Researchers identify genes linked to sex differentiation in giant Amazon fish

June 25 2019

Discoveries by Brazilian and German researchers may facilitate early sexing of pirarucu (*arapaima*) and its reproduction in captivity while also paving the way for genetic improvement. Credit: Léo Ramos Chaves/Pesquisa FAPESP

Brazilian and German scientists have completed a collaborative project to sequence and analyze the whole genome of *Arapaima gigas*, a giant freshwater fish known in Brazil as pirarucu and elsewhere as arapaima or paiche. Its growth rate is the fastest among known freshwater fish species. Its natural distribution covers most of the Amazon River basin in Peru and Brazil.

The research led to discoveries that help determine sex at an early stage, facilitating the separation of female and male fry for sex-specific breeding and sale. It also paves the way for further studies relating to genetic improvement of the species.

The findings of the research, which was supported by FAPESP, are published in *Scientific Reports*.

The collaboration began in 2015, when Manfred Schartl, a geneticist at the University of Würzburg in Germany, was contacted by biologist Rafael Henrique Nóbrega and his then Ph.D. student Marcos Antonio de Oliveira. Oliveira has since earned his doctorate from São Paulo State University's Aquaculture Center in Botucatu, Brazil.

Nóbrega, a professor at the university's Bioscience Institute (IB-UNESP), proposed collaboration on research into the mechanisms of sex determination and differentiation in *A. gigas*.

"It's the world's largest freshwater fish and an iconic Amazon species with considerable economic value, so Schartl was surprised that its genome was unknown at the time and that genetic markers hadn't been identified for sex determination," Nóbrega told.

Juveniles do not have sex-related secondary phenotypical differences, so males and females cannot be distinguished by morphology during early development, Nóbrega explained. Information on sex determination and differentiation mechanisms represents an important advance for the Brazilian aquaculture industry.

Schartl and his group specialize in identifying sex determination genes in fish and understanding sex differentiation mechanisms in this abundant and diverse group of vertebrates.

Early sex determination before juveniles reach maturity, when males and females differentiate morphologically and phenotypically, is a key step in the study of a fish species' reproductive cycle.

Advancing knowledge

A draft genome of *A. gigas* was published in September 2018 by researchers at the Federal University of Pará and Rio Grande do Norte, among other institutions.

The sequencing performed more recently by Nóbrega, Oliveira and collaborators led to important genetic discoveries linked to such characteristics as gigantism and rapid growth. It also showed that the sex determination and differentiation in the species are compatible with an XY chromosome sex determination system.

"Although *A. gigas* doesn't have heteromorphic sex chromosomes that can be detected cytologically [by tissue cell analysis], we identified exclusively male markers that support the existence of an XY chromosome sex determination system," Nóbrega said.

In 2015, Oliveira went to a fish farm called Peixes da Amazônia in Senador Guiomard, near Rio Branco, state capital of Acre in North Brazil, and collected small pieces of the fins of *A. gigas* from 60 adults (30 males and 30 females).

The material was sent to Schartl's laboratory, where DNA was sampled from each of the 60 specimens. The DNA was then sequenced in France by researchers at the National Institute for Agricultural Research in

Rennes and Toulouse and the University of Montpellier.

"Genomes of different sizes for males and females were obtained. The male genome has 666 million base pairs, while the female genome has 664 million. Both are quite small as fish genomes go, and only a fifth the size of the human genome, which has 3 billion base pairs," Oliveira said.

The genome of *A. gigas* was compared with that of another bonytongue, the Asian arowana *Scleropages formosus*, the only other species in the order Osteoglossiformes with a sequenced genome. It was also compared with the genomes of 10 other species belonging to various orders, such as the European eel (*Anguilla anguilla*), the Atlantic cod (*Gadus morhua*), the Nile tilapia (*Oreochromis niloticus*), the Torafugu (*Takifugu rubripes*), the Zebrafish (*Danio rerio*) and the primitive coelacanths (*Latimeria spp.*), which evolved 400 million years ago and since then have changed little or not at all.

"A phylogenetic tree was constructed indicating the probable divergence times for the ancestors of the species studied. The more closely related they are genetically, the shorter the time between lineage-splitting events. The order Osteoglossiformes, to which arapaimas and other bonytongues belong, evolved 138.4 million years ago, i.e., at the same time South America and Africa began separating prior to the opening of the South Atlantic," Nóbrega said.

The closest relative among the 10 species analyzed was *A. anguilla*, which 200 million years ago had a common ancestor with arapaimas and other bonytongues.

Adaptive success

The researchers also set out to identify positively selected genes in the genome of *A. gigas*, meaning genes that resulted from adaptive evolution of a lineage and frequently associated with newly enhanced or selected functions of the species.

"In fact, we identified 105 positively selected genes in *A. gigas*, some relating to growth and cell division," Nóbrega said.

The data suggested that the spectacular early growth and gigantism of this fish species were the key to its adaptive success. This characteristic was not observed in any other species the researchers studied.

Genetic analysis showed that the functions of these positively selected genes are associated mainly with muscle development and contribute to its large body size.

Aquaculture and sexual development

A. gigas can reach almost 3 m in length and weigh as much as 220 kg. Osteoglossiformes, the order to which it belongs, is an old group of freshwater fish species originating in Gondwana, the ancient supercontinent that began breaking up approximately 140 million years ago to form what are now South America, Africa, India, Antarctica and Australia.

As a result of the breakup and landmass drift lasting millions of years, extant lineages of Osteoglossiformes are now highly adapted to freshwater life, unable to survive in saltwater, and separated by vast oceans.

A. gigas is found only in the Amazon Basin. Its closest relatives, the 10 species of arowana, are found on four continents: South America (three species), Africa (one), Asia (four) and Australia (two).

"Juveniles weigh 10 to 15 kg a year after birth and their food conversion rate is extraordinarily efficient," Nóbrega said. They gain 700 g with every kg of food consumed.

Furthermore, arapaimas are obligate air breathers, obtaining up to 95% of their oxygen uptake from atmospheric air. Having degenerate gills has given them an adaptive advantage in that they tolerate extremely low oxygen levels in the waters they inhabit, where other species could not survive.

"This combination of unusual adaptations makes *A. gigas* a promising candidate for aquaculture. The Amazon region has many small fish farms, but large-scale production has proved unviable so far," Nóbrega said.

The difficulty is partly due to insufficient knowledge of its sexual development so that it can be successfully bred in captivity and to lack information about the molecular and biochemical mechanisms involved in its fast growth and gigantism.

"One thing we do know is that *A. gigas* becomes sexually mature by age four and a half [the species is believed to live as long as 100 years]. Sexual dimorphism appears in the reproductive stage, when scale color becomes redder in males to differentiate them from females," Oliveira said.

To find ways of determining the sex of juveniles as early as possible, Nóbrega and colleagues looked for "sex markers"—parts of the genome that are unequivocally associated with one sex or the other.

Because *A. gigas* does not have sex chromosomes (different chromosomes in males and females), the researchers focused on identifying molecular markers or genomic regions present in males and not in females or vice versa.

They first mapped restriction site-associated DNA (RAD) markers, a technique used to investigate population genetics, ecological genetics and evolution. "Based on an analysis of RAD markers present in the genetic material from 25 males and 25 females, 30 RAD markers were found to be present in most of the males but absent in most of the females," Nóbrega said.

Despite the lack of sex chromosomes, the researchers detected RAD markers capable of showing whether a juvenile is male in genetic testing. "In fact, the data point to a specific genomic region characteristic of males and not found in females, so that it can be considered compatible with an XY chromosome sex determination system," Nóbrega said.

Secretory organ

A. gigas displays morphological specialization linked to reproduction in the shape of a secretory organ reputedly used for parental care. This gland on the heads of both males and females lacks sex-specific morphological differences.

During the reproductive period the gland secretes a milky fluid that is thought to provide offspring with nutrients. "The fluid feeds larvae while they develop into fry," Nóbrega said.

Males engage in parental care and stay with offspring for up to three months, while females leave after about a month.

"We took samples of secretory organ tissue from males and females in order to analyze the transcriptome—the full set of transcribed sequences in the tissue, including messenger RNA, ribosomal RNA, transport RNA and microRNA," Nóbrega said.

"The results suggest the milky fluid released by the secretory organ contains compounds that prevent the female from entering a new reproductive cycle while parental care is ongoing. In addition, the fluid contains growth factors that may explain the very fast growth of fry."

More information: Kang Du et al, The genome of the arapaima (Arapaima gigas) provides insights into gigantism, fast growth and chromosomal sex determination system, *Scientific Reports* (2019). DOI: 10.1038/s41598-019-41457-x

Provided by FAPESP