THE RAFFLES BULLETIN OF ZOOLOGY 2012 **60**(2): 467–476
Date of Publication: 31 Aug.2012

PELVIC-FIN BROODING IN A NEW SPECIES OF RIVERINE RICEFISH (ATHERINOMORPHA: BELONIFORMES: ADRIANICHTHYIDAE) FROM TANA TORAJA, CENTRAL SULAWESI, INDONESIA

Fabian Herder
Zoologisches Forschungsmuseum Alexander Koenig, Sektion Ichthyologie, Adenauerallee 160, D-53113 Bonn, Germany
Email: f.herder.zfmk@uni-bonn.de

Renny Kurnia Hadiaty
Ichthyology Laboratory, Division of Zoology, Research Center for Biology, Indonesian Institute of Sciences (LIPI)
JL. Raya Bogor Km 46, Cibinong 16911, Indonesia
Email: rani_hadiaty@yahoo.com

Arne W. Nolte
Max-Planck-Institut fuer Evolutionsbiologie, Abteilung Evolutionsgenetik, August-Thienemannstrasse 2, 24306 Ploen
Email: nolte@evolbio.mpg.de

ABSTRACT. — A new species of ricefish is described from a hill stream in Tana Toraja, Sulawesi. *Oryzias eversi*, new species, is distinguished from all other adrianichthyids in Sulawesi by having a low number of fin rays in anal (17–18 (19)) and dorsal (10–12) fins, only 33–36 scales in lateral midline, ½14 transverse scale rows at dorsal fin origin, 30–32 (33) vertebrae, small eyes (28.2–35.5% of head length), a conspicuous blackish male courtship colouration, and pelvic brooding behaviour similar to lacustrine ricefishes. Female *Oryzias eversi* carry the eggs until the embryos hatch, and show a conspicuous abdominal concavity and extended pelvic fins, accommodating and holding the clutch of eggs. The eggs are connected to the female for the whole time of development by attaching filaments that protrude from the female's urogenital pore. A mitochondrial haplotype phylogeny suggests that the new species is closely related to another "pelvic brooder", the lake-dwelling *O. sarasinorum* from Lake Lindu in Central Sulawesi. However, the haplotype group of *O. eversi* and *O. sarasinorum* is nested within a clade of egg-depositing *Oryzias* from central, southwest, and southeast Sulawesi, whereas another pelvic brooder, *Adrianichthys oophorus* from Lake Poso, forms a distinct, second lineage of Sulawesi's ricefishes. Accordingly, the pelvic brooding strategy has probably evolved more than once and may be realised in river habitats, which represents a new evolutionary trajectory in the radiation of ricefishes in Sulawesi. The present discovery adds another new ricefish species to Sulawesi's still only partially known ichthyofauna, and highlights the island's role as hotspot of adrianichthyid diversity.

KEY WORDS. — *Oryzias*, taxonomy, freshwater fish, endemism, reproduction, sexual dimorphism

INTRODUCTION

Ricefishes (Adrianichthyidae) are small atherinomorph fishes with a remarkably diversity of reproductive modes, including ovoviviparity, facultative embryo retention, and maternal care (Parenti, 2005, 2008; Kinoshita et al., 2009). The Indonesian island Sulawesi is the major hotspot of ricefish species diversity, harbouring roughly half of the adrianichthyid species currently recognised. Most of the island's endemic species are restricted to single ancient lakes, i.e., Lake Poso, Lake Lindu, or the lakes of the Malili Lakes system (Kottelat, 1990a, 1990b; Parenti, 2008; Herder & Chapuis, 2010). In most of the ricefish species, females carry small bundles of fertilised eggs attached to the maternal fish by attaching filaments (Iwamatsu et al., 2007, 2008; Parenti, 2008). Carrying time varies within and among species, and does not exceed a few hours in most species of the genus *Oryzias* (Iwamatsu et al., 2007). However, data available on the reproductive biology of ricefishes are surprisingly restricted and partly in conflict, ranging from well-known (e.g., for the Medaka, *Oryzias latipes*, and a few other species that have been investigated so far; Iwamatsu et al., 2007, 2008), to poorly known in other species of the family (Kottelat, 1990a, 1990b; Parenti, 2005, 2008; Kinoshita et al. 2009).

In some species from Sulawesi's Lakes Poso and Lindu, the eggs are reportedly not deposited at all on submerged plants or other material, but kept by the female until hatching (Kottelat, 1990a). At least two of these species, *Adrianichthys oophorus* from Lake Poso and *Oryzias sarasinorum* from Lake Lindu, show clear adaptations for "pelvic brooding" (i.e., females have extended pelvic fins, covering the clutch of eggs kept in a more or less well developed abdominal concavity, attached to the female by attaching filaments; Kottelat, 1990a; Iwamatsu et al., 2007, 2008; Parenti, 2008). As pelvic brooders do not depend on substrate for completing their reproductive cycle, this strategy has been considered as a potential adaptation to a predominantly pelagic life style in lake habitats (Kottelat, 1990a; Parenti, 2008). In *O. sarasinorum*, the attaching filaments form a "plug" with epithelial cells at the female's urogenital pore, and there are indications that the developing embryos affect physiological pathways regulating the female's reproductive physiology (Iwamatsu et al., 2007, 2008); even the possibility of a placental function has been considered (Iwamatsu et al., 2008).

Since Parenti's comprehensive revision of ricefishes in 2008, additional species of *Oryzias* have been uncovered from lake and stream habitats in Sulawesi (Herder & Chapuis, 2010; Parenti & Hadiaty, 2010; Parenti et al., in prep.). Here we describe a new pelvic brooding ricefish species from a forest stream habitat in Tana Toraja (Central Sulawesi, South Sulawesi Province), the first record of this reproductive strategy (or *reproductive guild* sensu Kottelat, 1990a) in a stream habitat. As this discovery raises questions concerning the origin and evolutionary ecology of pelvic brooding, we present a mitochondrial haplotype phylogeny of Sulawesi's ricefishes and discuss the evolutionary origins of pelvic brooding. The present results provide further support for the still "under-described" (Roberts, 1998: 213) status of ricefishes, also highlighted by the recent discovery of new species from other Asian regions (Magtoon, 2010; Asai et al., 2011), and further emphasize our incomplete knowledge of the freshwater ichthyofauna of Sulawesi.

MATERIAL AND METHODS

Analyses of morphology and reproductive behaviour. — Specimens of the new *Oryzias* were obtained from a tributary of Salo Sadang (3°02.126S, 119°53.232E) about 8 km south of Rantepao, Tana Toraja, Central Sulawesi, Indonesia, in 24 Sep.2010. Fish were caught by snorkeling using hand nets. Single individuals of both sexes were photographed immediately after capture to document life colouration; a stock of living individuals was kept alive in aquaria to investigate reproductive biology, life colouration, and behaviour. The habitat characterisation is based on photographs and descriptions by H.-G. Evers (pers. comm.). Specimens for morphological examination were either preserved directly in 70% ethanol, or were kept for observations in the aquarium and were subsequently preserved in 4% formalin and later transferred to 70% ethanol for storage. Material examined is deposited in the Research Centre for Biology, the Indonesian Institute of Sciences (LIPI, formerly the Museum Zoologicum Bogoriense – MZB), Cibinong, Indonesia, the Zoologisches Forschungsmuseum Alexander Koenig, Bonn (ZFMK), Germany, and the National Museum of Natural History, Smithsonian Institution, Washington, DC (USNM), United States of America. Morphometric measurements and meristics follow Kottelat (1990b) and Parenti & Soeroto (2004). All measurements are taken from point to point, recorded to the nearest 0.1 mm with a digital caliper. Abbreviations used are SL – standard length, HL – head length. Counts of the unpaired fins and vertebrae (total = precaudal + caudal) were made from radiographs, using a digital X-ray device (Faxitron LX-60). Dorsal- and anal fin ray counts are reported in total, including the single first unbranched and a number of branched rays. Principal and procurrent caudal fin ray numbers are reported following Rosen & Parenti (1981) and Parenti (2008). For example, i,4/5,i, stands for one principal unbranched and four branched rays in the dorsal lobe and five branched and one principal unbranched ray in the ventral lobe, and 6/7 stands for six procurrent rays in the dorsal and seven procurrent rays in the ventral lobe. Scale counts follow Kottelat (1990b); the small scales on caudal fin base are not included. Scales in transverse row are counted from origin of dorsal fin to lateral row and continuing to anal fin origin; ½ refers to the scale in front of the dorsal fin. Meristic values in brackets are deviations from the type series present in 4 additional immature specimens examined.

Phylogenetic analyses. — Complementary to ricefish sequence data available from Takehana et al. (2005), a 16s rDNA fragment was sequenced using the primers L2606 5'-TTGACCGTGCAAAGGTAGCGCAATCACT-3' and H3056 5'-CTCCGGTCTGAACTCAGATCACGTAGG-3' from the same authors for *Oryzias eversi* and additional adrianichthyids from Sulawesi (Appendix 1). DNA was extracted using DNeasy Blood & Tissue Kit (Quiagen) and PCR amplified using the Quagen Multiplex PCR Kit following the protocols supplied by the provider. The following PCR program was used: 15 min at 95°C, 36 cycles of (30 s at 94°C, 90 s at 68°C, 90 s at 72°C) and a final extension of 10 min at 72°C. Products for sequencing were prepared for sequencing using an exo-sap protocol and sanger-sequenced in both directions on an ABI 3130xl Genetic Analyser. Sequences of representative adrianichthyid taxa from outside of Sulawesi and additional sequence data available for Sulawesi ricefishes were retrieved from genbank (http://www.ncbi.nlm.nih.gov/genbank/; see Appendix 1). All sequences were aligned using BioEdit (Hall, 1999) and electropherograms and alignments were checked by eye and edited after combining the data with reference sequences from GenBank. The final data was trimmed to 477 bp. We used MrModeltest (Nylander, 2004) to infer the most likely model of sequence evolution and used MrBayes (Ronquist et al., in press) to infer a bayesian phylogeny and posterior probabilities of the branching patterns.

TAXONOMY

Oryzias eversi, new species

(English common name: Evers' ricefish)
(Figs. 1–3)

Material examined. — *Holotype* – MZB 20780 (35.8 mm SL), male, Indonesia, Sulawesi: South Sulawesi Province, Tana Toraja; Salo

Sadang drainage, stream close by the village Tilanga, about 8 km S of Rantepao; 3°02.126S, 119°53.232E, elev. 859 m; coll. H.-G. Evers, J. Christian, P. Debold, T. Heinrich, 24 Sep.2010.

Paratypes – All collected with the holotype: MZB 20781, 1 female (28.0 mm SL), ZFMK 44938, 1 male (36.2 mm SL), ZFMK 44939, 1 female (34.9 mm SL), USNM 406817, 1 male (33.5 mm SL), USNM 406818, 1 female (38.4 mm SL).

Non-type material – ZFMK 44940-44943, 4 immature specimens (16.8–21.6 mm SL), collected with the holotype.

Diagnosis. — *Oryzias eversi* is distinguished from all other Adrianichthyidae in Sulawesi by the following combination of characters: 17–18 (19) fin rays in the anal fin; 10–12 fin rays in the dorsal fin; 33–36 scales along lateral midline; ½14 transverse scale rows at dorsal fin origin; 30–32 (33) total vertebrae; small eye size relative to head length (28.2–35.5% HL); absence of dark bluish or steel blue body colouration or brilliant red marks in both sexes; conspicuous blackish courtship colouration of males, including a blackish belly and posterior lateral body, presence of 6–9 blackish lateral bars, and presence of a narrow black line on a light brown background on dorsal surface; i,4/5,i principal caudal fin rays; and a conspicuous pelvic brooding behaviour associated with sexually dimorphic body depth and pelvic fin length.

Fig. 1. A, *Oryzias eversi*, new species, MZB 20780, holotype, male, 35.8 mm SL; B, ZFMK 44939, paratype, female, 34.9 mm SL.

Fig. 2. Radiograph of *Oryzias eversi*: A, *Oryzias eversi*, MZB 20780, holotype, male, 35.8 mm SL; B, ZFMK 44939, paratype, female, 34.9 mm SL.

Description. — See Fig. 3 for general appearance in lateral view, Fig. 2 for radiographs of male holotype and a female paratype, and Table 1 for morphometric data. Body compressed laterally, slender to somewhat deep-bodied, body depth 18.9–24.6% SL. Females with a pronounced abdominal concavity between pelvic fins and anal fin, covered by long (18.9–19.6% SL) pelvic fins. Mouth terminal, lower jaw extends slightly beyond upper jaw. No external teeth. Ventral body profile arching from head to anal fin origin; body depth at anal fin origin smaller in females than in males (18.9–22.1 vs. 23.9–24.6% SL). Dorsal body profile almost

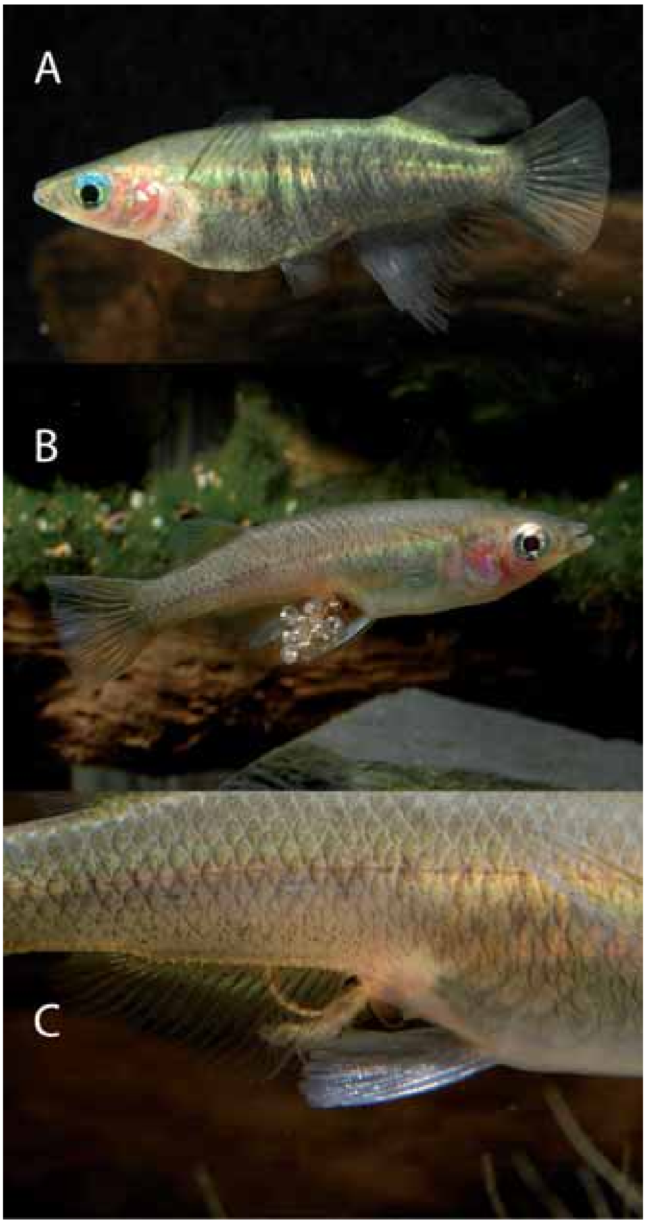

Fig. 3. A, *Oryzias eversi*, male, not preserved; B, *Oryzias eversi*, female carrying eggs, not preserved; C, urogenital pore of female *Oryzias eversi* with attaching filaments, after hatching of the embryos. (Photographs by: Hans-Georg Evers).

Table 1. Meristic and morphometric data of *Oryzias eversi*.

	Males			Females					
	MZB20780 Holotype	ZFMK44938 Paratype	USNM 406817 Paratype	USNM 406818 Paratype	ZFMK44939 Paratype	MZB20781 Paratype	**Min.**	**Max.**	**Mode**
Standard length (mm)	35.8	36.2	33.5	38.4	34.9	28	28	38.4	
Anal-fin rays (total)	17	18	18	18	18	17	17	18	18
Dorsal-fin rays (total)	11	11	12	11	11	10	10	12	11
Pelvic-fin rays	6	6	6	6	6	6	6	6	6
Pectoral-fin rays	10	10	10	10	10	10	10	10	10
Principal caudal-fin rays	i,4/5,i	i,4/5,i	i,4/5,i	i,4/5,i	i,4/5,i	i,4/5,i	i,4/5,i	i,4/5,i	i,4/5,i
Procurrent caudal-fin rays	6/7	6/7	7/7	6/7	6/8	6/6	6/6	7/8	6/7
Scales in lateral row	35	33	36	33	35	35	33	36	35
Scales in transverse row	14.5	14.5	14.5	14.5	14.5	*	14.5	14.5	14.5
Total vertebrae	30	31	32	30	32	32	30	32	32
% standard length							**Min.**	**Max.**	**Mean**
Total length	120.9	121.8	122.1	120.1	120.3	122.9	120.1	122.9	121.4
Head length	30.7	29.6	29.0	29.7	28.4	30.4	28.4	30.7	29.6
Head depth	20.7	19.9	19.4	19.8	19.2	18.6	18.6	20.7	19.6
Head width	20.67	20.44	15.52	19.53	18.91	16.43	15.5	20.7	18.6
Predorsal length	74.3	74.6	73.7	75.5	75.4	74.3	73.7	75.5	74.6
Prepelvic length	55.6	53.3	51.3	52.3	52.7	49.6	49.6	55.6	52.5
Preanal length	68.4	68.5	65.7	65.1	63.6	64.3	63.6	68.5	65.9
Body depth	24.6	24.6	23.9	20.3	18.9	22.1	18.9	24.6	22.4
Body width	20.4	18.2	11.9	18.0	19.2	11.8	11.8	20.4	16.6
Length of caudal peduncle	17.9	13.5	12.8	15.9	15.5	16.8	12.8	17.9	15.4
Depth of caudal peduncle	12.0	11.6	11.3	10.9	10.6	10.4	10.4	12.0	11.1
Length of dorsal fin	31.8	29.0	31.6	17.4	18.1	18.6	17.4	31.8	24.4
Length of dorsal fin base	12.8	12.2	13.7	11.2	12.0	12.1	11.2	13.7	12.4
Length of anal fin base	22.9	23.5	23.0	20.8	21.5	19.6	19.6	23.5	21.9
Length of pectoral fin	22.9	24.6	26.3	22.1	22.3	20.7	20.7	26.3	23.2
Length of pelvic fin	10.9	10.2	12.5	19.0	18.9	19.6	10.2	19.6	15.2
% head length									
Interorbital width	36.4	39.3	35.1	37.7	41.4	34.1	34.1	41.4	37.3
Eye diameter	28.2	35.5	33.0	31.6	31.3	31.8	28.2	35.5	31.9
Snout length	34.5	37.4	34.0	36.0	38.4	29.4	29.4	38.4	35.0

*missing transverse scale count data in a female paratype carrying a cluster of eggs; the eggs hide scale rows.

straight from nape to dorsal fin. Dorsal surface of head nearly straight to slightly convex just anterior the orbit. Head small to moderate, head length 28.4–30.7% SL. Eyes moderate to small, 28.2–35.5% HL. Orbits not or only slightly projecting beyond dorsal surface of head. Caudal peduncle 1.1–1.6 times longer than deep; caudal peduncle length 12.8–17.9% SL, caudal peduncle depth 10.4–12.0% SL.

Genital papilla small and tubular in males, large, bilobed and rounded in females. Scales: 33–36 cycloid scales along lateral midline, ½14 transverse rows at dorsal fin origin. Dorsal fin with 10–12 rays, its origin at vertical through anal fin rays 8–11. Dorsal fin rounded but small, not reaching caudal base in females, rounded and long, with pronounced individual fin rays, reaching or extending caudal base in males. Anal fin with 17–18 (19) rays, short and straight to slightly concave with rounded tips in females, with pronounced individual fin rays, reaching or extending caudal base in males. Pelvic fin with 6 rays, the last ray is connected to the body by a membrane over half of its length in males, whereas such a membrane is lacking in females. Pectoral fin with 10 (9) rays, reaching to (females) or slightly beyond (males) pelvic-fin origin. Caudal fin truncate, with i,4/5,i principal caudal-fin rays; 6–7/6–8 procurrent caudal-fin rays.

Live colouration. — See Fig. 3. Body whitish grey to light yellowish-brown with greenish sheen. Belly and throat white. Males in breeding mood with 6–9 blackish bars on lateral body, clearly distinct anterior to anal fin but less conspicuous in posterior body due to blackish to black background colouration. Males in breeding mood with blackish belly. Dorsal surface of head blackish, extending posteriorly as narrow black dorsal stripe on light cream-coloured background to dorsal fin; the black dorsal stripe may fade entirely. Opercle with silver bluish sheen. Iris golden with iridescent blue sheen. Fins hyaline, rays light cream coloured. Males in brooding mood with blackish to deep black dorsal, anal and pelvic fins, and blackish caudal fin with a narrow black margin. Pectoral fin hyaline, slightly blackish in males in breeding mood.

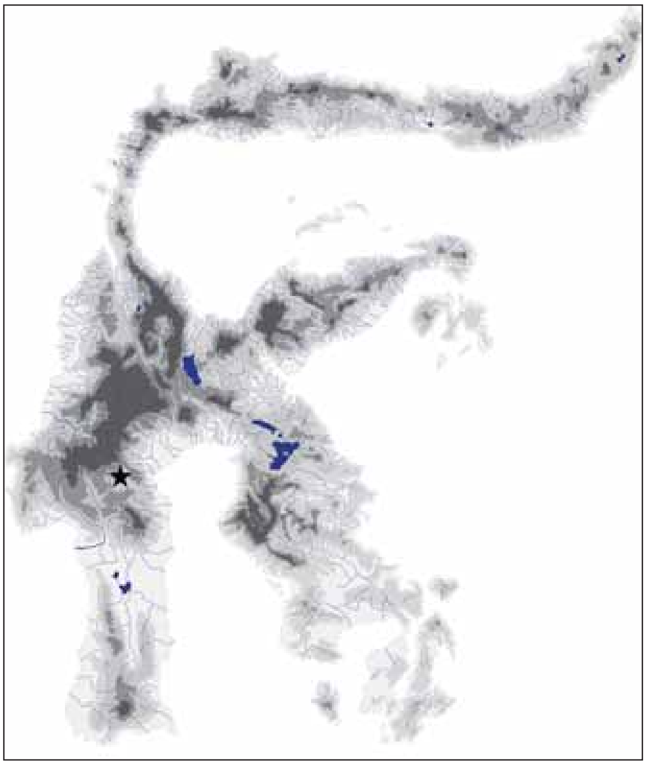

Fig. 4. Map of Sulawesi, with a black star highlighting the type locality of *Oryzias eversi* in the Toraja highlands (Map by: Thomas von Rintelen, modified).

Colouration in preservative. — See Fig. 1. In females and immature males, lateral body yellowish brown to grey, in mature males dusky grey to blackish. Males with 6–9 faint blackish bars on lateral body, females without such blackish bars or dusky grey to blackish colouration, but with a faint black lateral stripe on lateral midline, extending from uppermost posterior extremity of opercle to caudal base. Belly blackish grey in males, yellowish brown in females. Throat light yellowish brown. Dorsal surface of head brown to blackish, extending posteriorly as narrow blackish dorsal stripe to dorsal- and caudal fin. In females, fins dusky grey hyaline to light brown. In males, unpaired fins grey to blackish, with black caudal fin margins. Male pelvic fins blackish, male pectoral fins hyaline grey.

Sexual dimorphism. — Females grow slightly larger than males (maximum SL recorded: 36.2 mm in males, 38.4 mm in females) and differ in colouration in that they lack blackish breeding display or markings. Males have elongated, filamentous dorsal- and anal-fin rays (dorsal-fin length 29–31.8 in males vs. 17.4–18.6% SL in females), extending beyond caudal-fin base. Females have a pronounced abdominal concavity and significantly extended (18.9–19.6 vs. 10.2–12.5% SL) pelvic fins compared to males, together forming a "pouch" for pelvic brooding. Due to the less pronounced concavity, males have a deeper body at anal fin origin than females (23.9–24.6 vs. 18.9–22.1% SL), and also a deeper caudal peduncle (11.3–12 vs. 10.4–10.9% SL). The last ray of the pelvic fin is connected to the body by a membrane on half of its length in males, whereas such a membrane is lacking in female *O. eversi*. Preliminary field and aquarium observations suggest that adult males in breeding mood defend territories, but females form small schools in the natural habitat.

Fig. 5. Type locality of *Oryzias eversi*, about 8 km south of Rantepao, Tana Toraja, Central_Sulawesi, Indonesia. (Photograph by: Hans-Georg Evers).

Reproduction**.** — *Oryzias eversi* is a "pelvic brooder", a term coined by Kottelat (1990a) for the lake-dwelling *Oryzias* (*Xenopoecilus*) *oophorus* from Lake Poso in Central Sulawesi, which carry clusters of eggs accommodated in a belly concavity until they hatch. Female *O. eversi* were observed in the field and in aquaria to carry developing eggs and a preserved female (MZB 20781; compare Fig. 3B) carried about 30 eggs of approx. 1.4 mm diameter, with almost fully developed and partially pigmented embryos. Under aquarium conditions (24–25°C), females carry eggs until they hatch after 18–19 days. Aquarium observations showed that the females never deposit eggs on a substrate at all, even if various kinds of spawning substrate are available. Moreover, females change behaviour during brooding and hide among plants during the gestation period. The elongated pelvic fins apparently hold the egg clutch in position in the pronounced belly concavity, strongly resembling other known or suspected pelvic-brooding ricefishes (Iwamatsu et al., 2007). Like in *A. oophorus* and *O. sarasinorum* (Kottelat, 1990a; Iwamatsu et al., 2008), the eggs do not adhere to each other, and are suspended by attaching filaments to the female's urogenital pore. After hatching, the attaching filaments protrude out of the female's urogenital pore for some time (Fig. 3C).

Distribution and habitat**.** — *Oryzias eversi* is known to date only from the type locality in Tana Toraja, Central Sulawesi (Fig. 4). The type locality is an up to approx. 4 m deep karst pond of 30–40 m length and up to 10 m width, used by local people as natural "swimming pool" (Fig. 5). The water is calm and crystal clear, and had a water temperature of 21.5°C in Sep.2010. The pond has a single in- and outflow and is surrounded by rain forest. Sympatic fish species included native species of *Nomorhamphus* and introduced *Poecilia reticulata*. At time of sampling, adult *Oryzias eversi* were rare but juveniles were rather abundant. Males are solitary, whereas females occur in groups and inhabit the shallow habitat margins characterised by dense vegetation.

Etymology**.** — The specific name, *eversi*, honours Hans-Georg Evers who discovered this endemic ricefish while travelling to explore fishes and habitats in Sulawesi.

Comparisons**.** — *Oryzias eversi* is clearly distinguished by non-overlapping (33–36) lateral scale counts from *Adrianichthys* (Lake Poso, including "*Xenopoecilus*" *oophorus* and "*X.*" *poptae*; >62), *O. orthognathus* (Lake Poso, >44), *O. sarasinorum* (Lake Lindu, 70–75), *O. matanensis* (Lake Matano, >40) and *O. marmoratus* (Malili Lakes: Towuti, Mahalona, Lontoa, streams, 31–32) (Kottelat, 1990b; Parenti, 2008). Lateral scale counts overlap with *O. profundicola* (Lake Towuti, 32–34), *O. bonneorum* (36–39), *O. nebulosus* (32–36), *O. nigrimas* (34–37), *O. hadiatyae* (Lake Masapi, 27–31) and *O. woworae* (Muna Island, 30–33). *Oryzias eversi* has fewer (17–19) anal-fin rays than *O. orthognathus* (23–25), *O. nigrimas* (21–25), *O. nebulosus* (21–22), *O. matanensis* (20–25), *O. profundicola* (26–29), *O. marmoratus* (20–26), *A. oophorus* (20–22), *A. poptae* (24–26(27)), *O. sarasinorum* (21–23), *A. roseni* (25–26), and *A. kruyti* (24–25). Female *Oryzias eversi* shares with *Adrianichthys oophorus*, *A. poptae*, *A. roseni*, and *O. sarasinorum* an abdominal concavity between the pelvic fins and anal fin to carry the developing eggs (Parenti, 2008), a character absent in other ricefish species from Sulawesi. It is however distinguished from *Adrianichthys* by smaller adult size (largest adult specimen recorded: 38.4 mm SL, vs. adult size of nearly 200 mm SL) and by having only 30–32(33) total vertebrae (vs. 36–37; Parenti, 2008).

Oryzias eversi differs from *O. hadiatyae* among other characters in having more (½14 vs. ½10–12) transverse rows of lateral scales at dorsal-fin origin. *Oryzias eversi* lacks the pronounced concavity on the snout (vs. present in *O. hadiatyae*), has relatively longer pelvic fins in females (18.9–19.6 vs. 9.8–15.4% SL in *O. hadiatyae*), is characterised by rounded dorsal and anal fins with pronounced individual fin rays in males (vs. pointed dorsal fin and rather short anal fin, both without conspicuous individual rays in *O. hadiatyae*), and shows clear marginal stripes on the caudal fin (vs. absent in *O. hadiatyae*). From *O. celebensis*, *O. eversi* is distinguished by having more dorsal fin rays (10–12 vs. 8–10; Parenti, 2008, Herder & Chapuis, 2010), relatively smaller eyes (23.0–35.5 vs. 36.1–45.9% HL; Herder & Chapuis, 2010), conspicuously (vs. slightly) filamentous dorsal- and anal-fin rays in males, and presence (vs. absence) of blackish courtship colouration in adult males. Compared to *O. bonneorum*, *O. eversi* has among other characters less principal (i,4/5,i vs. i,5/6,i) and more procurrent (dorsal 6–7, ventral 6–8 vs. dorsal 5, ventral 5–6) caudal fin rays, less rays in dorsal- (10–12 vs. 12–13), anal- (17–18(19) vs. 19–20) and pectoral fin (10 vs. 11–12), and no pronounced abdominal concavity. *Oryzias eversi* is distinguished from *O. woworae* by its larger adult size (up to 38.4 mm vs. 28.0 mm SL; Parenti & Hadiaty, 2010), presence (vs. absence) of an abdominal concavity, and by features of adult male breeding colour pattern, most conspicuously the absence (vs. presence) of steel blue body colouration and absence (vs. presence) of red colouration in ventral surface of head and body anterior to pelvic fins, dorsal portion of pectoral fins, bases of dorsal and anal fin, and margins of caudal fin in both sexes.

Phylogenetic relationships**.** — The model that best reflected the evolutionary divergence of the 16s rDNA sequences (SYM+I+G) was selected by MrModeltest 2.3 and was used to infer the most likely Bayesian phylogeny (Fig. 6). The resulting Bayesian phylogeny is largely congruent with the consensus tree of Takehana et al. (2005) in all well supported branches, including monophyly of all known *Oryzias* from Sulawesi (the "*Oryzias celebensis* species group", distinguished also by karyological characters; Takehana et al., 2005). In agreement with shared pelvic brooding ecology and associated morphological characters, the present analyses clearly support a sister group relationship between *O. eversi* and *O. sarasinorum* from Lake Lindu in Central Sulawesi. These two pelvic brooders are more closely related to a number of egg-depositing species (i.e., *Oryzias matanensis*, *O. marmoratus*, *O. profundicola*, and *O. hadiatyae* endemic to the Mallili lakes, *O. nigrimas* from Lake Poso, and the riverine species *O. celebensis*, *O. woworae* and the hitherto undescribed *O.* cf. *woworae* [Parenti et al., in prep.]) than to the only other pelvic brooder (*Adrianichthys oophorus*) included in our analyses. The phylogenetic relationships among the egg-depositing taxa is not fully resolved and warrants further study. The Malili lakes endemics (*Oryzias matanensis*, *O. marmoratus*, *O. profundicola*, *O. hadiatyae*) share closely related haplotypes across species, including some individuals of *O. celebensis* from Larohea. The latter is a recently discovered ricefish population from a drainage west of Lake Matano in Central Sulawesi fitting the morphological concept of *O. celebensis*; this species was known until recently only from the south-western arm of the island (Herder & Chapuis, 2010). Conspicuously however, some of the *O. celebensis* from Larohea carry very distinct mitochondrial haplotypes most closely related to *O. sarasinorum* from Lake Lindu and to *O. eversi*.

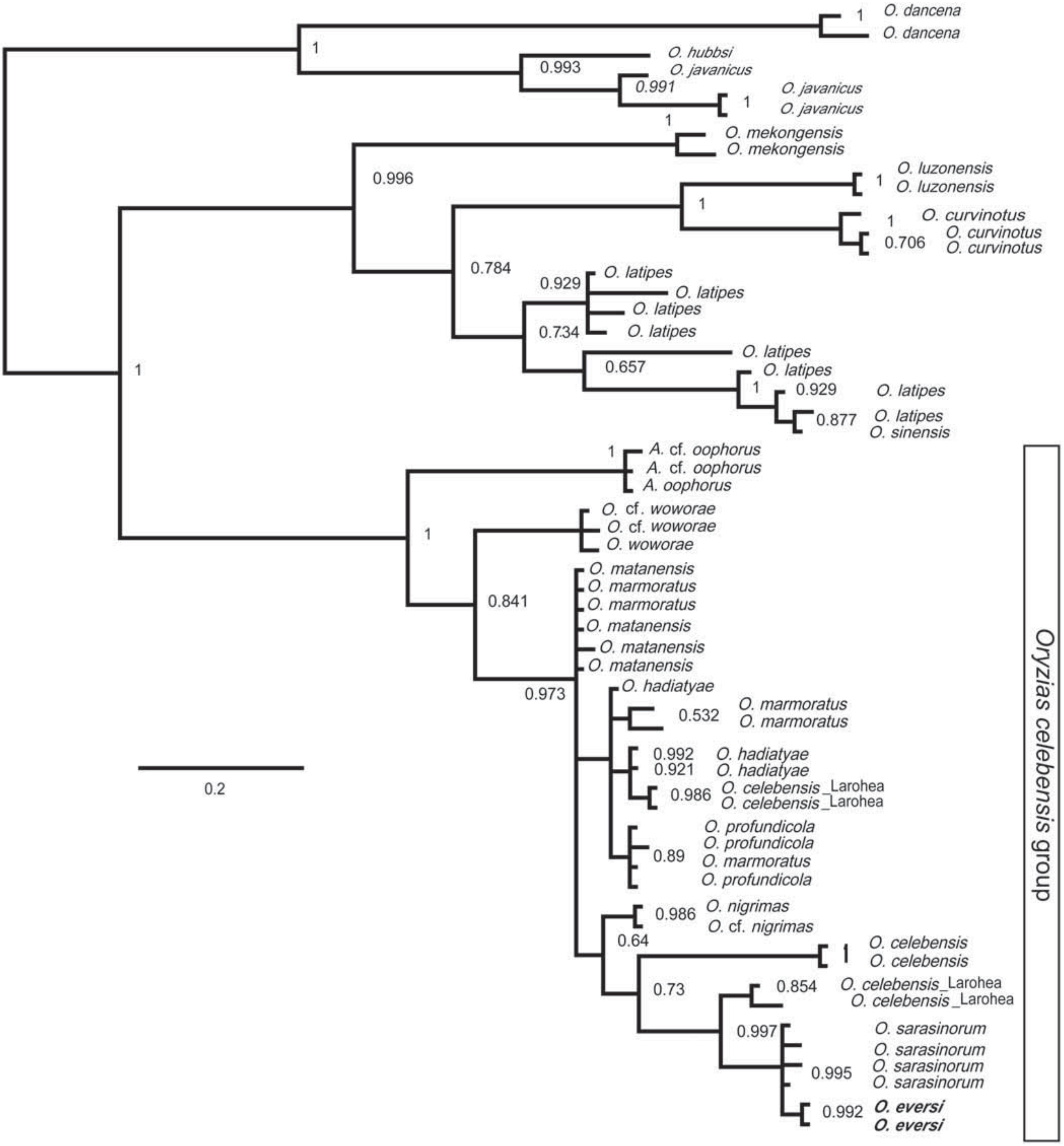

Fig. 6. Bayesian phylogeny of extant ricefishes based on 16s rDNA sequences. Scale bar indicates evolutionary distance and numbers at nodes represent posterior probabilities. The endemic radiation of ricefishes from Sulawesi is referred to as *Oryzias celebensis* group; *Oryzias eversi* specimens are highlighted.

DISCUSSION

Paternal care by pelvic brooding likely enhances offspring survival, which comes however at the cost of retarded oogenesis during embryo development, as demonstrated in the pelvic brooder *O. sarasinorum* (Iwamatsu et al., 2007, 2008). In lacustrine environments like in Lakes Poso and Lindu, pelvic brooding might also be a pathway for developing predominantly pelagic modes of reproduction, and for avoiding competition and predation at inshore habitats. In the stream dwelling *Oryzias eversi*, pelvic brooding may have evolved in response to egg-predation or other factors affecting survival of embryos developing at spawning substrates. The occasional occurrence of prolonged phases of egg-carrying in several ricefish species (Parenti, 2008), deviating from the common pattern of egg deposition after some few hours after fertilisation, suggests that there is potential for the rapid evolution of pelvic brooding in different lineages of the Adrianichthyidae. Interestingly, pelvic brooding in *O. eversi* is correlated with the same set of morphological adaptations observed also in female *A. oophorus* and *O. sarasinorum*, namely a pronounced abdominal concavity and extended pelvic fins (Iwamatsu et al., 2007; Parenti, 2008). In all three species, the pelvic fins cover the cluster of eggs carried in the abdominal concavity, attached to the female's urogenital pore by attaching filaments projecting from each egg. Abdominal concavities and elongated pelvic fins also characterise two other ricefish species (*A. poptae* and *A. roseni*; Parenti, 2008), but data on their reproductive biology is unavailable. Taken together, pelvic brooding occurs or is likely to occur in at least three (*A. oophorus*, *A. poptae*, *A. roseni*) ricefish species from Lake Poso, and one (*O. sarasinorum*) from Lake Lindu. In agreement with the morphological characters and the observed reproductive behavior of live fish, the mitochondrial haplotype phylogeny (Fig. 6) clearly revealed *O. eversi* and *O. sarasinorum* as sister taxa. Phylogenetic analyses by Takehana et al. (2005) and Parenti (2008) already suggested that pelvic brooding has evolved independently in *Adrianichthys* and in *Oryzias* in Sulawesi, a finding that is in full agreement with the present results.

Intriguingly, there is some evidence for haplotype sharing among morphologically well-defined egg depositing *Oryzias* species from lakes and streams across Sulawesi. We have previously described a case where hybridisation has blurred the relationships between gene trees and species trees of stream- and river-dwelling sailfin silversides (Herder et al., 2006) from the Mallili Lakes system, and it appears possible that similar processes also obscure the phylogenetic relationships among Sulawesi's ricefishes. It remains to be tested to what extent Sulawesi's endemic ricefishes, in particular the lineage that gave rise to *O. eversi* and *O. sarasinorum*, have evolved under the influence of hybridisation. To this end, *O. eversi* from Tana Toraja is the first record of a ricefish species characterised by obligate pelvic brooding in a stream habitat of Sulawesi. The case of *O. eversi* illustrates that pelvic brooding is not necessarily an adaptation to pelagic ecology in freshwater lakes, as it is the case in all other known pelvic brooders. This unexpectedly broad ecological potential might facilitate contact of lake- and stream-dwelling species, and may have facilitated reticulate evolution.

As several ricefish species new to science have been reported from Sulawesi during the last decade (e.g., Parenti & Soeroto, 2004; Parenti, 2008; Herder & Chapuis, 2010; Parenti & Hadiaty, 2010), and systematic surveys of the islands freshwaters are lacking in most areas, additional discoveries are not unlikely. *Oryzias eversi* was rare at the time of sampling in 2010, and is so far known only from the type locality. The new species might therefore be rare and potentially endangered in the wild. However, further investigations are required before its actual population sizes and distribution can be estimated.

Comparative material. — *Oryzias celebensis*, *O. hadiatyae*, *O. matanensis*, *O. marmoratus*, and *O. profundicola* as listed in Herder & Chapuis 2010; *Oryzias nigrimas*: ZFMK 41789, 30.4 mm SL, Indonesia, Sulawesi: Lake Poso near Tentena; A. Koch & E. Arida, 22 Aug.2007; *Oryzias* (*Xenopoecilus*) *oophorus*: ZFMK 41779–41783, 63.5–70.6 mm SL (incl. a female carrying eggs: 41781, 70.6 mm SL), Indonesia, Sulawesi: Lake Poso near Tentena; coll. A. Koch & E. Arida, 24 Aug.2007; *Oryzias* (*Xenopoecilus*) cf. *oophorus*: ZFMK 41784–41787, 60.8–74.8 mm SL, Indonesia, Sulawesi: Lake Poso near Tentena; coll. A. Koch & E. Arida, 24 Aug.2007; *Oryzias* (*Xenopoecilus*) cf. *oophorus*: ZFMK 41778, 62.4 mm SL, Indonesia, Sulawesi: Lake Poso near Tentena; coll. A. Koch & E. Arida, 24 Aug.2007; *Oryzias* cf. *orthognathus*: ZFMK 41788, 34.5 mm SL, Indonesia, Sulawesi: Lake Poso near Tentena; coll. A. Koch & E. Arida, 24 Aug.2007; *Oryzias* (*Xenopoecilus*) *sarasinorum*: ZFMK 41791–41800, 19.5–22.0 mm SL, Indonesia, Sulawesi: Lake Lindu at Rono, Desa Tomado, 19 Aug.2007; *Oryzias woworae*: MZB 15398 (holotype), male, 25.0 mm SL, Indonesia, Sulawesi Tenggara, Muna Island, Regency of Muna, District Parigi, Village Wakumoro, Mata air Fotuno, 5°04'39.7"S, 122°30'25.7"E, 13 Sep.2007, coll. D. Wowor; MZB 15397 (paratypes), 36 ind., 15.0–27.0 mm SL, same data as holotype. MZB 15399, 26 ind., 14.6–23.6 mm SL, same data as MZB 15398; *Oryzias* spez. affin. *woworae*: ZFMK 42955–42970, Indonesia, Sulawesi: Sumberasi waterfall near Kendari, 40°13'540"S, 122°44'169"E, 297 feet elev.; coll. H.-G. Evers, F. Evers, J. Ch. Panaddy, 25 Sep.2009.

ACKNOWLEDGEMENTS

We thank the Indonesian Institute of Sciences (LIPI) for the permit to conduct research in Indonesia. We are grateful to Hans-Georg Evers for providing the specimens of the species described herein, and for information on habitat and reproductive biology. We thank D. Schaller, Rainer Sonnenberg, and U. K. Schliewen for access to comparative material and for inspiring ricefish discussions, and Elke Bustorf and Serkan Güse for technical assistance. E. Arida and A. Koch collected comparative material in Sulawesi. Lynne Parenti is acknowledged for advice and for providing references. T. Huylebrouck helped improving graphics. Comments and suggestions by K. Conway and an anonymous referee helped improving the manuscript.

LITERATURE CITED

Asai, T., H. Senou & K. Hosoya, 2011. *Oryzias sakaizumii*, a new ricefish from northern Japan (Teleostei: Adrianichthyidae). *Ichthyological Exploration of Freshwaters*, **22**: 289–299.

Hall, T. A., 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, **41**: 95–98.

Herder, F. & S. Chapuis, 2010. *Oryzias hadiatyae*, a new species of ricefish (Atherinomorpha: Beloniformes: Adrianichthyidae) endemic to Lake Masapi, Central Sulawesi, Indonesia. *Raffles Bulletin of Zoology*, **58**: 269–280.

Herder, F., A. W Nolte, J. Pfaender, J. Schwarzer, R. K. Hadiaty & U. K. Schliewen, 2006. Adaptive radiation and hybridization in Wallace's Dreamponds: Evidence from sailfin silversides in the Malili Lakes of Sulawesi. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **273**: 2209–2217.

Iwamatsu, T., H. Kobayashi, Y. Shibata, M. Sato, N. Tsuji & K. Takakura, 2007. Oviposition cycle in the oviparous fish *Xenopoecilus sarasinorum*. *Zoological Science*, **24**: 1122–1127.

Iwamatsu, T., H. Kobayashi, M. Sato & M. Yamashita, 2008. Reproductive role of attaching filaments on the egg envelope in *Xenopoecilus sarasinorum* (Adrianichthidae [sic], Teleostei). *Journal of Morphology*, **269**: 745–750.

Kinoshita, M., K. Murata, K. Naruse & M. Tanaka, 2009. *Medaka: Biology, Management, and Experimental Protocols.* Wiley-Blackwell, Ames. 444 pp.

Kottelat, M., 1990a. Synopsis of the endangered buntingi (Osteichthyes: Adrianichthyidae and Oryziidae) of Lake Poso, Central Sulawesi, Indonesia, with a new reproductive guild and descriptions of three new species. *Ichthyological Exploration of Freshwaters*, **1**: 49–67.

Kottelat, M., 1990b. The ricefishes (Oryziidae) of the Malili Lakes, Sulawesi, Indonesia, with description of a new species. *Ichthyological Exploration of Freshwaters*, **1**: 151–166.

Magtoon, W., 2010. *Oryzias songkhramensis*, a new species of ricefish (Beloniformes; Adrianichthyidae) from Northeast Thailand and Central Laos. *Tropical Natural History*, **10**: 107–129.

Nylander, J. A. A., 2004. *MrModeltest v2. Program Distributed by the Author.* Evolutionary Biology Centre, Uppsala University.

Parenti, L. R., 2005. The phylogeny of atherinomorphs: Evolution of a novel reproductive system. In: Uribe, M. C. & H. J. Grier (eds.), *Viviparous Fshes: Proceedings of the I and II International Symposia on Livebearing Fishes.* New Life Press, Homestead, FL. Pp. 13–30.

Parenti, L. R., 2008. A phylogenetic analysis and taxonomic revision of ricefishes, *Oryzias* and relatives (Beloniformes, Adrianichthyidae). *Zoological Journal of the Linnean Society*, **154**: 494–610.

Parenti, L. R. & B. Soeroto, 2004. *Adrianichthys roseni* and *Oryzias nebulosus*, two new ricefishes (Atherinomorpha: Beloniformes: Adrianichthyidae) from Lake Poso, Sulawesi, Indonesia. *Ichthyological Research*, **51**: 10–19.

Parenti, L. R. & R. K. Hadiaty, 2010. A new, remarkably colorful, small ricefish of the genus *Oryzias* (Beloniformes, Adrianichthyidae) from Sulawesi, Indonesia. *Copeia*, **2010**: 268–273.

Roberts, T. R., 1998. Systematic observations on tropical Asian medakas or ricefishes of the genus *Oryzias*, with descriptions of four new species. *Ichthyological Research*, **45**: 213–224.

Ronquist, F., M. Teslenko, P. van der Mark, D. Ayres, A. Darling, S. Höhna, B. Larget, L. Liu, M. A. Suchard & J. P. Huelsenbeck, in press. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology.*

Rosen, D. E. & L. R. Parenti, 1981. Relationships of Oryzias, and the groups of atherinomorph fishes. *American Museum Novitates*, **2719**: 1–25.

Takehana, Y., K. Naruse & M. Sakaizumi, 2005. Molecular phylogeny of the medaka fishes genus *Oryzias* (Beloniformes: Adrianichthyidae) based on nuclear and mitochondrial DNA sequences. *Molecular Phylogenetics and Evolution*, **36**: 417–428.

Appendix 1. List of species included in phylogenetic analysis, including voucher specimens and GenBank accession numbers.

Species	**Voucher specimen**	**GenBank accession number**	**Origin**
Adrianichthys oophorus	ZFMK 41787	JX128118	this study
Adrianichthys oophorus	No voucher	gi_62005741	GenBank
Adrianichthys cf. *oophorus*	ZFMK 41786	JX128123	this study
Oryzias nigrimas	No voucher	gi_62005739	GenBank
Oryzias celebensis	ZFMK 47672	JX128107	this study
Oryzias celebensis	ZFMK 41557	JX128124	this study
Oryzias celebensis	ZFMK 41559	JX128125	this study
Oryzias celebensis	MZB 18518	JX128126	this study
Oryzias celebensis	No voucher	gi_62005718	GenBank
Oryzias celebensis	No voucher	gi_253960477	GenBank
Oryzias cf. *nigrimas*	ZFMK 41789	JX128112	this study
Oryzias cf. *woworae*	ZFMK 42956	JX128121	this study
Oryzias cf. *woworae*	ZFMK 42959	JX128122	this study
Oryzias curvinotus	No voucher	gi_283379405	GenBank
Oryzias curvinotus	No voucher	gi_62005720	GenBank
Oryzias curvinotus	No voucher	gi_62005719	GenBank
Oryzias dancena	No voucher	gi_261348399	GenBank
Oryzias dancena	No voucher	gi_253960463	GenBank
Oryzias eversi	ZFMK 47678	JX128120	this study
Oryzias eversi	No voucher	JX128128	this study
Oryzias hadiatyae	ZFMK 47675	JX128108	this study
Oryzias hadiatyae	ZFMK 47677	JX128110	this study
Oryzias hadiatyae	ZFMK 47673	JX128119	this study
Oryzias hubbsi	No voucher	gi_62005723	GenBank
Oryzias javanicus	No voucher	gi_62005725	GenBank
Oryzias javanicus	No voucher	gi_261348413	GenBank
Oryzias javanicus	No voucher	gi_253960435	GenBank
Oryzias latipes	No voucher	gi_284010098	GenBank
Oryzias latipes	No voucher	gi_317176277	GenBank
Oryzias latipes	No voucher	gi_283379404	GenBank
Oryzias latipes	No voucher	gi_283379402	GenBank
Oryzias latipes	No voucher	gi_283379401	GenBank
Oryzias latipes	No voucher	gi_283379398	GenBank
Oryzias latipes	No voucher	gi_84579629	GenBank
Oryzias latipes	No voucher	gi_84579587	GenBank
Oryzias luzonensis	No voucher	gi_62005732	GenBank
Oryzias luzonensis	No voucher	gi_253960393	GenBank
Oryzias marmoratus	ZFMK 41812	JX128114	this study
Oryzias marmoratus	ZFMK 41807	JX128116	this study
Oryzias marmoratus	No voucher	gi_42540604	GenBank
Oryzias marmoratus	No voucher	gi_42540603	GenBank
Oryzias marmoratus	No voucher	gi_62005733	GenBank
Oryzias matanensis	ZFMK 41830	JX128115	this study
Oryzias matanensis	No voucher	gi_11119799	GenBank
Oryzias matanensis	No voucher	gi_11119800	GenBank
Oryzias matanensis	No voucher	gi_62005734	GenBank
Oryzias mekongensis	No voucher	gi_62005735	GenBank
Oryzias mekongensis	No voucher	gi_62005736	GenBank
Oryzias profundicola	ZFMK 41819	JX128109	this study
Oryzias profundicola	ZFMK 41820	JX128113	this study
Oryzias profundicola	No voucher	gi_62005740	GenBank
Oryzias sarasinorum	ZFMK 41797	JX128106	this study
Oryzias sarasinorum	ZFMK 41799	JX128111	this study
Oryzias sarasinorum	ZFMK 41795	JX128117	this study
Oryzias sarasinorum	No voucher	gi_197304232	GenBank
Oryzias sinensis	No voucher	gi_261348385	GenBank
Oryzias woworae	No voucher	JX128127	this study