

GENOME SEQUENCING

The Complete Genome Sequences of three species from the killifish genus *Kryptolebias* (Rivulidae, Cyprinodontiformes)

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Biodiversity Genomes

The killifish genus *Kryptolebias* currently contains seven recognized species found in freshwater and mangrove microhabitats in South and Central America, the Caribbean, and Florida. *Kryptolebias* species have several unique features. Beyond its amphibious nature, two of the *Kryptolebias* species (*K. marmoratus* and *K. hermaphroditus sensu* Costa 2011) are the only known vertebrates capable of self-fertilization. Although reference genomes for self-fertilizing species are readily available, a complete understanding of the genomic basis leading to the transition in mating systems in the genus requires more genomic resources at a broader taxonomic level. Here, we present the complete genome sequences for three *Kryptolebias* species from Brazil: the endangered freshwater species *Kryptolebias brasiliensis* and *Kryptolebias gracilis* and the androdiecious but obligate outcrossing *Kryptolebias ocellatus sensu* Costa 2011). The raw data and assembled genomes are available in GenBank.

Introduction

Kryptolebias is a killifish genus (family Rivulidae) composed of seven currently valid species (Berbel-Filho et al. 2022), although the number of species in the genus is likely to change as some taxonomic debates are still ongoing (Berbel-Filho et al. 2022; Huber 2016). Phylogenetic analyses have indicated the presence of two distinct monophyletic clades within *Kryptolebias*, one of them composed of narrowly distributed freshwater species living in temporary streams and pools in South America: *K. campelloi* (Costa 1990) from North Brazil; *K. sepia* Vermeulen & Hrbek 2005 from Suriname; *K. gracilis* Costa 2007, and *K. brasiliensis* (Valenciennes 1821) from Southeast Brazil. The other clade is composed of three species living in mangrove forests along the tropical and subtropical western Atlantic basin, the ‘mangrove killifish clade’: *K. marmoratus* (Poey 1880), *K. hermaphroditus sensu* Costa 2011, and *K. ocellatus sensu* Costa 2011) (Berbel-Filho et al. 2022; Costa, Lima, and Bartolette 2010; Murphy, Thomerson, and Collier 1999; Tatarenkov et al. 2009, 2017).

Kryptolebias is a remarkable genus in many aspects. For instance, *K. marmoratus* and *K. hermaphroditus sensu* Costa 2011 are the only two vertebrates known to be capable of self-fertilization (Berbel-Filho et al. 2022), whereas *K. ocellatus sensu* Costa 2011) is a hermaphroditic but obligate outcrossing species (Berbel-Filho et al. 2020). This variation in mating systems makes *Kryptolebias* a unique vertebrate system for investigating the genomic, physiological, and behavioral changes involved in the transition from

outcrossing to selfing. In addition, *K. marmoratus*, the most well-studied *Kryptolebias* species, is considered a highly amphibious fish (Turko, Rossi, and Wright 2021), with extreme physiological and behavioral adaptations to live out of water, in some cases for months (Taylor 1990). The amphibious nature of *K. marmoratus* is also likely to be valid for other *Kryptolebias* species, providing unique opportunities for studying the phenotypic and genomic changes involved in the transition from aquatic to terrestrial habitats.

To avoid long-term taxonomic confusion, we would like to provide some background on the taxonomic status of *K. ocellatus* (*Sensu* Costa 2011), whose genome was sequenced here. Due to morphological similarities and syntopy between species, the taxonomic status of the mangrove killifish clade has been historically confusing, particularly in Southeast Brazil. Briefly, *Rivulus ocellatus* was initially described by Hensel (1868) using a single specimen from Rio de Janeiro, Brazil. Later, Seegers (1984) suggested the existence of two syntopic species in Rio de Janeiro: the hermaphroditic *R. ocellatus* as in Hensel (1868), and a yet undescribed species composed of hermaphrodites and males, named *R. caudomarginatus*. After taxonomic revision of the family Rivulidae, Costa (2004) reclassified some previously known *Rivulus* species (*Rivulus brasiliensis*, *R. campelloi*, *R. caudomarginatus*, *R. ocellatus*, and *R. marmoratus*) into a new genus called *Kryptolebias*. After morphological evaluation of the *K. ocellatus* holotype by Costa (2011) argued that the species originally described by Hensel as *K. ocellatus* was in fact *K. caudomarginatus* (as in Seegers (1984)). Therefore, *K. caudomarginatus* has become a junior synonym for *K. ocellatus*. The other syntopic species composed of selfing hermaphrodites was then named as *K. hermaphroditus* (Costa 2011). However, discussions on the taxonomic nomenclature of these mangrove killifish species are still ongoing (Huber 2016). This taxonomic connudrum is likely to be fully resolved only when the genetic data of the formalin-fixed *K. ocellatus* holotype, initially described by Hensel (1868), is available. For the genome generated here, we used the currently valid taxonomic classification, with the selfing species occurring from the Caribbean to Southeast Brazil, named *K. hermaphroditus sensu* Costa 2011, and the androdioceous outcrossing from South and Southeast Brazil, *K. ocellatus (sensu* Costa 2011) (Berbel-Filho et al. 2020, 2022).

Here we provide whole genome sequencing data for the mangrove killifish *K. ocellatus (sensu* Costa 2011) ([Fig. 1a](#)), and two freshwater *Kryptolebias* species: *K. brasiliensis* and *K. gracilis* ([Fig. 1b and c](#), respectively). Although *Kryptolebias ocellatus* has no current classification of its conservation status, *K. brasiliensis* and *K. gracilis* are categorized as endangered and critically endangered species, respectively, by the Brazilian list of threatened fish species (MMA 2022).

(a) *K. ocellatus* (*sensu* Costa 2011)



(b) *K. brasiliensis*



(c) *K. gracilis*



Figure 1. Specimens of (a) *Kryptolebias ocellatus* (*sensu* Costa 2011), (b) *K. brasiliensis*, and (c) *K. gracilis*.

Methods

Tissues from a single wild-collected individual per species were used in the present study. *Kryptolebias ocellatus* (*sensu* Costa 2011) was collected in the Piracão mangrove, Guaratiba, Rio de Janeiro, Brazil (23°0'1.90"S 43°34'51.50"W). *Kryptolebias brasiliensis* was sampled in puddles close to the RPPN Campo dos Escoteiros, Magé, RJ, Brazil (22°34'21.06" S 43°02'5.16" W). *Kryptolebias gracilis* was sampled at São Lourenço stream, Saquarema, RJ, Brazil (22°52'46.90"S 42°37'58.40"W). All sampling locations were either the type locality or within the type locality area for the species. All sampling was performed under the license ICMBio/SISBIO 57145-4.

DNA extraction was performed with the Qiagen DNAeasy Genomic Extraction Kit using a standard process. A paired-end sequencing library was constructed using the Illumina TruSeq kit, according to the manufacturer's instructions. The library was sequenced on an Illumina Hi-Seq platform in a paired-end, 2 × 150bp format. The resulting fastq files were trimmed of

adapter/primer sequences and low-quality regions using Trimmomatic v0.33 (Bolger, Lohse, and Usadel 2014). The trimmed sequence was assembled using SPAdes v2.5 (Bankevich et al. 2012) followed by a final step using Zanfona (Kieras, O'Neill, and Pirro 2021).

Results and Data Availability

All raw read data and assembled genomes are available in the GenBank database.

Species	Raw Data	Assembled Genomes
<i>Kryptolebias brasiliensis</i>	SRR17839738	JAQXNZ000000000
<i>Kryptolebias gracilis</i>	SRR17839737	JAOWBA000000000
<i>Kryptolebias ocellatus</i>	SRR17839743	JAOWBD000000000

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