGE--RYIEKFNKDKLSERGLI
Trout C3-2	-----GAQETVYHLDIVLKN-----SDRNATMTIDISILLTGFEVQDQLKLTGSTE--RYIQRFENKDKLSERGLI
Trout C3-3	-----EATETIKYLDIVLKN-----SDRNATMTIDISILLTGFEVQDQLKLTGSTE--RYIQRFENKDKLSERGLI
Flounder C3	-----DAKESFMTLIEVLKYK-----SDRNATMTIDISILLTGFEVQDQLKLTGSTE--RYIQRFENKDKLSERGLI
Wolfish C3	-----DEMVMYLRILIEVLYK-----SDRNATMTIDISILLTGFEVQDQLKLTGSTE--RYIQRFENKDKLSERGLI
Medaka C3-1	-----NQKRYKQIEVLYKYK-----SDRNATMTIDISILLTGFEVQDQLKLTGSTE--RYIQRFENKDKLSERGLI
Medaka C3-2	-----NQKRYKQIEVLYKYK-----SDRNATMTIDISILLTGFEVQDQLKLTGSTE--RYIQRFENKDKLSERGLI
Carp C3-81	-----GAIEYKTKIMDFYIKS-----DKDATTMTIDIGLITPGTFVDSRQDLQELSTGKE--RYIQKFNKDKLSERGLI
Carp C3-82	-----GAVASYTKIMDFYIKS-----DKDATTMTIDIGLITPGTFVDSRQDLQELSTGKE--RYIQKFNKDKLSERGLI
Carp C3-83	-----GAIEYKTKIMDFYIKS-----DKDATTMTIDIGLITPGTFVDSRQDLQELSTGKE--RYIQKFNKDKLSERGLI
Snfrafish C3	-----NQKRYKQIEVLYKYK-----DKDATTMTIDIGLITPGTFVDSRQDLQELSTGKE--RYIQKFNKDKLSERGLI
Catfish C3	-----DAQESVEIIDLKFDK-----SKSPATMTIDIGLITPGTFVDSRQDLQELSTGKE--RYIEKFNKDKLSERGLI
Lamprey C3	-----DIDGEGEVAEVNINLCTRYKPKRKL-----SKESGATIEIVNMLTGTFVDSRQDLQELSTGKE--RYIQKFNKDKLSERGLI
Hagfish C3	-----RRQSIENAFVNYFICRGYR-----RGRPGVMVMDISLPTGFEAKKQDLDMKNKVL--NYIVG--PG--RVR
Amphioxus C3	-----ROAEDDQSQHYFLVACTSYSGR-----GASNMADIDVGLISGFEFVKQDLQLNRNG--KKAVORYETNPP--SVI
Sea urchin C3	-----LMTVCTSYSGD-----GTTNMIIDVGLISGFEFVKQDLQLNRNG--KKAVORYETNPP--SVI

Human C3	ITLVKIVSTSEEDCLAFKVVYFVNLGIQGVAVVYVYNLEESCTFVYHEKDDGLNMLRCR--ELICRAEAGENCFTIQKS
Mouse C3	ILEVKSITHSEEDCLTFKVVQVFNVLGIQGVVSVYVYNLEESCTFVYHEKDDGLNMLRCR--ELICRAEAGENCFTIQKS
Human C3	ITLVKIVSTSEEDCLAFKVVYFVNLGIQGVAVVYVYNLEESCTFVYHEKDDGLNMLRCR--ELICRAEAGENCFTIQKS
Rat C3	ITLVKISHTLEEDCLISFKVYFVNLGIQGVVSVYVYNLEESCTFVYHEKDDGLNMLNCHK--EMCRAEAGENCFTFVKS
Chicken C3	ITLVKIVSTSEEDCLISFKVYFVNLGIQGVAVVSVYVYNLEESCTFVYHEKDDGLNMLNCHK--EMCRAEAGENCFTFVKS
Cobra C3	ITLVKIVSTSEEDCLISFKVYFVNLGIQGVAVVSVYVYNLEESCTFVYHEKDDGLNMLNCHK--EMCRAEAGENCFTFVKS
Xenopus C3	ITLVKIVSTSEEDCLISFKVYFVNLGIQGVAVVSVYVYNLEESCTFVYHEKDDGLNMLNCHK--EMCRAEAGENCFTFVKS
Trout C3-1	ITLVKIVSKLEDRISFKIHVQGVGLVQVPAASVYVEYDQ--TCQVFKVYHPQRKAGLLRLCRN--EECTCAEAGENSQHK
Trout C3-2	ITLVKIVSKLEDRISFKIHVQGVGLVQVPAASVYVEYDQ--TCQVFKVYHPQRKAGLLRLCRN--EECTCAEAGENSQHK
Trout C3-3	ITLVKIVSKLEDRISFKIHVQGVGLVQVPAASVYVEYDQ--TCQVFKVYHPQRKAGLLRLCRN--EECTCAEAGENSQHK
Trout C3-4	ITLVKIVSKLEDRISFKIHVQGVGLVQVPAASVYVEYDQ--TCQVFKVYHPQRKAGLLRLCRN--EECTCAEAGENSQHK
Flounder C3	ITLVKIVSKLEDRISFKIHVQGVGLVQVPAASVYVEYDQ--TCQVFKVYHPQRKAGLLRLCRN--EECTCAEAGENSQHK
Walleye fish C3	ITLVKIVSKLEDRISFKIHVQGVGLVQVPAASVYVEYDQ--TCQVFKVYHPQRKAGLLRLCRN--EECTCAEAGENSQHK
Medaka C3-1	ITLVKIVSKLEDRISFKIHVQGVGLVQVPAASVYVEYDQ--TCQVFKVYHPQRKAGLLRLCRN--EECTCAEAGENSQHK
Medaka C3-2	ITLVKIVSKLEDRISFKIHVQGVGLVQVPAASVYVEYDQ--TCQVFKVYHPQRKAGLLRLCRN--EECTCAEAGENSQHK
Carp C3-81	ITLVKIVSKLEDRISFKIHVQGVGLVQVPAASVYVEYDQ--TCQVFKVYHPQRKAGLLRLCRN--EECTCAEAGENSQHK
Carp C3-82	ITLVKIVSKLEDRISFKIHVQGVGLVQVPAASVYVEYDQ--TCQVFKVYHPQRKAGLLRLCRN--EECTCAEAGENSQHK
Carp C3-83	ITLVKIVSKLEDRISFKIHVQGVGLVQVPAASVYVEYDQ--TCQVFKVYHPQRKAGLLRLCRN--EECTCAEAGENSQHK
Rebafish C3	ITLVKIVSKLEDRISFKIHVQGVGLVQVPAASVYVEYDQ--TCQVFKVYHPQRKAGLLRLCRN--EECTCAEAGENSQHK
Catfish C3	ITLVKIVSKLEDRISFKIHVQGVGLVQVPAASVYVEYDQ--TCQVFKVYHPQRKAGLLRLCRN--EECTCAEAGENSQHK
Hampery C3	ITLVKIVSKLEDRISFKIHVQGVGLVQVPAASVYVEYDQ--TCQVFKVYHPQRKAGLLRLCRN--EECTCAEAGENSQHK
Amphipous C3	ITLVKIVSKLEDRISFKIHVQGVGLVQVPAASVYVEYDQ--TCQVFKVYHPQRKAGLLRLCRN--EECTCAEAGENSQHK
Sea urchin C3	ITLVKIVSKLEDRISFKIHVQGVGLVQVPAASVYVEYDQ--TCQVFKVYHPQRKAGLLRLCRN--EECTCAEAGENSQHK

14

Human C3 DD--KVTLERLDKACEPG---VDVYVKTLRKVKQLSNDFDEYIMAEIQTKSG-SDEVQVGQRTFIS-PKCREALK
Mouse C3 QE--KINLNVRLDKACEPG---VDVYVKTELNIELDDFDEYMTIQQVINSQ-SDEVQAGQRRFISH-ICRNRALK
Rat C3 QD--QVSLNERLDKACEPG---VDVYVKTLNLTIELSDDFDEYIMTIEQVINSQ-SDEVQAGQRRFISH-VGCRNALK
Pig C3 EE--EVTLDLRLERACEPG---VDVYVKTLRLKKLESDDFDDYIMVIEQIKSG-SDEVQVGQRRFISH-ICRREALK
Cattle C3 EE--EVTLEDRLDKACEPG---VDVYVKTLRLQKRLSDDFDEYIMVIENTIKSG-SDEVQVGQRRFISH-ICRREALK
Chicken C3 DM--PTTVNERLDLACKPG---VDVYVKVNVATETPSNDYIMVILTVINSQ-TDMFGGSGNRTFVSH-KQCRDALS
Cobra C3 QK--KIDLQLRLKACAGN---VDVYVKTLRLRIEKGNDIYPMVLEVIKGG-TORNAQAKARQVVSQ-RMCQEALN
Xenopus C3 IEG--KITPEMRINMACAPG---VDFVYKATLEVEQSENFNDYIMVIRKVIKQG-TDQPEEKTRNFISH-ICRNRALA
Trout C3-1 -G--EP-DVQRIDKACGAG---LDVYVKATVDSKLTHTTDTYTKIDLVIKPG-TDQEGVEGKRDPNGL-AYCREALG
Trout C3-3 HG--DMSKVKLLDKACEAG---MDVYFKASLVNATLESYTDYTLMEIKRIKEG-TDLLNGEKRLFLAH-PSCRALD
Trout C3-4 LG--DVSEIKLLDKACEH---MDVYFKASLVNATLESYTDYTLMEIKRIKEG-TDLVLEGGKRLFLAH-PSCREALD
Flounder C3 -G--KISNDQRIEKSCTTPTSKIDFVYKVLLEETDGGSTDTYIMRIEIKESGYDVAPOGKLATFLSY-QHCRESLD
Wolfish C3 -E--QVSGDQRTARVCETELMNKIDFAYRVGLLEETDGLSTDTYKRVVLQVINGSGKDVGPLNKERTLSY-PHCRRPDL
Medaka C3-1 -G--QISNDERTSKICESTESSKIEYAVKVLVEDVQVQPSIDYAMRVQDSINEGSTDVGPKKLRFPLSY-PHCRRALN
Medaka C3-2 -G--QISNDERTSKICESTESSKIEYAVKVLVEDVQVQPSIDYAMRVQDSINEGSTDVGPKKLRFPLSY-PHCRRALN
Carp C3-B1 -N--HIGDEERFNRACEAG---MDVYVKVNVGHDLQDQADIDYLEVEQVLEKQ-TDQVVEGKRRPFLAR-PTCRNHLG
Carp C3-B2 -N--RVSDERFNRACEAG---MDVYVKVNVGHDLQDQSDIYDMKVEQVLEKQ-TDQVVEGKRRPFLAR-PTCREYLG
Carp C3-S -N--HVEDDERFNRACEAG---MDVYVKVNVGHDLKQSDIYEMKVEQVLEKQ-TDQVVEGKRRPFLAR-PTCREHGL
Zebrafish C3 -G--GFKDEERNKACEAG---MDVYVKVNVGHDLAADADIDYQMEVEQVLEKQ-TDQVIEGQVKSFLGR-PSCKESLG
Catfish C3 -Q--KIDEKERENKACDAG---MDVYVKVNVGLDPSPTDIFYEMKIEEVLKEG-SDQVHDKVRNFMGH-ANCRSEFG
Lamprey C3 NFDTSLTVLHREAAACVAG---IDYAVGVINDNRTEVRSFVYTVNIQVTKSGGQDAIQPKAIRLFIVT-RSCDRLG
Hagfish C3 MAD--EDRNADRNACACAE---VYFVIGVTKVTKTASYINIHAAKTVLKKHMDQALNKGARRSFVIP-NHCGKNLN
Amphioxus C3 PKKLRELTQRDLLEKACH---DLQYAFVIVMEVREQGSFDRIIMVESPXKGLDIDVDEGERVFWRRKTCSGQLQVE
Sea urchin C3 DPCPGFYTRIDLEGTACAS---HSSYALKIRIDEVEIKEGFRICKETVLNPTNGDGVPHQAQRQLFNEGDCPKVKG

Human C3 LEEKKHVLMGLSSDLWGEKFN---LSYIIGKDTWVEHWPEEDECQDEE---NQKQCQDLGAFTESMVVFGCPN
Mouse C3 LQKGKKVLMGLSSDLWGEKFN---TSYIIGKDTWVEHWPEAEBCQDQK---YKQCQELGAFTESMVVFGCPN
Rat C3 LQKGKQVLMGLSSDLWGEKFN---TSYIIGKDTWVEHWPEAEBCQDQK---NQKQCQDLGAFTESMVVFGCPN
Pig C3 LKEGGHYLVGVSSDLWGEKFN---ISYIIGKDTWVEHWPEAEBCQDEE---NQKQCQDLGAFTESMVVFGCPN
Cattle C3 LKEGANYLVGVSSDLWGEKFN---ISYIIGKDTWVEHWPEAEBCQDEE---NQKQCQDLGAFTESMVVFGCPN
Chicken C3 LQKGQDYLWGLASDLWVTGSR---FSYIIGKDTWLEAWPLEECQDAD---LQPLCQDFTESDNLVLPFGPT
Cobra C3 LKLDNDYLVGLSSDLWPMKDD---ISYIITNMTWIERPNEDECQDEE---FQNLCDFAQLSNTLTIFGCP
Xenopus C3 MQLNRDYLWVTGDLWKPDPG---YSYIIGKDTWIEHWPNRECEQDRE---NLDLCDDEFTVSDNLEIVGCPN
Trout C3-1 LMQKTYLIMGKSEDHHRVEDKLLQYKYVLGEQTWIEYWPSEQECTSRD---YREVCGLIDEFINQITFGCPV
Trout C3-3 LEEGQSYLLIGDTADLIQS-VT---DRYVLGERTWIEYWPSTDECNMQDEKTRKRLIYDFVHEMQSEGCVN
Trout C3-4 LKEGQSYLLIGDTADLIQS-VT---DRYVLGERTWIEYWPSTDECNMQDEKTRKRLIYDFVHEMQSEGCVN
Flounder C3 LGKGRMYLIMGTSKDIHRDDQN---ESYHYVLGERTWIEYWPTEAECQTEE---HRPTCLGLQGMVQYLLFGCQQ
Wolfish C3 LRTGNMYLIMGASTDIYRDDQA---QSHQYVLGERTWIEYWPTEKQCQTEE---YHRTCLGNAEHVEQYRVFGCQQ
Medaka C3-1 LQKGKTYLIMGSSRDINHDEQ---LTYQYVLGERTWIEYWPTEAECQDGE---HRATCLGLDHEMLQFRVACQQ
Medaka C3-2 LKLGKTYLIMGSSRDINHDEK---QTYQYVLGERTWIEYWPTEAECQDGE---HRDTCLGLDHEMLQFRVACQQ
Carp C3-B1 LVKDKSYLIMGRSVDLPDLGGS---LQYIFGEQTWIEYWPTRQESQJRE---HRDRYIGISDLQNSLLKEGCAT
Carp C3-B2 LLEGKSYLIMGRSVDLPDLGGS---LQYIFGEQTWIEYWPTRQESQJRE---HRDRYIGISDLQNSLLKEGCAT
Carp C3-S LVKDKSYLIMGRSVDLPDLGGS---LQYIFGEQTWIEYWPTRQESQJRE---HRDRYIGISDLQNSLLKEGCAT
Zebrafish C3 FVQGSYLYMGKSTDLPLGGS---LQYILGEQTWIEYWPTEKQEGJKE---HRDRYIGISDLQNSLLKEGCAT
Catfish C3 FKQGSYLYMGKSTDLPLGGS---LQYILGEQTWIEYWPTEKQEGJKE---YKDSYIGISDLQNSLLKEGCAT
Lamprey C3 METFRQYLLMGRKGETKKNDR---FQYVLDSSSVWEQWPTDEKCNQPN---VQAFCAIKREYEFSSQIQGCS
Hagfish C3 VSPGDIYLVGKSHVNRNISR---TQYVLDSSSVWEQWPTDEKCNQPN---PASCQVSENFQKQSLG---
Amphioxus C3 G---TTVLWKGDKTYKTEQGE-DSFRYVITEGSPVAEWPTGAKEARK---PKRVADPQQLADKIFLGCTT
Sea urchin C3 RNIIGGTFLLVGKSLKYTTEQGE-ERYRYVYGPSTSKTEFWPATRKARNAA---VFDKLVAFQAQMAPASACSMEN

Fig. 2 (continued).

3.2. Sequence analysis of *C. macrocephalus* C8 γ cDNA

The full-length of C8 γ cDNA consisted of 886 bp comprising a 5' untranslated region (UTR) of 100 bp, an open reading frame of 633 bp, a stop codon of 3 bp and a 3' UTR of 150 bp containing the poly A tail. The 3' UTR contained a consensus polyadenylation signal (AATAAA) 16 bp upstream from the poly A tail (Fig. 3). The open reading frame encoded a protein consisting of 211 amino acid residues. N-terminal segment included a high proportion of hydrophobic amino acid residues and therefore the first 24 amino acid residues were predicted to be a signal peptide by the SignalP program. The mature *C. macrocephalus* C8 γ consisted of 187 amino acids with molecular mass of 21.64 kDa. No potential *N*-glycosylation site was observed in *C. macrocephalus* C8 γ after the NetNGlyc program analysis.

A BLASTX homology search revealed high similarity to C8 γ from fish and mammals, and much lower to other known lipocalins (Table 3). Multiple alignment of amino acid sequence alignment of *C. macrocephalus* C8 γ and other known C8 γ indicated three conserved cystein residues that involved in formation of disulphide bond in most C8 γ proteins (Fig 4).

3.3. Phylogenetic analysis of *C. macrocephalus* C3 and C8 γ

Phylogenetic analysis of *C. macrocephalus* C3 with C3 molecules from other species was performed using the Clustal software, based on the deduced amino acid sequence alignment of the C3 α -chains. The resulting tree showed that all the bony fish formed a cluster (Fig. 5). *C. macrocephalus* C3 were grouped with zebra fish and carp C3 indicating that the walking catfish (belong to order Siluriformes) and these two fish species (belong to order Cypriniformes) were phylogenetically close species. However, among these three species, zebra fish and carp, which belong to the same order, are strongly genetically related.

The amino acid sequence alignment of full-length *C. macrocephalus* C8 γ with other members of lipocalin family was used to generate a phylogenetic tree. The unrooted phylogenetic tree obviously indicated that *C. macrocephalus* C8 γ is more closely related to C8 γ of fishes and mammals than to other lipocalins (Fig. 6).

Table 3 Amino acid comparison between *C. macrocephalus* C8 γ and other known lipocalin proteins.

BLAST match	GenBank	% identity/
	Accession no.	similarity
C8 γ (Zebrafish, <i>Danio rerio</i>)	NM200863	50/68
C8 γ (Rainbow trout, <i>Oncorhynchus mykiss</i>)	NM001124408	44/68
C8 γ (Human, <i>Homo sapiens</i>)	NM000606	36/59
C8 γ (House mouse, <i>Mus musculus</i>)	NM027062	36/56
α -1-Macroglobulin (Cattle, <i>Bos taurus</i>)	BC102637	30/54
α -1-Macroglobulin (Human, <i>H. sapiens</i>)	NM001633	25/48
Prostaglandin D-synthase (Zebrafish, <i>D. rerio</i>)	NM213634	22/46

ACGCGGGGACAGGGCAACAGCAACCAACCAAACTGTAGCGGTGGTTTTAGTGCGATAAATAGA 60
GTGTTCTGTATTATAAATGAAAAGGTGGAATAAGTGTGGAGGTATGTTGCCGTGTGTGGCTCTA 120
M L R V C V Y
TTTGTCTCTAGTCAATTTGTTGGGTTTTAGTCTCTCTGGGAGCCTGTGGATGGAAGACGAAC 180
L S L V I L L G F S L M E P V D G R R T
TCGATACAGCCAAACCAACCCAAACCCAGAAAAAAGCCAATTGAGGAGATCGTTCCAGC 240
R Y S Q T N P N Q K K K P I E E I V P A
TCAAGACATTGATTTCAATAAGTTGAGTGGAAAAGTGGTATCTGCTAAGTGTGGCTTCAAG 300
Q D I D F N K L S G K W Y L L S V A S R
ATGTAAGTACCTTTTTAGAACATGGTTTTAAAGTGGAGGGGCACCGTCATAACTATGTTTGC 360
C K Y L L E H G F K V E G T V I T M F A
ACCTGACTCACCCAATGCACCAATCCAAGTGAGCACCTTCACCAAACTCAATTATCAGTG 420
P D S P N A P I Q V S T F T K L N Y Q C
TTGGGAGATCAAAACAGAAGTACGAGACCTCACAGAATTTGGGCCGCTTTTACTAAAAAGC 480
W E I K Q K Y E T S Q N L G R F L L K A
AAAGATACCAGTGAAGGAACACTGAGATCATTATTGTAGAAACCAAACTACAACTCTTACGC 540
K I P V K N T E I I I V E T N Y N S Y A
CATTTTACTTTACAAAGCAGATGAACAAGCTCCCCATGAAACTTTACGGCCGCACCCCCAGA 600
I L L V K Q M N K L P H K L Y G R T P E
GATCGCTGAAAGTATCGTGGATAAATTTGAAGACATGGCAAAAAAACAAGATCTGGGCCCT 660
I A E S I V D K F E D H A K K Q D L G L
GGATGTTGTTTTCCAGTTTCCTACTTATGGATTTTGTCTAGTCTGCAGACAAGGAACATAC 720
D V V F Q F P T Y G F C Q S A D K E H T
GCTCGTAATGGCCTGAAGAGGATTGTTTTGTGATAGAAAGTCTTTGGATATGACGGCTTCA 780
L V H A *
TCGTACCAGAATGCATAAGCACAAAGAACCGATTTCGACGTGGTAGAAATAATTAACAAT 840
AAACTCTGCCATTTCTGCCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA 886

Fig.3 Full-length nucleotide and deduced amino acid sequences of *C. macrocephalus* C8γ cDNA

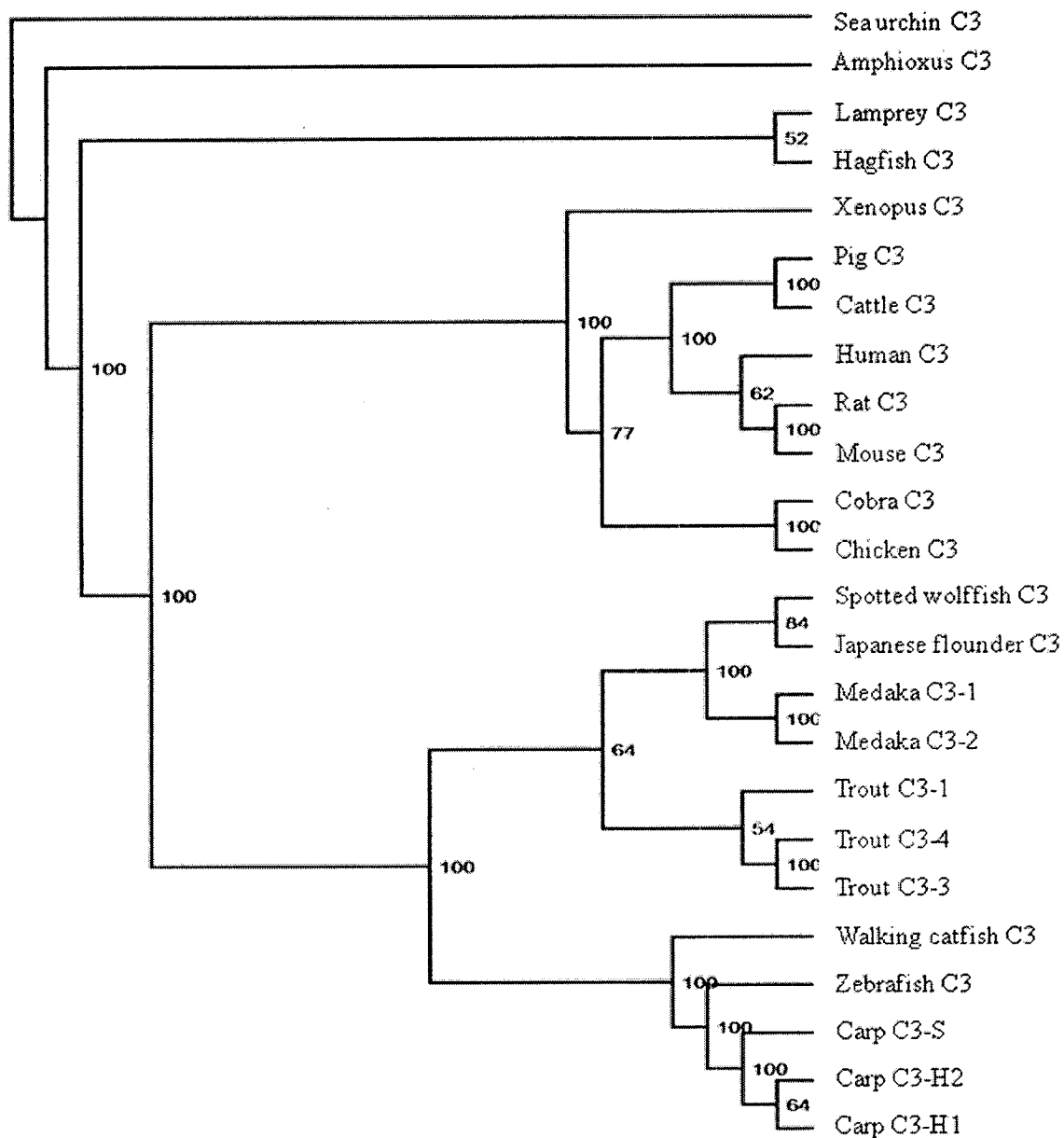
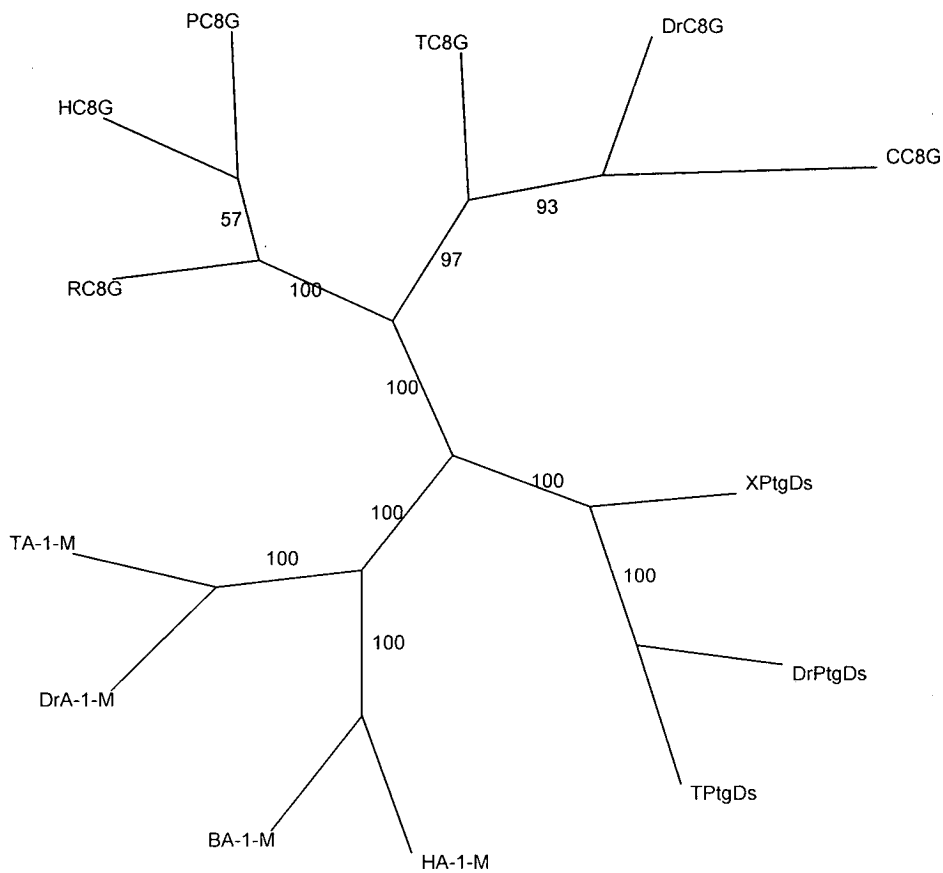


Fig. 5 Phylogenetic relationships of C3 proteins from different species. The tree was conducted by the neighbor-joining method based on the deduced amino acid sequences of C3s aligned in Fig. 2.



_10

Fig. 6 Unrooted phylogenetic tree various lipocalins. Amino acid sequence alignments of *Bos taurus* α -1-microglobulin (BA-1-M), *Danio rerio* α -1-Microglobulin (DrA-1-M), *Homo sapiens* α -1-microglobulin (HA-1-M), *Oncorhynchus mykiss* α -1-microglobulin (TA-1-M), *D. rerio* prostaglandin D-synthase (DrPtgDs), *Oncorhynchus mykiss* prostaglandin D-synthase (TPtgDs), *Xenopus laevis* prostaglandin D-synthase (XPTgDs), *Clarias macrocephalus* C8 γ (CC8G), *D. rerio* C8 γ (DrC8G), *H. sapiens* C8 γ (HC8G), *Sus scrofa* C8 γ (PC8G), *Orytolagus cuniculus* C8 γ (RC8G), *Oncorhynchus mykiss* C8 γ (TC8G) were used to generate the tree by the Phylip program.

2.5. Tissue distributions of *C. macrocephalus* C3 and C8 γ mRNA

Tissue expression profiles of *C. macrocephalus* C3 and C8 γ mRNA were studied by RT-PCR. As shown in Fig. 7 and Fig. 8, *C. macrocephalus* C3 and C8 γ mRNA expression was detected mainly in liver (lane 4). Expression was also observed at low level in brain (lane 1), heart (lane 2) and muscle (lane 7) for *C. macrocephalus* C3 and in kidney (lane 3), spleen (lane 5), intestine (lane 6) and muscle (lane 7) for C8 γ . Expression of β -actin used as internal control was observed in all tested tissues.

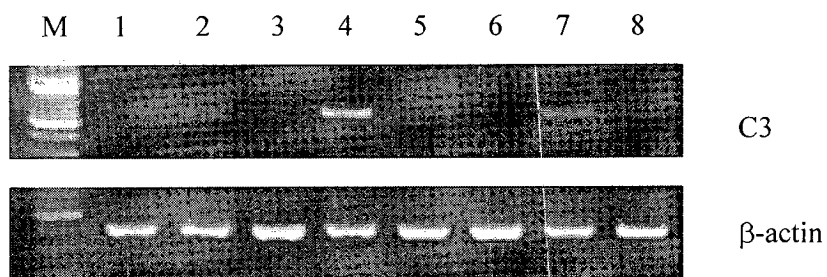


Fig. 7 Expression of *C. macrocephalus* C3 gene in various tissues. Lane 1, brain; lane 2, heart; lane 3, kidney; lane 4, liver; lane 5, spleen; lane 6, intestine; lane 7, muscle; lane 8, ovary. M indicates the molecular weight marker (100 bp. ladder).

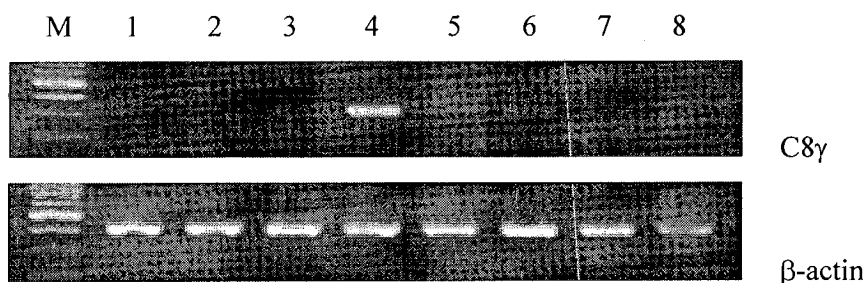


Fig. 8 Expression of *C. macrocephalus* C8 gene in various tissues. Lane 1, brain; lane 2, heart; lane 3, kidney; lane 4, liver; lane 5, spleen; lane 6, intestine; lane 7, muscle; lane 8, ovary. M indicates the molecular weight marker (100 bp. ladder).

2.6. Developmental expression of *C. macrocephalus* C3 and C8 γ mRNA

The expression levels of *C. macrocephalus* C3 and C8 γ mRNA were determined during catfish development at 1, 3, 5, 10, 20 and 30 days after hatching. *C. macrocephalus* C3 and C8 γ expressions were detected at all tested ages. Both *C. macrocephalus* C3 and C8 γ were found to be expressed at a low level at 1, 3 and 5 days post hatching. Strong expressions were observed at 20 and 30 days after hatching. Over all, transcripts of *C. macrocephalus* C3 and C8 γ gradually increased as development progressed (Fig. 9).

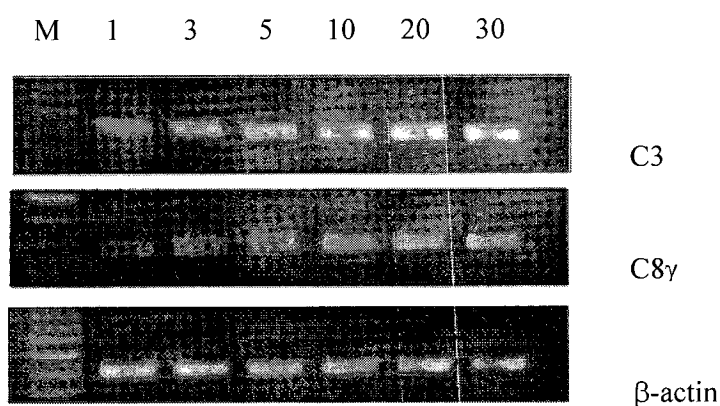


Fig. 9 Semi-quantitative RT-PCR analysis of C3 and C8 in the different larval stages of *C. macrocephalus*. M indicates the molecular weight marker (100 bp Ladder).

2.7. Expression of *C. macrocephalus* C3 and C8 γ mRNA in fish larvae

Localization of *C. macrocephalus* C3 and C8 γ mRNA expression in fish larvae were determined by *in situ* hybridization. Fish larval sections were prepared and hybridized with the sense and anti-sense probes for *C. macrocephalus* C3 and C8 γ mRNA. Hybridization performed with the *C. macrocephalus* C3 anti-sense probe showed staining of hepatocyte cells (dark purple hepatocytes) (Fig. 10), whereas no hybridization was observed in any

sections using the *C. macrocephalus* C8 γ anti-sense probe. There was no staining of the control sections hybridized with the *C. macrocephalus* C3 and C8 γ sense probes.

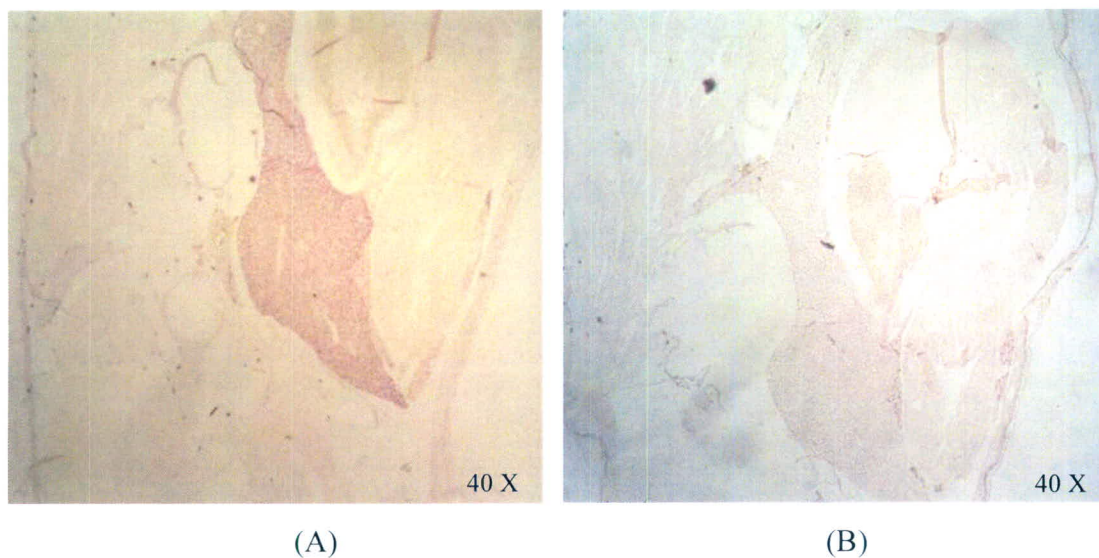


Fig. 10 Expression of *C. macrocephalus* C3 mRNA in liver hepatocytes performed by *in situ* hybridization with anti-sense probe (A) and sense probe (B).

2.8. The relative expression levels of *C. macrocephalus* C3 and C8 γ mRNA after β -glucan administration

The expression of *C. macrocephalus* C3 and C8 γ transcripts was examined in catfish fingerlings fed with β -glucan for 1, 3, 7 and 14 days. Semi-quantitative PCR analysis showed significant up-regulation of *C. macrocephalus* C3 mRNA in all β -glucan feeding groups (Fig. 11), compared to the 0 day unstimulated group ($P < 0.05$). The highest level of C3 gene expression was observed in catfish fingerlings fed with β -glucan for 7 days. There was no difference of the expression levels of *C. macrocephalus* C8 γ mRNA between β -glucan feeding groups and the 0 day unstimulated control (data not shown).

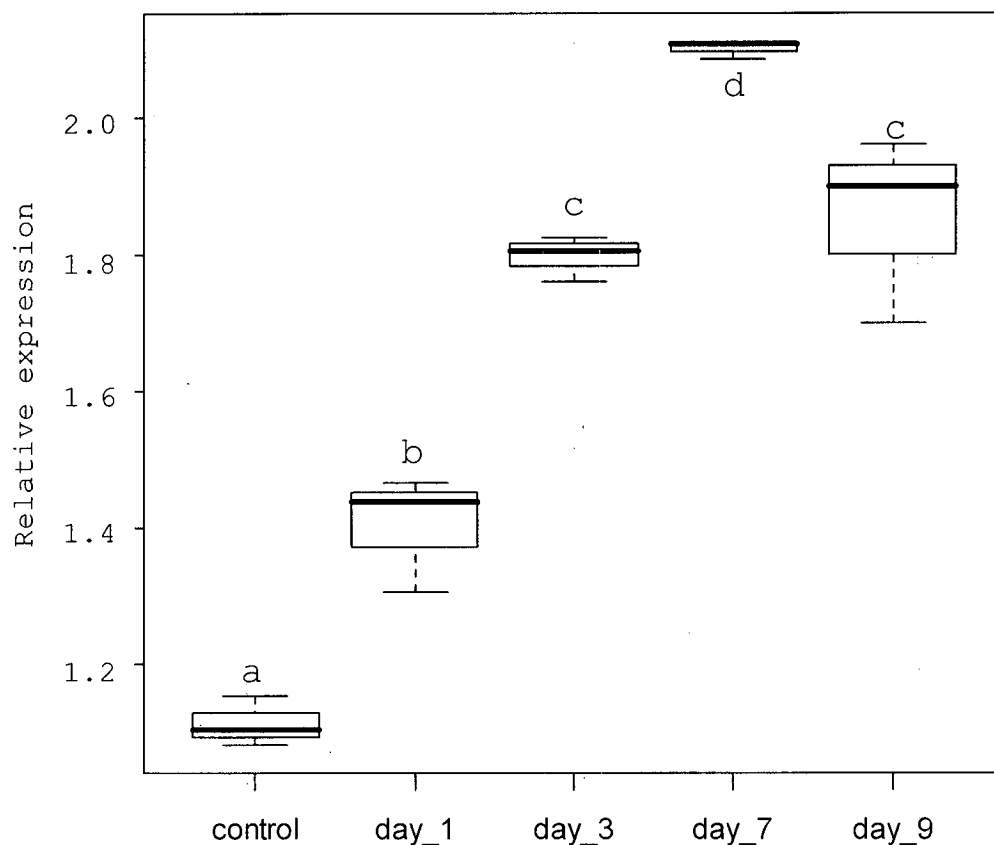


Fig. 11 Relative expression of *C. macrocephalus* C3 in liver from *C. macrocephalus* administered with β -glucan. Fish were collected at 1, 3, 7 and 9 days post β -glucan feeding. *C. macrocephalus* mRNA levels were determined by semi-quantitative RT-PCR and standardized according to the respective β -actin mRNA levels. Statistically significant differences are indicated by different letters ($p < 0.05$).