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## Two New Ricefishes of the Genus *Oryzias* (Atherinomorpha: Belontiiformes: Adrianichthyidae) Augment the Endemic Freshwater Fish Fauna of Southeastern Sulawesi, Indonesia

Lynne R. Parenti<sup>1</sup>, Renny K. Hadiaty<sup>2</sup>, Daniel Lumbantobing<sup>1,3</sup>,  
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# Two New Ricefishes of the Genus *Oryzias* (Atherinomorpha: Beloniformes: Adrianichthyidae) Augment the Endemic Freshwater Fish Fauna of Southeastern Sulawesi, Indonesia

Lynne R. Parenti<sup>1</sup>, Renny K. Hadiaty<sup>2</sup>, Daniel Lumbantobing<sup>1,3</sup>, and Fabian Herder<sup>4</sup>

*Oryzias asinua* and *O. wolasi* are two new species of ricefishes described from several disjunct inland freshwater habitats in the Indonesian province of Sulawesi Tenggara or southeastern Sulawesi. With *O. woworae*, the first described endemic ricefish of Sulawesi Tenggara, they comprise a group of small, colorful species characterized by orange to deep red dorsal and ventral margins of the caudal fin and the ventral margin of the caudal peduncle and at least the posterior portion of the base of the anal fin, and a bluish sheen on the body in both sexes that is most pronounced in live adult males. The two new species are distinguished from *O. woworae* by elongate middle dorsal-fin rays in adult males that reach the posterior extent of the first principal caudal-fin ray and by an orange-colored olfactory epithelium that marks each nasal organ in females. *Oryzias asinua* is relatively slender compared to *O. wolasi* and *O. woworae*: the body is narrow (21–25% SL, with a mean 22.9, in *O. asinua* versus 23–32% SL, mean 25.3 in *O. wolasi* and 22–30% SL, mean 26 in *O. woworae*). *Oryzias asinua* has fewer procurent caudal-fin rays in the lower lobe (4–5 versus 5–7 in *O. wolasi* and 5–6 in *O. woworae*). *Oryzias wolasi* is golden in life in both sexes and relatively deep-bodied, reaching 32% SL, and has a deeper caudal peduncle (11–12% SL, mean 11.2, versus 9–11, mean 10, in *O. asinua* and 8–11, mean 9.2, in *O. woworae*). Maximum parsimony and Bayesian inference analyses of the mitochondrial cytochrome c oxidase subunit 1 (COI) sequence, the DNA barcode, support our hypothesis that we sequenced representatives of three species. All species are allopatric. Description of *O. asinua* and *O. wolasi* brings the number of valid species in the beloniform family Adrianichthyidae to 35, 17 of which are endemic to Sulawesi. The two new species are just the second and third ricefishes described from the province of Sulawesi Tenggara, a neglected region in exploration and discovery of the freshwater fish fauna of the Indonesian islands of Sulawesi.

*Oryzias asinua* dan *O. wolasi* adalah dua jenis ikan padi yang dideskripsi dari beberapa, habitat air tawar di Provinsi Sulawesi Tenggara, Indonesia. Bersama *O. woworae*, ikan padi pertama dari Sulawesi Tenggara yang telah dideskripsi, ketiganya merupakan kelompok jenis berukuran kecil, berpola warna menarik, jingga hingga merah tua pada sirip punggung, tepi sirip ekor bagian bawah, tepi bawah batang ekor dan pada sedikit bagian belakang dari pangkal sirip anal, semburat sinar kebiruan terlihat pada tubuh ikan jantan dan betina, yang tampak jelas terlihat pada pada ikan jantan dewasa yang masih hidup. Ke dua spesies baru tersebut dapat dibedakan dari *O. woworae* dari jari-jari lemah sirip punggung bagian tengah pada ikan jantan dewasa, yang memanjang dan mencapai bagian posterior sirip ekor pertama, juga dari warna jingga pada *epithelium olfactory* yang menandai setiap organ nasal pada betina. *Oryzias asinua* tinggi tubuhnya relatif lebih kecil dibandingkan *O. wolasi* dan *O. woworae*: tubuhnya pendek (21–25% SL, rata-rata 22.9% pada *O. asinua* vs 23–32% SL, rata-rata pada *O. wolasi* dan 22–30% SL, rata-rata 26% pada *O. woworae*). *Oryzias asinua* memiliki lebih sedikit jari lemah tambahan pada cuping ekor bagian bawah (4–5 vs 5–7 pada *O. wolasi* dan 5–6 pada *O. woworae*). Pada saat masih hidup *Oryzias wolasi* berwarna keemasan baik pada jantan dan betina, tubuhnya relatif tinggi, mencapai 32% SL, dan batang ekor lebih tinggi (11–12% SL, rata-rata 11.2 vs 9–11, rata-rata 10, pada *O. asinua* dan 8–11, rata-rata 9.2, pada *O. woworae*). Maksimum parsimony dan analisa Bayesian inference pada sekuens mitochondrial cytochrome c oxidase subunit 1 (COI) atau sekuens DNA barcode selaras dengan hipotesis kami yang mensekuens sampel dari ketiga spesies tersebut. Ketiga spesies ini bersifat allopatrik. Dengan deskripsi dari *O. asinua* dan *O. wolasi* maka spesies valid pada beloniform famili Adrianichthyidae menjadi 35, 17 diantaranya endemik di Sulawesi. Dua spesies baru adalah ikan padi kedua dan ketiga yang dideskripsikan dari Provinsi Sulawesi Tenggara, wilayah yang terabaikan dari eksplorasi dan penemuan fauna ikan air tawar di Sulawesi, salah satu dari kepulauan di Indonesia.

THE extraordinarily colorful ricefish, *Oryzias woworae*, was discovered on Muna Island, Sulawesi Tenggara, by Indonesian carcinologist Daisy Wowor in 2007 and described in 2010 (Fig. 1). The discovery was notable for several reasons: ricefishes, the family Adrianichthyidae, had not been reported previously from the geologically distinct province of Sulawesi Tenggara; the new species was abundant, not rare; and, the new species was distinguished

by a vivid blue and red color pattern in life in both sexes (Parenti and Hadiaty, 2010).

That there had been no earlier scientific reports of ricefishes from Sulawesi Tenggara is odd. Sulawesi is home to an estimated 60 endemic freshwater fish species, 48 of which are atherinomorphs, including the two new species described herein (Parenti, 2011) and two recently described species of ricefish (Herder et al., 2012) and one halfbeak

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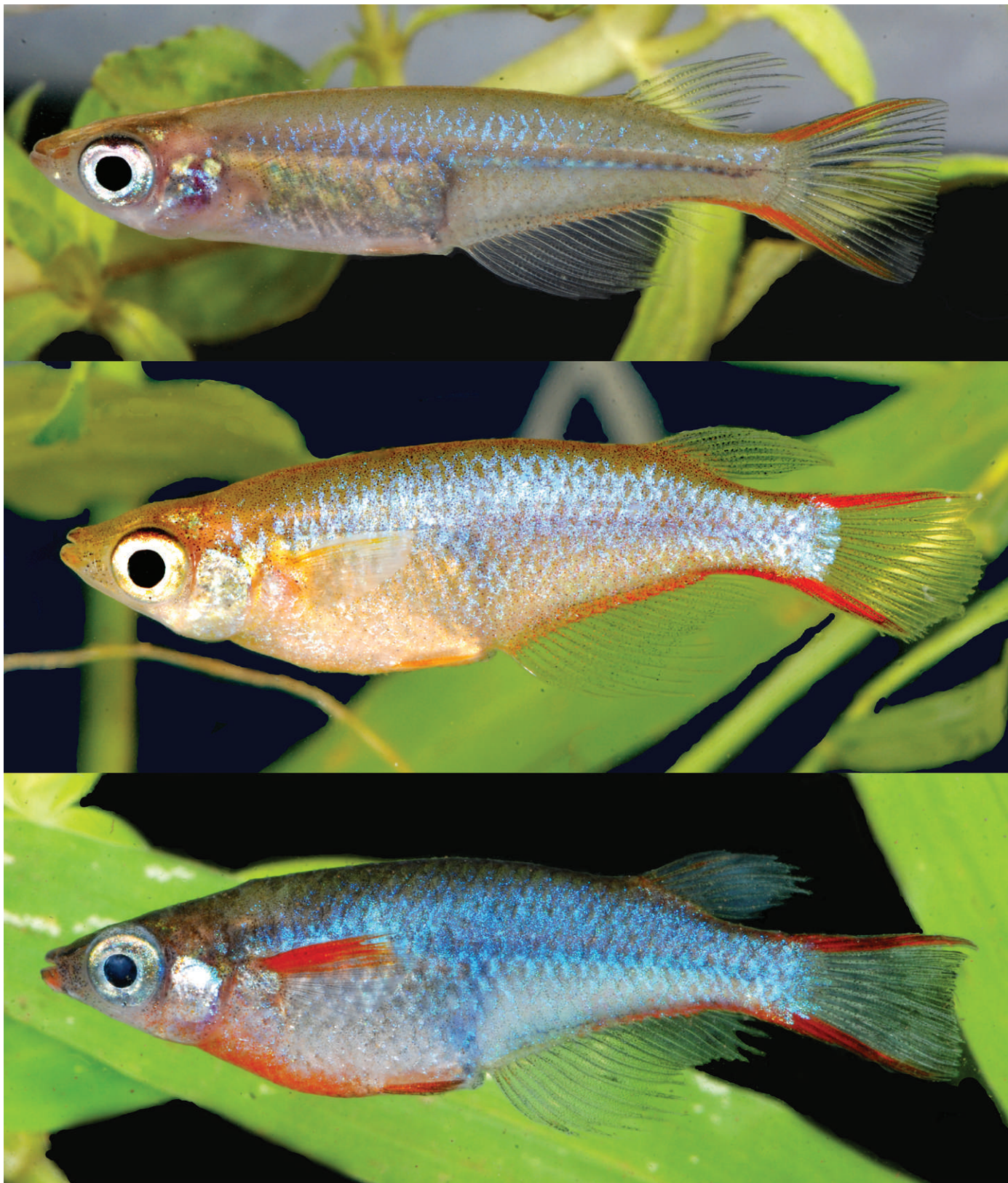
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**Fig. 1.** Live adult males, photographed just after capture, of the three described ricefishes from Sulawesi Tenggara. Top: *Oryzias asinua*, new species, paratype, USNM 406788. Middle: *O. wolasi*, new species, paratype, USNM 403660. Bottom: *Oryzias woworae*, topotype, USNM 399429. All individuals are approximately 24–25 mm SL.

(Huylebrouck et al., 2012). Sulawesi harbors nearly half of ricefish species diversity: 19 of the 35 valid recent atherinomorphic ricefishes live in Sulawesi and 17 are endemics (Parenti, 2011; Table 1). Perhaps because of the allure of the

central tectonic lake system (Vaillant et al., 2011) and its species flocks of ricefishes and atheriniform telmatherinids (Kottelat, 1990a, 1990b, 1990c, 1991; Herder et al., 2006; Herder and Chapuis, 2010), scientific collection of freshwa-



**Table 1.** Nineteen Valid Ricefishes of Sulawesi (Parenti, 2008, 2011). Sulawesi Province is given for endemic species. See Parenti (2008) for species description references.

Genus *Adrianichthys*

- A. kruyti* Weber, 1913 (Sulawesi Tengah)
- A. oophorus* (Kottelat, 1990a) (Sulawesi Tengah)
- A. poptae* (Weber and de Beaufort, 1922) (Sulawesi Tengah)
- A. roseni* Parenti and Soeroto, 2004 (Sulawesi Tengah)

Genus *Oryzias*

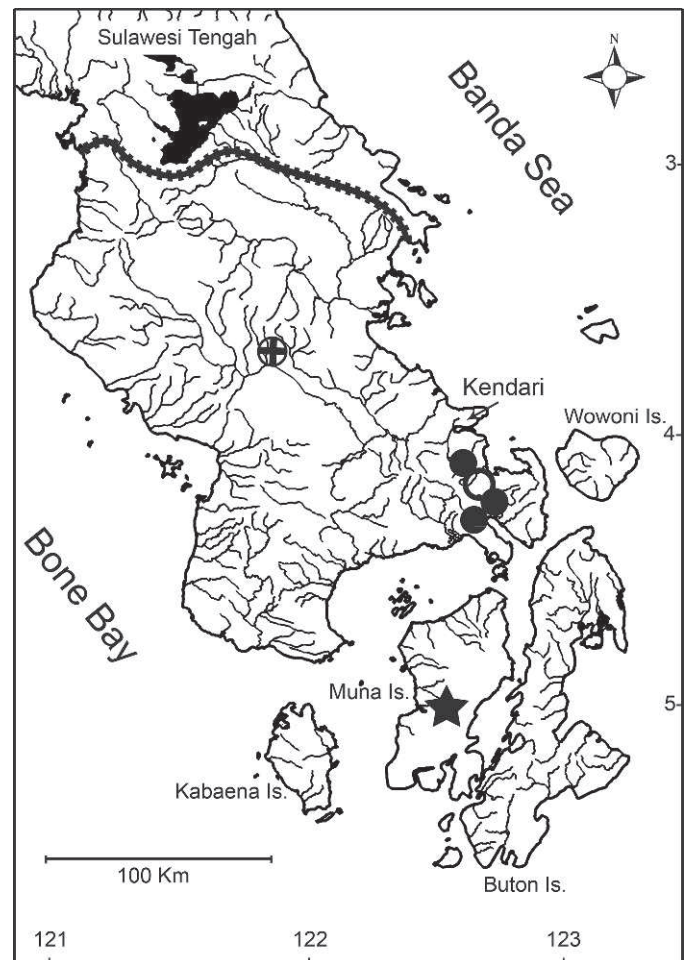
- O. asinua*, new species (Sulawesi Tenggara)
- O. bonneorum* Parenti, 2008 (Sulawesi Tengah)
- O. hadiatyae* Herder and Chapuis, 2010 (Sulawesi Selatan)
- O. eversi* Herder, Hadiaty and Nolte, 2012 (Sulawesi Selatan)
- O. marmoratus* (Aurich, 1935) (Sulawesi Selatan)
- O. matanensis* (Aurich, 1935) (Sulawesi Selatan)
- O. nebulosus* Parenti and Soeroto, 2004 (Sulawesi Tengah)
- O. nigrimas* Kottelat, 1990a (Sulawesi Tengah)
- O. orthognathus* Kottelat, 1990a (Sulawesi Tengah)
- O. profundicola* Kottelat, 1990b (Sulawesi Selatan)
- O. sarasinorum* (Popta, 1905) (Sulawesi Tengah)
- O. wolasi*, new species (Sulawesi Tenggara)
- O. woworae* Parenti and Hadiaty, 2010 (Sulawesi Tenggara)
- O. celebensis* (Weber, 1894) (Sulawesi and Timor)
- O. javanicus* (Bleeker, 1854) (broadly throughout Malay Peninsula and the Indo-Australian Archipelago)

ter fishes in Sulawesi has been focused on Sulawesi Selatan (southern Sulawesi) and Sulawesi Tengah (central Sulawesi), while the other provinces have been relatively neglected.

Although some ricefishes have bold strokes of yellow, blue, or even red, in life, they are generally known to be pallid (Seegers, 1997). Notice of the new, small colorful ricefish species from Muna has spread rapidly through the aquarium hobby (Walters, 2011), and possibly more new species from Sulawesi Tenggara have been documented (Evers et al., 2010). The discovery of *O. woworae* and, with it, the expectation that more endemic species await description prompted a joint field trip by US and Indonesian ichthyologists to Sulawesi Tenggara in June 2010 to carry out a preliminary survey of freshwater and coastal fishes. The Indonesian province of Sulawesi Tenggara or southeastern Sulawesi comprises the southeastern arm of the main island of Sulawesi and several smaller islands, including Muna, Buton, Kabaena, and Wowoni (Fig. 2) and covers a wide range of habitats from dramatic waterfalls to slow-flowing streams. Our fieldwork revealed a rich ricefish fauna in Sulawesi Tenggara, including the colorful *Oryzias woworae*-species group we review here: Daisy's Ricefish, *O. woworae*; a new slender-bodied species, *Oryzias asinua*; and a new golden species, *Oryzias wolasi*. All three species are allopatric and endemic to different drainages. Description of the new species and recognition of their areas of endemism augments the endemic freshwater fish fauna of Sulawesi Tenggara and begins to fill a void in our understanding of the natural history of Sulawesi.

## MATERIALS AND METHODS

Specimens were collected under a permit from the Sekretariat Perizinan Peneliti Asing (Secretariat of Foreign Research Permit), RISTEK: Kementerian Negara Riset dan Teknologi



**Fig. 2.** Localities of the *Oryzias woworae*-species group from Sulawesi Tenggara. *O. asinua*: circle with cross, type and only known locality (3°42'77.2"S, 121°47'92.1"E); *O. wolasi*: open and solid circles, open circle is type locality (4°15'05.2"S, 122°29'03.0"E); *O. woworae*: star, type and only known locality. See text for further details on these collection localities.

(State Ministry for Research and Technology), Republic of Indonesia, with the cooperation of Research Center for Biology-LIPI (Lembaga Ilmu Pengetahuan Indonesia), Indonesian Institute of Sciences. Specimens were collected according to the protocols of the National Museum of Natural History Institutional Animal Care and Use Committee (IACUC) using standard field techniques including seining and dipnetting. Specimens were preserved in 10% formalin or 95% ethanol. Tissue samples (dorsal musculature, fin clips) were taken from select specimens that were sedated with MS-222 (tricaine methanesulfonate) and preserved in 95% ethanol. Live fish that were photographed were maintained in a freshwater tank the day of their collection, then sedated with MS-222 and preserved in 10% formalin. All specimens preserved in 10% formalin were transferred through graded series of alcohol to 75% ethanol for long-term storage.

Morphological character descriptions, counts, and measurements follow Parenti (2008). Comparisons are made to characters described in that publication and to the material below. Measurements are reported as a range of percent of standard length, with the value for the holotype after the range in brackets. We follow a phylogenetic species concept (Rosen, 1978, 1979). Specimens were cleared and counterstained according to the protocol of Dingerkus and Uhler

**Table 2.** Tissues of the *Oryzias woworae*-Species Group and Outgroups Used in the COI Analyses. Specimen number and/or vial code refers to the identification of individual tissues or specimens as reported in Figures 3 and 4. All material is housed in the USNM under the listed catalog number. GenBank accession numbers for these tissues are listed under NCBI COI.

Taxon/specimen number	Vial code	USNM catalog number	GenBank NCBI COI
<i>O. asinua</i> 1	EX69	405299	JX311925
<i>O. asinua</i> 2	EX70	405300	JX311926
<i>O. asinua</i> 3	EX71	405301	JX311927
<i>O. asinua</i> 4	EX72	405302	JX311928
<i>O. asinua</i> 5	SES61	405329	JX311929
<i>O. wolasi</i> 1	SES16	405311	JX311930
<i>O. wolasi</i> 2	SES17	405312	JX311931
<i>O. wolasi</i> 3	SES18	405313	JX311932
<i>O. wolasi</i> 4	SES19	405314	JX311933
<i>O. wolasi</i> 5	SES22	405315	JX311934
<i>O. wolasi</i> 6	SES23	405318	JX311935
<i>O. wolasi</i> 7	SES81	405316	JX311936
<i>O. wolasi</i> 8	SES82	405309	JX311937
<i>O. wolasi</i> 9	SES83	405310	JX311938
<i>O. wolasi</i> 10	SES84	405317	JX311939
<i>O. woworae</i> 1	SES79	405327	JX311940
<i>O. woworae</i> 2	SES80	405328	JX311941
<i>O. celebensis</i>	SES42	399989	JX311942
<i>O. javanicus</i>	F87	405326	JX311943
<i>Chanos chanos</i>	SES04	399448	JX311944

(1977). Institutional abbreviations are as listed at <http://www.asih.org/node/204>.

The mitochondrial cytochrome *c* oxidase subunit 1 (COI), the DNA barcode (Ward et al., 2005), was sequenced from DNA extracted from a series of tissue samples (Table 2) to test species-level groups discovered via morphology. DNA extraction, amplification, and sequencing were all carried out in the Laboratory of Analytical Biology (LAB), National Museum of Natural History, Smithsonian Institution, by the third author. Total genomic DNA was extracted from approximately 20 mg of ethanol-fixed muscle or fin tissue via an automated phenol-chloroform extraction on AutoGenPrep 965 (AutoGen Inc., Holliston, MA) using the mouse-tail tissue protocol or via a Qiagen DNeasy Tissue Extraction Kit following the manufacturer's protocol. The polymerase chain reaction (PCR) was performed to amplify the targeted fragment for each individual sample in a total 10 µl reaction containing 3 µl of sterile water, 1 µl of the genomic DNA, 5 µl BIO-X-ACT Short DNA Polymerase (BioLine USA, Boston, MA), and 0.5 µl of 10 µM of a primer. PCR and DNA sequencing for the teleost fish DNA barcode sequence (mitochondrial cytochrome oxidase 1 gene or COI) were performed using the fish primers CO1LBC\_F [5'-TCAACYAATCAYAAAGATATYGGCAC-3'] and CO1HBC\_R [5'-ACTTCYGGGTGRCCRAARAATCA-3'] (Ward et al., 2005). The following PCR program was used: 1 cycle of initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 50–53°C for 60 s, and extension at 72°C for 60 s; it is ended by final extension at 72°C for 5 min. PCR products were cleaned up using ExoSAP-IT (USB, Cleveland, OH). Cycle sequencing reactions for both strands of amplified fragments were performed with a BigDye Terminator Cycle Sequencing Kit v.3.1 (Applied

Biosystems, Inc., Foster City, CA) and the PCR primers. Subsequent purification was conducted through gel filtration with Sephadex G-50 (Sigma-Aldrich Corp.). Purified products were sequenced using a 3730xl DNA analyzer (Applied Biosystems, Inc.). The program Sequencher 4.8 (GeneCodes, Ann Arbor, MI) was used to assemble and edit complementary chromatograms to produce the contiguous sequences (contigs) of COI.

Sequences were aligned via the multiple alignment procedure using the multi-integrated program SATé-II (Liu et al., 2012). The COI dataset contains a total of 665 nucleotide characters of which 205 are variable and 143 are parsimony informative. In a maximum parsimony (MP) analysis, phylogenetic trees were estimated using PAUP\* 4.0b (Swofford, 2002) with heuristic searches in 1,000 random addition replicates. Bootstrap supports were calculated out of 1,000 replicates in PAUP\* 4.0b. A bootstrap value for each node was calculated through a non-parametric bootstrap analysis using 100 pseudoreplicates. The Bayesian analysis was performed using MrBayes version 3.2.1 (Ronquist et al., 2012). TPM3uf + G was inferred as the best substitution model for the dataset of COI sequences using JModeltest version 2.0.2 (Posada, 2008) via the corrected version of Akaike Information Criterion (AICc). Two independent Markov chain Monte Carlo (MCMC) analyses with four simultaneous chains were run for 20 million generations and sampled every 1,000 generations. To obtain the final Bayesian tree, the "sumt" command was executed to output a summarized consensus tree with posterior probabilities that express nodal support. The computational tasks for searching the optimal trees (MP and BI) were performed on a high-performance Topaz cluster at LAB. The MP 50% majority-rule consensus of trees for populations of species in the *Oryzias woworae*-species group, analyzed with populations of the ricefishes, *O. celebensis* and *O. javanicus*, is depicted in Fig. 3; that of the corresponding BI consensus is in Fig. 4. The gonorhynchiiform, *Chanos chanos*, a distant outgroup, was used to root the trees. Data for the specimens used in the barcode analysis are summarized in Table 2 and include species, specimen number, vial code, USNM catalog number, and GenBank accession number.

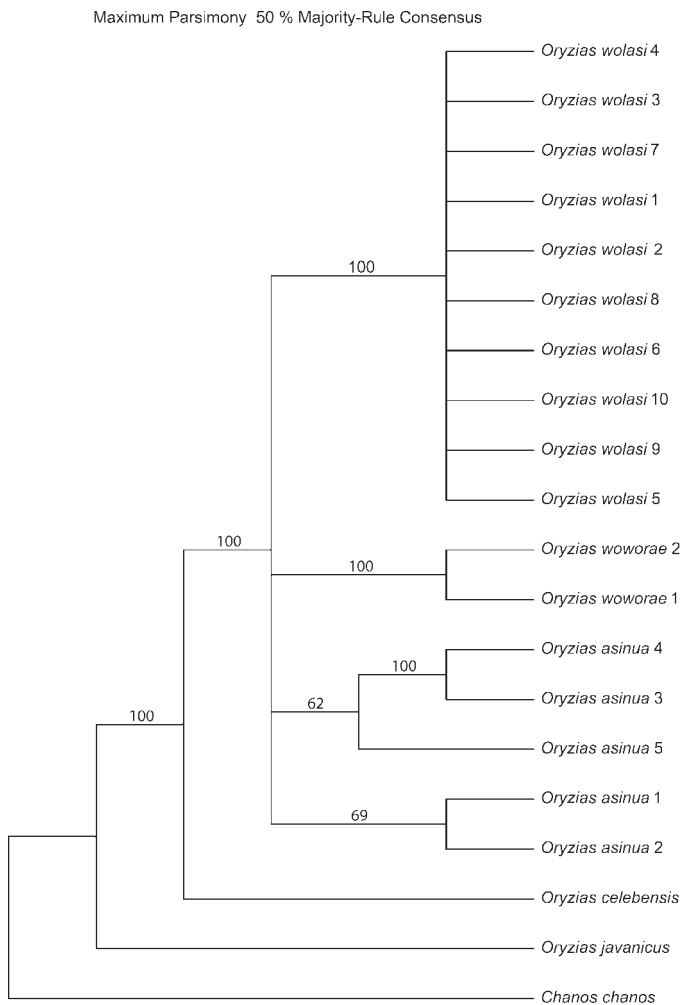
### *Oryzias asinua*, new species

Asinua Ricefish

Figures 1–7, Table 3

**Holotype.**—MZB 20782 (Fig. 5), male, 26.8 mm SL, Indonesia, Sulawesi Tenggara, Regency of Konawe, District of Asinua, Village of Asipako, Sungai Asinua near where crossed by bridge, 80 m alt., 3°42'77.2"S, 121°47'92.1"E, 18 June 2010, L. R. Parenti, R. K. Hadiaty, D. Lumbantobing, and S. Sauri.

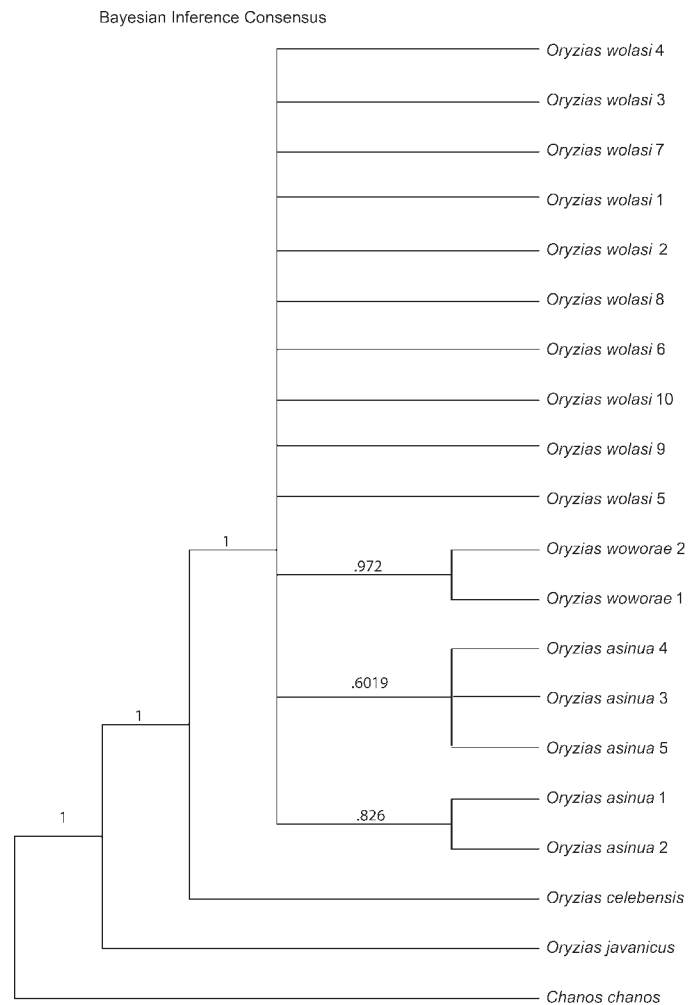
**Paratypes.**—ANSP 192931, 17.4–21 mm SL (2 alcoholic); MZB 20783, 12.5–23.5 mm SL (32 alcoholic); NSMT-P 111645, 20.5–21 mm SL (2 alcoholic); UF 183765, 17.8–18.5 mm SL (2 alcoholic); USNM 405299, 18 mm SL (EX69); USNM 405300, 17 mm SL (EX70); USNM 405301, 14.5 mm SL (EX71); USNM 405302, 15 mm SL (EX72); USNM 405329, 14 mm SL (SES61); USNM 406788, 14–22.9 mm SL (9 alcoholic, male and female cleared and counterstained); ZFMK 47666–47667, 20–22 mm SL (2 alcoholic); all collected with holotype.



**Fig. 3.** The 50% majority-rule consensus tree of a maximum parsimony analysis of the COI region among representative populations of each species in the *O. woworae* group, and ricefishes *O. celebensis* and *O. javanicus*. The gonorhynchiform, *Chanos chanos*, a distant outgroup, was used to root the tree. Numbers at nodes represent bootstrap support. Branch length is arbitrary. See text for further details.

**Non-type material.**—USNM 405298, 12.5–24 mm SL (13 alcoholic), collected with the holotype.

**Differential diagnosis.**—*Oryzias asinua* is a member of the *Oryzias woworae*-species group, which is distinguished by orange to deep red dorsal and ventral margins of the caudal fin and the ventral margin of the caudal peduncle and at least the posterior portion of the base of the anal fin, and a bluish sheen on the body in both sexes that is most pronounced in live adult males. *Oryzias asinua* and *O. wolasi* are distinguished from *O. woworae* by having elongate middle dorsal-fin rays in males that reach the posterior extent of the first principal caudal-fin ray and an orange-colored olfactory epithelium that marks each nasal organ in at least females in life (Fig. 5). *Oryzias asinua* is further distinguished by presence of such an orange-colored olfactory epithelium in both males and females in life and in preserved specimens. *Oryzias asinua* is a relatively slender, delicate species compared to *O. wolasi* and *O. woworae*: the body is narrow (21–25, mean 22.9, in *O. asinua* versus 23–32, mean 25.3 in *O. wolasi* and 22–30, mean 26 in *O. woworae*; Figs. 1, 3, 5). Further, *Oryzias asinua* has fewer procurent



**Fig. 4.** The consensus tree of the Bayesian inference analysis of the COI region among the same representative populations of each species in the *O. woworae* group, and ricefishes *O. celebensis* and *O. javanicus* as in Figure 3. The gonorhynchiform, *Chanos chanos*, a distant outgroup, was used to root the tree. Numbers at nodes represent posterior probabilities. Branch length is arbitrary. See text for further details.

caudal-fin rays in the lower lobe (4–5 versus 5–7 in *O. wolasi* and 5–6 in *O. woworae*; Tables 3, 4).

**Description.**—Small, maximum size of specimens examined 26.8 mm SL. Body elongate, slender, body depth 21–25 [25]. No pronounced abdominal concavity between pelvic fins and anal fin. Mouth subterminal, lower jaw extends slightly beyond upper jaw. Dorsal and ventral body profile relatively straight from head to dorsal- and anal-fin origins. Dorsal surface of head slightly convex just anterior to orbit. Head small to moderate, head length 25–30 [25]; snout short, length 6–9 [6]; eye large 10–12 [10], orbit projects somewhat beyond dorsal surface of head. Basal portion of dorsal and anal fin do not project significantly beyond primary body profile. Scales of moderate size, cycloid, and somewhat deciduous; 29–34 [34] in a lateral series. Elongate, filamentous dorsal- and anal-fin rays in males; middle dorsal-fin rays reach a vertical line through the distal extent of the first principal caudal-fin ray; anal-fin rays without bony contact organs. Pectoral fin rounded to somewhat pointed in larger specimens. Innermost pelvic-fin ray connected to body via a membrane along its proximal third. Caudal fin truncate.





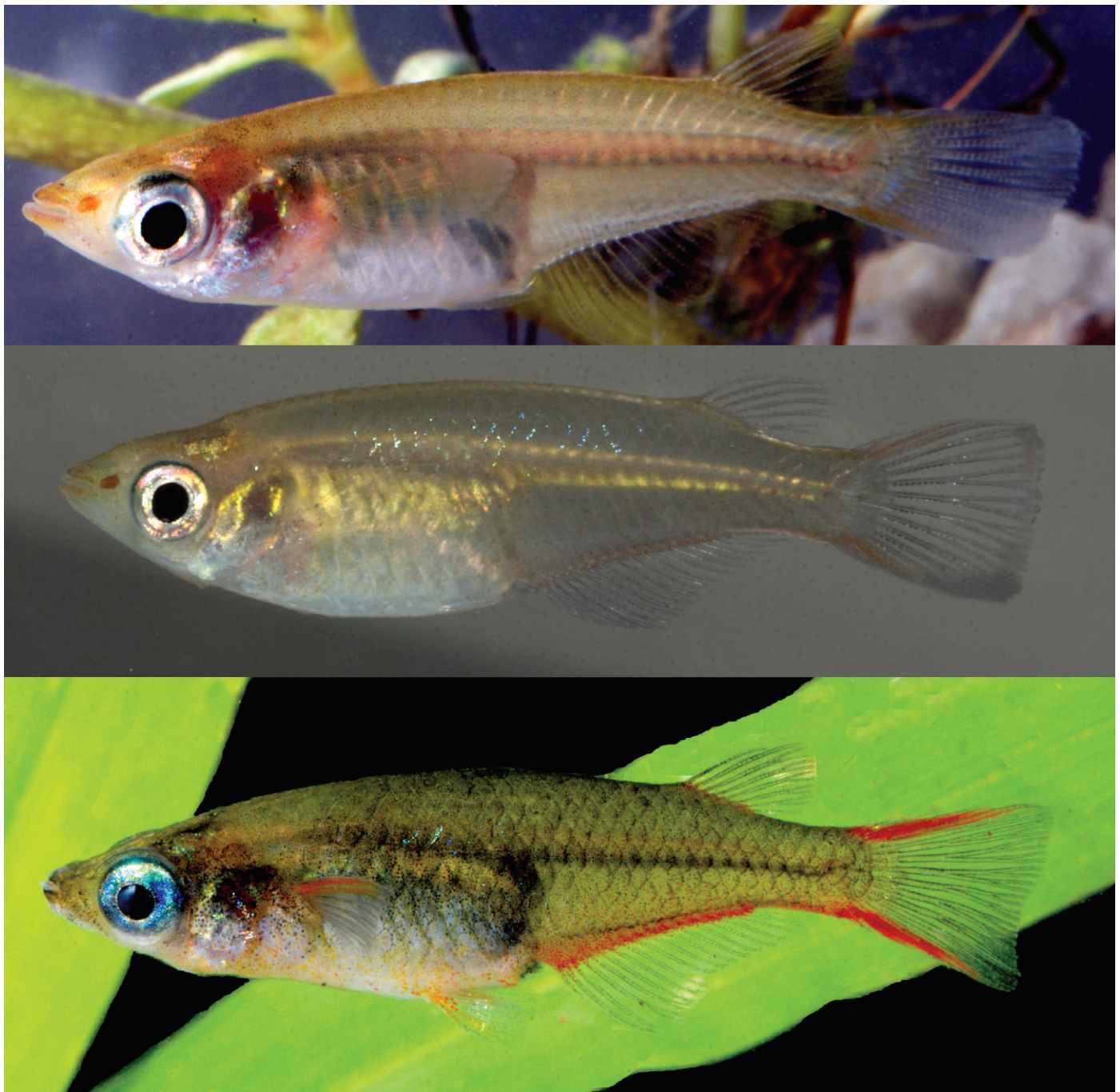
**Fig. 5.** Color photographs of preserved holotypes. Top: *Oryzias asinua*, new species, MZB 20782, holotype, male, 26.8 mm SL. Middle: *Oryzias wolasi*, new species, MZB 20784, holotype, male, 24.8 mm SL. Bottom: *Oryzias woworae*, MZB 15398, holotype, male, 25 mm SL.

Male with a short, slightly conical, tubular urogenital papilla; female with bilobed urogenital papilla.

Premaxilla short and broad with distinct, subtriangular ascending process; premaxilla and dentary with two irregular rows of caniniform teeth; males with two irregular rows of external conical teeth on the lateral portions of the upper and lower oral jaws. No pre-ethmoid cartilage; ossified portions of mesethmoid disc-shaped; anterior and lateral borders of ethmoid cartilage irregular. No flanges on the ventral surface of the palatine and the quadrate. Dorsal ramus of hyomandibula not distinctly bifid, single cartilage articulates with sphenotic and pterotic. Lacrimal sensory

canal carried in open, bony groove. First pleural rib on parapophysis of third vertebra; first epineural bone attaches to parapophysis of first vertebra dorsal to, and not in horizontal line with, posterior epineural bones; lateral process of pelvic bone attaches to third pleural rib (attached to fifth vertebra). Dorsal-fin origin over vertebra 21–23 [22]. Caudal skeleton with two epural bones; one ventral accessory bone. Anterior procurrent caudal-fin rays hooked slightly at their base. Fifth ceratobranchial toothplates subtriangular, with pavement dentition anteriorly, followed by five discrete rows of unicuspid teeth; no small, incomplete posterior row of teeth. Basihyal bone triangular,





**Fig. 6.** Live adult females, photographed just after capture, of the three described ricefishes from Sulawesi Tenggara. Top: *Oryzias asinua*, new species, paratype, USNM 406788. Middle: *Oryzias wolasi*, new species, paratype, USNM 403660. Bottom: *Oryzias woworae*, topotype, USNM 399429. All individuals are approximately 23–24 mm SL.

basihyal cartilage elongate and rectangular. Epibranchial elements incompletely to fully ossified; epibranchial two markedly smaller than the other epibranchial elements.

Dorsal-fin rays 7–9 [7]. Anal-fin rays 17–19 [18]. Pelvic-fin rays 6. Pectoral-fin rays 9–10 [9]. Principal caudal-fin rays i, 4/5, i. Procurent fin rays, dorsal 4–5 [4], ventral 4–5 [5]. Vertebrae 29–30 [30] (12–13[12]+17–18 [18]). Branchiostegal rays 5.

**Color in life.**—An orange-colored olfactory epithelium marks each nasal organ in both sexes (Fig. 6). Males are overall pale blue-gray; the posterior two-thirds of anal-fin base, caudal peduncle, and dorsal and ventral margins of caudal fin are

faint to bright orangish; midlateral scales from just posterior to the eye to the caudal-fin base and the body scales anterior to the anal fin and above midlateral scales reflect a faint silvery blue. The dorsal, anal, and caudal-fin interradyal membranes are speckled with minute melanophores; pectoral and pelvic fins are pale orange. The body is speckled with pinpoint melanophores that are less dense on the ventral surface of the body anterior to the anal fin and ventral surface of the head. Females are overall orangish, with a few scattered scales that reflect a faint silvery blue on the dorsal surface of the body; scattered pinpoint melanophores on body and fins; the posterior two-thirds of anal-fin



**Fig. 7.** Sungai Asinua at the village of Asipako (3°42'77.2"S, 121°47'92.1"E), District of Asinua, near where crossed by bridge, type locality of *O. asinua*.

base, caudal peduncle, and dorsal and ventral margins of caudal fin are pale orange.

**Color in alcohol.**—Ground color grayish-yellow, belly and ventral surface of head pale yellow. Dorsal surface of head and dorsal and lateral surface of body with diffuse to dense brown to black chromatophores. The orange pigment of the olfactory epithelium of each nasal organ in both sexes, vibrant in life, is retained in preserved specimens. A diffuse row of melanophores from the dorsal surface of the head to the dorsal-fin origin, a faint midlateral black line from the head to base of the caudal fin. Diffuse line of dark brown chromatophores from just posterior to anal-fin origin, along body just dorsal to anal-fin base to middle of caudal peduncle. Urogenital papilla cream in both sexes. Pectoral

and pelvic fins of females hyaline to dusky. Fins of males hyaline to dusky; dorsal and ventral margin of caudal fin with dark melanophores (Fig. 5).

**Distribution and habitat.**—The single known collection of *O. asinua*, the type locality, is from the Regency of Kendari, along the main channel of the Sungai Asinua, at the foothills of the Abuki mountains (Fig. 7). Emergent grasses were clustered in small “islands” over a substrate of mud, sand, and gravel. *Oryzias asinua* was living with a native species of halfbeak, *Nomorhamphus* sp., a possibly native *Clarias* sp., and the exotics *Puntius binotatus*, *Trichopodus trichopterus*, and *Oreochromis* sp. Specimens were collected from small pools and along the shore that was an extensive floodplain. The water was clear, with a slow to swift current and temperature of 26°C.

**Remarks.**—Ricefishes do not have the olfactory epithelium arranged in a rosette as is common for teleosts (Yamamoto, 1982; Hansen and Zielinski, 2005); instead, the olfactory epithelium lies flat on the floor of the olfactory chamber. The orange olfactory epithelium is quite bright in living *O. asinua* (Fig. 6), a pigment that is retained in preserved specimens more than two years after their collection. All five of the specimens of *O. asinua* used in the DNA barcode analysis retain the distinctive orange olfactory epithelium and are treated as paratypes. They may also be considered paragenotypes following Chakrabarty (2010). Several specimens have been catalogued as nontypes because of their relatively poor state of preservation. None retains an orange pigment of the olfactory epithelium; they may represent another new species, but this hypothesis cannot be tested without additional material.

Asinua Ricefish is chosen as a common name in English for the new species following Parenti (2008), in which common names were provided for all ricefishes.

**Etymology.**—The trivial name *asinua* to denote the occurrence of this species in the Sungai Asinua, the type locality.

**Table 3.** Morphometric and Meristic Data for Select Specimens of *Oryzias asinua*. Measurements are reported as a percentage of standard length. Number of examined paratypes (from USNM 406788 and MZB 20783) indicated in parentheses. Mean includes holotype.

Character	Holotype	Paratypes	Mean
Dorsal-fin rays	7	8–9 ( <i>n</i> = 12)	7.4
Anal-fin rays	18	17–19 ( <i>n</i> = 12)	17.8
Pelvic-fin rays	6	6 ( <i>n</i> = 10)	6
Pectoral-fin rays	9	9–10 ( <i>n</i> = 11)	9.8
Principal caudal-fin rays	i,4/5,i	i,4/5,i ( <i>n</i> = 8)	i,4/5,i
Procurent fin rays	4/5	4–5/4–5 ( <i>n</i> = 8)	4.4/4.6
Vertebrae	30 (12+18)	29–30 (12–13+17–18) ( <i>n</i> = 13)	29.6 (12.1+17.5)
Branchiostegal rays	5	5 ( <i>n</i> = 10)	5
Scales in lateral series	34	29–30 ( <i>n</i> = 10)	29.8
Head length	25	27–30 ( <i>n</i> = 10)	28.3
Snout length	6	7–9 ( <i>n</i> = 10)	7.6
Eye diameter	10	10–12 ( <i>n</i> = 10)	10.7
Body depth	25	21–24 ( <i>n</i> = 10)	22.9
Predorsal length	80	77–83 ( <i>n</i> = 10)	79.6
Preal length	55	57–63 ( <i>n</i> = 10)	58.4
Dorsal-fin base	11	8–9 ( <i>n</i> = 10)	8.4
Anal-fin base	30	25–29 ( <i>n</i> = 10)	27
Caudal peduncle depth	10	9–11 ( <i>n</i> = 10)	10
Standard length (mm)	26.8	15.5–23.5 ( <i>n</i> = 10)	20.4



**Table 4.** Morphometric and Meristic Data for Select Specimens of *Oryzias wolasi*. Measurements are reported as a percentage of standard length. Number of examined paratypes (USNM 403647, 403644) indicated in parentheses. Mean includes holotype.

Character	Holotype	Paratypes	Mean
Dorsal-fin rays	7	7–9 ( <i>n</i> = 11)	8
Anal-fin rays	19	17–20 ( <i>n</i> = 10)	18.7
Pelvic-fin rays	6	6 ( <i>n</i> = 10)	6
Pectoral-fin rays	9	9–10 ( <i>n</i> = 10)	9.8
Principal caudal-fin rays	i,4/5,i	i,4/5,i ( <i>n</i> = 10)	i,4/5,i
Procurrent fin rays	4/5	4–6/5–7 ( <i>n</i> = 10)	5.1/6.1
Vertebrae	29 (12+17)	29–30 (12+17–18) ( <i>n</i> = 10)	29.5(12+17.7)
Branchiostegal rays	5	5–6 ( <i>n</i> = 10)	5.1
Scales in lateral series	29	30–34 ( <i>n</i> = 10)	31.2
Head length	26	25–30 ( <i>n</i> = 10)	27.4
Snout length	6	7–9 ( <i>n</i> = 10)	7.4
Eye diameter	10	10–12 ( <i>n</i> = 10)	11.1
Body depth	32	23–30 ( <i>n</i> = 10)	25.3
Predorsal length	81	76–84 ( <i>n</i> = 10)	79.5
Preanal length	62	58–64 ( <i>n</i> = 10)	60.5
Dorsal-fin base	10	8–11 ( <i>n</i> = 10)	9.4
Anal-fin base	29	24–28 ( <i>n</i> = 10)	25.4
Caudal peduncle depth	12	11–12 ( <i>n</i> = 10)	11.2
Standard length (mm)	24.8	20.1–25.4 ( <i>n</i> = 10)	22.9

***Oryzias wolasi*, new species**

Wolasi Ricefish

Figures 1–6, 8; Table 4

*Oryzias* sp. “Sulawesi”.—Evers et al., 2010:62–65, figs.

**Holotype.**—MZB 20784 (Fig. 5), male, 24.8 mm SL, Indonesia, Sulawesi Tenggara, Regency of Konawe Selatan, District of Wolasi, Village of Andambao, Sungai Andambao near where crossed by road, 85 m alt., 4°15'05.2"S, 122°29'03.0"E, 13 June 2010, L. R. Parenti, R. K. Hadiaty, D. Lumbantobing, and S. Sauri.

**Paratypes.**—ANSP 192932, 18.6–23.6 mm SL (4 alcoholic); MZB 20785, 20.1–25.9 mm SL (20 alcoholic, 2 males, 2 females cleared and counterstained); NSMT-P 111646, 18–26.5 mm SL (4 alcoholic); UF 183766, 20.9–23.7 mm SL (4

alcoholic); USNM 403642, 21–28 mm SL (4 preserved in 95% ethanol); USNM 403644, 12–27.1 mm SL (103 alcoholic, 4 males, 3 females cleared and counterstained); USNM 405311 (SES 16); USNM 405312 (SES 17); USNM 405313 (SES 18); USNM 405314 (SES 19); USNM 405315 (SES 22); USNM 405316 (SES 81); USNM 405317 (SES 84); ZFMK 47668–47671, 19.3–25.9 mm SL (4 alcoholic); collected with holotype. USNM 403647, 16–26.7 mm SL (102 alcoholic); USNM 404347, 21.5–24.3 mm SL (5 preserved in 95% ethanol); collected at the type locality on 23 June 2010. USNM 403639, 22.7–24.8 mm SL (3 alcoholic), Sulawesi Tenggara, Regency of Konawe Selatan, District of Wolasi, Sungai Wolasi near where crossed by road, 167 m alt., 4°09'62.1"S, 122°29'56.1"E, 13 June 2010, L. R. Parenti, R. K. Hadiaty, D. Lumbantobing, and S. Sauri. USNM 403660, 20.1–28.6 mm SL (30 alcoholic); USNM 403640, 21.7–27.6 (5 preserved in 95% ethanol); USNM 405318 (SES 23); said to be from same locality, 14 June 2010, purchased from Ir. Hj Malani. USNM 403645, 11–27 mm SL (12 alcoholic); USNM 403646, 10 mm SL (1 preserved in 95% ethanol); Sulawesi Tenggara, Regency of Konawe Selatan, District of Moramo, Summersari Falls, 167–200 m alt., 4°13'16"S, 122°44'76.5"E, 14 June 2010, L. R. Parenti, R. K. Hadiaty, D. Lumbantobing, and S. Sauri. USNM 403643, 24 mm SL (1 alcoholic), Sulawesi Tenggara, Regency of Konawe Selatan, District of Laeya, Town of Ambolodangga, spring-fed coastal stream where crossed by road to Torobulu, 50 m alt., 4°18'83.7"S, 122°29'61.1"E, 23 June 2010, L. R. Parenti, R. K. Hadiaty, D. Lumbantobing, and S. Sauri.

**Non-type material.**—USNM 405308, 22.3–26.9 mm SL (8 alcoholic); USNM 405309, female, 21.8 mm SL (SES82); USNM 405310, male, 27.6 mm SL (SES83); aquarium material.

**Differential diagnosis.**—*Oryzias wolasi* is a species in the *Oryzias woworae*-species group which all share orange to

**Fig. 8.** Sungai Andambao near where crossed by road at the village of Andambao (4°15'05.2"S, 122°29'03.0"E), District of Wolasi, type locality of *O. wolasi*.



deep red dorsal and ventral margins of the caudal fin and the ventral margin of the caudal peduncle and at least the posterior portion of the base of the anal fin, and a bluish sheen on the body in both sexes that is most pronounced in live adult males. *Oryzias wolasi* and *O. asinua* are distinguished from *O. woworae* by having elongate middle dorsal-fin rays in males that reach the posterior extent of the first principal caudal-fin ray. *Oryzias wolasi* is golden in life in both sexes and relatively deep-bodied (Figs. 1, 5, 6, middle), reaching 32% SL, versus reaching 25% in *O. asinua* and 30% in *O. woworae*, has a deeper caudal peduncle (11–12, mean 11.2, versus 9–11, mean 10, in *O. asinua* and 8–11, mean 9.2, in *O. woworae*; Tables 3, 4).

**Description.**—Small, maximum size of specimens examined 28.6 mm SL. Body compressed laterally, slender to somewhat deep-bodied, body depth 23–32 [32]. No pronounced abdominal concavity between pelvic fins and anal fin. Mouth subterminal, lower jaw extends slightly beyond upper jaw. Dorsal and ventral body profile gently arching from head to dorsal- and anal-fin origins. Dorsal surface of head slightly convex just anterior to orbit. Head small to moderate, head length 25–30 [26]; snout short, length 6–9 [6]; eye moderate to large 10–12 [10], orbit projects somewhat beyond dorsal surface of head. Basal portion of dorsal and anal fin do not project significantly beyond primary body profile. Scales of moderate size, cycloid, and somewhat deciduous; 29–34 [29] in a lateral series. Elongate, filamentous dorsal- and anal-fin rays in males; middle dorsal-fin rays reach a vertical line through the distal extent of the first principal caudal-fin ray; anal-fin rays without bony contact organs. Pectoral fin rounded to somewhat pointed in larger specimens. Innermost pelvic-fin ray connected to body via a membrane along its proximal third. Caudal fin truncate. Male with a short, slightly conical, tubular urogenital papilla; female with bilobed urogenital papilla.

Premaxilla short and broad with distinct, subtriangular ascending process; premaxilla and dentary with two irregular rows of caniniform teeth; males with two irregular rows of external conical teeth on the upper and lower oral jaws. No pre-ethmoid cartilage; ossified portions of mesethmoid disc-shaped; anterior and lateral borders of ethmoid cartilage irregular. No flanges on the ventral surface of the palatine and the quadrate. Dorsal ramus of hyomandibula not distinctly bifid, single cartilage articulates with sphenotic and pterotic. Lacrimal sensory canal carried in open bony groove. First pleural rib on parapophysis of third vertebra; first epineural bone attaches to parapophysis of first vertebra dorsal to, and not in horizontal line with, posterior epineural bones; lateral process of pelvic bone attaches to third or fourth pleural rib (attached to fifth or sixth vertebra). Dorsal-fin origin over vertebra 21 or 22 [22]. Caudal skeleton with two epural bones; one ventral accessory bone. Anterior procurent caudal-fin rays hooked slightly at their base. Fifth ceratobranchial toothplates subtriangular, with pavement dentition anteriorly, followed by five discrete rows of unicuspid teeth; no small, incomplete posterior row of teeth. Basihyal bone triangular, basihyal cartilage elongate and rectangular. Epibranchial elements incompletely to fully ossified; epibranchial two markedly smaller than the other epibranchial elements.

Dorsal-fin rays 7–9 [7]. Anal-fin rays 17–20 [19]. Pelvic-fin rays 6. Pectoral-fin rays 9–10 [9]. Principal caudal-fin rays i,4/5,i. Procurent fin rays, dorsal 4–6 [4], ventral 5–7 [5]. Vertebrae 29–30 [29] (12 + 17–18 [17]). Branchiostegal rays 5–6 [5].

**Color in life.**—In males, the head and ventral surface of the body anterior to the anal fin and the dorsal-fin base are metallic orange; the lower jaw, approximately posterior two-thirds of anal-fin base, caudal peduncle, and dorsal and ventral margins of caudal fin are brilliant red-orange; midlateral scales from just posterior to the eye to the caudal-fin base and the body scales anterior to the anal fin and above and below midlateral scales reflect a silvery blue; in courting males, the silvery blue lateral coloration turns into a deep, slightly violet blue. The dorsal-, anal-, and caudal-fin interradial membranes are speckled with minute melanophores; pectoral and pelvic fins are pale orange. An orange-colored olfactory epithelium marks each nasal organ in females (Fig. 6). The body is speckled with pinpoint melanophores that are less dense on the ventral surface of the body anterior to the anal fin and ventral surface of the head. Females are overall golden, with scattered pinpoint melanophores on body and fins; the posterior two-thirds of anal-fin base, caudal peduncle, and dorsal and ventral margins of caudal fin are light orange.

**Color in alcohol.**—Ground color grayish-yellow, belly and ventral surface of head pale yellow. Dorsal surface of head and dorsal and lateral surface of body with diffuse brown to black chromatophores. A diffuse row of melanophores from the dorsal surface of the head to the dorsal-fin origin, a faint midlateral black line from the head to base of the caudal fin. Diffuse line of dark brown to black chromatophores from just posterior to anal-fin origin, along body just dorsal to anal-fin base to middle of caudal peduncle. Urogenital papilla pale brown to cream in both sexes. Pectoral and pelvic fins of females hyaline to dusky. Fins of males hyaline to dusky; dorsal and ventral margin of caudal fin with dark melanophores (Fig. 5, middle).

**Distribution and habitat.**—*Oryzias wolasi* was collected from several inland habitats in the Regency of Konawe Selatan (South Konawe), south of Kendari, the capital city of Sulawesi Tenggara (Fig. 2). The habitat of the holotype is a slow to swift-flowing freshwater stream with a substrate of mud, small rocks, and plant debris with emergent grasses and a shore of mud, rocks and grass (Fig. 8). *Oryzias wolasi* was also taken in a relatively cool (24°C), swift-flowing stream along Summersari Falls and a warmer (26°C), spring-fed coastal stream.

**Remarks.**—In captivity, *O. wolasi* usually mate in the early morning. Females carry a clutch of fertilized eggs usually not longer than a few hours after spawning; fertilized eggs are then deposited on a substrate such as aquatic plants (H.-G. Evers, pers. comm.; Fabian Herder, pers. obs.). Aquarium observations suggest that *Oryzias wolasi* differs slightly from *Oryzias woworae* in clutch-carrying time; some female *O. woworae* have been observed to carry the egg clutch for one or even two days (H.-G. Evers, pers. comm.).

This species has been reported on aquarium websites as “*Oryzias* sp. Neon (Kendari)” (www.aquabid.com; accessed on 30 August 2011). Wolasi Ricefish is chosen as a common name in English for the new species following Parenti (2008), in which common names were provided for all ricefishes.

**Etymology.**—The trivial name *wolasi* to denote the occurrence of this species in the District of Wolasi, the type locality.

## DISCUSSION

Ricefishes, the Asian and Southeast Asian beloniform family Adrianichthyidae, are classified in two genera, *Adrianichthys*, a Lake Poso, Sulawesi endemic, and *Oryzias*, which lives throughout the broad range of the family (Parenti, 2008). The two genera are readily distinguished by adult size and number of vertebrae, among other characters. Species of *Adrianichthys* are relatively large and reach over 60 mm SL, whereas no species of *Oryzias* reach 60 mm SL, and most species mature at a far smaller size. Among *Oryzias*, the *Oryzias woworae*-species group comprising *O. woworae* and the two new species described here, *O. asinua* and *O. wolasi*, is small-bodied, adults reach greater than 26 mm SL and less than 40 mm SL, according to the definition of body size groups of Parenti (2008). The maximum size recorded for all three species is 28.6 mm SL, slightly larger than the arbitrary 26 mm SL maximum for classification as a miniature species (Weitzman and Vari, 1988). The *Oryzias woworae*-species group has i,4/5,i, principal caudal-fin rays, contra Parenti and Hadiaty (2010) who reported i,5/6,i for *O. woworae*; a reduction to i,4/5,i, principal caudal-fin rays from i,5/6,i, is a synapomorphy of all *Oryzias* analyzed by Parenti (2008:fig. 30, node F) except for the two Lake Lindu endemics, *O. bonneorum* and *O. sarasinorum*. Within this clade, the *Oryzias woworae*-species group shares with the majority of species (Parenti, 2008:fig. 30, node I) a truncate caudal fin (versus a lunate or emarginate caudal fin).

The DNA barcode marker was analyzed from 17 representatives of the *O. woworae*-species group including the two species described here, two other species of *Oryzias* that live in Sulawesi, and *Chanos chanos* as a distant outgroup (Table 2) to test if barcodes are consistent with morphological species discrimination. The MP 50% majority-rule consensus tree (Fig. 3) shows that all ten specimens of *O. wolasi* clustered together, as did the two *O. woworae*. The five specimens of *O. asinua* sequenced formed two clusters that, in turn, form a polytomy with the two other species in the majority rule consensus. The BI consensus tree (Fig. 4) is less informative than the MP tree. The *Oryzias woworae* and *O. asinua* clusters of the MP tree are recovered in the BI tree, but there is no resolution among these clusters and the ten samples of *O. wolasi*. One possible interpretation of these analyses is that the specimens of *O. asinua* may represent two sympatric subpopulations or species, yet we have found no morphological evidence to support such a hypothesis. Further, there were no DNA barcode differences among the ten populations of *O. wolasi* that represented three collections, including material that had been collected for the aquarium trade. The barcode analyses support our hypothesis that we have sequenced representatives of three species.

The *Oryzias woworae*-species group represents a geographically distinct ricefish fauna endemic to Sulawesi Tenggara, which is geologically composed largely of the Sula Spur, an extension of the northern margin of the Australian plate that collided with the North Sulawesi volcanic arc in the Early Miocene (Spakman and Hall, 2010; Hall, 2011). One suture zone of the collision runs just south of the northern border of Sulawesi Tenggara (Fig. 2). These small fishes live in correspondingly restricted areas of endemism, as far as known, that further reflect Sulawesi Tenggara geology and topography. All three species are allopatric (Fig. 2). *Oryzias woworae* is known solely from the type locality, Mata air Fotuno, a freshwater stream on Muna Island. *Oryzias asinua* is also known solely from the type locality, the Sungai

Asinua, an upland tributary of the Sungai Pohara that flows into the Banda Sea north of Kendari. *Oryzias wolasi* is described from four proximate localities in the Regency of Konawe Selatan, two in the District of Wolasi (Sungai Wolasi and Sungai Andambao) and one each in the districts of Moramo (Sumbersari Falls) and Laeya (spring-fed coastal stream near Ambolodangga), all east of the Boroboro mountain range and south of the capital city of Kendari.

The *Oryzias woworae*-species group comprises the most colorful of ricefishes. Their beauty and relative ease of maintenance have quickly made them popular among aquarists, and they are likely to join their close relatives, such as the Medaka, *Oryzias latipes*, as widely used experimental biological organisms. At the same time, they are recognized as a valuable biodiversity resource well worth protecting. This discovery comes with the responsibility to manage endemic species effectively to maintain populations in their native habitat. Because these species may be bred easily in captivity, native stocks need not be depleted to satisfy the needs of the aquarium trade or of experimental biology. Attention has been drawn to Sulawesi Tenggara; biodiversity of the province will no longer be ignored. Its role in our understanding of the biogeography of Sulawesi will continue to be revealed.

## MATERIAL EXAMINED

*Adrianichthys oophorus*: USNM 348386, 4, cleared and counterstained, Indonesia, Sulawesi Tengah, Lake Poso.

*Chanos chanos*: USNM 399448 (SES04), Indonesia, Sulawesi Tenggara, Kendari Fish Market.

*Oryzias celebensis*: USNM 399989, 6 alcoholic, male and female cleared and counterstained, one specimen preserved in 95% ethanol (SES 42).

*Oryzias javanicus*: MZB 15400, 3, Indonesia, Sulawesi Tenggara, Parigi District, Muna Island; USNM 348513, 4, cleared and counterstained, Singapore, Sungai Buloh; USNM 391946, male and female cleared and counterstained, Indonesian Borneo, South Kalimantan; USNM 405326, one specimen preserved in 95% ethanol (F87), Indonesian Borneo, South Kalimantan.

*Oryzias woworae*: MZB 15398, holotype; MZB 15397, paratypes, 35, 1 male cleared and counterstained; USNM 391839, paratypes, 10, 1 male, 2 females cleared and counterstained; USNM 399429, topotypes, 68, 2 females and 2 males cleared and counterstained; MZB 20731 (ex. USNM 399429), topotypes(40); topogenotypes, USNM 405327 (SES 79), USNM 405328 (SES 80), Sulawesi Tenggara, Muna Island.

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