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Reproductive biology in males of the annual killifish *Millerichthys robustus* (Cyprinodontiformes: Cynolebiidae)

Omar Domínguez-Castanedo · **Mari Carmen Uribe**

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Abstract This study explored the male reproductive biology of the annual killifish *Millerichthys robustus*; the only annual fish distributed in North America. The structure of the testes was described, as well as the spermatogenesis, spermatozoon and reproductive cycle, using histological sections and gonadosomatic index. The testes of *M. robustus* correspond to the restricted spermatogonial type, with the spermatogenesis process of cystic and longitudinal type. Spermiation and mature spermatozoa were observed in the network of efferent ducts and in the central duct, uninterruptedly from the third week post hatching (WPH) until pool desiccation, during week 27 WPH. No testes were observed in regression or at rest, indicating the continuous active reproductive phase throughout its adult life. The gonadosomatic index showed that the reproductive potential of males increases towards the end of their life cycle prior to drying up of their pool, suggesting that fish prioritize their energy into reproduction in order to maximize the reproductive success up to the end of their life.

O. Domínguez-Castanedo (✉)
Departamento El Hombre y su Ambiente, Universidad Autónoma Metropolitana, Unidad Xochimilco. Calzada del Hueso No. 1100, Coyoacan, CDMX, Mexico
e-mail: dominguezcastanedo@gmail.com

M. C. Uribe
Laboratorio de Biología de la Reproducción. Departamento de Biología Comparada. Facultad de Ciencias, Circuito Exterior, Ciudad Universitaria, Universidad Nacional Autónoma de México, Insurgentes Sur 3000, Delegación Coyoacán, CDMX, Mexico

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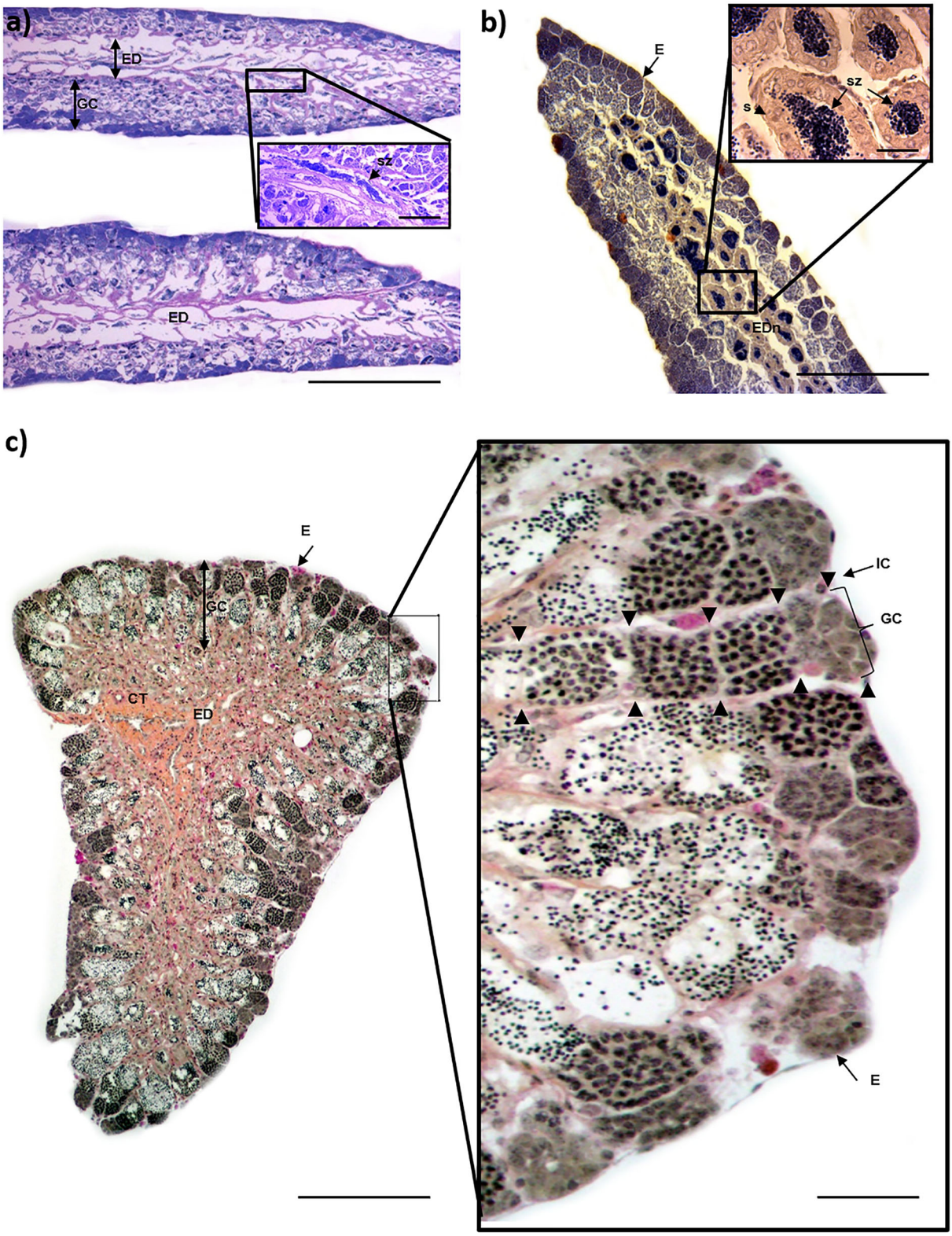
Introduction

Annual fish present a set of biological traits that allow them to establish permanent populations in temporary water bodies in locations with extreme environments in America and Africa (Murphy and Collier 1997; Loureiro and de Sá 2016). These ecosystems are subject to drastic, contrasting and unpredictable environmental changes that give rise to seasonal conditions that are alternately favorable and deleterious for development of the organisms that inhabit them (Berois et al. 2012, 2016; Furness 2015). During the favorable periods, the pools are filled with rainwater and annual fishes hatch, grow at fast rates and mature sexually at very early ages. Because the duration of this period –weeks to months– is liable to stochastic conditions, the mature fish reproduce constantly until natural death, creating a bank of embryos in the substrate of the pond (Gonçalves et al. 2011; Blaŵek et al. 2013; Domínguez-Castanedo et al. 2017). Over time, pools desiccate during the dry season because of high temperatures and lack of rainfall resulting in death of the adult fish. However, the populations persist through the embryos resistant to drought buried in the substrate, in a state of reversible metabolic depression (diapause), awaiting the return of favorable conditions brought by the rainy season in order to hatch and re-establish their population (Wourms 1972a, b, c; Berois et al. 2012; Furness et al. 2015; Pinceel et al. 2015).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◀ **Fig. 1** Histological structure of the testes of *Millerichthys robustus*. a) Longitudinal cut of the two testes. Germinal compartment (GC) and the central efferent duct (ED), Bar = 100 μm. The detail of the ED with spermatozoa (sz) is shown in the lumen, Bar = 20 μm. b) Longitudinal cut of the testes showing the germinal epithelium (E) on the periphery and the network of efferent ducts (EDn). Bar = 100 μm. Detail of ducts formed by the Sertoli cells (S) and spermatozoa (sz). Bar = 5 μm. c) Transversal cut of the testis, with the germinal epithelium (E) and the germinal compartment (GC) on the periphery and, in the interior, the central efferent duct (ED) and connective tissue (CT). Bar = 100 μm. Detail of the germinal compartment (GC) and the interstitial compartment (IC). Sides of a lobule (arrowheads). Bar = 15 μm. H-E

This annual life cycle was recently described on *Millerichthys robustus*, an annual killifish endemic of temporal water bodies located in coastal deserts, grasslands, intermittent wetland habitat; and, even, urbanized grasslands environments of Papaloapan and Coatzacoalcos basins rivers in the southeast of México. Therefore, it is the only species of annual fish, so far, distributed in North America. Also, female reproductive cycle was described, revealing the beginning of early reproduction, during the fourth week post-hatching and its prolongation throughout its adult life (Domínguez-Castanedo et al. 2013; Domínguez-Castanedo et al. 2017). However, the reproductive processes of males of this species have not been studied. The objective of this study was to describe the histological structure of the testis, the spermatogenesis and the spermatozoon of *M. robustus*, during an annual reproductive cycle, in order to identify elements of the male reproductive biology that can be related to those of the female, and are associated with their annual life history and ecology of the temporary water bodies.

Materials and methods

Three males of *M. robustus* were collected weekly during the first month post-hatching ($n = 12$) (September 2014) and five were collected monthly from the second month ($n = 30$) (October 2014) until the drying up of the pool (March 2015). The week of hatching was corroborated from the time the water body conserved the rainwater and the presence of newly hatched fish (for details, see Domínguez-Castanedo et al. 2017). The study population is located in the vicinity of the municipality of Tlacotalpan, Veracruz, Mexico (18°37′39.3“ N; 95°38’53.0” W).

This study was conducted according to the ethics for animal research. The collected fish were anaesthetized with 3% clove oil and euthanized by cerebral puncture. The fish and their testes were weighed (g) on an Ohaus Explorer® balance (± 0.0001); fixed with 4% formalin for 24 h and preserved in 70% alcohol until histological processing. The testes were dehydrated in increasing concentrations of alcohol (80, 95 and 100%), cleared with xylene and embedded in Paraplast© paraffin with melting point of 56 °C. Testes were then cut to 7 μm in thickness and dyed with hematoxylin-eosin (H-E), following Aguilar-Morales et al. (1996), and reticulin (RET) to highlight the basal membranes, dyed in black (Mazzoni et al. 2015). To describe the histological structure of the testes, the spermatogenesis and how they change from maturity until fish death, the criteria of Grier and Uribe (2009) and Uribe et al. (2014) were used. The photomicrographs and measurements were obtained using an Olympus® C5050Z digital camera coupled to an Olympus® CX31 microscope. Thirty cells of each stage of spermatogenesis were measured using the program ImagePro Plus 5.1®.

The male reproductive cycle was examined using Gonadosomatic index from data on testes weight and total weight collected regularly after their sexual maturity, following the formula given in De Vlaming et al. (1982):

$$GSI = \left(\frac{Wg}{Wt}\right)100$$

where:

GSI gonadosomatic index
Wg weight of testes (g)
Wt weight of individual (g).

In addition, semen was collected from three specimens during November 2014, following Domínguez-Castanedo et al. (2014) in order to describe the morphological characteristics of the spermatozoa. The semen samples were hydrated with 20 μL of water, dyed with eosin-nigrosin (E-N) and observed under fluorescence, de following Lahnsteiner and Patzen (2008). The photomicrographs and measurements of the samples were obtained from the equipment described above.

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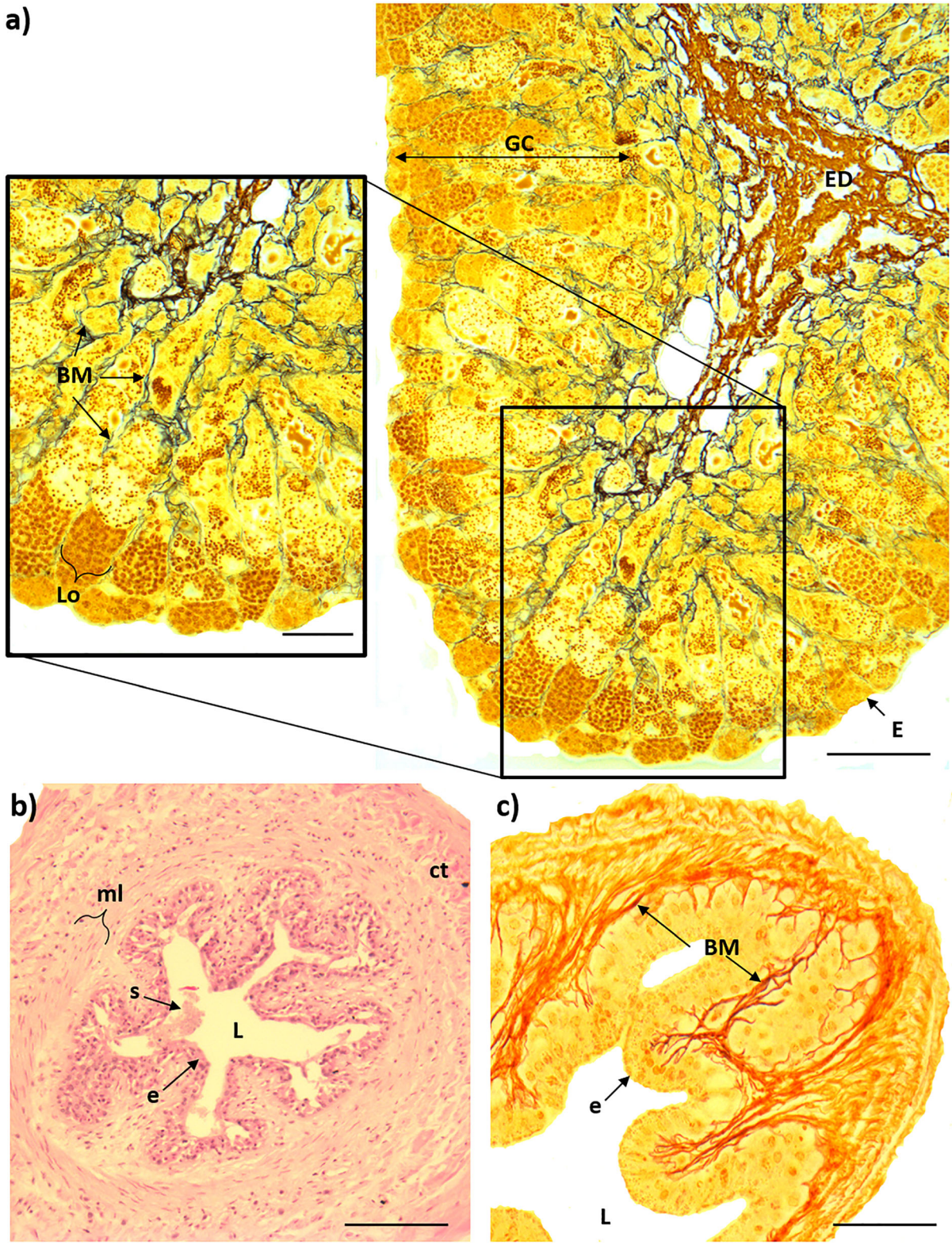
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◀ **Fig. 2** Histological structure of the testis and efferent duct of *Millerichthys robustus*. **a**) Transversal cut of a testis showing the germinal compartment (GC) with lobular structure (Lo) and the central efferent duct (ED), separated by the basal membrane (BM). Bar = 75 μm. RET. Detail of the lobular structure (Lo) and the disposition of the basal membrane (BM). Bar = 30 μm. **b**) Transversal section of efferent duct, with irregular lumen (L), in contact with the pseudostratified epithelium (e) secretions (s) towards the central lumen. On the periphery, the layer of smooth muscular (ml) and connective (ct) tissue is shown. Bar = 100 μm. H-E. **c**) Detail of the efferent duct, showing the basal membrane (BM) that separates the epithelium from the connective tissue of the duct (e) adjacent to the lumen (L). Bar = 30 μm. RET

Protección Ambiental, Dirección General de Vida Silvestre of SEMARNAT.

Results

Histological structure of the testis

Millerichthys robustus has two testes (Fig. 1a) attached to the dorsal wall by the mesorchium, and found between the swim bladder and the intestine. The testes are joined at their caudal region by the central efferent ducts that lead to the exterior by the genital pore. The efferent ducts are formed by a pseudostratified columnar epithelium, located on a basal membrane, which continue with the stroma formed by loose connective tissue surrounded by smooth muscle (Fig. 2b, c). The cephalic region of the testis presents an angular form (Fig. 3b). The testes form two compartments separated by a continuous basal membrane (Fig. 2a): (*i*) the germinal compartment, formed by lobular structures that contain germinal cells in all stages of spermatogenesis and epithelial somatic (Sertoli) cells (Figs. 1a, c; 2a), and (*ii*) the interstitial compartment, which surrounds the germinal compartment and is constituted by interlobular tissue, formed by loose connective tissue, blood and lymphatic vessels, nerve fibers and Leydig cells (Fig. 1a, c, 2a, 3c, 4a). No interlobular lumen was observed. The disposition of the spermatogonia is limited to the germinal epithelium, at the lobular distal extremes, on the periphery of the testis (Fig. 3a, 4a). During spermatogenesis, on being individually surrounded by the processes of the Sertoli cells, the germinal cells formed cysts and proliferate (Fig. 4b). These cysts, with different stages of spermatogenesis, migrated longitudinally through the interior of the lobules towards the network of efferent ducts (Fig. 1b). In the central efferent duct of the testes (Fig. 1c, 2a), cyst rupture occurred during spermiation, releasing the mature spermatozoa (Fig. 1a, b, 2a).

Spermatocytogenesis

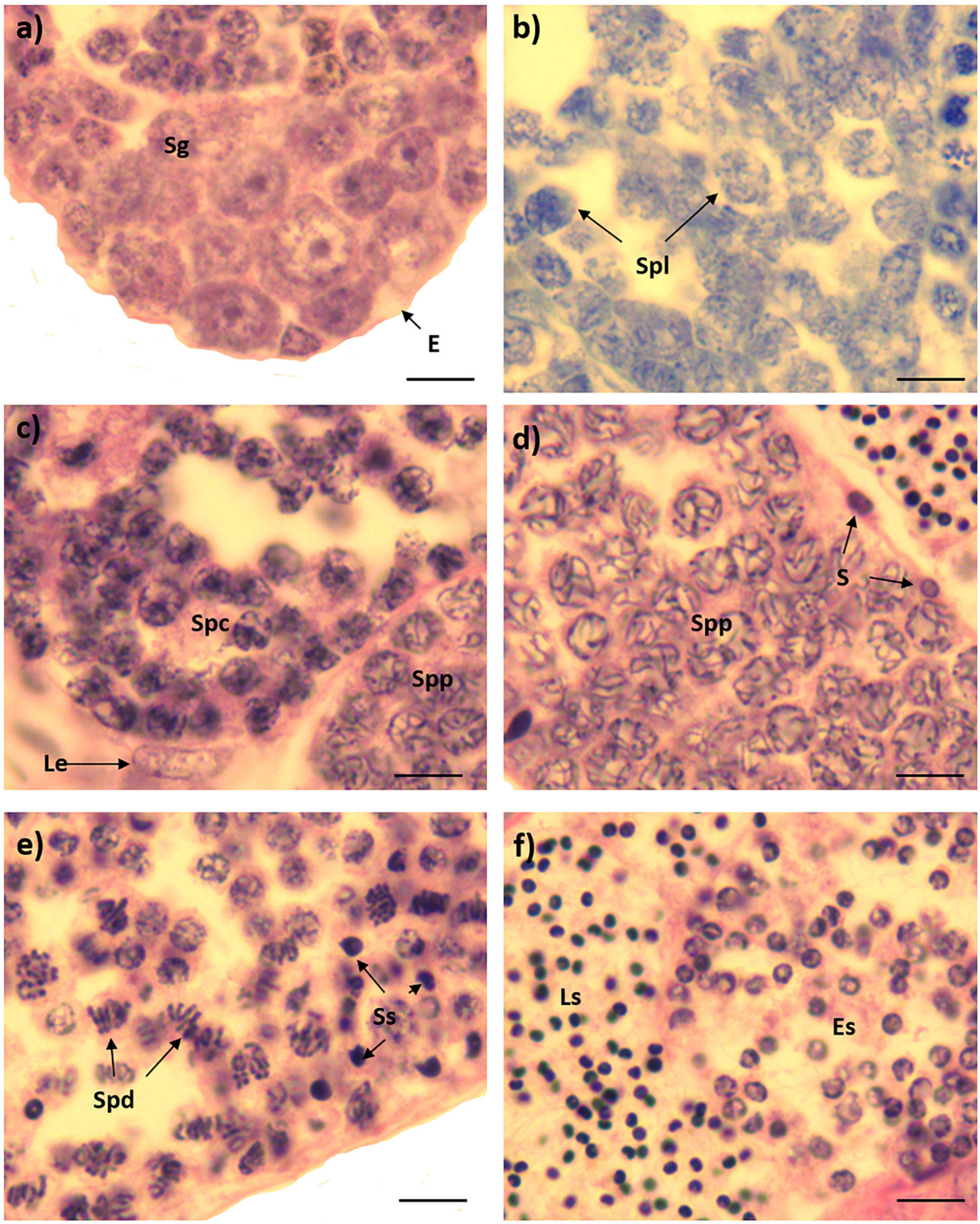
The primary and secondary spermatogonia of *M. robustus* were found distributed in small groups at the distal part of the lobules, in the germinal epithelium (Fig. 1c, 2a, 3a). Both types of spermatogonia have similar morphology: a single nucleolus and both the nucleus and the cytoplasm were hyaline. The primary and secondary spermatogonia had diameters of 8.45 ± 0.37 μm and 6.51 ± 0.36 μm, respectively. Sertoli cells were associated with the primary spermatogonia on the apical part of the lobules (Fig. 4b). Subsequently, multiple secondary spermatogonia were within the spermatocysts.

Meiosis

The secondary spermatogonia of *M. robustus* began the prophase I of meiosis, transforming into primary spermatocytes with diameter of 3.43 ± 0.18 μm and with the following characteristics in each phase: (*i*) leptotene, with the homogeneous nucleus (Fig. 3b); (*ii*) zygotene, in which the homologous chromosomes condense (Fig. 3c); (*iii*) pachytene, with the homologous chromosomes condensed and paired. This was the most prolonged phase, because the cells were most abundant in the germinal compartment (Fig. 3d); (*iv*) diplotene, in which the chiasmata of the homologous chromosomes appear (Fig. 3e). Following the first meiotic division, the secondary spermatocytes (Fig. 3e) had condensed nucleolus and basophilic cytoplasm. Their diameter was 2.84 ± 0.07 μm. On division, the secondary spermatocytes gave rise to early spermatids (Fig. 3f) with diameter of 2.5 ± 0.05 μm.

Spermiogenesis

The late spermatids gradually decreased in diameter to 2.0 ± 0.05 μm. Their nuclei were condensed and basophilic (Fig. 3f). At the end of the metamorphosis, the head of the spermatozoa was spherical, with a homogeneous nucleus and acidophilic flagellum; the flagella of the spermatozoa were observed to be parallel to each other, both in the cysts (Fig. 4b) and in the efferent ducts (Fig. 1b).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◀ **Fig. 3** Spermatogenesis of *Millerichthys robustus*. **a**) Proliferation of spermatogonia (Sg) in the germinal epithelium (E). H-E. **b**) Primary spermatocytes in the stage of leptotene (Spl) in which the condensation of the chromosomes is seen. Toluidine blue. **c**) Primary spermatocytes in the stages of zygotene (Spc) showing the pairing of the homologs and pachytene (Spp), in which appear the chiasmata. Leydig cell (Le) in the interlobular space. H-E. **d**) Cysts with primary spermatocytes in the pachytene stage (Spp), surrounded by Sertoli cells (S). H-E. **e**) Primary spermatocytes in the diplotene stage (Ssd), secondary spermatocytes (Ss). H-E. F) Early (Es) and late (Ls). Spermatids. H-E. Bars = 8 μm

Morphology of the spermatozoon

The spermatozoa of *M. robustus* are formed by a spherical head (4.08 ± 0.58 μm) with no acrosome, a reduced mid-piece integrated into the head (2.60 ± 0.44 μm) (Fig. 3d, e) and a flagellum (31.313 ± 2.16 μm). Together, these structures comprise a total length of 35.517 ± 2.29 μm (Fig. 5c).

Gonadal development

From the third week after hatching, fishes had small testes of 0.001 g, in which the germinal compartment presented all stages of development of spermatogenesis and spermatozoa in the network of efferent ducts and the central efferent duct (Fig. 5a, b).

From week six until week 27, the testes of 0.025 ± 0.002 g presented the same morphological characteristics: the germinal compartment had the same proportion of cysts in each stage of spermatogenesis and spermatozoa were present in the network of efferent ducts and the central efferent duct (Fig. 1a, b, 4b). Interestingly, at no time of the year were the testis observed to have accumulated spermatozoa.

The GSI presented an ascending pattern along the life cycle of *M. robustus*. The highest values were recorded during the final weeks of life. On the other hand, the total weight of individuals increases from hatching, decreasing in the last two months. The weight of the testes showed small variations over life cycle (Table 1).

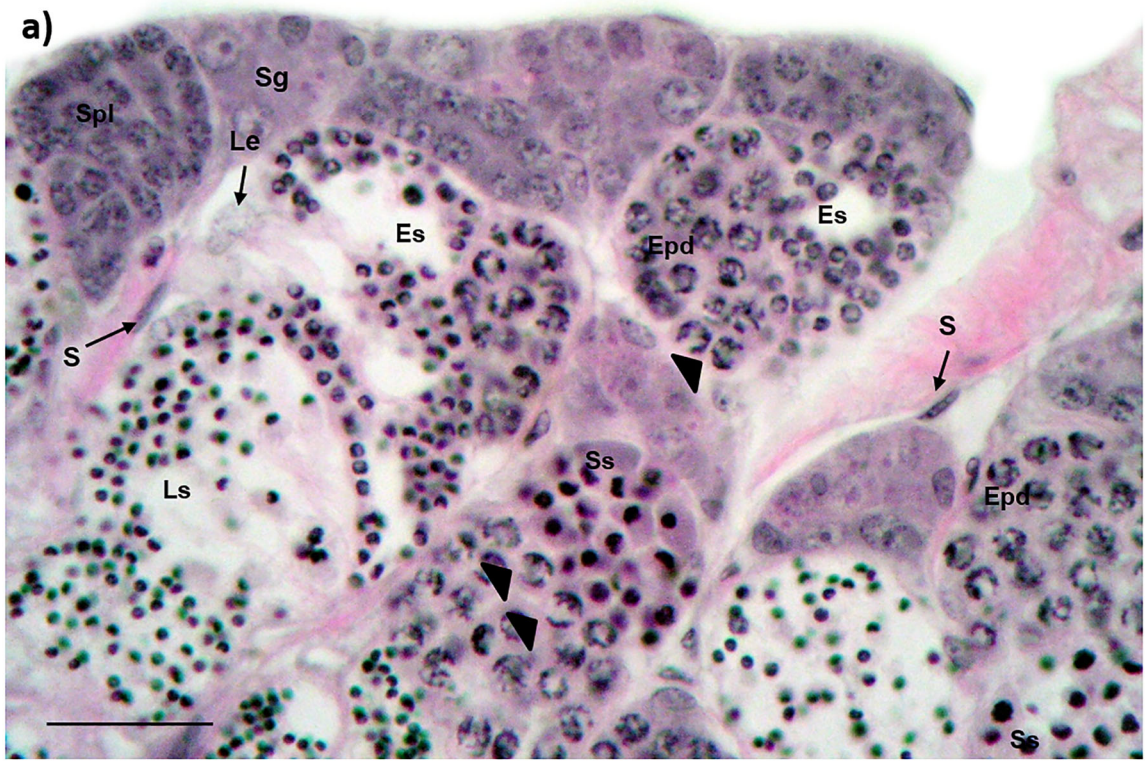
Discussion

The anatomical disposition of the testes of *M. robustus* was similar to that reported in other teleosts (Kobelkowsky 2007). Comparing our results with those in other annual fish (Arezo et al. 2007), the testicular structure and its development are similar. The histological organization of the testis of *M. robustus* corresponds to the lobular type with spermatogonia restricted to the periphery, as has been described in species of Atherinomorpha group (Atheriniformes; Cyprinodontiformes; Beloniformes; Parenti 2015) with longitudinal cystic spermatogenesis (Grier 1981; Grier and Uribe 2009; Uribe et al. 2014), but without regions for storage of spermatozoa, as in Goodeids (Uribe et al. 2010).

According to Mattei (1991) and Lahnsteiner and Patzen (2008), the spermatozoa of *M. robustus* correspond to type I, "aquasperm uniflagellate with no acrosome", as is the case in the majority of teleosts with external fertilization and in the Cynolebiidae *Kryptolebias marmoratus* (Kweon et al. 1998), *Austrolebias charrua*, *A. reicherti* and *A. viariatus* (Arezo et al. 2007; García et al. 2009), and in *Melanorivulus punctatus* (Cassel et al. 2014). Moreover, the size of the spermatozoa head coincides with the width of the micropyle of the ovarian follicles of *M. robustus* (3.92 μm) as expected (Domínguez-Castanedo et al. 2016).

The presence of mature spermatozoa both within the cysts and free in the efferent ducts and in the central duct indicates the sexual maturity of the fish between the second and third week of their life cycle. These results coincide with that observed in females of this species (Domínguez-Castanedo et al. 2017), where the beginning of vitellogenesis was recorded in the week three, but reproduction was confirmed in the fourth week. This gap could be explained by the following non-mutually exclusive hypotheses: (*i*) the males reach maturity slightly before the females, which allows them to have mature spermatozoa in readiness when the females begin to ovulate; (*ii*) the energy cost invested in the development of the testes is lower than that of the ovaries, for which reason it takes less time (Wootton 1991). Early sexual maturity in annual fishes is a fundamental feature that allows the development of crucial stages of life cycle of these species, allows their persistence of their populations in ephemeral environments that impose highly changing conditions (Vrtílek et al. 2018).

The reproductive characteristics of the males of *M. robustus* observed from their sexual maturation until natural death indicate that spermatogenesis is continuous, with no periods of rest, storage or regression. This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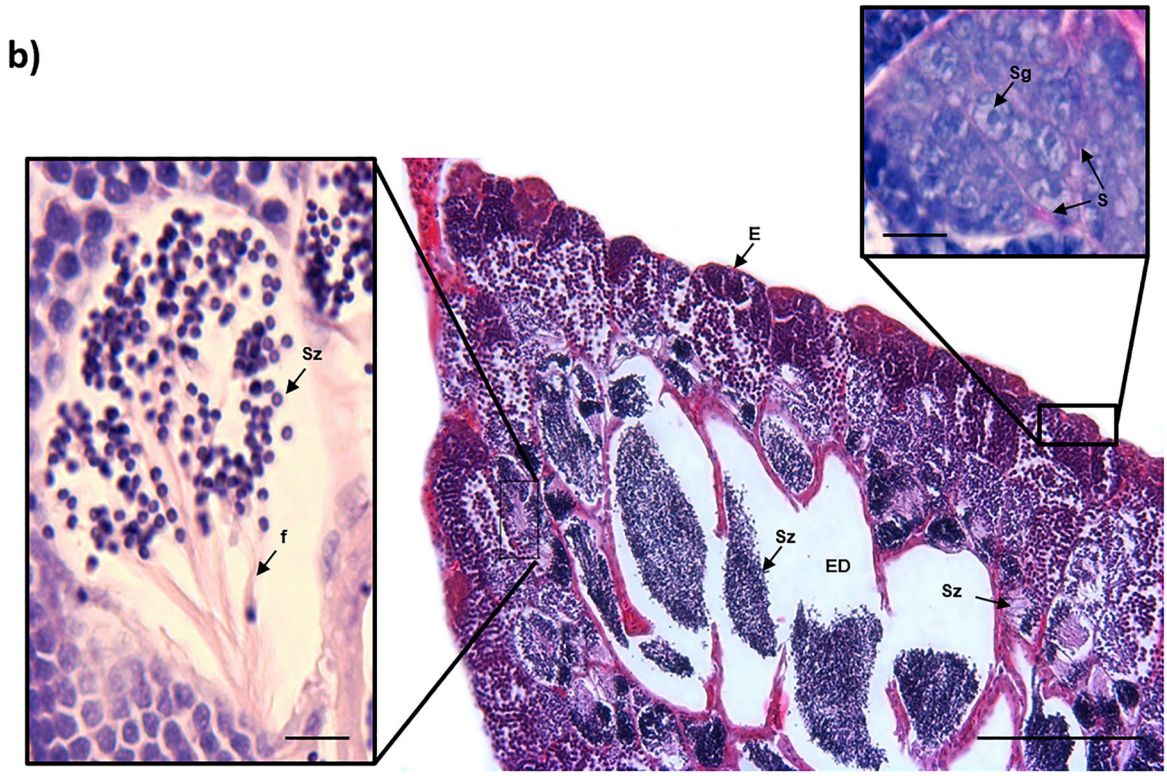
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◀ **Fig. 4** Panorama of the testis of *Millerichthys robustus*. **a**) Germinal compartment with several cysts in different stages of cellular development: spermatogonia (Sg) in the apical region, primary spermatocytes in leptotene (Spl) and diplotene (Spd), early (Se) and late (St) spermatids, secondary spermatocytes (SS), Sertoli (S) and Leydig (Le) cells. Spermatocytes in cellular division (arrowheads). Bar = 30 μm. H-N. **b**) Longitudinal section of the anterior region of the testis, showing the germinal epithelium (E) on the periphery and the central efferent duct (ED) with spermatozoa (Sz) in the lumen. Bar = 75 μm. Detail of numerous spermatogonia (Sg) surrounded by Sertoli cells (S) during the formation of a cyst in the apical part of the lobule. Bar = 10 μm. Spermatozoa (Sz) with parallel flagella (f). Bar = 5 μm. H-E

could be associated with the asynchronous follicular development documented in the ovaries of the females of this species, a condition that allows them to conduct continuous ovulation (Domínguez-Castanedo et al. 2017; Domínguez-Castanedo and Uribe 2019). Based on the above and, according to Uribe et al. (2014), the phase of testicular development of early spermatogenesis was observed during the third week, with a tendency toward the phase of late spermatogenesis observed from the fourth week until death. According to the criteria of Brown-Peterson et al. (2007), the characteristics of the testes of *M. robustus* were maintained in the phase of active reproduction from sexual maturity until natural death. Regression phases were not observed in any specimen.

Table 1 Total and gonad weight and *GSI* of *Millerichthys robustus* in each week post hatching (mean ± SD)

WPH	Total weight	Testes weight	*GSI*
3	0.24 ± 0.006	0.001 ± 0.000	0.417 ± 0.001
4	0.54 ± 0.034	0.001 ± 0.000	0.185 ± 0.021
6	1.080 ± 0.190	0.019 ± 0.000	1.759 ± 0.043
10	1.28 ± 0.137	0.022 ± 0.000	1.719 ± 0.011
14	1.402 ± 0.401	0.024 ± 0.000	1.712 ± 0.045
18	1.356 ± 0.170	0.024 ± 0.000	1.744 ± 0.061
22	1.373 ± 0.146	0.025 ± 0.000	1.848 ± 0.031
27	1.101 ± 0.260	0.018 ± 0.000	1.998 ± 0.011

GSI increased until the natural death of the fish. This aspect can be explained because fish notably lost body weight, but maintained the testes in a phase of active reproduction. During the final weeks of life, prior to the pool drying up, the water depth was observed to be very

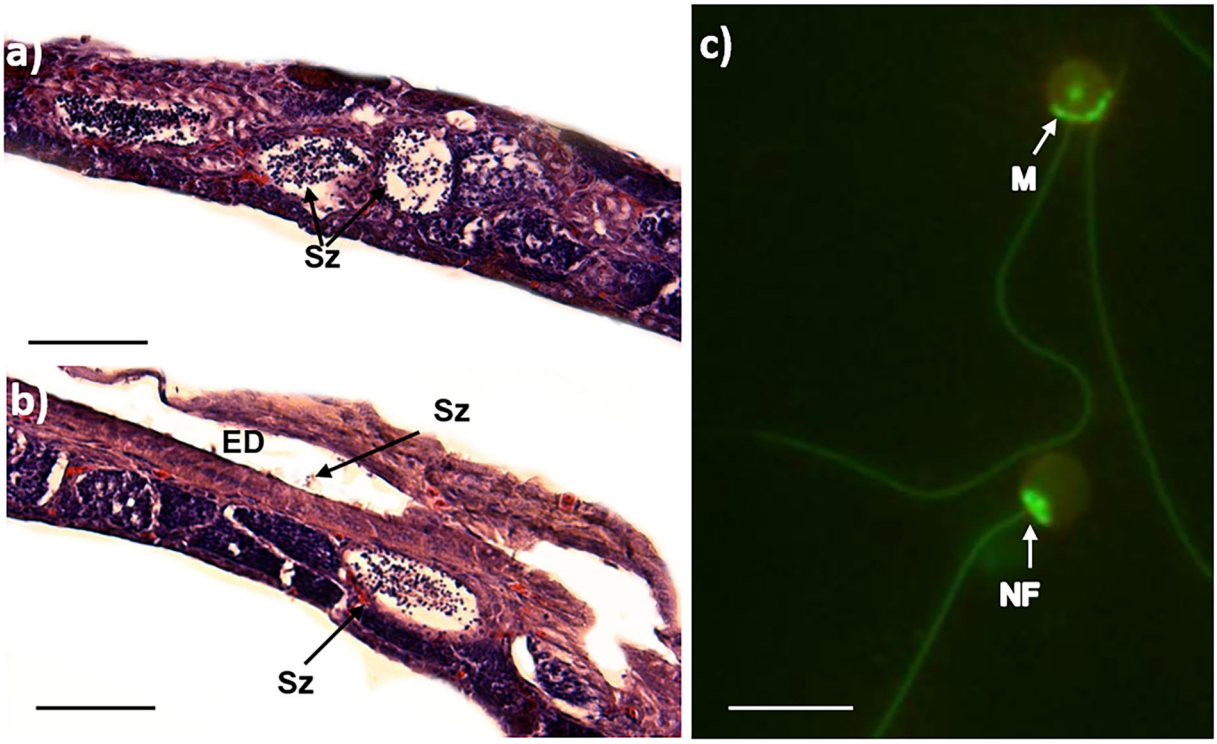

Fig. 5 **a**) and **b**) Testes during the third week after hatching, with the presence of spermatozoa (Sz) in cysts and in the central efferent duct (ED). **c**) Spermatozoa in which can be observed the mitochondria (M) on the periphery of the nucleus and the flagellum and a spermatozoa showing the mid region integrated into the head (NF). Bars 10 μm and 15 μm. H-N with fluorescence

shallow (<10 cm), a condition that could have increased both intra and interspecific competition for food, space and use of the habitat (Alonso-Fernández and Saborido-Rey 2012; Vrtílek and Reichard 2015). The presence of many individuals of the species *Xiphophorus maculatus*, *Gambusia sexradiata*, *Poecilia mexicana* and *Astyanax mexicanus* was observed in addition to that of *M. robustus,* trapped in the temporary pool by the loss of connection with other pools as a result of the drought (Domínguez-Castanedo, personal observation). This suggests that, despite the loss of body condition and age, the energetic investment remained directed towards reproduction and thus the constant production offspring, until the last possible time of the season. This plasticity is also documented in females of the African annual killifish *Nothobranchius furzeri* (Vrtílek and Reichard 2015; Vrtílek et al. 2018) and is similar to that documented in *N. wattersi*, a species that increases its reproductive potential at the end of its life cycle as a response to the desiccation risk of its pool (Grégoir et al. 2017).

Our work reveals crucial life history traits in males of *M. robustus*, such as early sexual maturity and uninterrupted reproduction; traits congruent with the biology of the females in an ecological context. In addition, it provides detailed information on the morphology of the male reproductive system of the only known annual species of fish in the north of America, thus allowing the comparison of its traits with those of other lineages of killifishes.

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References

Aguilar-Morales M, Coutiño BB, Rosales SP (1996) Manual general de Técnicas Histológicas y Citoquímicas. Facultad de Ciencias, UNAM, México D.F

Alonso-Fernández A, Saborido-Rey F (2012) Relationship between energy allocation and reproductive strategy in *Trisopterus luscus*. J Exp Mar Biol Ecol 416-417:8–16. https://doi.org/10.1016/j.jembe.2012.02.001

Arezo JM, D Alessandro S, Papa N, de Sá R, Berois N (2007) Sex differentiation pattern in the annual fish *Austrolebias charrua* (Cyprinodontiformes: Rivulidae). Tissue Cell 39:89–98. https://doi.org/10.1016/j.tice.2007.01.004

Berois N, Arezo JM, Papa GN, Clivo AG (2012) Annual fish: developmenal adaptations for an extreme environment. Wiley Interdiscip Rev Dev Biol 1:595–602. https://doi.org/10.1002/wdev.39

Berois N, Arezo MJ, Papa GN, Chalar C (2016) Life cycle, reproduction and development in annual fishes. In: Berois N, García G, De Sá OR (eds) Annual fishes: life history strategy, diversity and evolution. CRC Press, Taylor and Francis Group, pp 33–45

Blažek R, Polačik M, Reichard M (2013) Rapid growth, early maturation and short generation time in African annual fishes. EvoDevo 4:24. https://doi.org/10.1186/2041-9139-4-24

Brown-Peterson N, Lowerre-Barbieri S, Macewicz B, Saborido-Rey F, Tomkiewicz J, Wyanski D (2007) An improved and simplified terminology of reproductive classification in fishes. Join meeting of ichthyologist and herpetologist. USA, San Francisco

Cassel M, Ferreira A, Mehanna M (2014) Ultrastructural features of spermatogenesis in *Melanorivulus punctatus* (Cyprinodontiformes: Rivulidae). Micron 62:1–6. https://doi.org/10.1016/j.micron.2014.02.012

De Vlaming V, Grossman G, Chapman F (1982) On the use of the gonadosomatic index. Comp Biochem Physiol 73A(1):31–39

Domínguez-Castanedo O, Uribe MC (2019) Ovarian structure, folliculogenesis and oogenesis of the annual killifish *Millerichthys robustus* (Cyprinodontiformes: Cynolebiidae). J Morphol 280:316–328. https://doi.org/10.1002/jmor.20945

Domínguez-Castanedo O, Mosqueda-Cabrera MA, Valdesalici S (2013) First observations of annualism in *Millerichthys robustus* (Cyprinodontiformes: Rivulidae). Ichthyol Explor Fres 24(1):15–20

Domínguez-Castanedo O, Toledano-Olivares A, Martínez-Espinosa D, Ávalos-Rodríguez A (2014) Cambios morfológicos en gametos del barbo Tigre *Puntius tetrazona* (Cypriniformes: Cyprinidae) e implementación de la fertilización *in vitro*. Rev Biol Trop 62(4):1353–1363

Domínguez-Castanedo O, Uribe MC, Rosales-Torres AM (2016) Morphological development of the structures related to annualism in the ovarian follicle of the killifish *Millerichthys robustus* (Costa, 1995) (Teleostei: Cyprinodontiformes). J Morphol 277:1219–1230. https://doi.org/10.1002/jmor.20570

Domínguez-Castanedo O, Uribe MC, Rosales-Torres AM (2017) Life history strategies of annual killifish *Millerichthys robustus* (Cyprinodontiformes: Cynolebiidae) in a seasonal ephemeral water body in Veracruz, México. Environ Biol Fish 100(8):995–1006. https://doi.org/10.1007/s10641-017-0617-y

Furness IA (2015) The evolution of an annual life cycle in killifish: adaptation to ephemeral aquatic environments through embryonic diapause. Biol Rev 91(3):796–812. https://doi.org/10.1111/brv.12194

Furness IA, Lee K, Reznick ND (2015) Adaptation in a variable environment: phenotypic plasticity and bet-hedging during egg diapause and hatching in an annual killifish. Evolution 69(6):1461–1475. https://doi.org/10.1111/evo.12669

García G, Loureiro M, Berois N, Arezo M, Casanova G, Clivo G, Olivera A (2009) Pattern of differentiation in the annual killifish genus *Austrolebias* (Cyprinodontiformes: Rivulidae) from a biosphere reserve site in South America:

a multidisciplinary approach. Biol J Linn Soc 98:620–635. https://doi.org/10.1111/j.1095-8312.2009.01303.x

Gonçalves CS, Pereira SU, Volcan VM (2011) The opportunistic feeding and reproduction strategies of the annual fish *Cynopoecilus melanotaenia* (Cyprinodontiformes: Rivulidae) inhabiting ephemeral habitats on southern Brazil. Neotrop Ichthyol 9(1):191–200. https://doi.org/10.1590/S1679-62252011000100019

Grégoir FA, Philippe C, Pinceel T, Reniers J, Thoré JSE, Vanschoenwinkel B, Brendonck L (2017) Life stage dependent responses to desiccation risk in the annual killifish *Nothobranchius wattersi*. J Fish Biol 90(3):880–895. https://doi.org/10.1111/jfb.13385

Grier JH (1981) Cellular organization of the testis and spermatogenesis in fishes. Am Zool 21:345–357. https://doi.org/10.1093/icb/21.2.345

Grier JH, Uribe MC (2009) The testis and spermatogenesis in Teleosts. In: Jamieson BGM (ed) Reproductive biology and physiology of fishes (Agnsthans and bony fishes). Science Publishers, USA, pp119–142

Kobelkowsky A (2007) Diversidad Morfológica del sistema de conductos excretores de los peces teleósteos. Rev Mex Biodivers 78:105–116

Kweon SH, Park HE, Peters N (1998) Spermatozoon ultrastructure in the internally self-fertilizing hermaphroditic teleost, *Rivulus marmoratus* (Cyprinodontiformes, Rivulidae). Copeia 4:1101–1106

Lahnsteiner F, Patzen AR (2008) Sperm morphology and ultrastructure in fish. In: Mohammad HA, Cosson JJ, Coward K, Rafiee G (eds) Fish Spermatology. Alpha Science International Ltd, Oxford, pp 198–437

Loureiro M, de Sá OR (2016) Diversity of Aplocheiloidei. In: Berois N, García G, De Sá OR (eds) Annual fishes: life history strategy, diversity and evolution. CRC Press, Taylor and Francis Group, pp 3–31

Mattei X (1991) Spermatozoon ultrastructure and its systematic implications in fishes. Can J Zool 69(12):3038–3055. https://doi.org/10.1139/z91-428

Mazzoni TS, Grier HJ, Quagio-Grassiotto I (2015) The basement membrane and the sex establishment in the juvenile hermaphroditism during gonadal differentiation of the *Gymnocorymbus ternetzi* (Teleostei: Characiformes: Characidae). Anat Rec 298:1984–2010. https://doi.org/10.1002/ar.23270

Murphy WJ, Collier EG (1997) A molecular phylogeny for Aplocheiloid fishes (Atherinomorpha: Cyprinodontiformes): the role of vicariance and the origins of annualism. Mol Biol Evol 14(8):790–799. https://doi.org/10.1093/oxfordjournals.molbev.a025819

Pinceel T, Vanschoenwinkel B, Deckers P, Grégoir A, Ver Eecke T, Brendonck L (2015) Early and late developmental arrest as complementary embryonic bet-hedging strategies in African killifish. Biol J Linn Soc 114(4):941–948. https://doi.org/10.1111/bij.12474

Uribe MC, Grier JH, Mejía-Roa V, Yánez-García N, García-Alarcón A, De la Rosa-Cruz G, Aguilar-Morales M (2010) Functional structure of testis ans spermatogenesis of Goodeids and Poeciliids. In: Uribe MC, Grier J.H. (eds) Viviparous fishes II. New Life Publications, FL, pp. 151–172

Uribe MC, Grier JH, Mejia-Róa V (2014) Comparative testicular structure and spermatogenesis in bony fishes. Spermatogenesis 4(3):e983400. https://doi.org/10.4161/21565562.2014.983400

Vrtílek M, Reichard M (2015) Highly plastic resource allocation to growth and reproduction in females of an African annual fish. Ecol Freshw Fish 24:616–628. https://doi.org/10.1111/eff.12175

Vrtílek M, Žák J, Blažek R, Polačik M, Cellerino A, Reichard M (2018) Limited scope for reproductive senescence in wild populations of a short-lived fish. Sci Nat 105:68. https://doi.org/10.1007/s00114-018-1594-5

Wootton RF (1991) Ecoloegy of teleost fishes. Fish and Fisheries. Chapman & Hall, London

Wourms JP (1972a) Developmental biology of annual fishes. I Stages in the normal development of *Austrofundulus myersi* Dahl. J Exp Zool 182:143–168. https://doi.org/10.1002/jez.1401820202

Wourms JP (1972b) Developmental biology of annual fishes. II Naturally occurring dispersion and reaggregation of blastomers during the development of annual fish eggs. J Exp Zool 182:169–200. https://doi.org/10.1002/jez.1401820203

Wourms JP (1972c) Developmental biology of annual fishes. III Pre-embryonic and embryonic diapause of variable duration in eggs of annual fishes. J Exp Zool 182:389–414. https://doi.org/10.1002/jez.1401820310