

## THE NATURAL HISTORY OF MODEL ORGANISMS

# The untapped potential of medaka and its wild relatives

**Abstract** The medaka is a fish that has served as a model organism for over a century, yet there is still much to learn about its life in the wild. Here we summarize the current knowledge, highlight recent progress and outline remaining gaps in our understanding of the natural history of medaka. It has also become clear over time that rather than being a single species, medaka comprises an entire species complex, so disentangling the species boundaries is an important goal for future research. Moreover, medaka and other ricefishes exhibit striking functional diversity, little of which has been investigated to date. As such, there are opportunities to use the resources developed for medaka to study other ricefishes, and to learn more about medaka itself in an evolutionary context.

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## Introduction

Medaka (*Oryzias latipes* species complex) is a small, egg-laying freshwater fish from East Asia (**Figure 1**). It is often found in rice fields, which earned it its common English name of ricefish and the genus name of *Oryzias* (based on the genus name of rice, *Oryza*). The medaka has been kept as an ornamental fish in Japan from as early as the 17<sup>th</sup> century, and its central role in Japanese culture is exemplified by its appearance in pieces ranging from Edo era paintings (**Kinoshita et al., 2009; Naruse et al., 2011; Takeda and Shimada, 2010**) to modern-day children's songs (**Mamun et al., 2016**).

The first medaka species was described as *Poecilia latipes* by **Temminck and Schlegel (1846)** in the *Fauna Japonica* based on collections by the German physician and naturalist Philipp Franz von Siebold. It was reclassified in the genus *Oryzias* by **Jordan and Snyder (1906)** to become *Oryzias latipes*, and, more recently, it has become clear that the medaka comprises a species complex rather than a single species (**Table 1**).

The traditional breeding of naturally occurring color mutants led to experiments in the early 20<sup>th</sup> century that sparked the medaka's role as a model organism. It later proved to be an ideal laboratory organism that was easy to maintain due to its small body size, and its

simple dietary and habitat requirements. The combination of a sexual dimorphism, short generation times, large and transparent eggs with easily observable development, high tolerance to inbreeding and a comparably small genome size (~700 Mb) also made medaka suitable for a wide range of studies (**Braasch et al., 2015; Kasahara et al., 2007; Kirchmaier et al., 2015; Parenti, 2008; Wittbrodt et al., 2002**). Today, the medaka is a fully fledged model vertebrate that is deeply rooted within the life sciences (**Hori, 2011; Kirchmaier et al., 2015; Shima and Mitani, 2004; Takeda and Shimada, 2010; Wittbrodt et al., 2002**).

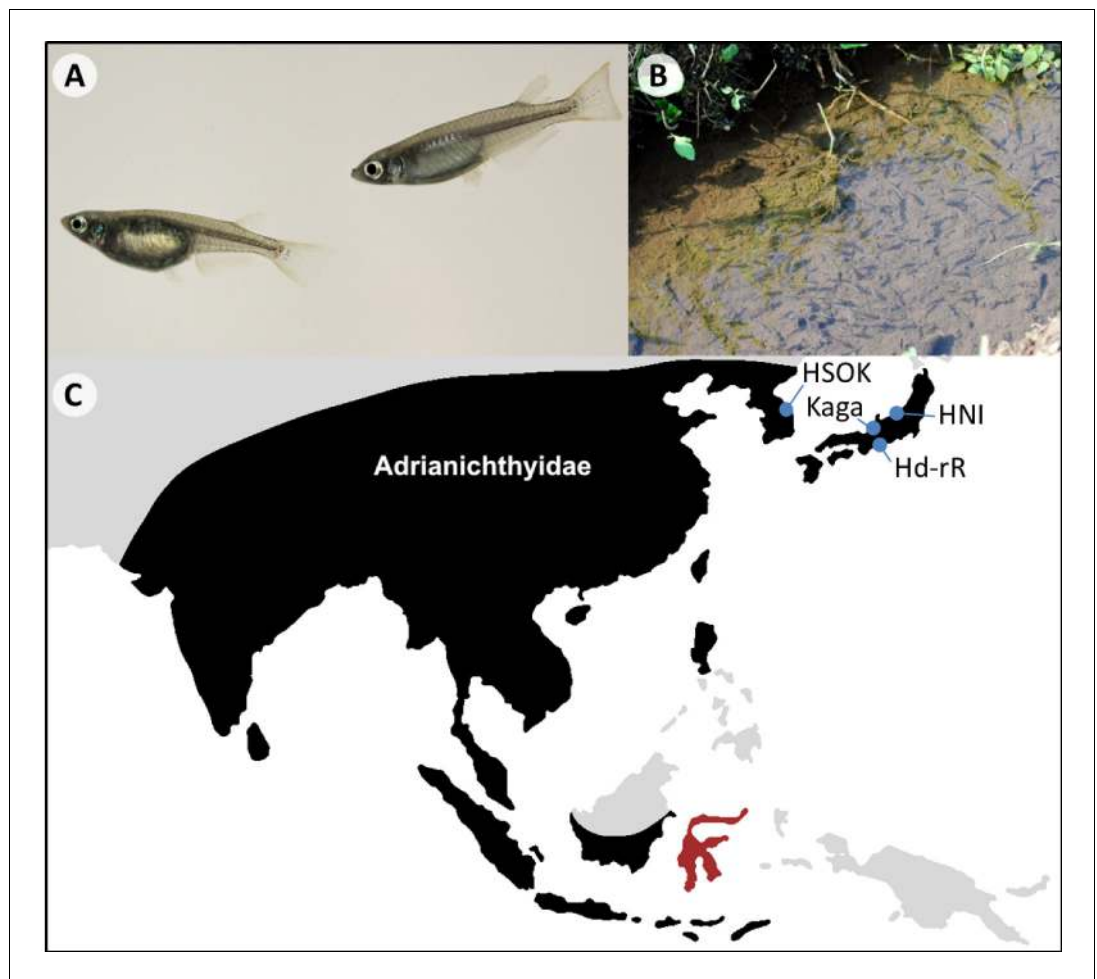
Early systematic crossing of color mutants of medaka provided evidence for Mendelian inheritance in vertebrates, sex limited inheritance and crossing over between sex chromosomes (**Aida, 1921; Ishikawa, 1913; Toyama, 1916**). Subsequently, the first hormone induced sex-reversal (**Yamamoto, 1958**) and the discovery of the first sex determining gene in a non-mammalian vertebrate (**Matsuda et al., 2002**) paved the way for the medaka as a model for sex determination and sex differentiation (**Kondo et al., 2009**). Scientific milestones achieved with the medaka further include the detection of the first active DNA-based transposable element (*To1*) in a vertebrate (**Koga et al., 1995**), which laid

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**Figure 1.** *Oryzias latipes*. (A) Male (right) and female (left) *Oryzias latipes* from Kiyosu (Photo by Felix Loosli). Males can be easily distinguished from females by their elongated anal and dorsal fins. (B) A school of medaka in their natural habitat. (C) Approximate distributional limits of the ricefishes family, the Adrianichthyidae (black), following Parenti (2008) with the locations from which some of the most famous medaka strains are derived (blue). The Indonesian island Sulawesi represents a ricefish biodiversity hotspot (red).

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the foundation for the development of several tools for genetic modification (Koga, 2011).

Over time, medaka developed into a model for development, toxicology, carcinogenesis and behavior and a growing number of inbred strains in combination with an expanding genetic toolbox provided ever growing resources for genetic studies (reviewed in Kirchmaier et al., 2015). Medaka's role as genomic model organism was consolidated when its genome became available as one of the first for any vertebrate (Kasahara et al., 2007). More recently, the gene-editing tool CRISPR/Cas9 was also established in medaka and employed in multiple studies (e.g. Gutierrez-Triana et al., 2018; Letelier et al., 2018; Murakami et al., 2017; Watakabe et al., 2018). In many fields,

including cancer research, medaka serves as complementary model to the well-established zebrafish (Schartl and Walter, 2016; Takeda and Shimada, 2010). In particular, the natural variation captured in several inbred strains and an isogenic panel offers a unique resource to study allelic variation including disease modifier genes (Schartl and Walter, 2016; Spivakov et al., 2014).

Due to its long and successful history as a model organism, several comprehensive reviews focus on the medaka and its role in different fields of research (Kinoshita et al., 2009; Kirchmaier et al., 2015; Naruse et al., 2011; Shima and Mitani, 2004; Takeda and Shimada, 2010; Wittbrodt et al., 2002; Yamamoto, 1975). In contrast, the natural history of

**Table 1.** Medaka nomenclature.

Common names	Scientific name
Japanese medaka, Southern (Japanese) population	<i>O. latipes</i>
Northern medaka, Northern (Japanese) population	<i>O. sakaizumii</i>
East Korean population	N/A
China–West Korean population	<i>O. sinensis</i>
Chinese medaka	<i>O. sinensis</i>
Taiwanese population, Taiwanese medaka	<i>O. cf. sinensis</i>

The model organism medaka comprises several species and deeply divergent lineages from the *Oryzias latipes* species complex. Hence, in this article we only use the term “medaka” when we collectively refer to lineages within the *Oryzias latipes* species complex in the context of the model system. Common or scientific species names are used to specifically refer to individual lineages as shown in this table.

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the medaka and its relatives has so far gained comparatively little attention. This is despite the fact that particularly Japanese biologists have been working on ricefishes for decades, which generated a more complete knowledge of *O. latipes* and its relatives than is available for many other fish species (Iwamatsu, 2006). However, some of this work is not available in English and thus is less accessible to the international research community. Furthermore, studies have highlighted a so far underexplored ricefish diversity in the wild (Katsumura et al., 2019; Parenti, 2008; Takehana et al., 2005; Uwa et al., 1988), which has already inspired new research agendas (e.g., Matsuda and Sakai-zumi, 2016; Mokodongan et al., 2018; Spivakov et al., 2014). The combination of available medaka resources and the diversity of its wild relatives represent a treasure trove to target various aspects of biology, which will equally expand our insight into the medaka as a model system. Here, we focus on the natural history of ricefishes and point out recent progress as well as remaining potential for studies focusing on their natural diversity.

### Medaka’s life in the wild

Medaka species are distributed in Japan, China, Taiwan and Korea (Shima and Mitani, 2004; Yamamoto, 1975), where they are mainly found in standing and slow-flowing water bodies such as rice paddies, ponds and agricultural channels (Fukuda et al., 2005, Figure 1B). The preferred habitats of medaka are shallow zones close to the shoreline with abundant plant cover where it feeds on microscopic organisms, including algae and zooplankton (Edeline et al., 2016; Fukuda et al., 2005). Although detailed studies on its position in the food web are lacking,

medaka is likely mainly preyed upon by dragonfly larvae, birds and predatory fishes. Throughout its life, medaka is parasitized by both ectoparasites and endoparasites from across the animal kingdom (catalogued in: Nagasawa, 2017), and females appear to be particularly susceptible to ectoparasites during the breeding season (Edeline et al., 2016). In the wild, most medaka have a lifespan of roughly one year during which males and females can reach a length between three and four centimeters (Leaf et al., 2011; Parenti, 2008; Shima and Mitani, 2004; Yamamoto, 1975). In captivity, medaka may live for more than four years (Egami and Etoh, 1969). As an inhabitant of the temperate zone, medaka is confronted with and tolerates a wide range of temperatures, from 4°C to 40°C (Kinoshita et al., 2009; Sampetean et al., 2009). When the water cools down, fish enter a state of hibernation during which they barely move and stop feeding (Edeline et al., 2016; Kinoshita et al., 2009). Cooling has been used in the lab to slow down the life cycle of medaka and store it for later use (Wittbrodt et al., 2002).

During the breeding season, medaka spawns every morning around sunrise (Leaf et al., 2011; Ueda and Oishi, 1982). Mating involves an intricate courtship dance during which males repeatedly approach females and exhibit diverse displays (Ono and Uematsu, 1957). If accepted, males hold on to the females with their elongated anal and dorsal fins, while females spawn up to 48 eggs (Leaf et al., 2011). Bundles of fertilized eggs stay attached to the mother’s ovarian cavity via attaching filaments for up to a few hours, before they are deposited (Parenti, 2005). Despite its practical use in the laboratory to easily harvest eggs from unambiguously identifiable

## Box 1. Open questions concerning the natural history of ricefishes:

- What is the general ecology of medaka species in the wild: What abiotic factors restrict their distribution; which species do they interact with and in what way?
- How much gene flow exists between *O. latipes* and *O. sakaizumii*?
- Which genes underlie local adaptation in the *O. latipes* species complex?
- How connected are populations of medaka species outside of Japan?
- How much ricefish diversity remains undiscovered?
- What are the major threats to ricefish populations?
- What mechanisms promote evolutionary divergence and functional diversity within medaka and across ricefishes in general?
- What are the molecular mechanisms underlying rapid sex chromosome turnover in ricefishes and what is its role for ricefish diversity?
- What are the processes underlying the remarkable ricefish diversity on Sulawesi, Indonesia?
- Which evolutionary scenarios gave rise to sympatric ricefish species flocks?
- How did pelvic brooding evolve: What is its physiological and genetic basis and what are the adaptive benefits?

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mothers, the reasons for the evolution of this unusual reproductive strategy remain unexplored. Juveniles finally hatch after 7–9 days and grow to maturity within about two and a half months (Iwamatsu, 2004; Takeda and Shimada, 2010).

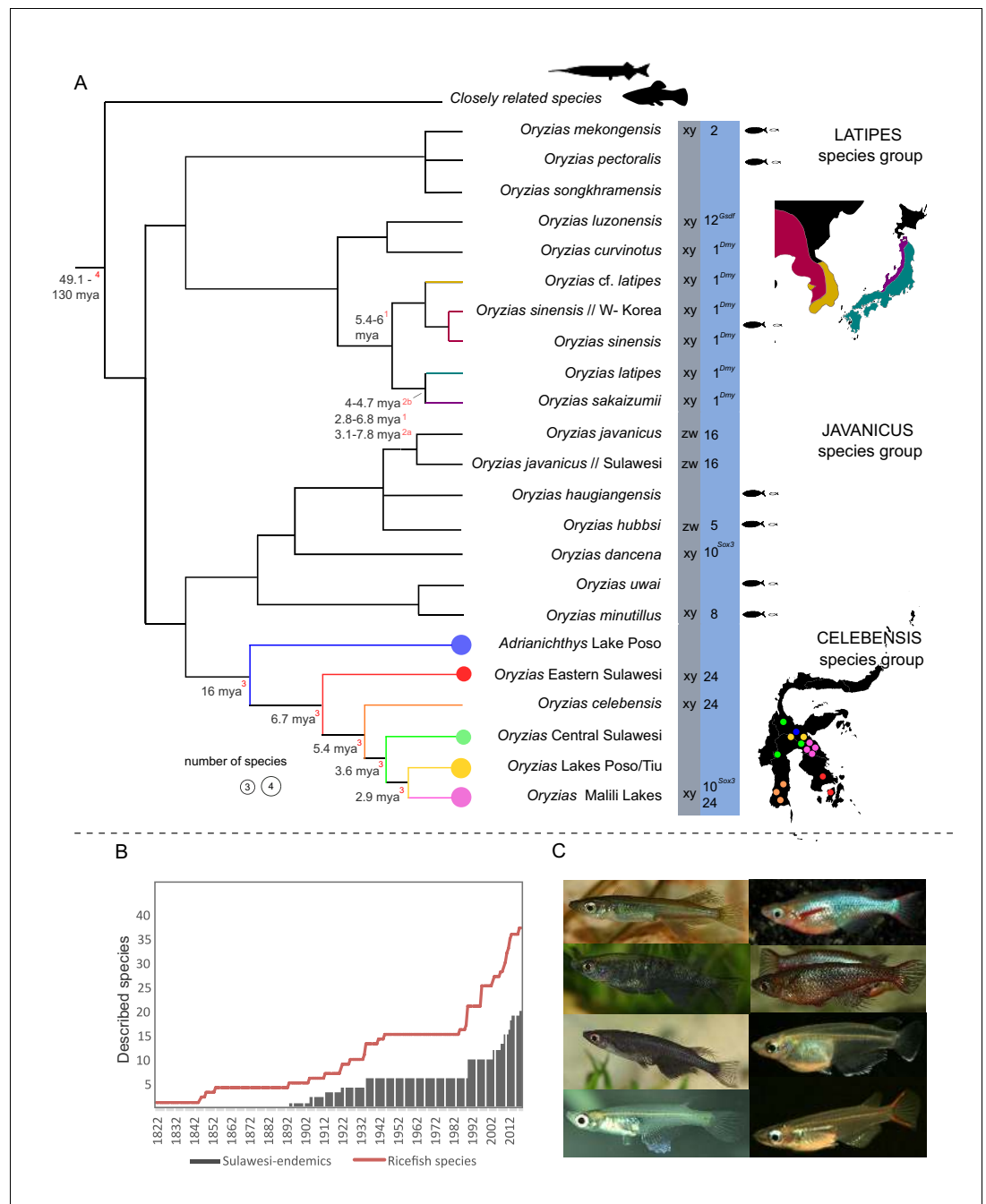
Overall, relatively little of our current knowledge on the biology of medaka has been

gathered from studies in the wild. Hence, similar to other model organisms, much remains to be discovered about the ecology and life history of wild medaka populations (Box 1), and the driving evolutionary forces (Alfred and Baldwin, 2015; Leaf et al., 2011; Parichy, 2015).

## Natural diversity of medaka

### The *Oryzias latipes* complex

Differences between Japanese medaka populations were recognized early on (Sakai-zumi, 1984), but it is only now that the scientific community has begun to fully appreciate the diversity summarized within the “medaka”. For a long time, the so-called northern and southern Japanese populations, from which the two most famous medaka inbred lines (Hd-rR and HNI) are derived, were regarded as a single species: *Oryzias latipes* (Figure 1C). Yet accumulating evidence suggested that these lines represent different species. Hence, the northern Japanese population was described as a new species: *Oryzias sakaizumii* by Asai et al., 2011, and medaka is now frequently referred to as “*Oryzias latipes* species complex” (Iguchi et al., 2018; Kawajiri et al., 2015; Takehana et al., 2016). Depending on the method, *Oryzias latipes* and *Oryzias sakaizumii* were estimated to have diverged between 3 and 8 million years ago (Figure 2A, Katsumura et al., 2019; Takehana et al., 2003; but see Setiamarga et al., 2009). They differ in a number of traits including craniofacial anatomy (Kimura et al., 2007), body coloration (Asai et al., 2011), aggressiveness (Kagawa, 2014) and the degree of sexual dimorphism of the dorsal fin (Asai et al., 2011), the last of which is likely mediated by different sex steroid levels during development (Katsumura et al., 2014; Kawajiri et al., 2015). Nonetheless, both species produce viable hybrid offspring in the laboratory (e.g. Murata et al., 2012) and current evidence also suggests limited gene flow in the wild (Iguchi et al., 2018; Katsumura et al., 2019). *Oryzias latipes* and *O. sakaizumii* thus offer the opportunity to gain insight into the evolution of physiological and behavioral trait disparity and their genetic bases. Recent insight into the biogeography of the medaka also suggests that *O. latipes* colonized the main island of Japan from the south (i.e., from Kyushu) and subsequently dispersed along the Pacific coast (Katsumura et al., 2019). In contrast, *Oryzias sakaizumii* likely originated on

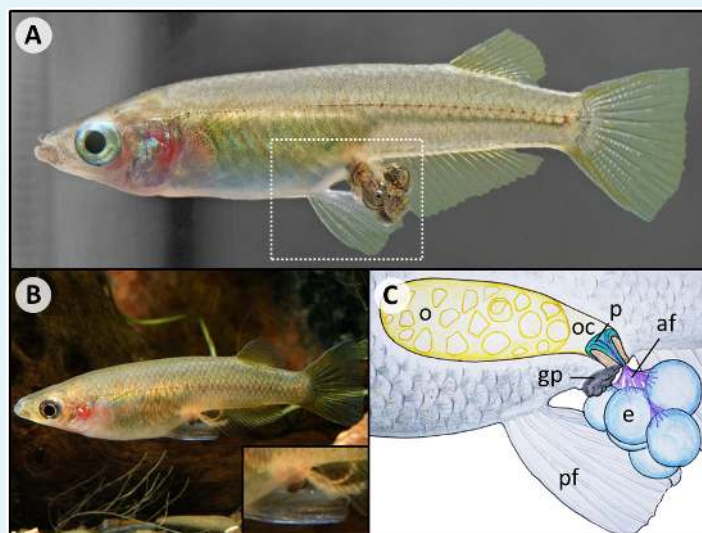


**Figure 2. Ricefish diversity.** (A) Combined phylogeny based on published studies from: *Katsumura et al., 2019*, *Parenti, 2008*; *Magtoon, 2010*; *Mokodongan and Yamahira, 2015*; *Takehana et al., 2005*. Sex determination systems and linkage groups with sex determination (SD) genes are given in the gray and blue columns, respectively (based on *Matsuda and Sakaizumi, 2016* and *Myosho et al., 2015*). Species defined as miniaturized (following *Parenti, 2008*) are marked with black/white fish. Detailed distribution maps are provided for the *O. latipes* species complex and for the celebensis species group. Divergence times in the tree are based on: <sup>1</sup>*Takehana et al., 2003*; <sup>2a</sup>*Katsumura et al., 2019*: scenario III; <sup>2b</sup>*Katsumura et al., 2019*: scenario IV; <sup>3</sup>*Mokodongan and Yamahira, 2015*; <sup>4</sup>*Hughes et al., 2018*. (B) Total number of described species from the first description of a ricefish in 1822 (*Hamilton, 1822*) until today (red line) in relation to described Sulawesi endemics (gray bars). The high number of newly described species in recent years especially on Sulawesi points towards a largely underexplored ricefish diversity. (C) Diversity in coloration and shapes of male *Oryzias*. From left to right and top to bottom: *O. sarasinorum*<sup>\*</sup>, *O. woworae*<sup>§</sup>, *O. eversi*<sup>\*</sup>, *O. wolasi*<sup>\*</sup>, *O. nigrimas*<sup>\*</sup>, *O. dancena*<sup>§</sup>, *O. minutillus*<sup>§</sup>, *O. mekongensis*<sup>§</sup> (Photos were taken by A Wagnitz<sup>\*</sup> and J Geck<sup>§</sup>).

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## Box 2. Pelvic brooding – a complex innovation.

Some ricefishes from Sulawesi exhibit a unique reproductive strategy referred to as “pelvic brooding” (**Kottelat, 1990**). While most ricefishes deposit fertilized eggs shortly after spawning, pelvic brooders instead carry an egg-clutch in a ventral concavity until the fry hatches (**Box 2—figure 1**). Pelvic brooding likely relies on a complex set of adaptations (**Iwamatsu et al., 2007; Iwamatsu et al., 2008; Kottelat, 1990; Parenti, 2008**). Elongated pelvic fins cover the eggs, while a plug structure that is connected to the eggs via attaching filaments anchors them inside the female’s ovarian cavity. Ovulation is delayed in pelvic brooders for as long as eggs are attached, and the plug was even assumed to allow the transfer of nutrients, akin to a placenta (**Iwamatsu et al., 2008**). Despite this complexity, pelvic brooding occurs in at least three species from two lineages, namely *Adrianichthys oophorus*, *Oryzias eversi* and *Oryzias sarasinorum*. This means it was either lost multiple times or evolved twice independently (**Mandagi et al., 2018; Mokodongan and Yamahira, 2015**). Pelvic brooding was suggested to have evolved in adaptation to the absence of suitable spawning substrates in pelagic habitats (**Herder et al., 2012a**). The recent discovery of the oviparous *O. dopingdopingensis* by **Mandagi et al. (2018)**, a sister species of the pelvic brooding *O. sarasinorum* and *O. eversi*, in a river in central Sulawesi might be considered as support for this hypothesis. However, the pelvic brooding *O. eversi* was described from a spring where potential spawning substrates are abundant (**Herder et al., 2012a**). Consequently, the eco-evolutionary dynamics promoting pelvic brooding as well as the underlying molecular mechanisms remain virtually unknown.



**Box 2—figure 1.** Overview of pelvic brooding. **(A)** The pelvic brooding ricefish *O. eversi* carries its eggs until the fry hatches (dashed box indicates section depicted in **(C)**). **(B)** Once the fry has hatched, only the filaments remain attached to the female (Photo by Hans-Georg Evers). **(C)** A schematic overview of anatomic structures that likely play a role in pelvic brooding. The eggs (e) stay attached to the female via attaching filaments (af). A plug (p) anchors the attaching filaments (af) inside the female’s ovarian cavity (oc) next to the ovary (o). Compared to their non-pelvic brooding relatives, pelvic brooding species further appear to have elongated pelvic fins (pf) that cover the eggs and a heavily pigmented, enlarged genital papilla (gp), whose role in pelvic brooding is unclear.

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the western side of the Japanese Alps, which still largely separate both species in their current distribution (**Figure 2A, Katsumura et al., 2019**).

The Korean medaka lineages are genetically divergent from the Japanese species and also highly structured (**Figures 1C and 2A**). They comprise at least two subgroups that have been

referred to as the East Korean (HSOK strain) and China–West Korean populations (**Ds et al., 2013; Kang et al., 2005; Katsumura et al., 2019; Parenti, 2008**). Although still frequently referred to as *O. latipes* (see **Katsumura et al., 2019; Spivakov et al., 2014; Takeda and Shimada, 2010**), the China–West Korean

population together with populations from Taiwan, likely represents a different species: *Oryzias sinensis* (see *Ds et al., 2013; Parenti, 2008; Tzeng et al., 2006*). While further data are required, mitochondrial phylogenies indicate the existence of a locally restricted ancient Taiwanese medaka lineage and two lineages derived from more recent invasion events (*Tzeng et al., 2006*). Continental *O. sinensis* are also genetically diverse and geographically distant populations exhibit signatures of divergence, for example, between Shanghai and Korea (*Katsumura et al., 2019*). However, comprehensive studies covering the morphological and genetic diversity in continental Asia are still lacking. Such studies will be quintessential to recognize species limits and distribution patterns in the future. Generally, whether or not divergent lineages within the *O. latipes* species complex represent different species is still treated controversially and inconsistently in the current literature (see *Katsumura et al., 2019; Parenti, 2008; Sasaki and Yamahira, 2016; Spivakov et al., 2014; Tzeng et al., 2006*). Aside from the underlying taxonomic debate, information about the degree of divergence and abundance of gene flow between lineages is fundamental for the design of experiments, as well as the interpretation and integration of their results.

### **Latitudinal adaptation in Japanese medaka species**

The wide range of Japanese medaka species has inspired research on latitudinal adaptation. For example, a recent study identified differences in courtship between populations of *O. sakaizumii*, which were inferred to result from stronger sexual selection at lower latitudes (*Sasaki and Yamahira, 2016*). Additionally, juveniles of *O. sakaizumii* from higher latitudes compensate for a shorter growing season further north with faster growth rates (*Yamahira and Takeshi, 2008*). However, faster growth, which is achieved by higher feeding rates, comes with a cost and is accompanied by higher vulnerability for predation by dragonfly larvae (*Suzuki et al., 2010*). Hence, different growth capacities in *O. sakaizumii* likely evolve in response to both predation pressure and length of the growing season (*Suzuki et al., 2010*). One study also found delayed fin development in northern populations of *O. sakaizumii* compared to the southernmost populations of *O. latipes*, which was speculated to have evolved in a trade-off for faster body growth (*Kawajiri et al., 2009*). However, this

study illustrates the caveats related to taxonomic uncertainties, because the authors unintentionally compared populations of different species and thus did not take other interspecific differences into consideration. Nonetheless, their interpretation might be accurate and interspecific diversity clearly represents a chance to gain insight into its evolution, if species boundaries are recognized.

## **The diversity of medaka's wild relatives**

### **Ricefish phylogeny**

Although mainly recognized for medaka, ricefishes comprise a considerable diversity with 36 described species in two genera (*Oryzias*: 32 species, *Adrianichthys*: four species, *Asai et al., 2011; Herder et al., 2012a; Herder and Chapuis, 2010; Magtoon, 2010; Mandagi et al., 2018; Mokodongan et al., 2014; Parenti, 2008; Parenti et al., 2013; Parenti and Hadiaty, 2010*). They form three major clades referred to as the latipes-, javanicus- and celebensis species group, the last of which diversified on Sulawesi, Indonesia (*Figure 2A, Mokodongan and Yamahira, 2015; Takehana et al., 2005*). With a total of 21 species and 19 local endemics, this island represents a hotspot of ricefish diversity (*Figures 1C and 2A–B*). Additionally, nine newly described species within the last decade, eight of them endemic to Sulawesi, suggest that both the global ricefish biodiversity as well as the ricefish fauna of Sulawesi are still underdescribed (*Figure 2A*). Nonetheless, the ricefishes known to science exhibit striking functional diversity, with relatively little of it understood or even investigated so far. In the following paragraphs we highlight research making use of ricefish diversity together with aspects that appear especially promising for future research endeavors.

### **Sex determination**

Sex determination mechanisms are highly variable in bony fishes (*Heule et al., 2014*) and genetic sex determination (SD) appears to be particularly diverse in ricefishes (*Kondo et al., 2009; Matsuda and Sakaizumi, 2016; Myosho et al., 2015; Takehana et al., 2007a; Takehana et al., 2007b*). So far, seven sex chromosomes and three different master SD genes have been identified in ricefish species (*Figure 2A*). For example, *O. latipes* has a young XY system in which *dmrt1bY (dmy)* on linkage

group 1 (LG1) acts as the male SD gene (*Mat-suda, 2005; Matsuda et al., 2002*). In contrast, *gsdf* on LG12 is the SD gene in *O. luzonensis* (*Myosho et al., 2012*), and *sox3* on LG10 serves as SD gene in *O. dancena*, *O. marmoratus* and *O. profundicola* (*Figure 2A, Myosho et al., 2015; Takehana et al., 2014*). *Oryzias hubbsi* and *O. javanicus* even exhibit a ZW, i.e. female heterogametic, system, albeit with different sex chromosomes (*Takehana et al., 2007b*). This astonishing diversity of young sex determination systems inspired ongoing research on the molecular mechanisms underlying rapid sex chromosome turnover and the rewiring of gene regulatory networks that is required to establish new master SD genes (*Herpin et al., 2010; Matsuda and Sakaizumi, 2016*).

### Salinity tolerance

Ricefishes live in hyper- and hypoosmotic environments and exhibit different levels of salinity tolerance (*Hayakawa et al., 2015; Inoue and Takei, 2003; Inoue and Takei, 2002*). While most *Oryzias* are primarily found in freshwater, *O. javanicus* and *O. dancena* (also referred to as *O. melastigma*, likely a synonym; *Parenti, 2008; Roberts, 1998*) occur in saltwater and brackish water, respectively. These species are being established as models for marine ecotoxicology to complement the medaka's role in freshwater (*Chen et al., 2011; Dong et al., 2014; Kang et al., 2013; Kim et al., 2018; Koyama et al., 2008*). Mobility between freshwater and saltwater depends on an organism's ability to adjust to the osmotic differences. Interestingly, *Oryzias latipes* can better adjust to saltwater when exposed to elevated levels of salinity earlier in life (*Miyanishi et al., 2016*). In contrast, species from some remote freshwater systems in Indonesia do not tolerate even small changes in osmolarity (*Inoue and Takei, 2002; Myosho et al., 2018*). The ability of *O. latipes* to acclimate to seawater was used to study the physiological and genetic basis of salt-water tolerance. Salinity tolerance in *Oryzias* is based on both genetic (*Myosho et al., 2018; Ogoshi et al., 2015*) and epigenetic factors, including an increase in the density of osmoregulating cells called ionocytes (*Liu et al., 2016; Miyanishi et al., 2016*) and an increase in activity of the same cells (*Kang et al., 2008*).

### Sulawesi: A natural laboratory of ricefish diversity

In addition to their species richness, Sulawesi ricefishes stand out due to their diversity in several traits (*Kottelat, 1990; Myosho et al., 2018; Myosho et al., 2015; Parenti, 2008*). While most ricefishes are largely pale in color, Sulawesi ricefishes exhibit several different fin and nuptial colorations and males in the *O. woworae* complex evolved an outstanding iridescent blue ornamentation (*Figure 2C, Parenti et al., 2013*). This diversity allows us to expand on the tremendous knowledge of medaka color mutants and to investigate, for example, its genetic basis and the evolutionary role of coloration disparity in ricefishes. Ricefishes further have a wide range of body shapes and sizes (*Parenti, 2008*). Within the *O. woworae* species complex there is variation in body depth (*Parenti et al., 2013*), which likely evolved in response to different flow regimes (*Mokodongan et al., 2018*) and raises the question of interspecific diversity and plasticity of this trait. The largest ricefish *A. poptae* from the ancient Lake Poso reaches more than ten times the size of miniature species such as *O. mekongensis* (see *Parenti, 2008*). Investigating the evolutionary mechanisms that gave rise to the sympatric ricefish species flocks from Lake Poso (namely *Oryzias orthognathus*, *O. nigrimas*, *O. nebulosus* and *Adrianichthys oophorus*, *A. kruyti*, *A. roseni*, *A. poptae*) appears particularly intriguing (*Mokodongan and Yamahira, 2015*). Virtually nothing is known about the ecology and evolution of these species, but striking differences in their craniofacial anatomy point at specialization in their feeding habits as a potential driver of lineage diversification (*Parenti, 2008; Parenti and Soeroto, 2004*). Finally, a new reproductive strategy referred to as "pelvic brooding" has evolved in at least two lineages from Sulawesi (*Herder et al., 2012a; Kottelat, 1990*). Pelvic brooders do not deposit fertilized eggs, but instead carry their clutch until the fry hatches (*Box 2, Iwamatsu, 2004; Iwamatsu et al., 2008; Parenti, 2008*). While this innovation likely relies on a complex set of behavioral, physiological and anatomical adaptations, the adaptive benefit of this female investment remains unexplored. The potentially repeated and likely relatively recent evolution of pelvic brooding in combination with the available resources for medaka make it a promising system to gain insight into the molecular basis of evolutionary innovations, which is a central goal



of evolutionary biology (Erwin, 2015; Shubin et al., 2009; Wagner and Lynch, 2010).

### New opportunities from technological advances

Technological progress continues to open up new opportunities to study the molecular basis of the natural diversity of ricefishes. Genome evolution can now be studied using long sequencing reads (Eid et al., 2009; Jain et al., 2018; Vaser et al., 2017) to shed light on the contribution of structural variants (see Sedlazeck et al., 2018) and methylation patterns (Simpson et al., 2017) to adaptation and speciation. The development of single cell transcriptome sequencing also enables insight into cell-specific gene expression patterns (Andrews and Hemberg, 2018; Fiers et al., 2018; Tang et al., 2010). Single cell transcriptome sequencing can, for example, be used to study the evolution of gene regulatory networks underlying sex determination. All of these technologies can help to identify candidate loci that may contribute to evolutionary processes of interest, while the phenotypic effects of natural genetic variation at these loci can now be assessed using gene-editing tools (Gutierrez-Triana et al., 2018; Letelier et al., 2018; Murakami et al., 2017; Watakabe et al., 2018). Together these technological advances have set the stage for scientists to begin converting the described natural diversity of ricefishes into a better understanding of the molecular basis of evolutionary processes.

### Threatened diversity

The world's freshwater biota is facing a biodiversity crisis and ricefishes are no exception. Only seven out of 21 assessed ricefish species are classified as least concerned according to the IUCN Red List. However, much of the currently available data needs to be treated with caution because the majority of species assessments based on sufficient data were carried out more than two decades ago (IUCN, 2019). What is known is that the high endemism among Sulawesi ricefishes, which are threatened by invasive species, habitat destruction, intensive fishing and pollution, makes them particularly vulnerable for extinction (Herder et al., 2012b; Hilgers et al., 2018; Mokodongan et al., 2014; Parenti, 2008). Accordingly, the Lake Poso endemics *A. roseni* and *A. kuyti*, which have not been caught since 1978 and 1983, respectively, are either on the brink of extinction or have

already gone extinct (Kottelat, 1990). However, severe threats are not restricted to medaka's relatives from Sulawesi. Populations of both Japanese ricefish species have reportedly been declining, particularly in densely populated regions and where exotic species have been introduced (Asai et al., 2011; Mamun et al., 2016; Parenti, 2008; Parenti and Soeroto, 2004). Ricefishes were even thought to have gone extinct in Taiwan, before they were rediscovered in 1993 (Parenti, 2008; Tzeng et al., 2006). In conclusion, current threats and their potential effect often remain speculative. Thus, new species assessments and conservation actions are urgently needed, and ultimately their success will also rely on a better understanding of medaka's life in the wild.

### Conclusions

Despite recent progress, there is much more we can learn about the natural history of medaka. The taxonomic debate around medaka that is fueled by recent insights clearly calls for further investigations, particularly targeting continental medaka species. Combining the tools and resources available for medaka with the diversity of its wild relatives harbors tremendous potential for evolutionary biology. In turn, putting our knowledge on medaka into an evolutionary context may inspire and inform further research in the fields that have traditionally used medaka as a model organism. Sulawesi appears to be an obvious focal point for studying the evolution of ricefish diversity. Conservation efforts targeting some of the ricefish species that are most interesting to science and which appear to be on the brink of extinction are urgently needed. Finally, exciting new research opportunities on medaka arise from the recent establishment of new gene-editing tools and the first isogenic population genetic panel for a vertebrate, which will allow interactions between genotype and environment to be dissected (Spivakov et al., 2014).

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## References

- Aida T.** 1921. On the inheritance of color in a freshwater fish, *Aplocheilichthys latipes* Temmick and Schlegel, with special reference to sex-linked inheritance. *Genetics* **6**:554–573. PMID: 17245975
- Alfred J, Baldwin IT.** 2015. New opportunities at the wild frontier. *eLife* **4**:e06956. DOI: <https://doi.org/10.7554/eLife.06956>
- Andrews TS, Hemberg M.** 2018. Identifying cell populations with scRNASeq. *Molecular Aspects of Medicine* **59**:114–122. DOI: <https://doi.org/10.1016/j.mam.2017.07.002>, PMID: 28712804
- Asai T, Senou H, Hosoya K.** 2011. *Oryzias sakaizumii*, a new ricefish from northern Japan (Teleostei: Adrianichthyidae). *Ichthyol Explor Freshwaters* **22**:289–299.
- Braasch I, Peterson SM, Desvignes T, McCluskey BM, Batzel P, Postlethwait JH.** 2015. A new model army: emerging fish models to study the genomics of vertebrate Evo-Devo. *Journal of Experimental Zoology Part B: Molecular and Developmental Evolution* **324**:316–341. DOI: <https://doi.org/10.1002/jez.b.22589>
- Chen X, Li L, Cheng J, Chan LL, Wang DZ, Wang KJ, Baker ME, Hardiman G, Schlenk D, Cheng SH.** 2011. Molecular staging of marine medaka: a model organism for marine ecotoxicity study. *Marine Pollution Bulletin* **63**:309–317. DOI: <https://doi.org/10.1016/j.marpolbul.2011.03.042>, PMID: 21708389
- Dong S, Kang M, Wu X, Ye T.** 2014. Development of a promising fish model (*Oryzias melastigma*) for assessing multiple responses to stresses in the marine environment. *BioMed Research International* **2014**:1–17. DOI: <https://doi.org/10.1155/2014/563131>
- Ds I, Choi ES, Yoon JD, Kim JH, Il MJ, Baek SH, Jang MH.** 2013. Cytochrome oxidase subunit I (COI) DNA sequence divergence between two cryptic species of *Oryzias* in South Korea. *Journal of Ecology and Environment* **36**:159–166. DOI: <https://doi.org/10.5141/ecoenv.2013.159>
- Edeline E, Terao O, Naruse K.** 2016. Empirical evidence for competition-driven semelparity in wild medaka. *Population Ecology* **58**:371–383. DOI: <https://doi.org/10.1007/s10144-016-0551-4>
- Egami N, Etoh H.** 1969. Life span data for the small fish, *Oryzias latipes*. *Experimental Gerontology* **4**:127–129. DOI: [https://doi.org/10.1016/0531-5565\(69\)90035-7](https://doi.org/10.1016/0531-5565(69)90035-7), PMID: 5353609
- Eid J, Fehr A, Gray J, Luong K, Lyle J, Otto G, Peluso P, Rank D, Baybayan P, Bettman B, Bibillo A, Bjornson K, Chaudhuri B, Christians F, Cicero R, Clark S, Dalal R, Dewinter A, Dixon J, Foquet M, et al.** 2009. Real-time DNA sequencing from single polymerase molecules. *Science* **323**:133–138. DOI: <https://doi.org/10.1126/science.1162986>, PMID: 19023044
- Erwin DH.** 2015. Novelty and innovation in the history of life. *Current Biology* **25**:R930–R940. DOI: <https://doi.org/10.1016/j.cub.2015.08.019>, PMID: 26439356
- Fiers M, Minnoye L, Aibar S, Bravo González-Blas C, Kalender Atak Z, Aerts S.** 2018. Mapping gene regulatory networks from single-cell omics data. *Briefings in Functional Genomics* **17**:246–254. DOI: <https://doi.org/10.1093/bfgp/elx046>, PMID: 29342231
- Fukuda S, Hiramatsu K, Mori M, Shikasho S.** 2005. An adaptive modeling technique for instream fish habitat preference of Japanese medaka (*Oryzias latipes*). *Faculty of Agriculture Kyushu University* **50**:363–373.
- Gutierrez-Triana JA, Tavhelidse T, Thumberger T, Thomas I, Wittbrodt B, Kellner T, Anlas K, Tsingos E, Wittbrodt J.** 2018. Efficient single-copy HDR by 5' modified long dsDNA donors. *eLife* **7**:e39468. DOI: <https://doi.org/10.7554/eLife.39468>, PMID: 30156184
- Hamilton F.** 1822. *An Account of the Fishes Found in the River Ganges and Its Branches*. Edinburgh: A. Constable and company.
- Hayakawa H, Le QD, Kinoshita M, Takehana Y, Sakuma K, Takeshima H, Kojima S, Naruse K, Inoue K.** 2015. Genetic similarity of the Hainan medaka populations collected from hyper- and hypo-osmotic environments in northern Vietnam. *Ocean Science Journal* **50**:231–235. DOI: <https://doi.org/10.1007/s12601-015-0020-6>
- Herder F, Hadiaty RK, Nolte AW.** 2012a. Pelvic-fin brooding in a new species of riverine ricefish (Atherinomorpha: Belontiiformes: Adrianichthyidae) from Tana Toraja, Central Sulawesi, Indonesia. *The Raffles Bulletin of Zoology* **60**:467–476.
- Herder F, Schliwen U, Geiger M, Hadiaty R, Gray S, McKinnon J, Walter R, Pfaender J.** 2012b. Alien invasion in Wallace's Dreamponds: records of the hybridogenic "flowerhorn" cichlid in Lake Matano, with an annotated checklist of fish species introduced to the Malili Lakes system in Sulawesi. *Aquatic Invasions* **7**:521–535. DOI: <https://doi.org/10.3391/ai.2012.7.4.009>
- Herder F, Chapuis S.** 2010. *Oryzias hadiatyae*, a new species of ricefish (Atherinomorpha: belontiiformes: adrianichthyidae) endemic to Lake Masapi, Central

- Sulawesi, Indonesia. *The Raffles Bulletin of Zoology* **58**: 269–280.
- Herpin A**, Braasch I, Kraeussling M, Schmidt C, Thoma EC, Nakamura S, Tanaka M, Scharl M. 2010. Transcriptional rewiring of the sex determining *dmrt1* gene duplicate by transposable elements. *PLoS Genetics* **6**:e1000844. DOI: <https://doi.org/10.1371/journal.pgen.1000844>, PMID: 20169179
- Heule C**, Salzburger W, Böhne A. 2014. Genetics of sexual development: an evolutionary playground for fish. *Genetics* **196**:579–591. DOI: <https://doi.org/10.1534/genetics.114.161158>, PMID: 24653206
- Hilgers L**, Herder F, Hadiaty RK, Pfaender J. 2018. Alien attack: trophic interactions of flowerhorn cichlids with endemics of ancient Lake Matano (Sulawesi, Indonesia). *Evolutionary Ecology Research* **19**:575–590.
- Hori H**. 2011. A Glance at the Past of Medaka Fish Biology. In: Naruse K, Tanaka M, Takeda H (Eds). *Medaka - a Model for Organogenesis, Human Disease, and Evolution*. Tokyo: Japan: Springer. p. 1–18. DOI: [https://doi.org/10.1007/978-4-431-92691-7\\_1](https://doi.org/10.1007/978-4-431-92691-7_1)
- Hughes LC**, Ortí G, Huang Y, Sun Y, Baldwin CC, Thompson AW, Arcila D, Betancur-R R, Li C, Becker L, Bellora N, Zhao X, Li X, Wang M, Fang C, Xie B, Zhou Z, Huang H, Chen S, Venkatesh B, et al. 2018. Comprehensive phylogeny of ray-finned fishes (Actinopterygii) based on transcriptomic and genomic data. *PNAS* **115**:6249–6254. DOI: <https://doi.org/10.1073/pnas.1719358115>, PMID: 29760103
- Iguchi Y**, Kume K, Kitagawa T. 2018. Natural hybridization between two Japanese medaka species (*Oryzias latipes* and *Oryzias sakaizumii*) observed in the Yura River basin, Kyoto, Japan. *Ichthyological Research* **65**:405–411. DOI: <https://doi.org/10.1007/s10228-018-0623-x>
- Inoue K**, Takei Y. 2002. Diverse adaptability in *Oryzias* species to high environmental salinity. *Zoological Science* **19**:727–734. DOI: <https://doi.org/10.2108/zsj.19.727>, PMID: 12149572
- Inoue K**, Takei Y. 2003. Asian medaka fishes offer new models for studying mechanisms of seawater adaptation. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* **136**:635–645. DOI: [https://doi.org/10.1016/S1096-4959\(03\)00204-5](https://doi.org/10.1016/S1096-4959(03)00204-5)
- Ishikawa C**. 1913. *Lectures on Zoology (In Japanese)*. Tokyo: Kanazashi-Horyu-Do.
- IUCN**. 2019. The IUCN red list of threatened species. <http://www.iucnredlist.org> [Accessed February 19, 2019].
- Iwamatsu T**. 2004. Stages of normal development in the medaka *Oryzias latipes*. *Mechanisms of Development* **121**:605–618. DOI: <https://doi.org/10.1016/j.mod.2004.03.012>, PMID: 15210170
- Iwamatsu T**. 2006. *The Integrated Book for the Biology of the Medaka*. 2nd. Tokyo, Japan: Daigaku Kyoiku.
- Iwamatsu T**, Kobayashi H, Shibata Y, Sato M, Tsuji N, Takakura K. 2007. Oviposition cycle in the oviparous fish *Xenopoeilus sarasinorum*. *Zoological Science* **24**: 1122–1127. DOI: <https://doi.org/10.2108/zsj.24.1122>, PMID: 18348613
- Iwamatsu T**, Kobayashi H, Sato M, Yamashita M. 2008. Reproductive role of attaching filaments on the egg envelope in *Xenopoeilus sarasinorum* (Adrianichthidae, teleostei). *Journal of Morphology* **269**:745–750. DOI: <https://doi.org/10.1002/jmor.10620>, PMID: 18302190
- Jain M**, Koren S, Miga KH, Quick J, Rand AC, Sasani TA, Tyson JR, Beggs AD, Dilthey AT, Fiddes IT, Malla S, Marriott H, Nieto T, O'Grady J, Olsen HE, Pedersen BS, Rhie A, Richardson H, Quinlan AR, Snutch TP, et al. 2018. Nanopore sequencing and assembly of a human genome with ultra-long reads. *Nature Biotechnology* **36**:338–345. DOI: <https://doi.org/10.1038/nbt.4060>, PMID: 29431738
- Jordan DS**, Snyder JO. 1906. A review of the poeciliidae or killifishes of Japan. *Proceedings of the United States National Museum* **31**:287–290. DOI: <https://doi.org/10.5479/si.00963801.31-1486.287>
- Kagawa N**. 2014. Comparison of aggressive behaviors between two wild populations of Japanese medaka, *Oryzias latipes* and *O. sakaizumii*. *Zoological Science* **31**:116–121. DOI: <https://doi.org/10.2108/zsj.31.116>, PMID: 24601772
- Kang TW**, Lee EH, Kim MS, Paik SG, Kim S, Kim CB. 2005. Molecular phylogeny and geography of Korean medaka fish (*Oryzias latipes*). *Molecules and Cells* **20**: 151–156. PMID: 16258254
- Kang C-K**, Tsai S-C, Lee T-H, Hwang P-P. 2008. Differential expression of branchial Na<sup>+</sup>/K<sup>+</sup>-ATPase of two medaka species, *Oryzias latipes* and *Oryzias dancena*, with different salinity tolerances acclimated to fresh water, brackish water and seawater. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* **151**:566–575. DOI: <https://doi.org/10.1016/j.cbpa.2008.07.020>
- Kang C-K**, Yang W-K, Lin S-T, Liu C-C, Lin H-M, Chen H-H, Cheng C-W, Lee T-H, Hwang P-P. 2013. The acute and regulatory phases of time-course changes in gill mitochondrion-rich cells of seawater-acclimated medaka (*Oryzias dancena*) when exposed to hypoosmotic environments. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* **164**:181–191. DOI: <https://doi.org/10.1016/j.cbpa.2012.08.010>
- Kasahara M**, Naruse K, Sasaki S, Nakatani Y, Qu W, Ahsan B, Yamada T, Nagayasu Y, Doi K, Kasai Y, Jindo T, Kobayashi D, Shimada A, Toyoda A, Kuroki Y, Fujiyama A, Sasaki T, Shimizu A, Asakawa S, Shimizu N, et al. 2007. The medaka draft genome and insights into vertebrate genome evolution. *Nature* **447**:714–719. DOI: <https://doi.org/10.1038/nature05846>, PMID: 17554307
- Katsumura T**, Oda S, Nakagome S, Hanihara T, Kataoka H, Mitani H, Kawamura S, Oota H. 2014. Natural allelic variations of xenobiotic-metabolizing enzymes affect sexual dimorphism in *Oryzias latipes*. *Proceedings of the Royal Society B: Biological Sciences* **281**:20142259. DOI: <https://doi.org/10.1098/rspb.2014.2259>
- Katsumura T**, Oda S, Mitani H, Oota H. 2019. Medaka population genome structure and demographic history described via genotyping-by-sequencing. *G3: Genes/Genomes/Genetics* **9**:217–228. DOI: <https://doi.org/10.1534/g3.118.200779>
- Kawajiri M**, Kokita T, Yamahira K. 2009. Heterochronic differences in fin development between latitudinal populations of the medaka *Oryzias latipes* (Actinopterygii: Adrianichthyidae). *Biological Journal of the Linnean Society* **97**:571–580. DOI: <https://doi.org/10.1111/j.1095-8312.2009.01233.x>

- Kawajiri M**, Uchida K, Chiba H, Moriyama S, Yamahira K. 2015. Variation in the ontogeny of sex steroid levels between latitudinal populations of the medaka. *Zoological Letters* **1**:31. DOI: <https://doi.org/10.1186/s40851-015-0032-1>
- Kim HS**, Lee BY, Han J, Jeong CB, Hwang DS, Lee MC, Kang HM, Kim DH, Lee D, Kim J, Choi IY, Lee JS. 2018. The genome of the marine medaka *Oryzias melastigma*. *Molecular Ecology Resources* **18**:656–665. DOI: <https://doi.org/10.1111/1755-0998.12769>, PMID: 29451363
- Kimura T**, Shimada A, Sakai N, Mitani H, Naruse K, Takeda H, Inoko H, Tamiya G, Shinya M. 2007. Genetic analysis of craniofacial traits in the medaka. *Genetics* **177**:2379–2388. DOI: <https://doi.org/10.1534/genetics.106.068460>, PMID: 18073435
- Kinoshita M**, Murata K, Naruse K, Tanaka M. 2009. *Medaka: Biology, Management, and Experimental Protocols*. John Wiley & Sons. DOI: <https://doi.org/10.1002/9780813818849>
- Kirchmaier S**, Naruse K, Wittbrodt J, Loosli F. 2015. The genomic and genetic toolbox of the teleost medaka (*Oryzias latipes*). *Genetics* **199**:905–918. DOI: <https://doi.org/10.1534/genetics.114.173849>, PMID: 25855651
- Koga A**, Inagaki H, Bessho Y, Hori H. 1995. Insertion of a novel transposable element in the tyrosinase gene is responsible for an albino mutation in the medaka fish, *Oryzias latipes*. *MGG Molecular & General Genetics* **249**:400–405. DOI: <https://doi.org/10.1007/BF00287101>, PMID: 8552044
- Koga A**. 2011. *Transposable Elements Tol1 and Tol2*. In: *Medaka: A Model for Organogenesis, Human Disease, and Evolution*. Tokyo: Japan: Springer.
- Kondo M**, Nanda I, Schmid M, Schartl M. 2009. Sex determination and sex chromosome evolution: insights from medaka. *Sexual Development* **3**:88–98. DOI: <https://doi.org/10.1159/000223074>, PMID: 19684454
- Kottelat M**. 1990. Synopsis of the endangered buntingi (Osteichthyes: adranichthyidae and oryziidae) of Lake Poso, Central Sulawesi, Indonesia, with a new reproductive guild and descriptions of three new species. *Ichthyol Explor Freshwaters* **1**:46–67.
- Koyama J**, Kawamata M, Imai S, Fukunaga M, Uno S, Kakuno A. 2008. Java medaka: a proposed new marine test fish for ecotoxicology. *Environmental Toxicology* **23**:487–491. DOI: <https://doi.org/10.1002/tox.20367>, PMID: 18246546
- Leaf RT**, Jiao Y, Murphy BR, Kramer JI, Sorensen KM, Wooten VG. 2011. Life-History characteristics of Japanese medaka *Oryzias latipes*. *Copeia* **2011**:559–565. DOI: <https://doi.org/10.1643/C1-09-190>
- Letelier J**, de la Calle-Mustienes E, Pieretti J, Naranjo S, Maeso I, Nakamura T, Pascual-Anaya J, Shubin NH, Schneider I, Martinez-Morales JR, Gómez-Skarmeta JL. 2018. A conserved *Shh* cis-regulatory module highlights a common developmental origin of unpaired and paired fins. *Nature Genetics* **50**:504–509. DOI: <https://doi.org/10.1038/s41588-018-0080-5>, PMID: 29556077
- Liu S-T**, Horng J-L, Chen P-Y, Hwang P-P, Lin L-Y. 2016. Salt secretion is linked to acid-base regulation of ionocytes in seawater-acclimated medaka: new insights into the salt-secreting mechanism. *Scientific Reports* **6**:1–13. DOI: <https://doi.org/10.1038/srep31433>
- Magtoon W**. 2010. *Oryzias songkhramensis*, A new species of ricefish (Beloniformes; Adrianichthyidae) from northeast Thailand and central Laos. *Tropical Natural History* **10**:107–129.
- Mamun MSA**, Tsuchida K, Onishi T, Hiramatsu K, Iwasawa A, Nishimura S. 2016. Medaka in Japanese agricultural water channels: genetic diversity and conservation. *Reviews in Agricultural Science* **4**:8–20. DOI: <https://doi.org/10.7831/ras.4.8>
- 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**:297–304. DOI: <https://doi.org/10.1643/C1-17-704>
- Matsuda M**, Nagahama Y, Shinomiya A, Sato T, Matsuda C, Kobayashi T, Morrey CE, Shibata N, Asakawa S, Shimizu N, Hori H, Hamaguchi S, Sakaizumi M. 2002. DMY is a Y-specific DM-domain gene required for male development in the medaka fish. *Nature* **417**:559–563. DOI: <https://doi.org/10.1038/nature751>, PMID: 12037570
- Matsuda M**. 2005. Sex determination in the teleost medaka, *Oryzias latipes*. *Annual Review of Genetics* **39**:293–307. DOI: <https://doi.org/10.1146/annurev.genet.39.110304.095800>, PMID: 16285862
- Matsuda M**, Sakaizumi M. 2016. Evolution of the sex-determining gene in the teleostean genus *Oryzias*. *General and Comparative Endocrinology* **239**:80–88. DOI: <https://doi.org/10.1016/j.ygcen.2015.10.004>, PMID: 26449160
- Miyanishi H**, Inokuchi M, Nobata S, Kaneko T. 2016. Past seawater experience enhances seawater adaptability in medaka, *Oryzias latipes*. *Zoological Letters* **2**:12. DOI: <https://doi.org/10.1186/s40851-016-0047-2>, PMID: 27307998
- Mokodongan DF**, Tanaka R, Yamahira K. 2014. A New Ricefish of the Genus *Oryzias* (Beloniformes, Adrianichthyidae) from Lake Tiu, Central Sulawesi, Indonesia. *Copeia* **2014**:561–567. DOI: <https://doi.org/10.1643/C1-13-081>
- Mokodongan DF**, Montenegro J, Mochida K, Fujimoto S, Ishikawa A, Kakioka R, Yong L, Mulis, Hadiaty RK, Mandagi IF, Masengi KWA, Wachi N, Hashiguchi Y, Kitano J, Yamahira K. 2018. Phylogenomics reveals habitat-associated body shape divergence in *Oryzias woworae* species group (Teleostei: Adrianichthyidae). *Molecular Phylogenetics and Evolution* **118**:194–203. DOI: <https://doi.org/10.1016/j.ympev.2017.10.005>, PMID: 29024751
- Mokodongan DF**, Yamahira K. 2015. Origin and intra-island diversification of Sulawesi endemic Adrianichthyidae. *Molecular Phylogenetics and Evolution* **93**:150–160. DOI: <https://doi.org/10.1016/j.ympev.2015.07.024>, PMID: 26256644
- Murakami Y**, Ansai S, Yonemura A, Kinoshita M. 2017. An efficient system for homology-dependent targeted gene integration in medaka (*Oryzias latipes*). *Zoological Letters* **3**:10. DOI: <https://doi.org/10.1186/s40851-017-0071-x>, PMID: 28694996
- Murata Y**, Oda S, Mitani H. 2012. Allelic expression changes in medaka (*Oryzias latipes*) hybrids between inbred strains derived from genetically distant populations. *PLOS ONE* **7**:e36875. DOI: <https://doi.org/10.1371/journal.pone.0036875>, PMID: 22590630
- Myosho T**, Otake H, Masuyama H, Matsuda M, Kuroki Y, Fujiyama A, Naruse K, Hamaguchi S, Sakaizumi M. 2012. Tracing the emergence of a novel sex-

- determining gene in medaka, *Oryzias luzonensis*. *Genetics* **191**:163–170. DOI: <https://doi.org/10.1534/genetics.111.137497>, PMID: 22367037
- Myosho T**, Takehana Y, Hamaguchi S, Sakaizumi M. 2015. Turnover of sex chromosomes in *Celebensis* group medaka fishes. G3: *Genes/Genomes/Genetics* **5**:2685–2691. DOI: <https://doi.org/10.1534/g3.115.021543>
- Myosho T**, Takahashi H, Yoshida K, Sato T, Hamaguchi S, Sakamoto T, Sakaizumi M. 2018. Hyperosmotic tolerance of adult fish and early embryos are determined by discrete, single loci in the genus *oryzias*. *Scientific Reports* **8**:1–8. DOI: <https://doi.org/10.1038/s41598-018-24621-7>, PMID: 29720646
- Nagasawa K**. 2017. A synopsis of the parasites of medaka (*Oryzias latipes*) of Japan (1929–2017). *Biosph Sci* **56**:71–85.
- Naruse K**, Tanaka M, Takeda H. 2011. *Medaka*. Tokyo: Japan: Springer.
- Ogoshi M**, Kato K, Sakamoto T. 2015. Effect of environmental salinity on expression of adrenomedullin genes suggests osmoregulatory activity in the medaka, *Oryzias latipes*. *Zoological Letters* **1**:12. DOI: <https://doi.org/10.1186/s40851-015-0012-5>, PMID: 26605057
- Ono Y**, Uematsu T. 1957. Mating ethogram in *Oryzias latipes* (With 1 Text-figure). *J Fac Sci Hokkaido Univ Ser VI Zool* **13**:197–202.
- Parenti LR**. 2005. The phylogeny of atherinomorphs: Evolution of a novel fish reproductive system. In: Uribe M. C, Grier H. J (Eds). *Viviparous Fishes*. New Life Publications, FL. p. 13–30.
- Parenti LR**. 2008. A phylogenetic analysis and taxonomic revision of ricefishes, *Oryzias* and relatives (Belontiiformes, Adrianichthyidae). *Zoological Journal of the Linnean Society* **154**:494–610. DOI: <https://doi.org/10.1111/j.1096-3642.2008.00417.x>
- Parenti LR**, Hadiaty RK, Lumbantobing D, Herder F. 2013. Two new ricefishes of the genus *Oryzias* (Atherinomorpha: Belontiiformes: Adrianichthyidae) augment the endemic freshwater fish fauna of Southeastern Sulawesi, Indonesia. *Copeia* **2013**:403–414. DOI: <https://doi.org/10.1643/Ci-12-114>
- Parenti LR**, Hadiaty RK. 2010. A new, remarkably colorful, small ricefish of the genus *Oryzias* (Belontiiformes, adrianichthyidae) from Sulawesi, Indonesia. *Copeia* **2010**:268–273. DOI: <https://doi.org/10.1643/Ci-09-108>
- Parenti LR**, Soeroto B. 2004. *Adrianichthys roseni* and *Oryzias nebulosus*, two new ricefishes (Atherinomorpha: Belontiiformes: Adrianichthyidae) from Lake Poso, Sulawesi, Indonesia. *Ichthyological Research* **51**:10–19. DOI: <https://doi.org/10.1007/s10228-003-0187-1>
- Parichy DM**. 2015. Advancing biology through a deeper understanding of zebrafish ecology and evolution. *eLife* **4**:e05635. DOI: <https://doi.org/10.7554/eLife.05635>
- Roberts TR**. 1998. Systematic observations on tropical Asian medakas or ricefishes of the genus *Oryzias*, with descriptions of four new species. *Ichthyological Research* **45**:213–224. DOI: <https://doi.org/10.1007/BF02673919>
- Sakaizumi M**. 1984. Rigid isolation between the northern population and the southern population of the medaka, *Oryzias latipes* (Genetics). *Zoological Science* **1**:795–800.
- Sampetean O**, Iida S, Makino S, Matsuzaki Y, Ohno K, Saya H. 2009. Reversible whole-organism cell cycle arrest in a living vertebrate. *Cell Cycle* **8**:620–627. DOI: <https://doi.org/10.4161/cc.8.4.7785>, PMID: 19182528
- Sasaki T**, Yamahira K. 2016. Variation in male courtship activeness between latitudinal populations of northern medaka. *Ichthyological Research* **63**:302–306. DOI: <https://doi.org/10.1007/s10228-015-0484-5>
- Schartl M**, Walter RB. 2016. *Xiphophorus* and medaka cancer models. In: Langenau D. M (Ed). *Cancer and Zebrafish, Advances in Experimental Medicine and Biology*. Cham: Springer International Publishing. p. 531–552. DOI: [https://doi.org/10.1007/978-3-319-30654-4\\_23](https://doi.org/10.1007/978-3-319-30654-4_23)
- Sedlazeck FJ**, Rescheneder P, Smolka M, Fang H, Nattestad M, von Haeseler A, Schatz MC. 2018. Accurate detection of complex structural variations using single-molecule sequencing. *Nature Methods* **15**:461–468. DOI: <https://doi.org/10.1038/s41592-018-0001-7>, PMID: 29713083
- Setiamarga DH**, Miya M, Yamanoue Y, Azuma Y, Inoue JG, Ishiguro NB, Mabuchi K, Nishida M. 2009. Divergence time of the two regional medaka populations in Japan as a new time scale for comparative genomics of vertebrates. *Biology Letters* **5**:812–816. DOI: <https://doi.org/10.1098/rsbl.2009.0419>, PMID: 19586967
- Shima A**, Mitani H. 2004. Medaka as a research organism: past, present and future. *Mechanisms of Development* **121**:599–604. DOI: <https://doi.org/10.1016/j.mod.2004.03.011>, PMID: 15210169
- Shubin N**, Tabin C, Carroll S. 2009. Deep homology and the origins of evolutionary novelty. *Nature* **457**:818–823. DOI: <https://doi.org/10.1038/nature07891>, PMID: 19212399
- Simpson JT**, Workman RE, Zuzarte PC, David M, Dursi LJ, Timp W. 2017. Detecting DNA cytosine methylation using nanopore sequencing. *Nature Methods* **14**:407–410. DOI: <https://doi.org/10.1038/nmeth.4184>, PMID: 28218898
- Spivakov M**, Auer TO, Peravali R, Dunham I, Dolle D, Fujiyama A, Toyoda A, Aizu T, Minakuchi Y, Loosli F, Naruse K, Birney E, Wittbrodt J. 2014. Genomic and phenotypic characterization of a wild medaka population: Towards the establishment of an isogenic population genetic resource in fish. G3: *Genes/Genomes/Genetics* **4**:433–445. DOI: <https://doi.org/10.1534/g3.113.008722>
- Suzuki Y**, Miyake T, Yamahira K. 2010. An acquisition trade-off with fast growth in a fish, the medaka *Oryzias latipes*: why do low-latitude ectotherms grow more slowly? *Evolutionary Ecology* **24**:749–759. DOI: <https://doi.org/10.1007/s10682-009-9331-3>
- Takeda H**, Shimada A. 2010. The art of medaka genetics and genomics: what makes them so unique? *Annual Review of Genetics* **44**:217–241. DOI: <https://doi.org/10.1146/annurev-genet-051710-151001>, PMID: 20731603
- Takehana Y**, Nagai N, Matsuda M, Tsuchiya K, Sakaizumi M. 2003. Geographic variation and diversity of the cytochrome *b* gene in Japanese wild populations of medaka, *oryzias latipes*. *Zoological Science* **20**:1279–1291. DOI: <https://doi.org/10.2108/zsj.20.1279>, PMID: 14569151
- Takehana Y**, Naruse K, Sakaizumi M. 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. DOI: <https://doi.org/10.1016/j.ympev.2005.01.016>, PMID: 15955519
- Takehana Y**, Demiyah D, Naruse K, Hamaguchi S, Sakaizumi M. 2007a. Evolution of different Y chromosomes in two medaka species, *Oryzias dancena* and *O. latipes*. *Genetics* **175**:1335–1340. DOI: <https://doi.org/10.1534/genetics.106.068247>, PMID: 17194774
- Takehana Y**, Naruse K, Hamaguchi S, Sakaizumi M. 2007b. Evolution of ZZ/ZW and XX/XY sex-determination systems in the closely related medaka species, *Oryzias hubbsi* and *O. dancena*. *Chromosoma* **116**:463–470. DOI: <https://doi.org/10.1007/s00412-007-0110-z>, PMID: 17882464
- Takehana Y**, Matsuda M, Myosho T, Suster ML, Kawakami K, Shin-I T, Kohara Y, Kuroki Y, Toyoda A, Fujiyama A, Hamaguchi S, Sakaizumi M, Naruse K. 2014. Co-option of Sox3 as the male-determining factor on the Y chromosome in the fish *oryzias dancena*. *Nature Communications* **5**:4157. DOI: <https://doi.org/10.1038/ncomms5157>, PMID: 24948391
- Takehana Y**, Sakai M, Narita T, Sato T, Naruse K, Sakaizumi M. 2016. Origin of boundary populations in medaka (*Oryzias latipes* species complex). *Zoological Science* **33**:125–131. DOI: <https://doi.org/10.2108/zs150144>, PMID: 27032677
- Tang F**, Barbacioru C, Nordman E, Li B, Xu N, Bashkurov VI, Lao K, Surani MA. 2010. RNA-Seq analysis to capture the transcriptome landscape of a single cell. *Nature Protocols* **5**:516–535. DOI: <https://doi.org/10.1038/nprot.2009.236>, PMID: 20203668
- Temminck JC**, Schlegel H. 1846. *Fauna Japonica*. **1846** Leiden: A. Arnz et Socios.
- Toyama K**. 1916. On some mendelian characters (in Japanese). *Rep Jap Breed Soc* **1**:1–9.
- Tzeng CS**, Lin YS, Lin SM, Wang TY, Wang FY. 2006. The phylogeography and population demographics of selected freshwater fishes in Taiwan. *Zoological Studies* **45**:285–297.
- Ueda M**, Oishi T. 1982. Circadian oviposition rhythm and locomotor activity in the medaka, *Oryzias latipes*. *Journal of Interdisciplinary Cycle Research* **13**:97–104. DOI: <https://doi.org/10.1080/09291018209359769>
- Uwa H**, Wang R-F, Chen Y-R. 1988. Karyotypes and geographical distribution of ricefishes from Yunnan, southwestern China. *Japanese J Ichthyol* **35**:332–340.
- Vaser R**, Sović I, Nagarajan N, Šikić M. 2017. Fast and accurate de novo genome assembly from long uncorrected reads. *Genome Research* **27**:737–746. DOI: <https://doi.org/10.1101/gr.214270.116>
- Wagner GP**, Lynch VJ. 2010. Evolutionary novelties. *Current Biology* **20**:R48–R52. DOI: <https://doi.org/10.1016/j.cub.2009.11.010>, PMID: 20129035
- Watakabe I**, Hashimoto H, Kimura Y, Yokoi S, Naruse K, Higashijima SI. 2018. Highly efficient generation of knock-in transgenic medaka by CRISPR/Cas9-mediated genome engineering. *Zoological Letters* **4**:1–11. DOI: <https://doi.org/10.1186/s40851-017-0086-3>, PMID: 29445519
- Wittbrodt J**, Shima A, Schartl M. 2002. Medaka—a model organism from the far east. *Nature Reviews Genetics* **3**:53–64. DOI: <https://doi.org/10.1038/nrg704>, PMID: 11823791
- Yamahira K**, Takeshi K. 2008. Variation in juvenile growth rates among and within latitudinal populations of the medaka. *Population Ecology* **50**:3–8. DOI: <https://doi.org/10.1007/s10144-007-0055-3>
- Yamamoto T**. 1958. Artificial induction of functional sex-reversal in genotypic females of the medaka (*Oryzias latipes*). *Journal of Experimental Zoology* **137**:227–263. DOI: <https://doi.org/10.1002/jez.1401370203>, PMID: 13575738
- Yamamoto T**. 1975. *Medaka (Killifish) Biology and Strains*. Tokyo: Keigaku Publishing Company.