CompCytogen 12(3): 267–284 (2018)
doi: 10.3897/CompCytogen.v12i3.25832
http://compcytogen.pensoft.net

RESEARCH ARTICLE

COMPARATIVE Cytogenetics
A peer-reviewed open-access journal
International Journal of Plant & Animal Cytogenetics, Karyosystematics, and Molecular Systematics

Karyotype characteristics and chromosomal polymorphism of *Chironomus* "annularius" sensu Strenzke (1959) (Diptera, Chironomidae) from the Caucasus region

Mukhamed Kh. Karmokov[1]

1 *Tembotov Institute of Ecology of Mountain territories RAS, I. Armand str., 37a, Nalchik 360051, Russia*

Corresponding author: *Mukhamed Kh. Karmokov* (lacedemon@rambler.ru)

Academic editor: *V. Golygina* | Received 14 April 2018 | Accepted 27 June 2018 | Published 30 July 2018

http://zoobank.org/E0798FE0-109A-4EC2-A987-4895D7038A8A

Citation: Karmokov MKh (2018) Karyotype characteristics and chromosomal polymorphism of *Chironomus* "annularius" sensu Strenzke (1959) (Diptera, Chironomidae) from the Caucasus region. Comparative Cytogenetics 12(3): 267–284. https://doi.org/10.3897/CompCytogen.v12i3.25832

Abstract

The study presents data on the karyotype characteristics and features of chromosomal polymorphism of *Chironomus* "annularius" sensu Strenzke (1959) (Diptera, Chironomidae) from three populations of the Caucasus region (South and Central Caucasus, and Eastern Ciscaucasia). We found 17 banding sequences in the Caucasian populations. We observed inversion polymorphism in almost all chromosome arms except for arm G. The genetic distances between all the studied populations of *Ch.* "annularius" were calculated using Nei criteria (1972). In spite of relative geographic proximity, the genetic distances between populations of the Caucasus are quite large, and they do not form a single cluster of Caucasian populations. The population of the South Caucasus goes to the European cluster, the population of the Central Caucasus goes to the Asian cluster and the population of Eastern Ciscaucasia does not belong to any of the outlined clusters. Principal component analysis (PCA) shows a similar picture. Two of the Caucasian populations do not follow Hardy-Weinberg expectation, there being a marked deficiency of heterozygotes in arms A, B and C, arguably, due to negative selection of heterozygotes or founder effect. All the obtained data are indicative of the complex genetic structure of Caucasian populations of *Ch.* "annularius" and total complexity microevolution processes occurring in the Caucasus region.

Keywords

Diptera, Chironomidae, *Chironomus annularius*, polytene chromosomes, chromosome polymorphism, Central Caucasus, South Caucasus, Eastern Ciscaucasia

Introduction

There are a great number of publications that mention the name of *Chironomus annularius* from the 18th century (Spies and Sæther 2004). According to Spies and Sæther there are several different species under this name and revision of the species described under the *Ch. annularius* name is necessary. The most complete descriptions of *Ch. annularius* morphology and karyotype were presented by Strenzke (1959), Keyl and Keyl (1959), Keyl (1962). For this reason, Spies and Sæther (2004) suggest using the name *Ch.* "annularius" sensu Strenzke (1959) until revision completion. According to the Fauna Europaea web source (http://www.faunaeur.org) the species is common in Western (British Isles, Norway, Sweden, Finland, French mainland, Germany, Spanish mainland, Italian mainland and so on) and Eastern Europe (Poland, Romania, Bulgaria, Ukraine and so on). Also, according to Kiknadze et al. (2016), the species is known from European Russia, the Ural, Western Siberia, the Republics of Altai, Tuva, and Sakha (Yakutia), Kazakhstan, the USA (several sites) and Canada (Alberta, Amisk Lake).

Keyl & Keyl (1959) described the karyotype of *Ch.* "annularius" sensu Strenzke (1959) from German populations. At first, Keyl (1962) and Kiknadze et al. (1991a) mapped chromosome arms A, E and F. Later, Kiknadze et al. (1996c, 2012) mapped arms C and D. Belyanina (1981), Petrova and Michailova (1986) presented some information on karyotype and chromosomal polymorphism of Palearctic *Ch.* "annularius" populations using an arbitrary system of chromosome mapping or without any mapping (Michailova 1989). The karyotype and chromosomal polymorphism of *Ch.* "annularius" from Nearctic populations were studied relatively later than that from Palearctic populations (Butler et al. 1995, Andreeva 1999, Kiknadze et al. 2008b, 2010, 2012).

Karmokov (2012) previously briefly described the karyotype and chromosomal polymorphism of *Ch.* "annularius" from one Central Caucasian population.

The aim of the work was to present the description of karyotype characteristics and chromosomal polymorphism of *Ch.* "annularius" from three Caucasian populations. In addition, it was also very important to compare the chromosomal polymorphism characteristics of *Ch.* "annularius" from the Caucasus with earlier studies.

Methods

We used fourth instar larvae of *Chironomus* in the karyological study. We provide the collection sites and abbreviations of earlier studied populations (Kiknadze et al. 2012) in Table 1. The Caucasus region served as larval collection sites and included one site from Republic of North-Ossetia-Alania (Russian Federation), one site from the Republic of Dagestan (Russian Federation) and one site from the Republic of Georgia (Table 2). Collection sites are marked on the map with dark dots (Fig. 1). The geographic division of the Caucasus follows Gvozdetskii (1963). The area to the west of Mount Elbrus considered as the West Caucasus. The area between Mount Elbrus and Mount Kazbek

Table 1. Collection sites and number of analyzed *Ch.* "annularius" larvae from the European, Siberian, Kazakhstan and Nearctic populations per Kiknadze et al. (2012).

Localities	Population abbreviation	Collection sites	Collection date	Number of specimens
European population	NL-NT-NT	The Netherlands	07.1998	16
Siberian populations	RU-OMS-IR	Omskaya Oblast': former riverbed or river Irtysh near Omsk	08.1996	39
	RU-NSK-EP	Reservoir near river Nizhnyaya Eltsovka	07.2006	26
	RU-NSK-BE	Pond in Berdsk	06.1998	52
Kazakhstan population	KZ-SIP-UB	Alma Ata, pond in the Botanical garden	09.1989	17
Nearctic populations	US-ND-WA	USA, Warsing Dam	09.05.96	16
	US-ND-IS	USA, Isabel Lake	02.1995	33

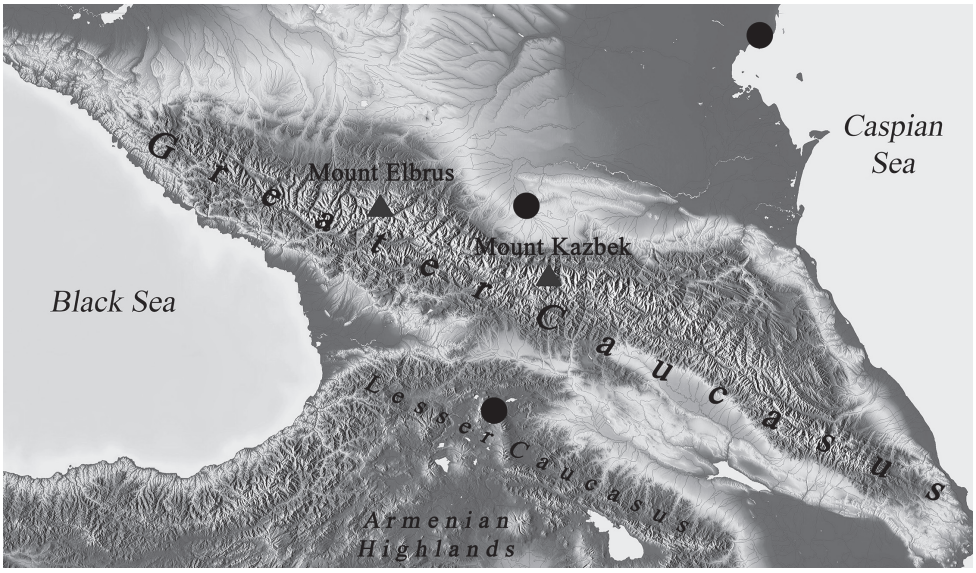

Figure 1. Collections sites of *Ch.* "annularius" in Caucasus region. Collection sites are marked with black dots.

considered as the Central Caucasus, and the area to the east of Mount Kazbek as the East Caucasus. The area, including the Kuban-Azov Lowland in the west, the Stavropol Upland in the middle and the Terek-Kuma Lowland in the east considered as Ciscaucasia. The area, including the Colchis Lowland, the Kura-Aras Lowland, the Lesser Caucasus, the Talysh mountains, the Lenkoran Lowland and eastern portion of the Armenian Highlands considered as the South Caucasus or Transcaucasia.

Consequently, the site from Republic of North-Ossetia-Alania belongs to the Central Caucasus, the site from the Republic of Dagestan belongs to the Eastern Ciscaucasia and the site from the Republic of Georgia belongs to South Caucasus or Transcaucasia.

Table 2. Collection sites and number of analyzed *Ch.* "annularius" larvae from the Caucasus region.

Localities	Population abbreviation	Collection sites	Collection date	Number of specimens
Central Caucasus	CC-OS-ZM	43°19.9067' N; 44°11.1333' E, Republic of North-Ossetia-Alania, puddle in the bed of drained pond, beside the Zmeiskaya settlement, altitude ca 310 m a.s.l.	05.05.10	32
Eastern Ciscaucasia	ECS-BK-ART	44°45.965' N; 46°48.2037' E, Republic of Dagestan, Tarumovsky District, ca 8 km southwest of "Biriuziak" holyday base, a puddle beside the artesian well, altitude ca -25 m b.s.l.	26.05.17	47
South Caucasus	SC-SJ-PA	41°19.3018' N; 43°45.5577' E, Republic of Georgia, Samtskhe-Javakheti region, ca 1 km north to the Sagamo settlement, one of branches of the Paravani river, altitude ca 2010 m a.s.l.	18.07.17	36

Regarding vertical zonation (Sokolov and Tembotov 1989), the first site belongs to the Terek variant, the second site belongs to the semi-steppe zone and the last one to the Javakheti-Armenian variant.

The head capsule and body of 25 larvae were slide mounted in Fora-Berlese solution. The specimens have been deposited in the Tembotov Institute of Ecology of Mountain territories RAS in Nalchik, Russia. We studied the karyotype and chromosomal polymorphism in 115 larvae from the Caucasus region.

We fixed the larvae for karyological study in ethanol-glacial acetic acid solution (3:1). The slides of the chromosomes were prepared using the ethanol-orcein technique (see Dyomin and Ilyinskaya 1988, Dyomin and Shobanov 1990). The banding sequences were designated per the accepted convention specifying the abbreviated name of the species, symbol of chromosome arm, and sequence number as in annA1, annA2, etc. (Keyl 1962, Wülker and Klötzli 1973).

We performed the identification of chromosome banding sequences for arms A, E and F using the photomaps of Kiknadze et al. (2012, 2016) in the system of Keyl (1962) and chromosome mapping for arms C and D as per Kiknadze et al. (1996c, 2012) in the system of Dévai et al. (1989).

We studied the chromosome slides using a Carl Zeiss Axio Imager A2 microscope and performed the statistical data processing using software packages PAST 3.18 (Hammer et al. 2001), GenALEx 6.503 (Peakall and Smouse 2006, 2012) and STATISTICA 10 (StatSoft).

We used the following parameters of chromosomal polymorphism characteristics for comparison: percentage of heterozygous larvae, number of heterozygous inversions per larvae, the number of banding sequences in a population and a number of genotypic combinations per population. We calculated the genetic distances between populations according to Nei criteria (Nei 1972) using Chironomus 1.0 software (Kazakov and Karmokov 2015) based on original data along with Kiknadze et al. (2012) research results.

We used the software package GenALEx 6.503 (Peakall and Smouse 2006, 2012) to check if the Caucasian populations follow Hardy-Weinberg expectation.

We performed a principal component analysis (PCA) of all the studied populations using original and previous data of Kiknadze et al. (1996c, 2012) to obtain a broader overview of the population genetic relationships (Fig. 5).

We measured the genetic distances (Table 6) between populations by Nei criteria (1972) based on original and previous data of Kiknadze et al. (1996c, 2012). Also, we constructed the tree dendrogram of genetic distances of studied populations using single-linkage clustering based on the obtained values (Fig. 6).

Results

We attributed the larvae of *Chironomus* in the studied sites to *Ch.* "annularius" by both morphological and chromosomal characteristics. The morphological larval characters of *Ch.* "annularius" from the Caucasian sites are similar to those previously described for this species by Kiknadze et al. (1996c, 2012).

Karyotype of *Ch.* "annularius" from the Caucasus region

The diploid number of chromosomes in *Ch.* "annularius" karyotype is 2n = 8, chromosome arm combination is AB, CD, EF, and G (the "thummi" cytocomplex) (Fig. 2). Chromosomes AB and CD are metacentric, EF is submetacentric, and G is telocentric. There are four permanent nucleoli (N) in karyotype: one nucleolus in arm C, two in the arm E and one in arm G. Besides permanent nucleoli there is a fluctuating nucleolus on arm A (region 2d-3a) that can be detected in most larvae of previously studied populations in homo- or heterozygous state (Kiknadze et al. 2012). The nucleolus on arm A is present in all the Caucasian populations (Fig. 2). There are four Balbiani rings (BR) in the karyotype: three in arm G and one in arm B (Fig. 2).

Banding sequences and chromosomal polymorphism of *Ch.* "annularius" from the Caucasus region.

Previously, Kiknadze et al. (2012) described 24 banding sequences in *Ch.* "annularius" banding sequences pool. In the studied populations, 15 of those sequences are present, and two banding sequences have been found for the first time, providing 17 banding sequences in the Caucasian populations (Table 3).

Arm A has four banding sequences: annA1, annA2, annA3, and annA5 (Figs 2–3, Table 3). The banding sequence annA1 and genotypic combination annA1.1 were predominant in populations of Eastern Ciscaucasia and South Caucasus (Tables 3, 4). In population of Central Caucasus, the banding sequence annA2 and genotypic combination

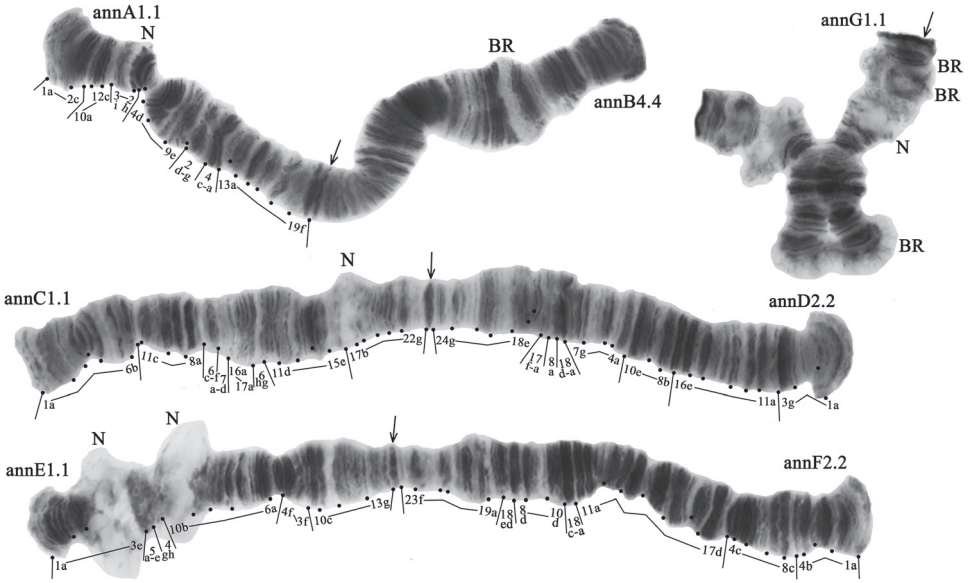

Figure 2. Karyotype of *Ch.* "annularius" from the Caucasus region; annA1.1, annD2.2 etc. – genotypic combinations of banding sequences; BR – Balbiani rings, N – nucleolus. Arrows indicate centromeric bands.

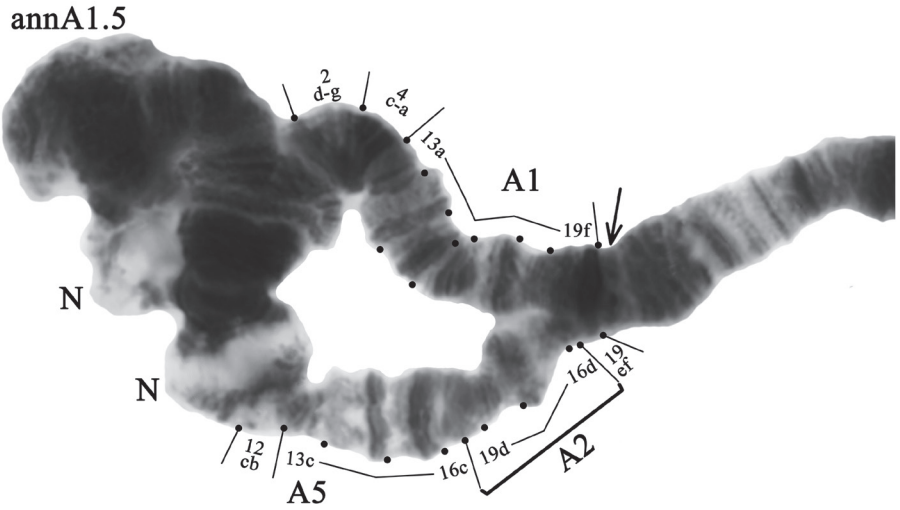

Figure 3. Heterozygous genotypic combination annA1.5. Designations as in Fig. 2.

annA2.2 were predominant. The banding sequence annA5 is new for the species and described for the first time (Fig. 3, Tables 3, 4). It differs from annA2 by one simple inversion step that involves regions 16d-19d:

annA5 1a-2c 10a-12a 13ba 4a-c 2g-d 9e-4d 2h-3i 12cb 13c-16c 19d-16d 19ef C

Table 3. Frequency of banding sequences in different populations of *Ch.* "annularius". N – the number of individuals, * – original data.

Banding sequences	European population	Caucasian populations			Siberian populations			Kazakhstan population	Nearctic populations	
	NL-NT-NT N=16	ECS-BK-ART N=47*	CC-OS-ZM N=32*	SC-SJ- PA N=36*	RU-OMS-IR N=39	RU-NSK-EP N=26	RU-NSK-BE N=52	KZ-AA-BG N=17	US-ND-WA N=16	US-ND-IS N=33
A1	0.438	0.766	0.313	0.708	0.910	0.769	0.865	0.736	0	0
A2	0.562	0.074	0.687	0.181	0.052	0.212	0.096	0.235	1	0.985
A3	0	0.160	0	0.042	0.038	0.019	0.039	0.029	0	0
A4	0	0	0	0	0	0	0	0	0	0.015
A5	0	0	0	0.069	0	0	0	0	0	0
B1	0.844	0	0	0.778	0.051	0.173	0.106	0	0	0
B2	0.156	0.596	0	0	0.949	0.827	0.894	0.706	1	0.985
B4	0	0.404	1	0.222	0	0	0	0.234	0	0
B5	0	0	0	0	0	0	0	0	0	0.015
C1	1	0.394	0.969	1	1	1	0.981	0.029	0	0
C2	0	0.606	0.031	0	0	0	0.019	0.971	0	0
C3	0	0	0	0	0	0	0	0	1	1
D1	1	0.085	0.156	0.944	0.538	0.788	0.673	0.588	0	0
D2	0	0.915	0.844	0.042	0.462	0.212	0.327	0.412	0	0
D3	0	0	0	0	0	0	0	0	1	1
D4	0	0	0	0.014	0	0	0	0	0	0
E1	1	0.170	0.875	0.806	0.500	0.538	0.462	0.794	1	0.970
E2	0	0.830	0.125	0.194	0.500	0.462	0.538	0.206	0	0.030
F1	0.156	0.723	0.141	0.153	0.243	0.173	0.163	0.206	0	0
F2	0.844	0.277	0.859	0.847	0.757	0.827	0.837	0.794	0.906	0.742
F3	0	0	0	0	0	0	0	0	0.094	0.258
G1	1	1	1	1	1	1	1	1	0	0
G3	0	0	0	0	0	0	0	0	1	1

Table 4. Frequency of genotypic combinations in different populations of *Ch.* "annularius". N – the number of individuals, * – original data.

Genotypic combinations	European population	Caucasian populations			Siberian populations			Kazakhstan population	Nearctic populations	
	NL-NT-NT N=16	ECS-BK-ART N=47*	CC-OS-ZM N=32*	SC-SJ- PA N=36*	RU-OMS-IR N=39	RU-NSK-EP N=26	RU-NSK-BE N=52	KZ-AA-BG N=17	US-ND-WA N=16	US-ND-IS N=33
A1.1	0.187	0.574	0.218	0.500	0.820	0.654	0.750	0.529	0	0
A1.2	0.500	0.064	0.188	0.250	0.103	0.193	0.153	0.353	0	0
A2.2	0.313	0.043	0.594	0.028	0	0.115	0.030	0.059	1	0.970
A1.3	0	0.319	0	0.028	0.077	0.038	0.077	0.059	0	0
A1.5	0	0	0	0.139	0	0	0	0	0	0
A2.3	0	0	0	0.055	0	0	0	0	0	0
A2.4	0	0	0	0	0	0	0	0	0	0.030
B1.1	0.687	0	0	0.611	0.103	0.115	0.038	0	0	0
B1.2	0.313	0	0	0	0	0.115	0.135	0	0	0
B1.4	0	0	0	0.333	0	0	0	0	0	0
B2.2	0	0.532	0	0	0.897	0.770	0.827	0.412	1	0.970
B2.4	0	0.128	0	0	0	0	0	0.588	0	0
B4.4	0	0.340	1	0.056	0	0	0	0	0	0
B2.5	0	0	0	0	0	0	0	0	0	0.030
C1.1	1	0.234	0.937	1	1	1	0.961	0	0	0
C1.2	0	0.319	0.063	0	0	0	0.039	0.059	0	0
C2.2	0	0.447	0	0	0	0	0	0.941	0	0
C3.3	0	0	0	0	0	0	0	0	1	1
D1.1	1	0	0	0.889	0.359	0.616	0.481	0.353	0	0
D1.2	0	0.17	0.313	0.083	0.359	0.346	0.385	0.470	0	0
D1.4	0	0	0	0.028	0	0	0	0	0	0
D2.2	0	0.83	0.687	0	0.282	0.038	0.134	0.177	0	0
D3.3	0	0	0	0	0	0	0	0	1	1
E1.1	1	0.043	0.750	0.639	0.256	0.308	0.250	0.706	1	0.940
E1.2	0	0.255	0.250	0.333	0.488	0.461	0.423	0.176	0	0.060

Genotypic combinations	European population	Caucasian populations			Siberian populations			Kazakhstan population	Nearctic populations	
	NL-NT-NT N=16	ECS-BK-ART N=47*	CC-OS-ZM N=32*	SC-SJ- PA N=36*	RU-OMS-IR N=39	RU-NSK-EP N=26	RU-NSK-BE N=52	KZ-AA-BG N=17	US-ND-WA N=16	US-ND-IS N=33
E2.2	0	0.702	0	0.028	0.256	0.231	0.327	0.118	0	0
F1.1	0	0.532	0	0	0	0	0	0.059	0	0
F1.2	0.313	0.383	0,281	0.306	0.487	0.346	0.327	0.294	0	0
F2.2	0.687	0.085	0.719	0.694	0.513	0.654	0.673	0.647	0.813	0.546
F2.3	0	0	0	0	0	0	0	0	0.187	0.394
F3.3	0	0	0	0	0	0	0	0	0	0.060
G1.1	1	1	1	1	1	1	1	1	0	0
G3.3	0	0	0	0	0	0	0	0	1	1

The banding sequence annA5 was found only in the population of the South Caucasus with relatively low frequency (annA5 – 0.069) and only in the heterozygous state (annA1.5 – 0.139) (Tables 3, 4).

Arm B has three banding sequences: annB1, annB2, and annB4 (Fig. 2; Tables 3, 4). The banding sequence annB1 and genotypic combination annB1.1 were predominant in the population of South Caucasus (Tables 3, 4). The banding sequence annB2 and genotypic combination annB2.2 were predominant in the population of Eastern Ciscaucasia. The banding sequence annB4 and genotypic combination annB4.4 were dominant in the population of Central Caucasus.

Arm C has two banding sequences: annC1 and annC2 (Fig. 2). The banding sequence annC1 and genotypic combination annC1.1 were predominant in populations of Central and South Caucasus (Tables 3, 4). In the population of Eastern Ciscaucasia, the banding sequence annC2 and genotypic combination annC2.2 were predominant.

Arm D has three banding sequences: annD1, annD2, and annD4 (Fig. 2). The banding sequence annD1 and genotypic combination annD1.1 were predominant in populations of Central and South Caucasus (Tables 3, 4). In the population of Eastern Ciscaucasia, the banding sequence annD2 and genotypic combination annD2.2 were predominant. The banding sequence annD4 is new for the species and described for the first time (Fig. 4, Tables 3, 4). It differs from annD1 by one simple inversion step that involves regions 3d-g 11a-c 12ab:

annD4 1a-3a-c 12ba 11c-a 3g-d 12dc 13a 10a 7a-4a 10e-b 13b-15e 20b-18e 17f-a 8a 18d-a 7g-b 9e-8b 16e-a 20c-24g C

The banding sequence annD4 was found only in the population of the South Caucasus with very low frequency (annD4 – 0.014) and only in the heterozygous state (annD1.4 – 0.028) (Tables 3, 4).

Arm E has two banding sequences: annE1 and annE2 (Fig. 2). The banding sequence annE1 and genotypic combination annE1.1 were predominant in populations of Central and South Caucasus (Tables 3, 4). In the population of Eastern Ciscaucasia, the banding sequence annE2 and genotypic combination annE2.2 were predominant.

Arm F has two banding sequences: annF1 and annF2 (Fig. 2). The banding sequence annF2 and genotypic combination annF2.2 were predominant in populations of Central and South Caucasus (Tables 3, 4). In the population of Eastern Ciscaucasia the banding sequence annF1 and genotypic combination annF1.1 were predominant.

Arm G was monomorphic with banding sequence annG1.1 (Fig. 2, Tables 3, 4).

Comparison of chromosomal polymorphism of *Ch.* "annularius" from the Caucasian populations with that of populations of other regions.

The data for European (Netherlands), Siberian, Kazakhstan and Nearctic (USA) populations are available due to Kiknadze et al. (1996c, 2012).

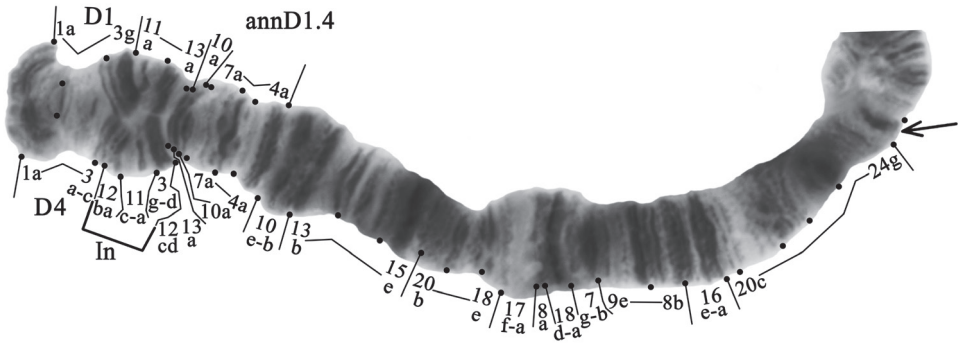

Figure 4. Heterozygous genotypic combination annD1.4. Designations as in Fig. 2.

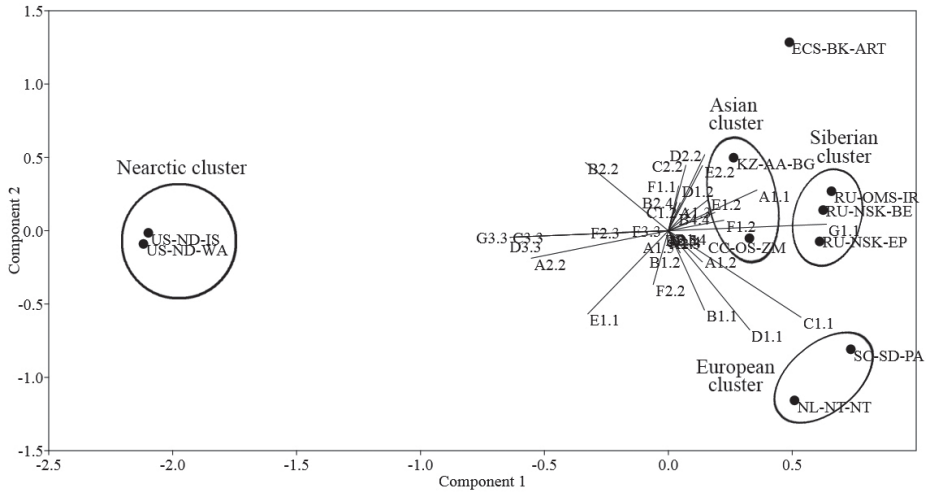

Figure 5. Principal component analysis (PCA) of genotypic combination frequencies in 10 *Ch.* "annularius" populations. For abbreviations of the populations, see Tables 1 and 2.

Arm A. Most earlier studied populations (Kiknadze et al. 1996c, 2012) were characterized by the presence of three banding sequences annA1, annA2 and annA3 (Table 3). The same picture was observed in the population of Eastern Ciscaucasia. In the European and Central Caucasian populations, two sequences were found, annA1 and annA2. In the Nearctic population of Warsing Dam (US-ND-WA) only sequence annA2 was present and in the second Nearctic population sequences annA2 and annA4 were present (Table 3). The most polymorphic population for this arm was the population of the South Caucasus, where four sequences (annA1, annA2, annA3 and annA5) and six genotypic combinations (annA1.1, annA1.2, annA1.3, annA1.5, annA2.2 and annA2.3) were present (Table 4). The banding sequence annA5 might be endemic for the region of South Caucasus (Table 3). In all the studied populations, sequences annA3, annA4 and A5 has been observed only in the heterozygote state (Table 4).

Arm B is polymorphic in most parts of studied populations, except for the Nearctic population of Warsing Dam where only banding sequence annB2 was present and the population of the Central Caucasus where also only sequence annB4 was found (Table 3). In European and Siberian populations, two sequences annB1 and annB2 were present, while in the first population sequence annB2 has been observed only in the heterozygote state (Table 4). In populations of Europe and South Caucasus, genotypic combination annB1.1 was predominant. The banding sequence annB2 in the homozygous state was predominant in populations of Eastern Ciscaucasia, Siberia and Nearctic population of Isabel Lake (US-ND-IS), while in the Kazakhstan population heterozygote annB2.4 was predominant (Tables 3, 4).

Arm C of *Ch.* "annularius" is polymorphic in two Caucasian populations (Eastern Ciscaucasia and Central Caucasus), Kazakhstan population and one Siberian population (Pond in Berdsk). The arm is monomorphic in populations of Europe, South Caucasus and rest of the Siberian populations, where only genotypic combinations annC1.1 was present. In addition, the arm is monomorphic in both Nearctic populations where the other genotypic combination annC3.3 was found (Table 4). In the population of the Eastern Ciscaucasia banding sequences annC1 and annC2 were present in both homozygous and heterozygous state with predominance of genotypic combination annC2.2 (Tables 3, 4). A similar picture observed in the population of Kazakhstan, where also both sequences annC1 and annC2 were found, but sequence annC1 was present only in heterozygous state and genotypic combination annC2.2 was dominant.

Arm D of *Ch.* "annularius" is polymorphic in most of the studied populations, except for both Nearctic populations, where only banding sequence annD3 was present and population of the Europe where only sequence annD1 was found (Table 3). In populations of Siberia and the population of South Caucasus genotypic combination annD1.1 was predominant, while in Kazakhstan population heterozygous combination annD1.2 was predominant. The banding sequence annD4 is probably endemic for the region of South Caucasus (Table 3). In populations of Eastern Ciscaucasia and Central Caucasus, two banding sequences annD1 and annD2 were found with predominance of genotypic combination annD2.2. The banding sequence annD1 in these populations was found only in the heterozygous state (Tables 3, 4).

Arm E of *Ch.* "annularius" is polymorphic in most part of the studied populations, except for the Nearctic population of Warsing Dam and the population of the Europe where only banding sequence annE1 was present (Table 3). In the second Nearctic population, Kazakhstan population and two Caucasian populations (Central and South Caucasus) banding sequences annE1 and annE2 are presented in both homozygous and heterozygous state with predominance of genotypic combination annE1.1. A similar picture is observed in Siberian populations, but here another combination annE1.2 was predominant (Tables 3, 4). In the population of Eastern Ciscaucasia unlike all other populations the genotypic combination annE2.2 was predominant (Table 4).

Arm F of *Ch.* "annularius" is polymorphic in all the studied populations. In most of them, with the exception of the population from Eastern Ciscaucasia, genotypic

combination annF2.2 was predominant (Table 4). In European, two Caucasian (Central and South Caucasus) and all Siberian populations the banding sequence annF1 was present only in the heterozygous state, while in Kazakhstan populations it was found both in homozygous and heterozygous state (Tables 3, 4). As noted earlier, in both Nearctic populations genotypic combination annF2.2 was predominant but also another banding sequence annF3 was present in both homozygous and heterozygous state (Tables 3, 4). Unlike all other populations, in population of Eastern Ciscaucasia the genotypic combination annF1.1 was predominant (Table 4).

Arm G is monomorphic in all the studied populations. However, there is an important difference. In Holarctic populations genotypic combination annG1.1 was dominant, while in Nearctic populations another combination annG3.3 was dominant.

The level of inversion polymorphism of Caucasian *Ch.* "annularius" populations is quite similar to those of previously studied Holarctic populations (Table. 5). The populations of the South Caucasus and Eastern Ciscaucasia are generally close to Asian populations (Siberia and Kazakhstan) by all the parameters of chromosomal polymorphism. The population of Central Caucasus is close to the European population by the average number of heterozygous inversions per larvae, number of banding sequences per population and number of genotypic combinations per population. The percentage of heterozygous larvae in population of Central Caucasus is lowest (72%) among all the Holarctic populations (81-90%) (Table 5).

On the dendrogram of genetic distances, there are four clear clusters that we conditionally assigned as European, Asian, Siberian and Nearctic clusters (Fig. 6). The European cluster is formed by populations of the Netherlands and South Caucasus. The Siberian populations form their own separate cluster and so do Nearctic ones. The populations of Central Caucasus and Kazakhstan form Asian cluster. The population of Eastern Ciscaucasia does not belong to any of the outlined clusters. In spite of relative geographic proximity, the genetic distances between Caucasian populations are quite large (Table 6), and they do not form a single cluster of Caucasian populations. The distance value between populations of Central and South Caucasus (0.3853) does not exceed the distance range (0.136–0.474) for different population of the one species (Gunderina 2001). At the same time, the distance value between populations of Central Caucasus and Eastern Ciscaucasia (0.5318) in one hand and the distance value between populations of Eastern Ciscaucasia and South Caucasus (0.8232) in other hand exceeds those ranges and fall in the distance range (0.474–2.815) for different subspecies (Gunderina 2001). One can see that separation of the population of Eastern Ciscaucasia from other Caucasian populations is relatively big and even reaches a level of subspecies.

The principal component analysis shows almost the same picture as the dendrogram of genetic distances (Fig. 6). One can see the dramatic separation of Holarctic and Nearctic populations. In addition, the separation of the European, Asian and Siberian clusters is quite clear. Moreover, the populations of the Nearctic cluster are characterized by a constant increase of genotypic combinations annA2.2, annC3.3, annD3.3, annG3.3, and annF2.3. The European cluster is characterized by increasing

Table 5. Cytogenetical characteristics of chromosomal polymorphism in different populations of *Ch.* "annularius". N – the number of individuals, * – original data.

Cytogenetical characteristics	European population	Caucasian populations			Siberian populations			Kazakhstan population	Nearctic populations	
	NL-NT-NT N=16	ECS-BK-ART N=47*	CC-OS-ZM N=32*	SC-SJ- PA N=36*	RU-OMS-IR N=39	RU-NSK-EP N=26	RU-NSK-BE N=52	KZ-AA-BG N=17	US-ND-WA N=16	US-ND-IS N=33
Heterozygous larvae, %	81	83	72	89	90	85	89	88	18	48
Average number of heterozygous inversions per larvae	1.1	1.6	1.2	1.6	1.6	1.5	1.5	1.0	0.2	0.5
Number of banding sequences per population	10	14	12	15	13	13	14	14	8	11
Number of genotypic combinations per population	11	19	13	19	15	17	18	18	8	12

Table 6. Values of genetic distances between the different populations of *Ch.* "annularius".

Population	NL-NT-NT	ECS-BK-ART	CC-OS-ZM	SC-SJ- PA	RU-OMS-IR	RU-NSK-EP	RU-NSK-BE	KZ-AA-BG	US-ND-WA	US-ND-IS
NL-NT-NT	0									
ECS-BK-ART	1.0628	0								
CC-OS-ZM	0.3919	0.5318	0							
SC-SJ-PA	0.0724	0.8232	0.3853	0						
RU-OMS-IR	0.4069	0.3421	0.4084	0.2454	0					
RU-NSK-EP	0.2589	0.4732	0.3952	0.1566	0.0272	0				
RU-NSK-BE	0.3422	0.3909	0.409	0.2124	0.0147	0.0084	0			
KZ-AA-BG	0.5162	0.4259	0.5787	0.4828	0.4121	0.3845	0.3807	0		
US-ND-WA	1.1784	2.0094	1.1094	1.5183	1.2745	1.1585	1.2025	1.1412	0	
US-ND-IS	1.2873	2.0136	1.2059	1.6637	1.3387	1.2404	1.2917	1.2424	0.0093	0

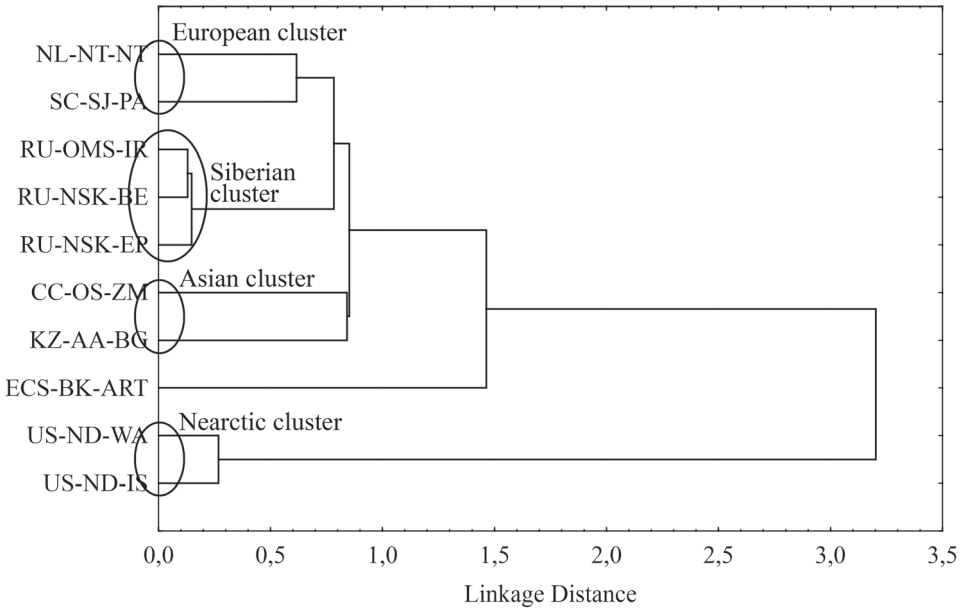

Figure 6. Tree dendrogram for 10 *Ch.* "annularius" populations, *single linkage, Euclidean distances.* For abbreviations of the populations, see Tables 1 and 2.

of other combinations annB1.1, annC1.1 and annD1.1. The Asian and Siberian clusters are closest to each other. The population of Eastern Ciscaucasia and populations of the European cluster are located on opposite sides of "cloud" of Holarctic populations.

Among Caucasian populations, the frequencies of genotypic combinations in all arms of *Ch.* "annularius" follow Hardy-Weinberg expectation only in the population of South Caucasus. In population of Central Caucasus, the frequencies of genotypic combinations in arm A do not follow Hardy-Weinberg expectation (χ^2 = 10.166, p – 0.001). The homozygotes annA1.1 were observed 2.22 times more frequently than it was expected and heterozygotes annA1.2 should be occurred 2.29 times more frequently than they were observed. One can observe an even more complex picture in the population of Eastern Ciscaucasia where the frequencies of genotypic combinations do not follow Hardy-Weinberg expectation across three arms: arm A (χ^2 = 16.046, p – 0.001), arm B (χ^2 = 25.388, p – 0.000), and arm C (χ^2 = 5.163, p – 0.023). In the arm A the heterozygotes annA1.2 should be occurred 1.78 times more frequently than they were observed, homozygotes annA2.2 were observed 7.17 times more frequently than it was expected, also expected combinations annA2.3 and annA3.3 were not found at all. In arm B the homozygotes annB2.2 and annB4.4 were observed 1.5/2.1 times more frequently than it was expected and heterozygotes annB2.4 should be occurred 3.77 times more frequently than they were observed. Finally, in arm C the homozygotes annC1.1 and annC2.2 were observed 1.5/1.2 times more frequently than it was expected and heterozygotes annC1.2 should be occurred 1.5 times more frequently than they were observed.

Discussion

We found the species *Ch.* "annularius" in the South Caucasus for the first time. Earlier (Karmokov 2017) we recorded the species for Eastern Ciscaucasia but without data on its karyotype and chromosomal polymorphism.

Overall, the Caucasian populations of the species can be characterized as relatively polymorphic. We found two new banding sequences annA5 and annD4 in the banding sequences pool of *Ch.* "annularius". We observed inversion polymorphism almost in all chromosome arms except for arm G, which was monomorphic in Caucasian populations.

Observed picture with Hardy-Weinberg expectation in the site from Eastern Ciscaucasia can be explained in several ways. First, it can be a negative selection of heterozygotes due to some adaptive processes that are still ongoing. Another possibility is that it is due to short time of existence of this population and founder effect.

The climate of Terek-Kuma lowland is much hotter and drier than in both other collection sites. We collected the larvae here from the puddle beside an active artesian well. This habitat is stable because it is constantly fed by water from the well. There are about 3 000 of such kind of wells (most of them still active), within the radius of ca 100 km. Most of them were drilled in the 50–60s of the 20th century for the aims of animal husbandry. Considering this, we can expect a lot of new records of this species from habitats situated beside those wells. The puddle that served as collection site is quite small (3×5m of water surface, max. depth about 0.5m) and thus the total size of the population is not so big. Possibly this population is relatively young and just over 50–60 years old. It can be presumed that initially a very small number of individuals from some nearby habitats established this population and the influx of new migrants is not so large. It is quite possible that most part of the larvae here could be relatives and so the inbreeding could occur quite often. Possibly, there was not enough time for the population to come to the equilibrium. Perhaps we see the founder effect that can also explain the observed picture with Hardy-Weinberg expectation.

All the obtained data are indicative of the complex genetic structure of Caucasian populations of *Ch.* "annularius" and total complexity of microevolution processes occurring in the Caucasus region. In spite of geographic proximity, one Caucasian population is separated from other populations of the Caucasus at the level of subspecies.

References

Andreeva EN (1999) Karyotypes and chromosomal polymorphism in Nearctic *Chironomus* species. Ph.D. Thesis, Institute of Cytology and Genetics SB RAS. Novosibirsk, 283 pp. [In Russian]

Belyanina SI (1981) Comparative karyological characteristics of *Chironomus annularius* (Diptera, Chironomidae) from different geographic zones. Zoologicheskiy Zhurnal 60: 1030–1039. [In Russian]

Butler MG, Kiknadze II, Cooper JK, Siirin MT (1995) Cytologically identified *Chironomus* species from lakes in North Dakota and Minnesota, USA. (p. 31-38). In: Cranston P. (Ed.) Chironomids: From Genes to Ecosystems. CSIRO Publications. Canberra, 482 pp.

Dévai Gy, Miskolczi M, Wülker W (1989) Standardization of chromosome arms B, C and D in *Chironomus* (Diptera, Chironomidae). Acta Biologica Debricina. Supplementum Oecologica Hungarica 2 (1): 79–92.

Dyomin SYu, Iliynskaya NB (1988) Change of the density of polytene chromosomes from different organs of *Chironomus plumosus* larvae. Tsitologiya 30 (4): 407–415. [In Russian]

Dyomin SYu, Shobanov NA (1990) Karyotype of *Chironomus entis* from the plumosus group (Diptera, Chironomidae) living in the European part of the Soviet Union. Tsitologiya 32 (10): 1046–1054. [In Russian]

Gunderina LI (2001) Genetic variation and evolution of Chironomids (Diptera, Chironomidae), Doctor of science thesis. Institute of Cytology and Genetics SB RAS. Novosibirsk, 360 pp. [In Russian]

Gvozdetskii NA (1963) The Caucasus. A Description of Its Nature. State Publishing House of Geographic Literature. Moscow, 261 pp. [In Russian]

Hammer O, Harper DAT, Ryan PD (2001) PAST: Paleontological Statistic software package for education and data analysis. Palaeontologia Electronica 4 (1): 9.

Karmokov MKh (2012) Genera *Chironomus* Meigen, 1803, *Camptochironomus* Keiffer, 1918 (Diptera, Chironomidae) from the Central Caucasus and Ciscaucasia: systematics, distribution and chromosomal polymorphism, Ph.D. Thesis, Saratov State Medical University. Saratov, 321 pp. [In Russian]

Karmokov MKh (2017) Non-biting midges of genus *Chironomus* (Diptera, Chironomidae) of Daghestan State Nature Reserve protected areas. Izvestia of Samara Scientific Center of the Russian Academy of Sciences 19 (6): 73–78. [In Russian with English summary]

Kazakov MA, Karmokov MKh (2015) The method to automate calculation of genetic similarity and genetic distance in chironomids of the genus *Chironomus* by the Nei criterion. Bulletin of the Adygeya State University 3: 142–145. [In Russian with English summary]

Keyl H–G, Keyl I (1959) Die cytologische Diagnostik der Chironomiden. I. Bestimmungstabelle für die Gattung Chironomus auf Grund der Speicheldrusenchromosomen. Archiv für Hydrobiologie 56 (1/2): 43–57.

Keyl H–G (1962) Chromosomenevolution bei *Chironomus*. II. Chromosomenumbauten und phylogenetische Beziehungen der Arten. Chromosoma 13 (4): 464–514. https://doi.org/10.1007/BF00327342

Kiknadze II, Shilova AI, Kerkis IE, Shobanov NA, Zelentsov NI, Grebenyuk LP, Istomina AG, Prasolov VA (1991a) Karyotypes and larval morphology in tribe Chironomini. Atlas. Nauka Publish. Novosibirsk, 115 pp. [In Russian with English summary]

Kiknadze II, Istomina AG, Gunderina LI, Aimanova KG, Salova TA, Savvinov DD (1996c) Banding sequence pools of chironomid of Yakutian Permafrost. Tribe Chironomini. Nauka Publish. Novosibirsk, 166 pp. [In Russian with English summary]

Kiknadze II, Gunderina LI, Butler MG, Wülker W, Martin J (2008b) Chromosomes and continents. In: Dobretsov N, Kolchanov N, Rosanov A, Zavarzin G (Eds) Biosphere origin and evolution. Springer Verlag, 427 pp. https://doi.org/10.1007/978-0-387-68656-1_25

Kiknadze II, Butler MG, Gunderina LI, Istomina AG, Gusev VD, Nemytikova LA (2010) Chromosomal evolution of Nearctic and Palearctic *Chironomus* species (Diptera, Chironomidae). (p. 203-221). In: Proc. XV Intern. Symp. on Chironomidae. L.C. Ferrington, (Ed.). University of Minnesota Saint Paul. Minnesota, 385 pp.

Kiknadze II, Istomina AG, Golygina VV (2012) The karyotype and chromosomal polymorphism of the Holarctic species *Chironomus* "annularius" sensu Strenzke (1959) (Diptera, Chironomidae). Euroasian Entomological Journal 11: 95–114.

Kiknadze II, Istomina AV, Golygina VV, Gunderina LI (2016) Karyotypes of Palearctic and Holarctic species of the genus *Chironomus* [Electronic resource]. Russian Academy of Sciences, Siberian Branch, Federal Research Center Institute of Cytology and Genetics. Novosibirsk: Academic Publishing House "GEO", 2016. 489 pp.

Michailova PV (1989) The polytene chromosomes and their significance to the systematics of the family Chironomidae, Diptera. Acta Zoologica Fennica 186: 1–107.

Nei M (1972) The genetic distance between populations. American Naturalist 106: 283–292. https://doi.org/10.1086/282771

Peakall R, Smouse PE (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes 6: 288–295. https://doi.org/10.1111/j.1471-8286.2005.01155.x

Peakall R, Smouse PE (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. Bioinformatics 28: 2537–2539. https://doi.org/10.1093/bioinformatics/bts460

Petrova NA, Michailova P (1986) Populational-karyological investigation of some chironomid species. Tsitologiya 28: 727–735. [In Russian]

Spies M, Sæther OA (2004) Notes and recommendations on taxonomy and nomenclature of Chironomidae (Diptera). Zootaxa 752: 1-90. https://doi.org/10.11646/zootaxa.752.1.1

Strenzke K (1959) Revision der Gattung *Chironomus* Meig. 1. Die Imagines von 15 noddeutshen Arten und Unterarten. Archiv für Hydrobiologie 56: 1–42.

Sokolov VE, Tembotov AK (1989) Mammals of Caucasus: Insectivores. Moscow, 548 pp. [In Russian with English summary]

Wülker W, Klötzli AM (1973) Revision der Gattung *Chironomus* Meig. IV. Arten des lacunarius-(commutatus-) complexes. Archiv für Hydrobiologie 72 (2): 474–489.

CompCytogen 12(3): 285–298 (2018)
doi: 10.3897/CompCytogen.v12i3.22590
http://compcytogen.pensoft.net

RESEARCH ARTICLE

COMPARATIVE Cytogenetics
A peer-reviewed open-access journal
International Journal of Plant & Animal Cytogenetics, Karyosystematics, and Molecular Systematics

Karyotypic variation in the long-whiskered catfish *Pimelodus blochii* Valenciennes, 1840 (Siluriformes, Pimelodidae) from the lower Tapajós, Amazonas and Trombetas Rivers

Ivanny Coelho da Fonseca[1,4], Luan Aércio Melo Maciel[2,4], Frank Raynner Vasconcelos Ribeiro[3], Luís Reginaldo Ribeiro Rodrigues[1,2,4]

1 *Graduate Program in Amazonian Natural Resources – PPGRNA, Federal University of Western Pará – UFOPA, Av. Mendonça Furtado, 2°Andar, Nº 2440, Bairro Aparecida CEP: 68040-050, Santarém, PA, Brazil* **2** *Graduate Program in BioSciences – PPGBio, Federal University of Western Pará – UFOPA, Av. Mendonça Furtado, 2°Andar, Nº 2440, Bairro Aparecida CEP: 68040-050, Santarém, PA, Brazil* **3** *Water Science and Technology Institute – ICTA, Federal University of Western Pará, Campus Amazonia, Av. Mendonça Furtado, 2°Andar, Nº 2946, Bairro Fátima CEP: 68040-050, Santarém, PA, Brazil* **4** *Genetics and Biodiversity Laboratory – LGBio, Federal University of Western Pará – UFOPA, Rua Vera Paz, s/n, Salé, 68035-110, Santarém, PA, Brazil*

Corresponding author: *Luís Reginaldo Ribeiro Rodrigues* (luisreginaldo.ufpa@hotmail.com)

Academic editor: *G. Furgala-Selezniow* | Received 28 November 2017 | Accepted 28 June 2018 | Published 2 August 2018

http://zoobank.org/E7737895-7F06-47EA-8C8A-7CF7D6BF491D

Citation: Fonseca IC, Maciel LAM, Ribeiro FRV, Rodrigues LRR (2018) Karyotypic variation in the long-whiskered catfish *Pimelodus blochii* Valenciennes, 1840 (Siluriformes, Pimelodidae) from the lower Tapajós, Amazonas and Trombetas Rivers. Comparative Cytogenetics 12(3): 285–298. https://doi.org/10.3897/CompCytogen.v12i3.22590

Abstract

The genus *Pimelodus* LaCépède, 1803 comprises 35 formally recognized species distributed along the major neotropical river basins. Despite conservatism in diploid number with 2n=56, an intense variation of chromosomal morphology (karyotypic formula) has been documented in *Pimelodus* species. In the present study, we analyzed karyotypes of 20 specimens, identified as *Pimelodus blochii* Valenciennes, 1840 and collected from the lower courses of the Tapajós, Amazonas and Trombetas Rivers. The karyotypes were characterized by Giemsa conventional staining, C-banding, silver staining (Ag-NOR) and fluorescent in situ hybridization (FISH) with 5S and 18S rDNA probes. The karyotypes showed 2n=56 chromosomes in fish from the Tapajós River. In contrast, fish from the Amazonas and Trombetas Rivers had 2n=58.

The nucleolus organizing regions were labeled on the short arm of an acrocentric chromosome as demonstrated by silver staining and FISH. Signals for 18S and 5S rDNA were co-localized on one chromosome pair. Our results demonstrate karyotypic divergence between Tapajós and Amazonas-Trombetas populations of *P. blochii*, interpreted as supporting the existence of a species complex in this taxon.

Keywords
Amazon basin, catfish, *Pimelodus*, rDNA, species complex

Introduction

The genus *Pimelodus* LaCépède, 1803 (Siluriformes, Pimelodidae) comprises 35 valid species exclusively distributed in neotropical freshwater drainages. It is commonly recorded in the Amazonas, Orinoco, Araguaia-Tocantins, São Francisco, and Paraná-Paraguay River basins. In the Amazon basin, seven species of *Pimelodus* have been recorded: *Pimelodus albofasciatus* Mees, 1974, *Pimelodus blochii* Valenciennes, 1840, *Pimelodus altissimus* Eigenmann & Pearson, 1942, *Pimelodus jivaro* Eigenmann & Pearson, 1942, *Pimelodus ornatus* Kner, 1858, *Pimelodus pictus* Steidachner, 1876, and *Pimelodus tetramerus* Ribeiro & Lucena, 2006 (Ferraris 2007, Eschmeyer and Fong 2017).

Cytogenetic analysis of 32 Pimelodidae family members revealed a conservative karyotypic macrostructure with 2n=56 chromosomes save for a few exceptional karyotypes (2n=50) in *Calophysus macropterus* Lichtenstein, 1819, *Pinirampus pirinampu* Spix & Agassiz, 1829 and *Luciopimelodus plati* Valenciennes, 1835 species (Ramirez-Gil et al. 1998; Sánchez et al. 2000; Vasconcelos and Santos 2000). Diploid chromosome number variations were also reported in *Pimelodus fur* Lütken, 1874 and *Megalonema platanum* Günther, 1880 species samples with 2n=54 and in *Pimelodus blochii* (Della-Rosa et al. 1980) species samples with 2n=58 (Sánchez et al. 2000; Garcia and Moreira-Filho 2008; Carvalho et al. 2011).

Diploid chromosome numbers of eleven previously investigated *Pimelodus* species showed variation from 54 to 58 with eleven distinct karyotype formula (see Table 2 in the Discussion section). Additionally, karyotype variation with B chromosomes had been reported in *P. ortmanni* and *Pimelodus* sp. (Borin and Santos 2004).

So far, two distinct karyotypes were reported for two *Pimelodus blochii* populations; 2n=56 for the Araguaia River, and 2n=58 for the Amazon River (Swarça et al. 2007). Although the chromosome numbers of *P. blochii* were declared in three meetings and published in abstracts by Swarça et al. (2007), no karyotype image of the species is available in any peer-reviewed literature. Therefore, the taxon has been considered as poorly described in the scientific literature.

Rocha (2006) suggested that the name *P. blochii* is arbitrarily assigned to many different long-whiskered catfish in the Brazilian part of the Amazon Basin. Based on morphometric and molecular data, a large collection of *P. blochii* specimens was examined by Rocha (2006). It was demonstrated that the Brazilian specimens are distinct from *P. blochii* topotypes from Suriname and possibly represent a species complex with six undescribed taxa.

In the present paper, we investigate the karyotype of *Pimelodus blochii* from the lower portions of the Tapajós, Amazonas and Trombetas Rivers in order to evaluate their chromosomal features and contribute the debate on the species taxonomy. The karyotypes were characterized by conventional Giemsa staining, C-banding, silver staining (Ag-NOR) and fluorescent in situ hybridization (FISH) technique with 5S and 18S rDNA probes.

Material and methods

Samples and collection sites

Twenty (20) specimens were collected from four localities in the Tapajós, Amazonas and Trombetas Rivers (Table 1). The fish were captured by local fishermen using hooks and gillnets. The specimens were transferred to plastic tanks (50 L capacity) filled with water from the collection site and aerated with an aquarium pump. After cytogenetic procedures, the specimens were photographed, fixed in 10% formalin for 48 h, washed with running water and preserved with 70% ethanol. The voucher specimens were deposited in the Fish Collection of the Water Science and Technology Institute at Federal University of Western Pará, Brazil. External morphology and coloration features are shown in Fig. 1. The experimental procedures were approved by the Ethical Committee of Animal Research at Federal University of Western Pará (CEUA/UFOPA) under Protocol N. 10001/2015.

Chromosome preparation

Intra-abdominal colchicine (0.0125%) injection was performed at 0.01 ml/g (Bertollo et al. 1978) to stop cell division. The exposed fish were placed in an aerated tank for 40 min and euthanized with water that contains a lethal concentration of clove oil. The posterior kidney tissue was removed and minced in 6 ml of hypotonic KCl solution (0.075 M, 5.6 g/L). The cell suspension was incubated at 37 °C for 20 min and then fixed with fresh methanol-acetic acid (3:1 v/v) solution; the fixative was changed three times.

Table 1. Samples and collection sites of *Pimelodus blochii* in the Amazon Basin.

River	Collection sites	GPS Coordinates (datum WGS84)	n
Tapajós	Itaituba	4°16'12.6"S, 55°58'37.1"W	6
Amazonas	Santarém	2°25'8.0"S, 54°44'28.6"W	6
	Chicaia River, Almeirim	1°38'15.6"S, 52°57'46.2"W	4
Trombetas	Oriximiná	1°45'52.2"S, 55°52'18.8"W	4

Specimens: PO-22, PO-25, PO-27, PO-28, ITB-11, ITB14-18, STXVI-1, STXVI-2, STXVI-9, ALC-1-4, PML-5-7.

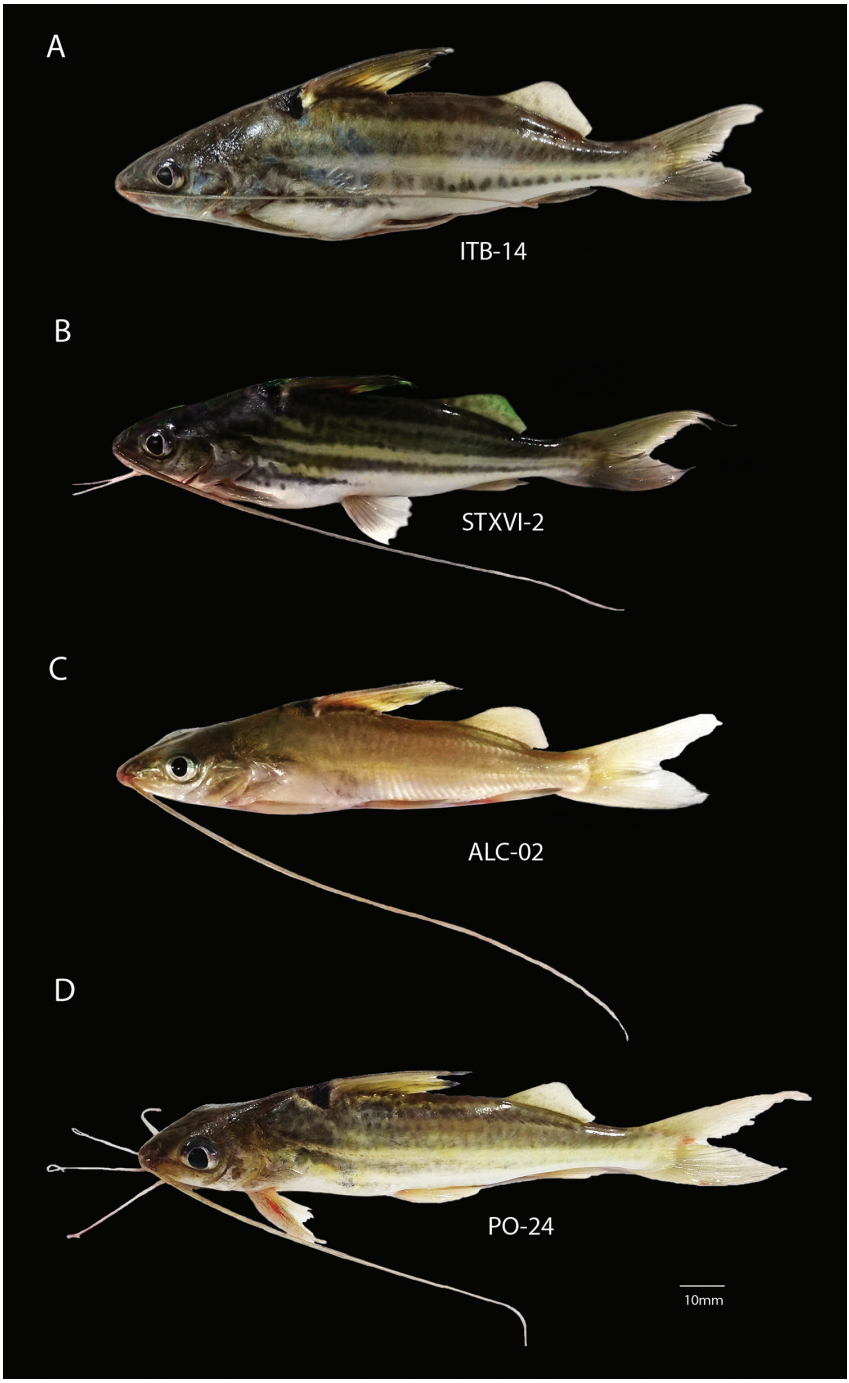

Figure 1. External morphology and coloration of *Pimelodus blochii* specimens examined in the present study. **A** specimen from the Tapajós River (ITB-14, SL=150 mm; W=55 g) **B** specimen from the lower Amazonas River at Santarém (STXVI-2, SL=145 mm; W=52 g) **C** specimen from the lower Amazonas River at Almeirim (ALC-2, SL=110 mm; W=9 g) **D** specimen from the Trombetas River (PO-22, SL=108 mm, W=33 g).

Chromosome staining, banding and FISH

Conventional staining was performed with 5% Giemsa solution (phosphate buffer, pH 6.8). The C-banding protocol from Sumner (1972) was followed with minor changes. The NORs were stained with silver nitrate following the Howell and Black (1980) technique.

FISH was used for mapping 18S and 5S rDNA loci (Pinkel et al. 1986). Double FISH experiments were processed with probes generated with 18Sf (5' CCG CTT TGG TGA CTC TTG AT 3') and 18Sr (5' CCG AGG ACC TCA CTA AAC CA 3') primers, as well as 5Sa (5-TAC GCC CGA TCT CGT CCG ATC) and 5Sb (5-CAG GCT GGT ATG GCC GTA AGC-3) PCR primers (Martins and Galetti 1999; Martins and Vicari 2012).

The PCR products were labeled by nick translation with biotin-14-dATP (BioNick Labeling System kit, Invitrogen/ThermoScientific, Waltham, Massachusetts, USA) and digoxigenin-11-dUTP (DIG-nick translation mix, Roche, Basel, Switzerland). The slides were treated with RNase solution (5 µl RNase 10 mg/mL diluted in 975 µl 2×SSC) for a short period of time. The fixed chromosomes were denatured in 70% formamide (pH 7.0 2×SSC) and heated at 70 °C for 5 min. The hybridization solution mixture was prepared with 20 µl formamide + 8 µl of 50% dextran sulfate + 4 µl of each probe + 4 µl of 20×SSC. The slides were incubated in 2×SSC solution in a humidified and heated (37 °C) chamber overnight.

Post-hybridization washes were performed with 15% formamide at 42 °C for 10 min, three washes in 0.1×SSC at 60 °C for 5 min, and 0.5% Tween20 at room temperature for 5 min. For signal detection, slides were placed in NFDM buffer (20 ml of 20×SSC, pH 7.0 + 5 g of powdered skim milk + 80 ml of distilled water) for 15 min, followed by two washes in 5% Tween20 for 5 min at room temperature.

The hybridized probes were applied in a mixture containing 20 µl anti-digoxigenin-rhodamine (1:200) (Roche, Basel, Switzerland) + 4 µl FITC-Avidin (1:100) (Sigma, St. Louis, Missouri, USA) + 26 µl of C buffer (0.1 M sodium bicarbonate, 0.15 M sodium chloride; pH 7.0) for 60 min. The slides were coated with anti-fading reagent Vectashield H-1000 (Vector Laboratories, Burlingame, California, USA) and chromosomes were counterstained with DAPI (1,2-diamidin-phenyl-indol).

Microscopy and karyotype analysis

At least 30 metaphases were counted to determine the diploid chromosome number. The best spread metaphase plates were photographed with a CCD camera (Moticam 10 MP) coupled to a Zeiss Axioskop40 microscope for conventional/banding images, and a Nikon Eclipse CI for FISH images. The contrast and brightness were adjusted with ADOBE PHOTOSHOP CS3. The chromosomes were arranged as metacentric (m), submetacentric (sm), subtelocentric (st) and acrocentric (t) following Levan et al. (1964).

Results

The diploid chromosome number was observed as 56 in the Tapajós River fish (Itaituba population). On the other hand, 2n=58 chromosomes were recorded from Amazonas (Santarém and Almeirim populations) and Trombetas Rivers (Oriximiná population) samples, with minor variation in the karyotypic formula, as 30m/sm+28a and 26m/sm+32a, respectively (Fig. 2).

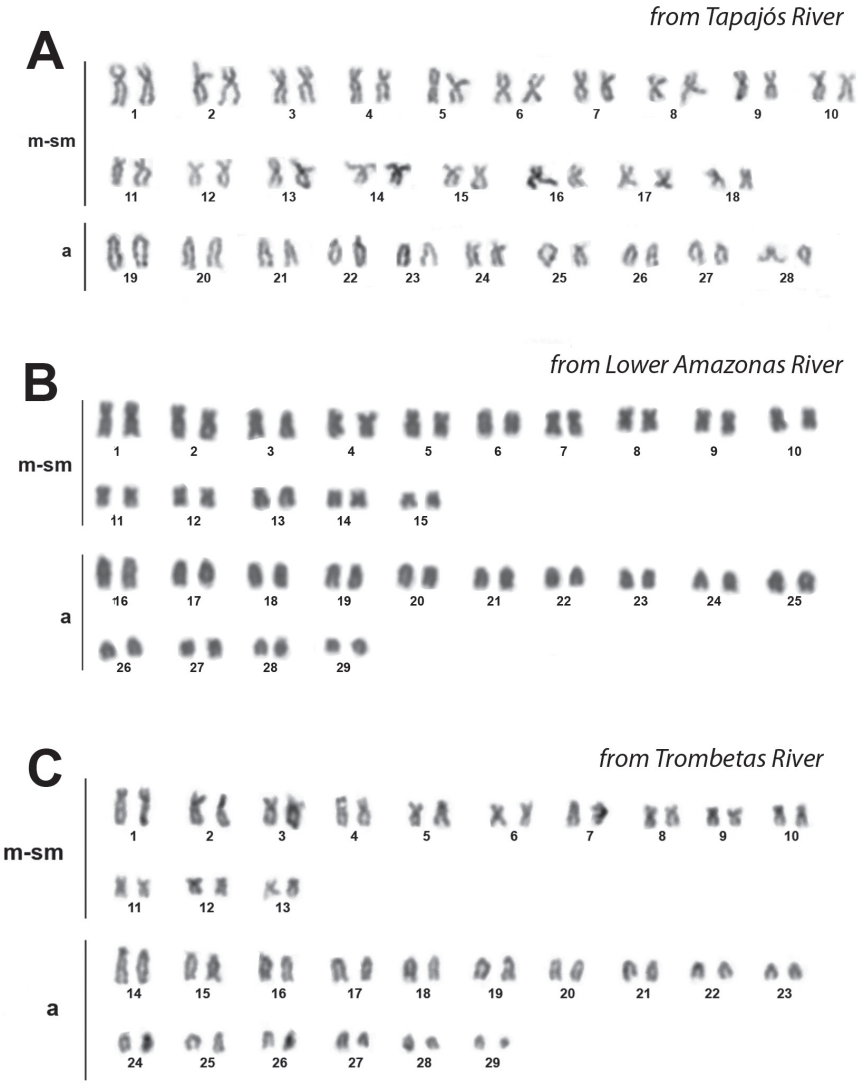

Figure 2. Giemsa-stained karyotypes of *Pimelodus blochii* from the Amazon Basin. **A** 2n=56 chromosomes (36m/sm + 20a) **B** 2n=58 chromosomes (30m/sm + 28a) **C** 2n=58 chromosomes (26m/sm + 32a). Scale bar: 10 μm.

The C-banding results showed small amounts of constitutive heterochromatin in the centromeres. Terminal C-bands were observed in 18 to 24 chromosome pairs from the Tapajós River specimens (Fig. 3). A single NOR-bearing chromosome pair was detected by silver staining in all samples and labeled as the centromeric position (Fig. 4a–d). These sites were compatible with the 18S rDNA locus as demonstrated by FISH (Fig. 4e–h).

The 5S rDNA probe showed distinct localizations among the samples. Co-localization of 5S and 18S rDNA to a single chromosome pair was detected in the Trombetas and Amazonas Rivers populations (Almeirim population); this syntenic pattern also occurred in the Santarém population but on just one homologous chromosome. The Tapajós River specimens' karyotypes showed a distinct position for 18S and 5S rDNA (Figs 4, 5).

Discussion

The karyotype macrostructure of *Pimelodus blochii* from the Tapajós, Amazonas and Trombetas Rivers are compatible with a previous report (Swarça et al. 2007) (Table 2). The diploid chromosome number was found to be 58 (30m/sm+28a) in the lower Amazonas population; this is compatible with the Solimões River population (Della-Rosa et al. 1980). The specimens from the Trombetas River conserved 2n=58 but shifted the

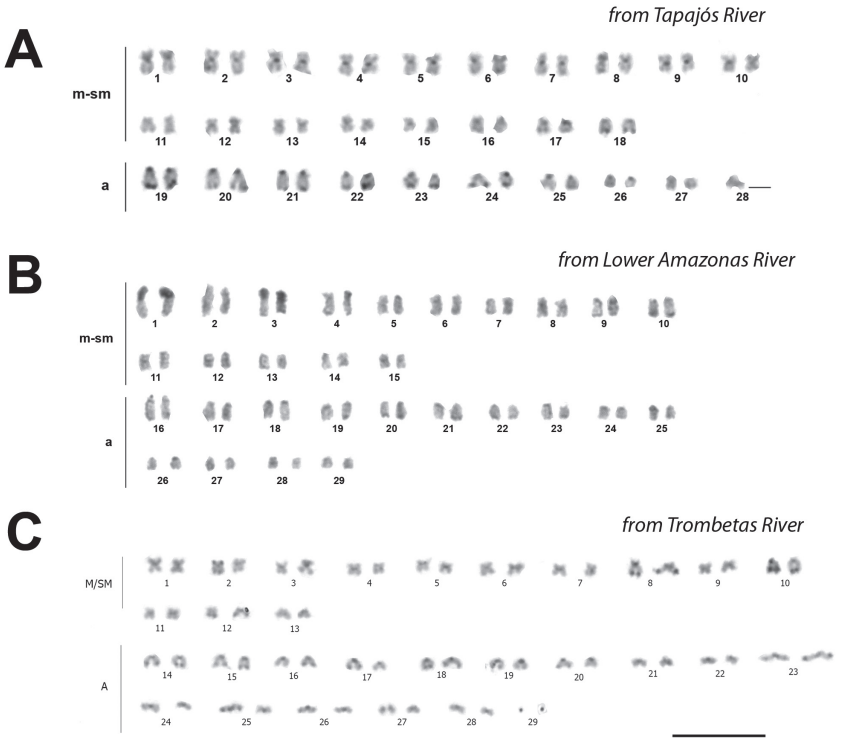

Figure 3. C-banded karyotypes of *Pimelodus blochii* from the Amazon River basin. Scale bar: 10 μm.

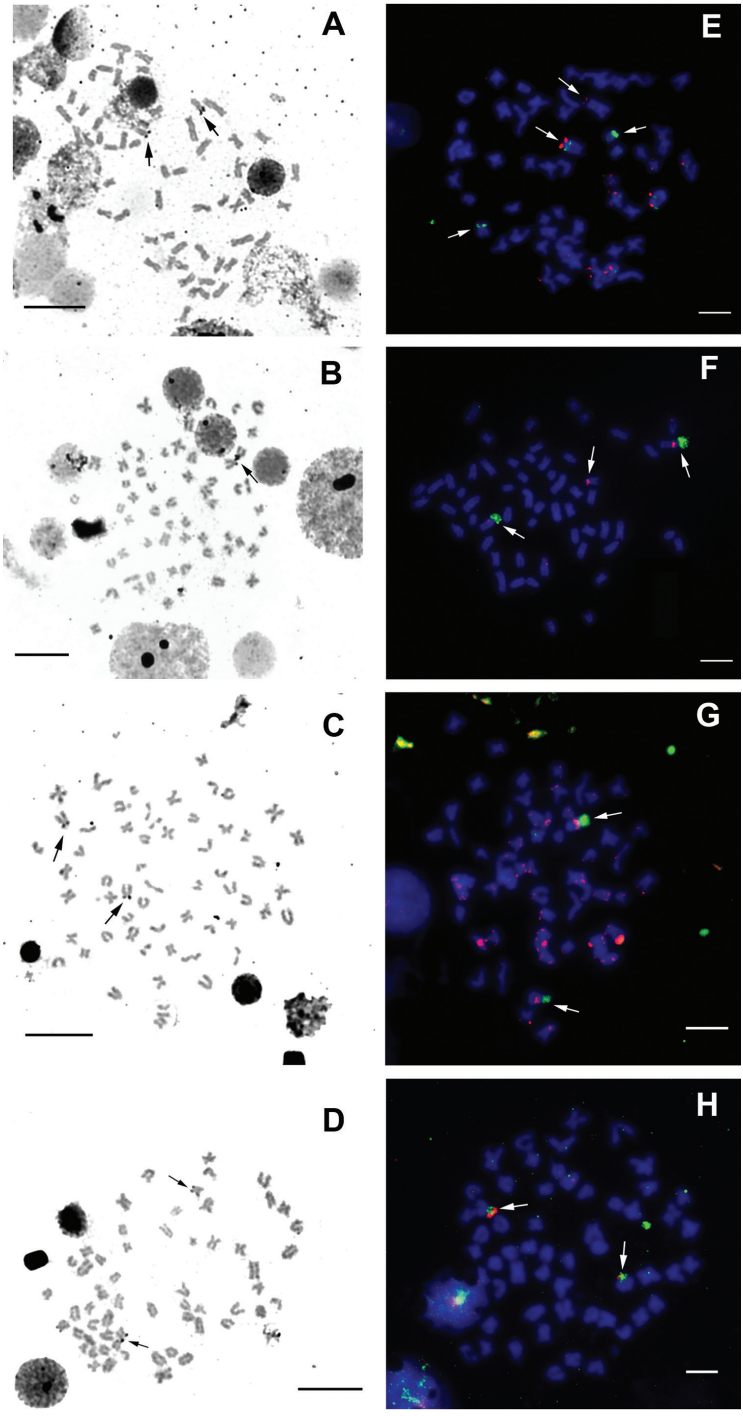

Figure 4. Metaphases of *Pimelodus blochii* showing NOR by silver staining (left) and double FISH of 18S rDNA (green) and 5S rDNA (red) (right). Specimens from the Tapajós River (**A, E**); from the Lower Amazonas River at Santarém (**B, F**), at Almeirim (**D, H**); and from the Trombetas River (**C, G**). Scale bar: 10 μm.

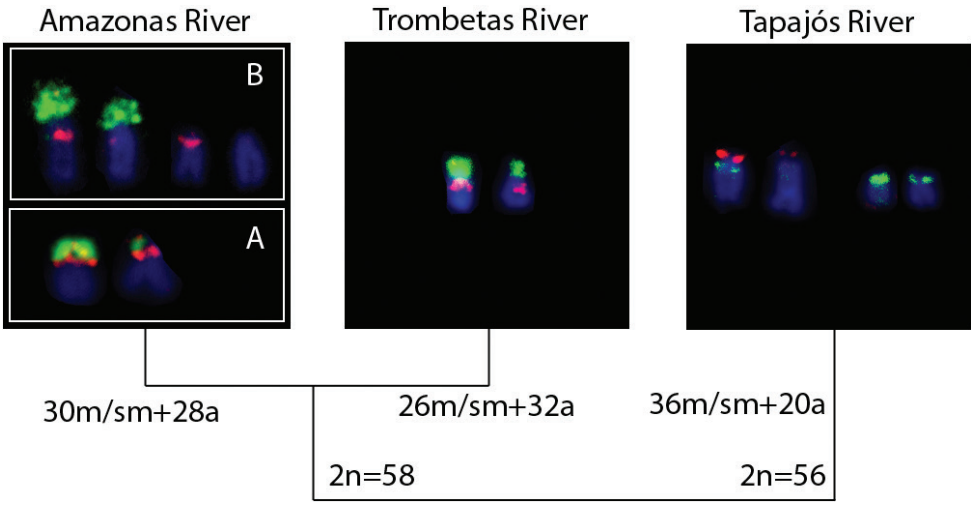

Figure 5. Variation of 18S rDNA (green signal) and 5S rDNA (red signal) chromosomal sites among *Pimelodus blochii* populations from Amazon basin. The samples from Amazonas River showed two distinct patterns: one observed in the Almeirim population (**A**) and other observed in the Santarém population (**B**).

karyotypic formula to 26m/sm+32a; this could be a result of pericentric inversions. On the other hand, *Pimelodus blochii* from the Tapajós River with 2n=56 (36m/sm+20a) showed a resemblance to the population from the Araguaia River (Barra do Garça, Mato Grosso State) (Farias et al. 2000).

Despite extensive conservatism in diploid number, variation in karyotypic formula has been frequently detected (Swarça et al. 2007). Pericentric inversions can explain such modifications of chromosomal morphology without alteration of the diploid number and have been previously demonstrated, such as in *Pimelodus maculatus* and *Pimelodus* sp. (Dias and Foresti 1993).

A single NOR (one pair) is the most common pattern observed in the *Pimelodus* karyotypes (Swarça et al. 2007). This pattern was confirmed for *P. blochii* in the present study. The Ag-NOR sites were coincident with the rDNA 18S FISH signal and showed co-localization with the 5S, a rare condition previously observed in *Pimelodus britskii* (Neto et al. 2011). In the Order Siluriformes, the synteny of 18S and 5S rDNA cistrons has been reported for *Pimelodus* (Neto et al. 2011; present study), *Imparfinis* Eigenmann et Norris 1900 (Ferreira et al. 2014), *Ancistrus* Kner 1854 (Favarato et al. 2016), *Hemibagrus* Bleeker 1862 (Supiwong et al. 2014), *Corydoras* LaCepède 1803 (Rocha et al. 2016), *Panaqolus* Isbrücker et Schraml 2001 (Ayres-Alves et al. 2017) and *Bunocephalus* Kner 1855 (Ferreira et al. 2017). This syntenic arrangement is interpreted as being less adaptive since in eukaryotes the 45S rRNA genes are transcribed by RNA polymerase I, whereas the 5S are transcribed by RNA polymerase III. This means both processes occur in separate nuclear territories (Amarasinghe and Carlson 1998). Additionally, a linked configuration of 18S and 5S rDNA arrays could favor an undesired disruption of both tandem repeats by means of unequal crossing-over (Martins and Wasko 2004).

Table 2. Compiled data from the literature on karyotypic traits in *Pimelodus* species. m=metacentric, sm=submetacentric, st=subtelocentric, a=acrocentric, q=long arm, t=terminal, c=centromeric, inter=interstitial, peri=pericentromeric.

Species	2n	Karyotypic formula	18S	5S	References
Pimelodus fur	54	32m+8sm+6st+8a	q, t, sm	q inter m, q peri sm	5
P. microstoma	56	22m+22sm+6st+6a	q, t, st	peri sm, q peri st	1; 2
P. argenteus	56	24m+16sm+12st+4a			3
P. britskii	56	24m+18sm+8st+6a	q, t, st	p inter sm, q t st	4
P. maculatus	56	32m+12sm+12st	q, t, sm	q inter m, q t sm q peri sm	5; 6
P. absconditus	56	24m+18sm+8st+6a	–	–	7
P. mysteriosus	56	26m+20sm+2st+8a	–	–	3; 8
P. ornatus	56	18m+22sm+6st+10a	–	–	9; 7
P. ortmanni	56	24m+18sm+8st+6a	–	–	10; 11; 12
P. paranaensis	56	22m+22sm+4st+8a	–	–	13
P. blochii	56/58	36m/sm+20st/a; 30m/sm+28a; 26m/sm+32a	q, c, a	q, c, a	14; 15; 16; 17

References: 1) Fenocchio et al. (1994); 2) Souza et al. (2004); 3) Souza et al. (2003); 4) Neto et al. (2011); 5) Garcia and Filho (2008); 6) Mazzuchelli et al. (2007); 7) Borin and Santos (2002); 8) Girardi (2015); 9) Abucarma and Santos (1996); 10) Borin and Santos (2004); 11) Terencio et al. (2001); 12) Margarido and Gavasso (2000); 13) Treco and Dias (2009); 14) Della-Rosa et al. (1980); 15) Farias et al. (2000); 16) Silva et al. (2004); 17 (present study).

Eigenmann (1912) recognized varieties (A and B) of *P. blochii* from Guyana. The A variety has an ashy body pigmentation without dots or stripes, whereas the B variety exhibits four lateral body stripes with the fourth stripe possibly absent or fragmented into dots. *Pimelodus albofasciatus* (Mees 1974) maintains a close resemblance to the B variety but is distinguished based on eye morphology and dorsal spine length (Ribeiro and Lucena 2006).

Our specimens collected from the Chicaia River, a tributary of the lower Amazonas (Almeirim population), have the typical pigmentation for the A variety, whereas the specimens from the Santarém population, collected at the confluence of the Amazonas and Tapajós Rivers, had the B variety pigmentation. Although both populations conserved the diploid number 2n=58 and karyotypic formula (30m/sm+28a), they diverged in their 18S and 5S rDNA locations (Fig. 5). The specimens from the Trombetas River also have the B variety pigmentation, but diverge in the karyotypic formula (26m/sm+32a) and 18S and 5S rDNA locations (Fig. 5). The most differentiated karyotype was observed in specimens from the Tapajós population; these had a clearly distinct diploid number, karyotypic formula and 18S and 5S location. In general, their coloration resembles the B variety, but their karyotypic distinctiveness leads us to suggest that these specimens may be a new, undescribed *Pimelodus* species.

Ribeiro and Lucena (2006), Azpelicueta et al. (2008), and Lucinda et al. (2016) discussed three distinct patterns of pigmentation among species of *Pimelodus*. According to Lucinda et al. (2016), among the species formally described, a striped-pattern is shared by *P. albicans*, *P. albofasciatus*, and *P. tetramerus*. However, our results, as well

as those of Ribeiro and Lucena (2006), suggest that the diversity of *Pimelodus* with blackish stripes along its flanks that inhabit the waters of the Amazonas River basin is greater than the diversity currently described. This includes undescribed species that have commonly been misidentified as *P. blochii*. *P. blochii* from the lower Amazonas River occurs as two morphotypes (A and B) distinguished through body pigmentation and characterized by a 2n=58 karyotype with minor variations. Additional studies of this group are needed in order to clarify the evolutionary dynamics of the 18S and 5S rRNA genes as well as to acquire morphological and molecular data to evaluate the taxonomy and phylogeny of *Pimelodus* species.

Conclusions

The populations of *Pimelodus blochii* from the lower courses of Amazonas, Tapajós and Trombetas rivers presented differentiated karyotypes based on variation in diploid number and chromosome morphology. The specimens collected from the Tapajós River, with 2n=56, are clearly distinguished from the others and may constitute a new, undescribed *Pimelodus* species.

Acknowledgments

This research was supported by grants from CAPES/PRO-AMAZÔNIA (Auxpe-3318/2013). The authors are grateful to the native fishermen (Haroldo Leite, Mr Naldo, Mr Nelson, William Vasconcelos, Janison Vasconcelos, Mr Claudenido) for helping us with fieldwork and to the LGBIO/UFOPA staff for help in the laboratory. ICF received Mastership from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). This work is part of the Project "Estudos Citogenéticos e Citogenômicos da Biodiversidade da Amazônia, com implementação de avanços técnicos" (Cytogenetic and cytogenomic studies of Amazonian biodiversity implementing technical advances) coordinated by Dra Cleusa Y. Nagamachi (UFPA).

References

Abucarma M, Santos ICM (1996) Caracterização cromossômica de duas espécies da família Pimelodidae (Pisces, Siluriformes). Proceedings of VI Simpósio de Citogenética Evolutiva Aplicada a Peixes Neotropicais, São Carlos, October 9–11, 1996, Brazil, 73.

Amarasinghe V, Carlson JE (1998) Physical mapping and characterization of 5S rRNA genes in douglas-fir. Journal of Heredity 89: 495–500. https://doi.org/10.1093/jhered/89.6.495

Ayres-Alves T, Cardoso AL, Nagamachi CY, de Sousa LM, Pieczarka JC, Noronha RCR (2017) Karyotypic evolution and chromosomal organization of repetitive DNA sequences in species of *Panaque*, *Panaqolus*, and *Scobinancistrus* (Siluriformes and Loricariidae) from the Amazon Basin. Zebrafish 14: 251–260. https://doi.org/10.1089/zeb.2016.1373

Azpelicueta MM, Lundberg JG, Loureiro M (2008) *Pimelodus pintado* (Siluriformes: Pimelodidae), a new species of catfish from affluent rivers of Laguna Merín, Uruguay, South America. Proceedings of the Academy of Natural Sciences of Philadelphia 157: 149–162. https://doi.org/10.1635/0097-3157(2008)157[149:PPSPAN]2.0.CO;2

Bertollo LAC, Takahashi CS, Filho OM (1978) Cytotaxonomic considerations on *Hoplias lacerdae* (Pisces, Erytrinidae). Revista Brasileira de Genética 2: 103–120.

Borin LA, Santos ICM (2002) Cytogenetic aspects in species of the genus *Pimelodus* (Pisces, Siluriformes, Pimelodidae) of the River Paraná Basin. Cytologia 67: 199–204. https://doi.org/10.1508/cytologia.67.199

Borin LA, Santos ICM (2004) Study on karyotype and occurrence of B chromosomes in two endemic species of the genus *Pimelodus* (Siluriformes, Pimelodidae) from the river Iguaçu. Hereditas 140: 201–209. https://doi.org/10.1111/j.1601-5223.2004.01812.x

Carvalho RA, Sanchez S, Swarça AC, Fenocchio AS, Santos ICM, Dias AL (2011) Chromosomal analyses in *Megalonema platanum* (Siluriformes: Pimelodidae), an endangered species from South American rivers. Neotropical Ichthyology 9(1): 177–182. https://doi.org/10.1590/S1679-62252011005000008

Della-Rosa VA, Bertollo LAC, Ferrari I, Takahashi CS, Moreira Filho O, Foresti F (1980) Estudos citogenéticos de peixes da Amazônia. II. Ordem Siluriformes. Ciência e Cultura 32: 735.

Dias AL, Foresti F (1993) Cytogenetic studies on fishes of the family Pimelodidae (Siluroidei). Revista Brasileira de Genética 16(3): 585–600.

Eigenmann CH (1912) The fresh-water fishes of British Guiana, including a study of the ecological grouping of species, and the relation of the fauna of the plateau to that of the lowlands. Memoirs of the Carnegie Museum 5(67): 1–578. https://doi.org/10.5962/bhl.part.14515

Eschmeyer WN, Fong JD (2017) Catalog of fishes. California Academy of Sciences. https://www.calacademy.org/scientists/projects/catalog-of-fishes [accessed 4 July 2018]

Farias AA, Brito JG, Venere PC (2000) Citogenética de Pimelodidae: Caracterização cromossômica de *Pimelodus blochii*, *Pimelodella cristata* e *Hemisorubim platyrhynchos* (Siluriformes) do médio Araguaia. Proceedings of VIII Simpósio de Citogenética e Genética de Peixes, Manaus, October 2000, Brazil.

Favarato RM, Silva M, Oliveira RR, Artoni RF, Feldberg E, Matoso DA (2016) Cytogenetic diversity and the evolutionary dynamics of rDNA genes and telomeric sequences in the *Ancistrus* genus (Loricariidae: Ancistrini). Zebrafish 13(2): 103–111. https://doi.org/10.1089/zeb.2015.1140

Fenocchio AS, Pastori MC, Lopez PA, Sanchez S, Alberdi AJ, Bordenave S, Dib MC (1994) Levantamento citogenético em peixes de água-doce da Argentina: resumo das espécies estudadas. Proceedings of V Simpósio de Citogenética Evolutiva Aplicada a Peixes Neotropicais, Botucatu, October 25–27, 1994, Brazil, 8.

Ferraris Jr CJ (2007) Checklist of catfishes, recent and fossil (Osteichthyes: Siluriformes), and catalogue of siluriform primary types. Zootaxa 1418: 1–628. https://doi.org/10.11646/zootaxa.1418.1.1

Ferreira M, Garcia C, Matoso DA, Jesus IS, Cioffi MB, Bertollo LAC, Zuanon J, Feldberg E (2017) The *Bunocephalus* coracoideus species complex (Siluriformes, Aspredinidae). Signs of a speciation process through chromosomal, genetic and ecological diversity. Frontiers in Genetics 8: 1–12. https://doi.org/10.3389/fgene.2017.00120

Ferreira M, Kavalco KF, Almeida-Toledo LF, Garcia C (2014) Cryptic diversity between two *Imparfinis* species (Siluriformes, Heptapteridae) by cytogenetic analysis and DNA barcoding. Zebrafish 11(4): 306–317. https://doi.org/10.1089/zeb.2014.0981

Garcia C, Filho OM (2008) Localization of ribosomal genes in three *Pimelodus* species (Siluriformes, Pimelodidae) of the São Francisco River: 5S genes as species markers and conservation of the 18S rDNA sites. Genetics and Molecular Biology 31(1): 261–264. https://doi.org/10.1590/S1415-47572008000200018

Girardi SC (2015) Citogenética Básica e Molecular em espécies de Pimelodidae (Siluriformes) coletadas nas Bacias do Rio Paraná e do Rio Uruguai: Uma Abordagem na Taxonomia e Sistemática. M.Sc. Dissertation, Cascavel, Brazil, State University of Western Paraná, 125 pp. [In Portuguese]

Howell WM, Black DA (1980) Controlled silver-staining of nucleolus organizer regions with a protective colloidal developer: a 1–step method. Experientia 36: 1014–1015. https://doi.org/10.1007/BF01953855

Levan A, Fredga K, Sandberg AA (1964) Nomenclature for centromeric position on chromossomes. Hereditas 52: 107–110.

Lucinda PHF, Ribeiro FRV, Lucena CAS (2016) *Pimelodus quadratus*, a new long-whiskered catfish from the rio Tocantins drainage, Brazil (Siluriformes: Pimelodidae). Ichthyological Exploration of Freshwaters 27: 337–346.

Margarido VP, Gavasso E (2000) Análise cariotípica em Pimelodus ortmanni (Pisces, Siluriformes, Pimelodidae) coletado no Rio Iguaçu – Bacia do Iguaçu. Proceedings of 46º Congresso Nacional de Genética. Águas de Lindóia, September 19–23, 2000, Brazil, 63.

Martins C, Galetti-Jr PM (1999) Chromosomal localization of 5S rDNA Genes in *Leporinus* fish (Anostomidae, Characiformes). Chromosome Research 7: 363–367. https://doi.org/10.1023/A:1009216030316

Martins C, Vicari MR (2012) Hibridização in situ em cromossomos de peixes. In: Guerra M (Ed.) Citogenética Molecular: Protocolos comentados. Ribeirão Preto, 59–87.

Martins C, Wasko A (2004) Organization and evolution of 5S ribosomal DNA in the Fish genome. In: Williams CR (Ed.) Focus on Genome Research. New York, 335–363.

Mazzuchelli J, Swarça AC, Dias AL (2007) Structural chromosome polymorphism in a *Pimelodus maculatus* La Cepéde, 1803 population (Siluriformes, Pimelodidae) from the Paranapanema River Basin, PR, Brazil. Brazilian Journal of Biology 67(4): 935–937. https://doi.org/10.1590/S1519-69842007000500018

Neto AM, Silva M, Matoso DA, Vicari MR, Almeida MC, Colares-Pereira MJ, Artoni RF (2011) Karyotype variability in Neotropical catfishes of the family Pimelodidae (Teleostei: Siluriformes). Neotropical Ichthyology 9(1): 97–105. https://doi.org/10.1590/S1679-62252011005000002

Pinkel D, Straume T, Gray JW (1986) Cytogenetic analysis using quantitative, high-sensitivity, fluorescence hybridization. Proceedings of the National Academy of Sciences of the United States of America 83(9): 2934–2938. https://doi.org/10.1073/pnas.83.9.2934

Ramirez-Gil H, Feldberg E, Almeida-Val VMF, Val AL (1998) Karyological, biochemical and physiological aspects of *Callophysus macropterus* (Siluriformes, Pimelodidae) from the Solimões and Negro rivers (Central Amazon). Brazilian Journal of Medical and Biological Research 31: 1449–1458. https://doi.org/10.1590/S0100-879X1998001100014

Ribeiro FRV, Lucena CAS (2006) Nova espécie de *Pimelodus* (Siluriformes, Pimelodidae) dos rios Tapajós e Tocantins, Brasil. Iheringia Serie Zoologia 96(3): 321–327. https://doi.org/10.1590/S0073-47212006000300008

Rocha MS (2006) Sistemática e Filogeografia de *Pimelodus blochii* (Siluriformes: Pimelodidae) da Amazônia. M.Sc. Dissertation, Manaus, Brazil: National Institute of Research on Amazonia, Federal University of Amazonas, 82 pp. [In Portuguese]

Rocha RH, Baumgärtner L, Paiz LM, Margarido VP, Fernandes CA, Gubiani EA (2016) An uncommon co-localization of rDNA 5S with major rDNA clusters in Callichthyidae (Siluriformes): a report case in *Corydoras carlae* Nijssen & Isbrücker, 1983. Comparative Cytogenetics 10(4): 603–613. https://doi.org/10.3897/compcytogen.v10i4.9507

Sánchez S, Fenocchio AS, Jorge LC (2000) Estudios citogenéticos en peces de la familia Pimelodidae (Pisces, Siluriformes) de la cuenca del río Paraná, Argentina. Análisis cromosómico de dos especies de Luciopimelodinae. Comunicaciones Científicas y Tecnológicas. Universidad Nacional del Nordeste. http://www.unne.edu.ar/unnevieja/Web/cyt/cyt/2000/4_veterinarias/v_pdf/v_050.pdf [Accessed 21 November 2017]

Silva LVB, Abreu MF, Souza IL, Venere PC (2004) Caracterização Cromossômica de *Hemisorubim platyrhynchos*, *Sorubim lima* e *Pimelodus blochii* (Pisces, Siluriformes, Pimelodidae) do Médio Araguaia. Proceedings of X Simpósio de Citogenética e Genética de Peixes. Natal, October 2004, Brazil, 132.

Souza L, Swarça AC, Dias AL (2004) Analysis of the nucleolus organizer regions in 5 species of the genus *Pimelodus* (Siluriformes, Pimelodidae), using $AgNO_3$, CMA3 and FISH with the 18S rDNA probe. Caryologia 57(2): 144–150. https://doi.org/10.1080/00087114.2004.10589384

Souza L, Giuliano-Caetano L, Dias AL (2003) Karyotypic Study of three species of *Pimelodus* (Pisces, Pimelodidae) from the Paraguai River Basin. Cytologia 68(4): 345–350. https://doi.org/10.1508/cytologia.68.345

Summer AT (1972) A simple technique for demonstrating centromeric heterocromatin. Experimental Cell Research 74: 304–306. https://doi.org/10.1016/0014-4827(72)90558-7

Supiwong W, Liehr T, Cioffi MB, Chaveerach A, Kosyakova N, Fan X, Tanee T, Tanomtong A (2014) Comparative cytogenetic mapping of rRNA genes among naked catfishes: implications for genomic evolution in the Bagridae family. Genetics and Molecular Research 13(4): 9533–9542. https://doi.org/10.4238/2014.November.12.2

Swarça AC, Fenocchio AF, Dias AL (2007) An update Cytogenetic Review for Species of the Families Pseudopimelodidae, Pimelodidae and Heptapteridae (Pisces, Siluriformes). Suggestion of a Cytotaxonomical Classification. Caryologia 60(4): 338–348. https://doi.org/10.1080/00087114.2007.10797957

Terencio ML, Almeida MC, Artoni RF (2001) Citogenética de *Pimelodus ortmanni*, uma espécie de mandí endêmica ao rio Iguaçu. Proceedings of 47º Congresso Nacional de Genética. Águas de Lindóia, October 2–5, 2001, Brazil, 135.

Treco FR, Dias AL (2009) Karyotypes of Two Species of the Genus *Pimelodus* (Siluriformes, Pimelodidae). Folia Biologica (Kraków) 57(1/2): 43–48.

Vasconcelos C, Santos ICM (2000) Chromosome polymorphism in species of the Pimelodidae (Teleostei, Siluriformes). Hereditas 132: 103–109. https://doi.org/10.1111/j.1601-5223.2000.00103.x

CompCytogen 12(3): 299–360 (2018)
doi: 10.3897/CompCytogen.v12i3.27448
http://compcytogen.pensoft.net

FORUM PAPER

COMPARATIVE Cytogenetics
A peer-reviewed open-access journal
International Journal of Plant & Animal Cytogenetics, Karyosystematics, and Molecular Systematics

23rd International Colloquium on Animal Cytogenetics and Genomics (23 ICACG)
June 9–12, 2018, Saint-Petersburg, Russia

Svetlana Galkina[1], Maria Vishnevskaya[1]

1 *St Petersburg State University, St. Petersburg, Russia*

Corresponding author: *Svetlana Galkina* (svetlana.galkina@mail.ru)

Academic editor: *E. Mikhailova* | Received 25 June 2018 | Accepted 3 August 2018 | Published 16 August 2018

http://zoobank.org/CA149494-A164-4B8A-9C77-2E8A3CC57589

Citation: Galkina S, Vishnevskaya M (2018) 23rd International Colloquium on Animal Cytogenetics and Genomics (23 ICACG). Comparative Cytogenetics 12(3): 299–360. https://doi.org/10.3897/CompCytogen.v12i3.27748

In memory of Ingemar Gustavsson

23rd International Colloquium on Animal Cytogenetics and Genomics (23 ICACG) took place in June 9–12, 2018 in Saint-Petersburg, Russia. Organized biennially, the Colloquium runs from 1970. From its very start this meeting is associated with the name of Ingemar Gustavsson to whom we dedicated the Colloquium 2018. The long and productive career of Ingemar Gustavsson had focused on chromosomes and their fundamental role in animal physiology, fertility, health and production in the context of agriculture and veterinary medicine. His meticulous analysis of breeding data performed back in 1964–69 resulted in the unequivocal identification of an association between heterozygosity for the 1/29 translocation in Swedish cattle and reduction in the fertility of the breed. Eventually, the argument in favor of selective elimination of bulls carrying the translocation from the breeding programs prevailed and the field of modern veterinary cytogenetics was established.

Participants from fourteen different countries attended the 23 ICACG in Russia, the country having long lasting traditions in cytogenetics and the Scientific schools of N.K. Koltzov, S.S. Chetverikov and A.S. Serebrovsky, geneticists who made important conceptual contributions to studies of chromosomes and genes, population genetics and evolutionary theory as early as in the beginning of the XX-th century.

All the abstracts received were subdivided between plenary and seven scientific sessions covering the issues in evolutionary and comparative cytogenetics, cytogenetics and genomes of domestic animals, meiosis studies, particular chromosome analyses, clinical cytogenetics, karyotypes and genomes of vertebrate

and invertebrate animals, chromatin studies. In the abstract text below each presentation is marked with a capital letter: „**L**" stands for lectures, „**O**" for oral presentations and „**P**" for poster presentations.

We gratefully acknowledge the support from the Saint-Petersburg Association of Scientists and Scholars (SPbSU), Veterinary Genetics Center ZOOGEN, Russian Foundation for Basic Research (RFBR), VEUK, Helicon, Axioma BIO, BioVitrum, Sartorius, DIA-M companies.

The current collected abstracts comprise written contributions of the presentations during the 23 ICACG and were edited by Svetlana Galkina and Maria Vishnevskaya.

The next Colloquium – 24 ICACG – will be held at the University of Kent in Canterbury (UK) in 2020.

Please, cite abstracts as follows:

Gall JG (2018) Giant chromosomes and deep sequences: what the amphibian egg tells us about transcription. In: Galkina SA, Vishnevskaya MS, Mikhailova EI (Eds) 23rd Inernational Colloquium on Animal Cytogenetics and Genomics (23rd ICACG), June 9–12, 2018, St Petersburg, Russia. Comparative Cytogenetics 12(3): p–p. https://doi.org/10.3897/CompCytogen.v12i3.27748

Plenary session

L1

Giant chromosomes and deep sequences: what the amphibian egg tells us about transcription

Joseph G. Gall

Department of Embryology, Carnegie Institution for Science, 3520 San Martin Drive, Baltimore MD 21218 USA (*gall@carnegiescience.edu*)

Lampbrush chromosomes (LBCs) occur in oocytes during prophase of the first meiotic division. They are characterized by extremely large transcription units (TUs), which extend as loops up to 100 mkm or more in length from an axis of condensed chromatin. The length of the TUs reflects the extraordinary close packing of RNA polymerase II molecules along the extended DNA, indicative of an extremely high rate of transcription. LBCs are of very wide occurrence phylogenetically, but are limited to large oocytes in which the oocyte nucleus is the sole source of maternal mRNA. Simple calculations show that a giant cell must either (1) become polyploid or (2) transcribe at an extraordinary rate to supply the needed mRNA in a reasonable time (days or weeks rather than years). Giant somatic cells are almost universally polyploid. Giant oocytes, on the other hand, cannot become polyploid, since they must undergo meiosis. It is probable, therefore, that LBCs represent the means by which a 4C oocyte nucleus can transcribe sufficient mRNA (in a reasonable time) to meet the needs of a giant oocyte.

Within this broad theoretical framework, there remain numerous unanswered questions about the LBC loops. What sequences are transcribed on the loops, as op-

posed to sequences that accumulate as stable mRNA in the cytoplasm? Where precisely are the promoter regions of the genes? Is there read-through transcription, especially on loops that contain two or more TUs? Although splicing is clearly co-transcriptional, are the introns shed from the loops before the end of the TU? What holds the bases of the loops together? What is the relationship, if any, of LBC loops to the TADs defined by Hi-C experiments?

In this lecture I will outline recent studies on LBCs and discuss new experimental approaches that can be applied to answer some of these outstanding questions.

L2

A moving landscape for comparative genomics in mammals

Stephen J. Obrien, Gaik S. Tamazian, Aleksey S. Komissarov, Pavel V. Dobrynin, Ksenia V. Krasheninnikova, Sergey F. Kliver, Nikolay A. Cherkasov, Klaus P. Koepfli

Theodosius Dobzhansky Center for Genome Bioinformatics, St Petersburg State University, 41 Sredniy Prospekt, St. Petersburg, Russia, 199004

Corresponding author: *Stephen J. Obrien* (*lgdchief@gmail.com*)

Today we count some 62,000 species of vertebrates (half are fishes) including some 550 species of mammals on earth. The genome sequencing of non-laboratory species in recent years is expanding our breadth and understanding of genetic bases of adaptation and evolution in varied and amazing ways.

Recent completion and inspection of whole genome sequence and assembly for over 200 species of mammals, from platypus to panda to human, offer the prospect of a better view of the patterns of changes within genome organization across the mammalian radiations. In 2009 my colleagues and I have created Genome-10K, an international consortium of scientist who have set a goal of gathering, sequencing, assembling, and annotating to high quality some 10,000 vertebrate genomes with 2nd and 3rd generation sequencing technology within the coming five years. These activities and advances provide an enormous Bioinformatics challenge whose solution will provide future zoologists of every persuasion a genome sequence resource for their favorite study animal. The applications and potential for the genome sequence in several research questions will be discussed.

Acknowledgements

The study has been supported by the grant from Russian Science Foundation (17-14-01138).

L3

Chromosome abnormalities in domestic bovids: a review

Leopoldo Iannuzzi

Institute for Animal Production System in Mediterranean Environment (*ISPAAM*), *National Research Council* (*CNR*), *via Argine 1085, Naples, 80147, Italy* (*leopiannuzzi949@gmail.com*)

After the discovery of rob(1;29) in the Swedish red cattle breed (Gustavsson and Rockborn 1964) and the demonstration of its deleterious effect on the fertility (Gustavsson 1969, 1971; Dyrendahl and Gustavsson 1979), the cytogenetics applied on domestic animals has been widely expanded in many laboratories to find relationships between chromosome abnormalities and phenotypic effects, especially on fertility. However, in the same years of this discovery, various groups of cytogeneticists published several reports on chromosome abnormalities, primarily involving sex chromosomes, underlining the importance of these types of abnormalities, often responsible for sterility, especially in the females. While numerical autosome abnormalities have been rarely reported, being animal phenotypes abnormal and easily eliminated directly by breeders, numerical sex chromosome abnormalities, as well as structural (and balanced) chromosome anomalies have been more frequently found in domestic bovids because they are often phenotypically invisible for breeders. For this reason, these chromosome abnormalities, without a cytogenetic control, escape selection leading to subsequent deleterious effects on the fertility, especially in females carrying sex chromosome abnormalities. In addition, chromosome abnormalities can be easily spread in the progeny, especially when an artificial insemination is utilized. Certainly, an advent of chromosome banding techniques and application of the FISH-mapping technique with the use of specific molecular markers (generally BAC-clones) and/or chromosome painting probes (Zoo-FISH), proved to be a powerful tool for cytogeneticists in their daily work of identification of the specific chromosomes affected by the abnormalities, especially when the banding pattern resolution is poor (as in many published papers, especially in the past). However, very few groups are actually involved in clinical cytogenetic analysis applied to domestic animal breeding. This problem needs to be addressed because clinical cytogenetics still remains one of the most important aspect of our work, especially for breeders and, in a longer run, for a genetic improvement of the livestock in general. In this brief review I will present a list of the most important chromosome abnormalities found in domestic bovids (mainly in cattle, sheep and river buffalo), and will suggest the strategies for the better detection of these rearrangements in animal populations by employment of efficient and simple banding techniques allowing to speed up the analyses and obtaining desirable results.

Acknowledgements

The study has been supported by the project PON1_486 GENOBU

L4

Viewing nuclear architecture through the eyes of nocturnal mammals

Irina Solovei

Department of Biology II, Biozentrum, Ludwig-Maximilians University Munich, Grosshadernerstrasse 2, 82152 Planegg-Martinsried, Germany (*irina.solovei@lrz.uni-muenchen.de*)

The mammalian nucleus displays spatial segregation of active euchromatic from inactive heterochromatic genomic regions. In conventional nuclei, euchromatin is localized in the nuclear interior, whereas heterochromatin lies at the nuclear periphery. In contrast, rod photoreceptors of nocturnal mammals have inverted nuclei, with a dense heterochromatic core and a thin euchromatic outer shell. This inverted architecture converts rod nuclei into microlenses reducing light scattering in thick nocturnal retinas and thus facilitating vision at scotopic conditions.

The unusual nuclear organization of nocturnal rods with a regular concentric arrangement of different chromatin classes offers a unique model to study various aspects of nuclear architecture. Within the last decade, studies of inverted nuclei helped us to understand several fundamental principles of nuclear architecture:

(i) We have demonstrated that although inverted nuclei are fully functional, inversion is not a favorable chromatin arrangement in mammalian cells (Solovei et al. 2009)
(ii) We have identified two major mechanisms of heterochromatin peripheral tethering that are responsible for establishing the conventional nuclear architecture (Solovei et al. 2013)
(iii) We have shown that the building of a functional nucleus is largely a self-organizing process based on mutual recognition of chromosome segments marked by certain repeats (van de Werken et al. 2017)
(iv) Finally, we have recently demonstrated that interactions among heterochromatic regions are central to phase separation of the active and inactive genome in inverted and conventional nuclei (Falk et al. 2018).

Evolutionary and comparative cytogenetics

L5

Chromosome-specific sequencing in comparative genomics

Malcolm A. Ferguson Smith

Cambridge University Department of Veterinary Medicine, Madingley Road, Cambridge CB3 0ES, UK (*maf12@cam.ac.uk*)

Twenty-five years ago the introduction of chromosome painting revealed chromosome homology between species and provided a powerful method to determine phylogenetic relationships among vertebrates. It showed that genome conservation occurs in large homologous syntenic blocks (HSBs) and that the assembly of HSBs in karyotypes differs between species. Chromosome sorting and next generation sequencing now provides a more precise method for comparative genomics. Sequence reads from chromosome-specific DNA can be aligned to total genome sequence of other species available from the genome database. This reveals chromosome homology at the nucleotide level and defines evolutionary recombination breakpoints. The procedure has helped in the determination of chromosome homology between human and camelids where conventional painting has proved challenging.

L6

Molecular cytogenetics in Zoo-FISH-studies – still urgently needed

Thomas Liehr

Jena University Hospital, Friedrich Schiller University, Institute of Human Genetics, Am Klinikum 1, D-07747 Jena, Germany (*Thomas.Liehr@med.uni-jena.de*)

Evolution can be observed, e.g. in different species of the same order or family, on different levels of resolution. Here I want to highlight the importance of all available approaches; chromosomal studies are the bases for all higher-resolution based approaches. Knowledge about chromosome number of a species, basic structure of chromosomes and (molecular) cytogenetically detectable gross chromosomal rearrangements compared to an outgroup is necessary first. Without that, wrong conclusions will be drawn from high resolution molecular data. This is among others due to the fact that high throughput sequencing still has problems in correct alignment of repetitive sequences.

L7

Evolutionary sex chromosome translocations in amniotes

Vladimir A. Trifonov[1,2], Artem P. Lisachov[3], Ilya G. Kichigin[1], Alexey I. Makunin[1], Jorge C. Pereira[4], Anna S. Druzhkova[1], Malcolm A. Ferguson-Smith[4], Massimo Giovannotti[5]

1 *Institute of Molecular and Cellular Biology, Russian Academy of Sciences, Siberian Branch, Novosibirsk 630090, Russia* **2** *Novosibirsk State University, Novosibirsk 630090, Russia* **3** *Institute of Cytology and Genetics, Russian Academy of Sciences, Siberian Branch, Novosibirsk 630090, Russia* **4** *Cambridge Resource Centre for Comparative Genomics, Department of Veterinary Medicine, University of Cambridge, Madingley Road, Cambridge*

CB3 0ES, UK **5** *Dipartimento di Scienze della Vita e dell'Ambiente, Università Politecnica delle Marche, via Brecce Bianche, 60131 Ancona, Italy*

Corresponding author: *Vladimir Trifonov* (*vlad@mcb.nsc.ru*)

High diversity of sex determination mechanisms in reptiles versus conserved sex chromosomes in therian mammals and birds is usually explained by a rapid turnover of sex determining pathways in cold-blooded vertebrates. However, pleurodont lizards represent an interesting exception, where an unusually conserved system of sex chromosomes has originated over 160 million years ago. Opposite to mammals and birds, where sex chromosomes only rarely translocate on autosomes, the ancestral sex chromosomes of dactyloid lizards have undergone fusions with one or several microchromosomes in different lineages, thus representing an interesting model to study the evolution of autosomal blocks after their transposition to sex chromosomes. Here, using low pass sequencing chromosome specific probes, we study the genetic content and evolution of sex chromosomal systems in several lineages of anoles (Dactyloidae). We demonstrate that autosomal elements undergo partial degeneration and accumulate specific repeated elements after their fusion with sex chromosomes. We postulate that the translocation of autosomal blocks onto sex chromosomes may have facilitated rapid degeneration of the pseudoautosomal region on the ancestral Y. The enrichment in repetitive DNA in chromosome specific DNA pools may serve as an indication of sex chromosomes in species with cytologically indistinguishable micro-sex chromosomes.

Acknowledgements

The work was financially supported by RSF grant No. 16-14-10009.

O1

Comparative genomics through the development of universal cross-species BAC sets

Rebecca E. O'Connor[1], Marta Farré[2], Lucas Kiazim[1], Rebecca Jennings[1], Joana Damas[2], Denis M. Larkin[2], Darren K. Griffin[1]

1 *University of Kent, Canterbury, CT2 7NJ, UK* **2** *Royal Veterinary College, University of London, London NW1 0TU, UK*

Corresponding author: *Rebecca E. O'Connor* (*ro215@kent.ac.uk*)

Comparative genomics using targeted BAC based FISH (fluorescence *in situ* hybridization) probes, is generally restricted to closely related species due to extensive sequence divergence between distantly related species. The ability to identify precise regions of

homology between species (for genome mapping, phylogenomics and genome organization studies) and the ability to anchor genomic sequence data to chromosomes (for chromosome assembly) is therefore restricted.

To overcome these difficulties, we developed a set of universal avian BAC probes, selected through the identification of evolutionary conserved regions. This BAC set was then used to upgrade the genomes of 5 avian species to a chromosome-level. Successful hybridisation of these probes to a further ~30 avian species revealed genome-wide patterns of chromosome stability and rearrangement between species. In addition, the probes successfully hybridized on non-avian reptile species (turtles and anole lizard) revealing a level of genome conservation extending far beyond birds.

Further, we applied the approach developed for avian probes to the selection of BACs from the cattle and human genome with the aim of generating a universal mammalian BAC set. Selection criteria were validated by testing probes on species at key nodes of the phylogenetic tree. Hybridisations were achieved on species as diverse as horse *Equus ferus* Boddaert, 1785, dolphin *Tursiops aduncus* Ehrenberg, 1833, bat *Lophostoma silvicolum* D'Orbigny, 1836 and lemur *Eulemur macaco* Linnaeus, 1766.

These preliminary results illustrate that our combined FISH-bioinformatics approach is also applicable to mammals. Development of a universal BAC set therefore permits cross-species sequence anchoring and comparative genomic research at a higher resolution than previously possible, providing new insight into the nature of genomic evolution and genomic stability.

O2

Pinniped karyotype evolution substantiated by comparative chromosome painting of 10 pinniped species (Pinnipedia, Carnivora)

Violetta R. Beklemisheva[1], Polina L. Perelman[1,2], Natalia A. Lemskaya[1], Anastasia I. Kulemzina[1], Anastasia A. Proskuryakova[1,2], Vladimir N. Burkanov[3,4], Steven J. O'Brien[5,6], Alexander S. Graphodatsky[1,2]

1 *Department of Comparative Genomics, Institute of Molecular and Cellular Biology, SB RAS, Acad. Lavrentiev Ave. 8/2, Novosibirsk, 630090, Russia* **2** *Novosibirsk State University, 1, Pirogova str., Novosibirsk, 630090, Russia* **3** *Department of Higher Vertebrates Ecology, Kamchatka Branch of Pacific Geographical Institute FEB RAS, 6, Partizanskaja str., Petropavlovsk-Kamchatski, 683000, Russia* **4** *National Marine Mammal Laboratory, AFSC, NMFS, 7600 Sand Point Way N.E., Building 4 Seattle, Washington, 98115, USA* **5** *Theodosius Dobzhansky Center for Genome Bioinformatics, 41A, Sredniy Av., St. Petersburg State University, St. Petersburg, 199034, Russia* **6** *Oceanographic Center, Nova Southeastern University, 3301 College Avenue, Fort Lauderdale, Florida 33314-7796, USA*

Corresponding author: *Violetta R. Beklemisheva* (*bekl@mcb.nsc.ru*)

Numerous Carnivora karyotype evolution investigations have been performed by classical and molecular cytogenetics and were supplemented by reconstructions of the Ancestral Carnivora Karyotype (ACK). However, the group of Pinnipedia was not studied in detail. Here we reconstruct pinniped karyotype evolution and refine ACK using published and our new painting data for 10 pinniped species. The combination of human (HSA) and domestic dog (CFA) whole-chromosome painting probes was used for the construction of the comparative chromosome maps for species from all three pinniped families: Odobenidae – *Odobenus rosmarus* Linnaeus, 1758, Phocidae – *Phoca vitulina* Linnaeus, 1758, *Pusa sibirica* Gmelin, 1788, *Erignathus barbatus* Erxleben, 1777, *Phoca largha* Pallas, 1811, *Phoca hispida* Schreber, 1775 and Otariidae – *Eumetopias jubatus* Schreber, 1775, *Callorhinus ursinus* Linnaeus, 1758, *Phocarctos hookeri* Gray, 1844, *Arctocephalus forsteri* Lesson, 1828. HSA and CFA autosome painting probes have delineated 32 and 68 conservative autosome segments in the studied genomes. The comparative painting in Pinnipedia supports monophyletic origin of pinnipeds, shows that pinniped karyotype evolution was characterized by slow rate of genome rearrangements (less then one rearrangement per 10 million years), provides strong support for refined structure of ACK with 2n = 38 and specifies plausible order of dog chromosome synthenic segments on ancestral Carnivora chromosomes. The heterochromatin, telomere and ribosomal DNA distribution was studied in all 10 species.

Acknowledgements

The work was financially supported by RFBR №17-00-00146, RSF №16-14-10009.

O3

X chromosome evolution in Cetartiodactyla

Anastasia A. Proskuryakova[1,2], Anastasia I. Kulemzina[1], Polina L. Perelman[1,2], Alexey I. Makunin[1], Natalya A. Lemskaya[1], Violetta R. Beklemisheva[1], Denis M. Larkin[3], Marta Farre[3] Anna V. Kukekova[4], Oliver A. Ryder[5], Stephen J. O'Brien[6], Alexander S. Graphodatsky[1,2]

1 *Institute of Molecular and Cellular Biology, SB RAS, Lavrentjeva ave 8/2, Novosibirsk, 630090, Russian Federation* **2** *Novosibirsk State University, Pirogova str. 1, Novosibirsk, 630090, Russian Federation* **3** *The Royal Veterinary College, Royal College Street, University of London, London NW1 0TU, UK* **4** *The University of Illinois at Urbana-Champaign, W. Gregory Drive Urbana 1207, Illinois, USA* **5** *Zoological Society of San Diego, Conservation and Research for Endangered Species, San Pasqual Valley Road 15600, Escondido, CA 92027, USA* **6** *Theodosius Dobzhansky Center for Genome Bioinformatics, Saint-Petersburg State University, Sredniy Av. 41A, Saint-Petersburg, 199034, Russia*

Corresponding author: *Anastasia A. Proskuryakova* (*andrena@mcb.nsc.ru*)

The mammalian X chromosome is characterized by high level of conservation. On the contrary the Cetartiodactyl X chromosome displays variation in morphology and G-banding pattern. It is hypothesized that X chromosome has undergone multiple rearrangements during Cetartiodactyla speciation. To investigate the evolution of this sex chromosome we have selected 26 BAC clones from cattle CHORI-240 library evenly distributed along the cattle X chromosome. High-resolution maps were obtained by fluorescence *in situ* hybridisation in a representative range of cetartiodactyl species from different families: pig (Suidae), gray whale (Eschrichtiidae), pilot whale (Delphinidae), hippopotamus (Hippopotamidae), Java mouse deer (Tragulidae), pronghorn (Antilocapridae), Siberian musk deer (Moschidae), giraffe (Giraffidae). To trace the X chromosome evolution during fast radiation in speciose families, we mapped more than one species in Cervidae (moose, Siberian roe deer, fallow deer and Pere David's deer) and Bovidae (musk ox, goat, sheep, sable antelope, nilgau, gaur, saola, and cattle). We have identified three major conserved synteny blocks and based on this data reconstructed the structure of putative ancestral cetartiodactyl X chromosome. We demonstrate that intrachromosomal rearrangements such as inversions and centromere reposition are main drivers of cetartiodactyl's chromosome X evolution.

Acknowledgements

The work was supported by the Russian Science Foundation (RSF #16-14-10009).

Cytogenetics and genomics of domestic animals

L8

Copy number variations in cattle and pigs: aging and reproduction

W. Allan King[1,2], Olutobi Oluwole[1], Tamas Revay[1]

1 *Department of Biomedical Sciences, University of Guelph, 50 Stone rd. Guelph, ON, Canada N1G2W1* **2** *Karyotekk Inc, University of Guelph, 50 Stone rd. Guelph, ON, Canada N1G2W1*

Corresponding author: *W. Allan King* (*waking@uoguelph.ca*)

Variability in genomes (including single nucleotide polymorphisms (SNP), copy number variations (CNV) and chromosomal rearrangements (CA)) is responsible for a significant proportion of the diverse phenotypes associated with many important traits including fertility and ageing. We have investigated copy number variation in Canadian Holstein bulls and Yorkshire, Landrace and Duroc boars. In bulls we studied *de novo* CNVs from two perspectives: somatic variability and ageing. In boars we

compared CNVs between those with very high or very low DBE (direct boar effect) on litter size.

Somatic tissues (blood, lung, heart, muscle, testis, brain) from four Holstein bulls were sampled, arrayed on the Bovine SNP50k chip and genome-wide CNVs analyzed from the probe intensity data. The results showed extensive copy number divergence among tissues of the same animal.

We have detected a median 31 CNVs per animal which were classified as either germ line origin (median 14), as they were constantly present in all investigated tissues of the animal or *de novo* somatic (median 18), those specific to one tissue. Thus, 57% of the total number of detected CNVs was the result of *de novo* somatic events. Additionally, CNVs from blood of 8 bulls at 3 different time points (14±3, 32±3, and 44±3 month of age) were examined, in just under 3 years *de novo* CNVs were generated in almost equal number to the number present at the start and throughout the study (107 vs. 111 or 49% vs. 51%, respectively), which were regarded as constant CNVs. Also, twice as many *de novo* CNVs emerged during the second half of the sampling schedule as in the first part (a total of 71 vs. 36, respectively).

Two groups of boars were selected from the upper and lower 10% of DBE value distribution of more than 38,000 animals and genotyped with the Porcine SNP60K chip. The CNV analysis identified 35 CNVs covering 36.5 Mb or ~1.3% of the porcine genome that were specific to the high fertility group (14) or to the low fertility group (19). These CNVs overlapped with 137 QTLs of reproductive traits and also with 50 genes significantly enriched in members of the innate immune system, various receptor signaling pathways and fatty acid oxidation. These genomic regions and physiological functions could be connected to fertility, thus might represent putative markers.

The results suggest that genome of cattle and pigs is dynamic and in constant change. Changes such as copy number variation are associated with fertility and aging.

Acknowledgements

Research support was obtained from NSERC, Agriculture and Agri-Food Canada, Ontario Ministry of Agriculture and Rural Affairs, and the Canada Research Chairs program.

L9

Whole genome genotyping and sequencing reveal history and signatures of adaptations in genomes of Russian native cattle breeds

Denis M. Larkin[1,2]

1 *The Federal Research Center Institute of Cytology and Genetics, The Siberian Branch of the Russian Academy of Sciences, Prospect Lavrientieva 10, Novosibirsk, 630090, Russia* **2** *Royal Veterinary College, University of London, 4 Royal College Street, NW1 0TU, London, UK* (*dmlarkin@gmail.com*)

In Russia ~20 native cattle breeds are currently being recognized. We focused on revealing history of their formation and signatures of genomic selection. We genotyped 274 animals from 18 Russian breeds on the GGP HD150K and 50K Bovine SNP arrays and combined the data with additional 135 cattle breeds. The data were clustered to reveal relationship between the breeds. Signatures of selection were detected using the hapFLK software and the de-correlated composite of multiple signals framework combining the FST, H1, H12, Tajima's D, nucleotide diversity statistics. To reveal specific nucleotide variants that could be related to economically important traits and adaptation to extreme environment, the two most distant breeds (Yakut, Kholmogory) were sequenced (40 animals) and signatures of selection/adaptation were detected. Four major clusters involving Russian breeds were identified. Yakut cattle were the most divergent amongst all taurine breeds in our dataset. The strongest signals of selection in Russian cattle were detected near the genes: LCORL/NCAPG, HMGA2, IMPAD1 (growth), KIT (coat colour), PLAG1 (reproduction). We further detected signatures of selection related to domestication (KITLG, EDN3, COPA), feed intake (XKR4, TMEM68), milk production (DGAT1, GHR, ABCG2, GLI2, LAP3, TRPV5, FKBP2), and reproduction (CSF2, BCL2, ANXA10, NPBWR1). Strong candidate genes for adaptation to cold climate and local environment were found under selection in the Yakut cattle: RETREG1, RPL7, TNKS, Kholmogory: AQP5, and multiple Russian breeds: ARRDC3, RAD50, SYK. Multiple regions under selection identified in the set of Russian native cattle breeds form a basis for future genomics-based selection and targeted breeding of Russian cattle.

Acknowledgements

This study was supported by the grant from the Russian Science Foundation (project no. 16–14-00090).

O4

An innovative method of gene co expression network inference reveals significant biological processes involved in fetal porcine muscle development

Maria Marti-Marimon[1], Nathalie Villa-Vialaneix[2], Valentin Voillet[1], Martine Yerle-Bouissou[1], Laurence Liaubet[1], Yvette Lahbib-Mansais[1]

1 *GenPhySE and* **2** *MIAT, University of Toulouse, INRA Occitanie-Toulouse, ENVT, 24 chemin de Borde-rouge 31326 Castanet Tolosan, France*

Corresponding author: *Yvette Lahbib-Mansais* (*yvette.lahbib-mansais@inra.fr*)

The integration of genetic information in the cellular and nuclear environments is crucial for deciphering how the genome functions in physiological conditions. By combining 3D nuclear mapping, high-flow transcriptomic data analyses, and statistical methods for the development of co-regulated gene networks, it becomes possible to develop an integrated approach to depict the regulation of gene expression. For this purpose, we focused on the mechanisms involved in the transcriptional regulation of genes expressed in muscle during late fetal development in pig (90 and 110 days), a critical period for survival. We published a muscle transcriptomic analysis performed during this perinatal period (Voillet et al. 2014). Data from this previous study obtained from two extreme genetic lines in terms of mortality at birth (Large White and Meishan), were used to construct networks of differentially co-expressed genes. As co-expressed genes are not necessary related to a common biological process, we used information of gene co-localizations (3D DNA FISH) to reinforce observed links in the co-expressed gene network. The innovative network inference method developed, sequentially incorporates biological knowledge on gene spatial co-localization to construct robust networks gathering co-regulated genes. Clustering of nodes (genes) becomes more and more biologically consistent in each iteration. Interestingly, by means of the final network, we particularly uncovered unexpected gene associations in the nuclear space between IGF2 and MYH3 suggesting that they could be subject to similar transcriptional regulation.

O5

Assessment of DNA damage in a rare case of ewe-buck hybrid using the comet assay

Alfredo Pauciullo[1], Georg Erhardt[2], Liliana Di Stasio[1], Isabel Wiedemann[3], Christoph Knorr[3]

1 *University of Turin, Department of Agricultural, Forest and Food Sciences, Largo Paolo Braccini 2, Grugliasco* (*To*), *Italy* **2** *Justus-Liebig-University of Giessen, Institute of Animal Breeding and Genetics, Ludwigstrasse 21b, Giessen, Germany* **3** *Georg-August-University, Department for Animal Science, Biotechnology and Reproduction of Livestock, Burckhardtweg 2, Göttingen, Germany*

Corresponding author: *Alfredo Pauciullo* (*alfredo.pauciullo@unito.it*)

Over the past decade, the Comet assay has been used in humans to study processes ranging from DNA repair to genotoxicity, mainly to assess the adverse effects of toxins on DNA. In contrast to humans, there are only few reports on comet assay in livestock. In the present study, we assessed the Comet assay to investigate the genome stability and detect primary DNA damages in a very rare case of an ewe-buck hybrid (2n=57, XX). Three

contemporary sheep (breed Leineschafe) (2n=54, XX) and three goats (Weiße Deutsche Edelziege) (2n=60, XX) belonging to the same flock were used as controls.

Blood samples were collected in K_2EDTAtubes. Comet assays (under alkaline conditions) were performed as described by Singh et al. (1988). Shortly, $1x10^5$ lymphocyte cells/mL were combined with molten LMAgarose (at 37 °C) at 1:10 (v/v) ratio. Alkaline electrophoresis was performed at 4 °C for 30 min at 300mA, followed by staining with SYBR® Green I. The slides were observed at 200x magnification with a Nikon Eclipse 80i fluorescence microscope equipped with FITC specific filter and provided with a LAS image-analysis system. Two slides per sample were analyzed and a total of 50 individual cells per animal were randomly chosen and screened per subject (25 cells from each slide). OpenComet software was used for scoring the comet characteristics, including tail length, tail moment, head area.

Departures from symmetry ($P<0.05$) were observed for each data distribution. Therefore, non-parametric tests (Kruskal-Wallis and Dunn) with Bonferroni correction were employed for the statistical analysis. Differences between means were considered statistically significant for $P<0.05$.

The hybrid animal showed significantly greater mean DNA tail length (16.42 ± 3.73 μm) than sheep ($P<0.0001$) and goat ($P<0.025$). In addition, the hybrid's tail moment (product of the tail length and the fraction of total DNA in the tail) was higher (4.76±2.68) compared to the sheep ($P<0.0001$) and goat ($P<0.010$). By contrast, the mean value of the head area (7095 ± 248 μm^2), was intermediate between sheep ($P<0.0001$) and goat ($P<0.0001$) was detected. The data reveal a higher DNA damage of the hybrid animal that is most likely due to the nature of its genetic diversity, especially as we consider the absence of the main environmental effects among the investigated animals (similar age, flock, feeding system). Reference data are not available to compare our analysis with the literature. Therefore, further investigations (more data from the comet assay and additional tests like SCE, CA, etc.) are necessary for deeper understanding the stability of the hybrid genome.

O6

Routine diagnostics in companion animals by digital PCR

Darja Krylova

Independent Veterinary Laboratory "Poisk", Repisheva street 13, Saint-Petersburg, Russia (*amenshenin1975@mail.ru*)

Some of the small domestic animal diseases were diagnosed until recently only by qualitative or semi-quantitative PCR methods. Furthermore, effectiveness of a given kind of treatment was evaluated by clinical scores, and sometimes it was affected by comorbidities. Development of the digital PCR method made it possible to precisely control the

influence of certain treatment mode on disease state at the molecular level. The benefits of using digital PCR are obvious as this method would allow to correct the chosen mode of treatment to make it more effective. We applied digital PCR method to quantitatively diagnose the disease state of *Feline leukemia virus* Jarrett, 1964, and *Mycoplasma haemofelis* Neimark, 2002. The effectiveness of treatment modes was evaluated by comparison of quantitative PCR data. The digital PCR method was applied to quantitatively diagnose Familiar Shar-Pei Fever as diagnostic test that might by useful to breeders.

Cytogenetics of meiotic cells

L10

Contributions of synaptonemal complex studies to avian cytogenetics

María Inés Pigozzi

Universidad de Buenos Aires, Facultad de Medicina, Paraguay 2155, Buenos Aires, Argentina (*mpigozzi@fmed.uba.ar*)

More than 40 years ago, the synaptic nature of the ZW pair in birds was established looking at the synaptonemal complexes in chicken oocytes. The ZW pair forms a synaptonemal complex with lateral elements of unequal lengths. The small pseudoautosomal region in the chicken remains elusive to full sequence characterization, but Z-autosome translocations and recombination nodules analyses at pachytene showed that this region comprises the terminal segment of Zp and Wp. Unlike mammals, the pseudoautosomal regions of birds are extremely variable in size, spanning over 80% of the W chromosome in the primitive ratites. The chromatin of the sex bivalent does not show signs of heteropicnosis during pachytene and, therefore condensation is not required for the pairing of the mainly heterologous Z and W chromosomes of Neognathae. Evidence comprising expression analysis of Z-linked genes and mapping of histone modifications seems to confirm that the chromatin of the ZW pair is not subject to meiosis-specific condensation and silencing in chicken oocytes. Nonetheless, this issue needs more comprehensive analyses using pure fractions of oocytes to measure gene expression of Z-linked genes vs. autosomal genes at specific meiotic stages. Recombination nodules and MLH1 focus mapping in whole pachytene nuclei revealed less interspecific variations of global recombination rates in birds. The mean recombination rate of the birds studied so far is in the range of 1.7–2.6 cM/Mb, while in eutherian mammals it is 0.5–1.1 cM/Mb. The differential organization of the chromatin loops along the synaptonemal complexes in birds and mammals might provide a structural basis for these differences in recombination levels. The changing morphology of the ZW pair in the chicken and other birds with highly differentiated sex chromosomes might be an excellent model to study the dynamics of synaptonemal complex proteins and its relationships with chromatin. Also,

the spatial arrangement of recombination nodules particularly found in birds and their relationship with synaptonemal complex components could be analyzed in depth using more recent approaches, such as super-resolution microscopy in combination with immunolocalization.

O7

Upgrade sperm FISH analysis of meiotic segregation in a river buffalo bull carrier a rob(1p;18)

Alessandra Iannuzzi[1], Chiara Di Dio[1], Angela Perucatti[1], Anna Caputi Jambrenghi[2], Francesco Giannico[2], Alfredo Pauciullo[3], Pietro Parma[4], Leopoldo Iannuzzi[1]

1 *Institute for Animal Production System in Mediterranean Environment* (*ISPAAM*), *National Research Council* (*CNR*), *via Argine 1085, Naples, 80147, Italy* **2** *Department of Animal Productions, Agricultural Faculty of Sciences, University of Bari, Campus Universitario „Ernesto Quagliariello", 70126 Bari – Italy* **3** *Dept. Agricultural, Forest and Food Sciences* (*DISAFA*), *University of Turin, Largo Paolo Braccini, 2 – 10095 GRUGLIASCO* (*TO*), *Italy* **4** *Dep.of Animal Science, Agricultural Faculty of Sciences, Milan, Via Celoria, 2 – 20133 Milan, Italy*

Corresponding author: *Alessandra Iannuzzi* (*alessandra.iannuzzi@cnr.it*)

An upgrade by triple-colour fluorescence *in situ* hybridization (FISH) in River Buffalo sperms was performed on chromosomes involved in the translocation (BBU1 and BBU18) using three different pools of specific bovine BAC (INRA library) probes, mapping in BBU1q, BBU1p and BBU18 (homologous to BTA1, BTA27 and BTA18, respectively). The meiotic segregation pattern was examined in the carrier and in the control counting 2.500 total sperms and 2.500 total motile sperms on both animals. The results revealed different frequencies of normal and chromosomally balanced sperms (alternate group) as follows: 24.1% and 11.4% (total of 35.5%) of total sperms in the carrier, while in the control there were 97.1% and 0.4% (total of 97.8%) respectively; 58.4% and 22.2% (total of 80.6%) of motile sperms in the carrier, while in the control there were 93.6% and 3.3% (total of 96.1%) respectively. These data have shown the increase of percentage in alternate group of motile sperms fraction that represents the real sperm population able to fertilize an oocyte. This result revealed a connection between sperm motility and DNA distribution, underling the importance of Sperm-FISH analysis in reproducers.

Acknowledgments

The research was supported by Project PON1_486-GENOBU.

O8

The plasticity of meiotic recombination and its implications for mammalian genome evolution

Aurora Ruiz-Herrera[1,2], Laia Capilla[1], Covadonga Vara[1,2], Guillermo Albert-Lizandra[1,2], Jeremy B. Searle[3], Jacint Ventura[4]

1 *Genome Integrity and Instability Group, Institut de Biotecnologia i Biomedicina, Universitat Autònoma de Barcelona, Campus UAB, 08193, Cerdanyola del Vallès, Barcelona, Spain* **2** *Departament de Biologia Cel.lular, Fisiologia i Immunologia, Universitat Autònoma de Barcelona, Campus UAB, 08193, Cerdanyola del Vallès, Barcelona, Spain* **3** *Department of Ecology and Evolution, Corson Hall, Cornell University, Ithaca, NY 14853-2701, USA* **4** *Departament de Biologia Animal, Biologia Vegetal i Ecologia, Universitat Autònoma de Barcelona, Campus UAB, 08193, Cerdanyola del Vallès, Barcelona, Spain*

Corresponding author: *Aurora Ruiz-Herrera* (*aurora.ruizherrera@uab.cat*)

Homologous chromosomes exchange genetic information through recombination, a process that increases genetic diversity and is fundamental to eukaryote organisms with sexual reproduction. A general appreciation of how this genetic variation is generated and maintained across different phylogenetic groups is relevant to our understanding of biodiversity. In an evolutionary framework, meiotic recombination can be modulated by genome reshuffling, thus contributing to chromosomal evolution. However, few empirical data are available regarding the mechanisms by which genome shuffling shapes recombination, especially in mammals. In this context, zones of chromosomal polymorphism of the Western European house mouse, *Mus musculus domesticus* Linnaeus, 1758, represent a natural model to study such processes. Here we present an empirical study on the impact of chromosomal fusions on genome-wide recombination in mice from wild populations by combining SNPs genotyping data and cytological analysis from meiotic crossovers. We detected a reduction in crossover frequencies in reorganized chromosomes when compared with acrocentric chromosomes. This observation was consistent with the higher genomic divergence detected in pericentromeric regions when compared to telomeric regions being suggestive of differential levels of gene flow associated to chromosomal fusions. Overall, we provide evidence for the contribution of genome reshuffling in modulating recombination landscapes.

O9

Interspecies gynogenesis – a way to avoid contamination with radiation induced paternal chromosome fragments?

Konrad Ocalewicz[1], Stefan Dobosz[2], Krzysztof Jagiełło[1], Marcin Polonis[1]

1 *University of Gdansk, Institute of Oceanography, Department of Marine Biology and Ecology, M. Piłsudskiego 46 Av., 81-378 Gdynia, Poland* **2** *Inland Fisheries Institute in Olsztyn, Department of Salmonid Research, 83-330 Żukowo, Poland*

Corresponding author: *Konrad Ocalewicz* (*konrad.ocalewicz@ug.edu.pl*)

Gynogenetic doubled haploids (DHs) as fully inbred fish are useful in developmental biology and aquaculture research. However, it has been noticed that incomplete UV-induced inactivation of the sperm nuclear DNA may result in "contamination" of the gynogenetic specimens with the radiation-induced chromosome fragments. Presumably, such fragments could be eliminated when gametes used for the induced gynogenesis originate from species whose hybrid offspring are lethal due to the genome incompatibility. In the present research gynogenetic development of rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792), brook trout (*Salvelinus fontinalis* Mitchill, 1814) and brown trout (*Salmo trutta* Linnaeus, 1758) was induced using UV irradiated homologous and heterologous spermatozoa. Eggs inseminated with the irradiated sperm were then subjected to high hydrostatic pressure (HHP) shock to inhibit the first cell cleavage and duplicate haploid sets of maternal chromosomes. Produced DH individuals were cytogenetically studied to confirm their ploidy and to detect any karyotypic abnormalities. Survival of gynogenetic DH embryos developing in eggs inseminated with irradiated homologous and heterologous sperm was comparable. Increased mortality among interspecies gynogenotes was observed after hatching. Cytogenetic analysis of DHs confirmed diploid chromosome complements for the studied egg donor species. No chromosome fragments – residues of the UV irradiated paternal genome were found in cells of interspecies gynogenetic DHs. It might be assumed that individuals with UV-irradiated chromosome fragments from heterologous spermatozoa were preferentially lost that could partially explain lower survival of the interspecies gynogenotes when compared to fish that hatched from eggs activated by the irradiated homologous sperm.

Alphabetical cytogenetics: B-, X-, Y-, W-, Z- etc. chromosome analysis

L11

Dosage-sensitive regulators are preferentially retained on vertebrate Y and W chromosomes

Daniel W. Bellott[1], Helen Skaletsky[1,3], Sahin Naqvi[1,2], Jennifer F. Hughes[1], David C. Page[1,2,3]

1 *Whitehead Institute, 455 Main Street, Cambridge, Massachusetts, USA* **2** *Department of Biology Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, MA, USAn* **3** *Howard Hughes Medical Institute, Whitehead Institute, 455 Main Street, Cambridge, MA, USA*

Corresponding author: *Daniel W. Bellott* (*bellott@wi.mit.edu*)

Across vertebrate lineages, sex chromosomes have repeatedly evolved from ordinary autosomes. In mammals, males are XY and females are XX, while in birds and snakes, males are ZZ and females are ZW. In each of these three lineages, a different set of ancestral autosomes evolved into highly differentiated sex chromosomes. This forms a natural experiment where the autosomes in two lineages serve as a control for the effects of sex chromosome evolution in the third. Sex-specific Y and W chromosomes have experienced extensive genetic decay, and the survivors are enriched for dosage-sensitive regulators of key cellular processes that are broadly expressed throughout adult tissues as well as developmental time. Moreover, survivors of W-decay in both birds and snakes are enriched for human orthologs implicated in congenital disorders caused by heterozygous loss-of-function mutations. We infer that the correct dosage of surviving Y and W genes is likely essential for the survival of the heterogametic sex. We also observe that ancestral differences in dosage sensitivity shaped the evolution of dosage compensation on X and Z chromosomes. Among genes without a surviving Y or W homolog, those that evolved dosage compensation are more sensitive to both under-expression and over-expression than those that did not. Studying the survival of genes on Y and W chromosomes, as well as the evolution of dosage compensation on X and Z chromosomes, may provide insights into key dosage sensitive genes and processes relevant to human health and disease.

L12

Evolution of W and Z sex chromosomes in moths and butterflies

František Marec

Biology Centre CAS, Institute of Entomology, Branišovská 31, 370 05 České Budějovice, Czech Republic (*marec@entu.cas.cz*)

Moths and butterflies (Lepidoptera) have sex chromosome systems with female heterogamety (WZ/ZZ or derived variants). Results of fluorescence *in situ* hybridization (FISH) with genomic, W-chromosome painting, and BAC (bacterial artificial chromosome) probes along with available sequence information suggest that lepidopteran W chromosomes are almost completely composed of repetitive sequences. The W chromosomes evolve rapidly, and their molecular composition differs considerably even between closely related species, as we have recently shown in the magpie moth, *Abraxas grossulariata* Linnaeus, 1758, an iconic species in which the female heterogamety was discovered, and its congeneric species *A. sylvata* Scopoli, 1763. On the contrary, Z chromosomes are highly conserved in Lepidoptera, as demonstrated by synteny mapping of Z-linked genes between distant species using BAC-FISH and linkage analyses. The W chromosome is an evolutionary novelty in Lepidoptera, as it is absent in the sister order Trichoptera (caddisflies) and in primitive moths such as Micropterigidae. Our recent data on the W presence/absence in lower Lepidoptera, together with conserved synteny of Z-linked genes, suggest the multiple origin of the W chromosome, although its single origin followed by repeated losses cannot be ruled out. Based on these new data, we have revised the hypothesis on the origin of the W chromosome. However, in a relatively large number of species, the WZ pair was altered by fusion with an autosome pair, resulting in neo-sex chromosomes or multiple sex chromosomes, if the fusion occurred in only one sex chromosome. Extreme cases are wood white butterflies of the genus *Leptidea* Billberg, 1820, with 3-4 W chromosomes and 3-6 Z chromosomes. We have established a battery of genomic tools in *L. juvernica* Williams, 1946, such as the transcriptome sequence, BAC library, and array-CGH. Here I demonstrate the application of these tools for the identification of sex-linked genes and genomic regions to determine the origin of multiple sex chromosomes in Leptidea species.

O10

B chromosomes in the light of functional analyses

Cesar Martins[1], Rafael T. Nakajima[1], Bruno E. Fantinnati[1], Diego F. Marques[1], Natália B Venturelli[1], Maryam Jehangir[1], Erica Ramos[1], Jordana Oliveira[1], Syed F. Ahmad[1], Luiz A. Bovolenta[1], Adauto L. Cardoso[1], Guilherme T. Valente[1]

1 *Sao Paulo State University* (*UNESP*), *Institute of Biosciences, Department of Morphology, 18618-689, Botucatu, SP, Brazil* **2** *Sao Paulo State University* (*UNESP*), *Agronomic Science School, Department of Bioprocess and Biotechnology, 18610-307, Botucatu, SP, Brazil*

Corresponding author: *Cesar Martins* (*cmartins@ibb.unesp.br*)

Supernumerary B chromosomes (Bs) are enigmatic dispensable elements extensively characterized in diverse eukaryote taxa including fungi, animals and plants. The

current concept on Bs states they are maintained and propagated by a parasitic-drive mechanism during cell cycle. Nevertheless, the molecular mechanism that governs the drive and other possible effects of B presence is still poorly understood. In this way, we have analyzed B chromosomes of the cichlid fish *Astatotilapia latifasciata* Regan, 1929, in the light of massive DNA and RNA sequencing, FISH-mapping, immunocytogenetics, meiotic transmission, RT-qPCR and epigenomics analysis in order to advance the undrestanding of the B chromosome biological role. We have detected thousands of gene fragments and few largely intact genes in the B chromosome genomic content. Furthermore, differentially expressed transcripts were detected in the presence of B chromosome, they might be transcribed by the B genome or emerge from the A genome under the B chromosome influence. Several of these transcribed sequences, including mRNAs, microRNAs and a possible lncRNA (BncRNA), presented a biased differential expression in the females carrying Bs. In the same way, our results demonstrated that the B chromosome may influence the transcription of DNA modification genes and lncRNAs with consequent epigenetic changes over the cell division regulation machinery. Meiotic analysis indicates a higher drive of Bs in females compared to males in *A. latifasciata* offspring. The integrated view of our data demonstrates B chromosomes can influence cell biology in a complex way, (i) favoring their own maintenance and perpetuation through the cell cycle and also (ii) influencing other important biological features like development and sex determination.

Acknowledgements

The work was financially supported by FAPESP 2013/04533-3; 2015/16661-1; CNPq 474684/2013-0;305321/2015-3.

Clinical cytogenetics

O11

Reciprocal copy number variations and new chromosomal diseases

Igor N. Lebedev

Research Institute of Medical Genetics, Tomsk National Research Medical Center, RAS, Naberezhnaya. r. Ushaiki, 10, Tomsk, 634050, Russia (*igor.lebedev@medgenetics.ru*)

Understanding the origin of chromosomal diseases often depends on the development of cytogenetic techniques that provide the precise detection of a wide range of chromosomal abnormalities. At the same time, the progress in investigation the molecular organization and variability of the human genome makes it possible to predict the appearance of recurrent chromosomal aberrations in distinct chromosomal regions. Reciprocal Copy Num-

ber Variations (CNVs) is a clear example of uniting theoretical and technological trends in modern human cytogenetics. Genomic sister-disorders were introduced recently as a novel class of chromosomal diseases mediated by duplications versus deletions of the same chromosomal region (Crespi et al. 2009). Currently, more than 60 chromosomal regions in human genome are linked to this type of disease. There is a growing list of reports about new microduplications, or even triplications, quadruplications, etc. for chromosomal regions previously known exclusively for microdeletion syndromes. The comparison of clinical features of patients with reciprocal CNVs provides better understanding of genotype-phenotype correlations for chromosomal diseases. As a result, mirrored, overlapped and unique phenotypes were designated for genomic sister-disorders. It is important, that this type of correlations is associated not only with gene content but also with the effect of chromosome rearrangement. Recently, we demonstrated a significant reduction in the level of CNTN6 expression in neurons differentiated from induced pluripotent stem cells of the patient with 3p26.3 microduplication affecting this single gene only (Gridina et al. 2018). Thus, single gene reciprocal CNVs is another way to clarify the genotype-phenotype correlations for known and new chromosomal diseases. They provide the possibility of phenotypic cleavage of chromosomal syndromes and blur the boundaries between single-gene and chromosomal disorders, creating a genetic continuum for human diseases.

Acknowledgements

This study is supported by Russian Science Foundation (14-15-00772).

O12

Patterns of gene expression in neurons derived from induced pluripotent stem cells of patients with reciprocal 3p26.3 microdeletion and microduplication

Maria E. Lopatkina[1], Veniamin S. Fishman[2], Maria M. Gridina[2], Nikolay A. Skryabin[1], Tatyana V. Nikitina[1], Anna A. Kashevarova[1], Lyudmila P. Nazarenko[1], Oleg L. Serov[2], Igor N. Lebedev[1]

1 *Research Institute of Medical Genetics, Tomsk National Research Medical Center, RAS, Naberezhnaya. r. Ushaiki, 10, Tomsk, 634050, Russia* **2** *Institute of Cytology and Genetics SB RAS, Lavrentiev 10, Novosibirsk, 630090, Russia*

Corresponding author: *Maria E. Lopatkina* (*lopatkina_maria@mail.ru*)

Microdeletions and microduplications of 3p26.3 were recently shown to be associated with neurodevelopmental disorders. Our goal was to reveal features of gene expression in neurons, derived from induced pluripotent stem cells (iPSC) of patients with intellectual disability and reciprocal aberrations affecting the copy number of *CNTN6* gene only.

Two iPSC clones with deletion, 2 clones with duplication and 3 wild-type clones were differentiated into cortical neurons. The neuronal RNA was examined using SurePrint G3 Human Gene Expression 8×60K Microarray Kit (Agilent Technologies).

It was revealed 676 upregulated and 188 downregulated common genes in neurons with deletion or duplication. Enrichment analysis of upregulated genes revealed their involvement in tissue development and cell adhesion, whereas downregulated genes were involved in the central nervous system and brain development, generation and differentiation of neurons, neurogenesis, axon genesis regulation, sterol and cholesterol biosynthesis. Importantly, 11 genes were upregulated in neurons with deletion but downregulated in duplication. They were involved in regulation of cell and anatomical structure morphogenesis, developmental processes and cell motility, hippocampus and limbic system development. In contrast, 49 genes were upregulated in neurons with duplication but downregulated in cells with deletion. They were involved in chemical synaptic transmission, anterograde trans-synaptic signaling, synaptic and trans-synaptic signaling, regulation of synaptic plasticity and glutamate secretion.

The obtained results provide evidence for a complex pattern of gene expression in iPSC-derived neurons of patients with single-gene genomic sister-disorders.

Acknowledgements

This study was supported by Russian Science Foundation (14-15-00772).

O13

DNA methylation in inherited copy number variations with incomplete penetrance

Stanislav A. Vasilyev, Nikolay A. Skryabin, Renata R. Savchenko, Ekaterina N. Tolmacheva, Anna A. Kashevarova, Ludmila P. Nazarenko, Elena O. Belyaeva, Igor N. Lebedev

Research Institute of Medical Genetics, Tomsk National Research Medical Center, RAS, Naberezhnaya. r. Ushaiki, 10, Tomsk, 634050, Russia

Corresponding author: *Stanislav A. Vasilyev* (*stas.vasilyev@gmail.com*)

Introduction: Chromosomal diseases caused by inherited copy number variations (CNV) are characterized by incomplete penetrance, but its mechanism remains unexplored. In our study, we analyzed differential DNA methylation of both promoter and intragenic CpG in probands with inherited CNV.

Materials and Methods: We have analyzed 15 probands with intellectual disability using aCGH. The presence of CNV in probands, parents and healthy siblings was investigated by realtime PCR. Methylation of promoter and intragenic CpG within a region of inherited CNV was analyzed using targeted bisulfite NGS.

Results: Various CNVs were identified in analyzed probands: dup12q24.12-q24.13 (2 probands from different families, both of maternal origin, ACAD10, ALDH2 and MAPKAPK5 genes); dup3p26.3 of paternal origin (CNTN6 gene); dup1q25.1-q25.2 of paternal origin (ASTN1, TDRD5 etc.); dup5q33.1 on maternal chromosome (AFAP1L1, GRPEL2 and PCYOX1L genes); dup17p13.3 of maternal origin (RPH3AL, VPS53, GEMIN4 etc.); dup18p11.32 of paternal origin (SMCHD1, METTL4 and NDC80 genes); del17q12 (2 probands from different families, both of maternal origin, SLFN11, SLFN13 genes); del9p21.1 (2 probands from different families, both of maternal origin, LINGO2 gene); del7q31.1 (3 probands from 2 different families, both of maternal origin, IMMP2L gene) and del12p11.1 of maternal origin (SYT10 gene). In families with dup18p11.32, dup3p26.3, dup1q25.1-q25.2 and del7q31.1, the methylation index of intragenic CpG was highest in probands and lowest in parents with CNV. Moreover, parents with CNV clustered distinctly from the rest of families.

Conclusion: Intragenic DNA methylation may be the cause of incomplete penetrance of inherited CNV.

Acknowledgements

This study was supported by Russian Science Foundation (grant № 16-15-10229).

Karyotyping and genomics of vertebrate and invertebrate animals

L13

Jurassic spark: Mapping the genomes of birds and other dinosaurs

Darren K. Griffin[1*], Rebecca E. O'Connor[1], Michael N. Romanov[1],Joana Damas[2], Marta Farré[2], Henry Martell[1], Lucas Kiazim[1], Rebecca Jennings[1], Anjali Mandawala[3], Sunitha Joseph[1], Katie E. Fowler[3], Eden A. Slack[2], Emily Allanson[2], Malcolm Ferguson-Smith[4], Paul M. Barrett[5], Nicole Valenzuela[6], Denis M. Larkin[2]

1 *School of Biosciences, University of Kent, Canterbury, CT2 7NJ, UK* **2** *Department of Comparative Biomedical Sciences, Royal Veterinary College, University of London, London, UK* **3** *School of Human and Life Sciences, Canterbury Christchurch University, Canterbury, Kent, UK* **4** *Department of Veterinary Medicine, Cambridge University, Cambridge, UK* **5** *Department of Earth Sciences, Natural History Museum, Cromwell Road, London SW7 5BD, UK* **6** *Department of Ecology, Evolution, and Organismal Biology, Iowa State University, USA*

Corresponding author: *Darren K. Griffin* (*d.k.griffin@kent.ac.uk*)

The ultimate aim of a genome assembly is to create a contiguous length of sequence from the p- to q- terminus of each chromosome. Most assemblies are however highly

fragmented, limiting their use in studies of gene mapping, phylogenomics and genomic organisation. To overcome these limitations, we developed a novel scaffold-to-chromosome anchoring method combining reference-assisted chromosome assembly (RACA) and fluorescence *in situ* hybridisation (FISH) to position scaffolds from *de novo* genomes onto chromosomes. Using RACA, scaffolds were ordered and orientated into 'predicted chromosome fragments' (PCFs) against a reference and outgroup genome. PCFs were verified using PCR prior to FISH mapping. A universal set of FISH probes developed through the selection of conserved regions were then used to map PCFs of peregrine falcon (*Falco peregrinus* Tunstall, 1771), pigeon (*Columba livia* Gmelin, 1789), ostrich (*Struthio camelus* Linnaeus, 1758), saker falcon (*Falco cherrug* Gray, 1834) the budgerigar (*Melopsittacus undulatus* Shaw, 1805). Using this approach, we were able to improve the N50 of genomes seven-fold. Results revealed that Interchromosomal breakpoint regions are limited to regions with low sequence conservation, shedding light on why most avian species have very stable karyotypes.

Our combined FISH and bioinformatics approach represents a step-change in the mapping of genome assemblies, allowing comparative genomic research at a higher resolution than was previously possible. The universal probe set facilitates research into avian karyotype evolution and the role of chromosome rearrangements in adaptation and phenotypic diversity in birds. Indeed, they have been used on over 20 avian species plus non-avian reptiles (including turtles), shedding light into the evolution of dinosaur species. Non-avian dinosaurs remain subjects of intense biological enquiry while pervading popular culture and the creative arts. While organismal studies focus primarily on their morphology, relationships, likely behaviour, and ecology there have been few academic studies that have made extensive extrapolations about the nature of non-avian dinosaur genome structure prior to the emergence of modern birds. We have used multiple avian whole genome sequences assembled at a chromosomal level, to reconstruct the most likely gross genome organization of the overall genome structure of the diapsid ancestor and reconstruct the sequence of inter and intrachromosomal events that most likely occurred along the Archosauromorpha-Archosauria-Avemetatarsalia-Dinosauria-Theropoda-Maniraptora-Avialae lineage from the lepidosauromorph-archosauromorph divergence ~275 million years ago through to extant neornithine birds.

L14

Cytogenetic and molecular background of disorders of sex development in domestic mammals

Marek Switonski

Poznan University of Life Sciences, Wolynska 33, 60-637 Poznan, Poland (*switonsk@up.poznan.pl*)

Disorders of sex development (DSD) can be caused by sex chromosome abnormalities, single gene mutations, non-genetic factors (e.g. placental anastomoses between heterosexual fetuses) or can have a multifactorial background. It is known that incidence of specific sex chromosome abnormalities is predominant in some species, e.g.: X monosomy in horses, XX/XY leukocyte chimerism in ruminants, XXY trisomy in cats etc. Our recent studies showed that the chimerism is also quite frequent in pigs and occasionally is observed in cats and dogs. Identification of the causative gene mutations in animals with DSD and a normal sex chromosome complement is a challenging task. There are only two monogenic XY DSDs in domestic animals, caused by known mutations. In horses three mutations of androgen receptor (AR) gene, responsible for androgen insensitivity syndrome (AIS), were reported. Secondly, a single mutation of Mullerian inhibiting substance receptor type 2 (MISR2) gene, causing persistent Mullerian duct syndrome (PMDS), is distributed in Miniature schnauzer dogs. In some species (pig, dog, goat, horse) testicular or ovotesticular XX DSD (lack of SRY gene) is quite common. The causative mutation for this DSD, affecting the expression of the FOXL2 crucial for ovarian development, has been identified in goats. Recent studies in dogs and pigs have shown that the mutations may be located near SOX9. Yet knowledge of the genetic background of multifactorial XY DSDs (cryptorchidism and hypospadias) is very scarce. It is known that, in some species the incidence of cryptorchidism is high (about 7% in dogs and horses, or up to 15% in some breeds), while hypospadias is rarely reported. There have been several unsuccessful attempts to find genetic markers associated with predispositions to these DSDs (horse, dog, cat). It can be foreseen that the use of new genomic tools will facilitate identification of new causative gene mutations in the near future.

Acknowledgements

The work was financially supported by the National Science Centre, Poland, grant no. 2016/23/B/NZ9/03424.

O14

Phylogenetic distribution of the canonical insect TTAGG telomeric repeat within the order Hymenoptera (Insecta)

Vladimir E. Gokhman[1], Valentina G. Kuznetsova[2]

1 *Botanical Garden, Moscow State University, Leninskie Gory, Moscow 119234, Russia*
2 *Zoological Institute, Russian Academy of Sciences, 1 Universitetskaya Naberezhnaya, St. Petersburg 199034, Russia*

Corresponding author: *Vladimir E. Gokhman* (*vegokhman@hotmail.com*)

Telomeres are terminal structures of eukaryotic chromosomes that contain repetitive DNA sequences. In the insect order Hymenoptera, both presence and absence of the canonical (TTAGG)n telomeric motif were previously reported. Nevertheless, all those reports investigated telomeric repeats in the suborder Apocrita, whereas DNA sequences of these regions in other hymenopterans were completely unknown. We have recently examined telomeric repeats in two sawfly species of the family Tenthredinidae that belongs to Symphyta, the only other suborder of the Hymenoptera which includes most basal members of the order. In both *Tenthredo omissa* Forster, 1844 and *Taxonus agrorum* Fallen, 1808, telomeres have clearly demonstrated presence of the (TTAGG) n motif, which constitutes the first report of this motif in the Tenthredinidae and Symphyta in general. Although the TTAGG telomeric repeat was long considered characteristic of the Hymenoptera, but, in fact, this repeat was detected until now only on chromosomes of certain aculeate Apocrita, i.e. ants and bees. Moreover, we have subsequently demonstrated absence of the (TTAGG)n telomeric motif in parasitoid Hymenoptera that belong to the same suborder. In addition, it was eventually shown that this motif was absent in all aculeate Hymenoptera except the families Formicidae and Apidae where it apparently reappeared independently. The most comprehensive phylogenetic reconstruction of the order nests the superfamily Tenthredinoidea together with Xyeloidea and Pamphilioidea within a separate clade Eusymphyta which represents a sister group to Unicalcarida, i.e. the remaining Hymenoptera. Furthermore, the (TTAGG)n telomeric motif was also found in many members of the clade Aparaglossata (= non-hymenopteran Holometabola), the sister group to Hymenoptera. Taken together, all these results therefore suggest the ancestral nature of this motif in the order Hymenoptera and its subsequent loss somewhere within Unicalcarida.

Acknowledgements

This study was partly supported by research grants no. 18-04-00611 and no. 17-04-00828-a from the Russian Foundation for Basic Research and no. 14-14-00541 from the Russian Science Foundation.

O15

Combined analysis of chromosomal and molecular markers reveals cryptic species: karyosystematics of the *Agrodiaetus* Hübner, [1822] blue butterflies

Maria S. Vishnevskaya[1], Vladimir A. Lukhtanov[1, 2], Alexandr V. Dantchenko[3], Alsu F. Saifitdinova[1, 4]

1 *Saint-Petersburg State University, Universitetskaya emb. 7/9, Saint-Petersburg, 199034, Russia* **2** *Zoological Institute Russian Academy of Sciences, Universitetskaya*

emb. 1, Saint-Petersburg, 199034, Russia **3** *Lomonosov Moscow State University, GSP-1, Leninskiye Gory 1/13, Moscow, 119991, Russia* **4** *Saint-Petersburg Scientist's Union, Universitetskaya emb. 5, Saint-Petersburg, 199034, Russia*

Corresponding author: *Maria S. Vishnevskaya* (*wishm@yandex.ru*)

Butterflies of the subgenus *Agrodiaetus* Hübner, 1822, genus Polyommatus Latreille, 1804, are the model system in studies of speciation and karyotype evolution. A unique feature of the subgenus is the highest diversity in chromosome numbers in the animal kingdom. In *Agrodiaetus* the number of chromosomes is stable within species and differentiated between species, therefore karyotypes are used for species description, delimitation and identification, although there are exceptions. The use of molecular markers provides an additional information for species delimitation. In our research for testing taxonomic hypotheses we used chromosomal markers in combination with the analysis of two genetically unlinked sequences: fragment of mitochondrial gene *COI* and nuclear spacer *ITS2*. This approach resulted in discovery of five cryptic species inhabiting the Balkan Peninsula – *P. ripartii* Freyer, 1830, *P. nephohiptamenos* Brown et Coutsis, 1978, *P. aroaniensis* Brown, 1976, *P. orphicus* Kolev, 2005 and *P. timfristos* Lukhtanov, Vishnevskaya et Shapoval, 2016 and three cryptic species inhabiting Azerbaijan and Iran – *P. valiabadi* Rose et Schurian, 1977, *P. rjabovianus* Koçak, 1980 and *P. pseudorjabovi* Lukhtanov, Dantchenko, Vishnevskaya et Saifitdinova, 2015 (Vishnevskaya et al. 2016, Lukhtanov et al. 2015). In general, the data obtained indicate that the genetic and taxonomic diversity of the subgenus *Agrodiaetus* is significantly higher than it was previously thought.

Acknowledgements

The study was supported by the grant N 14-14-00541 from the RSF to the Zoological Institute, RAS. The work was made on the base of "Chromas" Core Facility, Centre for Molecular and Cell Technologies (Saint-Petersburg State University, Research Park), and Department of Karyosystematics (Zoological Institute, RAS).

Chromatin studies

L15

Structure of holocentric chromosomes

Andreas Houben[1], Yi-Tsu Kuo[1], Stefan Heckmann[1], Andre Marques[2], Maja Jankowska[1], Veit Schubert[1], Jörg Fuchs[1], Wei Ma[1], Andrea Pedrosa-Harand[2], Jiri Macas[3], Pavel Neumann[3], Gerhard Wanner[4]

1 *Leibniz Institute of Plant Genetics and Crop Plant Research* (*IPK*) *Gatersleben, Corrensstrassse 3, 06466 Stadt Seeland, Germany* **2** *Laboratory of Plant Cytogenetics and Evolution, Department of Botany, Federal University of Pernambuco, Brazil* **3** *Laboratory of Molecular Cytogenetics, Institute of Plant Molecular Biology, Biology Centre AS CR, Ceske Budejovice, Czech Republic* **4** *Ultrastructural Research, Department Biology I, Biozentrum der Ludwig-Maximillians-Universität München, Großhadernerstr. 2-4, D-82152 Planegg-Martinsried, Germany*

Corresponding author: *Andreas Houben* (*houben@ipk-gatersleben.de*)

The centromere is the chromosome region where microtubules attach and the movement of newly formed chromatids to the daughter cells during mitosis and meiosis occurs. Most organisms contain one single size-restricted centromere per chromosome (monocentric chromosome) visible as a primary constriction during metaphase. It is generally accepted that an independent transition from mono- into holocentromeres occurred in total on at least 13 occasions in eukaryotic lineages (four times in plants). As holocentricity has arisen multiple times during evolution a striking question is whether the organization of centromeres differs in species having independently evolved holocentromeres. The lily *Chionographis japonica* Maximowicz, 1867 and the grasses *Luzula elegans* Lowe, 1838 and *Rhynchospora pubera* Boeckeler, 1872 were selected to analyse the organization of their holocentromeres. Strikingly, differences were found in the organization and dynamics of their holocentromeres. Thus, different types of holocentromeres exist.

O16

Breakthrough in understanding the phenomenon of lampbrush chromosomes

Alsu F Saifitdinova[1,2,3]

1 *Saint-Petersburg State University, Universitetskaya emb. 7-9, Saint-Petersburg, 199034, Russia* **2** *International Center of Reproductive Medicine, Komendantskiy av. 53/1, Saint Petersburg 197350, Russia* **3** *Saint Petersburg Association of Scientists and Scholars, Universitetskaya emb. 5, Saint Petersburg, 199034, Russia* (*saifitdinova@mail.ru*)

Lampbrush chromosomes (LBCs) are specific forms of giant meiotic chromosomes from oocytes of most vertebrates, including birds. They appear as half-bivalent with chromomere-loop organization. The prominent structure of the LBCs loops is maintained by a high density of transcripts. Despite years of research, the reason of such a transcription in meiotic diplotene is unknown. Some studies show that tandem repeats are transcribed in lateral loops, however most transcribed sequences themselves and

their biological role still need to be clarified. Contrary to the common opinion about the high speed of transcription on the LBC loops our data indicate a low transcription activity in these areas with blocking release of nascent transcripts from the DNA template. We found various types of repetitive sequences including transposable elements in the composition of the lateral loops on avian LBC whereas euchromatic sequences correspond to the chromomeric regions. Epigenetic markers of chromatin are removed in gametogenesis. Apparently, to regulate the activity of the whole genome in these cells, an alternative way based on the slowdown of transcription is utilized. Stuck transcripts are responsible for the formation of recognizable morphology of lateral loops. Among other things, incomplete transcription in these areas can also increase the level of co-transcriptional mutagenesis therein. It was shown that transcription, along with replication, makes a significant contribution to the level of mutagenesis in the gamete genome. It makes a significant contribution in the maintenance of a stable level of genome variability in populations. This can explain biological significance and widespread presence of LBCs in different groups of animals.

Acknowledgements

The study was supported by the Russian Foundation of Basic Research (grant #16-04-01823a). The equipment we used was provided by the "Chromas" Research Resource Center (Saint-Petersburg State University).

Poster session

P1

Selection of cell lines for the functional validation of 3D genomic interactions by genome editing

Florence Mompart, Yvette Lahbib-Mansais, Anne Calgaro, Hervé Acloque, Martine Bouissou-Matet Yerle

GenPhySE, University of Toulouse, INRA Occitanie-Toulouse, ENVT, 24 chemin de Borde-rouge 31326 Castanet Tolosan, France

Corresponding author: *Martine Bouissou-Matet Yerle* (*martine.bouissou-matet@inra.fr*)

Numerous studies have pointed out the major role of 3D nuclear architecture in the regulation of gene expression. Individual chromosomes occupy discrete territories in the nucleus but chromosomal regions often loop out and neighboring chromosomes can intermingle resulting in potential functional contacts between genomic regions from the same or different chromosomes. These cis- and trans-interactions could con-

tribute to gene expression regulation by facilitating the consolidation of co-regulated genes in specific transcription factories. Thus, their dynamic recruitment could result in activation or abatement of gene transcription. In this context, we investigated by means of DNA fluorescence *in situ* hybridization (FISH) the 3D genome organization in porcine fetal muscle cells particularly long-range chromatin interactions implicating the imprinted loci IGF2 (SSC2) and other loci located on different chromosomes (Lahbib-Mansais et al. 2017). We showed that IGF2 alleles associate with the reciprocally imprinted DLK1/MEG3 region (SSC7). We furthermore demonstrated that these trans-interactions preferentially occurred between the expressed alleles. To prove their suggested functional character, we tested cell lines in which we plan to delete the genomic region containing IGF2 to determine if this deletion compromises the occurrence of the interactions. The first step has consisted in analyzing different types of porcine cell lines (4 somatic cell hybrid clones, an iPS cell line, a primary muscle and a permanent cell lines) to determine if they could be a good cell model for this purpose. Both 2D and 3D FISH experiments were performed to verify the presence in these cell lines of: i) the target chromosomes/genes, ii) the genomic interactions between IGF2-DLK1 and IGF2-MEG3. The expression of all target genes was also analyzed. We were able to select one cell line for which we plan to delete IGF2 by CRISPR-Cas9 to determine if this implies the modification of the interactions.

P2

Gradual chromosome elimination via micronuclei formation during gametogenesis of di- and triploid interspecies hybrids from *Pelophylax esculentus* complex

Dmitry V. Dedukh[1], Sergey S. Rymin[1], Magdalena Chmielewska[2], Beata Rozenblut-Kościsty[2], Krzysztof Kolenda[2], Mikołaj Kaźmierczak[2], Maria Ogielska[2], Alla V. Krasikova[1]

1 *Department of Cytology and Histology, Faculty of Biological Sciences, Saint-Petersburg State University, 7/9 Universitetskaya emb., 199034 St. Petersburg, Russia* **2** *Department of Evolutionary Biology and Conservation of Vertebrates, Faculty of Biological Sciences, University of Wrocław, Sienkiewicza 21, 50-335, Wrocław, Poland*

Corresponding author: *Dmitry V. Dedukh* (*dmitrov89@yandex.ru*)

Hybrid sterility obstructs reproduction of interspecies hybrids. To cope with sterility some interspecific animal hybrids develop fascinating strategies. One of these strategies, called hybridogenesis, includes selective elimination of one parental genome from germ cells in order to prevent chromosomal conflict in meiosis. To find out how genome elimination is accomplished during hybrid gametogenesis we chose European water frogs complex (*Pelophylax esculentus* Linnaeus, 1758 complex) as a model. This

complex includes two parental species, *P. lessonae* Camerano, 1882 (LL), *P. ridibundus* Pallas, 1771 (RR), and their hybrids, *P. esculentus*. Hybrid frogs exist as a diploid (RL) and a triploid (RRL, LLR) forms which exploit genome elimination for their reproduction. After artificial hybridization experiments we found that diploid males and some females eliminate L genome while the majority of triploids with LLR genotype eliminate R genome. To detect elimination we performed analysis of gonads isolated from hybrid tadpoles with different ploidy. In gonads of hybrid tadpoles, we found micronuclei in cytoplasm of germ cells. Detection of kinetochore proteins using CREST antibodies revealed one signal per each micronucleus indicating that each micronucleus comprises one chromosome. FISH with probe specific to *P. ridibundus* centromeric sequences revealed that triploid LLR hybrids preferentially eliminate R chromosomes eventually forming micronuclei while diploid hybrid frogs preferentially eliminate L chromosomes. We conclude that genome elimination in gonads of diploid and triploid hybrids occurs via gradual elimination of individual chromosomes from one parental genome via micronuclei formation.

Acknowledgements

The work was partially performed using experimental equipment of the Research Resource Centers "Environmental Safety Observatory" and "Molecular and Cell Technologies" of St. Petersburg State University.

P3

Improving the knowledge of microchromosome repeated sequences in chicken by the sequencing of BAC clones with Pacific Bio technology

Valérie Fillon[1], Christophe Klopp[2], Yvette Lahbib-Mansais[1], Noémie Thebault[1], Céline Lopez-Roques[3], Nathalie Rodde[4], Stéphane Cauet[4] and Alain Vignal[1]

1 *GenPhySE, Université de Toulouse, INRA Occitanie-Toulouse, ENVT, 24 chemin de Borde-Rouge, 31326 Castanet Tolosan, France* **2** *Sygenae, INRA Occitanie-Toulouse, 24 chemin de Borde-Rouge, 31326 Castanet Tolosan, France* **3** *Get-Plage, INRA Occitanie-Toulouse, 24 chemin de Borde-Rouge, 31326 Castanet Tolosan, France* **4** *CNRGV, INRA Occitanie-Toulouse, 24 chemin de Borde-Rouge, 31326 Castanet Tolosan, France*

Corresponding author: *Valérie Fillon* (*valerie.fillon@inra.fr*)

Due to the chicken's (*Gallus gallus domesticus* Linnaeus, 1758) importance as a model organism and for agriculture, the whole genome assembly was published in 2004. Despite international efforts and many improvements, 138 Mb upon 1.21 Gb are still not assigned to chromosomes (virtual Chromosome Unknown), six microchromosomes

remain absent (Gallus_gallus-5.0), and three have a partial coverage. In order to contribute to the sequencing of microchromosomes, we decided to select six BAC clones showing painting signal by FISH on microchromosomes, thus probably containing repeated sequences potentially absent in the assembly.

We performed sequencing with a Pacific Bioscience RSII equipment (P4C6 chemicals). DNA extraction is a crucial step, as both high quantity and quality are needed for Pacbio sequencing. We obtained end sequences for the six BACs before pooling them in the smart cell. As expected, we obtained long reads (9 Kb) and high sequencing depth (953X). Three of these BACs were particularly resistant to sequencing and had to be sequenced separately, demonstrating that some sequence biases could be a possible explanation for the low coverage of microchromosomes in whole genome approaches.

The contigs were established using different assembler: HGAP3, Miniasm, MHAP and Canu. The vector was cleaned, the presence of *Escherichia coli* Migula, 1895, DNA was filtered and the contigs assigned using the Bac end sequences. The best results were obtained with HGAP3 but Miniasm gave very interesting results with some of the repeated sequences. The alignment against Gallus_gallus-5.0 has allowed to assign some of the Unknown contigs to microchromosomes.

P4

The use of interspecific hybrid chromosomes as a tool for precise comparative mapping analysis

Christelle Marrauld[1], Maria M. Kulak[2], Svetlana A. Galkina[2], Alain Vignal[1], David Gourichon[3], Francis Minvelle[3], Valérie Fillon[1]

1 *GenPhySE, Université de Toulouse, INRA Occitanie-Toulouse, ENVT, 24 chemin de Borde-Rouge, 31326 Castanet Tolosan, France* **2** *Saint-Petersburg University, Universitetskaya nab 7-9, 199034 Saint-Pétersburg, Russia* **3** *PEAT, INRA centre Val de Loire, 37380 Nouzilly, France*

Corresponding author: *Valérie Fillon* (*valerie.fillon@inra.fr*)

The chicken karyotype is considered to closely resemble that of the putative ancestor of modern Aves, presenting most of the characteristics typical of the 10% of avian species karyotyped to date. It possess a large number of chromosomes (2n=78), many of which are indistinguishable ‚microchromosomes' (30 pairs), females are heterogametic (ZW) whereas males are homogametic (ZZ). Moreover, the knowledge on the chicken genome is wide by comparison with other bird species. That is why the chicken genome is often used as a reference.

Evolutionary studies using zoo-FISH and BAC-FISH have demonstrated birds to have a more stable genome organization compared to mammals, with inter-chro-

mosomal rearrangements corresponding to rarely occurring fusion/fission events. In Galliformes, the karyotype is very stable but generation of high-resolution comparative maps and sequence data have revealed an unexpectedly high number of intra-chromosomal rearrangements if previous cytogenetic data is considered.

We took benefit of F1 interspecific hybrids to develop some precise macrochromosome comparisons between chicken and quail. In the 2000's in order to study feather coloration genes, a number of cocks (*Gallus gallus domesticus* Linnaeus, 1758) where mated with japanese quail females (*Coturnix japonica* Temminck et Schlegel, 1849). A few hybrids were obtained. It was possible to prepare cytogenetic samples from fibroblast cell cultures.

The analysis of the karyotype by classical cytogenetic methods (conventional giemsa staining and G-banding) has allowed the precise characterisation and comparison of macrochromosomes (length, centromere position, banding pattern) for each parental species at the same stage of chromatin condensation. We have also used this unique material for FISH localisation of specific repeated sequences. Thus, we were able to compare the cytogenetic distributions of these sequences in chicken and quail. The use of interspecific hybrid metaphases is a powerful tool for comparative mapping.

P5

Chromosome study of a fertile donkey x mare hybrid

Harmonie Barasc, Anne Calgaro, Nicolas Mary, Nathalie Mouney-Bonnet, Clémence Revel, Alain Ducos, Valérie Fillon, Alain Pinton

GenPhySE, University of Toulouse, INRA Occitanie-Toulouse, ENVT, 24 chemin de Borde-rouge 31326 Castanet Tolosan, France

Corresponding author: *Anne Calgaro* (*a.seguela@envt.fr*)

The natural mating of a donkey (*Equus asinus* Linnaeus, 1758, 2n = 62) with a mare (*Equus caballus* Linnaeus, 1758, 2n = 64) produces a male or female hybrid that is usually sterile. To date, very few cases of fertile mules have been reported (Rider et al. 1985, Jones and Johnsen 1985, Rong et al. 1988, Zong and Fan 1989) in contrast to the mullet for which no fertility cases have been reported. In fact, this hybrid belonging to the Equidae family has an intermediate chromosome number between that of the parental species, i.e. 63 chromosomes. This characteristic and the chromosomal differences between the parental species would be responsible for chromosome pairing problems during the first meiotic division and cause meiosis to stop. Nevertheless, a new case of a mule having farrowed a viable male has been identified in Morocco. The karyotype analysis of the latter showed that this hybrid was the product of mating between a mule and a donkey.

P6

Genetic analysis of canine mast cell tumors

Hana Hradska, Miluse Vozdova, Svatava Kubickova, Halina Cernohorska, Jan Fröhlich, Jiri Rubes

Central European Institute of Technology – Veterinary Research Institute, Hudcova 70, 621 00 Brno, Czech Republic

Corresponding author: *Hana Hradska* (*hradska@vri.cz*)

Being our pets, dogs (*Canis familiaris* Linnaeus, 1758) share our environment and develop similar diseases. Cancers cause as much morbidity and mortality in dogs, as they do in humans and are a leading cause of death in dogs over the age of 10. One of the most frequent skin neoplasias in dogs is the collect of mast cell tumors (MCTs) which account for up to 21% of all canine skin tumors. Despite the importance of MCTs in veterinary medicine, little is known about the genetic background of this disease.

To find associations between cytogenetic abnormalities, gene mutational status, histological grade and clinical outcome, we performed the cytogenetic analysis by FISH using a panel of the whole chromosome painting and BAC probes, and molecular analysis of c-kit and TP53 genes in fresh and FFPE samples of canine cutaneous MCTs. The cytogenetic abnormalities varied in the analysed cultured MCTs. However, chromosome 11 abnormalities were observed in all 3 analysed samples. BAC probes for individual canine chromosomes and selected oncogenes are used for detection of a copy number variants in interphase cells of the MCT samples. Mutation analysis of the c-kit (exones 8, 9 and 11), and TP53 (exones 5 and 6) revealed mutations in 18.8% and 6% of the 85 analysed MCT samples. Therefore, we used whole exome sequencing (WES) to search for other molecular aberrations in the coding part of the canine genome which might be causative of the MCT formation. Paired sample approach was used when tumor tissue and blood from the same animal were subjected to WES for elimination of non-causative genetic variants. The data analysis is in progress.

The identification of new MCT-associated genes will improve our understanding of the genetic background of canine MCT, provide data for accurate diagnosis and prognosis and offer a perspective of novel targeted treatment strategies.

P7

Analysis of segregation and aneuploidy in a hybrid boar heterozygous carrier of a rob(15;17) by dual-colour-sperm-FISH: preliminary studies

Caputi-Jambrenghi A.[1], Viviana Genualdo[2], Francesco Giannico[1], Bianca Castiglioni[3], Flavia Pizzi[3], Donata Marletta[4], Alessandra Iannuzzi[2]

1 *Department of Animal Productions, Agricultural Faculty of Sciences, University of Bari, Campus Universitario „Ernesto Quagliariello", 70126 Bari – Italy* **2** *Institute for Animal Production System in Mediterranean Environment* (*ISPAAM*)*, National Research Council* (*CNR*)*, via Argine 1085, Naples, 80147, Italy* **3** *Institute of Agricultural Biology and Biotechnology***,** *National Research Council* (*CNR***),** *U.O.S. di Lodi, Via Einstein, 26900 Lodi Lodi, Italy.*

Corresponding author: *Alessandra Iannuzzi* (*alessandra.iannuzzi@cnr.it*)

The Sicily black pig breed is an important autochthonous Italian breed. The animals of the breed are allowed to graze and forage over wide areas (extensive or semi-extensive systems), including woods, and this diet leads to the high quality and flavour of the meat. Crossing between domestic (2n=38) and wild (2n=36) pigs can result in fertile hybrids 2n=37. In fact, during a cytogenetic survey we found two boars with a heterozygous translocation of rob(15;17) (2n=37, XY), normally present in the wild pig. We decided to analyse the sperm segregation, in one of two hybrids, to evaluate the percentage of sperms carrying the translocation. Collected cryopreserved semen samples of 2 boars: one of the hybrid and a control (normal boar) were used for this study. We analysed the sperm segregation and aneuploidy in 2500 sperms, for both samples, by a dual-colour fluorescence *in situ* hybridization (FISH) analysis, using two sets of probe mixtures of specific porcine BAC probes (CHORI library), mapping on SSC15 and SSC17. The preliminary data have shown that the hybrid presents a higher percentage of balanced sperms (alternate II), responsible for transmission of the translocation in progeny, in comparison to the control. These data underline how important it is to conduct cytogenetic studies on reproducers, to preserve autochthonous pure pig breeds and to avoid the selection of hybrids as reproducers.

Acknowledgements

This study was supported by CISIA-Gene pig project, National Research Council (CNR) of Rome

P8

Genomic analysis on river buffalo (*Bubalus bubalis* Linnaeus, 1758, 2n=50) reared in different conditions: short-and long-term effects on DNA

Alessandra Iannuzzi[1], Angela Perucatti[1], Viviana Genualdo[1], Cristina Rossetti[1], Domenico Incarnato[1], Ciro Iorio[1], Maria Grazia Andreassi[2], Leopoldo Iannuzzi[1]

1 *Institute for Animal Production System in Mediterranean Environment* (*ISPAAM*)*, National Research Council* (*CNR*)*, via Argine 1085, Naples, 80147, Italy* **2** *CNR Institute of Clinical Physiology, c/o Area di Ricerca „S. Cataldo" Via Giuseppe Moruzzi, 1 – 56124 Pisa, Italy*

Corresponding author: *Alessandra Iannuzzi* (*alessandra.iannuzzi@cnr.it*)

DNA damage is the most important factor that induces genome instability and the exposure to exogenous agents (UV light, oxidative stress, chemical mutagens, and radiation) can lead to a variety of modifications of DNA constituents, resulting in genome alterations. The aim of this study is to verify the differences between long-term and short-term DNA damage by different genomic tests: Aberration Chromosomes (CA), Sister Chromatid Exchanges (SCEs) and Cytokinesis-Block Micronucleus (CBMN) for long-term DNA damage and the relative Telomere Lenght (TL), by Monochrome Multiplex Quantitative PCR (MMQPCR) method, for short-term DNA damage. We selected two groups of buffaloes (five for each homogeneous group for age and sex) raised in different environments: urban (group A) and extraurban (group B).

For CA test, we counted 100 cells for a sample with mean values of CA/cell of 0.06±0.26 (A) and 0.05±0.21 (B); for the SCE test, we elaborated 35 cells per sample with SCE-mean values being 9.06±3.73 and 9.02±3.92, in the A and B-groups, respectively. For CBMC test, we counted 500 cells for a sample: mean values of Nuclear Division Index (NDI) was 2.04±0.11 and 1.89±0.04 in the A and B- groups, while the Binucleated Cell Indexes (BCI) were 77.0±7.58 and 75.6±5.41 in the A- and B-groups, respectively. Mean values of the Bi-Nucleated cells with MN (BNMN) and MN for cell Bi-Nucleated they were 1.40±1.52 and 1.80±2.05 in the A- and B-groups, respectively. The TL value (expressed as telomere length relative to a single copy reference gene) was 0.98±0.57 (A) and 1.24±1.07 (B).

For each test no statistical differences were found between the two groups, but it is necessary to study a larger number of animals to validate the results in a better way.

Acknowledgments

The research was supported by Project PON1_486- GENOBU

P9

First description of karyotypes and localization of ribosomal genes in two sand lances (Perciformes: Ammodytidae); small sand eel (*Ammodytes tobianus* Linnaeus, 1758) and great sand eel (*Hyperoplus lanceolatus* (Le Sauvage, 1824))

Lech Kirtiklis[1], Katarzyna Mojsa[2], Konrad Ocalewicz[2]

1 *Department of Zoology, Faculty of Biology and Biotechnology, University of Warmia and Mazury in Olsztyn, M. Oczapowskiego5, 10-718 Olsztyn, Poland* **2** *Department of Marine Biology and Ecology, Institute of Oceanography, University of Gdansk, M. Piłsudskiego 46 Av., 81-378 Gdynia, Poland.*

Corresponding author: *Lech Kirtiklis* (*leo@uwm.edu.pl*)

The sand lances also known as sand eels are small fish belonging to family Ammodytidae. These elongated and slender fishes are able to dive into sand to escape predators. Ammodytidae consists of 31 species belonging to seven genera. Despite world-wide distribution, sand lances have rarely been objects of cytogenetic studies. In present research, chromosomes of small sand eel and great sand eel from Gulf of Gdansk (Baltic Sea) have been analysed. Karyotypes of both species were composed of 48 acrocentric chromosomes (FN= 48). Tiny DAPI positive sites were found in the pericentromeric locations of a few sand lances chromosomes. On the contrary, relatively large blocks of NOR-related DAPI-negative chromatin was observed on two chromosomes from small sand eel. Centromeric regions of both fishes studied were resistant to the Hinf I and Dde I restriction endonucleases digestion. Major and minor rDNA sites were observed on separate chromosome pairs in both examined species.

The presented results allowed us to describe the karyotypes of small sand eel and great sand eel, and brought new data regarding organization of the perciform genome related to the largest group of fishes in the world.

P10

Polymorphisms of *INSL3* and *ESR1* are not associated with cryptorchidism in dogs

Paulina Krzeminska[1], Maciej Gogulski[2], Tomasz Nowak[3], Katarzyna Sochon[4] and Marek Switonski[1]

1 *Department of Genetics and Animal Breeding, Poznan University of Life Sciences, Wolynska 33, 60-637 Poznan, Poland* **2** *University Centre for Veterinary Medicine, Poznan University of Life Sciences, Szydlowska 43, 60-656 Poznan, Poland* **3** *Department of Animal Reproduction, Poznan University of Life Sciences, Wolynska 35,60-637 Poznan, Poland* **4** *Vetka Veterinary Clinic, Modlinska 65, Warsaw, Poland*

Corresponding author: *Paulina Krzeminska* (*paulina.krzeminska@up.poznan.pl*)

Cryptorchidism, a multifactorial disorder of sex development (DSD), is common in dogs, with an average incidence of 7%. In humans, polymorphisms of 14 genes, including insulin-like protein 3 (*INSL3*) and estrogen receptor 1 (*ESR1*), have been reported as associated with this disorder. It is known that descent of the testes to the scrota depends on the growth of the gubernacula, which is controlled by *INSL3*, while *ESR1* plays an important role in sexual development and reproductive function. We analyzed coding (all exons) and flanking (fragments of introns, as well as 5'- and 3'-flanking regions) sequences of both genes in 19 cryptorchidic dogs, rep-

resenting 13 breeds. Thirty-four control dogs were also included in the study. Altogether, we sequenced 821 bp of *INSL3* and 5203 bp of *ESR1*. We found 3 SNPs in *INSL3* (g.45071105G>A; g.45071131G>A and g.45071193A>G), while in *ESR1* 1 indel (g.42084005GGGGCA[6_8]) and 6 SNPs (g.42131190T>C; g.42208686A>G; g.42359532G>A; g.42359539T>C; g.42359611A>C and g.42364093G>A) were observed. Two SNPs caused amino acid substitution: g.45071131G>A (Gly2Ser) in *INSL3* and g.42208686A>G (Ile327Val) in *ESR1*. The frequency of the minor variants varied from 0.03 to 0.4, and comparison of their distribution in the cryptorchid and control dogs did not reveal co-segregation with DSD phenotype. In conclusion, our initial study showed that the association of *INSL3* and *ESR1* polymorphism with cryptorchidism in dogs seems to be unlikely.

Acknowledgements

The work was financially supported by the Department of Genetics and Animal Breeding, Poznan University of Life Sciences, No. 508.534.01.0.

P11

New pericentromeric repeat identified in the genome of japanese quail

Maria M. Kulak[1], Alexander G. Dyomin[1], Aleksei S. Komissarov[1], Valerie Fillon[2], Alsu F. Saifitdinova[1], Olga A. Pavlova[1], Elena R. Gaginskaya[1], Svetlana A. Galkina[1]

1 *Saint-Petersburg State University, Universitetskaya emb, 7/9, Saint-Petersburg, 199034, Russia* **2** *GenPhySE, Université de Toulouse, INRA Toulouse-Occitanie, ENVT, 24 chemin de Borde-Rouge, 31326 Castanet Tolosan, France*

Corresponding author: *Maria Kulak* (*ontica@mail.ru*)

Pericentromeres are obligate structural chromosomal parts that guarantee proper chromosome segregation during cell division. Pericentromeric regions of many eukaryotes are enriched in tandem repeats, therefore they are the most difficult genome elements to analyze. The genome of Japanese quail *Coturnix japonica* Temminck et Schlegel, 1849, the species used in biomedical research and poultry farming, is relatively small (≈1.41 Gb) and packed into 39 chromosome pairs. 41-bp tandem repeats PO41 and BglII are found in centromere regions of CJA4, CJAW and some quail microchromosomes.

Here we report the deciphering of a new tandem repeat CjapSAT found within unassembled Japanese quail short raw reads (GeneBank accession number MH475922). In *C. japonica* genome there are several degenerated and truncated CjapSAT variants with basic repeat unit around 1180 bp and average A+T content of 68%. The CjapSAT repeat is specific to Japanese quail and is not found in any other avian genomes.

Meanwhile it has some similarity with centromere repeats of *C. chinensis* Linnaeus, 1766, and *Alectoris chukar* Gray, 1830. The presence of motifs homologous to LTR suggests its retroviral origin. FISH with CjapSAT specific probe revealed CjapSAT in pericentromeric heterochromatin on CJA 1-6 and two pairs of microchromosomes, as well as in p- and q-arms of CJAW. At lampbrush stage, the repeat transcribes in long transcription units on lateral loops extending from pericentromeric chromomeres of corresponding autosomes. Despite the species specificity of pericentromeric tandem repeats, their deciphering in sequenced genomes contributes to (1) filling in the gaps of genome assemblies and (2) revealing general patterns of organization of centromeric chromatin.

Acknowledgements

The work was done in "Chromas" Research Resource Center and Theodosius Dobzhansky Center for Genome Bioinformatics (SPbSU), with the support of Financial Program 4 of Saint Petersburg State University (#1.40.1625.2017).

P12

Loci-specific RNP-rich nuclear domains on lampbrush chromosomes: data pointing at RNA editing

Tatiana V. Kulikova[1], Lyudmila V. Kazakova[1], Antonina V. Maslova[1], Dmitry V. Dedukh[1], Irina L. Trofimova[1], Thomas Liehr[2], Alla V. Krasikova[1]

1 *Saint Petersburg State University, 7-9 Universitetskaya Emb., St Petersburg 199034, Russia* **2** *Jena University Hospital, Institute of Human Genetics, Am Klinikum 1, 07747 Jena, Germany*

Corresponding author: *Tatiana V. Kulikova* (*t.v.kulikova@gmail.com*)

Ubiquitous RNA-rich nuclear domains, such as nucleolus, paraspeckles and stress--induced domains form as a result of specific loci transcription. Nuclear domains of interphase nuclei disassemble in mitosis and loci of their association are hard to be traced on individual metaphase chromosomes. From this point lampbrush chromosomes (LBCs) – transcriptionally active meiotic bivalents of diplotene oocytes – represent promising model for studying nuclear domains at the loci of their formation. Loci--specific RNP-rich nuclear domains, in particular "lumpy loops" (LLs), are formed on LBCs of the majority studied avian and amphibian species. Earlier we developed an approach for the analysis of tiny chromosome loci using mechanical microdissection of individual chomomeres and lateral loops of LBCs followed by FISH probe generation and next-generation sequencing (Zlotina et al. 2016). Multiple LLs accumulating

splicing snRNPs form at specific loci of LBCs of marsh frog Pelophylaxridibundus (Dedukh et al. 2013). We have microdissected four LL-loci on marsh frog LBCs C, D, E and H and generated FISH probes. FISH on metaphase chromosomes revealed single major signals for each probe corresponding to the dissected loci, additional minor signals on other chromosomes and low dispersed signal. DNA+RNA FISH on LBCs revealed multiple signals in chromomeres and RNP-matrix of normal loops indicating the presence of repetitive sequences in the dissected loci. All used probes demonstrated cross-hybridization to the loci of other LLs, indicating that similar sequences may induce LL formation on different chromosomes. Interestingly, all probes hybridized only to the bases of loci-specific structures and adjacent chromomeres but not to the RNP--matrix of LLs. These data suggest that RNA in LLs undergoes promiscuous editing and thus is unable to hybridize with the DNA probe.

Acknowledgements

Equipment of SPBURRC "MCT" and "Observatory of environmental safety" was used for this study.

P13

A new mutation in the MC1R gene is responsible for golden and cornelian coat colours in the Kurilian Bobtails

Elina Bychkova[1,2], Anton Markov[2], Natalia Golubeva[2], Yulia Golovacheva[2], Elizaveta Filippova[2]

1 *Saint-Petersburg State University, Universitetsckaya emb., 7/9, Saint Petersburg, Russia*
2 *Zoogen, Karpovki emb., 5, Saint Petersburg, Russia*

Corresponding author: *Anton Markov* (*anton@zoogen.org*)

Golden colour occurs in many breeds of cats and has various forms of manifestation. The pigments eumelanin and pheomelanin take part in its formation. They are distributed along the length of the hair as two bands, so that pheomelanin is located at the base of the hair, and eumelanin is located on the tip of the hair. The Kurilian Bobtail is a breed of short-tailed cat of Russian origin that has both golden colour, and recessive yellow colour called „cornelian" in its phenotype. Two mutations in the melanocortin 1 receptor (MC1R) gene causing a recessive yellow colour have been described: *e* allele for Amber colour found in Norwegian Forest Cat and *er* allele for Russet colour found in Burmese. Until now, there has been no data for any breed of cats explaining the appearance of the golden colour. As a result of the full sequencing of the coding region of the MC1R gene, a new mutation was identified. We analyzed 68 Kurilian Bobtail

cats, of which 12, identified as gold, were found to be heterozygotes, and 16, identified as cornelian, were homozygous for the identified mutation. In the Kurilian Bobtails with other colours this mutation was not found. The gold cats of the other two breeds also did not have a mutation. The obtained data suggest that the mutant allele (*ec*) in the compound with the wild type allele leads to the formation of a golden colour, and in a double dose leads to the total absence of eumelanin in the hair, which manifests itself in the form of the cornelian colour. The results which explain the causes of the formation of the golden colour and the new colour called cornelian in the Kurilian Bobtail cats were obtained for the first time.

P14

Neo-X chromosome: independent origin and maintenance of syntenic blocks in highly rearranged karyotypes from genus *Proechimys* J.A.Allen, 1899 (Rodentia-Echimyidae)

Willam Oliveira da Silva[1], Marlyson J. Rodrigues da Costa[1], Julio C. Pieczarka[1], Jorge Rissino[1], Malcolm A. Ferguson-Smith[2], Jorge Pereira[2], Cleusa Y. Nagamachi[1]

1 *Laboratório de Citogenética, Centro de Estudos Avançados da Biodiversidade, Terreno 11, Parque de Ciência e Tecnologia do Guamá, Avenida Perimetral da Ciência, km 01 – Guamá, 66075-75, Belém – Pará – Brasil* **2** *Department of Veterinary Medicine, Madingley Road, Cambridge, CB3 0ES, United Kingdom*

Corresponding author: *Cleusa Y. Nagamachi* (*cleusanagamachi@gmail.com*)

Proechimys J. A. Allen, 1899 genus (Rodentia-Echimyidae) represents one of the most diverse group of Amazonian rodents at genetic and taxonomic levels, with more than 60 karyotypes associated with its 22 species, where Neo-X chromosomes are described. The aim of this study is to investigate the genome modification events in *Proechimys goeldii* Thomas, 1905 (2n=24♀,25♂/FN=42) and *Proechimys gr. goeldii* (2n=16♀,17♂/FN=16). Whole chromosome probes of *Proechimys roberti* Thomas, 1901 (2n=30/FN=54) were produced and used in comparative chromosomal mapping. Samples of *Proechimys roberti* (PRO, ♂), and *P. goeldii* (PGO, ♂) are from Abaetetuba, Pará, Brazil, and *Proechimys gr. goeldii* (PGG, ♀) from Parintins, Amazonas, Brazil. The chromosomes were obtained by fibroblast cell culture, and submitted to G-banding. Whole chromosome probes were produced by flow cytometry, from sorted chromosomes of PRO, and 18 peaks were identified by same species FISH experiments. Cross-species FISH with PRO probes showed 29 signals on PGO karyotype, and 27 signals on PGG karyotype. The comparative chromosome painting analysis between PGO and PGG karyotypes shows that they

differ due to 10 fusion/fission events and one inversion. Eight syntenic blocks are shared between the two taxa (PRO 5/2/3, 6/9/1, 9/5, 6/7, 14/1/4, 8, 11, and 12). Additionally, an independent origin of the Neo-X chromosomes of PGO (PRO 7/*/X) and PGG (PRO X/*/5/2/3) was revealed. Our data indicate a high plasticity of mechanisms that determined the chromosomal evolution of these taxa, and also highlight the potential role of rodents as a model to study the evolution of the sex chromosomes.

P15

A case of posterior limb malformation in Montbeliarde cattle

Ioana Nicolae[1], Daniela Maria Vidmichi[1], Mihai Bogdan[2], Cosmin Sonea[3]

1 *Research & Development Institute for Bovine, Sos.Bucuresti –Ploiesti, km.21, Baloteşti, Ilfov, 077015, Romania* **2** *Experimental Station Moara Domneasca, Kontszbuie street, no. 10, Ganeasa, Ilfov, 077102, Romania* **3** *University of gronomic Sciences and Veterinary Medicine, Bd. Marasti, no. 59, sector 1, Bucharest, 011464, Romania*

Corresponding author: *Ioana Nicolae* (*ioana_nicolae2002@yahoo.com*)

During the routine cytogenetic investigation carried out in 17 Montbeliarde females we identified 5 females with chromosomal instability. One of these females had a calf with posterior limb malformation, characterized by the lack of the left posterior leg. The chromosomal complement of the malformed calf and its mother was severely affected, the number of mono-and bi-chromatid breakages of autosomes and heterosomes, loss of chromosome fragments and gaps being much higher than in other four described cases. The SCEs test has been used and revealed a very high number of sister chromatid exchanges (9-15 SCEs/cell) and particularly, the presence of double interchromatid exchanges in one, two or even three chromosomes of the same metaphase.

To our knowledge, the malformed calf's mother was treated during the first months of gestation with antibiotic (amoxicillin, gentamicin) for an eye disease (palpebral ulcer) and, in the same period, there was a suspicion of aflatoxin pollution in the farm. Considering all this and knowing that the presence of a toxic chemical agent in the first months of gestation can induce fetal growth and development disorders, the etiology of this congenital malformation could be of a teratogenic nature.

Acknowledgements

This study was supported by the Sectorial Project 5.3.3./2015.

P16

Chromosome phylogeny of bats of subfamily Micronycterinae (Phyllostomidae) based on multidirectional chromosome painting data

Thayse C.M. Benathar[1]; Cleusa Y. Nagamachi[1]; Patricia C.M. O'Brien[2]; Fengtant Yang[3]; Malcolm A. Ferguson-Smith[2]; Luis R.R. Rodrigues[4]; Julio C. Pieczarka[1]

1 *Laboratório de Citogenética, Centro de Estudos Avançados da Biodiversidade, Terreno 11, Parque de Ciência e Tecnologia do Guamá, Avenida Perimetral da Ciência, km 01 – Guamá, 66075-75, Belém – Pará – Brasil* **2** *Department of Veterinary Medicine, Madingley Road, Cambridge, CB3 0ES, United Kingdom* **3** *Cytogenetics Facility, Welcome Sanger Institute, Wellcome Genome Campus, Hinxton, Cambridgeshire, CB10 1SA, United Kingdom* **4** *Laboratório de Genética e Biodiversidade, Universidade Federal do Oeste do Pará, Rua Vera Paz, s/n, Bairro Salé, 68035, Santarém, Pará, Brazil*

Corresponding author: *Julio C. Pieczarka* (*juliopieczarka@gmail.com*)

The Neotropical bat species of the subfamily Micronycterinae are classified in two genera, the monotypic *Lampronycteris* Sanborn, 1949, and the diversified *Micronycteris* Gray, 1866, with twelve species. We investigated the karyotype relationships of species of this subfamily using chromosomal banding and multidirectional chromosome painting with whole chromosome probes of *Carollia brevicauda* Wied-Neuwied, 1821 (CBR) and *Phyllostomus hastatus* Pallas, 1767 (PHA). A new cytotype was described for *M. megalotis* Gray, 1842 with 2n = 42, FN = 70, showing a fission of pair 4. Phylogenetic analyses were performed using parsimony (PAUP software) with *Macrotus californicus* Baird, 1858 (Macrotinae) as the outgroup. Few chromosome segments are shared between the *Lampronycteris* and *Micronycteris*, as well as among *Micronycteris* species, showing a high rate of karyotype evolution, with the fixation of a large number of rearrangements (inversions, fusions and fissions). The syntenic association CBR2q/Y2qis a chromosomal signature for Micronycterinae, while the associations PHA11p/3p and PHA4q/3p support the *Micronycteris* monophyly. We compared this chromosome map with 15 other species of Phyllostomidae previously studied to build the phylogeny. Most of the analyzed subfamilies each present a highly derived karyotype and different Phyllostomidae clades have different tendencies of karyotype evolution, including conservative, moderate and intense levels of chromosome reorganization. Our analysis is well supported and congruent with the molecular topologies on the relationship between *Lampronycteris* and *Micronycteris* as sister taxa, as well as the monophyly of *Micronycteris*.

P17

Meiotic and gene expression analyses in a case of t(1;15) azoospermic boar

Alain Pinton[1], Anne Calgaro[1], Nicolas Mary[1], Harmonie Barasc[1], Nathalie. Bonnet[1], Clémence Revel[1], Stéphane Ferchaud[2], Isabelle Raymond Letron[3], Thomas Faraut[1], Hervé Acloque[1], and Alain Ducos[1]

1 *GenPhySE, University of Toulouse, INRA Occitanie-Toulouse, ENVT, 24 chemin de Borde-rouge 31326 Castanet Tolosan, France* **2** *Génétique, Expérimentation et Système Innovants Poitou Charentes F-17700 Saint-Pierre-d'Amilly, France* **3** *LabHPEC (Laboratoire d'HistoPathologie Expérimentale et Comparée), ENVT, Université de Toulouse, France and STROMALab, Université de Toulouse, CNRS ERL5311, EFS, ENVT, Inserm U1031, UPS, Toulouse, France*

Corresponding author: *Alain Pinton* (*a.pinton@envt.fr*)

The systematic cytogenetic screening of young boars carried out for more than 20 years in our laboratory allowed us to accurately estimate the prevalence of balanced structural chromosomal rearrangements in the French pig populations (0.5%). Up to now, more than 39000 boars have been analyzed, and 180 new structural abnormalities have been identified. The most frequent were reciprocal translocations (87%). Contrary to humans, altered semen quality (oligo- or azoospermia) was detected in a few cases only: 4 Y-autosome translocations (Y/1, Y/9, Y/14, Y/16) and one autosome/autosome translocation (1/14).

Here, we report the case of a t(1;15) reciprocal translocation identified in an infertile zoospermic boar. Breakpoints position was determined by mate pair sequencing of microdissected translocated chromosomes. Meiotic pairing and recombination were investigated by immunostaining of the SCP1, SCP3, and MLH1 proteins, and analyzed by classical and super resolution microcopy. Finally, the impact of meiotic pairing impairments on SSC1 and SSC15, as well as SSCX and SSCY gene expression was investigated by qPCR.

Histological analysis revealed a total meiotic arrest at the spermatocyte I stage. The rearrangement was characterized by the translocation of a large part of the SSC15 onto the SSC1, leading to the formation of a tiny derivative chromosome 15. A quadrivalent was observed in 87% of the 113 spermatocytes analyzed, and a trivalent plus univalent in the remaining cells. 40% of the quadrivalents as as well as 33% of the trivalents were associated with the XY body. A γH2AX positive signal on SSC1 or SSC15 chromatin was observed in 87% of the spermatocytes analyzed. These results confirmed the impairment of meiotic process. We will also present on-going results on synaptonemal complex analysis by super-resolution microscopy and the expression of several genes located on SSC1, SSC15, SSCX and SSCY.

P18

Cytogenetics: a pertinent tool for analysis of therapeutic mesenchymal stem cells in regenerative medicine

Alain Pinton[1], Aude Noiret[1,2], Charlotte Bonnemaison[1,2], Sophie Ployon[1,2], Anne Calgaro[1], Isabelle Raymond-Letron[2]

1 *GenPhySE, University of Toulouse, INRA Occitanie-Toulouse, ENVT, 24 chemin de Borderouge 31326 Castanet Tolosan, France* **2** *LabHPEC* (*Laboratoire d'HistoPathologie Expérimentale et Comparée*), *ENVT, Université de Toulouse, France and STROMALab, Université de Toulouse, CNRS ERL5311, EFS, ENVT, Inserm U1031, UPS, Toulouse, France.*

Corresponding author: *Alain Pinton* (*a.pinton@envt.fr*)

Mesenchymal stem cells (MSCs) are adult stem cells that can be isolated from various human and animal tissue sources. They are multipotent, able to differentiate into osteocytes, adipocytes and chondrocytes lineage and offer large innovative therapeutic potential in regenerative medicine. Many clinical trials are running inhuman medicine, and veterinary use knows a rapid development. However, even if their therapeutic benefits are recognized, the biological mechanisms involved in regeneration are still not fully understood.

MSC, classified as ATMP (Advanced Therapy Medicinal Products), are used either as autologous or allogenic cells, freshly isolated cells or after culture expansion. Major scientific concerns address their tissue action mechanisms. Safety issues are linked to their biodistribution and long term tissue persistence.

To contribute these issues, we develop different cytogenetic tools:

An anti-human FISH probe was created in order to track human MSC in tissues sections of immunocompromised mouse models (efficacy proof of concept, safety studies). The Cot1 probe developed showed its efficiency for the precise tissue tracking of MSCs biodistribution after local or systemic administration.

In the same way, X and Y painting probes were developed to allow, in an immunocompetent canine model, the tracking of canine MSC after administration in an opposite sex host model.

Finally, chromosomal stability of MSCs, in particular in long term culture processes, is still controversial. Structural and numerical chromosomal abnormalities have been reported in Human MSC. Consequently, we aimed to analyze the chromosomal stability of the canine MSCs used in our studies. Preliminary results showed that 10% of these cells are polypoid (4n, 6n) at early passages (P2). Complementary researches are still in progress.

In conclusion, our work shows that cytogenetics provides original and pertinent tools to study MSC biology as well as for safety studies in regenerative medicine approaches for human or veterinary medicine.

P19

Molecular-cytogenetic characteristics of the *Gmelinoides fasciatus* Stebbing, 1899, karyotype

Eugene V. Potapenko, Elena I. Mikhailova, Svetlana A. Galkina, Larisa V. Barabanova

Department of Genetics and Biotechnology, Saint-Petersburg State University, Universitetskaya emb. 7/9, Saint-Petersburg, 199034, Russia

Corresponding author: *Eugene V. Potapenko* (*g.r.e.e.d@yandex.ru*)

The Baikal amphipod *Gmelinoides fasciatus* Stebbing, 1899, is of particular interest for population studies due to its unique adaptation abilities. In the middle of the last century, this indigenous species went through successful acclimation in quite a number of water bodies in Central and North-Western regions of Russia in the course of its direct introduction to enlarge food source for commercial fish. By virtue of the fact that comparatively short period of time elapsed since the start of *G. fasciatus* resettlement and that this species successfully adjusted to new habitat factors, it is possible to suggest that genetic mechanisms would underlay the adaptation process.

Peculiar features of the life cycle of amphipod *G. fasciatus* make it feasible to widely use cytogenetic and molecular-cytogenetic methods for investigation of genome changes at different levels of its organization. Having this objective in mind we analyzed frequencies of chromosomal aberrations in mitotically dividing embryo cells of *G. fasciatus* from different sample collection spots, but did not reveal considerable deviations of the parameter in the studied populations. We assumed that the time interval, starting from the point of introduction of the Baikal amphipod to new water systems, turned out to be sufficient to reach the optimal level.

Using individuals from the studied populations and routine acetoorcein-staining we determined the *G. fasciatus* diploid chromosome number to be equal to 2n=52. It became possible to construct a karyogram although we could not reveal specific morphological characteristics that would make every individual chromosome pair identification possible. Even so it became obvious that several pairs could be distinguished as being longer and having varied but similar within pairs chromatin appearance. DAPI-staining revealed A-T rich pericentromeric regions of mitotic chromosomes. FISH of telomeric repeats (TTAGG)n detected hybridization sites at chromosome ends, however some interstitial locations have been revealed as well. Hybridization of 18S rDNA molecular probe with *G. fasciatus* chromosomes manifested 4 distinct signals on 2 pairs of chromosomes in most of the nuclei. At the same time we have registered the presence of more than 4 (from 5 to 8) hybridization signals in several nuclei which indicates the variability of ribosomal cluster numbers in amphipod karyotype. Sequencing of 18S rRNA gene in particular conserved and variable fragments amplified using DNA of amphipod from indigenous Baikal population and those from dif-

ferent collection spots in the Gulf of Finland as well as from the Lake Ladoga showed no differences between specimens. Prospects of devising more molecular markers and *in situ* hybridization probes for the sake of disclosing more variation between *G. fasciatus* populations will be discussed with a view to getting insight into mechanisms of adaptation processes.

Acknowledgements

The research was partially supported by the grants, RFBR 15-29-02526 and NSH 9513.2016.4, and performed at the Research park of St.-Petersburg State University Centers for Molecular and Cell Technologies and "Chromas".

P20

Multiple highly amplified NORs co-localized with telomeric sequences in the parthenogenetic hybrid *Bacillus whitei* Nascetti et Bullini, 1982, (Insecta Phasmatodea)

Susanna Salvadori[1], Elisabetta Coluccia[1], Federica Deidda[1], Michela Sibiriu[1], Erica Costa[1], Anna Maria Deiana[1], Valerio Scali[2]

1 *Universiy of Cagliari Department of Life and Environmental Sciences* (*Università of Cagliari, Dipartimento di Scienze della Vita e dell'Ambiente*), *Via T. Fiorelli 1, 09126 Cagliari, Italia* **2** *Universiy of Bologna Department of BiGeA* (*Università di Bologna, Dipartimento BiGeA*), *Via Selmi 3, 40139 Bologna, Italia*

Corresponding author: *Susanna Salvadori* (*salvador@unica.it*)

Stick insects (Insecta, Phasmatodea) are a very interesting group for their reproductive biology. In fact most of them present bisexual reproduction but the lytokous parthenogenesis, hybridogenesis and androgenesisare are widespread among them. In fact, about 20% of the 3000 species are obligatory parthenogenetic, often as a consequence of hybridization between species. (reviewed in Scali 2009; Milani et al. 2015). Previous karyological investigations had evidenced extensive numerical and structural chromosome re-patterning in hybrid parthenogenetic taxa, leading to a variety of cytotypes, and a wide array of Ag-NOR bearing chromosomes in different species and even populations within species (Manaresi et al. 1992; 1993). In this work we analyzed by dual-FISH of 28S rDNA and telomeric pentameric sequences (TTAGG)n the karyological features in two populations of the parthenogenetic the lytokous *Bacillus whitei* Nascetti et Bullini, 1982 (2n=35 XX). This is a diploid hybrid, endemic of Sicily (Italy), between *B. rossius* (2n=36/35; XX/X0) and *B. grandii* Nascetti et Bullini, 1982 (2n=34/33, XX/X0) (Manaresi et al. 1992). Our results showed differences be-

tween the two populations in karyotype features such as chromosome rearrangements and the number/position of the highly amplified NORs. Furthermore, we constantly found a co-localization between rDNA and highly amplified telomeric sequences, as first evidenced in phasmid taxa of the genus *Leptynia* Pantel, 1890 (Scali et al. 2016). A similar interspersion has been also pointed out in five additional species of Phasmatodea belonging to distantly related genera (Liehr et al. 2017). The sharing of this type of amplification-interspersion of NOR and telomeric sequences in all phasmid species investigated by FISH strongly supports our opinion that their relationship could be not a casual one and also supports the early hypothesis that the NOR/telomere interspersion might constitute a hot spot of recombination (Salvadori et al. 1995), as actually shown by the variable NOR numbers and positions in *B. whitei*.

P21

Optimizating the analysis of porcine adipose tissue derived mesenchymal stem cells karyotyping

Raul Sánchez-Sánchez, Paloma De la Cruz Vigo, Ernesto Gómez Fidalgo, Sonia S. Pérez-Garnelo, Alexandra Calle, Miguel A. Ramirez, Mercedes Martin-Lluch

INIA. Avda. Puerta de Hierro Km. 5,9. Madrid, Spain

Corresponding author: *Raul Sánchez-Sánchez* (*raulss@inia.es*)

Cytogenetic studies are a reliable indicator of genetic stability of Adipose tissue-derived mesenchymal stemcells (ASCs), since these cells can lead to an accumulation of genetic and epigenetic alterations during their growing in *in vitro* culture. In particular, karyotype analys is isessential for the identification of both, numerical and structural chromosomal abnormalities.

There are several reasons for obtaining porcine ASCs. First, adipose tissue is abundant and easily accesible, second because pigs are used as a model for preclinical studies in human regenerative medicine, and for last, it may be interesting to store criopreserved porcine ASCs as a reserve of genetic material from pigs with chromosomal rearrangements for future research.

The aim of this study was to optimize the karyotyping method used in porcine ASCs to increase the number and the quality of obtained methaphases. Mesenchymal cells of abdominal adipose tissue were obtained from a boar with a chromosomal alteration (xy/xx mosaicism). Several tests were carried out on the established protocol for fibroblasts [The AGT CytogeneticLaboratory Manual], with modifications in colchicine and hypotonic solution concentrations, exposure times and fixation solution composition. Our results showed that metaphases quality was higher using porcine ASCs incubated with a final dilution of 0.1µl/ml colchicine for 18 hours at 37 °C,

treated with prewarmed hypotonic lysis solution (0.054M KCl) at room temperature for a time greater than 10 min. and fixed in methanol:aceticacid (3:1,v/v) solution. The adjustment to colchicine and hypotonic solution exposure is a critical step and thus, shorter periods of colchicine exposure were not enough to obtain a reasonable number of metaphases.On the other hand, higher exposure times and lower KCl concentrations provided better dispersion of methaphase chromosomes.

For that, standardization of karyotyping test is a crucial step to correctly interprete the results in porcine ASCs.

P22

Knockout of *ADAMTS1* gene by CRISPR/Cas9 affect the micronucleus frequency but not the DNA repair signaling in HeLa cell line

Renata R. Savchenko[1], Stanislav A. Vasilyev[1], Veniamin S. Fishman[2], Anastasiya A. Murashkina[3], AnastasiyaV. Dorofeeva[4], Igor N. Lebedev[1]

1 *Research Institute of Medical Genetics, Tomsk National Research Medical Center of the Russian Academy of Sciences, 10 Nab. Ushaiki, Tomsk, 634050, Russia* **2** *Institute of Cytology and Genetics of Siberian Branch of the Russian Academy of Sciences, 10 Lavrentyeva Prospect, Novosibirsk, 630090, Russia* **3** *National Research Tomsk State University, 36 Lenin Avenue, Tomsk, Russia* **4** *Siberian State Medical University, 2 Moskovsky trakt, Tomsk, Russia*

Corresponding author: *Renata R. Savchenko* (*savchenko_renata@mail.ru*)

Nowadays different DNA repair mechanisms are known, but the role of other indirect participants of the DNA damage response in maintaining the stability of the genome is insufficiently explored. Our previous transcriptome-wide experiments allowed us to identify differentially expressed genes in lymphocytes of individuals with different levels of repair of double-strand DNA breaks. The expression of several genes was correlated with the level of spontaneous γH2AX foci and the frequency of radiation-induced centromere-negative micronuclei not only in lymphocytes, but also in another cell type, namely human placental fibroblasts. The aim of this study was to analyze the effects of knockout of these genes on the DNA repair and genome integrity in model system *in vitro*.

We created HeLa cell lines with knockout of ADAMTS1, RBFOX2 and THBS1 genes using CRISPR/Cas9 technology. Level of spontaneous γH2AX and 53BP1 foci was assessed as a marker of signaling associated with DNA double-strand breaks. FISH-based micronucleus test was used to analyze the level of chromosome damage.

Knockout of all analyzed genes did not affect significantly the level of spontaneous γH2AX and 53BP1 foci. However, a significant increase in the level of micronuclei was observed inADAMTS1knockout cell line (21.7 ± 7.5 ‰) in comparison with the intact HeLa (5.0 ± 1.0 ‰, p = 0.019). We suppose that ADAMTS1can act as an

indirect participant of mechanisms of genome integrity maintenance without affecting DNA double-strand break repair signaling.

Acknowledgements

This study was supported by Grant of the President of Russian Federation MK-5944.2018.4.

P23

Identification of SNP polymorphism in the *growth hormone* and *myostatin* genes of the two Polish geese breeds

Grzegorz Smołucha[1], Anna Kozubska-Sobocińska[1], Anna Koseniuk[1], Mirosław Lisowski[2], Bartosz Grajewski[3], Igor Zubrzycki[1]

1 *National Research Institute of Animal Production, Krakowska 1, 32-020 Balice n. Cracow; Department of Animal Genomics and Molecular Biology. Poland* **2** *National Research Institute of Animal Production, Krakowska 1, 32-020 Balice n. Cracow; Department of Animal Reproduction Biotechnology, Poland* **3** *National Research Institute of Animal Production, Experimental Station in KołudaWielka, Waterfowl Genetic Resources Station in Dworzyska, 62-023 Dworzyska Poland*

Corresponding author: *Grzegorz Smołucha* (*grzegorz.smolucha@izoo.krakow.pl*)

Advances in molecular genetics of livestock animals allowed for the identification of genes which are responsible for production traits like lean body mass, as well as gene polymorphisms. So far, several genes were used as candidate genes for improvement of productive performance in animals. Among those, the growth hormone (GH) and myostatin (MSTN) play the paramount role. Growth hormone promotes muscle growth, bone formation and regulation of fat content. Myostatin is a major regulator of myogenesis expressed predominantly in skeletal muscle and a negative regulator of skeletal muscle growth and development. To determine the relationship between polymorphism and production performance rendered by the genes of growth hormone and myostatin the Single Nucleotide Polymorphism of the respective genes sequences was studied. The two breeds of Polish goose, i.e., Kielecka and Landes were selected for the study. An analysis of the GH sequence revealed the presence of three single nucleotide polymorphisms (SNP): nonsynonymous (V → A) SNPc.128C>T described previously and associated with the production features of many different breeds in China, and g.240108A> G and g.240247A> G of currently unknown phenotypic and physiological function. The two SNPs were also found in MSTN gene (exon 3): c.1124C>A resulted in amino acid exchange to stop codon and c.1231C>T in the 3`untranslated region.

P24

The role of nuclear architecture in regulating *PPARG* gene expression during porcine adipogenesis

Joanna M. Stachecka[1], Joanna Nowacka-Woszuk[1], Paweł A. Kolodziejski[2], Izabela M. Szczerbal[1]

1 *Department of Genetics and Animal Breeding, 33 Wolynska Street, 60-637 Poznan, Poland* **2** *Department of Animal Physiology and Biochemistry, Poznan University of Life Sciences, 35 Wolynska Street, 60-637 Poznan, Poland*

Corresponding author: *Joanna M. Stachecka* (*jstachec@up.poznan.pl*)

Adipogenesis in pig, as in other organisms, is mediated by a key transcription factor, peroxisome proliferator-activated receptor gamma, encoded by the *PPARG* gene located on porcine chromosome 13. Previous studies have shown that, during adipocyte differentiation, *PPARG* is repositioned from nuclear periphery to nuclear interior without relocation of chromosome territory. Here we examine in detail how changes in nuclear positioning of the *PPARG* gene affect its expression. An established *in vitro* adipocyte differentiation system from mesenchymal stem cells derived from bone marrow and adipose tissue was used. Differentiation was monitored for seven days and cells were examined using a 3D DNA/RNA/immuno-FISH approach. *PPARG* transcript level was measured by real-time PCR, and PPARG activity was detected by colorimetric assay. Changes in the nuclear location of the *PPARG* gene were seen when we compared undifferentiated mesenchymal stem cells with mature adipocytes. Also, the two *PPARG* alleles had different nuclear locations when measured in relation to the nuclear lamina. The RNA–DNA FISH approach has shown that differences in primary transcript production depend on the allele's nuclear positioning, with transcriptionally active alleles preferentially occupying the central part of the nucleus. The number of *PPARG* transcripts evaluated by RNA-FISH on the single-cell level corresponded to the transcript level and protein activity evaluated by the molecular approach. However, the advantage of FISH-based techniques was that temporal changes in mRNA production dynamics could be detected. Our study confirms the importance of nuclear architecture in regulating gene expression and thus in the establishment of adipose cell fate.

Acknowledgements

The work was financially supported by the National Science Center, Poland, grant no. 2012/07/E/NZ9/02573.

P25

Expression of key genes involved in DNA methylation during *in vitro* differentiation of porcine mesenchymal stem cells (MSCs) into adipocytes

Joanna M. Stachecka, Weronika Lemanska, Izabela M. Szczerbal

Department of Genetics and Animal Breeding, Poznan University of Life Sciences, 33 Wolynska Street, 60-637 Poznan, Poland

Corresponding author: *Joanna M. Stachecka* (*jstachec@up.poznan.pl*)

DNA methylation plays an important role in regulating gene expression and is essential for cell differentiation, including adipogenesis. The proper course of DNA methylation depends on the functioning of the major components of the DNA methylation machinery, including DNA methyltransferase enzymes and proteins that bind specifically to methylated DNA. It has been hypothesized that changes in DNA methylation during porcine adipocyte differentiation are related to changes in the transcription levels of genes crucial to the DNA methylation machinery. We selected five genes (*DNMT1*, *MeCP2*, *MBD1*, *MBD3* and *UHRF1*) and analyzed their expression using real-time PCR and immunofluorescence in undifferentiated mesenchymal stem cells derived from bone marrow (BM-MSC) and adipose tissue (AD-MSC) during the course of adipocyte differentiation. The transcript level of the *MeCP2* gene was found to be lower in AD-MSC differentiation system than in BM-MSC. At the initial days of adipogenesis, this gene was upregulated and its transcript level decreased during the terminal stages of differentiation. A low number of nuclei positive for the MeCP2 protein were also observed on day 7 of differentiation. The *DNMT1* gene was downregulated during the following days of adipocyte differentiation in both the studied systems. In case of *MBD1, MBD3,* and *UHRF1* genes we observed stable transcript levels during adipogenesis. The results indicate the significance of the DNA methylation machinery for proper adipocyte differentiation in pigs.

Acknowledgements

The work was financially supported by the National Science Center, Poland, grant no. 2012/07/E/NZ9/02573.

P26

Association of a known G-insertion upstream of *SOX9* with XX disorder of sex development in dogs is doubtful

J. Nowacka-Woszuk[1], I. Szczerbal[1], M. Stachowiak[1], S. Dzimira[2], W. Nizanski[2], R. Payan-Careira[3], E. Wydooghe[4], T. Nowak[1], K. Blasiak[5], A. Solecka[6], M. Switonski[1]

1 *Poznan University of Life Sciences, Wolynska 33, 60-637 Poznan, Poland* **2** *Wroclaw University of Environmental and Life Sciences, C.K. Norwida 31,* 50-375 Wrocław, Poland **3** *Wroclaw University of Environmental and Life Sciences, Pl. Grunwaldzki 49,* 50-366 Wroclaw, Poland **4** *Universidade de Trás-os-Montes e Alto Douro, Quinta de Prados, Apartado 1013, 5001-801 Vila Real, Portugal* **5** *Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium* **6** *VetMedica Veterinary Clinic, Zamenhofa 37, 64-100 Leszno, Poland* **7** *Medwetar Veterinary Clinic, Zeromskiego 105, 64-920 Pila, Poland*

Corresponding author: *Marek Switonski* (*switonsk@up.poznan.pl*)

Testicular or ovotesticular XX, SRY-negative (XX) disorder of sex development (DSD) is commonly diagnosed in numerous dog breeds, but its molecular background remains unclear. In a candidate region harboring SOX9, duplication of this gene, CNV, as well as an indel (–/G, rs852549625) located upstream of SOX9 has been suggested as associated with this DSD. We analyzed 32 unrelated XX (SRY-negative) DSD dogs, including 18 new cases, and 31 control females. We also studied two families in which 3 XX DSD cases had been identified. Cytogenetic analysis, molecular detection of the Y-linked genes (SRY and ZFY), and histological studies revealed, that among unrelated cases, ten were testicular XX DSD and eight were ovotesticular XX DSD, while the histology of the gonads was unknown in 14 cases. Sanger sequencing of the fragment (839 bp) harboring the G-insertion was performed. Apart from the analyzed indel, we identified five known and one unknown SNPs. In a cohort of unrelated XX DSD cases, the following genotypes were observed for the indel: G/G (n = 2), -/G (n = 8) and -/- (n = 22), while in control females, the number of genotypes were 0, 7, and 24, respectively. In a Pug family, two XX DSD siblings and their father had the G/G genotype, while the mother was -/G. In a Cane Corso family, two offspring were heterozygotes, including an XX DSD case, and the parents were G/G (mother) and -/G (father). We conclude that G-insertion upstream of the SOX9 gene is not associated with the affected phenotype in the studied cases.

Acknowledgements

The work was finencially supported by National Science Centre, Poland, grant no. 2016/23/B/NZ9/03424.

P27

Detection and quantification of leukocyte chimerism (XX/XY) using FISH and digital droplet PCR (ddPCR) in the offspring of highly prolific sows

Izabela Szczerbal[1], Joanna Nowacka-Woszuk[1], Stanislaw Dzimira[2], Anna Alama[3], Pawel Iskrzak[3], Marek Switonski[1]

1 *Department of Genetics and Animal Breeding, Poznan University of Life Sciences, Wolynska 33, 60-637 Poznan, Poland* **2** *Department of Pathology, Wroclaw University of Environmental and Life Sciences, C.K. Norwida 31, 50-375 Wroclaw, Poland* **3** *AgriPlus Poznan, Marcelinska 92/94, 60-324 Poznan, Poland*

Corresponding author: *Izabela Szczerbal* (*izabel@up.poznan.pl*)

Disorders of sex development (DSD) are a significant problem in pig production, since they lead to sterility and may affect meat quality through the presence of testicular tissue in animals of female karyotype. So far, testicular XX DSD (*SRY*-negative) has been recognized as the most common type of this disorder in pigs. In this study, we performed a complex cytogenetic and molecular analysis of 28 pigs with ambiguous external genitalia identified on a large commercial farm with highly prolific sows (~17 piglets/litter). We used standard Giemsa staining and FISH technique with X chromosome-specific probe (BAC clone CH242-156O11 for pseudoautosomal region). The *SRY*, *ZFX*, and *ZFY* genes were detected in blood samples, and in 20 cases in hair follicles, to exclude or confirm whole-body chimerism. Moreover, digital droplet PCR (ddPCR) was used to estimate X and Y chromosome copy numbers, based on copies of the *AMELX* and *AMELY* genes, in order to detect leukocyte chimerism. Among the examined animals, 20 were diagnosed as freemartins due to the presence of leukocyte chimerism (38,XX/38,XY), six had testicular XY DSD and two had XX DSD. The percentage of XX and XY cell lines in the freemartins varied over a wide range. The ddPCR approach turned out to be enough sensitive to detect the cell lines occurring at low frequency. Our study showed that high prolificacy is associated with the occurrence of freemartinism. Although the FISH approach is recommended as a gold standard in the cytogenetic diagnosis of freemartinism, the method is labor-intensive and time-consuming. We thus recommend ddPCR for rapid and reliable detection of the chimerism.

Acknowledgements

The work was financially supported the Department of Genetics and Animal Breeding, Poznan University of Life Sciences, No. 508.534.00.

P28

mtDNA variation of troglophilic crickets (Myrmecophilidae: Eremogryllodes), across Zagros Mountains and south of Iran

Mohadeseh S. Tahami[1], Mina Hojat Ansari[2], Saber Sadeghi[1], Alsu F. Saifitdinova[3]

1 *Entomology Research Laboratory, Biology Department, Adabiat St., Shiraz University, Shiraz, Iran, 7146713565* **2** *Gastroenterohepatology Research Center, Shiraz University of Medical Sciences, Khalili St., Shiraz, Iran, 71935-1311* **3** *Chromas Research Resource Center, Saint Petersburg State University, Oranienbaumskoye sh. 2, 198504, Saint Petersburg, Russia*

Corresponding author: *Mohadeseh S. Tahami* (*tahami.m@hotmail.com*)

Several new species from the genus *Eremogryllodes* Chopard, 1929 (Insecta: Orthoptera: Myrmecophilidae) inhabiting a number of caves located across Zagros Mountains and south of Iran, have been recently described based on morphology by the first author (Tahami et al. 2017). These species are *E. bifurcatus* Tahami et Gorochov, 2017, *E. dilutes* Tahami et Gorochov, 2017, *E. iranicus* Tahami et Gorochov, 2017 and *E. persicus* Tahami et Gorochov, 2017, and a number of subspecies. To our knowledge, the populations of those species spend most of their life span inside caves, as we have not encountered them outside the cave environment during our extensive surveys in Iran. The described species are morphologically very similar, which hampers the species identification. Therefore, molecular studies are essential to confirm their status. The absence of the distinct morphological characters, applicable for species separation, and the considerable genetic isolation can lead to the formation of the cryptic species. Therefore, our work has two main aims: (1) to test whether the morphological characters, published in Tahami et al. (2017), correlate with the molecular evidences; (2) to test for the presence of the cryptic species. To this extent, here we provided the molecular phylogenetic analysis based on 16S rDNA of the recently described species of *Eremogryllodes* for the first time. Overall, we included 37 specimens collected in 24 caves. Phylogenetic analyses, using RAxML and Bayesian inference methods, revealed four well-supported clusters. The resulting tree also shows one specimen, which does not cluster with the others, and can represent the potential cryptic species. This topology does not corroborate morphological taxonomy. The first cluster corresponds to *E. bifurcatus*. This also corresponds to the geographic distribution, as the specimens of this species were collected from the caves located in the north of Zagros Mts, whereas all other species were mostly collected in the middle and south of the region. The second cluster includes four specimens from *E. persicus*, however, other specimens from this species are intermixed with *E. iranicus* and *E. dilutus* within the third and fourth clusters. Two latter species are also intermixed with each other. Overall, the current investigation is not congruent with three previously described species, and indicates

that the morphological characters, although valuable, but cannot always be applicable to species identification in this genus by their own. However, further molecular work using more genes and more specimens from the same region is needed to confirm the status of the clusters provided by 16S and presence of the cryptic species.

Acknowledgements

The work was done in Chromas Core Facility Center of Saint-Petersburg State University.

P29

Some unusual properties of constitutive heterochromatic regions of chromosome from human extraembryonic tissues

Irina L. Trofimova[1,2,3], Natella I. Enukashvily [4], Mikhail A. Dobrynin[5], Tatyana V. Kuznetzova[6]

1 *Federal State Budgetary Institution V.A. Almazov Federal North-West Medical Research Centre of the Ministry of Health of the Russian Federation, Akkuratova street 2, 197341, Saint-Petersburg, Russia* **2** *International centre of reproductive medicine, Komendantskij prospect, 53k, 197350, Saint-Petersburg, Russia* **3** *Federal State Budget military educational institution of higher professional education "Military Medical Academy named after S.M. Kirov" under the Ministry of Defense of the Russian Federation, 6 Lebedeva street, St. Petersburg, 194044, Russia* **4** *Institute of Cytology of the Russian Academy of Sciences, Saint-Petersburg, Tikhoretsky ave. 4, 194064, Saint-Petersburg, Russia* **5** *Saint Petersburg State University, Universitetskaya nab. 7/9, 199034, Saint Petersburg, Russia* **6** *Federal State Budgetary Scientific Institution "The Research Institute of Obstetrics, Gynecology and Reproductology named after D. O. Ott", Mendeleevskaya street, 199034, Saint-Petersburg, Russia*

Corresponding author: *Irina L. Trofimova* (*il_trofimova@list.ru*)

Satellite DNA forms large arrays within heterochromatic regions of chromosomes and constitutes an essential part of the "non-coding landscape" of eukaryotic genomes. Satellite DNA of constitutive heterochromatin regions (CHRs) of autosomes 1, 9, 16 of human is of special interest and its functional value is discussed. These CHRs in chromosomes from cytotrophoblasts of chorion villi are characterized by decondensation, early replication, hypomethylation and DNAse 1 hypersensitivity.

We studied peculiarities of CHRs on direct and semi-direct slides from chorionic villi. After standard acridine orange (AO) staining of untreated direct chromosomal preparations CHRs manifest unusually bright red fluorescence in 1qh, 9qh, 16qh as typical for single-stranded DNA and RNA. The nature of this fluorescence was studied in direct chromosomal preparations pretreated with different enzymes (RNase A, RNase H, DNase I, DNA ligase T4) followed by AO staining. Results of this work show

that ssRNA and DNA*RNA hybrids are present in CHR of chromosome 1 (1q12) on fixed chromosomes.

We studied in details nuclear position and transcriptional activity of satellite 3 of CHR of 1q12. Our 3D-FISH results showed a significant repositioning of 1q12 towards the centre of the nucleus and near chromocenter in chorionic villi sample from early pregnancy (4-5 week). We found no changes in the position of 1q12 in chorionic villi from 5-6 week to 36 week pregnancy. Almost all FISH-signals were closer to the nuclear periphery and chromocenter. RT-PCR results showed polyadenilated non-coding RNAs of 1q12 in chorion on 6-15 weeks of gestation. The choice of the chain for transcription depends on gestation age.

The results confirm unusual conformational packaging of CHRs which can be associated with transcriptional activity of satellite DNA of 1qh in chorionic villi cells during early embryogenesis.

Acknowledgements

This research was supported by Russian Foundation for Basic Research (grant #18-34-00279).

P30

Two cases of equine X chromosome monosomy mosaicism

Bahareh Ahmadi[1], Tamas Revay[1], Ruby Yoana Murcia-Robayo[2], Ignacio Raggio[2], Daniel A.F. Villagomez[1,3], Mouhamadou Diaw[2], Keith Colquhoun[4], W. Allan King[1,5]

1 *Department of Biomedical Sciences, Ontario Veterinary College, University of Guelph, 50 Stone Rd E, Guelph, Ontario, Canada, N1G 4S7* **2** *Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Montréal, 3200 Rue Sicotte, Saint-Hyacinthe, Quebec, Canada, J2S 2M2* **3** *Departamento de Producción Animal, Universidad de Guadalajara, Camino Ramón Padilla Sánchez 2100, Zapopan, Jal. 44600, Mexico* **4** *Colquhoun Equine Veterinary Services, 5151 Wellington Road 29, Rockwood, ON, Canada, N0B 2K0* **5** *Karyotekk Inc, Box636 OVC, 50 Stone Rd E, Guelph, Ontario, Canada, N1G 4S7*

Corresponding author: *Daniel A.F. Villagomez* (*dvillago@uoguelph.ca*)

Monosomy of the X chromosome, the most frequent sex chromosome abnormality associated with infertility in mares, has been investigated since early 1970's. Affected mares may show one or more of the clinical symptoms such as short stature, undeveloped reproductive organs, and absent or irregular estrous cycle. It has been shown that some clinical features including fertility are correlated with the percentage of normal cells present. In our study, the presence of chromosomal mosaicism (XO/XX) in two mares

(Irish Cob maiden, Standardbred) with fertility problem was determined using three independent techniques. Case No. 1 was phenotypically normal with normal external genitalia appearance, normal ovaries, and two tubular structures compatible with cervices on the pelvic floor. Case No. 2 was normal size with flaccid uterus, very small ovaries, and atonic cervix of normal size. Using chromosome-counting technique, fluorescent staining of interstitial C-band of X chromosome, and fluorescence *in situ* hybridization revealed 14.6% and 95% of X monosomy for each case, respectively. As the percentage of X monosomy in the first mare is low and the ovaries are functional, the fertility problem could be related to a failure of maternal recognition of pregnancy in response to movements of the embryo being limited to one uterine horn. Therefore, there are some possibilities for the production of a foal in this case. The second horse with 95% of X monosomy and very small ovary is infertile. This report highlights the importance of molecular cytogenetic techniques in cases of equine infertility to decide whether to keep breeding from such individuals.

P31

Chicken tandem repeats Ggal10 and Ggal20 are specific to different microchromosomes

Valeriia A. Volodkina, Maria M. Kulak, Aleksei S. Komissarov, Svetlana A. Galkina, Elena R. Gaginskaya, Alsu F. Saifitdinova

Saint Petersburg State University, Universitetskaya emb. 7/9, Saint Petersburg, 199034, Russia

Corresponding author: *Valeriia Volodkina* (*leravolo94@gmail.com*)

Repeats are large and significant part of genome in many organisms and are proved to be functionally important. The chicken (*Gallus gallus* Linnaeus, 1758) genome is one of the smallest among vertebrates. Its haploid size has been estimated to be 1.25 pg (1.223 Gbp). The karyotype (2n=78) includes 10 pairs of macrochromosomes, 28 pairs of microchromosomes, and a pair of sex chromosomes ZZ or ZW. Although the content of repetitive DNA in avian genomes is considerably less than in other classes of vertebrates, interspersed and tandem repetitive sequences occupy 31-35 % of the chicken genome. Guizard et al. (2016) noticed the different distributions of repeats in avian chromosomes when compared to other vertebrate genomes investigated so far. Apart from the most abundant telomere repeat, in avian genomes (as exemplified primarily in the chicken genome studies) there are many different, small families of satellite DNAs, copious in small chromosomes, which might label each small chromosome with a "satellite DNA code". Since modern assembling techniques are not able to cope with tandem repeats, the way to solve the problem is to investigate them purposefully.

We have done research on identification and assessment of tandem repeats represented in the chicken *Gallus gallus* sequence raw archive (NCBI) for female (SRR958465) and male (SRR958466). The Lyrebird software was created for this purpose (Komissarov et al. 2018).

Isolated from raw reads, the new tandem repeat Ggal10 consists of 10-mers GCCCCATAGA. In female chicken genome this repeat constitutes a part of more than 2 Mb while in male genome it is less than 0.2 Mb. Published assembly of chicken genome and database of W-specific repeats don't have the sequence of Ggal10. FISH on mitotic chicken chromosomes with specific oligonucleotide probe revealed the only pair of microchromosomes bearing Ggal10 in male and female karyotypes. There was no FISH signal on W chromosome.

The polymorphic tandem repeat Ggal20 consists of 20-mer AAATCCATAGCCATCATTGT. Its AT content is about 65%. There is less than 0.2 Mb representing this repeat in female genome and approx. 0.3 Mb in male one. In the actual chicken genome assembly this repeat is located on Z chromosome, but there were no FISH signals on sex chromosomes. We have revealed dispersed signals corresponding to Ggal20 on a few chicken microchromosomes.

These data contribute to the complete deciphering of the chicken microchromosome content.

Acknowledgements

The work was financially and technically supported by "Chromas" Research Resource Center and Theodosius Dobzhansky Laboratory of Saint-Petersburg State University, RFBR grant # 16-04-01823.

P32

Early cytomolecular diagnostics of the Holstein-Friesian breed heifers from heterosexual multiple pregnancies

Anna Kozubska-Sobocińska, Grzegorz Smołucha, Barbara Danielak-Czech, Wojciech Witarski

Department of Animal Molecular Biology, National Research Institute of Animal Production, Krakowska 1, 32-083 Balice n. Krakow, Poland

Corresponding author: *Wojciech Witarski* (*wojciech.witarski@izoo.krakow.pl*)

Based on hitherto studies, 82 to 95% of heifers from heterosexual twin pregnancies are infertile freemartins defined by 60,XX/60,XY karyotype. The frequency of such, according to many authors is a function of the breed. This anomaly is a result of vascular

anastomosis and exchange of hematopoietic tissue between dizygotic twins of different sex and leads to extensive pathological changes in the female reproductive system, i.e., masculinization of sexual organs. The masculinization level is independent of the content of a cell line 60, XY adopted from the male sibling. The presented research is an attempt to determine the frequency of freemartinism in the HF-cattle population which can be further used for recommendations regarding early cytogenetic diagnostics and selection of heifers from heterosexual multiple pregnancies. In this study, we used 26 HF heifers (2 to 3 weeks old) and analyzed the metaphase chromosomes (minimum 100 metaphase plates per animal). In 24 heifers, leukocyte chimerism determined by the 60,XX/60,XY karyotype was revealed. The participation of the male cell line 60,XY in individual animals was in the range of 6% to 95%, whereas the normal karyotype, i.e., 60,XX was found in two heifers. The quality of cytogenetic analysis was confirmed by the study employing 12 microsatellite sequences, including BM1818, BM1824, BM2113, ETH3, ETH10, ETH225, INRA10, INRA23, SPS115, TGLA53, TGLA126, TGLA227, and heterosome markers such as AMELX and AMELY and SRY. The obtained results show that freemartinism is coupled to 92% of HF heifers from heterosexual multiple pregnancies and only 8% of heifers without the karyotype change can be qualified for further breeding and reproduction.

Acknowledgements

The work was financially supported from The Ministry of Agriculture and Rural Development funds, project no. 03-17-23-09.

P33

3D architecture of japanese quail DNA repeats in interphase nucleus

Anna M. Zlotina[1], Antonina V. Maslova[1], Nadezda V. Kosyakova[2], Thomas Liehr[2], Alla V. Krasikova[1]

1 *Saint Petersburg State University, 7-9 Universitetskaya Emb., St Petersburg 199034, Russia* **2** *Jena University Hospital, Institute of Human Genetics, Am Klinikum 1, 07747 Jena, Germany*

Corresponding author: *Anna M. Zlotina* (*anna-zlotina@yandex.ru*)

Japanese quail (*Coturnix c. japonica* Temminck et Schlegel, 1849), a galliform domestic species closely related to chicken, has typical avian karyotype that includes several pairs of macrochromosomes and numerous tiny microchromosomes (2n=78). The quail chromosomes are known to possess multiple heterochromatic segments such as centromeric regions and enigmatic blocks constituting short arms of submetacentric

microchromosomes. Using lampbrush chromosome mechanical microdissection we previously generated FISH-probes specific to centromeric repeats of macrochromosomes (1-5) and repetitive elements from short arms of microchromosomes. Their karyotype distribution and precise positions on giant lampbrush chromosomes were subsequently investigated. In the present study we got insights into three-dimensional organization of the isolated repeats in quail interphase nucleus from embryonic and adult tissues. In particular, we showed that prominent DAPI-positive chromocenters contain repressive H3K9Me3 and HP1β chromatin markers typical for constitutive heterochromatin and accumulate repetitive DNA-sequences from short arms of quail microchromosomes. In all types of cells, clusters of microchromosomal centromeric BglII-repeats rim the chromocenters. In contrast, the centromere repeats of the largest macrochromosomes (1 and 2) are predominantly located in perinuclear heterochromatin, while the rest macro-centromeres are observed as patches on the periphery of chromocenters. Some differences of spatial nucleus architecture between cultured embryonic fibroblasts and cells from adult tissues were noted. To conclude, well-known specific morphology of quail interphase nuclei as compared to chicken is due to clustering of large blocks of repeats from short arms of microchromosomes.

Acknowledgements

The research was supported by a grant of the President of the Russian Federation (MK-1630.2017.4). The work was partially performed using experimental equipment of the Research Resource Centers “Chromas” and “Molecular and Cell Technologies” of St. Petersburg State University.

CompCytogen 12(3): 361–372 (2018)
doi: 10.3897/CompCytogen.v12i3.27207
http://compcytogen.pensoft.net

RESEARCH ARTICLE

COMPARATIVE Cytogenetics
A peer-reviewed open-access journal
International Journal of Plant & Animal Cytogenetics, Karyosystematics, and Molecular Systematics

First cytogenetic analysis of lesser gymnures (Mammalia, Galericidae, *Hylomys*) from Vietnam

Svetlana V. Pavlova[1], Larisa S. Biltueva[2], Svetlana A. Romanenko[2,3], Natalya A. Lemskaya[2], Anton V. Shchinov[1], Alexei V. Abramov[4], Viatcheslav V. Rozhnov[1,5]

1 *A.N. Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, 33 Leninsky prosp., Moscow 119071, Russia* **2** *Institute of Molecular and Cellular Biology, Siberian Branch of the Russian Academy of Sciences, 8/2 Acad. Lavrentiev ave., Novosibirsk 630090, Russia* **3** *Novosibirsk State University, 2 Pirogova str., Novosibirsk 630090, Russia* **4** *Zoological Institute, Russian Academy of Sciences, 1 Universitetskaya nab., Saint Petersburg 199034, Russia* **5** *National Research Tomsk State University, 36 Lenin Avenue, Tomsk 634050, Russia*

Corresponding author: *Svetlana V. Pavlova* (swpavlova@mail.ru)

Academic editor: *Jan Zima* | Received 6 June 2018 | Accepted 1 August 2018 | Published 23 August 2018

http://zoobank.org/FC65675D-69F4-4D31-8C72-6B03391B96E7

Citation: Pavlova SV, Biltueva LS, Romanenko SA, Lemskaya NA, Shchinov AV, Abramov AV, Rozhnov VV (2018) First cytogenetic analysis of lesser gymnures (Mammalia, Galericidae, *Hylomys*) from Vietnam. Comparative Cytogenetics 12(3): 361–372. https://doi.org/10.3897/CompCytogen.v12i3.27207

Abstract

Gymnures are an ancient group of small insectivorous mammals and are characterized by a controversial taxonomic status and the lack of a description of karyotypes for certain species. In this study, conventional cytogenetic techniques (Giemsa, CBG- and GTG-banding, Ag-NOR), CMA_3-DAPI staining, and fluorescent *in situ* hybridization (FISH) with telomeric DNA probes were used to examine for the first time the karyotypes of lesser gymnures of group *Hylomys suillus* Müller, 1840 from northern and southern Vietnam. All studied specimens had karyotypes with 2*n*=48, NF*a*=64. C-positive heterochromatic blocks existed in centromeric regions of 7 bi-armed autosomes and the submetacentric X chromosome. The Y chromosome is a C-positive and dot-like. The nucleolus organizer regions resided terminally on the short arms of 2 small bi-armed pairs. Positive signals at the telomeres of all chromosomes were revealed by FISH. CMA_3-positive blocks were localized on the telomeric and pericentric regions of most bi-armed and acrocentric chromosomes. Despite the large genetic distances between *Hylomys* Müller, 1840, lesser gymnures from *H. suillus*-group from northern and southern Vietnam have similar karyotypic characteristics.

Keywords
cell culture, cryoconservation, FISH, insectivorous mammals, karyotype, telomeric sequence

Introduction

The order Erinaceomorpha is a diverse group of small insectivorous mammals that are widely distributed throughout Africa, Europe, and Asia. According to most current taxonomic systems, this order contains the single family Erinaceidae with 2 subfamilies: Erinaceinae (hedgehogs) and Galericinae (gymnures) (McKenna and Bell 1997, Hutterer 2005). However, their ancient origin, deep genetic divergence, and high morphological differentiation suggest that these 2 taxa should be ranked as families (Bannikova et al. 2014). Based on the latest multigene study, we consider gymnures to be representatives of a separate family, Galericidae.

The family Galericidae comprises 6 recent genera, with 6–12 species in total (Hutterer 2005, Bannikova et al. 2014). Karyotypes of Galericidae have been poorly studied (O'Brien et al. 2006) – only 3 gymnure species have been karyotyped: Mindanao gymnure (*Podogymnura truei* Mearns, 1905), endemic to Mindanao Island, Philippines, with 2*n*=40, NF=76 (Rickart 2003); shrew gymnure (*Neotetracus sinensis* Trouessart, 1909) from southern China, with 2*n*=32, NF=52 (Ye et al. 2006); and Hainan gymnure (*Neohylomys hainanensis* Shaw et Wong, 1959), endemic to Hainan Island, with 2*n*=32, NF=64 (Li et al. 2008). The karyotypes for lesser gymnures of the genus *Hylomys* Müller, 1840 remain unknown.

Lesser gymnures *Hylomys* spp. inhabit the Greater Sunda Islands, Indochina, and southern China (Hutterer 2005). In most current taxonomic systems, *Hylomys* s.str. contains 2 species: *H. parvus* Robinson et Kloss, 1916 is restricted to the highlands of Sumatra, and *H. suillus* Müller, 1840 is distributed throughout continental southeast Asia and the Sunda Islands (Frost et al. 1991, Corbet and Hill 1992, Ruedi and Fumagalli 1996, Hutterer 2005). According to current taxonomy, there are 7 subspecies (*H. s. suillus*, *H. s. dorsalis*, *H. s. maxi*, *H. s. microtinus*, *H. s. pequnensis*, *H. s. siamensis*, and *H. s. tionis*) of *H. suillus* (Hutterer 2005). A recent mtDNA analysis suggested that the taxon *H. suillus* (sensu Hutterer 2005) represents a paraphyletic association of 5 to 7 full species, including an undescribed taxon from southern Vietnam: *Hylomys* sp. (Bannikova et al. 2014). These authors also suggested that the name *Hylomys suillus* should be applied only to the Java population, whereas the lesser gymnures from northern Vietnam could be treated as distinct species: *Hylomys microtinus* Thomas, 1925 (Bannikova et al. 2014).

In this report, we characterized for the first time the karyotypes of lesser gymnures of *Hylomys suillus*-group from northern and southern Vietnam using a set of cytogenetic tools. Prior to comprehensive taxonomic revision of the group *H. suillus*, we use the name *Hylomys suillus microtinus* for the gymnures from northern Vietnam and *Hylomys* sp. for those from southern Vietnam (see Bannikova et al. 2014).

Material and methods

Specimens

Gymnures were collected during biodiversity surveys carried out by the Joint Vietnam-Russian Tropical Research and Technological Centre in 2013–2014. Voucher specimens are deposited in the Zoological Museum of Moscow State University (ZMMU), Moscow, Russia and the Zoological Institute of the Russian Academy of Sciences (ZIN), Saint Petersburg, Russia. Two specimens (male ZMMU S-193936 and female ZMMU S-199642) from Northern Vietnam, Phu Tho Province, Xuan Son National Park (21°08'12"N, 104°56'11"E), and one specimen (female ZIN 101915) from Southern Vietnam, Dak Lak Province, Chu Yang Sin National Park (12°25'26"N, 108°21'52"E) were karyotyped. The animals were caught alive using locally made cage traps (Abramov et al. 2008).

Cell cultures, preservation of cells, and chromosome preparations

Primary fibroblast cell cultures that were derived from tail biopsies of individuals from northern Vietnam were established and subsequently deposited to the cell banks of 2 cytogenetic laboratories (in Moscow and Novosibirsk, Russian Academy of Sciences). The cell cultures were established in parallel to prevent the loss of valuable material. As a result, the cell culture from a female (ZMMU S-199642) was deposited only to Moscow lab, whereas the Novosibirsk lab established the fibroblast cultures from a male (ZMMU S-193936) and female (ZMMU S-199642).

Each lab modified the standard cell culture protocol (Freshney 2010). Briefly, small pieces of tails were cultured in DMEM or αMEM (Invitrogen) that was supplemented with embryonic bovine serum (10% or 15%, respectively) with penicillin/streptomycin (5000 units/5 mg/ml or 10^5 U/L/100 mg/L, respectively) and amphotericin B (2.5 mg/L) at 37°C and 5% CO_2 for 3-4 weeks. In all cases, the cells were cryopreserved using a standard technique for mammalian fibroblast cell cultures, in which the cells were suspended in medium supplemented with a high concentration of serum (>40%) with a cryoprotectant, dimethyl sulfoxide (DMSO) (to a final concentration of 10%). Cryovials were kept in a freezer (-70°C) overnight and then transferred in an ultra-low-temperature container with liquid nitrogen for long-term storage.

Metaphase chromosome preparations from primary fibroblast cultures were made following the standard technique (Freshney 2010).

The standard field procedure for bone marrow cultures was used to obtain chromosome preparations for a female (ZIN 101915) from southern Vietnam.

Chromosome staining and microscope analysis

Air-dried chromosome spreads of all specimens were stained conventionally with 2% Giemsa for 4-5 minutes and then submitted to differential staining.

To determine the location of heterochromatin, C-banding was performed per the standard technique (Sumner 1972) with some modifications, as described in Gladkikh et al. (2016).

The fluorochromes chromomycin A3 (CMA_3) and 4,6-diamidino-2-phenylindole (DAPI) were applied to identify GC- and AT-rich heterochromatic regions, respectively (Lemskaya et al. unpubl.).

The standard trypsin-Giemsa staining technique (Graphodatsky and Radjabli 1988) with some modifications was used to identify homologies by G-bands. Chromosome spreads were treated with 0.25% trypsin solution (Paneco, Russia) at 25-30°C for 15-20 seconds, rinsed in 2xSSC buffer, and then stained with 2% Giemsa for 2-3 min.

Nuclear organizer regions (NORs) were detected by silver nitrate staining following Graphodatsky and Radjabli (1988).

To detect telomeric repeats, the G-banded metaphase chromosomes of a female from northern Vietnam was hybridized *in situ* with a fluorescein-conjugated peptide nucleic acid (PNA) probe from the Telomere PNA FISH Kit/FITC (K5325 from Dako, Glostrup, Denmark) following the manufacturer's instructions.

Images were captured with a ProgRes CCD (Jenoptic) camera mounted on an Axioscope 2 plus (Zeiss) microscope with filter sets for DAPI, FITC, and rhodamine, using VideoTesT-FISH 2.0 and VideoTesT-Karyo 3.1. (VideoTesT, Saint Petersburg, Russia) software. A Leica DFC-295 CCD camera mounted on a DM1000 (Leica) or Metasystems CCD (Zeiss) camera mounted on an Axioscope 2 (Zeiss) microscope were used to capture all other non-fluorescence images using a Metasystems Ikaros ver.5.3 and Leica Application ver.3.2 softwares, respectively.

Results

Karyotypes of gymnures from northern Vietnam (*H. suillus microtinus*)

The diploid chromosome number of the male and female karyotypes was 2*n*=48, NF*a*=64 (Fig. 1a). The chromosome set consists of 10 pairs of bi-armed chromosomes and 14 pairs of acrocentrics. A pair of the largest metacentrics (№ 1), 2 pairs of large submetacentrics (№ 2–3), 2 pairs of medium-sized submetacentrics (№ 4–5), 2 pairs of medium-sized metacentrics (№ 6, 8), 2 pairs of small submetacentrics (№ 7, 9), and large-to-small acrocentrics (№ 10-23) represent an autosome complement. After the G-banding pattern was assessed, 2 large submetacentrics in the female karyotype were identified as X chromosomes, and the smallest acrocentric in the male karyotype was the Y chromosome (Fig. 2).

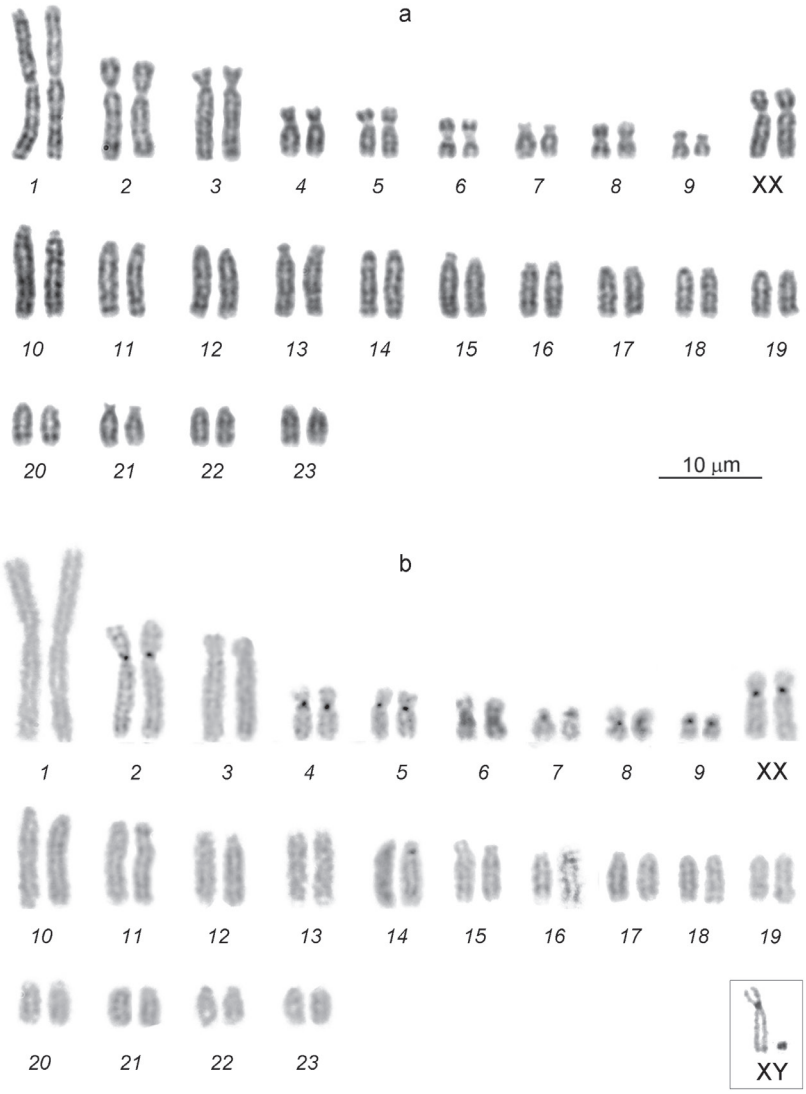

Figure 1. A female karyotype of the lesser gymnure *H. suillus microtinus* from northern Vietnam: conventional staining **(a)** and C-banding **(b)**. 2*n*=48, NF*a*=64. XX – the female sex chromosomes. C-banded sex chromosomes of a male (XY) are given in a frame.

C-heterochromatic blocks were revealed in the pericentric regions of 7 bi-armed autosomes (№ 2, 4–9) and the X chromosomes. The autosome 6 has the largest C-block (Fig. 1b). Slightly visible C-blocks were observed in the pericentric regions of certain acrocentrics. The dot-like Y chromosome was C-positive.

By silver nitrate staining the terminal localization of NORs was revealed on p-arms of 2 pairs of small autosomes in the female karyotype (Fig. 3).

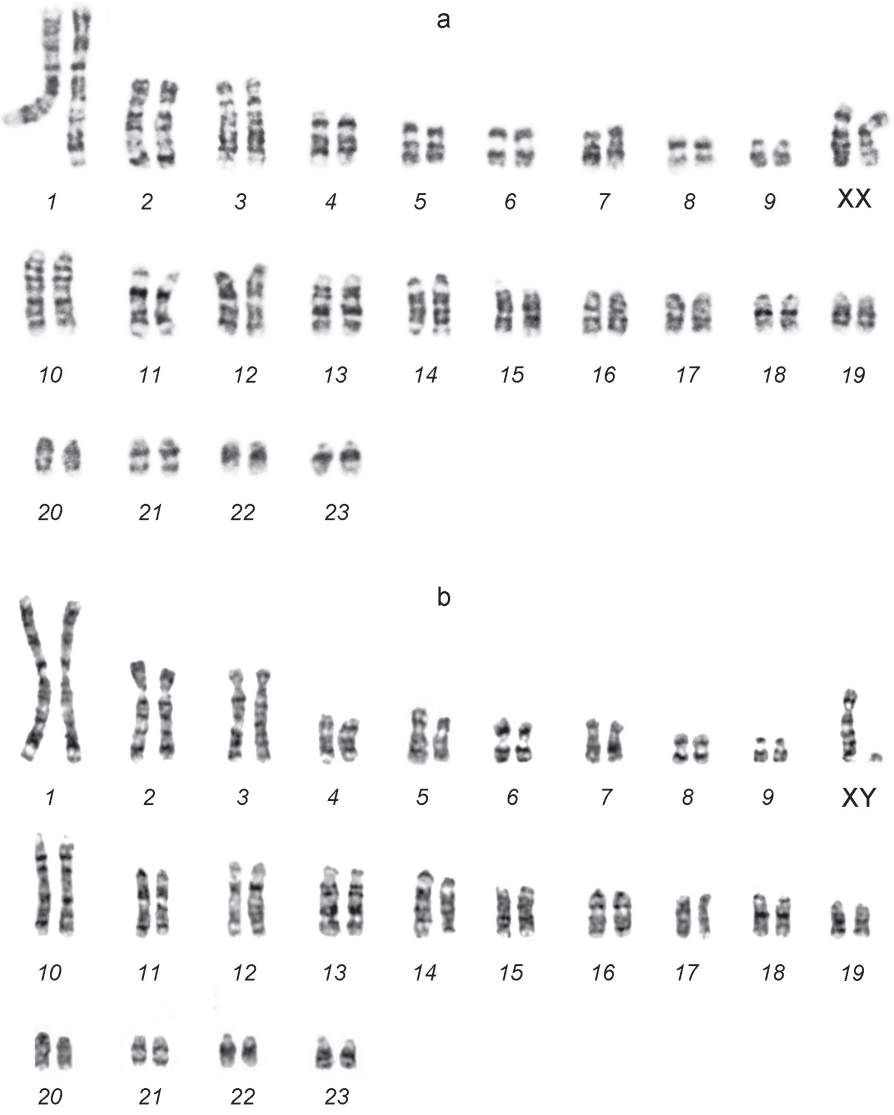

Figure 2. G-banded female (**a**) and male (**b**) karyotypes of the lesser gymnure *H. suillus microtinus* from northern Vietnam.

CMA_3-positive blocks were seen in the telomeric and pericentric regions of most bi-armed and acrocentric chromosomes (Fig. 4). However, the intensity of a signal varied between chromosomes – the brightest fluorescent signals were detected on bi-armed autosome pairs 6, 8, and 9. The Y chromosome had a CMA_3-positive signal.

The hybridization with the telomeric DNA (telDNA) probe revealed distinct signals at the telomeres of all chromosomes in the female karyotype (Fig. 5). No interstitial positive signals of telDNA were detected.

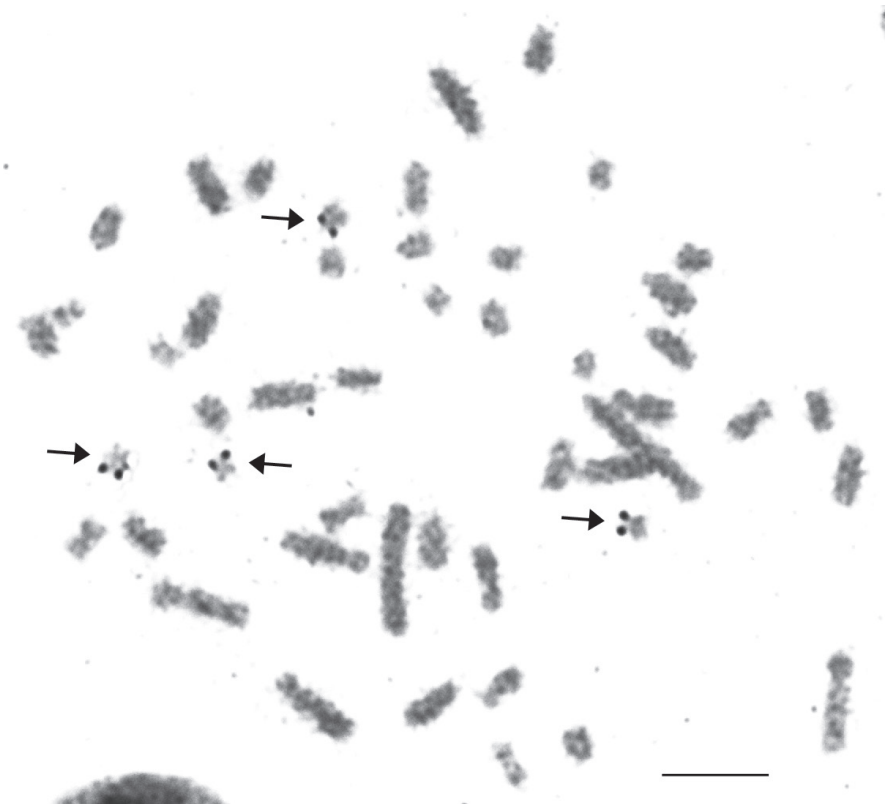

Figure 3. Ag-stained female karyotype of the lesser gymnure *H. suillus microtinus* from northern Vietnam. Black arrows indicate the localizations of NORs. Bar = 10 μm.

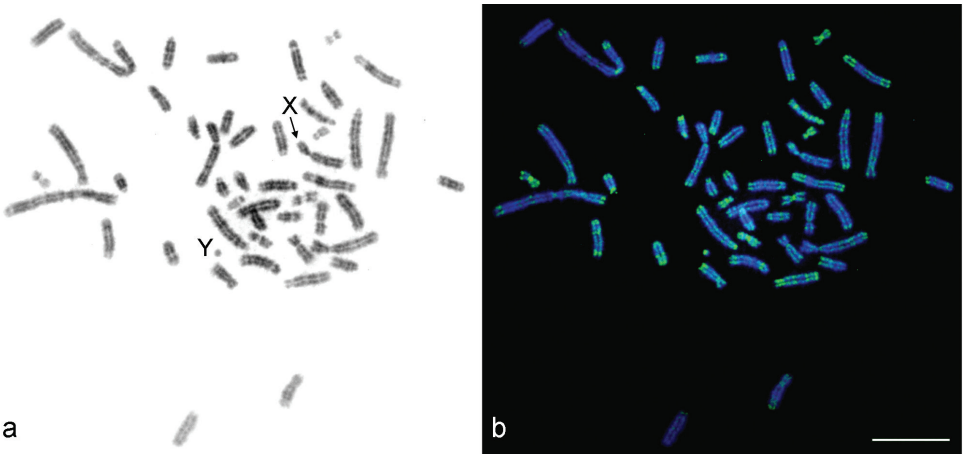

Figure 4. CMA_3-DAPI stained male karyotype of the lesser gymnure *H. suillus microtinus* from northern Vietnam: inverted DAPI **(a)** and CMA_3-DAPI staining **(b)**. X and Y indicate the sex chromosomes. Bar = 10 μm.

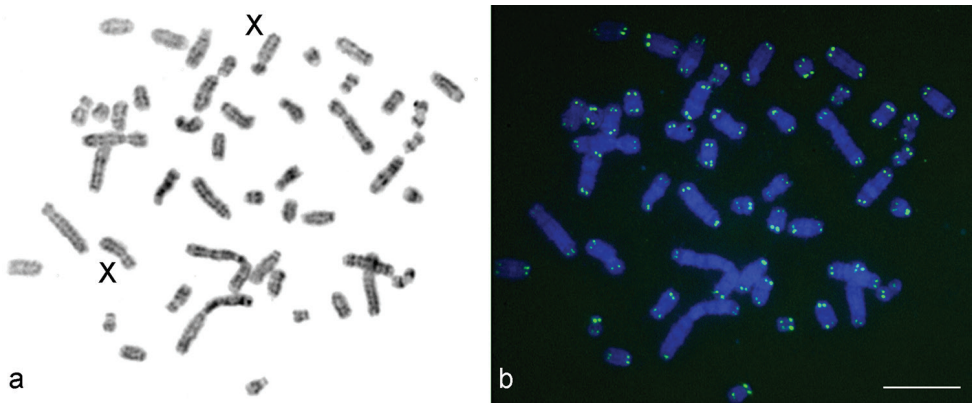

Figure 5. FISH on G-banded chromosomes **(a)** of the female of the lesser gymnure *H. suillus microtinus* from northern Vietnam using a fluorescein-conjugated PNA probe **(b)**. Chromosomes counterstained with DAPI (an image is inverted). XX – the sex chromosomes. Bar = 10 μm.

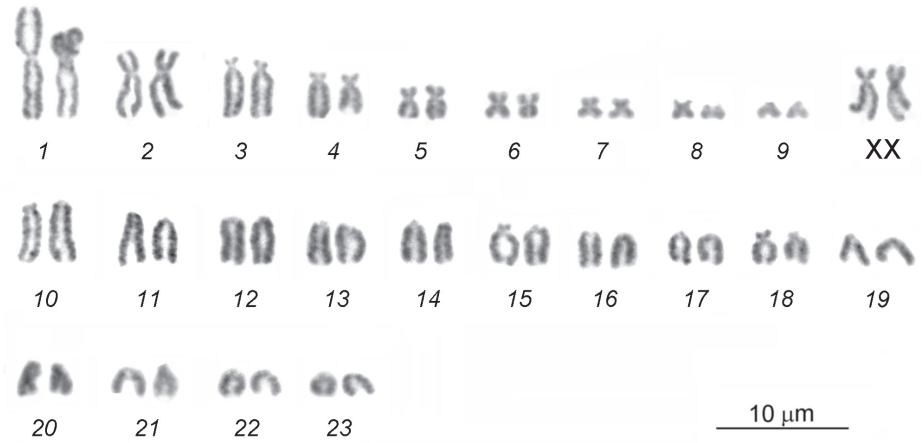

Figure 6. Routine stained female karyotype of the lesser gymnure *Hylomys* sp. from southern Vietnam. 2*n*=48, NF*a*=64.

Karyotype of gymnure from southern Vietnam (*Hylomys* sp.)

The karyotype of the female had the same number of chromosomes and chromosome arms as the karyotypes above: 2*n*=48, NF*a*=64 (Fig. 6). The quality of the chromosome suspension that was established in the field was too poor to perform differential stains. The silver nitrate staining revealed that at least one pair of small submetacentrics is bearing Ag-NORs. Nevertheless, the chromosome set appeared to be similar to the gymnure karyotypes from northern Vietnam. It consists of 10 pairs of bi-armed chromosomes including medium-sized submetacentric X chromosomes and 14 pairs of acrocentrics (at least 2 pairs had short arms).

Discussion

Karyotypes of several hundred mammalian species have been described in the several decades since the development of various methods for characterizing the chromosome sets (O'Brien et al. 2006). Until recently, the order Erinaceomorpha was unevenly studied cytogenetically. More attention was paid to the karyotypes of hedgehogs, whereas gymnures were omitted from even conventional cytogenetic analyses. Three previously studied gymnure species demonstrated large variations in diploid chromosome numbers (Rickart 2003, Ye et al. 2006, Li et al. 2008), implicating karyotype reorganization in the speciation of this clade.

The karyotypes of lesser gymnures from northern and southern Vietnam that we studied had 2*n*=48 and NF*a*=64. These data were first reported for the genus *Hylomys* and *H. suillus*-group. The karyotype structure and chromosome number differed from those of 3 karyotyped gymnure species: *P. truei*, *N. sinensis*, and *N. hainanensis* (Rickart 2003, Ye et al. 2006, Li et al. 2008). Their karyotypes have a smaller 2*n* (40, 32, and 32) and include up to 11 submeta/metacentric and 4-8 subtelo/telocentric autosomal pairs, whereas the studied *Hylomys* karyotypes consisted of 9 submeta/metacentric and 14 pairs of acrocentric autosomes. There are no data on the variation in C-heterochromatin between these 3 species, because only routine staining was applied to them, and *N. sinensis* and *N. hainanensis* were treated by G-banding (Rickart 2003, Ye et al. 2006, Li et al. 2008).

The sex chromosomes of all studied gymnure species, including *Hylomys,* had similar morphologies – the X chromosome is a mid-sized submetacentric, and the Y chromosome is the smallest acrocentric. However, notably, the Y chromosome in *N. hainanensis* is a small metacentric.

Based on an unpublished mtDNA analysis (Bannikova et al. in prep.), our specimens from northern Vietnam (ZMMU S-193936 and ZMMU S-199642) belong to the *H. s. microtinus* lineage. The specimen from southern Vietnam (ZIN 101915) clustered with the distinct genetic lineage of *Hylomys* sp. from southern Vietnam in Bannikova et al. (2014). The large genetic distances (~17% for cyt*b*) imply that this undescribed form of the lesser gymnure should be treated as a separate species, which appears to be the sister group to all taxa of *H. suillus*-group from Southeast Asia (Bannikova et al. 2014).

The diploid number in all spiny hedgehogs (Erinaceidae) studied so far appears to be 2*n*=48 (Orlov and Bulatova 1983, Reumer and Meylan 1986, Hübner et al. 1991, O'Brien et al. 2006), whereas the intrageneric variation in 2*n* in gymnures (Galericidae) is much higher (2*n*=32, 40, 48). This fact might also reflect the deep diversification of gymnures compared with spiny hedgehogs, as demonstrated by the molecular phylogenetic analysis (Bannikova et al. 2014). Despite the similarity in chromosome numbers, there is some variation in karyotype structure (the amount of C-heterochromatin, NORs) in hedgehog species (Gropp 1969, Sokolov et al. 1991). Our results showed that the 2*n* of *Hylomys* individuals from Vietnam are similar to that in spiny

hedgehogs; however, this difference might have resulted from the disparate ways of karyotypic evolution in these two divergent groups.

In conclusion, here we have provided the first karyotype description of 2 potential species – *Hylomys suillus microtinus* and *Hylomys* sp.–which are distributed throughout the northern and southern parts of Vietnam, respectively. A detailed characterization of the karyotype of *Hylomys* sp. from southern Vietnam by different chromosome staining is needed to provide a comprehensive comparison between these 2 forms.

Acknowledgments

We are grateful to the administration of the Xuan Son and Chu Yang Sin national parks for providing us with an opportunity to carry out field surveys. We thank A.N. Kuznetsov and Nguyen Dang Hoi (Joint Vietnam-Russian Tropical Research and Technological Centre, Hanoi) who made considerable efforts in preparing for the field works. We would like to express our sincere thanks to Drs Y.M. Kowalskaya, M.N. Erofeeva, I.V. Lukyanova, M.V. Rutovskaya, and V.M. Aniskin (all from the A.N. Severtsov IEE, Moscow) for their assistance in this study. Especial thanks to Dr. Eugeny Yu. Krysanov (A.N. Severtsov IEE, Moscow) who provided a telomeric probe, and gave us the permission for using the lab equipment for FISH analysis. Also we are very thankful to Dr. Anna A. Bannikova (Moscow State University) for her helpful advices on the manuscript. The Blue Pencil Science Company corrected English.

This study was funded in part by the Presidium of the Russian Academy of Sciences, Program № 41 «Biodiversity of natural systems and biological resources of Russia» and RSF № 16-14-10009.

References

Abramov AV, Shchinov AV, Rozhnov VV (2008) Study of insectivorous mammals in North Vietnam. Contemporary Problems of Ecology 1(5): 593–595. https://doi.org/10.1134/S1995425508050135

Bannikova AA, Lebedev VS, Abramov AV, Rozhnov VV (2014) Contrasting evolutionary history of hedgehogs and gymnures (Mammalia: Erinaceomorpha) as inferred from a multigene study. Biological Journal of Linnean Society 112(3): 499–519. https://doi.org/10.1111/bij.12299

Bulatova NSh, Searle JB, Nadjafova RS, Pavlova SV, Bystrakova NV (2009) Field protocols for the genomic era. Comparative Cytogenetics 3(1): 57–62. https://doi.org/10.3897/compcytogen.v3i1.9

Corbet GB, Hill JE (1992) The mammals of the Indomalayan region. Oxford University Press, Oxford, 488 pp.

Freshney RI (2010) Culture of animal cells: a manual of basic technique and specialized applications. Sixth edition. Wiley-Blackwell, Hoboken, 732 pp. https://doi.org/10.1002/9780470649367

Frost DR, Wozencraft WC, Hoffmann RS (1991) Phylogenetic relationships of hedgehogs and gymnures (Mammalia: Insectivora: Erinaceidae). Smithsonian Contributions to Zoology 518: 1–69. https://doi.org/10.5479/si.00810282.518

Gladkikh OL, Romanenko SA, Lemskaya NA, Serdyukova NA, O'Brien PCM, Kovalskaya JM, Smorkatcheva AV, Golenishchev FN, Perelman PL, Trifonov VA, Ferguson-Smith MA, Yang F, Graphodatsky AS (2016) Rapid karyotype evolution in *Lasiopodomys* involved at least two autosome – sex chromosome translocations. PLOS One. 11(12): e0167653. https://doi.org/10.1371/journal.pone.0167653

Graphodatsky AS, Radjabli SI (1988) Khromosomy sel'skokhozyaistvennykh i laboratornykh mlekopitayushchikh: Atlas (Chromosomes of farm and laboratory mammals: atlas). Nauka, Novosibirsk, 128 pp. [In Russian]

Gropp A (1969) Cytologic mechanisms of karyotype evolution in insectivores. In: Benirschke K (Ed.) Comparative mammalian cytogenetics. Springer-Verlag, New York, 247–266. https://doi.org/10.1007/978-3-642-85943-4_16

Hübner R, Maddalena T, Poduschka W (1991) The karyotype of the Middle-African hedgehog *Atelerix albiventris* Wagner, 1841 and its cytotaxonomical relationships to other Erinaceinae (Insectivora: Erinaceidae). Genetica 83(3): 243–246. https://doi.org/10.1007/BF00126230

Hutterer R (2005) Order Erinaceomorpha. In: Wilson DE, Reeder DM (Eds) Mammal species of the world. A taxonomic and geographic reference. Third edition. Johns Hopkins University Press, Baltimore, 212–219.

Li Y, Wu Y, Liang-Kong Lin, Harada M, Motokawa M (2008) First karyological and molecular study of the Hainan gymnure *Neohylomys hainanensis* (Erinaceomorpha: Erinaceidae). Mammalia 72: 344–346. https://doi.org/10.1515/MAMM.2008.037

McKenna MC, Bell SK (1997) Classification of mammals above the species level. Columbia University Press, New York, 631 pp.

O'Brien SJ, Menninger JC, Nash WG (2006) Atlas of mammalian chromosomes. John Wiley and Sons Ltd, New York, 714 pp. https://doi.org/10.1002/0471779059

Orlov VN, Bulatova NSh (1983) Comparative cytogenetics and karyosystematics of mammals. Nauka, Moscow, 405 pp. [In Russian]

Reumer JWF, Meylan A (1986) New developments in vertebrate cytotaxonomy. IX. Chromosome numbers in the order Insectivora (Mammalia). Genetica 70: 119–151. https://doi.org/10.1007/BF00123583

Rickart EA (2003) Chromosomes of Philippine mammals (Insectivora, Dermoptera, Primates, Rodentia, Carnivora). Proceedings of the Biological Society Washington 116: 69–-709.

Ruedi M, Fumagalli L (1996) Genetic structure of gymnures (genus *Hylomys*; Erinaceidae) on continental islands of Southeast Asia: historical effects of fragmentation. Journal of Zoological Systematic and Evolutionary Research 34:153–162. https://doi.org/10.1111/j.1439-0469.1996.tb00821.x

Sokolov VE, Aniskin VM, Lukyanova IV (1991) Karyological differentiation of the two hedgehog species of the genus *Erinaceus* (Insectivora, Erinaceidae) in the USSR. Zoologicheskii Zhurnal 70: 111–120 [In Russian]

Sumner AT (1972) A simple technique for demonstrating centromeric heterochromatin. Experimental Cell Research 75: 304–306.

Ye J, Biltueva L, Huang L, Nie W, Wang J, Jing M, Su W, Vorobieva N, Jiang X, Graphodatsky A, Yang F (2006) Cross-species chromosome painting unveils cytogenetic signatures for the Eulipotyphla and evidence for the polyphyly of Insectivora. Chromosome Research 14: 151–159. https://doi.org/10.1007/s10577-006-1032-y

CompCytogen 12(3): 373–386 (2018)
doi: 10.3897/CompCytogen.v12i3.25462
http://compcytogen.pensoft.net

RESEARCH ARTICLE

COMPARATIVE Cytogenetics
A peer-reviewed open-access journal
International Journal of Plant & Animal Cytogenetics, Karyosystematics, and Molecular Systematics

Cytogenetic studies in three octopods, *Octopus minor*, *Amphioctopus fangsiao*, and *Cistopus chinensis* from the coast of China

Jin-hai Wang[1,2], Xiao-dong Zheng[1,2]

1 *Institute of Evolution and Marine Biodiversity, Ocean University of China, Qingdao 266003, China* **2** *Key Laboratory of Mariculture, Ocean University of China, Ministry of Education, China*

Corresponding author: *Xiao-dong Zheng* (xdzheng@ouc.edu.cn)

Academic editor: *V. Specchia* | Received 3 April 2018 | Accepted 12 August 2018 | Published 4 September 2018

http://zoobank.org/4B6971F2-34FC-4A20-AA01-0D0EB22B75A8

Citation: Wang J-h, Zheng X-d (2018) Cytogenetic studies in three octopods, *Octopus minor*, *Amphioctopus fangsiao*, and *Cistopus chinensis* from the coast of China. Comparative Cytogenetics 12(3): 373–386. https://doi.org/10.3897/CompCytogen.v12i3.25462

Abstract

To provide markers to identify chromosomes in the genome of octopods, chromosomes of three octopus species were subjected to NOR/C-banding. In addition, we examined their genome size (*C* value) to submit it to the Animal Genome Size Database. Silver staining revealed that the number of Ag-nucleoli was 2 (*Octopus minor* (Sasaki, 1920)), 2 (*Amphioctopus fangsiao* (d'Orbigny, 1839)) and 1 (*Cistopus chinensis* Zheng et al., 2012), respectively, and the number of Ag-nucleoli visible was the same as that of Ag-NORs on metaphase plates in the same species. In all analyzed metaphases, Ag-NORs were mainly located terminally on the long arms of chromosomes 3 (3rd) of *O. minor* and on the short arms of chromosomes 4 (4th) of *A. fangsiao*, whereas only one of the chromosomes 23 (23rd) was found Ag-NORs of *C. chinensis*. C-bands were localized predominantly in the centromeric regions of chromosomes in the three species, while other conspicuous stable C-bands were observed in terminal regions, including the Ag-NORs. That means these three chromosome pairs (3rd, 4th and 23rd) could be considered species-specific cytogenetic markers. The mean *C* values of *O. minor*, *A. fangsiao* and *C. chinensis* were 7.81±0.39 pg (0.070 pg per unit length), 8.31±0.18 pg (0.068 pg per unit length) and 5.29±0.10 pg (0.038 pg per unit length), respectively, and results showed that *C* values of the three species were not proportional to the relative length of the chromosomes. These cytogenetic characteristics will provide more theoretical foundation for further researches on chromosome evolution in octopods.

Keywords

octopods, karyotype, Ag-NORs, C-bands, genome size, flow cytometry

Introduction

Genetics and cytology combine to establish cytogenetics, mainly from the perspective of cytology, especially from a chromosome structure and function as well as the relationship between chromosomes and other organelles, to elucidate the mechanism of inheritance and variation. Cytogenetic analysis usually includes chromosome karyotype, band type, flow karyotype analysis and fluorescence *in situ* hybridization. Previous published reviews on chromosomal studies of molluscs were greatly increased since cytogenetic techniques including silver-staining, C-and G-banding and have begun to be applied to molluscan chromosomes (Thiriot-Quiévreux 2003). Although these techniques have been widely used in the study of shellfish chromosomes, there are no reports on the cephalopods.

Octopods, such as *Octopus minor* (Sasaki, 1920), *Amphioctopus fangsiao* (d'Orbigny, 1839) and *Cistopus chinensis* Zheng et al., 2012 are cephalopod species. The previous chromosome analysis of cephalopods had revealed 2n=60 or 92 in most species studied, including *O. minor*, *A. fangsiao*, *C. chinensis*, two sepiids (*Sepia esculenta* and *S. lycidas* Gray, 1849) and three loliginids (*Heterololigo bleekeri* Natsukari, 1984, *Sepioteuthis lessoniana* Blainville, 1824 and *Photololigo edulis* (Hoyle, 1885)) (Gao and Natsukari 1990, Adachi et al. 2014, Wang and Zheng 2017), although *Nautilus macromphalus* Sowerby, 1849 had 2n=52 chromosomes (Bonnaud et al. 2004), and the studies led by Papan and Jazayeri reported the chromosome number of *S. arabica* Massy, 1916 and *S. pharaonis* Ehrenberg, 1831 was 2n=48 (Papan et al. 2010, Jazayeri et al. 2011). However, there are only a few studies on the cephalopod chromosomes in reported publications, and there are no researches on the band type in these species. Adachi et al. (2014) first tried to use fluorescence *in situ* hybridization on the cephalopod chromosomes and suggested that the telomere sequence of *O. areolatus* de Haan, 1839–1841 was (TTAGGG)n, but there was a lack of complete and clear metaphases in the report. Due to the restriction of the embryo acquisition, and the number of cephalopod chromosomes up to 60, it is difficult to obtain an ideal metaphase. All these factors seriously restrict the cytological study of cephalopods. In a previous published paper, we gave a detailed overview of the existing cephalopod chromosome information, including the genetic relationship analysis based on evolutionary distance (Wang and Zheng 2017). The present study used gills as materials, and through a large number of repeated tests, the ideal metaphases with NORs and C-bands were obtained based on the previous study.

As an important part of the study of cytogenetics, more and more genome sizes (*C* values) have been revealed. Although the genome sizes of 281 mollusks have been submitted to the Animal Genome Size Database (http://www.genomesize.com. Accessed December 25, 2017) while there just have been 6 species of cephalopod *C* values that can be obtained from the database: *O. bimaculatus* (Hinegardner 1974), *O. bimaculoides* (Albertin et al. 2015), *O. vulgaris* (Packard and Albergoni 1970), *Euprymna scolopes* (Adachi et al. 2014), *Loligo plei* (Hinegardner 1974) and Loliginidae sp. (Mirsky and Ris 1951). These *C* values were estimated based on bulk fluorometric assay and feulgen image analysis densitometry. As genomic and transcriptomic sequencing is carried out in cephalopods, more and more cephalopod genome sizes have been revealed

Table 1. The published information of cephalopod genome size. **BA**: Biochemical Analysis, **FCM**: Flow Cytometry, **BFA**: Bulk Fluorometric Assay, **CGS**: Complete Genome Sequencing, **FIA**: Feulgen Image Analysis Densitometry.

Species	Origin	Internal standard	Method	*C* value (pg)/ genome size (Gb)	References
O. vulgaris	sperm	Not specified	BA/CGS	5.15 pg/2.5–5 Gb	Packard and Albergoni 1970; Albertin et al. 2012
O. minor	Haemocytes	*G. domesticus*	FCM	7.82±0.56 pg	This study
O. bimaculatus	sperm	*Strongylocentrotus purpuratus*	BFA	4.30 pg	Hinegardner 1974
O. bimaculoides	Not specified	Not specified	BFA/CGS	2.93 pg/3.2 Gb	Albertin et al. 2012, 2015
A. fangsiao	Haemocytes	*G. domesticus*	FCM	8.23±0.42 pg	This study
C. chinensis	Haemocytes	*G. domesticus*	FCM	5.13±0.38 pg	This study
H. maculosa	–	–	CGS	4.5 Gb	Albertin et al. 2012
S. officinalis	–	–	CGS	4.5 Gb	Albertin et al. 2012
L. plei	sperm	*S. purpuratus*	BFA	2.80 pg	Hinegardner 1974
L. pealeii	–	–	CGS	2.7 Gb	Albertin et al. 2012
E. scolopes	Haemocytes, Sperm	*G. domesticus*	FIA/CGS	3.75 pg/3.7 Gb	Gregory 2013; Albertin et al. 2012
I. paradoxus	–	–	CGS	2.1 Gb	Yoshida et al. 2011
A. dux	–	–	CGS	4.5 Gb	Albertin et al. 2012
N. pompilius	–	–	CGS	2.8–4.2 Gb	Yoshida et al. 2011

by complete genome sequencing such as *N. pompilius*, *Architeuthis dux*, *Hapalochlaena maculosa*, *E. scolopes*, *Idiosepius paradoxus*, *L. pealeii*, *S. officinalis*, etc (Yoshida et al. 2011, Albertin et al. 2012) (Table 1). Besides, Adachi et al. (2014) examined the *C* values of *O. ocellatus* and *O. vulgaris* based on flow cytometry. Although there are many methods to detect *C* values, we choose flow cytometry for the convenience, rapid analysis and relative accuracy of the sample preparation (Gokhman et al. 2017).

To develop octopus chromosome markers, the present research has first completed NOR/C-banding. Also we examined their *C* values to submit to the Animal Genome Size Database. This is a basic work for molecular cytogenetic research of octopods. It is expected to lay a solid theoretical foundation for further researches on chromosome evolution in octopods.

Material and methods

Ag-staining of the NORs and C-bands

Collection of samples and chromosome slides preparation based on the previous published paper (Wang and Zheng 2017). The nucleoli in interphase and the NORs in metaphase were visualized using rapid silver nitrate staining using the previous meth-

ods (Howell and Black 1980). C banding were carried out following the protocols of Sumner (1972) with some modifications. The dyed chromosome slides were detected under a light microscope with an oil lens (Leica MC170 HD, Germany).

Genome size (*C* value)

Preparation of cell suspension

Ten individuals (5 males and 5 females) of each species were used for collecting hemolymph. All subjects were handled according to the guidelines put forth by the EU Directive 2010/63/EU for cephalopod welfare (Fiorito et al. 2014). Before dissecting, all octopods should be anesthetized using 7.5% magnesium chloride ($MgCl_2$) solution (Messenger et al. 1985) until unconscious.

Then hemolymph was withdrawn from the heart or cephalic aorta of each octopus using a disposable syringe, and the hemolymph was immediately transferred into a 1.5 ml centrifuge tube containing precooled (4 °C) phosphate-buffered saline (PBS) (v/v=1:1). Mixed samples were centrifuged for 12 min at 300g and 4 °C, and then the hemocytes were resuspended twice in PBS according to above. The last suspension containing 300 μl PBS was added to another tube containing 900 μl precooled (–20 °C) anhydrous ethanol (v/v=1:3), fixing at least 3 hours at 4 °C. The fixed cell was washed twice in PBS after it was suspended with PBS up to 1 ml. Before the flow cytometry detection, a moderate propidium iodide solution (PI, 20–30 μg/ml) was added to the suspensions, staining for 2 hours at 4 °C in dark.

Flow cytometry analysis

Previous studies have shown that the genome size (*C* value) of chickens (*Gallus domesticus*) was 1.25 pg (Tiersch et al. 1989, Adachi and Okumura 2012, Adachi et al. 2014). Here we determined *C* values of octopods using this chicken red blood cells as internal standard, and the standard sample was purchased from BD company (DNA QC Particles).

C value was measured using a model PA flow cytometer (Beckman Coulter Cytomics FC 500 MPL), in principle, at least 15,000 cells were measured in each sample. The blue light of 488 nm was first excited, and the fluorescence of PI was detected by the emission wave length of 625±10 nm. The present study used chicken standard sample as calibration instrument, and then used it as the internal standard, by comparing multiple relationships between the standard sample (chicken red blood cells) and the pending sample (octopus hemolymph) peak, calculating the *C* values.

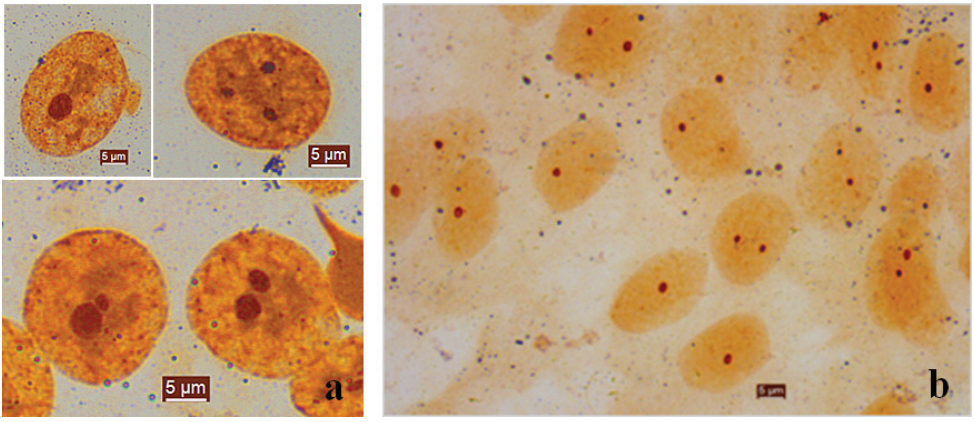

Figure 1. Ag-nucleoli of interphase. **a** 1, 2 or 3 normal nucleolus organizer regions (NORs) in interphase of *O. minor* and *A. fangsiao*, and the two species mainly contain 2 NORs **b** 1 or 2 NORs in interphase of *C. chinensis*, and most of them contain 1 NORs . Scale bar: 5 µm.

Results

Banding analysis

After silver staining was performed, the number of Ag-nucleoli was between 1–3 in interphase nuclei of three species (Fig. 1). We randomly selected 200 interphase nuclei to calculate the number of nucleolar organizer regions for each species. Among the scored interphases 24% had 1 nucleolus, and 61% had 2 nucleoli, and 10% had 3 nucleoli and 5% had more than 3 nucleoli in *O. minor* species. Twenty-four metaphases were counted indicating there were 2 Ag-NORs, then 7 of them were selected for karyotype analysis and Ag-NOR loci were located on the long arms of metacentric chromosomes 3 (3rd). In *A. fangsiao* species, 38 of 200 interphase nuclei showed 1 Ag-nucleolus, then 146 of them contained 2 nucleoli and 16 of them had 3 to 5 nucleoli. Eighteen metaphases were counted and 7 of them were selected for karyotype analysis, showing there were 2 Ag-NORs and Ag-NOR loci were located on the short arms of the metacentric chromosomes 4 (4th). For *C. chinensis* species, there were mainly 1 nucleolus (up to 68%), followed by 2 nucleoli accounting for about 21%, while a small proportion had 3–5 nucleoli. Selected 13 scored metaphases indicated that there were only 1 Ag-NOR and then 7 karyotypes were analyzed suggesting that Ag-NOR loci were located on long arms of the subtelomeric chromosome 23 (23rd) (Fig. 2).

The C-band karyotype analysis indicated that there were 31 C-bands (*O. minor*), 25 C-bands (*A. fangsiao*) and 25 C-bands (*C. chinensis*) in three species of octopods, respectively. C-bands were consistently localized in the centromeric regions of most chromosomes in the three species, but which varied in size (Fig. 3d–f), and in *C. chinensis*, the C-bands of long arms were smaller those of *O. minor* and *A. fangsiao*. In particular, several other steady C-bands were located on terminal region of chromosomes

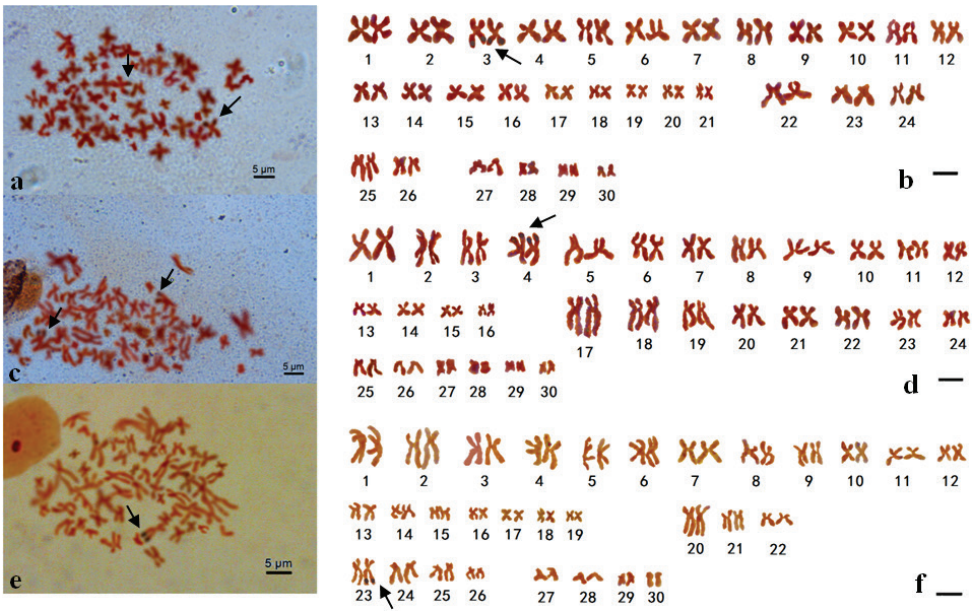

Figure 2. Karyotypes and NOR-bearing chromosomes from three species of octopod gills. **a** The metaphase plate of *O. minor* **b** Karyogram of *O. minor* from (**a**) showed that Ag-NOR loci were located on the long arms of metacentric chromosome 3 **c** The metaphase plate of *A. fangsiao* **d** Karyogram of *A. fangsiao* from (**c**) showed that Ag-NOR loci were located on the short arms of metacentric chromosome 4 **e** The metaphase plate of *C. chinensis* **f** Karyogram of *C. chinensis* from (**e**) showed that Ag-NOR loci were located on long arms of subtelomeric chromosome 23. Arrows indicate the NOR-bearing chromosomes. Scale bars: 5 μm.

3 (3rd) for *O. minor*, chromosomes 4 (4th) for *A. fangsiao* and chromosomes 23 (23rd) for *C. chinensis*, which was coincident with positive Ag-NOR loci, except for chromosomes 23 in *C. chinensis*, where only one of the chromosomes 23 was found to have an Ag-NOR (Fig. 2f). Besides, various C-bands were observed on the long arms of chromosome pairs 2 (proximal terminal region), 7 (interstitial region) and 25 (proximal terminal region) in *O. minor*, chromosome pairs 3 (interstitial region) and 17 (interstitial region) in *A. fangsiao* and chromosome pair 4 (terminal region) in *C. chinensis*.

Combined with the results of silver staining and C-banding, although C-bands were localized predominantly in the centromeric regions of chromosomes in the three octopus species, other conspicuous C-bands were observed in terminal regions, including the Ag-NORs. Therefore, we can select chromosome pairs 3, 4 and 23 as effective species-specific markers to distinguish the three octopods.

C value

In data analysis, we selected about 15,000 cells per sample, then fluorescence intensity representing the relative DNA content was recorded. Figure 4a showed the

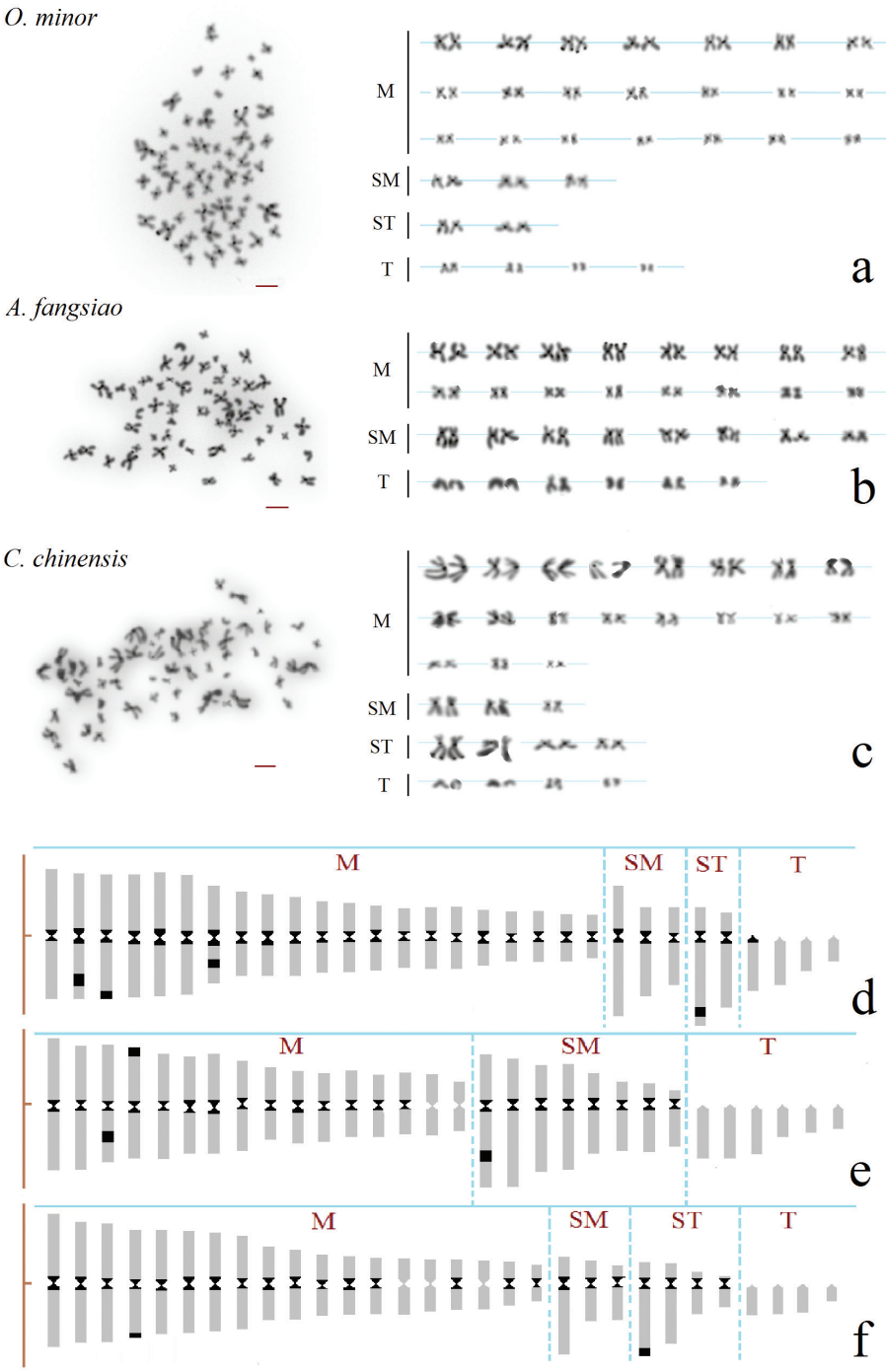

Figure 3. C-banding karyotypes arranged from mitotic metaphases of *O. minor* (**a**), *A. fangsiao* (**b**) and *C. chinensis* (**c**) and diagrams of C-banding (**d, e, f**). Black dots representing the chromosomes with C bands/heterochromatin blocks. Scale bars: 5 μm.

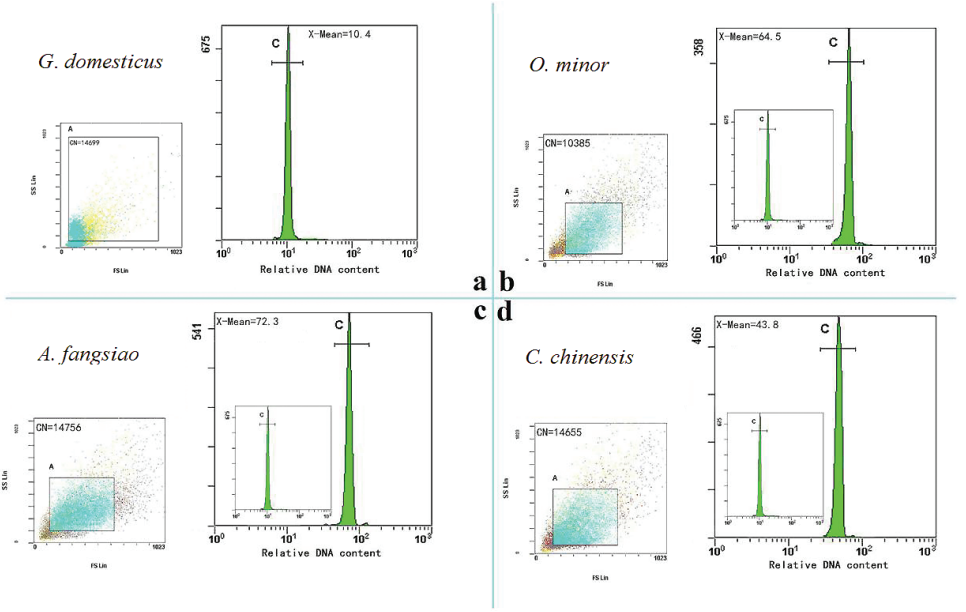

Figure 4. Flow cytometry profiles of relative fluorescence intensity of propidium iodide (PI) in octopus hemocytes of **b** *O. minor* **c** *A. fangsiao* and **d** *C. chinensis* with *G. domesticus* **a** as standard (St). Scatter plots display the quality and number of cell mass of standard samples and test samples, and the peak figures show the relative DNA content of each sample. **b**, **c** and **d** are just a representative graph of the three species of octopus samples. CN, the number of cells; X-MEAN, mean fluorescence intensity.

number of cells (CN=14699) and mean fluorescence intensity (X-Mean=10.4) of the internal standard and other three representative results of *O. minor* (CN=10385, X-Mean=64.5, Fig. 4b), *A. fangsiao* (CN=14756, X-Mean=72.3, Fig. 4c) and *C. chinensis* (CN=14655, X-Mean=43.8, Fig. 4d) also were enumerated to estimate the DNA content. The results showed the mean *C* values of *O. minor*, *A. fangsiao* and *C. chinensis* were 7.81±0.39 pg (male 7.85±0.47 pg, female 7.76±0.32 pg), 8.31±0.18 pg (male 8.33±0.25 pg, female 8.30±0.10 pg) and 5.29±0.10 pg (male 5.28±0.08 pg, female 5.29±0.12 pg), respectively. *C. chinensis* had the smallest *C* value, significantly lower than *O. minor* ($P < 0.05$) and *A. fangsiao* ($P < 0.05$) (Table 2).

Based on our previous studies, the chromosome total relative lengths of *O. minor*, *A. fangsiao* and *C. chinensis* were 112.33, 122.77 and 139.20. *C. chinensis* had the largest relative length, followed by *A. fangsiao* and *O. minor*, which was not proportional to the *C* value. Obviously, *C. chinensis* had the smallest *C* value but the largest chromosome relative length. The DNA content of the unit length chromosomes of the three octopods was about 0.070 pg (*O. minor*), 0.068 pg (*A. fangsiao*) and 0.038 pg (*C. chinensis*) respectively. Results suggested that there was no significant positive proportional relationship between the *C* value and the relative chromosome length. Besides, this study analyzed the *C* values of 14 species of cephalopods, ranging of 2.20 to 8.23 pg (2.10–7.86 Gb), of which *I. paradoxus* had the smallest genome size and

Table 2. The results of *C* values from three species of octopods.

Species		Sample no.	Fluorescence intensity	*C* value/pg	*C* value (X±SE) /pg	
G. domesticus		0	10.4	1.25	1.25	
O. minor	male	1	63.5	7.63	7.85±0.47	7.81±0.39
		2	60.1	7.22		
		3	70.4	8.46		
		4	67.2	8.08		
		5	65.5	7.87		
	female	6	61.3	7.37	7.76±0.32	
		7	63.5	7.63		
		8	67.2	8.08		
		9	66.1	7.94		
A. fangsiao	male	1	72.3	8.69	8.33±0.25	8.31±0.18
		2	68.9	8.28		
		3	67.5	8.11		
		4	68.4	8.22		
	female	5	70.2	8.44	8.30±0.10	
		6	68.8	8.27		
		7	68.3	8.21		
		8	69.0	8.29		
C. chinensis	male	1	43.8	5.26	5.28±0.08	5.29±0.10
		2	44.6	5.36		
		3	43.3	5.20		
	female	4	43.5	5.23	5.29±0.12	
		5	44.8	5.38		
		6	45.0	5.41		
		7	42.9	5.16		

the largest value from *A. fangsiao*. Overall, the average genome size of 6 species from Octopoda (3.35–8.23 pg) was higher than that of 8 species from Sepiida and Teuthida (2.20–4.71 pg).

Discussion

We first carried out silver staining (Ag-NOR) in octopus species, and the results showed that *C. chinensis* had only one nucleolus organizer region (NOR) which was located terminally at the long arms of a pair of homologous chromosomes. *O. minor* and *A. fangsiao* had two NORs which located terminally on a pair of homologous chromosomes. As an effective chromosome marker, polymorphisms in NORs can be observed in interspecies or intraspecies comparisons, including the number, location and size of sliver staining (Wang et al. 2015, Zaleśna et al. 2017), even the geographical location and habitat differences can cause the diversity. However, many studies have determined that the number of NORs in interphase is consistent with that on

metaphase plates of the same species (Iizuka et al. 2013, Zaleśna et al. 2017). According to the report of Okumura et al. (1999), NORs of the *Haliotis discus hannai* were located at the end of two pairs of chromosome long arms, and it was also found in the subcentral centromeric chromosome and the centromeric chromosome. Similarly, in the later studies of abalone chromosomes from Wang et al. (2015) showed NOR sites located on the 14th and 17th chromosomes, but at the end of the short arms of central and submetacentric chromosomes also were found the sites. From the two studies, the same species proved that the polymorphism of Ag-NOR bands was prevalent among most species, including crustaceans, teleost fish, reptiles, mammals and other mollusks (Babu and Verma 1985, Thiriot-Quiévreux and Insua 1992, Vitturi and Lafargue 1992, Cross et al. 2003, Britton-Davidian et al. 2011).

The number of C-bands in *O. minor* was larger than that of the other two octopus species, which was consistent with the Ag-NORs. Although *A. fangsiao* and *C. chinensis* had the same number of C-bands, while the former contained more interstitial C-bands. Taking these two points into account, it is confirmed that the *O. minor* is more advanced in evolution from the chromosome level. Almost all chromosomes of three species of octopods can show C-bands in centromere regions, and it means that heterochromatic blocks are evident in the pericentromeric regions of chromosomes, which is consistent with the traditional view that the centromere region is mainly consisted of heterochromatin. The C-banding results showed that the NOR regions of the three species were all deep-stained C-bands, which also coincided with the common assumption that the NOR regions were composed of heterochromatin. The stable C-bands can be used as markers for chromosome identification, while the higher polymorphic C-band is not suitable as a marker for distinguishing chromosomes, but it can be used as a genetic marker for the study of chromosome polymorphism. In present study, chromosomal markers for identifying three species of octopods were developed by Ag-NORs and C-bands, and it was effective means. Long before that, Martinez-Lage et al. (1995) managed to separate the chromosomes of three shellfish by banding techniques, which confirmed the reliability of this method.

In the present study, due to different survival pressure from geographical distribution (Zhang and Onozato 2004), the genome size of *C. chinensis* from South is significantly less than that of *O. minor* and *A. fangsiao* from North. Adachi et al. (2014) pointed out that the genome size of *O.* (*A.*) *areolatus* was 5.47 pg, then this study showed the value was about 8.23 pg, significantly higher than the former. The main reason may be the difference of samples or the existence of hidden species. Although some studies had shown (Rakic et al. 2014) that the genome size was related to ecological factors, that was not absolute. The diversity of genome size involved the interaction of multiple factors and can not simply attribute the differences to the external environmental factors.

DNA is linear on the chromosome. According to Adachi et al. (2014), genome size of *O.* (*A.*) *areolatus* and *O. vulgaris* was proportional to the relative chromosome length, they determined the values of the two octopods was 5.47 pg and 3.50 pg, respectively. The genome size of *O.* (*A.*) *areolatus* was about 1.5 times higher than

that of *O. vulgaris*, and this ratio coincided with the ratio of chromosome length to 122.60/66.30. Even so, we can not simply consider the existence of ploidy between the two, because the number of chromosomes is identical. Therefore, we speculate that genome duplication may occur during the evolution of *O. vulgaris*, which leads to the ploidy relationship. In contrast, current studies have found that the genome size of *O. minor*, *A. fangsiao* and *C. chinensis* have no obvious linear relationship with their chromosome length. Different methods to detect the genome size of the same species usually yield different results. In the reported cephalopod genomes, the genome size of *O. vulgaris*, *O. bimaculoides*, and *E. scolopes* have been determined by biochemical analysis, bulk fluorometric assay, complete genome sequencing and feulgen image analysis densitometry (Table 1). Regardless of the cephalopod species, the obtained genome size by complete genome sequencing is generally larger than other testing methods. For example, using biochemical analysis method to get *O. vulgaris* genome size is 5.15 pg (Packard and Albergoni 1970), and the result of genome sequencing is 2.5 to 5.0 Gb, about 2.62 to 5.24 pg (Albertin et al. 2012). The *O. bimaculoides* genome size is 2.93 pg/3.2 Gb (about 3.35 pg) by bulk fluorometric assay (Albertin et al. 2012) and genome sequencing (Albertin et al. 2015), respectively. Besides, the *E. scolopes* genome size by feulgen image analysis (3.75 pg) (Gregory 2013) is less than that of genome sequencing (3.7 Gb, about 3.87 pg) (Albertin et al. 2012). The main reason for these results is that genome size obtained by genome sequencing contains a complete set of nucleotide sequences, including non-coding sequences, and the increase of non-coding sequences largely obscures the correlation between genome size and species evolution complexity. In addition, the statistical analysis of cephalopod genome size is mainly based on the existing basic data. More cephalopod genomes are needed to be sequenced to further analyze the genomic characteristics of the population.

In conclusion, the present study combining a previously published paper (Wang and Zheng 2017) highlights our increased knowledge of cephalopod cytogenetic studies. Up to now, cytogenetic studies of the cephalopods have stepped forward: Thirteen species of cephalopod chromosome information have been reported, of which three are related to silver staining and C-banding, also fourteen species of cephalopod genome size or haploid DNA content have been revealed. What needs to be done next is the location of the functional genes (such as sex related genes) on the chromosomes to further deepen cytogenetic study of cephalopods.

Acknowledgments

We thank associate Prof. Mingzhuang Zhu, who helped us in the flow cytometer analysis. We thank Yaosen Qian, Qiaozhen Ke and Bing Cai for providing the *O. minor* and *C. chinensis* individuals from Rongcheng and Ningde, China. We thank Dr. Diego Orol Gómez for correcting the grammar of the article. This study was supported by research grants from National Natural Science Foundation of China (No31672257) and Key Development Plan of Shandong Province (2016GSF115014).

References

Adachi K, Ohnishi K, Kuramochi T, Yoshinaga T, Okumura SI (2014) Molecular cytogenetic study in *Octopus* (*Amphioctopus*) *areolatus* from Japan. Fisheries Science 80(3): 445–450. https://doi.org/10.1007/s12562-014-0703-4

Adachi K, Okumura SI (2012) Determination of genome size of *Haliotis discus hannai* and *H. diversicolor aquatilis* (Haliotidae) and phylogenetic examination of this family. Fisheries Science 78(4): 849–852. https://doi.org/10.1007/s12562-012-0518-0

Albertin CB, Bonnaud L, Brown CT, Crookes-Goodson WJ, da Fonseca RR, Di Cristo C, Dilkes BP, Edsinger-Gonzales E, Freeman RM, Hanlon RT, Koenig KM, Lindgren AR, Martindale MQ, Minx P, Moroz LL, Noedl MT, Nyholm SV, Ogura A, Pungor JR, Rosenthal JJC, Schwarz EM, Shigeno S, Strugnell JM, Wollesen T, Zhang GJ, Ragsdale CW (2012) Cephalopod genomics: a plan of strategies and organization. Standards in Genomic Science 7(1): 175–188. http://dx.doi.org/10.4056/sigs.3136559

Albertin CB, Simakov O, Mitros T, Wang ZY, Pungor JR, Edsinger-Gonzale E, Brenner S, Ragsdale CW, Rokhsar DS (2015) The octopus genome and the evolution of cephalopod neural and morphological novelties. Nature 524(7564): 220–224. https://doi.org/10.1038/nature14668

Babu K, Verma R (1985) Structural and functional aspects of nucleolar organizer regions (NORs) of human chromosomes. International Review of Cytology-a Survey of Cell Biology 94: 151–176. https://doi.org/10.1016/S0074-7696(08)60396-4

Bonnaud L, Ozouf-Costaz C, Boucher-Rodoni R (2004) A molecular and karyological approach to the taxonomy of *Nautilus*. Comptes Rendus Biologies 327(2): 133–138. https://doi.org/10.1016/j.crvi.2003.12.004

Britton-Davidian J, Cazaux B, Catalan J (2011) Chromosomal dynamics of nucleolar organizer regions (NORs) in the house mouse: micro-evolutionary insights. Heredity 108(1): 68–74. https://doi.org/10.1038/hdy.2011.105

Cross I, Vega L, Rebordinos L (2003) Nucleolar organizing regions in *Crassostrea angulata*: chromosomal location and polymorphism. Genetica 119(1): 65–74. https://doi.org/10.1023/A:1024478407781

Fiorito G, Affuso A, Anderson DB, Basil J, Bonnaud L, Botta G, Cole A, D'Angelo L, De Girolamo P, Dennison N, Dickel L, Di Cosmo A, Di Cristo C, Gestal C, Fonseca R, Grasso F, Kristiansen T, Kuba M, Maffucci F, Manciocco A, Mark FC, Melillo D, Osorio D, Palumbo A, Perkins K, Ponte G, Raspa M, Shashar N, Smith J, Smith D, Sykes A, Villanueva R, Tublitz N, Zullo L, Andrews P (2014) Cephalopods in neuroscience: regulations, research and the 3Rs. Invertebrate Neuroscience 14(1):13–36. http://dx.doi.org/10.1007/s10158-013-0165-x

Gao YM, Natsukari Y (1990) Karyological studies on seven Cephalopods. Venus 49: 126–145.

Gokhman VE, Kuhn KL, Woolley JB, Hopper KR (2017) Variation in genome size and karyotype among closely related aphid parasitoids (Hymenoptera, Aphelinidae). Comparative Cytogenetics 11(1): 97–117. https://doi.org/10.3897/CompCytogen.v11i1.10872

Gregory TR (2013) Animal Genome Size Database. http://www.genomesize.com

Hinegardner R (1974) Cellular DNA content of the Mollusca. Comparative Biochemistry and Physiology Part A 47(2): 447–460. https://doi.org/10.1016/0300-9629(74)90008-5

Howell WM, Black DA (1980) Controlled silver-staining of nucleolus organizer regions with a protective colloidal developer: a 1-step method. Experientia 36(8): 1014–1015. https://doi.org/10.1007/BF01953855

Iizuka K, Matsuda Y, Yamada T, Nakazato T, Sessions SK (2013) Chromosomal localization of the 18S and 28S ribosomal RNA genes using FISH and $AgNO_3$ banding in *Hynobius quelpaertensis*, *H. tsuensis* and *Onychodactylus koreanus* (Urodela: Hynobiidae). Current Herpetology 32(2): 89–101. https://doi.org/10.5358/hsj.32.89

Jazayeri A, Papan F, Motamedi H, Mahmoudi S (2011) Karyological investigation of Persian Gulf cuttlefish (*Sepia arabica*) in the coasts of Khuzestan province. Life Science Journal 8(2): 849–852.

Martínez-Lage A, González-Tizón A, Méndez J (1995) Chromosomal markers in three species of the genus *Mytilus* (Mollusca, Bivalvia). Heredity 74(4): 369–375. https://doi.org/10.1038/hdy.1995.55

Messenger JB, Nixon M, Ryan KP (1985) Magnesium chloride as an anaesthetic for cephalopods. Comparative Biochemistry and Physiology 82(1): 203–205. https://doi.org/10.1016/0742–8413(85)90230-0

Mirsky AE, Ris H (1951) The desoxyribonucleic acid content of animal cells and its evolutionary significance. Journal of General Physiology 34(4): 451–462. https://doi.org/10.1085/jgp.34.4.451

Okumura SI, Kinugawa S, Fujimaki A, Kawai W, Maehata H, Yoshioka K, Yoneada R, Yamamori K (1999) Analysis of karyotype, chromosome banding, and nucleolus organizer region of Pacific abalone, *Haliotis discus hannai* (Archaeo-gastropoda: *Haliotidae*). Journal of Shellfish Research 18: 605–609.

Packard A, Albergoni V (1970) Relative growth, nucleic acid content and cell numbers of the brain in *Octopus vulgaris* (Lamarck). Journal of Experimental Biology 52(3): 539–552.

Papan F, Jazayeri A, Ebrahimipour M (2010) The study of Persian Gulf cuttlefish (*Sepia pharaonis*) chromosome via incubation of blood cells. Journal of American Science 6(2): 162–164.

Rakic T, Siljak-Yakovlev S, Sinzar-Sekulic J, Lazarevic M, Stevanovic B, Stevanovic V, Lakusic D (2014) Morphological and genome size variations within populations of *Edraianthus graminifolius* "*Jugoslavicus*" (Campanulaceae) from the central Balkan peninsula. Archives of Biological Science 66(2): 743–763. https://doi.org/10.2298/ABS1402743R

Reed KM, Philips RB (1995) Molecular cytogenetic analysis of the double-CMA3 chromosome in lake trout (*Salvelinus namaycush*). Cytogenetics and Cell Genetics 70(1–2): 104–107. https://doi.org/10.1159/000134002

Sumner AT (1972) A simple technique for demonstrating centromeric heterochromatin. Experimental Cell Research 75: 304–306. https://doi.org/10.1016/0014-4827(72)90558-7

Thiriot-Quiévreux C (2003) Advances in chromosomal studies of gastropod molluscs. Journal of Molluscan Studies 69: 187–202. https://doi.org/10.1093/mollus/69.3.187

Thiriot-Quiévreux C, Insua A (1992) Nucleolar organiser region variation in the chromosomes of three oyster species. Journal of Experimental Marine Biology and Ecology 157(1): 33–40. https://doi.org/10.1016/0022-0981(92)90072-I

Tiersch TR, Chandler RW, Wachtel SS, Elias S (1989) Reference standards for flow cytometry and application in comparative studies of nuclear DNA content. Cytometry 10(6): 706–710. https://doi.org/10.1002/cyto.990100606

Vitturi R, Lafargue F (1992) Karyotype analyses reveal inter-individual polymorphism and association of nucleolus-organizer-carrying chromosomes in *Capros aper* (Pisces: Zeiformes). Marine Biology 112(1): 37–41. https://doi.org/10.1007/BF00349725

Wang HS, Luo X, You WW, Dong YW, Ke CH (2015) Cytogenetic analysis and chromosomal characteristics of the polymorphic 18S rDNA of *Haliotis discus hannai* from Fujian, China. PLoS One 10(2): e0113816. https://doi.org/10.1371/journal.pone.0113816

Wang JH, Zheng XD (2017) Comparison of the genetic relationship between nine Cephalopod species based on cluster analysis of karyotype evolutionary distance. Comparative Cytogenetics 11(3): 477–494. https://doi.org/10.3897/CompCytogen.v11i3.12752

Yoshida M, Ishikura Y, Moritaki T, Shoguchi E, Shimizu KK, Sese J, Ogura A (2011) Genome structure analysis of mollusks revealed whole genome duplication and lineage specific repeat variation. Gene 483(1–2): 63–71. https://doi.org/10.1016/j.gene.2011.05.027

Zaleśna A, Florek M, Rybacki M, Ogielska M (2017) Variability of NOR patterns in European water frogs of different genome composition and ploidy level. Comparative Cytogenetics 11(2): 249–266. https://doi.org/10.3897/CompCytogen.v11i2.10804

Zhang XL, Onozato H (2004) Hydrostatic pressure treatment during the first mitosis does not suppress the first cleavage but the second one. Aquaculture 240(1/4): 101–113. https://doi.org/10.1016/j.aquaculture.2004.07.004

CompCytogen 12(3): 387–402 (2018)
doi: 10.3897/CompCytogen.v12i3.25092
http://compcytogen.pensoft.net

RESEARCH ARTICLE

COMPARATIVE Cytogenetics
A peer-reviewed open-access journal
International Journal of Plant & Animal Cytogenetics, Karyosystematics, and Molecular Systematics

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

Eugene Krysanov[1], Tatiana Demidova[1]

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

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

Academic editor: *Rafael Noleto* | 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 biarmed 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).

Killishes 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, *Paranothobranchius* Seegers, 1985, *Plesiobranchius* Costa, 2018, and *Zononothobranchius* 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 *Cynobranchius* Costa, 2018					
N. microlepis (Vinciguerra, 1897)	24	26	2m+22st/a		Scheel 1990
N. fasciatus Wildekamp & Haas, 1992	34	46	12msm+22st/a		Scheel 1981
Subgenus *Plesiobranchius* Costa, 2018					
N. virgatus Chambers, 1984	32	32	32st/a	2♀/2♂	This study
Subgenus *Nothobranchius* Peters, 1868					
N. furzeri Jubb, 1971	38	60	14m+8sm+16st/a	4♀/5♂	This study, Scheel 1981,1990; Reichwald et al. 2009
N. kadleci Reichard, 2010	38	62	16m+8sm+14st/a	3♀/5♂	This study
N. krysanovi Shidlovskiy, Watters & Wildekamp, 2010	18	34	8m+8sm+2st/a	3♀/5♂	This study, Shidlovskiy et al. 2010; Safronova and Krysanov 2015
N. kuhntae (Ahl, 1926)	38	52	6m+8sm+24st/a	1♀/1♂	This study
N. orthonotus (Peters, 1844)	38	48	8m+2sm+28st/a	2♀/3♂	This study, Scheel 1990

Species	2n	NF	Karyotype structure	Number of specimens karyotyped	References
N. pienaari Shidlovskiy, Watters & Wildekamp, 2010	34	42	6m+2sm+26st/a	4♀/4♂	This study, Shidlovskiy et al. 2010
N. rachovii 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 *Paranothobranchius* Seegers, 1985					
N. ocellatus Seegers, 1985	30	40	2m+8sm+20st/a	2 larvae	This study
Subgenus *Zononothobranchius* Radda, 1969					
N. boklundi Valdesalici, 2010	36	46	6m+4sm+26st/a	2♀/3♂	This study
N. brieni Poll, 1938*	50♀ 49♂	50♀ 50♂	♀50st/a ♂1m+48st/a	4♀/5♂	This study, Krysanov et al. 2016
N. capriviensis Watters, Wildekamp & Shidlovskiy, 2015	36	58	4m+18sm+14st/a	1♀/2♂	This study
N. chochamandai Nagy, 2014	36	64	18m+10sm+8st/a	5♀/7♂	This study
N. flagrans Nagy, 2014	36	48	10m+2sm+24st/a	3♀/4♂	This study
N. hassoni Valdesalici & Wildekamp, 2004	36	52	8m+8sm+20st/a	3♀/5♂	This study
N. ivanovae Valdesalici, 2012	36	64	22m+6sm+8st/a	3♀/3♂	This study
N. kafuensis Wildekamp & Rosenstock, 1989	36	66	8m+22sm+6st/a	1♀/2♂	This study, Scheel 1981, 1990
N. kardashevi Valdesalici, 2012	36	52	6m+10sm+20st/a	2♀/3♂	This study, Valdesalici 2015
N. malaissei Wildekamp, 1978	48	62	4m+10sm+34st/a	3♀/3♂	This study
N. milvertzi Nagy, 2014	38	54	10m+6sm+22st/a	4♀/4♂	This study
N. neumanni (Hilgendorf, 1905)	36	70	18m+16sm+2st/a	4♀/5♂	This study
N. nubaensis Valdesalici, Bellemans, Kardashev & Golubtsov, 2009	36	62	14m+12sm+10st/a	3♀/4♂	This study, Valdesalici 2015
N. polli Wildekamp, 1978	36	60	10m+14sm+12st/a	2♀/3♂	This study
N. robustus Ahl, 1935	36	58	4m+18sm+14st/a	1♂	This study, Wildekamp 2004
N. rosenstocki Valdesalici & Wildekamp, 2005	36	62	14m+12sm+10st/a	1♀/2♂	This study
N. rubroreticulatus Blache & Miton, 1960	36	58	12m+10sm+14st/a	2♀/2♂	This study
N. seegersi Valdesalici & Kardashev, 2011	36	56	8m+12sm+16st/a	4♀/4♂	This study
N. steinforti Wildekamp, 1977	36	56	10m+10sm+16st/a	2♀/3♂	This study, Scheel 1981, 1990
N. streltsovi Valdesalici, 2016	36	48	6m+6sm+24st/a	3♀/3♂	This study
N. symoensi Wildekamp, 1978	36	68	20m+12sm+4st/a	2♀/3♂	This study
N. taeniopygus Hilgendorf, 1891	36	66	14m+16sm+6st/a	4♀/5♂	This study
N. ugandensis Wildekamp, 1994	36	58	8m+14sm+14st/a	3♀/3♂	This study, Wildekamp 1994, Valdesalici 2015
Subgenus *Adiniops* Myers, 1924					
N. albimarginatus Watters, Wildekamp & Cooper, 1998	36	38	2m+34st/a	3♀/5♂	This study
N. annectens Watters, Wildekamp & Cooper, 1998	28	36	8m+20st/a	5♀/7♂	This study
N. cardinalis Watters, Cooper & Wildekamp, 2008	36	38	2m+34st/a	8♀/12♂	This study
N. eggersi Seegers, 1982	36	40	4m+32st/a	5♀/6♂	This study, Scheel 1990
N. elongatus 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
N. flammicomantis Wildekamp, Watters & Sainthouse, 1998	20	38	18m+2st/a	5♀/8♂	This study
N. foerschi Wildekamp & Berkenkamp, 1979	34	46	10m+2sm+22st/a	3♀/5♂	This study, Scheel 1981, 1990; Ewulonu et al. 1985
N. fuscotaeniatus Seegers, 1997	38	40	2sm+36st/a	3♀/6♂	This study
N. geminus Wildekamp, Watters & Sainthouse, 2002	38	40	2sm+36st/a	2♀/3♂	This study
N. guentheri (Pfeffer, 1893) *	36♀ 35♂	40♀ 39♂	♀2m+2sm+32st/a ♂2m+2sm+31st/a	5♀/7♂	This study, Scheel 1990, Ewulonu et al. 1985
N. hengstleri Valdesalici, 2007	38	42	2m+2sm+34st/a	3♀/5♂	This study, Wildekamp et al. 2009
N. interruptus Wildekamp & Berkenkamp, 1979	36	50	8m+6sm+22st/a	2♀/3♂	This study
N. janpapi Wildekamp, 1977*	38♀ 37♂	48♀ 49♂	♀2m+8sm+28st/a ♂3m+9sm+25st/a	5♀/7♂	This study, Scheel 1990
N. jubbi Wildekamp & Berkenkamp, 1979	34	46	4m+8sm+22st/a	2♀/3♂	This study, Scheel 1981, 1990; Wildekamp 1982, Wildekamp et al. 1986
N. kilomberoensis Wildekamp, Watters & Sainthouse, 2002	32	46	8m+6sm+18st/a	2♀/4♂	This study
N. kirki Jubb, 1969	36	50	2m+12sm+22st/a	1♀/2♂	This study, Scheel 1981, 1990
N. korthausae Meinken, 1973	36	40	4m+32st/a	3♀/5♂	This study, Scheel 1981, 1990
N. lourensi Wildekamp, 1977*	28♀ 27♂	34♀ 34♂	♀6m+22st/a ♂7m+20st/a	2♀/3♂	This study
N. lucius Shidlovskiy, Watters & Wildekamp, 2010	36	58	6m+16sm+14st/a	2♀/3♂	This study, Wildekamp et al. 2009
N. luekei Seegers, 1984	38	40	2m+36st/a	2♀/2♂	This study
N. makondorum Shidlovskiy, Watters & Wildekamp, 2010	36	50	6m+8sm+22st/a	3♀/4♂	This study, Wildekamp et al. 2009
N. melanospilus (Pfeffer, 1896)	38	50	4m+8sm+26st/a	3♀/4♂	This study, Scheel 1981, 1990; Wildekamp et al. 2009
N. palmqvisti (Lönnberg, 1907)	36	42	6m+30st/a	2♀/2♂	This study, Ewulonu et al. 1985
N. patrizii (Vinciguerra, 1897)	36	52	4m+12sm+20st/a	2♀/2♂	This study, Ewulonu et al. 1985
N. rubripinnis Seegers, 1986	36	38	2m+34st/a	2♀/2♂	This study
N. ruudwildekampi Costa, 2009	36	38	2m+34st/a	3♀/4♂	This study
N. vosseleri Ahl, 1924	38	60	6m+16sm+16st/a	2♀/3♂	This study
N. wattersi Ng'oma, Valdesalici, Reichwald & Cellerino, 2013	36	40	4m+32st/a	2♀/2♂	This study, Scheel 1990
Unrecognized species					
N. ditte Nagy, 2018*	40♀ 39♂	♀64 ♂64	♀12m+12sm+16st/a ♂13m+12sm+14st/a	3♀/4♂	This study
N. torgashevi Valdesalici, 2015	36	46	6m+4sm+26st/a	3♀/4♂	This study, Valdesalici 2015
N. usanguensis Wildekamp, Watters & Shidlovskiy, 2014	36	54	6m+12sm+18st/a	1♀/2♂	This study
Genus *Fundulosoma* Ahl, 1924					
Fundulosoma thierryi (Ahl, 1924) *	44♀ 43♂	46♀ 45♂	♀2m+42st/a ♂1m+1sm+41st/a	2♀/4♂	This study
Genus *Pronothobranchius* Radda, 1969					
Pronothobranchius kiyawensis Ahl, 1928	28	30	2m+26st/a	2♂	This study

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

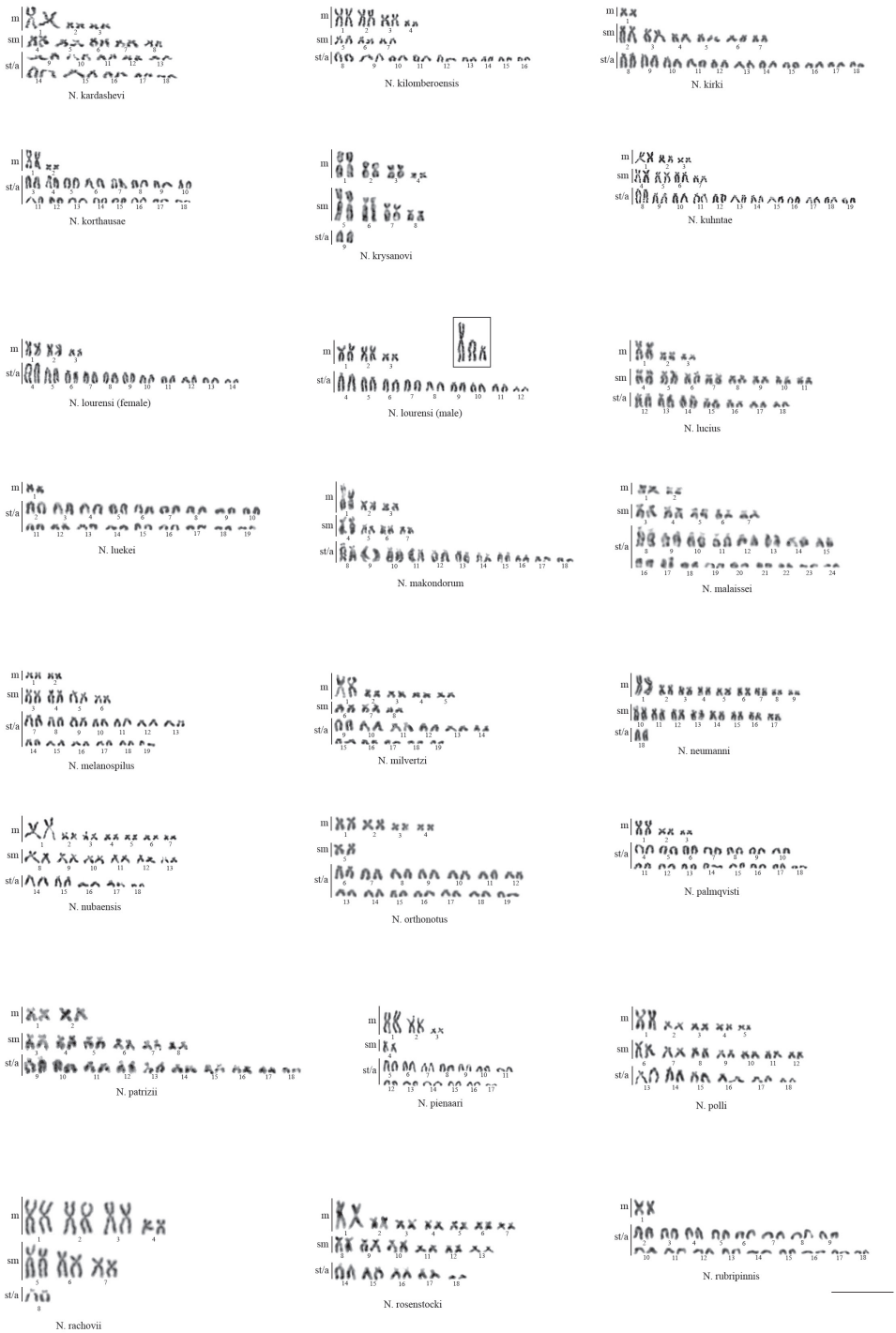

Figure 1. Continued. Karyotypes of species *Nothobranchius*. Scale bar: 10 μ.

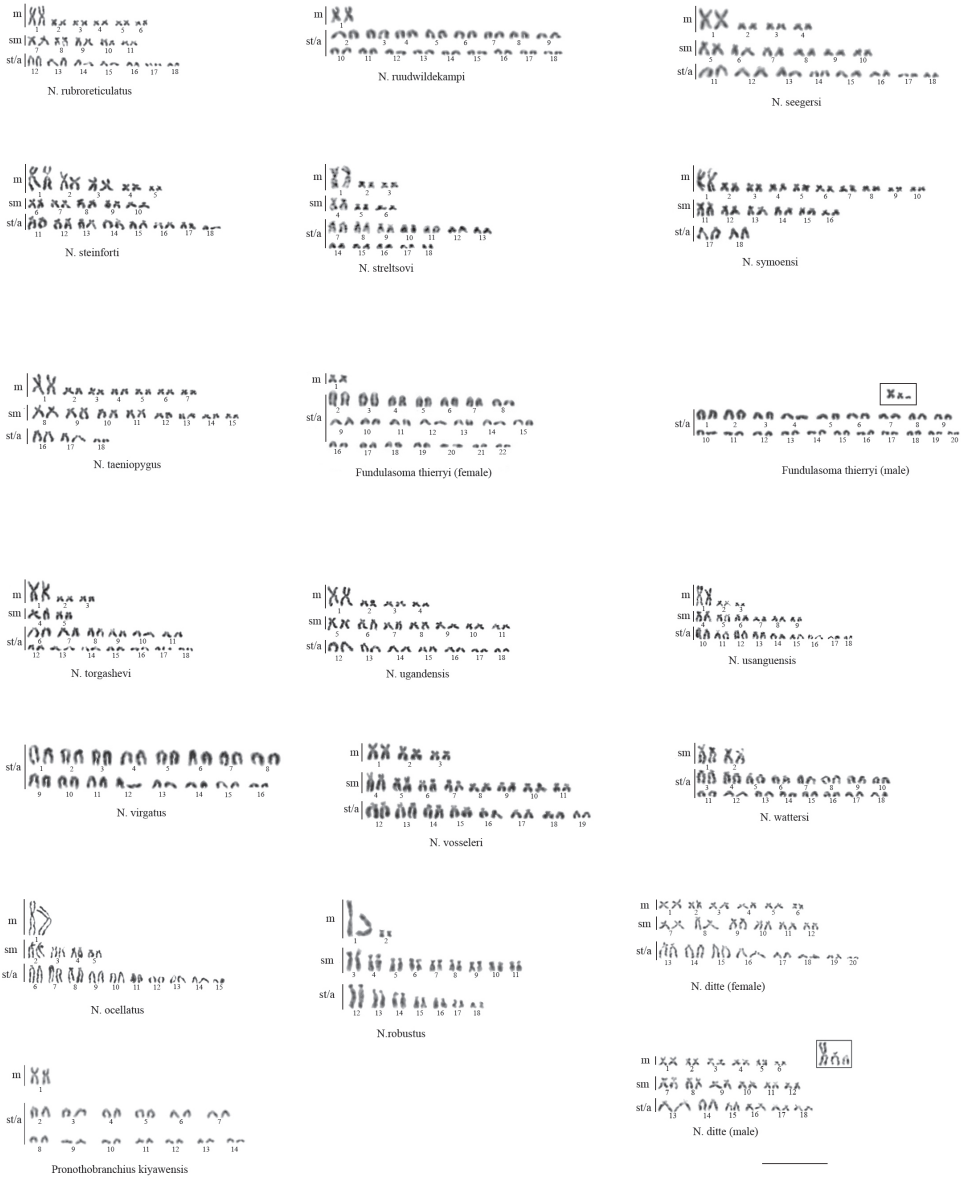

Figure 1. Continued. Karyotypes of species *Nothobranchius*. Scale bar: 10 μ.

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 Rs = -0.181 (Fig. 3).

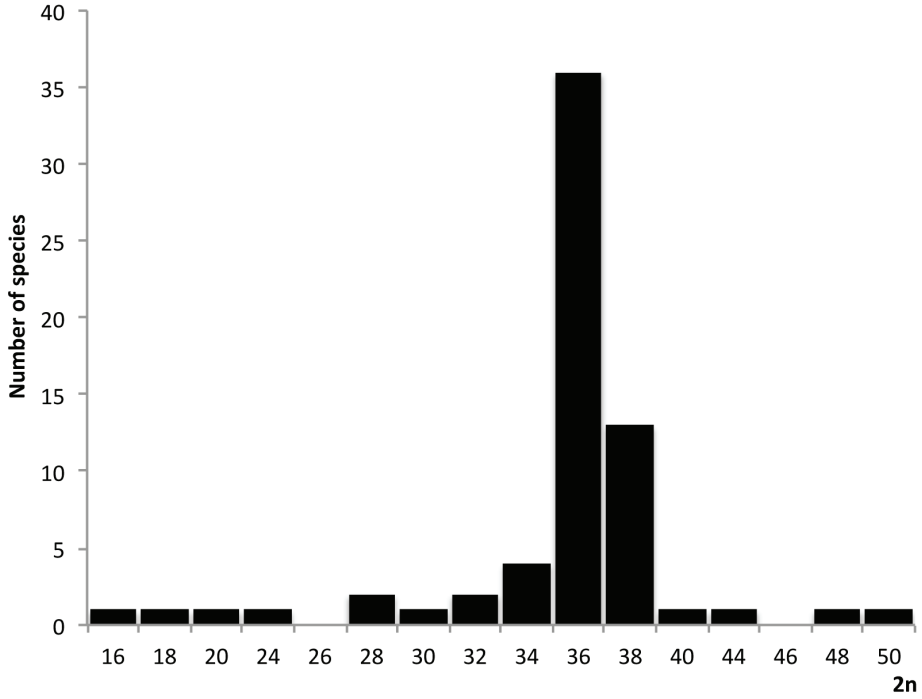

Figure 2. Histogram of the distribution of the diploid chromosome number (2n) in the genus *Nothobranchius*.

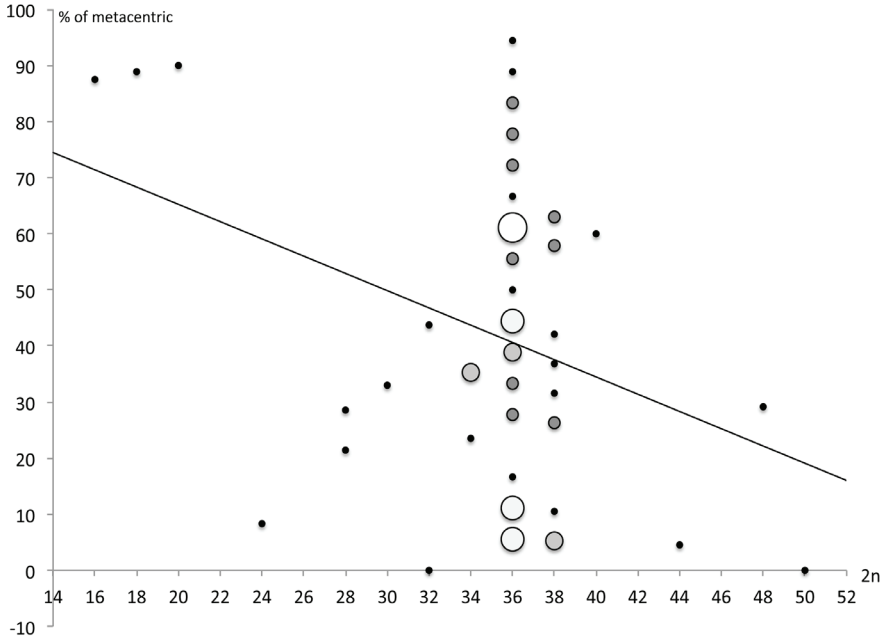

Figure 3. Scatter-plot of a diploid chromosome number (2n) and proportion of metacentric chromosomes with overall regression line. The diameter and color of circle indicate number of species from 1 to 5.

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 uniarmed with only one pair of biarmed chromosomes (NF = 26). *N. fasciatus* had 2n = 34 with 22 uniarmed and 12 biarmed chromosomes.

Subgenus *Plesiobranchius*

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

Subgenus *Nothobranchius*

Four species *N. furzeri*, *N. kadleci*, *N. orthonotus* and *N. kuhntae* possesed the 2n = 38. Biarmed elements dominated in karyotypes of *N. kadleci* (NF = 62) and *N. furzeri* (NF = 60), and uniarmed 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 uniarmed (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 at 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 uniarmed chromosomes dominated in the karyotype (NF = 40).

Subgenus *Zononothobranchius*

There are species in the subgenus possessing 2n higher than 38. The highest 2n = 49/50 among studied species was discovered in *N. brieni* (Krysanov et al. 2016) where all autosomes in the karyotype were acrocentric (NF = 50). *N. brieni* 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* possesed 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 $X_1X_1X_2X2/X_1X_2Y$ 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* (Agnèse 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 rarest 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 JF, 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 Actinopterygiian 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 Hystory 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

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 torgashevi* (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.

CompCytogen 12(3): 403–420 (2018)
doi: 10.3897/CompCytogen.v12i3.27307
http://compcytogen.pensoft.net

RESEARCH ARTICLE

COMPARATIVE Cytogenetics
A peer-reviewed open-access journal
International Journal of Plant & Animal Cytogenetics, Karyosystematics, and Molecular Systematics

New chromosome counts and genome size estimates for 28 species of *Taraxacum* sect. *Taraxacum*

Petra Macháčková[1], Ľuboš Majeský[1], Michal Hroneš[1], Eva Hřibová[2], Bohumil Trávníček[1], Radim J. Vašut[1]

1 *Department of Botany, Faculty of Science, Palacký University in Olomouc, Šlechtitelů 27, 783 71, Olomouc, Czech Republic* **2** *Institute of Experimental Botany, Centre of the Region Haná for Biotechnological and Agricultural Research, Šlechtitelů 31, 779 00 Olomouc, Czech Republic*

Corresponding author: *Radim J. Vašut* (radim.vasut@upol.cz)

Academic editor: *J.R. Daviña* | Received 11 June 2018 | Accepted 22 August 2018 | Published 18 September 2018

http://zoobank.org/AE192009-1A66-4B8B-8267-B6AC546BA057

Citation: Macháčková P, Majeský Ľ, Hroneš M, Hřibová E, Trávníček B, Vašut RJ (2018) New chromosome counts and genome size estimates for 28 species of *Taraxacum* sect. *Taraxacum*. Comparative Cytogenetics 12(3): 403–420. https://doi.org/10.3897/CompCytogen.v12i3.27307

Abstract

The species-rich and widespread genus *Taraxacum* F. H. Wiggers, 1780 (Asteraceae subfamily Cichorioideae) is one of the most taxonomically complex plant genera in the world, mainly due to its combination of different sexual and asexual reproduction strategies. Polyploidy is usually confined to apomictic microspecies, varying from 3x to 6x (rarely 10x). In this study, we focused on *Taraxacum* sect. *Taraxacum* (= *T.* sect. *Ruderalia*; *T. officinale* group), i.e., the largest group within the genus. We counted chromosome numbers and measured the DNA content for species sampled in Central Europe, mainly in Czechia. The chromosome number of the 28 species (*T. aberrans* Hagendijk, Soest & Zevenbergen, 1974*, T. atroviride* Štěpánek & Trávníček, 2008*, T. atrox* Kirschner & Štěpánek, 1997*, T. baeckiiforme* Sahlin, 1971*, T. chrysophaenum* Railonsala, 1957*, T. coartatum* G.E. Haglund, 1942*, T. corynodes* G.E. Haglund, 1943*, T. crassum* H. Øllgaard & Trávníček, 2003*, T. deltoidifrons* H. Øllgaard, 2003*, T. diastematicum* Marklund, 1940*, T. gesticulans* H. Øllgaard, 1978*, T. glossodon* Sonck & H. Øllgaard, 1999, *T. guttigestans* H. Øllgaard in Kirschner & Štěpánek, 1992*, T. huelphersianum* G.E. Haglund, 1935*, T. ingens* Palmgren, 1910, *T. jugiferum* H. Øllgaard, 2003*, T. laticordatum* Marklund, 1938*, T. lojoense* H. Lindberg, 1944 (= *T. debrayi* Hagendijk, Soest & Zevenbergen, 1972, *T. lippertianum* Sahlin, 1979), *T. lucidifrons* Trávníček, ineditus*, T. obtusifrons* Marklund, 1938*, T. ochrochlorum* G.E. Haglund, 1942*, T. ohlsenii* G.E. Haglund, 1936*, T. perdubium* Trávníček, ineditus*, T. praestabile* Railonsala, 1962*, T. sepulcrilobum* Trávníček, inedi-

tus, *T. sertatum* Kirschner, H. Øllgaard & Štěpánek, 1997, *T. subhuelphersianum* M.P. Christiansen, 1971, *T. valens* Marklund, 1938) is 2n = 3x = 24. The DNA content ranged from 2C = 2.60 pg (*T. atrox*) to 2C = 2.86 pg (*T. perdubium*), with an average value of 2C = 2.72 pg. Chromosome numbers are reported for the first time for 26 species (all but *T. diastematicum* and *T. obtusifrons*), and genome size estimates for 26 species are now published for the first time.

Keywords
Asteraceae, chromosome number, flow cytometry, karyology, *Taraxacum officinale*

Introduction

Taraxacum F. H. Wiggers, 1780 (Asteraceae subfamily Cichorioideae) is a species-rich genus of common and widespread perennial grassland herbs growing from the subtropics to subarctic (arctic/alpine) regions across the world. Rough estimates suggest the genus contains approximately 2,800 species in approximately 60 sections (Kirschner et al. 2015), with the higher diversity in the mountains of Eurasia (Ge et al. 2011); a total of 1,900 species in 35 sections are listed for Europe (Kirschner et al. 2007). The complexity of *Taraxacum* taxonomy is caused by its combination of different reproduction strategies, including sexual reproduction (mainly outcrossing, less frequently selfing) and apomixis (meiotic diplospory; Richards 1973, Asker and Jerling 1992, Kirschner and Štěpánek 1994, Kirschner et al. 1994, Majeský et al. 2017). The vast majority of *Taraxacum* taxa are apomictic polyploid microspecies, only a few species are sexual diploids. The phenomenon of apomixis itself (i.e. clonal reproduction by seeds) attracts the attention of plant systematists as well as plant breeders for its possible application in crop breeding.

The basic chromosome number in *Taraxacum* is x = 8, and it is constant across all the sections. The diploid number (2n = 2x = 16) is confined to only sexually reproducing species, and sexual species are nearly all diploids, with only a few exceptions of sexual tetraploids known in section *Piesis* (Kirschner and Štěpánek 1994, 1998a, Trávníček et al. 2013). In contrast, apomictic species are never diploids but always polyploids (Majeský et al. 2017), having one of the genes involved in regulation of apomixis (*DIPLOSPOROUS*) located on the NOR chromosome (Vašut et al. 2014). Most of the known chromosome numbers for apomictic *Taraxacum* species are at a triploid level (2n = 3x = 24), especially those of the widespread European sections *Taraxacum* sect. *Taraxacum* (Mártonfiová 2006, Kula et al. 2013), *T.* sect. *Erythrosperma* (Małecka 1967, 1969, Vašut 2003, Schmid et al. 2004, Vašut et al. 2005, Uhlemann 2007, 2010, Vašut and Majeský 2015, Wolanin and Musiał 2017), *T.* sect. *Palustria* (Małecka 1972, 1973, 1978, Kirschner and Štěpánek 1998b, Marciniuk et al. 2010) and *T.* sect. *Hamata* (Mogie and Richards 1983, Øllgaard 1983). However, tetraploids (2n = 4x = 32) also occur quite frequently in some sections, such as the European dandelions in sections *T.* sect. *Palustria* (e.g., *T. vindobonense* Soest, 1965, *T. brandenburgicum* Hudziok, 1969 and *T. portentosum* Kirschner & Štěpánek, 1998), *T.* sect. *Erythrosperma* (e.g., *T. tortilobum* Florström, 1914, *T. fulvum* Raunkiaer, 1906

and *T. bifurcatum* Hagendijk et al., ineditus), *T*. sect. *Naevosa* (e.g., *T. euryphyllum* (Dahlstedt, 1911) M. P. Christiansen, 1940 and *T. naevosum* Dahlstedt, 1900), *T*. sect. *Scariosa* and *T*. sect. *Celtica* (*T. unguilobum* Dahlstedt, 1912 and *T. fulvicarpum* Dahlstedt, 1927). Higher ploidy levels are uncommon in *Taraxacum*, while natural pentaploids (2n = 5x = 40; e.g., in the European species *T. skalinskanum* Małecka & Soest, 1972 and *T. zajacii* J. Marciniuk et P. Marciniuk, 2012 and 6 other species of section *Palustria*, *T. faeroense* Dahlstedt in H. H. Johnston, 1926 of *T*. sect. *Spectabilia*, *T. caledonicum* A. J. Richards, 1972 of section *Celtica* and *T. albidum* Dahlstedt, 1907 of section *Mongolica* from Japan), hexaploids (2n = 6x = 48 for *T. ranunculus* Kirschner & Štěpánek, 1998 of section *Palustria* and *T. nordstedtii* Dahlstedt, 1911 of section *Celtica*), and aberrant heptaploid (2n = 7x = 56) or decaploid (2n = 10x = 80) mutants of natural species have been documented (Richards 1969, Małecka 1973, Mogie and Richards 1983, Kirschner and Štěpánek 1984, 1998b, Sato et al. 2011, Marciniuk et al. 2012). The geographic distribution of diploids and polyploids in Europe is more or less separated, with polyploids mainly distributed in the colder regions of mountains in the north and with diploid sexuals distributed in warmer regions of the south, which results in the phenomenon of geographic parthenogenesis (den Nijs et al. 1990, den Nijs and van der Hulst 1988, Uhlemann 2001, Verduijn et al. 2004a).

Genome size estimation (plant genome C-value) (Greilhuber et al. 2005) is a rapid cytogenetic method that helps provide a better understanding of the evolutionary relationships among studied taxa. The method itself has methodological limitations (multiple factors can affect the measurements; the method does not provide any information on repetitive sequences involved; etc.); however, patterns of genome size estimates in species groups provide additional information on possible pathways of evolution (Soltis et al. 2003, Leitch et al. 2005, Šmarda et al. 2012). Although flow cytometry was widely used in *Taraxacum* research for rapidly identifying the ploidy level in mixed apomictic-sexual populations (e.g., Meirmans et al. 1999, Verduijn et al. 2004a, 2004b, Mártonfiová 2006, 2015, Mártonfiová et al. 2007, 2010) or in taxonomic revisions (e.g., Vašut 2003), genome size estimates are very limited. Genome size (C-value) in *Taraxacum* varies (in known species) between 2C = 1.74 pg in diploid *T. linearisquameum* Soest, 1966 and 2C = 6.91 pg in tetraploid *T. albidum* (Záveský et al. 2005, Siljak-Yakovlev et al. 2010); European triploid apomicts have a value of 2C ≈ 2.4–2.76 pg (Bennett et al. 1982, Záveský et al. 2005, Bainard et al. 2011, Iaffaldano et al. 2017). Considerable variation (~1.2-fold difference) in DNA content, measured as the C-value, was observed in *T. stenocephalum* Boissier et Kotschy ex Boissier, 1875 (Trávníček et al. 2013) and in a sample of an unidentified species of the *Taraxacum officinale* group in North America (Iaffaldano et al. 2017).

Taraxacum sect. *Taraxacum* (formerly known as *T*. sect. *Ruderalia*; generally known as *Taraxacum officinale* group; see Kirschner and Štěpánek 2011) has a strongly prevailing triploid ploidy level of 2n = 3x = 24, by which it differs from other closely related sections (*Erythrosperma*, *Palustria*, and *Celtica*) with known ploidies of 3x and 4x or even higher. In this study, we aimed to count the chromosome number of 28 species for which knowledge was lacking and to detect the ploidy level for these species. Fur-

thermore, we searched for variability in genome size among these species to determine whether we can detect variation in DNA content among species similar to that found in a sample of unidentified taxa of *T. officinale* group.

Material and methods

Plant Material

We studied a total of 28 *Taraxacum* species (25 formally described and three still undescribed, referred to by their working names) belonging to *Taraxacum* sect. *Taraxacum* (Table 1). Plants and achenes of the investigated species were collected in natural habitats of several localities of Central Europe in the period 2014–2016. A detailed description of the localities, date, and collectors of samples is provided in Table 1. The studied plant material was documented by herbarium specimens and is deposited in the herbarium of the Department of Botany, Palacký University in Olomouc, Czech Republic (OL). All studied species are apomictic (agamospermous); thus, maternal plants and offspring plants (grown from seeds) are taxonomically (genetically) identical.

For karyological analyses, achenes were sown in Petri dishes containing 1% agar solution and germinated at room temperature. Fresh young leaves for flow cytometric analyses were collected from juvenile plants cultivated in a greenhouse at the Department of Botany, Faculty of Science, Palacký University in Olomouc.

Karyology

For chromosome counts, we used mitotically active root tip meristems of dandelion seedlings. Seedlings of the investigated species with 1–2 cm long roots were collected in the morning. To obtain the desired metaphase index, the roots were pre-treated in a 2 mM solution of 8-hydroxyquinoline for two hours at room temperature and an additional two hours at 4 °C in the dark. Then, the material was fixed in Carnoy's fixative (a mixture (3:1, v/v) of absolute ethanol and acetic acid) and stored in a refrigerator (4 °C) until further processing (Hasterok and Maluszynska 2000). For slide preparation, a combination of protocols in Hasterok and Maluszynska (2000) and van Baarlen et al. (2000) was used with the following changes for the investigated species of dandelions. Fixed root tips were washed in citrate buffer (0.01 M, pH 4.8) for 5 min and then enzymatically digested in a mixture of 0.1% cellulose Onozuka RS (*Trichoderma* Persoon, 1794; Sigma), 0.1% pectolyase (*Aspergillus japonicus* Saito, 1906; Sigma) and 0.1% cytohelicase (*Helix pomatia* Linnaeus, 1758; Sigma) in the citrate buffer for 90 min at 37–40 °C. To remove trace amounts of the enzymatic mixture, the root tips were then gently washed in citrate buffer for 5 min. Only the mitotically active meristematic tissue of a root tip was cut off under a stereoscopic microscope, transferred into a drop of 50% acetic acid on a slide and covered by a cov-

Table 1. List of species used in this study, with sampling details. Country codes according to ISO 3166-1 alpha-2 (AT = Austria; CZ = Czechia, DE = Germany, HU = Hungary, IT = Italy, SK = Slovakia); Collectors: BT = Bohumil Trávníček; RJV = Radim Jan Vašut.

Taxon	Country	Locality; GPS; Date; Collector
T. aberrans Hagendijk, Soest & Zevenbergen, 1974	AT	Upper Austria, Obernberg am Inn town, lawn in the street of Therese-Riggle-Strasse; 48°19'14"N; 13°19'52"E; 10.05.2015; BT
T. atroviride Štěpánek & Trávníček, 2008	AT	Altaussee village (near Bad Aussee town), lawns and roadsides in the ski resort NNW from the village (valley of Augstbach brook); 47°39'42"N; 13°44'38"E; 08.05.2014; BT
T. atrox Kirschner & Štěpánek, 1997	IT	Cave del Predil settlement (S from Tarvisio town), lawns at the road no SP76 (at lake of Lago di Predil); 46°25'11"N; 13°33'42"E; 16.05.2015; BT
T. baeckiiforme Sahlin, 1971	HU	Felsöcsatár village (W from the Szombathely town), grassy roadsides at the road towards Vaskeresztes village; 250 m a.s.l.; 47°12'20"N; 16°26'51"E; 26.04.2015; BT
T. chrysophaenum Railonsala, 1957	CZ	Bartošovice village (near Nový Jičín town), lawns in park in central part of the village; 49°40'15"N, 18°02'59"E; 23.04.2014; BT
T. coartatum G. E. Haglund, 1942	CZ	Lubná village (near Polička town), grassy places at brook in E part of the village; 480 m a.s.l.; 49°46'26"N, 16°13'57"E; 17.05.2016; BT & RJV
T. corynodes G. E. Haglund, 1943	CZ	Hanušovice town, lawns at the railway station; 50°04'18"N, 16°55'52"E; 19.05.2015; BT
T. crassum H. Øllgaard & Trávníček, 2003	CZ	Nové Město na Moravě town, grassy places at brook in the town, ca 0.6 km ESE from railway station of "Nové Město na Moravě-zastávka"; 600 m a.s.l.; 49°33'45"N, 16°04'04"E; 17.05.2016; BT & RJV
T. deltoidifrons H. Øllgaard, 2003	CZ	Jimramov town, grassy places in the park of Bludník in N part of the town; 500 m a.s.l.; 49°38'19"N, 16°13'25"E; 17.05.2016; BT & RJV
T. diastematicum Marklund, 1940	CZ	Svratka village, meadows and grassy places at NW margin of the settlement of Česká Cikánka; 630 m a.s.l.; 49°42'35"N, 16°03'01"E; 17.05.2016; BT & RJV
T. gesticulans H. Øllgaard, 1978	CZ	Hanušovice town, lawns at the railway station; 50°04'18"N, 16°55'52"E; 19.05.2015; BT
T. glossodon Sonck & H. Øllgaard, 1999	CZ	Studnice village (N from Nové Město na Moravě town), meadow at road near the Paseky settlement ca 1 km NNW from the village; 780 m a.s.l.; 49°36'51"N, 16°05'17"E; 17.05.2016; BT & RJV
T. guttigestans H. Øllgaard in Kirschner & Štěpánek, 1992	CZ	Nové Město na Moravě town, grassy places at brook in the town, ca 0.6 km ESE from railway station of "Nové Město na Moravě-zastávka"; 600 m a.s.l.; 49°33'45"N, 16°04'04"E; 17.05.2016; BT & RJV
T. huelphersianum G. E. Haglund, 1935	CZ	Pekařov settlement (near Hanušovice town), lawns and meadows in the settlement; 50°04'41"N, 17°01'31"E; 19.05.2015; BT
T. ingens Palmgren, 1910	CZ	Svratka village, meadows and grassy places at NW margin of the settlement of Česká Cikánka; 630 m a.s.l.; 49°42'35"N, 16°03'01"E; 17.05.2016; BT & RJV
T. jugiferum H. Øllgaard, 2003	CZ	Jedlí village (NW from Zábřeh town), lawns and roadsides in central part of the village; 49°55'54"N, 16°47'45"E; 19.05.2015; BT
T. laticordatum Marklund, 1938	CZ	C Moravia, Hlinsko pod Hostýnem village, roadside at road towards Prusinovice village; 49°22'34"N, 17°36'47.8"E; 20.05.2016; BT
T. lojoense H. Lindberg, 1944 †	CZ	Úterý village (near Konstantinovy Lázně town), lawns at the brook on the eastern village margin; 510 m a.s.l.; 49°56'24"N, 13°00'21"E; 25.04.2014; BT
T. lucidifrons Trávníček, ineditus	CZ	Kunín village (near Nový Jičín town), lawns in chateau park; 49°38'39"N, 17°59'18"E, 23.04.2014; BT
T. obtusifrons Marklund, 1938	CZ	Lubná village (near Polička town), grassy places at brook in E part of the village; 480 m a.s.l.; 49°46'26"N, 16°13'57"E; 17.05.2016; BT & RJV

Taxon	Country	Locality; GPS; Date; Collector
T. ochrochlorum G. E. Haglund, 1942	CZ	Svratka village, meadows and grassy places at NW margin of the settlement of Česká Cikánka; 630 m a.s.l.; 49°42'35"N, 16°03'01"E; 17.05.2016; BT & RJV
T. ohlsenii G. E. Haglund, 1936	DE	Schönwald village (near Hof town), wet meadow and adjacent roadsides at the road (no. 15) towards Rehau village; 550 m a.s.l.; 50°13'37"N, 12°04'57"E; 27.04.2014; BT
T. perdubium Trávníček, ineditus	CZ	Záhlinice village (near Hulín town), wet meadow 1.3 km SSW from the railway station; 190 m a.s.l.; 49°16'52"N, 17°28'58"E; 20.04.2016; BT
T. praestabile Railonsala, 1962	IT	Sella Nevea settlement (SW from Tarvisio town), lawns near hotel of Canin, road no. SP76; 46°23'19"N, 13°28'25"E; 16.05.2015; BT
T. sepulcrilobum Trávníček, ineditus	CZ	Záhlinice village (near Hulín town), wet meadow 1.3 km SSW from the railway station; 190 m a.s.l.; 49°16'52"N, 17°28'58"E; 20.04.2016; BT
T. sertatum Kirschner, H. Øllgaard & Štěpánek, 1997	CZ	Svratka village, meadows and grassy places at NW margin of the settlement of Česká Cikánka; 630 m a.s.l.; 49°42'35"N, 16°03'01"E; 17.05.2016; BT & RJV
T. subhuelphersianum M. P. Christiansen, 1971	SK	Spišské Podhradie village (near Levoča town), lawn at road not far from Sivá brada travertine spring; 49°00'28"N, 20°43'26"E; 01.05.2014; BT
T. valens Marklund, 1938	HU	Szombathely town, lawns in the Szent István park (at the street of Jókai Mór); 225 m a.s.l.; 47°13'45"N, 16°36'15"E; 26.04.2015; BT

† The taxon traditionally identified as *T. lippertianum* Sahlin, 1979 in Central Europe and recently considered a synonym of *T. debrayi* Hagendijk, Soest & Zevenbergen, 1972. According to BT, both taxa are synonyms of *T. lojoense* (B. Trávníček unpubl., H. Øllgaard pers. comm.).

erslip. After heating the preparation to 42 °C for 1–2 min, cells were spread between a glass slide and coverslip in a drop of 50% acetic acid. The coverslip was mechanically removed by a razor blade after deep freezing in liquid nitrogen, and the slide was air dried. To increase the contrast of metaphase chromosomes for counting, the preparations were stained with DAPI (4',6-Diamidine-2'-phenylindole dihydrochloride; Vectashield Mounting Medium with DAPI, Vector Laboratories). For each species, at least ten metaphases were analysed to determine the chromosome number. Well-spread metaphase images were captured using Olympus BX 60 and Axio Imager Z.2 Zeiss fluorescence microscopes, both equipped with a CCD camera and ISIS software (Metasystems, Altlussheim, Germany).

Genome size estimation

The absolute genome size (2C-value; Doležel et al. 2007) of the fresh plant samples was quantified using a BD Accuri C6 flow cytometer (BD Biosciences, San Jose) equipped with a blue laser (488 nm, 20 mW, BD Accuri; BD Biosciences, San Jose). Sample preparation followed the standard protocol using LB01 isolation buffer supplemented with PVP (polyvinylpyrrolidone, 10 g/500 ml of buffer) to suppress interference of phenolic compounds with DNA staining (Doležel and Bartoš 2005, Doležel et al. 2007). Approximately 0.2 cm^2 of the plant tissue between secondary veins was chopped in 500 µl of LB01 buffer together with a similar amount of tissue

of an internal standard. Due to peak overlap in some accessions, *Solanum lycopersicum* Linnaeus, 1753 'Stupické polní rané' (2C = 1.96 pg; Doležel et al. 2007) served as the primary reference standard, and *Glycine max* (Linnaeus, 1753) Merrill, 1917 'Polanka' (2C = 2.33 pg, re-calculated against a primary standard) served as the secondary standard. The suspension was filtered through a 42 µm nylon mesh, supplemented with 20 µl of RNase A type II-A (with a final concentration of 50 µg/ml) and incubated at room temperature for approximately 10 min. The sample was then stained with 20 µl of propidium iodide (PI; final concentration of 50 µg/ml) and incubated with occasional shaking for approximately 5 min at room temperature. A flow-through fraction was then run on the flow cytometer, and the relative fluorescence intensity of at least 5,000 particles was recorded. Each sample was analysed at least three times. If the range of variation in the three measurements exceeded the 2% threshold, then the outlying value was discarded, and the sample was re-analysed. Only G0/G1 peaks with coefficients of variation < 4% were accepted. The 2C-value was calculated by multiplying the 2C-value of the standard with the sample/standard fluorescence ratio. Monoploid genome size (1Cx-value) was calculated by dividing the 2C-value by the inferred chromosome number.

Results

The chromosome number of all 28 studied species of *Taraxacum* sect. *Taraxacum* (*T. aberrans*, *T. atroviride*, *T. atrox*, *T. baeckiiforme*, *T. chrysophaenum*, *T. coartatum*, *T. corynodes*, *T. crassum*, *T. deltoidifrons*, *T. diastematicum*, *T. gesticulans*, *T. glossodon*, *T. guttigestans*, *T. huelphersianum*, *T. ingens*, *T. jugiferum*, *T. laticordatum*, *T. lojoense*, *T. lucidifrons*, *T. obtusifrons*, *T. ochrochlorum*, *T. ohlsenii*, *T. perdubium*, *T. praestabile*, *T. sepulcrilobum*, *T. sertatum*, *T. subhuelphersianum*, *T. valens*) was counted invariably as 2n = 3x = 24 (Figs 1, 2). With respect to the position of the centromere, the chromosomes of all studied species were predominantly submetacentric or metacentric. The chromosome sizes were relatively small (Figs 1, 2). The smallest chromosome size in this study was 1.02 µm (*T. ochrochlorum*), and the largest one was 4.94 µm (*T. baeckiiforme*).

The DNA content of the twenty-six studied *Taraxacum* species (two species, i.e., *T. chrysophaenum* and *T. subhuelphersianum*, were not analysed due to low-quality fresh material) ranged 1.08-fold from 2C = 2.60 pg in *T. atrox* to 2C = 2.86 pg in *T. perdubium* (Table 2). The average and median 2C-values for *Taraxacum* sect. *Taraxacum* (based on these 26 species) are 2.72 pg and 2.71 pg, respectively.

Discussion

Chromosome number variation differs among sections of the genus *Taraxacum* and more frequently occurs in sections such as *Palustria* or *Celtica*, whereas in section

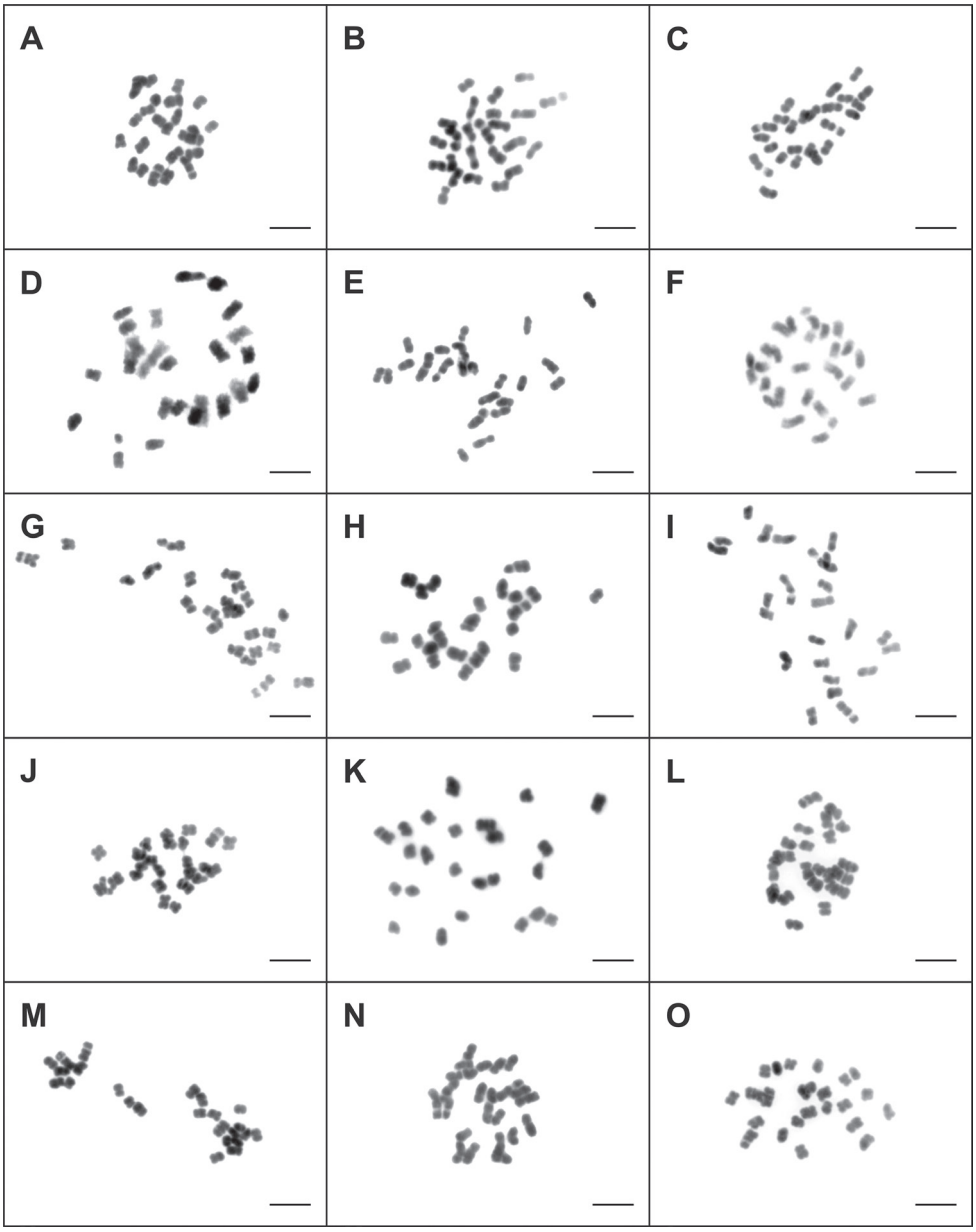

Figure 1. Mitotic metaphase chromosomes of studied triploid species (2n=3x=24) of *Taraxacum* sect. *Taraxacum*. **A** *T. aberrans* **B** *T. atroviride* **C** *T. atrox* **D** *T. baeckiiforme* **E** *T. chrysophaenum* **F** *T. coartatum* **G** *T. corynodes* **H** *T. crassum* **I** *T. deltoidifrons* **J** *T. diastematicum* **K** *T. gesticulans* **L** *T. glossodon* **M** *T. guttigestans* **N** *T. huelphersianum* **O** *T. ingens*. Scale Bar: 5μm.

Taraxacum (and also section *Hamata*), it is nearly unknown. In our study, we aimed to either find variation in ploidy or confirm the prevailing triploid level. Our findings confirmed previously published records of 2n = 3x = 24 for *T. diastematicum* and *T.*

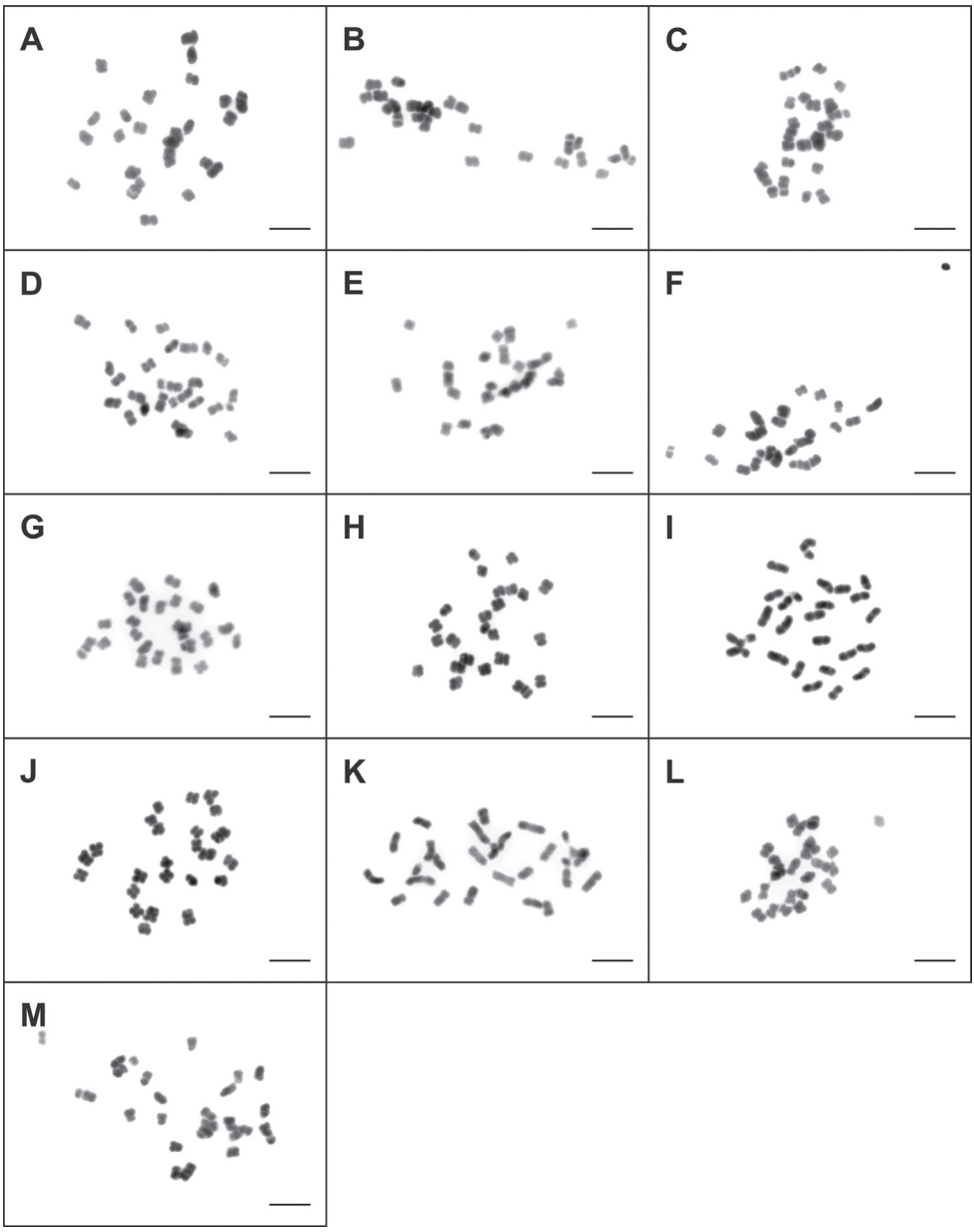

Figure 2. Mitotic metaphase chromosomes of studied triploid species (2n=3x=24) of *Taraxacum* sect. *Taraxacum*. **A** *T. jugiferum* **B** *T. laticordatum* **C** *T. lojoense* **D** *T. lucidifrons* **E** *T. obtusifrons* **F** *T. ochrochlorum* **G** *T. ohlsenii* **H** *T. perdubium* **I** *T. praestabile* **J** *T. sepulcrilobum* **K** *T. sertatum* **L** *T. subhuelphersianum* **M** *T. valens*. Scale Bar: 5μm.

obtusifrons (Uhlemann 2001, Salih et al. 2017); the chromosome numbers for all other 26 species are new findings. The ploidy level measured by flow cytometry was previously documented for 11 species (*T. atrox*, *T. baeckiiforme*, *T. corynodes*, *T. crassum*, *T.*

glossodon, *T. guttigestans*, *T. ingens*, *T. laticordatum*, *T. ohlsenii*, *T. sertatum* and *T. valens*; Trávníček et al. 2010); we now provide exact information on chromosome numbers and genome size estimations.

A tetraploid chromosome number (2n = 4x = 32) was counted for only a few species of the 165 species of *T.* sect. *Taraxacum* with known chromosome numbers in the Chromosome Counts Database (CDDB, version 1.45; Rice et al. 2015). None of the records can be considered fully reliable due to frequent misidentifications of the *Taraxacum* microspecies (lack of identification by specialists). Den Nijs and Sterk (1984) published two chromosome counts, i.e., triploid (2n = 3x = 24) and tetraploid (2n = 4x = 32), for species named as *T. lacistrum* Sahlin, 1982, and collected in France; however, the tetraploid number is listed as a question mark, and this chromosome number must therefore be considered dubious (due to the apomictic behaviour of microspecies, it is implausible to have 2 different ploidy levels for the same species). The chromosome number for a species from the High Atlas, *T. atlantis-majoris* H. Lindberg, 1932 was counted as tetraploid, but the species identification is mentioned as "*T.* cf. *atlantis-majoris*", and misidentification as other species (even from other sections, such as *Piesis*) cannot be excluded (Oberprieler and Vogt 1993). The tetraploid record for *T. albertshoferi* Sahlin, 1984 (Sahlin 1984) cannot be accepted without doubt either, because in the same paper, *T. franconicum* Sahlin, 1984 (which is now considered a synonym of *T. plumbeum* Dahlstedt, 1911) is also described with a tetraploid chromosome number, which was confirmed to be erroneous; the correct one is triploid (e.g., Vašut 2003). The tetraploid record for *T. mediterraneum* Soest, 1954 (Cardona and Contandriopoulos 1983; identified as *T. balearicum* Soest, 1961) does not refer how the taxon was determined. Rousi et al. (1985) published a tetraploid record for *T. penicilliforme* H. Lindberg, 1907 as a member of *T.* sect. *Vulgaria* (= *T.* sect. *Taraxacum*), but this species belongs to *T.* sect. *Borea*. Thus, the only somewhat reliable record of a tetraploid in *Taraxacum* sect. *Taraxacum* is for the alpine species *T. venticola* A. J. Richards, 1972 (Richards 1972).

Our list of species of *T.* sect. *Taraxacum* mainly includes typical members of the section, which differ slightly in their eco-geographic preferences. Some species have (in Central Europe) a preference for wet and sub-oceanic regions (such as *T. corynodes*, *T. chrysophaenum*, *T. lucidifrons* and *T. ochrochlorum*); on the other hand, some occupy more xerothermic regions (e.g., *T. atrox*, *T. baeckiiforme,* and *T. lojoense*). Some species resemble members of *T.* sect. *Celtica* (*T. lucidifrons*) or *T.* sect. *Palustria* (*T. perdubium* and *T. sepulcrilobum*). However, although the species in our study differ somewhat in ecology and geography, there is no variation in their ploidy levels. This is in agreement with previous studies in which only a triploid level was undoubtedly recorded for Nordic ("Atlantic") and Pannonian or Mediterranean species.

Genome size estimates in *Taraxacum* sect. *Taraxacum* are very limited. Only a few papers dealt with its genome size (Bennett et al. 1982, Záveský et al. 2005, Bainard et al. 2011, Iaffaldano et al. 2017), but none of these papers studied known apomictic microspecies; only unknown species of the *T. officinale* group were measured. Gener-

Table 2. Nuclear DNA content of studied *Taraxacum* sect. *Taraxacum* species (Lyc = *Solanum lycopersicon* 'Stupické polní rané'; Gly = *Glycine max* 'Polanka'; n.a. = not analysed, N = number of plants analysed; 1Cx = monoploid genome size, 2C = DNA amount/ploidy level).

Species	2C DNA amount [pg] (mean ± s.d.)	N	Ploidy	1Cx [pg]	Standard
T. aberrans	2.71 ± 0.010	3	3x	0.90	Lyc
T. atroviride	2.70 ± 0.020	2	3x	0.90	Lyc
T. atrox	2.60 ± 0.002	2	3x	0.87	Lyc
T. baeckiiforme	2.62 ± 0	1	3x	0.87	Lyc
T. chrysophaenum	n.a.	n.a.	3x	n.a.	n.a.
T. coartatum	2.72 ± 0.070	2	3x	0.91	Lyc
T. corynodes	2.67 ± 0.001	2	3x	0.89	Lyc
T. crassum	2.62 ± 0.020	2	3x	0.87	Lyc
T. deltoidifrons	2.69 ± 0.007	3	3x	0.90	Lyc
T. diastematicum	2.67 ± 0	1	3x	0.89	Lyc
T. gesticulans	2.83 ± 0.040	2	3x	0.94	Lyc
T. glossodon	2.77 ± 0.010	2	3x	0.92	Lyc
T. guttigestans	2.74 ± 0.004	2	3x	0.91	Lyc
T. huelphersianum	2.79 ± 0.006	2	3x	0.93	Lyc
T. ingens	2.68 ± 0.013	3	3x	0.89	Gly + Lyc
T. jugiferum	2.71 ± 0.001	2	3x	0.90	Lyc
T. laticordatum	2.84 ± 0.008	2	3x	0.95	Lyc
T. lojoense	2.62 ± 0.020	4	3x	0.87	Lyc
T. lucidifrons	2.81 ± 0	1	3x	0.94	Lyc
T. obtusifrons	2.75 ± 0.03	2	3x	0.92	Lyc
T. ochrochlorum	2.67 ± 0	1	3x	0.95	Gly
T. ohlsenii	2.63 ± 0	1	3x	0.88	Lyc
T. perdubium	2.86 ± 0	1	3x	0.95	Lyc
T. praestabile	2.73 ± 0.050	3	3x	0.91	Lyc
T. sepulcrilobum	2.72 ± 0	1	3x	0.91	Lyc
T. sertatum	2.69 ± 0.010	2	3x	0.90	Lyc
T. subhuelphersianum	n.a.	n.a.	3x	n.a.	n.a.
T. valens	2.70 ± 0	1	3x	0.90	Lyc

Table 3. Genome size estimates of *T. officinale* group in literature record. Values with asterisk (*) indicate re-calculated values according to conversion rate of 1 pg ~ 9.78×10^8 bp (Doležel et al. 2003).

Literature	2C [pg]	2C [Gbp]
Bennett et al. 1982	2.55	2.49*
Záveský et al. 2005	1.74–2.70	1.70–2.64*
Vidic et al. 2009	2.56*	2.50
Temsch et al. 2010	2.51	2.45*
Bainard et al. 2011	2.67	2.61*
Iaffaldano et al. 2017	1.65–3.09* (2.45–2.76*)	1.61–3.02 (2.40–2.70)
this study	2.60–2.86	2.54–2.80*

ally, the genome size of the *T. officinale* group varies between 2C = 1.65 pg and 2C = 3.09 pg (Bennett et al. 1982, Záveský et al. 2005, Vidic et al. 2009, Temsch et al. 2010, Bainard et al. 2011, Iaffaldano et al. 2017; summarized in Table 3); values between 2C = 1.65–1.74 pg (Záveský et al. 2005, Iaffaldano et al. 2017) are equal to a diploid ploidy level (i.e., the species *T. linearisquameum*). The genome size of triploid apomicts apparently ranges from 2C = 2.45 pg to 2.76 (3.09) pg (see literature above). Our results are among the highest recorded values. The overall variation in recorded values is approximately 16 % (excluding the highest value of 2C = 3.09 pg, which may represent an aneuploid or tetraploid plant). Such variation can reflect real genome size variation among different species (individuals). Within a single species, *Taraxacum stenocephalum* (*T*. sect. *Piesis*), an ~1.2-fold difference in DNA content is documented (1.194-fold difference for DAPI and 1.219-fold difference for PI; Trávníček et al. 2013). Greater variation in DNA content can be attributed to the sexual reproduction of the species (in contrast to the apomictic reproduction of the species in our study). Even greater variation in DNA content was documented in *Picris hieracioides* Linnaeus, 1753 (Asteraceae, Cichorioideae, Cichorieae); in diploid sexual species, it ranged from 2C = 2.26 to 3.11 pg (1.37-fold difference; Slovák et al. 2009). In other genera of Asteraceae with the occurrence of apomictic taxa, such as *Hieracium* Linnaeus, 1753 and *Pilosella* Hill, 1756 DNA content variation is considerably larger than the known variation in *Taraxacum* sect. *Taraxacum*, i.e., 2.37-fold and 4.3-fold, respectively (Suda et al. 2007, Chrtek et al. 2009).

Genome size estimates vary in all taxa. Multiple factors can affect the measurement of genome size, e.g., differences in instrument settings among the instruments used (Doležel et al. 1998), using inadequate dye (DAPI vs. PI; Doležel et al. 1992), interactions between the dye and other molecules that lead to cytosolic effects (Noirot et al. 2000), and discrepancies in standardization (Doležel and Greilhuber 2010). Applying different laboratory procedures to the same species can lead to up to <10% variation; in the *T. officinale* group, different treatments led to a difference of up to 8.7% (Bainard et al. 2011). Therefore, at least part of the difference among published records can be attributed to a bias due to differences in laboratory procedures. We used a standardized procedure (buffers, tissue treatments, etc.) in our lab; therefore, the observed variation among the species used in this study likely reflects the real variation in DNA content.

Our study provided new data for 26 species of *T*. sect. *Taraxacum*, which confirmed no variation in chromosome number and ploidy level (2n = 3x = 24) and revealed only minor variation in DNA content that roughly equalled a possible methodological bias. The species sampled cover variation within the section: a sample of typical *T*. sect. *Taraxacum* species (most of the studied species) but also species that by morphology or ecology are intermediates of other sections, i.e., *T. perdubium* and *T. sepulcrilobum*, which are morphological and ecological intermediates between the studied section and *T*. sect. *Palustria*; or *T. lucidifrons*, which is morphologically similar to *T*. sect. *Celtica* or species resembling members of *T*. sect. *Borea* (*T. ohlsenii*, *T. lojoense* and *T. atrox*). Two species in our list are apolliniferous (*T. atrox* and *T. subhuelphersianum*). Such unusual homogeneity among species in *T*. sect. *Taraxacum*

rather than great morphological (and ecological) variability might reflect a young evolutionary origin, which is likely in contrast to sections *Palustria*, *Erythrosperma* and others that may partly consist of evolutionarily older species (Wittzell 1999, Majeský et al. 2012, Kirschner et al. 2015). Although there is no evidence for the potential evolutionary scenario in European *Taraxacum* sections, we can speculate that the origin of apomictic species of *T.* sect. *Taraxacum* (*T. officinale* group) may be a result of "recent" hybridization between triploid apomicts and diploid sexuals in the sexual-asexual cycle in a mixed dandelion population, a phenomenon experimentally described in this group (Tas and van Dijk 1999, van Dijk 2003, van Dijk and Vijverberg 2005). In a mixed population (2x and 3x cytotypes; sexual and apomictic types), triploids are results of hybridization between triploid apomicts (diploid pollen) and diploid sexuals (haploid egg cell); however, a rare occurrence of tetraploidy (probably of temporary occurrence) can accelerate the formation of novel triploids (Verduijn et al. 2004b). These tetraploids probably occur in nature as a (rare) product of hybridization in mixed populations (probably discovered in the papers of Sato et al. 2014 or Iaffaldano et al. 2017; Ľ. Majeský, unpublished results) and function as a bridge in the formation of novel stable apomictic microspecies, but probably no such temporary tetraploid hybrids evolved in stable microspecies.

Acknowledgements

This study was supported by the Internal Grant Agency of Palacký University (IGA PrF-2018-001), the European Social Fund, Education for Competitiveness Operational Programme (CZ.1.07/2.2.00/28.0158) and by the National Program of Sustainability I (award LO1204).

References

Asker SE, Jerling L (1992) Apomixis in Plants. CRC Press, Boca Raton, Ann Arbor, London, Tokyo, 298 pp.

Bainard JD, Husband BC, Baldwin SJ, Fazekas A, Gregory TR, Newmaster SG, Kron P (2011) The effects of rapid desiccation on estimates of plant genome size. Chromosome Research 19: 825–842. https://doi.org/10.1007/s10577-011-9232-5

Bennett MD, Smith JB, Lewis Smith RI (1982) DNA amounts of angiosperms from the Antarctic and South Georgia. Environmental and Experimental Botany 22: 307–318. https://doi.org/10.1016/0098-8472(82)90023-5

Cardona MA, Contandriopoulos J (1983) IOPB chromosome number reports LXXIX. Taxon 32(2): 323–324.

Chrtek Jr J, Zahradníček J, Krak K, Fehrer J (2009) Genome size in *Hieracium* subgenus *Hieracium* (Asteraceae) is strongly correlated with major phylogenetic groups. Annals of Botany 104: 161–178. https://doi.org/10.1093/aob/mcp107

den Nijs JCM, Kirschner J, Štěpánek J, van der Hulst A (1990) Distribution of diploid plants of *Taraxacum* sect. *Ruderalia* in East-Central Europe, with special reference to Czechoslovakia. Plant Systematics and Evolution 170: 71–84. https://doi.org/10.1007/BF00937850

den Nijs JCM, Sterk AA (1984) Cytogeography of *Taraxacum* section *Taraxacum* and section *Alpestria* in France and adjacent parts of Italy and Switzerland, including some taxonomic remarks. Acta Botanica Neerlandica 33(1): 1–24. https://doi.org/10.1111/j.1438-8677.1984.tb01769.x

den Nijs JCM, van der Hulst A (1988) Cytogeography of *Taraxacum* section *Erythrosperma*: Diploid sexuals in SE and SW Europe. Botanische Jahrbücher für Systematik 110: 83–93.

Doležel J, Bartoš J (2005) Plant DNA flow cytometry and estimation of nuclear genome size. Annals of Botany 95: 99–110. https://doi.org/10.1093/aob/mci005

Doležel J, Bartoš J, Voglmayr H, Greilhuber J (2003) Nuclear DNA content and genome size of trout and human. Cytometry Part A 51A: 127–128. https://doi.org/10.1002/cyto.a.10013

Doležel J, Greilhuber J (2010) Nuclear genome size: Are we getting closer? Cytometry Part A 77A(7): 635–642. https://doi.org/10.1002/cyto.a.20915

Doležel J, Greilhuber J, Lucretti S, Meister A, Lysák MA, Nardi L, Obermayer R (1998) Plant Genome Size Estimation by Flow Cytometry: Inter-laboratory Comparison. Annals of Botany 82(suppl. 1): 17–26. https://doi.org/10.1093/oxfordjournals.aob.a010312

Doležel J, Greilhuber J, Suda J (2007) Estimation of nuclear DNA content in plants using flow cytometry. Nature Protocols 2: 2233–2244. https://doi.org/10.1038/nprot.2007.310

Doležel J, Sgorbati S, Lucretti S (1992) Comparison of three DNA fluorochromes for flow cytometric estimation of nuclear DNA content in plants. Physiologia Plantarum 85(4): 625–631. https://doi.org/10.1111/j.1399-3054.1992.tb04764.x

Ge XJ, Kirschner J, Štěpánek J (2011) 71. *Taraxacum* Wiggers FH, Prim Fl Holsat 56. 1780. In: Wu ZY, Raven PH, Hong DY (Eds) Flora of China (Vol. 20–21). Science Press (Beijing) and Missouri Botanical Garden Press, St. Louis, 270–325. http://www.efloras.org [Accessed 11. April 2018]

Greilhuber J, Doležel J, Lysák M, Bennett MD (2005) The origin, evolution, and proposed stabilisation of the terms 'genome size' and 'C-value' to describe nuclear DNA contents. Annals of Botany 95: 255–260. https://doi.org/10.1093/aob/mci019

Hasterok R, Maluszynska J (2000) Cytogenetic markers of *Brassica napus* L. chromosomes. Journal of Applied Genetics 41(1): 1–10.

Iaffaldano BJ, Zhang YX, Cardina J, Cornish K (2017) Genome size variation among common dandelion accessions informs their mode of reproduction and suggests the absence of sexual diploids in North America. Plant Systematics and Evolution 303(6): 719–725. https://doi.org/10.1007/s00606-017-1402-2

Kirschner J, Štěpánek J (1984) *Taraxacum* (*Spectabilia*) *nordstedtii* Dahlst. in Central Europe (Studies in *Taraxacum*). Folia Geobotanica et Phytotaxonomica 19(3): 287–297. https://doi.org/10.1007/BF02853093

Kirschner J, Štěpánek J (1994) Clonality as a part of the evolution process in *Taraxacum*. Folia Geobotanica et Phytotaxonomica 29: 265–275. https://doi.org/10.1007/BF02803800

Kirschner J, Štěpánek J (1998a) A revision of *Taraxacum* sect. *Piesis* (Compositae). Folia Geobotanica 33: 391–414. https://doi.org/10.1007/BF02803642

Kirschner J, Štěpánek J (1998b) A Monograph of *Taraxacum* sect. *Palustria*. Průhonice, 281 pp.

Kirschner J, Štepánek J, Greuter W (2007-onwards) *Taraxacum*. In: Greuter W, Raab-Straube E. von (Eds) Compositae, Euro+Med Plantbase – the information resource for Euro-Mediterranean plant diversity. http://www.emplantbase.org/home.html [Accessed 11. April 2018]

Kirschner J, Štěpánek J, Tichý M, Krahulcová A, Kirschnerová L, Pellar L (1994) Variation in *Taraxacum bessarabicum* and allied taxa of the section *Piesis* (*Compositae*): Allozyme diversity, karyotype and breeding behaviour. Folia Geobotanica et Phytotaxonomica 29: 61–83. https://doi.org/10.1007/BF02807777

Kirschner J, Záveská-Drábková L, Štěpánek J, Uhlemann I (2015) Towards a better understanding of the *Taraxacum* evolution (Compositae-Cichorieae) on the basis of nrDNA of sexually reproducing species. Plant Systematics and Evolution 301: 1135–1156. https://doi.org/10.1007/s00606-014-1139-0

Kirschner J, Štěpánek J (2011) Typification of *Leontodon Taraxacum* L. (≡ *Taraxacum officinale* F.H.Wigg.) and the generic name *Taraxacum*: A review and a new typification proposal. Taxon 60: 216–220.

Kula A, Grabowska-Joachimiak A, Kasjaniuk M, Legutko J, Marciniuk P, Musiał K (2013) Chromosome numbers in 10 *Taraxacum* species from Poland. Acta Biologica Cracoviensia series Botanica 55(2): 153–157. https://doi.org/10.2478/abcsb-2013-0030

Leitch IJ, Soltis DE, Soltis PS, Bennett MD (2005) Evolution of DNA amounts across land plants (Embryophyta). Annals of Botany 95: 207–217. https://doi.org/10.1093/aob/mci014

Majeský Ľ, Krahulec F, Vašut RJ (2017) How apomictic taxa are treated in current taxonomy: A review. Taxon 66: 1017–1040. https://doi.org/10.12705/665.3

Majeský Ľ, Vašut RJ, Kitner M, Trávníček B (2012) The pattern of genetic variability in apomictic clones of *Taraxacum officinale* indicates the alternation of asexual and sexual histories of apomicts. PLoS One 7(8): e41868. https://doi.org/10.1371/journal.pone.0041868

Małecka J (1967) Cyto-embryological studies in *Taraxacum scanicum* Dt. Acta Biologica Cracoviensia Series Botanica 10: 195–208.

Małecka J (1969) Further cyto-taxonomic studies in the genus *Taraxacum* section *Erythrosperma* Dt. I. Acta Biologica Cracoviensia Series Botanica 12: 57–72.

Małecka J (1972) Further cyto-taxonomic studies in the genus *Taraxacum* section *Palustria* Dahlstedt. Acta Biologica Cracoviensia Series Botanica 15: 113–126.

Małecka J (1973) Problems of the mode of reproduction in microspecies of *Taraxacum* section *Palustria* Dahlstedt. Acta Biologica Cracoviensia Series Botanica 16: 37–84.

Małecka J (1978) Further cyto-taxonomic studies in the genus *Taraxacum* section *Palustria* Dahlstedt, Part III. Acta Biologica Cracoviensia Series Botanica 21: 109–115.

Marciniuk J, Rerak J, Grabowska-Joachimiak A, Jastrząb I, Musiał K, Joachimiak AJ (2010) Chromosome numbers and stomatal cell length in *Taraxacum* sect. *Palustria* from Poland. Acta Biologica Cracoviensia Series Botanica 52(1): 117–121. https://doi.org/10.2478/v10182-010-0015-7

Marciniuk P, Musiał K, Joachimiak AJ, Marciniuk J, Oklejewicz K, Wolanin M (2012) *Taraxacum zajacii* (Asteraceae), a new species from Poland. Annales Botanici Fennici 49(5): 387–390. https://doi.org/10.5735/085.049.0611

Mártonfiová L (2006) Possible pathways of the gene flow in *Taraxacum* sect. *Ruderalia*. Folia Geobotanica 41(2): 183–201. https://doi.org/10.1007/BF02806478

Mártonfiová L (2015) Hybridization in natural mixed populations of sexual diploid and apomictic triploid dandelions (*Taraxacum* sect. *Taraxacum*): Why are the diploid sexuals not forced out? Folia Geobotanica 50(4): 339–348. https://doi.org/10.1007/s12224-015-9231-y

Mártonfiová L, Majeský Ľ, Mártonfi P (2007) Polyploid progeny from crosses between diploid sexuals and tetraploid apomictic pollen donors in *Taraxacum* sect. *Ruderalia*. Acta Biologica Cracoviensia Series Botanica 49(1): 47–54.

Mártonfiová L, Mártonfi P, Šuvada R (2010) Breeding behavior and its possible consequences for gene flow in *Taraxacum* sect. *Erythrosperma* (H. Lindb.) Dahlst. Plant Species Biology 25(2): 93–102. https://doi.org/10.1111/j.1442-1984.2010.00270.x

Meirmans PG, Calame FG, Bretagnolle F, Felber F, den Nijs JHC (1999) Anthropogenic disturbance and habitat differentiation between sexual diploid and apomictic triploid *Taraxacum* sect. *Ruderalia*. Folia Geobotanica 34(4): 451–469. https://doi.org/10.1007/BF02914922

Mogie M, Richards AJ (1983) Satellite chromosomes, systematics and phylogeny in *Taraxacum* (Asteraceae). Plant Systematics and Evolution 141(3–4): 219–229. https://doi.org/10.1007/BF00989003

Noirot M, Barre P, Louarn J, Duperray C, Hamon S (2000) Nucleus-cytosol interactions – A source of stoichiometric error in flow cytometric estimation of nuclear DNA content in plants. Annals of Botany 86: 309–316. https://doi.org/10.1006/anbo.2000.1187

Oberprieler C, Vogt R (1993) Chromosome numbers of North African Phanerogams II. Willdenowia 23(1/2): 211–238.

Øllgaard H (1983) *Hamata*, a new section of *Taraxacum* (Asteraceae). Plant Systematics and Evolution 141: 199–217. https://doi.org/10.1007/BF00989002

Rice A, Glick L, Abadi S, Einhorn M, Kopelman NM, Salman-Minkov A, Mayzel J, Chay O, Mayrose I (2015) The Chromosome Counts Database (CCDB) – a community resource of plant chromosome numbers. New Phytologist 206(1): 19–26. https://doi.org/10.1111/nph.13191

Richards AJ (1969) Chromosome Number Reports XXIII. Taxon 18(5): 560–562.

Richards AJ (1972) The karyology of some *Taraxacum* species from alpine regions of Europe. Botanical Journal of the Linnean Society 65(1): 47–59. https://doi.org/10.1111/j.1095-8339.1972.tb00923.x

Richards AJ (1973) The origin of *Taraxacum* agamospecies. Botanical Journal of the Linnean Society 66: 189–211. https://doi.org/10.1111/j.1095-8339.1973.tb02169.x

Rousi A, Huttunen H, Hyrkäs-Lyytikäinen K (1985) Chromosomes and reproductive behaviour of Finnish *Taraxacum* agamospecies. Nordic Journal of Botany 5(2): 127–141. https://doi.org/10.1111/j.1756-1051.1985.tb02082.x

Sahlin CI (1984) Zur *Taraxacum*-Flora Bayerns. Berichte der Bayerischen Botanischen Gesellschaft 55: 49–57.

Salih RHM, Majeský Ľ, Schwarzacher T, Gornall R, Heslop-Harrison P (2017) Complete chloroplast genomes from apomictic *Taraxacum* (Asteraceae): Identity and variation between three microspecies. PLoS One 12(2): e0168008. https://doi.org/10.1371/journal.pone.0168008

Sato K, Yamazaki T, Iwatsubo Y (2011) Cytogeography of *Taraxacum albidum* (Asteraceae) in Japan. Cytologia 76(2): 201–212. https://doi.org/10.1508/cytologia.76.201

Sato K, Yamazaki T, Iwatsubo Y (2014) Chromosome Diversity of Japanese *Taraxacum officinale* Weber ex F. H. Wigg. s.l. (Common Dandelion; Asteraceae). Cytologia 79(3): 395–408. https://doi.org/10.1508/cytologia.79.395

Schmid M, Vašut RJ, Oosterveld P (2004) *Taraxacum prunicolor* spec. nova, a new species of the *Taraxacum scanicum* group (sect. *Erythrosperma*). Feddes Repertorium 115: 220–229. https://doi.org/10.1002/fedr.200311038

Siljak-Yakovlev S, Pustahija F, Šolić EM, Bogunić F, Muratović E, Bašić N, Catrice O, Brown SC (2010) Towards a Genome Size and Chromosome Number Database of Balkan Flora: C-Values in 343 Taxa with Novel Values for 242. Advanced Science Letters 3(2): 190–213. https://doi.org/10.1166/asl.2010.1115

Slovák M, Vít P, Urfus T, Suda J (2009) Complex pattern of genome size variation in a polymorphic member of the Asteraceae. Journal of Biogeography 36(2): 372–384. https://doi.org/10.1111/j.1365-2699.2008.02005.x

Šmarda P, Bureš P, Šmerda J, Horová L (2012) Measurements of genomic GC content in plant genomes with flow cytometry: a test for reliability. New Phytologist 193: 513–521. https://doi.org/10.1111/j.1469-8137.2011.03942.x

Soltis DE, Soltis PS, Bennett MD, Leitch IJ (2003) Evolution of genome size in the angiosperms. American Journal of Botany 90: 1596–1603. https://doi.org/10.3732/ajb.90.11.1596

Suda J, Krahulcová A, Trávníček P, Rosenbaumová R, Peckert T, Krahulec F (2007) Genome size variation and species relationships in *Hieracium* subgenus *Pilosella* (Asteraceae) as inferred by flow cytometry. Annals of Botany 100: 1323–1335. https://doi.org/10.1093/aob/mcm218

Tas ICQ, van Dijk PJ (1999) Crosses between sexual and apomictic dandelions (*Taraxacum*). I. The inheritance of apomixis. Heredity 83(6): 707–714. https://doi.org/10.1046/j.1365-2540.1999.00619.x

Temsch EM, Temsch W, Ehrendorfer-Schratt L, Greilhuber J (2010) Heavy metal pollution, selection, and genome size: The species of the Žerjav study revisited with flow cytometry. Journal of Botany 2010: 596542. https://doi.org/10.1155/2010/596542

Trávníček B, Kirschner J, Štěpánek J, Vašut RJ (2010) 23. *Taraxacum* Wigg. In: Štěpánková J (Ed.) Flora of the Czech Republic (Vol. 8). Academia Praha, Praha, 23–269.

Trávníček P, Kirschner J, Chudáčková H, Rooks F, Štěpánek J (2013) Substantial Genome Size Variation in *Taraxacum stenocephalum* (Asteraceae, Lactuceae). Folia Geobotanica 48(2): 271–284. https://doi.org/10.1007/s12224-013-9151-7

Uhlemann I (2001) Distribution of reproductive systems and taxonomical concepts in the genus *Taraxacum* F. H. Wigg. (Asteraceae, Lactuceae) in Germany. Feddes Repertorium 112: 15–35. https://doi.org/10.1002/fedr.20011120105

Uhlemann I (2007) New species of the genus *Taraxacum* (Asteraceae, Cichorieae) from Croatia. Willdenowia 37(1): 115–121. https://doi.org/10.3372/wi.37.37105

Uhlemann I (2010) New species of the genus *Taraxacum* (Asteraceae, Cichorieae) from Croatia II. Willdenowia 40(2): 179–182. https://doi.org/10.3372/wi.40.40203

van Baarlen P, van Dijk PJ, Hoekstra RF, de Jong JH (2000) Meiotic recombination in sexual diploid and apomictic triploid dandelions (*Taraxacum officinale* L.). Genome 43(5): 827–835. https://doi.org/10.1139/g00-047

van Dijk PJ (2003) Ecological and evolutionary opportunities of apomixis: insights from *Taraxacum* and *Chondrilla*. Philosophical Transactions of The Royal Society of London Series B-Biological Sciences 358(1434): 1113–1120. https://doi.org/10.1098/rstb.2003.1302

van Dijk PJ, Vijverberg K (2005) The significance of apomixis in the evolution of the angiosperms: a reappraisal. Regnum Vegetabile 143: 101–116. https://doi.org/10.13140/2.1.4567.9042

Vašut RJ (2003) *Taraxacum* sect. *Erythrosperma* in Moravia (Czech Republic): Taxonomic notes and the distribution of previously described species. Preslia 75(4): 311–338.

Vašut RJ, Štěpánek J, Kirschner J (2005) Two new apomictic *Taraxacum* microspecies of section *Erythrosperma* from Central Europe. Preslia 77: 197–210.

Vašut RJ, Vijverberg K, van Dijk PJ, de Jong H (2014) Fluorescent in situ hybridization shows DIPLOSPOROUS located on one of the NOR chromosomes in apomictic dandelions (*Taraxacum*) in the absence of a large hemizygous chromosomal region. Genome 57(11/12): 609–620. https://doi.org/10.1139/gen-2014-0143

Vašut RJ, Majeský Ľ (2015) *Taraxacum pudicum*, a new apomictic microspecies of *T.* section *Erythrosperma* (Asteraceae) from Central Europe. Phytotaxa 227(3): 243–252. https://doi.org/10.11646/phytotaxa.227.3.3

Verduijn MH, van Dijk PJ, van Damme JM (2004a) Distribution, phenology and demography of sympatric sexual and asexual dandelions (*Taraxacum officinale* s.l.): geographic parthenogenesis on a small scale. Biological Journal of the Linnean Society 82(2): 205–218. https://doi.org/10.1111/j.1095-8312.2004.00325.x

Verduijn MH, van Dijk PJ, van Damme JMM (2004b) The role of tetraploids in the sexual-asexual cycle in dandelions (*Taraxacum*). Heredity 93(4): 390–398. https://doi.org/10.1038/sj.hdy.6800515

Vidic T, Greilhuber J, Vilhar B, Dermastia M (2009) Selective significance of genome size in a plant community with heavy metal pollution. Ecological Applications 19(6): 1515–1521. https://doi.org/10.1890/08-1798.1

Wittzell H (1999) Chloroplast DNA variation and reticulate evolution in sexual and apomictic sections of dandelions. Molecular Ecology 8: 2023–2035. https://doi.org/10.1046/j.1365-294x.1999.00807.x

Wolanin MM, Musiał K (2017) Chromosome numbers in 11 species of *Taraxacum* section *Erythrosperma* Dt. from Poland. Acta Biologica Cracoviensia Series Botanica 59(2): 77–82. https://doi.org/10.1515/abcsb-2017-0008

Záveský L, Jarolímová V, Štěpánek J (2005) Nuclear DNA content variation within the genus *Taraxacum* (Asteraceae). Folia Geobotanica 40(1): 91–104. https://doi.org/10.1007/BF02803047

CompCytogen 12(3): 421–438 (2018)
doi: 10.3897/CompCytogen.v12i3.28736
http://compcytogen.pensoft.net

REVIEW ARTICLE

COMPARATIVE Cytogenetics
A peer-reviewed open-access journal
International Journal of Plant & Animal Cytogenetics, Karyosystematics, and Molecular Systematics

The karyotypes and evolution of ZZ/ZW sex chromosomes in the genus *Characidium* (Characiformes, Crenuchidae)

Marcela Baer Pucci[1], Viviane Nogaroto[2], Luiz Antonio Carlos Bertollo[1], Orlando Moreira-Filho[1], Marcelo Ricardo Vicari[2]

1 *Departamento de Genética e Evolução, Universidade Federal de São Carlos, Rodovia Washington Luís, Km 235, 13565-905, São Carlos, São Paulo State, Brazil* **2** *Departamanento de Biologia Estrutural, Molecular e Genética, Universidade Estadual de Ponta Grossa, Av. Carlos Cavalcanti, 4748, 84030-900, Ponta Grossa, Paraná State, Brazil*

Corresponding author: *Marcelo Ricardo Vicari* (vicarimr@uepg.br)

Academic editor: *I. Kuznetsova* | Received 30 July 2018 | Accepted 7 September 2018 | Published 2 October 2018

http://zoobank.org/D2BC52AE-7F91-49EE-970B-19B80B93A796

Citation: Pucci MB, Nogaroto V, Bertollo LAC, Moreira-Filho O, Vicari MR (2018) The karyotypes and evolution of ZZ/ZW sex chromosomes in the genus *Characidium* (Characiformes, Crenuchidae). Comparative Cytogenetics 12(3): 421–438. https://doi.org/10.3897/CompCytogen.v12i3.28736

Abstract

Available data on cytotaxonomy of the genus *Characidium* Reinhardt, 1867, which contains the greatest number of species in the Characidiinae (Crenuchidae), with 64 species widely distributed throughout the Neotropical region, were summarized and reviewed. Most *Characidium* species have uniform diploid chromosome number (2n) = 50 and karyotype with 32 metacentric (m) and 18 submetacentric (sm) chromosomes. The maintenance of the 2n and karyotypic formula in *Characidium* implies that their genomes did not experience large chromosomal rearrangements during species diversification. In contrast, the internal chromosomal organization shows a dynamic differentiation among their genomes. Available data indicated the role of repeated DNA sequences in the chromosomal constitution of the *Characidium* species, particularly, in sex chromosome differentiation. Karyotypes of the most *Characidium* species exhibit a heteromorphic ZZ/ZW sex chromosome system. The W chromosome is characterized by high rates of repetitive DNA accumulation, including satellite, microsatellite, and transposable elements (TEs), with a varied degree of diversification among species. In the current review, the main *Characidium* cytogenetic data are presented, highlighting the major features of its karyotype and sex chromosome evolution. Despite the conserved karyotypic macrostructure with prevalent 2n = 50 chromosomes in *Characidium*, herein we grouped the main cytogenetic information which led to chromosomal diversification in this Neotropical fish group.

Keywords
Chromosomal differentiation, Cryptic species, Repetitive DNA, Speciation genes

Introduction

Crenuchidae (Teleostei: Characiformes) include 18 genera and 95 species (Eschmeyer et al. 2018), grouped in Crenuchinae and Characidiinae (Buckup 1999). *Characidium* Reinhardt, 1867 is the most species-rich genus of Characidiinae, containing 64 valid species, which are morphologically very similar (Buckup 1993), and broadly distributed across the Neotropical region (Eschmeyer et al. 2018). These fishes are small-sized, reaching 15 cm of length at adulthood (Buckup 1999), and some are commercially used in aquarium hobbies. They usually live in streams and can be found in both lentic and lotic habitats (Buckup 1999). Their elongated body shape and ventrally extended pectoral and pelvic fins enable them to attach tightly to the substrate, allowing them to resist to the water flow and capture food (Aranha et al. 2000). *Characidium* can be classified as autochthonous and insectivorous (Aranha et al. 2000, Bastos et al. 2013, Fernandes et al. 2017) and usually do not exhibit morphological sexual dimorphism (Buckup 1999). *Characidium satoi* Melo & Oyakawa, 2015 is an exception, where males develop a seasonal darker and uniform pigmentation of the body and head vs. the vertical bars exhibited in females (Melo and Oyakawa 2015).

Phylogenetic analysis removed these fishes from the Characidae along with the Crenuchinae, and this group was organized in a new monophyletic family, the Crenuchidae (Buckup 1998). Phylogenetic relationships are available for most taxa in this family (Buckup 1993). According to available molecular and morphological data, *Characidium* is a monophyletic group, and its most recent common ancestor (Crenuchidae) likely originated during the Eocene, approximately 50.2 Mya. The geological events during this period boosted South American ichthyofauna diversity (Poveda-Martínez et al. 2016).

Based on morphological data, *Characidium zebra* Eigenmann, 1909 is the most ancestral species of the genus as well as also of Characidiinae (Buckup 1993). An integrative study using cytogenetic data combined to partial *Cytochrome oxidase C subunit 1* (*COI*) and *Cytochrome B* sequences (*Cyt B*) for molecular phylogenetic analyses was applied in some *Characidium* species (Pansonato-Alves et al. 2014). This analysis proposed *Characidium* into two main groups of species: *i)* those which do not exhibit sex chromosomes heteromorphism; and *ii)* those with a ZZ/ZW sex chromosome heteromorphism with a partial or total heterochromatinization of the W chromosome (Pansonato-Alves et al. 2014). In addition, these data suggested: *i)* that the origin of sex chromosomes in analyzed *Characidium* species was unique and considered an apomorphic state and; *ii)* that B chromosomes present in some *Characidium* species presumably showed independent origins (Pansonato-Alves et al. 2014).

Another common characteristic in cytogenetic data of *Characidium* is the occurrence of cryptic species (Vicari et al. 2008, Machado et al. 2011, Pucci et al. 2014). This is suggested to be due to some populations of the same nominal taxa carrying the

Z and W chromosomes at different stages of differentiation and apparent flow gene isolation (Vicari et al. 2008). Hence, new *Characidium* species are frequently described in the scientific literature (Melo and Oyakawa 2015, Zanata and Camelier 2015, Zanata and Ohara 2015) and, the genus needs a critical revision.

General chromosomal characteristics in Characidium

Table 1 summarizes the recognized *Characidium* individuals/populations with cytogenetic data. The first cytogenetic investigation of this genus was performed by Miyazawa and Galetti (1994), who analyzed four species and some populations of *C.* cf. *zebra*, *Characidium* sp., *Characidium* cf. *lagosantensis* Travassos, 1947 and *Characidium pterostictum* Gomes, 1947, all of which had 2n = 50 chromosomes (Table 1). In fact, phylogenetically basal *C. zebra*, already possesses such chromosomal plesiomorphic features in the genus (2n = 50; 32m + 18sm), including the absence of heteromorphic sex chromosomes (Vicari et al. 2008, Machado et al. 2011, Pazian et al. 2013). This karyotype pattern occurs in most *Characidium* species (Table 1, Fig. 1), although rare spontaneous triploids have been detected among specimens of *Characidium gomesi* Travassos, 1956 (Centofante et al. 2001) and *C.* cf. *zebra* (Pansonato-Alves et al. 2011a). The evolutionary history of this genus revealed no large chromosomal rearrangements (Machado et al. 2011, Pucci et al. 2014, Scacchetti et al. 2015a, 2015b). However, occasional changes in the karyotypic formula can be found due to differences in the autosome morphology (Table 1).

Interstitial telomeric sites (ITS), which are usually correlated with chromosomal fusions, were identified in the karyotypes of *Characidium schubarti* Travassos, 1955, *Characidium lanei* Travassos, 1967, *Characidium lauroi* Travassos, 1949, *Characidium timbuiense* Travassos, 1946, *Characidium serrano* Buckup & Reis, 1997, and two populations of *C. pterostictum* (Scacchetti et al. 2015c). The varied locations of ITS regions in the karyotypes were ascribed to their probable association with satellite DNA through transposition events and ectopic recombinations (Scacchetti et al. 2015c).

Generally, the constitutive heterochromatin has a preferential distribution in the pericentromeric regions in the most *Characidium* chromosomes, but some large interstitial and terminal blocks were also observed. Chromosomal mapping of 18S and 5S rDNAs showed varied autosomal positions among *Characidium* genomes, ranging from single to multiple sites (Table 1). Nucleolar organizing regions (NORs) were probably related to the origin of the ZZ/ZW sex chromosome system that characterizes many *Characidium* species (Table 1), as commented below.

Distribution of repetitive DNAs in the Characidium genome

In fishes, tandem or dispersed repetitive DNA sequences are relevant markers for clarifying karyotype evolution and sex chromosome differentiation (Schemberger et al. 2011, Barbosa et al. 2017, do Nascimento et al. 2018, Glugoski et al. 2018). Their

Table 1. Review of *Characidium* cytogenetic studies until 2018. The variation in the diploid number (2n) is due to the presence of B chromosomes. "Unknown" signifies that the data was not available in the original study. NOR: Nucleolar Organizer Region; M: Metacentric; SM: Submetacentric; ST: Subtelocentric; A: Acrocentric. * The chromosome pairs are not indicated in the original publication.

Species	Localization	2n	Sex chromosome system	Karyotype formula	rDNA 18S	rDNA 5S	References
C. alipioi Travassos, 1955	Ribeirão Grande Stream, SP, Brazil	50	ZZ/ZW	30M+20SM	Pair 16 (NOR)	Unknown	Centofante et al. (2003)
	Ribeirão Grande Stream, SP, Brazil	50–54	ZZ/ZW	32M+18SM	Pair 18	Pair 20	Serrano et al. (2017)
C. fasciatum Reinhardt, 1867	Rio São Francisco, MG, Brazil	50	ZZ/ZW	32M+18SM	Unknown	Unknown	Pazian et al. (2014)
C. cf. *fasciatum*	Rio das Velhas Stream, MG, Brazil	50	ZZ/ZW	Unknown	Unknown	Unknown	Pazian et al. (2013)
C. gomesi Travassos, 1956	Paiol Grande Stream, SP, Brazil	50	ZZ/ZW	♂ 32 M+18 SM ♀ 31 M+19SM	Pair 18	Unknown	Centofante et al. (2001)
C. gomesi (cited like *C.* cf. *fasciatum*)	Paranapanema, SP, Brazil	50–54	ZZ/ZW	32M+18SM	Three autossomic pairs*	Unknown	Maistro et al. (1998)
C. gomesi	Pardo River, SP, Brazil	50–54	ZZ/ZW	32M+18SM	Pair 17 and an additional chromosome (NOR)	Unknown	Maistro et al. (2004), Serrano et al. (2016)
	Machado River, MG, Brazil	50	Absent	32M+18SM	Pair 17 (NOR)	Unknown	da Silva and Maistro (2006)
C. cf. *gomesi*	Quebra Perna Stream, PR, Brazil	50	ZZ/ZW	♂ 32 M+18 SM ♀31M+18SM+1ST	Pairs 4, 7 and 17	One autosomal pair*	Vicari et al. (2008), Pucci et al. (2014),.
	Alambari Stream, SP, Brazil	50	ZZ/ZW	♂ 32 M+18 SM ♀ 31 M+19SM	ZW	Pairs 20 and 25	Machado et al. (2011) Pansonato-Alves et al. (2011b), Pazian et al. (2014)
	Novo River, SP, Brazil	50–54	ZZ/ZW	♂ 32 M+18 SM ♀ 31 M+19SM	Pair 18	Pair 25	Pansonato-Alves et al. (2011b, 2014)
C. gomesi	Verde River, PR, Brazil	50	ZZ/ZW	♂ 32 M+18 SM ♀31+18SM+1ST	Pairs 17, 22 and in one of the homologous of the pairs 1 and 20	Unknown	Machado et al. (2011)
C. cf. *gomesi*	Rio da Cachoeira Stream, GO, Brazil	50	ZZ/ZW	32M+18SM	Unknown	Unknown	Pazian et al. (2013, 2014)
	Magdalena Stream, SP, Brazil	50–52	ZZ/ZW	32M+18SM	Unknown	Unknown	Pazian et al. (2014)
C. gomesi	Grande River, SP, Brazil	50	ZZ/ZW	32M+18SM	Pair 17	Unknown	Machado et al. (2011)
	Minhoca Stream, MG, Brazil	50	ZZ/ZW	32M+18SM	Pair 17	Unknown	Machado et al. (2011)
	Tietê River, SP, Brazil	50	ZZ/ZW	32M+18SM	ZW	Unknown	Pansonato-Alves et al. (2014)
	São Domingos River, MG, Brazil	50	ZZ/ZW	32M+18SM	Pair 17	Unknown	Pansonato-Alves et al. (2014)
	Vermelho River, MT, Brazil	50	ZZ/ZW	32M+18SM	Pair 17	Unknown	Pansonato-Alves et al. (2014)
	São João River, PR, Brazil	50	ZZ/ZW	♂ 32 M+18 SM ♀31M+18SM+1ST	Pairs 10 and 17	Unknown	Pucci et al. (2016)

Species	Localization	2n	Sex chromosome system	Karyotype formula	rDNA 18S	rDNA 5S	References
C. heirmostigmata da Graça & Pavanelli, 2008	Barra Grande River, PR, Brazil	50	ZZ/ZW	32M+18SM	Pair 4	Pair 19	Pucci et al. (2014)
C. lagosantense Travassos, 1947	Amendoim Stream, MG, Brazil	50	Absent	Unknown	Unknown	Unknown	Pazian et al. (2013)
C. cf. *lagosantense*	Infernao Lagoon, SP, Brazil	50	Unknown	32M+18SM	Unknown	Unknown	Miyazawa and Galetti (1994)
C. lanei Travassos, 1967	Barroca River, PR, Brazil	50	ZZ/ZW	32M+16SM+2A	ZW	One autosomal pair*	Noleto et al. (2009)
	Cari Stream, PR, Brazil	50	ZZ/ZW	32M+18SM	ZW (NOR)	One autosomal pair*	Pansonato-Alves et al. (2010), Scacchetti et al. (2015b, c),
C. lauroi Travassos, 1949	Grande River, SP, Brazil	50	ZZ/ZW	♂ 32 M+18 SM ♀31M+18SM+1ST	ZW (NOR)	Unknown	Centofante et al. (2003) Pansonato-Alves et al. (2010), Machado et al. (2011)
C. oiticicai Travassos, 1967	Pairaitinguinha River, SP, Brazil	50–53	ZZ/ZW	32M+18SM	ZW (NOR)	Unknown	Pansonato-Alves et al. (2010, 2014)
C. orientale Buckup & Reis, 1997	Chasqueiro Stream, RS, Brazil	50	ZZ/ZW	32M+18SM	ZW	Pairs 1, 3, 5, 6, 20 and W	Scacchetti et al. (2015a)
C. pterostictum Gomes, 1947	Betari River, SP, Brazil	50–53	ZZ/ZW	32M+16SM+2A	ZW	Unknown	Pansonato-Alves et al. (2010, 2014)
	Faú River, SP, Brazil	50	ZZ/ZW	32M+16SM+2A	ZW	Unknown	Pansonato-Alves et al. (2014)
	Cari River, PR, Brazil	50	ZZ/ZW	32M+16SM+2A	ZW	Unknown	Pansonato-Alves et al. (2014)
	Jacareí River, PR, Brazil	50	ZZ/ZW	32M+16SM+2A	ZW	Unknown	Pansonato-Alves et al. (2014)
	Itapocu River, SC, Brazil	50	ZZ/ZW	32M+16SM+2A	ZW	Unknown	Pansonato-Alves et al. (2014)
	Pairiquera-Açú River, SP, Brazil	50	ZZ/ZW	32M+16SM+2A	ZW	Pairs 9, 11 and 13	Pucci et al. (2014)
	Jacuí River, RS, Brazil	50	ZZ/ZW	32M+16SM+2A	ZW	Three autosomal pairs*	Scacchetti et al. (2015b)
	Itapeva Lagoon, RS, Brazil	50	ZZ/ZW	32M+16SM+2A	Unknown	Unknown	Scacchetti et al. (2015c)
	Carlos Botelho Ecological Station, SP, Brazil	50	Unknown	32M+16SM+2ST	Unknown	Unknown	Miyazawa and Galetti (1994)
C. rachovii Regan, 1913	Cabeças Stream, RS, Brazil	50	ZZ/ZW	32M+18SM	ZW	Pairs 1, 3 ,5, 17, 20 and W	Scacchetti et al. (2015a)
C. schubarti Travassos, 1955	Cinco Réis River, PR, Brazil	50	ZZ/ZW	32M+18SM	ZW (NOR)	Unknown	Pansonato-Alves et al. (2010), Scacchetti et al. (2015c)
C. serrano Buckup & Reis, 1997	Canoinha Stream, RJ, Brazil	50	ZZ/ZW	32M+16SM+2A	Unknown	Unknown	Scacchetti et al. (2015c)
C. stigmosum Melo & Buckup, 2002	Ave Maria River, GO, Brazil	50	Absent	32M+18SM	Pair 23	Pairs 1, 7 and 17	Scacchetti et al. (2015a)
C. tenue (Cope, 1894)	Chuí Stream, SC, Brazil	50	Absent	32M+18SM	Pair 23	Pairs 1 and 7	Scacchetti et al. (2015a)
C. timbuiense Travassos, 1946	Valsugana Velha Stream, ES, Brazil	50	ZZ/ZW	32M+16SM+2A	ZW	Three autosomal pairs*	Scacchetti et al. (2015b)

Species	Localization	2n	Sex chromosome system	Karyotype formula	rDNA 18S	rDNA 5S	References
C. vestigipinne Buckup & Hahn, 2000	Caraguatá River, RS, Brazil	50	ZZ/ZW	32M+18SM	ZW	Pairs 1, 17 and 20	Scacchetti et al. (2015a)
C. vidali Travassos, 1967	Bananeiras Stream, RJ, Brazil	50	ZZ/ZW	32M+18SM	One autosomal pair*	W chromosome and in one autosomal pair*	Scacchetti et al. (2015b, c)
C. aff. *vidali*	Bananeiras Stream, RJ, Brazil	50–54	ZZ/ZW	32M+18SM	Pair 21	Pairs 5, 12 and 20	Scacchetti et al. (2015a)
C. xavante da Graça, Pavanelli & Buckup, 2008	Xingu River, MT, Brazil	50	Absent	32M+18SM	Pair 23	Pairs 1, 7 and 17	Scacchetti et al. (2015a)
C. zebra Eigenmann, 1909	Jatai Reservoir, SP, Brazil	50	Unknown	32M+18SM	Pair 25 (NOR), with 1 to 2 additional pairs	Unknown	Miyazawa and Galetti (1994)
C. cf. *zebra*	Passa Cinco River, SP, Brazil	50	Unknown	32M+18SM	Pair 23	Pair 17	Miyazawa and Galetti (1994) Machado et al. (2011), Pucci et al. (2014)
	Passa Cinco River, SP, Brazil	50–51	Unknown	Unknown	Unknown	Unknown	Venere et al. (1999)
	Piracicaba River, SP, Brazil	50	Unknown	32M+18SM	Pair 25 (NOR)	Unknown	Miyazawa and Galetti (1994)
	Ribeirão Claro Stream, SP, Brazil	50	Absent	Unknown	Unknown	Unknown	Pazian et al. (2013)
	Pairaitinga River, SP, Brazil	50	Absent	32M+18SM	Pair 23	Pairs 1, 6, and 17	Pansonato-Alves et al. (2010, 2011a), Scacchetti et al. (2015b, 2015c)
	Paiol Grande Stream, SP, Brazil	50	Absent	32M+18SM	Pair 23 (NOR)	Unknown	Centofante et al. (2001), Pucci et al. (2016)
	Machado River, MG, Brazil	50	Absent	32M+18SM	Pair 23 (NOR)	Unknown	da Silva and Maistro (2006)
	Alambari River, SP, Brazil	50	Absent	32M+18SM	Pair 23	Pair 17	Pansonato-Alves et al. (2011a)
	Novo River, SP, Brazil	50	Absent	32M+18SM	Pair 23	Pair 17	Pansonato-Alves et al. (2011a)
	Araquá River, SP, Brazil	50	Absent	32M+18SM	Pair 23	Pair 17	Pansonato-Alves et al. (2011a)
	Duas Antas Stream, MT, Brazil	50	Absent	32M+18SM	Pair 23	Pairs 1 and 17	Scacchetti et al. (2015a)
	Juba River, MT, Brazil	50	Absent	32M+18SM	Pair 23	Pairs 1, 6, 9, 17 and 18	Pansonato-Alves et al. (2011a)
C. aff. *zebra*	Corredeira Stream, SP, Brazil	50	Absent	32M+18SM	Pairs 4, 7 and 23	Pair 17	Pucci et al. (2014)
	Corredeira Stream, SP, Brazil	50	Absent	32M+18SM	Pairs 2, 4, 7, 20, 23 and 17	Pair 17	Pucci et al. (2014)
Characidium sp.	Preto River, SP, Brazil	50	ZZ/ZW	32M+18SM	ZW (NOR)	Unknown	Pansonato-Alves et al. (2010)
	Lagoon of the Corredeira Stream, SP, Brazil	50	ZZ/ZW	32M+16SM+2A	ZW	Pairs 3, 7, 8, 23 and 24	Pucci et al. (2014)
Characidium sp.2	Vermelho River, MT, Brazil	50	ZZ/ZW	32M+18SM	W and pair 7	Pair 17	Scacchetti et al. (2015a)
Characidium sp.	Formoso River, GO, Brazil	50	ZZ/ZW	32M+18SM	Unknown	Unknown	Pazian et al. (2013, 2014)
	Inferno Lagoon, SP, Brazil	50	Unknown	32M+18SM	Unknown	Unknown	Miyazawa and Galetti (1994)

Species	Localization	2n	Sex chromosome system	Karyotype formula	rDNA 18S	rDNA 5S	References
Characidium sp.1	Russo River, MT, Brazil	50	ZZ/ZW	32M+18SM	Pair 7	Pair 17	Scacchetti et al. (2015a)
Characidium sp.3	Arinos River, MT, Brazil	50	ZZ/ZW	32M+18SM	Pair 1	Pair 1	Scacchetti et al. (2015a)
Characidium sp.4	Nanay River, Peru	50	ZZ/ZW	32M+18SM	Pair 7	Pair 18	Scacchetti et al. (2015a)
Characidium sp.5	Canoinha Stream, RS, Brazil	50	ZZ/ZW	32M+18SM	Pair 19	Pairs 1, 5 and 6	Scacchetti et al. (2015a)

accumulation is a key factor for the morphogenesis and the differentiation process of sex chromosomes, and the induction of gene erosion (Matsunaga 2009, Schemberger et al. 2014, Ziemniczak et al. 2014).

Despite the highly conserved karyotype structure, the genomes of *Characidium* species display a dynamic pattern of their internal chromosomal composition (Table 1, Fig. 2). Phylogenetics studies using mitochondrial DNA in *Characidium* were used to anchor a comparative cytogenetic analysis using telomeric DNA probe. This data indicated that the ITS signals found in genomes of some *Characidium* species (Fig. 2a) do not have relation with chromosome fusions but, on contrary, are associated with repetitive DNAs dispersion (Scacchetti et al. 2015c). Probably the ITS have origin in the evolutionary lineage of the genus in related hydrographic drainages (Scacchetti et al. 2015c), although some relationship species, such as *C. zebra* and *C. gomesi*, do not harbor such sequences. U2 small nuclear RNA (*snRNA U2*) had a highly conserved distribution in the first m pair in the most species (Fig. 2b), except for *Characidium* sp. aff. *Characidium vidali* Travassos, 1967, *Characidium* sp. 1 and *Characidium alipioi* Travassos, 1955, in which *snRNA* U2 site was located in the first submetacentric (sm) pair (Scacchetti et al. 2015b, Serrano et al. 2017).

Distinct microsatellites also had a wide distribution in autosomal pairs (Fig. 2c), probably due to their association with TEs (Scacchetti et al. 2015b, Pucci et al. 2016), such as Tc1-Mariner (Fig. 2d). This pattern was also corroborated by Serrano et al. (2017), evidencing $(CA)_{15}$ and $(GA)_{15}$ autosomal accumulation in the *C. alipioi* genome, as well as of several other microsatellites in *C. zebra* and *C. gomesi*. The molecular characterization and chromosome mapping of the histone genes H1, H3 and H4 were described for *C. zebra* and *C. gomesi* (Pucci et al. 2018). These three histone sequences appear to be associated with TEs and, *in situ* localization, revealed that they are dispersed throughout the autosomes, but they are not involved in the differentiation of the specific region of the W sex chromosome in *C. gomesi* (Pucci et al. 2018).

The available data point to the substantial role of repeated DNA sequences in the chromosomal constitution of *Characidium* species. However, due to the extension of the existing repetitive elements, additional investigations must address their significance in the evolutionary history of *Characidium* and, particularly, in sex chromosome differentiation.

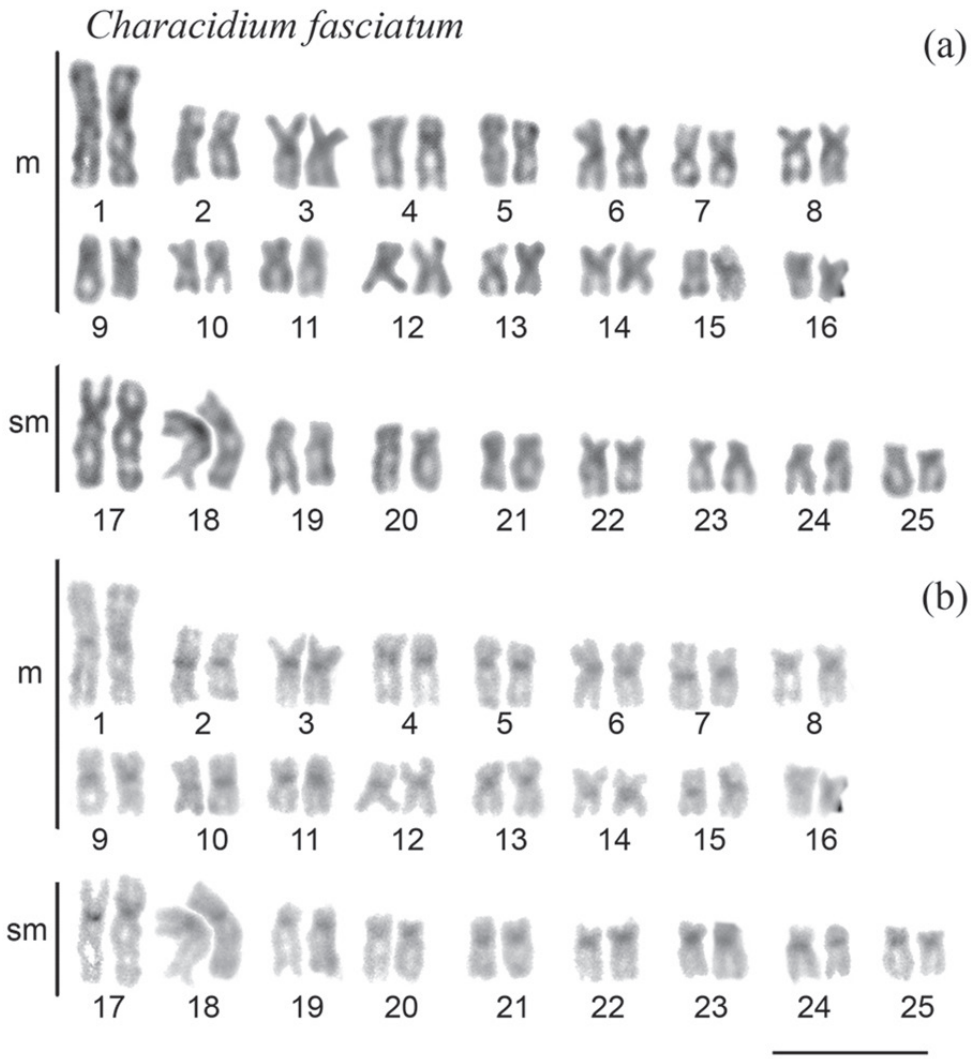

Figure 1. Representative karyotype of *Characidium fasciatum* with 2n = 50 chromosomes. Cytogenetic data revealed 32 m + 18 sm, without heteromorphic sex chromosomes: **a** conventionally Giemsa-stained **b** sequentially C-banded chromosomes. Scale bar: 5 μm.

Supernumerary and sex chromosomes in Characidium

Several Neotropical fish species are carriers of supernumerary or B chromosomes (Carvalho et al. 2008). Additionally, due to the variety of simple or multiple sex chromosome systems in these fishes, differentiated karyotypes exist between sexes (Moreira-Filho et al. 1993, Almeida-Toledo et al. 2001).

B chromosomes, ranging from one to four chromosomes, were described in several *Characidium* species (Table 1). They are hypothesized to have different and independ-

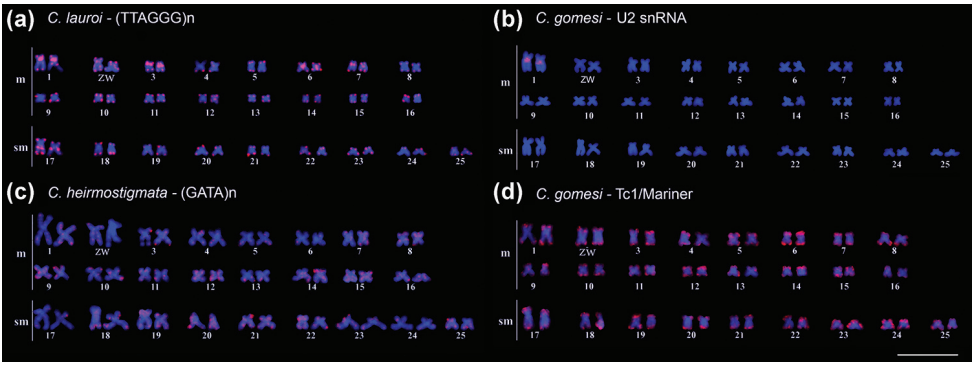

Figure 2. Fluorescence *in situ* hybridization using distinct classes of repeated DNA sequences as probes: In **a** karyotype of *C. lauroi* submitted to $(TTAGGG)_n$ probing (red) **b** karyotype of *C. gomesi* evidencing U2 snRNA sites (red) **c** Karytype of *C. heirmostigmata* submitted to $(GATA)_n$ probing (red) and **d** karyotype of *C. gomesi* evidencing Tc1/Mariner mapping (red). Scale bar: 10 μm.

ent origins in evolutionary history of the species. To explain the origin, frequency and evolution of B chromosomes it was hypothesized that these elements are derivate from autosomes followed by gene silencing, heterochromatinization, and accumulation of repetitive DNA and transposons (Camacho et al. 2000, Vicari et al. 2011). In some species, B chromosomes are related to sex chromosomes due to share the same repetitive elements (Scacchetti et al. 2015a). In fact, genomes of *C. gomesi*, *C. pterostictum* and *Characidium* sp. aff. *C. vidali* displayed similar repetitive DNA sequences among B and sex chromosomes (Pansonato-Alves et al. 2014, Pazian et al. 2014, Scacchetti et al. 2015a, Serrano et al. 2016), while *Characidium oiticicai* Travassos, 1967 and *C. alipioi* did not show such shared sequences (Pansonato-Alves et al. 2014, Serrano et al. 2017, respectively). Despite their molecular homology, it was demonstrated that B and W chromosomes do not form multivalent pairings during meiosis in male and female *C. gomesi* individuals.

Meiotic analyses revealed the bivalent pairing of the ZW chromosomes, as well as the bivalent plus one univalent formation in specimens carrying three B chromosomes (Serrano et al. 2016). Chromosome pairing does not always indicate complete homology between chromosomes (Ramsey and Schemske 2002). In fact, the Z and W sex chromosomes in *Characidium* species possesses differences in 45S rDNA chromosomal localization and in heterochromatin blocks extension (Fig. 3). Chromosomal localization differences of the repetitive sequences among *Characidium* species are also observed, such as in $(TTA)_{10}$, $(GAG)_{10}$, $(CG)_{15}$ and $(GATA)_n$ sequences (Scacchetti et al. 2015b, Pucci et al. 2016). In *C. gomesi* it was shown that the short arm of the W chromosome keeps homology with the terminal region of the Z chromosome in relation to the $(CG)_{15}$, $(GATA)_n$, and $(TAA)_{10}$ sequences (Pucci et al. 2016). $(GATA)_n$ and $(TAA)_{10}$ homology is also present in the centromeric region of the *C. gomesi* (Pucci et al. 2016). These data help to explain ZW chromosome pairing and its bivalent formation in *Characidium* species.

The occurrence of a ZZ/ZW sex chromosome system is another karyotypic characteristic of *Characidium* genomes. It was first described by Maistro et al. (1998) in *Characidium* cf. *fasciatum* Reinhardt, 1867 (Table 1), but it is also present in most *Characidium* species studied. The sex chromosomes in *Characidium* show a high degree of differentiation among species by chromosomal size, morphology, heterochromatin accumulation and presence or absence of rDNA sites (Maistro et al. 1998, 2004, Centofante et al. 2001, 2003, Vicari et al. 2008, Noleto et al. 2009, Pansonato-Alves et al. 2010, 2011b, 2014, Machado et al. 2011, Pazian et al. 2013, 2014, Pucci et al. 2014, 2016, Scacchetti et al. 2015a, 2015b, 2015c, Serrano et al. 2017), as exemplified in Fig. 3. Interestingly, the W chromosome can possess distinct cytotypes among *C. gomesi* populations, such as sm (Centofante et al. 2001, Pansonato-Alves et al. 2011b) or subtelocentric (Vicari et al. 2008, Pucci et al. 2014, 2016).

The majority of microsatellites sites were located in the terminal region of the Z chromosome and in the terminal/centromeric regions of W chromosome. The exception is $(TTA)_{10}$, which was widely distributed throughout the whole W chromosome, and $(GAG)_{10}$, which had a preferential accumulation in the W and B chromosomes of *C. alipioi* (Scacchetti et al. 2015b). $(CG)_{15}$ and $(GATA)_n$ sequences were mainly found on the short arm of W chromosome in genomes of *C. zebra* and *C. gomesi*. It was suggested that these regions are enriched with sex-specific genes (Pucci et al. 2016), since the $(GATA)_n$ sequences are known as a motif for sex- and tissue-specific GATA-binding proteins. However, this pattern was not found in *Characidium heirmostigmata* da Graça & Pavanelli, 2008 (Fig. 2).

18S rDNA sequences are also particular components of many *Characidium* sex chromosomes, occupying the short and the long arms of Z and W chromosomes, respectively, or the long arms of both sex chromosomes (Table 1, Fig. 3). These ribosomal sequences were likely associated with the origin of the protosex chromosome. It is likely that the NORs of the sm pair 23 (an ancestral pattern) were translocated to opposite arms of the second metacentric (m) pair (Machado et al. 2011, Pucci et al. 2014).

Later differentiations in such protosex chromosomes were gradually acquired by isolated populations, leading to deletions and duplications in the rearranged regions due to meiotic pairing failures. Thus, recombination suppression mechanisms (rearrangements, heterochromatinization, repeated DNA accumulation and gene erosion) were naturally selected, giving rise to distinct heteromorphic W chromosomes (Machado et al. 2011, Pucci et al. 2014). Such modifications also promoted the accumulation of the so-called "speciation genes", particularly in linked Z chromosome loci (Pucci et al. 2014). These genes established meiotic barriers and post-zygotic isolation mechanisms, along with the morphological variations of W chromosome (Fig. 4).

The current sympatric occurrence of some *Characidium* species does not display hybridization events among them. Sympatric and syntopic pairs of *Characidium* species, with the presence or absence of sex chromosomes, had already been described, namely *C. alipioi* and *Characidium* sp. cf. *C. lauroi* (Centofante et al. 2003), and *C.* cf. *zebra* and *C. gomesi* (da Silva and Maistro 2006). Thus, it is likely that NOR displacements throughout the genome was a key factor linked to W chromosome differ-

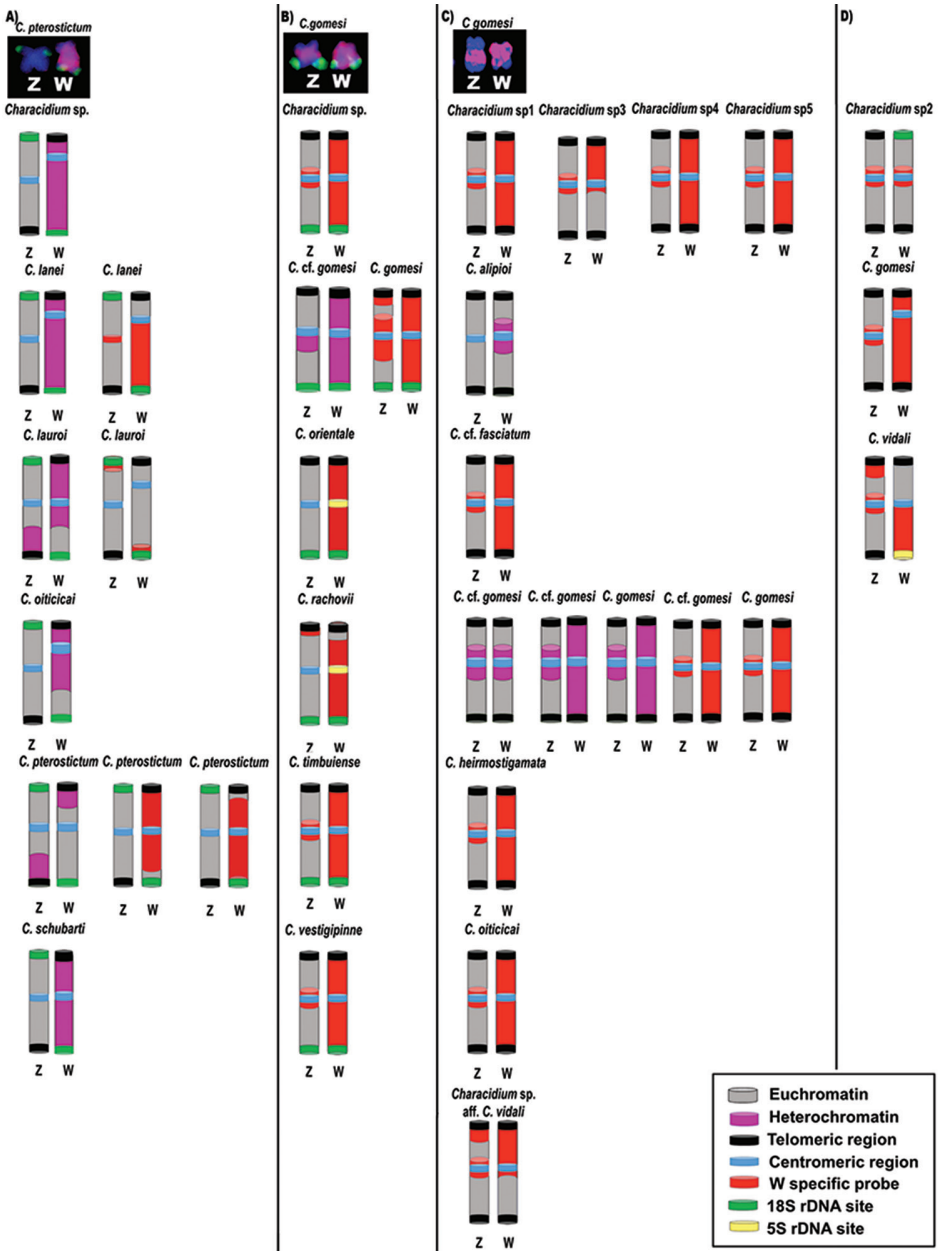

Figure 3. Idiograms showing main characteristics already identified for the ZZ/ZW sex chromosome system in *Characidium* species. It was highlighted the position of the centromere, distribution of euchromatin and heterochromatin, W-specific probes, and rDNA sites. The **a** column detaches the species carrying 18S rDNA sites on the short and long arms of the Z and W chromosomes, respectively; the **b** column highlights the species bearing 18S rDNA sites on the long arms of both Z and W chromosomes; the **c** column shows the species that do not present 18S rDNA sequences on either Z or W chromosomes; the **d** column presents the species bearing Z and W chromosomes with unusual characteristics, including morphology, 18S and 5S rDNA sites, and W-specific probe distribution.

entiation in Crenuchidae. Usually, when the W chromosome is partially heterochromatic, it is still a NOR bearing chromosome; but in totally heterochromatic chromosomes, NORs are found in different autosomes (Table 1, Fig. 3). Restriction-site associated DNA sequencing (RAD-seq) was applied to study the sex chromosomes of *C. gomesi* (Utsonomia et al. 2017). This application identifies 26 female-specific RAD loci, putatively located on the W chromosome, as well as 148 sex-associated SNPs showing significant differentiation. The use of W markers validated for *in situ* localization in other populations and species of the genus *Characidium* suggested a rapid turnover of W-specific repetitive elements (Utsonomia et al. 2017). This finding corroborates the inference that modifications on sex chromosomes also promote the accumulation of the "speciation genes", leading to chromosomal speciation mechanisms in *Characidium*.

Perspectives on Characidium investigations

Fish cytogenetic and molecular studies have improved over the last few years, especially with regard to better identification of the karyotypic evolution and sex chromosome differentiation among different groups of fish, as well as genes or specific regions related to sex determination. W-specific repetitive probes were already constructed for *Characidium* using microdissection from female metaphase chromosomes and degenerate oligonucleotide-primed PCR (DOP-PCR) or whole genome amplification (WGA) protocols. These probes were later applied to chromosome painting in *Characidium* using a *C. gomesi* W-specific probe (Machado et al. 2011, Pazian et al. 2013, 2014, Pansonato-Alves et al. 2014, Pucci et al. 2014). This was followed by investigations of homologous regions between the sex pairs, B chromosomes and autosomes (Machado et al. 2011, Pazian et al. 2013, 2014, Pansonato-Alves et al. 2014, Pucci et al. 2014, 2016, Scacchetti et al. 2015a, 2015b, Serrano et al. 2016, 2017), and the cloning of a W-specific sequence that generated the CgW9 clone, which is similar to the zebrafish *Helitron* transposon (Pazian et al. 2014).

The ZZ/ZW sex chromosome system is well-known and described. The repeated DNA classes related to gene erosion and differentiation of W chromosome, as well as regions or genes implicated in sex determination and gonadal differentiation, have not yet been properly investigated in most species. It has been demonstrated that the repeated DNA sequences are closely related to the regulatory genes network, particularly TEs, in a process called molecular co-option or exaptation (Feschotte 2008). In this sense, future studies concerning the dynamics of mobile elements and molecular co-option in the regulatory system of *Characidium* will be relevant contributions to this research area. Sequencing and comparisons between male and female genomes of different *Characidium* species will contribute to highlighting the genic and/or repetitive sequences that are sex-restricted.

In other pathways, sequencing procedures of particular W fractions is needed for investigating specific genes related to sex determination and differentiation. Indeed, integrating cytogenetic, genomic, molecular, and bioinformatic tools will be essential for a better understanding of sex determination and differentiation processes in fishes, with applications in ecological and evolutionary studies.

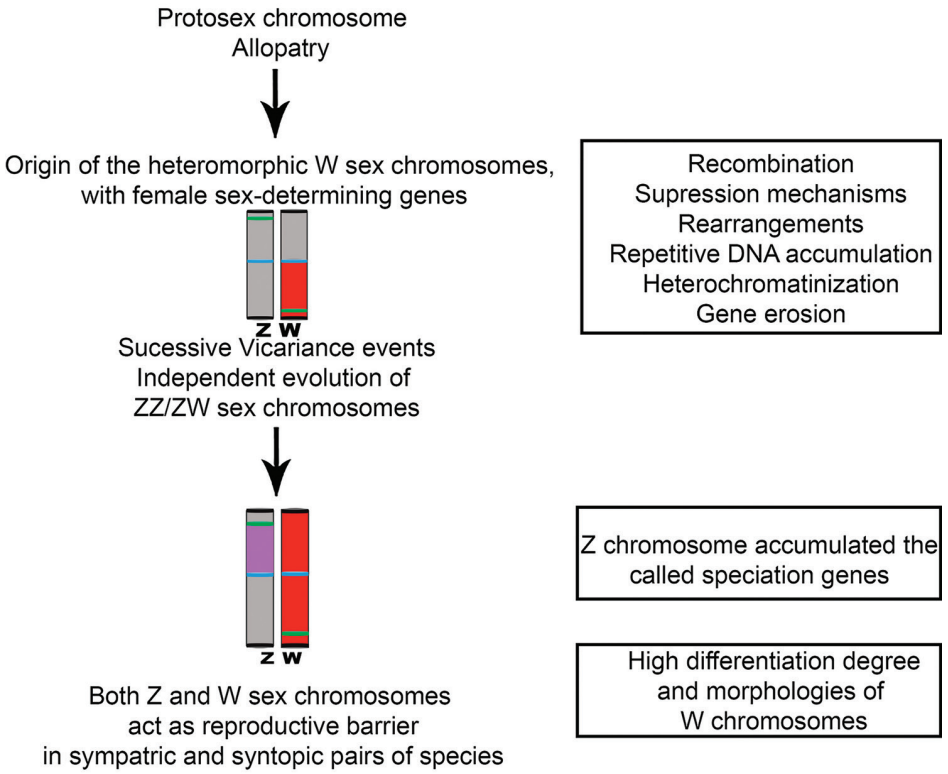

Figure 4. Schematic idiograms showing some steps proposed in the differentiation process of the ZZ/ZW sex pair. The origin of the ZZ/ZW sex pair from the protosex chromosome of the *Characidium* species. Centromeric region (blue); 18S rDNA site (green); W specific probe region (red); probable Z speciation genes region (purple).

Conclusion

Chromosomal diversification in *Characidium* here revised show a diversified karyotype microstructure despite its conserved karyotypic macrostructure with prevalent 2n of 50 chromosomes arranged in 32 m + 18 sm. Differences in the number of rDNA sites, in heterochromatin blocks, in B chromosomes number and, in sex chromosomes sizes, as well as an interesting dynamic of repetitive DNAs on the chromosomes are observed among species, leading to chromosomal diversification and speciation. The data showed that different microsatellite expansions are involved in the sex chromosome differentiation in *Characidium*. In addition, the microsatellite $(TTA)_{10}$ play an important role in gene degeneration and erosion on the W chromosome in some *Characidium* species. These data are important for the molecular characterization of the W and B chromosomes, to karyotype structures determination and comprehension of cryptic species. Future studies integrating cytogenetic, genomic and molecular data open perspectives to understand the sex determination, B chromosome composition and, "speciation genes" in *Characidium* genomes.

Acknowledgements

The authors are grateful to Instituto Chico Mendes de Conservação da Biodiversidade (protocol number SISBIO 15117) for authorizing the capture of specimens. This study was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), Secretaria de Ciência e Tecnologia do Estado do Paraná (SETI), and Fundação Araucária de Apoio ao Desenvolvimento Científico e Tecnológico do Estado do Paraná (Fundação Araucária).

References

Almeida-Toledo LF, Foresti F, Péquignot EV, Daniel-Silva MFZ (2001) XX:XY sex chromosome system with X heterochromatinization: an early stage of sex chromosome differentiation in the Neotropic electric eel *Eigenmannia virescens*. Cytogenetics and Cell Genetics 95: 73–78. https://doi.org/10.1159/000057020

Aranha JMR, Gomes JHC, Fogaça FNO (2000) Feeding of two sympatric species of *Characidium*, *C. lanei* and *C. pterostictum* (Characidiinae) in a coastal stream of Atlantic Forest (Southern Brazil). Brazilian Archives of Biology and Technology 43: 527–531. http://dx.doi.org/10.1590/S1516-89132000000500013

Barbosa P, Pucci MB, Nogaroto V, Almeida MC, Artoni RF, Vicari MR (2017) Karyotype analysis of three species of *Corydoras* (Siluriformes: Callichthyidae) from southern Brazil: rearranged karyotypes and cytotaxonomy. Neotropical Ichthyology 15: e160056. http://dx.doi.org/10.1590/1982-0224-20160056

Bastos RF, Miranda SF, Garcia AM (2013) Dieta e estratégia alimentar de *Characidium rachovii* (Characiformes, Crenuchidae) em riachos de planície costeira do sul do Brazil. Iheringia 103: 335–341. http://dx.doi.org/10.1590/S0073-47212013000400001

Buckup PA (1993) Phylogenetic interraletionships and reductive evolution in neotropical characidiin fishes (Characiformes, Ostariophysi). Cladistics 9: 305–341. https://doi.org/10.1111/j.1096-0031.1993.tb00227.x

Buckup PA (1998) Relationships of the Characidiinae and phylogeny of characiform fishes (Teleostei: Ostariophysi). In: Malabarba LR, Reis RE, Vari RP, et al. (Eds) Phylogeny and classification of neotropical fishes. Edipucrs, Porto Alegre, 123–144.

Buckup PA (1999) Ecologia de peixe de riachos. Série Oecologia Brasiliensis, vol. VI. PPGE-UFRJ. In: Caramaschi EP, Mazzoni R, Peres Neto PR (Eds) Chapter 3, Sistemática e Biogeografia de Peixes de Riachos. Rio de Janeiro, 91–138.

Camacho JP, Sharbel TF, Beukeboom LW (2000) B-chromosome evolution. Philosophical Transactions of the Royal Society B: Biological Sciences 355: 163–178. https://doi.org/10.1098/rstb.2000.0556

Carvalho PA, Martins-Santos IC, Dias AL (2008) B-chromosomes: an update about its occurrence in freshwaters Neotropical fishes (Teleostei). Journal of Fish Biology 72: 1907–1932. https://doi.org/10.1111/j.1095-8649.2008.01835.x

Centofante L, Bertollo LAC, Buckup PA, Moreira-Filho O (2003) Chromosomal divergence and maintenance of sympatric *Characidium* fish species (Crenuchidae, Characidiinae). Hereditas 138: 213–218. https://doi.org/10.1034/j.1601-5223.2003.01714.x

Centofante L, Bertollo LAC, Moreira-Filho O (2001) Comparative cytogenetics among sympatric species of *Characidium* (Pisces, Characiformes). Diversity analysis with the description of a ZW sex chromosome system and natural triploidy. Caryologia 54: 253–260. https://doi.org/10.1080/00087114.2001.10589233

da Silva AR, Maistro EL (2006) Cytogenetic divergence between two sympatric species of *Characidium* (Teleostei, Characiformes, Crenuchidae) from the Machado River, Minas Gerais, Brazil. Genetics and Molecular Biology 29: 459–463. http://dx.doi.org/10.1590/S1415-47572006000300010

do Nascimento VD, Coelho KA, Nogaroto V, Almeida RB, Ziemniczak K, Centofante L, Pavanelli CS, Torres RA, Moreira-Filho O, Vicari MR (2018) Do multiple karyomorphs and population genetics of freshwater darter characines (*Apareiodon affinis*) indicate chromosomal speciation? Zoologischer Anzeiger 272: 93–103. https://doi.org/10.1016/j.jcz.2017.12.006

Eschmeyer WN, Fricke R, van der Laan R (Eds) (2018) Catalog of fishes: genera, species, references [Internet]. http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp [updated 2018 Apr 30, cited 2018 May 21]

Fernandes S, Leitão RF, Dary EP, Guerreiro AIC, Zuanon J, Bührnheim CM (2017) Diet of two syntopic species of Crenuchidae (Ostariophysi: Characiformes) in an Amazonian rocky stream. Biota Neotropica 17(1): e20160281. http://dx.doi.org/10.1590/1676-0611-bn-2016-0281

Feschotte C (2008) Transposable elements and the evolution of regulatory networks. Nature Reviews Genetics 9: 397–405. http://doi.org/10.1038/nrg2337

Glugoski L, Giuliano-Caetano L, Moreira-Filho O, Vicari MR, Nogaroto V (2018) Co-located hAT transposable element and 5S rDNA in an interstitial telomeric sequence suggest the formation of Robertsonian fusion in armored catfish. Gene 650: 49–50. https://doi.org/10.1016/j.gene.2018.01.099

Machado TC, Pansonato-Alves JC, Pucci MB, Nogaroto V, Almeida MC, Oliveira C, Foresti F, Bertollo LAC, Moreira-Filho O, Vicari MR (2011) Chromosomal painting and ZW sex chromosomes differentiation in *Characidium* (Characiformes, Crenuchidae). BMC Genetics 12: 65. https://doi.org/10.1186/1471-2156-12-65

Maistro EL, de Jesus CM, Oliveira C, Moreira-Filho O, Foresti F (2004) Cytogenetic analysis of A-, B-chromosomes and ZZ/ZW sex chromosomes of *Characidium gomesi* (Teleostei, Characiformes, Crenuchidae). Cytologia 69: 181–186. https://doi.org/10.1508/cytologia.69.181

Maistro EL, Mata EP, Oliveira C, Foresti F (1998) Unusual occurrence of a ZZ/ZW sex-chromosome system and supernumerary chromosomes in *Characidium* cf. *fasciatum* (Pisces, Characiformes, Characidiinae). Genetica 104: 1–7. https://doi.org/10.1023/A:1003242020259

Matsunaga S (2009) Junk DNA promotes sex chromosome evolution. Heredity 102: 525–526. https://doi.org/10.1038/hdy.2009.36

Melo MRS, Oyakawa OT (2015) A new species of *Characidium* Reinhardt (Characiformes, Crenuchidae) with a distinctively dimorphic male. Copeia 103: 281–289. https://doi.org/10.1643/CI-14-073

Miyazawa CS, Galetti Jr PM (1994) First cytogenetical studies in *Characidium* species (Pisces: Characiformes, Characidiinae). Cytologia 59: 73–79. https://doi.org/10.1508/cytologia.59.73

Moreira-Filho O, Bertollo LAC, Galetti Jr PM (1993) Distribution of sex chromosome mechanisms in Neotropical fish and description of a ZZ/ZW system in *Parodon hilarii* (Parodontidae). Caryologia 46: 115–125. https://doi.org/10.1080/00087114.1993.10797253

Noleto RB, Amorim AP, Vicari MR, Artoni RF, Cestari MM (2009) An unusual ZZ/ZW sex chromosome system in *Characidium* fishes (Crenuchidae, Characiformes) with the presence of rDNA sites. Journal of Fish Biology 75: 448–453. https://doi.org/10.1111/j.1095-8649.2009.02342.x

Pansonato-Alves JC, Oliveira C, Foresti F (2011a) Karyotypic conservatism in samples of *Characidium* cf. *zebra* (Teleostei, Characiformes, Crenuchidae): Physical mapping of ribosomal genes and natural triploidy. Genetics and Molecular Biology 34: 208–213. http://dx.doi.org/10.1590/S1415-47572011005000005

Pansonato-Alves JC, Paiva LRS, Oliveira C, Foresti F (2010) Interespecific chromosomal divergences in the genus *Characidium* (Teleostei: Characiformes: Crenuchidae). Neotropical Ichthyology 8: 77–86. http://dx.doi.org/10.1590/S1679-62252010000100010

Pansonato-Alves JC, Serrano EA, Utsunomia R, Camacho JPM, Silva GJC, Vicari MR, Artoni RF, Oliveira C, Foresti F (2014) Single origin of sex chromosomes and multiple origins of B chromosomes in fish genus *Characidium*. Plos One 9: e107169. https://doi.org/10.1371/journal.pone.0107169

Pansonato-Alves JC, Vicari MR, Oliveira C, Foresti F (2011b) Chromosomal diversification in populations of *Characidium* cf. *gomesi* (Teleostei, Crenuchidae). Journal of Fish Biology 78: 183–194. https://doi.org/10.1111/j.1095-8649.2010.02847.x

Pazian MF, Oliveira C, Foresti F (2014) Sex chromosome composition revealed in *Characidium* fishes (Characiformes: Crenuchidae) by molecular cytogenetic methods. Biologia 69: 1410–1416. https://doi.org/10.2478/s11756-014-0434-0

Pazian MF, Shimabukuro-Dias CK, Pansonato-Alves JC, Oliveira C, Foresti F (2013) Chromosome painting of Z and W sex chromosomes in *Characidium* (Characiformes, Crenuchidae). Genetica 141: 1–9. https://doi.org/10.1007/s10709-013-9701-1

Poveda-Martínez D, Sosa CC, Chacón-Vargas K, García-Merchán VH (2016) Historical biogeography of five *Characidium* fish species: Dispersal from the amazon paleobasin to southeastern South America. PLoS One 11: e0164902. https://doi.org/10.1371/journal.pone.0164902

Pucci MB, Barbosa P, Nogaroto V, Almeida MC, Artoni RF, Pansonato-Alves JC, Foresti F, Moreira-Filho O, Vicari MR (2014) Population differentiation and speciation in the genus *Characidium* (Characiformes: Crenuchidae): effects of reproductive and chromosomal barriers. Biological Journal of Linnean Society 111: 541–553. https://doi.org/10.1111/bij.12218

Pucci MB, Barbosa P, Nogaroto V, Almeida MC, Artoni RF, Pansonato-Alves JC, Scacchetti PC, Foresti F, Moreira-Filho O, Vicari MR (2016) Chromosomal spreading of microsatellites and $(TTAGGG)_n$ sequences in the *Characidium zebra* and *C. gomesi* genomes (Characiformes: Crenuchidae). Cytogenetic and Genome Research 149: 182–190. https://doi.org/10.1159/000447959

Pucci MB, Nogaroto V, Moreira-Filho O, Vicari MR (2018) Dispersion of transposable elements and multigene families: Microstructural variation in *Characidium* (Characiformes: Crenuchidae) genomes. Genetics and Molecular Biology 41: 585–592. http://dx.doi.org/10.1590/1678-4685-GMB-2017-0121

Ramsey J, Schemske DW (2002) Neopolyploidy in flowering plants. Review of Ecology and Systematics 33(1): 589–639. https://doi.org/10.1146/annurev.ecolsys.33.010802.150437

Scacchetti PC, Utsunomia R, Pansonato-Alves JC, Vicari MR, Artoni RF, Oliveira C, Foresti F (2015b) Chromosomal mapping of repetitive DNAs in *Characidium* (Teleostei, Characiformes): Genomic organization and diversification of ZW sex chromosomes. Cytogenetic and Genome Research 146: 136–143. https://doi.org/10.1159/000437165

Scacchetti PC, Utsunomia R, Pansonato-Alves JC, Silva GJC, Vicari MR, Artoni RF, Oliveira C, Foresti F (2015a) Repetitive DNA sequences and evolution of ZZ/ZW sex chromosomes in *Characidium* (Teleostei: Characiformes). PLoS One 10: e0137231. https://doi.org/10.1371/journal.pone.0137231

Scacchetti PC, Utsunomia R, Pansonato-Alves JC, da Costa-Silva GJ, Oliveira C, Foresti F (2015c) Extensive spreading of interstitial telomeric sites in the chromosomes of *Characidium* (Teleostei, Characiformes). Genetica 143: 263–270. https://doi.org/10.1007/s10709-014-9812-3

Schemberger MO, Bellafronte E, Nogaroto V, Almeida MC, Schühli GS, Artoni RF, Moreira-Filho O, Vicari MR (2011) Differentiation of repetitive DNA sites and sex chromosome systems reveal closely related group in Parodontidae (Actinopterygii: Characiformes). Genetica 139: 1499–1508. https://doi.org/10.1007/s10709-012-9649-6

Schemberger MO, Oliveira JIN, Nogaroto V, Almeida MC, Artoni RF, Cestari MM, Moreira-Filho O, Vicari MR (2014) Construction and characterization of a repetitive DNA library in Parodontidae (Actinopterygii: Characiformes): A genomic and evolutionary approach to the degeneration of the W sex chromosome. Zebrafish 11: 518–527. https://doi.org/10.1089/zeb.2014.1013

Serrano EA, Araya-Jaime C, Suárez-Villota EY, Oliveira C, Foresti F (2016) Meiotic behavior and H3K4m distribution in B chromosomes of *Characidium gomesi* (Characiformes, Crenuchidae). Comparative Cytogenetics 10: 255–268. https://doi.org/10.3897/CompCytogen.v11i1.10886

Serrano EA, Utsunomia R, Scudeller OS, Oliveira C, Foresti F (2017) Origin of B chromosomes in *Characidium alipioi* (Characiformes, Crenuchidae) and its relationship with supernumerary chromosomes in other *Characidium* species. Comparative Cytogenetics 11: 81–95. https://doi.org/10.3897/CompCytogen.v11i1.10886

Utsunomia R, Scacchetti PC, Hermida M, Fernández-Cebrián R, Taboada X, Fernández C, Bekaert M, Mendes NJ, Robledo D, Mank JE, Taggart JB, Oliveira C, Foresti F, Martínez P (2017)

Evolution and conservation of *Characidium* sex chromosomes. Heredity 119: 237–244. https://doi.org/10.1038/hdy.2017.43

Vicari MR, Artoni RF, Moreira-Filho O, Moreira-Filho O (2008) Diversification of a ZZ/ZW sex chromosome system in *Characidium* fish (Crenuchidae, Characiformes). Genetica 134: 311–317. https://doi.org/10.1007/s10709-007-9238-2

Vicari MR, Pistune HFM, Castro JP, Almeida MC, Bertollo LAC, Moreira-Filho O, Camacho JPM, Artoni RF (2011) New insights on the origin of B chromosomes in *Astyanax scabripinnis* obtained by chromosome painting and FISH. Genetica 139: 1073–1081. https://doi.org/10.1007/s10709-011-9611-z

Zanata AM, Camelier P (2015) Two new species of *Characidium* Reinhardt (Characiformes: Crenuchidae) from northeastern Brazilian coastal drainages. Neotropical Ichthyology 13: 487–498. http://dx.doi.org/10.1590/1982-0224-20140106

Zanata AM, Ohara WM (2015) A new species of *Characidium* Reinhardt (Ostariophysi: Characiformes: Crenuchidae) from headwaters of rio Pacaás Novos, rio Madeira basin, Rondônia, Brazil. Zootaxa 4021: 368–376. http://dx.doi.org/10.11646/zootaxa.4021.2.7

Ziemniczak K, Traldi JB, Nogaroto V, Almeida MC, Artoni RF, Moreira-Filho O, Vicari MR (2014) In situ localization of $(GATA)_n$ and $(TTAGGG)_n$ repeat DNAs and W sex chromosome differentiation in Parodontidae (Actinopterygii: Characiformes). Cytogenetic and Genome Research 144: 325–332. https://doi.org/10.1159/000370297

CompCytogen 12(3): 439–443 (2018)
doi: 10.3897/CompCytogen.v12i3.29648
http://compcytogen.pensoft.net

SHORT COMMUNICATION

COMPARATIVE Cytogenetics
A peer-reviewed open-access journal
International Journal of Plant & Animal Cytogenetics, Karyosystematics, and Molecular Systematics

First illustration of chromosomes and genetic system of Lecanodiaspidinae (Homoptera, Coccinea, Asterolecaniidae s.l.)

Ilya A. Gavrilov-Zimin[1]

1 *Zoological Institute, Russian Academy of Sciences, Universitetskaya nab. 1, St. Petersburg, 199034, Russia*

Corresponding author: *I.A. Gavrilov-Zimin* (coccids@gmail.com)

Academic editor: *V. Kuznetsova* | Received 10 September 2018 | Accepted 25 September 2018 | Published 4 October 2018

http://zoobank.org/FF0DD4A1-3DF4-47D2-9D33-316B0D3C31C6

Citation: Gavrilov-Zimin IA (2018) First illustration of chromosomes and genetic system of Lecanodiaspidinae (Homoptera, Coccinea, Asterolecaniidae s.l.) Comparative Cytogenetics 12(3): 439–443. https://doi.org/10.3897/CompCytogen.v12i3.29648

Abstract

The karyotype of *Psoraleococcus multipori* (Morrison, 1921) was studied for the first time, based on material from Indonesia (Sulawesi). The diploid chromosome number was found to be 18 in both males and females, but some cells contained also additional small chromosomal elements, probably B chromosomes. About 50 % of the studied embryos demonstrated paternal genome heterochromatinization of one haploid set of chromosomes (PGH) suggesting presence of a Lecanoid genetic system. The embryos with PGH are known to be always the male embryos in scale insects and so, bisexual reproduction may be presumed for *P. multipori*. The information provided represents the first probative cytogenetic data for the subfamily Lecanodiaspidinae Targioni Tozzetti, 1896 as a whole. A detailed morphological figure and photos of female and male embryonic cells are given. Additionally, it was discovered that the females of *P. multipori* exhibit complete ovoviviparity.

Keywords

Psoraleococcus multipori, scale insects, morphology, karyotype, Lecanoid system

The subfamily Lecanodiaspidinae Targioni Tozzetti, 1896 comprises 12 genera and about 80 species in the world fauna (Ben-Dov 2006). Most species are delicate insects with vestigial or partly reduced legs in adult females, covered by a resinous translucent or semi-translucent protective test. All species are obligate phytophages on angiosperm plants, most frequently on trees and shrubs. The subfamily is often considered as a separate family (see, for example, Koteja 1974, Ben-Dov 2006, Hodgson and Williams 2016 and others). However, Lecanodiaspidinae along with Asterolecaniinae Cockerell, 1896 and Cerococcinae Balachowsky, 1942 share a well-defined apomorphic character – the presence of so-called 8-shaped pores – the peculiar wax glands scattered on dorsum and/or venter of adult females and larvae (Fig. 1). Due to this character all three groups are traditionally considered as subfamilies of Asterolecaniidae s.l. (see, for example, Brown and McKenzie 1962, Danzig 1980, Danzig and Gavrilov-Zimin 2014, and Gavrilov-Zimin 2018 and references therein).

Lecanodiaspidinae was almost unstudied previously in respect of cytogenetics. The chromosomal number (2n=14) was reported for one species only, *Anomalococcus indicus* Ramakrishna Ayyar, 1919 by Parida and Moharana (1982) without karyotype photo or information about genetic system. Two other subfamilies of Asterolecaniidae are also very poorly studied cytogenetically with only one analyzed species for subfam. Asterolecaniinae (Gavrilov 2007) and 3 species for subfam. Cerococcinae (Brown 1959, Brown and McKenzie 1962); the chromosome numbers of these species vary from 2n=6 to 2n=24 (l.c.).

During an expedition in Sulawesi Is. (Indonesia) the author was able to collect the series of adult females of *Psoraleococcus multipori* (Morrison, 1921): K 923, vicinity of Kendari, on branch of undetermined dicotyledonous tree, inside of ant gallery, 10.XI.2011, I. Gavrilov-Zimin (deposited at Zoological Institute RAS, St. Petersburg). These females appeared to be suitable for preparing both morphological and chromosomal slides. The method of preparation of the morphological slides and method of squashing of the embryonic cells in lactoacetorcein for chromosomal studies see, for example, in Gavrilov-Zimin (2018).

The diploid chromosomal number of *P. multipori* is 18 in both sexes (Figs 2a, f). The karyotype consists of chromosomes gradually differing in size (Fig. 2f). A similar gradual pattern of chromosome size variation (for karyotype 2n=14) was reported for *Anomalococcus indicus* by Parida & Moharana (1982). Some cells of *P. multipori* contain additional small chromosomal elements (Fig. 2b), probably B-chromosomes, which are also known in some scale insects from different families (see for review Gavrilov 2007), but were not reported previously for any Asterolecaniidae s.l. Totally, nine cleavage stage embryos were found in the two dissected females (in addition to numerous embryos at later stages) and five of them demonstrated characteristic Lecanoid heterochromatinization of one haploid set of chromosomes (Fig. 2c–d) that suggests a Lecanoid genetic system and is known for many other neococcids (superfamily Coccoidea) (Nur 1980, Gavrilov 2007, Gavrilov-Zimin 2016). In particular, within Asterolecaniidae s.l., such a system (including both “Lecanoid” and “Comstockioid” variants of spermatogenesis) was demonstrated previously by Brown (1959) for *Cerococcus quercus* Comstock, 1882 and for *Mycetococcus ehrhorni* (Cockerell, 1895) by Brown and McKenzie (1962) (both species are from the subfamily Cerococcinae). The embryos with such hetero-

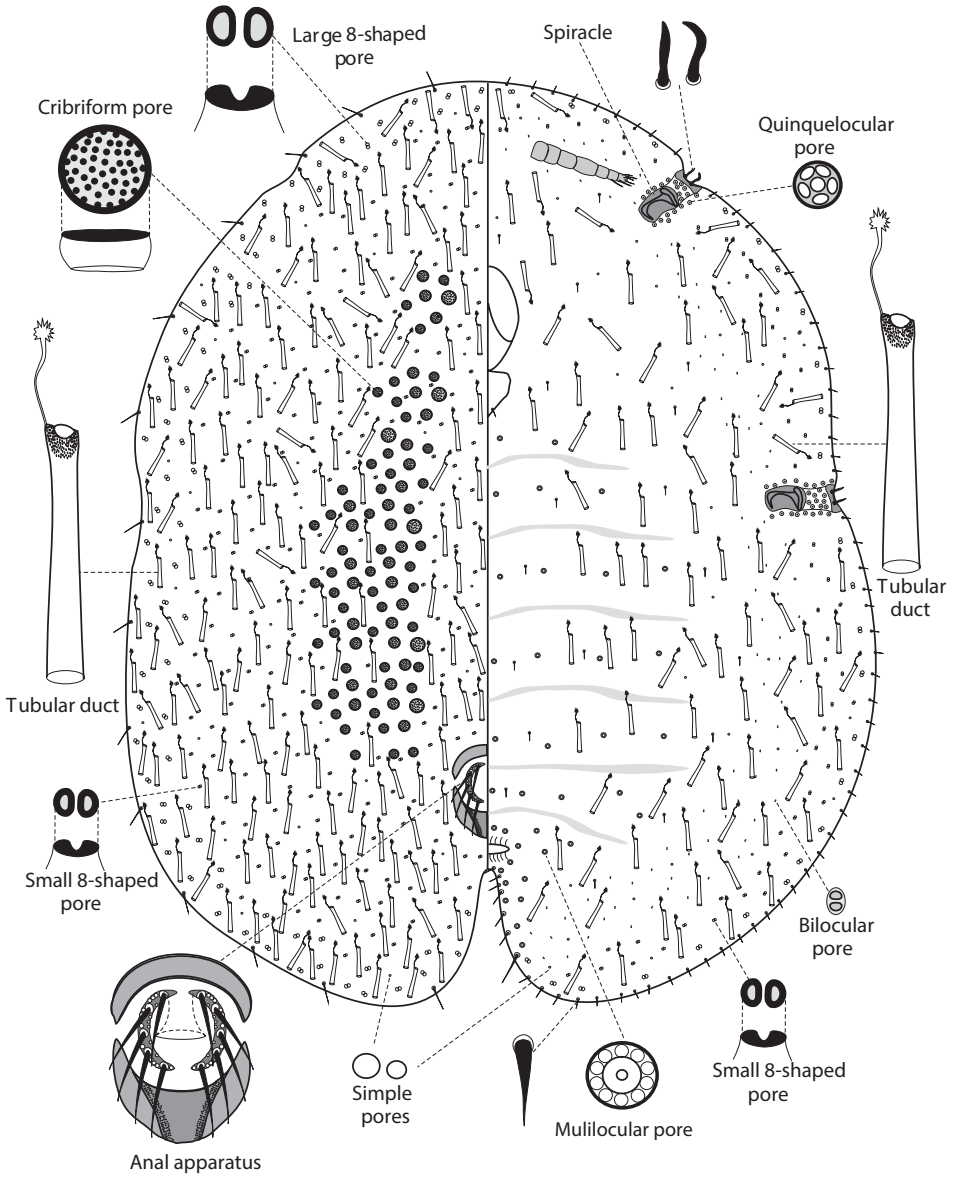

Figure 1. Morphology of *Psoraleococcus multipori*, adult female, Indonesia (Sulawesi).

chromatinization are always the males and so, bisexual reproduction may be presumed in *Psoraleococcus multipori*. On the other hand, the adult males or male larvae have not been collected up to now in any species of the genus *Psoraleococcus* Borchsenius, 1960. This situation is probably connected with separate lives of female and male colonies on different parts of a host plant or even on different plants. Moreover, all species of *Psoraleococcus* live in symbiosis with ants (Lambdin and Kosztarab 1973) which may transport different instars of scale insects inside hidden underground galleries, which significantly impedes their detection and collection.

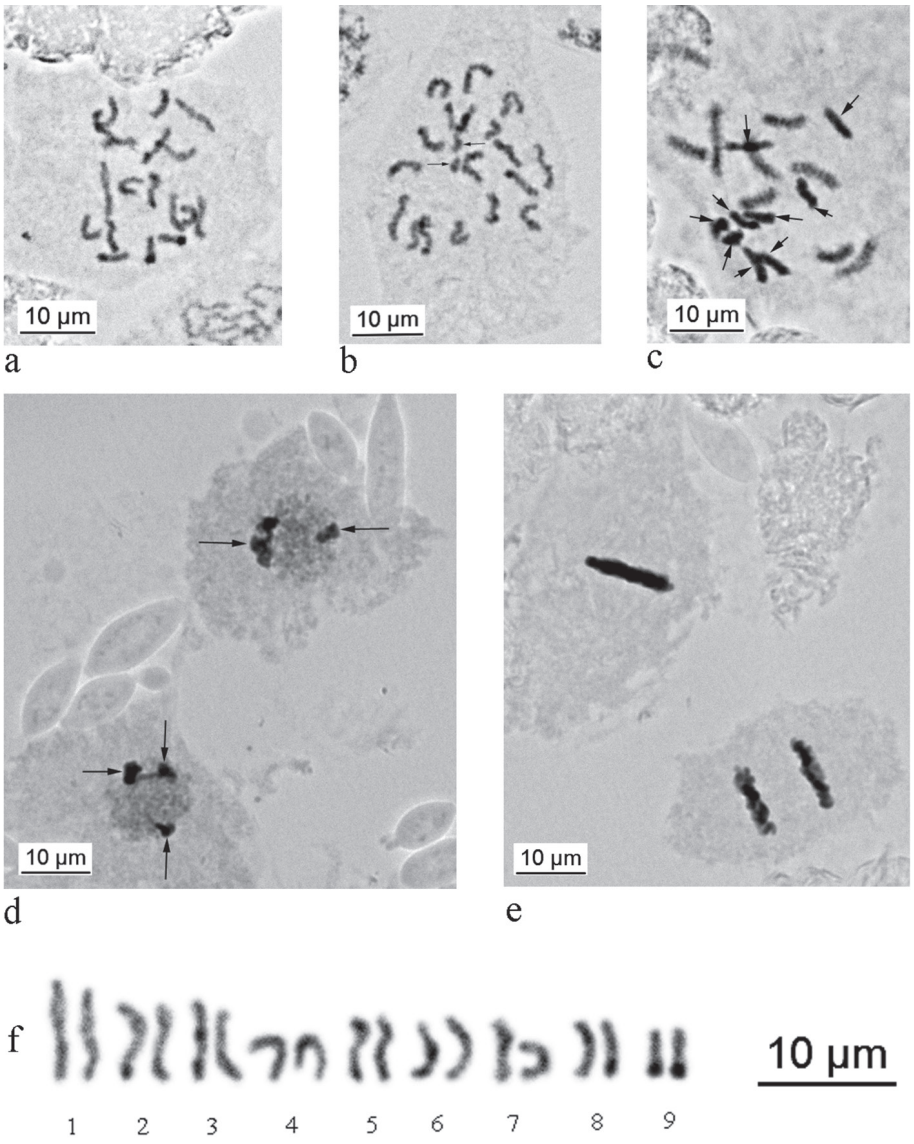

Figure 2. Embryonic cells of *Psoraleococcus multipori*. **a** female embryo, 2n = 18 **b** female embryo, 2n = 18 + 2B (B-chromosomes arrowed) **c** male embryo, paternal set of chromosomes begins heterochromatinization (arrowed) **d** Lecanoid heterochromatinization (arrowed) in interphase cells of male embryo; **e** metaphase and anaphase in female embryo showing no lagging chromosomes (Bs) **f** karyogram, prepared basing on the Fig. 2a.

The females of *P. multipori* exhibit complete ovoviviparity, i.e. all stages of embryonic development occur inside the maternal body (see a review of reproductive strategies of scale insects and appropriate terminology in Gavrilov-Zimin 2018).

The work was performed in the frame of the state research project no. AAAA-A17-117030310018-5 at Zoological Institute, Russian Academy of Sciences.

References

Ben-Dov Y (2006) A systematic catalogue of eight scale insect families (Hemiptera: Coccoidea) of the World. Amsterdam, 368 pp.

Brown SW (1959) Lecanoid chromosome behavior in three more families of the Coccoidea (Homoptera). Chromosoma (Berlin) 10: 278–300. https://doi.org/10.1007/BF00396575

Brown SW, McKenzie HL (1962) Evolutionary patterns in the armored scale insects and their allies (Homoptera: Coccoidea: Diaspididae, Phoenicococcidae, and Asterolecaniidae). Hilgardia 33: 141–170. https://doi.org/10.3733/hilg.v33n04p141

Danzig EM (1980) Koktsydy Dal'nego Vostoka SSSR (Homoptera, Coccinea) s analizom filogenii koktsyd mirovoy fauny. Leningrad, 367 pp. [In Russian]. English Edition: Danzig EM (1986) Coccids of the Far-Eastern USSR (Homoptera: Coccinea). Phylogenetic analysis of coccids in the world fauna. New Delhi, 450 pp.

Danzig EM, Gavrilov-Zimin IA (2014) Palaearctic mealybugs (Homoptera: Coccinea: Pseudococcidae). Part 1. Subfamily Phenacoccinae. St Petersburg, 678 pp. [Fauna of Russia and neighbouring countries. New series, № 148. Insecta: Hemiptera: Arthroidignatha]

Gavrilov IA (2007) A catalogue of chromosome numbers and genetic systems of scale insects (Homoptera: Coccinea) of the world. Israel Journal of Entomology 37: 1–45.

Gavrilov-Zimin IA (2016) Cytogenetic and taxonomic studies of some legless mealybugs (Homoptera, Coccinea, Pseudococcidae). Comparative Cytogenetics 10(4): 587–601. https://doi.org/10.3897/compcytogen.v10i4.10503

Gavrilov-Zimin IA (2018) Ontogenesis, morphology and higher classification of archaecococcids (Homoptera: Coccinea: Orthezioidea). Zoosystematica Rossica (Supplementum 2). 260 pp.

Hodgson CJ, Williams DJ (2016) A revision of the family Cerococcidae Balachowsky (Hemiptera: Sternorrhyncha, Coccomorpha) with particular reference to species from the Afrotropical, western Palaearctic and western Oriental Regions, with the revival of *Antecerococcus* Green and description of a new genus and fifteen new species, and with ten new synonomies. Zootaxa 4091(1): 1–175.

Koteja J (1974) On the phylogeny and classification of the scale insects (Homoptera, Coccinea) (discussion based on the morphology of the mouthparts). Acta Zoologica Cracoviensia 19: 267–325.

Lambdin PL, Kosztarab MP (1973) A revision of the seven genera related to *Lecanodiaspis* (Homoptera: Coccoidea: Lecanodiaspididae). Research Division Bulletin Virginia Polytechnic Institute and State University (Blacksburg) 83: 1–110.

Nur U (1980) Evolution of unusual chromosome systems in scale insects (Coccoidea: Homoptera). In: Blackman RL, Hewitt GM, Ashburner M (Eds) Insect Cytogenetics. London, 97–117.

Parida BB, Moharana S (1982) Studies on the chromosome constitution in 42 species of scale insects (Coccoidea: Homoptera) from India. Chromosome Information Service (Tokyo) 32: 18–20.