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A Global Community Effort to Decipher the Unique Biology of Annual Killifish

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Over the past 50 years, annual killifishes arose as alternative model organisms for studies of vertebrate biology. The annual fish offers exceptional advantages for studies of genetics, genomics, developmental biology, population dynamics, ecology, biogeography, and evolution. They inhabit extremely variable freshwater environments in Africa and South America, have a short lifespan and a set of unique and fascinating developmental characteristics. Embryos survive within the dry substrate during the dry season, whereas the adult population dies. Thus, the survival of the populations is entirely dependent on the buried embryos that hatch the next rainy season. Although Old and New World species share similarities in their life cycle, they also have different adaptive responses associated with climate-related selective pressures. Therefore, contrasting different species from these areas is essential to understand unique adaptations to heterogeneous environment. A network of laboratories (United States, Czech Republic, Italy, Brazil, Chile, and Uruguay) is working and collaborating on many aspects of the biology of annual fishes. Participating researchers share projects and cross-training undergraduate and graduate students. These efforts resulted in two International Symposia (2010 and 2015) that took place in Montevideo and an international book. Herein, we summarize the progress made by this global community of scientists. *Developmental Dynamics* 246:807–811, 2017. © 2017 Wiley Periodicals, Inc.

Key words: extremophile; interdisciplinary; research and education

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Introduction

Annual killifishes are placed within the order Cyprinodontiformes, suborder Aplocheiloidei (Costa, 2013; Nelson et al., 2016). The suborder comprises a diversified clade of small and primary freshwater teleost fishes that inhabit tropical and subtropical regions of the Americas, Africa, and South-eastern Asia. The Aplocheiloidei currently consists of 620 described species that occur in shallow freshwater or, less frequently, brackish environments (Costa, 2013). Many species are common in the pet trade as aquarium fishes; a few species, both African and Neotropical genera, are emerging model systems for laboratories and field studies (e.g., Harrington and Kallman, 1968; Wourms, 1972a; Park and Kim, 1984). Annual killifishes have also been the focus of debate among biogeographers contrasting dispersal and vicariance approaches.

Evolutionary relationships among aplocheiloid lineages began to be studied in mid-20th century (Myers, 1931, 1952), which formally recognized killifishes as members of the currently defined suborder Aplocheiloidei. Later, Parenti (1981) analyzed relationships concluding that Asian taxa are more closely related to African than to Neotropical taxa. Furthermore, this author suggested that the group had a Gondwanan origin and diversified through

vicariance events along with the breakup of the continent during the Mesozoic. An alternative hypothesis suggested that the present distribution pattern of aplocheiloid fishes is the result of marine dispersal favored by the occurrence of some taxa in brackish water habitats (Lundberg, 1993).

Furthermore, this author suggested that the Neotropical region was the center for aplocheiloid dispersal. An early molecular phylogeny for 25 aplocheiloid species and two cyprinodontiform outgroups also supported a vicariance model that is consistent with the Gondwana drift model (Murphy and Collier, 1997). A more recent biogeographical analysis (Costa, 2013) supports vicariance events chronologically compatible with the break-up of Gondwana, including separation of the Madagascar, India, South America, and Africa plates (about 121–84 Ma) and to paleogeographical events within the African plate. In addition, the biogeographical reconstruction is congruent with geodispersal events related to the colonization of the Greater Antilles (about 35–33 Ma) and Central America (3.7–3.4 Ma) by aplocheiloids through land connections and dispersal events through Zaire, East Africa, Amazon, and Eastern Brazil (Costa, 2013).

Myers coined the term “annual fishes” in 1952. These fish inhabit African and South American temporal ponds with extremely variable conditions. They have a short lifespan and exhibit a set of unique developmental characteristics that are key

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for their survival. Whereas the entire adult population dies during the dry season, developing embryos remain within the substrate and survive the dry months. Thus, the survival of the populations, and hence of the species, is entirely dependent on the buried embryos that hatch the next rainy season, once the ponds are flooded. The unique combination of features, e.g., ecology, lifespan, reproduction behavior, etc., drew the attention of early researchers that described the embryology of some African and South American species (Wourms 1964, 1972a–c). Early work also described the occurrence of embryonic diapauses (Peters, 1963; Wourms, 1964), a key feature in the evolutionary success of annual fishes. Diapauses are developmental arrests of variable length present in South American and African annual species.

In the Neotropical region, a researcher from Uruguay, Raúl Vaz Ferreira (1918–2006), focused his attention in diverse aspects of the ecology and ethology of Neotropical species (Vaz Ferreira et al., 1964; Vaz Ferreira and Sierra, 1973). The focus of this research was the subfamily Cynolebiatinae (Rivulidae), an endemic and speciose group of annual fishes occurring in the southern Neotropical region (Argentina, Uruguay, Southern and central Brazil).

Literature on the single or multiple origins of annualism is relatively extensive and was recently reviewed (de Sá et al., 2016). The complex development and evolution of a diapausing egg led early researchers to conclude that diapausing eggs likely did not evolve multiple times (Murphy and Collier, 1997). In addition, annualism might have been favored by the prolonged development of cyprinodontiform eggs (pg. 1214, Hberk and Larson, 1999). Currently, most researchers adhere to independent and multiple origins of annualism (Hrbek and Larson, 1999; Costa, 2009a, 2015; Furness et al., 2015). The lack of understanding of the mechanisms and processes involved in the repeated evolution of the complex diapausing embryo, rapid growth of juveniles, and early senescence undoubtedly precluded the acceptance of convergence as a driving evolutionary process in the diversification of these clades.

Annual fishes differ from other teleosts by having a mid-late blastula stage where deep cells dispersed over the syncytial cell layer at the same time that other embryonic layers undergo epiboly. This is called the dispersed phase. Subsequently, these deep cells migrate and aggregate over the surface, forming an overall discoidal plate. This is called the re-aggregation phase (Wourms, 1972b,c). Furthermore, the cell dispersion and re-aggregation phases, key processes in gastrulation of the embryo, may be considered derived states (Berois et al., 2014) of processes previously reported in another teleost (e.g., *Oryzias latipes*, Yokoya, 1966; *Salmo* and *Salvelinus*, Ballard and Dodes, 1968). In addition in addition to the dispersion-re-aggregation stages an obligate and prolonged diapause is a trait of annualism that may have been favored by natural selection under harsh environmental conditions (de Sá et al., 2016).

Members of the subfamily Cynolebiatinae vary greatly in morphology (Vaz-Ferreira and Melgarejo, 1984; Loureiro and de Sá 1998; Costa, 1998a) and genetic and karyological patterns (García et al., 1993, 1995, 2001, 2002). Genome size for 16 diploid *Austrolebias* species was studied and revealed an unusually high DNA content ($2C = 5.95 \pm 0.45$ picograms) per diploid cell (mean C -value = 2.98 pg; García et al., 2014). Whereas in other rivulids, e.g., *Cynopocilus melanotaenia* (García et al., 2014), *Kryptolebias marmoratus* (Kelley et al., 2016), *Rivulus urophthalmus* (Hinegardner and Rosen, 1972), as well as in old world annual killifish, e.g.,

Nothobranchius (Reichwald et al., 2009), the genomes sizes are within the average range of other diploid actinopterygian fishes (2–3.00 pg/diploid cell) (Carvalho et al., 1998; Mank and Avise, 2006).

The giant genome size may be related to a greater instability of the genome of *Austrolebias* (García, 2006). In addition, the large genome could explain the high morphological diversity described among *Austrolebias* species (Loureiro and de Sá, 1998). Furthermore, the *Cynopocilus melanotaenia* species complex is the sister group to *Austrolebias* and constitutes a morphologically homogeneous group (Costa et al., 2016). Furthermore, comparatively there is a relative morphological uniformity among the 62 species of *Nothobranchius*, species that are distinguished mostly on the exceptionally diverse and vivid male nuptial coloration (Dorn et al., 2014).

A recent comparative analysis of repetitive DNA content by means of NGS technique revealed that the proportion of moderately repetitive DNA in *A. charrua* (45%) is approximately twice than in *C. melanotaenia* (25%). Particularly, these data could suggest that class I and II of transposable elements (ETs) could have played a preponderant role in the remarkable genome increase. Furthermore, these events could have occurred associated to the differentiation and speciation of the genus *Austrolebias* within the Rivulidae (García et al., 2015).

Developmental biology studies have focused on two genera of Neotropical annual fishes: *Austrofundulus* and *Austrolebias*. The stages and timing of development, physiological conditions, and the alternative developmental trajectory of “escape embryos” (i.e., those embryos that bypass diapauses) in *Austrofundulus* have been described (Podrabsky, 1999; Podrabsky et al., 2001, 2010; Hand et al., 2016). The stages and timing of early development (Arezo et al., 2005), the pattern of sex differentiation (Arezo et al., 2007), and the overall life cycle (Berois et al., 2012, 2014) has been described for *Austrolebias*. The genus *Nothobranchius* is the most commonly used and studied African genus of annual fishes since the 1970s. Several species were analyzed to understand early stages of development, morphogenesis, and diapauses (Lesseps et al., 1975, 1979; Van Haarlem, 1979; Van Haarlem et al., 1981, 1983a,b; Inglima et al., 1981; Levels et al., 1986; Levels and Denucé, 1988). More recently, *N. furzeri* has been the focus of developmental research primarily due to its extremely short lifespan (Valdesalici and Cellerino, 2003; Blažek et al., 2013). This species is considered a good model to study the effects of genetic and nongenetic factors on aging and its related pathologies using approaches from morphology to molecular and diverse “omic” approaches (Cellerino, 2016).

Overall, research on different areas of the developmental and evolutionary biology of annual killifishes expanded starting in the 1990s. Most research on Neotropical annual killifishes has been focused on *Austrolebias* at the School of Science of the Universidad de la República, Uruguay (UdelaR) and on *Austrofundulus* at Portland State University (PSU), Oregon. The work at UdelaR is organized in an interdisciplinary way. It was generated the “Evolutionary Biology of Annual Fishes Group”, interested in developmental biology, evolutionary genetics, ethology, systematics, and neurobiology. The research at PSU has been focused on examining different aspects of the physiology and eco-physiology of the diapausing embryo. Considering that Old and New World annual killifishes share many similarities in their life cycles, they are good models to compare species from temperate to tropical regions and to understand unique adaptations in heterogeneous

environments as well as their adaptive responses to climate-related selection pressures.

Currently, the collaborative research network has grown and consists of laboratories around the world (United States, Czech Republic, Italy, Brazil, Chile, and Uruguay) working complementary on different areas of the biology of annual killifishes. Researchers in this network have published several articles (examples: Arezo et al., 2007; García et al., 2008, 2009; Passos et al., 2013, 2014; Berois et al., 2014), offered undergraduate and graduate courses, tutored MS and PhD theses, applied and secured financial support to pursue research projects, and organized two international symposia (2010 and 2015, a third symposium is scheduled for 2020) in Uruguay. The symposia brought together researchers, faculty, and students (undergraduates and graduate), working both on African and South American annual killifishes and served as catalytic venues to present recent research, exchange ideas, and develop new collaborations.

The second symposium coincided with the release of the first book on this emerging vertebrate model (Berois et al., 2016). The book included chapters on life cycle, developmental biology, evolutionary ecology, reproductive behavior and sexual selection, genetics and speciation, neurobiology, and integrative and conservation strategies. The goal of the book was to bring together colleagues working in different research areas of annual fishes, summarizing previous work on the group, providing an overview of current research, and highlighting promising areas of research for future generations of students and researchers. The symposia and the book facilitate and enhance the international collaborative efforts to get a deeper understanding of the unique biology of annual killifishes. Currently, one of the main efforts of the research network is focused on elucidating the genomes, transcriptomes, and proteomes of *Austrofundulus* and *Austrolebias*. The genome of *Nothobranchius*, with the later assembly and annotation (*Nothobranchius furzeri* Information Network Genome Browser, NFINGb) has an essential value to compare African and Neotropical species.

Research on the “annual fish model” has the potential to contribute in the understanding of the current biodiversity of the group, not only as usually estimated by number of species, genera, etc., but also to improve the comprehension of the different levels and patterns of diversification from which such biodiversity arises. This includes deciphering the role and interplay of genetic diversity, ranging from genome size to gene expression, and how the genetic and molecular diversity contribute to the plasticity of developmental pathways, e.g., diapause and “escape embryos,” and the role that such plasticity may play in variable adaptation to ephemeral environments. This physiological condition may be critical for the survival of species threatened by unpredictable climate change conditions. Much research is required to understand the biology and conservation efforts needed for the survival of annual fish species, several of them likely under severe threats due to habitat degradation and loss. Developmental biologists have led to better understanding the biology of annual fishes in the early stages of the life cycle. In addition, annual fishes provide unique opportunities for a wide range of evolutionary biology questions.

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