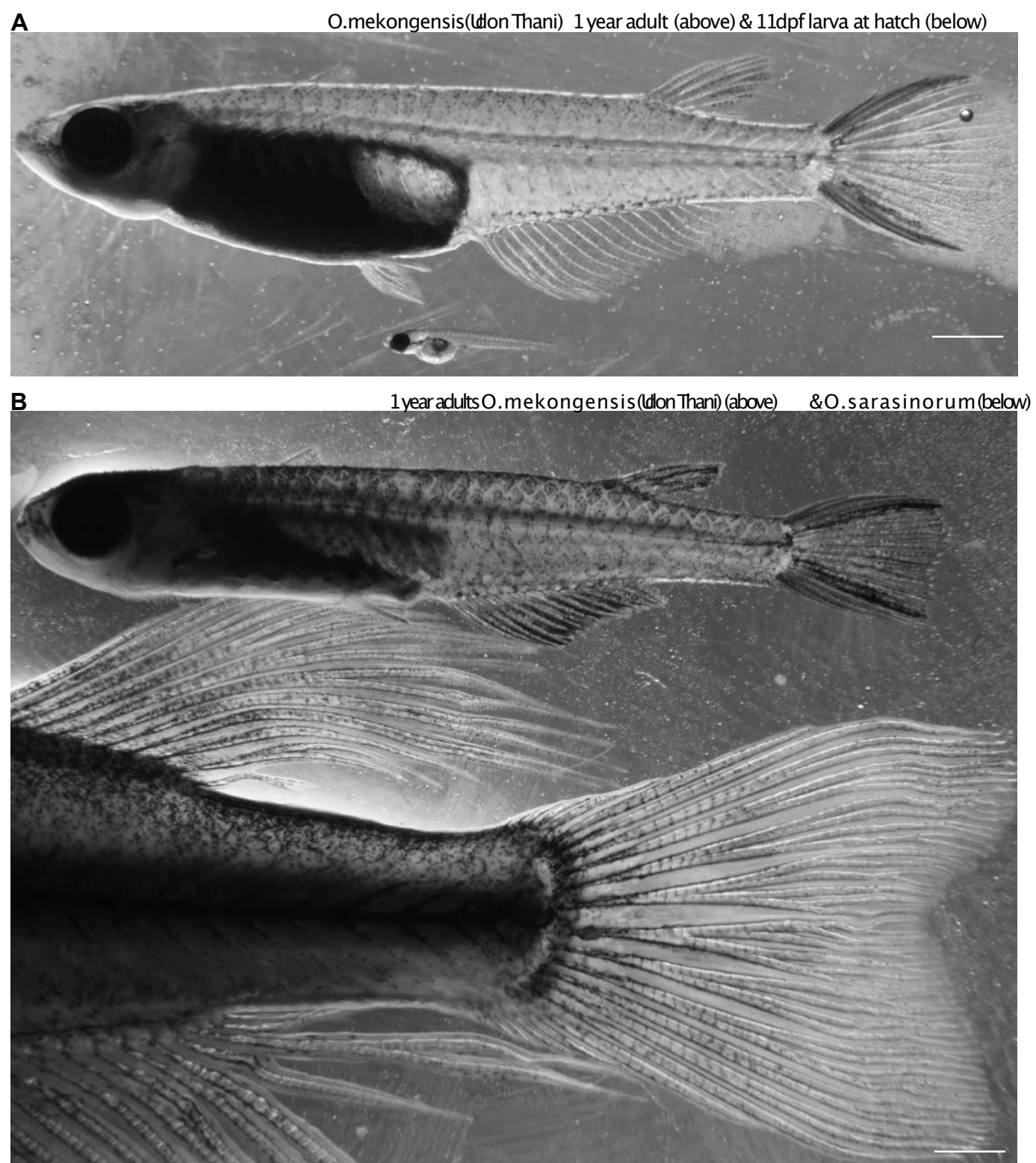


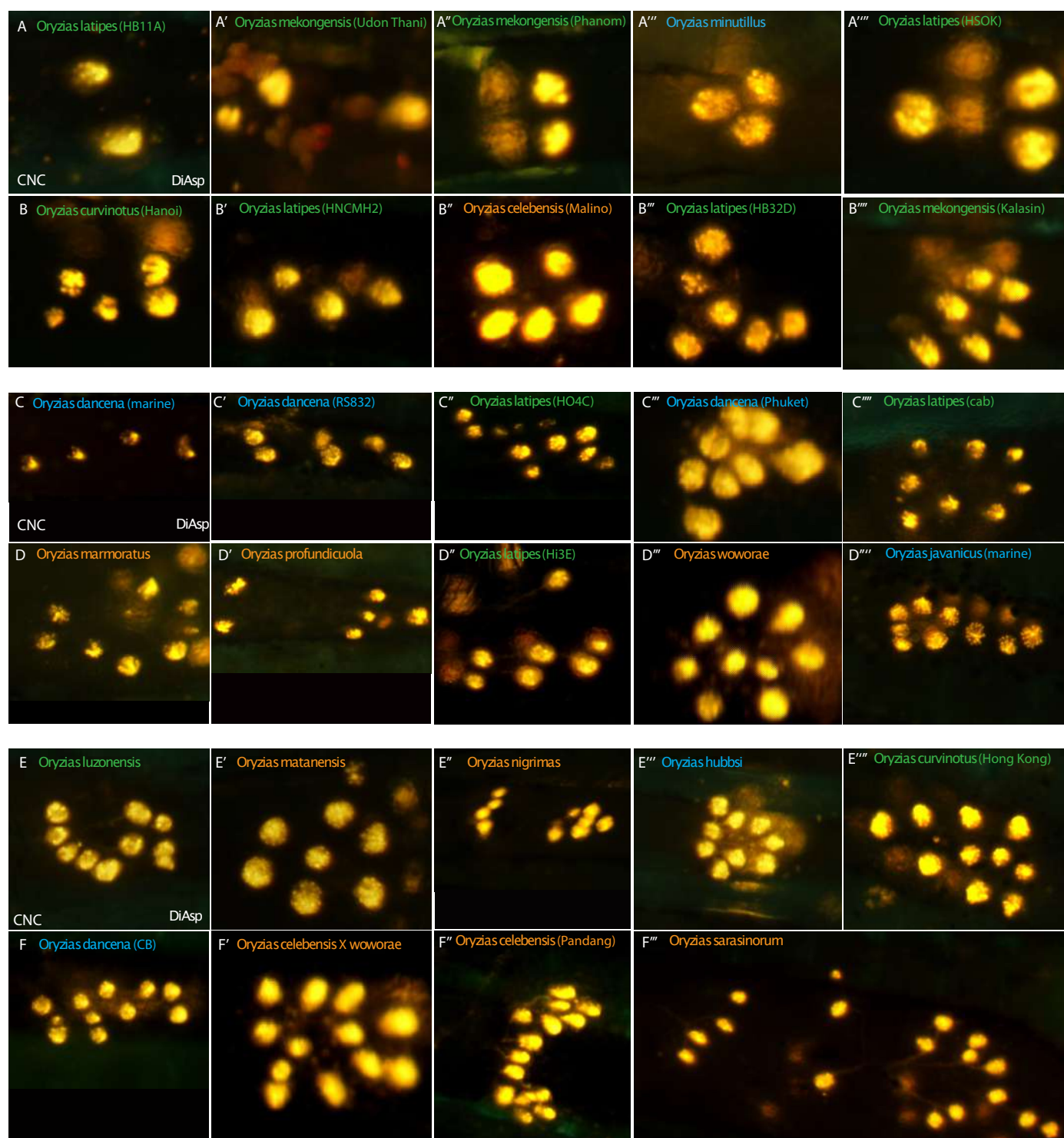
**Fig. S1. Habitats of different *Oryzias* species**

Natural environment for the different species in the *Oryzias* genus. **(S1A)** Typical habitats of *O. latipes* in Japan. *O. latipes* live in the small creeks, rice paddy fields (S1A1, 3) swims near surface water and tend to live in relatively shallow areas (Fig S1A 2 and 4). **(S1B)** Habitats of *O. mekongensis* in Thailand. *O. mekongensis* inhabits ponds covered with water plants. **(S1C)** Habitats of *O. minutillus* in Thailand. *O. minutillus* lives in ponds and small creeks (S1C 1-3) but in the open water area - compare with *O. mekongensis* (Pictures are courtesy of Yusuka Takehana, Nagahama Institute of Bio-Science and Technology). **(S1D)** Habitats of *O. javanicus* in Malaysia. *O. javanicus* lives in brackish water (S1D2, 4) to seawater area (S1D 1 and 3) in the mangrove forests (Fig. S1D1, 4). **(S1E)** Habitats of *O. dancena* in Malaysia, which is similar with *O. javanicus*, but with lower salinity values, from freshwater (S1D 1) to brackish water (S1D 2) (Pictures are courtesy of Koji Inoue, University of Tokyo & Masato Kinoshita, Kyoto University). **(S1F)** Habitats of *O. nigrimas* in the Lake Poso, Sulawesi island. *O. nigrimas* live in near shore of the lake Poso (Fig S1F 1, 4) where it is important for fisheries activity in the lake (Fig S1F 2). **(S1G)** Habitats of *O. sarasinorum* in the lake Lindu, Sulawesi island. *O. sarasinorum* inhabit the relatively pelagic area in the lake Lindu. **(S1H)** Habitats of *O. matanensis* in the lake Matana, Sulawesi island. *O. matanensis* inhabits near the shore. **(S1I)** Habitats of *O. woworae* typically in clear springs in Mauna island, Sulawesi island. **(S1J)** Habitats of *O. celebensis*, in relatively fast running rivers. *Oryzias* species in Sulawesi island (*O. sarasinorum*, *O. nigrimas*, *O. matanensis*, *O. marmoratus* and *O. profundicola*) are relatively unique compared with other *Oryzias* species, since these species mainly live in deep tectonic lakes like Poso, Lindu, Matana, Towti and Maholona.



**Fig. S2. Size differences between *Oryzias mekongensis* and *O. sarasinorum***

(A) Bright-field images showing differences in size between *O. mekongensis* (Udon Thani) one-year old adult (above) compared to an 11dpf larva (below). (B) Bright-field images showing differences in size between *O. mekongensis* (Udon Thani) one year old adult (above) and *O. sarasinorum* one year old adult (below). Scale bar in (A) and (B) = 1.6 mm. Anterior is to the left and dorsal is up in all panels.



**Fig. S3. Caudal neuromast cluster (CNC) organ numbers and distribution in the *Oryzias* group - extended**

(A-F''') CNC organ numbers and patterns in 1 year old adult *Oryzias* group members obtained via fluorescent imaging of Diasp-2 treated fish. Images are arranged from the least elaborate to the most elaborate: (A) *O. latipes* (HB11A), (A') *O. mekongensis* (Udon Thani), (A'') *O. mekongensis* (Nakhon Phanom), (A''') *O. minutillus*, (A''''') *O. latipes* (HSOK)sb, (B) *O. curvinotus* (Hanoi), (B') *O. latipes* (HNCMH2), (B'') *O. celebensis* (Malino), (B''') *O. latipes* (HB32D), (B''''') *O. mekongensis* (Kalasin), (C) *O. dancena* (marine), (C') *O. dancena* (RS832), (C'') *O. latipes* (HO4C), (C''') *O. dancena* (Phuket), (C''''') *O. latipes* (Cab), (D) *O. marmoratus*, (D') *O. profundicuola*, (D'') *O. latipes* (Hi3E), (D''') *O. woworae*, (D''''') *O. javanicus* (Penang), (E) *O. luzonensis*, (E') *O. matanensis*, (E'') *Oryzias nigrimas*, (E''') *Oryzias hubbsi*, (E''''') *Oryzias curvinotus* (Hong Kong), (F) *Oryzias dancena* (Chidambaram), (F') F1 Hybrid *Oryzias woworae* X *celebensis* (Ujung Pandang), (F'') *O. celebensis* (Ujung Pandang), (F''') *O. sarasinorum*. Notice the differences in organ numbers and patterns between the different species and strains with *O. latipes* (HB11A), *O. mekongensis* and *O. minutillus* having the least elaborate CNCs while *O. sarasinorum* and *O. celebensis* (Ujung Pandang) having the most elaborate. Anterior is to the left and dorsal is up in all panels. Species names are colour coded according to sub-group (as in Figure 1); *O. latipes* complex in green, *O. celebensis* complex in orange and *O. javanicus* complex in blue.

**Table S1.** Accession numbers of *ND2* and *cyt b* sequences used in the mtDNA phylogeny.

Species	Locality / Collection site	Classification by molecular phylogeneny	Accession number for mtDNA phylogeny		Strain	NBRP strain ID	Comments
			ND2	cyt b			
<i>Oryzias celebensis</i>	Malino River, Lake Tempe, South Sulawesi, Indonesia	<i>celebensis</i> species group	LC051717	LC051664	Malino	RS279	
					Ujung Pandang	RS278	
<i>Oryzias curvinotus</i>	So Lo Pun, Hong Kong. Bang La, Do Son and Dai Hop Vietnam	<i>latipes</i> species group	LC051739	LC051686	Hanoi	RS268	
					Hong Kong	RS269	
<i>Oryzias dancena</i>	Chidambaram, Tamil Nadu, India and Phuket, Thailand	<i>javanicus</i> species group	AB498069	AB498069	Chidambaram	RS274	
					HK-1	-	Maintained in seawater
					Phuket	RS275	
					RS832	RS832	
<i>Oryzias hubbsi</i>	Tangerang, Indonesia	<i>javanicus</i> species group	LC497921	N.A.	-	RS273	
<i>Oryzias javanicus</i>	Penang, Malaysia to Pangkajene, Sulawesi, Indonesia	<i>javanicus</i> species group	LC051734	LC051681	Penang	RS831	Maintained in seawater
<i>Oryzias latipes</i>	Japan, China and Korea	<i>latipes</i> species group	LC051738	LC051685	Cab	MT830	
					HB11A	IB185	Inbred strain
					HB32D	IB184	Inbred strain
					Hi3E	IB186	Inbred strain
					HNCMH2	IB182	Inbred strain
					HO4C	IB180	Inbred strain
					HSOK	IB183	Inbred strain
<i>Oryzias luzonensis</i>	Solsona, Ilocos Norte Province, Philippines	<i>latipes</i> species group	AB498064	AB498064	-	RS270	
<i>Oryzias marmoratus</i>	Lake Towuti, South Sulawesi, Indonesia	<i>celebensis</i> species group	LC051709	LC051656	-	RS280	
<i>Oryzias matanensis</i>	Lake Matano, South Sulawesi, Indonesia	<i>celebensis</i> species group	LC051705	LC051652	-	RS281	
<i>Oryzias mekongensis</i>	Udon Thani, Thailand. Along Mekong river	<i>latipes</i> species group	LC497922	AB084756	Kalasin	RS271	
					Nakhon Phanom	RS272	
					Udon Thani	RS915	
<i>Oryzias minutillus</i>	Chai Nat, Thailand	<i>javanicus</i> species group	AB498068	AB498068	-	RS277	
<i>Oryzias nigrimas</i>	Lake Poso, Central Sulawesi, Indonesia	<i>celebensis</i> species group	LC051707	LC051654	-	RS282	
<i>Oryzias profundicuola</i>	Lake Towuti, South Sulawesi, Indonesia	<i>celebensis</i> species group	LC051697	LC051644	-	RS283	
<i>Oryzias sarasinorum</i>	Lake Lindu, Central Sulawesi, Indonesia	<i>celebensis</i> species group	LC051691	LC051638	-	RS284	
<i>Oryzias woworae</i>	Fotuno Fountain, Muna Island, Indonesia	<i>celebensis</i> species group	LC051723	LC051670	-	-	
<i>Hyporhamphus sajori</i>	-	-	AB370892	AB370892	-	-	Outgroup for phylogeny
<i>Cololabis saira</i>	-	-	AP002932	AP002932	-	-	Outgroup for phylogeny

**Table S2.** Number of adult males analysed per species & strains, and maximum reported body size for each species.

Species	Species Group	Strain	N Adults Analysed	Reported adult length
<i>Oryzias celebensis</i>	<i>celebensis</i>	Ujung Pandang	3	3,3 cm
<i>Oryzias curvinotus</i>	<i>latipes</i>	Hanoi	3	nd
		Hong Kong	3	2,6 cm
<i>Oryzias dancena</i>	<i>javanicus</i>	Chidambaram	2	3,3 cm
		HK-1	4	nd
		Phuket	4	3,2 cm
<i>Oryzias hubbsi</i>	<i>javanicus</i>	-	2	2,2 cm
<i>Oryzias javanicus</i>	<i>javanicus</i>	Penang	4	nd
<i>Oryzias latipes</i>	<i>latipes</i>	Cab	5	3,2 cm
		HB11A	3	nd
		HB32D	2	nd
		Hi3E	2	nd
		HNCMH2	3	nd
		HO4C	3	nd
		HSOK	2	nd
		-	2	nd
<i>Oryzias luzonensis</i>	<i>latipes</i>	-	4	3,4 cm
<i>Oryzias marmoratus</i>	<i>celebensis</i>	-	3	4,2 cm
<i>Oryzias matanensis</i>	<i>celebensis</i>	-	6	4,3 cm
<i>Oryzias mekongensis</i>	<i>latipes</i>	Kalasin	6	2,1 cm
		Nakhon Phanom	2	nd
		Udon Thani	4	nd
<i>Oryzias minutillus</i>	<i>javanicus</i>	-	2	2 cm
<i>Oryzias nigrimas</i>	<i>celebensis</i>	-	5	4,3 cm
<i>Oryzias profundicuola</i>	<i>celebensis</i>	-	4	4,2 cm
<i>Oryzias sarasinorum</i>	<i>celebensis</i>	-	4	4,8 cm
<i>Oryzias woworae</i>	<i>celebensis</i>	-	3	2,8 cm

**Table S3.** Number of samples analysed per species and per LL region, together with the corresponding 95 CI mean.

Species	aLL				pLL				CNC			
	n	mean	sd	95CI mean	n	mean	sd	95CI mean	n	mean	sd	95CI mean
<i>Oryzias celebensis</i> (Pandang)	11	9,5	3,2	7.2 - 11.7	20	8,5	1,8	7.7 - 9.3	4	13,2	1,5	10.5 - 16
<i>Oryzias curvinotus</i> (Hanoi)	6	3,2	1,2	1.8 - 4.5	16	2,9	1	2.4 - 3.5	4	4	0,8	2.5 - 5.5
<i>Oryzias curvinotus</i> (Hong Kong)	7	4,7	1	3.8 - 5.7	17	4,8	1,4	4.1 - 5.6	4	9,2	1	7.5 - 11
<i>Oryzias dancena</i> (CB)	11	4,7	1,1	3.9 - 5.5	20	4	1,4	3.3 - 4.6	4	10,5	1	8.7 - 12.3
<i>Oryzias dancena</i> (marine)	9	3,8	1,6	2.5 - 5.1	11	2,5	0,9	1.9 - 3.2	6	5,5	1,4	3.9 - 7.1
<i>Oryzias dancena</i> (phuket)	11	6,2	1,6	5.1 - 7.3	17	5,4	1,7	4.5 - 6.2	2	7	1,4	11 - 25
<i>Oryzias hubbsi</i>	11	4,6	1,4	3.7 - 5.6	16	5	1,3	4.3 - 5.7	3	9,7	1,2	6.2 - 13.2
<i>Oryzias javanicus</i> (marine)	8	5,4	1,7	3.9 - 6.9	16	4	1,3	3.3 - 4.7	4	8	2,2	4 - 12
<i>Oryzias latipes</i> (Cab)	8	3,9	1,2	2.8 - 5	15	5,3	1,1	4.6 - 5.9	9	7,1	1,6	5.8 - 8.4
<i>Oryzias luzonensis</i>	11	3,2	1,5	2.1 - 4.3	17	2,4	0,9	1.9 - 2.8	4	8,2	1,7	5.1 - 11.4
<i>Oryzias marmoratus</i>	7	5,9	1,6	4.3 - 7.4	19	4,9	1,7	4.1 - 5.8	4	6,8	1,5	4 - 9.5
<i>Oryzias matanensis</i>	9	7	1,7	5.6 - 8.4	20	4,7	1,6	3.9 - 5.5	4	8,8	1,7	5.6 - 11.9
<i>Oryzias mekongensis</i> (Kalasin)	5	2	1,2	0.3 - 3.7	11	4,5	0,7	4 - 4.9	9	5,1	2,3	3.3 - 7
<i>Oryzias mekongensis</i> (N.Phanom)	9	1,3	0,5	0.9 - 1.7	11	2,7	0,8	2.2 - 3.3	2	2,5	0,7	6.5 - 11.5
<i>Oryzias mekongensis</i> (U.Thani)	8	1,5	0,5	1 - 2	11	1,7	0,6	1.3 - 2.2	8	1,8	1,2	0.7 - 2.8
<i>Oryzias minutillus</i>	6	1,7	0,5	1.1 - 2.3	11	2,3	1,2	1.4 - 3.1	2	2,5	0,7	6.5 - 11.5
<i>Oryzias nigrimas</i>	11	5,5	1,3	4.5 - 6.4	17	4,6	1,2	4 - 5.2	6	8,7	0,8	7.7 - 9.6
<i>Oryzias profundicuola</i>	7	6,6	1,7	4.9 - 8.3	17	5,1	1,5	4.3 - 5.9	3	7,3	3,5	3.4 - 18
<i>Oryzias sarasinorum</i>	9	7,8	2	6.2 - 9.4	20	4,3	0,8	3.9 - 4.7	2	17,5	0,7	8.5 - 26.5
<i>Oryzias woworae</i> (indonesian)	10	9	3,5	6.4 - 11.6	17	6,7	1,8	5.7 - 7.7	4	8,2	1,5	5.5 - 11