

Research article

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(Beloniformes: Adrianichthyidae: *Oryzias*)
from Lake Kalimpa'a, Sulawesi, Indonesia****Abdul Gani¹, Novian Suhendra², Fabian Herder³, Julia Schwarzer⁴, Jan Möhring⁵, Javier Montenegro⁶,
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Abstract. *Oryzias kalimpaaensis* sp. nov. is a new species of the genus *Oryzias* Jordan & Snyder, 1906, endemic to Lake Kalimpa'a in Lore Lindu National Park, Central Sulawesi, Indonesia. The species is a pelvic-brooder, a non-monophyletic group of ricefishes in which females carry an egg cluster until hatching. Pelvic-brooding is known from only a few taxa, and the new species reported here is only the fifth pelvic-brooding species known so far. *Oryzias kalimpaaensis* sp. nov. differs from all others *Oryzias* species by the following combination of characters: 61–67 scales in lateral line, 11–13 dorsal-fin rays, 11–13 pectoral-fin rays, body depth 16.0–22.2% SL, and length of head 30.1–33.7% SL. It is distinguished from all species of the genus *Adrianichthys* Weber 1913, by its small size (max. 52.8 mm SL). A molecular phylogeny based on mitochondrial ND2 sequences supports the distinctiveness of *O. kalimpaaensis* sp. nov. *Oryzias kalimpaaensis* sp. nov. is closely related to pelvic-brooding *O. eversi* Herder, Hadiaty & Nolte 2012 endemic to Tilanga Pond in Tana Toraja, and to the two species of Lake Lindu in Central Sulawesi, *O. sarasinorum* (Poeta 1905) and *O. bonneorum* Parenti 2008. As Lake Kalimpa'a is a popular destination for nature tourism, anthropogenic pressure is high. The presence of invasive fish species in the lake and parasites on collected specimens support this assumption.

Key words. Endemic, *Oryzias*, freshwater fish, Kalimpa'a, pelvic-brooder.

INTRODUCTION

Sulawesi is one of the five largest islands in Indonesia, and harbors a rich fauna of freshwater fishes, including endemic lake- and stream-dwelling species (Kottelat 1990a, 1990b; Miesen et al. 2016; Hadiaty 2018). The island is a hotspot of ricefish diversity (Adrianichthyidae; reviewed by Hilgers & Schwarzer 2019), and almost all ricefish species known from it are endemic. Distribution areas of most species are small, typically restricted to sin-

gle lakes like those of the Malili Lakes system, Lake Lindu, or Lake Poso; some ricefishes are even restricted to tiny pools or ponds, or stretches of rivers (Parenti 2008; Herder & Chapuis 2010; Parenti & Hadiaty 2010; Herder et al. 2012; Parenti et al. 2013; Mokodongan et al. 2014). The genus *Oryzias* Jordan & Snyder, 1906 is the most diverse and consists of thirty-two species; four species restricted to Lake Poso make up the endemic genus *Adrianichthys* Weber, 1913.

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Fig. 1. Lake Kalimpa'a: type locality of *Oryzias kalimpaaensis* sp. nov., in Central Sulawesi, Indonesia.

Reproduction of Sulawesi ricefishes can be assigned to two general strategies, transfer-brooders, i.e., species that deposit fertilized eggs on a substrate, as it is the case with most adrianichthyids, and pelvic-brooders (Parenti 2008; Spanke et al. 2021). In pelvic-brooding ricefishes, females carry the fertilized eggs until the fry hatches, protected in a ventral concavity, and covered by elongated pelvic fins (Parenti 2008; Parenti & Hadiaty 2010; Parenti et al. 2013; Mokodongan et al. 2014; Mandagi et al. 2018; Spanke et al. 2021). Interestingly, pelvic-brooding occurs in species of both genera, *Oryzias* and *Adrianichthys*. So far, a total of four pelvic-brooding ricefishes has been reported: *A. oophorus* (Kottelat, 1990), *A. poptae* Weber & de Beaufort, 1922, *O. sarasinorum* (Popta, 1905) and *O. eversi* Herder, Hadiaty & Nolte, 2012 (Kottelat 1990a; Parenti 2008; Herder et al. 2012; Mokodongan & Yamahira 2015).

Here we describe a new species of pelvic-brooding *Oryzias* endemic to a small lake in Central Sulawesi, Lake Kalimpa'a, which is located within Lore Lindu National Park, close to Lake Lindu. This discovery brings the number of recognized ricefish species on Sulawesi to 23, including at least five pelvic brooders (Parenti 2008; Herder & Chapuis 2010; Parenti & Hadiaty 2010; Herder et al. 2012; Parenti et al. 2013; Mokodongan et al. 2014; Mandagi et al. 2018; Utama et al. 2022).

MATERIAL AND METHODS

Species description and specimens of the new *Oryzias* were collected from Lake Kalimpa'a (Fig. 1) in Central Sulawesi, at 1°19'36.1" S, 120°18'26.1" E, approximately 23 km east of Lake Lindu (Fig. 2). The fish were caught with a hand net 13 Sep. 2020. Immediately after

capture, specimens of both sexes were photographed in a small tank to document life coloration. For morphological investigations, 18 individuals (10 males and 8 females) were anesthetized using ice slurry, preserved in 5% formalin for morphological analyses and later transferred to 70% ethanol for storage. Specimens are deposited in the fish collections of Museum Zoologicum Bogoriense (MZB), BRIN in Cibinong; Museum Koenig, Bonn (ZFMK); the Zoological Reference Collection (ZRC) of the Lee Kong Chian Natural History Museum, National University of Singapore.

Following Parenti (2008) we distinguished the specimens as genus *Oryzias* by several characters including maximum size in adult fish 52.8 mm SL. Specimens were compared to all known Sulawesi *Oryzias* species, with emphasis to those from Central Sulawesi and other pelvic-brooding species (Lake Lindu: *O. sarasinorum* and *O. bonneorum*; Tilanga Pond: *O. eversi*; Lake Poso: *O. nebulosus* Parenti & Soeroto 2004, *O. nigrimas* Kottelat 1990, *O. orthognathus* Kottelat 1990, and Lake Tiu: *O. soeroto* Mokodongan, Tanaka & Yamahira 2014). Assessment of morphological traits follows Herder et al. (2012). Number of fin rays and scales were counted directly from each individual using a stereo-microscope (SWIFT- S306S-20-2L).

To investigate phylogenetic relationships, NADH dehydrogenase subunit 2 (ND2) gene region was sequenced using two primers ND2L (5'-GGGCCCCAT-ACCCCAAACATGTTGG-3') and ND2H (5'-TTAAT-TAAAGTGTCTGTTTTGC-3') following Mokodongan and Yamahira (2015). DNA was extracted from the right pectoral fin of two different uncatalogued individuals of *O. kalimpaaensis* sp. nov. using standard protocol Qiagen DNeasy Blood & Tissue Kit. PCR conditions were as following: 94 °C for 3 min for denaturation, 35 cycles

of 94 °C for 30 s, 50 °C for 30 s, and 72 °C for 60 s, with a final extension of 72 °C for 2 min. Positive amplification was verified on a 1% agarose electrophoresis gel. PCR products were then sent to PT. Genetika Science in Jakarta (<http://actagen.com>) for sequencing. Sequence information for the new species were aligned together with sequences of 21 other species (two *Adrianichthys* and 19 *Oryzias*) obtained from Mokodongan & Yamahira (2015); accession numbers LC051687-LC051739. Alignments were done via ClustalW ver. 1.4 (Thompson et al. 1994) and manually curated. The final alignment consisted of 1046 bp. Sequence data of *O. kalimpaaensis* sp. nov. and *O. bonneorum* were deposited in DNA Data Bank of Japan (DDBJ) with accession number LC669549-LC669550, and LC685422-LC685423, respectively. Phylogenetic reconstructions using Maximum Likelihood were performed in raxmlGUI 2.0.6 (Edler et al. 2021), following the substitution model selected as best fitting (GTR-I-G) by ModelTest-NG (Darriba et al. 2019) and with 1000 bootstrap replicates conducted. *Cololabis saira* (Brevoort 1856) (Beloniformes: Scomb-

eresocidae) was used as outgroup with DDBJ accession number AP002932.

Taxonomy

Oryzias kalimpaaensis sp. nov.

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Lake Kalimpa'a Ricefish

Figs 3–4

Holotype

MZB. 26462 (Figs 3 (top), 4), ♂, 41.9 mm SL, Indonesia, Sulawesi Tengah, Regency of Lore Utara, District Poso, Lake Kalimpa'a, 13 Sep. 2020, Abdul Gani and Novian Suhendra.

Paratypes

MZB 26463–26466; 26528–26529, 4 ♂♂ (44.1–50.2 mm SL), 2 ♀♀ (41.1–42.4 mm SL); ZFMK ICH-128486–128492, 4 ♂♂ (43.7–49.1 mm SL), 3 ♀♀ (41.8–43.6 mm SL); ZRC 62531, 2 ♂♂ (47.3–47.6 mm SL), 2 ♀♀ (46.9–52.8 mm SL), collected with the holotype.

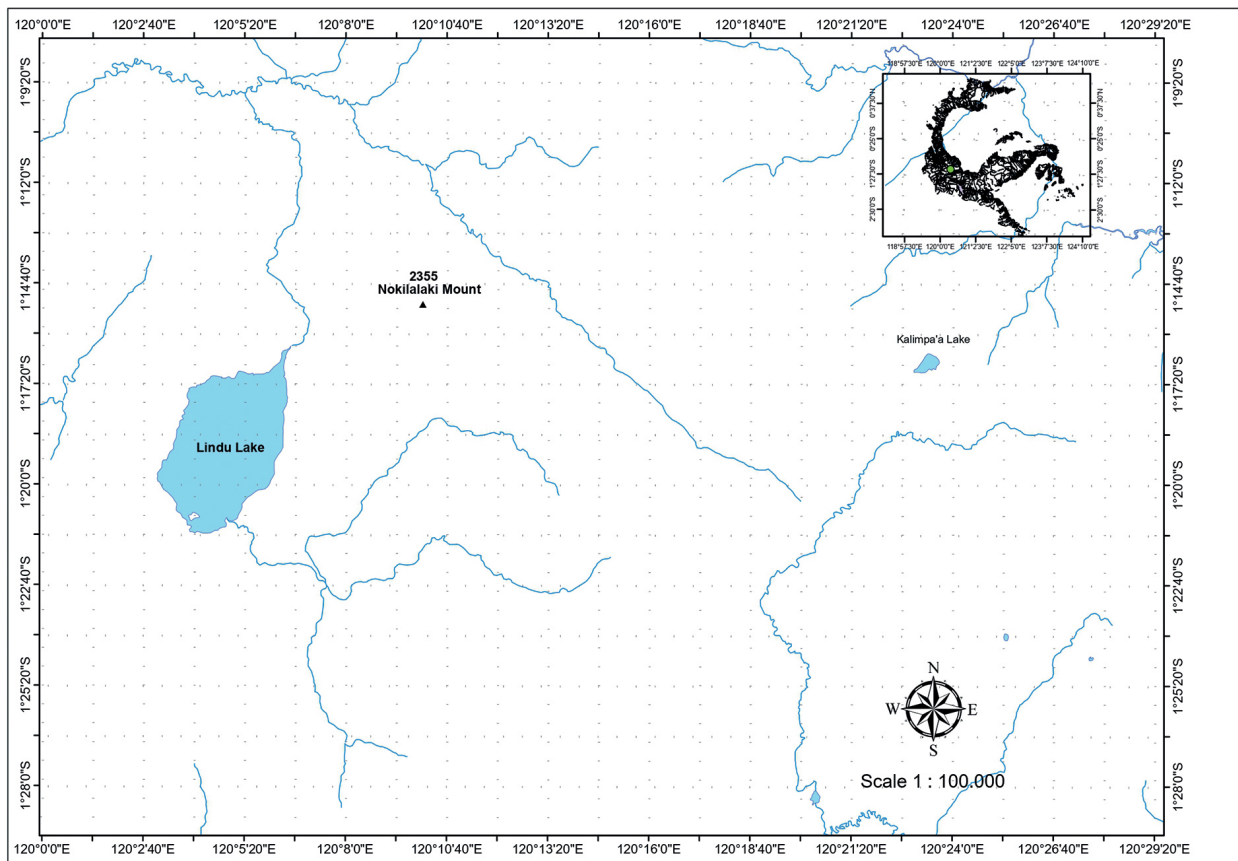


Fig. 2. Map of streams and lakes in Lore Lindu national park (Central Sulawesi, Indonesia) including the type locality of *Oryzias kalimpaaensis* sp. nov. (map by Moh. Arif Rahman). The location of the lake Kalimpa'a in Sulawesi is marked by a green dot on the map in the upper right corner.

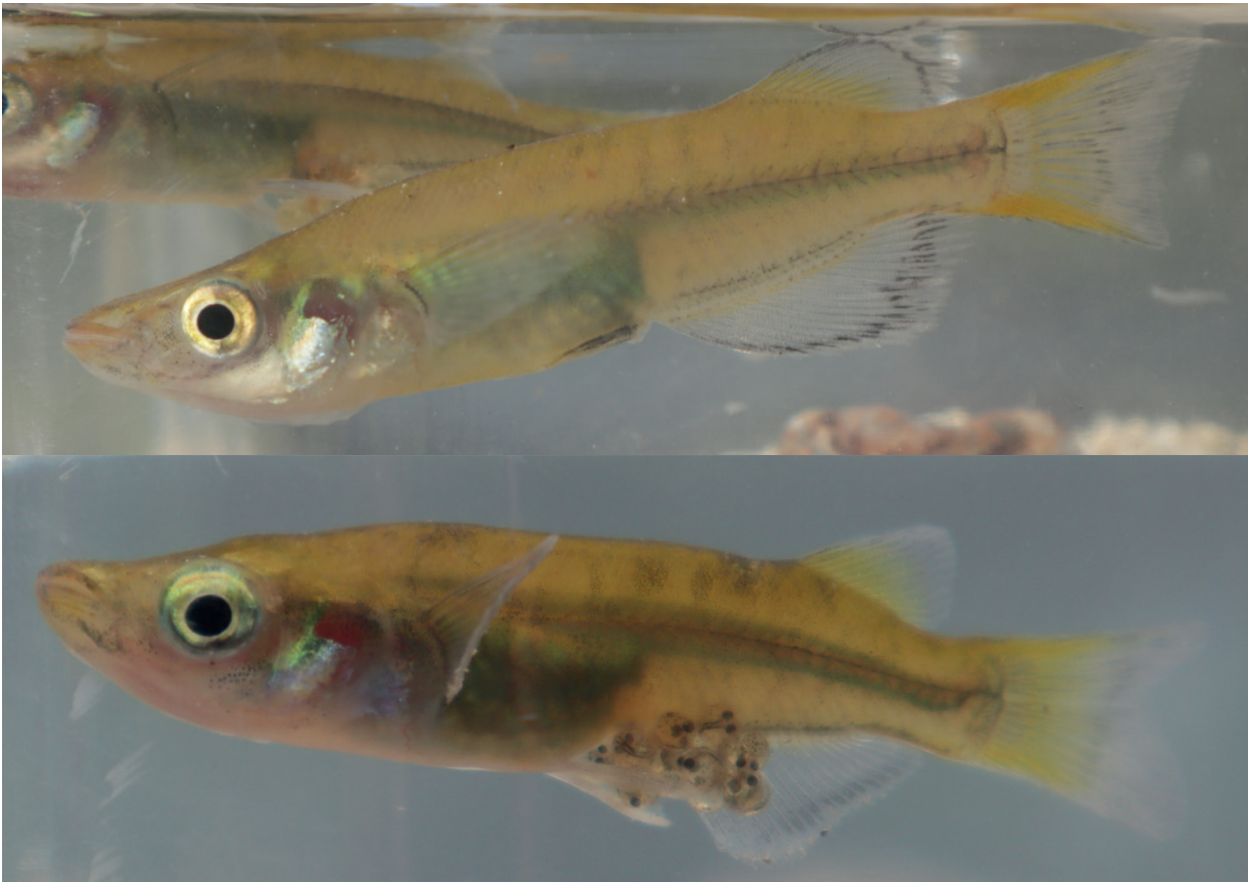


Fig. 3. Living specimens of *Oryzias kalimpaaensis* sp. nov., ♂ (top) and ♀ (bottom) from Lake Kalimpa'a, Central Sulawesi, Indonesia.

Diagnosis. *Oryzias kalimpaaensis* sp. nov. is a pelvic-brooding ricefish with pronounced sexual dimorphism. Females share with females of other pelvic-brooding ricefishes (*O. eversi*, *O. sarasinorum*, *A. oophorus*) morphological structures that enable the maternal fish to carry bundles of fertilized eggs. The eggs remain connected to the female by filaments, and are carried in a ventral concavity present in females but not in males.

Oryzias kalimpaaensis sp. nov. differs from all other *Oryzias* species from Sulawesi by unique lateral line scale counts (60–67 in *O. kalimpaaensis* sp. nov. vs. 70–75 in *O. sarasinorum*, vs. <58 in all remaining Sulawesi *Oryzias*). It has a deeper body than *O. sarasinorum* (16.0–22.2% SL vs. 13–15% SL). *Oryzias kalimpaaensis* sp. nov. has more dorsal-fin rays (11–13) than *O. hadiatyae* Herder & Chapuis 2010 (8–10), *O. celebensis* (Weber 1894) (8–10), *O. woworae* Parenti & Hadiaty 2010 (8), *O. asinua* (7–9), *O. wolasi* Parenti, Hadiaty, Lumbantobing & Herder 2013 (7–9) and *O. dopingdopingensis* Mandagi, Mokodongan, Tanaka & Yamahira 2018 (8–9), and more pectoral-fin rays (11–13) than *O. eversi* (10). In contrast to ricefishes of the *O. woworae* species group (SE Sulawesi: *O. woworae*, *O. asinua*, *O. wolasi*) *O. kalimpaaensis* sp. nov. does not exhibit red color

in fins or bluish body coloration. Black blotches or lines on the lateral side of body are characteristic for *O. celebensis* (Parenti, 2008), but absent in *O. kalimpaaensis* sp. nov.. *Oryzias kalimpaaensis* sp. nov. has a relatively long head (30.1–33.7% SL) compared to *O. sarasinorum* (29% SL), *O. eversi* (28.4–30.7% SL), *O. soerotoi* (21.4–25.4% SL), *O. orthognathus* (22–26.1% SL), *O. nigrimas* (21.5–25% SL), *O. nebulosus* (23–26% SL), *O. marmoratus* (Aurich 1935) (24.0–27.2% SL), *O. matanensis* (Aurich 1935) (25–29% SL), *O. profundicola* Kottelat 1990 (22–25.4% SL), *O. celebensis* (24–26% SL), *O. woworae* (24–29% SL), *O. asinua* (25–30% SL), *O. wolasi* (25–30% SL) and *O. dopingdopingensis* (25.8–29.1% SL). Its anal-fin base is short (19.2–24.0% SL) compared to *O. soerotoi* (24.1–30.5% SL), *O. orthognathus* (26.2–31.6% SL), *O. nigrimas* (24.5–29.9% SL), *O. nebulosus* (25–29% SL), *O. marmoratus* (31.4–36.9% SL), *O. matanensis* (30.5–35.0% SL) and *O. profundicola* (37.4–41.4% SL). The snout concavity characterizing *O. hadiatyae* and *O. orthognathus* is absent in *O. kalimpaaensis* sp. nov..

Oryzias kalimpaaensis sp. nov. is distinguished from *Adrianichthys* spp. by its smaller adult body size (maximum size 52.8 mm SL, vs. 200 mm) (Parenti, 2008),



Fig. 4. Preserved specimens of *Oryzias kalimpaaensis* sp. nov., holotype (MZB 26462), ♂, 41.9 mm SL (top); paratype (MZB 26466), ♀, 42.4 mm SL (bottom).

and differs from all *Adrianichthys* except *A. oophorus* by having less anal-fin rays (20–22 vs. >23). From *A. oophorus*, it is distinguished by having more dorsal-fin rays (11–13 vs. 10) and longer head (30.1–33.7% SL vs. 25–27% SL (Parenti, 2008)).

Etymology. The species epithet, '*kalimpaaensis*', denotes the occurrence of this species in Lake Kalimpa'a, Central Sulawesi, the type locality.

Description. See Fig. 3 and Fig. 4 for general appearance in lateral view and table 1 for morphometric data. Female with pronounced abdominal concavity between pelvic fin and anal fin covered by adpressed pelvic fins (11.7–19.0% SL). Body compressed laterally, body depth 16.0–22.2% SL. Caudal fin truncate; principal caudal-fin rays i,4/5,i; procurrent caudal-fin rays 6/6–6/8. Mouth supra-terminal, lower and upper jaw equal or lower jaw slightly longer than upper jaw. Length of caudal peduncle 10.3–14.4% SL, depth of caudal peduncle 10.0–12.1% SL. 11–13 dorsal-fin rays; 20–22 anal-fin rays; 5–6 pelvic-fin rays; 11–13 pectoral-fin rays.

Head length 30.1–33.7% SL and eye diameter 25.0–29.6% HL. Dorsal head profile appears slightly concave just above the orbit to the nape. Dorsal body profile relatively straight without noticeable arch from nape to dorsal-fin origin. Ventral body profile relatively straight, with slight arching from head to anal. Genital papilla single lobed in both sexes.

Live coloration. Body yellowish-brown with a brown-greenish lateral line, more pronounced in females than in males. Belly and throat light yellowish to white.

7–10 faint blackish bars on lateral side of body. Dorsal surface of head blackish, extending posteriorly as narrow black dorsal stripe to dorsal-fin origin. Opercle with silver greenish sheen. Membranes of paired and unpaired fins hyaline, rays light cream colored. Base of dorsal fin yellowish. Anal-fin base with narrow blackish stripe. Males with black submargin in dorsal and anal fin. Females with blackish submarginal band in anal fin, submarginal marking in dorsal fin black, submargin lacking in females. Caudal-fin base yellowish, followed posteriorly by a faint blackish bar. Dorsal and ventral caudal-fin margins yellowish to orange, more pronounced in males than in females. Courtship coloration unknown (Fig. 3).

Color in alcohol. Body of males and females light yellowish-brown. 7–10 faint and irregular dark brown to blackish bars on lateral body in both sexes. Males and females with a faint blackish lateral stripe on lateral midline, extending from uppermost posterior opercle to caudal-fin base. Belly blackish grey in males, whitish in females. Throat whitish in males and females. Dorsal surface of head blackish in males and females, extending posteriorly as narrow blackish dorsal stripe. Unpaired fins whitish. Anal-fin base with narrow blackish stripe. Faint blackish submargin in anal fin. Blackish submargin present in dorsal fin in males, but not in females. Base of caudal fin yellowish in both sexes (Fig. 4).

Sexual dimorphism. Females have a slightly wider body compared to males (body width: 12.3–14.1% SL in females vs. 11.7–13.4% SL in males). The single-lobed genital papilla of males is tubular and slender compared

to the more rounded female papilla. Adult males have elongated rays in dorsal and anal fin, a typical sexual dimorphism in ricefishes. Length of dorsal fin extends beyond caudal-fin base in adult males (21.2–34.4% SL in males vs. 14.4–24.9% SL in females). Elongated fin-rays are absent in dorsal and anal fins of females. Females have longer (13.7–19.0% SL vs. 10.3–12.7% SL) pelvic fins compared to males and a pronounced abdominal concavity between anal fin and pelvic fin; egg clusters in the concavity are supported by the pelvic fins (Fig. 3; Fig. 4). Due to the less pronounced concavity, males have a larger relative body depth at anal-fin origin than females (19.3–22.2% SL vs. 16.0–20.3% SL). See ‘Live coloration’ for coloration of male and female specimens.

Reproduction. *Oryzias kalimpaaensis* sp. nov. is a ‘pelvic-brooder’. Pelvic-brooding is defined by females carrying a cluster of eggs, connected by attaching filaments to their gonoduct, until the fry hatches (Kottelat 1990a). All pelvic-brooding ricefish species described to date (*A. oophorus*, *A. poptae*, *O. eversi* and *O. sarasinorum*) share certain external female-specific morphological characteristics like elongated pelvic fins (compared to their respective males and to all transfer-brooding species) and the presence of a ventral concavity (Herder et al. 2012; Mokodongan & Yamahira 2015; Spanke et al. 2021). Female specimens of *O. kalimpaaensis* sp. nov. carry their eggs on the abdominal concavity covered by the pelvic fins. As described for the other pelvic-brooding species (Kottelat 1990a; Iwamatsu et al. 2008; Herder et al. 2012), the eggs do not adhere to each other, and are suspended by attaching filaments to the female’s genital pore. Approximately 24 eggs were counted from one female specimen depicted in Fig. 4. The eggs are clearly developed and partially pigmented with embryos visible. The size of each fertilized egg is about 2.19 ± 0.10 mm in diameter.

Distribution and habitat. *Oryzias kalimpaaensis* sp. nov. is known only from Lake Kalimpa’a, about 22 km from Lake Lindu, Central Sulawesi, situated at ca. 1,660 m above sea level. The lake is relatively small, with the longest distance across the surface being approximately 300 meters from Southwest to Northeast. The lake is used by local people as a place for nature tourism. At time of sampling, most female *O. kalimpaaensis* sp. nov. observed were carrying eggs. The lake habitat is characterized by calm water with sandy and muddy substrate with vegetation dominated by the weed *Phragmites karka* (Retz.) (Cyperales: Poaceae). The depth of Lake Kalimpa’a is about 11 meters (D. H. Kristianto & Wantoko Staff of BBTNLL, pers. comm.) and water temperature was 22 °C with pH 5–6.5 and DO 13.9 Mg/L, measured during daytime. There is a main inlet of Basakura Stream near the collection site and four more inlets of small streams around the lake. The Lake outlet is connected to the Sopus River, a tributary of the Palu River, which drains into the Makassar Strait. Other

species present in the Lake Kalimpa’a were introduced african tilapia *Oreochromis* sp. (Cichliformes: Cichlidae) and snakeskin gourami *Trichopodus pectoralis* Regan 1910 (Anabantiformes: Osphronemidae), and native eel *Anguilla* sp. (Anguilliformes: Anguillidae).

Phylogenetic relationships. The resulting ML phylogeny (Fig. 5) is largely congruent with the consensus tree of Mokodongan & Yamahira (2015) in all well supported branches, including the monophyly of all known *Oryzias* species from Sulawesi (the “*Oryzias celebensis* species group”). The two *O. kalimpaaensis* sp. nov. individuals (DDBJ accession numbers: LC669549–LC669550) cluster together and form the sister-group to the two other pelvic-brooding *Oryzias* species (*O. eversi* and *O. sarasinorum*) and *O. bonneorum*. *Oryzias sarasinorum* appears paraphyletic based on our phylogeny, as one individual clusters with *O. bonneorum*. Sister-group to this clade is the river-dwelling transfer-brooding species *O. dopingdopingensis*. Interestingly, *O. bonneorum* formed a clade together with the pelvic-brooding *Oryzias* species, although it is still unclear whether this species is a pelvic-brooder or not.

DISCUSSION

Ricefishes (Beloniformes: Adrianichthyidae) are a strikingly diverse group of fishes, inhabiting riverine, lacustrine, and brackish water habitats (Parenti 2008; Hilgers & Schwarzer 2019). On Sulawesi, they show a great diversity in coloration, shape, adaptations to divergent habitat conditions and belong to two genera (*Adrianichthys* and *Oryzias*) with two main reproductive strategies: transfer-brooding and pelvic-brooding. To date pelvic-brooding is described in four species (*A. oophorus*, *A. poptae*, *O. sarasinorum* and *O. eversi*), contrasted by all other ricefish species that are either transfer-brooding or have an unknown reproductive status (i.e., *O. bonneorum*, *A. roseni* Parenti & Soeroto 2004 and *A. kruyti* Weber 1913). More undiscovered diversity appears likely, as ricefishes tend to inhabit small isolated habitats (see Parenti & Hadiaty 2010). Several species of Sulawesi ricefishes are known only from water bodies with small surface area, i.e., *O. soerotoi* from Lake Tiu (Mokodongan et al. 2014), *O. hadiatyae* from Lake Masapi, a small satellite lake of Lake Towuti (Herder & Chapuis 2010), *O. eversi* from Tilanga pond, a tiny karst pool (Herder et al. 2012), as well as the new species described herein.

Oryzias kalimpaaensis sp. nov. is known only from the Lake Kalimpa’a, a small upland lake at ~1660 m above sea level. This is the highest altitude at which ricefishes have so far been recorded in Sulawesi. Distance between Lake Kalimpa’a and the closest habitat of other pelvic-brooding ricefishes – Lake Lindu, the habitat of *O. sarasinorum* and *O. bonneorum* – is only ~22 km (Fig. 2). However, both are separated by a substantial

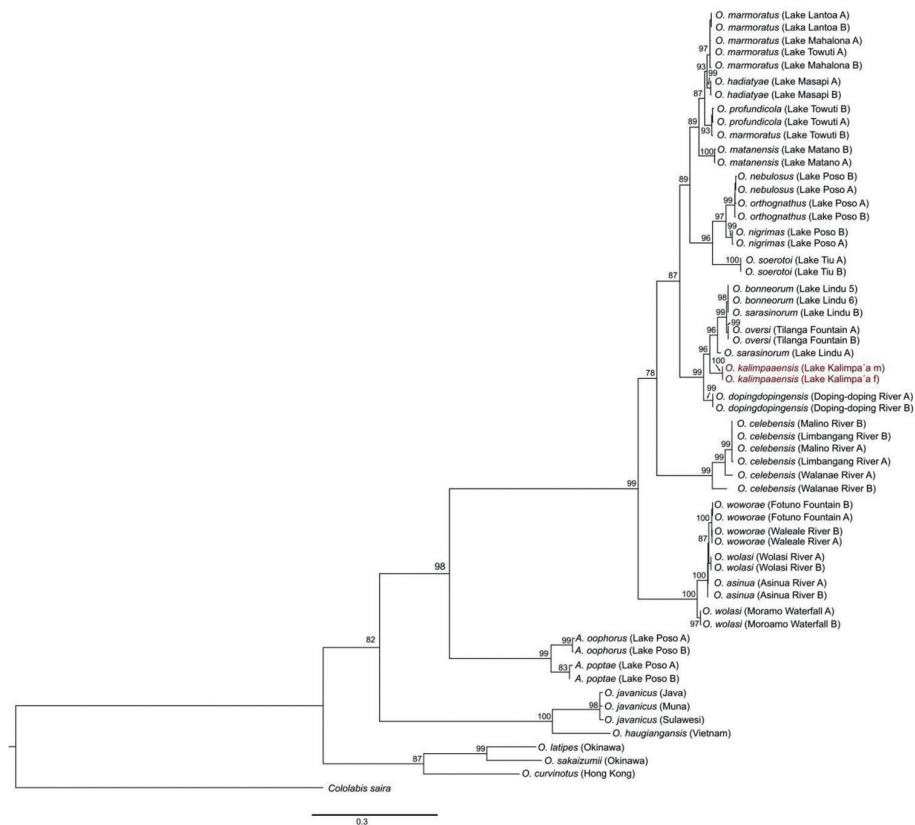


Fig. 5. Maximum Likelihood (ML) phylogenetic tree with the bootstrap values from 1000 bootstrap replicates. *Oryzias kalimpaaensis* sp. nov. is marked in red.

barrier, the Nokilalaki mountain (2357 m elevation). Geographic distance to pelvic-brooding *Adrianichthys* exceeds 56 km (Lake Poso: *A. oophorus*, *A. poptae*) and to *O. eversi* (Tilanga Pond) the distance is 194 km.

Based on our phylogenetic analyses, *O. kalimpaaensis* sp. nov. is closely related to both, the Lake Lindu ricefishes, and *O. eversi* (Fig. 5). Its placement within the clade of pelvic-brooding *Oryzias* appears plausible; however, the reproductive biology of *O. bonneorum* remains undocumented (Parenti 2008). The new species is distinguished from other ricefish species by several morphological characters, supporting that *O. kalimpaaensis* sp. nov. is a distinct species.

Although the reproductive ecology of *O. kalimpaaensis* sp. nov. has not been studied in detail yet, records of female *O. kalimpaaensis* sp. nov. with developing eggs on their belly (Fig. 3, Fig. 4 bottom) indicate – in line with the phylogenetic and morphological evidence – that it is a pelvic-brooding species. As in the other pelvic-brooding species, females show a set of adaptations enabling brooding, namely elongated pelvic fins, presence of a ventral concavity and long filaments attaching the eggs to the genital pore.

Lake Kalimpa'a is located in the Lore Lindu National Park, and is a popular destination for nature tourism. Protection of the flora and fauna inhabiting the lake should

be implemented, especially with regard to endemic species, like *O. kalimpaaensis* sp. nov.. The most substantial threats to Sulawesi's endemic freshwater fishes are exotic fish introduction, eutrophication, overfishing, and pollution (Parenti & Soeroto 2004, Herder et al. 2022). Lake Kalimpa'a already contains non-native fishes such as African tilapia (*Oreochromis* sp.) and snakeskin gourami (*Trichopodus pectoralis*) (A. Gani & N. Suhendra, pers. obs.), which might affect together with the direct anthropogenic impact (e.g., through nature tourism) the lake flora and fauna. We found anchor worms *Lernaea* sp. in a female *O. kalimpaaensis* sp. nov., which indicates that the population might already suffer stressful conditions; parasites and disease are one of the causes of the decline in other endemic lake fishes in Sulawesi (Kottelat 1990a; Herder et al. 2022).

Comparative material examined

Oryzias asinua: MZB 21464, 2, Indonesia, Sulawesi Tenggara, District Asinua; *Oryzias bonneorum*: MZB 15499, holotype, Indonesia, Sulawesi Tengah, Lake Lindu; *Oryzias celebensis*: MZB 2688, 4, MZB 5862, 3, Indonesia, Sulawesi Selatan, Regency of Maros Indonesia; *Oryzias eversi*: MZB 20780, holotype, MZB 20781, 1 paratype, Indonesia, Sulawesi Selatan, District Rante-

Table 1. Meristic and morphometric data of *Oryzias kalimpaaensis* sp. nov. from Lake Kalimpa'a. Data of the holotype are presented in a separate column.

	males				females		
	holotype	paratypes (N=10)			paratypes (N=7)		
	MZB26462	min.	max.	mode	min.	max.	mode
anal-fin rays (total)	21	20	22	20	20	22	22
dorsal-fin rays (total)	12	11	13	11	11	12	12
pelvic-fin rays	5	6	6	6	6	6	6
pectoral-fin rays	13	11	13	12	12	12	12
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
procurrent caudal-fin rays	6/7	6/6	6/8	6/7	6/6	6/7	6/7
scales in lateral row	66	62	67	66	61	66	64
		min.	max.	mean (SD)	min.	max.	mean (SD)
standard length (mm)	41.9	44.1	50.2	47.5 (±2.2)	41.1	52.8	44.4 (±4.1)
% standard length							
total length	122.3	119.3	122.9	120.8 (±1.3)	119.0	121.9	120.1 (±1.0)
head length	33.7	30.1	33.1	31.4 (±1.0)	30.6	33.2	32.0 (±1.0)
head depth	33.7	16.7	32.1	20.7 (±5.9)	18.7	32.9	26.5 (±6.6)
head width	19.5	18.0	21.7	20.0 (±1.1)	18.5	22.4	20.0 (±1.6)
predorsal length	75.4	74.4	78.0	75.4 (±1.1)	73.3	75.9	74.9 (±1.0)
prepelvic length	49.7	49.7	51.8	50.5 (±0.8)	49.2	52.9	51.19 (±1.4)
preanal length	62.6	61.7	64.6	62.7 (±1.0)	61.8	65.7	63.29 (±1.4)
body depth	21.0	19.3	22.2	21.0 (±1.0)	16.0	20.3	18.49 (±1.5)
body width	11.7	12.1	13.4	12.7 (±0.3)	12.3	14.1	13.49 (±0.6)
length of caudal peduncle	10.6	10.3	13.3	11.9 (±1.0)	10.7	14.4	12.09 (±1.3)
depth of caudal peduncle	10.1	10.9	11.5	11.2 (±0.2)	10.0	12.1	11.09 (±0.8)
length of dorsal fin	34.4	21.2	29.1	24.5 (±2.4)	14.4	16.1	15.29 (±0.8)
length of dorsal fin base	12.8	11.3	13.9	12.7 (±0.7)	10.7	13.1	11.89 (±0.8)
length of anal fin base	23.9	19.2	24.0	22.7 (±1.4)	19.4	22.5	21.99 (±1.1)
length of pectoral fin	22.7	17.5	21.7	19.2 (±1.5)	17.3	19.8	18.69 (±0.9)
length of pelvic fin	10.6	10.3	12.6	11.4 (±0.7)	13.7	18.9	15.89 (±1.8)
% head length							
interorbital width	29.4	27.8	36.5	33.0 (±2.6)	30.6	34.2	32.79 (±1.5)
eye diameter	26.9	25.0	28.4	26.7 (±1.1)	26.3	29.6	28.09 (±1.0)
snouth length	34.7	31.5	39.2	35.5 (±2.3)	32.5	39.6	35.29 (±2.3)

pao; *Oryzias hadiatyae*: MZB 18491, holotype, MZB 18503, 1 paratype, MZB 18504, 1 paratype, MZB 18505, 1 paratype, MZB 18506, 1 paratype, Indonesia, Sulawesi Selatan, Lake Masapi; *Oryzias marmoratus*: MZB 2686, 7, MZB 2690, 1, Indonesia, Sulawesi Selatan, Lake Wawontoa; MZB 2695, 5, MZB 2697, 5, Indonesia, Sulawesi Selatan, Lake Towuti; *Oryzias matanensis*: MZB 21379, 1, Indonesia, Sulawesi Selatan, Lake Matano; *Oryzias nebulosus*: MZB 21381, 2, Indonesia, Sulawesi

Tengah, Lake Poso; *Oryzias nigrimas*: MZB 5859, holotype, MZB 5872, 5 paratypes, Indonesia, Sulawesi Tengah, Lake Poso; *Oryzias orthognathus*: MZB 5870, holotype, Indonesia, Sulawesi Tengah, Lake Poso; *Oryzias profundicola*: MZB 5868, holotype, MZB 5861, 1 paratype, MZB 5866, 1 paratype, MZB 5867, 2 paratypes, Indonesia, Sulawesi Selatan, Lake Towuti; *Oryzias wolasi*: MZB 21465, 2, Indonesia, Sulawesi Tenggara, District

Wolasi; *Oryzias woworae*: MZB 21380, 1, Indonesia, Sulawesi Tenggara, Muna Island, District Parigi.

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REFERENCES

- Darriba D, Posada D, Kozlov AM, Stamatakis A, Morel B, Flouri T (2019) ModelTest-NG: A New and Scalable Tool for the Selection of DNA and Protein Evolutionary Models. *Molecular Biology and Evolution* 37 (1): 291–294
- Elder D, Klein J, Antonelli A, Silvestro D (2021) raxmlGUI 2.0: A graphical interface and toolkit for phylogenetic analyses using RAxML. *Methods in Ecology and Evolution* 12 (2): 373–377
- Hadiaty RK (2018) Taxonomical status of endemic freshwater ichthyofauna of Sulawesi. *Jurnal Ikhtologi Indonesia* 18 (2): 175–190. <https://doi.org/10.32491/jii.v18i2.428>
- Herder F, Hadiaty RK, Nolte AW (2012) Pelvic-Fin Brooding in a New Species of Riverine Ricefish (Atherinomorpha: Beloniformes: Adrianichthyidae) from Tana Toraja, Central Sulawesi, Indonesia. *Raffles Bulletin of Zoology* 60 (2): 467–476
- Herder F, Chapuis S (2010) *Oryzias hadiatyae*, a new species of ricefish (Atherinomorpha: Beloniformes: Adrianichthyidae) endemic to Lake Masapi, Central Sulawesi, Indonesia. *Raffles Bulletin of Zoology* 58 (2): 269–280
- Herder F, Möhring J, Flury JM, Utama IV, Wantania L, Wowor D, Boneka FB, Stelbrink B, Hilgers L, Schwarzer J, Pfaender J (2022) More non-native fish species than natives, and an invasion of Malawi cichlids, in ancient Lake Poso, Sulawesi, Indonesia. *Aquatic Invasions* 17 (1): 72–91
- Hilgers L, Schwarzer J (2019) The untapped potential of medaka and its wild relatives. *Elife* 8: e46994. <https://doi.org/10.7554/eLife.46994>
- Iwamatsu T, Kobayashi H, Sato M, Yamashita M (2008) Reproductive role of attaching filaments on the egg envelope in *Xenopoecilus sarasinorum* (Adrianichthyidae, Teleostei). *Journal of Morphology* 269 (6): 745–750
- Kottelat M (1990a) Synopsis of the endangered Buntingi (Osichthyidae: 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
- Mandagi IF, Mokodongan DF, Tanaka R, Yamahira K (2018) A New Riverine Ricefish of the Genus *Oryzias* (Beloniformes, Adrianichthyidae) from Malili, Central Sulawesi, Indonesia. *Copeia* 106 (2): 297–304
- Miesen FW, Droppelmann F, Hüllen S, Hadiaty RK, Herder F (2016) An annotated checklist of the inland fishes of Sulawesi. *Bonn Zoological Bulletin* 64 (2): 77–106
- Mokodongan DF, Tanaka R, Yamahira K (2014) A New Ricefish of the Genus *Oryzias* (Beloniformes, Adrianichthyidae) from Lake Tiu, Central Sulawesi, Indonesia. *Copeia* 3: 561–567
- Mokodongan DF, Yamahira K (2015) Origin and intra-island diversification of Sulawesi endemic Adrianichthyidae. *Molecular Phylogenetics and Evolution* 93: 150–160
- Parenti LR (2008) A phylogenetic analysis and taxonomic revision of ricefishes, *Oryzias* and relatives (Beloniformes, Adrianichthyidae). *Zoological Journal of the Linnean Society* 154 (3): 494–610
- Parenti LR, Hadiaty RK, Lumbantobing D, Herder F (2013) Two New Ricefishes of the Genus *Oryzias* (Atherinomorpha: Beloniformes: Adrianichthyidae) Augment the Endemic Freshwater Fish Fauna of Southeastern Sulawesi, Indonesia. *Copeia* 3: 403–414
- Parenti LR, Soeroto B (2004) *Adrianichthys roseni* and *Oryzias nebulosus*, two new ricefishes (Atherinomorpha: Beloniformes: Adrianichthyidae) from Lake Poso, Sulawesi, Indonesia. *Ichthyological Research* 51 (1): 10–19
- Parenti LR, Hadiaty RK (2010) A New, Remarkably Colorful, Small Ricefish of the Genus *Oryzias* (Beloniformes, Adrianichthyidae) from Sulawesi, Indonesia. *Copeia* 2: 268–273
- Spanke T, Hilgers L, Wipfler B, Flury JM, Nolte AW, Utama IV, Misof B, Herder F, Schwarzer J (2021) Complex sexually dimorphic traits shape the parallel evolution of a novel reproductive strategy in Sulawesi ricefishes (Adrianichthyidae). *BMC Ecology and Evolution* 21
- Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic acids research* 22: 4673–4680
- Utama IV, Mandagi IF, Lawelle SA., Masengi KW, Watanabe K, Sawada N, Nagano AJ, Kusumi J, Yamahira K (2022). Deeply divergent freshwater fish species within a single river system in central Sulawesi. *Molecular Phylogenetics and Evolution* 173: 107519

APPENDIX I

(electronic supplement, available at www.zoologicalbulletin.de)

Table S1. All measurements and counts of examined *O. kalimpaaensis*; separated in ♂♂ in ♀♀. *holotype.