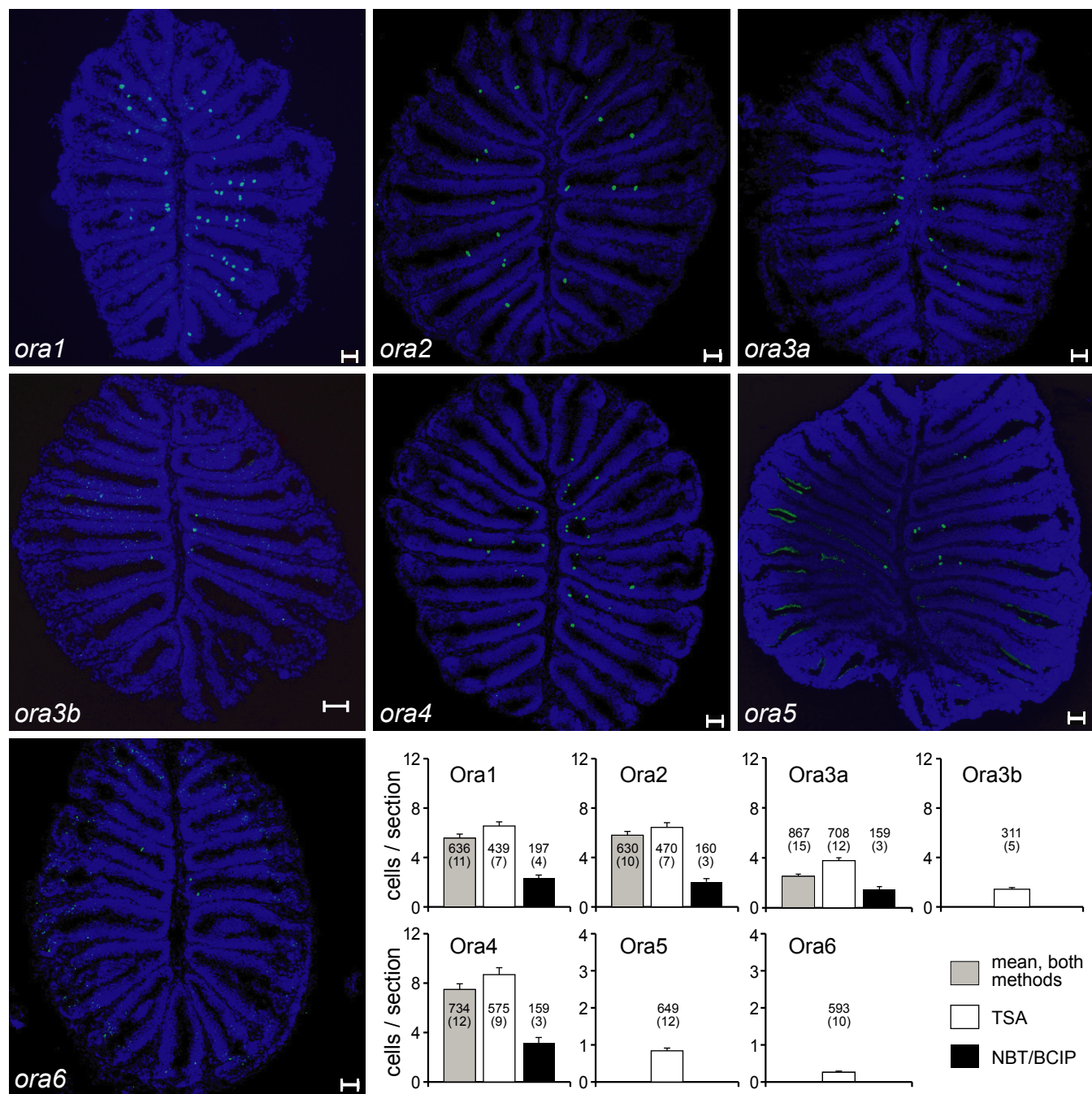


Spatial organization of olfactory receptor gene choice in the complete V1R-related ORA family of zebrafish

Daniel Kowatschew, Shahrzad Bozorg Nia, Shahzaib Hassan,
Jana Ustinova, Franco Weth, Sigrun I. Korsching

SI Figure 1: TSA-labeling for the entire ORA family and comparison of labeling efficacy for TSA and chromogenic labeling methods for in situ hybridisation

Horizontal sections of adult zebrafish olfactory epithelium were hybridized with probes for ORA1, ORA2, ORA3a, ORA3b, ORA4, ORA5 and ORA6. Note that for better visualization sections with above average frequency are shown. Bottom right panel, expression frequencies for different labeling methods are compared. Missing bars, numbers too small for accurate determination. Mean values and SEM are shown; black bars, chromogenic labeling with NBT/BCIP (only for ORA1, ORA2, ORA3a, ORA4); white bars, TSA labeling (all 7 genes); grey bars, numbers/section obtained for both methods were averaged. Numbers within/above bars refer to the number of sections analysed for the respective bar, in parentheses the number of organs analysed is given.



Spatial organization of olfactory receptor gene choice in the complete V1R-related ORA family of zebrafish

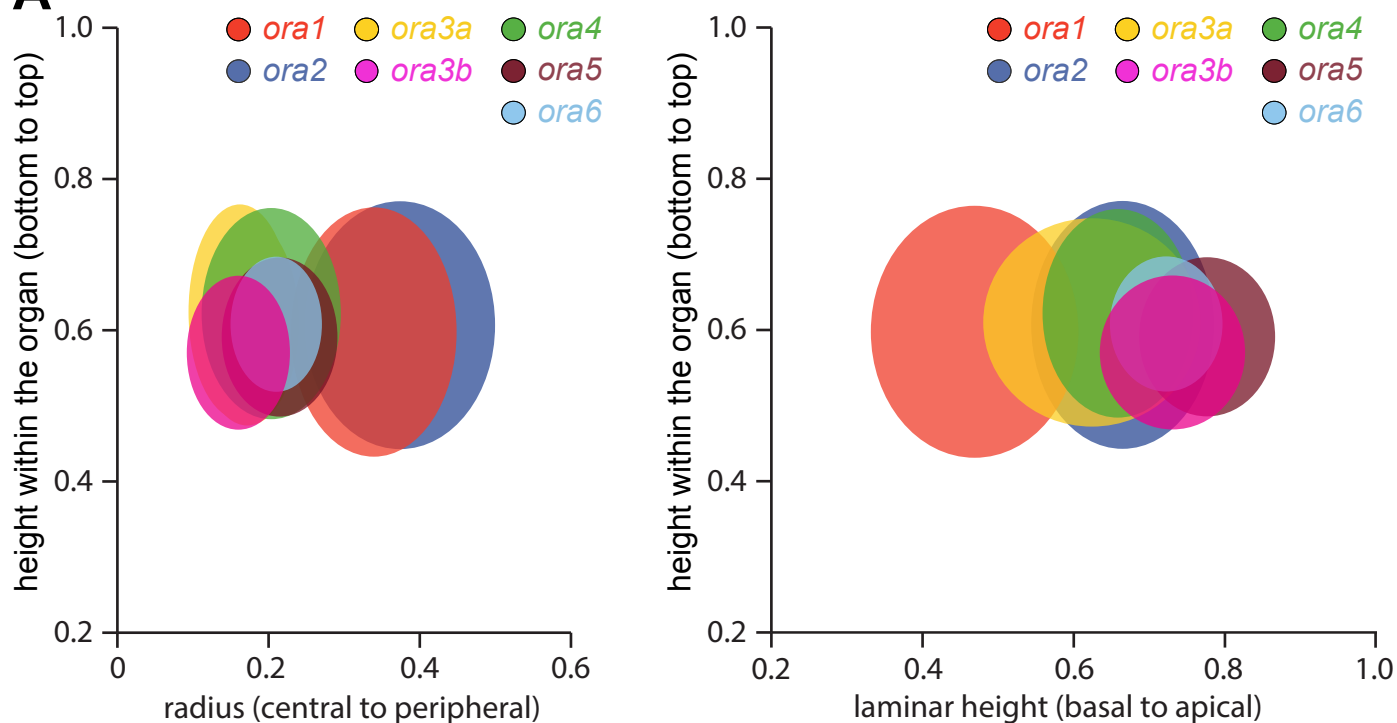
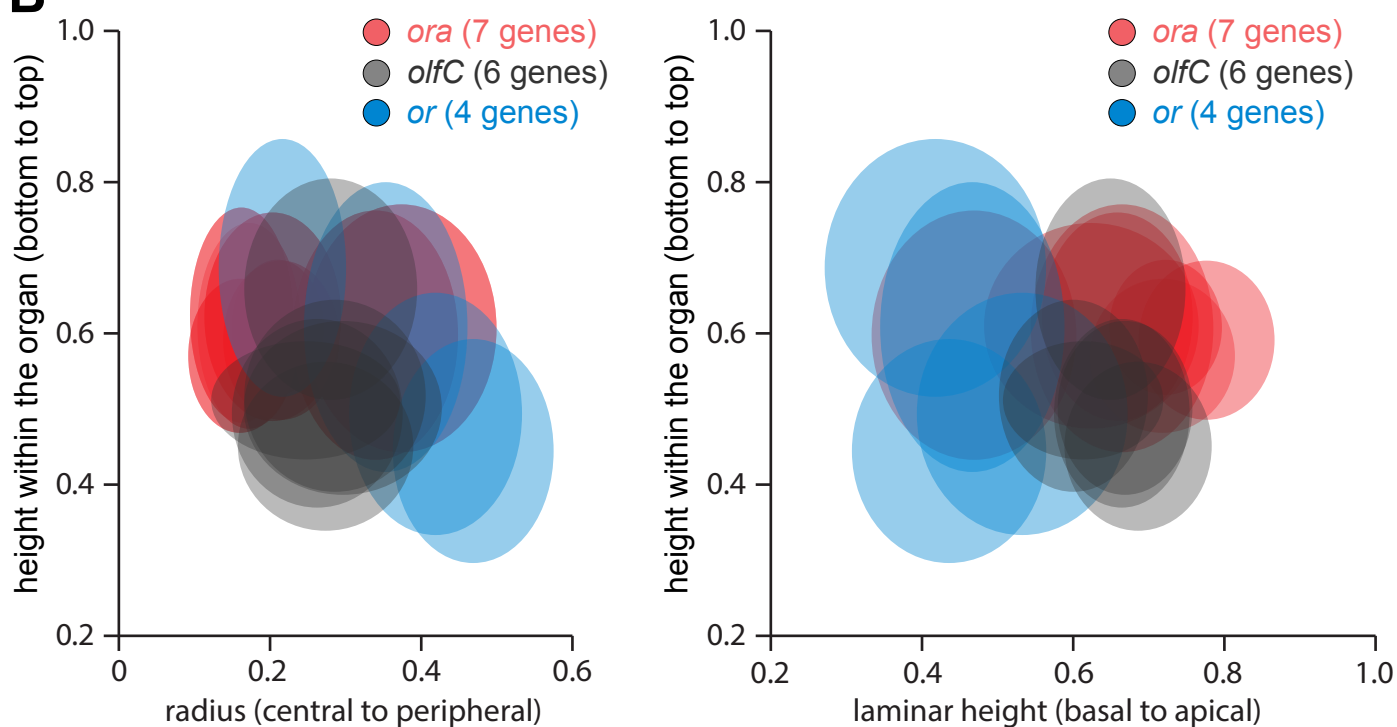
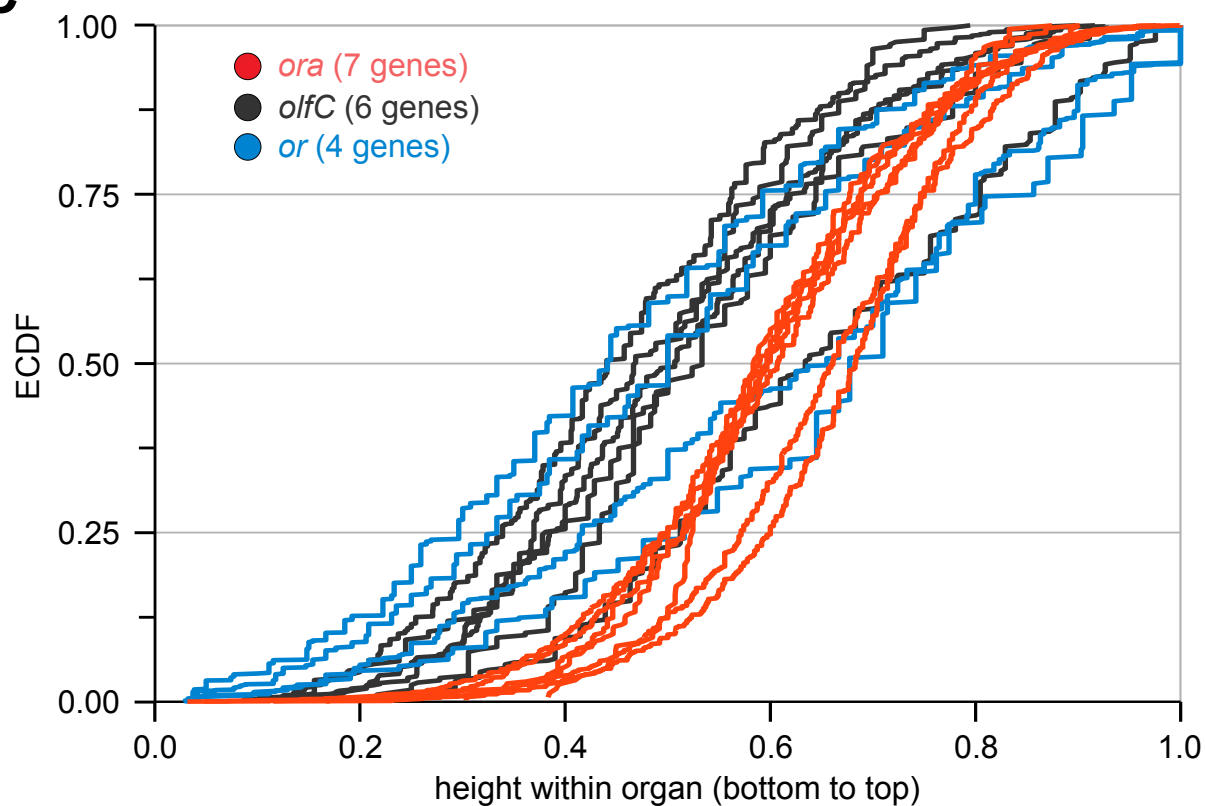
Daniel Kowatschew, Shahrzad Bozorg Nia, Shahzaib Hassan,
Jana Ustinova, Franco Weth, Sigrun I. Korsching

SI Figure 2: Multidimensional analysis of spatial expression patterns: No correlation between preferred positions for height-within-the-organ and the other two parameters, radius and laminar height, but clear segregation from two other olfactory receptor families for height-within-the-organ

A) Comparison of spatial distribution parameters between *ora* genes. Schematic representation of spatial distributions for all *ora* genes by ellipses ranging from the 1st to the 3rd quartile value for height-within-the-organ (y axis) and radial or laminar height (x axis) parameter. Color code for *ora* genes as indicated.

B) Comparison of distributions for *ora* versus *olfC* and odorant receptor (*or*) genes using the same coordinates and representation as in A). Note that the color code is changed compared to panel A). The raw data for *olfC* genes are taken from [11], raw data for *or* genes are from [7] [16]. For names and quartile values of *or* and *olfC* genes see SI Table 1.

C) Distributions of *ora*-expressing OSNs for height-within-the-organ segregate from those of other olfactory receptor families despite broad overlap. Distributions are shown as ECDFs. Red lines, *ora* genes; black, *olfC* genes; blue, *or* genes.

A**B****C**

Spatial organization of olfactory receptor gene choice in the complete V1R-related ORA family of zebrafish

Daniel Kowatschew, Shahrzad Bozorg Nia, Shahzaib Hassan,
Jana Ustinova, Franco Weth, Sigrun I. Korsching

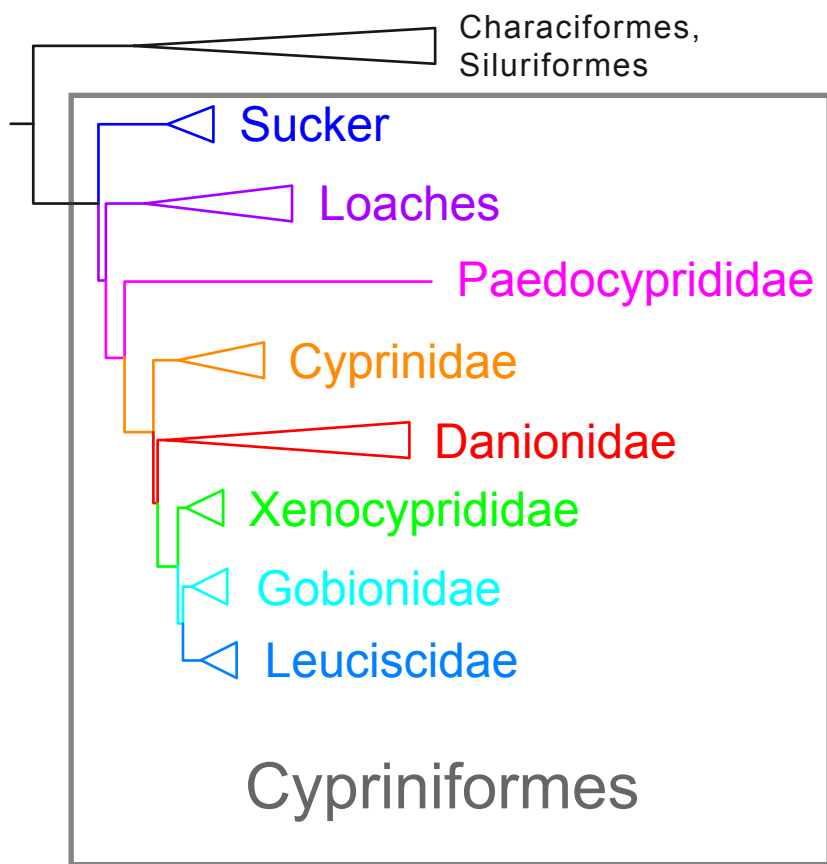
SI Figure 3: Origin of the ORA3 duplication in the MRCA of cypriniformes

A) Taxonomic tree of Cypriniformes redrawn from [Stout et al., 2016]. Only those families and larger groups are included, for which genomes were available, color code as indicated. Two adjacent orders (Characiformes and Siluriformes) are also shown. B) Maximum likelihood tree of the entire ORA family (amino acid sequences) from four reference species - two early ray-finned species (*Acipenser ruthenus* and *Lepisosteus oculatus*), zebrafish and a neoteleost (medaka) - and all validated Cypriniformes, Characiformes and Siluriformes sequences of the ORA3,4 clade obtained from tblastn searches in the WGS of the respective orders. Search strategy and tree construction as described in [17]. The rainbow-colored triangle corresponds to the subtree shown in C. C) Maximum likelihood tree showing all *ora3* genes of Cypriniformes, Characiformes (collapsed) and Siluriformes (collapsed). Color code for fish families and larger groups as indicated. Zebrafish ORA3a and ORA3b are indicated by red arrows. Two separate clades for ORA3a and ORA3b include already the earliest deriving Cypriniformes (sucker and loaches), suggesting an origin of this duplication in the MRCA of Cypriniformes.

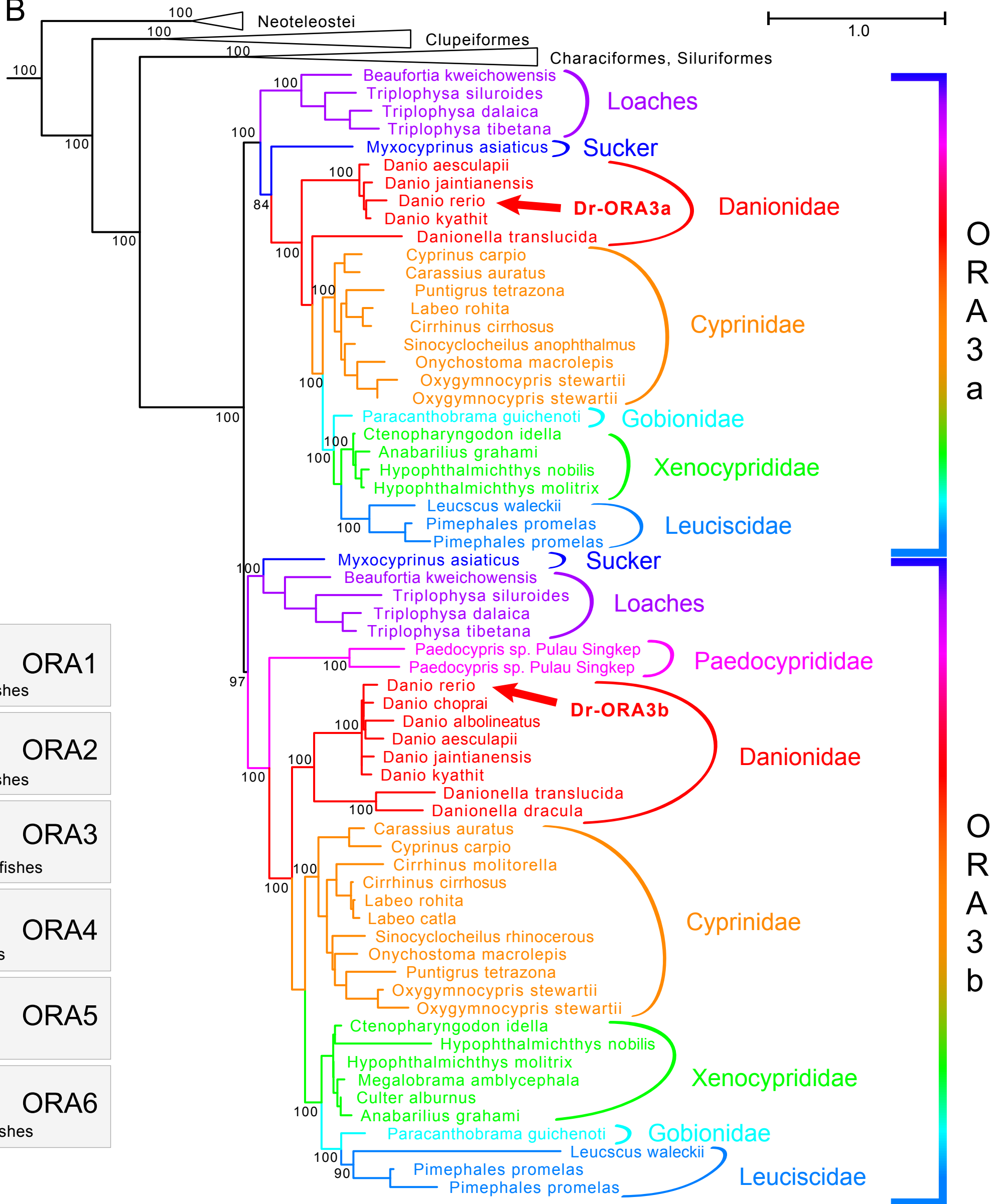
Supplementary Reference

Stout, C. C., Tan, M., Lemmon, A. R., Lemmon, E. M. & Armbruster, J. W. Resolving Cypriniformes relationships using an anchored enrichment approach. *BMC Evol Biol* **16**, 244 (2016).

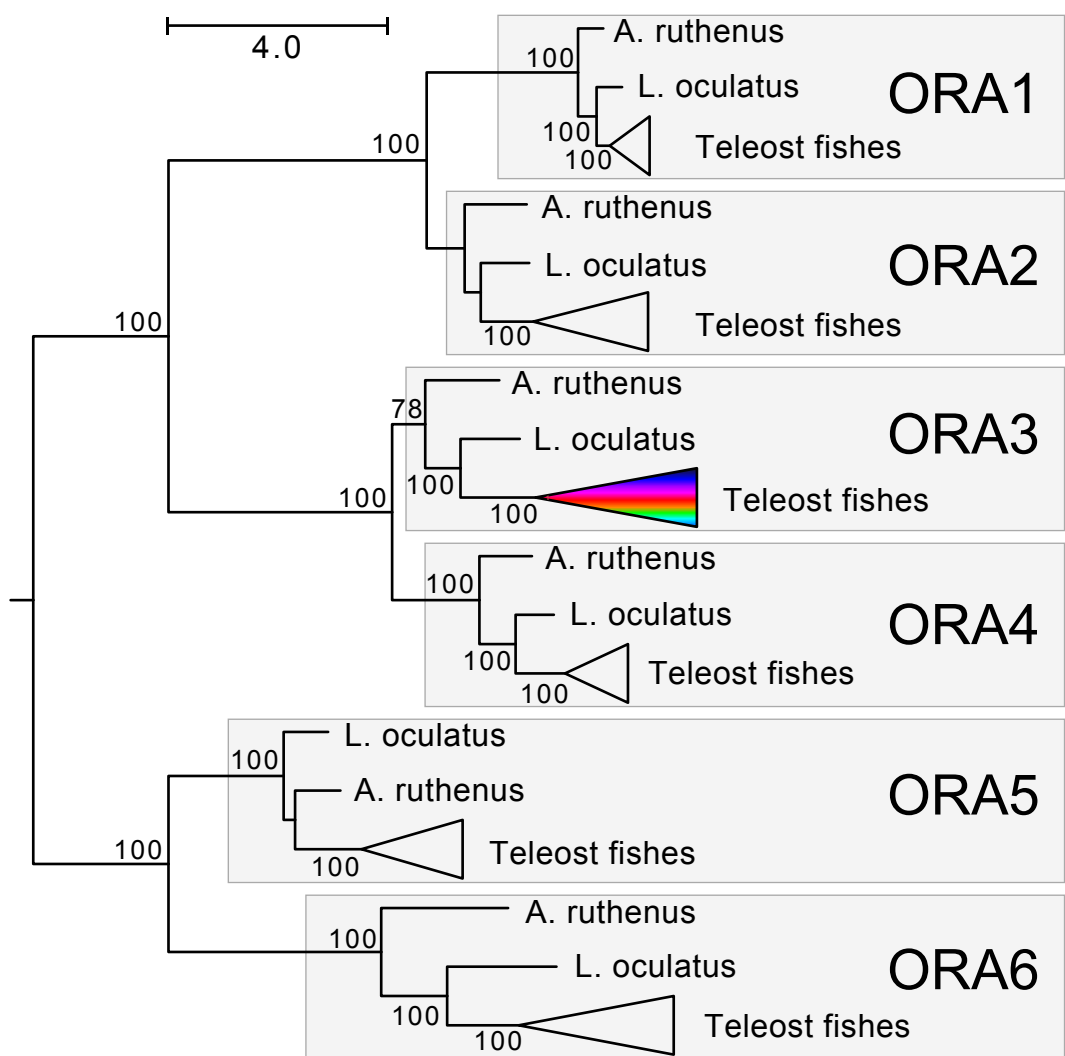
A



B



C



Spatial organization of olfactory receptor gene choice in the complete V1R-related ORA family of zebrafish

Daniel Kowatschew, Shahrzad Bozorg Nia, Shahzaib Hassan,
Jana Ustinova, Franco Weth, Sigrun I. Korsching

SI Table 1 Quantitative parameters of distributions for all ora genes

Sheet 1, Accession numbers and synonyms for all zebrafish *ora* genes and those *or* and *olfC* genes used in this study. Sheet 2, Parameter values for the first, second and third quartile of the distributions, i.e., for the radial, laminar height and z-axis coordinates. Half width of distributions was determined as 3rd quartile-1st quartile difference. Sheet 3, Maximal vertical distance in pairwise comparisons for all seven *ora* genes, for all four *or* genes and for *ora* genes with similar distribution to genes from other families in at least one dimension. Sheet 4, Evaluation of significant differences by KS test for pairwise comparisons.

Gene accession and synonyms

gene	Synonyms	Ensembl Gene ID	NCBI
<i>ora1</i>	V1R2, V1r-like, vn1r1l, vn1r2l, zgc:19431	ENSDARG00000098601	NM_001130668.1
<i>ora2</i>	V1R1	not annotated	NM_001098395.1
<i>ora3a</i>	ora3, V1R3	ENSDARG00000059315	XM_009295769.2
<i>ora3b</i>	-	ENSDARG00000091638	XM_009295770.3
<i>ora4</i>	V1R4	ENSDARG00000078223	XM_005168282.4
<i>ora5</i>	V1R5	ENSDARG00000078257	XM_002663460.5
<i>ora6</i>	V1R6	not annotated	XM_003200787.2
<i>olfcc1</i>	v2rl1	ENSDARG00000005942	XM_009293770
<i>olfcg1</i>		ENSDARG00000104552	NM_001159840
<i>olfcn1</i>	si:ch73-125k17.2	ENSDARG00000088029	XM_009293708, XM_009293709
<i>olfcq1</i>	v2rh14	ENSDARG00000068520	NM_001083099
<i>olfcj1</i>		ENSDARG00000104430	NM_001110291
<i>olfct1</i>		not present in z10	XM_021473301
<i>olfcu1</i>	v2rx1	ENSDARG00000040632	NM_001082899
<i>or102-1</i>	ZOR5	ENSDARG00000041005	XM_009291682
<i>or112-1</i>	ZOR6	ENSDARG00000077211	XM_003200004
<i>or103-1</i>	ZOR8	ENSDARG00000094080	NM_131588
<i>or107-1</i>	ZOR9	ENSDARG00000041032	XM_009291685

Expression of V1R-related *ora* genes in zebrafish olfactory epithelium:
Distribution of three coordinates, radius, laminar height, and height within the organ

Radial coordinate Half width :=
3rd -1st quartile
Values are normalized radial length (r/ro)

receptor	1 st quartile	2 st quartile	3 rd quartile	half width
<i>ora1</i>	0,230	0.340	0.449	0.219
<i>ora2</i>	0.248	0.376	0.500	0.251
<i>ora3a</i>	0.094	0.147	0.230	0.136
<i>ora3b</i>	0.092	0.143	0.228	0.136
<i>ora4</i>	0.113	0.192	0.294	0.181
<i>ora5</i>	0.138	0.204	0.291	0.153
<i>ora6</i>	0.150	0.223	0.270	0.121
<i>zor5</i>	0.362	0.494	0.575	0.213
<i>zor6</i>	0.132	0.198	0.301	0.169
<i>zor8</i>	0.305	0.436	0.533	0.229
<i>zor9</i>	0.245	0.363	0.461	0.216
<i>olfcc1</i>	0.233	0.342	0.458	0.225
<i>olfcg1</i>	0.157	0.262	0.389	0.232
<i>olfcj1</i>	0.122	0.210	0.372	0.250
<i>olfcn1</i>	0.149	0.247	0.375	0.226
<i>olfcq1</i>	0.165	0.283	0.427	0.262
<i>olfct1</i>	0.166	0.299	0.406	0.240
<i>olfcu1</i>	0.154	0.259	0.382	0.229

Laminar height coordinate
Values are normalized laminar length (h/ho)

Gene	1 st quartile	2 st quartile	3 rd quartile	half width
<i>ora1</i>	0.333	0.469	0.604	0.271
<i>ora2</i>	0.544	0.672	0.785	0.242
<i>ora3a</i>	0.482	0.650	0.766	0.284
<i>ora3b</i>	0.634	0.747	0.826	0.192
<i>ora4</i>	0.559	0.671	0.758	0.199
<i>ora5</i>	0.687	0.808	0.866	0.180
<i>ora6</i>	0.648	0.722	0.797	0.149
<i>zor5</i>	0.307	0.436	0.564	0.257
<i>zor6</i>	0.271	0.395	0.564	0.293
<i>zor8</i>	0.393	0.529	0.672	0.279
<i>zor9</i>	0.345	0.457	0.588	0.244
<i>olfcc1</i>	0.541	0.658	0.757	0.216
<i>olfcg1</i>	0.588	0.714	0.783	0.195
<i>olfcj1</i>	0.507	0.603	0.719	0.212
<i>olfcn1</i>	0.575	0.678	0.754	0.179
<i>olfcq1</i>	0.579	0.676	0.758	0.178
<i>olfct1</i>	0.502	0.615	0.699	0.197
<i>olfcu1</i>	0.550	0.647	0.749	0.199

Z-axis coordinate
Values are normalized height within organ (section no/total # sections)

Gene	1 st quartile	2 st quartile	3 rd quartile	half width
<i>ora1</i>	0.500	0,607	0.705	0.205
<i>ora2</i>	0.500	0.589	0.689	0.189
<i>ora3a</i>	0.571	0.661	0.750	0.179
<i>ora3b</i>	0.515	0.597	0.677	0.163
<i>ora4</i>	0.600	0.683	0.746	0.146
<i>ora5</i>	0.518	0.599	0.705	0.187
<i>ora6</i>	0.525	0.584	0.689	0.164
<i>zor5</i>	0.296	0.441	0.593	0.296
<i>zor6</i>	0.516	0.677	0.857	0.341
<i>zor8</i>	0.333	0.500	0.654	0.321
<i>zor9</i>	0.417	0.655	0.800	0.383
<i>olfcg1</i>	0.339	0.441	0.563	0.224
<i>olfcj1</i>	0.433	0.500	0.590	0.156
<i>olfcn1</i>	0.370	0.469	0.619	0.249
<i>olfcq1</i>	0.387	0.507	0.616	0.230
<i>olfct1</i>	0.390	0.533	0.644	0.254
<i>olfcu1</i>	0.512	0.634	0.805	0.293

Pairwise comparison of spatial patterns for expression of different *ora* genes in zebrafish olfactory epithelium: maximal vertical distance between distributions (determined in Kolmogorov-Smirnov test)

Three coordinates were evaluated: radius, laminar height, and height within the organ (z-axis)

		Maximal vertical distance between the two distributions compared		
Gene 1	Gene 2	Radius	Laminar Height	Z-axis
		r/ro	h/ho	z/zo
<i>ora1</i>	<i>ora2</i>	0.110	0.409	0.065
<i>ora1</i>	<i>ora3a</i>	0.516	0.404	0.174
<i>ora1</i>	<i>ora3b</i>	0.512	0.404	0.085
<i>ora1</i>	<i>ora4</i>	0.371	0.432	0.248
<i>ora1</i>	<i>ora5</i>	0.377	0.628	0.065
<i>ora1</i>	<i>ora6</i>	0.430	0.558	0.137
<i>ora2</i>	<i>ora3a</i>	0.550	0.057	0.219
<i>ora2</i>	<i>ora3b</i>	0.550	0.207	0.066
<i>ora2</i>	<i>ora4</i>	0.422	0.081	0.286
<i>ora2</i>	<i>ora5</i>	0.436	0.323	0.067
<i>ora2</i>	<i>ora6</i>	0.490	0.205	0.142
<i>ora3a</i>	<i>ora3b</i>	0.057	0.206	0.226
<i>ora3a</i>	<i>ora4</i>	0.197	0.077	0.099
<i>ora3a</i>	<i>ora5</i>	0.267	0.354	0.197
<i>ora3a</i>	<i>ora6</i>	0.313	0.201	0.240
<i>ora3b</i>	<i>ora4</i>	0.189	0.236	0.311
<i>ora3b</i>	<i>ora5</i>	0.257	0.195	0.063
<i>ora3b</i>	<i>ora6</i>	0.305	0.126	0.125
<i>ora4</i>	<i>ora5</i>	0.120	0.400	0.275
<i>ora4</i>	<i>ora6</i>	0.146	0.200	0.302
<i>ora5</i>	<i>ora6</i>	0.075	0.286	0.104
<i>zor5</i>	<i>zor6</i>	0.602	0.087	0.438
<i>zor5</i>	<i>zor8</i>	0.162	0.196	0.132
<i>zor5</i>	<i>zor9</i>	0.347	0.084	0.324
<i>zor6</i>	<i>zor8</i>	0.523	0.268	0.366
<i>zor6</i>	<i>zor9</i>	0.399	0.152	0.148
<i>zor8</i>	<i>zor9</i>	0.194	0.158	0.259
<i>zor5</i>	<i>ora1</i>	0.374	0.074	0.276
<i>zor6</i>	<i>ora1</i>	0.359	0.141	0.221
<i>zor8</i>	<i>ora1</i>	0.232	0.140	0.186
<i>zor9</i>	<i>ora1</i>	0.078	0.049	0.100
<i>olfcc1</i>	<i>ora1</i>	0.028	0.402	n.d.
<i>olfcn1</i>	<i>ora2</i>	0.267	0.104	0.250
<i>olfcq1</i>	<i>ora2</i>	0.210	0.100	0.250
<i>olfcn1</i>	<i>ora4</i>	0.191	0.047	0.334
<i>olfcq1</i>	<i>ora4</i>	0.244	0.070	0.320
<i>olfcu1</i>	<i>ora4</i>	0.181	0.071	0.080

Expression patterns of V1R-related *ora* genes in zebrafish olfactory epithelium:

Distribution of radial coordinate, laminar height, and height within organ (z axis)

Pairwise comparison of distributions: p-value determined by Kolmogorov-Smirnov test.

Significant p values are overlayed grey (p<0.01 is considered significant for 100<n<500 and p<0.001 for n>500).

Gene 1	Gene 2	p value	p value	p value
		Radius	Laminar Height	Z-axis
<i>ora1</i>	<i>ora2</i>	4.90E-011	2.20E-016	7.81E-007
<i>ora1</i>	<i>ora3a</i>	2.20E-016	2.20E-016	2.20E-016
<i>ora1</i>	<i>ora3b</i>	2.20E-016	2.20E-016	0.006393
<i>ora1</i>	<i>ora4</i>	2.20E-016	2.20E-016	2.20E-016
<i>ora1</i>	<i>ora5</i>	2.20E-016	2.20E-016	0.1191
<i>ora1</i>	<i>ora6</i>	2.20E-016	2.20E-016	0.007076
<i>ora2</i>	<i>ora3a</i>	2.20E-016	0.013	2.20E-016
<i>ora2</i>	<i>ora3b</i>	2.20E-016	1.40E-009	0.0669
<i>ora2</i>	<i>ora4</i>	2.20E-016	1.63E-007	2.20E-016
<i>ora2</i>	<i>ora5</i>	2.20E-016	2.20E-016	0.09496
<i>ora2</i>	<i>ora6</i>	2.20E-016	6.90E-005	0.004451
<i>ora3a</i>	<i>ora3b</i>	0.475	1.40E-008	2.20E-016
<i>ora3a</i>	<i>ora4</i>	2.20E-016	1.00E-004	3.33E-016
<i>ora3a</i>	<i>ora5</i>	2.20E-016	2.20E-016	2.06E-011
<i>ora3a</i>	<i>ora6</i>	2.60E-010	2.00E-004	6.47E-008
<i>ora3b</i>	<i>ora4</i>	3.50E-008	1.75E-012	2.20E-016
<i>ora3b</i>	<i>ora5</i>	4.30E-009	1.90E-005	0.398
<i>ora3b</i>	<i>ora6</i>	1.60E-007	0.120	0.05134
<i>ora4</i>	<i>ora5</i>	0.0003352	2.20E-016	2.20E-016
<i>ora4</i>	<i>ora6</i>	0.1076	1.07E-004	1.38E-012
<i>ora5</i>	<i>ora6</i>	0.6647	4.50E-007	0.181

<i>zor5</i>	<i>zor6</i>	2.20E-016	0.09894	2.20E-016
<i>zor5</i>	<i>zor8</i>	1.35E-006	2.20E-016	3.24E-012
<i>zor5</i>	<i>zor9</i>	2.20E-016	0.00107	2.20E-016
<i>zor6</i>	<i>zor8</i>	2.20E-016	5.38E-010	2.20E-016
<i>zor6</i>	<i>zor9</i>	2.20E-016	0.0002161	2.77E-006
<i>zor8</i>	<i>zor9</i>	1.64E-011	9.50E-013	2.20E-016

<i>zor5</i>	<i>ora1</i>	2.20E-016	0.001342	2.20E-016
<i>zor6</i>	<i>ora1</i>	2.20E-016	0.000423	8.37E-009
<i>zor8</i>	<i>ora1</i>	2.20E-016	7.26E-013	2.53E-009
<i>zor9</i>	<i>ora1</i>	0.001517	0.08032	0.007384
<i>olfcc1</i>	<i>ora1</i>	0.9721	6.16E-013	n.d.
<i>olfcn1</i>	<i>ora2</i>	5.55E-016	0.002907	2.63E-011
<i>olfcq1</i>	<i>ora2</i>	2.49E-014	0.002541	2.30E-013
<i>olfcn1</i>	<i>ora4</i>	1.88E-008	0.5118	2.20E-016
<i>olfcq1</i>	<i>ora4</i>	2.20E-016	0.07049	2.20E-016
<i>olfcu1</i>	<i>ora4</i>	2.64E-007	0.1839	0.2301