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Contents

Editorial

This is the fourth edition, and concluding issue of the first volume, of Killi-Data News and I am happy that it has been well received by readers. At 25 pages this issue is a bit thinner than the last but this is because we agreed to make the cut-off for submissions the 1st of December so we could get this edition out by the New Year.

This is an exciting edition full of new species descriptions and analyses that will keep taxonomists busy for years to come. Costa has given us two molecular phylogenies on *Melanorivulus* as well as an exciting paper on *Kryptolebias*. Two new *Nothobranchius* are described and both papers will no doubt pave the way for further descriptions. There are two new publications on Mexican killifish. One is very depressing reading and serves to highlight the plight of Mexican killifish. Perhaps there is a partner for conservation in Rescuing Profundulus oaxacae?

This edition we are joined by Mark Peterson who has agreed to review ecology papers. Help is still needed with the following topics:

- Phylogenetics and Genomics research papers
- Ecotoxicology
- Behavioral research

If you would like to join the editorial team please email me: killiflash-newsletter@killi-data.org. As papers are published they will be made available to the reviewers; or if you come across a paper send me a review. You can use this issue as

a guide to the workload per paper. I know we professional researchers are constantly reading, and probably reading the interesting papers in this edition before they see it here. It doesn't take long to lets us know what you learnt from the paper and why it would be important to others to read.

The Molecular Platform project is growing steadily (http://www.killi-data.org/list-names-molecular.php). We received several rare specimens of South American Annual from Didier Pillet as well a specimen of *Pronothobranchius seymouri* from Amer Faour—which has already been shared with two research labs. We have also been offered specimens from several big breeders: Lou Hersch (AKA), Willem-Jan Hoetmer (KFN), Morgens Juhl (SKS), Jurij Phunkner (BKA) and Jeff Wasley (AKA). We are optimistic that the project will continue to grow and more researchers will make use of this facility.

I hope you will enjoy this issue and continue your support of KDI. May you have a prosperous new year!

[*Tyrone Genade*]

Questions to KDI

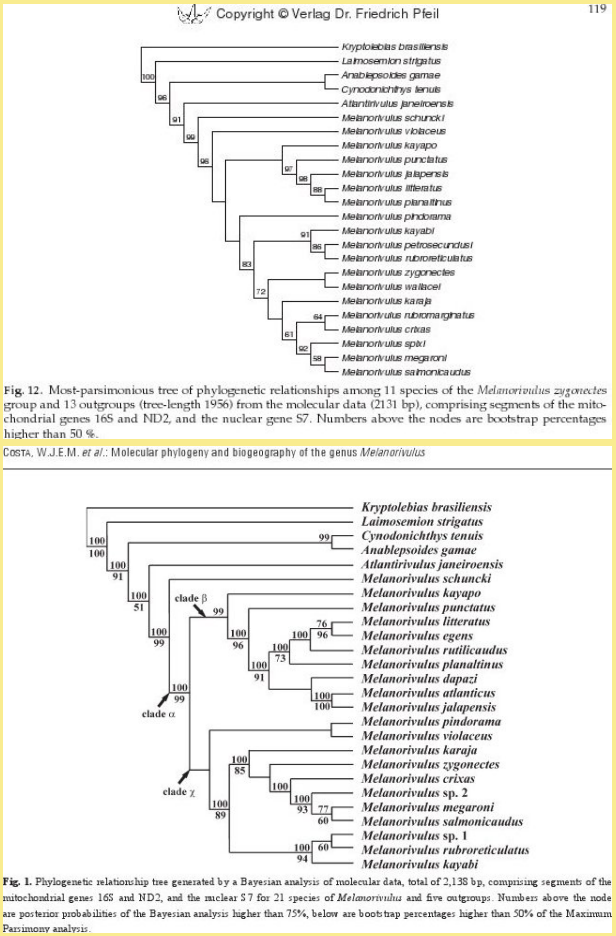
I am interested in killifish research with all its novelties and surprises. And I want to know your analysis on tools and techniques using genetics and killifish at the species-level, notably those so easy to understand horizontal trees (I know that you discussed many times the same topic at the genus-level). Isn't it already the end of the game? (Anonymous, Brazil, December 2016)

Your question is a frequent item in direct messages (mails sent to the editor) that are herein received and usually the answer is general and balanced (and not published herein), because the question is too complex to be dealt with seriously in a few lines and because the question is too general (wide) to have an appropriate universal answer (only for killifish the situation is so complicated from the very-very little we know with some comfort that it would be better to pass the question to the next generation of researchers, say in 30 years); therefore, the answer to your interesting question could be easily finished at this point; but it happens by hazard that 2 new simultaneously distributed publications with comparative data allow to answer your question with more solid and exemplary arguments than in the past. Still, let's start by some basic evidence.

Yes, molecular data (a topic much narrower than simply genetics) have revolutionized killifish research output since the last 24 years (first paper in 1992; and remember that, before, electrophoretic studies were the golden standard, now outdated) and yes that molecular evolution is great (even if morphological and osteological techniques of the old times are still valid in modern research notably when they use computerized softwares), and herein, in Killi-Data (see www.killi-data.org/list-names-molecular.phplist-names-molecular.php), it is so much an evidence and a priority that a molecular list of all studied so far species-populations has been lately created (to overcome the limits of GenBank) and is continuously updated, and, that a molecular bank of ethanol-fixed specimens available freely to researchers has been built, in order to boost research in that field (many, many species have not yet been studied with molecular techniques, and some species have been studied only by using 1 or 2 segments of the genome…; and since we know for sure that multigene studies, i.e. studies with longer and multiple sequences of genome, with the ultimate target of the entire genome, mitochondrial and nuclear, give much better results, you see, it is not the end of the molecular studies with killifish, and researchers can be easily compared to Sisyphus!). Obviously some—rare—-species have been studied using distinct specimens and populations by distinct researchers and they have given sometimes different results pushing to a reserved attitude (and also to consider that the molecular techniques

require high skills from the concerned researcher in order to align sequences properly and get correct results).

Anyhow, now with the many collections of live killifish and with the costs of molecular techniques that have fallen sharply, molecular techniques are available to any student with access to a PCR machine (also much cheaper) and this is no surprise to see that in your own country (Brazil) several new teams have recently started to molecularly study killifish, and this brings me to the 2 new publications that change the perspective of your question: those 2 publications of 2016 are led by a single author Wilson Costa (herein reviewed on page 71 &72) both studies are exactly using the same genetic segments [ND2, 16S rRNA, S7], are linked to an homogeneous timing (both distributed with less than 3 weeks gap) and are using mainly the same specimens (21), consisting of related species of the same group *Melanorivulus* and using the same outgroup species (for comparison), then results should be the same? But actually they are not, and by far the difference lies only in the slightly distinct sample of species corresponding to 2 executions of the computer programs analyzed by Costa's team; precisely, in the Vertebrate Zoology paper there are more studied species (and 1 less, *wallacei*) so that 4 newcomers [*Rivulus* (*Melanorivulus*) *atlanticus*, *dapazi*, *egens*, *rutilicaudus*] are added and indeed it reshuffles the obtained results, i.e. the 2 obtained trees are very different from each other, with (1) a distinctive topography, the tree in Ichthyological Exploration of Freshwaters having 9 branches, while the tree in Vertebrate Zoology has 4–5 branches, with (2) species *violaceus* placed directly below the primitive *schuncki* in Ichthyological Exploration of Freshwaters and down the tree closely related to *pindorama* in Vertebrate Zoology (itself isolated in Ichthyological Exploration of Freshwaters), with (3) species *jalapensis* placed directly below *punctatus* (huge geographical gap) in Ichthyological Exploration of Freshwaters and further down the tree closely related to *atlanticus* in Verebrate Zoology (small geographical gap, but still 2 distinct regions, coast and plateau), and so on.



Phylogenetic trees constructed by Costa et al. Top image from Ichthylogical Exploration of Freshwaters paper; and the bottom image from Vertebrate Zoology publication.

In conclusion, yes the molecular technique is a real asset in the tools available to killifish research (in this case, species

relationships), but clearly, no, any technique, even with those today's genetic data (erroneously tagged as ultimate by some people!), cannot be the end of the game, and there are no risk to predict that future trees, either in that group (both publications use only half of the total of over 46+ species, described for that group, nearly all by Costa) or elsewhere in killifish, with more sequences and more species in a given group will be different from present ones. Research is moving progressively and there is no end of the game, even with molecular data (not to speak of other techniques we simply do not have any idea of, today)

Jean Huber, finalized December 2016

Killifish Collecting Reports

International Collecting Expedition 2016 Colombia by with Ken Normandin (USA), Daniel Mejia (Colombia) and Frans Vermeulen (Aruba). Field code ICE 2016-**

From 16 November till 4 December 2016 an expedition was held to reach several points of interest in Colombia and the neighboring Ecuador. The first part of the trip was done by Ken and me only. First goal was to visit Ecuador to find *R. limoncochae* at its type locality along Rio Napo but due to strong advises not to cross the border we skipped that plan and collected around Mocoa, Florencia and finally reached Morelia to successfully collect *R. taeniatus* for the first time after its description at the type locality. We then went to a village named Curillo at the upper Caquetá where we did find *R. erberi* and by that expanded its known range by hundreds of kilometers. We then drove all the way to the North meanwhile collecting in the mountains around Caceres and Puerto Triunfo and found *R. elegans* and *R. azurescens* on their type locality. . . then to the Sinu area further North visiting towns like Monteria and Sincelejo for collecting annuals like *Austrofundulus myersi* and *Rachovia splendens*. We were able to find *R. splendens* but again we did not find the *Austrofundulus*. From Sincelejo we went westwards and crossed the Rio Magdalena to visit Bucaramanga for collecting annuals like *R. brevis* without success this time because it was out-of-season in that part of Colombia and return to Puerto Triunfo to deepen the search for *Rivulus* over there.

We were able to collect *R. ribesrubrum* at its type locality near San Miguel, this time not using a boat but found the spot over land by car, and further finding new locations of *Rivulus* aff. *xi* and others not known to science.

The second part of the survey Daniel joined us to collect in the Llanos near Puerto Lopez to find live specimens of *R. tessellatus* for the first time after its discovery in 1971 by J. Boehlke, N. Foster & J. Thomerson and its description by J. Huber in 1992. I like to thank Neal Foster for his help as he is the only remaining person alive that discovered this species. Finding this taxon was one of the main goals of the survey and we did find a species similar to the description. Still need to confirm its status. From there to the South into virgin areas for Killi research, areas that were inaccessible for decades to scientist as result of civil war. The goal was to reach San Jose de Guaviare and find new species of *Rivulus* and annuals. We did discover several stunning species on which we will report later.

This part was the end of the trip for Ken that had his flight home 2 days before my flight which was planned for December 4 2016.

Frans Vermeulen, 23 December 2016.

Internet Review

Interesting Websites

The FB account of Killifish Association of Bulgaria (EU) (in English and Bulgarian) has been more active re-

cently and it holds now several hundreds of photos and close-ups of live Killifish by the renown photographer Hristo Hristov (alias Aquasaur) at https://www.facebook.com/KilliAssociationBulgaria/timeline and his personal account at https://www.facebook.com/hristo.hristov.3150807?hc_ref=PAGES_TIMELINE (congratulations Hristo!), worth regular visits. [*Jean Huber*©]

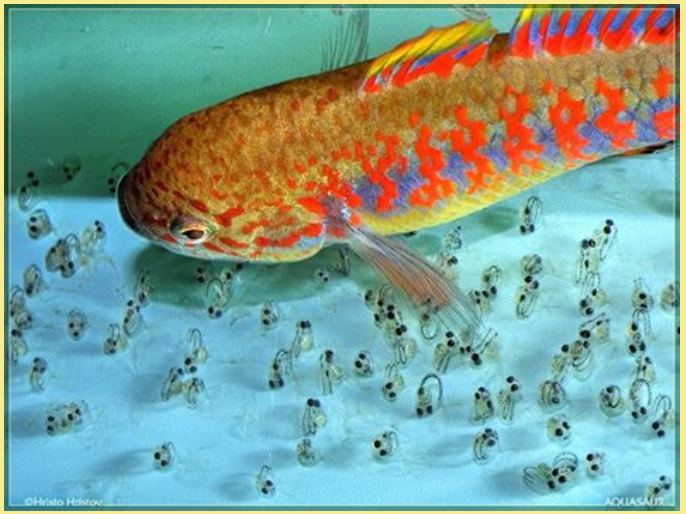


Photo of male *Tateurndina ocellicauda* tending its eggs. It isn't a killifish but does make a for a great photo. Photo by Hristo Hristov.

In The News

The BKA show made its way into Practical Fishkeeping magazine: http://www.practicalfishkeeping.co.uk/news/fishkeeping-news/articles/2016/12/12/a-weekend-of-killies.

Review of new research publications

Book Reviews

Annual Fishes: Life History Strategy, Diversity, and Evolution edited by Nibia Berois, Graciela García, and Rafael O. de Sá. Turner BJ. *The Quarterly Review of Biology*, 91:523–525, 2016. DOI http://dx.doi.org/10.1086/689534.

This is an Open Access paper which can be accessed at the above link. Bruce Turner offers an extensive review of the contents of the book which will be informative to those interested in annual killifish. [*Tyrone Genade*]

Systematics, Taxonomy & Distribution

***Papiliolebias habluetzeli* (Cyprinodontiformes: Cynolebiidae) a new miniature annual fish from the upper Rio Mamoré, Bolivia**. Valdesalici S; Nielsen D; Brousseau R; & Phunkner J. *Aqua, Journal of Ichthyology and Aquatic Biology*, 22:155–164, 2016. URL http://www.aqua-aquapress.com/product/aqua-22-4_/

The authors describe a new species of the genus *Papiliolebias*. *P. habluetzeli* is distinguished from other species of *Papiliolebias* based on having pointed dorsal and anal fins that are whitish with broad dark red stripes. The caudal fin has transverse rows of dark red spots and a metallic gold patch on the humeral region. Specifically, it differs from the similar looking *P. francescae* by the presence of 11–13 orange red bars on the flanks (vs 9–10 dark red), the number of red proximal stripes on the anal fin (5–8 vs 5), as well as the presence of extensive white pigmentation at the proximal area of the anal fin and the absence of a white rim on the anal fin. *P. habluetzeli* was found sympatric with *Pterolebias longipinnis* and several non-annual fish. The habitat was small pools that

were heavily overgrown by aquatic vegetation and grass. It is known from several locations within the Rio Mamoré basin, north-east and south of Trinidad, Bolivia. The fish is named for Pascal István Hablútzel who was the first to document this species while researching Bolivian ichthyofauna. This species has been distributed in the hobby under the name *Papiliolebias* sp. Trinidad BP 2013-04 and Nord Trinidad ABPV 13-7 . [*Tyrone Genade*]

Photo of *Papiliolebias habluetzeli* Trinidad BP 2013-04 (top, photo by Roger Brousseau©.) and *Papiliolebias francescae* (bottom, photo by Stefano Valdesalaci©).

Description of two new species of *Anablepsoides* (Cyprinodontiformes: Cynolebiidae) from Rio Madeira, Amazon drainage, Rondônia state and from Rio Itapecurú, Maranhão state, Brazil. Nielsen DTB. *Aqua, Journal of Ichthyology and Aquatic Biology*, 22:165–176, 2016. URL http://www.aqua-aquapress.com/product/22-4_anablepsoides/

The authors describe two new species of *Rivulus*[1]: *Anablepsoides luitalimae* and *vieirai*. The former can be distinguished from other members of the *limoncochae* by having light blue pelvic fins, a reticulated caudal fin and an anal fin with a reticulated base of light blue and red-brown dots. The dorsal fin origin is posterior to the last anal-fin ray. It originates from flooded areas within forest fragments along the Rio Madeira near Porto Velho, Rondônia state, Brazil. The habitat had dark stained water of pH 6 and low conductivity. There was a dense bed of leaf litter in the pond. The pond had no aquatic vegetation and no other species of fish were present. The fish is named for Luita Lima. It was formely known as *Rivulus* aff. *taeniatus*.

Anablepsoides vieirai differs from all other members of the *urophthalmus* species group by the presence of black spots in the head and opercular regions, the four centralmost rays have pale gray spots, the caudal fin has small distal filaments and the dorsal fin originates between neural spines of vertebrae 27 & 28. This species is only known from the type location near Duque Bacelar, rio Parnaíba basin, Maranhão state, Brazil. The habitat is a spring that forms a Buritizal (wetland dominated by *Mauritia flexuosa* palm). The spring forms a small stream that is damned to form a pond. The pH ranges from 5.0–6.5 at the source to 6.0–7.5 at the pond. The fish were found in the flooded area below the Buritizal in a stream that flows into the Riacho Joana. The fish is named for the environmentalist Gilberto da Silva Vieira who discovered the species. [*Tyrone Genade*]

[1] KDI considers *Anablepsoides* a subgenus of *Rivulus*.

Molecular phylogeny and biogeography of the South American savanna killifish genus *Melanorivulus* (Teleostei:Aplocheilidae). *Vertebrate Zoology*, 66:267–273, 2016. URL http://www.senckenberg.de/root/index.php?page_id=18450

This is the first of Costa's papers on *Melanorivulus*[2] in this issues of K-D News. In this paper Costa et al construct a phylogeny for the entire genus. The range of the species is set as the Cerrado biome between Oiapoque river basin in the north, the coastal plains of north-eastern Brazil in the east, the Uruguay river basin in northern Argentina in the south, and the Paraguay river basin in the west. Most species are found living in shallow streams draining the South American savannas. The exceptions are *M. schuncki* of the lowland savannas of Amapá and Marajó, *M. punctatus* of the northeasten Chaco and Pantanal as well as *M. decoratus* and *M. atlanticus* from the savanna enclaves within the semi-arid Caatinga and coastal plains of northeastern Brazil. It is stated that most species are less than 50 mm in length. The authors sampled 19 described and two undescribed species[3] of *Melanorivulus* and constructed a molecular phylogeny based on 16s, ND2 ad S7 DNA sequences. The sequences were also used to perform a biogeographical analysis. The authors concluded that the most recent common ancestor to the group was from eastern Amazon savanna of the Amazon-Cerrado ecotone. The fish distribution is the product of a series of dispersal events and vicariance as climatic changes causes repeated expansion and contraction of the rain forest. [*Tyrone Genade*]

Three species of *Rivulus* (*Melanorivulus*). From top to bottom: *Riv. punctatus* aquarium strain from Paraguay (photo by Frans Vermeulen©); *Riv. zygonectes* Porto dos Gaúchos HvdB 2015-15 (photo by Jurij Phunkner©); *Riv. rubromarginatus* Mato Grosso HvdB 2014-23 (photo by Jurij Phunkner©).

[2] KDI considers *Melanorivulus* a subgenus within *Rivulus*.

[3] Described in the next paper by Costa, sp. 1 = *petrosecundi* and sp. 2 = *spixi*.

Comparative morphology, phylogenetic relationships, and taxonomic revision of South American killifishes of the *Melanorivulus zygonectes* species group (Cyprinodontiformes: Rivulidae). Costa WJM. *Ichthyological Exploration of Freshwaters*, 27:107–152, 2016. URL http://www.pfeil-verlag.de/04biol/pdf/ief27_2_02.pdf

In this highly detailed and thorough paper Costa reviews the history, distribution, systematics and biogeography of the *Melanorivulus zygonectes*[4] species group. The *zygonectes* group is composed of: *M. crixas*, *M. imperatrizensis*, *M. javaeh*, *M. karaja*, *M. kayabi*, *M. megaroni*, *M. modestus*, *M. parnaibensis*, *M. petrisecundi*, *M. rubromarginatus*, *M. rubroreticulatus*, *M. salmonicaudus*, *M. spixi*, *M. wallacei* and *M. zygonectes*. Each species (except *imperatrizensis*) is illustrated with color photographs. A phylogenetic tree based on 16s and ND2 mitochondrial DNA as well as S7 nuclear DNA sequences is presented along with a phylogenetic tree based on combined DNA and morphological data. Highly informative traits in the morphological tree were male and female caudal and anal fin coloration as well as flank coloration and patterning.

The general habitat of the fish is described as shallow streams flowing through *Mauritia flexuosa* palms in the transition zones between Amazon rain forest and Cerrado savannah. The pH is reported to vary between 5.5 and 6.5. The fish are found in areas of moderate to bright light. The author notes that none of the species of this group are threatened with extinction.

Three new species are described. *Melanorivulus petrisecundi* is found in small streams and floodplains along the lower Rio Araguaia (and possibly the streams draining the Serra dos Carajás), southern Rio Amazonas basin, Estado do Pará, northern Brazil. It is named for Emperor Dom Pedro of Brazil (1841–1889) who during his reign was a strong patron of the natural sciences. It can be distinguished from other members of the group (except *M. rubroreticulatus*) by having a series of oblique red dots along the midline of the flanks. It differs from *M. rubroreticulatus* in having an orange caudal fin without bars and an anal fin without intense bright blue but with short red bars along the base. The caudal fin of females has a reticulate pattern in the middle portion.

M. spixi is from the Rio das Mortes drainage (a tributary of the Rio Araguaia) and is found in the swamps and on the flood plains. This is a robust species. It is distinguished from other members of the group by the females having a reddish gray spot at the base of the caudal fin. It has a deeper body compared to *M. salmonicaudatus* and *megaroni* (its closest relatives) by having a shorter caudal fin (without red bars), deeper body, red dots along the flank that are clearly defined and spaced out. This species is named for the zoologist Johann Baptist von Spix who partook in the first major ichthyological collections in the Rio Amazonas basin in 1817–1820.

M. wallacei is from the middle section of the Rio Tocantins drainage and adjacent area of the Rio Araguaia drainage. It can be distinguished from other members of the group (except *M. parnaibensis*) by possessing irregularly arranged red spots on the flank that are half the diameter of the pupil. It differs from *M. parnaibensis* by the absence of red spots in the middle portion of the male caudal fin and the presence of 5–8 gray to black bars in the female caudal fin (versus 3–5). In males the anal fin doesn't have red bars and in females there is a rounded black spot in the basal portion of the caudal fin that is smaller than the eye.

The author discusses the biogeography and the importance of this unexpected assemblage of species in the same drainage. The author notes that *M. imperatrizensis* was described by Nielsen & Pinto (2015) after this manuscript had been submitted, explaining why it isn't included in the analysis. He continues to note that the distribution of this species is at the

[4] KDI considers *Melanorivulus* a subgenus of *Rivulus*.

Four undescribed (or unidentified) species of *Rivulus* (*Melanorivulus*). From top to bottom: *Rivulus* sp, San Matías HvdB 2015-04; *Rivulus* sp. Nova Xavantina HvdB 2014-29, State of Mato Grosso, Brazil; *Rivulus* sp. Água Boa HvdB 2014-27; *Rivulus* sp Cocalinho HvdB 2014-24, State of Mato Grosso, Brazil. Photos by Jurij Phunkner©

northern limit of the range of *M. wallacei* and that the two species can be distinguished by the pattern of oblique rows of red dots along the flank and its orange caudal fin. A distribution map and identification key is provided. [*Tyrone Genade*]

Literature Cited

Nielsen & Pinto (2015) Aqua International Journal of Ichthyology, 21:136–143.

Colouration, taxonomy and geographical distribution of mangrove killifishes, the *Kryptolebias marmoratus* species group, in southern Atlantic coastal plains of Brazil (Cyprinodontiformes: Rivulidae). Costa WJEM. *Ichthyol. Explor. Freshwaters*, 27:183–192, 2016. URL http://www.pfeil-verlag.de/04biol/e9902d27.php

This paper concerns the *Kryptolebias marmoratus* species group which includes the species *marmoratus*, *hermaphroditus* and *ocellatus* (formally *caudomarginatus*[5]). All members of this group produce hermaphrodites. In *marmoratus* and *hermaphroditus* the hermaphrodites dominate the populations with very few males encountered. In *ocellatus* (sensu Costa) both sexes are encountered in the wild with equal frequency. In this paper Costa reports the collection of males of *hermaphroditus* from Coqueiral Beach (Espírito Santo state) and Divinéia mangrove.

Males occurred in two color forms: a dark morph and a light morph. Of 20 fish collected at the first location, three were males. Of 18 fish collected from the second location, two were males. Both morphs were present in each population. The color pattern of the males was unlike that of *marmoratus* which tends to have a yellow dorsum surface and a blue venter surface. The flanks are punctuated with red-brown or red-orange spots. In *hermaphroditus* males the dorsum was greenish brown and the venter white. The flank was

[5]KDI currently maintains all three names: *caudomarginatus*, *hermaphroditus* and *ocellatus*.

dark brownish purple with vertical bluish silver spots in the dark morph and bluish silver with dark red spots in the light morph. The caudal of *marmoratus* is orange with a thin black marginal band along the ventral margin while *hermaphroditus* has a caudal fin with an orange upper had, central white portion and a wide black marginal border.

K. ocellatus also occurred as a dark and light color morph. Male *hermaphroditus* differed from *ocellatus* in that the former lacked the well defined caudal spot and submarginal markings in the anal fin. The markings on the flanks of *ocellatus* are clearly arranged in vertical pattern while the markings and blotches appear random in the light morph of *hermaphroditus* while in the dark morph the color pattern is inverted with white spots and blotches over a dark flank.

Costa compares the *hermaphroditus* to that of the Brazil populations of *marmoratus* and demonstrate that they are clearly different fish. Costa notes that between Maceió (the most southern population of *marmoratus* and Coqueiral Beach (the most northern location for *hermaphroditus*) no *marmoratus* group *Kryptolebias* have been found even though thorough sampling efforts have been made.

The male *Kryptolebias* reported by Berbel-Filho et al (2016) collected from Ceará-Mirim River estuary, Rio Grande do Norte State, is clearly different to the male *hermaphroditus* shown by Costa. Tatarenkov (2011, 2015) showed that *Kryptolebias* from the Turks and Caicos, Panama, southern Cuba and Puerto Rico were genetically more similar to *Kryptolebias hermaphroditus* (what he called *ocellatus* in the paper). *Kryptolebias* sp. aff *marmotatus* from Brazil needs further research to determine its taxnomic status now that there is evidence for *hermaphroditus* and the Caribbean *Kryptolebias* being separate species to that of *Kryptolebais marmoratus* (Poey, 1880). Costa's paper is Open Access. [*Tyrone Genade*]

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Tatarenkov et al. *J. Fish. Biol.* 79:2095–2105 (2011). DOI https://dx.doi.org/10.1111/j.1095-8649.2011.03155.x.

A male *Kryptolebias* of the Punta Maracayo PR 2007 population. Photo by Tyrone Genade©.

Validity and redescription of *Profundulus balsanus* Ahl, 1935 (Cyprinodontiformes: Profundulidae). Jamangape O JA; Velazquez-Velazquez E; Martinez-Ramirez E; Anzueto-Calvo MJ; Gomez EL; Dominguez-Cisneros SE; Mcmahan CD; & Matamoros WA. *Zootaxa*, 4173:55–65, 2016. DOI http://dx.doi.org/10.11646/zootaxa.4173.1.5

Ernst Ahl described *Profundulus balsanus* in 1935 based on 12 specimens collected in the Malinaltepec river, Guerrero, Mexico. The description provided minimal morphometric and meristic data and in 1955 Miller synonymised *balsanus* with *Profundulus punctatus*. This decision was based on only two poorly preserved specimens of *balsanus* and the uncertainty of whether the species actually comes from the Balsas River system (it does not, this was an error in Ahl's description). In 1999 Doadrio concluded that *Profundulus punctatus* is a complex of species. This year Morcillo et al produced a

phylogeny based on two mitochondrial (ATPs 8-6 and ND2) and one nuclear gene (S7) that also showed that *balsanus* is a distinct species. In this paper Jamangapé et al redescribe *P. balsanus* and compare its morphology and meristics to that of the closely related *P. punctatus* and *P. oaxacae*. They also provide a phylogenetic tree based on the cyt b and COI genes that confirms that these are distinct species. The authors report that *P. balsanus* differs from all species of *Tlaloc* based on the presence of a humoral spot, a preorbital bone covered with well developed scales and more than half of the anal fin covered with scales. It can be distinguished from all other members of the genus *Profundulus* by the possession of 33 scales along the lateral line. It can be distinguished from *P. punctatus* by a transparent anal fin without any marks or dark lines extending across the entire fin, as in the case of *punctatus*. A detailed identification key and distribution map are included in the paper. The range of *P. balsanus* stretches from the Arroyo Inzcuinatoyac river in the north to the Huatulco river in the south. There are no report of *balsanus* being found in the Balsas river. The paper is Open Access and includes color maps and photos of the fish. [*Tyrone Genade*]

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Morcillo et al Mol. Phylogenet. Evol., 94:241–251 (2016).

Diversity and status of Mexican killifishes. Lozano-Vilano ML & De La Maza-Benignos M. *Journal of Fish Biology*, Epub ahead of print, 2016. ISSN 1095-8649, DOI http://dx.doi.org/10.1111/jfb.13186

An important paper best read under sunny skies and pleasant mood, because its contents are depressive. Oh cry, cry, killifish! Human nature is a misery! The high extinction vulnerability of killifishes of Mexico is reflected in the current conservation status of about 87% of the 52 species that occur, including 37 endemic to Mexico. Seventeen of these endemic species, not including species already extinct, are confined to a single spring, spring complex or lake (e.g. *Profundulus* (*Tlaloc*) *hildebrandi*, the Laguna de Chichancanab species flock (7 species), *Cyprinodon julimes*, *Cyp. fontinalis* and *Fundulus philpisteri*) and 13 species are restricted to narrowly defined geographic areas (e.g. *Prf. oaxacae*, *Cyp. pisteri*, *Cyp. bifasciatus*, *F. lima* and *Lucania interioris*). Only 15 (about 29%) species have distribution ranges that extend beyond Mexico's borders. Full evaluation of the conservation status of Mexican killifishes, together with conservation action is an urgent matter. To date, 7 species (about 14% of killifish diversity) are extinct; 20 (about 39%) are endangered, including 13 (about 25%) critically endangered or effectively extinct (e.g. *Cyp. simus*). Overall, it can be said that only about 7 species (about 13%) of killifish are thriving in Mexico (e.g., *Rivulus tenuis*). Which means that 45 species are at critical short-term risk or even extinct because of excessive watering by human activities (hence drying out biotopes), because of artificial introduction by man of predator aliens such as *Hemichromis guttatus*, because of building of recreation sites for man! Shame on us! May we encourage (support and endorse) Mexican researchers activities (e.g. those of the 2 co-authors) who try to explain to local authorities and inhabitants (farmers) how to act and behave differently and who build up conservation strategies in association with them (e.g., Pronatura Noreste). [*Jean Huber*]

This review paper focused on secondary freshwater killifish of the order Cyprinodontiformes from Mexico, representing four extant families, Rivulidae (3 species), Profundulidae (5 species), Fundulidae (10 species), and Cyprinodontidae (34 species) from a wide variety of habitats. The review paper has excellent color images of the species and some habitat images. This paper also reviews the current classification of Mexico killifishes, as well as aspects of their distribution, biology, ecology and current population conservation status. This is a must for scientists and aquarist alike! [*Mark Peterson*]

This paper is available through www.researchgate.net.

Checklist of the continental fishes of the state of Chiapas, Mexico, and their distribution. Velázquez-Velázquez E; López-Vila JM; Gómez-González AE; Romero-Berny EI; Lievano-Trujillo JL; & Matamoros WA. *ZooKeys*, 632:99–120, 2016. DOI https://doi.org/10.3897/zookeys.632.9747

The authors provide a checklist of fish species for the Mexican state of Chiapas. This will no doubt interest fish collectors. [*Tyrone Genade*]

***Nothobranchius streltsovi*, a new species of annual killifish with two male color morphs from the Malagarasi drainage, central Tanzania (Teleostei: Cyprinodontiformes: Nothobranchiidae)**. Valdesalici S. *Ichthyological Exploration of Freshwaters*, 27:153–161, 2016. URL http://www.pfeil-verlag.de/04biol/pdf/ief27_2_03.pdf

Valdesalici publishes the description of a new species: *Nothobranchius streltsovi*. This species was first collected by Lothar Seegers in 1994 and given the name sp. Limba Limba for the river system it came from. In 2010, 2012 and 2014 specimens were collected from the Nkululu river in central Tanzania by Konstantin Shidlovskiy, Sergey Torgashev, Sergey Streltsov and Nikita Savelov. It has been circulated in the hobby as sp. TSTS 10-5, TSTS 12-05 and TNT 14-07[6]. The species is named for Sergey Streltsov who collected this species.

The distinctiveness of this species was determined by a combination of morphological and meristic characters and employing Principle Component Analysis. The species can be distinguished from other species of the *ugandensis* group by the possession of scales with orange margins, an orange caudal fin with proximal spotting, a spotted pectoral fin, an anal and pelvic fins with red dots that are blue proximally and yellow or creamy white distally as well as several other meristic characters. The species exists as two color morphs: a white and a yellow morph.

It was found together with *N. seegersi* in shallow pools without vegetation. The water is turbid from a clay suspension with pH 7.9 and 150 μS·cm^{-1} conductivity. [*Tyrone Genade*]

Photo of *Nothobranchius streltsovi* TNT 2014-07. Photo by Sergey Torgashev©.

Nothobranchius sainthousei, a new species of annual killifish from the Luapula River drainage in northern Zambia (Teleostei: Cyprinodontiformes). Nagy B; Cotterill FPD; & Bellstedt DU. *Ichthyological Exploration of Freshwaters*, 27:233–254, 2016. URL http://www.pfeil-verlag.de/04biol/pdf/ief27_3_04.pdf

Nagy et al describe *Nothobranchius* sp. Mweshi ZM 12-19 as a new species: *N. sainthousei* in honor of Ian Sainthouse (BKA). This species hails from the Congo River basin which has a high level of species endemism. The Luapula River, which is fed by the Chambeshi swamps and flows into Lake Mweru, is already known by hobbyists as the basin housing *N. malaissei* (from the lower Luapula system), *chochamandai* (from the middle Luapula system), as well as *N. symoensi* and

[6] Collection details are available at http://www.nmg.nothos.org/htm/TSTS10.htm and http://www.nmg.nothos.org/htm/TNKS12.htm.

rosenstocki (from the upper part of the river system).

Photos of *N. sainthousei*, top; and *N. rosenstocki*, below. Photos by Bela Nagy©.

Based on combination of morphometric and meristic variables, together with Principle Component Analysis and molecular phylogenetics (using the ND2 gene), *sainthousei* could be distinguished from the other species. *N. sainthousei* can be differentiated from other species of the *Nothobranchius* species (except *rosenstocki*) by having broad orange-brown scale margins on the trunk and an anal fin with orange-brown margin. It is distinguished from *N. rosenstocki* by having orange-brown spots in the dorsal and anal fins. The type location is the flood plain at the confluence of the Luapula and Luongo rivers where it was collected from the ephemeral pools formed in the riverbed of the Chimbembe River. It is reported, by the locals, to also be present in the flood plain between the Chimbembe and Kusake rivers. Other non-annual fish species were collected with the *N. sainthousei*. The water was brown and turbid with pH 6.98, temperature 23.0 °C and the hardness was 15 ppm. The embryonic development took three to four months at 22–24 °C. Stomach contents of wild collected fish consisted of small aquatic crustaceans, worms, insect larvae and zooplankton. The paper goes on at length to discuss endemism in the river system, predict new species discoveries and note that further exploration of the Luapula River basin is needed. The authors provide a valuable identification key. [*Tyrone Genade*]

A very well done, comprehensive and emblematic of best-in-class description with all details except detailed osteological data. Ian Sainthouse is a noted KDI member, collector, specialist and describer of new *Nothobranchius*, to whom a better health is wished cheerfully. [*Jean Huber*]

Killifish Biology: Ecology & Physiology

The genomic landscape of rapid repeated evolutionary adaptation to toxic pollution in wild fish. Reid NM; Proestou DA; Clark BW; Warren WC; Colbourne JK; Shaw JR; Karchner SI; Hahn ME; Nacci D; Oleksiak MF; Crawford DL; & Whitehead A. *Science*, 354:1305–1308, 2016. DOI http://dx.doi.org/10.1126/science.aah4993

A team led by Whitehead shows, in 384 whole genome sequences of *Fundulus heteroclitus* s.l., living off the east coast of USA, mutations 8,000 times more resistant to toxic waste than normal fish [a breakthrough published in Science] with a possible plus (better survival) and a risk (reduced diversity) for the species. Atlantic killifish populations, like many organisms, have evolved tolerance to natural and human-generated toxins, i.e. they have rapidly adapted to normally lethal levels of pollution in 4 urban estuaries (heavily polluted sites with a mix of dioxins, polychlorinated biphenyl or PCB and heavy metals). Through analysis of whole killifish genome sequences (not limited sequences) and comparative transcriptomics in 4 pairs of sensitive and tolerant populations, the authors identify the aryl hydrocarbon receptor-based signaling pathway as a shared target of selection (they show similar mutations have shown up independently in the same genes within each population). This suggests evolutionary constraint on adaptive solutions to complex toxicant mixtures at each site and opens a pathway to better understanding in mutational/evolutionary modes. More details in a didactic tone at http://www.sciencealert.com/scientists-discover-mutated-fish-that-have-become-8-000-times-more-resistant-to-toxic-waste. [*Jean Huber*]

Sequencing and characterization of the complete mitochondrial genome of the endangered Devils Hole pupfish *Cyprinodon diabolis* (Cyprinodontiformes: Cyprinodontidae). Lema SC; Wilson KP; Senger BL; & Simons LH. *Mitochondrial DNA Part B*, 1:705–707, 2016. DOI http://dx.doi.org/10.1080/23802359.2016.1225526

Lema et al report on the sequencing and characterization of the mitochondrial genome of *Cyprinodon diabolis*. The mitochondrial genome is 16'499 base pairs in length and encodes 13 protein subunits, 22 transfer RNAs and two ribosomal RNAs as well as non-coding RNAs. Phylogenetics confirmed the close relationship to *Cyprinodon nevadensis* subspecies *pectoralis* (99.436% identity) and *amargosae* (99.382% identity). Gene variations were identified in the cytochrome c oxidase subuit 2 and NADH dehydrogenase subunits *nd1*, *nd2*, *nd3*, and *nd4*. Both of these genes are important in energy metabolism and the deviations from the other species could be adaptations to the harsh conditions of Devil's Hole or the result of founder effects and bottlenecks when the fish got trapped in Devil's Hole 830 to 105 years ago (Martin et al, 2016). [*Tyrone Genade*]

Literature Cited

Martin et al, (2016) Proc. R. Soc. B. http://dx.doi.org/10.1098/rspb.2015.2334

Metabolic and regulatory responses involved in cold acclimation in Atlantic killifish, *Fundulus heteroclitus* Healy TM; Chung DJ; Crowther KG; & Schulte PM. *Journal of Comparative Physiology B*, Epub ahead of print, 2016. DOI http://dx.doi.org/10.1007/s00360-016-1042-9.

The authors set out to determine how *Fundulus heteroclitus* acclimates to cold. The response of southern (*F. heteroclitus heteroclitus*) and northern (*F. heteroclitus macrolepidotus*) were compared.

Southern fish had adapted their resting muscle O_2 consumption within 24 hours of exposure to 5 °C water; but there was no difference between the 15 and 5 °C fish with respect to their MO_2 max (the highest O_2 consumption rate without developing anoxia in tissues). There was no effect of cold on the expression mitochondrial genes, Cytochrome

C Oxidase II, Cytochrome C Oxidase Subunit 4I1; nor Citrate Synthase enzyme activity. Expression changes of Nuclear Respiratory Factor 1 and Peroxisome Proliferator-Activated Receptor Gamma, Coactivator 1 Alpha and Betas were studied. Effects were observed for both gradually and abruptly cold-water acclimated fish. Nuclear Respiratory Factor 1 increased in both northern and southern fish when exposed to the cold and the difference in the increase was significant between the populations (the effect being larger for northern fish).

The authors discuss the absence of cold acclimation effecting metabolic rate in the fish. The authors note that a 10 °C drop in temperature will decrease metabolic rate by 2 to 3 times; and that this could explain the drop in resting muscle O_2 consumption. The authors note that there is no metabolic compensation to the change in temperature. The changes that did occur did so over the course of days and all tissues didn't respond the same. The authors state that changes in mitochondrial volume and function were changed after several weeks and that this could be due to shifts towards the metabolism of lipids which is a common response in low temperature fish.

If these observations are a conserved phenomenon in killifish it could explain many of the challenges of keeping coolkillies (such as *Diapteron* species) in captivity. The fish simply can't adapt its mitochondrial metabolism to changes in temperature. Going from 18 to 24 °C could greatly increase oxygen demand in the fish while there is no concomitant increase in the enzymes needed to process the oxygen or energy from food. In the early 1990s Monty Lehman (AKA) experimented with *Diapteron* at high temperatures (27 °C) [7]. He reasoned that incorrect feeding at higher temperature was causing a stomach upset in his fish. In his case he switched from *Artemia* nauplii to sieved *Daphnia* believing that the *Artemia* was too high in protein compared to the *Daphnia*. This isn't actually the case: *Artemia* nauplii and *Daphnia* have almost equivalent amounts of protein (56 vs 51% dry weight) but *Artemia* nauplii have much more fat (12% vs 2.4% dry weight)[8]. In addition, the *Daphnia*, also provide enzymes that can break down food and so doing aid in digestion. If fish switch from protein to fat metabolism when going from high to low temperature then a switch from fat to protein is needed going from cool to warm temperatures. This is exactly what Monty did and at 27 °C he achieved large egg and fry yields. As the fish may not be able to acclimate metabolically to changes in temperature a change in diet and/or oxygen supply might be needed to ensure good health. [*Tyrone Genade*]

Effects of increasing temperature due to aquatic climate change on the self-fertility and the sexual development of the hermaphrodite fish, *Kryptolebias marmoratus* Park CB; Kim YJ; & Soyano K. *Environmental Science and Pollution Research*, Epub ahead of print, 2016. DOI https://dx.doi.org/10.1007/s11356-016-7878-4.

Park et al set out to determine how temperature effects the reproduction and sexual development of *Kryptolebias marmoratus*. Previous research by Turner et al demonstrated that temperature effects the sexual development of this species. The authors, concerned by the prospect of global warming, hypothesized the higher temperatures would impair reproductive fitness in this species. They set out to determine the effect of normal (25 °C) and warm (30 °C) water on the percentage of fertilized eggs, reproductive endocrine activity and alteration of sexual development under. Higher temperatures suppressed self-fertilization, caused a decline in egg quality and quantity as well as delayed spawning. Warm-water fish had significantly lower gonad mass as a proportion of the total body mass and produced less vitellogenin (needed

[7] http://www.chesapeakekillifish.org/page.php?17
[8] http://goo.gl/t8YW3u.

for form eggs). There were no changes in the levels of 17β-estradiol, testosterone, and ratio of testicular tissue to total gonadal tissue. No increase in the frequency of male fish was observed between the experimental groups (only one male was record and it was among the warm group). An interesting behavioral question unanswered by the authors is whether warm-water hermaphrodites would show less aggression to male fish or perhaps even seek them out for spawning. Further research involving different populations might prove insightful. My *Krytolebias* from the Island of Guadeloupe only begin to spawn at a higher temperature (above 25 °C) and produce males more easily. The fish from Puerto Rico I used to have would regularly produce male fish at 24 °C and spawned at the same temperature. [*Tyrone Genade*]

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Turner et al. Evol. Ecol. Res., 8:1475–1486 (2006)

Metazoan parasites of African annual killifish (Nothobranchiidae): abundance, diversity, and their environmental correlates. Nezhybová V; Reichard M; Blažek R; & Ondračková M. *Biotropica*, Epub ahead of print, 2016. DOI http://dx.doi.org/10.1111/btp.12396

The authors sought to better understand the relationship between parasites and their hosts, using *Nothobranchius* as a model system because these fish ocur in isolated pools across a wide range of environmental conditions. The authors put forward four hypotheses. (1) Parasite communities do not differ between host killifish species living in sympatry and that (2) host killifish parasites are generalists. (3) Parasite diversity would increase from dry to humid regions due to differences in pool duration that would allow for a longer life cycle. (4) That there would be a strong association between the characteristics of the parasite communities and environmental factors and that this would manifest as a higher parasite diversity in pools whether other fish and invertebrate species are present.

The authors investigated several pools in Mozambique and the species *N. furzeri*, *N. kadleci*, *N. orthonotus* and *N. pienaari*. The authors recorded 17 parasite taxa from 205 fish captured for sampling. Only 12 fish were free of parasites and almost all the parasites were endoparasites. Some parasites required another host organism to complete their life cycle. 13 different parasites were found in pools with sympatric killifish communities. 11 of these were generalists and two were specialists parasites effected *N. furzeri* (*Apatemon* sp.) and *N. orthonotus* (*Digenea* sp.). Two generalists species were found in high abundance and prevalence in *N. furzeri*, suggesting a tendency to host preference. Camallanidae were found to infect all four species of fish.

Parasite diversity and abundance were found to correlate with fish species diversity and the intermediate aridity region. The authors explain the lack of ectoparasites as being due to the temporary nature of the habitat. The authors confirmed the positive role of pool size and aquatic vegetation on parasite diversity but not parasite abundance. No effect was observed as to invertebrate diversity effecting parasite community structure. This paper should serve as a warning to collectors: your fish are infected with parasites! A general antihelminth treatment, such a flubendazole, should be applied to new imports. [*Tyrone Genade*]

Another study, in the same sense, reports 100% infection in wild *Aphanius sophiae* on collection: Gholami et al. Journal of Parasitic Diseases, 40:1030–1032 (2016). [*Jean Huber*]

Repeated intra-specific divergence in lifespan and ageing of African annual fishes along an aridity gradient. Blažek R; Polačik M; Kačer P; Cellerino A; Řežucha R; Methling C; Tomášek O; Syslová K; Tozzini ET; Albrecht T; Vrtílek, M. & Reichard, M. *Evolution*, Epub ahead of print, 2016. DOI http://dx.doi.org/10.1111/evo.13127

This is an excellent paper on *Nothobranchius* aging. A long-standing hypotheses among *Nothobranchius* researchers

is that lifespan has evolved to suit the duration of the temporary habitat the fish live in. In this paper the authors set out to determine if high condition-dependent mortality can select for extended, rather than reduce, lifespan or, in other words, whether a longer lasting environment would favor and select fish that remained healthier for longer. Several studies have indicated that aging patterns within a species (i.e. between different populations) can evolve in the opposite direction to the pattern among species.

The authors set out to determine the captive lifespan of four *Nothobranchius* species (*furzeri*, *kadleci*, *orthonotus* and *pienaari*) that occurred often sympatrically as well as in short- or long-duration pools. The authors measured the level of oxidative stress, incidence of neoplasias (i.e. tumors) and reproductive success. The authors decided to test the "pace-of-life-syndrome" hypothesis which purports that rapid life history would be associated with high metabolic rate, higher activity, aggressive and bold behavior. To this end the authors measured oxygen consumption, spontaneous locomotion and aggression.

The authors found that populations from drier regions had a higher mortality rate than wet-region populations. Dry region populations also had higher levels of oxidative stress that increased faster with chronological age. Unexpectedly, this increase was not as pronounced in brain tissue as for heart and liver. The dry-populations also had a higher incidence of neoplasia and other tissue degeneration. The total number of eggs per spawning decreased with age in the dry-population fish but tended to increase among the wet-population fish. Fertilization success declined for all populations with age but more so in the dry- than the wet-population fish. Pace-of-life-syndrome wasn't confirmed among the species or populations.

The authors put forward that in *Nothobranchius* longer lifespan is achieved through a lower baseline mortality, i.e. the normal day to day risk of death; rather than by a slower aging rate. The authors show that the rate of biological aging between the populations was similar but the mortality rate was lower in the wet-populations. Interestingly, while *N. orthonotus* populations showed clear differences with respect to functional and reproductive aging there was no difference in lifespan. The authors conclude that the pattern of life history among the Mozambican *Nothobranchius* was not consistent with their hypothesis of concerted evolution of aging and life history and that there was no support for pace-of-life syndrome.

This paper provides lots of fascinating data and raises many interesting questions. In my personal experience most *Nothobranchius* have shown a decline in fertility with age. Five months old *N.* sp. Nyando River were totally infertile, looking thin with skin lesions. Even though the fish was obviously old it persisted for several more weeks before dying. Its biological age did not necessarily determine its likelihood of death. On the other hand one *N. ruudwildekampi* male reached an age of at least 18 months and was still fully fertile, spawning with his daughters and did not look nearly as haggard as the afore mentioned sp. Nyando River. Expanding this study to other species of *Nothobranchius* could prove interesting, especially comparing *pienaari* to *krysanovi* and *rachovii*; and *korthausae*, *lourensi* and *ruudwildekampi*. These come from areas with different aridity and are more closely related to each other than other species. In addition, the longer lifespan might allow for fine measurement of the various biological-aging parameters used by the authors. Assaying the immune system using blood smears or PCNA/L-plastin[9] double labeling of immunogenic tissue might also prove valuable. Evolutionary patterns may be more consistent between them. More frequent sampling and performing a necropsy on each dead fish to determine cause of death could be very informative. A very interesting

[9]PCNA is a marker of recently divided cells while L-plastin labels fish immune cells; new immune cells are born in the kidneys of fish.

paper that will no doubt prove very influential in the field of aging research. [*Tyrone Genade*].

Aging asymmetry: systematic survey of changes in age-related biomarkers in the annual fish *Nothobranchius guentheri*. Dong Y; Cui P; Li Z; & Zhang S. *Fish Physiology and Biochemistry*, Epub ahead of print, 2016. DOI http://dx.doi.org/10.1007/s10695-016-0288-1

In this paper the authors described evidence for cellular aging in several tissues. They show that the tissues (brain, eye, heart, intestine, liver, muscle, skin, spleen, and testis) accumulate aging-related damage at different rates. Levels of protein oxidation and lipid peroxidation were assayed as well as the activity of the anti-oxidant enzymes catalase, glutathione peroxidase and superoxide dismutase. All the tissues showed large changes with age but the change was not consistent from tissue type to tissue type, nor from assay to assay. For example, the brain showed increased protein oxidation but not lipid peroxidation; both catalase and superoxide dismutase declined with age but there was no change in glutathione peroxidase. Interestingly, the expression of the DNA excision repair protein, ERCC-1, in the spleen, eye and heart was not different in young, middle-aged and old fish but did differ in other tissues. This is an interesting paper that will be of great benefit to those studying aging in *Nothobranchius* fish. [*Tyrone Genade*]

A low cost and adaptable benchtop recirculating aquarium system for *Nothobranchius* fish. Genade T. *Journal of the American Killifish Association*, 49:2–13, 2016. URL https://www.researchgate.net/publication/310088351_A_low_cost_and_adaptable_benchtop_recirculating_aquarium_system_for_Nothobranchius_fish

In this article is described a very interesting recirculating aquarium system that can be easily set up on a laboratory bench but also on hobbyist fish room because it occupies little space and can be assembled at low cost. The system is at the end a tank divided in many section by foam that works as biological filter and separator. The water is pumped from last portion in one side to the opposite side (where a home made filter "cleaning" the inlet water). Many "separated" aquariums can be interconnected by PVC piping giving a very stable environment. To limit disease a UV lamp is connected to water line. The system has been proven suitable for raising and spawning *Nothobranchius* (for experimental purposes) because it is also adaptable to the needs and can be up- or down-scaled as needed. The author describe also his Nothobranchius spawning system, using granulated peat which is sieved to collect eggs. Fry care is also described in the context of the system. Disease and water quality management strategies are also discussed. [*Stefano Valdesalici*]

Photo of tanks connected in series on a recirculating system. Photo by Tyrone Genade©.

Patterns of reproductive behaviour in *Millerichthys robustus* (Cyprinodontiformes: Cynolebiidae). Valdesalici S; Dominguez O; & Mosqueda M. *Aqua, Journal of Ichthyology and Aquatic Biology*, 22:177–180, 2016. URL http://www.aqua-aquapress.com/volume-22-issue-4-22-october-2016/

The material has been collected not far from type locality from a pool with several sympatric non-annual fishes, non killifish, plus *Rivulus* (*Cynodonichthys*) *tenuis*, and viviparous killifishes in Poeciliidae (*Gambusia sexradiata*, *Xiphophorus*

maculatus, *Poecillia sphenops*, *Poeciliopsis gracilis*, *Belonesox belizanus*). The study is a lab experiment with each trial being recorded for a period varying from 1 to 6 minutes by a Nikon coolpix s6100 video. This is a descriptive behavioral study as an annual diver, distinctive from all annual killifish by the fact that female, not male, dives first into substratum camera [herein photos by Omar Dominguez, (a), female first diving, (b) female emerging first]; 7 qualitative steps are reported in breeding behavior, (1) alternating lateral displays by male, (2) female responding by following male above spawning site, (3) alternating lateral displays with vibration by male, with folding of Dorsal and Anal fins and sigmoid displays above substrate, (4) female responding by diving, when three fourth to almost completely inside substrate, (5) male following into substrate, (6) female emerging, and (7) male emerging a few seconds later. [*Jean Huber*]

Delayed impacts of developmental exposure to 17-α-ethinylestradiol in the self-fertilizing fish *Kryptolebias marmoratus*. Voisin AS; Fellous A; Earley RL; & Silvestre F. *Aquatic Toxicology*, 180:247 – 257, 2016. DOI http://dx.doi.org/10.1016/j.aquatox.2016.10.003

The estrogen-disrupting compound, 17-α-ethinylestradiol, is both one of the most potent estrogen-disruptors and resists degradation. EE2 is widely used in medicine (e.g. oral contraceptive) and agriculture and is a commonly encountered as a pollutant in aquatic environments. Concentrations within the pg-ng/L range are able to negatively effect reproduction and survival in aquatic organisms. Voisin et al set out to investigate the effects of early-life 17-α-ethinylestradiol on *Kryptolebias marmoratus*. Fish were exposed to a 4 and 120 ng/L concentration for 28 days after hatching. The 4 ng/L dose is comparable to that found in some polluted surface waters. At the age of 168 days after hatching growth, reproduction and hormone levels were assayed. The high dose of 17-α-ethinylestradiol initially decreased the size of the fish but this effect vanished after 91 days. 17-α-ethinylestradiol did not effect survival but treated fish began to reproduce at an earlier age. Fish exposed to the lower dose laid significantly fewer eggs than the other fish. No difference was found between the control and treatment groups for cortisol and estradiol concentration but the 120 ng/L treated fish had higher levels of testosterone (at the 91 and 168 day time

Spawning behavior of *Millerichthys robustus*. Photos by Omar Dominguez©.

points) and 11-ketotestosterone (at the 168 day time point). The authors hypothesize that the lower dose is too low to activate the compensatory mechanisms of the fish as the high dose does, explaining the lower fecundity of the low dose fish. That the low dose has effects on reproduction several months after an acute exposure is troubling and it would be interesting to know what the effect of chronic exposure would be. The critical finding in this study is that EE2 concentrations recorded from polluted environments are high enough to effect reproductive biology and growth several months after the initial exposure. In addition, the higher the dose the longer it took the fish to recover from the exposure. *Kryptolebias* exhibit effects of EE2 exposure at only 4 ng/L while *Fundulus heteroclitus* and Medaka require higher doses. The authors hypothesize that low levels of exposure are not severe enough to activate the fish's compensatory mechanisms, as in the 120 ng/L dosed fish. For aquarists, we should be careful with plastics and other sources of estrogen-signaling-disruptor. However, the molecule leached by plastics, BPA, is a weaker estrogen dysrupter than EE2 (Shyu et al). At the concentrations it is found in plastic water bottles (4 to 8 ng/L), *Kryptolebias* probably have nothing to fear. [*Tyrone Genade*]

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Shyu et al Toxicol. Appl. Pharmacol., 250:322–326, 2011.

Amphibious fish jump better on land after acclimation to a terrestrial environment. Brunt E; Turko AJ; Scott GR; & Wright PA. *Journal of Experimental Biology*, Epub ahead of print, 2016. DOI http://dx.doi.org/10.1242/jeb.140970

Brunt et al set out to determine whether *Kryptolebias marmoratus* jump better on land after a short acclimation period. Fish were divided into three groups: a group kept in water and fasted for 14 days, a group kept out of water and fasted for 14 days, and a group kept out of water and then returned to the water and fasted for 14 days. The fish were not fed during this period because *Kryptolebias marmotatus* cannot feed out of water. Experimental fish were placed in an experimental box and then gently prodded to cause them to jump. They were prodded until exhausted and then left to recover where upon more jumping was encouraged until the fish reached exhaustion. During the jumps the entire body of the fish left the ground. Fish that had been acclimated to the terrestrial environments performed 1.6 times more jumps than unacclimatized fish. Their jumps were also twice as far and they could perform 1.7 times more jumps before becoming exhausted. Physiologically, they produced less lactate during the exercise and histological analysis revealed that they had almost 1.5 times more oxidative muscle (the muscle type needed for endurance exercise) and a higher density of capillaries per muscle fiber. Fish that were returned to the water showed normal levels of jumping and muscles mass as compared to the unacclimatized control fish. [*Tyrone Genade*]

Osmotic versus adrenergic control of ion transport by ionocytes of *Fundulus heteroclitus* in the cold. Tait JC; Mercer EW; Gerber L; Robertson GN; & Marshall WS. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 203:255–261, 2017. DOI http://dx.doi.org/10.1016/j.cbpa.2016.10.003

Fundulus heteroclitus can withstand temperatures from –1.5 °C to 38 °C. The authors had previously demonstrated that at 5°*C* the secretion of NaCl slows down but doesn't stop. Tait et al set out to discover whether the neural or local osmotic responses regulate NaCl at low temperatures. In the experiment, fish were acclimated to 5°*C* and maintained there for 30 days. Experiments showed that the neural control over NaCl secretion was hindered at low temperature and that the local control mechanism, responsive to differences in osmolarity, were sufficient to regulate osmolarity of the fish's blood and tissues. These local controls were more sensitive under conditions of hypertonicity than hypotonicity. [*Tyrone Genade*]

Posters & Conference Abstracts

Upregulation of microRNA29 family protects from cardiac hypertrophy and fibrosis modulating DNA methyltransferases in the novel animal aging model *Nothobranchius furzeri*. Spallotta F; Heid J; Atlante S; Ripa R; Cencioni C; Baumgart M; Milano G; Scopece A; Kuenne C; Guenther S; Rossi G; Braun T; Pompilio G; Zeiher AM; Cellerino A; & Gaetano C. 2016. URL http://circ.ahajournals.org/content/134/Suppl_1/A18790

In this conference abstract the authors present the hypothesis that changes in the expression of microRNA miR-29 is involved in the aging-associated degeneration of the heart of *N furzeri*. Changes in the expression of 34 microRNAs were discovered between young and old fish. To test their observations in *N. furzeri* the authors genetically manipulated zebrafish so they would present the same age-related changes in microRNA. Very excitingly! the genetically manipulated zebrafish developed the same age-related pathology as *N. furzeri*. [*Tyrone Genade*]

Theses & Dissertations

Central regulation of food intake peptides during ageing in the teleost fish *Nothobranchius furzeri*. Montesano A. Doctoral thesis, *Università degli Studi di Napoli Federico II*, 2016. URL http://www.fedoa.unina.it/id/eprint/10704

Montesano studied the effect of aging on the expression of hormones involved in the resultion of hunger and satiety. These hormones, neuropeptide-Y, orexin and nesfatin-1 are known to play important rolls in whole-organism energy metabolism. *N. furzeri* of the MZM 04-10 and Gonarezhou strains were starved at different ages and the abundance of the hormones measured by gene expression and protein abundance. Sections of the fish brains were also labeled with antibody probes against the peptides. The author shows that during aging neuropeptide-Y and orexin are increased in young fish after a period of starvation but not in old fish. No changes in the expression of nesfatin-1 were observed. The author was able to determine the anatomical location of the expression of these hormones in the brains of the fish. [*Tyrone Genade*]

Hydraulic geometry and fish habitat in semi-alluvial bedrock controlled rivers. Ferguson S. Doctoral thesis, *Université d'Ottawa/University of Ottawa*, 2016. URL https://www.ruor.uottawa.ca/bitstream/10393/35313/1/Ferguson_Sean_2016_thesis.pdf

In this thesis the author described his findings as per habitat preference for several species of fish. He found that *Fundulus diaphanus* prefers streams and rivers that flow over bedrock. [*Tyrone Genade*]

Evolution in light of mitonuclear landscapes an examination of mitochondrial replacement in killifish (*Fundulus* SPP.). Flanagan SD. 2016. URL http://aquila.usm.edu/cgi/viewcontent.cgi?article=1922&context=dissertations

The *Fundulus notatus* species complex is an emerging model for evolutionary study. The author set out to determine the genetic population structure of two species in the *Fundulus notatus* group: *olivaceus* and *euryzonus*. They used a combination of molecular biology methods: restriction length polymorphisms and microsatellites; and, together with morphological data, determined whether mitochondrial introgression had taken place (i.e. how many genes had been exchanged between two species). The authors confirmed hybridization between the two species in the Tangipahoa species. The author goes on to discuss the utility of this river/species system to study how mitochondrial introgression influences evolution. [*Tyrone Genade*]

Insulin-like Growth Factor Pathway Described in *Austrofundulus limnaeus* Diapause and Escape Embryos. Woll SC. Masters thesis, , 2016. URL http://pdxscholar.library.pdx.edu/open_access_etds/3207/

Insulin and Insulin-like Growth Factor signaling regulates growth in all animals so far studied. In invertebrates it is known to regulate the entrance into diapause. In his research, Steven Woll, explored whether insulin-like Growth Factor played a role in the diapause of *Austrofundulus limnaeus* by means of messenger RNA expression levels. He found stage-specific expression patterns in Insulin-like Growth Factor and insulin signaling proteins. Using inhibitors of the Insulin-like Growth Factor receptor he was able to force eggs into diapause under conditions they would ordinarily develop. He identified the RAS-MAPK-ERK signaling pathway as being involved in the recommencement of development after diapause. In mammals this same pathway is implicated in growth and development and its deregulation with cancer. The Insulin-like Growth Factor Binding Protein was also implicated in the escape from diapause. He concludes that the Insulin-like Growth Factor pathway is critical to diapause and that the TFG-beta and SMAD pathways also needs to be investigated as they have been found to be involved in developmental arrest in other animal species. [*Tyrone Genade*]

Stress Response and Acclimation in the Adult Turquoise Killifish *Nothobranchius furzeri*. Henderson DW. Masters thesis, *Southern Illinois University, Carbondale*, 2016. URL http://opensiuc.lib.siu.edu/theses/1941/

Henderson set about to determine whether the hormonal stress response could be measured in *N. furzeri*. Cortisol levels as well as the levels of corticotropin releasing hormone, mineralocorticoid receptor and glucocorticoid receptor mRNA were assayed. Fish were sampled in an unstressed state and then at several time points after the initiation of a stressful event (a confinement event). Differences in plasma cortisol were observed after 30 minutes of confinement. Whole-body cortisol was significantly elevated after only 15 minutes. A sex difference was found in the expression of mineralocorticoid receptor level at 15 minutes for males. Repeated stress resulted in a decrease in baseline cortisol levels as well as post-stress levels, showing that there is physiological acclimation to stress. Together with a drop in cortisol levels there was an increase in the expression of corticotropin releasing hormone and mineralocorticoid receptor levels. The physiological acclimation was detected after only one week of experimentation. This thesis is the first report of stress response in this species, making it a very import for the research of *N. furzeri*. [*Tyrone Genade*]

Interesting research on other fish

Cytochrome P450 1A mRNA in the guppy *Phalloceros caudimaculatus* and response to beta-naphthoflavone and environmental samples. Ferreira RS; da Cruz Chivittz C; dos Santos GS; & Zanette J. *Aquatic Toxicology*, 181:86 – 93, 2016. DOI http://dx.doi.org/10.1016/j.aquatox.2016.10.023

In this paper Ferreira et al use the expression of Cytochrome P450 A1 enzyme (CYPA1), which is responsible for the detoxification of many chemical pollutants in the body, as measure of the pollution in an environment. For their research they chose to use *Phalloceros* because of its abundance in the environment, wide geographic distribution and adaptability. They set out to clone the gene for CYP1A and then measure how its expression changes when the fish are exposed to a toxin (beta-naphthoflavone). They found that they could assay the change in CYP1A expression in several tissues, including fin-clips. Next they took soil samples of polluted and unpolluted environments and then exposed fish to the elutriate of these samples. Fish exposed to soil from polluted environments shows a 47–199 fold increase in CYP1A expres-

sion depending on the tissue studied. Anal fins showed a large response to the toxins. The expression change remained elevated for at least 96 hours after exposure. These results will be of great value to ecotoxicologists studying endangered species. There is no longer a need to kill fish to obtain samples for toxicological analysis. Fin clips can be taken from wild fish and analyzed later. There is high identity between in the gene sequences of fish of the order Cyprinodontiformes so this method could easily be modified to suit different killifish species. [*Tyrone Genade*]

Developmental bisphenol A exposure impairs sperm function and reproduction in zebrafish. Chen J; Saili KS; Liu Y; Li L; Zhao Y; Jia Y; Bai C; Tanguay RL; Dong Q; & Huang C. *Chemosphere*, 169:262–270, 2016. DOI https://dx.doi.org/10.1016/j.chemosphere.2016.11.089

This paper reports on the effect of longterm exposure of zebrafish to the chemical bisphenol A. Bisphenol A is the compound used to synthesize hard plastics such as polycarbonate. It is a common environmental pollutant with concentrations as high as 21 μg/L in steams and 17.2 mg/L in landfill leachate. Bisphneol A is a confirmed estrogen-signaling disruptor. The authors report that concentrations as low as 0.3 μg/L have effects on fish reproduction. Eggs exposed to 0.3 μg/L from 6 days post fertilization reduced the weight of testes, sperm volume, sperm density, sperm motility and sperm swimming velocity as well as increased the number malformed embryos and embryonic mortality in the next generation. Bisphenol A can reach concentrations of 5 μg/L in water stored in polycarbonate containers. Few of us hobbyists use such polycarbonate containers. Lee's Critter Keepers are made of acrylic and I haven't found any data showing that these leach estrogen-signaling disrupting compounds. Most food containers, such as ice-cream and margarine tubes are made from polypropylene plastics. These containers find their way into fishrooms (at least they do into mine). There is evidence for these leaching estrogen-signaling disruptors but the concentrations are low (0.6–4.21 μg/L in bio-17β-estradiol equivalents) (Vigren, D. 2015). Other studies have reported 0.5 ng/L for polypropylene, 0.3 ng/L for high density polyethylene and 0.004 pg/nL for polyethylene terephthalate water bottles (Karlsson, F. 2014). For comparison, bisphenol A is one ten thousandth as potent as 17β-estradiol, so the 0.3 μg/L BPA used by Chen et al is 3 mg/L bio-17β-estradiol equivalents (Xu et al, 2016). It is several thousands more potent than the molecules leached by polypropylene and there is probably nothing to worry about. [*Tyrone Genade*]

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Xu et al. Environmental Pollution, 215:103–112, (2016). DOI http://dx.doi.org/10.1016/j.envpol.2016.04.090.

To each his own: no evidence of Gyrodactylid parasite host switches from invasive poeciliid fishes to *Goodea atripinnis* Jordan (Cyprinodontiformes: Goodeidae), the most dominant endemic freshwater goodeid fish in the Mexican Highlands. Rubio-Godoy M; Razo-Mendivil U; García-Vásquez A; Freeman MA; Shinn AP; & Paladini G. *Parasites & Vectors*, 9:604, 2016. DOI http://dx.doi.org/10.1186/s13071-016-1861-2

It is well established the alien fish can serve as carriers for non-indigenous parasites which can go on to infect indigenous fish with severe ecological consequences. In this paper the authors report on their study to determine whether non-indigenous poeciliid fish infected indigenous *Goodea atripinnis* with *Gyrodactylus* (flukes). Previous research had demon-

strated that some species of *Gyrodactylus* are very invasive. The authors report that there is no evidence of any *Gyrodactylus* switching host from invasive fish to *G. atripinnis*. They report that *G. atripinnis* is host to at least six different species of *Gyrodactylus* and describe one new species of *Gyrodactylus*. [*Tyrone Genade*]

Phylogenetic analyses of the subgenus *Mollienesia* (Poecilia, Poeciliidae, Teleostei) reveal taxonomic inconsistencies, cryptic biodiversity, and spatio-temporal aspects of diversification in Middle America. Palacios M; Voelker G; Rodriguez LA; Mateos M; & Tobler M. *Molecular Phylogenetics and Evolution*, 103:230–244, 2016. DOI http://dx.doi.org/10.1016/j.ympev.2016.07.025

The authors sought to gain better insight into the evolution of *Mollienesia* species. This subgenus of the genus *Poecilia* inhabits a large area from northern South America to the eastern United States. Previous phylogenies have the genera *Limia*, *Pamphorichthys*, and other minor genera nested inside the genus *Poecilia*. As consequence the phylogeny and taxonomy of this group is very complex.

The authors asked several questions. They sought to know whether the taxonomy based on morphological characters agree with molecular phylogenetic hypotheses. They also wanted to know to what extent molecular phylogenetics can identify cryptic species. The main goal was to build a robust phylogeny and determine if this, together with dated geological processes and the fossil record, can shed light on the evolution and diversification of this group.

The authors used nine gene sequences to build their phylogeny. Two were mitochondrial: cytochrome b, NADH subunit 2; and six were nuclear genes: recombination activating gene-1, rhodopsin, myosin heacy polypeptide 6, ectodermal-neural cortex 1 like protein, glycosyltransferase, SH3 and PX domain containing 3 and tyrosine kinase. Their phylogeny also showed *Limia* and several other genera nested between *Acanthophacelus* (e.g. *reticulata*, the guppy) and *Mollienesia mexicana*. Their tree, with robust sampling (41 species or populations) reveals many cryptic species among the *Mollienesia* and other groups. Using cytochrome b mutation rate as a molecular clock set the origin of the *Mollienesia* group at ≈5.8 million years; but using fossils to calibrate the tree suggests an origin date of ≈1.28 million years ago. The authors discuss this difference and, based on dated geological events believe that the 1.28 million year is more accurate than the cytochrome b date; and that cytochrome b molecular dating is probably overestimating the age of the lineage.

This is a major paper with broad ramifications for the field of ichthyology and molecular phylogenetics. The authors state that intraspecific morphological variation is often greater than interspecific morphological variation which presents taxonomists, that choose to pursue the crytpic species revealed by the phylogeny, with a perplexing puzzle. [*Tyrone Genade*]

Photo by Hristo Hristov©

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How to join Killi-Data International

Killi-Data presents top quality information on Killies or oviparous Cyprinodontiformes fishes, in a Data Base, with full ichthyological, ecological and historical coverage of each taxon with all details of systematics, morphology, genetics, patterns of each species and, with nearly 3000 color photos and maps for over 1100 valid species (male and female); as well as with all aquarium information for maintenance and breeding of each species.
Killi-Data is now nested in non-profit association "K-D-I" for services to their members (access to Data Base, Killiflash and PDFs of K-D-S journal) for a modest fee (a once-off € 10 Euro registration fee, + € 6 per year) with all revenues redistributed in grants (for researchers and collecting aquarists).
Email editor@killi-data.org for help.