

Extensive karyotype variability of African fish genus *Nothobranchius* (Cyprinodontiformes)

Eugene Krysanov¹, Tatiana Demidova¹

¹ *Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, Moscow, 119071 Russia*

Corresponding author: *Tatiana Demidova* (demidovatanya@mail.ru)

Academic editor: *Rafael Noletto* | Received 16 March 2018 | Accepted 18 August 2018 | Published 10 September 2018

<http://zoobank.org/659877B4-CE79-474C-A2E9-F6D75E8C71E3>

Citation: Krysanov E, Demidova T (2018) Extensive karyotype variability of African fish genus *Nothobranchius* (Cyprinodontiformes). *Comparative Cytogenetics* 12(3): 387–402. <https://doi.org/10.3897/CompCytogen.v12i3.25092>

Abstract

Karyotypes of 65 species of the genus *Nothobranchius* Peters, 1868 were reviewed and of those 35 examined first time. The results of present study have shown that fishes of the genus *Nothobranchius* possessed highly diverse karyotypes. The diploid chromosome number ($2n$) ranged from 16 to 50. The most frequent $2n$ was $2n = 36$ (in 35 species) while the second one $2n = 38$ (in 13 species). Proportion of banded chromosomes varied from 0 to 95% between species. Diploid chromosome number variability apparently exists as a result of chromosomal fusions or fissions and extensive karyotypic formula alterations promoting by inversions. Multiple sex chromosomes of system $X_1X_1X_2X_2/X_1X_2Y$ type were found only in karyotypes of 5 species. The extensive karyotype variability, unusual for teleosts, of genus *Nothobranchius* can be likely associated with the characteristics of its life cycle and inhabiting under unstable environment of East African savannah temporal pools.

Keywords

African killifishes, fish cytogenetics, karyotype differentiation

Introduction

More than a half of teleost fish examined had diploid chromosomes number $2n = 48-50$ (Mank and Avise 2006, Molina et al. 2014). Karyotypes containing either high or low proportions of acrocentrics tend to be more frequent than those with balanced numbers of acrocentric and metacentric chromosomes (Molina et al. 2014). According to

Naruse et al. 2004, Galetti et al. 2006 and Molina et al. 2014 the karyotype of teleost fishes is stable but intrachromosomal rearrangements such as inversions and centromere shift are common. The association of chromosome rearrangements with speciation is known, especially inversions which can promote the local adaptation due to suppression of recombination and thus accumulation of linked adaptive genes. These then favour the accumulation of genetic incompatibilities between species, reduce fertility of hybrids contributing to reproductive isolation and speciation (Navarro and Barton 2003, Kirkpatrick and Barton 2006, Noor et al. 2001, Rieseberg 2001, Hooper & Price 2017). A higher degree of karyotype variation for freshwater fish species inhabiting a more unstable environment compared to that of marine ones has been demonstrated (Nirchio et al. 2014).

Killifishes of the genus *Nothobranchius* Peters, 1868 comprise 76 valid species (Eschmeyer et al. 2018, FishBase 2018). The main life-style characteristics of killifishes reside in fact that species and their populations inhabiting in ephemeral pools of East Africa are isolated both geographically and temporarily due to extremely short life cycle (Wildekamp 2004; Reichard 2016).

Phylogenetic data based on molecular markers demonstrated that the genus *Nothobranchius* is a monophyletic assemblage and it includes four geographically separated clades (Dorn et al. 2014). Costa (2018) performed taxonomy analysis of the genus on the basis of morphology and phylogenetic data. Six subgenera were recognised: *Adiniops* Myers, 1924, *Cynobranchius* Costa, 2018, *Nothobranchius* Peters, 1868, *Paranotobranchius* Seegers, 1985, *Plesiobranchius* Costa, 2018, and *Zononotobranchius* Radda, 1969.

Karyotypes of 30 species were described earlier and high karyotype variability was revealed (summarized in Arai 2011). The diploid chromosome number ($2n$) of *Nothobranchius* species varies from 16 to 50 (Scheel 1990, Krysanov et al. 2016). Two species *N. guentheri* (Pfeffer, 1893) and *N. brieni* Poll, 1938 had multiple chromosome system (Ewulonu et al. 1985, Krysanov et al. 2016). Thus, the representatives of the genus *Nothobranchius* is a good model for studying karyotype differentiation due to high karyotype variability and features of the life cycle.

The aim of the study was to characterize karyotype diversity of the genus *Nothobranchius* and conduct cytogenetic comparison among different species. In present study, we i) reviewed all available data dealing with cytogenetic study of *Nothobranchius* species and ii) analyzed 35 other species not studied as yet for $2n$ and karyotype composition using conventional cytogenetic protocol.

Material and methods

Specimens collection

Individuals of *Nothobranchius* species were collected either from wild populations of East Africa or provided by killifish hobbyists. Geographical data and coordinates are given in supplements.

Cytogenetic analysis

Chromosomes were prepared according to the method of Kligerman and Bloom (1977). The chromosome preparations were obtained from anterior kidney tissue. Briefly, individuals were injected intraperitoneally with 0.1% colchicine solution for 3-4 hours. The hypotonization in 0.075 M KCl was 20-30 min at room temperature. Then tissue samples were fixed in 3:1 methanol: acetic acid for 24 hours.

Slides were air dried and then stained with 2% Giemsa solution in phosphate buffer a (pH 6.8) for 10 min. Chromosomes were analyzed under microscope "AxioImager" Karl Zeiss (Germany) equipped with CCD camera and "KaryoImage" Metasystems Software (Germany). Chromosome morphology was determined according to Levan et al. (1964) and classified as metacentric (m), submetacentric (sm), subtelocentric (st) and acrocentric (a). To determine the fundamental number (NF), chromosomes of the m and sm groups were considered biarmed and those of group st/a uniarmed.

Statistical analysis was done using IBM SPSS 20 package. Data were tested for normality. Regression between the rate of biarmed chromosomes and diploid chromosome number, and the Spearman correlation were calculated.

Results

Karyological data of 65 species of the genus *Nothobranchius* and two species of sister taxa *Fundulosoma* Ahl, 1924 and *Pronothobranchius* Radda, 1969 (according to Costa, 2018) are provided in Table 1 and Fig. 1.

Table 1. Diploid chromosome numbers (2n), fundamental numbers (NF) and karyotype structures of analysed species. [*sex chromosome system of $X_1X_1X_2X_2/X_1X_2Y$ type]

Species	2n	NF	Karyotype structure	Number of specimens karyotyped	References
Subgenus <i>Cynobranchius</i> Costa, 2018					
<i>N. microlepis</i> (Vinciguerra, 1897)	24	26	2m+22st/a		Scheel 1990
<i>N. fasciatus</i> Wildekamp & Haas, 1992	34	46	12msm+22st/a		Scheel 1981
Subgenus <i>Plesiobranchius</i> Costa, 2018					
<i>N. virgatus</i> Chambers, 1984	32	32	32st/a	2♀/2♂	This study
Subgenus <i>Nothobranchius</i> Peters, 1868					
<i>N. furzeri</i> Jubb, 1971	38	60	14m+8sm+16st/a	4♀/5♂	This study, Scheel 1981,1990; Reichwald et al. 2009
<i>N. kadleci</i> Reichard, 2010	38	62	16m+8sm+14st/a	3♀/5♂	This study
<i>N. krysanovi</i> Shidlovskiy, Watters & Wildekamp, 2010	18	34	8m+8sm+2st/a	3♀/5♂	This study, Shidlovskiy et al. 2010; Safronova and Krysanov 2015
<i>N. kubntae</i> (Ahl, 1926)	38	52	6m+8sm+24st/a	1♀/1♂	This study
<i>N. orthonotus</i> (Peters, 1844)	38	48	8m+2sm+28st/a	2♀/3♂	This study, Scheel 1990

Species	2n	NF	Karyotype structure	Number of specimens karyotyped	References
<i>N. pienaar</i> Shidlovskiy, Watters & Wildekamp, 2010	34	42	6m+2sm+26st/a	4♀/4♂	This study, Shidlovskiy et al. 2010
<i>N. nachovii</i> Ahl, 1926	16	30	8m+6sm+2st/a	10♀/12♂	This study, Ewulonu et al. 1985; Krysanov 1992; Shidlovskiy et al. 2010; Safronova and Krysanov 2015
Subgenus <i>Paranotothobranchius</i> Seegers, 1985					
<i>N. ocellatus</i> Seegers, 1985	30	40	2m+8sm+20st/a	2 larvae	This study
Subgenus <i>Zonotothobranchius</i> Radda, 1969					
<i>N. boklundi</i> Valdesalici, 2010	36	46	6m+4sm+26st/a	2♀/3♂	This study
<i>N. brieni</i> Poll, 1938*	50♀ 49♂	50♀ 50♂	♀ 50st/a ♂ 1m+48st/a	4♀/5♂	This study, Krysanov et al. 2016
<i>N. capriviensis</i> Watters, Wildekamp & Shidlovskiy, 2015	36	58	4m+18sm+14st/a	1♀/2♂	This study
<i>N. chochamandai</i> Nagy, 2014	36	64	18m+10sm+8st/a	5♀/7♂	This study
<i>N. flagrans</i> Nagy, 2014	36	48	10m+2sm+24st/a	3♀/4♂	This study
<i>N. hassoni</i> Valdesalici & Wildekamp, 2004	36	52	8m+8sm+20st/a	3♀/5♂	This study
<i>N. ivanovae</i> Valdesalici, 2012	36	64	22m+6sm+8st/a	3♀/3♂	This study
<i>N. kafuensis</i> Wildekamp & Rosenstock, 1989	36	66	8m+22sm+6st/a	1♀/2♂	This study, Scheel 1981, 1990
<i>N. kardashevi</i> Valdesalici, 2012	36	52	6m+10sm+20st/a	2♀/3♂	This study, Valdesalici 2015
<i>N. malaissei</i> Wildekamp, 1978	48	62	4m+10sm+34st/a	3♀/3♂	This study
<i>N. milwertzi</i> Nagy, 2014	38	54	10m+6sm+22st/a	4♀/4♂	This study
<i>N. neumanni</i> (Hilgendorf, 1905)	36	70	18m+16sm+2st/a	4♀/5♂	This study
<i>N. nubansis</i> Valdesalici, Bellemans, Kardashev & Golubtsov, 2009	36	62	14m+12sm+10st/a	3♀/4♂	This study, Valdesalici 2015
<i>N. polli</i> Wildekamp, 1978	36	60	10m+14sm+12st/a	2♀/3♂	This study
<i>N. robustus</i> Ahl, 1935	36	58	4m+18sm+14st/a	1♂	This study, Wildekamp 2004
<i>N. rosenstocki</i> Valdesalici & Wildekamp, 2005	36	62	14m+12sm+10st/a	1♀/2♂	This study
<i>N. rubroreticulatus</i> Blache & Miton, 1960	36	58	12m+10sm+14st/a	2♀/2♂	This study
<i>N. seegersi</i> Valdesalici & Kardashev, 2011	36	56	8m+12sm+16st/a	4♀/4♂	This study
<i>N. steinforti</i> Wildekamp, 1977	36	56	10m+10sm+16st/a	2♀/3♂	This study, Scheel 1981, 1990
<i>N. strelsovi</i> Valdesalici, 2016	36	48	6m+6sm+24st/a	3♀/3♂	This study
<i>N. symoensi</i> Wildekamp, 1978	36	68	20m+12sm+4st/a	2♀/3♂	This study
<i>N. taeniopygus</i> Hilgendorf, 1891	36	66	14m+16sm+6st/a	4♀/5♂	This study
<i>N. ugandensis</i> Wildekamp, 1994	36	58	8m+14sm+14st/a	3♀/3♂	This study, Wildekamp 1994, Valdesalici 2015
Subgenus <i>Adiniops</i> Myers, 1924					
<i>N. albimarginatus</i> Watters, Wildekamp & Cooper, 1998	36	38	2m+34st/a	3♀/5♂	This study
<i>N. annectens</i> Watters, Wildekamp & Cooper, 1998	28	36	8m+20st/a	5♀/7♂	This study
<i>N. cardinalis</i> Watters, Cooper & Wildekamp, 2008	36	38	2m+34st/a	8♀/12♂	This study
<i>N. eggersi</i> Seegers, 1982	36	40	4m+32st/a	5♀/6♂	This study, Scheel 1990
<i>N. elongatus</i> Wildekamp, 1982	38	48	8m+2sm+28st/a	1♀/2♂	This study, Wildekamp 1982, Scheel 1990

Species	2n	NF	Karyotype structure	Number of specimens karyotyped	References
<i>N. flammicomantis</i> Wildekamp, Watters & Sainthouse, 1998	20	38	18m+2st/a	5♀/8♂	This study
<i>N. foerschi</i> Wildekamp & Berkenkamp, 1979	34	46	10m+2sm+22st/a	3♀/5♂	This study, Scheel 1981, 1990; Ewulonu et al. 1985
<i>N. fuscotaeniatus</i> Seegers, 1997	38	40	2sm+36st/a	3♀/6♂	This study
<i>N. geminus</i> Wildekamp, Watters & Sainthouse, 2002	38	40	2sm+36st/a	2♀/3♂	This study
<i>N. guentheri</i> (Pfeffer, 1893) *	36♀ 35♂	40♀ 39♂	♀2m+2sm+32st/a ♂2m+2sm+31st/a	5♀/7♂	This study, Scheel 1990, Ewulonu et al. 1985
<i>N. hengstleri</i> Valdesalici, 2007	38	42	2m+2sm+34st/a	3♀/5♂	This study, Wildekamp et al. 2009
<i>N. interruptus</i> Wildekamp & Berkenkamp, 1979	36	50	8m+6sm+22st/a	2♀/3♂	This study
<i>N. janpapi</i> Wildekamp, 1977*	38♀ 37♂	48♀ 49♂	♀2m+8sm+28st/a ♂3m+9sm+25st/a	5♀/7♂	This study, Scheel 1990
<i>N. jubbi</i> Wildekamp & Berkenkamp, 1979	34	46	4m+8sm+22st/a	2♀/3♂	This study, Scheel 1981, 1990; Wildekamp 1982, Wildekamp et al. 1986
<i>N. kilomberoensis</i> Wildekamp, Watters & Sainthouse, 2002	32	46	8m+6sm+18st/a	2♀/4♂	This study
<i>N. kirki</i> Jubb, 1969	36	50	2m+12sm+22st/a	1♀/2♂	This study, Scheel 1981, 1990
<i>N. korthausae</i> Meinken, 1973	36	40	4m+32st/a	3♀/5♂	This study, Scheel 1981, 1990
<i>N. lowrensi</i> Wildekamp, 1977*	28♀ 27♂	34♀ 34♂	♀6m+22st/a ♂7m+20st/a	2♀/3♂	This study
<i>N. lucius</i> Shidlovskiy, Watters & Wildekamp, 2010	36	58	6m+16sm+14st/a	2♀/3♂	This study, Wildekamp et al. 2009
<i>N. luekei</i> Seegers, 1984	38	40	2m+36st/a	2♀/2♂	This study
<i>N. makondorum</i> Shidlovskiy, Watters & Wildekamp, 2010	36	50	6m+8sm+22st/a	3♀/4♂	This study, Wildekamp et al. 2009
<i>N. melanospilus</i> (Pfeffer, 1896)	38	50	4m+8sm+26st/a	3♀/4♂	This study, Scheel 1981, 1990; Wildekamp et al. 2009
<i>N. palmqvisti</i> (Lönnerberg, 1907)	36	42	6m+30st/a	2♀/2♂	This study, Ewulonu et al. 1985
<i>N. patrizii</i> (Vinciguerra, 1897)	36	52	4m+12sm+20st/a	2♀/2♂	This study, Ewulonu et al. 1985
<i>N. rubripinnis</i> Seegers, 1986	36	38	2m+34st/a	2♀/2♂	This study
<i>N. ruudwildekampi</i> Costa, 2009	36	38	2m+34st/a	3♀/4♂	This study
<i>N. vosseleri</i> Ahl, 1924	38	60	6m+16sm+16st/a	2♀/3♂	This study
<i>N. wattersi</i> Ng'oma, Valdesalici, Reichwald & Cellerino, 2013	36	40	4m+32st/a	2♀/2♂	This study, Scheel 1990
Unrecognized species					
<i>N. ditte</i> Nagy, 2018*	40♀ 39♂	♀64 ♂64	♀12m+12sm+16st/a ♂13m+12sm+14st/a	3♀/4♂	This study
<i>N. torgashevi</i> Valdesalici, 2015	36	46	6m+4sm+26st/a	3♀/4♂	This study, Valdesalici 2015
<i>N. usanguensis</i> Wildekamp, Watters & Shidlovskiy, 2014	36	54	6m+12sm+18st/a	1♀/2♂	This study
Genus <i>Fundulosoma</i> Ahl, 1924					
<i>Fundulosoma thieryi</i> (Ahl, 1924) *	44♀ 43♂	46♀ 45♂	♀2m+42st/a ♂1m+1sm+41st/a	2♀/4♂	This study
Genus <i>Pronothobranchius</i> Radda, 1969					
<i>Pronothobranchius kiyawensis</i> Ahl, 1928	28	30	2m+26st/a	2♂	This study



Figure 1. Karyotypes of species *Nothobranchius*. Scale bar: 10 μ.

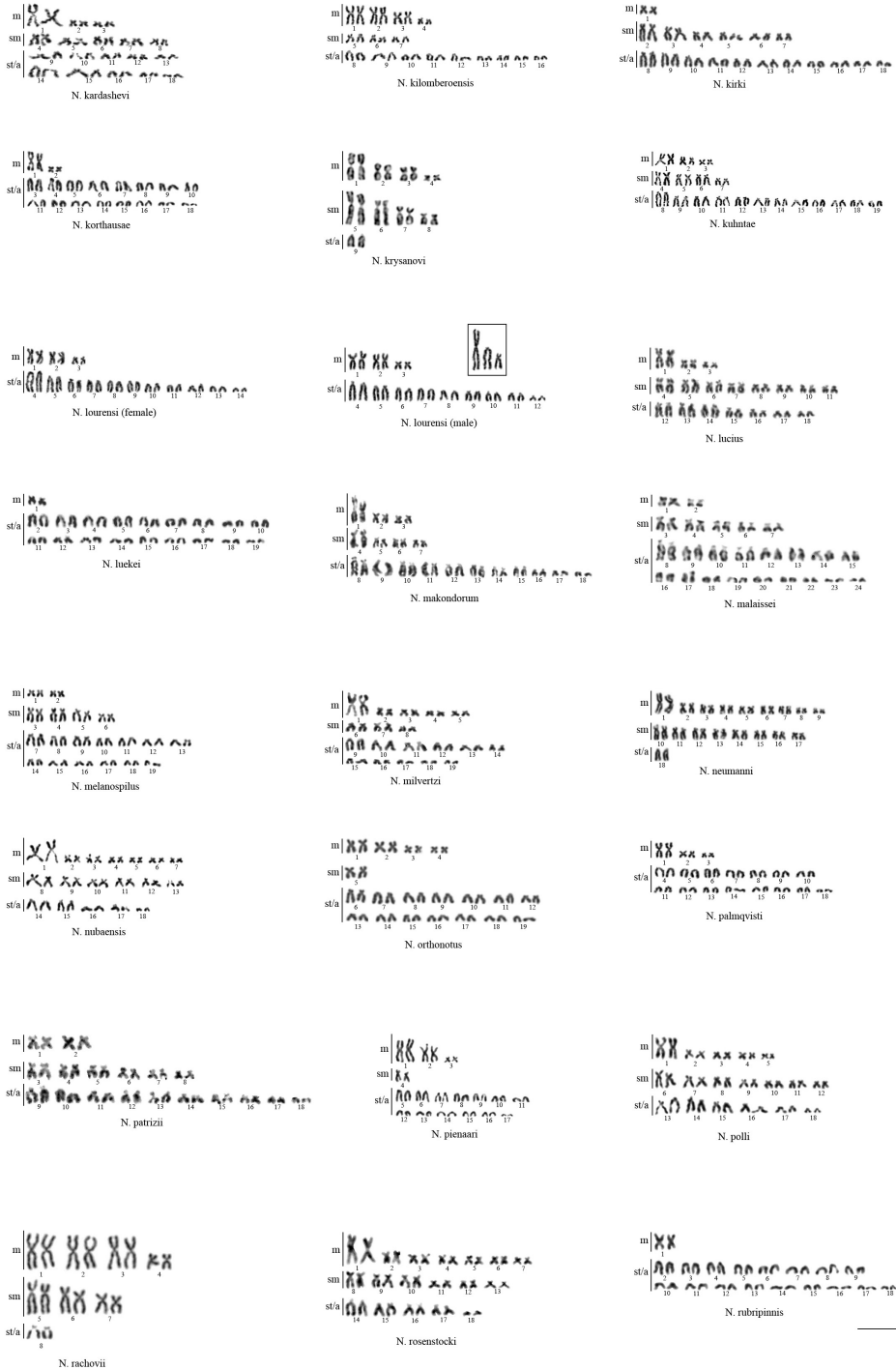


Figure 1. Continued. Karyotypes of species *Nothobranchius*. Scale bar: 10 μ .

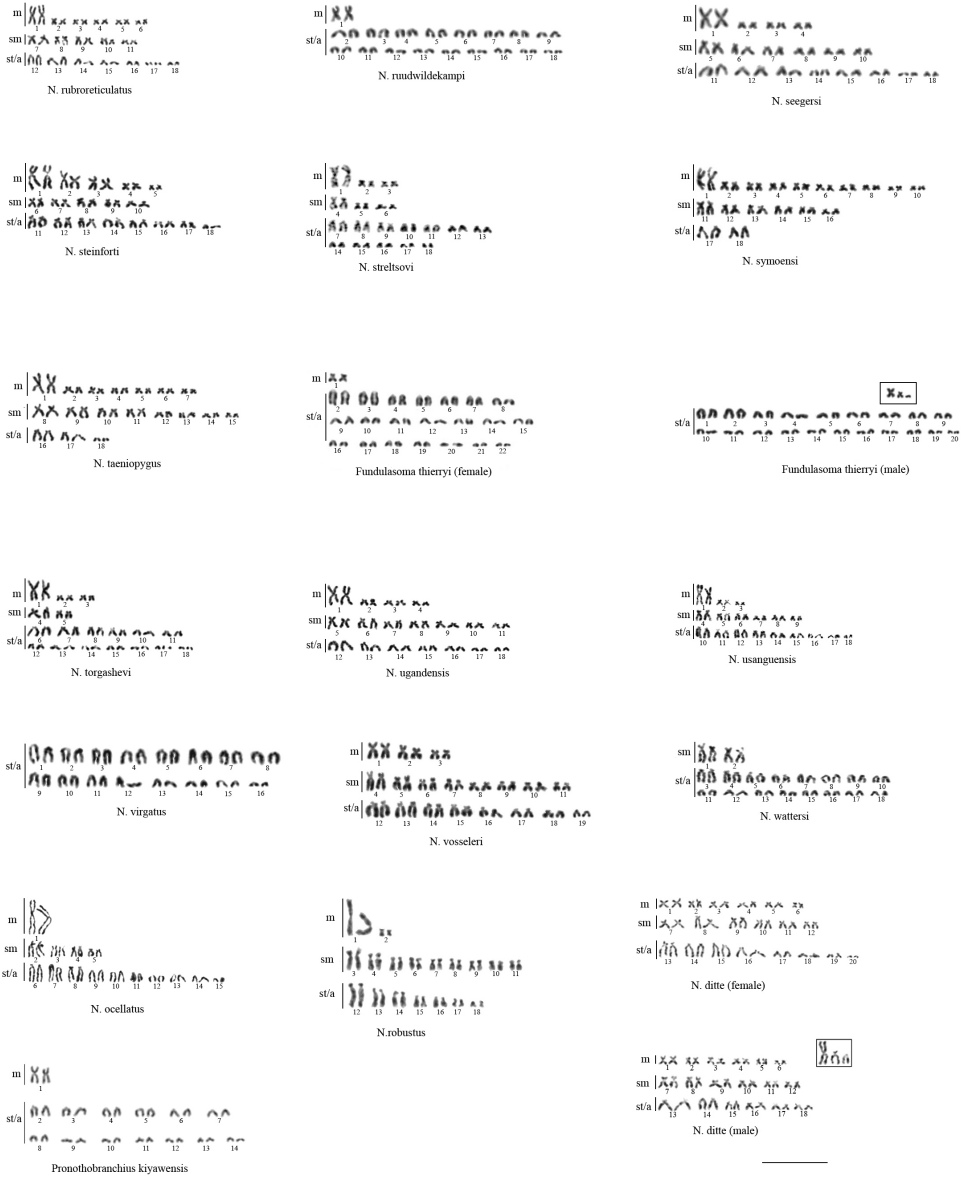


Figure 1. Continued. Karyotypes of species *Nothobranchius*. Scale bar: 10 μ .

As evident, the number and morphology of chromosomes varied widely between karyotypes of analyzed species $2n$ ranged from 16 to 50 where the most frequent was $2n = 36$ and second $2n = 38$ (Fig. 2).

Our data showed that the proportion of biarmed chromosomes in the karyotype of the species varied widely from 0 to 95%. Regression between the rate of biarmed chromosomes and $2n$ was $y = -1.607x + 96.863$, $R^2 = 0.29$ and the Spearman correlation was $R_s = -0.181$ (Fig. 3).

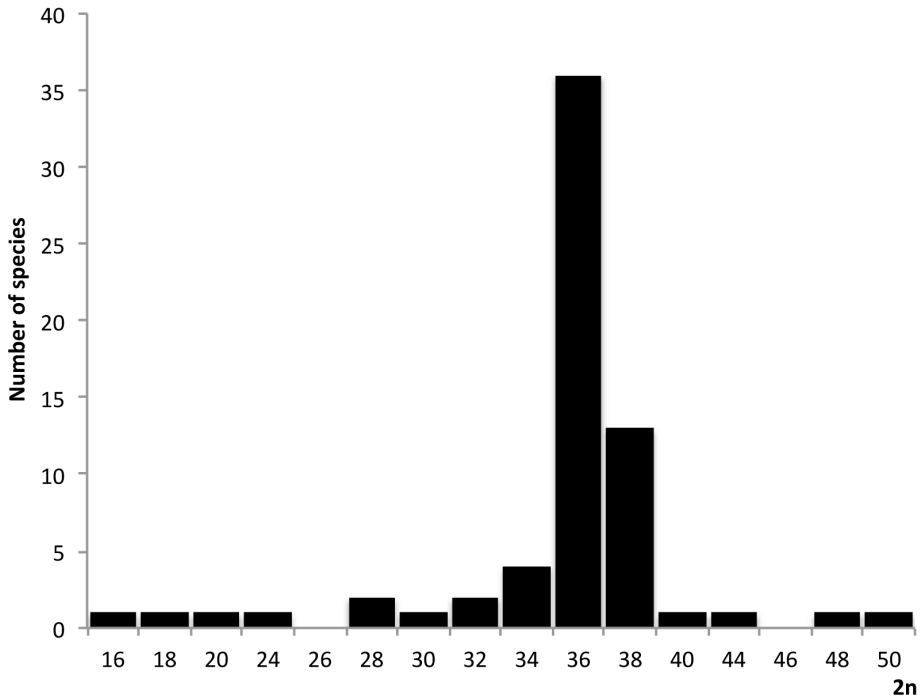


Figure 2. Histogram of the distribution of the diploid chromosome number ($2n$) in the genus *Nothobranchius*.

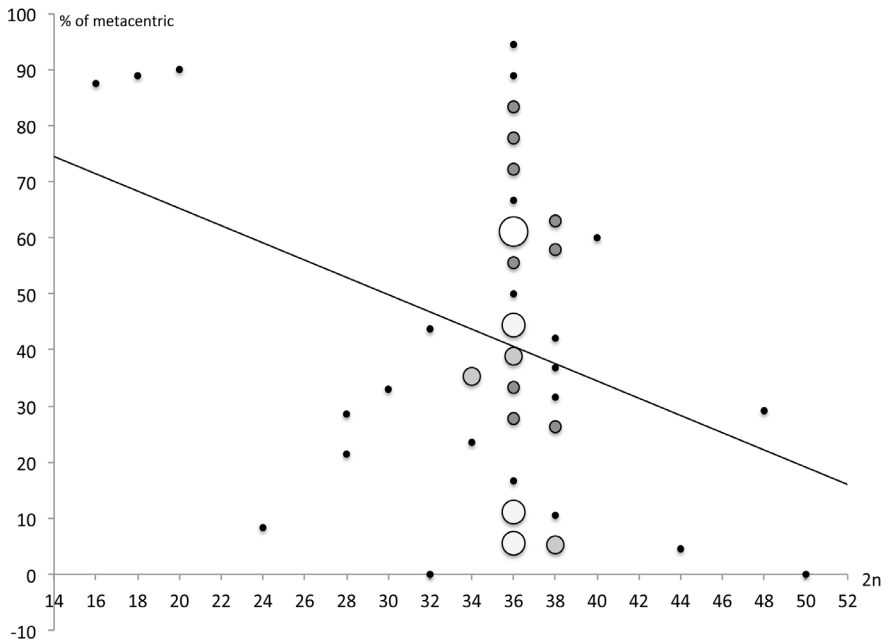


Figure 3. Scatter-plot of a diploid chromosome number ($2n$) and proportion of metacentric chromosomes with overall regression line. The diameter and color of circle indicate number of species from 1 to 5.

Subgenus *Cynobranchius*

Karyotypes of two species belonging to this subgenus were described by Scheel (1981, 1990). The karyotype of *N. microlepis* had the $2n = 24$ and most chromosomes in the karyotype were unarmed with only one pair of biarmed chromosomes (NF = 26). *N. fasciatus* had $2n = 34$ with 22 unarmed and 12 biarmed chromosomes.

Subgenus *Plesiobranchius*

The only species in the subgenus *N. virgatus* has $2n = 32$ unarmed chromosomes (NF = 32).

Subgenus *Nothobranchius*

Four species *N. furzeri*, *N. kadleci*, *N. orthonotus* and *N. kuhntae* possessed the $2n = 38$. Biarmed elements dominated in karyotypes of *N. kadleci* (NF = 62) and *N. furzeri* (NF = 60), and unarmed chromosomes dominated in karyotypes of *N. kuhntae* (NF = 52) and *N. orthonotus* (NF = 48).

The karyotype of *N. pienaari* had $2n = 24$ and most of chromosomes were unarmed (NF = 42).

The lowest $2n$ was found in two closely related species *N. rachovii* ($2n = 16$, NF = 30) and *N. krysanovi* ($2n = 18$, NF = 34). Most of chromosomes in their karyotypes were metacentric elements with only one pair of acrocentric chromosomes as described earlier (Scheel 1990, Shidlovskiy et al. 2010). Both species had similar karyotype structure and were distinguished by one additional pair of metacentric chromosomes in *N. krysanovi*.

Subgenus *Paranothobranchius*

The only species in the subgenus *N. ocellatus* has $2n = 30$ and unarmed chromosomes dominated in the karyotype (NF = 40).

Subgenus *Zonothobranchius*

There are species in the subgenus possessing $2n$ higher than 38. The highest $2n = 49/50$ among studied species was discovered in *N. brienii* (Krysanov et al. 2016) where all autosomes in the karyotype were acrocentric (NF = 50). *N. brienii* had karyotype with differentiated heteromorphic sex chromosomes $X_1X_1X_2X_2/X_1X_2Y$ type (Krysanov

et al. 2016). The karyotype of *N. malaissei* had diploid numbers $2n = 48$ and uniarmed chromosomes dominated in the karyotype (NF = 62).

N. milvertzi had the $2n = 38$ with karyotype formulae $10m+6sm+22st/a$ (NF = 54).

The rest species in subgenus had diploid chromosome numbers $2n = 36$ (see table 1). The ratio of uniarmed and biarmed chromosomes differed among species. The most uniarmed chromosomes number was found for *N. boklundi* (NF = 46) which had 26 uniarmed and 10 biarmed chromosomes ($6m+4sm+26st/a$) and the least uniarmed chromosomes number was found for *N. neumanni* (NF = 70) with only two uniarmed and 34 biarmed chromosomes ($18m+16sm+2st/a$). Other species had karyotypes with uniformly decreasing numbers of uniarmed chromosomes from 24 to 4 and numbers of biarmed chromosomes increased correspondingly.

Subgenus *Adiniops*

Eight species had the $2n = 38$ with different ratio of uniarmed and biarmed chromosomes. Karyotypes of three species *N. fuscotaeniatus*, *N. geminus* and *N. luekei* possessed 36 uniarmed and only two biarmed chromosomes (NF = 40) while *N. vosseleri* (NF = 60) karyotype had only 16 uniarmed and 22 biarmed chromosomes. Other species had karyotypes with uniformly decreasing numbers of uniarmed chromosomes from 34 to 26 and numbers of biarmed chromosomes increased correspondingly. Females of *N. janpapi* had more chromosome than males $2n = 38/37$ and multiple sex chromosome system $X_1X_1X_2X_2/X_1X_2Y$ type was revealed.

The modal diploid chromosome number $2n = 36$ was found for 14 species. Four sister species *N. albimarginatus*, *N. cardinalis*, *N. rubripinnis* and *N. ruudwildekampi* had similar karyotypes with 34 uniarmed and only 2 biarmed chromosomes (NF = 38). Karyotypes of three species *N. eggersi*, *N. korthausae*, and *N. wattersi* possessed 32 uniarmed and 4 biarmed chromosomes (NF = 40). Females of *N. guentheri* had more chromosome than males $2n = 36/35$ and multiple sex chromosome system $X_1X_1X_2X_2/X_1X_2Y$ type was revealed.

Karyotypes of other species had uniformly decreasing numbers of uniarmed chromosomes from 30 to 14 and numbers of biarmed chromosomes increased correspondingly.

Two species *N. foerschi* and *N. jubbi* had the $2n = 34$ with 22 uniarmed and 12 biarmed chromosomes (NF = 46).

Only one species *N. kilomberoensis* possessed the $2n = 32$ with karyotype formulae $8m+6sm+18st/a$ and NF = 46.

In karyotypes of two species *N. annectens* ($2n = 28$, NF = 36) and *N. lourensi* ($2n = 27/28$, NF = 34) uniarmed chromosomes dominated over biarmed ones. *N. lourensi* possessed multiple sex chromosome system $X_1X_1X_2X_2/X_1X_2Y$ type.

N. flammicomantis possessed the lowest diploid numbers in the subgenus $2n = 20$. The karyotype of *N. flammicomantis* consisted mainly of biarmed chromosomes with one pair of uniarmed chromosomes (NF = 38).

Discussion

Karyotype characteristics of representatives of the genus *Nothobranchius*

Karyotypes of 65 species of the genus *Nothobranchius* were overviewed and those of 35 species reported here for first time.

The results of present work have shown that representatives of the genus *Nothobranchius* possess a highly diverse karyotype. The $2n$ ranged from 16 to 50. The most frequent was $2n = 36$ (35 species) and the second was $2n = 38$ (13 species). similar karyotype diversity was found only for one closely related genus *Aphyosemion* Myers, 1924 among the family Cyprinodontiformes (Völker et al. 2008).

It has been shown that karyotypes of teleost fish consisted mainly of uniarmed or biarmed chromosomes (Molina et al. 2014). We did not find a similar trend in karyotype structure within the genus *Nothobranchius*. Fully acrocentric or metacentric karyotypes occurred as frequently as intermediate type. Such a high diversity of $2n$ and karyotype structure could be the result of many inter- and intrachromosomal rearrangements.

Scheel (1990) assumed that the karyotype evolution of the Old World Cyprinodontidae proceeded by decreasing the $2n$ while increasing the proportion of biarmed chromosomes in the karyotype by means of centric fusions. The correlation between the proportion of biarmed chromosomes and $2n$ was non-significant for the representatives of the genus *Nothobranchius* in contrast to those of the genus *Aphyosemion* (Agnès et al. 2006) since pericentric inversions played essential role in the chromosome evolution of the genus.

Sex chromosomes

Most of the studied species did not display morphologically distinguished sex chromosomes. Sex chromosomes were found only in six species, namely *N. guentheri* (Ewulonu et al. 1985), *N. brieni* (Krysanov et al. 2016), *N. lourensi*, *N. janpapi*, *N. ditte* and *F. thierryi* (this study) where multiple sex chromosome system of $X_1X_1X_2X_2/X_1X_2Y$ type was found. Neo-Y chromosome likely originated through Robertsonian fusion of the original Y chromosome and autosome as was shown for another fish species (Kitano and Peichel 2012). *Nothobranchius* species with multiple sex chromosomes were found in two subgenera *Zononothobranchius* (*N. brieni*) and *Adiniops* (*N. guentheri*, *N. lourensi* and *N. janpapi*) (Costa 2018). According to molecular data *N. guentheri* and *N. janpapi* are not closely related (Dorn et al. 2014). We suppose that multiple sex chromosomes originated in these species independently.

Chromosome evolution of *Nothobranchius* subgenera

Subgenera *Cynobranchius* and *Plesiobranchius* form basal Northern phylogenetic clade (sensu Dorn et al., 2014). It is noteworthy that the species with the most dis-

tinctive $2n$ and karyotype structures, namely *N. virgatus* and *N. microlepis* belonged to the basal clade.

Subgenus *Nothobranchius* corresponds well with the Southern clade (sensu Dorn et al 2014). Karyotype alterations by pericentric inversions were main trends in the karyotype evolution of species with $2n = 38$. Four species *N. furzeri*, *N. kadleci*, *N. orthonotus* and *N. kuhntae* distinguished from each other by the ratio of uniarmed and biarmed chromosomes.

Reductions of diploid chromosomes number by fusions were probably characteristic of species with $2n$ lower 38. Biarmed chromosomes dominated in the karyotypes of species (*N. rachovii* and *N. krysanovi*) with the lowest diploid numbers (16 and 18) in the genus.

Only the species *N. ocellatus* from the subgenus *Paranothobranchius* with a distinctive karyotype structure is included in the Southern clade.

Subgenus *Zononothobranchius* corresponds well with the Inland clade (sensu Dorn et al 2014). Karyotypes of all species except *N. malaissei*, *N. brieni* and *N. milvertzi* have $2n = 36$ and ratio of biarmed and uniarmed chromosomes differs among species. The karyotype evolution of the species with the $2n = 36$ probably proceeded mainly by pericentric inversions.

Two species *N. malaissei* ($2n=48$), *N. brieni* ($2n=49/50$) had the highest diploid chromosome numbers among all species of the genus and high percent of uniarmed chromosomes.

Therefore, karyotype evolution of the subgenus proceeded mainly by pericentric inversions or rare chromosome fusions (or fissions).

Subgenus *Adiniops* corresponds well with the Coastal clade (sensu Dorn et al 2014). Most species of the subgenus have diploid chromosomes number 36 or 38. And four species have diploid number lower than 36. Karyotype diversity is a result of chromosome fusions, fissions and pericentric inversions. Moreover, three species *N. guentheri*, *N. janpapi* and *N. lourensi* have multiple sex chromosome system.

Thus, two main trends were revealed in chromosome evolution of the genus: chromosome fusions (or rare fissions) and pericentric inversions.

Conclusions

According to our data species of the genus *Nothobranchius* possess high variability of karyotype structure and diploid chromosome numbers. Such variability exists as a result of chromosome fusions or fissions and pericentric inversion, which is especially characteristic for the species with $2n$ equal 36 and 38. Centromere fusion apparently took place in formation of karyotypes with reduced $2n$ (less than 36).

In our opinion, variability of *Nothobranchius* karyotypes is associated with the characteristics of its life cycle and inhabiting in ephemeral partly isolated pools of East African savannah. Karyotype flexibility of *Nothobranchius* individuals may play adaptive role for survival under unstable conditions.

Acknowledgements

This work was partially supported by research grants of Russian foundation for Basic Research № 16-04-01102 and № 17-04-01899. We are grateful to A. Nikiforov, S. Streltsov, S. Torgashov, B. Nagy and R. Wildekamp for collecting, keeping and providing fishes. We also thank Petr Rab, Viktor Vasiliev and other reviewers for providing feedback that greatly improved the quality of the manuscript.

References

- Agnèse JE, Zentz F, Legros O, Sellos D (2006) Phylogenetic relationships and phylogeography of the Killifish species of the subgenus *Chromaphyosemion* (Radda, 1971) in West Africa, inferred from mitochondrial DNA sequences. *Molecular Phylogenetics and Evolution* 40(2): 332–346. <https://doi.org/10.1016/j.ympev.2006.03.018>
- Arai R (2011) Fish karyotypes – a Check List. Springer, 340 pp. <https://doi.org/10.1007/978-4-431-53877-6>
- Costa WJEM (2018) Comparative morphology, phylogeny and classification of African seasonal killifishes of the tribe Nothobranchiini (Cyprinodontiformes: Aplocheilidae). *Zoological Journal of the Linnean Society* 184(1): 115–135. <https://doi.org/10.1093/zoolinnean/zlx102>
- Dorn A, Musilova Z, Platzer M, Reichwald K, Cellerino A (2014) The strange case of East African annual fish: aridification correlates with diversification for a savannah aquatic group? *BMC Evolutionary Biology* 14: 210. <https://doi.org/10.1186/s12862-014-0210-3>
- Eschmeyer WN, Fricke R, van der Laan R [Eds] (2018) Catalog of Fishes: Genera, Species, References. <http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp> [accessed 27.08.2018; version edited by Bill Eschmeyer]
- EWulonu UV, Haas R, Turner BJ (1985) A multiple sex chromosome system in the annual killifish, *Nothobranchius guentheri*. *Copeia* 2: 503–508. <https://doi.org/10.2307/1444868>
- FishBase (2018) FishBase. <http://www.fishbase.org> [accessed 27.08.2018]
- Galetti Jr PM, Molina WF, Affonso PR, Aguilar CT (2006) Assessing genetic diversity of Brazilian reef fishes by chromosomal and DNA markers. *Genetica* 126(1–2): 161–177. <https://doi.org/10.1007/s10709-005-1446-z>
- Hooper DM, Price TD (2017) Chromosomal inversion differences correlate with range overlap in passerine birds. *Nature Ecology and Evolution* 1: 1526–1534. <https://doi.org/10.1038/s41559-017-0284-6>
- Kirkpatrick M, Barton N (2006) Chromosome inversions, local adaptation and speciation. *Genetics* 173(1): 419–434. <https://doi.org/10.1534/genetics.105.047985>
- Kitano J, Peichel C (2012) Turnover of sex chromosomes and speciation in fishes. *Environmental Biology of Fishes* 94: 549–558. <https://doi.org/10.1007/s10641-011-9853-8>
- Kligerman AD, Bloom SE (1977) Rapid chromosome preparations from solid tissues of fishes. *Journal of the Fisheries Research Board of Canada* 34(2): 266–269. <https://doi.org/10.1139/f77-039>

- Krysanov E, Demidova T, Nagy B (2016) Divergent karyotypes of the annual killifish genus *Nothobranchius* (Cyprinodontiformes, Nothobranchiidae). *Comparative Cytogenetics* 10(3): 439–445. <https://doi.org/10.3897/CompCytogen.v10i3.9863>
- Krysanov EY (1992) Aneuploidy in postnatal ontogenesis of fishes. *Acta Zoologica Fennica* 191: 177–182.
- Levan A, Fredga K, Sandberg A (1964) Nomenclature for centromeric position on chromosomes. *Hereditas* 52: 201–220. <https://doi.org/10.1111/j.1601-5223.1964.tb01953.x>
- Mank J, Avise J (2006) Phylogenetic conservation of chromosome numbers in Actinopterygian fishes. *Genetica* 127(1–3): 321–327. <https://doi.org/10.1007/s10709-005-5248-0>
- Molina WF, Martinez PA, Bertollo LAC, Bidau CJ (2014) Evidence for meiotic drive as an explanation for karyotype changes in fishes. *Marine Genomics* 15(Supplement C): 29–34. <https://doi.org/10.1016/j.margen.2014.05.001>
- Nagy B (2018) *Nothobranchius ditte*, a new species of annual killifish from the Lake Mweru basin in the Democratic Republic of the Congo (Teleostei: Nothobranchiidae). *Ichthyological Exploration of Freshwaters* 28 (2): 115–134.
- Naruse K, Tanaka M, Mita K, Shima A, Postlethwait J, Mitani H (2004) A Medaka gene map: the trace of ancestral vertebrate proto-chromosomes revealed by comparative gene mapping. *Genome Research* 14(5): 820–828. <https://doi.org/10.1101/gr.2004004>
- Navarro A, Barton NH (2003) Chromosomal speciation and molecular divergence – accelerated evolution in rearranged chromosomes. *Science* 300(5617): 321–323. <https://doi.org/10.1126/science.1080600>
- Nirchio M, Rossi AR, Foresti F, Oliveira C (2014) Chromosome evolution in fishes: a new challenging proposal from Neotropical species. *Neotropical Ichthyology* 12(4): 761–770. <https://doi.org/10.1590/1982-0224-20130008>
- Noor MAF, Grams KL, Bertucci LA, Reiland J (2001) Chromosomal inversions and the reproductive isolation of species. *Proceedings of the National Academy of Sciences* 98(21): 12084–12088. <https://doi.org/10.1073/pnas.221274498>
- Reichard M (2016) The evolutionary ecology of african annual fishes. In: Berios N, Garcia G, De Sa RO (Eds) *Annual Fishes Life History Strategy, Diversity, and Evolution*. 133–158.
- Reichwald K, Lauber C, Nanda I, Kirschner J, Hartmann N, Schories S, Gausmann U, Taudien S, Schilhabel MB, Szafransky K, Glöckner G, Schmid M, Cellerino A, Schartl M, Englert C, Platzer M (2009) High tandem repeat content in the genome of the short-lived annual fish *Nothobranchius furzeri*: a new vertebrate model for aging research. *Genome Biology* 10(2): 1–17. <https://doi.org/10.1186/gb-2009-10-2-r16>
- Rieseberg LH (2001) Chromosomal rearrangements and speciation. *Trends in Ecology & Evolution* 16(7): 351–358. [https://doi.org/10.1016/s0169-5347\(01\)02187-5](https://doi.org/10.1016/s0169-5347(01)02187-5)
- Safronova LD, Krysanov EY (2015) Synaptonemal complex of two species of the genus *Nothobranchius* (Cyprinodontidae). *Genetika* 51(10): 1203–1206. <https://doi.org/10.1134/S1022>
- Scheel JJ (1981) Notes of certain groups of killifish-1. *Killi-News* 185: 55–58.
- Scheel JJ (1990) *Atlas of killifishes of the Old World*. TFH Publications, Neptune, 448 pp.

- Shidlovskiy KM, Watters BR, Wildekamp RH (2010) Notes on the annual killifish species *Nothobranchius rachovii* (Cyprinodontiformes; Nothobranchiidae) with the description of two new species. *Zootaxa* 2724: 37–57. <https://doi.org/10.5281/zenodo.199938>
- Valdesalici S (2015) *Nothobranchius torgasbevi* (Cyprinodontiformes: Nothobranchiidae) a new species of annual killifish with two male colour morphs from the Lake Eyasi Basin, Central Tanzania. *Killi-Data Series* 2015: 15–30.
- Völker M, Ráb P, Kullmann H (2008) Karyotype differentiation in *Chromaphyosemion* killifishes (Cyprinodontiformes, Nothobranchiidae): patterns, mechanisms and evolutionary implications. *Biological Journal of the Linnean Society* 94: 143–153. <https://doi.org/10.1111/j.1095-8312.2008.00967.x>
- Wildekamp RH (1982) Die *Nothobranchius*-Arten des Küstengebietes Kenias. *Deutsche Aquarien- und Terrarien-Zeitschrift* 35 (9): 333–339.
- Wildekamp RH, Romand R, Scheel JJ (1986) Cyprinodontidae. In: Daget J, Gosse J-P, Thys van den Audenaerde DFE (Eds) *Check-List of the Freshwater Fishes of Africa (CLOFFA)*. Vol. 2. ISNB, Brussels, MRAC, Tervuren and ORSTOM, Paris., 165–276.
- Wildekamp RH (1994) The *Nothobranchius* species from Uganda, with description of a new polymorphic species (Cyprinodontiformes: Aplocheilidae). *Ichthyological Exploration of Freshwaters*. 5(3): 193–206.
- Wildekamp RH (2004) *A World of Killies – Atlas of the Oviparous Cyprinodontiform Fishes of the World (Vol. 4)*. The American Killifish Association, Elyria, 398 pp.
- Wildekamp RH, Shidlovskiy KM, Watters BR (2009) Systematics of the *Nothobranchius melanospilus* species group (Cyprinodontiformes: Nothobranchiidae) with description of two new species from Tanzania and Mosambique. *Ichthyology Exploration Freshwaters* 20(3): 237–254.