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BRIEF COMMUNICATION

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Multiple CC chemokines in channel catfish and blue catfish as revealed by analysis of expressed sequence tags

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Abstract Chemokines represent a superfamily of chemotactic cytokines involved in recruitment, activation and adhesion of a variety of leukocyte types to inflammatory foci, as well as in the organization and maintenance of lymphoid organ architecture and in normal developmental processes. Nearly all chemokines have been identified in human and mouse, but only a handful of fish chemokines have been identified. Here we describe 14 distinct chemokines from channel catfish and blue catfish identified by analysis of 30,000 expressed sequence tags. Based on sequence analysis, sequence similarity, and the arrangement of the conserved cysteine residues, all 14 chemokines were identified as members of the CC subfamily. Phylogenetic analysis did not reveal clear evidence of orthology of the catfish and human or mouse chemokines. Similarity analysis indicated that nine of the 14 CC chemokines were identified for the first time in fish. The availability of this pool of catfish CC chemokines should facilitate rapid identification and phylogenetic analysis of CC chemokines from other fish and related species.

Keywords Chemokine · Gene expression · Innate immunity · Fish · Catfish

Chemokines can be functionally grouped into homeostatic and induced categories. The homeostatic chemokines are produced and secreted constitutively, and are involved in lymphocyte trafficking, immune surveillance and localization of lymphocytes with antigen in the lymphatic system. The induced chemokines are produced during infection and are involved in recruitment, activation and adhesion of leukocytes to the site of infection or injury (Neville et al. 1997; Secombes et al. 2001). They are structurally related small peptides, with the majority containing four conserved cysteine residues (for a recent review, see Laing and Secombes 2004). Based on the arrangement of these conserved cysteine residues (Ahuja and Murphy 1996; Murphy et al. 2000), chemokines were divided into four subfamilies: CXC, CC, C, and CX3C. Corresponding to these subfamilies of chemokine proteins, their coding genes were designated by SCY (for small inducible cytokines) followed by a letter, A, B, C, or D (for CC, CXC, C, and CX3C, respectively). The CXC and CC are the two major subfamilies. A large number of chemokines have been identified from mammalian species including 16 CXC, 28 CC, two C, and one CX3C chemokines (Bacon et al. 2003).

Identification of teleost fish chemokines has been slow. To date, only a handful of fish chemokines have been identified and reported (CC chemokines summarized in Table 1, reviewed by Laing and Secombes 2004). Low sequence conservation during evolution, coupled with the small size of chemokines, has prohibited molecular cloning using hybridization-based approaches or amplification using PCR. The lack of fish chemokine gene sequences hinders accurate phylogenetic analysis of orthology. Efficient means for the discovery of fish chemokines are demanded for the studies of comparative immunology and for understanding the evolutionary processes of the immune systems of fish in general. In recent years, the adoption of genomic approaches, especially through the analysis of expressed sequence tags (ESTs), has allowed faster discovery of fish chemokines (Kuroda et al. 2003; Baoprasertkul et al. 2004). A number of fish chemokines have been described and phylogenetically analyzed in a recent publication by Huising and co-workers (2003a). A CXC chemokine most similar to the mammalian CXCL10 was reported from carp (Savan et al. 2003). A second carp CXC chemokine,

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Table 1 A summary of previously reported fish CC chemokines

Species	CC chemokine	References
Rainbow trout	*O. mykiss* CK1	Dixon et al. (1998)
	O. mykiss CK2	Liu et al. (2002)
	O. mykiss CC	Sangrador-Vegas et al. unpublished
Carp	*C. carpio* CCL1	Fujiki et al. (1999)
Japanese flounder	CC chemokine	Nam et al. (2003)
	P. olivaceus CC	Kono et al. (2003)
	P. olivaceus CCL4	Khattiya et al. (2004)
Cichlids	*M. auratus* SCYA101, *M. auratus* SCYA102, *P. chilotes* SCYA101, *P. chilotes* SCYA103, *P. chilotes* SCYA104, *P. chilotes* SCYA105, *P. chilotes* SCYA106	Kuroda et al. (2003)
Cat shark	*S. canicula* SCYA107	Kuroda et al. (2003)

likely involved in phagocyte chemotaxis, has been recently characterized by Huising and co-workers (2003b). In addition, two CXC chemokines have been reported from the model species zebrafish (Long et al. 2000; Doitsidou et al. 2002). By the analysis of sequences in the dbEST database, Laing and Secombes (2004) observed three CXC chemokines from channel and blue catfish, two CC chemokines from Japanese flounder, one CC chemokine from rainbow trout, five CC and one CXC chemokines from zebrafish, and one CC chemokine from pufferfish. After the observation of multiple chemokines in the EST databases, Laing and Secombes (2004) concluded that fish likely have multiple chemokines similar to the situation in mammals, though lower in number. In this work, we provide direct evidence for the presence of multiple chemokines in fish. We used a genomic approach through EST analysis for the identification of catfish chemokines. Here we report 14 CC chemokines from channel catfish (*Ictalurus punctatus*) and blue catfish (*I. furcatus*), closely related members of the same genus. Our previous studies indicated that their gene sequences were well conserved with a divergence rate of 1.32 base pairs (bp) per 100 bp (He et al. 2003). In spite of the current inability of phylogenetic analysis to concretely assign orthology to the putative CC chemokines, the availability of this pool of chemokines from catfish will provide an important framework for resolving the status of chemokines in fish.

Fifteen individuals each of the channel catfish and the blue catfish were used for the collection of various tissues used for the extraction of RNA. As part of a different project, various cDNA libraries were constructed as we previously reported (Ju et al. 2000; Cao et al. 2001; Karsi et al. 2002; Kocabas et al. 2002). From these libraries, a total of 30,000 ESTs were generated. These ESTs served as the sources for the identification of the catfish chemokines reported in this paper.

Plasmid DNA was prepared by the alkaline lysis method (Sambrook et al. 1989) using Qiagen Spin Column Miniplasmid kits. Chain termination sequencing was performed using Thermosequenase kit (Amersham, Piscataway, NJ). Sequences were analyzed on an automatic LI-COR DNA Sequencer Long ReadIR 4200, LI-COR DNA Analyzer Gene ReadIR 4200, or ABI PRISM 3700 automatic sequencers. BLAST searches (Altschul et al. 1990; Gish and States 1993) were conducted to determine gene identities. Procedures for establishing homologues were the same as we previously described (Cao et al. 2001; Karsi et al. 2002). After a first round of BLASTX searches with a cut-off P value for significant similarities set at 1×10^{-5}, a second round was conducted at a cut-off P value=0.1 to determine if additional clones similar to chemokines could be identified. This lower stringency of similarity was necessary for genes with relatively rapid divergence rates. After the identification of homologues, all the ESTs belonging to the "chemokine" group based on gene ontology analysis were clustered to identify the contigs. After clustering, a list of unique catfish ESTs with putative identities of CC chemokines was generated. Representative clones from each contig and all the singletons were completely sequenced. These CC chemokine candidate clones were further analyzed with DNASTAR software package (Lasergene, Madison, Wis.) and the Vector NTI software package (Informax, Carlsbad, Calif). Open reading frames were identified, and translated. The translated amino acid sequences were used for sequence alignments.

To better understand the identities of the CC chemokines, the complete cDNA sequences were analyzed using BLASTX searches. Attention was given to whether homologous sequences were of mammalian origin, fish origin, or both. The relevant sequences were then retrieved from GenBank for multiple sequence alignments using CLUSTAL X (Thompson et al. 1994). Percentage of amino acid identities was recorded after all multiple alignments. Phylogenetic trees were drawn by the neighbor-joining method (Saitou and Nei 1987) using amino acid sequence p-distances, or by the maximum parsimony method using the PAUP program (Swofford 2002). The topological stability of the neighbor-joining trees was evaluated by bootstrapping with 1,000 replications.

A total of 30 EST clones representing 14 unique genes had significant similarity to CC chemokines in the GenBank databases. These 14 genes were designated as SCYA101 to SCYA114 with either Icpu or Icfu prefixes to

Table 2 Summary of sequence information of the 14 CC chemokines. Numbers under 5′-untranslated region (*UTR*) and *3′UTR* are base pairs (*bp*). Open reading frame (*ORF*) indicates the size of ORFs in amino acids (*aa*). *Typical polyA signal* refers to AAUAAA polyadenylation signal sequences, and the numbers following the *slash* indicate the number of base pairs between the polyA signal and the polyA sequences

CC chemokine	Completely sequenced clones	Species identified from	Accession numbers	Most similar mammalian CCL	5′ UTR (bp)	ORF (aa)	3′ UTR (bp)	Typical polyA signal
Icfu-SCYA101	AUF_IfHdk_245_f18	Blue catfish	AY555498	None	15	114	920	ATTAAA/14
Icpu-SCYA102	AUF_IpTrk_25_o02, AUF_IpTrk_27_n24	Channel catfish	AY555499	CCL8	27	100	711	Yes/15
Icfu-SCYA103	AUF_IfSpn_231_o14	Blue catfish	AY555500	CCL21	60	86	614	Yes/19
Icfu-SCYA104, Icpu-SCYA104	AUF_IfHdk_245_a21, AUF_IpSpn_69_b09, AUF_IpSpn_64_c06	Blue catfish, Channel catfish, Channel catfish	AY555501, AY555512, AY555513	CCL3	16	95, 95, 99	178, 176, 179	Yes/22
Icpu-SCYA105	AUF_IpHdk_41_g02	Channel catfish	AY555502	CCL14	–	>79	299	Yes/10
Icfu-SCYA106	AUF_IfHdk_241_b01	Blue catfish	AY555503	CCL21, CCL19	113	110	405	Yes/9
Icfu-SCYA107	AUF_IfHdk_241_m03	Blue catfish	AY555504	None	64	119	261	Yes/14
Icpu-SCYA108	AUF_IpSto_11_n06	Channel catfish	AY555505	CCL14, CCL3	136	101	233	TATATA/21
Icfu-SCYA109	AUF_IfHdk_244_l22	Blue catfish	AY555506	CCL19	–	>79	336	Yes/16
Icfu-SCYA110	AUF_IfSpn_236_h01	Blue catfish	AY555507	CCL8	109	94	272	Yes/16
Icpu-SCYA111	AUF_IpSpn_69_h23	Channel catfish	AY555508	CCL18*	–	>76	158	Yes/13
Icpu-SCYA112	AUF_IpInt_52_h06	Channel catfish	AY555509	CCL20	0	124	399	ATTAAA/15
Icpu-SCYA113	AUF_IpTrk_26_n02	Channel catfish	AY555510	CCL19	–	>85	408	Yes/16
Icfu-SCYA114	AUF_IfHdk_243_c14	Blue catfish	AY555511	CCL24	14	111	287	Yes/17

Table 3 Summary of sequence similarities of the newly identified catfish CC chemokines. The catfish CC chemokines are listed in three groups based on their similarities only to non-fish chemokines (*Group 1*), only to fish chemokines (*Group 2*), and to both fish and non-fish chemokines (*Group 3*)

Catfish	Species	Genes	Accession #	BLAST *P* value	Amino acid identity	References
Group 1						
Icpu-SCYA104	Bovine	CCL3	NP_776936	2e-09	32.3%	Unpublished
Icfu-SCYA104	Human	CCL3	A30574	2e-09	30.4%	Zipfel et al. (1989)
	Horse	CCL5	AAM34212	3e-07	29.7%	Unpublished
	Human	CCL14	NP_116738	3e-07	20.4%	Wells and Peitsch (1997)
Icpu-SCYA105	Human	CCL14	NP_116738	2e-10	34.6%	Wells and Peitsch (1997)
	Chicken	SCYA4	AAD48772	5e-10	33.3%	Unpublished
Icfu-SCYA106	Rhesus monkey	CCL21	AAN76079	3e-08	30%	Basu et al. (2002)
	Human	CCL19	NP_006265	4e-08	31.6%	Rossi et al. (1997)
	Human	CCL21	O00585	5e-08	30%	Hromas et al. (1997)
Icpu-SCYA108	Human	CCL14	NP_116738	2e-09	25.7%	Pardigol et al. (1998)
	Chicken	SCYA4	AAD48772	6e-09	32.2%	Unpublished
	Human	CCL3	NP_002974	8e-09	30.4%	Zipfel et al. (1989)
Icfu-SCYA110	Mouse	CCL8	NP_067418	8e-08	32.6%	Okazaki et al. (2002)
	Swine	CCL8	CAA88371	2e-07	32.6%	Hosang et al. (1994)
	Rat	CCL11	AAB65775	1e-06	37%	Unpublished
Icpu-SCYA111	Rat	CCL22	AAL30397	0.032	22.1%	Unpublished
	Human	CCL18	NP_002979	0.032	25%	Adema et al. (1997)
Group 2						
Icfu-SCYA101	Japanese flounder	JPCCL4	BAD04055	2e-14	37.5%	Khattiya et al. (2004)
	Rainbow trout	CC chemokine with stalk CK2	AAM09300	9e-08	22.6%	Unpublished
IcfuSCYA107	Japanese flounder	JFCCL4	BAD04055	9e-15	38.5%	Khattiya et al. (2004)
	Rainbow trout	CC chemokine	CAC45063	1e-09	29.4%	Unpublished
Group 3						
Icpu-SCYA102	*Scyliorhinus canicula*	SCYA107	AAO21210	7e-14	40%	Kuroda et al. (2003)
	P. chilotes	SCYA106	AAO21208	6e-04	26.4%	Kuroda et al. (2003)
	Carp	CC chemokine-1	BAA31459	0.003	20.8%	Fujiki et al. (1999)
	Mouse	CCL8	NP_067418	0.021	19.6%	Okazaki et al. (2002)
Icfu-SCYA103	*P. chilotes*	SCYA105	AAO21207	6e-17	47.7%	Kuroda et al. (2003)
	Human	CCL21	XP_216379	6e-08	40.7%	Nagira et al. (1997)
Icfu-SCYA114	Carp	Chemokine-1	BAA31459	2e-15	43.6%	Fujiki et al. (1999)
	Rainbow trout	CC Chemokine	AAM09300	4e-15	42.3%	Liu et al. (2002)
	Japanese flounder	JFCCL4	BAD04055	4e-11	31.7%	Khattiya et al. (2004)
	Human	CCL24	NP_002982	6e-04	27.9%	Patel et al. (1997)
	Mouse	CCL19	O70460	0.001	25.9%	Ngo et al. (1998)
	Mouse	CCL7	Q03366	0.003	22.7%	Okazaki et al. (2002)
Icfu-SCYA109	Mouse	CCL19	AAH51472	2e-08	34.2%	Ngo et al. (1998)
	Human	CCL19	NP_006265	5e-08	34.2%	Rossi et al. (1997)
	Human	CCL21	NP_002980	3e-06	34.2%	Nagira et al. (1997)
	P. chilotes	SCYA104	AAO21206	3e-06	32.9%	Kuroda et al. (2003)
Icpu-SCYA112	Chicken	ah189	AAK84434	7e-11	36%	Hughes et al. (2001)
	Mouse	CCL20	O89093	6e-10	35.1%	Utans-Schneitz et al. (1998)
	Rainbow trout	CK1	AAD25977	2e-07	29%	Dixon et al. (1998)
Icpu-SCYA113	*P. chilotes*	SCYA104	AAO21206	4e-09	29.4%	Kuroda et al. (2003)
	Human	CCL19	NP_006265	2e-08	36.5%	Rossi et al. (1997)
	Mouse	CCL19	AAH51472	4e-08	34.1%	Strausberg et al. (2002)

indicate channel catfish or blue catfish, respectively (Table 2). Representative clones from all contigs and all the singletons were completely sequenced and their sequences have been deposited to GenBank with accession numbers summarized in Table 2.

Several characteristics of chemokines were used as criteria for the identification of CC chemokines, including: (1) a significant level of sequence similarities to existing CC chemokines; (2) the presence of the four invariable cysteine residues with the first two being adjacent to each other; (3) small molecular mass. Genomic organizations of the chemokine genes have been sometimes used to identify CC chemokines since most CC chemokines contain three exons and two introns (for review, see Laing and Secombes 2004). Considering the large numbers of genes involved in this report, here we only used the first three criteria because genomic sequences for the putative catfish CC chemokines are not yet available. Based on the three criteria, all 14 cDNAs were identified as chemokines belonging to the CC subfamily. BLASTX search results divide the 14 CC chemokines into three distinct groups: those that exhibit similarities only to CC chemokines from non-fish species, those that exhibit similarities only to fish chemokines, and those that exhibit similarities to both fish and non-fish chemokines (Table 3).

The first group of catfish chemokines included those that showed similarity to only non-fish chemokines (Table 3). These included Icpu-SCYA104/Icfu-SCYA104, Icpu-SCYA105, Icfu-SCYA106, Icpu-SCYA108, Icfu-SCYA110, and Icpu-SCYA111. Clearly, these six sequences represented chemokines identified for the first time from fish with no fish equivalents currently in the GenBank. The availability of these catfish CC chemokine cDNAs should facilitate rapid identification of their counterparts from other fish species.

The second group of the catfish chemokines included those that showed significant similarities to only fish chemokines (Table 3). These included Icfu-SCYA101 and Icfu-SCYA107. Considering that essentially all human and mouse CC chemokines have been identified, the lack of significant similarities to any of the mammalian CC chemokines may indicate that these chemokines could be specific for fish or related taxa. Alternatively, the evolutionary divergence rate of these chemokines could be more rapid than that of other CC chemokines such that similarities could no longer be detected using computational approaches. It is not possible to differentiate between the two hypotheses at present because the CC chemokines are far from being completely identified in a wide range of organisms between fish and mammals. Nonetheless, future studies of the functions of this group of potentially fish-specific CC chemokines could provide very important information concerning comparative immunology.

The third group of the catfish CC chemokines exhibited similarities to both fish and non-fish chemokines (Table 3). Intuitively, this group would represent catfish chemokines whose counterparts have been identified from fish and non-fish species. This could be true for Icpu-SCYA102, Icfu-SCYA103, and Icfu-SCYA114 because their similarity *P* values were very low when compared with fish chemokines, but high when compared with non-fish chemokines.

The similarity significance *P* values do not support this simple interpretation for the remaining three catfish chemokines, Icfu-SCYA109, Icpu-SCYA112, and Icpu-SCYA113 (Table 3). The evolutionary time-frame between fish and mammals is approximately 450 million years, while the evolutionary time-frame for the entire spectrum of teleost fish is approximately 150 million years. Accordingly, the similarity significance *P* values should be significantly lower between a pair of orthologous genes of fish than between a pair of orthologous genes of a fish and of a mammal. The catfish CC chemokines Icfu-SCYA109, Icpu-SCYA112, and Icpu-SCYA113 violate this basic assumption. Our preliminary conclusion, therefore, is that these sequences may also represent chemokines identified here for the first time in fish. This conclusion was supported by the fact that these catfish CC chemokines were more similar to mammalian or bird CC chemokines than to fish chemokines (for Icfu-SCYA109 and Icpu-SCYA112). Icpu-SCYA113, although most similar to a fish CC chemokine SCYA104 from *P. chilotes* (an African cichlid), shares similarly high similarity to the mammalian CC chemokine CCL19. Combining these chemokines with the first group of catfish CC chemokines, this work has tentatively identified nine chemokines for the first time in fish.

Phylogenetic analysis of the 14 catfish chemokines with previously known chemokines from mammals, fish, and other species failed to provide much information about orthologies (Fig. 1). Only Icpu-SCYA112 was placed into a clade containing mammalian chemokine CCL20 and chicken chemokine ah189; all the remaining 13 catfish chemokines were placed into clades containing only themselves or other fish chemokines (Fig. 1). Four apparent clades were formed with fish chemokines. The first clade included Icfu-SCYA106, Icfu-SCYA109, and Icpu-SCYA113; the second clade included Icfu-SCYA103 and three previously identified fish chemokines; the third clade included four catfish CC chemokines identified from this study, two SCYA104 variants, Icpu-SCYA105, and Icpu-SCYA108; the fourth clade included the newly identified Icfu-SCYA101, Icpu-SCYA102, Icfu-SCYA107, and Icfu-SCYA114, and most of the previously known fish chemokines (Fig. 1). Although their co-existence within a single clade by phylogenetic analysis indicated their relatedness in structures, their orthologues cannot be determined at present because many members of CC chemokines from other fish species are yet to be discovered.

Orthologous relationships are more difficult to establish for genes involving large numbers of families. To date, a total of 28 CC chemokines have been identified from mammals (Laing and Secombes 2004). They are structurally highly related. The human CC chemokines share various levels of similarities among themselves with some

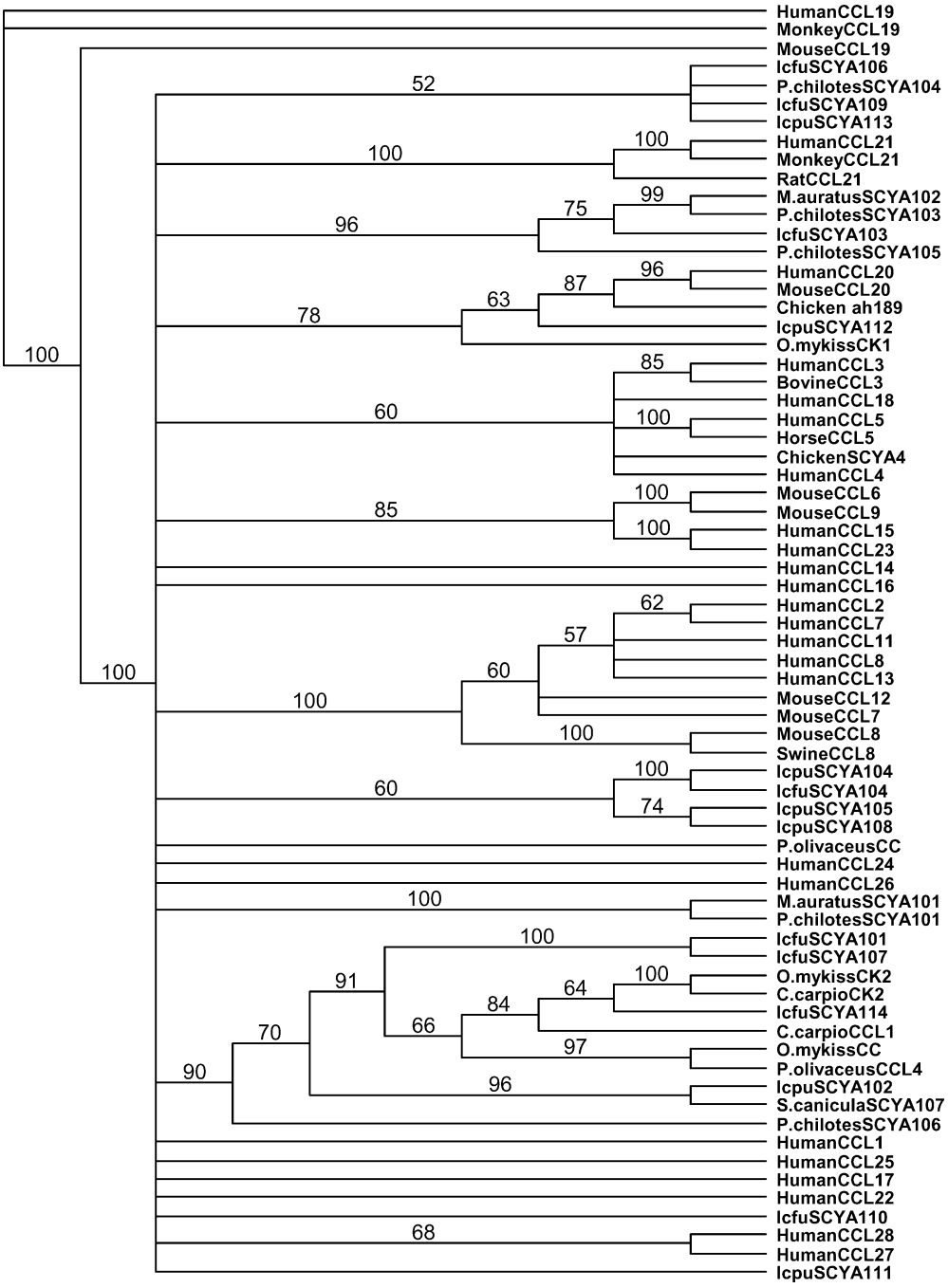

Fig. 1 Unrooted phylogenetic tree constructed using the neighbor-joining method of PAUP after sequence alignments with CLUSTAL X. Numbers on the dendrogram are percentages from bootstrapping of 1,000 replications. The accession numbers for previously known chemokines are: bovine CCL3, NP_776936; *C. carpio* CCL1, BAA31459; *C. carpio* CK2, AAF66446; chicken ah189, AAK84434; chicken SCYA4, AAD48772; horse CCL5, AAM34212; human CCL1, P22362; human CCL2, P13500; human CCL3, NP_002974; human CCL4, P13236; human CCL5, P13501; human CCL7, NP_006264; human CCL8, NP_005614; human CCL11, AAH17850; human CCL13, Q99616; human CCL14, NP_116738; human CCL15, Q16663; human CCL16, O15467; human CCL17, Q92583; human CCL18, NP_002979; human CCL19, NP_006265; human CCL20, P78556; human CCL21, O00585; human CCL22, O00626; human CCL23, P55773; humanCCL24, O00175; human CCL25, O15444; human CCL26, Q9Y258; human CCL27, NP_006655; human CCL28, Q9NRJ3; *M. auratus* SCYA101, AAO21202; *M. auratus* SCYA102, AAO21203; monkey CCL19, AAN76077; monkey CCL21, AAN76079; mouse CCL6, NP_033165; mouse CCL7, AAH61126; mouse CCL8, NP_067418; mouse CCL9, P51670; mouse CCL12, Q62401; mouse CCL19, NP_036018; mouse CCL20, O89093; *O. mykiss* CC, CAC45063; *O. mykiss* CK1, AF093812; *O. mykiss* CK2, AAM09300; *P. chilotes* SCYA101, AAO21204; *P. chilotes* SCYA103, AAO21205; *P. chilotes* SCYA104, AAO21206; *P. chilotes* SCYA105, AAO21207; *P. chilotes* SCYA106, AAO21208; *P. olivaceus* CC, AU090535; *P. olivaceus* CCL4, BAD04055; rat CCL21, XM_216379; *S. canicula* SCYA107, AAO21210; swine CCL8, CAA88371; All accession numbers for the catfish CC chemokines identified from this study are as shown in Table 2

Table 4 Amino acid identities (%) among the 14 catfish CC chemokines. All *numbers* in the first row and the first column refer to the catfish CC chemokine SCYA as listed in Table 2. For SCYA104, Icfu-SCYA104 was used. *Italicized numbers* indicate those sharing 30% or greater amino acid identities

SCYA	102	103	104	105	106	107	108	109	110	111	112	113	114
101	25.0	19.8	15.8	10.1	16.4	*59.6*	16.8	7.6	13.8	11.8	14.0	15.3	25.2
102	–	19.8	14.7	15.2	17.0	22.0	15.0	11.4	20.2	6.6	16.0	14.1	19.0
103		–	18.6	26.6	*33.7*	22.1	25.6	26.6	23.3	18.4	24.4	23.5	15.1
104			–	*35.4*	18.9	14.7	*43.2*	21.5	23.4	19.7	14.7	25.9	10.5
105				–	20.3	15.2	*46.8*	19.0	22.8	17.1	26.6	19.0	8.9
106					–	12.7	20.8	*36.7*	23.4	6.6	19.1	*30.6*	13.6
107						–	11.9	15.2	11.7	18.4	16.0	22.4	*32.4*
108							–	13.9	24.5	22.4	19.8	17.6	12.9
109								–	26.6	13.2	21.5	*41.8*	13.9
110									–	14.5	27.7	20.0	16.0
111										–	6.6	14.5	9.2
112											–	20.0	16.2
113												–	17.6

of them being extremely similar. For instance, human CCL8 and CCL11 share 70.1% amino acid identities. Clearly, much more sequence information is needed from a wide range of species between mammals and fish, as well as from various fish in order to establish phylogenetic relationships. While the inclusion of 15 individual fish in the construction of cDNA libraries added greater possibilities of allelic variations, the 14 CC chemokines were likely encoded by distinct genes. This conclusion is supported by the low amino acid identities among the 14 CC chemokines (Table 4). The most similar pair of the 14 CC chemokines is SCYA101 and SCYA107 with 59.6% amino acid identities. Additionally, the sequence divergence of the 14 CC chemokines was much greater than the "baseline" allelic variations, even among the various alleles of both channel catfish and blue catfish (1.32%; He et al. 2003).

Functional analysis would be required to have insight into the roles of these CC chemokines and their relationships with other known CC chemokines. It is obvious that these catfish CC chemokines represent a large fraction of all the fish CC chemokines reported to date and nine of the 14 catfish chemokines represent chemokines identified for the first time in fish. Two more chemokines were quite unique in fish with no detectable similarities to any known mammalian CC chemokines, making their phylogenetic analysis more difficult. The availability of this pool of catfish CC chemokines should facilitate rapid identification and analysis of CC chemokines in fish, which in turn will help resolve the issues related to evolution and functions of the CC chemokines in fish.

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