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Research Article

Annotation of the Nuclear Receptors in an Estuarine Fish species, *Fundulus heteroclitus*

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Abstract. The nuclear receptors (NRs) are ligand-dependent transcription factors that respond to various internal as well as external cues such as nutrients, pheromones, and steroid hormones that play crucial roles in regulation and maintenance of homeostasis and orchestrating the physiological and stress responses of an organism. We annotated the *Fundulus heteroclitus* (mummichog; Atlantic killifish) nuclear receptors. Mummichog are a non-migratory, estuarine fish with a limited home range often used in environmental research as a field model for studying ecological and evolutionary responses to variable environmental conditions such as salinity, oxygen, temperature, pH, and toxic compounds because of their hardiness. *F. heteroclitus* have at least 74 NRs spanning all seven gene subfamilies. *F. heteroclitus* is unique in that no RXRα member was found within the genome. Interestingly, some of the NRs are highly conserved between species, while others show a higher degree of divergence such as PXR, SF1, and ARα. Fundulus like other fish species show expansion of the RAR (NR1B), Rev-erb (NR1D), ROR (NR1F), COUPTF (NR2F), ERR (NR3B), RXR (NR2B), and to a lesser extent the NGF (NR4A), and NR3C steroid receptors (GR/AR). Of particular interest is the co-expansion of opposing NRs, Reverb-ROR, and RAR/RXR-COUPTF.

Keywords: RXR, phylogenetics, nuclear receptors, stress, endocrinology

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1. Introduction

Fundulus heteroclitus (mummichogs) are an atlantic killifish species found from Nova Scotia, Canada to northern Florida, USA. In some estuaries, they can account for as much as 25% of the total macrofauna [1]. Mummichog are noted for their hardiness, which includes the ability to survive a wide variety of temperatures, large salinity fluctuations, low dissolved oxygen, and heavily polluted ecosystems [1–7]. The ability to adapt and acclimate to these different conditions has made mummichogs a popular research subject in a number of fields, including ecology, evolution, physiology, and toxicology [1, 3, 8–12].

Coastal population growth, urban runoff from increased impervious cover, and industrial pollution results in significant anthropogenic stress in estuaries. The abundance of *F. heteroclitus* in estuaries, their association with sediments due to hiding from predators or searching for food, and their non-migratory nature makes them an excellent bioindicator species. *Fundulus* species have been used as bioindicators at contaminated sites [1, 2, 5, 13, 14] and to follow the evolution of pollution resistance in a vertebrate [3, 10, 11, 15–17]. Thus, mummichogs are an excellent vertebrate bioindicator species for understanding rapid adaptation to environmental change in

feral populations [5, 8], including adaptations in transcription factors such as estrogen receptor alpha (ERα) splice variants [18], and evolutionary convergence in the aryl hydrocarbon receptor pathway (AHR1a, AHR2a, CYP1A) [5, 8].

NRs consist of five modules: A/B, C, D, E, and F. The A/B module contains activation function-1 (AF-1) sites crucial in binding coactivators. The C module contains the DNA-binding domain (DBD) that encompasses the zinc-fingers necessary for binding DNA at response elements and mediating the basic transcriptional functions of a NR. The DBD is also highly conserved between orthologs and in turn is used in phylogenetic analysis [19, 20]. The D module contains the hinge region and nuclear localization sites. The E module contains the ligand-binding domain (LBD) domain and AF-2 function crucial in binding coactivators. This module mediates receptor activity by sensing the cellular environment, binding ligand and responding by activating transcription [21, 22]. The LBD is larger than the DBD, moderately conserved among the orthologs of different species, and therefore used in phylogenetic analysis [20, 23]. The F module is of unknown function and is missing in some NRs [21, 24].

NRs regulate multiple physiological pathways such as cell differentiation, resource allocation, reproduction, development, and maintenance of homeostasis. They regulate these diverse physiological conditions by responding to both internal or external cues such as nutrients, steroids, heme, or xenobiotics [25–29]. Thus, NRs are considered conduits that help in the maintenance of homeostatic conditions by responding to various internal and external cues. NRs occupy crucial roles in the disciplines of environmental physiology, endocrinology, pharmacology, toxicology, nutrition, biochemistry, gene regulation, ecology, chemistry, and other fields of study [30].

In this study, we annotated the *F. heteroclitus* NRs through phylogenetic analysis. *F. heteroclitus* are keystone species in the estuaries of the eastern seaboard of North America that are hardy and able to thrive in various conditions of toxic insult, salinity, and temperature [1]. Therefore, we anticipate that analysis of the NRs will ultimately be useful in providing novel insight regarding their unique abilities to withstand diverse stressors and the subsequent transcriptional and physiological responses of these fish.

2. Methods

2.1. Identification of *Fundulus heteroclitus* nuclear receptors

F. heteroclitus NRs were identified using a Basic Local Alignment Search Tool (BLAST) [31] with NR DNA binding domains (DBDs) from human, *Takifugu rubripes*, or *D. magna* [20] against the assembled *F. heteroclitus* genome (`https://my.mdibl.org/display/FGP/Home`) as described previously [22]. Positive BLAST hits were confirmed as nuclear receptors with BLASTp on the NCBI database as were percent identity determinations between orthologous NRs [32].

2.2. Phylogenetics

Phylogenetic analysis was performed using methods described previously [20, 33]. The *F. heteroclitus* sequences are predicted protein sequences from the genome browser, and non-killifish sequences used for phylogenetic analysis were derived from the NCBI database, or the Takifugu genome brower (`http://www.fugu-sg.org/`). Phylogenetic comparisons included *F. heteroclitus, Takifugu rubripes, Danio rerio, and Homo sapiens.* A list of nuclear receptors used in comparisons to *F. heteroclitus* is provided in Additional File 1 available online at `http://www.agialpress.com/journals/nurr/2017/101285/`.

Phylogenetic analysis was performed using only the highly conserved DBD and moderately conserved LBD of each receptor. These domains were identified and isolated using the pfam00105 (Zf-C4) and pfam00104 (hormone receptor) designations on the conserved domain database CDD [34] as described previously [20, 23, 35]. The DBD and LBD from 246 NRs from three fish species and humans were aligned using ClustalX default parameters [36] (Additional File 2 available online at `http://www.agialpress.com/journals/nurr/2017/101285/`). Phylogenetic analysis was performed with Maximum Likelihood (ML) using MEGA 6.0 [37]. "Find Best Model" was used to determine the parameters for Maximum Likelihood. Further analysis was performed using the Bootstrap method with 500 replications, the JTT model with Gamma distributed rates among sites [3]. Tree inference options included SPR level 3, BIONJ with a very strong branch filter.

Maximum parsimony and distance parameters were used to provide additional support for the phylogenetic relationships observed. Distance parameters were measured using PAUP 4.0b10 with default characteristics (mean character difference and among site rate variation), and full heuristic searches. Branch support was measured by bootstrap analysis with 1000 replicates. Parsimony was constructed using PAUP version 4.0b10 with heuristic searches, tree-bisection-reconnection, topological constraints not enforced, and multiple tree option in effect with an initial maximum tree setting at 100,000. Branch support was measured by bootstrapping with 10,000 replicates [20, 38]. Maximum Parsimony trees were visualized with PAUP 4.0b10 and Neighbor-Joining trees were visualized with FigTree (`http://tree.bio.ed.ac.uk/software`).

3. Results and Discussion

3.1. Nuclear receptor isolation from Fundulus

Analysis of the *F. heteroclitus* genome found 74 NRs, which include representatives of all seven subfamily members. Additional File 3 (available online at `http://www.agialpress.com/journals/nurr/2017/101285/`) provides links to the scaffold for each NR, its cDNA and its protein sequence. Seventy-four NRs are in range of what has been found in other fish species as 68 NRs were found in *Takifugu rubripes* [39] and 72 NRs in *Tetraodon nigriviridis* [40] although recent improvements in the Takifugu genome indicates 73 NRs [23]. The number of NRs in teleost genomes is significantly greater than those found in invertebrates and mammals because of whole genome duplication events [41]. The number of NRs in the common carp (*Cyprinus carpio*) is even greater (137) than other fish species because of an additional genome

duplication event [23]. Genome duplication is often followed by loss of some genes, gain of function of some genes (neofunctionalization) including differences in spatial or temporal gene expression between paralogs, or partitioning of ancient functions on duplicated genes (subfunctionalization) [42].

3.2. Phylogenetics

Phylogenetic analysis by Maximum Likelihood (ML) confirms the presence of all seven NR subfamilies in *F. heteroclitus*, and demonstrates that there are 2 NR0 members, 30 NR1 members, 17 NR2 members, 15 NR3 members, 5 NR4 members, 4 NR5 members, and 1 NR6 member (Figure 1). This file is also available as an expandable pdf (Additional File 4 available online at `http://www.agialpress.com/journals/nurr/2017/101285/`). The percentage of each subfamily of NRs is relatively similar between *F. heteroclitus*, the fish species examined and humans with minor exceptions. The three different phylogenetic models used (Maximum Likelihood, Maximum Parsimony, Neighbor-Joining) agreed at the group level and often at the subfamily level, but there are differences at the base of the phylogram; primarily the relationship of the 0-subfamily to the 5 and 6 subfamilies. However, the ancient bootstrap values are typically not significant using any of the analysis with Maximum Parsimony rarely being able to resolve distinct clades on the left hand side of the tree. The Neighbor-Joining and Maximum Parsimony phylogenetic trees are provided as additional files (Additional Files 5, 6 available online at `http://www.agialpress.com/journals/nurr/2017/101285/`).

3.3. NR0 Subfamily

The NR0 subfamily contains two groups, NR0A and NR0B. NR0A receptors lack a ligand-binding domain (LBD) and NR0B receptors lack a DNA-binding domain (DBD). Similar to other vertebrates, there are no NR0A members (knirps) in *F. heteroclitus* [20, 43]. Mummichogs contain two NR0B members, SHP and DAX (Figure 1; Table 1). We found that DAX/SHP fall within the 6 subfamily based on Maximum Likelihood but with low posterior probabilities (50%) (Figure 1). Maximum parsimony resolved the 2, 6, and 0 subfamilies as separate subfamilies but without resolving their evolutionary relationship to each other (Additional File 5 available online at `http://www.agialpress.com/journals/nurr/2017/101285/`). Previous work using parsimony and NJ suggested with 73% confidence that the NR0B group is evolutionarily related to 2 subfamily [44]. Bayesian Inference also indicated that these NRs evolved as part of the 2 subfamily [22]. However, most studies performed do not include them as part of a phylogenetic tree because of the reduced molecular information due to the lack of the LBD and subsequent uncertainty in the analysis [20, 45, 46].

Overall, the resolution of the 0 subfamily is poor. NR0B members clearly form their own group based on the lack of a C domain; however, their evolutionary relationship to other NRs is questionable. In turn, unlike other receptors, NR0 members are not named for their phylogenetic position but instead for their lack of a key domain [47]. The NR0B receptors SHP and DAX primarily work as co-regulators as they both contain LxxLL domains typically found in coregulators [48–50]. DAX is involved in reproductive development and steroidogenesis; SHP regulates cholesterol and glucose homeostasis [51].

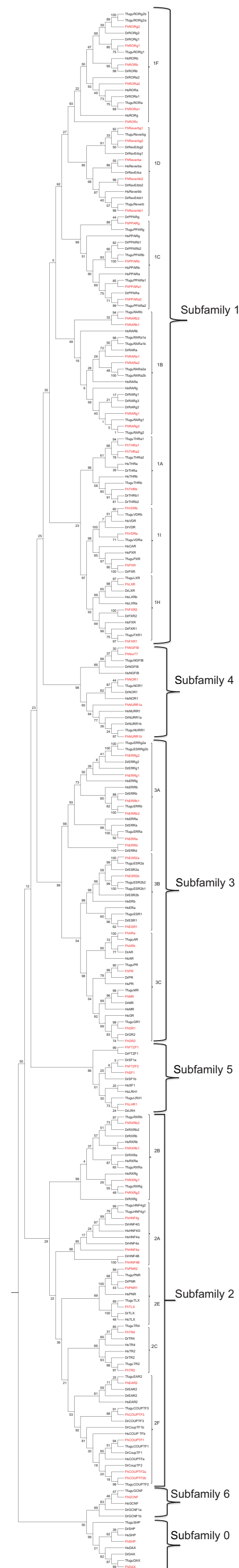

Figure 1: Phylogenetic relationship of nuclear receptors as determined by ML. The phylogenetic tree is shown with bootstrap support values (frequency of occurrence) from ML at each node. Species included are *Takifugu rubripes (Tfugu), H. sapiens (Hs), Danio rerio (Dr),* and *Fundulus heteroclitus (Fh)*. All *F. heteroclitus* sequences are in red.

Table 1: Nuclear receptors from Fundulus compared to those described in other species.

Group	*F. heteroclitus*	*H. sapiens*	*D. rerio*	*T. rubripes*	*T. nigroviridis*	*D. pulex*	*D. melanogaster*
0A						KNR-R1	KNI
						KNR-R2	KNRL
							EGON
0B	DAX	DAX	DAX	DAX			
	SHP	SHP	SHP	SHP			
1A	THRα1	THRα	THRα	THRα1	THRα1	THRL11	
	THRα2			THRα2	THRα2		
	THRβ	THRβ	THRβ1	THRβ	THRβ		
			THRβ2				
1B	RARα1	RARα	RARα	RARα1a	RARα1	RARL10	
	RARα2			RARα1b	RARα2	(unresolved may be member of 1M family)	
				RARα2a			
				RARα2b			
	RARβ1	RARβ		RARβ			
	RARβ2						
	RARγ1	RARγ	RARγ1	RARγ1	RARγ		
	RARγ2		RARγ2	RARγ2			
			RARγ3				
1C	PPARα1	PPARα		PPARα1	PPARα1		
	PPARα2		PPARα2	PPARα2	PPARα2		
	PPARβ	PPARβ	PPARβ1	PPARβ	PPARβ		
			PPARβ2				
	PPARγ	PPARγ	PPARγ	PPARγ	PPARγ		
1D	Rev-erb-α	Rev-erb-α	Rev-erb-α	Reb-erb-α	Rev-Erb-α1	E75	E75
					Rev-Erb-α2		
	Rev-erb-β1	Rev-erb-β	Rev-erb-β1	Rev-erb-β	Rev-erb-β1		
	Rev-erb-β2		Rev-erb-β2		Rev-erb-β2		
	Rev-erb-γ1		Rev-erb-γ1	Rev-erb-γ	Rev-erb-γ		
	Rev-erb-γ2		Rev-erb-γ2				
1E						E78	E78
1F	RORα1	RORα	RORα1	RORα	RORα1	HR3	DHR3
	RORα2		RORα2		RORα2		
	RORβ	RORβ	RORβ	RORβ	RORβ		
					RORβ		
	RORγ1	RORγ	RORγ1	RORγ1	RORβ		
	RORγ2		RORγ1b	RORγ2a			
	RORc			RORγ2b			

Group	*F. heteroclitus*	*H. sapiens*	*D. rerio*	*T. rubripes*	*T. nigroviridis*	*D. pulex*	*D. melanogaster*
1H	LXRα	LXRα	LXRα	LXRα	LXRα	EcRα	EcR
		LXRβ				EcRβ	
	FXR1	FXR	FXR1	FXR1	FXRa		
	FXR2		FXR2	FXR2	FXRa		
					FXRb		
1I	VDR-A	VDR	VDR-A	VDR-A	VDR-A1		
	VDR-B			VDR-B	VDR-A2		
					VDR-B		
	PXR	PXR	PXR	PXR	PXR		
		CAR					
1J						HR96	DHR96
1L						HR97a	
						HR97b	
						HR97g	
2A	HNF4α	HNF4α	HNF4α	HNF4α	HNF4α	HNF4	HNF4
	HNF4β		HNF4β				
	HNF4γ	HNF4γ	HNF4γ	HNF4γ1	HNF4γ		
				HNF4γ2			
2B		RXRα	RXRα	RXRα	RXRα	RXR	USP
	RXRβ-p				RXRα		
	RXRβ1	RXRβ	RXRβ1	RXRβ	RXRβ		
	RXRβ2		RXRβ2		RXRβ		
	RXRγ1	RXRγ	RXRγ	RXRγ	RXRβ		
	RXRγ2						
2C	TR2	TR2	TR2	TR2	TR2		
	TR4	TR4	TR4	TR4	TR4		
2D						HR78	DHR78
2E	TLX	TLX	TLX	TLX	TLX	TLL	TLL
	PNR 1	PNR	PNR	PNR	PNR	PNR	PNR
	PNR 2					DSF	DSF
						NR2E6	FAX-1
2F	COUP-TF1	COUP-TFa	COUP-TF1a	COUP-TF1	COUP-TFa	SVP	SVP
		COUP-TFb	COUP-TF1b	COUP-TF1	COUP-TFa		
	COUP-TF2a		COUP-TF2	COUP-TF2	COUP-TFb		
	COUP-TF2b				COUP-TFb		
	COUP-TF3		COUP-TF3	COUP-TF3	COUP-TFg		
	EAR2	EAR2	EAR2	EAR2	EAR2		
			EAR2	EAR2	EAR2		
3A	ESR1	ESR1	ESR1	ER1	ESR1		
	ESR2a	ESR2	ESR2a	ERb	ESR2a		
	ESR2b		ESR2b	ER2a	ESR2b		
				ER2b			

Group	*F. heteroclitus*	*H. sapiens*	*D. rerio*	*T. rubripes*	*T. nigroviridis*	*D. pulex*	*D. melanogaster*
3B	ERRα	ERRα	ERRα	ERRα	ERRα	ERR	ERR
	ERRβ1	ERRβ	ERRβ	ERRβ	ERRβ1		
	ERRβ2				ERRβ2		
	ERRδ		ERRδ				
	ERRγ1	ERRγ	ERRγ1	ERRγ2a	ERRγ		
	ERRγ2		ERRγ2	ERRγ2b	ERRγ		
				ERRγ	ERRγ		
					ERRγ		
3C	GR	GR	GR	GR	GR		
	GR2			GR2	GR2		
	MR	MR	MR	MR	MR		
	PR	PR	PR	PR	PR		
	ARα	AR	AR	ARα	ARα		
	ARβ			ARβ	ARβ		
4A	NGF1B	NGFIB	NGFIB	NGFIBa	NGF1B	HR38	DHR38
	Nur77				NGF1B		
	NURR1a	NURR1	NURR1a	NURR1	NURR1		
	NURR1b		NURR1b				
	NOR1	NOR1	NOR1	NOR1	NOR1		
5A	SF1	SF1	SF1a	FTZ-F2	SF1	FTZ-F1	FTZ-F1
	FTZ-F2/SF1b		SF1b				
	LHR1	LRH1	LRH1	LRH1	LRH1		
	FTZ-F1		FTZF1	FTZF1	FTZ-F1		
5B						DpHR39	DHR39
6A	GCNF	GCNF	GCNF1a	GCNF	GCNF	DpHR4	DHR4
			GCNF1b				

3.4. NR1 Subfamily

The NR1 subfamily is the largest subfamily in *F. heteroclitus* with 30 members. 40.5 Percent of mummichog NRs are in the 1-subfamily; 36-40% of NRs are in the 1-subfamily of other fish species and 40% of NRs are 1 subfamily members in humans. The 1-subfamily separates into two distinct clades; one clade includes the NR1B (RAR), NR1C (PPAR), NR1D (Reverb), and NR1F (ROR) groups, and the other one includes the NR1A (THR), NR1H (LXR/FXR), and NR1I (VDR) groups (Figure 1). There is disagreement in some of our different phylogenetic analysis at the extreme left of the trees where bootstrap values may drop as low as 23 indicating poor resolution.

The fish species, including *F. heteroclitus* show significant expansion of NR1B (RAR), NR1D (Rev-erb), and NR1F (ROR) groups relative to humans (Table 1). Interestingly, all of these NRs are found in the same NR1 clade. There are 6 members of the *F. heteroclitus* RAR group. Humans have 3 members and there are 3–7 RAR members in the other fish species investigated. There are 5 members of the Rev-erb clade and 6 members of the ROR groups in *F. heteroclitus*.

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Five members in the Rev-erb group are typical in fish; however, the other fish species examined only have 5 ROR members. In comparison, humans have 2 Rev-erb and 3 ROR members (Table 1).

The RAR, Rev-erb, and ROR groups are important in lipid and glucose metabolism, gas-response, development, promoting T-cell differentiation, inflammation, and circadian rhythms [52–54]. Several genes are coordinately regulated by Rev-erbα and RORα as they share the same response elements but exert opposing effects on transcription. Significant expansion of these two opposing sets of NRs in fish species including *F. heteroclitus* appears synchronous. It is thought that the crosstalk between the two receptor groups helps regulate their transcriptional and physiological networks, including circadian rhythms, lipid and glucose metabolism, and inflammation [55, 56].

In contrast, the NR1C (PPAR) group shows little expansion in *F. heteroclitus* or other fish species. There are three members in humans and four in each of the fish species investigated (Table 1). PPARs are crucial in the regulation of lipids [27, 57]. The pufferfish *(Takifugu* and *Tetraodon)* genomes both show two PPARα NRs similar to *F. heteroclitus*, while the Atlantic salmon (*Salmo salar*) and zebrafish (*D. rerio*) genomes have two members of the PPARβ/δ group [41, 58, 59]. *Danio* are ancient Ostariophysi, *Salmo* are Salmoniformes, and *Fundulus* and *Takifugu* are both modern Percomorphs [60]. Because of whole genome duplication in teleosts [42], it is most likely that an ancient relative lost PPARβ/δ in the Percomorphs and PPARα in the early teleosts. However, it cannot be completely ruled out that separate duplication events occurred in the Percomorphs and early teleosts as individual gene duplication events have occurred multiple times including cytochrome P450s, opsins, and NRs [20, 35, 61–63]. The organ distribution of PPARs differs from mammals. For example, pufferfish show wide tissue distribution of the PPARs; in mammals only PPARβ/δ is widely distributed [58]. Changes in organ distribution or domain structure and function is common in neofunctional retained duplicated receptors. Therefore, we performed pairwise comparisons between human NRs and their *F. heteroclitus* orthologs with duplications (Table 2). Mummichog PPARα2 shows greater differences in its LBD than PPARα1 compared to human PPARα suggesting neofunctionalization (Table 2).

Table 2: Percent Identity comparisons of the DNA binding domain (DBD) and ligand binding domain (LBD) of orthologous human and F. heteroclitus nuclear receptors with Blastp.

Nuclear Receptor[a]	DBD	LBD	Ligands[b]
SHP		51	
DAX		51	
THRα1-110	92	84	Thyroid hormones [114]
THRα2-10098	96	91	
PPARα1	86	85	Leukotriene B4,
			1-palmityl-2-oleolyl-sn-glycerol-3-phosphocholine,
PPARα2	86	73	Fatty acids [115]
Rev-Erb-β1-10090	94	83	Heme [114]
Rev-Erb-β2-10062	93	77	
RORα-9958	99	93	
RORα-369	91	75	
RORγ-9885	90	43	

Nuclear Receptor[a]	DBD	LBD	Ligands[b]
RORγ-10005	90	47	
FXR1-10028	96	72	5β-bile acids [83]
FXR2-9861	84	35	
VDR-A	93	79	1,25-OH vitamin D3, bile salts [116, 117]
VDR-B	94	80	
PXR	72	58	Xenobiotics, bile salts [73, 80]
RXRβ1-483	94	88	9-cis retinoic acid, rexinoids [114, 118]
RXRβ2-9867	90	79	
RXRγ1-9885	94	95	
RXRγ2-9880	94	95	
PNR1-9949	93	74	
PNR2-195	91	59	
ESR1	96	66	17β-estradiol [119]
ESR2α-10024	96	68	
ESR2β-10026	96	65	
ERRβ-9910	100	76	
ERRβ-3	100	84	
ERRγ-10140	97	94	
ERRγ2-9925	91	81	
GR-0	96	74	Cortisol [120]
GR2-10031	96	74	
ARα-9941	65	59	Testosterone, 11-ketotestosterone, 17α-methyltestosterone [121]
ARβ-9884	91	70	
NGF1B	99	69	
Nurr77	93	66	
NURR1a-9995	96	95	
NURR1b-114	97	58	
SF1a	72	58	Phosphatidylinositol, phosphatidylcholine [122]
FTZ-F2/SF1b-224	84	nd	

[a]Scaffold is often provided after the receptor to aid in determining the receptor in question.

[b]Known or putative endogenous physiological ligands for fish (or human if fish unknown) are included for the receptors that show show high divergence from humans or have a duplicated receptor with potential neofunctionalization.

The presence of PPARs in fish may make them sensitive to peroxisome proliferation or perturbations in lipid allocation and homeostasis. For example, peroxisome proliferation or induction of biomarkers of peroxisome proliferation has been measured in fish exposed to PAHs, pharmaceuticals, phthalates, alkylphenols, and pesticides [64–69]. Peroxisome proliferation has been measured in mummichogs following 2,4-D exposure [70].

Furthermore, pharmaceuticals such as the PPARα activator, gemfibrozil reduced n-3 fatty acids in rainbow trout (*Oncorhynchus mykiss*), which may reduce the nutritional quality of the fish, perturb their ability to acclimate to changes in water temperature, and repress their ability to reproduce following migration [64]. The PPARγ activator, TBT activated an obesogen

response by increasing body weight and whole-lipid content in Chinook salmon (*Oncorhynchus tshawytscha*) and condition factor, triglycerides, and hepatosomatic index in zebrafish [71]. Interestingly, toxicants that activate PPARs may enhance condition factor while also increasing other stress responses typically associated with poor physiological conditions [72].

The other NR1 clade includes the NR1A (THR), NR1H (LXR/FXR), and NR1I (VDR/PXR) groups. THR is split into two subgroups, THRα and THRβ. There are three THR members in each of the fish species examined compared to two in humans that only contain one THRα and THRβ member. Pufferfish and mummichogs contain two THRα members and one THRβ members; *D. rerio* contains one THRα member and two THRβ members (Table 1).

The NR1I group in fish that contains the vitamin D receptor (VDR), PXR, and CAR, has been relatively well studied because of the roles of PXR and CAR in acclimation to foreign chemicals [21, 73]. PXR was previously cloned in *F. heteroclitus* from a PCB polluted site and this PXR is nearly identical to the PXR sequenced during the genome project with the exception of a three amino acid region at amino acid 190 that is missing in the *F. heteroclitus* genome sequence [74].

PXR, but not CAR, has been identified in teleost species [39, 75]. Originally it was thought that CAR diverged from PXR at some later evolutionary point because CAR had only been found in mammals. However, recent phylogenetic data, examination of Saurapsid genomes such as reptiles and birds [76] and the lobe-finned fish Coelocanth indicates that CAR was lost in teleosts and PXR was lost in reptiles and birds [77]. Therefore, it is thought that the lobe-finned fish that arose 400 million years ago during the Devonian period and are the ancestors of all tetrapods, contained CAR and PXR [78, 79].

The DBD and LBD of *F. heteroclitus* PXR are highly diverged from humans with pairwise comparisons showing 72% identity to human DBD and 58% to human LBD (Table 2). This is typical of fish PXRs as previous comparisons to human PXR show 61-73% sequence identity to the DBD and 52-57% identity to the LBD [78]. Only ARα (65%) and SF1a (72%) show as much divergence in the DBD as PXR; only RORγ, SF1, FXR2, SHP, and DAX show similar divergence within the LBD when compared to mammals [78]. However, with the exception of the NR0 members, SHP and DAX, all the other receptors showing high divergence have duplicated members and at least one of the duplicates appears to show neofunctionalization based on the large change in amino acid identity (Table 2).

The large differences in LBD sequences for PXR provides biochemical support for the significant differences in activation profiles between different fish species and between fish and humans, especially for fenvalerate and several organochlorine insecticides [73]. Ligand activation of zebrafish PXR matched only 30% of the representative human PXR ligands further indicating the divergence of fish and human PXR [75]. Ligands tested include primarily bile acids, steroids and pharmaceuticals. Of these, Phenobarbital, clotrimazole, dihydrotestosterone, androstanol and 5β-pregnane-3,20-dione all activated the zebrafish PXR. In general, human PXR can be activated by many bile salts, including both C_{24} and C_{27} bile salts. However, the zebrafish PXR was only activated by C_{27} bile salts with a strong preference for sulfated bile alcohols [80]. The C_{27} pathway is an ancient pathway found in early fish and amphibians and it has been hypothesized that PXR has evolved to deal with the increasingly complex bile salt synthetic pathways [76]. This is in contrast to other theories that suggest that the reason for the great divergence in the LBD of PXR between species, including zebrafish and *Fugu*,

is in response to the different sets of xenobiotic/environmental challenges encountered by the different species [81].

The FXR/LXR group (NR1H) in *F. heteroclitus* contains one LXR member, LXRa, and two FXR members. The FXR/LXR group is related to the ecdysone receptors in invertebrates (Table 1) [20, 82]. Humans have two LXR members and one FXR that is more closely aligned with FXR1 (Figure 1). The existence of two or more FXR members is common among fish species. The green pufferfish (*T. nigroviridis) and* Japanese medaka (Oryzias latipes) have at least 3 FXR members because they have two similar FXRa members (Table 1; Figure 1). In medaka, FXRa2 is activated by C24 bile acids and GW4064, but FXRa1 is not activated by these common ligands probably due to differences in the A/B domain [83]. Interestingly, in *Fundulus*, FXR2's LBD has the lowest percent identity when compared to humans (Table 2), indicating neofunctionalization and a different activation profile than humans.

In addition, there is significant diversity in FXR ligand responses in fish species [84]. Tetraodon FXR has a ligand selectivity profile very similar to human FXR, with activation by the synthetic ligand GW4064 and the bile acid, chenodeoxycholic acid. Furthermore, modeling and docking studies suggest that Tetraodon's ligand-binding pocket more similar to mammalian FXRs than to lamprey or zebrafish FXRs [85], which are activated by 5α-bile alcohols but not by the evolutionarily more recent 5β-bile acids [86]. Based on phylogenetics, *Fundulus* FXR is more like Tetraodon and medaka than zebrafish (Figure 1) [78].

3.5. NR2 Subfamily

The NR2 subfamily is the second largest subfamily of NRs in *F. heteroclitus* with 18 NRs. NR2 is divided into 5 groups A, B, C/D, E and F. Of these, Group 2B (RXR) has expanded the most of the fish species investigated relative to humans, and group 2F (COUP) has expanded in all of the fish species investigated relative to human NRs (Table 1). In addition, *F. heteroclitus* has three Group 2E members, which are involved in eye development in zebrafish [87, 88], because it has two PNR receptors (Table 1). To our knowledge, other fish species only have one PNR with the recent exception of Nile tilapia (*Oreochromis niloticus)* and medaka [23, 78]. Considering the phylogenetic distance of the receptors it is unlikely that this is a recent event (Figure 1) and instead it is more likely that the second PNR was not lost following the genome wide duplication in fishes [41]. Interestingly, invertebrate species often contain more group 2E members than vertebrates (Table 1) [20].

RXRs bind to retinoids, are crucial for growth and development, and are key heterodimeric partners with several other NRs [89, 90]. COUP-TFs are necessary for growth and development, including venous and lymphatic development in zebrafish [91, 92]. Interestingly, COUP-TF members have also been shown to interact with a few other NRs including RXRα, RAR, THR, ERRγ, ERα, and other COUP-TF members as hetero- and homodimers [90]; however they typically repress transcription including RAR/RXR responses [93]. Thus, the NRs that have shown expansion may have a repressive counterpart that also expanded.

There are three RXR members in humans and 4-5 members in *F. heteroclitus* of which none appear to be RXRα members. The exact number of RXR members is unknown because of the brevity of scaffold 2083 (Additional Figure 3 available online at `http://www.agialpress.`

com/journals/nurr/2017/101285/), which ends at 17,995 bp and in turn cuts off a potential RXR member at the AB domain (AF-1). Therefore, this RXR member appears to be a pseudogene but may not be. This partial gene contains a start site, two introns, and a stop codon. It is also structurally different than the other RXRβ genes that have two relatively close exons near the 5'-end. However given that the scaffold ends shortly after the stop codon it cannot be completely ruled out that there might be other splice sites and exons following. Interestingly, scaffold's 2083 gene fragment aligns well with RXRβ members from several fish species. If it is a RXRβ member than phylogenetic analysis would indicate that *F. heteroclitus* has 3 RXRβ, 2 RXRγ, and 0 RXRα members (Table 1; Figure 1). Initial examination of the genome by BLAST suggested that the RXR at scaffold 9880 may be a RXRα member; however phylogenetic analysis indicates that this NR is an RXRγ member with a weak posterior probability of 37. While the posterior probability is weak, Maximum Likelihood (Figure 1), Maximum Parsimony (Additional File 5 available online at http://www.agialpress.com/journals/nurr/2017/101285/), and Neighbor-Joining (Additional File 6 available online at http://www.agialpress.com/journals/nurr/2017/101285/) all agree that complete RXR members are found within RXRβ or RXRγ subgroups and therefore at this time no RXRα member was found in *F. heteroclitus*. If the RXR at scaffold 2380 is a complete RXRα member than the *F. heteroclitus* genome will have at least 75 NRs. The lack of an RXRα member would be surprising if not unprecedented as we did not find other fish or mammalian species lacking RXRα.

Group 2A (HNF) is an ancient group [46] that expanded greatly in *C. elegans* [94]. HNF4 contains 3 members, which is typical of most fish species (Table 1). The HNF group of receptors has been highly studied in fish species, but what work has been done indicates that similar to mammals the HNF4 receptors are enrich in the liver and regulate liver enriched gene expression [95] sometimes in conjunction with other HNFs including HNF1 and COUP-TF1 [95, 96].

3.6. NR3 subfamily

There are 15 NR3 subfamily members in *F. heteroclitus*. In comparison, there are 15 in *T. rubripes*, 16 in *T. nigroviridis*, and 9 in humans. There are 6 estrogen related receptors (ERRs; NR3B) in *F. heteroclitus*, while most fish species contain 5-7 and humans contain 3 (Table 1). There are 3 estrogen receptors (ERs) (NR3A) in *F. heteroclitus*, while most fish species contain 3-4 receptors and humans contain 2 (Table 1). Overall, there is a significant expansion of the 3A and 3B group in *F. heteroclitus* and other fish species relative to humans. Interestingly, *Fundulus* chronically exposed to estrogenic pollutants developed heritable splicing variants that show different transcriptional responses than wild-type ER potentially as an adaptive mechanism to the estrogens found in the polluted environment [11, 18].

There are 6 NR3C (glucocorticoid, mineralcorticoid, androgen, and progesterone receptors) members in *F. heteroclitus*. There are also six members in *Tetraodon* and *Takifugu*, but only 4 NR3C members in zebrafish and humans (Table 1). *F. heteroclitus* has two glucocorticoid receptors (GR), two androgen receptors (AR), and one mineralocorticoid (MR) and one progesterone receptor (PR) within the NR3C group. This is consistent with several other fish species such as *Tetraodon* and *Takifugu* (Figure 1; Table 1) that have two GRs and two ARs [39, 40].

In addition, medaka, stickleback (*Gasterosteus aculeatus*), common carp (*Cyprinus carpio*) and rainbow trout (*Oncorynchus mykiss*) all have two GRs [97, 98]; however, rainbow trout and common carp have undergone an additional round of genome duplication that may explain why these species have two GRs. Zebrafish (*Danio rerio*), only has one GR, GR2, that is missing a 9 amino acid sequence between the zinc fingers of the DBD [98, 99]. It is interesting to speculate that the increase in GRs are crucial in salt balance, especially in marine or estuarine species [12, 100, 101] as GR regulates salt balance in part by regulating the transcription of the sodium-chloride cotransporter and sodium-potassium ATPase [102, 103]. Carp, like zebrafish, are Ostariophysi and they contain two GRs. Thus, it appears that zebrafish unlike most other teleosts, lost a GR after the genome wide duplication in fish [104, 105] and carp (and rainbow trout) have two GR because of the second genome duplication.

The loss of the second AR occurred in several fish species unlike the loss of GR2, which occurred in only a few species. *F. heteroclitus, Takifugu* and *Tetraodon* all have two ARs. However, Cypriniformes such as zebrafish, Siluriformes such as catfish, and Salmoniformes, such as salmon and trout all have one AR. Interestingly, early teleosts such as Osteoglossiformes (arowana, knifefish) and Anguilliformes (eels) contain two very similar ARs, while Percomorphs such as pufferfish and *Fundulus* show divergence between their ARs [106] with different binding affinities for steroids and xenobiotics [107]. ARα which shows high divergence relative to humans and ARβ (Table 2), also has higher transactivation activity confirming neofunctionalization [108].

3.7. NR subfamilies 4-6

In *F. heteroclitus*, subfamilies 4-6 account for nine NRs; one more than *T. nigroviridis* because *F. heteroclitus* contains 5 NR4 members. Overall, there is a small expansion of the 4-subfamily as most fish species contain 4 members; however *F. heteroclitus* and Nile tilapia (*O. niloticus*) contain 5 members. Humans contain three NR4 members and most invertebrates contain one [20, 22]. Some NR4 members are crucial in brain differentiation, cell cycle, inflammation, and atherosclerosis [109–111]. The NR4 subfamily is related to the NR1 subfamily, and is an ancient family that is ligand independent [46, 112]. Several of the NR4 members show relatively large differences between mummichog and human LBDs. This divergence is unique to the duplicated NR4A2 member, Nurr1b, but occurs is both NR4A1 member (Table 2).

The NR5 subfamiy contains FTZ members that regulate development. There are four members of the NR5 subfamily. SF-1 members are crucial in sex determination and down-regulated upon exposure to anthropogenic estrogenic chemicals during sex reversal [113]. SF1a and FTZ-F2 show very high divergence relatively to human SF1 (Table 2). GCNF, which is the only NR6 subfamily member, is involved in growth and maturation [112]. Zebrafish have two GCNF members but other fish species, *Daphnia*, and humans have one (Table 1) [23].

4. Conclusions

There are at least 74 full length NRs in the *F. heteroclitus* genome spanning all seven gene subfamilies of which 40% are in the NR1 subfamily often involved in circadian rhythms, development, energy metabolism and resource allocation. Fish species show expansion of the RAR

(NR1B), Reverb (NR1D), ROR (NR1F), COUPTF (NR2F), ERR (NR3B), RXR (NR2B), and to a lesser extent the NGF (NR4A), and NR3C steroid receptors (GR/AR). Of particular interest is the co-expansion of opposing NRs, Reverb-ROR, and RAR/RXR-COUPTF, and the potential lack of an RXRα member in the *F. heteroclitus* genome.

Competing Interests

The authors declare that they have no competing interests.

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References

[1] K. G. Burnett, L. J. Bain, W. S. Baldwin et al., "Fundulus as the premier teleost model in environmental biology: Opportunities for new insights using genomics," *Comparative Biochemistry and Physiology-Part D: Genomics and Proteomics*, vol. 2, no. 4, pp. 257–286, 2007.

[2] J. A. Roling, L. J. Bain, J. Gardea-Torresdey, P. B. Key, and W. S. Baldwin, "Using mummichog (Fundulus heteroclitus) arrays to monitor the effectiveness of remediation at a superfund site in Charleston, South Carolina, USA," *Environmental Toxicology and Chemistry*, vol. 26, no. 6, pp. 1205–1213, 2007.

[3] A. M. Reitzel, S. I. Karchner, D. G. Franks et al., "Genetic variation at aryl hydrocarbon receptor (AHR) loci in populations of Atlantic killifish (Fundulus heteroclitus) inhabiting polluted and reference habitats," *BMC Evolutionary Biology*, vol. 14, no. 1, article no. 6, 2014.

[4] J. A. Roling, L. J. Bain, and W. S. Baldwin, "Differential gene expression in mummichogs (Fundulus heteroclitus) following treatment with pyrene: Comparison to a creosote contaminated site," *Marine Environmental Research*, vol. 57, no. 5, pp. 377–395, 2004.

[5] N. M. Reid, D. A. Proestou, B. W. Clark et al., "The genomic landscape of rapid repeated evolutionary adaptation to toxic pollution in wild fish," *Science*, vol. 354, no. 6317, pp. 1305–1308, 2016.

[6] J. M. Mancera and S. D. McCormick, "Influence of cortisol, growth hormone, insulin-like growth factor I and 3,3′,5-triiodo-L-thyronine on hypoosmoregulatory ability in the euryhaline teleost Fundulus heteroclitus," *Fish Physiology and Biochemistry*, vol. 21, no. 1, pp. 25–33, 1999.

[7] J. E. Podrabsky and G. N. Somero, "Changes in gene expression associated with acclimation to constant temperatures and fluctuating daily temperatures in an annual killifish Austrofundulus limnaeus," *Journal of Experimental Biology*, vol. 207, no. 13, pp. 2237–2254, 2004.

[8] N. M. Reid, C. E. Jackson, D. Gilbert et al., "The Landscape of Extreme Genomic Variation in the Highly Adaptable Atlantic Killifish," *Genome Biology and Evolution*, vol. 9, no. 3, pp. 659–676, 2017.

[9] J. A. Roling, L. J. Bain, J. Gardea-Torresdey, J. Bader, and W. S. Baldwin, "Hexavalent chromium reduces larval growth and alters gene expression in mummichog (Fundulus heteroclitus)," *Environmental Toxicology and Chemistry*, vol. 25, no. 10, pp. 2725–2733, 2006.

[10] M. E. Hahn, S. I. Karchner, D. G. Franks, and R. R. Merson, "Aryl hydrocarbon receptor polymorphisms and dioxin resistance in Atlantic killifish (Fundulus heteroclitus)," *Pharmacogenetics*, vol. 14, no. 2, pp. 131–143, 2004.

[11] K. A. Cotter, D. Nacci, D. Champlin, J. Chuprin, and G. V. Callard, "Cloning of multiple ERα mRNA variants in killifish (fundulus heteroclitus), and differential expression by tissue type, stage of reproduction, and estrogen exposure in fish from polluted and unpolluted environments," *Aquatic Toxicology*, vol. 159, pp. 184–197, 2015.

[12] J. R. Shaw, K. Gabor, E. Hand et al., "Role of glucocorticoid receptor in acclimation of killifish (Fundulus heteroclitus) to seawater and effects of arsenic," *American Journal of Physiology-Regulatory Integrative and Comparative Physiology*, vol. 292, no. 2, pp. R1052–R1060, 2007.

[13] A. Whitehead, B. Dubansky, C. Bodinier et al., “Genomic and physiological footprint of the Deepwater Horizon oil spill on resident marsh fishes,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 109, no. 50, pp. 20298–20302, 2012.

[14] B. Dubansky, A. Whitehead, J. T. Miller, C. D. Rice, and F. Galvez, “Multitissue molecular, genomic, and developmental effects of the deepwater horizon oil spill on resident Gulf killifish (Fundulus grandis),” *Environmental Science and Technology*, vol. 47, no. 10, pp. 5074–5082, 2013.

[15] S. M. Bello, D. G. Franks, J. J. Stegeman, and M. E. Hahn, “Acquired resistance to Ah receptor agonists in a population of Atlantic killifish (Fundulus heteroclitus) inhabiting a marine Superfund site: In vivo and in vitro studies on the inducibility of xenobiotic metabolizing enzymes,” *Toxicological Sciences*, vol. 60, no. 1, pp. 77–91, 2001.

[16] J. N. Meyer, D. E. Nacci, and R. T. Di Giulio, “Cytochrome P4501A (CYP1A) in killifish (Fundulus heteroclitus): Heritability of altered expression and relationship to survival in contaminated sediments,” *Toxicological Sciences*, vol. 68, no. 1, pp. 69–81, 2002.

[17] J. V. Wojyoldo, W. Vogelbein, L. J. Bain, and C. D. Rice, “AHR-related activities in a creosote-adapted population of adult atlantic killifish, Fundulus heteroclitus, two decades post-EPA superfund status at the Atlantic Wood Site, Portsmouth, VA USA,” *Aquatic Toxicology*, vol. 177, pp. 74–85, 2016.

[18] K. A. Cotter, D. Nacci, D. Champlin, A. T. Yeo, T. D. Gilmore, and G. V. Callard, “Adaptive significance of ERα splice variants in killifish (fundulus heteroclitus) resident in an estrogenic environment,” *Endocrinology*, vol. 157, no. 6, pp. 2294–2308, 2016.

[19] C. Helsen and F. Claessens, “Looking at nuclear receptors from a new angle,” *Molecular and Cellular Endocrinology*, vol. 382, no. 1, pp. 97–106, 2014.

[20] E. J. Litoff, T. E. Garriott, G. K. Ginjupalli et al., “Annotation of the Daphnia magna nuclear receptors: Comparison to Daphnia pulex,” *Gene*, vol. 552, no. 1, pp. 116–125, 2014.

[21] J. P. Hernandez, L. C. Mota, and W. S. Baldwin, “Activation of CAR and PXR by dietary, environmental and occupational chemicals alters drug metabolism, intermediary metabolism, and cell proliferation,” *Current Pharmacogenomics and Personalized Medicine*, vol. 7, no. 2, pp. 81–105, 2009.

[22] S. A. Thomson, W. S. Baldwin, Y. H. Wang, G. Kwon, and G. A. LeBlanc, “Annotation, phylogenetics, and expression of the nuclear receptors in Daphnia pulex,” *BMC Genomics*, vol. 10, article no. 1471, p. 500, 2009.

[23] Y.-Y. Cheng, W.-J. Tao, J.-L. Chen et al., “Genome-wide identification, evolution and expression analysis of nuclear receptor superfamily in Nile tilapia, Oreochromis niloticus,” *Gene*, vol. 569, no. 1, pp. 141–152, 2015.

[24] A. Chawta, J. J. Repa, R. M. Evans, and D. J. Mangelsdorf, “Nuclear receptors and lipid physiology: opening the x-files,” *Science*, vol. 294, no. 5548, pp. 1866–1870, 2001.

[25] S. A. Kliewer, J. M. Lehmann, and T. M. Willson, “Orphan nuclear receptors: Shifting endocrinology into reverse,” *Science*, vol. 284, no. 5415, pp. 757–760, 1999.

[26] J.-M. Pascussi, S. Gerbal-Chaloin, C. Duret, M. Daujat-Chavanieu, M.-J. Vilarem, and P. Maurel, “The tangle of nuclear receptors that controls xenobiotic metabolism and transport: Crosstalk and consequences,” *Annual Review of Pharmacology and Toxicology*, vol. 48, pp. 1–32, 2008.

[27] T. Mello, M. Materozzi, and A. Galli, “PPARs and Mitochondrial Metabolism: From NAFLD to HCC,” *PPAR Research*, vol. 2016, pp. 1–18, 2016.

[28] H. Yang and H. Wang, “Signaling control of the constitutive androstane receptor (CAR),” *Protein and Cell*, vol. 5, no. 2, pp. 113–123, 2014.

[29] X. C. Kretschmer and W. S. Baldwin, “CAR and PXR: Xenosensors of endocrine disrupters?” *Chemico-Biological Interactions*, vol. 155, no. 3, pp. 111–128, 2005.

[30] R. M. Evans, “The nuclear receptor superfamily: A Rosetta stone for physiology,” *Molecular Endocrinology*, vol. 19, no. 6, pp. 1429–1438, 2005.

[31] S. F. Altschul, W. Gish, W. Miller, E. W. Myers, and D. J. Lipman, “Basic local alignment search tool,” *Journal of Molecular Biology*, vol. 215, no. 3, pp. 403–410, 1990.

[32] A. Marchler-Bauer and S. H. Bryant, “CD-Search: Protein domain annotations on the fly,” *Nucleic Acids Research*, vol. 32, pp. W327–W331, 2004.

[33] B. R. Hannas, Y. H. Wang, W. S. Baldwin, Y. Li, A. D. Wallace, and G. A. LeBlanc, “Interactions of the crustacean nuclear receptors HR3 and E75 in the regulation of gene transcription,” *General and Comparative Endocrinology*, vol. 167, no. 2, pp. 268–278, 2010.

[34] A. Marchler-Bauer, J. B. Anderson, M. K. Derbyshire et al., “CDD: a conserved domain database for interactive domain family analysis,” *Nucleic Acids Research*, vol. 35, no. 1, pp. D237–D240, 2007.

[35] Y. Li, G. K. Ginjupalli, and W. S. Baldwin, “The HR97 (NR1L) group of nuclear receptors: A new group of nuclear receptors discovered in Daphnia species,” *General and Comparative Endocrinology*, vol. 206, pp. 30–42, 2014.

[36] E. Long, G. Scott, J. Kucklick et al., “Magnitude and extent of sediment toxicity in selected estuaries of South Carolina and Georgia,” *NOAA Technical Memorandum NOS ORCA*, p. 178, 1997.

[37] K. Tamura, G. Stecher, D. Peterson, A. Filipski, and S. Kumar, “MEGA6: Molecular Evolutionary Genetics Analysis version 6.0,” *Molecular Biology and Evolution*, vol. 30, no. 12, pp. 2725–2729, 2013.

[38] P. Lemey, M. Salemi, and A. Vandamme, *The Phylogenetic Handbook*, Cambridge University Press, Cambridge, 2009.

[39] J. M. Maglich, J. A. Caravella, M. H. Lambert, T. M. Willson, J. T. Moore, and L. Ramamurthy, “The first completed genome sequence from a teleost fish (Fugu rubripes) adds significant diversity to the nuclear receptor superfamily,” *Nucleic Acids Research*, vol. 31, no. 14, pp. 4051–4058, 2003.

[40] R. P. R. Metpally, R. Vigneshwar, and R. Sowdhamini, “Genome inventory and analysis of nuclear hormone receptors in Tetraodon nigroviridis,” *Journal of Biosciences*, vol. 32, no. 1, pp. 43–50, 2007.

[41] M. Robinson-Rechavi, O. Marchand, H. Escriva et al., “Euteleost fish genomes are characterized by expansion of gene families,” *Genome Research*, vol. 11, no. 5, pp. 781–788, 2001.

[42] S. M. K. Glasauer and S. C. F. Neuhauss, “Whole-genome duplication in teleost fishes and its evolutionary consequences,” *Molecular Genetics and Genomics*, vol. 289, no. 6, pp. 1045–1060, 2014.

[43] T. Naggan Perl, B. G. M. Schmid, J. Schwirz, and A. D. Chipman, “The evolution of the knirps family of transcription factors in arthropods,” *Molecular Biology and Evolution*, vol. 30, no. 6, pp. 1348–1357, 2013.

[44] V. Laudet, “Evolution of the nuclear receptor superfamily: Early diversification from an ancestral orphan receptor,” *Journal of Molecular Endocrinology*, vol. 19, no. 3, pp. 207–226, 1997.

[45] S. Bertrand, F. G. Brunet, H. Escriva, G. Parmentier, V. Laudet, and M. Robinson-Rechavi, “Evolutionary genomics of nuclear receptors: From twenty-five ancestral genes to derived endocrine systems,” *Molecular Biology and Evolution*, vol. 21, no. 10, pp. 1923–1937, 2004.

[46] J. T. Bridgham, G. N. Eick, C. Larroux et al., “Protein evolution by molecular tinkering: Diversification of the nuclear receptor superfamily from a ligand-dependent ancestor,” *PLoS Biology*, vol. 8, no. 10, Article ID e1000497, 2010.

[47] P. Germain, B. Staels, C. Dacquet, M. Spedding, and V. Laudet, “Overview of nomenclature of nuclear receptors,” *Pharmacological Reviews*, vol. 58, no. 4, pp. 685–704, 2006.

[48] Y. Bae, J. K. Kemper, and B. Kemper, “Repression of CAR-Mediated Transactivation of CYP2B Genes by the Orphan Nuclear Receptor, Short Heterodimer Partner (SHP),” *DNA and Cell Biology*, vol. 23, no. 2, pp. 81–91, 2004.

[49] A. Bavner, S. Sanyal, J.-A. Gustafsson, and E. Treuter, “Transcriptional corepression by SHP: molecular mechanisms and physiological consequences,” *Trends in Endocrinology & Metabolism*, vol. 16, no. 10, pp. 478–488, 2005.

[50] L. Johansson, J. S. Thomsen, A. E. Damdimopoulos, G. Spyrou, J.-Å. Gustafsson, and E. Treuter, “The orphan nuclear receptor SHP inhibits agonist-dependent transcriptional activity of estrogen receptors ERα and ERβ,” *Journal of Biological Chemistry*, vol. 274, no. 1, pp. 345–353, 1999.

[51] A. Ehrlund and E. Treuter, “Ligand-independent actions of the orphan receptors/corepressors DAX-1 and SHP in metabolism, reproduction and disease,” *Journal of Steroid Biochemistry and Molecular Biology*, vol. 130, no. 3–5, pp. 169–179, 2012.

[52] M. Mark, N. B. Ghyselinck, and P. Chambon, “Function of retinoic acid receptors during embryonic development,” *Nuclear Receptor Signaling*, vol. 7, article e002, 2009.

[53] A. M. Jetten, “Retinoid-related orphan receptors (RORs): critical roles in development, immunity, circadian rhythm, and cellular metabolism,” *Nuclear Receptor Signaling*, vol. 7, article e003, 2009.

[54] S. N. Ramakrishnan and G. E. O. Muscat, “The orphan Rev-erb nuclear receptors: a link between metabolism, circadian rhythm and inflammation?” *Nuclear Receptor Signaling*, vol. 4, article e009, 2006.

[55] B. M. Forman, J. Chen, B. Blumberg et al., “Cross-talk among RORα 1 and the Rev-erb family of orphan nuclear receptors,” *Molecular Endocrinology*, vol. 8, no. 9, pp. 1253–1261, 1994.

[56] J. Delezie and E. Challet, “Interactions between metabolism and circadian clocks: Reciprocal disturbances,” *Annals of the New York Academy of Sciences*, vol. 1243, no. 1, pp. 30–46, 2011.

[57] F. Echeverría, M. Ortiz, R. Valenzuela, and L. A. Videla, “Long-chain polyunsaturated fatty acids regulation of PPARs, signaling: Relationship to tissue development and aging,” *Prostaglandins Leukotrienes and Essential Fatty Acids*, vol. 114, pp. 28–34, 2016.

[58] M. J. Leaver, E. Boukouvala, E. Antonopoulou et al., “Three peroxisome proliferator-activated receptor isotypes from each of two species of marine fish,” *Endocrinology*, vol. 146, no. 7, pp. 3150–3162, 2005.

[59] M. J. Leaver, M. T. Ezaz, S. Fontagne, D. R. Tocher, E. Boukouvala, and G. Krey, “Multiple peroxisome proliferator-activated receptor β subtypes from Atlantic salmon (Salmo salar),” *Journal of Molecular Endocrinology*, vol. 38, no. 3-4, pp. 391–400, 2007.

[60] M. J. Benton, *Vertebrate Paleontology*, Wiley-Blackwell, 4th edition, 2014.

[61] F. Cortesia, Z. Musilová, S. M. Stieb et al., “Ancestral duplications and highly dynamic opsin gene evolution in percomorph fishes,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 112, no. 5, pp. 1493–1498, 2015.

[62] W. S. Baldwin, P. B. Marko, and D. R. Nelson, “The cytochrome P450 (CYP) gene superfamily in Daphnia pulex,” *BMC Genomics*, vol. 10, article no. 169, 2009.

[63] D. R. Nelson, “Comparison of P450s from human and fugu: 420 Million years of vertebrate P450 evolution,” *Archives of Biochemistry and Biophysics*, vol. 409, no. 1, pp. 18–24, 2003.

[64] J. S. Prindiville, J. A. Mennigen, J. M. Zamora, T. W. Moon, and J.-M. Weber, “The fibrate drug gemfibrozil disrupts lipoprotein metabolism in rainbow trout,” *Toxicology and Applied Pharmacology*, vol. 251, no. 3, pp. 201–208, 2011.

[65] M. P. Cajaraville, I. Cancio, A. Ibabe, and A. Orbea, “Peroxisome proliferation as a biomarker in environmental pollution assessment,” *Microscopy Research and Technique*, vol. 61, no. 2, pp. 191–202, 2003.

[66] H. Arnold, H.-J. Pluta, and T. Braunbeck, “Simultaneous exposure of fish to endosulfan and disulfoton in vivo: ultrastructural, stereological and biochemical reactions in hepatocytes of male rainbow trout (Oncorhynchus mykiss),” *Aquatic Toxicology*, vol. 33, no. 1, pp. 17–43, 1995.

[67] J. R. Pedrajas, J. López-Barea, and J. Peinado, “Dieldrin induces peroxisomal enzymes in fish (Sparus aurata) liver,” *Comparative Biochemistry and Physiology-C Pharmacology Toxicology and Endocrinology*, vol. 115, no. 2, pp. 125–131, 1996.

[68] T. Ye, M. Kang, Q. Huang et al., “Accumulation of Di(2-ethylhexyl) Phthalate Causes Endocrine-Disruptive Effects in Marine Medaka (Oryzias melastigma) Embryos,” *Environmental Toxicology*, vol. 31, no. 1, pp. 116–127, 2016.

[69] H. F. Olivares-Rubio and A. Vega-López, “Fatty acid metabolism in fish species as a biomarker for environmental monitoring,” *Environmental Pollution*, vol. 218, pp. 297–312, 2016.

[70] J. T. Ackers, M. F. Johnston, and M. L. Haasch, “Immunodetection of hepatic peroxisomal PMP70 as an indicator of peroxisomal proliferation in the mummichog, Fundulus heteroclitus,” *Marine Environmental Research*, vol. 50, no. 1-5, pp. 361–365, 2000.

[71] A. Lyssimachou, J. G. Santos, A. André et al., “The mammalian ”obesogen” tributyltin targets hepatic triglyceride accumulation and the transcriptional regulation of lipid metabolism in the liver and brain of zebrafish,” *PLoS ONE*, vol. 10, no. 12, Article ID 0143911, 2015.

[72] A. O. Adeogun, O. R. Ibor, F. Regoli, and A. Arukwe, “Peroxisome proliferator-activated receptors and biotransformation responses in relation to condition factor and contaminant burden in tilapia species from Ogun River, Nigeria,” *Comparative Biochemistry and Physiology Part-C: Toxicology and Pharmacology*, vol. 183-184, pp. 7–19, 2016.

[73] M. R. Milnes, A. Garcia, E. Grossman et al., “Activation of steroid and xenobiotic receptor (SXR, NR1/2) and its orthologs in laboratory, toxicologic, and genome model species,” *Environmental Health Perspectives*, vol. 116, no. 7, pp. 880–885, 2008.

[74] J. Gräns, B. Wassmur, M. Fernández-Santoscoy et al., “Regulation of pregnane-X-receptor, CYP3A and P-glycoprotein genes in the PCB-resistant killifish (Fundulus heteroclitus) population from New Bedford Harbor,” *Aquatic Toxicology*, vol. 159, pp. 198–207, 2015.

[75] L. B. Moore, J. M. Maglich, D. D. McKee et al., “Pregnane X receptor (PXR), constitutive androstane receptor (CAR), and benzoate X receptor (BXR) define three pharmacologically distinct classes of nuclear receptors,” *Molecular Endocrinology*, vol. 16, no. 5, pp. 977–986, 2002.

[76] M. D. Krasowski, K. Yasuda, L. R. Hagey, and E. G. Schuetz, “Evolutionary selection across the nuclear hormone receptor superfamily with a focus on the NR1I subfamily (vitamin D, pregnane X, and constitutive androstane receptors),” *Nuclear Receptor*, vol. 3, article no. 2, 2005.

[77] M. Mathäs, O. Burk, H. Qiu et al., “Evolutionary history and functional characterization of the amphibian xenosensor CAR,” *Molecular Endocrinology*, vol. 26, no. 1, pp. 14–26, 2012.

[78] Y. Zhao, K. Zhang, J. P. Giesy, and J. Hu, “Families of nuclear receptors in vertebrate models: characteristic and comparative toxicological perspective,” *Scientific Reports*, vol. 5, article 8554, 2015.

[79] D. George and A. Blieck, “Rise of the earliest tetrapods: An Early Devonian origin from marine environment,” *PLoS ONE*, vol. 6, no. 7, Article ID e22136, 2011.

[80] M. D. Krasowski, K. Yasuda, L. R. Hagey, and E. G. Schuetz, “Evolution of the pregnane X receptor: Adaptation to cross-species differences in biliary bile salts,” *Molecular Endocrinology*, vol. 19, no. 7, pp. 1720–1739, 2005.

[81] J. Zhang, W. Huang, M. Qatanani, R. M. Evans, and D. D. Moore, “The constitutive androstane receptor and pregnane X receptor function coordinately to prevent bile acid-induced hepatotoxicity,” *Journal of Biological Chemistry*, vol. 279, no. 47, pp. 49517–49522, 2004.

[82] S. Fiorucci, A. Zampella, and E. Distrutti, “Development of FXR, PXR and CAR agonists and antagonists for treatment of liver disorders,” *Current Topics in Medicinal Chemistry*, vol. 12, no. 6, pp. 605–624, 2012.

[83] D. L. Howarth, L. R. Hagey, S. H. W. Law et al., “Two farnesoid X receptor alpha isoforms in Japanese medaka (Oryzias latipes) are differentially activated in vitro,” *Aquatic Toxicology*, vol. 98, no. 3, pp. 245–255, 2010.

[84] M. D. Krasowski, A. Ni, L. R. Hagey, and S. Ekins, “Evolution of promiscuous nuclear hormone receptors: LXR, FXR, VDR, PXR, and CAR,” *Molecular and Cellular Endocrinology*, vol. 334, no. 1-2, pp. 39–48, 2011.

[85] M. D. Krasowski, N. Ai, L. R. Hagey et al., “The evolution of farnesoid X, vitamin D, and pregnane X receptors: Insights from the green-spotted pufferfish (Tetraodon nigriviridis) and other non-mammalian species,” *BMC Biochemistry*, vol. 12, no. 1, article no. 5, 2011.

[86] E. J. Reschly, N. Ai, S. Ekins et al., “Evolution of the bile salt nuclear receptor FXR in vertebrates,” *Journal of Lipid Research*, vol. 49, no. 7, pp. 1577–1587, 2008.

[87] S. S. Kitambi and G. Hauptmann, “The zebrafish orphan nuclear receptor genes nr2e1 and nr2e3 are expressed in developing eye and forebrain,” *Gene Expression Patterns*, vol. 7, no. 4, pp. 521–528, 2007.

[88] S. M. Nelson, R. A. Frey, S. L. Wardwell, and D. L. Stenkamp, “The developmental sequence of gene expression within the rod photoreceptor lineage in embryonic zebrafish,” *Developmental Dynamics*, vol. 237, no. 10, pp. 2903–2917, 2008.

[89] T. Wang, L.-J. Zhao, P. Li et al., “Hepatoprotective effects and mechanisms of dehydrocavidine in rats with carbon tetrachloride-induced hepatic fibrosis,” *Journal of Ethnopharmacology*, vol. 138, no. 1, pp. 76–84, 2011.

[90] G. D. Amoutzias, E. E. Pichler, N. Mian et al., “A protein interaction atlas for the nuclear receptors: Properties and quality of a hub-based dimerisation network,” *BMC Systems Biology*, vol. 1, article no. 34, 2007.

[91] X. L. Aranguren, M. Beerens, W. Vandevelde, M. Dewerchin, P. Carmeliet, and A. Luttun, “Transcription factor COUP-TFII is indispensable for venous and lymphatic development in zebrafish and Xenopus laevis,” *Biochemical and Biophysical Research Communications*, vol. 410, no. 1, pp. 121–126, 2011.

[92] M. R. Swift, V. N. Pham, D. Castranova, K. Bell, R. J. Poole, and B. M. Weinstein, “SoxF factors and Notch regulate nr2f2 gene expression during venous differentiation in zebrafish,” *Developmental Biology*, vol. 390, no. 2, pp. 116–125, 2014.

[93] P. Tran, X.-K. Zhang, G. Salbert, T. Hermann, J. M. Lehmann, and M. Pfahl, “COUP orphan receptors are negative regulators of retinoic acid response pathways,” *Molecular and Cellular Biology*, vol. 12, no. 10, pp. 4666–4676, 1992.

[94] M. Robinson-Rechavi, C. V. Maina, C. R. Gissendanner, V. Laudet, and A. Sluder, “Explosive lineage-specific expansion of the orphan nuclear receptor HNF4 in nematodes,” *Journal of Molecular Evolution*, vol. 60, no. 5, pp. 577–586, 2005.

[95] W. Cheng, L. Guo, Z. Zhang et al., “HNF factors form a network to regulate liver-enriched genes in zebrafish,” *Developmental Biology*, vol. 294, no. 2, pp. 482–496, 2006.

[96] A. McNair, S. Cereghini, H. Brand, T. Smith, C. Breillat, and F. Gannon, “Synergistic activation of the Atlantic salmon hepatocyte nuclear factor (HNF) 1 promoter by the orphan nuclear receptors HNF4 and chicken ovalbumin upstream promoter transcription factor I (COUP-TFI),” *Biochemical Journal*, vol. 352, no. 2, pp. 557–564, 2000.

[97] S. Miyagawa, A. Lange, S. Tohyama et al., “Characterization of Oryzias latipes glucocorticoid receptors and their unique response to progestins,” *Journal of Applied Toxicology*, vol. 35, no. 3, pp. 302–309, 2015.

[98] N. R. Bury, “The evolution, structure and function of the ray finned fish (Actinopterygii) glucocorticoid receptors,” *General and Comparative Endocrinology*, epub ahead of print, 2017.

[99] Y. Kumai, D. Nesan, M. M. Vijayan, and S. F. Perry, “Cortisol regulates Na+ uptake in zebrafish, Danio rerio, larvae via the glucocorticoid receptor,” *Molecular and Cellular Endocrinology*, vol. 364, no. 1-2, pp. 113–125, 2012.

[100] E. G. Notch, J. R. Shaw, B. A. Coutermarsh, M. Dzioba, and B. A. Stanton, “Morpholino gene knockdown in adult Fundulus heteroclitus: Role of SGK1 in seawater acclimation,” *PLoS ONE*, vol. 6, no. 12, Article ID e29462, 2011.

[101] G. R. Scott, D. W. Baker, P. M. Schulte, and C. M. Wood, “Physiological and molecular mechanisms of osmoregulatory plasticity in killifish after seawater transfer,” *Journal of Experimental Biology*, vol. 211, no. 15, pp. 2450–2459, 2008.

[102] C.-H. Lin, H.-J. Hu, and P.-P. Hwang, “Cortisol regulates sodium homeostasis by stimulating the transcription of sodium-chloride transporter (NCC) in zebrafish (Danio rerio),” *Molecular and Cellular Endocrinology*, vol. 422, pp. 93–102, 2016.

[103] A.-M. Flores and J. Mark Shrimpton, “Differential physiological and endocrine responses of rainbow trout, Oncorhynchus mykiss, transferred from fresh water to ion-poor or salt water,” *General and Comparative Endocrinology*, vol. 175, no. 2, pp. 244–250, 2012.

[104] S. Hoegg, H. Brinkmann, J. S. Taylor, and A. Meyer, “Phylogenetic timing of the fish-specific genome duplication correlates with the diversification of teleost fish,” *Journal of Molecular Evolution*, vol. 59, no. 2, pp. 190–203, 2004.

[105] H. R. Crollius and J. Weissenbach, “Fish genomics and biology,” *Genome Research*, vol. 15, no. 12, pp. 1675–1682, 2005.

[106] V. Douard, F. Brunet, B. Boussau et al., “The fate of the duplicated androgen receptor in fishes: A late neofunctionalization event?” *BMC Evolutionary Biology*, vol. 8, no. 1, article no. 336, 2008.

[107] T. S. Sperry and P. Thomas, “Identification of two nuclear androgen receptors in kelp bass (Paralabrax clathratus) and their binding affinities for xenobiotics: Comparison with atlantic croaker (Micropogonias undulatus) androgen receptors,” *Biology of Reproduction*, vol. 61, no. 4, pp. 1152–1161, 1999.

[108] Y. Ogino, S. Kuraku, H. Ishibashi et al., "Neofunctionalization of androgen receptor by Gain-of-Function mutations in teleost fish lineage," *Molecular Biology and Evolution*, vol. 33, no. 1, pp. 228–244, 2016.

[109] M. Blin, W. Norton, L. Bally-Cuif, and P. Vernier, "NR4A2 controls the differentiation of selective dopaminergic nuclei in the zebrafish brain," *Molecular and Cellular Neuroscience*, vol. 39, no. 4, pp. 592–604, 2008.

[110] L. Pei, A. Castrillo, and P. Tontonoz, "Regulation of macrophage inflammatory gene expression by the orphan nuclear receptor Nur77," *Molecular Endocrinology*, vol. 20, no. 4, pp. 786–794, 2006.

[111] M. A. Maxwell and G. E. O. Muscat, "The NR4A subgroup: immediate early response genes with pleiotropic physiological roles," *Nucl Recept Signal*, vol. 4, p. e002, 2006.

[112] K. King-Jones and C. S. Thummel, "Nuclear receptors—A perspective from Drosophila," *Nature Reviews Genetics*, vol. 6, no. 4, pp. 311–323, 2005.

[113] R.-R. Dong, S.-J. Yang, R.-J. Feng et al., "Complete feminization of catfish by feeding Limnodilus, an annelid worm collected in contaminated streams," *Environmental Research*, vol. 133, pp. 371–379, 2014.

[114] F. M. Sladek, "What are nuclear receptor ligands?" *Molecular and Cellular Endocrinology*, vol. 334, no. 1-2, pp. 3–13, 2011.

[115] G. A. LeBlanc, D. O. Norris, W. Kloas, S. W. Kullman, W. S. Baldwin, and J. M. Greally, "Detailed Review Paper on the State of the Science on Novel," *In Vitro and In Vivo Screening and Testing Methods and Endpoints for Evaluating Endocdrine Disruptors Series on Testing Assessment: No. 178. Organisation for Economic Co-operation and Development*, p. 213, 2012.

[116] E. M. Kollitz, G. Zhang, M. B. Hawkins, G. K. Whitfield, D. M. Reif, and S. W. Kullman, "Molecular cloning, functional characterization, and evolutionary analysis of vitamin D receptors isolated from basal vertebrates," *PLoS ONE*, vol. 10, no. 4, Article ID e0122853, 2015.

[117] E. J. Reschly and M. D. Krasowski, "Evolution and function of the NR1I nuclear hormone receptor subfamily (VDR, PXR, and CAR) with respect to metabolism of xenobiotics and endogenous compounds," *Current Drug Metabolism*, vol. 7, no. 4, pp. 349–365, 2006.

[118] I. Tzameli, S. S. Chua, B. Cheskis, and D. D. Moore, "Complex effects of rexinoids on ligand dependent activation or inhibition of the xenobiotic receptor, CAR," *Nuclear Receptor*, vol. 1, article no. 2, 2003.

[119] A. Menuet, E. Pellegrini, I. Anglade et al., "Molecular characterization of three estrogen receptor forms in zebrafish: Binding characteristics, transactivation properties, and tissue distributions," *Biology of Reproduction*, vol. 66, no. 6, pp. 1881–1892, 2002.

[120] E. H. Stolte, B. M. L. Verburg van Kemenade, H. F. J. Savelkoul, and G. Flik, "Evolution of glucocorticoid receptors with different glucocorticoid sensitivity," *Journal of Endocrinology*, vol. 190, no. 1, pp. 17–28, 2006.

[121] P. P. de Waal, D. S. Wang, W. A. Nijenhuis, R. W. Schulz, and J. Bogerd, "Functional characterization and expression analysis of the androgen receptor in zebrafish (Danio rerio) testis," *Reproduction*, vol. 136, no. 2, pp. 225–234, 2008.

[122] E. P. Sablin, R. D. Blind, I. N. Krylova et al., "Structure of SF-1 bound by different phospholipids: Evidence for regulatory ligands," *Molecular Endocrinology*, vol. 23, no. 1, pp. 25–34, 2009.

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