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Short Sequence Report

Molecular characterization of two isoforms of piscidin 4 from the hybrid striped bass (*Morone chrysops* × *Morone saxatilis*)

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Antimicrobial peptides (AMPs), components of innate immunity, serve as a first line of defense against potential pathogens. AMPs have a broad spectrum of activity against microorganisms such as bacteria, fungi, parasites, and viruses [1–3]. They have been identified in virtually all groups of organisms, from bacteria to eukaryotic plants and animals. It also has been postulated that they may be involved in other functions, such as chemotaxis and opsonization. Their ubiquity and potent activity suggest that they are critical to immune health.

One class of AMPs are small, linear, α-helical, amphipathic polypeptides [1]. A major family of peptides in this class are the piscidins. Piscidin peptides are less than 26 residues in length, except for the 44 amino acid piscidin 4, which was recently purified from the gill of hybrid striped bass (white bass, *Morone chrysops*, × striped bass, *Morone saxatilis*) [4]. Along with the three other piscidins isolated from hybrid striped bass, piscidin 4 shares a conserved N-terminal domain rich in histidine and phenylalanine. Originally isolated from striped bass, white bass, and their hybrid [5,6], there is evidence that piscidins are present in a wide range of teleost taxa, including the families Moronidae, Sciaenidae, Siganidae, Belontidae, Cichlidae, Percichthyidae [5,7] and Latridae [8]. The chrysophsins, antimicrobial peptides isolated from red sea bream (*Chrysophrys major*), a member of the family Sparidae, are also similar to the piscidins [9]. Piscidin genes have also been cloned as cDNA and characterized in striped bass and white bass [6], European seabass (*Dicentrarchus labrax*) [10], mandarin fish (*Siniperca chuatsi*) [11], and Atlantic cod (*Gadus morhua*) [12]. Furthermore, genomic evidence suggests that the pleurocidins, found in many flatfish species, may also be members of the piscidin family [11], as may be epinecidins of the grouper, *Epinephelus coioides* [13]. Also, these genes share a common pre-propeptide structure consisting of a 22 amino acid signal peptide, followed by the mature peptide and a C-terminal prodomain of varying lengths [6,14]. Specifically regarding piscidin 4, there is biochemical and immunochemical evidence for its widespread presence in fish, including members of the families Moronidae, Latidae, Sparidae, and Sciaenidae [15]. Piscidin 4 has strong activity against both fish and human Gram-positive and Gram-negative bacterial pathogens [4].

Here we present for the first time the complete coding sequence (CDS) of a piscidin 4 peptide, cloned from the gill and intestine of hybrid striped bass. We also describe a novel mRNA transcript similar to that of piscidin 4 cloned from intestine. This latter sequence shares an identical putative signal peptide with and 89% nucleotide identity to piscidin 4.

Hybrid striped bass (20–35 g body weight) provided by Castle Hayne Fisheries, Inc., Aurora, NC, USA, were held at 14 °C for at least 6 mo in 50 L aquaria fitted with a high flow rate sand filter and bioreactor. The fish were fed a commercial diet (2 mm Finfish Starter pellets, Zeigler Bros., Inc., Gardners, PA, USA). Water temperature was monitored daily with ammonia, nitrite, pH, and salinity levels checked biweekly. Water changes were performed once a week. During sampling, the fish were carefully netted from their aquarium and euthanized with buffered tricaine methansulfonate (MS-222; 100 mg/mL). Fifty to hundred milligram tissue samples of gill and foregut sections of the intestine were carefully removed and briefly rinsed with deionized water to remove any surface debris. The samples were stored in liquid nitrogen until extraction was performed. Total RNA and genomic DNA were extracted using Trizol Reagent (Invitrogen, Carlsbad, CA, USA) and quantified by Nanodrop® spectrophotometry (Thermo Fisher Scientific, Wilmington, DE, USA).

A partial coding sequence of piscidin 4 was obtained by amplification of genomic DNA from hybrid striped bass using primers designed from European seabass (*D. labrax*) sequences. In December 2009, four seabass expressed sequence tags (ESTs)

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Table 1
Ambion RLM-RACE and gene-specific primers used to clone the two piscidin 4 isoforms.

Primer name	Primer sequence	Use
5′ RLM-RACE outer primer	5′ GCTGATGGCGAT GAATGAACACTG 3′	5′ RLM-RACE primary primer
3′ RLM-RACE outer primer	5′ GCGAGCACAGA ATTAATACGACT 3′	3′ RLM-RACE primary primer
5′ RLM-RACE inner primer	5′ CGCGGATCCGAACACTG CGTTTGCTGGCTTTGATG 3′	5′ RLM-RACE nested primer
3′ RLM-RACE inner primer	5′ CGCGGATCCGAATTAA TACGACTCACTATAGG 3′	3′ RLM-RACE nested primer
P4 5 F	5′ GTTATGATCTTTTTG GTGTTGACA 3′	Initial cloning of partial genomic DNA sequences
P4 5 R	5′ GCAGATAGTTTTGA AGCAAATTTT 3′	Initial cloning of partial genomic DNA sequences
RP4 F1	5′ CTGGTCGTCCT CATGGCTGAA 3′	Gene specific 5′ RLM-RACE primary primer
RP4 F2	5′ CAGACACTTATTCA GAGGGGCCAA 3′	Gene specific 5′ RLM-RACE nested primer
RP4 F3	5′ GGGAGGGTTTGATC GGAAGCTTATT 3′	Gene specific 5′ RLM-RACE nested primer
RP4 F4	5′ GGTCTTATTTCAA GGTGCCAGGCA 3′	Gene specific 5′ RLM-RACE nested primer
RP4 R1	5′ AAAAAGAACAGA TTCAGCGCT 3′	Gene specific 3′ RLM-RACE primary primer
RP4 R2	5′ GGTAGTAGATCAC AGGTCTTCGAAC 3′	Gene specific 3′ RLM-RACE nested primer
RP4 R3	5′ GATTGTTGTCCG TCTCAGGAAC 3′	Gene specific 3′ RLM-RACE nested primer

encoding polypeptides of high similarity to piscidin 4 were identified following a TBLASTN search [16] using the hybrid striped bass piscidin 4 polypeptide (Noga et al., 2009) as the reference sequence: NCBI Accession No. FM019965 (E-value = 9e-11), FM025254 (E-value = 1e-10), FM019301 (E-value = 7e-07), and FM022266 (E-value = 3e-05). These ESTs were aligned using Vector NTI software (Invitrogen) and PCR primers were designed to target conserved regions in the seabass ESTs (primer pair P4 5, see Table 1). Using hybrid striped bass genomic DNA extracted from gill as the template, PCR amplification was performed with these primers on a *Hybaid Px2* Thermal Cycler (Thermo Fisher Scientific, Inc., Waltham, MA, USA). The cycling protocol was as follows: 1 cycle, 95 °C for 1 min; 30 cycles, 94 °C for 30 s, 52.2 °C for 30 s, and 68 °C for 1 min; final extension, 68 °C for 5 min. The amplified products were electrophoresed (3% agarose, 10 mg/mL EtBr), and all bands were excised and purified using a GeneClean II kit (MP Biomedicals, Solon, OH, USA) according to the kit protocol. Purified amplicons were cloned into a pCR 2.1-TOPO vector and transformed into chemically competent TOP10 *Escherichia coli* using a TOPO TA Cloning kit (Invitrogen) and protocol. Transformant colonies were selectively grown (LB media with 50 mg/mL ampicillin) for plasmid extraction (PureLink Quick Plasmid Miniprep Kit; Invitrogen) and sequencing at the University of Chicago Cancer Research Center DNA Sequencing Facility (Chicago, IL, USA). Forward Sanger sequencing was performed on an Applied Biosystems 3730XL DNA sequencer using the M13 universal forward primer. All resulting sequences were aligned using the ClustalW algorithm [17].

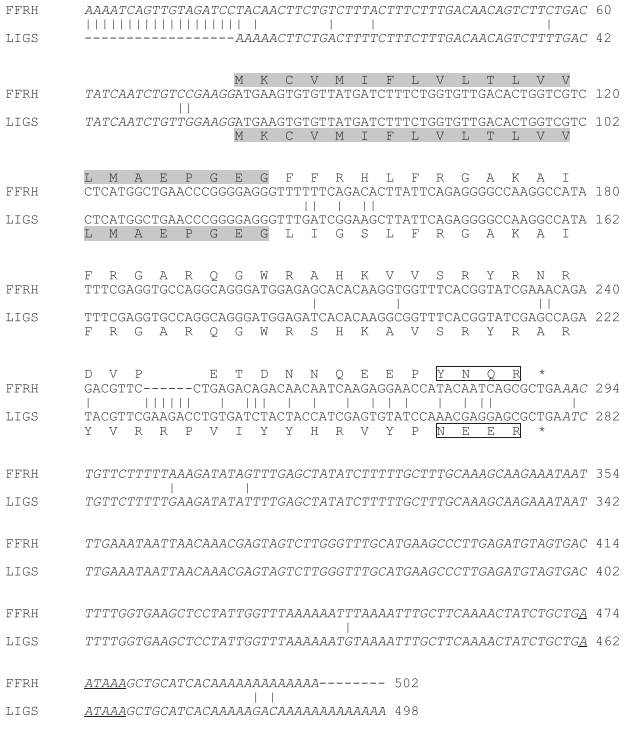

Fig. 1. Nucleotide and predicted amino acid sequences for the two putative isoforms of piscidin 4 (FFRH and LIGS). There was 89% similarity between the two nucleotide sequences. The predicted 22 amino acid signal peptide, shown by the gray shading, was identical in both isoforms. The 4 amino acid prodomains are boxed. Major differences between the two isoforms were in the first 11 nucleotides encoding the predicted mature peptide and in the C-terminal regions. Both the 5′ and 3′ untranslated regions (shown in italics) were similar in both isoforms. The polyadenylation signals are underlined. (| = nucleotide differences, * = stop codons).

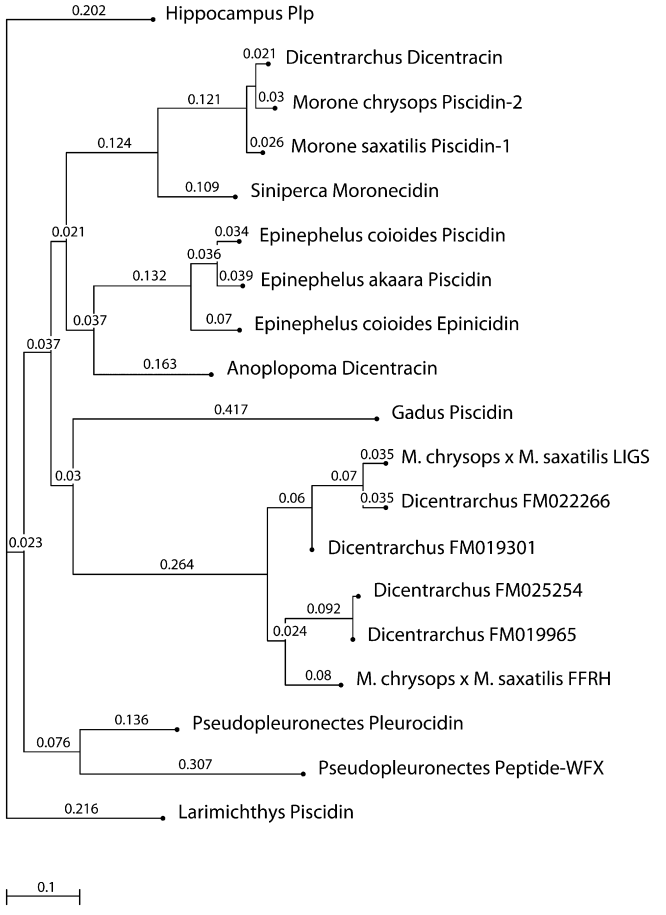

Fig. 2. ClustalW-formatted dendrogram showing relationships among representative piscidins, dicentracins, moronecidins, and pleurocidins based on their complete polypeptide sequences deduced from cDNA (Genbank accession numbers are listed in Table 2). Numbers above each branch represent the p-distances (proportion of differences between sequences). The two piscidin 4 isoforms (LIGS and FFRH) from hybrid striped bass (*M. chrysops* × *M. saxatilis*) cluster into two distinct branches that are dissimilar to the previously identified *Morone* piscidins and *Dicentrarchus* dicentracin.