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Random chromosome distribution in the first meiosis of F1 disomic substitution line 2R(2D) x rye hybrids (ABDR, 4× = 28) occurs without bipolar spindle assembly

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Abstract

The assembly of the microtubule-based spindle structure in plant meiosis remains poorly understood compared with our knowledge of mitotic spindle formation. One of the approaches in our understanding of microtubule dynamics is to study spindle assembly in meiosis of amphyhaploids. Using immunostaining with phH3Ser10, CENH3 and α-tubulin-specific antibodies, we studied the chromosome distribution and spindle organisation in meiosis of F_1 2R(2D)xR wheat-rye hybrids (genome structure ABDR, 4× = 28), as well as in wheat and rye mitosis and meiosis. At the prometaphase of mitosis, spindle assembly was asymmetric; one half of the spindle assembled before the other, with simultaneous chromosome alignment in the spindle mid-zone. At diakinesis in wheat and rye, microtubules formed a prospindle which was subsequently disassembled followed by a bipolar spindle assembly. In the first meiosis of hybrids 2R(2D)xR, a bipolar spindle was not found and the kinetochore microtubules distributed the chromosomes. Univalent chromosomes are characterised by a monopolar orientation and maintenance of sister chromatid and centromere cohesion. Presence of bivalents did not affect the formation of a bipolar spindle. Since the central spindle was absent, phragmoplast originates from "interpolar" microtubules generated by kinetochores. Cell plate development occurred with a delay. However, meiocytes in meiosis II contained apparently normal bipolar spindles. Thus, we can conclude that: (1) cohesion maintenance in centromeres and between arms of sister chromatids may negatively affect bipolar spindle formation in the first meiosis; (2) 2R/2D rye/wheat chromosome substitution affects the regulation of the random chromosome distribution in the absence of a bipolar spindle.

Keywords
immunostaining, cohesion, kinetochore microtubules, pro-spindle, monopolar orientation, phragmoplast, univalents, wheat-rye amphyhaploids

Introduction

Flawless chromosome segregation to daughter cells during mitotic and meiotic division is necessary to maintain the viability of organisms and their progeny. Due to the importance of these processes, cell division is controlled by multiple genes (Masoud et al. 2013, Zamariola et al. 2014, Mercier et al. 2015). Chromosome disjunction involves a multitude of processes, of importance amongst which is the formation of the division apparatus. In a plant cell, the division apparatus is comprised of membranes, kinetochores, microtubules (MTs) and actin microfilaments (Baskin and Cande 1990). MTs and actin microfilaments are elements of the cytoskeleton - a dynamic structure that changes in the course of division. MTs form bipolar spindles and interact with sister chromatids through a large protein complex - kinetochore, which is formed in centromeric regions (Perpelescu and Fukagawa 2011). As a result, MTs attach sister chromatids/homologous chromosomes to opposite poles, while chromosomes align in the spindle mid-zone and then move to the poles.

During animal cell division, the spindle is formed by special organelles - centrosomes (Walczak et al. 2010, Prosser and Pelletier 2015), in liverwort - with centrosome-like MT-organising centres, called polar organisers (PO) (Shimamura et al. 2004). Centrosomes are absent in oocytes of some animals including humans (Severson et al. 2016, Bennabi et al. 2016), as well as in cells of higher plants (Yamada and Goshima 2015).

Most of our knowledge about MTs and chromosome dynamics in higher plants was obtained while studying mitosis. MT nucleation sites are the inner surface of the plasma membrane, chromosomes and nuclear envelope (Baskin and Cande 1990, Canaday et al. 2004, Masoud et al. 2013, de Keijzer et al. 2014, Chabout and Schmit 2016, Lee and Liu 2019). The main structures formed by MTs in dividing cells during mitosis are interphase cortical or radial networks, preprophase band (PPB), prophase spindle and phragmoplast (De Mey et al. 1982, Baskin and Cande 1990, Hepler et al. 1993, Smirnova and Bajer 1992, 1994, 1998). PPB is a structure consisting of parallel MT arrays beneath the cell cortex. PPB ensures bipolarity of prophase spindles. In plants, along with PPB development, the MTs surrounding the nucleus are gradually organised into a spindle-like structure called the “prophase spindle” (“pro-spindle” or “polar caps”) (Baskin and Cande 1990, De Mey et al. 1982, Smirnova and Bajer 1992, 1994, 1998). The pro-spindle is formed with MTs that nucleate from γTuRCs sites and/or H1 histone complexes on the nucleus surface (Lee and Liu 2019). Motor and non-motor proteins (MAP) associated with microtubules, re-organise microtubules through sliding, cross-linking and severing into MT arrays, specific to the cell cycle phases (Chabout and Schmit 2016).

After nuclear envelope breakdown (NEB), MTs growing from polar caps become a source of interpolar spindle MTs; simultaneously, regardless of the pro-spindle during prometaphase, MT nucleation around the chromosome/kinetochore depends on the RanGTP gradient or aurora kinase. Those MTs are then organised into an overall bipolar configuration (Yamada and Goshima 2015). The mitotic spindle develops from two half-spindles, plus-ends are orientated at the mid-zone and minus-ends at the poles (Dhonukshe et al. 2006). At the mid-zone, plus-end-directed motors kinesin–5 push the poles apart by cross-linking anti-parallel microtubules and travel to their plus ends; while at the pole, minus-end-directed motors kinesin–14 draw the spindle halves together and focus the poles (Yamada and Goshima 2015). The mid-zone represents the region of overlap between the two halves of the spindle, where microtubule plus-ends terminate at chromosomal kinetochores (kinetochore microtubules) or inter-digitate in an anti-parallel manner with microtubules from the opposite pole (interpolar microtubules). The robust spindle is barrel-shaped rather than fusiform, as the pole is not tightly focused at one point; multiple kinetochore and non-kinetochore MTs are converged or cross-linked locally and, thus, multiple mini-poles are observed (Smirnova and Bajer 1992). With the start of the anaphase, sister chromatids are separated and then segregated to the pole by kinetochore MT depolymerisation, analogous to animal spindles (Yamada and Goshima 2015). The MT-based arrays assembled after sister chromatid separation are called phragmoplasts. The central factors for MT generation in the phragmoplast are γTuRC and augmin, whereas MAP65 is an essential MT cross-linker that ensures phragmoplast bipolarity (Yamada and Goshima 2015).

Assembly and functioning of the MT-based spindle in plant meiosis have been studied in less detail than in mitosis. In maize meiocytes, a "self-assembly" model for spindle formation was proposed (Chan and Cande 1998). Xue et al. (2019) postulate that the transition from multipolar spindles into bipolar spindles is a common process in both monocots and dicots. According to another model, a specific structure in late prophase is a ring-shaped perinuclear cytoskeleton system, which is destroyed at the beginning of the prometaphase and forms a chaotic bipolar array which is then focused and orientated into the spindle (Shamina 2005). Spindle development in the first meiosis of *Arabidopsis thaliana* (Linnaeus, 1753) is also accompanied by a specific distribution of MT arrays (Prusicki et al. 2019). A half-moon is formed in the prophase and then transforms into full-moon MT arrays surrounding the nucleus. Dense MT arrays around the nucleus, called the prophase spindle, are similar to what is observed in mitosis. When the nuclear envelope is destroyed, the prophase spindle disassembles and a robust bipolar spindle forms (Prusicki et al. 2019).

The *Arabidopsis* genome encodes two kinesin–14A genes: *Atk5/AtKIN14b* (Ambrose and Cyr 2007, Quan et al. 2008), which affects mitotic spindle pole formation and *Atk1/AtKIN14a* (Chen et al. 2002, Quan et al. 2008), which primarily affects meiotic spindle pole formation and chromosome segregation. It was found that *Dv1* is not required for the formation of bipolar spindles, but is specifically required for focusing the spindle pole to a fine point (Higgins et al. 2016). Recently, it has been observed that OsMTOPVIB, an initiation factor of homologous recombination, plays a crucial role in meiotic bipolar spindle assembly (Xue et al. 2019).

Desynaptic mutants and haploids are the target of the research of spindle assembly in the absence of bivalents. Bi-orientation of sister kinetochores in a univalent is essential for bipolar spindle formation when homologous recombination is absent in meiosis of maize asynaptic mutants and rice haploids (Chan and Cande 1998, Xue et al. 2019). Meiosis of distant hybrids is also characterised by the absence of bivalents. First-generation hybrids between species/genera of different taxa constitute a complex organism; its nucleus unites several haploid genomes. Previously, our group described four types of meiotic chromosome behaviour in hybrids (2n = 4× = 28, ABDR) between the disomic wheat-rye substitution lines 1Rv(1A), 2R(2D), 5R(5D), 6R(6A) and rye (Silkova and Loginova 2016). Two of them are significantly different from each other. The first type is a mitotic-like division in hybrids 1Rv(1A), 5R(5D) and 6R(6A) with rye. Data on MT dynamics and kinetochore architecture in univalent chromosomes indicate that the robust bipolar spindle is formed and back-to-back sister kinetochores anchor spindle microtubules followed by sister chromatid separation during the first and only meiotic division, resulting in meiotic restitution and the restoration of fertility. The second type is known as the reductional division. It is characterised by the side-by-side positioning of sister kinetochores within a random univalent distribution at anaphase I, followed by the second meiotic division. Only sterile pollen is produced as a result of such chromosome behaviour. The 2R(2D)xR genotype tends to promote reductional division in most cases (95.99±1.59 and 85.55±1.46% in different vegetations) (Silkova et al. 2007, 2011). Chromosome behaviour in the meiocytes of androgenic haploids of line 2R(2D) (Silkova et al. 2007a) was similar, 98.22 ± 1.46%, to that of the hybrids between 2R(2D) and rye (Silkova et al. 2007, 2011).

Thus, a bipolar spindle is formed in meiosis of wheat-rye hybrids when bipolar-directed kinetochores are present (Silkova and Loginova 2016), similar to asynaptic mutants and haploids (Chan and Cande 1998, Xue et al. 2019). Little is known, so far, as to how the spindle is formed and chromosomes are distributed to daughter cells in meiosis with monopolar-directed kinetochores. The study is aimed at obtaining answers to these questions. In-depth analysis was undertaken for MT cytoskeleton dynamics in meiosis of F_1 2D(2R)xR wheat-rye hybrids compared to MT dynamics in mitosis and meiosis of *Secale cereale* (Linnaeus, 1753) rye and *Triticum aestivum* (Linnaeus, 1753) hexaploid wheat. We did not find a robust bipolar spindle in hybrids. The presence of bivalents also does not affect the formation of a bipolar spindle. The main hybrid feature was a random distribution of chromosomes in the first division. The univalents characterised monopolar orientation and maintained cohesion. Therefore, cohesion release may affect bipolar spindle formation in meiosis. Hence, when there are no bipolar-orientated chromosomes, there is no issue with 'release of cohesion' and the system of motor proteins and MT kinetochores can distribute chromosomes. The minus-ends of the kinetochore microtubules were focused and formed poles. Thus, the Anaphase Promotion Complex (APC/C^{CDC20}) is activated only when a bipolar spindle is formed. At anaphase I, MT bundles could connect kinetochores as bridges and kinetochores generated "interpolar" microtubules. Phragmoplast and cell plate development occurred with a delay. However, meiocytes in meiosis II contained apparently normal bipolar spindles.

Material and methods

Plant material

In this study, wheat cultivar *T. aestivum* cv. Saratovskaya 29 (cv S29, BBAADD, 2n=42), rye cultivar *S. cereale* cv. Onokhoiskaya (RR, 2n=14) and wheat-rye F_1 hybrid (ABDR, 4×=28) plants were used. The parental plants of the wheat-rye hybrid included a disomic single chromosome wheat-rye substitution line (2n=42): 2R(2D) (*T. aestivum* cv. Saratovskaya 29/Novosibirskaya 67/*S. cereale* (Linnaeus, 1753) cv. Onokhoiskaya) (Silkova et al. 2006). The line was crossed as female to the diploid rye and is hereafter called 2R(2D)xR. F_1 hybrids, wheat and rye plants were grown in greenhouse conditions at a temperature of 24/18 °C during day/night and under a day/night cycle of 16/8 h.

Meiotic conventional analysis

For the analysis of MT dynamics in 2R(2D)xR meiosis, spikes estimated to be entering meiosis were fixed in modified Navashin's fixative (Wada and Kusunoki 1964), which consisted of a mixture of A and B solutions (1:1). Solution A consisted of 1.1 g $CdCl_2$, 10 ml glacial acetic acid and 65 ml distilled H_2O. Solution B consisted of 40 ml CH_2O (40%) and 35 ml distilled H_2O. Spikes were fixed in Navashin's fixative for two days and then the mixture was replaced with a new one. The fixed material was stored at 4 °C. Pollen mother cells (PMCs) were stained with 3% acetocarmine.

Chromosome pairing in 2R(2D)xR meiosis was examined on squashed preparations stained with 3% acetocarmine. Anthers containing PMCs at metaphase I-anaphase I were fixed in a 1:3 (v/v) mixture of acetic acid:ethanol for 24 h and stored in 70% ethanol in a refrigerator. All anthers with PMCs at metaphase I-anaphase I were analysed. Each anther was examined individually and all scorable PMCs were assayed (Table 1).

All slides were examined under a Leica DM 2000 (Leica Microsystems) microscope and images were recorded with a DFC 295 (Leica Microsystems) camera.

Meiotic chromosome preparation and fluorescence *in situ* hybridisation

Fluorescence *in situ* hybridisation (FISH) was performed according to Silkova and Loginova (2016). Spikes were fixed in 45% acetic acid for 2 to 4 h at room temperature, anthers with meiocytes at MI-AI were selected, squashed and slides were frozen in liquid nitrogen, dehydrated through a series of alcohols with increasing concentrations of 70%, 90% and 96% and stored at –20 °C until needed. Each anther was examined individually and all scorable PMCs were assayed (Table 1).

The centromere structure of chromosomes was examined by *in situ* hybridisation using the centromere-specific probe *Aegilops tauschii* (Cosson, 1849) pAct 6–09 specific for rye, wheat, rice and barley centromere repeats (Zhang et al. 2004, Qi et al.

Table 1. Materials analysed in the study.

Hybrids / rye and wheat	Conventional analysis Navashin's / acetic acid:ethanol fixatives		FISH		Immunostaining		
	Plants	Meiocytes	Plants	Meiocytes	Plants	Meiocytes	Mitotic cells
2R(2D)xR	13/15	2268/7506	9	169	13	365	–
T. aestivum / S29	–	–	–	–	5	151	200
S. cereale / Onokhoiskaya	–	–	–	–	6	232	384

2013). The samples of DNA, containing the corresponding repeats, were kindly provided by Dr. A. Lukaszewcki (UCR, CA, USA). *In situ* hybridisation with labelled DNA probes was performed according to A. Houben (Houben et al. 2006). For its use as probes, the total genomic DNA of rye was labelled with biotin 16–dUTP or digoxigenin 11–dUTP by nick translation and the centromere-specific probes were labelled with digoxigenin 11–dUTP or biotin 16–dUTP by the polymerase chain reaction (PCR). The two probes were used alone or combined together in various proportions and mixed with blocking wheat DNA. Chromatin was stained using 1 mg/ml DAPI in Vectasheild anti-fade solution (Vector Laboratories). All slides were examined under an Axio Imager M1 (Karl Zeiss) microscope, images were recorded with a ProgRes MF camera (Meta Systems, Jenoptic) in the Center of Microscopic Analysis of Biological Objects (SB RAS) and processed using the Adobe Photoshop CS2 software.

Immunolabelling

The slide preparation of mitotic and meiotic cells and immunostaining with primary and secondary antibodies was performed according to Silkova and Loginova (2016). All scorable PMCs and mitoic cells were assayed (Table 1). Root tips or anthers were fixed in fresh 8% paraformaldehyde in PBS for 2 h in a humid chamber, washed 4 × 15 min in phosphate-buffered saline (PBS) solution and digested at room temperature for 5 to 15 min in a mixture of 1% pectinase, 1% cellulase Onozuka R–10 and 1% pectolyase Y–23 dissolved in PBS. Root tips or anthers were then washed 3 × 5 min in PBS. The material was separated on poly-L-lysine-coated slides after freezing for 15 min at –70 °C and blocking for 30 min in 3% bovine serum albumin (BSA)/PBS/non-fat milk. Three primary antibodies used were anti-phH3Ser10 (1:1000; Active Motif), which specifically recognised histone H3 phosphorylated at Ser 10; anti-CENH3 (kindly provided by Dr. A. Houben, IPK Gatersleben, Germany and diluted at 1:850), which specifically recognised the centromeric histone H3 variant; and monoclonal anti-α-tubulin (Sigma, No.T5168, diluted 1:1000), which detects the α-tubulin of microtubules. The secondary antibodies to anti-phH3S10 and anti-CENH3 were anti-rabbit IgG conjugated with rhodamine (Sigma, diluted 1:100); the secondary antibody to anti-α-tubulin was anti-mouse IgG conjugated with FITC (Sigma, diluted at 1:100). Incubation with the primary antibodies was completed overnight at 4 °C. Then, slides were washed 4 × 15 min in PBS and incubated with the secondary antibody at room temperature for

1 h. After 4 × 15 min washes in PBS, the slides were counterstained with 4',6–diamidino–2–phenylindole (DAPI) and mounted in anti-fade Vectashield medium.

Slides were examined under an Axio Imager M1 (Carl Zeiss AG, Germany) microscope and the images were recorded with a ProgRes MF camera (Meta Systems, Jenoptic, Germany) with Isis software (Meta Systems, Jenoptic, Germany) or under a confocal laser scanning microscope LSM 780 NLO (Zeiss) with a monochrome digital camera AxioCam MRm (Zeiss) and ZEN software (Zeiss) in the Center of Microscopic Analysis of Biological Objects, SB RAS. The images were processed using Adobe Photoshop CS2 software.

Results

Dynamics of MT cytoskeleton in mitosis of bread wheat *T. aestivum* L. (2n=42) and rye *S. cereale* L. (2n=14)

Analysis of microtubule dynamics in wheat and rye mitosis was performed using antibodies to phH3Ser10 and α-tubulin. Phosphorylation of H3Ser10 histone in mitosis has a particular dynamic (Fig. 1). At prometaphase, metaphase phH3Ser10 is localised in centromeric regions (Fig. 1d, k). It can be used to mark kinetochores to study their interaction with MTs.

MTs in wheat and rye mitosis aggregated mainly into interphase cortical or radial networks (Fig. 1a, h), pre-prophase band (Fig. 1b, i), prophase spindle (Fig. 1c, j), metaphase spindle (Fig. 1d, k) and phragmoplast (Fig. 1g, n). These structures did not differ from those described earlier for other objects (De Mey et al. 1982, Baskin and Cande 1990).

In the early prometaphase, the pro-spindle structure changes radically after the destruction of the nuclear envelope. MT distribution changes were described in mitosis *of Haemanthus katherinae* (Martyn, 1795) (Baker) Friis et Nordal 1976 (De Mey et al.

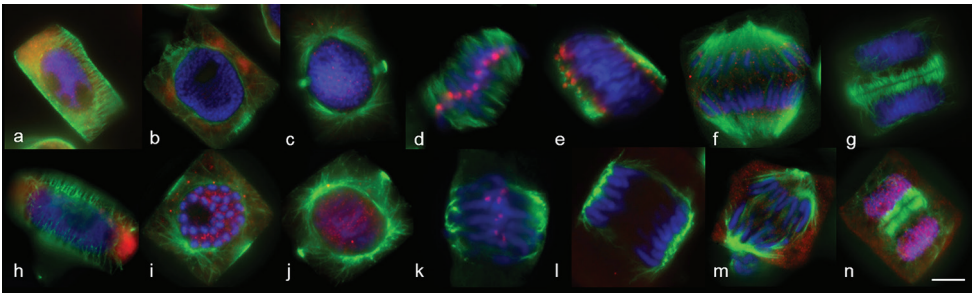

Figure 1. MT dynamics in wheat (**a–g**) and rye (**h–n**) mitosis. **a, h** interphase **b, c, i, j** prophase **d, k** metaphase **e, f, l, m** anaphase **g, n** telophase. Immunostaining was undertaken with anti–α–tubulin (green) and anti–histone phH3Ser10 (red) antibodies, DNA staining with DAPI (blue). Scale bar: 10 μm.

1982). Distinct bundles of MTs are formed, a number of these bundles ending at kinetochores in the spindle mid-zone (De Mey et al. 1982). The structure of pro-spindle in wheat and rye also changed radically after the destruction of the nuclear envelope. We found cells at early wheat prometaphase where chromosomes were Rabl-orientated (Fig. 2b, c). A metaphase-like spindle was found in such cells (Fig. 2b). One pole was tightly focused at one point with this pole being located on the kinetochore side. In other cells, the regions with more intensive nucleation and tight MT arrays were also located from the kinetochore side (Fig. 2c), MTs being arranged from kinetochores towards subtelomeric regions of chromosomes (Fig. 2d). Local MT nucleation sites were also found between chromosomes with arms at the sides and subtelomeric regions, which was registered with bright massive signals of anti-α-tubulin (Fig. 2c). Inside a prometaphase spindle, chromosomes were moving and kinetochores aggregated near the spindle mid-zone (Fig. 2e).

Rye MT re-organisation in prometaphase differed from wheat. At the pro-spindle stage, chromosomes were Rabl-orientated (Fig. 3a, b). Rabl-orientation remained after nuclear envelope destruction (Fig. 3c). The loci of nucleation and MT growth were near kinetochores and on them, wherein MT polymerisation was unidirectional, from the bottom upwards, from kinetochores to chromosome subtelomeres (Fig. 3c). Such MT polymerisation looked like flares (Fig. 3c, d, e). As a result, the region of tighter MT arrays was registered from the subtelomere side, no tight MT arrays being found near kinetochores (Fig. 3f). Additional autonomous sites of MT nucleation emerged near kinetochores in the cells, where chromosome movement started (which was registered by the absence of Rabl-orientation). The intensity and density of anti-α-tubulin signals on such sites varied widely (Fig. 3g). MT bundles were arranged chaotically in different directions (Fig. 3g).

The later spindle had a form similar to the metaphase; however, the second pole was not developed and kinetochores were not yet bipolar-orientated (Fig. 3h). Pole convergence took place in the late prometaphase and kinetochore assembly in the spindle mid-zone was completed (Fig. 3i)

Poles in wheat and rye metaphase were transformed into several microtubule convergence centres - minipoles (Fig. 1d, k) and a mitosis-specific bipolar barrel-like (anastral) cleavage spindle was formed that aligned kinetochores on the mid-section (Fig. 1d, k). At anaphase, the kinetochore MT bundles shortened, chromosome separa-

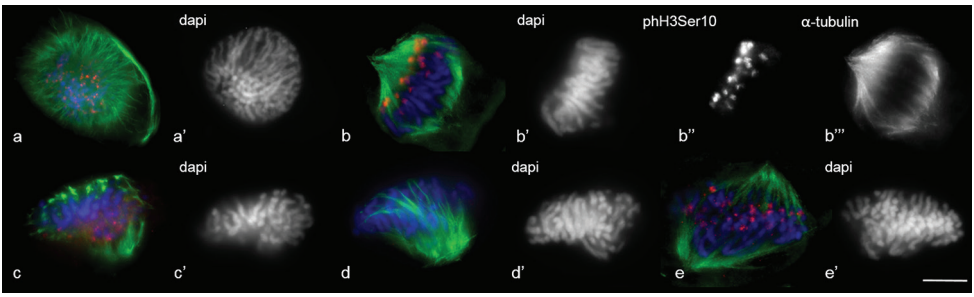

Figure 2. MT dynamics in wheat prometaphase. Immunostaining was undertaken with a primary antibody specific to α–tubulin (green) and histone phH3Ser10 (red). (**a**) prophase, PPB break (**b–e**) prometaphase. DAPI counterstaining (**a'–e'**). Scale bar: 10 μm.

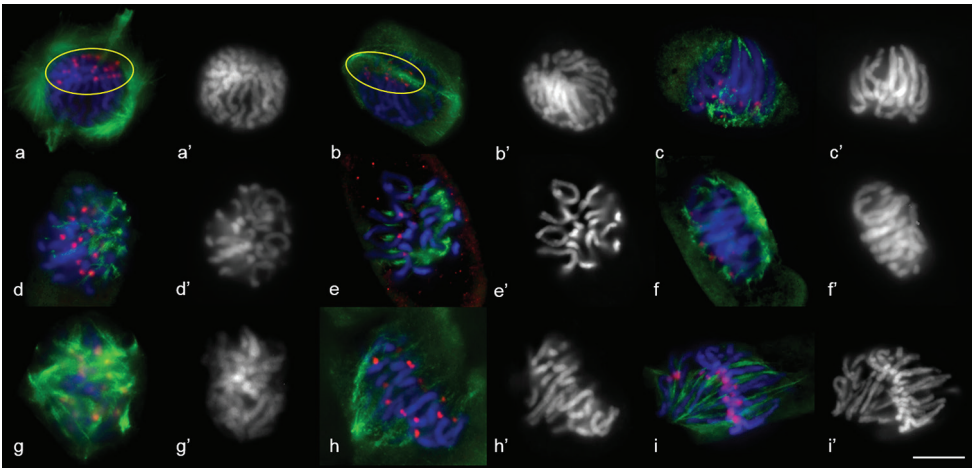

Figure 3. MT dynamics in rye prometaphase. **a, b** pro–spindle **c–i** MT re–organisation in prometaphase. Ovals indicate the accumulation of kinetochores. Immunostaining was undertaken with anti–α–tubulin (green) and anti–histone phH3Ser10 (red) antibodies, DNA staining with DAPI (blue). DAPI counterstaining (**a'–e'**). Scale bar: 10 μm.

tion began and, after chromosome separation was completed, minipoles moved closer and banded, forming tightly focused poles (Fig. 1f, m). Mitosis ended with phragmoplast formation (Fig. 1g, n) and the building of a new cell wall.

Dynamics of MT cytoskeleton in meiosis of *Triticum aestivum* L. bread wheat (2n=42) and *Secale cereale* L. rye (2n=14)

MT dynamics in wheat and rye meiosis were analysed using antibodies to phH3Ser10, CENH3 and α-tubulin. CENH3 is localised on kinetochores and phH3Ser10 on the entire chromosome in the first meiosis, while a more intensive signal is registered on the centromere at diakinesis and prometaphase. Transformation of a reticular system of MT arrays, formed around the nucleus in interphase, was observed in early prophase (leptotene, zygotene). MT polymerisation took place in different directions, a tight round-up of MT arrays formed around the nucleus (Fig. 4a) and tangential MT re-orientation resulted in development of a cortical ring near the cell membrane (Fig. 4b). The MT ring shifted to the nucleus envelope in pachytene (Fig. 4c). The MT ring remained in diplotene, while nuclei migrated to the cell edge (Fig. 4d). A perinuclear ring of microtubules formed in diakinesis (Fig. 4e), the nucleus shifting to the central position. MT structures, similar to mitotic pole caps or pro-spindles were found at diakinesis (Figs 4f, g, 5b). Rye also formed a pro-spindle-like structure at diakinesis as a result of re-organising MT arrays (Fig. 5a).

Destruction of a nuclear envelope was accompanied by its "invagination" (Fig. 4h). The perinuclear MT ring remained, but its shape changed (Fig. 4h). After the destruction of the nuclear envelope, MTs still surrounded chromosomes along the outline of the former nucleus (Fig. 4i), while interpole and kinetochore MT bundles developed simul-

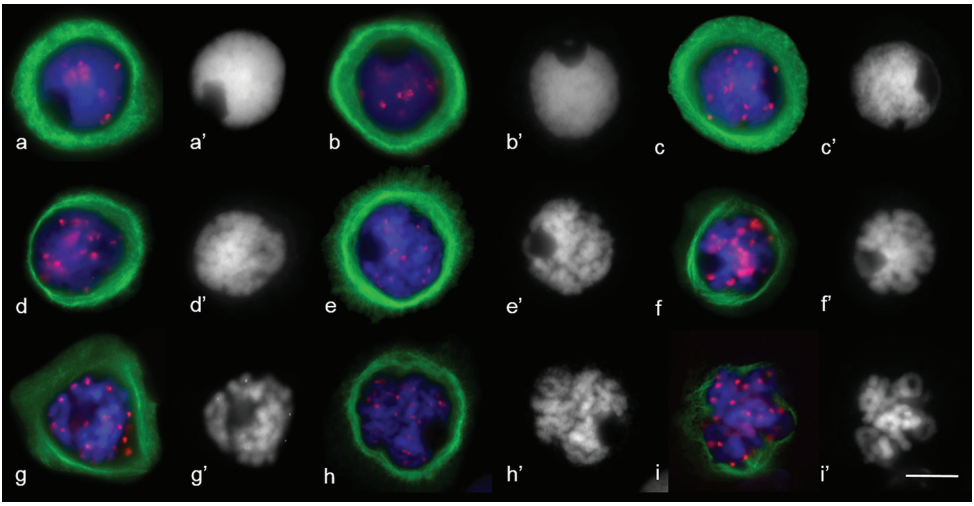

Figure 4. MT cytoskeleton dynamics in wheat prophase **a** Zygotene **b** pachytene **c** diplotene **d–h** diakinesis **i** prometaphase I. Immunostaining was undertaken with antibodies specific to α–tubulin (green) and CENH3 (red), DAPI counterstaining (**a'–i'**). DAPI (blue). Scale bar: 10 μm.

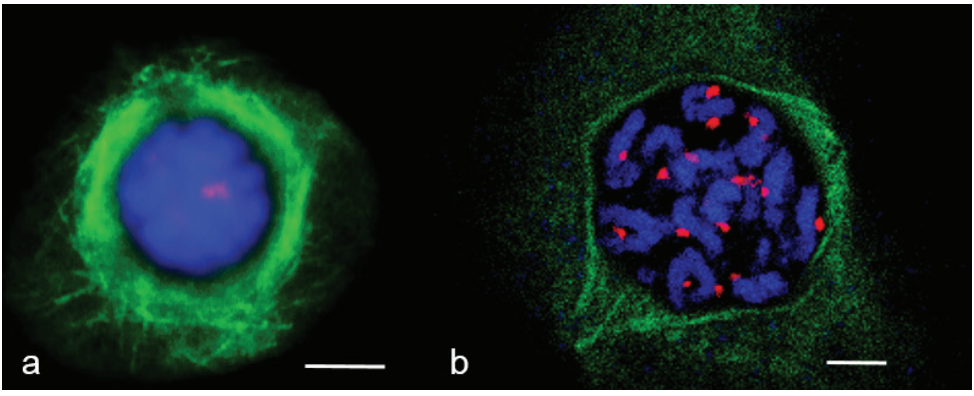

Figure 5. Pro–spindle formation at diakinesis in rye (**a**) and wheat (**b**). Immunostaining was undertaken with anti–α–tubulin (green) and anti–CENH3 (red) antibodies, DNA staining with DAPI (blue). Scale bar: 5 μm.

taneously in the prometaphase (Fig. 6a, b). Bivalents were present in meiocytes at the late prometaphase outside the metaphase plate, while homologue kinetochores formed MT bundles towards the spindle mid-zone or spindle poles (Fig. 6d, e). All bivalents at MI were positioned in the mid-region (Fig. 6f), the bipolar spindle being formed with kinetochore and interpole MTs (Fig. 6f (1, 2)). Unlike the mitotic spindle, the meiotic one had convergent poles (Fig. 6e, f). Kinetochore MT depolymerised at AI, with homologues separating to the opposite poles. Inter-regional microtubule systems could be observed in mid-late AI (Fig. 6h), which was involved in phragmoplast formation at telophase I (Figs 6h, i, 7a, b).

In prometaphase II, MTs nucleated near chromosomes and on kinetochores, where anti-phH3Ser10 was localised (Fig. 6j). Interpole and kinetochore MTs con-

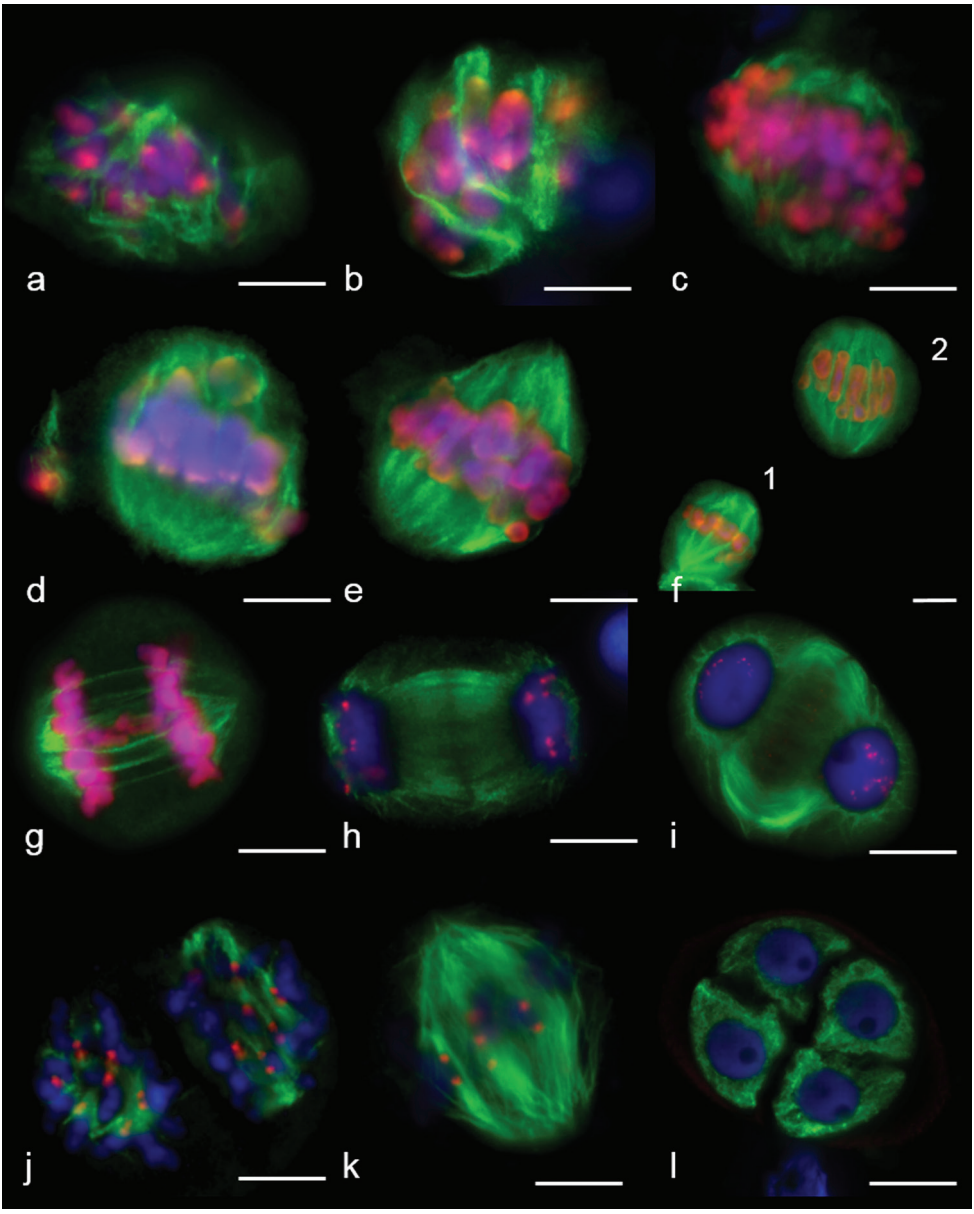

Figure 6. MT arrays in the first and second meiosis of wheat and rye (**j**). **a, b** early prometaphase I **c–e** prometaphase I **f** metaphase I, 1 – kinetochore MTs in the focus, 2 – interpolar MTs in the focus **g** anaphase I **h–i** telophase I **j** prometaphase II **k** one half of metaphase II **l** telophase II. Immunostaining was undertaken with antibodies specific to α–tubulin (green) and histone phH3Ser10 (red), DNA staining with DAPI (blue). Scale bar: 10 μm.

tinued polymerisation in the late prometaphase and then they converged at the poles and re-organised into a bipolar spindle (Fig. 6k). Meiosis ended with cytokinesis and development of four microspores (Fig. 6l).

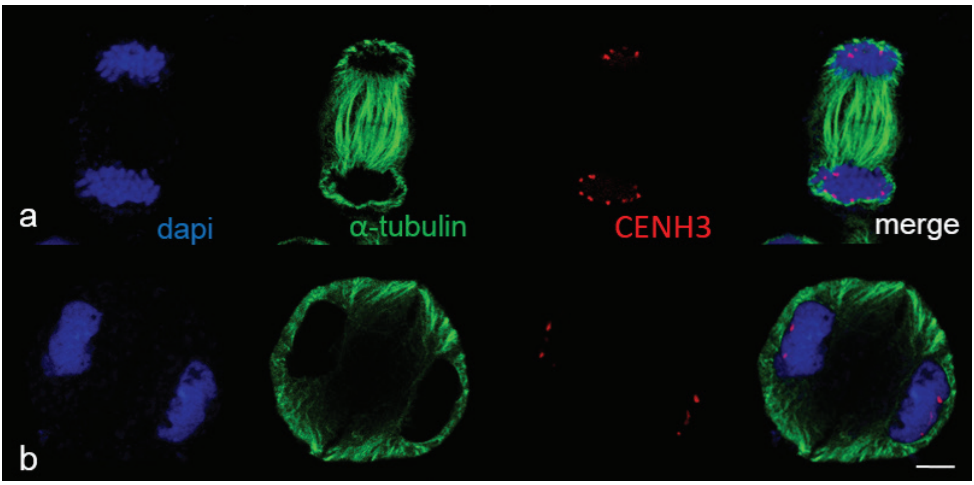

Figure 7. Phragmoplast expansion at anaphase I (**a**) and telophase I (**b**) in wheat. DNA was undertaken by staining with DAPI (blue). Z–stacks, confocal microscopy. Immunostaining was undertaken with anti–α–tubulin (green) and anti–CENH3 (red) antibodies. Scale bar: 5 μm.

Chromosome segregation in meiosis of 2R(2D)xR amphihaploids with reductional division

The main hybrid feature was a random distribution of univalent chromosomes between poles in the first division, while bivalents, whether rod or ring, lagged at the equatorial plane (Figs 8, 13c). Bivalents formed in 60.35±2.05% of meiocytes (Table 2). The average number of bivalents was 1.18±0.06 per PMC undergoing the reductional division and 0.15±0.03 per PMC undergoing equational+reductional division (Table 2). Amongst meiocytes with reductionally-dividing chromosomes, 41.11% of PMCs had no bivalents, while 18.48, 28.63, 10, 1.49, 0.29% of PMCs had 1, 2, 3, 4 and 5 bivalents, respectively (Fig. 8).

To understand the meiotic mechanisms of chromosome divergence in hybrids, the formation and functioning of the division apparatus are analysed. An analysis of MTs dynamics in meiosis, using Navashin fixation, showed MT re-organisation in pachytene: first, MTs were positioned cortically (Fig. 9a) and then perinuclear (Fig. 9b); a tight MT perinuclear ring was formed in diplotene (Fig. 9c). The ring could not be visualised after the destruction of the nuclear envelope; isolated MT arrays were observed (Fig. 9d). At metaphase I, chromosomes were in the cell centre in close contact with each other, but without the formation of the classic metaphase plate (Fig. 9e). MT developed bundles, but no bipolar spindle was found (Fig. 9e). At anaphase I, chromosomes were mostly arranged into two groups (Fig. 9f, g). The spindle had a curved form in 66.65±3.35% meiocytes (Table 3) (Fig. 9f, g, l) and the spindle was straight (as usual) in 29.35±3.0% (Table 3) (Fig. 9h, k) or chaotic MT bundles were present in a cell (Fig. 9i, j). Phragmoplast developed at the end of the first division (Fig. 9m-o), cytokinesis occurred and a cell wall was formed (Fig. 9p). In some cases, a cell plate was not observed, chromosomes remained condensed and

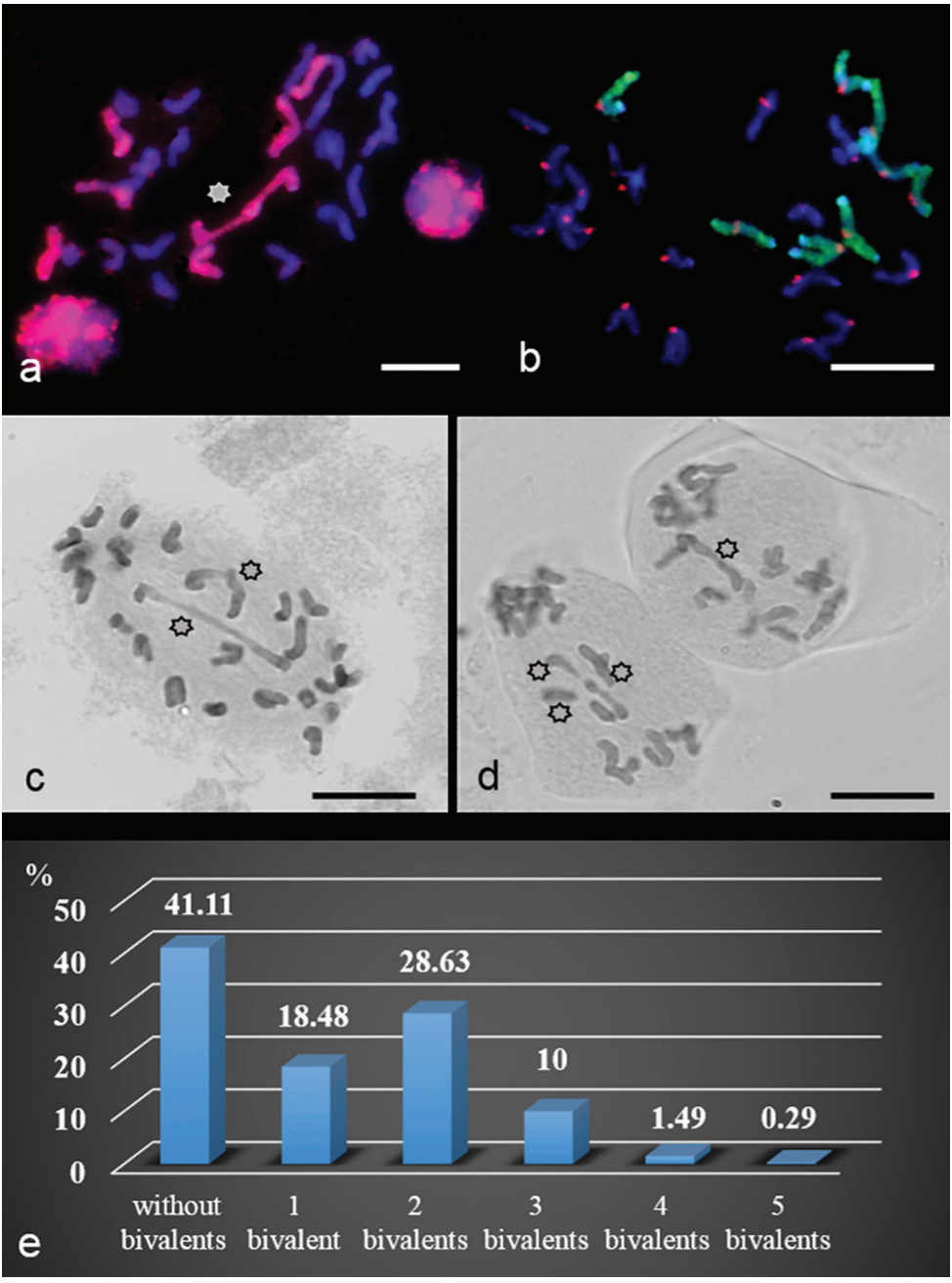

Figure 8. Bivalent formation in hybrids 2R(2D)xR. **a, c, d** bivalent formation (*sun*) **b** bivalent lacking **c** The distribution of meiocytes with different numbers of bivalent chromosomes. Rye chromosomes labelled red (**a**) and green (**b**), centromeres labelled red (**b**). Scale bar: 10 μm.

telophase groups were not formed (Fig. 9n). A MT bipolar spindle was assembled in the second division (Fig. 9q), phragmoplast developed after chromosome separation (Fig. 9r) and cytokinesis occurred.

Table 2. Bivalent formation according to chromosome division type in 2R(2D)xR hybrids.

The mean number of bivalent per cell			The percent of meiocytes with bivalent chromosomes		
reductional	**equational + reductional**	**overall**	**reductional**	**equational + reductional**	**overall**
1.18±0.06	0.15±0.03	1.09±0.05	60.35±2.05	3.06±0.9	63.41±1.69

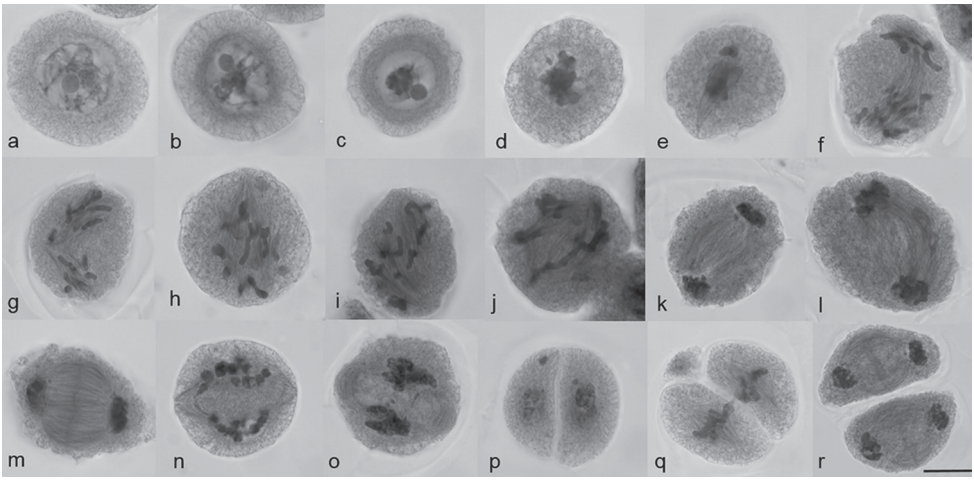

Figure 9. MTs dynamics in meiosis of hybrids 2R(2D)xR. **a–c** prophase I **d** prometaphase I **e** metaphase I **f–j** anaphase I **k–o** telophase I **p** interkinesis **q** metaphase II **r** telophase II. Scale bar: 10 μm.

Immunostaining with anti-α-tubulin revealed the specifics of MT dynamics in the first meiosis. Kinetochores were visualised using antibodies to CENH3 as a means to distinguish chromosomes from one another. Given that phosphorylation of histone H3Ser10 residue in plants is cell-cycle dependent and related to cohesion maintenance, we used anti-H3Ser10ph as a marker of cohesion upon sister chromatid segregation and to visualise meiotic stages.

At the early stages of prophase (leptotene-zygotene), meiocytes contained networks of cytoplasmic MTs (Fig. 10a). These MTs appear to be randomly arranged and tangential to the nuclear surface, a tight narrow perinuclear ring forming near the nuclear envelope (Fig. 10a). Both tight and more disperse cortical MT arrays were found at pachytene in different meiocytes (Fig. 10b, c).

The presence of a bright α-tubulin halo around the nucleus was a common feature of meiocytes at diplotene (Fig. 10d). MTs were arranged unevenly inside the halo, while autonomous circular bright and tight α-tubilin signals were found. As diakinesis progressed, MT arrays demonstrated different shape, density and distribution (Fig. 10e – h). MT arrays crossed over each other or were organised in a parallel manner (Fig. 10e). Before the nuclear envelope breakdown, some meiocytes contained noticeable triangular MT arrays with one focused pole (Fig. 10h). Destruction of the nuclear envelope at diakinesis was accompanied by its invagination (Fig. 10i). The

Table 3. The percentage of meiocytes with different forms of spindle in the first meiosis of 2R(2D)xR hybrids.

Curved spindle	Straight spindle	3–poles spindle
66.65±3.35	29.35±3.0	0.68±0.41

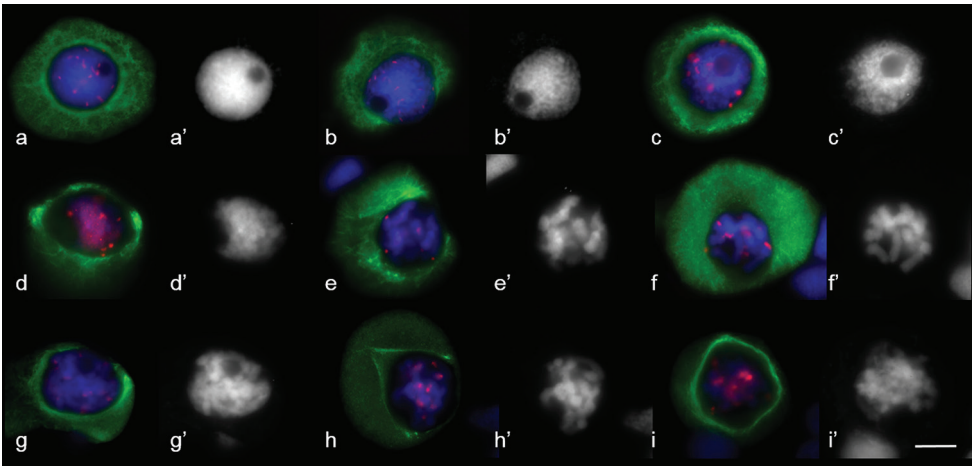

Figure 10. MT cytoskeleton dynamics in 2R(2D)xR hybrids prophase. **a, b** zygotene **c** pachytene **d** diplotene **e–h** diakinesis **i** prometaphase I. Immunostaining was undertaken with anti–α–tubulin (green) and anti–CENH3 (red) antibodie,. DAPI counterstaining **(a' – i').** DAPI (blue). Scale bar: 10 μm.

perinuclear MTs ring changed its shape. The specific feature of prophase substages in hybrids was the migration of nuclei to the cell edge.

At prometaphase, the microtubules appeared to nucleate from multiple sites in the cells and surround the chromatin (Fig. 11a, b). Microtubules even appeared to emanate directly from the chromosome surface. Later, we detected large branched bundles of microtubules associated with kinetochores (Fig. 11c, d). Chromosomes are not aligned at the metaphase plate; they were located close to each other in the centre of the meiocyte (Fig. 11c, d). MT bundles elongated and attempted to cross-link (Fig. 11c, d).

We identified meiocytes where chromosomes were divided up into groups as anaphase I (Fig. 11e–h). Since interpolar MTs were not found (Fig. 12c), the standard bipolar spindle was not formed and we marked the sites where kinetochore MTs focused as "pole". At this stage, MTs' minus-ends attempted to focus or were "searching" the pole site (Fig. 11e, f, h). Although MT bundles could connect kinetochores as bridges, perhaps they compensated for the absence of inter-pole MTs (Figs 11a, 12c, e). MT kinetochore bundles cross-linked and grew to the "pole" (Fig. 12a). In some cases, MT bundles did not cross-link and were not focused and chromosomes were not grouped (Fig. 11g). Due to the presence of bright strong signals of α-tubulin, kinetochores seem to be MT nucleation sites (Figs 12a–c, 13a–d). In all described cases, chromosome kinetochores were monopolar-orientated and, at anaphase I, we observed single tight pin-pointed anti-CENH3

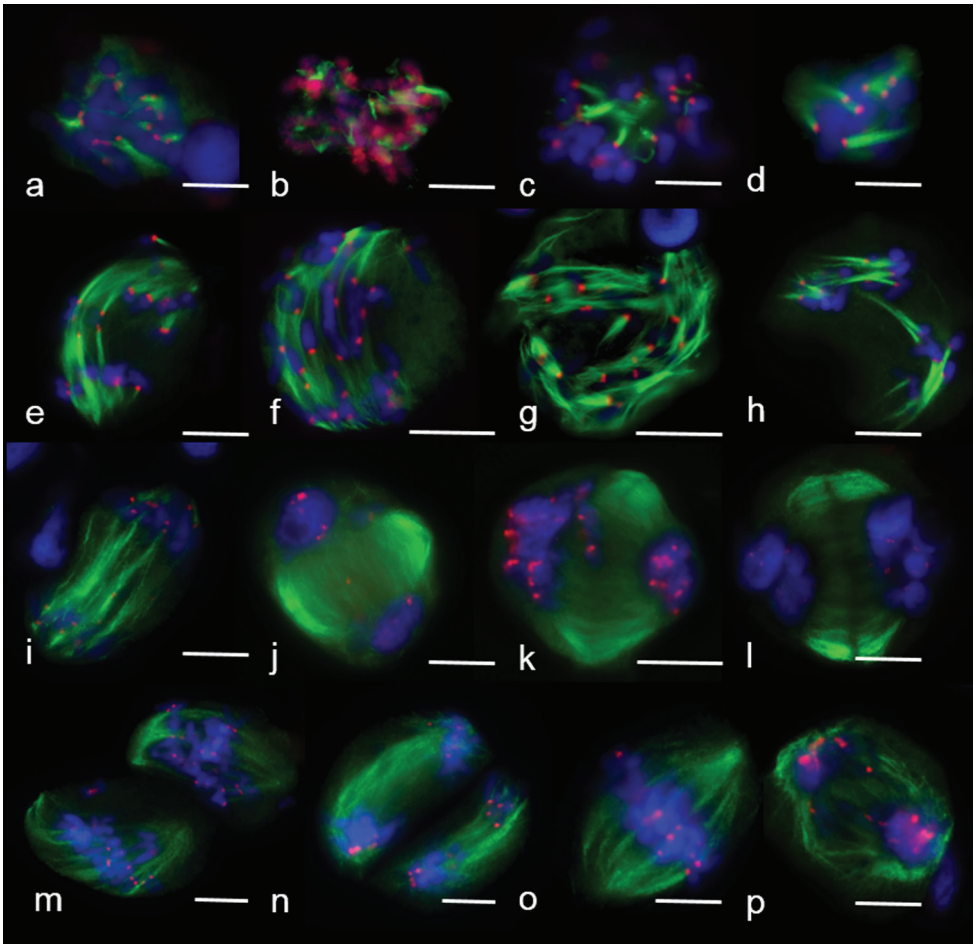

Figure 11. MT arrays in the first and second meiosis of 2R(2D)xR hybrids. **a, b** early prometaphase I **c, d** metaphase I **e–h** anaphase I **i** the late anaphase I **j–l** telophase I **m** metaphase II **n** anaphase II **o** one–half of meiocytes at metaphase II **p** one–half of meiocytes at anaphase II. Immunostaining was undertaken with antibodies specific to α–tubulin (green) and CENH3 (red) **(a, c–t)** and **(b)** histone phH3Ser10 (red), DNA staining with DAPI (blue). Scale bar: 10 μm.

signals, matching the integrated kinetochore of sister chromatids (Figs 11e–h, 12a–c). We did not see changes in monopolar orientation of kinetochores to bipolar (re-orientation). Sometimes, chromosomes with two anti-CENH3 signals, from which MT bundles grew to opposite poles, were found in meiocytes (Figs 11e, 12b). Such chromosomes were delayed on the mid plane after the divergence of univalents with a monopolar orientation. If bivalents were present in meiocytes, first, univalents were arranged between "poles" while the bivalent was delayed in the spindle mid-zone (Fig. 13b).

Other specifics of reductional chromosome separation include the absence of division of sister chromatids at anaphase I, which was identified by the absence of

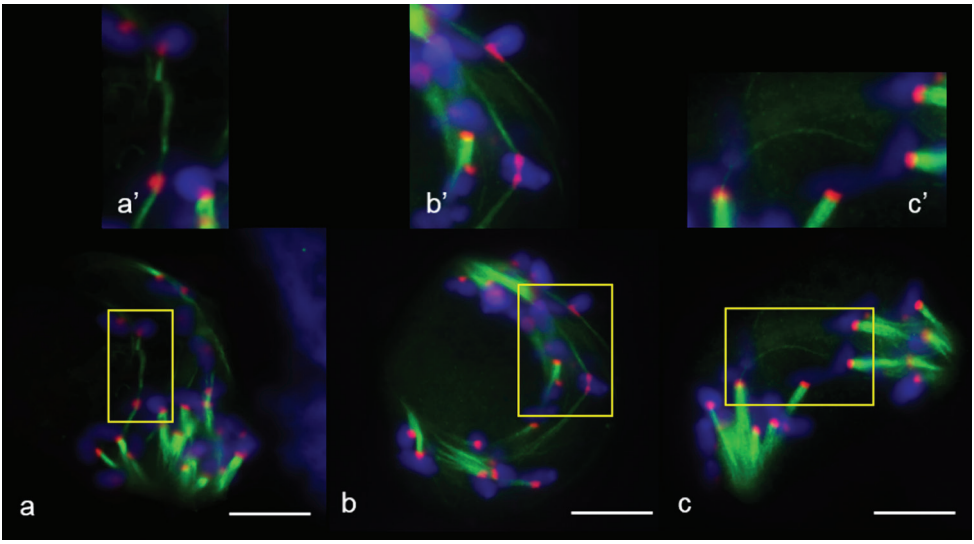

Figure 12. Bipolar and monopolar kinetochore orientation at anaphase I in meiosis of 2R(2D)xR hybrids. **a** kinetochores linked by microtubules **b** bi–polar kinetochore orientation **c** thin kinetochore MT bundle – like interpolar MT bundle. Immunostaining was undertaken with anti–α–tubulin (green) and anti– CENH3 (red) antibodies, DNA staining with DAPI (blue). Scale bar: 10 μm.

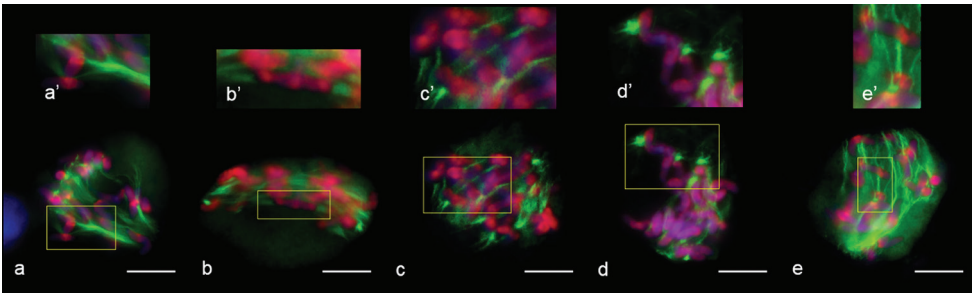

Figure 13. Patterns of MT arrays distribution in the first meiosis of 2R(2D)xR hybrids. **a** cross–link of three MT kinetochore bundles, one of them with bipolar orientation **b** bivalent lies in the metaphase plate **c, e** MT bridges between kinetochores **d** kinetochores as sites of MT nucleation. Immunostaining was undertaken with antibodies specific to α–tubulin (green) and histone phH3Ser10 (red). Scale bar: 10 μm.

"x" shaped chromosomes. phH3Ser10 localisation during chromosome separation in meiosis I was characterised by more intensive staining of centromeres compared to chromosome arms (Fig. 13). After chromosome separation, between telophase groups, inter-zonal MTs assembled (Fig. 11i). It seems they were formed by kinetochore microtubules (Fig. 11i). Phragmoplasts without a formed cell plate were found (Fig. 11j); however, cytokinesis occurs in most meiocytes (Fig. 11k, l). Meiocytes were capable of progressing through meiosis II. These cells also contained apparently normal bipolar spindles (Fig. 11m–p); however, chromosomes did not always segregate properly.

Discussion

Specifics of the MT-based spindle assembly in mitosis of rye and wheat

In mitosis after the nuclear envelope breakdown (NEB), MTs growing from polar caps become a source of MTs of the interpolar spindle. At the same time, regardless of the pro-spindle, MTs nucleate near the chromosomes/kinetochores during the prometaphase (nucleation depends on RanGTP gradient or aurora kinase) and those MTs are then organised into an overall bipolar configuration (Yamada and Goshima 2015). We discovered asymmetric MT arrangements after NEB in the prometaphase of rye and wheat mitosis. The common feature at the onset of prometaphase was nucleation and MT polymerisation near the kinetochores. At this stage, chromosomes maintained their Rabl-orientation. Wheat formed a pole from the kinetochores side and MT polymerisation was towards chromosome telomeres. It seems that chromosome relocation and continued spindle assembly took place simultaneously. Kinetochores were also the site of MT nucleation in rye; further MT polymerisation had a flame-like shape. As a result, a tight MT array formed on the telomere side. Subsequently, the spindle assembly occurred similarly to the wheat assembly. Such asymmetry in bipolar spindle assembly was registered using live imaging of microtubules in *A. thaliana* (Komis et al. 2017). Pro-spindle re-organisation began before PPB disruption, MT re-arrangement started from one PPB side (one half of spindle) and then the second half of the spindle assembled simultaneously with PPB disassembly (Komis et al. 2017, video 8). The same time was needed to align chromosomes in the spindle mid-zone and form a robust bipolar spindle as for assembling a prometaphase bipolar spindle. Multipolar, apolar and monopolar prophase spindles are relatively common in *Haemanthus katherinae* (Smirnova and Bajer 1992). During the prometaphase, these three types of spindle differentiate invariably into the bipolar metaphase spindle (Smirnova and Bajer 1992).

Meiotic spindle assembly in rye and wheat

Meiotic spindle assembly was studied for several species of dicotyledon and monocotyledon plants (Chan and Cande 1998, Shamina 2005, Xue et al. 2019). In maize meiocytes, a 'self-assembly' model for spindle formation was proposed (Chan and Cande 1998). According to the model, MTs initially appear around the chromosomes during the prometaphase, followed by self-organisation of the MTs into a bipolar spindle (Chan and Cande 1998). In both monocots (rice, maize) and dicots (tobacco, *Arabidopsis* Heynhold, 1842), a multipolar spindle has been found at early metaphase I and this re-organises into a bipolar spindle at the metaphase I (Xue et al. 2019). The authors postulate that the transition from multipolar spindles into bipolar spindles is a common process in both monocots and dicots (Xue et al. 2019). According to another model, the MTs' perinuclear ring is the structure specific to the late prophase (Shamina 2005). At the beginning of the prophase, the ring degrades. MTs interact with chromosome kinetochores and with each other and form a chaotic bipolar array which is then focused and orientated into the spindle (Shamina 2005). Spindle development

in the first *A. thaliana* meiosis is accompanied by a specific arrangement of MT arrays (Prusicki et al. 2019). Half-moon MT arrays are found in late prophase and these are later transformed into full-moon ones surrounding the nucleus. The tightening of MT arrays around the nucleus was called the "prophase spindle", similar to what is observed in mitosis. When a nuclear envelope degrades, the prophase spindle is then disassembled and a robust bipolar spindle is formed (Prusicki et al. 2019).

We found in wheat that the prophase MTs arranged similarly to that which was described by Shamina (2005). A perinuclear ring also formed at diakinesis; then, however, the MTs that form the ring re-orientated and structures that looked like mitotic pole caps or pro-spindle developed. In rye diakinesis, MTs arrays formed a diamond-shape prophase spindle, similar to the pro-spindle of *A. thaliana* (Prusicki et al. 2019). Rye and wheat showed simultaneous development of interpolar and kinetochore MT bundles in the prometaphase. We did not find multipolar spindles either in the later prometaphase or in the early metaphase. Perhaps, assembly of the meiotic spindle in wheat and rye occurs similarly to the mitotic spindle assembly, while the pro-spindle "poles" mark the sites of future poles of the metaphase spindle. In plant mitosis, ɣ-tubulin exhibits bipolar localisation from prophase to anaphase (Binarová et al. 2000). TPX2 participates in the pro-spindle formation; it concentrates in the polar caps (Vos et al. 2008).

The function of Anaphase Promotion Complex APC/C^{cdc20} fails in 2R(2D) xR hybrids

Chromosome separation in normal meiosis I has its specifics. Single DNA replication occurs in the S-phase, when DNA copies (sister chromatids) are captured by a ring-shaped protein complex called cohesin. In the meiosis I prophase, homologues pair and become joined by a synaptonemal complex and then exchange DNA reciprocally during crossover recombination, forming chiasms (Petronczki et al. 2003, Zamariola et al. 2014, Ohkura 2015, Zickler and Kleckner 2016). MTs of a meiotic spindle establish attachments to the bivalents. Two sister kinetochores of each homologue are captured by the same pole, not by opposite poles as occurs during meiosis II and mitosis. As soon as bipolar homologue attachment takes place and bivalents align on the metaphase plane, meiotic cohesion between chromosome arms is destroyed, allowing the homologues to segregate at the opposite poles (Petronczki et al. 2003, Ohkura 2015). Two sister kinetochores of each homologue remain tethered by the surviving cohesin complexes before meiosis II. In meiosis II, sister kinetochores are captured by the opposite poles and move apart when the remaining cohesin complexes are destroyed.

Random distribution of chromosomes in meiosis I of 2R(2D)xR hybrids was characterised by monopolar kinetochore orientation and their side-by-side geometry, as well as maintained cohesion between sister chromatids in the anaphase. Normally, absence of bipolar attachments of kinetochores and their tension between the poles cause an anaphase delay due to insertion of a spindle assembly checkpoint (SAC). Components of this complex include evolutionally conservative proteins Chromosome Passenger Complex (CPC): the Ser/Thr kinases monopolar spindle 1 (MPS1),

Aurora B and Budding Uninhibited by Benomyl 1 (BUB1) and BUB3 and the non-kinase components Mitotic Arrest Deficient 1 (MAD1), MAD2, BUB1 Related kinase 1 (BUBR1), Cell Division Cycle 20 (CDC20) (Kang and Yu 2009, Lara-Gonzalez et al. 2012, Zamariola et al. 2014). All these proteins localise to unattached kinetochores and generate a kinetochore signal that inhibits the anaphase-promoting complex/cyclosome. APC/C^{CDC20} remains inactive until all sister chromatids are attached to the mitotic spindle and under tension indicating an equal alignment of the chromosomes in the metaphase plate (Kang and Yu 2009, Lara-Gonzalez et al. 2012, Wijnker and Schnittger 2013). APC/C is activated twice in meiosis: at anaphase I and anaphase II (Yamamoto et al. 2008).

Absence of APC/C activity can be one of the reasons for chromosome separation with monopolar orientation and maintaining cohesion with sister chromatids. APC/C can be inactive due to the absence or disrupted signal transfer from SAC proteins. Homologues of SAC proteins are involved in plant meiosis, including maize MAD2 (Yu et al. 1999), rice BRK1 (BUBR1) (Wang et al. 2012) and *A. thaliana* Aurora kinases (Demidov et al. 2014). MAD2 localises on an outer kinetochore and is necessary for sensing the amount of tension at a kinetochore (Yu et al. 1999). MAD2 plays the key role in SAC, since it bonds with CDC20 on a kinetochore with abnormal attachment to MTs (Kang and Yu 2009, Lara-Gonzalez et al. 2012). It is known that to activate APC/C, one of the two CDC20 or Cdh1 co-factors is necessary (Lara-Gonzalez et al. 2012). In wheat, the MAD2 gene is mapped on the chromosomes of the 2nd homoeologous group (Kimbara et al. 2004). It is assumed that MAD2 protein is involved in SAC control in wheat mitosis as, in the course of colchicine treatment of dividing cells, MAD2 remains on kinetochores in the metaphase, but normally it is absent (Kimbara et al. 2004). Perhaps, the genotype of the disomic substitution line 2R(2D), used in rye crossing, has the MAD2 gene. In this case, MAD2 protein in the complex with CDC20 is presumably localised on kinetochores and, in the absence of kinetochore tension due to its monopolar orientation, the MAD2/CDC20 complex is maintained and APC/C is not activated. As a result, the cohesion between the kinetochores and arms of sister chromatids does not cleave.

Otherwise, release of cohesion may also be impossible due to activation of Aurora B kinase. Aurora B controls multiple aspects of cell division and plays a key role in bipolar spindle assembly (Zhang and Dawe 2011, Joukov 2011). H3Ser10 histone in plants is a substrate of AtAurora3 (Kawabe et al. 2005) and it is shown that phH3Ser10 and AtAurora3 are localised in centromeric regions of mitotic chromosomes (Demidov et al. 2005). For most plant species, phosphorylation of H3Ser10 is typical for only pericentromeric regions in mitosis and the second division of meiosis and along the entire length of the chromosomes in the first meiotic division (Loginova and Silkova 2017). Based on these data, a conclusion is reached that localisation of phH3Ser10 in plants is associated with maintained cohesion (Kaszas and Cande 2000, Manzanero et al. 2000, Houben et al. 2007). Therefore, phosphorylation of H3Ser10 can indicate involvement of Aurora3 (Aurora B) in maintaining cohesion on kinetochores. In 2R(2D)xR hybrids, maintaining cohesion was identified indirectly by localisation of

phH3Ser10 along all chromosomes (Fig. 13). phH3Ser10 localisation during chromosome separation in meiosis I was characterised by more intensive staining of centromeres compared to chromosome arms (Fig. 13). Probably, Aurora-kinase is localised on monopolar-orientated kinetochores and cohesion is maintained. It turned out that the CDC20, APC/C co-factor, regulates Aurora localisation on chromosomes in meiosis of Arabidopsis (Niu et al. 2015). Abnormal distribution of H3Ser10 and H3Thr3 histones, phosphorylated with Aurora-kinase, is found in meiocytes of *cdc20.1* mutant (Niu et al. 2015). In meiosis of *cdc20.1* mutants, chromosomes align asynchronously and segregate unequally and the metaphase I spindle has aberrant morphology (Niu et al. 2015). These findings indicate the involvement of CDC20.1 in SAC-dependent segregation of meiotic chromosomes (Niu et al. 2015).

On the other hand, why are monopolar-orientated kinetochores in 2R(2D)xR hybrids unable to re-orientate bipolarly at metaphase I? Bi-orientation of sister kinetochores in a univalent is essential for bipolar spindle formation when homologous recombination is absent (Chan and Cande 1998, Xue et al. 2019). We found bipolar orientation in hybrids with 1Rv(1A)xR, 5R(5D)xR and 6R(6A)xR lines (Silkova and Loginova 2016). In 2R(2D)xR meiocytes, there were only rare univalents on the mid section with two CENH3 signals and bipolar orientated MT bundles or univalents near poles had extended kinetochores (dumb-bells) and two attached MT bundles. What can affect the inability of kinetochores for bipolar re-orientation? The reasons for the monopolar orientation in 2R(2D)xR hybrids may be the formation of the MIS12 – NDC80 bridge at the kinetochore (Li and Dawe 2009) plus the protection of centromeric cohesion of univalents by SGO1 from destruction (Kitajima et al. 2004, Zamariola et al. 2013). SGO1 recruits PP2A at centromeres to dephosphorylate REC8, making it resistant to separase cleavage.

Apart from CPC proteins, γ-tubulin complex protein 3–interacting proteins (GIPs) are essential for the proper recruitment and/or stabilisation of centromeric proteins, as well as for centromeric cohesion in somatic cells (Batzenschlager et al. 2015).

The bipolar spindle in meiosis of asynaptic mutants of maize and rice haploids is formed regardless of the presence of bivalents (Chan and Cande 1998, Xue et al. 2019). On the contrary, in 2R(2D)xR hybrids, a bipolar spindle is not formed in the presence of bivalents. Therefore, cohesion release, but not the presence of bivalents, may affect bipolar spindle formation in meiosis. Cohesion between univalent arms and sister chromatids of bivalents can be maintained due to the CDC20 co-factor related to CPC proteins, while cohesion in the centromeric region may be protected by SGO1.

Chromosome segregation without bipolar spindle in 2R(2D)xR hybrids

MT arrangements throughout prophase in 2R(2D)xR hybrids deviated from the norm. The main features were nucleus migration at all stages of prophase and uneven distribution of cortical MTs. MTs formed a triangular pro-spindle in diakinesis and bright tight signals of α-tubulin were localised in the triangle angles, perhaps at the sites of MT nucleation. At the prometaphase, the microtubules appeared to nucleate

from kinetochores and to surround the chromatin. MT bundles were evident from the chromosome mass at metaphase I. Zhang and Dawe (2011) have also observed small kinetochore fibres in barley (*Hordeum vulgare* Linnaeus, 1753), formed immediately after NEB, suggesting that plant kinetochores may initiate their own kinetochore fibres early in the prometaphase. Many proteins involved in MT nucleation and spindle assembly interact with kinetochores. It has been demonstrated that RanGAP1 associates with kinetochores in mitosis (Lipka et al. 2015). TPX2 may both catalyse new MT nucleation from ɣ-TuRCs around chromatin in a GTP-Ran dependent pathway and stabilise kinetochore fibres (Aguirre-Portoles et al. 2012). In meristem cells of *Vicia faba* (Linnaeus, 1753), an association of ɣ-tubulin with kinetochores and kinetochore fibres has been described after release from amiprophos-methyl treatments (antimicrotubular drugs) and on isolated chromosomes (Binarova et al. 1998), suggesting the possibility of MT nucleation at kinetochores.

It is also unclear why the metaphase I stage was not blocked. On the contrary, a tight chromosome mass with protuberant MT bundles was able to arrange chromosomes. Perhaps, when there are no bipolar-orientated chromosomes, there is no issue with 'release of cohesion' and the system of motor proteins and MT kinetochores can arrange chromosomes in 2R(2D)xR hybrids. At the beginning of anaphase I, univalents of 2R(2D)xR hybrids were arranged mainly into two groups and their kinetochore MTs cross-linked and focused. The single MT bundle was polymerised on kinetochores and inter-regional MT arrays were not present. Few interpolar microtubule bundles could be found in meiocytes, which were very thin compared to massively-wide MT bundles of kinetochores. Kinetochore MT bundles could be generated by the γ-TuRC-Augmin-mediated nucleation (Murata and Hasebe 2007, Lee et al. 2017). Chromosome separation and spindle poles focusing could occur through the functioning of kinesin–14A motor protein. The *Arabidopsis* kinesin–14A Atk1/AtKIN14a is involved in the assembly of the meiotic spindle and is needed for organising MTs at the two poles at metaphase and anaphase I and II (Chen et al. 2002) and the *divergent spindle–1(dv1)* gene encodes kinesin–14A that is specifically required for focusing the spindle pole to a fine point (Higgins et al. 2016).

A cell plate is formed after chromosome separation in a plant cell (De Storme and Geelen 2013). The cell plate is synthesised by a specialised structure called the phragmoplast, which consists of microtubules, actin filaments, membrane compartments and associated proteins (Murata et al. 2013, Smertenko et al. 2018). The phragmoplast forms between daughter nuclei during the transition from anaphase to telophase and originates from the remnants of the central spindle (Seguí-Simarro et al. 2007, Murata et al. 2013). The cases of *Arabidopsis* and *Physcomitrella paten* (Hedwig, 1801) moss show that MAP65 isotypes are localised in the mid-zone, where they stabilise the phragmoplast structure by cross-linking anti-parallel microtubules (Smertenko et al. 2000, Muller et al. 2004, Van Damme et al. 2004, Kosetsu et al. 2017), which presumes the need for anti-parallel microtubules to build phragmoplast.

MT bundles linking kinetochores were found at anaphase I of 2R(2D)xR between separated chromosome groups. Probably those MT arrays replaced inter-zonal MTs,

as the bipolar spindle did not assemble in hybrids. Despite the absence of anti-parallel microtubules, a phragmoplast formed after chromosome separation. According to Smertenko et al. (2018), three zones form with distinct patterns of microtubule behaviour in the phragmoplast: the outer leading zone, the transition zone and an inner lagging zone. New MTs are formed in the outer leading zone; here cell plate assembly is initiated. A cell plate acquires the standard appearance in the transition zone through vesicle joining and migrating; the balance of microtubule polymerisation and depolymerisation is maintained here. In the inner lagging zone, the cell plate is practically formed and microtubules are depolymerised. As cytokinesis progresses and the phragmoplast array expands, microtubules are lost from the central region of the cell. Phragmoplast formation in hybrids, however, was delayed; it was found only in telophase and not in all meiocytes at the same time. Meiocytes were found where the leading edge of the phragmoplast reached the plasma membrane, but in a lagging zone, a cell plate was not formed. Nevertheless, phragmoplast was present in most cases, since 96% of meiocytes had tetrads at telophase II (Silkova et al. 2011). In wheat male meiosis, cytokinesis is successive, when each meiotic cell division is directly followed by a cytokinesis. Dyads are generated after meiosis I and tetrads are formed after meiosis II.

It is conceivable, perhaps, that replacing inter-zonal microtubules with the kinetochore ones in hybrids assumes another way/regulation of phragmoplast formation. Anti-parallel microtubules in normal meiosis constitute a phragmoplast "blank" and their absence in hybrids delays and interrupts phragmoplast expansion. However, meiocytes in meiosis II contained apparently normal bipolar spindles.

Conclusions

Currently there is no universal model of spindle formation in plant meiosis. We discovered new structures in wheat and rye meiotic prophase and preprophase spindle. Based on it, we propose that chromatin– and pro-spindle-based cooperative mechanisms are needed to form a bipolar spindle in meiosis. Spindle assembly and pole marking in meiosis I take place similarly to mitosis. Probably, location sites of polar caps, for example, through ɣ-tubulin (Binarová et al. 2000, Canaday et al. 2004), retain memory to organise MTs in poles and their focusing.

Bipolar spindle in meiosis of asynaptic mutants of maize and rice haploids is formed regardless of the presence of bivalents (Chan and Cande 1998, Xue et al. 2019). On the contrary, in 2R(2D)xR hybrids, a bipolar spindle is not formed in the presence of bivalents. Therefore, cohesion release, but not the presence of bivalents, may affect bipolar spindle formation in meiosis. An anaphase promotion complex is activated only when a bipolar spindle is formed. Based on current data on the regulation of chromosome distribution during cell division, the sequence of possible events and their participants during chromosome segregation in the first meiosis of 2R(2D)xR amphihaploids can be represented as follows. At the prometaphase, the MAD2 protein in the complex with CDC20 is localised on kinetochores and MTs nucleate around

the chromosome/kinetochore (nucleation depends on RanGTP gradient or aurora kinase). Only kinetochore MT bundles polymerise. In the absence of kinetochore tension due to its monopolar orientation, the MAD2/CDC20 complex is maintained. Aurora-kinase is localised on monopolar-orientated kinetochores. APC/C is not activated. Overall, the cohesion between the kinetochores and arms of sister chromatids does not cleave and cohesion is maintained. Thus, cohesion between univalent arms can be maintained due to the CDC20 co-factor related to CPC proteins, while cohesion in the centromeric region may be protected by SGO1. At anaphase I, univalents separate and their kinetochore microtubules are cross-linked and focused through the functioning of the kinesin–14A motor protein. At telophase I, kinetochore MT arrays replaced inter-zonal MTs and phragmoplast formation must be modified.

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References

Aguirre-Portoles C, Bird A, Hyman A, Canamero M, Perez de Castro I, Malumbres M (2012) *Tpx2* controls spindle integrity, genome stability, and tumor development. Cancer Research 72(6): 1518–1528. https://doi.org/10.1158/0008-5472.CAN-11-1971

Ambrose JC, Cyr R (2007) The Kinesin ATK5 functions in early spindle assembly in *Arabidopsis*. The Plant Cell. 19(1): 226–236. http://www.plantcell.org/lookup/doi/10.1105/tpc.106.047613

Baskin TI, Cande WZ (1990) The structure and function of the mitotic spindle in flowering plants. Annual Review of Plant Physiology and Plant Molecular Biology 41: P277–315. http://doi.org/10.1146/annurev.pp.41.060190.001425

Batzenschlager M, Lermontova I, Schubert V, Fuchs J, Berr A, Koini MA, Houlné G, Herzog E, Rutten T, Alioua A, Fransz P, Schmit AC, Chabouté ME (2015) Arabidopsis MZT1 homologs GIP1 and GIP2 are essential for centromere architecture. Proceedings of the National Academy of Sciences of the United States of America 112(28): 8656–8660. https://doi.org/10.1073/pnas.1506351112

Bennabi I, Terret ME, Verlhac MH (2016) Meiotic spindle assembly and chromosome segregation in oocytes. Journal of Cell Biology 215(5): 611–619. https://doi.org/10.1002/9780470687970.ch10

Binarová P, Cenklová V, Hause B, Kubátová E, Lysák M, Dolezel J, Bögre L, Dráber P (2000) Nuclear γ-tubulin during acentriolar plant mitosis. The Plant Cell 12(3): 433–42. https://doi.org/10.1105/tpc.12.3.433

Binarova P, Hause B, Dolezel J, Draber P (1998) Association of gamma-tubulin with kinetochore/centromeric region of plant chromosomes. The Plant Journal 14(6): 751–757. https://doi.org/10.1046/j.1365-313×.1998.00166.x

Canaday J, Brochot AL, Seltzer V, Herzog E, Evrard JL, Schmit AC (2004) Microtubule assembly in higher plants. In: Pandalai SG (Eds), Recent Research Developments in Molecular Biology, Research Signpost, Trivandrum, India, 103–119 https://www.researchgate.net/publication/230817995

Chabout ME, Schmit AC Unique characteristics of cell division in vascular plants. (2016) In: Encyclopedia of Life Sciences. John Wiley & Sons Ltd (Eds): Chichester, 1–8. https://doi.org/10.1002/9780470015902.a0001686.pub3

Chan A, Cande WZ (1998) Maize meiotic spindles assemble around chromatin and do not require paired chromosomes. Journal of Cell Silences 111(23): 3507–3515. https://jcs.biologists.org/content/111/23/3507

Chen C, Marcus A, Li W, Hu Y, Calzada JP, Grossniklaus U, Cyr RJ, Ma H (2002) The *Arabidopsis ATK1* gene is required for spindle morphogenesis in male meiosis. Development. 129(10): 2401–2409. https://dev.biologists.org/content/129/10/2401

de Keijzer J, Mulder BM, Janson ME (2014) Microtubule networks for plant cell division. Systems and Synthetic Biology 8(3): 187–194. https://doi.org/10.1007/s11693-014-9142-x

De Mey J, Lambert AM, Bajer AS, Moeremans M, De Brabander M (1982) Visualization of microtubules in interphase and mitotic plant cells of *Haemanthus* endosperm with the immuno-gold staining method. Proceedings of the National Academy of Sciences of the United States of America 79(6): 1898–1902. https://doi.org/10.1073/pnas.79.6.1898

De Storme N, Geelen D (2013) Cytokinesis in plant male meiosis. Plant Signaling and Behavior 8: e23394. http://doi.org/10.4161/psb.23394

Demidov D, Van Damme D, Geelen D, Blattner FR, Houben A (2005) Identification and dynamics of two classes of aurora-like kinases in *Arabidopsis* and other plants. The Plant Cell. 17(3): 836–848. https://doi.org/10.1105/tpc.104.029710

Demidov D, Lermontova I, Weiss O, Fuchs J, Rutten T, Kumke K, Sharbel TF, Van Damme D, De Storme N, Geelen D, Houben A (2014) Altered expression of Aurora kinases in *Arabidopsis* results in aneu– and polyploidization. The Plant Journal 80(3): 449–461 https://doi.org/10.1111/tpj.12647

Dhonukshe P, Vischer N, Gadella T (2006) Contribution of microtubule growth polarity and flux to spindle assembly and functioning in plant cells. Journal of Cell Science 119(15): 3193–3205. https://doi.org/10.1242/jcs.03048

Hepler PK, Cleary AL, Gunning BES, Wadsworth P, Wasteneys GO, Zhang DH (1993) Cytoskeletal dynamics in living plant cells. Cell Biology International 17(2): 127–142. https://doi.org/10.1006/cbir.1993.1050

Higgins DM, Nannas NJ, Dawe RK (2016) The Maize *Divergent spindle–1 (dv1)* gene encodes a kinesin–14A motor protein required for meiotic spindle pole organization. Frontiers in Plant Sciences 7: 1277. https://doi.org/10.3389/fpls.2016.01277

Houben A, Demidov D, Caperta AD, Karimi R, Agueci F, Vlasenko L (2007) Phosphorylation of histone H3 in plants—a dynamic affair. Biochimica et Biophysica Acta 1769(5–6): 308–315. https://doi.org/10.1016/j.bbaexp.2007.01.002

Houben A, Orford SJ, Timmis JN (2006) *In Situ* Hybridization to Plant Tissues and Chromosomes. In: Darby IA, Hewitson TD (Eds) In Situ Hybridization Protocols. Methods in Molecular Biology™, V. 326. Humana Press, 203–218. https://doi.org/10.1385/1-59745-007-3:203

Joukov V (2011) Aurora kinases and spindle assembly: variations on a common theme? Cell Cycle 10(6): 895–903. https://doi.org/10.4161/cc.10.6.14909

Kang J, Yu H (2009) Kinase signaling in the spindle checkpoint. The Journal of Biological Chemistry 284(23): 15359–15363. https://doi.org/10.1074/jbc.R900005200

Kaszas E, Cande WZ (2000) Phosphorylation of histone H3 is correlated with changes in the maintenance of sister chromatid cohesion during meiosis in maize, rather than the condensation of the chromatin. Journal of Cell Sciences 113(18): 3217–3226. https://jcs.biologists.org/content/113/18/3217

Kawabe A, Matsunaga S, Nakagawa K, Kurihara D, Yoneda A, Hasezawa S, Uchiyama S, Kiichi F (2005) Characterization of plant Aurora kinases during mitosis. Plant Molecular Biology 58(1): 1–13. https://doi.org/10.1007/s11103-005-3454-x

Kimbara J, Endo T, Nasuda S (2004) Characterization of the genes encoding for MAD2 homologues in wheat. Chromosome Research 12(7): 703–714. https://doi.org/10.1023/B:CHRO.0000045760.42880.8c

Kitajima TS, Kawashima SA, Watanabe Y (2004) The conserved kinetochore protein shugoshin protects centromeric cohesion during meiosis. Nature. 427(6974): 510–517 https://doi.org/10.1038/nature02312

Komis G, Luptovciak I, Ovecka M, Samakovli D, Samajová O, Samaj J (2017) Katanin effects on dynamics of cortical microtubules and mitotic arrays in *Arabidopsis thaliana* revealed by advanced live-cell imaging. Frontiers in Plant Sciences 24(8): 866. https://doi.org/10.3389/fpls.2017.00866

Kosetsu K, Murata T, Yamada M, Nishina M, Boruc J, Hasebe M, Van Damme D, Goshima G (2017) Cytoplasmic MTOCs control spindle orientation for asymmetric cell division in plants. Proceedings of the National Academy of Sciences of the United States of America 114(42): E8847–E8854. https://doi.org/10.1073/pnas.1713925114

Lara-Gonzalez P, Westhorpe FG, Taylor SS (2012) The spindle assembly checkpoint. Current Biology 22(22): R966–80. https://doi.org/10.1016/j.cub.2012.10.006

Lee YJ, Liu B (2019) Microtubule nucleation for the assembly of acentrosomal microtubule arrays in plant cells. New Phytologist 222: 1705–1718. https://doi.org/10.1111/nph.15705

Lee YJ, Hiwatashi Y, Hotta T, Xie T, Doonan JH, Liu B (2017) The mitotic function of Augmin is dependent on its microtubule-associated protein subunit EDE1 in *Arabidopsis thaliana*. Current Biology 27(24): 3891–3897.e4. https://doi.org/10.1016/j.cub.2017.11.030

Li X, Dawe R (2009) Fused sister kinetochores initiate the reductional division in meiosis I. Nature Cell Biology 11: 1103–1108. https://doi.org/10.1038/ncb1923

Lipka E, Herrmann A, Mueller S (2015) Mechanisms of plant cell division. Wiley Interdisciplinary Reviews: Developmental Biology 4(4): 391–405. https://doi.org/10.1002/wdev.186

Loginova DB, Silkova OG (2017) H3Ser10 histone phosphorylation in plant cell division. Russian Journal of Genetics: Applied Research 7: 46–56. https://doi.org/10.1134/S2079059717010087

Manzanero S, Arana P, Puertas MJ, Houben A (2000) The chromosomal distribution of phosphorylated histone H3 differs between plants and animals at meiosis. Chromosoma 109(5): 308–317. https://doi.org/10.1007/s004120000087

Masoud K, Herzog E, Chabouté ME, Schmit AC (2013) Microtubule nucleation and establishment of the mitotic spindle in vascular plant cells. The Plant Journal 75(2): 245–57. https://doi.org/10.1111/tpj.12179

Mercier R, Mezard C, Jenczewski E, Macaisne N, Grelon M (2015) The molecular biology of meiosis in plants. Annual Review of Plant Biology 66: 297–327. https://doi.org/10.1146/annurev-arplant-050213-035923

Muller S, Smertenko A, Wagner V, Heinrich M, Hussey PJ, Hauser M-T (2004) The plant microtubule-associated protein AtMAP65–3/PLE is essential for cytokinetic phragmoplast function. Current Biology 14(5): 412–417. https://doi.org/10.1016/j.cub.2004.02.032

Murata T, Hasebe M (2007) Microtubule-dependent microtubule nucleation in plant cells. Journal of Plant Research 120: 73–78. http://link.springer.com/10.1007/s10265-006-0054-z

Murata T, Sano T, Sasabe M, Nonaka S, Higashiyama T, Hasezawa S, Machida Y, Hasebe M (2013) Mechanism of microtubule array expansion in the cytokinetic phragmoplast. Nature Communications 4.1967. https://doi.org/10.1038/ncomms2967

Niu B, Wang L, Zhang L, Ren D, Ren R, Copenhaver GP, Ma H, Wang Y (2015) Arabidopsis *cell division cycle 20.1* is required for normal meiotic spindle assembly and chromosome segregation.The Plant Cell 27: 3367–3368. https://doi.org/10.1105/tpc.15.00834

Ohkura H (2015) Meiosis: an overview of key differences from mitosis. Cold Spring Harbor Perspectives in Biology 7(5): a015859. https://doi.org/10.1101/cshperspect.a015859

Qi LL, Wu JJ, Friebe B, Qian C, Gu YQ, Fu DL, Gill BS (2013) Sequence organization and evolutionary dynamics of *Brachypodium* specific centromere retrotransposons. Chromosome Research 21:507–521. https://doi.org/10.1007/s10577-013-9378-4

Perpelescu M, Fukagawa T (2011) The ABCs of CENPs. Chromosoma 120(5): 425–46. https://doi.org/10.1007/s00412-011-0330-0

Petronczki M, Siomos MF, Nasmyth K (2003) Un menage a quatre: the molecular biology of chromosome segregation in meiosis. Cell 112(4): 423–440. https://doi.org/10.1016/S0092-8674(03)00083-7

Prosser SL, Pelletier L (2015) Centrosome biology: the ins and outs of centrosome assembly. Current Biology 25(15): R656–R659. https://doi.org/10.1016/j.cub.2015.06.038

Prusicki MA, Keizer EM, van Rosmalen RP, Komaki S, Seifert F, Müller K, Wijnker E, Fleck C, Schnittger A (2019) Live cell imaging of meiosis in *Arabidopsis thaliana*. Elife 8:e42834. https://doi.org/10.7554/eLife.42834.001

Quan L, Xiao R, Li W, Oh SA, Kong H, Ambrose JC, Malcos JL, Cyr R, Twell D, Ma H (2008) Functional divergence of the duplicated *AtKIN14a* and *AtKIN14b* genes: Critical roles in Arabidopsis meiosis and gametophyte development. The Plant Journal 53(6): 1013–1026. https://doi.org/10.1111/j.1365-313X.2007.03391.x

Seguí-Simarro JM, Otegui MS, Austin II JR, Staehelin LA (2007) Plant cytokinesis – insights gained from electron tomography studies. In: Verma DPS, Hong Z (Eds) Cell Division Control in Plants. Plant Cell Monographs. Springer, Berlin, Heidelberg, 9: 25–87. https://doi.org/10.1007/7089_2007_131

Severson AF, von Dassow G, Bowerman B (2016) Oocyte meiotic spindle assembly and function. In: Wassarmann P (Eds). Essays on Developmental Biology Part A. Current Topics in Developmental Biology 116: 65–98. https://doi.org/10.1016/bs.ctdb.2015.11.031

Shamina NV (2005) Formation of division spindles in higher plant meiosis. Cell Biology International 29(4): 307–318. https://doi.org/10.1016/j.cellbi.2004.12.012

Shimamura M, Brown RC, Lemmon BE, Akashi T, Mizuno K, Nishihara N, Tomizawa K, Yoshimoto K, Deguchi H, Hosoya H, Horio T, Mineyuki Y (2004) γ-Tubulin in basal land plants: Characterization, localization, and implication in the evolution of acentriolar microtubule organizing centers. The Plant Cell 16(1): 45–59. https://doi.org/10.1105/tpc.016501

Silkova OG, Dobrovolskaya OB, Dubovets NI, Adonina IG, Kravtsova LA, Roder MS, Salina EA, Shchapova AI, Shumny VK (2006) Production of wheat-rye substitution lines and identification of chromosome composition of karyotypes using C-banding, GISH, and SSR markers. Russian Journal of Genetics 42(6): 645–653. https://doi.org/10.1134/S1022795406060093

Silkova O G, Shchapova A I, Shumny V K (2007) Role of rye chromosome 2R from wheat-rye substitution line 2R(2D)$_1$ (*Triticum aestivum* L. cv. Saratovskaya 29–*Secale cereale* L. cv. Onokhoiskaya) in genetic regulation of meiotic restitution in wheat-rye polyhaploids. Russian Journal of Genetics 43(7): 805–814. https://doi.org/10.1134/S1022795407070137

Silkova OG, Dobrovolskaya OB, Shchapova AI, Shumny VK (2007a) Features of the regulation of meiotic restitution in androgenic haploids of wheat-rye substitution lines 2R(2D)1, 2R(2D)3, and 6R(6A) (*Triticum aestivum* L., cultivar Saratovskaya29/*Secale cereale* L., cultivar Onokhoiskaya). Russian Journal of Genetics 45(9): 1062–1066. https://doi.org/10.1134/S1022795409090063

Silkova OG, Loginova DB (2016) Sister chromatid separation and monopolar spindle organization in the first meiosis as two mechanisms of unreduced gametes formation in wheat-rye hybrids. Plant Reproduction 29. 199–213. https://doi.org/10.1007/s00497-016-0279-5

Silkova OG, Shchapova AI, Shumny VK (2011) Patterns of meiosis in ABDR amphihaploids depend on the specific type of univalent chromosome division. Euphytica 178(3): 415–426. https://doi.org/10.1007/s10681-010-0325-6

Smertenko A, Hewitt SL, Jacques CN, Kacprzyk R, Liu Y, Marcec MJ, Moyo L, Ogden A, Oung HM, Schmidt S, Serrano-Romero EA (2018) Phragmoplast microtubule dynamics – a game of zones. Journal of Cell Silences 31: jcs203331. https://doi.org/10.1242/jcs.203331

Smertenko A, Saleh N, Igarashi H, Mori H, Hauser-Hahn I, Jiang C-J, Sonobe S, Lloyd CW, Hussey PJ (2000) A new class of microtubule associated proteins in plants. Nature Cell Biology 2: 750–753. https://doi.org/10.1038/35036390

Smirnova EA, Bajer AS (1998) Early stages of spindle formation and independence of chromosome and microtubule cycles in *Haemanthus* endosperm. Cell Motil Cytoskeleton 40(1): 22–37. https://doi.org/10.1002/(SICI)1097-0169(1998)40:1<22::AID-CM3>3.0.CO;2-H

Smirnova EA, Bajer AS (1994) Microtubule converging centers and reorganization of the interphase cytoskeleton and the mitotic spindle in higher plant *Haemanthus*. Cell Motility and the Cytoskeleton. 27(3): 219–233. https://doi.org/10.1002/cm.970270304

Smirnova EA, Bajer AS (1992) Spindle poles in higher plant mitosis. Cell Motility and the Cytoskeleton 23(1): 1–7. https://doi.org/10.1002/cm.970230102

Van Damme D, Van Poucke K, Boutant E, Ritzenthaler C, Inze D, Geelen D (2004) In vivo dynamics and differential microtubule-binding activities of MAP65 proteins. Plant Physiology 136(4): 3956–3967. https://doi.org/10.1104/pp.104.051623

Vos JW, Pieuchot L, Evrard JL, Janski N, Bergdoll M, de Ronde D, Perez LH, Sardon T, Vernos I, Schmit AC (2008) The plant tpx2 protein regulates prospindle assembly before nuclear envelope breakdown. The Plant Cell 20(10): 2783–2797. https://doi.org/10.1105/tpc.107.056796

Wada B, Kusunoki F (1964) Spindle membrane in meiosis of pollen mother cells of *Tradescantia* and in mitosis of endosperm cells of *Zephyranthes*. Cytologia (Tokyo) 29(1): 109–117. https://doi.org/10.1508/cytologia.29.109

Walczak CE, Cai S, Khodjakov A (2010) Mechanisms of chromosome behaviour during mitosis. Nature Reviews Molecular Cell Biology 11(2): 91–102. https://doi.org/10.1038/nrm2832

Wang L-I, Das A, McKim KS (2019) Sister centromere fusion during meiosis I depends on maintaining cohesins and destabilizing microtubule attachments. PLoS Genetics 15(5): e1008072. https://doi.org/10.1371/journal.pgen.1008072

Wang M, Tang D, Luo Q, Jin Y, Shen Y, Wang K, Cheng Z (2012) BRK1, a Bub1–related kinase, is essential for generating proper tension between homologous kinetochores at metaphase I of rice meiosis. The Plant Cell 24(12): 4961–4973. https://doi.org/10.1105/tpc.112.105874

Wijnker E, Schnittger A (2013) Control of the meiotic cell division program in plants. Plant Reproduction 26(3): 143–158. https://doi.org/10.1007/s00497-013-0223-x

Xue Z, Liu C, Shi W, Miao Y, Shen Y, Tang D, Li Y, You A, Xu Y, Chong K, Cheng Z (2019) OsMTOPVIB is required for meiotic bipolar spindle assembly. Proceedings of the National Academy of Sciences of the United States of America 116(32): 15967–15972. https://doi.org/10.1073/pnas.1821315116

Yamada M, Goshima G (2017) Mitotic spindle assembly in land plants: Molecules and mechanisms. Biology. 6(1): 6. https://doi.org/10.3390/biology6010006

Yamamoto A, Kitamura K, Hihara D, Hirose Y, Katsuyama S, Hiraoka Y (2008) Spindle checkpoint activation at meiosis I advances anaphase II onset via meiosis-specific APC/C regulation. Journal of Cell Biology 182(2): 277–288. https://doi.org/10.1083/jcb.200802053.

Yu H, Muszynski MG, Dawe RK (1999) The maize homologue of the cell cycle checkpoint protein MAD2 localization patterns. Journal of Cell Biology 145(3): 425–435. https://doi.org/10.1083/jcb.145.3.425

Zamariola L, De Storme N, Tiang CL, Armstrong SJ, Franklin FC, Geelen D. (2013) SGO1 but not SGO2 is required for maintenance of centromere cohesion in *Arabidopsis thaliana* meiosis. Plant Reproduction 26(3): 197–208. https://doi.org/10.1007/s00497-013-0231-x

Zamariola L, Tiang CL, De Storme N, Pawlowski, W Geelen D (2014) Chromosome segregation in plant meiosis. Frontiers in Plant Sciences 5: 279. https://doi.org/10.3389/fpls.2014.00279

Zhang H, Dawe R (2011) Mechanisms of plant spindle formation. Chromosome Research 19(3): 335–344. https://doi.org/10.1007/s10577-011-9190-y

Zhang P, Wanlong L, Fellers J, Friebe B, Gill BS (2004) BAC-FISH in wheat identifies chromosome landmarks consisting of different types of transposable elements. Chromosoma 112: 288–299. https://doi.org/10.1007/s00412-004-0273-9

Zickler D, Kleckner N (2016) A few of our favorite things: Pairing, the bouquet, crossover interference and evolution of meiosis. Seminars in Cell & Developmental. Biology 54: 135–148. https://doi.org/10.1016/j.semcdb.2016.02.024

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RESEARCH ARTICLE

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DNA-barcoding and a new data about the karyotype of *Myotis petax* (Chiroptera, Vespertilionidae) in the Russian Far East

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Abstract

The DNA-barcoding and chromosomal study of the eastern water bat, *Myotis petax* Hollister, 1912, from the earlier unexplored localities in the Russian Far East are carried out. The COI barcoding obtained for 18 from a total of 19 individuals captured in five localities in the Russian Far East showed the low nucleotide variability with the prevalence of the central, the most abundant haplotype. The chromosomal characteristics of eight *M. petax* specimens (2n = 44, NFa = 52) in the Russian Far East are clarified. The number and localization of NOR in karyotype of *M. petax* is described at the first time and differ from distributional patterns of NOR in the sibling species *M. daubentonii* Kuhl, 1819 that can be used as diagnostic feature. The considerable intraspecific variability in the distribution of heterochromatin material revealed is not typical of the genus *Myotis*, but it has been found in other species of the family Vespertilionidae.

Keywords

bats, chromosome, COI, heterochromatin, *Myotis*, NOR

Introduction

The eastern water bat, *Myotis petax* Hollister, 1912, is a common Eastern Palaearctic bat species. The range of *M. petax* includes the near-water habitats throughout forest, forest-steppe and steppe zones from Western Siberia to the Russian Far East (including Sakhalin and the Kuril Islands) and, outside of Russia – in northern Mongolia, NE China, Korea and Japan (Kruskop 2012). It was first described as a distinct species from the village Kosh-Agach in the Altai Mountains (Hollister 1912). However, starting from Ognev (1928) and until the early 2000s, *M. petax* had been considered as part of the widespread polytypic species *Myotis daubentonii* Kuhl, 1819 which had included about 3 to 6 subspecies according to various estimates (Kuzaykin 1950; Gromov 1963; Tiunov 1984, 1997; Yoshiyuki 1989; Bogdanowicz 1994; Koopman 1994).

The morphological heterogeneity and the presence of two major groups of forms in *M. daubentonii* complex: the "western" and the "eastern" (including the Altai form *M. d. petax*) has been shown by Kruskop (2004). The species rank of *M. petax* was finally confirmed by the genetic and morphological data with the using SINEs as genetic markers. A total of 6 specimens of *M. daubentonii* and 7 specimens of *M. petax* (including only one bat from the Far East) were examined by molecular method (Matveev et al. 2005). It was shown by the molecular studies based on cyt b and ND1 sequences that *M. petax* is closer related to *M. macrodactylus* (Temminck, 1840), *M. pilosus* Peters, 1869 and *M. fimbriatus* Peters, 1871 than *M. daubentonii*. The closest related species for *M. daubentonii* are *M. bechsteinii* Kuhl, 1817, *M. longicaudatus* Ognev, 1927 and *M. frater* G. Allen, 1823 (Kawai et al. 2002, 2003; Ruedi et al. 2013; Ruedi et al. 2015).

The DNA-barcoding based on 657 bp length sequences of cytochrome c oxidase I (COI) gene has been studied for the 23 *M. petax* individuals including 6 specimens from the Far East, i.e. 5 bats from Sakhalin and 1 animal from the Primorsky Krai. It was revealed that the intraspecific distances for *M. petax* are amounted to 0.28% to 1.16% while interspecific distance between *M. petax* and *M. daubentonii* is 12% (Kruskop et al. 2012). The differences between cyt b sequences of *M. petax* from the Far East (n = 1) and China (n = 17) were amounted to 0.2% (Wang et al. 2010). In addition, the partial sequence of control region for one specimen from China had been studied (Zhang et al. 2009) and the full mitochondrial genomes of 4 individuals from South Korea had been obtained (Hwang et al. 2016). Otherwise, the genetics of *Myotis petax* in Far Eastern populations still remains poorly studied.

Karyotype features are essential diagnostic characteristics of many mammalian species (Vorontsov 1958; Matthey 1973; Orlov and Bulatova 1983; Mazzoleni et al. 2018). The chromosomal data are successfully applied to clarify species affinity and interspecific relationships between species of the order Chiroptera (Volleth 1987; Volleth and Heller 1994, 2012; Volleth et al. 2001; Kearney et al. 2002; Volleth et al. 2006). It was shown by our review that the karyology of Far Eastern bats is studied insufficiently (Gorobeyko and Kartavtseva 2019).

For the genus *Myotis* Kaup, 1829 the position and number of the nucleolus organizer regions (NORs) and the amount and location of heterochromatic material

on chromosomes are species-specific characteristics (Harada and Yosida 1978; Ando et al. 1980; Volleth 1987; Ando et al. 1987; Ono and Obara 1994; Volleth and Heller 2012). The NOR distribution has been studied for 4 out of 6 Far Eastern *Myotis* species and varied from 5 to 13 centromeric NORs on the acrocentric chromosomes (Ono and Obara 1994). Only 3 NORs were found in karyotype of *M. daubentonii* on acrocentric pairs Nos. 8 to 10 (Volleth 1987; Volleth and Heller 2012). It is likely that the number and location of NOR on the *M. petax* and *M. daubentonii* chromosomes should be different. A small intercalary heterochromatic band in the proximal part of the long arm of X chromosome and largely heterochromatic submetacentric Y chromosome was detected in *Myotis daubentonii* karyotype (Volleth and Heller 2012). The pattern of heterochromatic material on *M. petax* chromosomes is still unknown.

Only conventional staining karyotypes of *M. petax* have been studied from the Primorsky Kray, Russian Far East (Korablev et al. 1989), and from South Korea (Yoo and Yoon 1992). The diploid number of *M. petax* did not differ from other *Myotis* species (2n = 44), but the number of autosomal arms (NFa) was different in two works and amounted to 50 or 52, respectively. The feature of genus *Myotis* the fundamental number of autosomal arms is 52 due to the short euchromatic arms on the autosomal pair No. 7 (Volleth and Heller 1994, 2012)

Thus, the aim of present paper is to study DNA barcodes and chromosomes of *Myotis petax* from the localities of the Russian Far East that are not covered by previous studies, and to compare obtained results with these data for the species. It is important to investigate the position and number of the NORs and the amount and location of heterochromatic material on chromosomes to clarify chromosomal characteristics of *M. petax* and to find the differences with the karyotypes of other *Myotis* species.

Materials and methods

There are 19 specimens of *M. petax* caught in the Primorsky Krai (n = 7), Khabarovsky Krai (n = 4), Amur Oblast (n = 8) studied in this paper. Bats were caught using mist nets (6–7 m × 2.5 m, Ecotone, Poland) in swarming site and near summer roosts, handling in hibernation sites. The geographical origin of the examined animals and coordinates listed in Table 1. The other collecting data see Suppl. file 1. The samples used in the present study are deposited in the Genetic Mammalian Tissue Collection of the Federal Scientific Center of the East Asia Terrestrial Biodiversity, Far East Branch, Russian Academy of Sciences (Vladivostok, Russia). All applicable international, national and institutional ethics statements when using animals in research have been followed.

In addition, the 26 COI sequences of *Myotis petax* (Table 1) and 19 COI sequences of five Far Eastern *Myotis* species (*M. macrodactylus*, *M. longicaudatus*, *M. bombinus* Thomas, 1906, *M. ikonnikovi* Ognev, 1912, *M. sibirica* Kastschenko, 1905) and *M. daubentonii* from GenBank were analyzed. The COI sequence of *Murina hilgendorfi* Peters, 1880 was used as outgroup in phylogenetic analysis.

Table 1. Sampling localities and GenBank sequencing data of *Myotis petax*. specimen – the number of animal in Genetic Mammalian Tissue Collection of the FSCEATB FEB RAS or in Collection of Zoological Museum of Moscow University. 2n/ NFa – the diploid number of chromosome and the fundamental number of autosomal arms, X and Y – morphology of sex chromosomes: M – metacentric, SM – submetacentric, M-SM – biarmed chromosome, A – acrocentric, conv – conventional staining.

Code	Locality	Coordinates	GenBank	Specimen	Sex	2n/ NFa	X	Y	Chromosomal stainings
1	Primorsky Krai, Primorsky Velican Cave	43°17.133'N, 133°36.8'E	MT383996	3240	m				-
			MT383997	3400	f	44/52	M-SM	-	conv, GTG, AgNOR
			MT383998	3865	f	44/52	M-SM	-	GTG, CBG
			–	3867	m	44/52	M-SM	A	GTG, CBG
			MT383999	3869	f				-
2	Primorsky Krai, Spasskaya Cave	44°34.883'N, 132°46.083'E	MT384000	3258	m	44/52	M-SM	A	conv, AgNOR
			MT384001	3259	m	44/52	M-SM	A	conv, GTG, CBG, AgNOR
3	Khabarovsky Krai, Komsomolsk Nature Reserve	50°50.1'N, 137°28.7'E	MT384002	UG16-18	m				-
			MT384004	UG21-18	m				-
4	Khabarovsky Krai, Komsomolsk-on-Amur City	50°42.114'N, 137°12.291'E	MT384003	UG28-18	m				-
			MT384005	UG36-18	f				-
5	Amur Oblast, Zeya City	53°41.767'N, 127°4.317'E	MT384006	3332	m				-
			MT384007	3333	m	44/52	M-SM	A	conv
			MT384008	3334	f				-
			MT384009	3335	f				-
			MT384010	3336	f	44/52	M-SM	-	conv, CBG, AgNOR
			MT384011	3337	m				-
			MT384012	3338	f	44/52	M-SM	-	conv, GTG, CBG, AgNOR
			MT384013	3339	f				-

GenBank sequencing data of *Myotis petax*

Code	Locality	Coordinates	GenBank	Specimen	Sex	Reference
6	Primorsky Krai, Priiskovaya Cave	44°22.767'N, 133°12.283'E	JF443025	S173255	m	Kruskop et al. 2012
7	Sakhalin Oblast	46°22.3'N, 141°52.217'E	JF443019, JF443032–JF443035	S175221-25	-	Kruskop et al. 2012
8	Transbaikal Krai	53°22.5'N, 121°10.38'E	JF443026	S182081	m	Kruskop et al. 2012
9	Transbaikal Krai	53°25.2'N, 120°19.8'E	JF443028	S175362	m	Kruskop et al. 2012
10	Mongolia	47°5.783'N, 102°46.38'E	JX008075–JX008077	S187466-68	-	Kruskop et al. 2012
11	Tuva Republic	50°02'N, 95°04'E	JF443020, JF443029–JF443031, JF443036–JF443038	S167627, S167738, S168602-03, S168637, S168648-49	-	Kruskop et al. 2012
12	Altai Republic	51°22.2'N, 84°43.8'E	JF443024	S171621	m	Kruskop et al. 2012
13	Altai	51°21.9'N, 84°42.9'E	JF443021	S171624	f	Kruskop et al. 2012
14	Altai	51°17.22'N, 84°43.92'E	JF443039, JF443040	S184155-56	2f	Kruskop et al. 2012
15	South Korea	36°31'N, 127°48'E	KT199099–KT199102	KW001-004	-	Hwang et al. 2016

M. macrodactylus: HQ580337, HQ580338 (International Barcode of Life, 2010), KT862813, KT862814 (GenBank), *M. longicaudatus*: JF442982, JF442983, JF442989 (Kruskop et al. 2012), *M. bombinus*: HQ580336 (International Barcode of Life, 2010), JF442874, JF442876 (Kruskop et al. 2012), *M. ikonnikovi*: HQ974651, HQ974652 (International Barcode of Life, 2010), JF442993 (Kruskop et al. 2012), *M. sibirica*: JF442902, JF442905, JF442926 (Kruskop et al. 2012), *M. daubentonii*: JF442939, JF442942, JF442943 (Kruskop et al. 2012), and *Murina hilgendorfi*: JF442830 (Kruskop et al. 2012).

DNA extraction, amplification and sequencing

Total DNA was isolated from ethanol-fixed tissues by the method of saline extraction (Aljanabi and Martinez 1997). For the DNA-barcoding we used the part of COI from 49 to 705 nucleotides, 657 bp length. The COI gene was amplified and sequenced by polymerase chain reaction and sequenced using the forward MPCO+ (5'-ATTTGCAATTCAATGTGTATT-3') and reverse MPCO- (3'-ATAGCTCATACCATTCCTAT-5'). The both primers were designed in this study. Amplification was carried out in a 25 μL reaction mixture, which included 3–4 μg of total DNA, 2.5 μL 10× buffer, 2.5 μL of 20 mM dNTP mixture, 2 μL of each primer, 0.5 units Taq-polymerase (Sibenzim, Russia), and deionized water. The COI gene was amplified under the following conditions: 5 min DNA denaturation at 95 °C, 35 cycles of amplification (95 °C for 10 s, 47.5 °C for 60 s and 72 °C for 60 s) and 7 min chain completion at 72 °C. PCR products were purified and sequenced with the forward and reverse primers using the Big Dye Terminator series 3.1 kit (Applied Biosystems, United States). The nucleotide sequences were analyzed with the ABI Prizm 3130 sequencer (Applied Biosystems, United States) in the Federal Scientific Center of the East Asia Terrestrial Biodiversity, Far East Branch, Russian Academy of Sciences (Vladivostok, Russia).

Phylogenetic analysis

All sequences were aligned using the software program BioEdit, version 7.0.9.0 and deposited in the GenBank database. The accession numbers of our and sequences obtained from GenBank are reported in the Table 1.

The interspecific nucleotide diversity (π) and haplotype diversity (P) were calculated using DnaSP6 (Hall 1999). A search for the best model of nucleotide evolution was performed using Modeltest: Hasegava-Kishino-Yano including invariant sites (HKY+I) (Nei and Kumar 2000, Kumar et al. 2018). The phylogenetic analysis was based on Maximum Likelihood (ML) method and run in MEGA-X 10.1.7 with 1000 bootstrap replicates (Kumar et al. 2018). To calculate pairwise genetic p-distances the MEGA-X 10.1.7 software used. To construct the haplotype network by the "median joining" method the Network 10 software used (https//www.fluxus-engineering.com).

Chromosomal analysis

Chromosome preparations were obtained from *in vivo* bone marrow method (Ford and Hamerton 1956), as well as from short-term cell cultures established from spleen and bone marrow (Graphodatsky and Rajabli 1988). GTG-banding procedure was carried out according to Seabright (1971). Chromosomes were numbered using Bickham's scheme, in which ordinal numbers have been given to all of the autosomal arms based on GTG-banding patterns (Bickham 1979). The locations of nucleolus organizer regions (NORs) were detected by sequential using of silver staining method (Bloom and Goodpasture 1976) and GTG-banding of chromosomes. Heterochromatic material was detected using C-banding (Sumner 1972). To determine the locations of heterochromatic bands on chromosomes, we used sequential GTG-staining and C-staining. The mean value of active NORs per chromosomal pair and cell was calculated according to Volleth (1987), where each distinct NOR was counted as 1.0 and indistinct one as 0.5. The greatest possible value of NORs per chromosomal pair and cell was 2.0 (Volleth 1987).

The results of differential staining were analyzed with an AXIOSKOP 2 Plus microscope (Zeiss). The microimage registration and adjustment was performed with a CCD camera with software (META Systems GmbH, Germany) of the Joint-Use Center «Biotechnology & Genetic Engineering» in the Federal Scientific Center of the East Asia Terrestrial Biodiversity Far East Branch Russian Academy of Sciences (Vladivostok, Russia).

Results and discussion

DNA-barcoding and phylogenetic analysis

The DNA barcodes are obtained for 18 from a total of 19 *M. petax* individuals captured in five localities in the Russian Far East. To identify the species the sequences have compared with the 45 DNA barcodes of 7 *Myotis* species (*M. petax*, *M. macrodactylus*, *M. longicaudatus*, *M. bombinus*, *M. ikonnikovi*, *M. sibirica*, *M. daubentonii*) from GenBank. All of the obtained sequences have highest similarity with sequences of *M. petax* from GenBank (Fig. 1).

A pairwise genetic distances between the specimens of *M. petax* studied vary from 0 to 0.8%. The obtained values are within the range of interspecific distances (0.28–1.16%) previously described for *M. petax* (Kruskop et al. 2012). A mean genetic p-distances between the individuals from the Primorsky Krai and South Korea is 0.54% (less than 1000 km), while a mean genetic p-distances between the Altai Mountains and the Primorsky Krai specimens is only 0.26% (approximately 3000–3500 km). This means that a geographically closer South Korean population is genetically more distant from the population of *M. petax* in the Primorsky Krai.

The nucleotide diversity for the whole species is amounted to 0.00227±0.00032 with the haplotype diversity $P = 0.801 \pm 0.040$. The nucleotide diversity and hap-

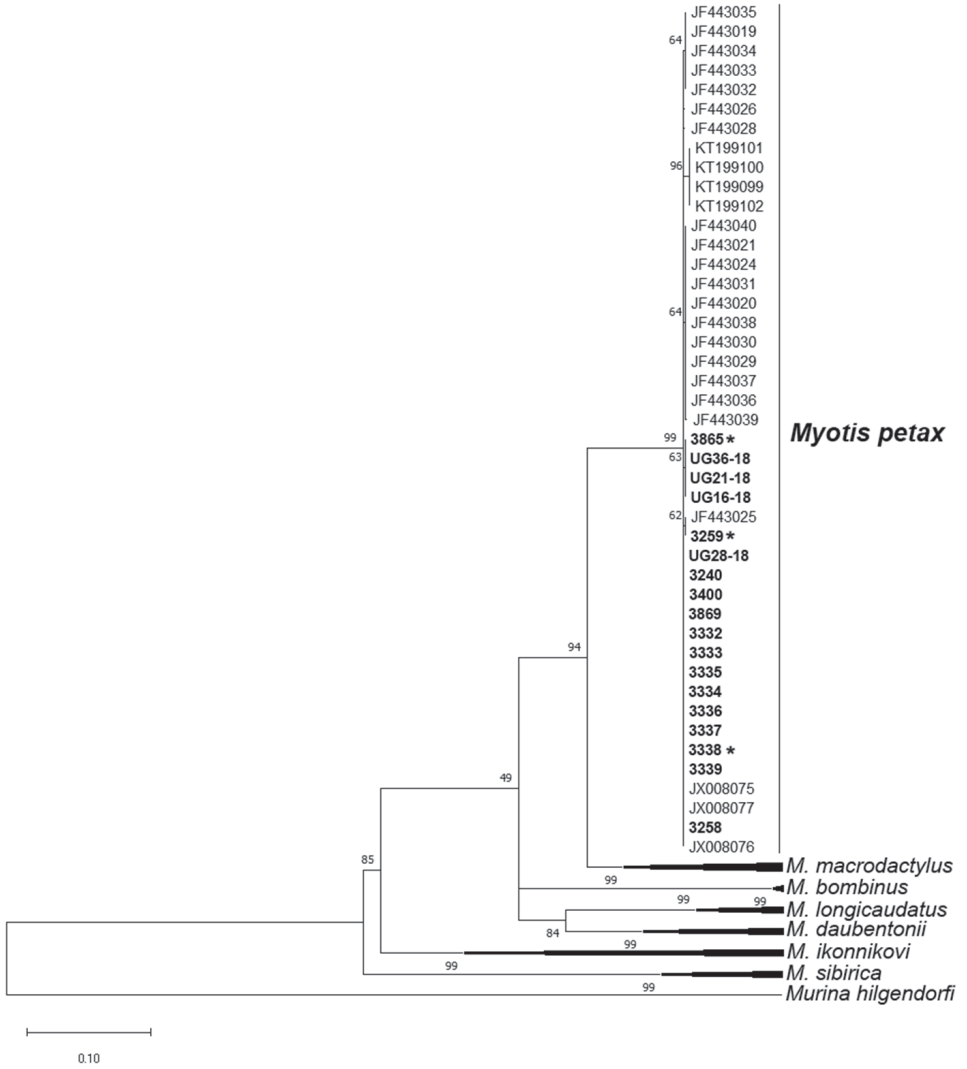

Figure 1. Maximal Likelihood tree of the cytochrome oxidase I gene. ML tree based on 64 COI sequences of *Myotis* species and outgroup. The bold numbers marked our data. Asterisks marked individuals for which the CBG-banding karyotype is studied.

lotypic diversity for specimens from the mainland part of the Russian Far East are amounted to P = 0.503 ± 0.113, π = 0.00084 ± 0.00022. These values are close to the values of haplotype diversity for the COI gene described for *M. ikonnikovi* from South Korea (P = 0.5–0.8667) which are characterized by high genetic diversity of mitochondrial genes compared to other *Myotis* species (Park et al. 2019). The similar values of haplotype diversity have found for control region of two Northern American bat species *M. lucifugus* (P = 0.812–0.845) and *M. septentrionalis* (P = 0.827–0.910) (Johnson et al. 2015). At the same time the haplotype diversity of cyt b gene described

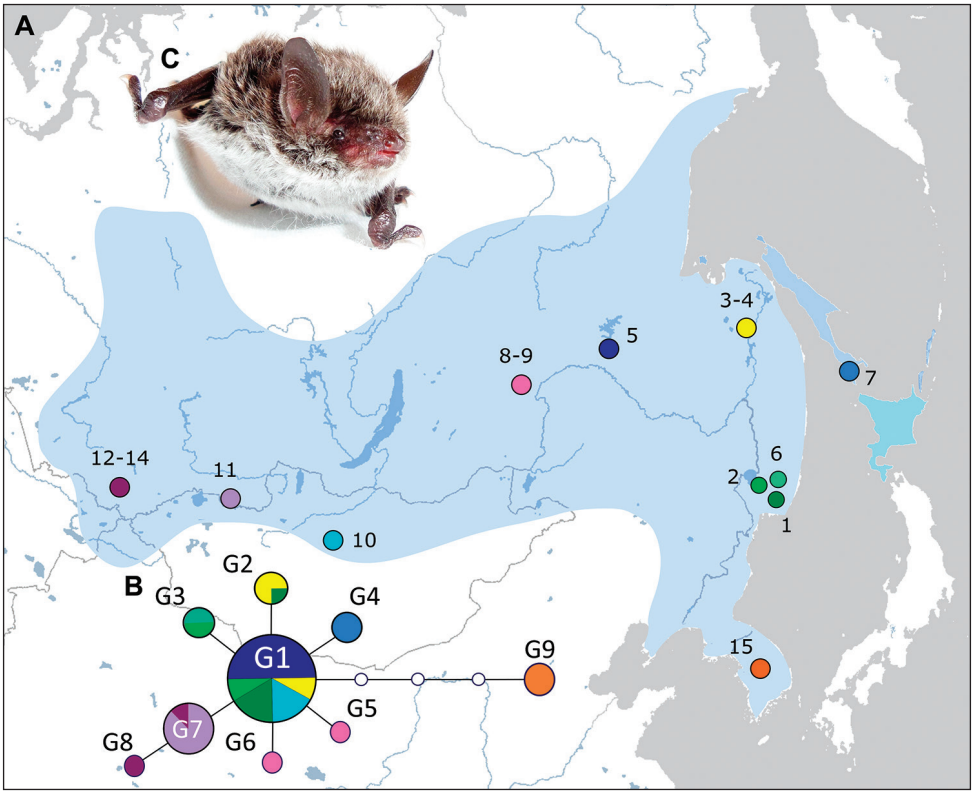

Figure 2. Distributional range and COI haplotypes of *Myotis petax* **A** map showing approximate range and capture sites of *M. petax* (for this paper and previous studies) **B** median-joining network of COI haplotypes are colour-coded based on capture sites, circle size corresponds to number of samples **C** *M. petax* (Russia, Buryatia Republic, 2014), photo by Denis V. Kazakov.

for European *M. myotis* was amounted to $P = 0.491$ (Ruedi and Castella 2003), and for *M. dasycneme* was P = 0.335–0.868 (Andersen et al. 2018). On the other hand, the nucleotide diversity of *M. petax* is lower than that of *M. ikonnikovi* (π between 0.00163 to 0.00878) and is comparable with the nucleotide diversity of cyt b of *M. myotis* (π between 0.0003 to 0.0028) and *M. dasycneme* (π between 0.0004 to 0.0029) (Ruedi and Castella 2003, Andersen et al. 2018, Park et al. 2019).

A total of 9 COI haplotypes found in all specimens of *M. petax* studied including GenBank data (G1–9) but only 3 COI haplotypes detected in 18 *M. petax* individuals from the Russian Far East (G1–3). The G2 haplotype revealed at the first time.

The relationship among a total of 9 haplotypes reflected in the median-joining network (Fig. 2) revealed a close relationship between the all *M. petax* studied, expect the Korean bats which are more distantly related to other populations. The most common haplotype, G1, is observed in the waist territory from Baikal Lake to Pacific Ocean coast. It is found in 16 of the 44 specimens studied. Khabarovsky Krai and Primorsky Krai shared haplotype G2 which is found in the 4 individuals. The third haplotype observed

in the Primorsky Krai is a G3 haplotype found in 2 specimens. Two individuals from Transbaikal Krai have two different haplotype G5 and G6, and 5 bats from the Sakhalin Island have G4 haplotype.

Haplotypes G7 and G8 form a separate branch on the network and are found only in 8 specimens from Tuva and the Altai. The G8 haplotype revealed in the one specimen from the Altai differed from G7 on one nucleotide substitution and from G1 on two nucleotide substitution. The spread of G7 and G8 is apparently coincides with the distribution of nominative subspecies.

The other differential branch on the network is a G9 haplotype differed from G1 on three nucleotide substitution. It is found only in 4 individuals from South Korea. The distinct subspecies for *M. petax* from Korea has not been described previously.

Most of the haplotypes represented in the samples are separated by G1 just one mutation creating a starlike network characteristic for expanding populations that have been through a bottleneck or been founded recently. However, COI gene is conservative and is not suitable for studying population events.

Karyotype, differential staining and chromosomal polymorphism *of M. petax* from the Far East

The conventional staining karyotypes of eight *M. petax* specimens from Primorsky Krai and Amur Oblast have no differences and shows 2n = 44 with the NFa = 52 (Table 1). There are composed of three large (1/2, 3/4, 5/6) and one small (16/17) metacentric pair, 17 acrocentric-subtelocentric autosomal pairs and one pair of sex chromosomes (X, Y). The X is a medium-sized biarmed chromosome. The small-sized Y chromosome is acrocentric and largely heterochromatic.

It was previously reported for the specimens from the Primorsky Krai the fundamental number of autosomal arms was 50 (Korablev et al. 1989). We already noted that variations of fundamental number in the different studies can be explained by different approaches to the taking into account short euchromatic arms on the seventh autosomal pair or the including the additional heterochromatic short arms on 24 or 25 pairs of acrocentrics in NFa (Kartavtseva et al. 2014, Gorobeyko and Kartavtseva 2019). While the karyotype of *M. petax* from South Korea (NFa = 52) showed short arms on 24 or 25 pair of acrocentric (Yoo and Yoon 1992), the all Far Eastern specimens studied have no short arms on these autosomal pairs. The image of *M. petax* chromosomes from the Primorsky Krai is not given and there is no mention of the presence or absence short arms on any autosomal pairs in the paper (Korablev et al. 1989).

The X chromosome is biarmed and it was not possible to determine whether this is a submetacentric or metacentric. At the same time the previously examined individuals from the Primorsky Krai have shown clearly a metacentric X chromosome. It is possible that these karyotypic differences are due to the methodological difficulties, such as the various spiralization of metacentric chromosomes or the lack of metaphase plates on the preparation often occurred in the analysis of chromosome suspensions obtained *in vivo*.

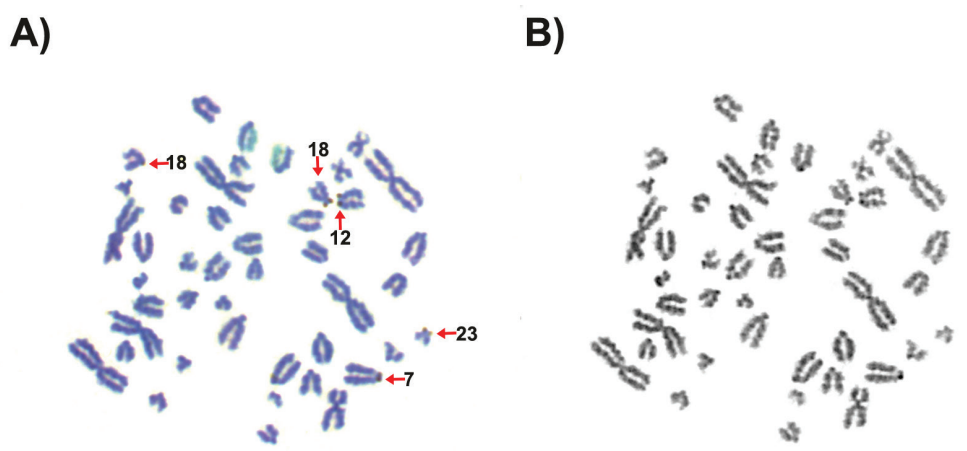

Figure 3. The sequential GTG- and AgNOR-banding of *Myotis petax* chromosomes **A** the AgNOR-banded karyotype of male 3259. Arrows indicate the NOR-bearing xcrocentric chromosome. Ordinal numbers indicate autosomal arm numbers revealed by GTG-banding **B** the GTG-banded karyotype of male 3259.

Table 2. Distribution of nucleolus organizer regions: mean value of active NORs per chromosomal arm and cell. ID – identification number of specimen. No cells – number of cells analyzed. The numbers before ID (1, 2, 5) indicate sampling localities, the abbreviations see in Table 1.

ID	No cells	chromosomal arm no.																
		7	8	9	10	11	12	13	14	15	18	19	20	21	22	23	24	25
1-3400	**11**	0,41		0,5	0,27		0,14	0,09		0,27	0,86		0,55	0,27		0,27	0,27	0,09
2-3259	**20**	0,78		0,93	0,48		0,2	0,33		0,45	1		0,8	0,8		0,13	0,2	0,2
5-3336	**22**	0,16		0,38	0,13		0,19	0,17		0,27	0,36		0,33	0,38		0,38	0,14	0,2
5-3338	**63**	0,34		0,63	0,41		0,45	0,32		0,68	0,9		0,88	0,56		0,18	0,1	0,06

The patterns of NOR and the heterochromatic segments in karyotype of *M. petax* are described at the first time. Figure 3 demonstrated the sequential GTG- and AgNOR-banding of *Myotis petax* chromosomes. The distribution of active NORs in the four *M. petax* specimens is shown in Table 2. All four specimens showed active NORs in the minute short arms of chromosomes Nos. 7, 9, 10, 12, 13, 15, 18, 20, 21, 23-25.

On average only 4.7 NOR per cell from 24 potential sites is detected that illustrated the low NOR activity of all the specimens studied. In many cells only one homologue of a chromosomal pair is shown to bear an active NOR. A similar low NOR-activity was shown for *M. myotis*, *M. capaccinii*, *M. bechsteinii* (Volleth 1987) and for *M. bombinus*, *M. longicaudatus*, *M. macrodactylus* (Ono and Obara 1994). All these species including *M. petax* have small multiple centromeric NORs on chromosomes.

M. petax is clearly differ on the number and localization of NORs as from the other Far Eastern *Myotis* species as from the sibling species *M. daubentonii*. The comparison of the NOR distributions in the karyotypes of the Far Eastern *Myotis* species

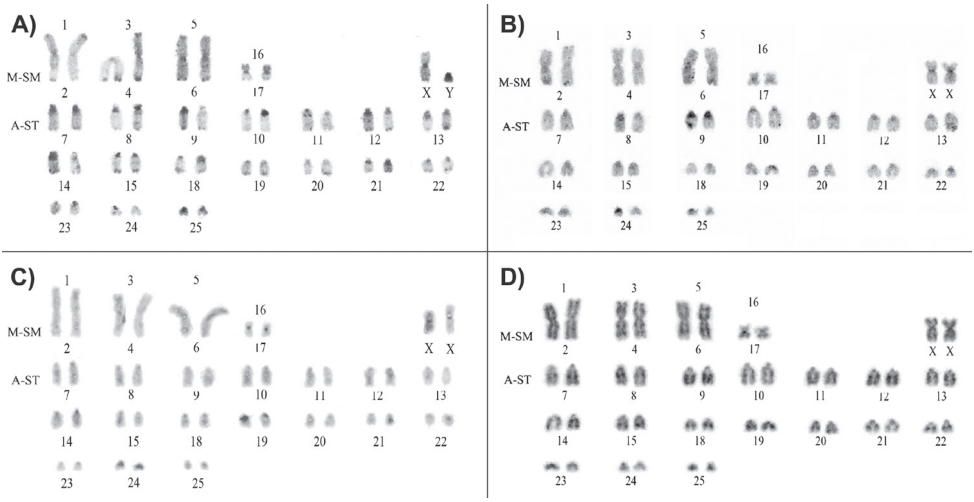

Figure 4. Comparison of C-banded karyotypes of far eastern *Myotis petax* **A** CBG-banded karyotype of specimen 3259 (locality 2) **B** CBG- banded karyotype of 3865 (locality 1) **C** C-banded karyotype of specimen 3338 (locality 5) **D** GTG-banded karyotype of 3865 (locality 1). The abbreviations see in Table 1.

is shown in Table 3. The NOR-distribution of the one Far Eastern species *M. sibirica* (*gracilis*) is still unknown. The conventional staining karyotype of this species (2n = 44, NFa = 50) was published by Kartavtseva and Dokuchaev (1998).

The amounts and localizations of heterochromatin bands on chromosomes of three *M. petax* from the Primorsky Krai and Amur region presented in Figure 4A–C and are clearly different.

1) The male *M. petax* (3259) from Spasskaya Cave (locality 2) showed centromeric heterochromatic bands on most of chromosomal pairs. The one or two homologues in chromosome pairs Nos. 7–10, 12–14 and 25 bore large centromeric heterochromatic segment. Small but distinct telomeric heterochromatic bands are found on all biarmed chromosomal pairs and seven acrocentric pairs from 7 to 22. Large intercalary heterochromatic segments are located on chromosome 8, 11 and 18. A heteromorphism in localization of heterochromatin blocks found in nine autosomal pairs 8–12, 14, 18, 21, 24.
2) The female *M. petax* (3865) from the Primorsky Velican Cave (locality 1) showed centromeric heterochromatic bands on most of the acrocentric pairs, on the metacentric pair 16/17 and X chromosome. The large heterochromatic centromeric segments are found in 8 and 9 autosomal pairs. The telomeric heterochromatic segments are presented on all biarmed chromosomal pairs and acrocentric pairs Nos. 11 and 21. A heteromorphism in localization of heterochromatin blocks is found in four autosomal pairs 8, 25 and 16/17. There were no intercalary heterochromatic bands in karyotype of *M. petax* from the Primorsky Velican Cave. The GTG-banded karyotype of 3865 showed in Figure 4D.

3) In karyotype of the female *M. petax* (3338) from Zeya (locality 5) small and slightly stained heterochromatic centromeric bands are found on nine acrocentric pairs from 7 to 25, metacentric pair 16/17 and X chromosome. Three autosomal pairs 7, 14 and 22 showed a heteromorphism on amount heterochromatic material. This specimen had no telomeric or intercalary heterochromatic bands.

The distinct telomeric heterochromatic segments found on several chromosomes of both individuals from the Primorsky Krai were previously described only for the Chinese *Myotis* species such as *M. altarium* Thomas, 1911 (Li et al. 2007), *M.* cf. *siligorensis* (published as "*M. dividii*), *M.* cf. *daubentonii* (Peng et al. 2011), *M. fimbriatus* (Peters, 1871) (Wang et al. 2009). The intercalary heterochromatic segments were observed in karyotypes of Eurasian *Myotis* species (Volleth and Heller 2012), but no one have intercalary heterochromatin bands on acrocentric pairs Nos. 8, 11, 18 found in the specimen 3259.

All individuals studied had the heteromorphic chromosome pairs. The similar intraspecific heteromorphism of several heterochromatic segments was previously observed in a few Eurasian *Myotis* species (Harada and Yosida 1978; Volleth and Heller 2012). Intraspecific polymorphism of the several heterochromatic segments in karyotypes of a few Eurasian *Myotis* species is illustrated in the Table 4. Nevertheless, a variability of the heterochromatic material as found in karyotype *M. petax* is not typical for the most of the Eurasian *Myotis* species. We have already noted the same significant polymorphism in the amount and location of the heterochromatin bands in the karyotype of two *Pipistrellus*-like species: *Pipistrellus abramus* (Temminck, 1840) and *Vespertilio sinensis* Peters, 1880 (Ando et al. 1980; Harada et al. 1987; Ando et al. 1987; Ono and Obara 1994; Ono and Yoshida 1997; Lin et al. 2002; Wu et al. 2009; Gorobeyko and Kartavtseva 2019).

The individuals differing in the amounts and localizations of heterochromatin bands on chromosomes are also belonged in different COI haplotypes. The specimen 3331 from Amur Oblast is showed G1 haplotype, while the bats 3259 and 3865 from the Primorsky Krai are belonged to G3 and G2, respectively. Nevertheless, the number of *M. petax* individuals studied and the differences between the COI haplotypes are insufficient to draw conclusions regarding the relationship between chromosomal and COI variability.

Conclusion

The COI barcoding showed the presence of only 3 COI haplotypes (G1–3) in the Russian Far East from 9 COI haplotypes (G1–9) found in *M. petax*. The G2 haplotype detected at the first time. This species showed to have the low nucleotide variability with the prevalence of the central, the most abundant haplotype. The distances between individuals do not exceed 0.8%.

The chromosomal characteristics of *M. petax* from the Russian Far East are clarified. The distributional patterns of NOR and heterochromatic segments on the chro-

Table 3. Distribution of NORs in karyotypes of the Far Eastern *Myotis* species. * – distribution of NORs of European species, *Myotis daubentonii*, is shown to comparison with distribution of NORs in karyotype of *M. petax*. The abbreviations see in Table 1. **cmc** – centromere-cap NORs, ST – subtelocentric chromosome.

Species	2n	NFa	*X*	*Y*	Chromosome arm no.																NOR	Source
					7	8	9	10	11	12	13	14	15	18	19	20	21	22	23	24	25	
Myotis bombinus	44	52	M	A	+	+	+	+	+	+	+	+	+		+			+				11 cmc	Ono and Obara 1994
M. longicaudatus	44	52	M	ST		+	+	+	+		+	+	+	+	+	+	+	+	+			13 cmc	Ono and Obara 1994
M. ikonnikovi	44	52	M	A	+						+	+						+	+			5 cmc	Ono and Obara 1994
M. macrodactylus	44	52	M	A										+	+	+	+	+	+			6 cmc	Ono and Obara 1994
M. petax	44	52	M-SM	A	+		+	+		+	+		+	+		+	+		+	+	+	12 cmc	this study
*M. daubentonii**	44	52	SM	SM		+	+	+														3 cmc	Volleth 1987, Volleth and Heller 2012

Table 4. Intraspecific variations of heterochromatic material in karyotypes of *Myotis* species. specimen – identification number (ID) of specimen. The numbers before ID (1, 4, 5) indicate sampling localities, the abbreviations see in Table 1. Symbols: o – totally euchromatic chromosomes, • – totally heterochromatic chromosomes. + – small heterochromatic band in vicinity of the centromere on both homologues in pair, ++ – large heterochromatic band in vicinity of the centromere on both homologues in pair, x/xx – heteromorphic heterochromatic bands in vicinity of the centromere: small and absent / large and small or absent. tel – heterochromatic segment in vicinity of the telomere, int – interstitial heterochromatic band, arm – heterochromatic secondary arm. ***Bold Italic*** – heteromorphic chromosomal pair. The abbreviations see in Table 1.

Specimen/Species	2n	NFa	Chromosome arm no.																				X	Y	Sourse
			1/2	3/4	5/6	16/17	7	8	9	10	11	12	13	14	15	18	19	20	21	22	23	24	25			
1-3338	44	50	o	o	o	+	x	o	o	+	o	+	o	x	o	+	+	o	o	x	+	++	o	+	-	**Present study**
4-3259	44	50	tel	tel	+, tel	+, tel	++, tel	+, ***int***	xx, tel	xx	+, ***int***	xx, tel	+, tel	xx	+, tel	+, ***int***	+, tel	o	xx	+, tel	+	x	++	+	•, A	**Present study**
5-3865	44	50	tel	tel	tel	+, ***tel***	o	xx	++	+	+, tel	+	+	o	+	+	+, tel	+	+, tel	+	+	+	x	+	-	**Present study**
M. m. bulgaricus	44	52	+	+	+	+, int	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+, ***arm***	+	•, SM	Volleth and Heller 2012
M. daubentonii	44	52	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	int , ***int***	•, ***SM/ST***	Volleth and Heller 2012
M. ikonnikovi	44	52	++	++	++	++	++	+	++	++	++	++	++	+	+	+	+	++	+	+	x	x	arm, ***A/M***	++	-	Harada and Yoshida 1978
M. macrodactylus	44	52	xx	+	xx	+	+	++	++	+	++	+	+	+	++	++	+	+	+	++	+	+	arm, ***SM/M***	++	•, A	Harada and Yoshida 1978

mosomes *M. petax* are described at the first time. The number and localization of NOR in karyotypes of sibling species *M. petax* and *M. daubentonii* is different and can be used as diagnostic feature. The significant intraspecific variability in the heterochromatin distribution of revealed in Far Eastern *M. petax* was not described for the genus *Myotis*, but it had been found in other vespertilionid species.

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References

Aljanabi S, Martinez I (1997) Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. Nucleic Acids Research 25: 4692–4693. https://doi.org/10.1093/nar/25.22.4692

Andersen LW, Dirksen R, Nikulina EA, Baagøe HJ, Petersons G, Estók P, Orlov OL, Orlova MV, Gloza-Rausch F, Göttsche M, Fjederholt ET, Krüger F, Elmeros M (2018) Conservation genetics of the pond bat (*Myotis dasycneme*) with special focus on the populations in northwestern Germany and in Jutland, Denmark. Ecology and Evolution 9: 5292–5308. https://doi.org/10.1002/ece3.5119

Ando K, Tagawa T, Uchida TA (1980) The C-banding pattern of 6 Japanese species of vespertilionine bats (Mammalia: Chiroptera). Experientia 36: 653–653. https://doi.org/10.1007/BF01970118

Ando K, Harada M, Uchida TA (1987) A karyological study on five Japanese species of *Myotis* and *Pipistrellus*, with special attention to composition of their C-band materials. Journal of the Mammalogical Society of Japan 12(1–2): 25–29.

Bickham J (1979) Chromosomal Variation and Evolutionary Relationships of Vespertilionid Bats. Journal of Mammalogy 60(2): 350–363. https://doi.org/10.2307/1379807

Bloom SE, Goodpasture C (1976) An improved technique for selective silver staining of nucleolar organizer regions in human chromosomes. Human Genetics 34: 199–206. https://doi.org/10.1007/BF00278889

Bogdanowic W (1994) *Myotis daubentonii*. Mammalian Species 43: 1. https://doi.org/10.2307/3504215

Ford CE, Hamerton JL (1956) A colchicine, hypotonic citrate, squash sequence for mammalian chromosomes. Stain Technology 31: 247–251. https://doi.org/10.3109/10520295609113814

Gorobeyko UV, Kartavtseva IV (2019) Karyology of the Bats from the Russian Far East. In: Larramendy M, Soloneski S (Eds) Cytogenetics – Past, Present and Further Perspectives. IntechOpen, 14 pp. https://doi.org/10.5772/intechopen.78767

Graphodatsky AS, Radjabli SI (1988) Chromosomes of Agricultural and Laboratory Animals: Atlas. Nauka Press, Novosibirsk, 127 pp.

Gromov IM, Gureev AA, Novikov GA, Sokolov AI, Strelkov PP, Chapsky PP (1963) Mammal Fauna of the USSR, part 1. Keys to the Fauna of Russia, Published by the Zoological Institute of the USSR Academy of Sciences. Moscow, Leningrad, Publishing House of the USSR Academy of Sciences, 640 pp.

Hall TA (1999) BioEdit: A User-Friendly Biological Sequence Alignment Editor and Analysis Program for Windows 95/98/NT. Nucleic Acids Symposium Series 41: 95–98.

Harada M, Yoshida TH (1978) Karyological study of four Japanese *Myotis* bats (Chiroptera, Mammalia). Chromosoma (Berlin) 65: 283–291. https://doi.org/10.1007/BF00327623

Harada M, Ando K, Uchida TA, Takada S (1987) Karyotypic evolution of two Japanese *Vespertilio* species and its taxonomic implication (Chiroptera: Mammalia). Caryologia 40(3): 175–184. https://doi.org/10.1080/00087114.1987.10797821

Hollister N (1912) New mammals from the highlands of Siberia. Smithsonian miscellaneous collection 60: 1–6. https://doi.org/10.5962/bhl.part.7637

Hwang JY, Jin GD, Park J, Lee SG, Kim EB (2016) Complete sequences of eastern water bat, *Myotis petax* (Chiroptera; Microchiroptera; Vespertilionidae) mitogenome. Mitochondrial DNA Part A DNA Mapping, Sequencing, and Analysis 27(5): 3715–3716. https://doi.org/10.3109/19401736.2015.1079871

Johnson LNL, McLeod BA, Burns LE, Arseneault K, Frasier TR, Broders HG (2015) Population Genetic Structure Within and among Seasonal Site Types in the Little Brown Bat (*Myotis lucifugus*) and the Northern Long-Eared Bat (*M. septentrionalis*). PLoS ONE 10(5): e0126309. https://doi.org/10.1371/journal.pone.0126309

Kartavtseva IV, Gorobeiko UV, Tiunov MP (2014) The current status of chromosomal investigations of bats (Chiroptera) from the Russian Far East. Russian Journal of Zoology. 93(7): 887–900.

Kawai K, Nikaido M, Harada M, Matsumura S, Lin LK, Wu Y, Hasegawa M, Okada N (2002) Intra- and interfamily relathionships of Vespertilionidae inferred by various molecular markers including SINE insertion data. Journal of Molecular Evolution 5: 284–301. https://doi.org/10.1007/s00239-002-2326-0

Kawai K, Nikaido M, Harada M, Matsumura S, Lin LK, Wu Y, Hasegawa M, Okada N (2003) The status of the Japanese and East Asian bats of the genus *Myotis* (Vespertilionidae) based on mitochondrial sequences. Molecular Phylogenetics and Evolution 28(2): 297–307. https://doi.org/10.1016/S1055-7903(03)00121-0

Kearney TC, Volleth M, Contrafatto G, Taylor PG (2002) Systematic implications of chromosome GTG-band and bacula morphology for Southern African *Eptesicus* and *Pipistrellus* and several other species of Vespertilioninae (Chiroptera: Vespertilionidae). Acta Chiropterologica. 4(1): 55–76. https://doi.org/10.3161/001.004.0107

Korablev BP, Yakimenko LV, Tiunov MP (1989) Karyotypes of bats in the Russian Far East. In: Kryukov AP, Chelomina GN, Pavlenko MV (Eds) The present-day approached to studies of variability: collection of scientific papers. Vladivostok: The Far Eastern Branch Academy of Sciences of the USSR, 95–98.

Kruskop SV (2004) Subspecific structure of *Myotis daubentonii* (Chiroptera, Vespertilionidae) and composition of the "*daubentonii*" species group. Mammalia 68(4): 299–306. https://doi.org/10.1515/mamm.2004.029

Kruskop SV (2004) Order Chiroptera. In: Pavlinov IY, Lissovsky AA (Eds) The Mammals of Russia: A Taxonomic and Geographic Reference. Moskow: KMK Sci. Press, 73–126.

Kruskop SV, Borisenko AV, Ivanova NV, Lim BK, Eger JL (2012) Genetic diversity of north-eastern Palaearctic bats as revealed by DNA barcodes. Acta Chiropterologica 14(1): 1–14. https://doi.org/10.3161/150811012X654222

Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Molecular Biology and Evolution 35: 1547–1549. https://doi.org/10.1093/molbev/msy096

Kuzyakin AP (1950) Bats. Moscow: Soviet science, 443 pp.

Li N, Ao L, He SY, Gu XM (2007) G-bands and C-bands in 3 species of Vespertilionidae. Chinese Journal of Zoology 42(2): 96–101.

Lin LK, Motokawa M, Harada M (2002) Karyological study of the house bat *Pipistrellus abramus* (Mammalia: Chiroptera) from Taiwan with comments on its taxonomic status. The Raffles Bulletin of Zoology 50(2): 507–510.

Matthey R (1973) The chromosome formulae of eutherian mammals. In: Cytotaxonomy and Vertebrate Evolution. London: Academic Press, 531–616. https://doi.org/10.1515/mamm.1973.37.3.394

Matveev VA, Kruskop SV, Kramerov DA (2005) Revalidation of *Myotis petax* Hollister, 1912 and its new status in connection with *M. daubentonii* (Kuhl, 1817) (Vespertilionidae, Chiroptera). Acta Chiropterologica 7(1): 23–37. https://doi.org/10.3161/1733-5329(2005)7[23:ROMPHA]2.0.CO;2

Mazzoleni S, Rovatsos M, Schillaci O, Dumas F (2018) Evolutionary insight on localization of 18S, 28S rDNA genes on homologous chromosomes in Primates genomes. Comparative Cytogenetics 12(1): 27-40. https://doi.org/10.3897/compcytogen.v12i1.19381

Nei M, Kumar S (2000) Molecular Evolution and Phylogenetics. Oxford University Press, New York.

Ognev SV (1928) Animals of Eastern Europe and North Asia. T. 1. Moscow, Leningrad, GIZ, 631 pp.

Ono T, Obara Y (1994) Karyotypes and Ag-NOR variations in Japanese vespertilionid bats (Mammalia: Chiroptera). Zoological Science 11(3): 473–484.

Ono T, Yoshida MC (1997) Differences in the chromosomal distribution of telomeric (TTAGGG)n sequences in two species of the vespertilionid bats. Chromosome Research 5: 203–212. https://doi.org/10.1023/A:1018403215999

Orlov VN, Bulatova NSh (1983) Comparative cytogenetics and karyosystematics of mammals: a training manual. Moscow: Science, 406 pp.

Park S, Noh P, Choi YS, Joo S, Jeong G, Kim SS (2019) Population genetic structure based on mitochondrial DNA analysis of Ikonnikov's whiskered bat (*Myotis ikonnikovi*, Chiroptera: Vespertilionidae) from Korea. Journal of Ecology and Environment 43: 1–45. https://doi.org/10.1186/s41610-019-0140-5

Peng YQ, Li GH, Gu XM (2011) Study on the karyotypes, G-bands and C-bands of two *Myotis* species. Sichuan Journal of Zoology 30: 882–885.

Ruedi M, Castella V (2003) Genetic consequences of the ice ages on nurseries of the bat *Myotis myotis*: a mitochondrial and nuclear survey. Molecular Ecology 12(6): 1527–1540. https://doi.org/10.1046/j.1365-294X.2003.01828.x

Ruedi M, Stadelmann B, Gager Y, Douzery EJP, Francis CM, Lin LK, Guillén-Servent A, Cibois A (2013) Molecular phylogenetic reconstructions identify East Asia as the cradle for the evolution of the cosmopolitan genus *Myotis* (Mammalia, Chiroptera). Molecuar Phylogenetics and Evolution 69: 437–449. https://doi.org/10.1016/j.ympev.2013.08.011

Ruedi M, Csorba G, Lin LK, Chou CH (2015) Molecular phylogeny and morphological revision of *Myotis* bats (Chiroptera: Vespertilionidae) from Taiwan and adjacent China. Zootaxa 3920(1): 301–342. https://doi.org/10.11646/zootaxa.3920.2.6

Seabright M (1971) A rapid banding technique for human chromosomes. Lancet 2: 971–972. https://doi.org/10.1016/S0140-6736(71)90287-X

Sumner AT (1972) A simple technique for demonstraiting centrometric heterochromatin. Experimental Cell Research 83: 438–442. https://doi.org/10.1016/0014-4827(74)90366-8

Tiunov MP (1997) Bats of the Russian Far East. Vladivostok: Dal'nauka Press, 134 pp. Tiunov MP (1984) Order Chiroptera Blumenbach, 1779 – Bats. In: Krivosheev VG (Eds) Terrestrial mammals of the Far East. Nauka, Moscow, 73–102.

Volleth M, Heller KG (2012) Variations on a theme: Karyotype comparison in Eurasian *Myotis* species and implications for phylogeny. Vespertilio 16: 329–350. ISSN 1213-6123

Volleth M, Bronner G, Gopfert MC, Heller KG, von Helversen O, Yong HS (2001) Karyotype comparison and phylogenetic relationships of *Pipistrellus*-like bats (Vespertilionidae; Chiroptera; Mammalia). Chromosome Research 9: 25–46. https://doi.org/10.1023/A:1026787515840

Volleth M, Heller KG, Fahr J (2006) Phylogenetic relationships of three "Nycticeiini" genera (Vespertilionidae, Chiroptera, Mammalia) as revealed by karyological analysis. Mammalian Biology – Zeitschrift für Säugetierkunde 71(1): 1–12. https://doi.org/10.1016/j.mambio.2005.09.001

Volleth M (1987) Differences in the location of nucleolus organizer regions in European vespertilionid bats. Cytogenetics and Cell Genetics 44: 186–197. https://doi.org/10.1159/000132371

Volleth M, Heller KG (1994) Phylogenetic relationships of vespertilionid genera (Mammalia: Chiroptera) as revealed by karyological analysis. Zeitschrift für Zoologische Systematik und Evolutionsforschung 32: 11–34. https://doi.org/10.1111/j.1439-0469.1994.tb00467.x

Vorontsov NN (1958) The importance of chromosomal sets for mammalian taxonomy. Bulletin of the Moscow Society of Naturalists 6(2): 5–36.

Wang H, Li N, Ao L, Gu XM (2009) A study on karyotypes, G-bands and C-bands of *Myotis fimbriatus*. Journal of Guizhou Normal University (Natural Science) 27 (2): 13–14. https://doi.org/10.7150/ijbs.5.659 [In Chinese with English summary]

Wang L, Jiang TL, Sun KP, Wang YX, Tiunov MP, Feng J (2010) Morphological description and taxonomical status of *Myotis petax*. Acta Zootaxonomica Sinica 35 (2): 360–365.

Wu Y, Motokawa M, Li YC, Harada M, Chen Z, Lin LK (2009) Karyology of eight species of bats (Mammalia: Chiroptera) from Hainan Island, China. International Journal of Biological Sciences 5: 659–666.

Yoo DH, Yoon MH (1992) A karyotypic study on six Korean vespertilionid bats. Korean Journal of Zoology 35(4): 489–496.

Yoshiyuki M (1989) A Systematic Study of the Japanese Chiroptera. National Science Museum, Tokyo, 242 pp.

Zhang Z, Tan X, Sun K, Liu S, Xu L, Feng J (2009) Molecular systematics of the Chinese *Myotis* (Chiroptera, Vespertilionidae) inferred from cytochrome-b sequences. Mammalia 73: 323–330. https://doi.org/10.1515/MAMM.2009.058

Supplementary material 1

The other collecting data for *Myotis petax*

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REVIEW ARTICLE

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A checklist of chromosome numbers and a review of karyotype variation in Odonata of the world

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Abstract

The ancient insect order Odonata is divided into three suborders: Anisoptera and Zygoptera with approximately 3000 species worldwide each, and Anisozygoptera with only four extant species in the relict family Epiophlebiidae. An updated list of Odonata species studied regarding chromosome number, sex chromosome mechanism and the occurrence of m-chromosomes (= microchromosomes) is given. Karyotypes of 607 species (198 genera, 23 families), covering approximately 10% of described species, are reported: 423 species (125 genera, 8 families) of the Anisoptera, 184 species (72 genera, 14 families) of the Zygoptera, and one species of the Anisozygoptera. Among the Odonata, sex determination mechanisms in males can be of X(0), XY and X_1X_2Y types, and diploid chromosome numbers can vary from 6 to 41, with a clear mode at 2n = 25(60%) and two more local modes at 2n = 27(21%) and 2n = 23(13%). The karyotype 2n = 25(24A + X) is found in each of the three suborders and is the most typical (modal) in many families, including the best-covered Libellulidae, Corduliidae (Anisoptera), Lestidae, Calopterygidae, and Platycnemididae (Zygoptera). This chromosome set is considered ancestral for the Odonata in general. Chromosome rearrangements, among which fusions and fissions most likely

predominated, led to independent origins of similar karyotypes within different phylogenetic lineages of the order. The karyotype 2n = 27(26A + X) prevails in Aeshnidae and Coenagrionidae, whereas the karyotype 2n = 23(22A + X) is modal in Gomphidae and Chlorocyphidae, in both pairs of families one being from the Anisoptera while the other from the Zygoptera.

Keywords
Chromosome numbers, damseldragons, damselflies, dragonflies, m-chromosomes, sex chromosome mechanisms

Introduction

The order Odonata, which comprises slightly more than 6,000 described species worldwide, is one of the most ancient among winged insects (Pterygota), dating from the Permian (Grimaldi and Engel 2005). Extant Odonata include two main suborders with approximately 3,000 species each, the Zygoptera or damselflies with about 308 genera and the Anisoptera or true dragonflies with about 344 genera. Within these suborders, up to 21 and 11 families (and sometimes more), respectively, are currently recognized. The third suborder, the Anisozygoptera or damseldragons, includes only one genus *Epiophlebia* Calvert, 1903 with four extant species in the relict family Epiophlebiidae. A substantial body of evidence indicates that Anisoptera and Zygoptera are each monophyletic, and Zygoptera are sister to *Epiophlebia* plus Anisoptera (Rehn 2003; Kalkman et al. 2008; Dijkstra et al. 2013, 2014; Schorr and Paulson 2020).

The field of Odonata cytogenetics was heavily influenced by Bastiaan Kiauta, who has published dozens of papers and analyzed karyotypes of about 260 species and subspecies of this group (see References and Table 1). During the years that have passed since the publication of chromosome number checklist of Odonata (Kiauta 1972c), approximately 90 chromosome papers have been published. The number of examined species has since increased by more than 2.3 times, and now it seems appropriate to publish an updated list. In this review article, all data available today are presented in two tables and one figure. Table 1 includes all species studied so far cytogenetically and compiles data on their chromosome numbers, sex chromosome mechanisms and the occurrence of the so-called m-chromosomes (= microchromosomes). Table 2 summarizes data presented in Table 1 and shows the family-level variability of the above-mentioned traits (except m-chromosomes, since data on their presence or absence in specific species are often questionable) together with the most characteristic (modal) karyotypes for each of the families explored. On the Fig. 1, the modal karyotypes are mapped onto phylogenetic tree of Odonata families taken from Bybee et al. (2016) who in turn redrawn and synthesized it from Dijkstra et al. (2014) and Carle et al. (2015). In the final section of the review, the main characteristics of Odonata karyotypes are briefly discussed and prospects for future research are outlined.

Table 1. Cytogenetically analyzed species of Odonata and their main karyotype characteristics (chromosome numbers, sex chromosomes, m-chromosomes).

Taxon		Karyotype formula 2n ♂	m-chromosomes	Country	References
Anisozygoptera					
Epiophlebioidea					
Epiophlebiidae					
1.	***Epiophlebia*** *superstes* Selys, 1889	25(24A+X)	–	Japan	Oguma 1951
Anisoptera					
Aeshnoidea					
Aeshnidae					
2.	***Aeshna*** *caerulea* (Ström, 1783)	24(22A+neo-XY)	–	Finland	Oksala 1943
3.	*A. canadiensis* Walker, 1908	27(26A+X)	+	USA	Cruden 1968
4.	*A. clepsydra* Say, 1839	27(26A+X)	+	USA	Hung 1971
5.	*A. crenata* Hagen, 1856	27(26A+X)	+	Finland	Oksala 1939a, 1943, 1944, 1952
		– » –	–	Russia	Perepelov and Bugrov 2002
6.	*A. cyanea* (Müller, 1764)	27(26A+X)	+	Finland	Oksala 1943
		– » –	+	Netherlands	Kiauta 1969a
7.	*A. grandis* (Linnaeus, 1758)	27(26A+X)	+	Former USSR	Fuchsówna and Sawczyńska 1928
		25(24A+X)	+	Former USSR	Makalowskaja 1940
		26(24A+neo-XY)	+	Finland	Oksala 1939a, 1943, 1944, 1945
		– » –	+	Netherlands	Kiauta 1967a–d 1968a, b, 1969a
		– » –	+	Russia	Perepelov and Bugrov 2002
		25(24A+X)	–	Finland	Nokkala et al. 2002
8.	*A. isoceles* (Müller, 1767)	27(26A+X)	–	USA	Kiauta 1978 as *Anaciaeschna isosceles* (Müller, 1767)
		25(24A + X)	+	Russia	Kuznetsova et al. 2020b
9.	*A. juncea* (Linnaeus, 1758)	26(24A+neo-XY)	+	Finland	Oksala 1939a, 1943, 1944
		– » –	+	Former USSR	Makalowskaja 1940
		27(26A+X)	+	Italy	Kiauta 1971a
		26(24A+neo-XY)	+	Russia	Perepelov and Bugrov 2002
10.	*A. mixta* Latreille, 1805	27(26A+X)	+	Netherlands	Kiauta 1969a
		25(24A+X)	+	India	Sandhu and Malhotra 1994a
		– » –	+	India	Sharma and Durani 1995
		27(26A+X)	+	Russia	Perepelov and Bugrov 2001b
11.	*A. nigroflava* Martin, 1909	27(26A+X)	+	Japan	Katatani 1987
		– » –	–	Russia	Perepelov and Bugrov 2002
12.	*A. palmata* Hagen, 1856	27(26A+X)	+	USA	Cruden 1968
13.	*A. serrata* Hagen, 1856	26(24A+neo-XY)	+	Finland	Oksala 1943 as *A. osiliensis* Mierzejewski, 1913 and *A. s. fennica* Valle, 1938
14.	*A. subarctica* Walker, 1908	27(26A+X)	+	USA	Oksala 1939a, 1943, 1952 as *A. s. elisabethae* Djakonov, 1922
		– » –	+	Switzerland	Kiauta and Kiauta 1980a as *A. s. elisabethae*
15.	*A. umbrosa* Walker, 1908	27(26A+X)	+	USA	Cruden 1968 as *A. u. occidentalis* Walker, 1908 and *A. u. umbrosa* Walker, 1908
16.	*A. verticalis* Hagen, 1861	27(26A+X)	+	USA	Hung 1971
17.	*A. viridis* Eversmann, 1836	26(24A+neo-XY)	+	Finland	Oksala 1943
		– » –	+	Russia	Perepelov et al. 1998
18.	*A. walkeri* Kennedy, 1917	27(26A+X)	+	USA	Cruden 1968
19.	***Anaciaeschna*** *jaspidea* (Burmeister, 1839)	27(26A+X)	+	India	Walia and Sandhu 1999
20.	***Anax*** *amazili* (Burmeister, 1839)	27(26A+X)	–	Argentina	Capitulo et al. 1991
		– » –	+	Argentina	Mola et al. 1999
21.	*A. concolor* Brauer, 1865	27(26A+X)	+	Surinam	Kiauta 1979a
22.	*A. ephippiger* (Burmeister, 1839)	13(12A+X)	+	India	Seshachar and Bagga 1962 as *Hemianax ephippiger* (Burmeister, 1839)
		14(12A+neo-XY)	+	India	Kiauta 1969a as *H. ephippiger*

Taxon		Karyotype formula 2n ♂	m-chromosomes	Country	References
23.	*A. guttatus* (Burmeister, 1839)	15(14A+X)	+	Nepal	Kiauta and Kiauta 1982
24.	*A. immaculiformis* Rambur, 1842	27(26A+X)	+	India	Sangal and Tyagi 1982
		– » –	+	India	Walia et al. 2018
25.	*A. imperator* Leach, 1815	27(26A+X)	+	France	Kiauta 1965, 1969a
		– » –	–	Kenya	Wasschner 1985
		– » –	+	Russia	Perepelov and Bugrov 2002
26.	*A. junius* (Drury, 1773)	27(26A+X)	+	USA	McGill 1904, 1907
		– » –	+	USA	Lefevre and McGill 1908
		– » –	–	Japan	Kichijo 1942a
		– » –	+	USA	Cruden 1968
		– » –	–		
27.	*A. longipes* Hagen, 1861	27(26A+X)	+	USA	Cruden 1968
28.	*A. nigrofasciatus* Oguma, 1915	27(26A+X)	+	Nepal	Kiauta 1974, 1975 (*A. n. nigrolineatus* Fraser, 1935)
		25(24A+X)	+	India	Sandhu and Malhotra 1994a (*A. n. nigrolineatus*)
		27(26A+X)	+	India	Walia and Sandhu 1999 (*A. n. nigrolineatus*)
		– » –	+	India	Walia et al. 2018 (*A. n. nigrolineatus*)
29.	*A. papuensis* (Burmeister, 1839)	27(26A+X)	+	Australia	Kiauta 1968c, 1969a as *Hemianax papuensis* (Burmeister, 1839)
30.	*A. parthenope* (Selys, 1839)	27(26A+X)	+	Japan	Omura 1957 as *A. parthenope julius* Brauer, 1865
		– » –	+	India	Thomas and Prasad 1986
		– » –	+	China	Zhu and Wu 1986 as *A. p. julius*
		25(24A+X)	+	Japan	Suzuki and Saitoh 1990 as *A. p. julius*
		27(26A+X)	+	India	Sandhu and Malhotra 1994a
31.	***Andaeschna*** *unicolor* (Martin, 1908)	27(26A+X)	+	Bolivia	Cumming 1964 as *Aeshna* cf. *unicolor* Martin, 1908
32.	***Austroaeschna*** *anacantha* Tillyard, 1908	27(26A+X)	+	Australia	Kiauta 1968c as *Acanthaeschna anacantha* (Tillyard, 1908)
33.	*A. multipunctata* (Martin, 1901)	27(26A+X)	+	Australia	Kiauta 1968c as *Acanthaeschna multipunctata* (Martin, 1901)
34.	***Basiaeschna*** *janata* (Say, 1939)	25(24A+X)	–	USA	Cruden 1968
35.	***Boyeria*** *maclachlani* (Selys, 1883)	27(26A+X)	+	Japan	Omura 1957
36.	*B. vinosa* (Say, 1839)	27(26A+X)	–	USA	Cruden 1968
37.	***Caliaeschna*** *microstigma* (Schneider, 1845)	16(14A+neo-XY)	+	Greece	Kiauta 1972a
38.	***Castoraeschna*** *castor* (Brauer, 1865)	27(26A+X)	+	Brazil	Kiauta 1972b
39.	***Cephalaeschna*** *orbifrons* Selys, 1883	25(24A+X)	+	Nepal	Kiauta 1975
40.	*Cephalaeschna* sp.	25(24A+X)	+	India	Sandhu and Malhotra 1994a
41.	***Coryphaeschna*** *adnexa* (Hagen, 1961)	27(26A+X)	–	Bolivia	Cumming 1964
42.	*C. perrensi* (McLachlan, 1887)	25(24A+X)	–	Argentina	Capitulo et al. 1991
		27(26A+X)	+	Argentina	Mola et al. 1999
		– » –	+	Argentina	De Gennaro et al. 2008
43.	*C. viriditas* Calvert, 1952	23(22A+X)	+	Surinam	Kiauta 1979a
44.	***Gynacantha*** *bayadera* Selys, 1891	25(24A+X)	+	India	Walia 2007 as *G. milliardi* Fraser, 1936
		27(26A+X)	+		
45.	*G. hyalina* Selys, 1882	28(26A+XX)*	+	India	Tyagi 1978a, b
46.	*G. interioris* Williamson, 1923	26(24A+neo-XY)	+	Surinam	Kiauta 1979a
		– » –	+	Brazil	Ferreira et al. 1979
47.	*G. japonica* Bartenev, 1909	27(26A+X)	+	Japan	Omura 1957
48.	***Gynacanthaeschna*** *sikkima* (Karsch, 1891)	27(26A+X)	+	India	Walia et al. 2016
49.	***Oplonaeschna*** *armata* (Hagen, 1861)	27(26A+X)	+	Mexico	Kiauta 1970a
50.	***Planaeschna*** *milnei* (Selys, 1883)	27(26A+X)	+	Japan	Kiauta 1968c, 1969a
51.	***Remartinia*** *luteipennis* (Burmeister, 1839)	25(24A+X)	+	Surinam	Kiauta 1979a as *Coryphaeschna l. luteipennis* Burmeister, 1839
		27(26A+X)	+	Brazil	Ferreira et al. 1979 as *C. l. luteipennis*
52.	***Rhionaeschna*** *bonariensis* (Rambur, 1842)	26(24A+neo-XY)	+	Argentina, Uruguay	Mola and Papeschi 1994 as *Aeschna bonariensis* Rambur, 1842

Taxon		Karyotype formula 2n ♂	m-chromosomes	Country	References
52.	***Rhionaeschna*** *bonariensis* (Rambur, 1842)	– » –	+	Argentina, Uruguay	Mola 1995 as *A. bonariensis*
53.	*Rh. californica* (Calvert, 1895)	27(26A+X)	+	Canada	Kiauta 1973a as *Aeshna californica* Calvert, 1895
54.	*Rh. confusa* (Rambur, 1842)	27(26A+X)	+	Argentina, Uruguay	Mola and Papeschi 1994 as *Aeshna confuse* Rambur, 1842
		– » –	+	Argentina, Uruguay	Mola 1995 as *A. confuse*
55.	*Rh. diffinis* (Rambur, 1842)	21(20A+X)	+	Bolivia	Cumming 1964 as *Aeshna d. diffinis* Rambur, 1842
56.	*Rh. intricata* (Martin, 1908)	19(18A+X)	+	Bolivia	Cumming 1964 as *Aeshna intricata* Martin, 1908
57.	*Rh. peralta* (Ris, 1918)	27(26A+X)	+	Bolivia	Cumming 1964 as *Aeshna peralta* Ris, 1918
58.	*Rh. planaltica* (Calvert, 1845)	16(14A+neo-XY)	+	Argentina	Mola and Papeschi 1994 as *Aeschna cornigera planaltica* Calvert, 1952
		– » –	+	Argentina	Mola 1995 as *A. c. planaltica*
59.	***Staurophlebia*** *reticulata* (Burmeister, 1839)	27(26A+X)	+	Brazil	Souza Bueno 1982 (*S. r. reticulata* (Burmeister, 1839))
Petaluroidea					
Petaluridae					
60.	***Tachopteryx*** *thoreyi* (Hagen, 1857)	19(18A+X)	+	USA	Cumming 1964
61.	***Tanypteryx*** *hageni* (Selys, 1879)	17(16A+X)	+	USA	Cruden 1968
62.	*T. pryeri* (Selys, 1889)	17(16A+X)	+	Japan	Kichijo 1939, 1942a
63.	***Uropetala*** *carovei* (White, 1846)	17(16A+X)**	+	New Zealand	Wolfe 1953
		25(24A+X)	+	New Zealand	Jensen and Mahanty 1978
		– » –	+	New Zealand	Jensen 1980
Gomphoidea					
Gomphidae					
64.	***Anisogomphus*** *bivittatus* (Selys, 1854)	23(22A+X)	+	India	Das 1956
		– » –	+	India	Walia and Chahal 2020
65.	*A. occipitalis* (Selys, 1854)	23(22A+X)	–	Nepal	Kiauta 1974, 1975
66.	***Aphylla*** *edentata* Selys, 1869	23(22A+X)	–	Bolivia	Cumming 1964
67.	*A. producta* Selys, 1854	23(22A+X)	–	Bolivia	Cumming 1964
68.	*A. theodorina* (Navas, 1933)	23(22A+X)	+	Surinam	Kiauta 1979a
		– » –	+	Brazil	Ferreira et al. 1979
69.	*A. williamsoni* (Gloyd, 1936)	23(22A+X)	+	USA	Kiauta and Brink 1978
70.	*Aphylla* sp.	23(22A+X)	+	Argentina	Mola 2007
71.	***Arigomphus*** *lentulus* (Needham, 1902)	23(22A+X)	–	USA	Cruden 1968 as *Gomphus lentulus* Needham, 1902
72.	*A. pallidus* (Rambur, 1842)	23(22A+X)	–	USA	Cumming 1964 as *Gomphus pallidus* Rambur, 1842
73.	*A. submedianus* (Williamson, 1914)	23(22A+X)	–	USA	Cruden 1968 as *Gomphus submedianus* Williamson, 1914
74.	***Asiagomphus*** *melaenops* (Selys, 1854)	23(22A+X)	+	Japan	Toyoshima and Hirai 1953 as *Gomphus melaenops* Selys, 1854
		– » –	+	Japan	Hirai 1956 as *G. melaenops*
		– » –	+	USA	Cruden 1968 as *G. melaenops*
75.	***Burmagomphus*** *pyramidalis* Laidlaw, 1922	23(22A+X)	+	India	Tyagi 1977
76.	***Davidius*** *nanus* (Selys, 1869)	23(22A+X)	–	Japan	Kichijo 1939, 1942a
77.	***Dromogomphus*** *spinosus* (Selys, 1854)	23(22A+X)	+	USA	Cruden 1968
78.	*D. spoliatus* (Hagen, 1857)	23(22A+X)	+	USA	Cruden 1968
79.	***Epigomphus*** *llama* Calvert, 1903	23(22A+X)	–	Bolivia	Cumming 1964
80.	***Erpetogomphus*** *designatus* Hagen, 1857	23(22A+X)	+	USA	Cumming 1964
81.	*E. diadophis* Calvert, 1905	23(22A+X)	–	USA	Cumming 1964
82.	*E. ophibolus* Calvert, 1905	23(22A+X)	+	Mexico	Kiauta 1970a
83.	***Gomphoides*** sp.	23(22A+X)	–	Bolivia	Cumming 1964
84.	***Gomphus*** *confraternus* Selys, 1873	23(22A+X)	+	USA	Cruden 1968
85.	*G. exilis* Selys, 1854	23(22A+X)	+	USA	Cruden 1968
		– » –	+	Canada	Kiauta 1969a
86.	*G. graslini* Rambur, 1842	12(10A+neo-neo-XY)	+	France	Kiauta 1968d, 1969a

Taxon		Karyotype formula 2n ♂	m-chromosomes	Country	References
87.	*G. pulchellus* Selys, 1840	23(22A+X)	+	France	Kiauta 1973b
88.	*G. vulgatissimus* (Linnaeus, 1758)	23(22A+X)	–	Russia	Perepelov et al. 2001
89.	***Ictinogomphus*** *rapax* (Rambur, 1942)	23(22A+X)	+	India	Asana and Makino 1935
		– » –	+	India	Makino 1935
		– » –	+	India	Kichijo 1942a
		– » –	+	India	Omura 1949, 1952, 1953
		– » –	+	India	Dasgupta 1957
90.	***Nepogomphus*** *modestus* (Selys, 1878)	23(22A+X)	–	India	Walia et al. 2006
		– » –	–	India	Walia and Chahal 2014
91.	***Nihonogomphus*** *ruptus* (Selys, 1858)	23(22A+X)	–	Russia	Perepelov et al. 2001
92.	*N. viridis* Oguma, 1926	23(22A+X)	+	Japan	Omura 1957
93.	***Nychogomphus*** *duaricus* (Fraser, 1924)	22(20A+neo-XY)	+	India	Tyagi 1977
94.	***Octogomphus*** *specularis* (Hagen, 1859)	23(22A+X)	–	USA	Cruden 1968
95.	***Onychogomphus*** *forcipatus* (Linnaeus, 1758)	25(24A+X)	–	Finland	Oksala 1945
		22(20A+neo-XY)	–	Austria	Kiauta 1969a
		25(24A+X)	–		
96.	*O. saundersii* Selys, 1854	22(20A+neo-XY)	+	India	Tyagi 1977 (*O. s. duaricus* Fraser, 1924)
97.	***Ophiogomphus*** *bison* Selys, 1873	23(22A+X)	–	USA	Cruden 1968
		25(24A+X)	–		
98.	*O. cecilia* (Fourcroy, 1785)	24(22A+XX)*	–	Finland	Oksala 1945
		23(22A+X)	–	Russia	Perepelov et al. 1998
		– » –	–	Russia	Perepelov and Bugrov 2001a
99.	*O. colubrinus* Selys, 1854	23(22A+X)	–	USA	Cruden 1968
100.	*O. obscurus* Bartenev, 1909	23(22A+X)	–	Russia	Perepelov and Bugrov 2001b
101.	*O. occidentalis* Hagen, 1882	23(22A+X)	–	USA	Cruden 1968
102.	*O. rupinsulensis* (Walsh, 1862)	23(22A+X)	–	USA	Cruden 1968
103.	***Phanogomphus*** *lividus* (Selys, 1854)	23(22A+X)	+	USA	Cruden 1968 as *Gomphus lividus* Selys, 1854
104.	*Ph. militaris* (Hagen, 1858)	23(22A+X)	–	USA	Cruden 1968 as *Gomphus militaris* Hagen, 1858
105.	*Ph. spicatus* (Selys, 1854)	23(22A+X)	+	USA	Cruden 1968 as *Gomphus spicatus* Selys, 1854
106.	***Paragomphus*** *lineatus* (Selys, 1850)	23(22A+X)	–	Nepal	Kiauta 1974, 1975
		– » –	–	India	Walia and Chahal 2014
107.	*P. capricornis* (Förster, 1914)	23(22A+X)	–	Thailand	Kiauta and Kiauta 1983
108.	***Phyllocycla*** *propinqua* Belle, 1972	21(20A+X)	–	Argentina	De Gennaro 2004
109.	*Phyllocycla* sp.	23(22A+X)	–	Bolivia	Cumming 1964
110.	*Phyllocycla* sp. 1	23(22A+X)	+	Argentina	Mola 2007
111.	*Phyllocycla* sp. 2	23(22A+X)	–	Argentina	Mola 2007
112.	***Phyllogomphoides*** *undulatus* (Needham, 1944)	23(22A+X)	+	Surinam	Kiauta 1979a
113.	***Progomphus*** *borealis* McLachlan, 1873	23(22A+X)	–	USA	Cruden 1968
114.	*P. intricatus* (Hagen, 1857)	23(22A+X)	–	Bolivia	Cumming 1964
115.	*P. obscurus* (Rambur, 1842)	23(22A+X)	–	USA	Cruden 1968
116.	*P. phyllochromus* Ris, 1918	23(22A+X)	+	Bolivia	Cumming 1964
117.	***Scalmogomphus*** *bistrigatus* (Hagen, 1854)	23(22A+X)	–	Nepal	Kiauta 1974, 1975 as *Onychogomphus bistrigatus* (Hagen, 1854)
118.	***Shaogomphus*** *postocularis* (Selys, 1869)	23(22A+X)	+	Japan	Omura 1957 as *Gomphus postocularis* Selys, 1869
		– » –	–	Russia	Perepelov et al. 2001 as *Gomphus epophtalmus* Selys, 1872
119.	***Sieboldius*** *albardae* Selys, 1886	23(22A+X)	+	Japan	Omura 1957
120.	***Stylogomphus*** *suzukii* (Matsumura, 1926)	23(22A+X)	+	Japan	Oguma 1930
		– » –	+	Japan	Kichijo 1942a
121.	***Stylurus*** *flavipes* (Charpentier, 1825)	23(22A+X)	+	Russia	Perepelov and Bugrov 2001b
122.	*S. plagiatus* (Selys, 1854)	23(22A+X)	+	USA	Cruden 1968 as *Gomphus plagiatus* Selys, 1854
123.	*S. scudderi* (Selys, 1873)	23(22A+X)	–	USA	Cruden 1968 as *Gomphus scudderi* Selys, 1873

Taxon		Karyotype formula 2n ♂	m-chromosomes	Country	References
124.	*S. townesi* Gloyd, 1936	22(20A+neo-XY)	–	USA	Kiauta and Brink 1978 as *Gomphus townesi* Gloyd, 1936
125.	***Temnogomphus** bivittatus* (Selys, 1854)	23(22A+X)	+	Nepal	Kiauta 1975
126.	***Trigomphus** citimus* (Needham, 1931)	21(20A+X)	+	Japan	Toyoshima and Hirai 1953 (*T. c. tabei* Asahina, 1949)
		– » –	+	Japan	Hirai 1956 (*T. c. tabei*)
127.	*T. interruptus* (Selys, 1854)	19(18A+X)	+	Japan	Oguma 1930
		– » –	+	Japan	Toyoshima and Hirai 1953
		– » –	+	Japan	Hirai 1956
		– » –	+	Japan	Omura 1957
128.	*T. melampus* (Selys, 1869)	21(20A+X)	–	Japan	Oguma 1930, 1942 as *T. unifasciatus* (Oguma 1926)
129.	***Zonophora** callipus* Selys, 1869	23(22A+X)	+	Surinam	Kiauta 1979a
Libelluloidea					
Macromiidae					
130.	***Didymops** transversa* (Say, 1839)	25(24A+X)	+	USA	Cruden 1968
131.	***Epophthalmia** frontalis* (Selys, 1871)	25(24A+X)	+	India	Dasgupta 1957 (*E. f. frontalis* (Selys, 1871))
132.	***Macromia** daimoji* Okumura, 1949	25(24A+X)	–	Japan	Katatani 1987
133.	*M. amphigenia* Selys, 1871	25(24A+X)	–	Russia	Perepelov and Bugrov 2001b (*M. a. fraenata* Martin, 1906)
134.	*M. magnifica* (McLachlan, 1874)	25(24A+X)	+	USA	Cruden 1968
		– » –	–		
135.	*M. moorei* Selys, 1874	25(24A+X)	+	Nepal	Kiauta 1977
		– » –	+	India	Walia and Chahal 2018
Corduliidae					
136.	***Cordulia** aenea* (Linnaeus, 1758)	25(24A+X)	–	Finland	Oksala 1939a
		– » –	–	Former USSR	Makalowskaja 1940
		– » –	–	Netherlands	Kiauta 1968b, 1969a
		– » –	–	Russia	Perepelov et al. 1998
		– » –	–	Bulgaria	Grozeva and Marinov 2007
		– » –	–	Russia	Kuznetsova et al. 2018
137.	*C. shurtleffi* Scudder, 1866	25(24A+X)	+	USA	Cruden 1968
		– » –	+	Canada	Kiauta 1973a
138.	***Dorocordulia** libera* (Selys, 1871)	11(10A+X)	–	USA	Cruden 1968
		13(12A+X)	–		
		14(12A+neo-XY)	–	USA	Kiauta 1969a
		13(12A+X)	–		
139.	***Epicordulia** princeps* (Hagen, 1861)	25(24A+X)	+	USA	Hung 1971
140.	***Epitheca** bimaculata* (Charpentier, 1825)	25(24A+X)	–	Russia	Perepelov 2003
		– » –	–	Russia	Kuznetsova et al. 2018
141.	*E. canis* McLachlan, 1886	25(24A+X)	+	USA	Cruden 1968
142.	*E. cynosura* (Say, 1839)	19(18A+X)	–	USA	Cruden 1968
		21(20A+X)	–		
143.	*E. petechialis* (Muttkowski, 1911)	21(20A+X)	–	USA	Cumming 1964 as *Tetragoneuria petechialis* Muttkowski, 1911
144.	*E. semiaquea* (Burmeister, 1839)	25(24A+X)	–	USA	Cruden 1968
145.	*E. spinigera* (Selys, 1871)	25(24A+X)	+	USA	Cruden 1968
		27(26A+X)	–	USA	Hung 1971 as *Tetragoneuria spinigera* (Selys, 1871)
146.	***Procordulia** grayi* (Selys, 1871)	25(24A+X)	+	New Zealand	Jensen 1980
147.	*P. smithii* (White, 1846)	25(24A+X)	+	New Zealand	Jensen 1980
148.	***Rialla** villosa* Rambur, 1842	25(24A+X)	+	Argentina	De Gennaro 2004
149.	***Somatochlora** alpestris* (Selys, 1840)	25(24A+X)	–	Switzerland	Kiauta and Kiauta 1980a
		27(26A+X)	+		
150.	*S. arctica* (Zetterstedt, 1840)	25(24A+X)	+	Russia	Perepelov 2003
151.	*S. borisi* Marinov, 2001	20(18A+XY)	–	Bulgaria	Grozeva and Marinov 2007
152.	*S. flavomaculata* (Van der Linden, 1825)	25(24A+X)	–	Former USSR	Makalowskaja 1940
		– » –	–	Russia	Perepelov 2003
		– » –	+	Russia	Kuznetsova et al. 2020b

Taxon		Karyotype formula 2n ♂	m-chromosomes	Country	References
153.	*S. graeseri* Selys, 1887	25(24A+X)	–	Russia	Perepelov et al. 2001
154.	*S. meridionalis* Nielsen, 1935	25(24A+X)	–	Slovenia	Kiauta and Kiauta 1995
		– » –	–	Bulgaria	Grozeva and Marinov 2007
155.	*S. metallica* (Van der Linden, 1825)	26(24A+XX)*	–	Finland	Oksala 1945
		25(24A+X)	–	Finland	Nokkala et al. 2002
		– » –	–	Finland	Grozeva and Marinov 2007
		– » –	–	Russia	Perepelov and Bugrov 2001b
156.	*S. semicircularis* (Selys, 1871)	25(24A+X)	–	USA	Cruden 1968
157.	*S. uchidai* Fürster, 1909	25(24A+X)	+	Japan	Oguma 1915, 1930
		– » –	+	Japan	Kichijo 1942b
158.	*S. viridiaenea* (Uhler, 1858)	25(24A+X)	–	Japan	Oguma 1915, 1930
		– » –	–	Japan	Kichijo 1942b
Libellulidae					
159.	***Acisoma*** *panorpoides* Rambur, 1842	25(24A+X)	+	Bangladesh, India	Dasgupta 1957 (*A. p. panorpoides* Rambur, 1842)
		– » –	+	Nepal	Kiauta 1975 (*A. p. panorpoides*)
		– » –	+	Thailand	Kiauta and Kiauta 1983 (*A. p. panorpoides*)
		– » –	+	India	Tyagi 1982
160.	***Aethriamanta*** *brevipennis* (Rambur, 1842)	25(24A+X)	+	India	Dasgupta 1957
161.	***Anatya*** *guttata* (Erichson, 1848)	25(24A+X)	–	Surinam	Kiauta 1979a
162.	***Atoconeura*** *biordinata* Karsch, 1899	21(20A+X)	+	Sudan	Wasscher 1985
163.	***Brachydiplax*** *chalybea* Brauer, 1868	25(24A+X)	+	India	Dasgupta 1957
		– » –	+	India	Taygi 1982
		– » –	+	Thailand	Kiauta and Kiauta 1983
		– » –	+	India	Prasad and Thomas 1992
164.	*B. farinosa* Krueger, 1902	25(24A+X)	+	India	Dasgupta 1957
		– » –	+	India	Taygi 1982
		– » –	–	Thailand	Kiauta and Kiauta 1983
165.	*B. sobrina* (Rambur, 1842)	25(24A+X)	+	India	Ray Chaudhuri and Dasgupta 1949
		– » –	+	India	Taygi 1982
		– » –	+	Nepal	Kiauta and Kiauta 1982
166.	***Brachymesia*** *furcata* (Hagen, 1861)	25(24A+X)	+	Surinam	Kiauta 1979a
		– » –	+	Argentina	Agopian and Mola 1988
		– » –	–	Brazil	Ferreira et al. 1979
		– » –	–	Brazil	Souza Bueno 1982
167.	*B. gravida* (Calvert, 1890)	25(24A+X)	+	USA	Cruden 1968 as *Cannacria gravida* (Calvert, 1890)
168.	*B. herbida* (Gundlach, 1889)	25(24A+X)	+	Jamaica	Cumming 1964 as *Cannacria herbida* (Gundlach, 1889)
169.	***Brachythemis*** *contaminata* (Fabricius, 1793)	25(24A+X)	+	India	Asana and Makino 1935
		– » –	+	India	Makino 1935
		– » –	+	India	Kichijo 1942b
		– » –	+	India	Dasgupta 1957
		– » –	+	Nepal	Kiauta 1975
		– » –	+	India	Tyagi 1982
		– » –	+	Thailand	Kiauta and Kiauta 1983
170.	*B. lacustris* (Kirby, 1899)	25(24A+X)	+	Sudan	Wasscher 1985
171.	***Bradinopyga*** *cornuta* Ris, 1911	25(24A+X)	+	Republic of South Africa	Boyes et al. 1980
172.	*B. geminata* (Rambur, 1842)	25(24A+X)	+	India	Dasgupta 1957
		– » –	+	India	Tyagi 1982
173.	***Brechmorhoga*** *mendax* (Hagen, 1861)	25(24A+X)	+	USA	Cruden 1968
		– » –	–		
174.	*B. nubecula* (Rambur, 1842)	25(24A+X)	+	Bolivia	Cumming 1964
175.	*B. pertinax* (Hagen, 1861)	25(24A+X)	–	Bolivia	Cumming 1964 (*B. p. peruviana* Ris, 1913)
176.	***Cannaphila*** *vibex* (Hagen, 1861)	25(24A+X)	+	Bolivia	Cumming 1964
177.	***Celithemis*** *amanda* (Hagen, 1861)	25(24A+X)	+	USA	Kiauta and Brink 1978
178.	*C. elisa* (Hagen, 1861)	25(24A+X)	+	USA	Cruden 1968
179.	*C. fasciata* Kirby, 1889	25(24A+X)	+	USA	Cruden 1968

Taxon		Karyotype formula 2n ♂	m-chromosomes	Country	References
180.	*C. ornata* (Rambur, 1842)	25(24A+X)	+	USA	Kiauta and Brink 1978
181.	***Crocothemis*** *erythraea* (Brulle, 1832)	25(24A+X)	+	India	Dasgupta 1957
		– » –	+	Kenya	Kiauta 1969b
		– » –	+	Italy	Kiauta 1971a
		– » –	+	India	Prasad and Thomas 1992
		– » –	+	Republic of South Africa	Boyes et al. 1980
		– » –	+	India	Tyagi 1982
182.	*C. sanguinolenta* (Burmeister, 1839)	25(24A+X)	+	Kingdom of Eswatini (Former Swaziland)	Boyes et al. 1980
183.	*C. servilia* (Drury, 1773)	25(24A+X)	+	India	Asana and Makino 1935
		– » –	+	India	Makino 1935
		– » –	+	India	Kichijo 1942b
		– » –	+	India	Ray Chaudhuri and Dasgupta 1949
		– » –	+	Nepal	Kiauta 1975
		– » –	+	Philippines	Kiauta and Kiauta 1980b
		– » –	+	Nepal	Kiauta and Kiauta 1982
		– » –	+	India	Tyagi 1982
		– » –	+	Thailand	Kiauta and Kiauta 1983
		– » –	+	Japan	Katatani 1987
		– » –	+	Japan	Higashi and Kayano 1993
		– » –	+	Japan, Taiwan	Higashi et al. 2001
		24(22A+neo-XY)	+	Japan	Omura 1955 (*C. s. mariannae* Kiauta, 983)
		– » –	–	Japan	Kiauta 1983 (*C. s. mariannae*)
		– » –	–	Japan	Katatani 1987 (*C. s. mariannae*)
		– » –	–	Japan	Higashi et al. 2001 (*C. s. mariannae*)
184.	***Dasythemis*** *esmeralda* Ris, 1910	25(24A+X)	+	Bolivia	Cumming 1964
185.	*D. mincki* (Karsch, 1890)	25(24A+X)	+	Brazil	Souza Bueno 1982
186.	*D. venosa* (Burmeister, 1839)	25(24A+X)	+	Brazil	Kiauta and Boyes 1972
187.	***Diastatops*** *intensa* Montgomery, 1940	25(24A+X)	+	Bolivia	Cumming 1964
188.	*D. obscura* (Fabricius, 1775)	25(24A+X)	+	Bolivia	Cumming 1964
189.	*D. pullata* (Burmeister, 1839)	23(22A+X)	+	Surinam	Kiauta 1979a
190.	***Diplacodes*** *bipunctata* (Brauer, 1865)	25(24A+X)	+	Australia	Kiauta 1969b
		29(28A+X)	+		
191.	*D. haematodes* (Burmeister, 1839)	25(24A+X)	+	Australia	Kiauta 1969b
		23(22A+X)	–		
192.	*D. lefebvrei* (Rambur, 1842)	25(24A+X)	+	Madagascar	Kiauta 1968c, 1969b
193.	*D. nebulosa* (Fabricius, 1793)	25(24A+X)	+	India	Dasgupta 1957
		– » –	+	India	Kiauta and Kiauta 1982
		– » –	+	India	Tyagi 1982
194.	*D. trivialis* (Rambur, 1842)	25(24A+X)	+	India	Asana and Makino 1935
		– » –	+	India	Makino 1935
		– » –	+	India	Dasgupta 1957
		– » –	+	Australia	Kiauta 1969c
		– » –	+	Nepal	Kiauta 1975
		– » –	+	India	Tyagi 1982
195.	***Dythemis*** *fugax* Hagen, 1861	25(24A+X)	+	USA	Cruden 1968
196.	*D. multipunctata* Kirby, 1894	25(24A+X)	+	Surinam	Kiauta 1979a
		– » –	+	Brazil	Ferreira et al. 1979
197.	*D. rufinefris* (Burmeister, 1839)	25(24A+X)	+	Jamaica	Cumming 1964
198.	*D. velox* Hagen, 1861	25(24A+X)	+	Bolivia	Cumming 1964
		– » –	+	Peru	Kiauta and Boyes 1972
199.	***Elasmothemis*** *cannacrioides* (Calvert, 1906)	21(20A+X)	–	Bolivia	Cumming 1964 as *Dythemis cannacrioides* Calvert, 1906
		23(22A+X)	+	Surinam	Kiauta 1979a as *D. cannacrioides*
		– » –	+	Brazil	Ferreira et al. 1979
200.	*E. williamsoni* (Ris, 1919)	22(20A+neo-XY)	–	Surinam	Kiauta 1979a as *Dythemis williamsoni* (Ris, 1919)
		25(24A+X)	–		

Taxon		Karyotype formula 2n ♂	m-chromosomes	Country	References
201.	***Erythemis*** *attala* (Selys, 1857)	25(24A+X)	–	Bolivia	Cumming 1964
		– » –	+	Argentina	Agopian and Mola 1988
202.	*E. collocata* (Hagen, 1861)	25(24A+X)	+	USA	Cruden 1968
203.	*E. credula* (Hagen, 1861)	25(24A+X)	+	Surinam	Kiauta 1979a
204.	*E. haematogastra* (Burmeister, 1839)	25(24A+X)	–	Surinam	Kiauta 1979a
205.	*E. peruviana* (Rambur, 1842)	25(24A+X)	–	Surinam	Kiauta 1979a
206.	*E. plebeja* (Burmeister, 1839)	25(24A+X)	–	Bolivia	Cumming 1964
207.	*E. simplicicollis* (Say, 1839)	25(24A+X)	+	USA	Cruden 1968
208.	*E. vesiculosa* (Fabricius, 1775)	25(24A+X)	–	Bolivia	Cumming 1964 as *Lepthemis vesiculosa* (Fabricius, 1775)
		– » –	–	Surinam	Kiauta 1979a as *L. vesiculosa*
		– » –	+	Brasil	Ferreira et al. 1979 as *L. vesiculosa*
209.	***Erythrodiplax*** *anomala* (Brauer, 1865)	25(24A+X)	+	Brazil	Souza Bueno 1982
210.	*E. atroterminala* Ris, 1911	25(24A+X)	+	Uruguay	Goni and Abenante 1982
		– » –	+	Argentina	Mola 1996
211.	*E. attenuata* (Kirby, 1889)	25(24A+X)	+	Surinam	Kiauta 1979a
		– » –	+	Brasil	Ferreira et al. 1979
212.	*E. basalis* (Kirby, 1897)	25(24A+X)	–	Bolivia	Cumming 1964
		– » –	+	Surinam	Kiauta 1979a (*E. b. basalis* (Kirby, 1897))
		– » –	+	Brasil	Ferreira et al. 1979 (*E. b. basalis*)
213.	*E. berenice* (Drury, 1770)	25(24A+X)	–	USA	Cruden 1968
		27(26A+X)	+	USA	Hung 1971
		25(24A+X)	+		
214.	*E. castanea* (Burmeister, 1839)	25(24A+X)	–	Bolivia	Cumming 1964
215.	*E. chromoptera* Borror, 1942	23(22A+X)	+	Uruguay	Goni and Abenante 1982
216.	*E. cleopatra* Ris, 1911	25(24A+X)	+	Peru	Kiauta and Boyes 1972
217.	*E. connata* (Burmeister, 1839)	25(24A+X)	+	Chile	Kiauta and Boyes 1972 (*E. c. connata* (Burmeister, 1839))
		– » –	+	USA	Kiauta and Brink 1978 (*E. c. minuscula* (Rambur, 1842))
218.	*E. coralline* (Brauer, 1865)	25(24A+X)	+	Argentina	Mola 1996
219.	*E. famula* (Erichson, 1848)	25(24A+X)	+	Brazil	Souza Bueno 1982
220.	*E. fusca* (Rambur, 1842)	25(24A+X)	–	Bolivia	Cumming 1964 as *E. connata fusca* (Rambur, 1842)
		– » –	–	Guatemala	Cruden 1968 as *E. c. fusca*
		– » –	+	Surinam	Kiauta 1979a as *E. c. fusca*
		– » –	+	Brazil	Ferreira et al. 1979 as *E. c. fusca*
		– » –	+	Brazil	Souza Bueno 1982
		– » –	+	Argentina	Mola 1996
221.	*E. fervida* (Erichson, 1848)	25(24A+X)	+	Jamaica	Cumming 1964
222.	*E. justiniana* (Selys, 1857)	25(24A+X)	+	Jamaica	Cumming 1964
223.	*E. juliana* Ris, 1911	25(24A+X)	+	Brazil	Souza Bueno 1982
224.	*E. latimaculata* Ris, 1911	25(24A+X)	+	Surinam	Kiauta 1979a
		– » –	+	Brasil	Ferreira et al. 1979
225.	*E. lygaea* Ris, 1911	25(24A+X)	+	Argentina	Capitulo et al. 1991
		– » –	+	Argentina	Mola 1996
226.	*E. media* Borror, 1942	21(20A+X)	+	Bolivia	Cumming 1964
		22(20A+XX)*	+	Brazil	Kiauta and Boyes 1972
		21(20A+X)	+	Surinam	Kiauta 1979a
		– » –	+	Brasil	Ferreira et al. 1979
		22(20A+neo-XY)	+	Argentina	Mola 1996
227.	*E. melanorubra* Borror, 1942	25(24A+X)	+	Bolivia	Cumming 1964
		– » –	+	Venezuela	Kiauta and Boyes 1972
		– » –	+	Argentina	Capitulo et al. 1991
		– » –	+	Argentina	Mola 1996
228.	*E. minuscula* (Rambur, 1842)	25(24A+X)	+	USA	Kiauta and Brink 1978
		22(20A+neo-XY)	+	Argentina	Mola and Agopian 1985
229.	*E. nigricans* (Rambur, 1842)	25(24A+X)	+	Uruguay	Goni and Abenante 1982

Taxon		Karyotype formula 2n ♂	m-chromosomes	Country	References
229.	*E. nigricans* (Rambur, 1842)	– » –	+	Argentina	Mola 1996
		– » –	–	Argentina	De Gennaro 2004
		– » –	+	Argentina	De Gennaro et al. 2008
230.	*E. ochracea* (Burmeister, 1839)	25(24A+X)	+	Argentina	Mola 1996
231.	*E. paraguayensis* (Foerster, 1904)	23(22A+X)	+	Bolivia	Cumming 1964
		– » –	+	Surinam	Kiauta 1979a
232.	*E. umbrata* (Linnaeus, 1758)	25(24A+X)	+	Bolivia	Cumming 1964
		– » –	+	Dominica	Cruden 1968
		– » –	+	Surinam	Kiauta 1979a
		– » –	+	Brazil	Ferreira et al 1979
		– » –	+	Argentina	Mola 1996
233.	*E. unimaculata* (DeGeer, 1773)	25(24A+X)	+	Bolivia	Cumming 1964
		– » –	+	Surinam	Kiauta 1979a
234.	***Hydrobasileus*** *croceus* (Brauer, 1867)	25(24A+X)	+	India	Prasad and Thomas 1992
235.	***Ladona*** *julia* (Uhler, 1857)	25(24A+X)	+	USA	Cruden 1968
236.	***Lathrecista*** *asiatica* (Fabricius, 1798)	25(24A+X)	+	India	Dasgupta 1957
		– » –	+	India	Tyagi 1982
237.	***Leucorrhinia*** *albifrons* (Burmeister, 1839)	25(24A+X)	+	Former USSR	Makalowskaja 1940
238.	*L. dubia* (Van der Linden, 1825)	26(24A+XX)*	–	Finland	Oksala 1939a, 1945
		25(24A+X)	+	Russia	Kuznetsova et al. 2020b
239.	*L. frigida* Hagen, 1890	21(20A+X)	–	USA	Cruden 1968
		23(22A+X)	+		
240.	*L. glacialis* Hagen, 1890	25(24A+X)	+	USA	Cruden 1968
241.	*L. hudsonica* (Selys, 1850)	25(24A+X)	+	USA	Cruden 1968
		– » –	–		
242.	*L. intacta* (Hagen, 1861)	25(24A+X)	+	USA	Cruden 1968
		– » –	–		
243.	*L. pectoralis* (Charpentier, 1825)	26(24A+XX)*	–	Finland	Oksala 1945
244.	*L. proxima* Calvert, 1890	25(24A+X)	+	USA	Cruden 1968
245.	*L. rubicunda* (Linnaeus, 1857)	25(24A+X)	–	Finland	Oksala 1939a
		– » –	–	Former USSR	Makalowskaja 1940
		– » –	–	Russia	Kuznetsova et al. 2018
246.	***Libellula*** *angelina* Selys, 1883	25(24A+X)	+	Japan	Oguma 1915, 1930
		– » –	+	Japan	Kichijo 1942a
247.	*L. auripennis* Burmeister, 1839	25(24A+X)	+	USA	Kiauta and Brink 1978
248.	*L. axilena* Westwood, 1837	23(22A+X)	–	USA	Cumming 1964
249.	*L. basalis* (Say, 1840)	25(24A+X)	–	USA	Smith 1916
250.	*L. composita* (Hagen, 1873)	25(24A+X)	+	USA	Cruden 1968
251.	*L. croceipennis* Selys, 1868	25(24A+X)	+	USA	Cruden 1968
252.	*L. cyanea* Fabricius, 1775	25(24A+X)	–	USA	Cruden 1968
253.	*L. depressa* Linnaeus, 1758	23(22A+X)	–	Belgium	Carnoy 1885
		– » –	–	England	Hogben 1921
		25(24A+X)	+	Austria	Kiauta 1968c, 1969b
		23(22A+X)	–		
		25(24A+X)	+	France	Kiauta 1973b
		– » –	+	Croatia	Francovič and Jurečic 1986, 1989
		– » –	+	Russia	Perepelov et al. 1998
		– » –	+	Russia	Kuznetsova et al. 2018
254.	*L. flavida* Rambur, 1842	25(24A+X)	+	USA	Cruden 1968
255.	*L. forensis* Hagen, 1861	25(24A+X)	+	USA	Cruden 1968
256.	*L. fulva* Muller, 1764	25(24A+X)	+	Switzerland	Kiauta and Kiauta 1979
		27(26A+X)	+	Croatia	Francovič and Jurečic 1986, 1989
257.	*L. insecta* Hagen, 1861	25(24A+X)	–	USA	Cumming 1964
		– » –	–	USA	Cruden 1968
258.	*L. luctuosa* Burmeister, 1839	25(24A+X)	–	USA	Smith 1916
259.	*L. pulchella* Drury, 1773	25(24A+X)	+	USA	Cruden 1968
		– » –	+	Canada	Kiauta 1969a
260.	*L. quadrimaculata* Linnaeus, 1758	25(24A+X)	+	Japan	Oguma 1915, 1930 (*L. q. asahinai* Schmidt, 1957)

Taxon		Karyotype formula 2n ♂	m-chromosomes	Country	References
260.	*L. quadrimaculata* Linnaeus, 1758	25(24A+X)	+	Japan	Kichijo 1942d (*L. q. asahinai*)
		– » –	+	Japan	Omura 1955 (*L. q. asahinai*)
		– » –	+	Japan	Kiauta 1968b, c (*L. q. asahinai*)
		– » –	+	Former USSR	Fuchsówna and Sawczyńska 1928 (*L. q. quadrimaculata* Linnaeus, 1758)
		– » –	+	Finland	Oksala 1939a, b, 1945 (*L. q. quadrimaculata*)
		– » –	+	Former USSR	Makalowskaja 1940 (*L. q. quadrimaculata*)
		– » –	+	Netherlands	Kiauta 1968b, c (*L. q. quadrimaculata*)
		– » –	+	USA	Cruden 1968 (*L. q. quadrimaculata*)
		– » –	+	Russia	Perepelov et al. 1998 (*L. q. quadrimaculata*)
		– » –	+	Russia	Kuznetsova et al. 2018 (*L. q. quadrimaculata*)
261.	*L. saturata* Uhler, 1857	25(24A+X)	+	USA	Cruden 1968
262.	*L. semifasciata* Burmeister, 1839	25(24A+X)	+	USA	Cruden 1968
263.	*L. vibrans* Fabricius, 1793	25(24A+X)	+	USA	Cruden 1968
264.	***Lyriothemis*** *pachygastra* (Selys, 1878)	25(24A+X)	–	Japan	Omura 1955
265.	***Macrothemis*** *declivata* Calvert, 1909	23(22A+X)	+	Brazil	Kiauta and Boyes 1972
266.	*M. hemichlora* (Burmeister, 1839)	6(4A+neo-XY)	–	Bolivia	Cumming 1964
267.	*M. imitans* Karsch, 1890	25(24A+X)	+	Brazil	Kiauta and Boyes 1972 (*M. i. imitans* Karsch, 1890)
268.	*M. mortoni* Ris, 1913	25(24A+X)	+	Bolivia	Cumming 1964
269.	*M. musiva* Calvert, 1898	25(24A+X)	+	Bolivia	Cumming 1964
270.	*Macrothemis* sp.	25(24A+X)	+	Argentina	Mola 2007
271.	***Miathyria*** *artemis* (Selys, 1857)	25(24A+X)	+	Surinam	Kiauta 1979a
272.	*M. marcella* (Selys, 1857)	25(24A+X)	+	Bolivia	Cumming 1964
		– » –	+	Surinam	Kiauta 1979a
		– » –	+	Argentina	Mola and Agopian 1985
		– » –	+	Brazil	Ferreira et al. 1979
273.	***Micrathyria*** *artemis* Ris, 1911	25(24A+X)	+	Brazil	Ferreira et al. 1979
		– » –	+	Brazil	Souza Bueno 1982
274.	*M. atra* (Martin, 1897)	25(24A+X)	+	Bolivia	Cumming 1964
275.	*M. catenata* Calvert, 1909	25(24A+X)	+	Brazil	Souza Bueno 1982
		– » –	+	Argentina	Mola 2007
276.	*M. didyma* (Selys, 1857)	25(24A+X)	+	Jamaica	Cumming 1964
277.	*M. exima* Kirby, 1897	25(24A+X)	+	Surinam	Kiauta 1979a
278.	*M. hagenii* Kirby, 1890	25(24A+X)	+	Jamaica	Cumming 1964
279.	*M. hesperis* Ris, 1911	25(24A+X)	+	Surinam	Kiauta 1979a
		– » –	+	Brazil	Ferreira et al. 1979
		– » –	+	Argentina	Mola et al. 1999
280.	*M. hypodydima* Calvert 1906	23(22A+X)	+	Brazil	Souza Bueno 1982
		25(24A+X)	+	Argentina	Agopian and Mola 1988
281.	*M. iheringi* Santos, 1946	23(22A+X)	+	Bolivia	Cumming 1964
282.	*M. laevigata* Calvert, 1909	25(24A+X)	+	Bolivia	Cumming 1964
		– » –	+	Brazil	Kiauta and Boyes 1972
283.	*M. longifasciata* Calvert, 1909	24(22A+neo-XY)	–	Argentina	Agopian and Mola 1988
284.	*M. ocellata* (Martin, 1897)	25(24A+X)	+	Bolivia	Cumming 1964 (*M. o. dentiens* Calvert, 1909)
285.	*M. spuria* (Selys, 1900)	25(24A+X)	+	Bolivia	Cumming 1964
		– » –	+	Argentina	Mola et al. 1999
286.	*M. stawiarskii* Santos, 1953	25(24A+X)	+	Brazil	Souza Bueno 1982
287.	*M. ungulata* Foerster, 1907	23(20A+X_1X_2Y)	–	Argentina	Mola et al. 1999
288.	*M.* cf. *eximia* Kirby, 1879	21(20A+X)	–	Bolivia	Cumming 1964
289.	*M.* sp. (*ungulata* Foerster, 1907-group)	23(22A+X)	–	Bolivia	Cumming 1964
290.	***Nannothemis*** *bella* (Uhler, 1857)	25(24A+X)	+	USA	Cruden 1968
291.	***Nesciothemis*** *farinosa* (Foerster, 1898)	25(24A+X)	+	Kenya	Kiauta 1969c
		– » –	+	Kenya	Wasscher 1985

Taxon		Karyotype formula 2n ♂	m-chromosomes	Country	References
292.	***Nesogonia*** *blackburni* (McLachlan, 1883)	25(24A+X)	+	Hawaii	Kiauta 1969d
293.	***Neurothemis*** *fulvia* (Drury, 1773)	25(24A+X)	+	Nepal	Kiauta 1974, 1975
294.	*N. intermedia* (Rambur, 1842)	25(24A+X)	+	Nepal	Kiauta 1974, 1975 (*N. i. intermedia* (Rambur, 1842))
		– » –	+	Nepal	Kiauta and Kiauta 1982 (*N. i. degener* (Sel, 1842))
295.	*N. terminata* Ris, 1911	25(24A+X)	+	Philippines	Kiauta and Kiauta 1980b
296.	*N. tullia* (Drury, 1773)	28(26A+neo-XY)	+	India	Ray Chaudhuri and Dasgupta 1949
		– » –	+	India	Kiauta 1969a (*N. t. tullia* (Drury, 1773))
		– » –	+	India	Tyagi 1982 (*N. t. tullia*)
		25(24A+X)	+	Thailand	Kiauta and Kiauta 1983
297.	***Oligoclada*** *amphinome* Ris, 1919	25(24A+X)	+	Surinam	Kiauta 1979a
298.	*O. laetitia* Ris, 1911	23(22A+X)	+	Argentina	Mola and Agopian 1985
		21(20A+X)	–	Brazil	Souza Bueno 1982
299.	*O. monosticha* Borror, 1931	23(22A+X)	+	Surinam	Kiauta 1979a
		– » –	+	Brazil	Ferreira et al. 1979
300.	*O. pachystigma* Karsch, 1890	23(22A+X)	+	Brazil	Souza Bueno 1982
301.	***Orthemis*** *aequilibris* Calvert, 1909	12(10A+neo-XY)	–	Surinam	Kiauta 1979a
302.	*O. ambinigra* Calvert, 1909	12(10A+neo-XY)	–	Argentina	Agopian and Mola 1984
303.	*O. biolleyi* Calvert, 1906	23(22A+X)	+	Bolivia	Cumming 1964
304.	*O. cultiformis* Calvert, 1906	23(22A+X)	+	Bolivia	Cumming 1964
		– » –	+	Surinam	Kiauta 1979a
		– » –	+	Brazil	Ferreira et al. 1979
305.	*O. discolor* Burmeister, 1839	23(22A+X)	+	Argentina	Mola 2007
306.	*O. ferruginea* (Fabricius, 1775)	10(8A+neo-XY)***	–	Bolivia	Cumming 1964
		23(22A+X)	–	USA	
		– » –	+	Guatemala, Dominica	Cruden 1968
		– » –	+	Peru	Kiauta 1969a, 1971c
		– » –	+	Peru	Kiauta and Boyes 1972
		23(22A+X)	+	Surinam	Kiauta 1979a
		25(24A+X)	+		
		23(22A+X)	+	Brazil	Ferreira et al. 1979
		23(22A+X)	–	Brazil, Argentina	Mola and Agopian 1985
		24(22A+XX)*	+		
307.	*O. levis* Calvert, 1906	6(4A+neo-XY)***	–	Bolivia	Cumming 1964
		8(6A+neo-XY)***	–		
308.	*O. nodiplaga* Karsch, 1891	41(40A+X)	–	Argentina	Agopian and Mola 1984
309.	***Orthetrum*** *abbotti* Calvert, 1892	25(24A+X)	+	Kingdom of Eswatini (Former Swaziland)	Boyes et al. 1980
310.	*O. albistylum* (Selys, 1848)	25(24A+X)	+	Italy	Kiauta 1971a (*O. a. albistylum* (Selys, 1848))
		– » –	+	Russia	Perepelov et al. 1998
		– » –	+	Japan	Oguma 1915, 1917, 1930 (*O. a. speciosum* (Uhler, 1858))
		– » –	+	India	Kichijo 1942b (*O. a. speciosum*)
		– » –	+	Japan	Omura 1955 (*O. a. speciosum*)
311.	*O. azureum* (Rambur, 1842)	25(24A+X)	+	Madagascar	Kiauta 1969b, c
312.	*O. brachiale* (Beauvois, 1805)	21(20A+X)	–	Kenya	Kiauta 1969b, c
		25(24A+X)	+	Burkina Faso (Former Voltiac Republic)	Kiauta and Ochssée 1979 (*O. b. brachiale* (Beauvois, 1805))
313.	*O. brunneum* (Fonscolombe, 1837)	25(24A+X)	+	Italy	Kiauta 1971a
		– » –	+	Russia	Perepelov et al. 1998
314.	*O. cancellatum* (Linnaeus, 1758)	25(24A+X)	+	Finland	Oksala 1939a
		– » –	+	India	Dasgupta 1957
		– » –	+	Netherlands	Kiauta 1969a, b
		– » –	+	India	Tyagi 1982
		– » –	+	Russia	Kuznetsova et al. 2018

Taxon		Karyotype formula 2n ♂	m-chromosomes	Country	References
315.	*O. chrysostigma* (Burmeister, 1839)	25(24A+X)	+	Burkina Faso (Former Voltiac Republic)	Kiauta and Ochssée 1979
		– » –	+	Kingdom of Eswatini (Former Swaziland)	Boyes et al. 1980
		– » –	+	Kenya	Wasscher 1985
316.	*O. coerulescens* (Fabricius, 1798)	25(24A+X)	+	Austria	Kiauta 1969c
		23(22A+X)	–		
		25(24A+X)	+	Italy	Kiauta 1971a
		27(26A+X)	+		
317.	*O. glaucum* (Brauer, 1865)	25(24A+X)	+	India	Dasgupta 1957
		– » –	+	India	Tyagi 1978a, b
		– » –	+	India	Handa and Batra 1980
		– » –	+	India	Tyagi 1982
		– » –	+	India	Handa et al. 1984
		– » –	+	India	Walia and Sandhu 2002
		– » –	+	India	Kumari and Gautam 2017
318.	*O. guineese* (Ris, 1909)	25(24A+X)	+	Burkina Faso (Former Voltiac Republic)	Kiauta and Ochssée 1979
319.	*O. japonicum* (Uhler, 1858)	25(24A+X)	+	Japan	Oguma 1917, 1930 (*O. j. internum* McLachlan, 1894)
		– » –	+	Japan	Kichijo 1942b (*O. j. internum*)
		– » –	+	Japan	Omura 1955 (*O. j. internum*)
		– » –	+	Nepal	Kiauta 1975 (*O. j. internum*)
		– » –	+	Nepal	Kiauta and Kiauta 1976 (*O. j. internum*)
320.	*O. julia* Kirby, 1900	25(24A+X)	+	Kingdom of Eswatini (Former Swaziland)	Boyes et al. 1980 (*O. j. falsum* (Longfeild, 1955))
		– » –	+	Kenya	Wasscher 1985 (*O. j. falsum*)
321.	*O. luzonicum* (Brauer, 1868)	25(24A+X)	+	Nepal	Kiauta 1975
		– » –	+	Nepal	Kiauta and Kiauta 1982
		– » –	+	India	Thomas and Prasad 1981
		– » –	+	India	Prasad and Thomas 1992
322.	*O. melania* (Selys, 1883)	25(24A+X)	+	Japan	Oguma 1917
		– » –	+	Japan	Omura 1955
		– » –	+	Russia	Perepelov 2003
323.	*O. monardi* (Schmidt, 1951)	25(24A+X)	+	Burkina Faso (Former Voltiac Republic)	Kiauta and Ochssée 1979
324.	*O. poecilops* (Ris, 1916)	25(24A+X)	+	Japan	Suzuki et al. 1991 (*O. p. miyajimaensis* Yuki et Doi, 1938)
325.	*O. pruinosum* (Burmeister, 1839)	25(24A+X)	+	India	Dasgupta 1957 (*O. p. neglectum* (Rambur, 1842))
		– » –	+	Taiwan	Kiauta 1969a, c (*O. p. neglectum*)
		– » –	+	India	Tyagi 1982 (*O. p. neglectum*)
		– » –	+	India	Prasad and Thomas 1992 (*O. p. neglectum*)
		– » –	+	India	Tyagi 1978a, b (*O. p. neglectum*)
		– » –	+	Nepal	Kiauta and Kiauta 1982 (*O. p. neglectum*)
		– » –	+	India	Walia and Sandhu 2002 (*O. p. neglectum*)
		– » –	+	India	Kumari and Gautam 2017 (*O. p. neglectum*)
326.	*O. sabina* (Drury, 1773)	25(24A+X)	+	India	Asana and Makino 1935
		– » –	+	India	Makino 1935
		– » –	+	India	Kichijo 1942b
		– » –	+	India	Ray Chaudhuri and Dasgupta 1949
		– » –	+	Nepal	Kiauta 1975

Taxon		Karyotype formula 2n ♂	m-chromosomes	Country	References
326.	*O. sabina* (Drury, 1773)	– » –	+	India	Tyagi 1982
		– » –	+	India	Prasad and Thomas 1992
		– » –	+	India	Walia and Sandhu 2002 (*O. s. sabina* (Drury, 1773))
327.	*O. taeniolatum* (Schneider, 1845)	25(24A+X)	+	Greece	Kiauta 1972a
		– » –	+	Nepal	Kiauta 1975
		– » –	+	India	Tyagi 1978a, b
		– » –	+	India	Handa and Batra 1980
		– » –	+	India	Tyagi 1982
		– » –	+	India	Handa et al. 1984
		– » –	+	India	Thomas and Prasad 1986
		– » –	+	India	Walia and Sandhu 2002a
		– » –	+	India	Walia et al. 2015
328.	*O. testaceum* (Burmeister, 1839)	25(24A+X)	+	Nepal	Kiauta and Kiauta 1982
329.	*O. triangulare* (Selys, 1878)	25(24A+X)	+	Japan	Omura 1955 (*O. t. melania* (Selys, 1883))
		– » –	+	Taiwan	Kiauta 1969a, b (*O. t. triangulare* (Selys, 1878))
		– » –	+	Nepal	Kiauta 1975 (*O. t. triangulare*)
		– » –	+	India	Tyagi 1978a, b (*O. t. triangulare*)
		– » –	+	India	Handa and Batra 1980 (*O. t. triangulare*)
		– » –	+	India	Tyagi 1982 (*O. t. triangulare*)
		– » –	+	India	Walia and Sandhu 2002 (*O. t. triangulare*)
330.	***Pachydiplax*** *longipennis* (Burmeister, 1839)	25(24A+X)	–	USA	Cumming 1964
		– » –	+	USA	Cruden 1968
		– » –	+	USA	Kiauta and Brink 1978
331.	***Palpopleura*** *jucunda* Rambur, 1842	25(24A+X)	+	Kingdom of Eswatini (Former Swaziland)	Boyes et al. 1980
332.	*P. lucia* (Drury, 1773)	25(24A+X)	+	Burkina Faso (Former Voltiac Republic)	Kiauta and Ochssée 1979 (*P. l. portia* (Drury, 1773))
		– » –	+	Kenya	Wasscher 1985 (*P. l. portia*)
333.	*P. sexmaculata* (Fabricius, 1787)	25(24A+X)	+	Nepal	Kiauta 1974, 1975
		– » –	+	India	Tyagi 1982 (*P. s. sexmaculata* (Fabricius, 1787))
334.	***Pantala*** *flavescens* (Fabricius, 1798)	25(24A+X)	+	India	Asana and Makino 1935
		– » –	+	India	Makino 1935
		– » –	+	India	Kichijo 1942b
		– » –	+	India	Dasgupta 1957
		– » –	+	India	Seshachar and Bagga 1963
		– » –	+	Bolivia	Cumming 1964
		– » –	+	Madagascar	Kiauta 1969b
		– » –	+	Surinam	Kiauta 1979a
		– » –	+	Brazil	Ferreira et al. 1979
		– » –	+	Kingdom of Eswatini (Former Swaziland)	Boyes et al. 1980
		– » –	+	Brazil	Souza Bueno 1982
		– » –	+	Argentina	Agopian and Mola 1988
		– » –	+	India	Prasad and Thomas 1992
		– » –	+	Russia	Perepelov and Bugrov 2001b
		23(22A+X)	+	India	Walia et al. 2011
335.	*P. hymenaea* (Say, 1836)	25(24A+X)	+	Bolivia	Cumming 1964
		– » –	+	USA	Cruden 1968
336.	***Perithemis*** *cornelia* Ris, 1910	25(24A+X)	–	Bolivia	Cumming 1964
337.	*P. domitia* (Drury, 1773)	25(24A+X)	+	Jamaica	Cumming 1964
338.	*P. electra* Ris, 1928	25(24A+X)	–	Bolivia	Cumming 1964
339.	*P. icteroptera* (Selys in Sagra, 1857)	25(24A+X)	+	Argentina	Mola and Agopian 1985

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340.	*P. lais* (Petry, 1834)	17(16A+X)	–	Bolivia	Cumming 1964
		– » –	–	Surinam	Kiauta 1979a
		– » –	–	Brazil	Ferreira et al. 1979
341.	*P. mooma* Kirby, 1889	25(24A+X)	+	Bolivia	Cumming 1964
		– » –	+	Surinam	Kiauta 1979a
		– » –	–	Brazil	Ferreira et al. 1979
		– » –	+	Argentina	Mola and Agopian 1985
342.	*P. tenera* (Say, 1839)	25(24A+X)	+	USA	Kiauta and Brink 1978
343.	*P. seminole* Calvert, 1907	25(24A+X)	+	USA	Cumming 1964
344.	*Perithemis* sp.	25(24A+X)	–	Bolivia	Cumming 1964
345.	***Planiplax*** *erythropyga* (Karsch, 1891)	25(24A+X)	+	Argentina	Mola et al. 1999
		– » –	+	– » –	De Gennaro 2004
346.	*P. sanguiniventris* (Calvert, 1907)	25(24A+X)	+	USA	Cruden 1968
347.	***Plathemis*** *lydia* (Drury, 1773)	25(24A+X)	+	USA	McGill 1907
		– » –	+	USA	Cruden 1968
348.	***Potamarcha*** *congener* (Rambur, 1842)	25(24A+X)	+	India	Asana and Makino 1935 as *P. obscura* (Rambur, 1842)
		– » –	+	India	Makino 1935 as *P. obscura*
		– » –	+	India	Kichijo 1942b as *P. obscura*
		– » –	+	India	Dasgupta 1957 as *P. obscura*
		– » –	+	India	Tyagi 1982 as *P. obscura*
		– » –	+	India	Prasad and Thomas 1992
		– » –	+	India	Sandhu and Walia 1995
349.	***Pseudothemis*** *zonata* (Burmeister, 1839)	24(22A+neo-XY)	–	Japan	Omura 1955
350.	***Pseudotramea*** *prateri* Fraser, 1920	25(24A+X)	+	Nepal	Kiauta 1974, 1975
351.	***Rhodopygia*** *cardinalis* (Erichson, 1848)	25(24A+X)	+	Bolivia	Cumming 1964
352.	*R. geijskesi* Belle, 1964	25(24A+X)	+	Surinam	Kiauta 1979a
353.	***Rhodothemis*** *rufa* (Rambur, 1842)	25(24A+X)	+	India	Prasad and Thomas 1992
354.	***Rhyothemis*** *fuliginosa* Selys, 1883	25(24A+X)	+	Japan	Toyoshima and Hirai 1953
		– » –	+	Japan	Omura 1955
		– » –	+	Japan	Hirai 1956
		25(24A+X)	+	Japan	Kiauta 1969c
		23(22A+X)	+		
355.	*R. variegata* (Linnaeus et Johansson, 1763)	25(24A+X)	+	India	Ray Chaudhuri and Dasgupta 1949
		– » –	+	Nepal	Kiauta 1975
356.	***Scapanea*** *frontalis* (Burmeister, 1839)	25(24A+X)	+	Jamaica	Cumming 1964
357.	***Sympetrum*** *commixtum* (Selys, 1884)	25(24A+X)	–	India	Tyagi 1978a, b, 1982
358.	*S. corruptum* (Hagen, 1861	25(24A+X)	+	USA	Cruden 1968 as *Tarnetrum corruptum* (Hagen, 1861)
		– » –	+	USA	Kiauta 1969a, c as *T. corruptum*
359.	*S. costiferum* (Hagen, 1861)	25(24A+X)	+	USA	Cruden 1968
360.	*S. croceolum* (Selys, 1840)	25(24A+X)	+	Russia	Perepelov 2003
361.	*S. danae* (Sulzer, 1776)	25(24A+X)	+	Former USSR	Makalowskaja 1940
		– » –	+	Finland	Oksala 1945
		– » –	+	USA	Cruden 1968
		– » –	+	Russia	Perepelov 2003
		– » –	+	Russia	Kuznetsova et al. 2018
362.	*S. eroticum* (Selys, 1883)	21(20A+X)	–	Japan	Kichijo 1942b, c
		– » –	–	Japan	Hirai 1956
		– » –	–	Japan	Kiauta 1969c
363.	*S. flaveolum* (Linnaeus, 1758)	25(24A+X)	+	Former USSR	Makalowskaja 1940
		– » –	+	Russia	Perepelov 2003
364.	*S. fonscolombii* (Selys, 1840)	25(24A+X)	+	Russia	Perepelov 2003
365.	*S. frequens* (Selys, 1883)	23(22A+X)	–	Japan	Oguma 1917, 1930
		– » –	–	Japan	Kichijo 1942a, b
		– » –	–	Japan	Kiauta 1969c
366.	*S. infuscatum* (Selys, 1883)	25(24A+X)	+	Russia	Perepelov 2003
367.	*S. internum* Montgomery, 1943	27(26A+X)	+	Canada	Kiauta 1973a
368.	*S. madidum* (Hagen, 1861)	25(24A+X)	+	USA	Cruden 1968

Taxon		Karyotype formula 2n ♂	m-chromosomes	Country	References
368.	*S. madidum* (Hagen, 1861)	– » –	+	Canada	Kiauta 1973a
369.	*S. meridionale* (Selys, 1841)	25(24A+X)	+	Switzerland	Kiauta 1966
370.	*S. obtrusum* (Hagen, 1867)	25(24A+X)	+	USA	Cruden 1968
371.	*S. parvulum* Bartenev, 1912	25(24A+X)	+	Japan	Kiauta 1968c
372.	*S. pedemontanum* Müller in Allioni, 1766	25(24A+X)	+	Japan	Oguma 1917, 1930 (*S. p. elatum* (Selys, 1872))
		– » –	+	Japan	Kichijo 1942b (*S. p. elatum*)
		– » –	+	Japan	Kiauta and Brink 1975 (*S. p. elatum*)
		– » –	+	Switzerland	Kiauta and Brink 1975 (*S. p. pedemontanum* (Müller, 1766))
		– » –	+	Russia	Perepelov et al. 1998 (*S. p. pedemontanum*)
		– » –	+	Russia	Perepelov and Bugrov 2001b
373.	*S. rubicundulum* (Say, 1839)	25(24A+X)	+	USA	Cruden 1968
374.	*S. sanguineum* (Müller, 1764)	25(24A+X)	+	Italy	Kiauta 1971a
		– » –	+	Russia	Perepelov and Bugrov 2001b
375.	*S. semicinctum* (Say, 1839)	25(24A+X)	+	USA	Smith 1916
		– » –	+	USA	Cruden 1968
376.	*S. striolatum* (Charpentier, 1840)	25(24A+X)	–	Luxembourg	Kiauta 1966
377.	*S. vicinum* (Hagen, 1861)	25(24A+X)	+	USA	Cruden 1968
378.	*S. vulgatum* (Linnaeus, 1758)	25(24A+X)	+	Netherland	Kiauta 1972c
		– » –	+	Russia	Perepelov 2003
		– » –	+	Russia	Kuznetsova et al. 2018
379.	***Tarnetrum** illotum* (Hagen, 1861)	25(24A+X)	+	Jamaica	Cumming 1964
		– » –	+	USA	Cruden 1968
380.	***Tauriphila** australis* (Hagen, 1867)	25(24A+X)	+	Bolivia	Cumming 1964
381.	*T. azteca* Calvert, 1906	25(24A+X)	+	Mexico	Cruden 1968
382.	*T. risi* Martin 1896	25(24A+X)	+	Argentina, Uruguay	Mola and Agopian 1985
383.	***Tholymis** citrina* Hagen, 1867	25(24A+X)	+	Surinam	Kiauta 1979a
		– » –	+	Brazil	Ferreira et al. 1979
384.	*Th. tillagra* (Fabricius, 1798)	25(24A+X)	+	India	Prasad and Thomas 1992
		– » –	+	Nepal	Kiauta and Kiauta 1982
		– » –	+	Thailand	Kiauta and Kiauta 1983
385.	***Tramea** abdominalis* (Rambur, 1842)	25(24A+X)	–	Bolivia	Cumming 1964
386.	*T. basilaris* (Palisot de Beauvois, 1817)	25(24A+X)	+	India	Das 1956 (*T. b. burmeisteri* (Kirby, 1889))
		– » –	+	India	Dasgupta 1957 (*T. b. burmeisteri*)
		– » –	+	Nepal	Kiauta and Kiauta 1982 (*T. b. burmeisteri*)
		– » –	+	India	Prasad and Thomas 1992 (*T. b. burmeisteri*)
387.	*T. binotata* (Rambur, 1842)	25(24A+X)	+	Surinam	Kiauta 1979a
		– » –	–	Brazil	Ferreira et al. 1979
388.	*T. carolina* (Linnaeus, 1763)	25(24A+X)	–	USA	Cumming 1964
		– » –	–	USA	Cruden 1968
389.	*T. cophysa* (Hagen, 1867)	25(24A+X)	+	Bolivia	Cumming 1964
390.	*T. lacerata* (Hagen, 1861)	25(24A+X)	–	USA	Cruden 1968
391.	*T. limbata* (Desjardins, 1832)	25(24A+X)	+	India	Asana and Makino 1935
		– » –	+	India	Makino 1935
		– » –	+	India	Kichijo 1942b
392.	*T. virginia* (Rambur, 1842)	25(24A+X)	+	India	Oguma and Asana 1932
		– » –	+	India	Kichijo 1942b
		– » –	+	India	Dasgupta 1957
393.	***Trithemis** annulata* (Palisot de Beauvois, 1805)	25(24A+X)	–	Republic of South Africa	Boyes et al. 1980
		– » –	+	Kenya	Wasscher 1985
394.	*T. arteriosa* (Burmeister, 1839)	25(24A+X)	+	Kingdom of Eswatini (Former Swaziland)	Boyes et al. 1980

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395.	*T. atra* Pinhey, 1961	25(24A+X)	+	Burkina Faso (Former Voltiac Republic)	Kiauta and Ochssée 1979
396.	*T. aurora* (Burmeister, 1839)	25(24A+X)	+	India	Oguma and Asana 1932
		– » –	+	Nepal	Kiauta 1975
		– » –	+	India	Tyagi 1982
397.	*T. dorsalis* (Rambur, 1842)	25(24A+X)	+	Kingdom of Eswatini (Former Swaziland)	Boyes et al. 1980
398.	*T. festiva* (Rambur, 1842)	25(24A+X)	+	Nepal	Kiauta 1974, 1975
		– » –	+	India	Tyagi 1982
		– » –	+	India	Prasad and Thomas 1992
399.	*T. furva* Karsch, 1899	25(24A+X)	+	Sudan	Wasscher 1985
400.	*T. imiata* Pinhey, 1961	25(24A+X)	–	Burkina Faso (Former Voltiac Republic)	Kiauta and Ochssée 1979
401.	*T. kirbyi* Selys, 1891	25(24A+X)	–	Burkina Faso (Former Voltiac Republic)	Kiauta and Ochssée 1979 (*T. k. ardens* Gerstaecker, 1891)
		– » –	+	Kenya	Wasscher 1985 (*T. k. ardens*)
402.	*T. pallidinervis* (Kirby, 1889)	25(24A+X)	+	India	Asana and Makino 1935
		– » –	+	India	Makino 1935
		– » –	+	India	Kichijo 1942b
		– » –	+	India	Dasgupta 1957
		– » –	+	Philippines	Kiauta and Kiauta 1980b
403.	*T. werneri* Ris, 1912	25(24A+X)	+	Kenya	Wasscher 1985
404.	***Uracis** imbuta* (Burmeister, 1839)	25(24A+X)	+	Surinam	Kiauta 1979a
		– » –	+	Brazil	Ferreira et al. 1979
405.	*U. ovipositrix* Calvert, 1909	25(24A+X)	+	Surinam	Kiauta 1979a
		– » –	–	Brazil	Ferreira et al. 1979
406.	***Urothemis** edwardsi* (Selys, 1849)	25(24A+X)	+	Sudan	Wasscher 1985
407.	*U. signata* (Rambur, 1842)	25(24A+X)	+	India	Das 1956 (*U. s. signata* (Rambur, 1842))
		– » –	+	India	Dasgupta 1957 (*U. s. signata*)
		– » –	+	Nepal	Kiauta 1975
		– » –	+	India	Prasad and Thomas 1992
408.	***Zenithoptera** fasciata* (Linnaeus, 1758)	25(24A+X)	+	Surinam	Kiauta 1979a
409.	*Z. lanei* Santos, 1941	25(24A+X)	+	Surinam	Kiauta 1979a
		– » –	+	Brazil	Ferreira et al. 1979
410.	*Z. viola* Ris, 1910	25(24A+X)	+	Bolivia	Cumming 1964
411.	***Zygonyx** iris* Kirby, 1900	23(22A+X)	+	Thailand	Kiauta and Kiauta 1983 (*Z. i. malayanus* (Laidlaw, 1902))
412.	*Z. torrida* (Kirby, 1889)	25(24A+X)	+	India	Tyagi 1978a, b
413.	***Zyxomma** petiolatum* (Rambur, 1842)	25(24A+X)	+	India	Prasad and Thomas 1992
Cordulegastroidea					
Chlorogomphidae					
414.	***Watanabeopetalia** atkinsoni* (Selys, 1878)	25(24A+X)	+	India	Walia and Chahal 2019
Cordulegastridae					
415.	***Anotogaster** basalis* Selys, 1854	23(22A+X)	–	India	Sandhu and Malhotra 1994b
416.	*A. kuchenbeiseri* (Förster, 1899)	25(24A+X)	+	China	Zhu and Wu 1986
417.	*A. sieboldii* (Selis, 1854)	25(24A+X)	+	Japan	Oguma 1930
		– » –	+	Japan	Kichijo 1942a
		– » –	+	Japan	Kiauta 1969a
		– » –	+	Russia	Perepelov et al. 2001
418.	***Cordulegaster** boltoni* (Donovan, 1807)	25(24A+X)	+	Finland	Oksala 1939a, b
		– » –	–	Austria	Kichijo 1942a
		– » –	+	Sweden	Kiauta 1968d, e, 1969a
419.	*C. brevistigma* Selys, 1854	25(24A+X)	+	India	Walia and Chahal 2019
420.	*C. diastatops* (Selys, 1854)	25(24A+X)	+	USA	Cruden 1968
421.	*C. dorsalis* Hagen, 1857	25(24A+X)	+	USA	Cruden 1968

Taxon		Karyotype formula 2n ♂	m-chromosomes	Country	References
422.	*C. maculata* Selys, 1854	25(24A+X)	+	USA	Cruden 1968
423.	***Neallogaster*** *hermionae* (Fraser, 1927)	25(24A+X)	+	Nepal	Kiauta and Kiauta 1976
Zygoptera					
Lestoidea					
Lestidae					
424.	***Austrolestes*** *colensonis* (White, 1846)	25(24A+X)	+	New Zealand	Jensen 1980
425.	***Chalcolestes*** *viridis* (Van der Linden, 1825)	25(24A+X)	+	Netherlands	Kiauta 1969a
426.	***Indolestes*** *cyaneus* (Selys, 1862)	25(24A+X)	+	Nepal	Kiauta and Kiauta 1976 as *I. cyanea* (Selys, 1862)
427.	***Lestes*** *barbarus* (Fabricius, 1798)	25(24A+X)	+	Former Yugoslavia	Kiauta 1972a
428.	*L. congener* Hagen, 1861	25(24A+X)	+	USA	Cruden 1968
429.	*L. disjunctus* Selys, 1862	25(24A+X)	–	USA	Cruden 1968
430.	*L. dorothea* Fraser, 1924	25(24A+X)	+	Nepal	Kiauta 1974, 1975
431.	*L. dryas* Kirby, 1890	25(24A+X)	–	USA	Cruden 1968
		– » –	+	Russia	Perepelov and Bugrov 2001b
432.	*L. forcipatus* Rambur, 1842	21(20A+X)	–	USA	Cruden 1968
433.	*L. forficula* Rambur, 1842	25(24A+X)	+	Jamaica	Cumming 1964
434.	*L. paulistus* Calvert, 1909	25(24A+X)	+	Brazil	Souza Bueno 1982
435.	*L. rectangularis* Say, 1839	25(24A+X)	+	USA	Cruden 1968
436.	*L. similatrix* McLachlan, 1895	25(24A+X)	+	Madagascar	Kiauta 1969b
437.	*L. sponsa* (Hansemann, 1823)	25(24A+X)	–	Former USSR	Makalowskaja 1940
		– » –	+	Japan	Kichijo 1941, 1942a, d, e
		– » –	+	Russia	Perepelov and Bugrov 2001b
438.	*L. stultus* Hagen, 1861	25(24A+X)	+	USA	Cruden 1968
439.	*L. vidua* Hagen, 1861	25(24A+X)	+	USA	Cumming 1964
440.	*L. vigilax* Selys, 1862	19(18A+X)	–	USA	Kiauta and Brink 1978
441.	*L. virens* Charpentier, 1825	25(24A+X)	+	Netherlands	Kiauta 1969a (*L. v. vestalis* Rambur, 1842)
442.	***Sympecma*** *fusca* (Van der Linden, 1823)	25(24A+X)	+	Japan	Kichijo 1941, 1942d, e
443.	*S. paedisca* (Brauer, 1877)	25(24A+X)	+	Netherlands	Kiauta and Kiauta-Brink 1975 (*S. annulata braueri* (Bianchi, 1904))
		– » –	+	Russia	Perepelov 2003 (*S. a. braueri*)
Synlestidae					
444.	***Megalestes*** *major* Selys, 1862	25(24A+X)	–	Nepal	Kiauta 1974, 1975
Platystictoidea					
Platystictidae					
445.	***Drepanosticta*** sp.	25(24A+X)	–	Nepal	Kiauta and Kiauta 1976
446.	*Drepanosticta* sp.	25(24A+X)	–	India	Tyagi 1978a, b
447.	***Palaemnema*** *paulina* (Drury, 1773)	25(24A+X)	+	Costa Rica	Cumming 1964
448.	***Protosticta*** sp.	25(24A+X)	–	Tailand	Kiauta and Kiauta 1983
Calopterygoidea					
Calopterygidae					
449.	***Atrocalopteryx*** *atrata* (Selys, 1853)	25(24A+X)	+	Japan	Oguma 1930 as *Calopteryx atrata* Selys, 1853
		– » –	+	Japan	Kichijo 1942d as *C. atrata*
		– » –	+	Japan	Omura 1957 as *C. atrata*
450.	***Calopteryx*** *aequabilis* Say, 1839	25(24A+X)	+	USA	Cruden 1968
451.	*C. cornelia* (Selys, 1853)	25(24A+X)	+	Japan	Oguma 1930 as *Anaciagrion cornelia* (Selys, 1853)
		– » –	+	Japan	Kichijo 1942a as *A. cornelia*
452.	*C. dimidiata* Burmeister, 1839	25(24A+X)	+	USA	Kiauta and Brink 1978
453.	*C. japonica* Selys, 1869	25(24A+X)	+	Japan	Kichijo 1942a
		– » –	+	Japan	Hirai 1956
		– » –	+	Japan	Omura 1957
		– » –	+	Japan	Kiauta 1968e, f
454.	*C. maculata* (Beauvois, 1805)	25(24A+X)	+	USA	Cumming 1964a
		– » –	+	USA	Cruden 1968
455.	*C. splendens* (Harris, 1780)	25(24A+X)	+	Turkey	Kiauta 1972a (*C. s. amasina* Bartenev, 1912)

Taxon		Karyotype formula 2n ♂	m-chromosomes	Country	References
455.	*C. splendens* (Harris, 1780)	– » –	+	Italy	Kiauta 1971a (*C. s. caprai* Conci, 1956)
		– » –	–	Former USSR	Makalowskaja 1940 (*C. s. splendens* (Harris, 1782))
		– » –	–	Finland	Oksala 1945 (*C. s. splendens*)
		– » –	–	Germany	Kiauta 1969a, 1971b (*C. s. splendens*)
		– » –	–	France	Kiauta 1973b (*C. s. splendens*)
		– » –	–	Russia	Perepelov et al. 1998 (*C. s. splendens*)
		– » –	+	Russia	Kuznetsova et al. 2020b
456.	*C. virgo* (Linnaeus, 1758)	25(24A+X)	+	Spain	Kiauta 1971b (*C. v. meridionalis* Selys, 1873)
		27(26A+X)	+		
		25(24A+X)	+	Slovenija	Kiauta 1967a, 1968b, c (*C. v. padana* Conci, 1956)
		– » –	+	Austria	Kiauta 1967a, 1968b, c (*C. v. padana*)
		– » –	–	Belgium	Carnoy 1885 (*C. v. virgo* (Linnaeus, 1758))
		– » –	+	Finland	Oksala 1939 (*C. v. virgo*)
		– » –	+	Former USSR	Makalowskaja 1940 (*C. v. virgo*)
		– » –	+	Germany, Luxembourg	Kiauta 1968e, f (*C. v. virgo*)
		– » –	+	Netherlands	Kiauta 1972c (*C. v. virgo*)
		– » –	+	Russia	Kuznetsova et al. 2020b
457.	***Hetaerina*** *americana* (Fabricius, 1798)	25(24A+X)	+	USA	Cumming 1964
		– » –		USA	Cruden 1968
458.	*H. charca* Calvert, 1909	25(24A+X)	+	Bolivia	Cumming 1964
459.	*H. longipes* (Hagen in Selys, 1853)	25(24A+X)	+	Brazil	Souza Bueno 1982 as *H. carnifex* Hagen in Selys, 1853
		– » –	+	Brazil	Agopian and Mola 1984 as *H. carnifex*
460.	*H. rosea* Selys, 1853	27(26A+X)	+	Bolivia	Cumming 1964
		– » –	+	Bolivia	Kiauta 1969c
		25(24A+X)	–	Brazil	Ferreira et al. 1979
		27(26A+X)	+		
461.	*H. sanguinea* Selys, 1853	25(24A+X)	–	Bolivia	Cumming 1964
462.	*H. titia* (Drury, 1773)	25(24A+X)	+	USA	Cumming 1964
		– » –	+	Mexico	Kiauta 1970a as *H. tricolor* (Burmeister, 1839)
463.	*H. vulnerata* (Selys, 1853)	25(24A+X)	+	Mexico	Kiauta 1970a
464.	***Matrona*** *basilaris* Selys, 1853	25(24A+X)	–	Taiwan	Kiauta 1968c
465.	***Mnais*** *costalis* Selys, 1869	25(24A+X)	+	Japan	Oguma 1930
		– » –	+	Japan	Kichijo 1942a
466.	*M. pruinosa* Selys, 1853	25(24A+X)	+	Japan	Oguma 1930 as *M. strigata* Selys, 1853
		– » –	+	Japan	Kichijo 1942a as *M. strigata*
		– » –	+	Japan	Omura 1957 as *M. strigata*
467.	***Neurobasis*** *chinensis* (Linnaeus, 1758)	23(22A+X)	–	Nepal	Kiauta 1975 (*N. c. chinensis* (Linnaeus, 1758))
		25(24A+X)	–		
		23(22A+X)	–	India	Tyagi 1978b (*N. c. chinensis*)
		– » –	+	Nepal	Kiauta and Kiauta 1982 (*N. c. chinensis*)
		– » –	–	Thailand	Kiauta and Kiauta 1983 (*N. c. chinensis*)
		– » –	+	India	Walia and Sandhu 2002 (*N. c. chinensis*)
		– » –	–	India	Walia et al. 2016 (*N. c. chinensis*)
		– » –	–	India	Walia and Katnoria 2018 (*N. c. chinensis*)
468.	***Phaon*** *iridipennis* (Burmeister, 1839)	25(24A+X)	+	Republic of South Africa	Boyes et al. 1980
Chlorocyphidae					
469.	***Aristocypha*** *fenestrella* Rambur, 1842	23(22A+X)	–	Thailand	Kiauta and Kiauta 1983 as *Rhinocypha fenestrella* Rambur, 1842

Taxon		Karyotype formula 2n ♂	m-chromosomes	Country	References
470.	*A. quadrimaculata* (Selys, 1853)	23(22A+X)	+	India	Chatterjee and Kiauta 1973 as *Rhinocypha quadrimaculata* Selys, 1853
		– » –	+	Nepal	Kiauta and Kiauta 1982 as *Rh. quadrimaculata*
471.	*A. trifasciata* (Selys, 1853)	23(22A+X)	–	India	Tyagi 1978a, b as *Rhinocypha trifasciata* Selys, 1853
		– » –	+	Nepal	Kiauta and Kiauta 1982 as *Rh. trifasciata*
472.	***Heliocypha*** *biforata* (Selys, 1859)	23(22A+X)	–	India	Tyagi 1978 a, b as *Rhinocypha biforata beesoni* Selys, 1859
473.	*H. biseriata* (Selys, 1859)	23(22A+X)	–	Thailand	Kiauta and Kiauta 1983 as *Rhinocypha b. biforata* Selys, 1859
474.	***Libellago*** *lineata* (Burmeister, 1839)	23(22A+X)	–	India	Walia et al. 2018 (*L. l. lineata* (Burmeister, 1839))
		25(24A+X)	–		
475.	***Paracypha*** *unimaculata* (Selys, 1879)	23(22A+X)	+	Nepal	Kiauta 1974, 1975 as *Rhinocypha unimaculata* Selys, 1879
		– » –	+	Nepal	Kiauta and Kiauta 1982 as *Rh. unimaculata*
476.	***Rhinocypha*** *colorata* Selys, 1869	23(22A+X)	–	Philippines	Kiauta and Kiauta 1980b
		25(24A+X)	–		
477.	***Vestalis*** *gracilis* (Rambur, 1842)	25(24A+X)	+	Thailand	Kiauta and Kiauta 1983
Polythoridae					
478.	***Cora*** *irene* Ris, 1918	23(22A+X)	–	Bolivia	Cumming 1964
479.	***Polythore*** *boliviana* (McLachlan, 1878)	23(22A+X)	–	Bolivia	Cumming 1964
Euphaeidae					
480.	***Anisopleura*** *comes* Hagen, 1880	25(24A+X)	+	Nepal	Kiauta and Kiauta 1976, 1982
481.	***Bayadera*** *indica* (Selys, 1853)	25(24A+X)	+	Nepal	Chatterjee and Kiauta 1973
		– » –	+	Nepal	Kiauta 1975
482.	***Euphaea*** *guerini* Rambur, 1842	25(24A+X)	–	Thailand	Kiauta and Kiauta 1983
483.	***Epallage*** *fatime* (Charpentier, 1840)	25(24A+X)	–	Greece	Kiauta 1970b
		– » –	–	Greece	Chatterjee and Kiauta 1973
Megapodagrionidae					
484.	***Allopodagrion*** *contortum* (Selys, 1862)	25(24A+X)	+	Brazil	Kiauta 1972b as *Megapodagrion contortum* (Selys, 1862)
485.	***Teinopodagrion*** *macropus* (Selys, 1862)	25(24A+X)	–	Bolivia	Cumming 1964 as *Megapodagrion macropus* (Selys, 1862)
486.	*T. setigerum* (Selys, 1886)	25(24A+X)	–	Bolivia	Cumming 1964 as *Megapodagrion setigerum* Selys, 1886
Heteragrionidae					
487.	***Heteragrion*** *flavidorsum* Calvert, 1909	25(24A+X)	–	Bolivia	Cumming 1964
488.	*H. inca* Calvert, 1909	25(24A+X)	+	Bolivia	Cumming 1964
Philogeniidae					
489.	***Philogenia*** *carrillica* Calvert, 1907	25(24A+X)	+	Costa Rica	Cumming 1964
Hypolestidae					
490.	***Hypolestes*** *clara* (Calvert, 1891)	l7(16A+X)	–	Jamaica	Cumming 1964
Coenagrionoidea					
Platycnemididae					
491.	***Calicnemia*** *miniata* (Selys, 1886)	25(24A+X)	+	Nepal	Kiauta and Kiauta 1982
492.	*C. pulverulans* (Selys, 1886)	25(24A+X)	–	Nepal	Kiauta 1975
493.	*Calicnemia* sp.	25(24A+X)	–	Nepal	Kiauta 1975
494.	*Calicnemia* sp.	25(24A+X)	–	India	Tyagi 1978b
495.	***Coeliccia*** *chromothorax* (Selys, 1891)	25(24A+X)	–	India	Walia and Devi 2020b
496.	*C. bimaculata* (Laidlaw, 1914)	25(24A+X)	–	India	Walia and Devi 2020b
497.	*C. didyma* (Selys, 1863)	25(24A+X)	–	India	Walia and Devi 2020b
498.	*C. fraseri* (Laidlaw, 1932)	25(24A+X)	–	India	Walia and Devi 2020b
499.	*C. renifera* (Selys, 1886)	25(24A+X)	–	Nepal	Kiauta 1974, 1975
		– » –	–	India	Walia and Devi 2020b
500.	***Copera*** *annulata* (Selys, 1863)	25(24A+X)	+	Japan	Kichijo 1941, 1942a, c
		– » –	+	India	Dasgupta 1957
		– » –	–	Thailand	Kiauta and Kiauta 1983
		– » –	+	India	Walia and Devi 2018

Taxon		Karyotype formula 2n ♂	m-chromosomes	Country	References
501.	*C. marginipes* (Rambur, 1842)	25(24A+X)	–	India	Tyagi 1978a, b
		– » –	–	Thailand	Kiauta and Kiauta 1983
		– » –	+	India	Walia and Devi 2018
502.	*C. vittata* (Selys, 1863)	25(24A+X)	+	India	Walia and Devi 2018
		– » –	+	India	Walia and Devi 2018 (*C. v. assamensis* (Laidlaw, 1914))
503.	***Disparoneura*** *quadrimaculata* (Rambur, 1842)	25(24A+X)	–	India	Walia and Devi 2020a
504.	***Esme*** *cyaneovittata* Fraser, 1922	25(24A+X)	–	India	Walia and Devi 2020a
505.	*E. longistyla* Fraser, 1931	25(24A+X)	–	India	Walia and Devi 2020a
506.	***Onychargia*** *atrocyana* (Selys, 1865)	25(24A+X)	–	Thailand	Kiauta and Kiauta 1983
507.	***Platycnemis*** *pennipes* (Pallas, 1771)	25(24A+X)	–	Finland	Oksala 1945
		– » –	–	Italy	Kiauta 1971a
		– » –	–	Russia	Perepelov and Bugrov 2001b
508.	***Prodasineura*** *autumnalis* (Fraser, 1922)	25(24A+X)	+	Thailand	Kiauta and Kiauta 1983
509.	*P. nigra* (Fraser, 1922)	25(24A+X)	–	India	Walia and Devi 2020a
510.	*P. verticalis* (Selys, 1860)	25(24A+X)	–	India	Walia and Devi 2020a
511.	*Prodasineura* sp.1	25(24A+X)	–	Thailand	Kiauta and Kiauta 1983
512.	*Prodasineura* sp.2	25(24A+X)	–	Thailand	Kiauta and Kiauta 1983
Coenagrionidae					
513.	***Acanthagrion*** *ascendens* Calvert, 1909	27(26A+X)	+	Bolivia	Cumming 1964
514.	*A. chacoense* Calvert, 1909	27(26A+X)	+	Bolivia	Cumming 1964
515.	*A. gracile* (Rambur, 1842)	27(26A+X)	–	Surinam	Kiauta 1979a (*A. g. minarum* Selys, 1876)
		– » –	–	Brazil	Ferreira et al. 1979 (*A. g. minarum* Selys, 1876)
516.	***Aeolagrion*** inca Selys, 1876	27(26A+X)	–	Bolivia	Cumming 1964 as *A. foliaceum* (Sjöstedt, 1918)
517.	***Agriocnemis*** *clauseni* Fraser, 1922	27(26A+X)	+	India	Tyagi 1978a, b
518.	*A. femina* (Brauer, 1868)	27(26A+X)	–	Philippines	Kiauta and Kiauta 1980b
		– » –	+	Thailand	Kiauta and Kiauta 1983
519.	*A. pygmaea* (Rambur, 1842)	27(26A+X)	–	India	Tyagi 1978b
		– » –	+	Thailand	Kiauta and Kiauta 1983
520.	***Amphiagrion*** *abbreviatum* (Selys, 1876)	27(26A+X)	–	USA	Cruden 1968
521.	***Amphiallagma*** *parvum* (Selys, 1876)	27(26A+X)	+	India	Handa and Kochhar 1985 as *Enallagma parvum* Selys, 1876
522.	***Argia*** *apicalis* (Say, 1839)	37(36A+X)	–	USA	Kiauta and Kiauta 1980b
523.	*A. fumipennis* (Burmeister, 1839)	27(26A+X)	–	USA	Kiauta and Kiauta 1980c (*A. f. atra* Gloyd, 1968)
		– » –	–	USA	Kiauta and Brink 1978 (*A. f. fumipennis* (Burmeister, 1839))
		– » –	–	USA	Kiauta and Kiauta 1980c (*A. f. fumipennis*)
		– » –	+	Canada	Kiauta and Kiauta 1980c (*A. f. violacea* (Hagen, 1861))
524.	*A. funebris* (Hagen, 1861)	27(26A+X)	–	USA	Kiauta 1972b
		28(26A+XX)*	–	Mexico	Kiauta and Kiauta 1980c
525.	*A. immunda* (Hagen, 1861)	27(26A+X)	–	USA	Kiauta and Kiauta 1980c
526.	*A. moesta* (Hagen, 1861)	25(24A+X)	–	Canada	Kiauta 1978
		– » –	–	USA	Kiauta and Kiauta 1980c
527.	*A. nahuana* Calvert, 1902	25(24A+X)	–	USA	Kiauta and Kiauta 1980c
528.	*A. sedula* (Hagen, 1861)	27(26A+X)	–	USA	Cruden 1968
		– » –	–	USA	Kiauta and Kiauta 1980c
529.	*A. tibialis* (Rambur, 1842)	37(36A+X)	–	USA	Kiauta and Kiauta 1980c
530.	*A. translata* Hagen, 1865	25(24A+X)	+	USA	Kiauta and Kiauta 1980c
531.	*A. violacea* (Hagen, 1861)	27(26A+X)	–	USA	Cruden 1968
532.	*A. vivida* (Hagen, 1861)	27(26A+X)	–	USA	Cruden 1968
533.	***Ceriagrion*** *auranticum* Fraser, 1922	27(26A+X)	+	Thailand	Kiauta and Kiauta 1983 as *C. latericium* Lieftinck, 1951
534.	*C. azureum* (Selys, 1891)	27(26A+X)	–	Nepal	Kiauta 1974, 1975
535.	*C. cerinomelas* Lieftinck, 1927	27(26A+X)	–	Nepal	Kiauta 1974, 1975

Taxon		Karyotype formula 2n ♂	m-chromosomes	Country	References
536.	*C. cerinorubellum* (Brauer, 1866)	27(26A+X)	+	India	Dasgupta 1957
		– » –	+	India	Prasad and Thomas 1992
537.	*C. coromandelianum* (Fabricius, 1798)	27(26A+X)	+	India	Ray Chaudhuri and Dasgupta 1949
		– » –	+	India	Srivastava and Das 1953
		– » –	+	India	Das 1956
		– » –	+	Nepal	Kiauta and Kiauta 1982
		– » –	+	India	Prasad and Thomas 1992
538.	*C. fallax* Ris, 1914	27(26A+X)	+	Republic of South Africa	Dasgupta 1957
539.	*C. glabrum* (Burmeister, 1839)	27(26A+X)	–	Kingdom of Eswatini (Former Swaziland)	Boyes et al. 1980
540.	*C. rubiae* Laidlaw, 1916	27(26A+X)	+	India	Asana and Makino 1935
		– » –	+	India	Makino 1935
		– » –	+	India	Kichijo 1942a
541.	*C. tenellum* (Villers, 1789)	27(26A+X)	+	Italy	Kiauta 1971a (*C. t. tenellum* (Villers, 1789))
542.	***Chromagrion** conditum* (Hagen, 1876)	27(26A+X)	–	USA	Cruden 1968
543.	***Coenagrion** armatum* (Charpentier, 1840)	27(26A+X)	–	Finland	Oksala 1939a
		– » –	–	Former USSR	Makalowskaja 1940
544.	*C. hastulatum* (Charpentier, 1825)	27(26A+X)	–	Former USSR	Makalowskaja 1940
		– » –	–	Russia	Perepelov and Bugrov 2001b
545.	*C. hylas* (Trybom, 1889)	27(26A+X)	–	Austria	Kiauta and Kiauta 1991 (*C. h. freyi* (Bilek, 1954))
546.	*C. lunulatum* (Charpentier, 1840)	27(26A+X)	+	Russia	Perepelov and Bugrov 2001b
547.	*C. pulchellum* (Vander Linden, 1823)	27(26A+X)	–	Former USSR	Makalowskaja 1940
		– » –	–	Netherlands	Kiauta 1969c
		– » –	+	Russia	Kuznetsova et al. 2020b
548.	*C. puella* (Linnaeus, 1758)	27(26A+X)	+	Russia	Kuznetsova et al. 2020b
549.	*C. resolutum* (Hagen, 1876)	27(26A+X)	–	USA	Cruden 1968
550.	*Coenagrion* sp.	27(26A+X)	+	Japan	Kichijo 1941, 1942d, e
551.	***Diceratobasis** macrogaster* (Selys, 1875)	27(26A+X)	+	Jamaica	Cumming 1964
552.	***Enallagma** aspersum* (Hagen, 1861)	27(26A+X)	–	USA	Cruden 1968
553.	*E. boreale* Selys, 1875	27(26A+X)	–	USA	Cruden 1968
554.	*E. carunculatum* Morse, 1895	27(26A+X)	–	USA	Cruden 1968
555.	*E. circulatum* Selys, 1883	27(26A+X)	+	Russia	Perepelov and Bugrov 2001b
556.	*E. civile* (Hagen, 1861)	27(26A+X)	–	USA	Cruden 1968
557.	*E. cyathigerum* (Charpentier, 1840)	27(26A+X)	–	Finland	Oksala 1939a, 1945
		– » –	–	Former USSR	Makalowskaja 1940
		– » –	+	USA	Brink and Kiauta 1964
		27(26A+X),	–	USA	Cruden 1968
		29(28A+X)	–		
		27(26A+X)	+	Netherlands	Kiauta 1969a, c
		29(28A+X)	+		
558.	*E. ebrium* (Hagen, 1861)	27(26A+X)	–	USA	Cruden 1968
559.	*E. praevarum* (Hagen, 1861)	27(26A+X)	–	USA	Cruden 1968
560.	***Erythromma** lindeni* (Selys, 1840)	27(26A+X)	+	Italy	Kiauta 1971a
561.	*E. najas* (Hansemann, 1823)	27(26A+X)	–	Finland	Oksala 1939a
		– » –	–	Former USSR	Makalowskaja 1940
		– » –	–	Netherlands	Kiauta 1969a
		– » –	–	Russia	Perepelov and Bugrov 2001b
		– » –	+	Russia	Kuznetsova et al. 2020b
562.	***Homeoura** chelifera* (Selys, 1876)	27(26A+X)	+	Surinam	Kiauta 1979a as *Enallagma cheliferum* (Selys, 1876)
		– » –	+	Brazil	Ferreira et al. 1979 as *E. cheliferum*
563.	***Ischnura** aurora* (Brauer, 1865)	27(26A+X)	–	Nepal	Kiauta 1974, 1975
		– » –	–	India	Handa and Kochhar 1985
564.	*I. capreola* (Hagen, 1861)	27(26A+X)	–	Bolivia	Cumming 1964 as *Ceratura capreola* (Hagen, 1861)

Taxon		Karyotype formula 2n ♂	m-chromosomes	Country	References
565.	*I. cervula* Selys, 1876	27(26A+X)	–	USA	Cruden 1968
566.	*I. denticollis* (Burmeister, 1839)	27(26A+X)	–	USA	Cruden 1968
567.	*I. elegans* (Van der Linden, 1823)	27(26A+X)	–	Finland	Oksala 1939a, 1945
		– » –	–	Netherlands	Kiauta 1969a
		– » –	–	Russia	Perepelov 2003
568.	*I. fluviatilis* Selys, 1876	27(26A+X)	–	Bolivia	Cumming 1964
569.	*I. forcipata* Morton, 1907	27(26A+X)	–	Nepal	Kiauta 1974, 1975
570.	*I. nursei* (Morton, 1907)	25(24A+X)	+	India	Tyagi 1978b as *Rhodischnura nursei* (Morton, 1907)
571.	*I. pumilio* (Charpentier, 1825)	27(26A+X)	+	Netherlands	Kiauta 1979b
572.	*I. perparva* Selys, 1876	27(26A+X)	–	USA	Cruden 1968
573.	*I. ramburii* (Selys, 1850)	27(26A+X)	+	USA	Kiauta and Brink 1978
574.	*I. rufostigma* Selys, 1876	27(26A+X)	–	Nepal	Kiauta 1974, 1975 (*I. r. annandalei* Laidlaw, 1919)
575.	*I. senegalensis* (Rambur, 1842)	27(26A+X)	+	Japan	Kichijo 1941, 1942d, e
		– » –	+	India	Dasgupta 1957
		– » –	+	Ethiopia	Kiauta 1969b
		– » –	+	Philippines	Kiauta and Kiauta 1980b
		– » –	–	Thailand	Kiauta and Kiauta 1983
		– » –	+	India	Prasad and Thomas 1992
576.	*I. verticalis* (Say, 1839)	27(26A+X)	–	USA	Cruden 1968
577.	*I. ultima* Ris, 1908	27(26A+X)	–	Bolivia	Cumming 1964
578.	***Leptagrion*** *macrurum* (Burmeister, 1839)	30(28A+neo-XY)	–	Brazil	Kiauta 1971c, 1972d
579.	***Mecistogaster***. sp. 1	29(28A+X)	+	Bolivia	Cumming 1964
580.	*Mecistogaster* sp. 2	12(10A+neo-XY)	–	Bolivia	Cumming 1964
581.	***Megalagrion*** *oahuense* (Blackburn, 1884)	27(26A+X)	+	Hawaii	Kiauta 1969b
582.	***Mortonagrion*** *selenion* (Ris, 1916)	27(26A+X)	+	Japan	Kichijo 1941, 1942a, d, e
583.	***Nehalennia*** *irene* (Hagen, 1861)	27(26A+X)	–	USA	Cruden 1968
584.	*N. speciosa* (Charpentier, 1840)	28(26A+XX)*	–	Finland	Oksala 1945
585.	***Oxyagrion*** *hempeli* Calvert, 1909	27(26A+X)	–	Brazil	Souza Bueno 1982
586.	*O. terminale* Selys, 1876	27(26A+X)	–	Surinam	Kiauta 1979a
		– » –	–	Brazil	Ferreira et al. 1979
587.	***Paracercion*** *hieroglyphicum* (Brauer, 1865)	27(26A+X)	+	Japan	Kichijo 1941, 1942d, e as *Coenagrion hieroglyphicum* (Brauer, 1865)
588.	*P. malayanum* (Selys, 1876)	27(26A+X)	+	Nepal	Kiauta 1974, 1975
589.	***Proischnura*** *subfurcata* (Selys, 1876)	27(26A+X)	–	Kenya	Wasscher 1985 as *Enallagma subfurcatum* Selys, 1876
590.	***Pseudagrion*** *acaciae* Förster, 1906	27(26A+X)	+	Republic of South Africa	Boyes et al. 1980
591.	*P. australasiae* Selys, 1876	27(26A+X)	+	India	Dasgupta 1957
592.	*P. decorum* (Rambur, 1842)	27(26A+X)	+	India	Dasgupta 1957
593.	*P. kersteni* (Gerstaker, 1869)	27(26A+X)	–	Kingdom of Eswatini (Former Swaziland)	Boyes et al. 1980
594.	*P. microcephalum* (Rambur, 1842)	27(26A+X)	+	India	Dasgupta 1957
		– » –	+	Philippines	Kiauta and Kiauta 1980b
595.	*P. pruinosum* (Burmeister, 1839)	27(26A+X)	+	Thailand	Kiauta and Kiauta 1983
596.	*P. rubripes* (Selys, 1876)	27(26A+X)	+	India	Dasgupta 1957
		– » –	+	Philippines	Kiauta and Kiauta 1980b
		– » –	+	Thailand	Kiauta and Kiauta 1983
597.	*P. salisburyense* Ris, 1921	27(26A+X)	+	Kingdom of Eswatini (Former Swaziland)	Boyes et al. 1980
598.	*P. spencei* Fraser, 1922	27(26A+X)	+	India	Dasgupta 1957
599.	*P. whellani* Pinhey, 1956	25(24A+X)	+	Burkina Faso (Former Voltiac Republic)	Kiauta and Ochssée 1979
600.	***Pyrrhosoma*** *nymphula* (Sutzer, 1776)	28(26A+XX)*	–	Finland	Oksala 1945
601.	***Telebasis*** *carmesina* Calvert, 1909	27(26A+X)	–	Surinam	Kiauta 1979a
		– » –	–	Brazil	Ferreira et al. 1979
602.	***Tigriagrion*** *aurantinigrum* Calvert, 1909	27(26A+X)	–	Bolivia	Cumming 1964

Taxon		Karyotype formula 2n ♂	m-chromo somes	Country	References
603.	***Xanthocnemis*** *zealandica* (McLachlan, 1873)	27(26A+X)	–	New Zealand	Jensen 1980 as *X. zelandica* (McLachlan, 1873)
604.	***Zoniagrion*** *exclamationis* (Selys, 1876)	27(26A+X)	–	USA	Cruden 1968
Protoneuridae					
605.	***Caconeura*** *autumnalis* Fraser, 1922	25(24A+X)	+	India	Tyagi 1978b
606.	***Epipleoneura*** sp.	27(26A+X)	–	Bolivia	Cumming 1964
607.	***Protoneura*** *rubriventris* (Selys, 1860)	27(26A+X)	+	Bolivia	Cumming 1964 as *Neoneura rubriventris* Selys, 1860

* In the original publication, the female karyotype is given.
** Jensen (1980) considers these data as erroneous (but see section "Concluding remarks and future directions" in the present paper).
*** Karyotype formula is extrapolated based on vague descriptions by Cumming (1964).

Table 2. The diversity of chromosome numbers and sex chromosome mechanisms, and modal karyotypes in 23 families of Odonata: a summary.

Taxa (N of species/genera described*)		N of species/ genera studied	Male karyotypes	Modal karyotype	N of species/genera with modal karyotype (occurrence in percent)
Anisozygoptera					
Epiophlebioidea	Epiophlebiidae (4/1)	1/1	25, X0	24A + X	1 (100) / 1 (100)
Anisoptera					
Aeshnoidea	Aeshnidae (456/51)	58/18	13, X0; 14, neo-XY; 15, X0; 16, neo-XY; 19, X0; 21, X0; 24, neo-XY; 25, X0; 26, neo-XY; 27, X0	26A + X	44 (76) / 14 (78)
Petaluroidea	Petaluridae (10/5)	4/3	17, X0; 19, X0; 25, X0	16A + X	3 (75) / 2 (67)
Gomphoidea	Gomphidae (980/87)	66/31	12, neo-neo-XY; 21, X0; 22, neo-XY; 23, X0; 24, neo-XY; 25, X0	22A + X	57 (86) / 28 (90)
Libelluloidea	Macromiidae (125/4)	6/3	25, X0	24A + X	6 (100) / 3 (100)
	Corduliidae (154/20)	23/7	10, neo-XY; 11, X0; 13, X0; 14, neo-XY, 20, XY; 21, X0; 25, X0; 26, neo-XY; 27, X0	24A + X	19 (83) / 6 (86)
	Libellulidae (1037/142)	255/59	6, neo-XY; 6 neo-XY; 8, neo-XY; 10, neo-XY; 12, neo-XY; 17, X0; 21, X0; 22, neo-XY; 23, X0; 23, X1X2Y; 24, neo-XY; 25, X0; 27, X0; 28, neo-XY; 29, X0; 41, X0	24A + X	227 (89) / 57 (97)
Cordulegastroidea	Cordulegastridae (46/3)	9/3	23, X0; 25, X0	24A + X	8 (89) / 3 (100)
	Chlorogomphidae (47/3)	1/1	25, X0	24A + X	1 (100) / 1 (100)
Zygoptera					
Lestoidea	Lestidae (151/9)	20/5	19, X0; 21, X0; 25, X0	24A + X	18 (90) / 5 (100)
	Synlestidae (39/9)	1/1	25, X0	24A + X	1 (100) / 1 (100)
Platystictoidea	Platystictidae (224/6)	4/3	25, X0	24A + X	4 (100) / 3 (100)
Calopterygoidea	Calopterygidae (185/21)	20/8	23, X0; 25, X0; 27, X0	24A + X	20 (100) / 8 (100)
	Chlorocyphidae (144/19)	9/6	23, X0; 25, X0	22A + X	8 (89) / 5 (84)
	Polythoridae (59/7)	2/2	23, X0	22A + X	2 (100) / 2 (100)
	Euphaeidae (68/12)	4/4	25, X0	24A + X	4 (100) / 4 (100)
	Megapodagrionidae (296/42)	3/2	25, X0	24A + X	3 (100) / 2 (100)
	Heteragrionidae (57/2)	2/1	25, X0	24A + X	2 (100) / 1 (100)
	Philogeniidae (40/2)	1/1	25, X0	24A + X	1 (100) / 1 (100)
	Hypolestidae (6/4)	1/1	17, X0	16A + X	1 (100) / 1 (100)
Coenagrionoidea	Platycnemididae (404/40)	22/8	25, X0	24A + X	19 (100) / 7 (100)
	Coenagrionidae (1267/114)	92/28	12, neo-XY; 25, X0; 27, X0; 29, X0; 30, neo-XY; 37, X0	26A + X	81 (89) / 26 (90)
	Protoneuridae (260 / 25)	3/3	25, X0; 27, X0	26A + X	2 (70) / 2 (70)

*Taken from Dijkstra et al. 2013

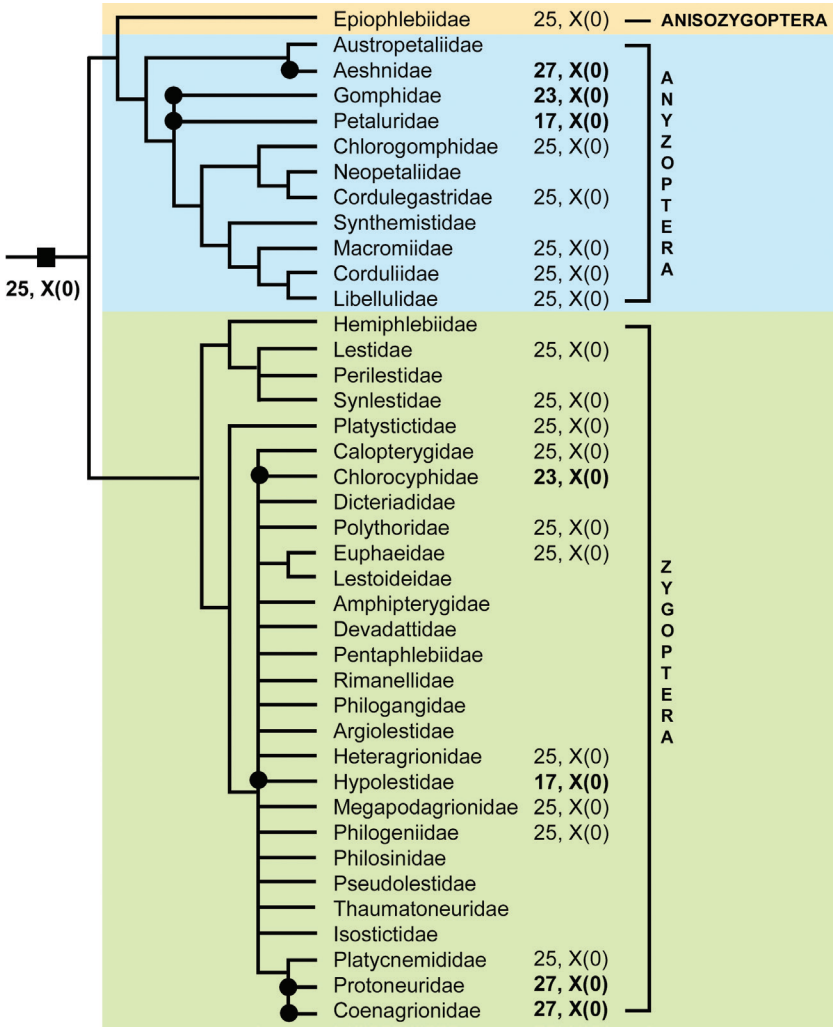

Figure 1. Mapping of modal karyotypes onto phylogenetic tree of Odonata families. The phylogenetic tree is taken from Bybee et al. (2016) who synthesized it based on trees from Dijkstra et al. (2014) and Carle et al. (2015). Plesiomorphic karyotype state is indicated by a black solid **square** (■), apomorphic karyotype states are indicated by black solid **circles** (●).

Concluding remarks and future directions

In total, karyotypes of 607 species (198 genera, 23 families) of Odonata are studied up to now. Table 1, presented in our work, includes 423 species (125 genera, 8 families) of the Anisoptera, 184 species (72 genera, 14 families) of the Zygoptera, and one species of the Anisozygoptera. Thus, the presently available karyotype data cover about 10% of the world species diversity of the order in general.

Within Odonata, chromosome numbers in males vary over a relatively wide range, from 2n = 6 in *Macrothemis hemichlora* and *Orthemis levis* to 2n = 41 in *O. nodiplaga*. Both low chromosome number species are suggested to have an evolutionarily secondary neo-XY system (Cumming 1964; Kiauta 1972c) that could have arisen through an X-autosome fusion from an X(0) system. All three of the above species belong to the largest dragonfly family Libellulidae, in which nearly 89% of studied species (255 in total) have the karyotype 2n = 25(24A + X). The last one is the most common in Odonata in general: it occurs in each of the three suborders, Zygoptera, Anisoptera and Anisozygoptera, and in all families with the exception of two damselfly families, the Polythoridae with only two studied species sharing 2n = 23(22A + X) and a monotypic family Hypolestidae with 2n = 17(16A + X) in male *Hypolestes clara*. Besides Libellulidae, the karyotype 2n = 25(24A + X) is currently the presumed modal one in 14 other families, such being the case at least in six better covered (at species and/or generic level) families, i.e. the dragonfly families Corduliidae, Cordulegastridae, and Macromiidae, and the damselfly families Lestidae, Calopterygidae, and Platycnemididae (Table 2, Fig. 1). This chromosome set is suggested to be an ancestral one for the order Odonata in general (Oguma 1930; Kuznetsova et al. 2020b) although this suggestion remains questionable at this stage.

Chromosomal rearrangements, among which fission and fusions apparently predominated (Kiauta 1969c, 1972c), led to the appearance of divergent karyotypes in the evolution of Odonata. As a result, in many dragonfly and damselfly families, other karyotypes, when occurring, are of secondary origin as indicated by either a diverged number of autosomes or a secondary sex chromosome system of an XY-type or both (e.g. Cumming 1964; Kiauta 1969a, c; Agopian and Mola 1984, 1988; Mola et al. 1999; Perepelov and Bugrov 2002). Some interesting examples of this kind can be found in the family Libellulidae, in which 2n = 25(24A + X) is most likely an evolutionarily initial karyotype (e.g. Agopian and Mola 1988). These examples are as follows (see Table 1): *Orthemis nodiplaga* and *O. ambinigra* with 2n = 41(40A + X) and 2n = 12(10A + neo-XY), respectively; *Erythrodiplax media* and *E. minuscula*, both with 2n = 22(20A + neo-XY); *Micrathyria longifasciata* and *M. ungulata* with 2n = 24(22A + neo-XY) and 2n = 23(20A + X_1X_2Y), respectively. In some families, any of these presumably derived karyotypes not only occurs but also prevails and may be considered modal (see Table 2 and Fig. 1). Within Anisoptera, such families are Aeshnidae (2n = 26A + X) and Gomphidae (2n = 22A + X), whereas within Zygoptera, these are Chlorocyphidae (2n = 22A + X) and Coenagrionidae (2n = 26A + X). Thus, Odonata, despite the fact that they have holokinetic chromosomes (Nokkala et al. 2002), demonstrate rather high karyotypic stability, with most species showing 2n = 25 (found in 60% of studied species), 2n = 27(21%) and 2n = 23(13%) which may point to some selective constraints acting to stabilize chromosome number in their evolution (Kuznetsova et al. 2020b).

There are the species for which different authors give various karyotypes that are sometimes difficult to interpret (see Table 1). In some cases, this might be due to

misidentifications of a particular species or an error in determining the karyotype. For example, Wolfe (1953) reported 2n = 17(16A + X) for males of *Uropetala carovei* (Petaluridae, Anisoptera) from New Zealand. However, according to later studies of this species in the same locality (Jensen and Mahanty 1978; Jensen 1980), it has 2n = 25(24A + X), and Jensen (1980) therefore considers the Wolfe data as erroneous. We cannot exclude, however, that the above authors studied different *U. carovei* subspecies, *U. c. carovei* White, 1846 and *U. c. chiltoni* Tillyard, 1921, that may indeed have different karyotypes. In other cases, the chromosome number difference between geographic populations might be indicative of the inter-population variation within the bounds of one taxonomic species or even the existence of a species complex with several morphologically cryptic species. For example, 4 of the 17 studied species of the dragonfly genus *Aeshna* Fabricius, 1775 were reported to have different karyotypes in different populations. These are: *Aeshna grandis* – 2n = 26A + X (former USSR), 2n = 24A + X (former USSR, Finland), and 2n = 24A + neo-XY (Netherlands, Finland); *A. isoceles* – 2n = 26A + X (USA) and 2n = 24A + X (Russia); *A. juncea* – 2n = 26A + X (Italy) and 2n = 24A + neo-XY (Finland, former USSR, Italy); *A. mixta* – 2n = 26A + X (Netherlands) and 2n = 24A + X (India) (Table 1). In all such cases, special studies involving a combined analysis of karyotypes, morphology, distribution patterns and molecular markers are needed.

Approximately 80% of Odonata species have a pair of very small chromosomes, i.e. microchromosomes or m-chromosomes (Mola 2007, Table 1). A number of speculations have been forwarded to explain the origin of these chromosomes in Odonata. Kiauta (1968e) suggested m-chromosomes to be fragments of "normal" chromosomes, whereas Oguma (1930) considered them the remnants of an autosome pair in the process of its elimination by progressive loss of chromatin. The size of the smaller chromosome pair was shown to be variable within different species (Kiauta 1968e; see Mola 2007 for other references) which is consistent with both hypotheses. Closely related species and different populations of the same species often differ from each other in the presence/absence of m-chromosomes (Table 1). This is most likely due to the lack of clear criteria for the identification of a small chromosome pair as m-chromosomes in a particular karyotype (Mola 2007; Kuznetsova et al. 2020b).

Most cytogenetic studies of Odonata have been made only to determine the chromosome number and sex chromosome mechanism for which the routine staining was used. Although a considerable amount of such data was obtained (Table 1, 2), standard karyotypes of many Odonata taxa remain totally unknown (Fig. 1). Lack of data on more "primitive" families of Zygoptera (e.g. Hemiphlebiidae) and Anisoptera (e.g. Austropetaliidae and Neopetaliidae) makes difficult understanding karyotype evolution of the order in general.

During the last decades, karyotypes of a few dozen Odonata species were studied using various techniques of differential staining of chromosomes such as C-banding, AgNOR-staining and DNA specific fluorochrome banding visualiszing constitutive heterochromatin, nucleolus organizing regions (NORs) and AT- and GC-rich chromosome segments, respectively. Such data can be found in the following publica-

tions: Thomas and Prasad (1986), Prasad and Thomas (1992), Perepelov et al. (1998), Perepelov and Bugrov (2001a, b, 2002), Grozeva and Marinov (2007), De Gennaro et al. (2008), Walia et al. (2011, 2018), Walia and Chahal (2014, 2018), Walia and Devi (2018), Walia and Katnoria (2018), Walia and Devi (2020a, b). Unfortunately, these data alone did not shed much light on the karyotypic evolution of Odonata.

Although the classical cytological techniques remain necessary starting points for cytogenetic studies of Odonata to get an overview of their genomes, the future of Odonata cytogenetics must be coupled with the application of new cytogenetic molecular techniques that enable the localization of specific DNA sequences in chromosomes and the identification of individual chromosomes in karyotypes. In the article by Frydrychová et al. (2004) and, on a larger scale, in two of our recent publications (Kuznetsova et al. 2018, 2020b), the fluorescence *in situ* hybridization (FISH) technique was used for the first time for analyzing Odonata karyotypes. Several species belonging to the Anisoptera (from the families Aeshnidae, Libellulidae, and Corduliidae) and the Zygoptera (from the families Coenagrionidae and Calopterygidae) were studied regarding the occurrence of the TTAGG telomeric repeats and the distribution of the *18S rRNA* genes in their karyotypes. The TTAGG repeats proved to be the canonical motif of telomeres in the class Insecta in general, which, however, was repeatedly lost in the evolution of different phylogenetic lineages (Kuznetsova et al. 2020a). It was shown in the listed Odonata publications that the $(TTAGG)_n$ motif does not occur in all but one (*Sympetrum vulgatum*) species, and the *18S* is located on one of the largest pairs of autosomes in all studied dragonfly species but on m-chromosomes in all studied damselfly species (Kuznetsova et al. 2020b).

The results obtained showed great promise of the combined use of FISH and classical and banding cytogenetics in order to identify new chromosomal markers, reveal differences between species, particularly when they share the same or very close karyotypes, and speculate about the mechanisms involved in the karyotype evolution of Odonata (Kuznetsova et al. 2020b). Another promising line of future research could be to test hypotheses (Mola and Papeschi 1994; Ardila-Garcia and Gregory 2009) about whether there is a relationship between karyotype evolution and genome size diversity in the Odonata or there is no such relationship.

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References

Agopian SS, Mola LM (1984) An exceptionally high chromosome number in *Orthemis nodiplaga* Kersch (Anisoptera, Libellulidae). Notulae Odonatologicae 2(3): 45.

Agopian SS, Mola LM (1988) Intra and interspecific karyotype variability in five species of Libellulidae (Odonata, Anisoptera). Caryologia 41(1): 69–78. https://doi.org/10.1080/00087114.1988.10797849

Ardila-Garcia AM, Gregory TR (2009) An exploration of genome size diversity in dragonflies anddamselflies (Insecta: Odonata). Journal of Zoology 278: 163–173. https://doi.org/10.1111/j.1469-7998.2009.00557.x

Asana JJ, Makino S (1935) A comparative study of the chromosomes in the Indian dragonflies. Journal of the Faculty of Science, Hokkaido University Series 6, Zoology 4(2): 67–86.

Boyes JW, van Brink JM, Kiauta B (1980) Sixteen dragonfly karyotypes from the republic of South Africa and Swaziland, with evidence on the possible hybrid nature of *Orthetrum julia falsum* Longfeild (Anisoptera: Libellulidae). Odonatologica 9: 131–145.

Bybee S, Córdoba-Aguilar A, Duryea MC, Futahashi R, Hansson B, Lorenzo-Carballa MO, Schilder R, Stoks R, Suvorov A, Svensson EI, Swaegers J, Takahashi J, Watts PC, Wellenreuther M (2016) Odonata (dragonflies and damselflies) as a bridge between ecology and evolutionary genomics. Frontiers in Zoology 13: e46. https://doi.org/10.1186/s12983-016-0176-7

Capitulo RA, Mola LM, Agopian SS (1991) Species catalogue and chromosomal data of Odonata from Argentina. Revista de la Sociedad Entomológica Argentina 49(1–4): 59–72.

Carle FL, Kjer KM, May ML (2015) A molecular phylogeny and classification of Anisoptera (Odonata). Arthropod Systematics and Phylogeny 73(2): 281–301. https://entomology.rutgers.edu/news/docs/Carle-2015-Anisoptera-Phylogeny-Classification.pdf

Carnoy BJ (1885) La cytodierese chez les arthropodes. IV. Pseudo-Nevropteres. Cellule 1: 279–282.

Chatterjee K, Kiauta B (1973) Male germ cell chromosomes of two Calopterygoidea from the Darjeeling Himalaya (Zygoptera: Chlorocyphidae, Euphaeidae). Odonatologica 2(2): 105–108.

Cruden RW (1968) Chromosome numbers of some North American dragonflies (Odonata). Canadian Journal of Genetics and Cytology 10: 200–214. https://doi.org/10.1139/g68-029

Cumming RB (1964) Cytogenetic studies in the order Odonata. PhD thesis, University of Texas, Austin, 93 pp.

Das C (1956) Studies on the association between non-homologous chromosomes during meiosis in four species of the Indian dragonflies (Odonata). Journal of the Zoological Society of India 8(2): 119–132.

Dasgupta J (1957) Cytological studies of some Indian dragonflies. II: A study of the chromosomes during meiosis in thirty species of Indian Odonata (Insecta). Proceedings of the Zoological Society of Calcutta 10: 1–65.

De Gennaro D (2004) Análisis meiótic y caracterización de la heterocromatina en species argentinas de Anizoptera (Odonata). Tesis de Licenciatura. Facultad de Ciencias Exactas y Naturales de la Universidad de Buenos Aires. Buenos Aires, 66 pp.

De Gennaro D, Rebagliati PJ, Mola LM (2008) Fluorescent banding and meiotic behaviour in *Erythrodiplax nigricans* (Libellulidae) and *Coryphaeschna perrensi* (Aeschnidae) (Anisoptera, Odonata). Caryologia 61: 60–67. https://doi.org/10.1080/00087114.2008.10589610

Dijkstra K-DB, Bechly G, Bybee SM, Dow RA, Dumont HJ, Fleck G, Garrison RW, Hämäläinen M, Kalkman VJ, Karube H, May ML, Orr AG, Paulson DR, Rehn AC, Theischinger G, Trueman JWH, van Tol J, Ellenrieder N, Ware J (2013) The classification and diversity of dragonflies and damselflies (Odonata). In: Zhang ZQ (Ed.) Animal Biodiversity: An Outline of Higher-level Classification and Survey of Taxonomic Richness (Addenda 2013). Zootaxa 3703: 36–45. https://doi.org/10.11646/zootaxa.3703.1.9

Dijkstra K-DB, Kalkman VJ, Dow RA, Stokvis FR, van Tol J (2014) Redefining the damselfly families: a comprehensive molecular phylogeny of Zygoptera (Odonata). Systematic Entomology 39: 68–96. https://doi.org/10.1111/syen.12035

Ferreira A, Kiauta B, Zaha A (1979) Male germ cell chromosomes of thirty-two Brazilian dragonflies. Odonatologica 8: 5–22.

Francovič M, Jurečic R (1986) Prilog citogenetickim i citotaksonomskim istrazivanjima vrste *Libellula depressa* L. (Odonata, Libellulidae). Plenarni Referati VII Kongres Biologa Jugoslavije, Budva, 341 pp.

Francovič M, Jurečic R (1989) Comparative cytogenetic analysis of karyotype morphology and organization in males of species *Libellula depressa* L. and *L. fulva* Müll. (Insecta: Odonata). Periodicum Biologorum 91(1): 32–33.

Frydrychová R, Grossmann P, Trubač P, Vítková M, Marec F (2004) Phylogenetic distribution of TTAGG telomeric repeats in insects. Genome 47: 163–178. https://doi.org/10.1139/g03-100

Fuchsówna J, Sawczyńska J (1928) Zachowanie sie heterochromosomóv podczam spermatogenezy u ważek (Odonata). Cz. I. *Aeschna grandis* L. *Libellula quadrimaculata* L. Archiwum Towarzystwa naukowego we Lwowie (III) 4(9): 177–197. [In Polish]

Goni B, de Abenante YP (1982) Cytological notes on five dragonfly species from Uruguay. Odonatologica 11(4): 323–329.

Grimaldi D, Engel MS (2005) Evolution of the Insects. Cambridge University Press, Cambridge, 755 pp.

Grozeva SM, Marinov MG (2007) Cytogenetic study of *Somatochlora borisi* Marinov, 2001 (Odonata: Corduliidae), and three relative species. Acta Zoologica Bulgarica 59(1): 53–58.

Handa SM, Batra HN (1980) Cytology of ten species of dragonflies (Anisoptera: Odonata). Proceedings of the 67th Indian Science Congress, Part III, Calcutta, 103 pp.

Handa SM, Kochhar N (1985) Chromosomal architecture in two species of damselflies from Chandigarh and its surrounding areas. National Seminar on Current Trends in Chromosome Dynamics, Chandigarh, 34 pp.

Handa SM, Mittal OP, Batra HN (1984) Chromosomes in ten species of dragonflies (Anisoptera: Odonata). Research Bulletin of the Panjab University (Science) 35: 65–75.

Higashi K, Kayano H (1993) The distribution of distinct karyomorphs of *Crocothemis servilia* Drury (Anisoptera, Libellulidae) in Kyushu and the south-western islands of Japan. Japanese Journal of Entomology 61: 1–10.

Higashi K, Lee CE, Kayano H, Kayano A (2001) Korea strait delimiting distribution of distinct karyomorphs of *Crocothemis servilia* (Drury) (Anisoptera: Libellulidae). Odonatologica 30(3): 265–270.

Hirai H (1956) Chromosomes of six species of dragonflies. Zoological Magazine, Tokyo 65: 198–202.

Hogben L (1921) Studies on synapsis, III. The nuclear organisation of the germ cells in *Libellula depressa*. Philosophical Transactions of the Royal Society of London Series B 92: 60–80. https://doi.org/10.1098/rspb.1921.0006

Hung ACF (1971) Cytological studies of five dragonflies (Odonata: Anisoptera). Entomological News 82: 103–106.

Jensen AL (1980) The karyotypes of five species of Odonata endemic to New Zealand. Odonatologica 9: 29–33.

Jensen AL, Mahanty HK (1978) A preliminary note on the chromosome number of *Uropetala carovei* (White) (Anisoptera: Petaluridae). Odonatologica 7: 385–386.

Kalkman VJ, Clausnitzer V, Dijkstra K-DB, Orr AG, Paulson DR, van Tol J (2008) Global diversity of dragonflies (Odonata) in freshwater. Hydrobiologia 595: 351–363. https://doi.org/10.1007/s10750-007-9029-x

Katatani N (1987) On the chromosomes of dragonflies, 1. Synopsis on the studies in some Japanese dragonflies. Aeschna 20: 21–31.

Kiauta B (1965) The chromosome behaviour in spermatogenetic meiosis of *Anax imperator* Leach (Odonata: Aeshnidae). Tombo 7(3–4): 18–21.

Kiauta B (1966) The chromosome behaviour in spermatogenetic meiosis of the dragonfly *Sympetrum striolatum* (Charp.) (Odonata: Libellulidae) from Luxembourg. Bulletin de la Société des Naturalistes Luxembourgeois 69: 54–60.

Kiauta B (1967a) Evolution of the chromosome complement in Odonata. Genen en Phaenen 11(4): 56–61.

Kiauta B (1967b) Abstract. Evolution of the chromosome complement in Odonata. Genetica 38(3): 403–404. https://doi.org/10.1007/BF01507474

Kiauta B (1967c) A new hypothesis on the evolution of the chromosome complement in Odonata. Tombo 10(1–4): 29–33.

Kiauta B (1967d) Considerations on the evolution of the chromosome complement in Odonata. Genetica 38(4): 430–446. https://doi.org/10.1007/BF01507474

Kiauta B (1967e) Meiotic chromosome behaviour in the male damselfly, *Calopteryx virgo* (Linnaeus), with a discussion on the value of chromosome numbers and karyotype morphology in odonate systematics. Deutsche Entomologische Zeitschrift 14(3–4): 339–348. https://doi.org/10.1002/mmnd.19670140312

Kiauta B (1968a) Evolution of the chromosome complement in Odonata. Entomologische Berichten, Amsterdam 28(5): 97–100.

Kiauta B (1968b) Morphology and kinetic behaviour of the odonate sex chromosomes, with a review of the distribution of sex determining mechanisms in the order. Genen en Phaenen 12(1): 21–24.

Kiauta B (1968c) The chromosome numbers of eight Old World dragonflies (Odonata). Chromosome Information Service, Tokyo 9: 3–4.

Kiauta B (1968d) The chromosomes of the male dragonfly *Cordulegaster boltoni* (Donovan, 1807) (Odonata: Cordulegasteridae). Biološki Vestnik: glasilo slovenskih biologov 16: 87–94.

Kiauta B (1968e) Variation in size of the m-chromosome of the dragonfly, *Calopteryx virgo* (L.), and its significance for the chorogeography and taxonomy of the *Calopteryx virgo superspecies*. Genen en Phaenen 12(1): 11–16.

Kiauta B (1968f) Variation in size of the dragonfly m-chromosome, with considerations on its significance for the chorogeography and taxonomy of the order Odonata, and notes on the validity of the rule of Reinig. Genetica 39(1): 64–74. https://doi.org/10.1007/BF02324456

Kiauta B (1969a) Sex chromosomes and sex determining mechanisms in Odonata, with a review of the cytological conditions in the family Gomphidae, and reference to the karyotypic evolution in the order. Genetica 40(2): 127–157. https://doi.org/10.1007/BF01787346

Kiauta B (1969b) The chromosomes of eight dragonfly species from continental Africa and Madagascar (Odonata). Arnoldia (Rhodesia) 4(15): 1–8.

Kiauta B (1969c) Autosomal fragmentations and fusions in Odonata and their evolutionary implications. Genetica 40(2): 158–180. https://doi.org/10.1007/BF01787347

Kiauta B (1969d) The chromosomes of the Hawaiian endemic dragonflies, *Megalagrion oahuense* (Blackburn) (Coenagrionidae: Pseudagrioninae) and *Nesogonia blackburni* (McLachlan) (Libellulidae: Sympetrinae), with a note on the cytotaxonomic affinities between the genera *Nesogonia* Kirby and *Sympetrum* Newman (order Odonata). Proceedings of the Hawaiian Entomological Society 20(2): 429–433.

Kiauta B (1970a) The chromosomes of four Neotropical dragonflies from Mexico. Chromosome Information Service, Tokyo 11: 8–9.

Kiauta B (1970b) The karyotype of the damselfly, *Epallage fatime* (Charpentier, 1840) (Odonata, Zygoptera: Epallagidae), with a note on the cytotaxonomic affinities in the superfamily Calopterygoidea. Genetica 41: 390–397. https://doi.org/10.1007/BF00958931

Kiauta B (1971a) Studies on the germ cell chromosome cytology of some cytotaxonomically interesting or hitherto not studied Odonata from the autonomous region Friuli-Venezia Giulia (northern Italy). Atti del Museo civico di Storia naturale di Trieste 27: 65–127.

Kiauta B (1971b) An unusual case of precocious segregation and chromosome fragmentation in the primary spermatocytes of the damselfly, *Calopteryx virgo meridionalis* (Selys, 1873), as evidence for a possible hybrid character of some populations of the *Calopteryx-virgo*-complex (Odonata, Zygoptera: Calopterygidae). Genen en Phaenen 14(2): 32–40.

Kiauta B (1971c) Cytotaxonomic peculiarities in the neotropical odonate genera *Leptagrion* Selys, *Orthemis* Hagen and *Macrothemis* Hagen. Abstracts of papers read at the 1st European Symposium on Odonatology, Gent, 27–28.

Kiauta B (1972a) Notes on new or little known dragonfly karyotypes, 2. Male germ cell chromosomes of four East Mediterranean species: *Lestes barbarus* (Fabricius), *Calopteryx splendens amasina* Bartenev (Zygoptera: Lestidae, Calopterygidae), *Caliaeschna microstigma* (Schneider) and *Orthetrum taeniolatum* (Schneider) (Anisoptera: Aeshnidae, Libellulidae). Genen en Phaenen 15: 95–98.

Kiauta B (1972b) Notes on new or little known dragonfly karyotypes, 1. The germ cell chromosomes of three Latin American species: *Argia funebris* (Hagen), *Megapodagrion contortum* (Selys) (Zygoptera: Coenagrionidae, Megapodagrionidae) and *Castoraeschna castor* (Brauer) (Anisoptera: Aeshnidae). Genen en Phaenen 15: 23–26.

Kiauta B (1972c) Synopsis on the main cytotaxonomic data in the order Odonata. Odonatologica 1(2): 73–102.

Kiauta B (1972d) The karyotype of the damselfly, *Leptagrion macrurum* (Burmeister, 1839), and its possible origin, with a note on the cytotaxonomic affinities of the genus (Zygoptera: Coenagrionidae). Odonatologica 1(1): 31–35.

Kiauta B (1973a) Notes on new or little known dragonfly karyotypes. III. Spermatocyte chromosomes of four Nearctic anisopterans: *Aeshna californica* Calvert (Aeshnidae), *Cordulia shurtleffi* Scudder (Corduliidae), *Sympetrum internum* Montgomery, and *S. madidum* (Hagen) (Libellulidae). Genen en Phaenen 16(1): 7–12.

Kiauta B (1973b) Notes on new or little known dragonfly karyotypes. IV. Spermatocyte chromosomes of *Calopteryx splendens splendens* Harris (Zygoptera: Calopterygidae), *Gomphus pulchellus* Selys, *Libellula depressa* Linnaeus (Anisoptera: Gomphidae, Libellulidae) from northern France. Genen en Phaenen 16(2): 55–60.

Kiauta B (1974) Introduction to insect cytotaxonomy. Lectures delivered at the Tribhuvan University, Kathmandu, Vol. 1. Nepal Research Center, Kathmandu, 81 pp.

Kiauta B (1975) Cytotaxonomy of dragonflies, with special reference to the Nepalese fauna. Lectures delivered at the Tribhuvan University, Kathmandu, Vol. 2. Nepal Research Center, Kathmandu, 78 pp.

Kiauta B (1977) Notes on new or little known dragonfly karyotypes.V. The male germ cell chromosomes of *Macromia moorei* Selys from Nepal (Anisoptera: Corduliidae, Epophthalmiinae). Genen en Phaenen 19: 49–51.

Kiauta B (1978) Two cytotaxonomically interesting cases of irreversible autosome fusion in dragonflies *Agria modesta* (Hagen) (Zygoptera: Coenagrionidae) and *Anaciaeschna isosceles* (Müller) (Anizoptera: Aeshnidae). Notulae Odonatologicae 1(1): 7–9.

Kiauta B (1979a) The karyotypes of some Anisoptera from Surinam. Odonatologica 2: 267–283.

Kiauta B (1979b) The karyotype of *Ischnura pumilio* (Charp.) (Zygoptera: Coenagrionidae). Notulae Odonatologicae 1(3): 47–48.

Kiauta B (1983) The status of the Japanese *Crocothemis servilia* (Drury) as revealed by karyotypic morphology (Anisoptera: Libellulidae). Odonatologica 12: 381–388.

Kiauta B, Boyes JW (1972) Cytology of ten South American Libellulidae, with cytophylogenetic consideration of the genera *Orthemis* Hagen and *Erythrodipax* Brauer (Odonata, Anisoptera). Genetica 43(3): 407–421. https://doi.org/10.1007/BF00156136

Kiauta B, Brink JM (1975) Cytotaxonomic notes on the *Sympetrum pedemontanum* complex (Anisoptera: Libellulidae). Odonatologica 4(4): 249–254.

Kiauta B, Brink JM (1978) Male chromosome complements of some Florida dragonflies, United States. Odonatologica 7(1): 155–25.

Kiauta B, Kiauta MAJE (1976) The chromosomes of some dragonflies from the Langtang Valley, Central Nepal. Odonatologica 5(4): 347–354.

Kiauta B, Kiauta MAJE (1979) The karyotype of *Libellula fulva* Müll, from Switzerland (Anisoptera: Libellulidae). Notulae Odonatologicae 1(4): 73–74.

Kiauta B, Kiauta MAJE (1980a) The karyotypes of *Aeshna subarctica elisabethae* Djak. and *Somatochlora alpestris* (Sel.) from Switzerland (Anisoptera, Aeshnidae, Corduliidae). Notulae Odonatologicae 1(6): 104–105.

Kiauta B, Kiauta MAJE (1980b) On a small collection of dragonfly karyotypes from the Philippines. Odonatologica 9(3): 237–245.

Kiauta B, Kiauta MAJE (1980c) Introduction to the cytotaxonomy of the odonate genus *Agria* Rambur (Zygoptera: Coenagrionidae). Odonatologica 9(1): 35–56.

Kiauta B, Kiauta MAJE (1982) The chromosome numbers of sixteen dragonfly species from the Arun Valley, Eastern Nepal. Notulae Odonatologicae 9(1): 143–146.

Kiauta B, Kiauta MAJE (1983) The chromosome numbers of some Odonata from Thailand. Notulae Odonatologicae 2(2): 17–32.

Kiauta B, Kiauta MAJE (1991) Biogeographic considerations on *Coenagrion hylas freyi* (Bilek, 1954), based mainly on the karyotype features of a population from North Tyrol, Austria (Zygoptera: Coenagrionidae). Odonatologica 20(4): 417–431.

Kiauta B, Kiauta MAJE (1995) The karyotypes of *Somatochlora meridionalis* Nielsen from Slovenia and *S. metallica* (Vander L.) from Switzerland, with a tentative note on the origin of Central European *S. metallica* (Odonata: Corduliidae). Opuscula zoologica fluminensia 137: 1–5.

Kiauta B, Kiauta-Brink MAJE (1975) Chromosomes of the dragonfly, *Sympecma annulata braueri* (Yakobson & Bianki, 1905) from the Netherlands, with a note on the classification of the family Lestidae (Odonata, Zygoptera). Genen en Phaenen 18(2–3): 39–48.

Kiauta B, Ochssée BV (1979) Some dragonfly karyotypes from the Voltiac Republic (Haute Volta), West Africa. Odonatologica 8: 47–54.

Kichijo H (1939) Chromosomes of *Tachopteryx pryeri* and *Gomphus hakiensis* (Odonata, Aeshnidae). Japanese Journal of Genetics 15: 287–289. https://doi.org/10.1266/jjg.15.287

Kichijo H (1941) Chromosomes of seven species of insects belonging to the order of dragonflies, suborder of damselfies. Nagasaki Medical Journal 19(10): 2033–2041. [In Japanese]

Kichijo H (1942a) Insect chromosomes. IV. Order of dragonflies, Pt. 2. Nagasaki Medical Journal 20(10): 1639–1648. [In Japanese]

Kichijo H (1942b) Insect chromosomes. III. Order of dragonflies, Pt. 1. Nagasaki Medical Journal 20(7): 1084–1092. [In Japanese]

Kichijo H (1942c) Chromosomes of *Sympetrum eroticum eroticum* (Odonata). Japanese Journal of Genetics 18: 195–196. https://doi.org/10.1266/jjg.18.195

Kichijo H (1942d) A comparative study of seven species of Zygoptera from Japan. Acta medica Nagasakiensi 3(2): 95–97.

Kichijo H (1942e) On the chromosomes of some species of the zygopterous dragonflies (Odonata, Zygoptera). Japanese Journal of Genetics 18: 273–276. https://doi.org/10.1266/jjg.18.273

Kumari U, Gautam DC (2017) Karyotypic studies on two species of *Orthetrum* (Anisoptera: Odonata) from Himachal Pradesh. The Journal of Cytology and Genetics 18: 1–7.

Kuznetsova V, Grozeva S, Gokhman V (2020a) Telomere structure in insects: A review. Journal of Zoological Systematics and Evolutionary Research 58: 127–158. https://doi.org/10.1111/jzs.12332

Kuznetsova VG, Maryańska-Nadachowska A, Shapoval NA, Anokhin BA, Shapoval AP (2018) Cytogenetic characterization of eight Odonata species originating from the Curonian Spit (the Baltic Sea, Russia) using C-banding and FISH with 18S rDNA and telomeric (TTAGG)n n probes. Cytogenetic and Genome Research 153: 147–157. https://doi.org/10.1159/000486088

Kuznetsova VG, Maryańska-Nadachowska A, Anokhin BA, Shapoval NA, Shapoval AP (2020b) Chromosomal analysis of eight species of dragonflies (Anisoptera) and damselflies (Zygoptera) using conventional cytogenetics and FISH: insights into the karyotype evolution of the ancient insect order Odonata. Journal of Zoological Systematics and Evolutionary Research 58, 00: 1–13. https://doi.org/10.1111/jzs.12429 [in press]

Lefevre G, McGill C (1908) The chromosomes of *Anasa tristis* and *Anax junius*. The American Journal of Anatomy 7(4): 469–487. https://doi.org/10.1002/aja.1000070404

Makalowskaja WN (1940) Comparative karyological studies of dragonflies (Odonata). Archives russes d'Anatomie, d'Histologie et d'Embryologie 25: 24–39.

Makino S (1935) A comparative study of the chromosomes in the Indian dragonflies. Japanese Journal of Genetics 11: 234–235. https://doi.org/10.1266/jjg.11.234

McGill C (1904) The spermatogenesis of *Anax junius*. University of Missouri Studies 2: 236–250.

McGill C (1907) The behavior of the nucleoli during oogenesis of the dragonfly with special reference to synapsis. Zoologische Jahrbücher. Abteilung für Anatomie und Ontogenie der Tiere 23: 207–230.

Mola LM (1995) Post-reductional meiosis in *Aeshna* (Aeshnidae, Odonata). Hereditas 122: 47–55. https://doi.org/10.1111/j.1601-5223.1995.00047.x

Mola LM (1996) Meiotic studies in nine species of *Erythrodiplax* (Libellulidae, Odonata). Neo-XY sex chromosome system in *Erythrodiplax media.* Cytologia 61: 349–357. https://doi.org/10.1508/cytologia.61.349

Mola LM (2007) Cytogenetics of American Odonata. In: Tyagi BK (Ed.) Odonata: Biology of Dragonflies. Scientific Publishers, India, 153–173.

Mola LM, Agopian SS (1985) Observations on the chromosomes of four South American Libellulidae (Anisoptera). Odonatologica 14(2): 115–125.

Mola LM, Papeschi AG (1994) Karyotype evolution in *Aeshna* (Aeshnidae: Odonata). Hereditas 121: 185–189. https://doi.org/10.1111/j.1601-5223.1994.00185.x

Mola LM, Papeschi AG, Carrillo ET (1999) Cytogenetics of seven species of dragonflies. Hereditas 131: 147–153. https://doi.org/10.1111/j.1601-5223.1999.00147.x

Nokkala S, Laukkanen A, Nokkala C (2002) Mitotic and meiotic chromosomes in *Somatochlora metallica* (Corduliidae, Odonata). The absence of localized centromeres and inverted meiosis. Hereditas 136: 7–12. https://doi.org/10.1034/j.1601-5223.2002.1360102.x

Oguma K (1915) A study of the chromosomes of dragonflies. Zoological Magazine 27: 241–250. [In Japanese]

Oguma K (1917) Entomology and cytology. In: Nagano K (Ed.) A Collection of Essays for Mr. Yasushi Nawa, Written in Commemoration of His Sixtieth Birthday, October 8, 1917. Gifu, 105–114.

Oguma K (1930) A comparative study of the spermatocyte chromosome in allied species of the dragonfly. Journal of Faculty of Sciences, Hokkaido University VI: 1–32.

Oguma K (1942) Observationes de formis compositionibusque chromosomatum et dispositionibus eorum in tempore divisionis atque propositio aliquorum novorum terminorum. Japanese Journal of Genetics 18: 205–216. https://doi.org/10.1266/jjg.18.205

Oguma K (1951) The chromosomes of *Epiophlebia superstes* Selys (dragonfly). Iden-no-Sogo-Kenkyu 2: 23–26.

Oguma K, Asana JJ (1932) Additional data to our knowledge on the dragonfly chromosome with a note on the occurrence of X-Ychromosome in the ant-lion (Neuroptera). Journal of Faculty of Sciences, Hokkaido University 1(4): 133–142.

Oksala T (1939a) Über Tetraploidie der Binde- und Fettgewebe bei den Odonaten. Hereditas 25: 132–144. https://doi.org/10.1111/j.1601-5223.1939.tb02690.x

Oksala T (1939b) Über die somatische Polyploidie bei Insekten. Annales Entomologici Fennici 5(3): 208–218.

Oksala T (1943) Zytologische Studien an Odonaten I. Chromosomenverhältnisse bei der Gattung *Aeschna* mit besonderer Berücksichtigung der postreduktionellen Teilung der Bivalente. Annales Academiae Scientiarum Fennicae (A) IV, Biologica (4): 1–64.

Oksala T (1944) Zytologische Studien an Odonaten. II. Die Enstehung der Meiotischen Präkozität. Annales Academiae Scientiarum Fennicae (A) IV, Biologica (5): 1–33.

Oksala T (1945) Zytologische Studien an Odonaten. III. Die Ovogenese. Annales Academiae Scientiarum Fennicae (A) IV, Biologica (9): 1–132.

Oksala T (1952) Chiasma formation and chiasma interference in the Odonata. Hereditas 38: 449–480. https://doi.org/10.1111/j.1601-5223.1952.tb02937.x

Omura T (1949) On at-random connection of chromosomes in the aeschnid dragonfly, *Ictinus rapax*. Japanese Journal of Genetics 24: 162–165. https://doi.org/10.1266/jjg.24.162

Omura T (1952) The spermatogenesis of an Indian dragonfly, *Ictinus rapax* (Rambur) with special reference to the behaviour of the spermatozoa in the cyst. Biological Journal of Okayama University 1(1–2): 103–146.

Omura T (1953) On the abnormal spermatogenesis in an Indian dragonfly, *Ictinus rapax* (Rambur). Biological Journal of Okayama University 1(3): 163–170.

Omura T (1955) A comparative study of the spermatogenesis in the Japanese dragonflies. I. Family Libellulidae. Biological Journal of Okayama University 2(2–3): 95–135.

Omura T (1957) A comparative study of the spermatogenesis in the Japanese dragonfly II: Family Aeschnidae, Gomphidae and Calopterygidae. Biological Journal of Okayama University 3: 1–86.

Perepelov EA (2003) Karyotype evolution of Odonata (Insecta) of Northern Palearctics. Ph.D. Dissertation, Novosibirsk, Russian Federation: Institute of Systematics and Ecology of Animals of Siberian Branch of Russian Academy of Sciences, 144 pp. [In Russian] https://www.dissercat.com/ content/evolyutsiya-kariotipov-strekoz-insecta-odonata-severnoi-palearktiki

Perepelov E, Bugrov AG (2001a) C-heterochromatin in chromosomes of *Ophiogomphus Cecilia cecilia* (Four.) (Anisoptera: Gomphidae) with notes on the sex chromosome origin in the species. Caryologia 54(2): 169–172. https://doi.org/10.1080/00087114.2001.10589224

Perepelov E, Bugrov AG (2001b) The constituent geterochromatin in karyotypes of dragonflies. Belyshevia 1(1): 10–13. [In Russian]

Perepelov E, Bugrov AG (2002) Constitutive heterochromatin in chromosomes of some Aeshnidae, with notes on the formation of the neo-XY/neo-XX mode of sex determination in *Aeshna* (Anisoptera). Odonatologica 31(1): 77–83.

Perepelov EA, Bugrov AG, Warchałowska-Śliwa E (1998) C banded karyotypes of some dragonfly species from Russia. Folia biologica (Kraków) 46: 137–142.

Perepelov EA, Bugrov AG, Warchalowska-Sliwa E (2001) C-banded karyotypes of some dragonfly species from Russia. II. The families Cordulegasteridae, Corduliidae and Gomphidae. Folia biologica (Kraków) 49(3–4): 175–178.

Prasad K, Thomas KI (1992) C-band pattern homogeneity in dragonflies (Odonata). Caryologia 45: 57–68. https://doi.org/10.1080/00087114.1992.10797211

Ray Chaudhuri SP, Dasgupta J (1949) Cytological studies on the Indian dragonflies I. Structure and behaviour of chromosomes in six species of dragonflies (Odonata). Proceedings of the Zoological Society of Bengal 2: 81–93.

Rehn AC (2003) Phylogenetic analysis of higher-level relationships of Odonata. Systematic Entomology 28: 181–240. https://doi.org/10.1046/j.1365-3113.2003.00210.x

Sandhu R, Malhotra I (1994a) Karyological studies of four aeshnid dragonflies from the states of Jammu and Kashmir and Himachal Pradesh (India). In: Srivastava VK (Ed.) Advances in Oriental Odonatology: Proceedings of IV South Asian Symposium of Odonatology, Allahabad, India October 10–12, 1992, Cherry publications, Allahabad, 111–115.

Sandhu R, Malhotra I (1994b) New chromosome count in male dragonfly, *Anatogaster s. basalis*. Bionature 14: 69–70.

Sandhu R, Walia GK (1995) A note on the karyotype of *Potamarcha congener* (Anisoptera: Libellulidae). Chromosome Information Service 58: 24–25.

Sangal SK, Tyagi BK (1982) The spermatocyte chromosomes of *Anax immaculifrons* Rambur from India (Anisoptera: Aeshnidae). Notulae odonatologicae 1(9): 154–155.

Schorr M, Paulson D (2020) World Odonata List. https://www.pugetsound.edu/academics/academic-resources/slater-museum/biodiversity-resources/dragonflies/world-odonata-list2/

Seshachar BR, Bagga S (1962) Chromosome number and sex-determining mechanism in dragonfly *Hemianax ephippiger* (Burmeister). Cytologia 27: 443–449. https://doi.org/10.1508/cytologia.27.443

Seshachar BR, Bagga S (1963) A cytochemical study of oogenesis in the dragonfly *Pantala flavescens* (Fabricius). Growth 27: 225–246.

Sharma OP, Durani S (1995) A study on the chromosomes of three species of dragonflies (Odonata: Anisoptera). National Academy Science Letters 18(5–6): 97.

Smith EA (1916) Spermatogensis of the dragonfly *Sympetrum semicinctum* with remarks upon *Libellula basalis*. Biological Bulletin 31: 269–290. https://doi.org/10.2307/1536236

Souza Bueno AM (1982) Estudos cromossomicos na ordem Odonata. M. Sc. Thesis, Universidad Estatal Paulista, 140 pp.

Srivastava MDL, Das CC (1953) Heteropycnosis in the autosome segments of *Ceriagrion coromandelianum* (Odonata). Nature 172: 765–766. https://doi.org/10.1038/172765b0

Suzuki KJ, Saitoh K (1990) A revised chromosome study of Japanese Odonates (I). Chromosomes of 14 species belonging to nine families. The Science Reports of the Hirosaki University 37: 38–49.

Suzuki KJ, Saitoh K, Sawano J (1991) Male germ-line chromosomes of *Orthetrum poecilops miyajimaensis* Yuki et Doi, 1938 (Libellulidae: Odonata). Tombo 34: 29–30.

Toyoshima H, Hirai H (1953) Studies on chromosomes of four dragonflies from Kagawa Prefecture. Kagawa Biology 1: 17–19. [In Japanese]

Thomas KI, Prasad R (1981) The chromosomes of five Indian dragonflies (Odonata). Perspectives in Cytology and Genetics 3: 629–632.

Thomas KI, Prasad R (1986) A study of the germinal chromosomes and C-band patterns in four Indian dragonflies (Odonata). Perspectives in Cytology and Genetics 5: 125–131.

Tyagi BK (1977) A note on the karyotypes of *Burmagomphus pytamidalis* Laidlow and *Onychogomphus saundersi duaricus* Faser (Anizoptera; Gomphidae). Odonatologica 6(4): 277–282.

Tyagi BK (1978a) The chromosome numbers and sex-determining mechanisms newly recorded in thirteen Indian dragonflies (Odonata). Chromosome Information Service, Tokyo 25: 5–7.

Tyagi BK (1978b) Studies on the chromosomes of Odonata of Dun Valley (Dehradun, India). PhD thesis, University of Garhwal, Srinagar.

Tyagi BK (1982) Cytotaxonomy of Indian dragonflies. Indian Review of Life Sciences 2: 149–161.

van Brink JM, Kiauta B (1964) Notes on chromosome behaviour in the spermatogenesis of the damselfly *Enallagma cyathigerum* (Charp.) (Odonata: Coenagrionidae). Genetica 35: 171–174. https://doi.org/10.1007/BF01804885

Walia GK (2007) Cytomorphological studies on *Gynacantha milliardi* Fraser of the family Aeschnidae (Anisoptera: Odonata). Cytologia 72(1): 57–62. https://doi.org/10.1508/cytologia.72.57

Walia GK, Chahal SS (2014) Distribution of constitutive heterochromatin and nucleolar organizer regions in two species of family Gomphidae (Odonata: Anisoptera). Nucleus 57: 223–227. https://doi.org/10.1007/s13237-014-0122-z

Walia GK, Chahal SS (2018) Cytogenetic characterization of *Macromia moorei* Selys, 1874 of family Macromiidae (Odonata: Anisoptera) from India by C-banding, silver nitrate staining and sequence specific staining. International Journal of Life Sciences Research 6(2): 64–68.

Walia GK, Chahal SS (2019) Cytogenetic report on *Cordulegaster brevistigma* and *Watanabeopetalia atkinsoni* (Odonata: Cordulegastridae, Chlorogomphidae). Odonatologica 48(1–2): 101–113.

Walia GK, Chahal SS (2020) Linear differentiation of chromosomes of *Anisogomphus bivittatus* Selys, 1854 from India (Odonata: Anisoptera: Gomphidae). International Journal of Entomology 5(2): 120–122.

Walia GK, Chahal SS, Babu R (2016) Cytogenetic report on *Gynacanthaeschna sikkima* from India (Odonata: Aeshnidae). Odonatologica 45: 87–94.

Walia GK, Chahal SS, Somal DS (2018) Chromosome observations based on C-banding, Ag-NOR and sequence-specific staining in two *Anax* species from India (Odonata: Aeshnidae). Odonatologica 47(1–2) 2018: 145–160.

Walia GK, Devi M (2018) Distribution of constitutive heterochromatin in four species of genus *Copera* of family Platycnemididae (Odonata: Zygoptera) from India. International Journal of Life Sciences 6(2): 457–461.

Walia GK, Devi M (2020a) Cytogenetic characterization of five species of genus *Coeliccia* of family Platycnemididae (Odonata: *Zygoptera*) using C-banding, silver nitrate staining and sequence specific staining. Nucleus (2020). https://doi.org/10.1007/s13237-020-00314-3

Walia GK, Devi M (2020b) Cytogenetic data of subfamily Disparoneurinae (Odonata: Zygoptera: Platycnemididae) based on localization of C-heterochromatin, AgNOR's and AT-GC regions. International Journal of Entomology Research 5(2): 70–73.

Walia GK, Katnoria N (2018) Morphological variation in the chromosome complement of *Neurobasis chinensis chinensis* of family Calopterygidae (Odonata: Zygoptera). International Journal of Life Sciences Research 6(4): 260–266.

Walia GK, Katnoria N, Gill JK (2018) Chromosomes of *Libellago lineata lineata* (Chlorocyphidae: Odonata). Indian Journal of Entomology 80(3): 737–740. https://doi.org/10.5958/0974-8172.2018.00118.9

Walia GK, Kaur H, Kaur J (2011) Karyotypic variations in the chromosome complement of *Pantala flavescens* (Fabricius) of the family Libellulidae (Anisoptera: Odonata). Cytologia 76(3): 301–307. https://doi.org/10.1508/cytologia.76.301

Walia GK, Kaur H, Kaur J (2015) Karyomorphological variations in the chromosome complement of *Orthetrum taeniolatum* of family Libellulidae (Odonata: Anisoptera). Cytologia 80(1): 95–99. https://doi.org/10.1508/cytologia.80.95

Walia GK, Sandhu R (1999) Karyotypic study of two species of family Aeschnidae (Anisoptera: Odonata). Chromosome Science 3: 45–47.

Walia GK, Sandhu R (2002) Chromosomal data on seven species of genus *Orthetrum* (Libellulidae: Anisoptera: Odonata). Bionature 22: 7–12.

Walia GK, Sandhu R, Goyal S (2006) Cytogenetical analysis of *Nepogomphus modestus* from Palampur area of Himachal Pradesh, India (Gomphidae: Anisoptera). Chromosome Science 9(3): 99–100.

Wasscher M (1985) The karyotypes of some dragonflies from Kenya and Sudan. Notulae odonatologicae 2(6): 105–106.

Wolfe LS (1953) A study of the genus *Uropetala* Selys (order Odonata) from New Zealand. Transactions and Proceedings of the Royal Society of New Zealand 80(3–4): 245–275.

Zhu H, Wu J (1986) Notes on the male germ cell karyotypes of some Odonata from the Shanxi Province, China. Notulae odonatologicae 2: 118–120.

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RESEARCH ARTICLE

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First cytogenetic information on four checkered beetles (Coleoptera, Cleridae)

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Abstract

The karyotypes of four species of Cleridae (Coleoptera): *Trichodes favarius* (Illiger, 1802), *Trichodes quadriguttatus* Adams, 1817, *Trichodes reichei* (Mulsant et Rey, 1863), and *Tilloidea transversalis* (Charpentier, 1825) were reported for the first time with this study. The chromosome numbers of these four species were determined as 2n = 18, sex chromosome system Xy_p, and all chromosomes were metacentric (the except y chromosome). Together with this study, the chromosome data of only 17 species are available in this family. It is remarkable that all of them display the same chromosome number and similar karyotypes. This may make the effect of karyotypical features important in interpreting the evolutionary process of Cleridae.

Keywords

Chromosome, Cleridae, Coleoptera, cytogenetic, *Tilloidea*, *Trichodes*

Introduction

The Cleroidea containing 16 families and including approximately 10,000 taxonomically defined species is an important superfamily of Coleoptera (Gimmel et al. 2019). After Melyridae, Cleridae is the second largest Cleroid family with almost 3700 species and 350 genera in 13 subfamilies described so far (Opitz 2010; Bulak et al. 2012; Gunter et al. 2013; Gerstmeier 2018). Cleridae are widespread in all continents (ex-

cept for the Antarctic) and has the highest diversity in the tropics (Gunter et al. 2013). Former analyses of phylogenetic and taxonomic relationships of Cleridae were especially based on morphology (Gerstmeier and Eberle 2011; Opitz 2012; Gunter et al. 2013). Therefore, these relationships were generally determined according to morphological characters with traditional classification systems. The molecular phylogeny of the family is extensively discussed in Gunter et al. (2013).

The data given by chromosomal characters may help to understand the evolutionary relationships of species or higher taxa. Karyological data from the studies in recent years present important findings of genetic structure, life cycle, ecological characteristics, evolution, taxonomy, and phylogeny of insects (Shaarawi and Angus 1991; Gokhman and Kuznetsova 2006). For those reasons, karyotypic features may be referable as a taxonomic character in solving taxonomic problems, assessing relationships, and phylogenetic classification. (Dobigny et al. 2004; Gokhman and Kuznetsova 2006; Miao and Hua 2017).

Although the Cleroidea have a large representative and wide distribution area, only 18 species (13 Cleridae, 5 Melyridae) of the superfamily have been cytogenetically studied so far. The 13 species of Cleridae in five genera (*Enoclerus* Gahan, 1910, *Priocera* Kirby, 1818, *Thanasimus* Latreille, 1806, *Trichodes* Herbst, 1792, and *Necrobia* Olivier, 1795) display monotypic chromosome number as "2n = 18", the basic sex chromosome system for Coleoptera as Xy_p, and metacentric/submetacentric morphology for all chromosomes (Smith and Virkki 1978; Schneider et al. 2007; Mendes-Neto et al. 2010).

This study was carried out to support cytogenetic data of the family Cleridae. The chromosomal first data belonging to four species, *Trichodes favarius* (Illiger, 1802), *Trichodes quadriguttatus* Adams, 1817, *Trichodes reichei* (Mulsant et Rey, 1863), and *Tilloidea transversalis* (Charpentier, 1825) were given in this study.

Material and methods

The localities of collected adult specimens are as follows: 16 *Trichodes favarius* (Illiger, 1802): Hıdırbey village of Samandağ county in Hatay province, 36°8'19"N, 35°58'49"W; 13 *T. quadriguttatus* Adams, 1817: Göksun county in Kahramanmaraş province 37°59'50"N, 36°31'50"W; 8 *T. reichei*: Sıddıklı town in Kırşehir province 39°7'55"N, 33°54'57"W and 14 *Tilloidea transversalis* (Charpentier, 1825): Kesikköprü town in Kırşehir province 38°57'39"N, 34°11'48"W (Leg: A.Y. Okutaner). The specimens were identified by Hüseyin Ozdikmen and were stored in Zoology Lab of Kırsehir Ahi Evran University.

Living beetles were transferred to the laboratory. The gonads and midguts were dissected and isolated from abdominal contents with the aid of a stereomicroscope microscope. The chromosomal preparation procedure was performed according to the method described by Rozek (1994) with partial modifications. The chromosomal preparation procedure in this study was based on the method described by Rozek (1994) with some modifications. The tissues were treated 15–30 min at room temperature with a hypotonic solution containing 1% sodium citrate and 0.005% w/v colchicine. Tissue

samples were transferred to cryotubes including 3:1 ethanol: acetic acid solution and stored in the freezer. Each treated sample was placed on a clean slide and disintegrated lightly. With the subsequent addition of the acetic acid: distilled water (1:1) solution, another slide was firmly covered over this slide. These slides were immediately frozen in liquid nitrogen and uncoupled to be stained in 4% Giemsa solution.

The chromosomes of females were obtained only from *Trichodes favarius*. Meiotic chromosome sets of all species were obtained from testis tissues. The chromosome sets fixed on the slides were photographed at 100X magnification with Olympus BX53F microscope equipped with a camera. Chromosome measurements were calculated in terms of μm using the "ImageJ" program with the "levan" plug-in. The chromosome measurements were made from different meiosis metaphase plates of each species and the ideograms were formed with the average for these measurements.

Results and discussion

The number of the diploid chromosome complement was determined as 2n = 18 and the sex chromosome system as Xy_p for each species: *Trichodes favarius*, *Trichodes quadriguttatus*, *Trichodes reichei*, and *Tilloidea transversalis*. The males of these four species display n = 8 + Xy_p meioformula. Their chromosome sets (autosomes and X chromosomes) consist of metacentric chromosomes except for subtelocentric y chromosome. Sex chromosome system (association of Xy_p) in meiosis I, and the presence of y chromosome in meiosis II were clearly demonstrated (Figs 1, 2).

The idiogram shows that the first two chromosome pairs of the species belonging to the genus *Trichodes* are larger than others and a gradual decrease in size in the karyotype of *Tilloidea transversalis* (Fig. 2).

In the previous literature, there is cytogenetic information of only 13 checkered beetles (2 subfamilies, 5 genera). Additionally, cytogenetic data of 4 different species were presented for the first time in this study. After all given data, the diploid chromosome numbers have been presented as 2n = 18 and the sex chromosome system as Xy_p of all these 17 Cleridae species. However, four species of Melyridae have observed different chromosome numbers and two different sex chromosome systems XO and Xyp, the chromosome morphologies of these four species are metacentric except for the y chromosome as similar to the Cleridae (Table 1).

Diploid chromosome number 20 and sex chromosome system Xyp are considered ancestral cytogenetic features of Coleoptera, especially the Polyphaga (Smith and Wirkki 1978). According to the limited number of previous studies, it can be said that 2n = 18 chromosome numbers formed by decreasing the ancestral chromosome set (2n = 20) and Xy_p sex chromosome system belonging to Cleridae family are quite conservative.

Although it shows variation in the family Melyridae, the numerical changes of chromosomes may not have an important role in the karyotypic evolution of the family Cleridae. Except for the Y chromosome, the metacentric/submetacentric form of all chromosomes may have created a balance for the karyotype of the species. The

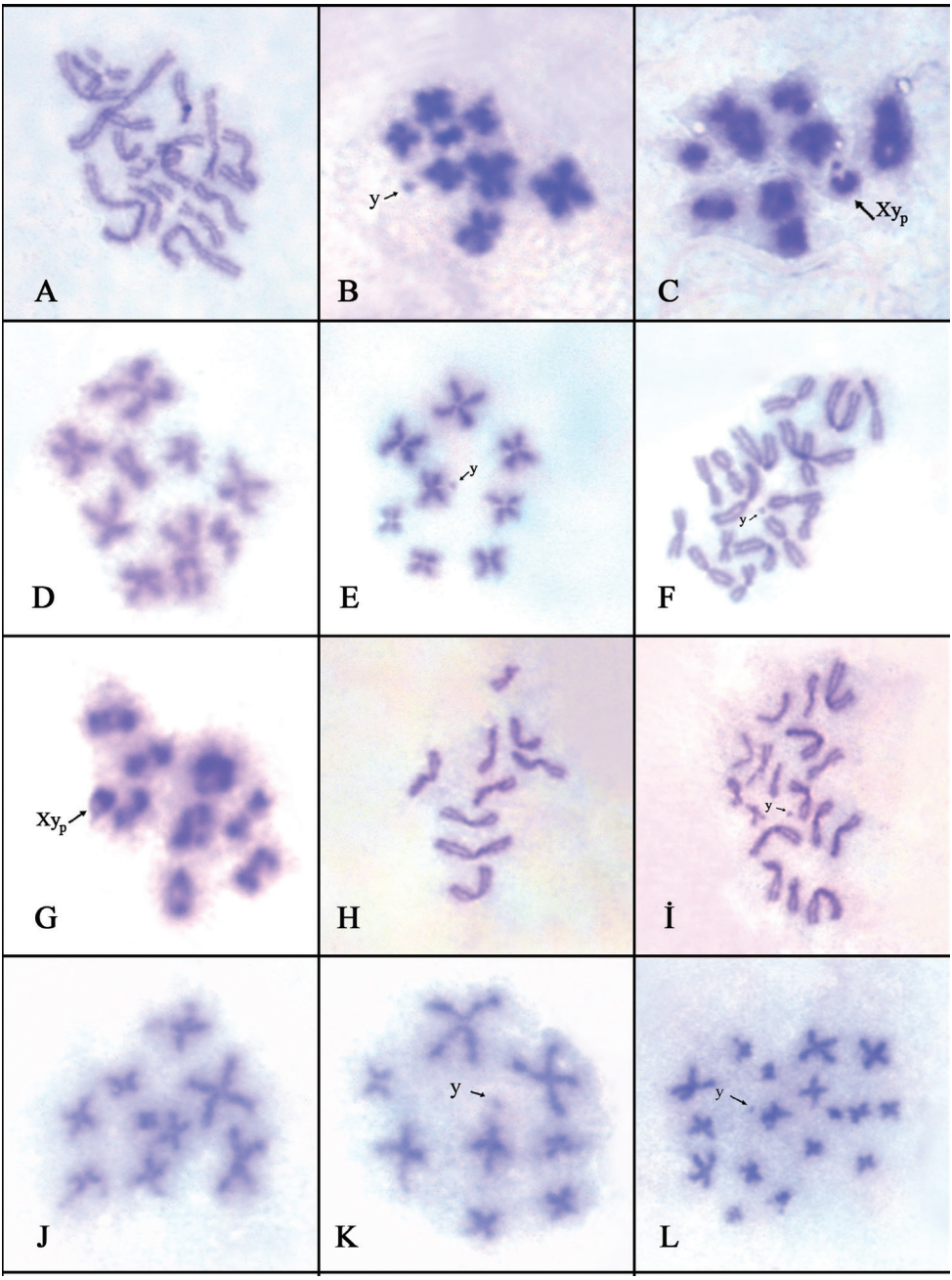

Figure 1. A Female Mitotic metaphase of *Trichodes favarius* **B, C** male meiotic metaphases of *Trichodes favarius* (**B** meiosis II; **C** meiosis I) **D, E** male meiotic metaphases of *Trichodes quadriguttatus* (**D, E** meiosis II) **F** male mitotic metaphase of *Trichodes quadriguttatus* **G, H** male meiotic metaphases of *Trichodes reichei* (**G** meiosis I; **H** meiosis II) **I** male mitotic metaphase of *Trichodes reichei* **J, K** male meiotic metaphases of *Tilloidea transversalis* (**J, K** meiosis II) **L** male mitotic metaphase of *Tilloidea transversalis*.

Table 1. The chromosome data of the Cleridae and Melyridae.

Taxa	Haploid Formula	Diploid Number/Formula	Citations
Cleridae			
Thanasimus dubius (Fabricius, 1777) (Clerinae)	$8+Xy_p$		Smith (1950)
Trichodes nutalli (Kirby, 1818) (Clerinae)	$8+Xy_p$		Smith (1953)
Enoclerus nigripes rujiventris (Spinola, 1844) (Clerinae)	$8+Xy_p$	18	
Enoclerus sp. (Clerinae)	$8+Xy_p$	18	Smith (1960)
Trichodes ornatus (Linsley et MacSwain, 1943) (Clerinae)	$8+Xy_p$	18	
Thanasimus formicarius (Linnaeus, 1758) (Clerinae)	$8+Xy_p$	18	Virkki (1960)
Trichodes apiarius (Linnaeus, 1758) (Clerinae)	$8+Xy_p$	18	
Enoclerus sp. (Clerinae)	$8+Xy_p$		Virkki (1963)
Priocera spinosa (Fabricius, 1801) (Clerinae)	$8+Xy_p$		
Enoclerus moestus (Klug, 1842) (Clerinae)	$8+Xy_p$	18	Smith and Virkki (1978)
Thanasimus undatulus (Say, 1835) (Clerinae)	$8+Xy_p$		
Necrobia ruficollis (Fabricius, 1775) (Corynetinae)	$8+Xy_p$	18	Yadav and Dange (1989)
Necrobia rujipes (De Geer, 1775) (Corynetinae)	$8+Xy_p$	18	
Trichodes favarius (Illiger, 1802) (Clerinae)	$8+Xy_p$	18	This Study
Trichodes quadriguttatus Adams, 1817 (Clerinae)	$8+Xy_p$	18	
Trichodes reichei (Mulsant et Rey, 1863) (Clerinae)	$8+Xy_p$	18	
Tilloidea transversalis (Charpentier, 1825) (Tillinae)	$8+Xy_p$	18	
Melyridae			
Endeodes collaris LeConte, 1853 (Malachiinae)		18+X0	Smith and Virkki (1978)
Collops sp. (Malachiinae)		16+X0	
Hoppingiana hudsonica LeConte 1866 (Dasytinae)		$12+Xy_p$	
Astylus variegatus (Germar, 1824) (Melyrinae)		$16+Xy_p$	Schneider at all (2007)
Astylus antis (Perty, 1830) (Melyrinae)	$8+X_{p\,or}\,y_p$	$16+Xy_p$	de Oliveira Mendes-Neto et al. (2010)

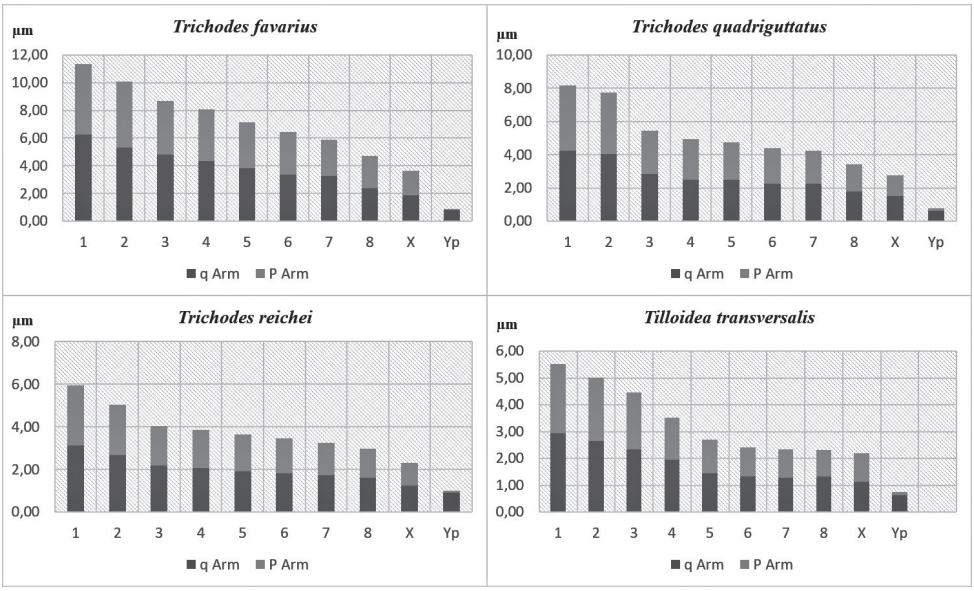

Figure 2. Ideograms of the haploid chromosomes.

absence of acrocentric and telocentric chromosomes can reduce the possibility of new centric fusions such as Robertsonian Translocation (Schubert 2007; Chmátal et al. 2014). On the other hand, being resistant to mechanism of chromosome aberration such as chromosome breaks and euploidy may also have created chromosome number stability in the evolutionary process of the family.

In all these respects, the stability of the chromosome set of the family Cleridae is quite remarkable. If these results can be supported by expanding further studies, the cytogenetic features of Cleridae would be very useful taxonomic and evolutionary characters.

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References

Bulak Y, Yıldırım E, Gerstmeier R (2012) Contribution to the knowledge of the Cleridae (Coleoptera) fauna of Turkey. Entomofauna 33(23): 325–332.

Chmátal L, Gabriel SI, Mitsainas GP, Martínez-Vargas J, Ventura J, Searle JB, Schultz RM, Lampson MA (2014) Centromere strength provides the cell biological basis for meiotic drive and karyotype evolution in mice. Current biology 24(19): 2295–2300. https://doi.org/10.1016/j.cub.2014.08.017

de Oliveira Mendes-Neto E, Vicari MR, Campaner C, Nogaroto V, Artoni RF, Almeida MC (2010) Cytogenetic analysis of Astylus antis (Perty, 1830) (Coleoptera, Melyridae): Karyotype, heterochromatin and location of ribosomal genes. Genetics and Molecular Biology 33(2): 237–243. https://doi.org/10.1590/S1415-47572010005000050

Dobigny G, Ducroz JF, Robinson TJ, Volobouev V (2004) Cytogenetics and cladistics. Systematic Biology 53(3): 470–484. https://doi.org/10.1080/10635150490445698

Gerstmeier R, Eberle J (2011) Definition and Revision of the Orthrius-group of genera (Coleoptera, Cleridae, Clerinae). ZooKeys 92: 35–60. https://doi.org/10.3897/zookeys.92.1157

Gerstmeier R (2018) Checklist of the checkered beetles of Namibia (Coleoptera, Cleridae). Namibian Journal of Environment 2: 7–21.

Gimmel ML, Bocakova M, Gunter NL, Leschen RAB (2019) Comprehensive phylogeny of the Cleroidea (Coleoptera: Cucujiformia). Systematic Entomology 44: 527–558. https://doi.org/10.1111/syen.12338

Gokhman VE, Kuznetsova VG (2006) Comparative insect karyology: current state and applications. Entomological Review 86(3): 352–368. https://doi.org/10.1134/S0013873806030110

Gunter NL, Leavengood JM, Bartlett JS, Erıc G, Chapman EG, Cameron S (2013) A molecular phylogeny of the checkered beetles and a description of Epiclininae a new subfamily (Coleoptera: Cleroidea: Cleridae). Systematic Entomology 38: 626–636. https://doi.org/10.1111/syen.12019

Miao Y, Hua BZ (2017) Cytogenetic comparison between *Terrobittacus implicatus* and *Bittacus planus* (Mecoptera: Bittacidae) with some phylogenetic implications. Arthropod Systematics & Phylogeny 75(2): 175–183. Opitz W (2012) Classification, Natural History, and Evolution of Tarsosteninae (Coleoptera: Cleridae) Part I: Generic Composition of the Subfamily and Key and Phylogeny of Genera. Psyche 2012: 1–35. https://doi.org/10.1155/2012/752402

Rozek M (1994) A new Chromosome preparation technique for Coleoptera (Insecta). Chromosome Research 2(1): 76–78. https://doi.org/10.1007/BF01539458

Schneider MC, Carraro BP, Cella DM, Matiello RR, Artoni RF, Almeida MC (2007) *Astylus variegatus* (Coleoptera, Melyridae): Cytogenetic study of a Population exposed to agrochemical products. Genetics and Molecular Biology 30(3): 640–645. https://doi.org/10.1590/S1415-47572007000400023

Schubert I (2007) Chromosome evolution. Current Opinion in Plant Biology 10: 109–115. https://doi.org/10.1016/j.pbi.2007.01.001

Shaarawi FA, Angus RB (1990) A chromosomal investigation of five European species of Anacaena Thomson (Coleoptera: Hydrophilidae). Insect Systematic and Evolution 21(4): 415–426. https://doi.org/10.1163/187631290X00319

Smith SG (1950) The cytotaxonomy of Coleoptera. The Canadian Entomologist 82: 58–68. https://doi.org/10.4039/Ent8258-3

Smith SG (1953) Chromosome numbers of Coleoptera. Heredity 7: 31–48. https://doi.org/10.1038/hdy.1953.3

Smith SG (1960) Chromosome numbers of Coleoptera. Canadian Journal of Genetics and Cytology 2: 66–88. https://doi.org/10.1139/g60-007

Smith SG, Virkki N (1978) Animal Cytogenetics. V. 3, Insecta 5. Coleoptera. Gebrüder Borntraeger, Berlin, 366 pp.

Virkki N (1960) Cytology of male meiosis in certain European forest beetles of the families Scolytidae, Cleridae, and Anobiidae. Annales Academiae Scientiarum Fennicae / A / 4B 49: 1–18.

Virkki N (1963) On the cytology of some Neotropical Cantharoids (Coleoptera). Academiae Scientiarum Fennicae / A / 4B 65: 1–17.

Yadav JS, Dance MP (1989) On the cytology of two species of *Necrobia* (Oliv.) (Coleoptera: Cleridae). Genome 32: 165–167. https://doi.org/10.1139/g89-424

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RESEARCH ARTICLE

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Karyotypes diversity in some Iranian Pamphagidae grasshoppers (Orthoptera, Acridoidea, Pamphagidae): new insights on the evolution of the neo-XY sex chromosomes

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Abstract

For the first time, cytogenetic features of grasshoppers from Iran have been studied. In this paper we conducted a comparative cytogenetic analysis of six species from the family Pamphagidae. The species studied belong to subfamilies Thrinchinae Stål, 1876 (*Eremopeza bicoloripes* (Moritz, 1928), *E. saussurei* (Uvarov, 1918)) and Pamphaginae (*Saxetania paramonovi* (Dirsh, 1927), *Tropidauchen escalerai* Bolívar, 1912, *Tropidauchen* sp., and *Paranothrotes citimus* Mistshenko, 1951). We report information about the chromosome number and morphology, C-banding patterns, and localization of ribosomal DNA clusters and telomeric $(TTAGG)_n$ repeats. Among these species, only *S. paramonovi* had an ancestral Pamphagidae karyotype (2n=18+X0♂; FN=19♂). The karyotypes of the remaining species differed from the ancestral karyotypes. The karyotypes of *E. bicoloripes* and *E. saussurei*, despite having the same chromosome number (2n=18+X0♂) had certain biarmed chromosomes (FN=20♂ and FN=34♂ respectively). The karyotypes of *T. escalerai* and *Tropidauchen* sp. consisted of eight pairs of acrocentric autosomes, one submetacentric neo-X chromosome and one acrocentric neo-Y chromosome in males (2n=16+neo-X neo-Y♂). The karyotype of *P. citimus* consisted of seven pairs of acrocentric autosomes, submetacentric the neo-X_1 and neo-Y and acrocentric the neo-X_2 chromosomes (2n=14+neo-X_1 neo-X_2 neo-Y♂). Comparative analysis of the localization and size of C-positive regions, the position of ribosomal clusters and the telomeric DNA

motif in the chromosomes of the species studied, revealed early unknown features of their karyotype evolution. The data obtained has allowed us to hypothesize that the origin and early phase of evolution of the neo-Xneo-Y♂ sex chromosome in the subfamily Pamphaginae, are linked to the Iranian highlands.

Keywords
C-banding, FISH, karyotypes, neo-sex chromosomes, Pamphagidae grasshoppers, ribosomal DNA repeats, telomeric repeat $(TTAGG)_n$

Introduction

Among Pamphagidae grasshoppers, over 300 species inhabit the desert, semidesert and mountainous landscapes of the Palaearctic Region. All of them belong to the subfamilies Thrinchinae and Pamphaginae (Uvarov 1966; Massa 2013; Ünal 2016). Until recently, the Pamphagidae grasshoppers did not attract the attention of cytogenetic researchers. Poor cytogenetic studies of Pamphagidae were associated not only with the low density of their populations, but also with the uniformity of their karyotypes. White (1973) reported a conservative karyotype consisting of 19 acrocentric chromosomes in males and 20 in females with X0♂/XX♀ sex chromosome system. This was confirmed by further studies in Pamphagidae species from Europe, South Africa and China (Hewitt 1979; Camacho et al. 1981; Santos et al. 1983; Cabrero et al. 1985; Fossey 1985; Fu Peng et al. 1989; Mansueto and Vitturi 1989; Vitturi et al. 1993; Warchałowska-Śliwa et al. 1994). Pamphagidae species with the neo-X neo-Y/neo-X neo-X sex chromosome system from Central Asia (Bugrov 1986) has drawn our attention to this family. Cytogenetic information concerning species of *Asiotmethis* Uvarov, 1943 and *Glyphotmethis* Bey-Bienko, 1951 genera (Thrinchinae) and representatives of Nocarodeini tribe (Pamphaginae) from Central Asia, the Caucasus and Transcaucasia, Bulgaria and Turkey have shown variation of sex chromosome systems (Bugrov 1986, 1996; Bugrov and Warchałowska-Śliwa 1997; Bugrov and Grozeva 1998; Bugrov et al. 2016; Jetybayev et al. 2017a). Those variations modified the organization of standard karyotypes, with species showing eight pairs of acrocentric autosomes, one metacentric neo-X chromosome and acrocentric neo-Y chromosome in males (2n♂=18; 16+neo-Xneo-Y) and two metacentric neo-X chromosomes in females (2n♂=18; 16+neo-X neo-X). This karyotype originated from an ancestral Pamphagidae chromosome set, as a result of a Robertsonian translocation of a large acrocentric autosome and acrocentric X chromosome (Bugrov 1986, 1996; Bugrov and Warchałowska-Śliwa 1997; Bugrov and Grozeva 1998; Bugrov et al. 2016).

Moreover, the neo-Y chromosomes found in previously studied Thrinchinae (*Asiotmethis* and *Glyphotmethis* genera) and Pamphaginae (Nocarodeini tribe) species varies in size and content of constitutive heterochromatin. In the karyotypes of some *Glyphotmethis* and *Asiotmethis* species, the neo-Y chromosome is similar in size to its homologous XR-arm of the neo-X chromosome. But unlike the XR-arm of the neo-X

chromosome the neo-Y chromosome showed two small interstitial C-bands near the pericentromeric region. In the karyotypes of all Nocarodeini species, the neo-Y chromosome is smaller than the XR-arm of the neo-X chromosome. But unlike the XR-arm of the neo-X chromosome the neo-Y chromosome showed a large pericentromeric C-band and two or three large subproximal interstitial C-bands located close to each other (Bugrov and Grozeva 1998; Bugrov et al. 2016; Jetybayev et al. 2017b). Based on these results it was suggested that neo-Y chromosomes arose independently in two different evolutionary lineages (Thrinchinae and Pamphaginae) and underwent a significant degradation process in Nocarodeini (Jetybayev et al. 2017b). Further evolution of the neo sex chromosomes in the Nocarodeini tribe is associated with the origination of the neo-X_1X_2Y♂/neo-$X_1X_1X_2X_2$♀ sex chromosome system. Such neo-sex chromosome system was observed in *Paranothrotes opacus* (Brunner von Wattenwyl, 1882) as a result of a Robertsonian translocation of the neo-Y chromosome with an autosome (Bugrov et al. 2016).

Analysis of the geographical distribution of Pamphagidae species with neo-sex chromosomes allowed the assumption that the origin of this type of sex chromosome system may occur in the Western Asian region (Jetybayev et al. 2017a). To test this hypothesis, we acquired data on karyotypes of previously unstudied Pamphagidae species from Iran (Fars, Khorosan-e Razavi and Qazvin provinces) (Table 1). Iran is one of the main centres of species diversity of Pamphagidae grasshoppers. Currently near 110 species from 21 genera of Pamphagidae, belonging to the Thrinchinae and Pamphaginae subfamilies, originate from this area (Mistshenko 1951; Shumakov 1963; Mirzayans 1998; Hodjat 2012; Ünal 2016). The diversity of Iranian Pamphagidae is most significant in the Palearctic Region compared with Europe (52 species), North Africa (101 species), Asia Minor (66 species) and Central Asia (almost 78 species) (Bey-Bienko and Mistshenko 1951; Shumakov 1963; Sergeev 1995; Massa 2013; Ünal 2016).

The present study reports the results of our comparative analysis of the karyotypes, C-banding patterns, distribution of clusters of telomeric $(TTAGG)_n$ repeats and ribosomal DNA (rDNA) in the chromosomes of the species studied. We hope that this study will provide the motivation for further cytogenetic study of Iranian grasshoppers.

Material and methods

Material collection

Males of the *Eremopeza saussurei* (Uvarov, 1918), *E. bicoloripes* (Moritz, 1928) belonging to the Thrinchinae, and *Saxetania paramonovi* (Dirsh, 1927), *Tropidauchen escalerai* Bolívar, 1912, *Tropidauchen* sp. (Tropidauchenini), *Paranothrotes citimus* Mistshenko, 1951 (Nocarodeini) from the subfamily Pamphaginae were collected in the early summer season (1st to 12th June, 2018) in mountain and semidesert landscapes in Iran (Table 1).

Table 1. List of species, collection locations and number of specimens of the studied Pamphagidae species.

Taxa	Species	Location	Number of males
Thrinchinae Thrinchini	*Eremopeza saussurei* (Uvarov, 1918)	Iran, Fars Prov., Zagros Range, 1433 m. asl. 29°25'54.9"N, 052°46'20.0"E	7
	Eremopeza bicoloripes (Moritz, 1928)	Iran, Khorosan-e Razavi Prov., 60 km, N. of Mashhad, Ferizi vil. vicinities, ~1800 m. asl.	5
Pamphaginae Nocarodeini	*Paranothrotes citimus* Mistshenko, 1951	Iran, Qazvin Prov., Alborz Range, Qazvin town vicinities, 2380 m. asl. 36°7'29.0"N, 50°40' 25"E	1
Pamphaginae Tropidauchenini	*Saxetania paramonovi* (Dirsh, 1927)	Iran, Khorosan-e Razavi Prov., 60 km, N. of Mashhad, Ferizi vil. vicinities, ~1800 m. asl.	10
	Tropidauchen escalerai Bolívar, 1912	Iran, Fars Prov., Zagros Range, Estahban, Runiz town vicinities, 1800 m. asl.	1
	Tropidauchen sp.	Iran, Fars Prov., Zagros Range, 2800 – 3200 m. asl. 30°23'10.1"N, 51°55'35.2"E	7

Methods

Fixation, chromosome preparations, C-banding and fluorescence *in situ* hybridization (FISH)

The 0.1% colchicine solution was injected into the abdomens of collected males. After 1.5–2 hours, their testes were dissected and placed into a 0.9% solution of sodium citrate for 20 minutes. Then the testes were fixed in 3:1 (ethanol : glacial acetic acid) for 15 minutes. Thereafter, fixed testes were stored in 70% ethanol in a refrigerator at 4 °C until used. Air-dried chromosome preparations were made by squashing testis follicles in 45% acetic acid and subsequently freezing them in dry ice.

The constitutive heterochromatin was identified by C-banding, using the technique described by Sumner (1972) with minor modifications. Slides were treated with 0.2 N HCl for 15–20 minutes at room temperature then incubated in a saturated solution of $Ba(OH)_2$ at 61°C for three to five minutes, rinsed in tap water and incubated in 2×SSC at 61 °C for 60 minutes. After washing in distilled water, the slides were stained with 2% Giemsa solution on Sorensen's phosphate buffer for 30 to 60 minutes.

Fluorescence *in situ* hybridization (FISH) with telomeric $(TTAGG)_n$ DNA probes and rDNA genes on meiotic chromosomes was carried out according to the protocol by Pinkel (1986) with modifications as described in previous studies (Rubtsov et al. 2000). Telomeric repeats $(TTAGG)_n$ were generated by the non-template PCR method with 5'-TAACCTAACCTAACCTAACC-3' and 5'-TTAGGTTAGGTTAGGTTAGG-3' primers. Further labelling with Tamra-dUTP (Biosan, Novosibirsk, Russia) was performed in 33 additional cycles of PCR as described previously (Sahara et al. 1999). The ribosomal DNA probe was obtained as previously described (Buleu et al. 2017; Jetybayev et al. 2017a). An unlabelled rDNA probe was generated by the polymerase chain reaction (PCR) according to Jetybayev (2017a). The fragments of the 18S rDNA and 28S rDNA genes were labelled in additional PCR cycles with Fluorescein-12-dUTP (Biosan, Novosibirsk, Russia) and mixed together into a single rDNA probe. For the description of karyotype structure, location and size of C-positive regions in chromosomes, the nomenclature previously proposed for Pamphagidae grasshoppers was used (King and John 1980; Santos et al. 1983; Cabrero and Camacho 1986). According to this nomenclature, autosomes were numbered in order of decreasing size (1–9) and classified into three size

groups: L – large, M – medium and S – small. The neo-sex chromosomes were named after White (1940). The arms of the neo-X chromosome were referred to as XL and XR. The XL-arm corresponds to the original acrocentric X chromosome and the XR-arm to the translocated acrocentric autosome. The other non-translocated autosome, homologous to the XR-arm, remains acrocentric and is the neo-Y chromosome (White 1940; Hewitt 1979). In the multiple $X_1X_2Y/X_1X_1X_2X_2$ sex chromosome system, the neo-X_1 is formed by the XL- and the XR-arms of the neo-X chromosome described above. The neo-Y is biarmed. The short YL-arm corresponds to the neo-Y chromosome described above and the long YR-arm is formed by a second Robertsonian translocation with a second acrocentric autosome. The homologous of non-translocated autosome from this second pair is referred to as the neo-X_2 chromosome (White 1940; Hewitt 1979).

Microscopic analysis was performed at the Centre for Microscopy of Biological Objects of SB RAS (Novosibirsk, Russia). Chromosomes were studied with an Axio-Imager M1 (Zeiss, Germany) fluorescence microscope equipped with filter sets #49, #46HE, #43HE and a ProgRes MF (MetaSystems GmbH, Germany) CCD camera. The ISIS5 software package was used for image capture and analysis.

Results

Eremopeza bicoloripes (Moritz, 1928)

The karyotype of *E. bicoloripes* consisted of nine pairs of acrocentric autosomes and one subacrocentric X chromosome in males ($2n♂=19$; 18AA+X) (Fig. 1). The male meiotic karyotype was represented by four large (L_1–L_4), three medium (M_5–M_7) and two small (S_8–S_9) autosome bivalents and a medium-sized X univalent (Fig. 1).

Distinct pericentromeric C-bands were revealed in all chromosomes of the complement (Fig. 1). Telomeric C-bands were localized in the M_6 autosome bivalent (Fig. 1).

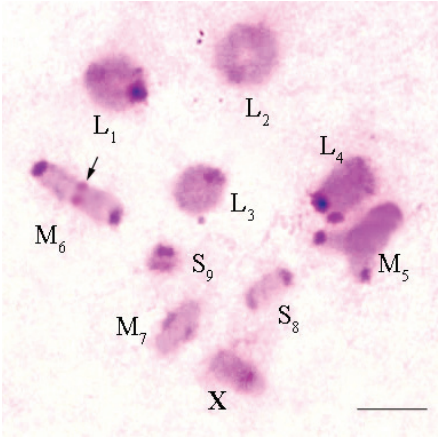

Figure 1. C-banded metaphase I of *Eremopeza bicoloripes*. Arrow indicate the telomeric C-bands in M_6 autosome bivalent. Scale bar: 10 μm.

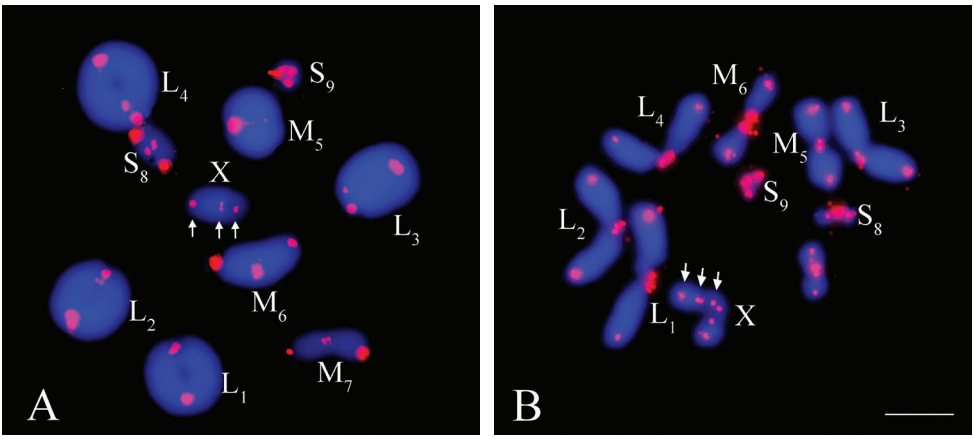

Figure 2. FISH with telomeric $(TTAGG)_n$ probe (red) in male meiotic chromosomes of *Eremopeza bicoloripes* **A** metaphase I **B** metaphase II. Chromosomes were counterstained with DAPI (blue). Arrows indicate the telomere hybridization signals at the terminal and pericentromeric regions of X chromosome

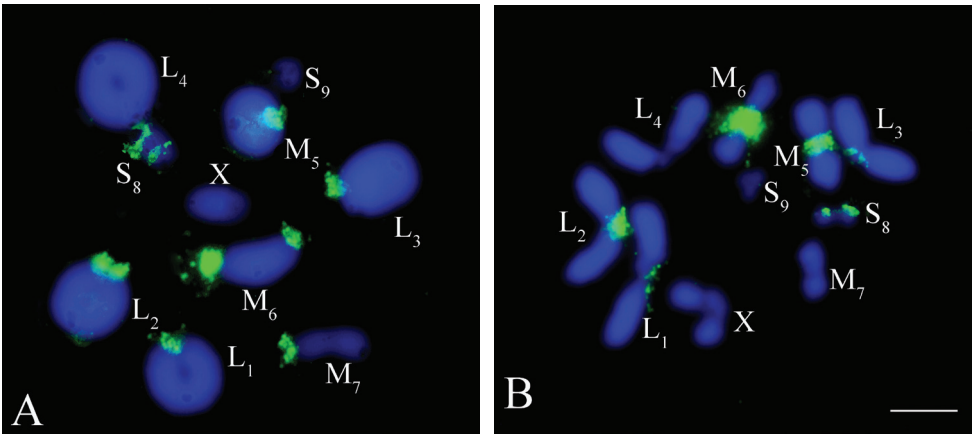

Figure 3. FISH with rDNA genes (green) in male meiotic chromosomes of *Eremopeza bicoloripes* **A** metaphase I **B** metaphase II. Chromosomes were counterstained with DAPI (blue). Scale bar: 10 μm.

Telomeric DNA repeats were hybridized at the terminal region of all chromosomes (Fig. 2). In the subacrocentric X chromosome, additional clusters of telomeric DNA were observed in the distal area of the pericentromeric region of the long arm (Fig. 2B).

The clusters of rDNA genes were located at pericentromeric regions in most autosome bivalents, except the L_4 and S_9 autosome bivalents and the X chromosome (Fig. 3). In the L_3 and the M_7 bivalents, the clusters of ribosomal DNAs were only detected in one of the homologues (Fig. 3A).

Eremopeza saussurei (Uvarov, 1918)

The karyotype of *E. saussurei* consisted of nine pairs of autosomes and the X chromosome in males ($2n♂=19$; 18AA+X). Four autosome bivalents were large (L_1–L_4),

three were medium (M_5–M_7) and two were small (S_8–S_9). The X chromosome was of medium-sized. All large autosome bivalents, the M_5, the M_7, and the X chromosome were subacrocentric. The M_6 autosome bivalent was submetacentric. Small (S_8–S_9) autosome bivalents were acrocentric (Fig. 4).

Distinct pericentromeric C-bands were revealed in all chromosomes of the complement (Fig. 4). Telomeric C-bands were revealed in long arm of the L_2 autosome bivalent (Fig. 4).

Telomeric DNA repeats were observed at terminal regions of all chromosomes (Fig. 5A). Telomeric hybridization signals were also found at pericentromeric regions

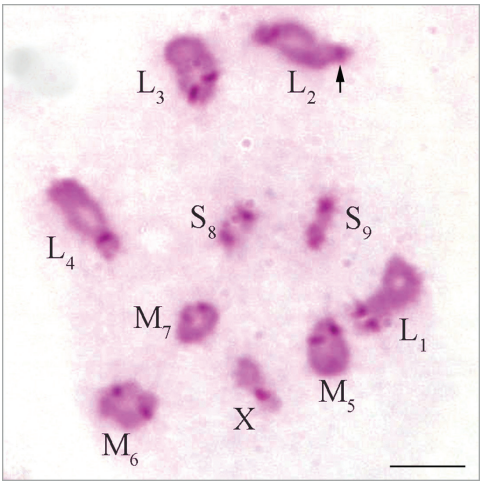

Figure 4. C-banded diakinesis of *Eremopeza saussurei*. Arrow indicates the telomeric C-band in L_2 autosome bivalent. Scale bar: 10 μm.

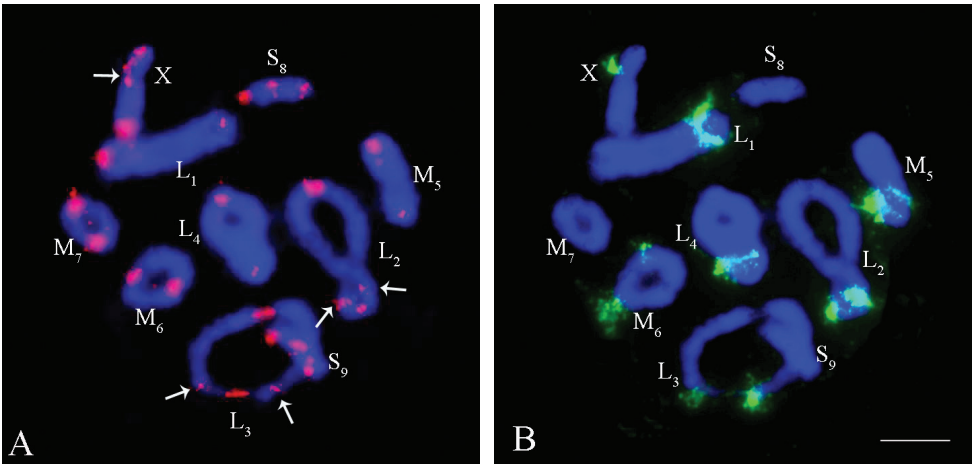

Figure 5. FISH with telomeric $(TTAGG)_n$ probe (red) (**A**) and rDNA genes (green) (**B**) in cell at diakinesis of *Eremopeza saussurei*. Arrows indicate the telomere hybridization signals at pericentromeric regions of L_2 and L_3 bivalents and X chromosome. Chromosomes were counterstained with DAPI (blue). Scale bar: 10 μm.

of two large (L_2, L_3) bivalents and the X chromosome (Fig. 5A). Clusters of rDNA genes were observed at pericentromeric regions of six autosome bivalents (L_1–L_4, M_5–M_6) and X univalent (Fig. 5B).

Saxetania paramonovi (Dirsh, 1927)

The karyotype of *S. paramonovi* consisted of nine pairs of acrocentric autosomes and an acrocentric X chromosome in males (2n♂=19; 18AA+X). The male meiotic karyotype was represented by four large (L_1–L_4), three medium (M_5–M_7) and two small (S_8–S_9) autosome bivalents. The X chromosome was of medium-sized (Fig. 6A).

Pericentromeric C-bands were revealed in all autosome bivalents and the X chromosome (Fig. 6A). The pericentromeric C-band on one of the homologues in the L_1 bivalent was noticeably larger than in the other homologue (Fig. 6A). Telomeric C-bands were observed in the M_7, S_8 and S_9 autosome bivalents (Fig. 6A).

Telomeric DNA repeats were only observed in the terminal regions of all chromosomes (Fig. 6B). The clusters of rDNA genes were detected in the pericentromeric region of the L_2 and L_4 autosome bivalents and in the proximal interstitial region of the L_3 autosome bivalent (Fig. 6B).

Tropidauchen escalerai Bolívar, 1912

The karyotype of *T. escalerai* consisted of 18 acrocentric chromosomes (2n=16+neo-Xneo-Y♂): four large (L_1–L_4), two medium (M_5, M_6) and two small sized (S_7, S_8) autosome bivalents (Fig. 7). The neo-X chromosome was metacentric (Fig. 7A, inset). The neo-Y chromosome was acrocentric. During meiosis, the XR-arm of the

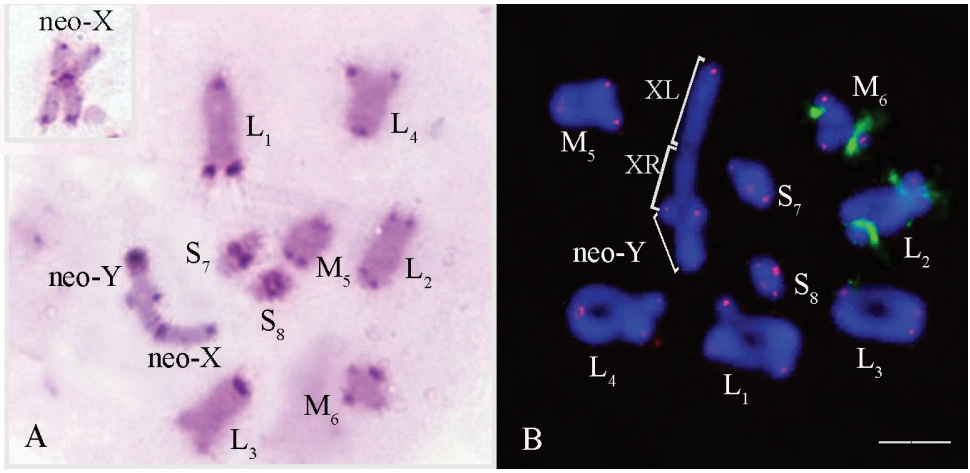

Figure 6. *Saxetania paramonovi* **A** C-banded metaphase I **B** FISH with telomeric $(TTAGG)_n$ probe (red) and rDNA genes (green) in cell at diakinesis. Chromosomes were counterstained with DAPI (blue) (**B**). Scale bar: 10 μm.

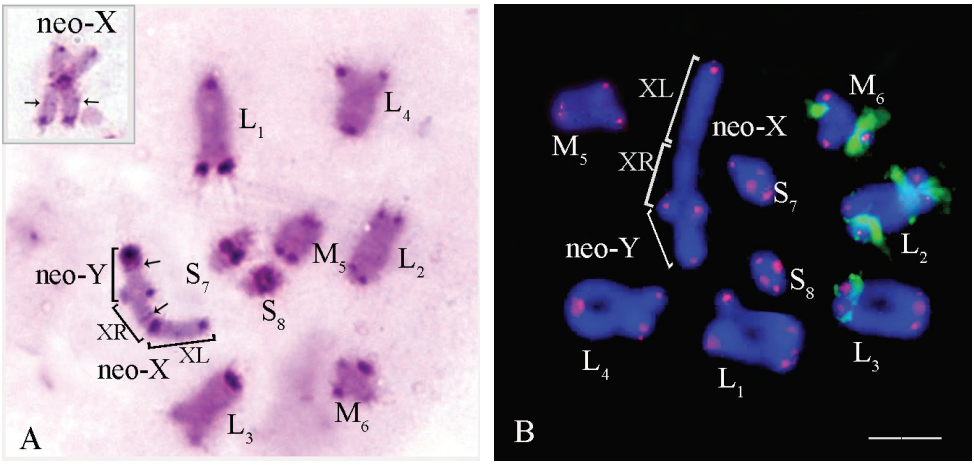

Figure 7. *Tropidauchen escalerai* **A** C-banded metaphase I. The inset in the right upper corner shows the neo-X chromosome in meiotic metaphase II. Arrows indicate the C-bands in the XR-arm of the neo-X and neo-Y chromosomes **B** FISH with telomeric $(TTAGG)_n$ probe (red) and rDNA genes (green) in cell at metaphase I. Chromosomes were counterstained with DAPI (blue) (**B**). Scale bar: 10 μm.

neo-X and neo-Y usually forms one chiasma at interstitial or subterminal positions (Fig. 7).

Distinct pericentromeric C-bands were found in all autosome bivalents and in the neo-X chromosome (Fig. 7A). The pericentromeric region of the neo-Y chromosome showed a large C-block (Fig. 7A). Tiny interstitial C-bands were observed in the proximal positions in the XR-arm of the neo-X and in the neo-Y chromosomes (Fig. 7A). Telomeric C-bands were detected in the L_1, L_2 and L_4 autosome bivalents and in both arms of the neo-X chromosome (Fig. 7A).

Telomeric DNA repeats were located only at terminal regions of all chromosomes (Fig. 7B). Clusters of rDNA genes were observed in three autosome bivalents (Fig. 7B). Two clusters of rDNA genes were observed in the L_2 autosome bivalent: the first one located in the proximal interstitial region and the second one in the distal interstitial region (Fig. 7B). In the L_3 bivalent, the rDNA cluster was localized in the distal area of the pericentromeric region. In the M_6 bivalent, the cluster of rDNA genes was observed in the interstitial position (Fig. 7B).

Tropidauchen sp.

The karyotype of the *Tropidauchen* sp. consisted of 18 chromosomes (2n=16+neo-Xneo-Y♂): three large (L_1, L_2, L_3), two medium (M_5, M_6) and two small (S_7, S_8) acrocentric autosome bivalents (Fig. 8). The L_4 autosome bivalent was subacrocentric (Fig. 8A, inset). The neo-X chromosome was metacentric (Fig. 8A). The neo-Y chromosome was acrocentric. During meiosis, the XR-arm of the neo-X and neo-Y usually forms one chiasma at interstitial or subterminal positions (Fig. 8).

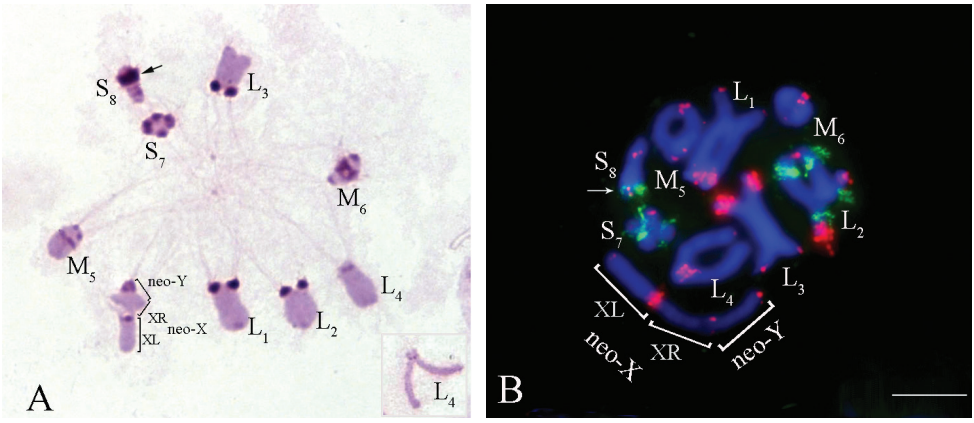

Figure 8. *Tropidauchen* sp. **A** C-banded metaphase I. The inset in the lower left corner shows the L_4 chromosome in meiotic metaphase II. Arrows indicate the interstitial C-band in the S_8 autosome bivalent; **B** FISH with rDNA (green) and telomeric $(TTAGG)_n$ (red) probes in cell at metaphase I. Arrows indicate the cluster of rDNA genes in the S_8 autosome bivalent. Chromosomes were counterstained with DAPI (blue) (**B**). Scale bar: 10 μm.

Pericentromeric C-bands were detected in all chromosomes (Fig. 8A). Interstitial C-bands were identified in the M_5 and S_8 autosome bivalents (Fig. 8A). In the S_8 autosome bivalent, one of the homologues had a huge interstitial C-band. The other homologue a thin C-band in the same position (Fig. 8A). Telomeric C-positive block was revealed in the M_6 and S_7 autosome bivalents (Fig. 8A).

Telomeric DNA repeats in *Tropidauchen* sp. were localized only at terminal regions of the all autosomes (Fig. 8B). Additional clusters of telomeric repeats were observed in the pericentromeric region of the neo-X chromosome (Fig. 8B). The clusters of rDNA genes were localized in the L_2, S_7, and S_8 autosome bivalent (Fig. 8B). Two clusters of rDNA repeats were observed in the L_2 bivalent: the first one located in the proximal interstitial region and the second one in the distal interstitial region (Fig. 8B). In the S_7 autosome bivalent, the cluster of rDNA repeats was revealed at the interstitial region (Fig. 8B). In the S_8 autosome bivalent, the clusters of rDNA genes were detected only in one homologue (Fig. 8B). This cluster was localized in the proximal position on the border of the C-positive huge band and C-negative chromatin (Fig. 8A, B).

Paranothrotes citimus Mistshenko, 1951

The karyotype of *P. citimus* consisted of 14 autosomes and three neo-sex chromosomes (2n=14+neo-X_1neo-X_2neo-Y♂). The karyotype structure was represented by two large (L_1–L_2), four medium (M_3–M_6) and one small (S_7) acrocentric autosome bivalents and three neo-sex chromosomes (Fig. 9A). The neo-X_1 and the neo-Y chromosomes were submetacentric. The neo-X_2 chromosome was acrocentric. During prophase I of male meiosis the sex chromosomes formed a trivalent consisting of the neo-X_1, neo-X_2 chromosomes and the neo-Y chromosome (Fig. 9A).

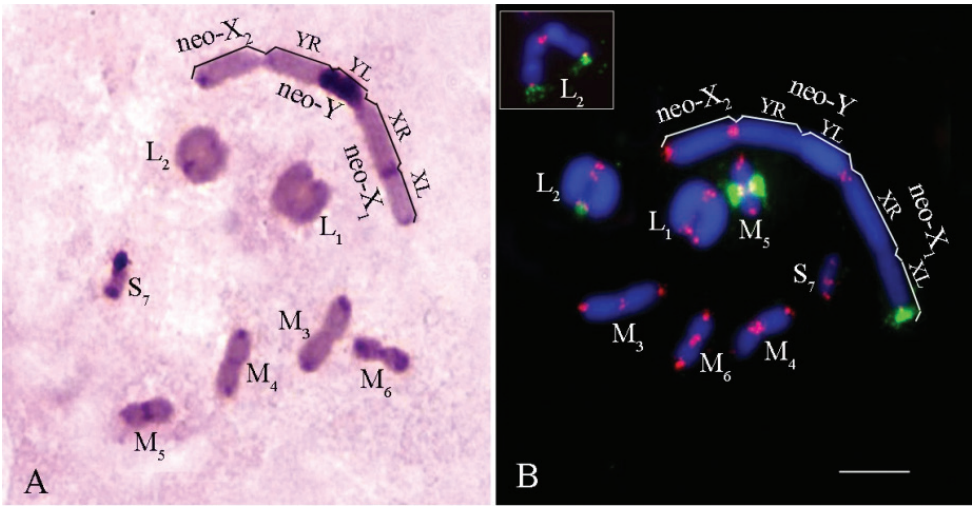

Figure 9. *Paranothrotes citimus* **A** C-banded chromosome in metaphase I **B** FISH with rDNA (green) and telomeric $(TTAGG)_n$ (red) probes in cell at metaphase I. The inset in the right upper corner shows the L_2 chromosome in meiotic metaphase II. Chromosomes were counterstained with DAPI (blue) (**B**). Scale bar: 10 μm.

Distinct pericentromeric C-positive blocks were observed in all chromosomes. The YL-arm of the neo-Y chromosome was completely C-positive (Fig. 9A).

FISH signals of telomeric DNA probe were observed in the terminal regions of all chromosomes (Fig. 9B). The clusters of rDNA genes were localized near the pericentromeric region of the M_5 and at distal position of L_2 autosomal bivalents, and at terminal region in the X_1L-arm of the neo-X_1 chromosome (Fig. 9B).

The chromosome number, morphology, sex chromosome system, distribution of heterochromatin (C-bands) and location of rDNA and tDNA genes in the studied Pamphagidae species presented in Table 2.

Discussion

A comparative cytogenetic analysis of Iranian Pamphagidae provides new information about the karyotype evolution in this group of grasshoppers. Two species from the *Eremopeza* Saussure, 1888 genus (Thrinchinae) have the fundamental chromosome number of the Pamphagidae karyotype (2n=19♂). However, unlike the standard Pamphagidae karyotype, in which all chromosomes are acrocentric, in *Eremopeza* subacrocentric chromosomes were found. Early, biarmed chromosomes were found in *Eremopeza festiva* (Saussure, 1888) from Armenia (Bugrov et al. 2016). Two possible paths of the origin of biarmed chromosomes in *Eremopeza* genus may suggested: a) amplification of repetitive elements; b) pericentric inversion. It was shown that in *E. festiva* the presence of all biarmed chromosomes (FN=38) was associated with invasion and amplification of

Table 2. The chromosome number, chromosomal morphology, sex chromosome system, distribution of constitutive heterochromatin (C-bands) and location of rDNA and tDNA genes in the studied Pamphagidae species.

Taxa	2n♂; FN	SD	KS	CM	C-bands	rDNA	tDNA
Thrinchinae, Thrinchini							
Eremopeza bicoloripes	19; 20	X0 XX	L_1–L_4, M_5-M_7, S_8–S_9, X	all a X sm	p all; t 1,4, 5,6,9	p1,2,3*,4,5, 6,7*,8	d all dpd X
Eremopeza saussurei	19; 34	X0 XX	L_1–L_4, M_5–M_7, S_8–S_9, X	1-4,5,7, X sa; 6 sm; 8,9 a	p all; t 2	p1-4,5,6,7,X	d all dpd 2,3,X
Pamphaginae, Nocarodeini							
Paranothrotes citimus	14;18	neo-X_1X_2Y neo-$X_1X_1X_2X_2$	L_1–L_2, M_3–M_6 S_7 neo-X_1 neo-X_2 neo-Y	all a neo-X_1 sm neo-X_2 a neo-Y sm	p all; t X_1L	p5; d2; X_1L	d all
Pamphaginae, Tropidauchenini							
Saxetania paramonovi	19;19	X0 XX	L_1–L_4, M_5-M_7, S_8–S_9 X	all a	p all; t 7,9,8	p2,4;i3	d all
Tropidauchen escalerai	18;19	neo-XY neo-XX	L_1–L_4, M_5,M_6 S_7, S_8 neo-X neo-Y	all a neo-X m neo-Y a	p all; i XR, neo-Y; t 1,2,4, neo-X	p2i2; p3;i6	d all
Tropidauchen sp.	18;19	neo-XY neo-XX	L_1–L_4, M_5,M_6 S_7, S_8 neo-X neo-Y	1-3,5,6,7,8 a, 4 sa neo-X sm neo-Y a	p all; i 5,8; t 6,7	ip2, id2; i7; 8*	d all dpd neo-X

FN=fundamental number of chromosome arms; SD=sex chromosome system; KS=karyotype structure; L=large; M=medium; S=small; CM=morphology of chromosomes; a=acrocentric; sa=subacrocentric; sm=submetacentric; p=pericentromeric, i=interstitial, t=telomeric; rDNA=clusters of ribosomal DNA; tDNA=telomeric DNA repeats; d=distal; *=in one of the homologues; XR=XR-arm neo-X chromosome; X_1L=X_1L-arm of the neo-X_1 chromosome.

rDNA repeats (Bugrov et al. 2016). In species of *Eremopeza* analyzed in this article not all chromosomes in the sets are biarmed. In *E. bicoloripes*, the X is the only biarmed chromosome and has no clusters of rDNA genes. In *E. saussurei*, most chromosomes in the karyotype have small second arms. The rDNA clusters in this species are located only in pericentromeric regions on biarmed chromosomes, while small arms were not enriched by the rDNA repeats. These observations indicate that the formation of the second arms in *E. bicoloripes* and *E. saussurei* are not associated with the amplification of rDNA repeats. Also, the presence of interstitial telomeric sites in pericentromeric region of some biarmed chromosomes is a strong argument in favor of the inversion hypothesis.

The discovery of some Pamphagidae species with neo-sex chromosome systems supports our hypothesis that the origin of this unusual sex chromosome system is the West Asian region (Jetybayev et al. 2017a). The two species with the neo-sex chromosomes belong to the Tropidauchenini tribe. Previously, the karyotype of only one species, *Saxetania cultricollis* (Saussure, 1887), from this tribe was described. In this species a neo-XY sex chromosome system was found (Bugrov and Warchałowska-Śliwa 1997). Thus, in the Tropidauchenini tribe both the X0 (*S. paramonovi*) and neo-XY sex chromosome systems (*S. cultricollis*, *T. escalerai* and *Tropidauchen* sp.) exist (Figs 6–8). It should be noted that in *S. cultricollis* and *Tropidauchen*, the neo-Y chromosome is very similar to the XR-arm of the neo-X chromosome. During meiosis, these homolo-

gous chromosomes form a sex bivalent with one or two chiasmata. The localization of the C-positive regions in the neo-Y chromosome in these species, also does not differ from its homologue, namely the XR-arm of the neo-X. These features indicate that in Tropidauchenini we found the initial stage of neo-XY sex chromosome evolution in the Pamphaginae subfamily. All early studied species of the Nocarodeini tribe (Pamphaginae) possessed a neo-sex chromosome system. In these works, it was emphasized that in Nocarodeini tribe the neo-Y is significantly shorter than the XR and shows a significantly larger heterochromatic region. In the meiosis prophase I, the XR and the neo-Y chromosome of the Nocarodeini species were associated only with the distal region. These features indicate that the Nocarodeini tribe demonstrate the advanced stage of the neo-Y chromosome evolution in Pamphaginae (Bugrov and Grozeva 1998; Bugrov et al. 2016; Jetybayev et al. 2017a, b).

The fluorescence *in situ* hybridization (FISH) with telomeric probe and rDNA genes is a very useful tool for comparative analysis of karyotype in Orthoptera insects (Warchałowska-Śliwa et al. 2020). In addition, the determination of the position of telomeric and rDNA repeats in chromosomes of many groups of insects made it possible to identify the mechanisms of structural rearrangements (Kuznetsova et al. 2019). It is known that telomeres play an important role in the stability of the eukaryotic karyotype. Basically, telomeric repeats are located at the physical ends of chromosomes in the form of tandem arrays that protect the ends of the chromosomes from attack by exonucleases, degradation and prevent chromosome fusion (Bolzán 2017; Kuznetsova et al. 2019). In chromosome rearrangements the clusters of telomeric repeats may be transferred to interstitial chromosome locations so-called interstitial telomeric sequences (ITSs). Therefore, ITSs may constitute good markers of the occurrence of chromosome rearrangements. We expected to observe ITS in the pericentromeric regions of the neo-X chromosomes in the *Tropidauchenini* tribe. However, the telomeric motif in the Robertsonian translocation site between the X chromosome and the autosome in *T. escalerai* was not observed. Similar results were previously shown in the vast majority of species belonging to the Nocarodeini tribe (Pamphaginae) (Jetybayev et al. 2017a). Additionally, we performed FISH of the telomeric $(TTAGG)_n$ probe in chromosomes of the *Saxetania cultricollis* from Turkmenistan. It was also discovered that in *S. cultricollis*, there was no telomeric repeats in the pericentromeric region of the neo-X chromosome (Fig. 10). The absence of telomeric repeats in the pericentromeric region of the neo-X chromosome of these species may indicate that the Robertsonian translocation of the X chromosome and the autosome was accompanied by the deletion of a chromosome fragment containing telomeric DNA repeats. Nevertheless, in *Tropidauchen* sp. we observed telomeric repeats in the pericentromeric region of the neo-X chromosome (Fig. 8B). Previously, the presence of these repeats in the pericentromeric region of the neo-X chromosomes was detected in two species of the *Paranocarodes* Bolívar, 1916 genera (Jetybayev et al. 2017a). It is hardly possible, that in the aforementioned *Paranocarodes* species and *Tropidauchen* sp. the origin of the neo-XY sex system, was different from that of other XY species of Pamphaginae. We suggest that the ITS in these species could occur after pericentric inversion in the neo-X chromosome.

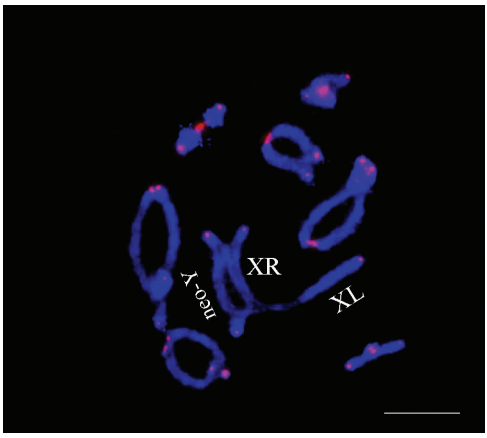

Figure 10. *Saxetania cultricollis*: FISH with telomeric repeats (red) in cells at diakinesis. Chromosomes were counterstained with DAPI (blue). Scale bar: 10 μm.

The distribution of rDNA clusters in the chromosomes of the *Saxetania* and *Tropidauchen* species was similar to the distribution of rDNA in the chromosomes of previously studied Pamphaginae species (Vitturi et al. 2008; Bugrov et al. 2016; Jetybayev et al. 2017a). The rDNA clusters were localized on two or three autosome bivalents at the pericentromeric and interstitial regions. One large pair of autosomes carried two rDNA clusters at interstitial position in the proximal and distal regions. Multiple rDNA sites on a single chromosome is a very rare type of rDNA cluster distribution among Acridoid grasshoppers (Cabrero and Camacho 2008; Jetybayev et al. 2012; Palacios-Gimenez et al. 2013). This feature has only been detected in species of the family Pamphagidae (Vitturi et al. 2008; Bugrov et al. 2016; Jetybayev et al. 2017a; Buleu et al. 2019). Our results thus confirm a special type of rDNA cluster localization in the Pamphagidae grasshoppers.

The neo sex chromosome systems were observed in two subfamilies (Thrinchinae and Pamphaginae) of the Pamphagidae grasshoppers. Based on the analysis of the chromosome features (karyotype, C-banding, telomeric $(TTAGG)_n$ and rDNA genes) we see that the neo-sex chromosome system in the genera *Saxetania* and *Tropidauchen* in the subfamily Pamphaginae is at a similar level of chromosome evolution to the neo-sex chromosomes in the genera *Glyphotmethis* and *Asiotmethis* of the subfamily Thrinchinae (Bugrov 1996; Jetybayev et al. 2017a). However, the neo-XY system was observed only in several species of the genera *Asiotmethis* and *Glyphotmethis* and no advanced stages of the neo-Y differentiation were observed in this subfamily. Conversely, in the subfamily Pamphaginae, the neo-Y chromosome was observed at different stages of its evolution from the chromosome that is homologous to the autosome (in the tribe Tropidauchenini) to the small heteromorphic mostly heterochromatic (in the tribe Nocarodeini). Furthermore, in the tribe Nocarodeini, we observed an additional stage of the structural evolution of the neo-sex chromosomes: formation of the multiple neo-X_1X_2Y♂ sex chromosome system. Previously, this kind of sex chromosome system was identified in the *Paranothrotes opacus* from Armenia (Bugrov et al. 2016). In this

Figure 11. Distribution of Pamphaginae grasshoppers with the X0 (blue circles) and neo-sex chromosomes (red circles). 1 – North Caucasus (Russia). 2 – Armenia.

paper, we report on a second species with the same type of neo-sex chromosome system and other cytogenetic characters – *Paranothrotes citimus*. It is possible that the evolutionary divergence of the species in the genus *Paranothrotes* could occur on the basis of the neo-X_1X_2Y♂sex chromosome system.

Analysis of the geographic distribution of Pamphaginae grasshoppers with different types of the sex chromosome systems (Alicata et al. 1976; Camacho et al. 1981; Cabrero et al. 1985; Vitturi et al. 1993; Warchałowska-Śliwa et al. 1994; Bugrov 1996; Bugrov and Warchałowska-Śliwa 1997; Bugrov and Grozeva 1998; Bugrov et al. 2016; Jetybayev et al. 2017a; Buleu et al. 2019) confirmed that species with the neo-sex chromosomes widespread mainly in Western Asia (Fig. 11). The finding of species with the sex chromosome X0 (*Saxetania paramonovi*) and with the neo-XY chromosomes at initial stages of chromosomal evolution (*Tropidauchen* species) in Iranian fauna of Pamphaginae grasshoppers allow us to suggest that translocation between an autosome and the original X chromosome in the karyotype evolution in this subfamily originated in the Iranian highlands.

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References

Alicata P, Messina A, Oliveri S (1976) Frequenza e distribuzione dei chiasmi in *Pamphagus marmoratus* Burm., *Acinipe calabra* (Costa) e *Ocneridia canonica* (Fish.) (Orthoptera Pamphagidae). Animalia 3: 171–193.

Bey-Bienko GYa, Mistshenko LL (1951) Locusts and Grasshoppers. Part 1. Fauna of the USSR and Adjacent Countries, Vol. 38, 400 pp. [In Russian]

Bolzán AD (2017) Interstitial telomeric sequences in vertebrate chromosomes: Origin, function, instability and evolution. Mutation Research 773: 51–65. https://doi.org/10.1016/j.mrrev.2017.04.002

Bugrov AG (1986) Neo-XY sex-chromosome determination in grasshoppers *Asiotmethis heptapotamicus heptapotamicus* (Zub) and *Atrichotmethis semenovi* (Zub) (Orthoptera Pamphagidae). Tsitologiya 28: 117–119. [In Russian]

Bugrov AG (1996) Karyotypes of the short-horned Orthopteran insects (Orthoptera, Caeliferа) from Russia, Kazakhstan, Central Asia, and the Caucasus. Folia Biologica (Krakow) 44(1–2): 15–25.

Bugrov AG, Jetybayev IE, Karagyan GH, Rubtsov NB (2016) Sex chromosome diversity in Armenian toad grasshoppers (Orthoptera, Acridoidea, Pamphagidae). Comparative Cytogenetics 10: 45–59. https://doi.org/10.3897/CompCytogen.v10i1.6407

Bugrov AG, Warchałowska-Śliwa E (1997) Chromosome numbers and C-banding patterns in some Pamphagidae grasshoppers (Orthoptera, Acrididae) from the Caucasus, Central Asia, and Transbaikalia. Folia Biologica (Krakow) 45(3–4): 133–138.

Buleu OG, Jetybayev IY, Bugrov AG (2017) Comparative analysis of chromosomal localization of ribosomal and telomeric DNA markers in three species of Pyrgomorphidae grasshoppers. Comparative Cytogenetics 11(4): 601–611. https://doi.org/10.3897/compcytogen.v11i4.14066

Buleu OG, Jetybayev IY, Chobanov DP, Bugrov AG (2019) Comparative analysis of C-heterochromatin, ribosomal and telomeric DNA markers in chromosomes of Pamphagidae grasshoppers from Morocco. Comparative Cytogenetics 13(1): 61–74. https://doi.org/10.3897/CompCytogen.v13i1.32039

Cabrero J, Camacho JPM (1986) Cytogenetic Studies in Gomphocerine Grasshoppers. I. Comperative Analysis of Chromosome C-banding pattern. Heredity 56: 365–372. https://doi.org/10.1038/hdy.1986.58

Cabrero J, Camacho JPM (2008) Location and expression of ribosomal RNA genes in grasshoppers: Abundance of silent and cryptic loci. Chromosome Research 16(4): 595–607. https://doi.org/10.1007/s10577-008-1214-x

Cabrero J, Camacho JPM, Pascual F (1985) Cytotaxonomic studies on pamphagids genus *Eumigus*. Detection of two chromosomal races in *E. monticola* (Rambur) (Insecta, Orthoptera). Caryologia 38(1): 1–12. https://doi.org/10.1080/00087114.1985.10797724

Camacho JPM, Cabrero J, Viseras E (1981) C-heterochromatin variation in the genus *Eumigus* (Orthoptera, Pamphagoidea). Genetica 56(3): 185–188. https://doi.org/10.1007/BF00057558

Fossey A (1985) Cytogenetic Research of the Short-Horned Orthoptera Insect from South Africa. Dr Sci. Dissertation, Pretoria University, Pretoria, 106 pp.

Fu P et al. (1989) Karyotype C-banding staining on two species of the genus *Sinotmethis* B.-Bienko (Orthoptera, Pamphagidae). Hereditas (China) 11(3): 26–28.

Hewitt GM (1979) Grasshoppers and cricket. In: John B (Ed.) Animal Cytogenetics, 3. Insecta I. Orthoptera. Borntraeger, Berlin, Stuttgart, 170 pp. https://doi.org/10.3897/compcytogen.v5i4.2307

Hodjat SH (2012) An update list of Pamphagidae Brumster 1840 (Insecta: Orthoptera) of Iran with a key to genera. Journal of Crop Protection 1(3): 261–270.

Jetybayev IE, Bugrov AG, Karamysheva TV, Camacho JPM, Rubtsov NB (2012) Chromosomal localization of ribosomal and telomeric DNA provides new insights on the evolution of Gomphocerinae grasshoppers. Cytogenetic and Genome Research 138(1): 36–45. https://doi.org/10.1159/000341571

Jetybayev IE, Bugrov AG, Ünal M, Buleu OG, Rubtsov NB (2017a) Molecular cytogenetic analysis reveals the existence of two independent neo-XY sex chromosome systems in Anatolian Pamphagidae grasshoppers. BMC Evolutionary Biology 17(1): 1–20. https://doi.org/10.1186/s12862-016-0868-9

Jetybayev IY, Bugrov AG, Buleu OG, Bogomolov AG, Rubtsov NB (2017b) Origin and evolution of the neo-sex chromosomes in Pamphagidae grasshoppers through chromosome fusion and following heteromorphization. Genes 8(323). https://doi.org/10.3390/genes8110323

King M, John B (1980) Regularities and restrictions governing C-band variation in acridoid grasshoppers. Chromosoma (Berlin) 76(2): 123–150. https://doi.org/10.1007/BF00293413

Kuznetsova V, Grozeva S, Gokhman V (2019) Telomere structure in insects: A review. Journal of Zoological Systematics and Evolutionary Research 58: 127–158. https://doi.org/10.1111/jzs.12332

Mansueto C, Vitturi R (1989) NORs location and C-banding pattern in spermatogenesis of Pamphagus ortolanii (Orthoptera, Acrididae). Caryologia 42: 303–311. https://doi.org/10.1080/00087114.1989.10796978

Massa B (2013) Pamphagidae (Orthoptera: Caelifera) of North Africa: key to genera and the an- notated check-list of species. Zootaxa 3700(3): 435–475. https://doi.org/10.11646/zootaxa.3700.3.7

Mirzayans (1998) Insects of Iran: the list of Orthoptera in insect collection of Plant pests & diseases Research Institute: Orthoptera (X), Pamphagidae (8) and Pyrgomorphidae (10). Tehran, 40 pp.

Mistshenko LL (1951) Revision of Orthoptera of the genus Tropidauchen Sauss. (Saltatoria – Orthoptera, Acrididae) and related genera. Dokladij Akademii Nauk USSR 57: 737–740. [In Russian]

Palacios-Gimenez OM, Castillo ER, Martí DA, Cabral-de-Mello DC (2013) Tracking the evolution of sex chromosome systems in Melanoplinae grasshoppers through chromosomal mapping of repetitive DNA sequences. BMC Evolutionary Biology 13(1): 1–167. https://doi.org/10.1186/1471-2148-13-167

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: 2934–2938. https://doi.org/10.1073/pnas.83.9.2934

Rubtsov N, Karamysheva T, Astakhova N, Liehr T, Claussen U, Zhdanova N (2000) Zoo-FISH with region-specific paints for mink chromosome 5q: delineation of inter- and intra-chromosomal rearrangements in human, pig and fox. Cytogenetics and Cell Genetics 90: 268–270. https://doi.org/10.1159/000056786

Sahara K, Marec F, Traut W (1999) TTAGG telomeric repeats in chromosomes of some insects and other arthropods. Chromosome Research 7: 449–60. https://doi.org/10.1023/A:1009297729547

Santos JL, Arana P, Giraldez R (1983) Chromosome C-banding patterns in Spanish Acridoidea. Genetica 61: 65–74. https://doi.org/10.1007/BF00563233

Sergeev MG (1995) The general distribution of Orthoptera in the eastern parts of the Saharan-Gobian and Scythian Subregions. Acta Zoologica Cracoviensia 38(2): 213–256.

Shumakov EM (1963) Acridoidea of Afghanistan and Iran. Academie des Science de L' URSS. 284 pp.

Ünal M (2016) Pamphagidae (Orthoptera: Acridoidea) from the Palaearctic Region: taxonomy, classification, keys to genera and a review of the tribe Nocarodeini I. Bolívar. Zootaxa 4206(1): 1–223. https://doi.org/10.11646/zootaxa.4206.1.1

Uvarov BP (1966) Grasshoppers and locusts. A handbook of general acridology (Vol. 1). London, Cambridge University Press, 481 pp.

Vitturi R, Lannino A, Mansueto C, Mansueto V, Stella M (2008) Silver-negative NORs in *Pamphagus ortolaniae* (Orthoptera: Pamphagidae). European Journal of Entomolology 105: 35–39. https://doi.org/10.14411/eje.2008.004

Vitturi R, Mansueto C, Ficarella P (1993) Heterochromatin variation in four species of the genus *Pamphagus* (Orthoptera: Pamphagidae) analyzed by C-banding. Biologisches Zentralblatt 112: 335–341.

Warchałowska-Śliwa E, Maryańska-Nadachowska A, Massa B (1994) Some new data on C-banding and NORs in three species of Pamphagidae (Orthoptera). Folia Biologica (Krakow) 42(1–2): 13–18.

Warchałowska-Śliwa E, Grzywacz B, Maryańska-Nadachowska A, Heller K-G, Hemp C (2020) Rapid chromosomal evolution in the bush-cricket *Gonatoxia helleri* Hemp, 2016 (Orthoptera, Phaneropterinae). Comparative Cytogenetics 14(3): 417–435. https://doi.org/10.3897/CompCytogen.v14i3.54422

White MJD (1940) The origin and evolution of multiple sex-chromosome mechanisms. Journal of Genetics Springer 40: 303–36. https://doi.org/10.1007/BF02982496

White MJD (1973) Animal Cytology and Evolution. Cambridge, 961 pp.

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SHORT COMMUNICATION

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Karyotype and DNA barcode of *Polyommatus* (*Agrodiaetus*) *cyaneus* (Staudinger, 1899) from its type locality: implication for taxonomic and evolutionary research in *Polyommatus* blue butterflies (Lepidoptera, Lycaenidae)

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Abstract

Chromosomal and molecular analyses of rapidly evolving organisms such as *Polyommatus* Latreille, 1804 blue butterflies are essential for understanding their taxonomy and evolutionary history, and the studies of populations from their type localities are crucially important for resolving problems of nomenclature and species identity. Here we present data on the topotypical population of the blue butterfly species described as *Lycaena damone* var. *cyanea* Staudinger, 1899. This taxon was described from Khankendi (Nagorno-Karabakh, Caucasus), and rediscovered at the type locality for the first time since it was collected there in 1869. The specimens were found on dry stony meadows with a predominance of *Onobrychis radiata* Bieberstein, 1810, on upper border of oak forests. Their haploid chromosome number (n) was established as n = 17. Chromosomal and mitochondrial DNA barcode analyses of the studied samples from type-locality provided an opportunity for the critical taxonomic re-examination of Caucasian species of the subgenus *Agrodiaetus* Hübner, 1822 of the genus *Polyommatus* Latreille, 1804. The obtained data support the interpretation of the *P.* (*A.*) *cyaneus* (Staudinger, 1899) and *P.* (*A.*) *carmon* (Herrich-Schäffer, 1851) as

two different, not closely related species complexes as previously hypothesized by Hugo de Lesse. On the contrary, the treatment by Walter Forster who considered these taxa as two groups of conspecific populations was not supported by our data.

Keywords
Agrodiaetus, chromosome, karyosystematics, taxonomy

Introduction

The species-rich butterfly subgenus *Agrodiaetus* Hübner, 1822 of the genus *Polyommatus* Latreille, 1804 has become a model system for studying speciation and chromosomal evolution (Lukhtanov et al. 2005; Wiemers et al. 2009; Dincă et al. 2013; Lukhtanov et al. 2020a). However, despite the attention from biologists, numerous taxonomic and nomenclatural problems remain unresolved in the subgenus. In particular, this concerns the taxon known as *P.* (*A.*) *cyaneus* (Staudinger, 1899), which is a polytypic species (or even a complex of closely related species) (Eckweiler and Bozano 2016). This taxon was initially described as a "variation" *Lycaena damone* var. *cyanea* Staudinger, 1899, based on specimens collected in 1866 by Josef Haberhauer in Hankynda (now Khankendi, Nagorno-Karabakh) and in Akhalzich (now Akhaltsikhe, Georgia) (Lederer 1870; Staudinger 1899). In the first detailed revision of the subgenus *Agrodiaetus* Hübner, 1822, Walter Forster (1956) treated it as a separate genus, designated a specimen from Hankynda as the lectotype of *Lycaena damone* var. *cyanea*, and regarded this taxon as subspecies *Agrodiaetus carmon cyanea* (Forster, 1956). However, after the chromosomal studies of Hugo de Lesse (1960, 1963), *Agrodiaetus carmon* (Herrich-Schäffer, 1851) and *A. cyaneus* are usually considered as two different species. At the same time, it is important to emphasize that these studies (de Lesse 1960, 1963) and consequent studies on karyosystematics and molecular taxonomy of the *P.* (*A.*) *carmon* and *P.* (*A.*) *cyaneus* species groups (Wiemers 2003; Lukhtanov et al. 2014) dealt with butterflies from Iran and Turkey and never affected the population from Nagorno-Karabakh.

Accordingly to the lectotype designation (Forster 1956), Khankendi in Nagorno-Karabakh is treated as the type locality of *P. cyaneus*. It is generally accepted that the knowledge of karyotype characters of topotypical populations is an essential requirement for revealing species identity in the subgenus *Agrodiaetus* (Lukhtanov and Dantchenko 2002a; Kandul et al. 2004). As it was shown, the cytological approach using DNA data for certain type populations led to dramatic taxonomic rearrangements on the species level (Lukhtanov et al. 2006, 2008, 2015; Lukhtanov and Dantchenko 2017). In the case of *P. cyaneus* such study of the population from Nagorno-Karabakh seemed especially important because the study of the lectotype specimen (Fig. 1) revealed that the latter differed significantly from the Iranian and Turkish butterflies (e.g. see the figures in Hesselbarth et al. 1995; Eckweiler and Bozano 2016), which were previously attributed to this species.

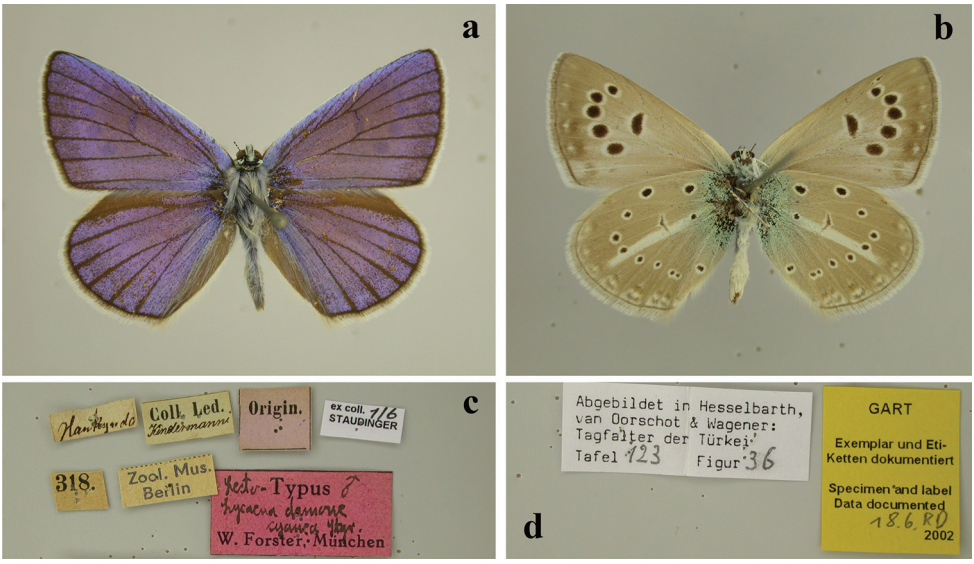

Figure 1. Lectotype of *Lycaena damone* var. *cyanea* Staudinger, 1899. In collection of Humboldt-Universität zu Berlin. Photo: V. Lukhtanov **a** upperside **b** underside **c** labels **d** additional labels.

Here we present the first karyotype description of *P.* (*A.*) *cyaneus* exactly from its type locality. As suggested previously (Lukhtanov and Iashenkova 2019), we also provide the DNA barcodes for the chromosomally studied samples to avoid the possible problems of inaccurate species identification.

Material and methods

The specimens of *P.* (*A.*) *cyaneus* (5 males and 2 females) were collected by the third author, Karine Balayan, in vicinity of Stepanakert (Khankendi, Nagorno-Karabakh) and near Kanachtala village (20 km to the west from Stepanakert). The collection of the specimens was carried out during July of three summer seasons: in 2015, 2016 and 2018. The collecting places are dry stony glades in oak forest with dominating *Onobrychis radiata* Bieberstein, 1810 (Fabacaea). For chromosomal analysis, testes were extracted from the butterfly abdomens and fixed in a mixture of glacial acetic acid and 96% ethyl alcohol (1: 3). The fixed material was stored at + 4 °C for 5–24 months. For molecular analysis, a single leg was sampled from each collected specimen. Standard *COI* barcodes (658-bp 5' segment of mitochondrial cytochrome oxidase subunit I) were obtained using primers and protocols described by Shapoval et al. (2017).

The Bayesian majority rule consensus tree of the analyzed samples (Fig. 2) was constructed as previously described (Przybyłowicz et al. 2014; Lukhtanov and Iashenkova 2019) using the sequences obtained in this study as well as the published sequences

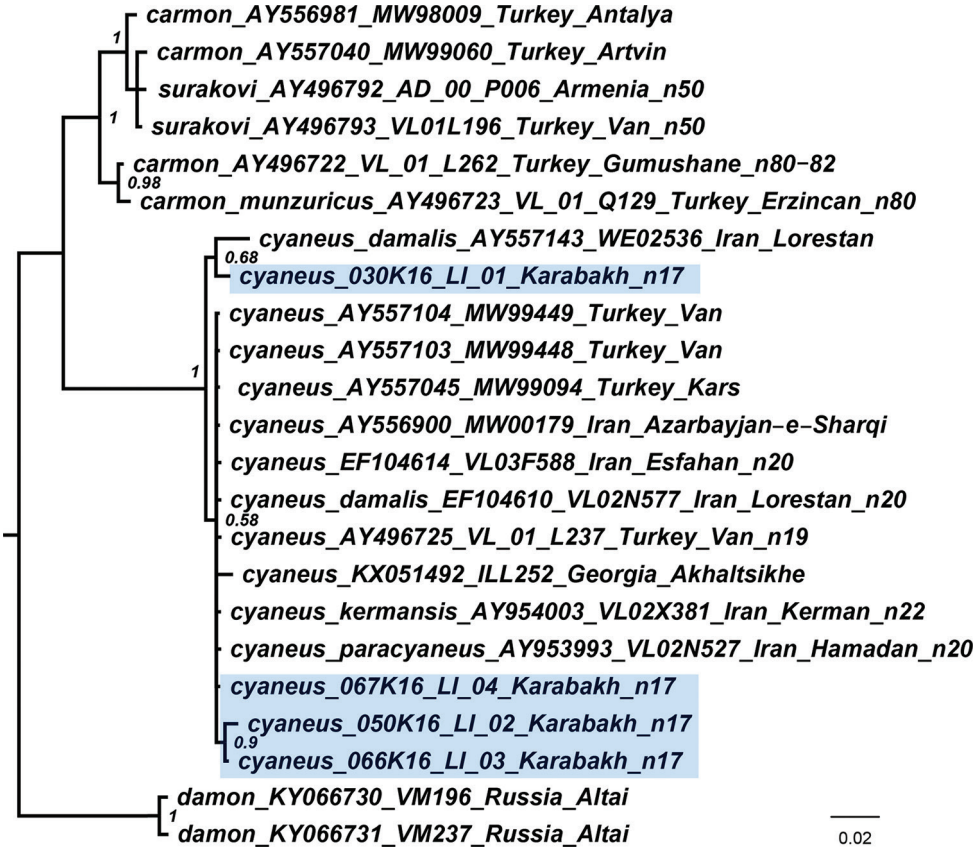

Figure 2. The Bayesian majority rule consensus tree of the analyzed samples of *Polyommatus* (*Agrodiaetus*) inferred from *COI* sequences. *Polyommatus damon* (Denis et Schiffermüller, 1775) is used to root the tree. Species and subspecies names, GenBank accession numbers, museum ID numbers, localities and haploid chromosome numbers (if known) are shown to the right of the branches. Bayesian posterior probabilities higher than 0.5 are shown next to the recovered branches.

downloaded from GenBank (Wiemers 2003; Kandul et al. 2004, 2007; Lukhtanov et al. 2005; Vishnevskaya et al. 2016). Briefly, sequences were aligned using the BioEdit (Hall 1999) and edited manually. The Bayesian analysis was performed using the program MrBayes 3.2 (Ronquist et al. 2012) with default settings as suggested by Mesquite (Maddison and Maddison 2015): burn-in = 0.25, nst = 6 (GTR + I + G). Two runs of 10,000,000 generations with four chains (one cold and three heated) were performed. We checked runs for convergence and proper sampling of parameters [effective sample size (ESS) > 200] using the program tracer v1.7.1 (Rambaut et al. 2018). The first 25% of each run was discarded as burn-in. The consensus of the obtained trees was visualised using FigTree 1.3.1 (http://tree.bio.ed.ac.uk/software/figtree/).

For chromosomal analysis, the testes were stained with 2% orcein acetic acid for 8–30 days as previously described (Lukhtanov 2019). The stained material was placed in a drop of 40% lactic acid on a glass slide. The testes were macerated with thin pins.

The slide was covered with a coverslip and the macerated testes were squashed between the two glasses. Excess lactic acid was removed with filter paper.

Karyotypes were studied in 5 males. Haploid chromosome number (n) was counted at metaphase I (MI), metaphase II (MII) and prometaphase I cells. For determination of karyotype parameters, 79 metaphase plates (MI and MII) of the highest quality and 11 cells at the stage of prometaphase I were selected. Cells in which the chromosomes were not located on the same plane, as well as cells with overlapping or touching chromosomes and/or bivalents, were rejected and not used for analysis. In some cases, diploid chromosome number (2n) was counted in atypical male meiosis which represent a kind of asynaptic meiosis (Lorković 1990; Lukhtanov and Dantchenko 2017; Lukhtanov et al. 2020b).

A Leica DM2500 light microscope equipped with HC PL APO 100x/1,44 Oil CORR CS lens and S1/1.4 oil condenser head was used for bright-field microscopy analysis. A Leica lens HC PL APO 100x/1,40 OIL PH3 was used for phase-contrast microscopy analysis.

Results and discussion

DNA-barcode analysis demonstrated that the studied samples collected exactly in the type locality and nearby the type locality are almost identical with the previously studied samples collected in Iran and Turkey (p-distance from 0 to 1.6%) (Fig. 2). *Polyommatus* (*Agrodiaetus*) *cyaneus* and *P.* (*A.*) *carmon* species complexes were found to be strongly diverged (p-distance = 6.3%) confirming previous data (Wiemers 2003; Kandul et al. 2004, 2007).

In karyotype, at the MI stage, 17 chromosome bivalents were observed in four studied males (Fig. 3a–c, e–f). At the MII stage, 17 chromosome elements were observed (Fig. 3d). The bivalents at the MI and the elements at the MII were found to form a gradient size row in which the largest element was approximately one and a half times larger than the smallest element. In the fifth male, the diploid chromosome number was established as 2n = 34 in male asynaptic meiosis (Table 1). No variation in chromosome number was found.

In terms of chromosome numbers and karyotype structure, the studied populations from Nagorno-Karabakh fit well into the previously described variability within *P. cyaneus* (from n = 16–17 to 22) (de Lesse 1963; Lukhtanov 1989; Lukhtanov et al. 1998). De Lesse (1963), based mainly on his chromosomal studies, divided *Agrodiaetus carmon* (Herrich-Schäffer, 1851) (sensu Forster 1956) into two different species: *A. carmon* with n = 80–82 and *A. cyaneus* with chromosome numbers varying from n = 16 to n=22 in different populations in Iran and Turkey.

Over the next years, the following important additions were made to the taxonomy and cytogenetics of these two species complexes. (i) Chromosome numbers supporting the findings of de Lesse (1963), were established for additional populations (Lukhtanov 1989; Lukhtanov et al. 1998; Kandul et al. 2007). (ii) *Polyommatus* (*Agrodiaetus*) *carmon* was divided in two allopatric, chromosomally diverged species: *P.* (*A.*) *carmon* sensu stricto (n = 80–82) and *P.* (*A.*) *surakovi* Dantchenko et Lukhtanov, 1994 (n = 50)

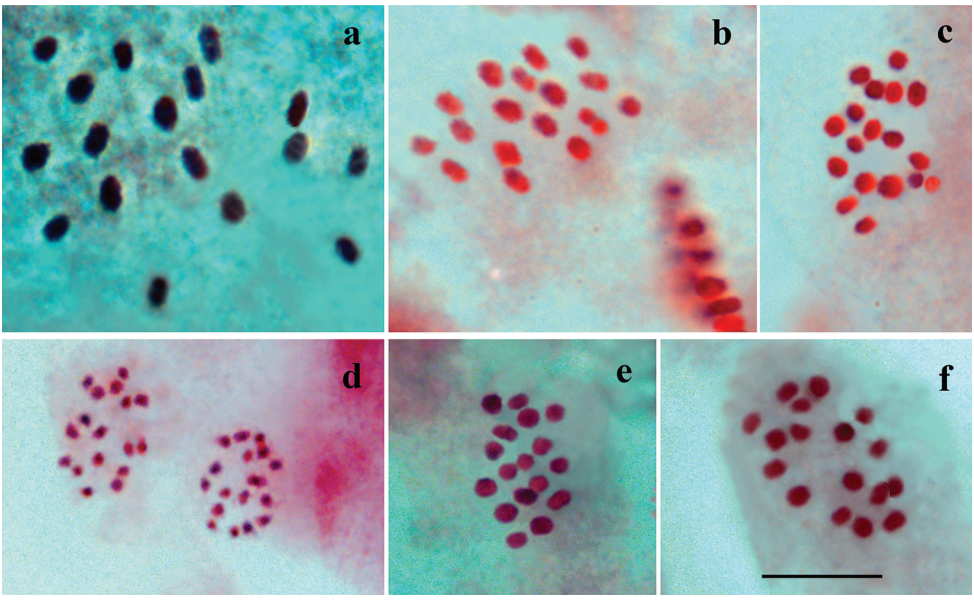

Figure 3. Karyotypes of *Polyommatus* (*Agrodiaetus*) *cyaneus* from Nagorno-Karabakh, Caucasus **a** sample 047K18, Khankendi, prometaphase I, n = 17, phase-contrast **b** sample 047K18, Khankendi, MI, n = 17 **c** sample 047K18, Khankendi, MI, n = 17 **d** sample 030K16, Kanachtala, two MII cells displaying n = 17 **e** sample 050K16, Kanachtala, MI, n = 17 **f** sample 066K16, Kanachtala, MI, n = 17. Scale bar: 10 μ.

Table 1. Chromosome number in studied samples of *P.* (*A.*) *cyaneus* from its type locality (Nagorno-Karabakh).

Field ID	Lab Id	GenBank#	Chromosome number	Locality
030K16A	L1-01	MW094230	n = 17	near Kanachtala
050K16A	L1-02	MW094231	n = 17	near Kanachtala
066K16A	L1-03	MW094232	n = 17	near Kanachtala
067K16A	L1-04	MW094233	2n = 34	near Kanachtala
047K18A	n/a-	n/a-	n = 17	vicinity of Stepanakert

(Lukhtanov and Dantchenko 2002b). (iii) *P.* (*A.*) *carmon* and *P.* (*A.*) *cyaneus* were found as distantly related species complexes, not sister species (Wiemers 2003; Vershinina and Lukhtanov 2017). However, all of the above conclusions were imperfect in terms of zoological nomenclature, since the karyotype of *P. cyaneus* from its type locality was not studied. Our data on topotypes, both in terms of karyotypes and mitochondrial DNA, solve this problem, confirming the taxonomic hypothesis of de Lesse (1963) that *P.* (*A.*) *carmon* and *P.* (*A.*) *cyaneus* as two distinct species complexes.

At the same time, one should note the high chromosomal variability within the taxon, which is now called *P. cyaneus*, as well as the confinement of certain karyotypes to geographic regions. For example, there is a clear tendency that lower chromosome numbers are found in the northern half of the complex's geographic distribution, and higher ones in the southern half. It is therefore expectable that subsequent studies will shed light on finer taxonomic and phylogeographic structure of this complex.

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References

de Lesse H (1960) Speciation et variation chromosomique chez les Lepidopteras Rhopaloceras. Annales des Sciences Naturelles Zoologie et Biologie Animale, 12e série 2: 1–223.

de Lesse H (1963) Variation chromosomique chez *Agrodiaetus carmon* H. S. et *A. cyanea* Stgr. Revue Française d'Entomologie 30: 177–181.

Dincă V, Runquist M, Nilsson M, Vila R (2013) Dispersal, fragmentation and isolation shape: the phylogeography of the European lineages of *Polyommatus* (*Agrodiaetus*) *ripartii* (Lepidoptera: Lycaenidae). Biological Journal of the Linnean Society 109: 817–829. https://doi.org/10.1111/bij.12096

Eckweiler W, Bozano GC (2016) Guide to the Butterflies of the Palearctic region. Lycaenidae part IV. Omnes artes, Milano, 132 pp.

Forster W (1956) Bausteine zur Kenntnis der Gattung *Agrodiaetus* Scudd. (Lep. Lycaen.). Wiener Entomologische Geselschaft 41: 42–61.

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41: 95–98.

Hesselbarth G, Oorschot H, Wagener S (1995) Die Tagfalter der Türkei unter Berücksichtigung der Angrenzenden Länder (Vol. 1–3). Selbstverlag Siegbert Wagener, Bocholt, 1354 pp.

Kandul NP, Lukhtanov VA, Dantchenko AV, Coleman JWS, Sekercioglu CH, Haig D, Pierce NE (2004) Phylogeny of *Agrodiaetus* Hübner 1822 (Lepidoptera: Lycaenidae) Inferred from mtDNA Sequences of *COI* and *COII* and Nuclear Sequences of *EF1-α*: Karyotype Diversification and Species Radiation. Systematic Biology 53(2): 278–298. https://doi.org/10.1080/10635150490423692

Kandul NP, Lukhtanov VA, Pierce NE (2007) Karyotypic diversity and speciation in *Agrodiaetus* butterflies. Evolution 61(3): 546–559. https://doi.org/10.1111/j.1558-5646.2007.00046.x

Lederer J (1870) Contributions a la Faune des Lepidopteres de la Transcaucasie. Annales de la Société entomologique de Belgique 13: 17–54.

Lorković Z (1990) The butterfly chromosomes and their application in systematics and phylogeny. In: Kudrna O, ed. Butterflies of Europe (Vol. 2). Introduction to Lepidopterology. Wiesbaden: Aula Verlag, 332–396.

Lukhtanov VA (1989) Karyotypes of some blue butterflies of the *Agrodiaetus* species groups. Annales Entomologici Fennici 55: 137–144.

Lukhtanov VA (2015) The blue butterfly *Polyommatus* (*Plebicula*) *atlanticus* (Lepidoptera, Lycaenidae) holds the record of the highest number of chromosomes in the non-polyploidy eukaryotic organisms. Comparative Cytogenetics 9(4): 683–690. https://doi.org/10.3897/CompCytogen.v9i4.5760

Lukhtanov VA (2019) Two types of highly ordered micro- and macrochromosome arrangement in metaphase plates of butterflies (Lepidoptera). Comparative Cytogenetics 13(1): 19–25. https://doi.org/10.3897/CompCytogen.v13i1.32614

Lukhtanov VA, Dantchenko AV (2002a) Descriptions of new taxa of the genus *Agrodiaetus* Hübner, [1822] based on karyotype investigation (Lepidoptera, Lycaenidae). Atalanta 33(1/2): 81–107. [224–225.]

Lukhtanov VA, Dantchenko AV (2002b) Principles of highly ordered metaphase I bivalent arrangement in spermatocytes of *Agrodiaetus* (Lepidoptera). Chromosome Research 10(1): 5–20. https://doi.org/10.1023/A:1014249607796

Lukhtanov VA, Dantchenko AV (2017) A new butterfly species from south Russia revealed through chromosomal analysis of the *Polyommatus* (*Agrodiaetus*) *damonides* complex (Lepidoptera, Lycaenidae). Comparative Cytogenetics 11(4): 769–795. https://doi.org/10.3897/compcytogen.v11i4.20072

Lukhtanov VA, Iashenkova Y (2019) Linking karyotypes with DNA barcodes: proposal for a new standard in chromosomal analysis with an example based on the study of Neotropical Nymphalidae (Lepidoptera). Comparative Cytogenetics 13(4): 435–449. https://doi.org/10.3897/CompCytogen.v13i4.48368

Lukhtanov VA, Kandul NP, De Prins WO, van der Poorten D (1998) Karyology of species of *Polyommatus* (*Agrodiaetus*) from Turkey: new data and their taxonomic consequences (Lepidoptera: Lycaenidae). Holarctic Lepidoptera 5(1): 1–8.

Lukhtanov VA, Kandul NP, Plotkin JB, Dantchenko AV, Haig D, Pierce NE (2005) Reinforcement of pre-zygotic isolation and karyotype evolution in *Agrodiaetus* butterflies. Nature 436(7049): 385–389. https://doi.org/10.1038/nature03704

Lukhtanov VA, Vila R, Kandul NP (2006) Rearrangement of the *Agrodiaetus dolus* species group (Lepidoptera, Lycaenidae) using a new cytological approach and molecular data. Insect Systematics and Evolution 37(3): 325–334. https://doi.org/10.1163/187631206788838563

Lukhtanov VA, Shapoval NA, Dantchenko AV (2008) *Agrodiaetus shahkuhensis* sp. n. (Lepidoptera, Lycaenidae), a cryptic species from Iran discovered by using molecular and chromosomal markers. Comparative Cytogenetics 2(2): 99–114.

Lukhtanov VA, Shapoval NA, Dantchenko AV (2014) Taxonomic position of several enigmatic *Polyommatus* (*Agrodiaetus*) species (Lepidoptera, Lycaenidae) from Central and Eastern Iran: insights from molecular and chromosomal data. Comparative Cytogenetics 8(4): 313–322. https://doi.org/10.3897/CompCytogen.v8i4.8939

Lukhtanov VA, Dantchenko AV, Vishnevskaya MS, Saifitdinova AF (2015) Detecting cryptic species in sympatry and allopatry: analysis diversity in *Polyommatus* (*Agrodiaetus*) butterflies (Lepidoptera, Lycaenidae). Biological Journal of the Linnean Society 116: 468–485. https://doi.org/10.1111/bij.12596

Lukhtanov VA, Dantchenko AV, Khakimov FR, Sharafutdinov D, Pazhenkova EA (2020a) Karyotype evolution and flexible (conventional versus inverted) meiosis in insects with holocentric chromosomes: a case study based on *Polyommatus* butterflies. Biological Journal of the Linnean Society 130(4): 683–699. https://doi.org/10.1093/biolinnean/blaa077

Lukhtanov VA, Dincă V, Friberg M, Vila R, Wiklund C (2020b) Incomplete sterility of chromosomal hybrids: implications for karyotype evolution and homoploid hybrid speciation. Frontiers of Genetics. https://doi.org/10.3389/fgene.2020.583827

Maddison WP, Maddison DR (2015) Mesquite: a modular system for evolutionary analysis. Version 3.04. http://mesquiteproject.org

Przybyłowicz Ł, Lukhtanov V, Lachowska-Cierlik D (2014) Towards the understanding of the origin of the Polish remote population of *Polyommatus* (*Agrodiaetus*) *ripartii* (Lepidoptera: Lycaenidae) based on karyology and molecular phylogeny. Journal of Zoological Systematics and Evolutionary Research 52(1): 44–51. https://doi.org/10.1111/jzs.12040

Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA (2018) Posterior summarisation in Bayesian phylogenetics using Tracer 1.7. Systematic Biology 67: 901–904. https://doi.org/10.1093/sysbio/syy032

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Hohna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61: 539–542. https://doi.org/10.1093/sysbio/sys029

Shapoval NA, Yakovlev RV, Kuftina GN (2017) The taxonomic status of *Cossus cossus afghanistanus* (Lepidoptera, Cossidae) from Afghanistan: insights from molecular and morphological data. Ukrainian Journal of Ecology 7(3): 134–138. https://doi.org/10.15421/2017_61

Staudinger O (1899) Ueber die Arten and Formen der *Lycaena damon*-Gruppe. Deutsche Entomologische Zeitschrift Iris 12: 137–155.

Vershinina AO, Lukhtanov VA (2017) Evolutionary mechanisms of runaway chromosome number change in *Agrodiaetus* butterflies. Scientific Reports 7(8199): 1–9. https://doi.org/10.1038/s41598-017-08525-6

Vishnevskaya MS, Saifitdinova AF, Lukhtanov VA (2016) Karyosystematics and molecular taxonomy of the anomalous blue butterflies (Lepidoptera, Lycaenidae) from the Balkan Peninsula. Comparative Cytogenetics 10(5): 1–85. https://doi.org/10.3897/CompCytogen.v10i5.10944

Wiemers M (2003) Chromosome differentiation and the radiation of the butterfly subgenus *Agrodiaetus* (Lepidoptera: Lycaenidae: *Polyommatus*): a molecular phylogenetic approach. PhD Thesis, University of Bonn, Bonn, 203 pp.

Wiemers M, Keller A, Wolf M (2009) ITS2 secondary structure improves phylogeny estimation in a radiation of blue butterflies of the subgenus *Agrodiaetus* (Lepidoptera: Lycaenidae: *Polyommatus*). BMC Evolutionary Biology 9(300): 1–27. https://doi.org/10.1186/1471-2148-9-300

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SHORT COMMUNICATION

COMPARATIVE Cytogenetics
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New cytogenetic data for three species of Pentatomidae (Heteroptera): *Dichelops melacanthus* (Dallas, 1851), *Loxa viridis* (Palisot de Beauvois, 1805), and *Edessa collaris* (Dallas, 1851)

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Abstract

In this paper, we present new cytogenetic data for three species of the family Pentatomidae: *Dichelops melacanthus* (Dallas, 1851), *Loxa viridis* (Palisot de Beauvois, 1805), and *Edessa collaris* (Dallas, 1851). All studied species presented holocentric chromosomes and inverted meiosis for the sex chromosomes. *D. melacanthus* has $2n$ = 12 (10A + XY); *L. viridis* showed $2n$ = 14 (12A + XY); and *E. collaris* showed $2n$ = 14 (12A + XY). C-banding was performed for the first time in these species and revealed terminal and interstitial heterochromatic regions on the autosomes; DAPI/CMA_3 staining showed different fluorescent patterns. In all species, fluorescence *in situ* hybridization (FISH) with 18S rDNA probe identified signals on one autosomal bivalent, this being the first report of FISH application in the species *D. melacanthus* and *L. viridis*. The results obtained add to those already existing in the literature, enabling a better understanding of the meiotic behavior of these insects.

Keywords

Heterochromatin, Holocentric chromosome, Meiosis, Pentatomidae, rDNA-FISH

Introduction

The suborder Heteroptera has approximately 40,000 species distributed in seven infraorders (Enicocephalomorpha, Dipsocoromorpha, Gerromorpha, Nepomorpha, Leptopodomorpha, Cimicomorpha, and Pentatomomorpha) and is considered the largest and most diverse group of hemimetabolous insects (Štys and Kerzhner 1975; Weirauch and Schuh 2011). Although many of these insects play an important role as indicators of environmental quality (Brown 1997), other species are responsible for significant economic importance as vectors of diseases (Alevi et al. 2015) and agricultural pests (Schaefer and Panizzi 2000).

Pentatomidae are considered the fourth largest family in the suborder Heteroptera, with approximately 900 genera and almost 4,800 species classified in 10 subfamilies with a worldwide distribution (Rider 2011). The Neotropical region, which includes Brazil, is known for its vast biodiversity (Goldani 2012), where 230 genera and more than 1,400 Pentatomidae species have already been described (Grazia et al. 2015).

Several cytogenetic studies have been conducted on this insect family, where more than 300 species have been analyzed (Ueshima 1979; Rebagliati et al 2005; Kaur and Semahagn 2010; Kerisew 2012; Souza et al. 2007, 2011; Grozeva et al. 2011; Souza and Itoyama 2011; Bardella et al. 2013a, 2016a). The diploid numbers ranging from $2n = 6$ in *Rhytidolomia senilis* (Say, 1832) to $2n = 27$ in *Thyanta calceata* (Say, 1832) with a predominance of $2n = 14$ and sex chromosome system XX/XY. These insects like the rest species of Heteroptera have specific cytogenetic features, such as (i) holocentric chromosomes; (ii) kinetic activity located in the terminal region of chromosomes during meiosis (telokinetic activity); (iii) chiasmatic autosomal bivalents, in contrast to the sex chromosomes that are achiasmatic; and (iv) inverted meiosis in the sex chromosomes, which is different from the typical equation reduction-sequence observed in the meiosis of organisms with monocentric chromosomes (Ueshima 1979; Nokkala and Nokkala 1984, 1997; Nokkala and Grozeva 2000; Pérez et al. 2000; Papeschi et al. 2003; Poggio et al. 2009; Melters et al. 2012).

Because of the importance and diversity of the family Pentatomidae, we present cytogenetic data for three species of Pentatomidae in this paper: *Dichelops melacanthus* (Dallas, 1851), *Loxa viridis* (Palisot de Beauvois, 1805), and *Edessa collaris* (Dallas, 1851).

Methods

Chromosomal preparations and conventional staining

For this study, only male adults were used (Table 1). Specimens were collected with the authorization of the ICMBio (31946-4). The insects were anesthetized and dissected in a physiological solution for insects (7.5 g NaCl, 2.38 g Na_2HPO_4, and 2.72 g KH_2PO_4 in 1 l of distilled water). The gonads were washed with tap water and fixed in methanol and acetic acid (3:1, v:v). The slides were prepared based on the protocol of Pijnacker

Table 1. Studied species and collection sites.

Species	Number of samples (N)	Collection site
Dichelops melacanthus	40	District of Maravilha, Londrina, Paraná, Brazil (23°28'03"S, 51°00'46.3"W)
Loxa viridis	15	Iguaçu National Park in Foz do Iguaçu, Paraná, Brazil (25°04'–25°41"S, 53°58'–25°04"W)
Edessa collaris	15	Iguaçu National Park in Foz do Iguaçu, Paraná, Brazil (25°04'–25°41"S, 53°58'–25°04"W)

and Ferwerda (1984), using a portion of the testes, which was macerated in 45% acetic acid and then dried on a hot plate at 45–50 °C. These preparations were stained using conventional staining with Giemsa 3%.

C-banding and fluorochromes

The slides were submitted to C-banding following the protocol of Sumner (1972) with the modifications of Grozeva et al. (2011). The slides were treated with 0.2 N HCl solution at room temperature for 30 min, incubated in 5% barium hydroxide solution at room temperature for 8 min, and then incubated in 2 × SSC saline at 60 °C for 1 h. The slides were washed with distilled water; some were stained with propidium iodide according to Lui et al. (2012), and others were stained with the fluorochromes 4'6-diamidino-2-phenylindole (DAPI), which identify AT-rich regions, and chromomycin A3 (CMA_3), which identify GC-rich regions (Schweizer 1980).

DNA extraction and isolation of the 18S rDNA probe

Total DNA was extracted using the phenol-chloroform method of Sambrook and Russel (2006). The 18S rDNA probe was obtained via a polymerase chain reaction (PCR) using the primers Forward 5'-CCTGAGAAACGGCTACCACATC-3' and Reverse 5'-GAGTCTCGTTCGTTATCGGA-3', as described by Whting et al. (1997). The PCR was performed with a final volume of 25 µl containing 100 ng of genomic DNA (1 µl), 10 mM primer (1 µl each), 10 mM dNTP mix (1 µl), 50 mM $MgCl_2$ (1.5 µl), and 10 × PCR buffer (2.5 µl); Taq polymerase at 5 U/µl (0.5 µl) was added to ultrapure water to complete the reaction. The PCR was used in the following conditions: first step at 94 °C for 2 min, followed by 35 cycles at 94 °C for 1 min, 58 °C for 1 min, 72 °C for 1 min, and a final extension of 72 °C for 5 min. Amplified 18S rDNA probes were labeled using digoxigenin-11-dUTP (Roche Applied Science, Indianapolis, EUA).

Fluorescence *in situ* hybridization (FISH)

FISH was based on the protocol of Schwarzacher and Heslop-Harrison (2000): the slides were treated with RNase (0.4% RNAse/2 × SSC) and pepsin (0.005%) for 1 h and 10 min, respectively, both at 37 °C, and dehydrated in ethanol series (75% and 100%) for 3 min each. Subsequently, 40 µl of the hybridization mix containing 100% formamide (20 µl),

50% polyethylene glycol (8 µl), 20 × SSC (4 µl), 10% sodium dodecyl sulfate (SDS) (1 µl), and 100 ng of probes (4 µl) was added to ultra-pure water to complete the reaction. The hybridization mix was denatured at 75 °C for 10 min and then was transferred to the ice. After this time, the mix was applied onto the slides and taken to the thermocycler for denaturing/renaturing following the steps (90 °C, 56 °C, and 38 °C for 10 min each); hybridization occurred at 37 °C in a humidified chamber overnight. After probe detection with anti-digoxigenin-rhodamine (Roche Applied Science, Indianapolis, EUA), the chromosomes were counterstained with DAPI and mounted in Vectashield (Thermo Fisher Scientific, Massachusetts, EUA) and left in the dark for 24 h before analysis.

The slides were analyzed in an epifluorescence microscope (Leica DM 2000), which was equipped with a digital camera Moticam Pro 282B. The images were captured using Motic Images Advanced software, version 3.2. The chromosome images were acquired separately with specific filters for each fluorophore or in light field.

Results

The stink bug *D. melacanthus* had $2n = 12$ (10A + XY) (Fig. 1a, b) with one pair of autosomes larger than the other autosomes. *L. viridis* (Fig. 1g, h) and *E. collaris* (Fig. 1m, n) had chromosomes of homogeneous size and $2n = 14$ (12A + XY), in both species. The sex chromosome system of all species was simple (XY male). The analysis of the meiotic behavior revealed a kinetic activity in the terminal regions of the chromosomes owing to their positioning and migration to the opposite poles. In metaphase II, it was possible to observe a radial plate the autosomes forming a ring and the sex chromosomes positioned in the center of the ring in a brief association, known as touch-and-go-pairing in all species (Fig. 1b, h, n).

In all analyzed species, a heterochromatic region corresponding to sex chromosomes was observed, which are associated in the early stages of meiosis (Fig. 1c, i, o). In *D. melacanthus*, the DAPI/CMA_3 staining was homogeneous for all chromosomes (Fig. 1d, e). In *L. viridis* presented interstitial and terminal heterochromatic regions in some autosomes (Fig. 1i), and after staining with fluorochromes, the Y chromosome stood out as $DAPI^+$ (Fig. 1j). A similar pattern was observed in one of the autosomes but with more discrete coloration; the $DAPI^+$ interstitial regions were not so evident in another autosome (Fig. 1j). Staining with CMA_3 showed very weak terminal dots in a bivalent (Fig. 1k). The specie *E. collaris* showed terminal and interstitial heterochromatic bands in the autosomal bivalents in addition to the sex chromosomes that were associated (Fig. 1o). The DAPI/CMA_3 staining revealed the presence of several interstitial and a terminal $DAPI^+$ bands (Fig. 1p) as well as CMA_3^+ bright terminal dots in a bivalent, and the $DAPI^+/CMA_3^+$ sex chromosomes (Fig. 1p, q).

FISH revealed the following distribution patterns of 18S rDNA among species: *D. melacanthus* showed discrete dots in the terminal region of the larger bivalent at metaphase I (Fig. 1f); two 18S rDNA sites in the terminal region of a bivalent were observed in the initial meiotic phases of *L. viridis* and *E. collaris* (Fig. 1l, r).

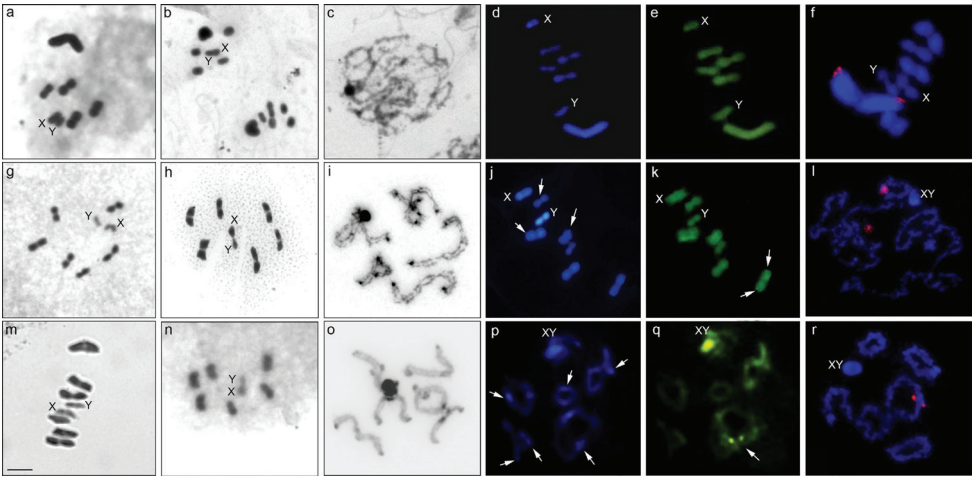

Figure 1. Meiotic stages of *Dichelops melacanthus* (**a–f**), *Loxa viridis* (**g–l**) and *Edessa collaris* (**m–r**). (**a, g** and **m**) metaphase I by Giemsa conventional staining; (**b, h** and **n**) metaphase II by Giemsa conventional staining; (**c, i** and **o**) C-banding pachytenes; (**d** and **j**) metaphase I by DAPI staining; (**p**) diplotene by DAPI staining; (**e** and **k**) metaphase I by CMA_3 staining; (**q**) diplotene by CMA_3 staining; (**f, l** and **r**) Fluorescence *in situ* hybridization with digoxigenin-labeled 18S rDNA probe and counterstained with DAPI. X and Y correspond to the sex chromosomes. Arrows show heterochromatic marks in autosomes. Scale bar: 10 μm.

Discussion

Conventional staining analysis performed here confirmed the presence of holocentric chromosomes and kinetic activity localized in the terminal region during meiosis, as observed in most Heteroptera (Ueshima 1979; Nokkala and Nokkala 1984, 1997; Nokkala and Grozeva 2000; Pérez et al. 2000; Papeschi et al. 2003; Poggio et al. 2009; Melters et al. 2012). In addition, it was possible to observe the occurrence of inverted meiosis for the sex chromosomes, as evidenced by the presence of these chromosomes as univalents in metaphase I and the presence of heteromorphic chromatids with touch-and-go-pairing behavior in metaphase II, a feature already reported in other species of Pentatomidae (Viera et al. 2009).

According to data available for Pentatomidae (Rebagliati et al. 2005; Bardella et al. 2013a), a diploid number conservation of *L. viridis* ($2n$ = 14) was observed. *D. melacanthus* presented $2n$ = 12 (10A + XY), as previously observed by other authors (Rebagliati et al. 2002, 2005; Souza et al. 2011). The population of *E. collaris* analyzed in this study had $2n$ = 14 (12A + XY); however, Souza et al. (2011) reported $2n$ = 12 (10A + XY) for the same species, which may indicate an interpopulation polymorphism because the collection sites were distinct. Another explanation of the difference in the chromosome number of *E. collaris* could be an error in species identification because of the morphological similarity between species in this genus, making identification difficult (Fernandes and Doesburg 2000).

The stink bug *D. melacanthus* was the only species in the study that presented $2n = 12$ (10A + XY), a result confirming previous observations reported for other populations of this species (Rebagliati et al. 2002, 2005; Souza et al. 2011). This diploid number is described in nine other Pentatomidae species: *Euschistus crassus* Dallas, 1851 (Foot and Strobell 1912 according to Ueshima 1979, Hughes-Schrader and Schrader 1956); *Oechalia patruelis* Stål, 1859 (Heizer 1950), *Scotinophara* sp. (Jande 1959 and 1960c according to Rebagliati et al. 2005), *Scotinophara coarctata* Fabricius, 1979 (Satapathy et al. 1990 according to Rebagliati et al. 2005), *Dichelops furcatus* Fabricius, 1775 (Rebagliati et al. 2001), *Mecocephala maldonadensis* Schwertner, Grazia, and Fernandes, 2002 (Rebagliati et al. 2005), *Acledra bonariensis* Stål, 1859 (Rebagliati and Mola 2010), *Edessa collaris* (Souza et al. 2011) and *Cahara confusa* Distant, 1879 (Kaur and Sharma 2015).

Apart from the differences in the diploid number, *D. melacanthus* was distinguished by the presence of a pair of autosomal chromosomes of large size in relation to the other chromosomes. According to Souza et al. (2011), the presence of this visibly larger autosomal pair suggests that this reduced karyotype originated through a fusion between two autosomes. The same characteristic has also been observed in other species of the family with $2n = 12$, as in *E. crassus*, *D. furcatus*, *M. maldonadensis*, and *A. bonariensis* (Hughes-Schrader and Schrader 1956; Ueshima 1979; Rebagliati et al. 2001, 2005; Rebagliati and Mola 2010), which supports the hypothesis of the fusions resulting in a reduction of the diploid number in this group.

In all species studied, the location and composition of heterochromatin was first performed. In relation to the characteristics of heterochromatin in the autosomes, we can classify the species studied into two distinct patterns: (i) presence of AT-rich heterochromatin as in *D. melacanthus* and (ii) predominance of $DAPI^+$ blocks and few CMA_3^+ blocks as in *L. viridis* and *E. collaris*. According to Poggio et al. (2011), most of the reports concerning the characterization of heterochromatin in the autosomes in species of the order Hemiptera are described as $DAPI^+$, as was reported by Bressa et al. (2005) in *Athaumastus haematicus* (Stål, 1860), *Leptoglossus impictus* (Stål, 1859), *Phthia picta* (Drury, 1770) (Coreidae), *Largus rufipennis* (Laporte, 1832) (Largidae) and *Jadera sanguinolenta* (Fabricius, 1775) (Rhopalidae), and by Franco et al. (2006) in *Spartocera batatas* (Fabricius, 1798) (Coreidae).

The heterogeneity of heterochromatin in chromosomes was observed. In *D. melacanthus* and *L. viridis*, the sex Y chromosome was completely heterochromatic and $DAPI^+$, while the sex X chromosome in these two species showed homogeneous staining with both DAPI and CMA_3. In most species of Heteroptera, the Y chromosome presents a large amount of heterochromatin, sometimes being completely heterochromatic (Grozeva and Nokkala 2001). This has been reported for the subfamily Triatominae (Panzera et al. 1995), in species of Belostomatidae (Papeschi 1988) and in three species of pentatomids of the genus *Antiteuchus* (Dallas, 1851) (Lanzone and Souza 2006). Although studies in Reduviidae show that the DAPI positive Y chromosome is quite common, particularly in species of the Triatomini tribe (Bardella et al. 2016b), it is not observed with the same frequency in Pentatomidae,

being pointed out only in *Halys serrigera* (Westwood, 1837) and *Perillus bioculatus* (Fabricius, 1775) (Kerisew 2012).

In this study, *E. collaris* presented associated sex chromosomes and $DAPI^+/CMA_3^+$ in early meiotic phases. This has also been reported in *Nabis viridulus* Spinola, 1837 (Grozeva et al. 2004) and in species of the genus *Edessa* in *E. meditabunda* (Fabricius, 1974) *and E. rufomarginata* (De Geer, 1773) (Rebagliati et al. 2003).

Studies on the characterization and localization of heterochromatin are important because in addition to the numerous functions that it performs during the cell cycle, it is related to karyotype evolution since chromosomal breaks and rearrangements occur frequently in these regions (Huisinga et al. 2006; Grewal and Jia 2007). The occurrence of small CMA_3^+ blocks and/or dots related to co-localization with the nucleolus-organizing regions is a common feature (Camacho et al. 1985; Rebagliati et al. 2001; Bardella et al. 2013b). We confirmed this for *E. collaris*, where the heterochromatic dots showed specificity to the fluorochrome CMA_3, and subsequently by 18S rDNA hybridization. A higher percentage of CG repeats in the nucleotide composition of the 18S gene has already been observed by Bargues et al. (2000) in study with triatomines.

Signals of 18S rDNA in a single bivalent were observed for all species of this study, and this pattern is commonly found in the species of the Pentatomidae (Papeschi et al. 2003; Grozeva et al. 2015). Most studies of the Pentatomidae report the presence of this cluster on an autosomal pair (Papeschi et al. 2003; Cattani and Papeschi 2004; Cattani et al. 2004; Bressa et al. 2008, 2009; Grozeva et al. 2011, 2015; Bardella et al. 2013a, 2016a; Souza-Firmino et al. 2020).

In this study, first data on FISH with the 18S rDNA probe with *D. melacanthus* and *L. viridis* are presented and both species showed terminal blocks in autosomes, being the larger bivalent in *D. melacanthus*. This terminal location is highly conserved in the infraorder Pentatomomorpha, even in related species that exhibit wide variations in chromosome number; chromosome position of the 18S rDNA sites is commonly subterminal (Bardella et al. 2013a). The species *E. collaris* showed two signals of hybridization in an autosomal bivalent, as previously reported by Souza-Firmino et al. (2020).

Our results confirm the karyotype conservation of the family and present original cytogenetic data for three species: (i) analysis of heterochromatin in all species; and (ii) FISH with 18S rDNA probe data for *D. melacanthus* and *L. viridis*. In conclusion, we present new data for future studies that can collaborate in the evolutionary study of the Pentatomidae family.

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References

Alevi KCC, Rodas LAC, Tartarotti E, Azeredo-Oliveira MTV, Guirado MM (2015) Entoepidemiology of Chagas disease in the Western region of the State of São Paulo from 2004 to 2008, and cytogenetic analysis in *Rhodnius neglectus* (Hemiptera, Triatominae). Genetics and Molecular Research 14(2): 5775−5784. https://doi.org/10.4238/2015.may.29.9

Bardella VB, Fernandes T, Vanzela ALL (2013a) The conservation of number and location of 18S sites indicates the relative stability of rDNA in species of Pentatomomorpha (Heteroptera). Genome 56: 425−429. https://doi.org/10.1139/gen-2013-0140

Bardella VB, Grazia J, Fernandes AM, Vanzela ALL (2013b) High Diversity in CMA3/DAPI-Banding Patterns in Heteropterans. Cytogenetic and Genome Research 142: 46−53. https://doi.org/10.1159/000355214

Bardella VB, Fernandes JAM, Cabral-de-Mello DC (2016a) Chromosomal evolutionary dynamics of four multigene families in Coreidae and Pentatomidae (Heteroptera) true bugs. Molecular Genetics and Genomics 291: 1919−1925. https://doi.org/10.1007/s00438-016-1229-5

Bardella VB, Pita S, Vanzela ALL, Galvão C, Panzera F (2016b) Heterochromatin base pair composition and diversification in holocentric chromosomes of kissing bugs (Hemiptera, Reduviidae). Memórias do Instituto Oswaldo Cruz 111(10): 614−624. https://doi.org/10.1590/0074-02760160044

Bargues MD, Marcilla A, Ramsey J, Dujardin JP, Schofield CJ, Mas-Coma S (2000) Nuclear rDNA-based molecular clock of the evolution of Triatominae (Hemiptera: Reduviidae), vectors of Chagas disease. Memórias do Instituto Oswaldo Cruz 95: 567−573. https://doi.org/10.1590/s0074-02762000000400020

Bressa MJ, Larramendy ML, Papeschi AG (2005) Heterochromatin characterization in five species of Heteroptera. Genetica 124: 307−317. https://doi.org/10.1007/s10709-005-4524-3

Bressa MJ, Franco MJ, Toscani MA, Papeschi AG (2008) Heterochromatin heteromorphism in *Holhymenia rubiginosa* (Heteroptera: Coreidae). European Journal of Entomology 105: 65−72. https://doi.org/10.14411/eje.2008.009

Bressa MJ, Papeschi AG, Vitková M, Kubícková S, Fuková I, Pigozzi MI, Marec F (2009) Sex chromosome evolution in cotton stainers of the genus *Dysdercus* (Heteroptera: Pyrrhocoridae). Cytogenetic and Genome Research 125: 292−305. https://doi.org/10.1159/000235936

Brown KS (1997) Diversity, disturbance, and sustainable use of Neotropical forest: insects as indicators for conservation monitoring. Journal of Insect Conservation 1(1): 25−42. https://doi.org/10.1023/A:1018422807610

Camacho JPM, Belda J, Cabrejo J (1985) Meiotic behavior of the holocentric chromosomes of *Nezara viridula* (Insecta, Heteroptera) analysed by C-band and silver impregnation. Canadian Journal of Genetics and Cytology 27: 490−497. https://doi.org/10.1139/g85-073

Cattani MV, Papeschi AG (2004) Nucleolus organizing regions and semipersistent nucleolar during meiosis in *Spartocera fusca* (Thunberg) (Coreidae: Heteroptera). Hereditas 140: 105−111. https://doi.org/10.1111/j.1601-5223.2004.01752.x

Cattani MV, Greizerstein EJ, Papeschi AG (2004) Male meiotic behaviour and nucleolus organizing regions in *Camptischium clavipes* (Fabr.) (Coreidae, Heteroptera) analyzed by

fluorescent banding and in situ hybridization. Caryologia 57: 267–273. https://doi.org/10.1080/00087114.2004.10589403

Fernandes JAM, Doesburg van PH (2000) The *E. dolichocera*-group of *Edessa* Fabricus, 1803 (Heteroptera: Pentatomidae: Edessinae). Zoologische Mededelingen 73: 305–315.

Franco MJ, Bressa MJ, Papeschi AG (2006) Karyotype and male meiosis in *Spartocera batatas* and meiotic behaviour of multiple sex chromosomes in Coreidae (Heteroptera). European Journal of Entomology 103: 9–16. https://doi.org/10.14411/eje.2006.002

Goldani A (2012) Neotropical historical biogeography: parsimony analysis of endemicity with distributional data of fish. Revista Eletrônica de Biologia 5(3): 12–41.

Grazia J, Panizzi AR, Greve C, Schwertner CF, Campos LA, Garbelotto TA, Fernandes JAM (2015) Stink Bugs (Pentatomidae). Chapter 22 In: Panizzi AR, Grazia J (Eds) True bug (Heteroptera of the Neotropics). Entomology in Focus 2, Springer, 681–756. https://doi.org/10.1007/978-94-017-9861-7_22

Grewal SI, Jia S (2007) Heterochromatin revisited. Nature Reviews Genetics 8(1): 35–46. https://doi.org/10.1038/nrg2008

Grozeva SM, Nokkala S (2001) Chromosome numbers, sex determining systems, and patterns of the C-heterochromatin distribution in 13 species of lace bugs (Heteroptera, Tingidae). Folia Biologica 49: 29–41.

Grozeva S, Kuznetsova V, Nokkala S (2004) Patterns of chromosome banding in four nabid species (Heteroptera, Cimicomorpha, Nabidae) with high chromosome number karyotypes. Hereditas 140: 99–104. https://doi.org/10.1111/j.1601-5223.2004.01782.x

Grozeva S, Kuznetsova VG, Anokhin BA (2011) Karyotypes, male meiosis and comparative FISH mapping of 18S ribosomal DNA and telomeric (TTAGG)n repeat in eight species of true bugs (Hemiptera, Heteroptera). Comparative Cytogenetics 5(4): 355–374. https://doi.org/10.3897/compcytogen.v5i4.2307

Grozeva, S, Anokhin, BA, Kuznetsova, VG (2015) Bed bugs (Hemiptera). Chapter 8 In: Sharakhov I (Ed.) Protocols for Cytogenetic Mapping of Arthropod Genomes, Boca Raton: CRC press, https://doi.org/10.1201/b17450-9

Heizer P (1950) The chromosome cytology of two species of the Pacific genus *Oechalia* (Pentatomidae, Hemiptera-Heteroptera), *Oechalia patruelis* Stal and *Oechalia pacifica* Stal. Journal of Morphology 87: 179–226. https://doi.org/10.1002/jmor.1050870105

Hughes-Schrader S, Schrader F (1956) Polyteny as a factor in the chromosomal evolution of the Pentatomini (Hemiptera). Chromosoma 8: 135–151. https://doi.org/10.1007/BF01259497

Huisinga KL, Brower-Toland B, Elgin SCR (2006) The contradictory definitions of heterochromatin: transcription and silencing. Chromosoma 115: 110–122. https://doi.org/10.1007/s00412-006-0052-x

Kaur H, Semahagn BK (2010) Meiotic behaviour of chromosomes in three predator species of the subfamily Asopinae (Heteroptera: Pentatomidae). Comparative Cytogenetics 4(2): 133–139. https://doi.org/10.3897/compcytogen.v4i2.47

Kaur H, Sharma K (2015) First report on meiotic behavior of chromosomes in three species of subfamily Pentatominae (Pentatomidae: Heteroptera). Zootaxa 3929(2): 393–400. https://doi.org/10.11646/zootaxa.3920.2.10

Kerisew B (2012) Cytogenetic Investigations on Some Species of the Family Pentatomidae (Insecta: Hemiptera: Heteroptera), p 169. Ph.D. Thesis, India: Punjabi University.

Lanzone C, Souza MJ (2006) C-banding, fluorescent staining and NOR location in holocentric chromosomes of bugs of the Neotropical genus *Antiteuchus* (Heteroptera: Pentatomidae: Discocephalinae). European Journal of Entomology 13: 239–243. https://doi.org/10.14411/eje.2006.026

Lui RL, Blanco DR, Moreira-Filho O, Margarido VP (2012) Propidium iodide for making heterochromatin more evident in the C-banding technique. Biotechnic & Histochemistry 87(7): 433–438. https://doi.org/10.3109/10520295.2012.696700

Melters DP, Paliulis LV, Korf IF, Chan WL (2012) Holocentric chromosomes: convergente evolution, meiotic adaptations, and genomic analysis. Chromosome Research 20: 579–593. https://doi.org/10.1007/s10577-012-9292-1

Nokkala S, Nokkala C (1984) The occurrence of the XO sex chromosome system in *Dictyonota tricornis* (Schr.) (Tingidae, Hemiptera) and its significance for concepts of sex chromosome system evolution in Heteroptera. Hereditas 100: 299–301. https://doi.org/10.1111/j.1601-5223.1984.tb00130.x

Nokkala S, Nokkala C (1997) The absence of chiasma terminalization and inverted meiosis in males and females of *Myrmus miriformis* Fn. (Corizidae, Heteroptera). Heredity 78: 561–566. https://doi.org/10.1038/hdy.1997.87

Nokkala S, Grozeva S (2000) Achiasmatic male meiosis in *Myrmedobia coleoptrata* (Fn.) (Heteroptera, Microphysidae). Caryologia 53(1): 5–8. https://doi.org/10.1080/00087114.2000.10589176

Panzera F, Perez R, Panzera Y, Alvarez F, Scvortzoff E, Salvatella R (1995) Karyotype evolution holocentric chromosomes of three related species of triatomines (Hemiptera-Reduviidae). Chromosome Research 3: 143–150. https://doi.org/10.1007/bf00710707

Papeschi AG (1988) C-banding and DNA content in three species of *Belostoma* (Heteroptera) with large differences in chromosome size and number. Genetica 76: 43–51. https://doi.org/10.1007/BF00126009

Papeschi AG, Mola LM, Bressa MJ, Greizerstein EJVL, Poggio L (2003) Behaviour of ring bivalents in holokinetic systems: alternative sites of spindle attachment in *Pachylis argentinus* and *Nezara viridula* (Heteroptera). Chromosome Research 11: 725–733. https://doi.org/10.1023/B:CHRO.0000005740.56221.03

Pérez R, Rufas JS, Suja JA, Page J, Panzera F (2000) Meiosis in holocentric chromosomes: orientation and segregation of anautosome and sex chromosomes in *Triatoma infestans* (Heteroptera). Chromosome Research 8(1): 17–25. https://doi.org/10.1023/a:1009266801160

Pijnacker LP, Ferwerda MA (1984) Giemsa C-banding of potato chromosomes. Canadian Journal of Genetics and Cytology 26(4): 415–419. https://doi.org/10.1139/g84-067

Poggio MG, Bressa MJ, Papeschi AG (2011) Cytogenetics male meiosis, heterochromatin characterization and chromosomal location of rDNA in *Microtomus lunifer* (Berg, 1900) (Hemiptera: Reduviidae: Hammacerinae). Comparative Cytogenetics 5(1): 1–22. https://doi.org/10.3897/compcytogen.v5i1.1143

Poggio MG, Bressa MJ, Papeschi AG, Di Iorio O, Turienzo PN (2009) Insects found in birds' nests from Argentina: cytogenetic studies in Cimicidae (Hemiptera) and its taxo-

nomical and phylogenetic implications. Zootaxa 2315: 39–46. https://doi.org/10.11646/zootaxa.2315.1.4

Rebagliati PJ, Mola LM (2010) Meiotic behavior and karyotypic variation in *Acledra* (Pentatomidae, Heteroptera). Genetics and Molecular Research 9(2): 739–749. https://doi.org/10.4238/vol9-2gmr763

Rebagliati PJ, Mola LM, Papeschi AG (2001) Karyotype and meiotic behaviour of the holokinetic chromosomes of six Argentine species of Pentatomidae (Heteroptera). Caryologia 54: 339–347. https://doi.org/10.1080/00087114.2001.10589245

Rebagliati PJ, Papeschi AG, Mola LM (2002) Cytogenetics of Pentatomidae. In: Kerzhner IM (Eds). 2nd Quadrennial Meeting of the Heteropterists Society. St Petersburg, Abstracts book, 1–49.

Rebagliati PJ, Papeschi AG, Mola LM (2003) Meiosis and fluorescent banding in *Edessa meditabunda* and *E. rufomarginata* (Heteroptera, Pentatomidae, Edessinae). European Journal of Entomology 100: 11–18. https://doi.org/10.14411/eje.2003.002

Rebagliati PJ, Mola LM, Papeschi AG, Grazia J (2005) Cytogenetic studies in Pentatomidae (Heteroptera): A review. Journal of Zoological Systematics and Evolutionary Research 43(3): 199–213. https://doi.org/10.1111/j.1439-0469.2005.00312.x

Rider DA (2011) Pentatomoidea Home page. North Dakota State University. http://www.ndsu.nodak.edu/ndsu/rider/Pentatomoidea/. Access in: 05 Jan 2019.

Sambrook J, Russell DW (2006) Purification of nucleic acids by extraction with phenol: chloroform. Cold Spring Harbor Protocols (1): pdb.prot4455. https://doi.org/10.1101/pdb.prot4455

Schaefer CW, Panizzi AR (2000) Economic Importance of Heteroptera: A General View. In: Schaefer CW, Panizzi AR (Eds.) Heteroptera of economic importance. Boca Raton: CRC press, 3–8. https://doi.org/10.1201/9781420041859.ch1

Schwarzacher T, Heslop-Harrison JS (2000) Pratical *in situ* hybridization. BIOS Scientific Publishers Ltda, Oxford, 199 pp.

Schweizer D (1980) Simultaneous fluorescente staining of R bands and specific heterochromatic regions (DA-DAPI bands) in human chromosomes. Cytogenetics and Cell Genetics 27: 190–193. https://doi.org/10.1159/000131482

Souza HV, Itoyama MM (2011) Comparative analysis of heteropyknotic chromatin and silver-stained material in *Platycarenus umbractulatus* (Pentatomidae: Discocephalinae). Invertebrate Reproduction & Development 55(2): 65–72. https://doi.org/10.1080/07924259.2010.548637

Souza HV, Bicudo HEMC, Costa LAA, Itoyama MM (2007) Study of meiosis and spermiogenesis in the testicular lobes of *Antiteuchus tripterus* (Heteroptera: Pentatomidae). European Journal of Entomology 104: 353–362. https://doi.org/10.14411/eje.2007.053

Souza HV, Murakami AS, Moura J, Almeida EC, Marques IFG, Itoyama MM (2011) Comparative analysis of the testes and spermatogenesis in species of the Family Pentatomidae (Heteroptera). European Journal of Entomology 108: 333–345. https://doi.org/10.14411/eje.2011.041

Souza-Firmino TS, Alevi KCC, Itoyama MM (2020) Chromosomal divergence and evolutionary inferences in Pentatomomorpha infraorder (Hemiptera, Heteroptera) based on

the chromosomal location of ribosomal genes. PLoS One 15(2): e0228631. https://doi.org/10.1371/journal.pone.0228631

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

Štys P, Kerzhner I (1975) The rank and nomenclature of higher taxa in recent Heteroptera. Acta Entomologica Bohemoslovaca 72: 65–79.

Ueshima N (1979) Hemiptera II: Heteroptera. In: Bernard J (Eds) Animal cytogenetics, Insecta Berlin (Germany) 3, 117pp.

Viera A, Page J, Rufas JS (2009) Inverted meiosis: the true bugs as a model to study. Genome Dynamics 5: 137–156. https://doi.org/10.1159/000166639

Weirauch C, Schuh R (2011) Systematics and Evolution of Heteroptera. Annual Review of Entomology 56: 487–510. https://doi.org/10.1146/annurev-ento-120709-144833

Whiting MF, Carpenter JC, Wheeler QD, Wheeler WC (1997). The Strepsiptera problem: phylogeny of the holometabolous insect orders inferred from 18S and 28S ribosomal DNA sequences and morphology. Systematic Biology 46(1): 1–68. https://doi.org/10.1093/sysbio/46.1.1

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SHORT COMMUNICATION

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Homologous series by Nikolai Vavilov in the phylogeny of Homoptera*

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Abstract

The paper briefly discusses the most impressive examples of the Nikolai Vavilov's "Law of homologous series" in the evolution of one of the largest animal groups, homopterous insects, which comprise about 65,000 recent species in the world fauna. Different taxonomic and phylogenetic characters (morpho-anatomical, cytogenetic, reproductive and others) are considered at the taxonomic ranks of the order, suborder, superfamily and family.

Keywords

Aphids, cicadas, homologous variability, parallel evolution, psyllids, scale insects, whiteflies

Introduction

The famous geneticist and evolutionist Nikolai I. Vavilov (1887–1943) manifested his "Law of homologous series in variation" one hundred years ago (Vavilov 1920; Kolchinsky 2017; Bulatova 2020a). The Law was described as a universal rule which is applicable to all plants, animals and microorganisms, although, N.I. Vavilov was primarily a botanist and illustrated his findings mainly by examples from plant morphology, physiology and genetics. In view of this fact, it is not surprising that the Law

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was subsequently cited predominantly in the works of botanists, geneticists and historians interested in biology (see, for example, Goncharov 2014; Kolchinsky 2017). Zoological illustrations of the Law, although rather rare, could be found, for example, in the paper on the evolution of the morphological characters of Echinodermata in paleontological material (Rozhnov 2006), in the discussion of fur color variability in farm bred American mink (Trapezov 2007), in the article on natural chromosomal variability of the common shrew (Bulatova 2020b) and some others scientific publications. It also worth to mention, that some of the evolutional trends in the Animalia described with such terms as "arthropodization", "ornithization", "mammalization" (see for review Markov 2020) could also be considered in the frames of the Law of homologous series.

The present paper will briefly describe Vavilov's Law of homologous series in the evolution of one of the largest animal groups, homopterous insects. The order Homoptera comprises about 65,000 recent species in the world fauna. It is subdivided into five recent suborders: Aphidinea (about 6,000 species), Coccinea (8,000 species), Psyllinea (3,500 species), Aleyrodinea (1,500 species) and Cicadinea (47,000 species). More detailed information about general classification and nomenclature of these taxonomic groups can be found, for example, in the monographs of Danzig and Gavrilov-Zimin (2014: 36) or Gavrilov-Zimin (2018: 21). There are also numerous publications addressing the phylogeny of these organisms. One of the main problems uniting majority of those publications is poor understanding of the differences between synapomorphic characters, inherited from the common ancestor, and parallelisms or homologous characters in N. Vavilov's sense. The last characters evolve independently in the related organisms because their related genomes demonstrate similar response to a similar environment pressure.

Homologous characters at the order level

Some authors (for example, Emeljanov 1987: 22) identify several morphological structures of the wing apparatus as synapomorphies of Cicadinea and true bugs (order Heteroptera). If those characters were really inherited by both groups from the common ancestor, it would be a proof of the paraphyly in the order Homoptera, which has Heteroptera evolved inside the group. Although, both Cicadinea and Heteroptera are characterized by deep adaptations for the perfect flight, all other suborders of Homoptera as well as the members of the related order Thysanoptera (thrips) have significantly reduced wing structure and are able to fly rather badly or not at all (for example, females of all 8,000 species of scale insects and many species of aphids completely lost their wings). In a view of this fact, it is not unlikely that the progressive adaptations of Cicadinea and Heteroptera to the flight evolved independently during the parallel evolution as a result of the homologous changes in the related groups of genes. Unfortunately, it is impossible now to unequivocally prove or reject this assumption without detailed molecular studies of the appropriate genes. Moreover, even the structure of

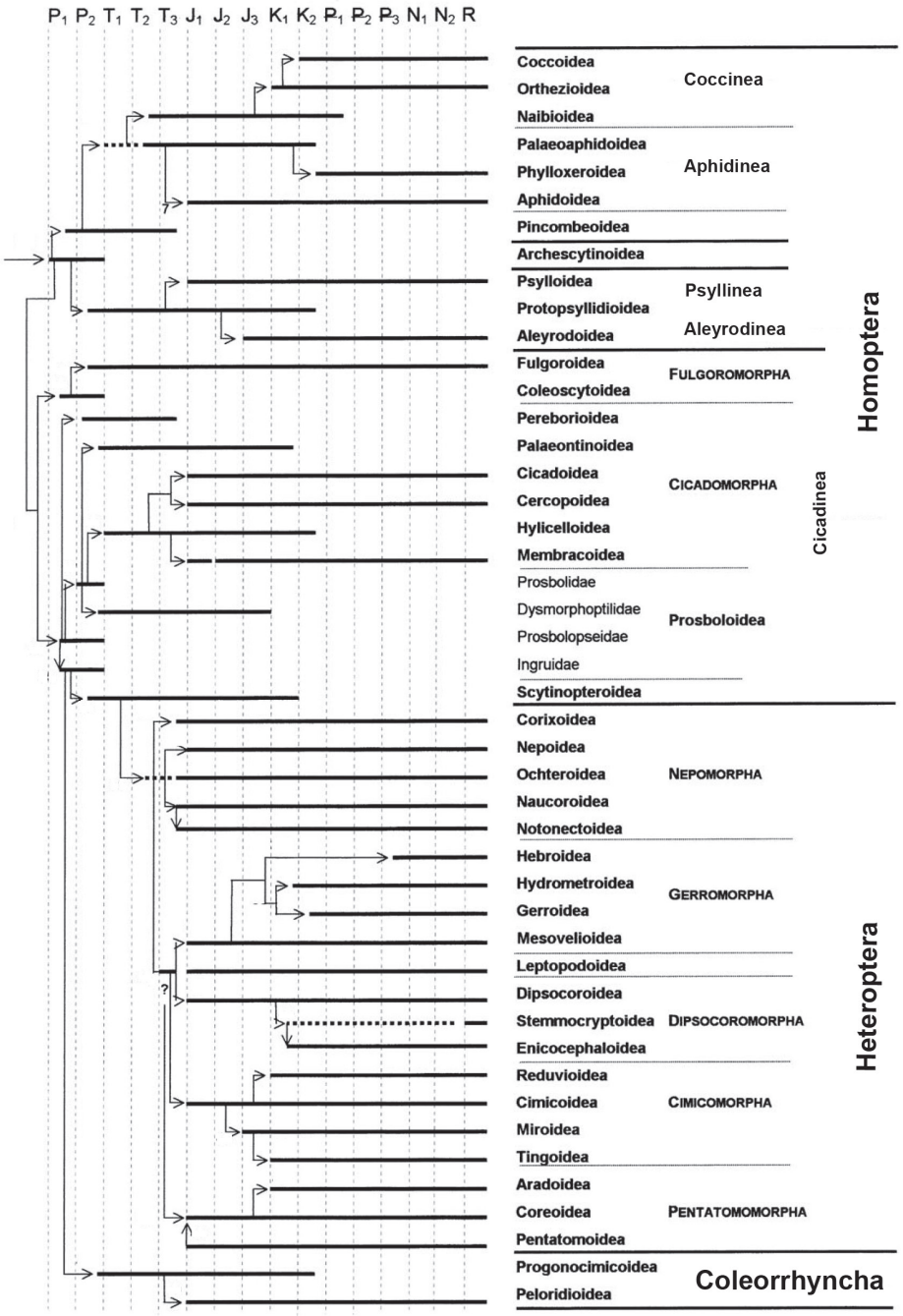

Figure 1. Reconstruction of the phylogeny of Homoptera and related insects, placed on geochronological scale (after Shcherbakov and Popov 2002: 146, with changes). Time periods $\mathbf{P_1}$, $\mathbf{P_2}$ Early (Lower) and Late (Upper) Permian $\mathbf{T_1}$, $\mathbf{T_2}$, $\mathbf{T_3}$ Early, Middle and Late Triassic $\mathbf{J_1}$, $\mathbf{J_2}$, $\mathbf{J_3}$ Early, Middle and Late Jurassic $\mathbf{K_1}$, $\mathbf{K_2}$ Early and Late Cretaceous $\mathbf{P_1}$ Palaeocene $\mathbf{P_2}$ Eocene $\mathbf{P_3}$ Oligocene $\mathbf{N_1}$ Miocene $\mathbf{N_2}$ Pliocene **R** present time (Holocene).

the wing coupling, which is one of the characters, the most studied phylogenetically, is considered by some authors (for example, D'Urso 2002) in the opposite sense compared to the Emeljanov's (l.c.) conclusions. Several additional morphological characters, not related to the wing apparatus, were also hypothesized as synapomorphies of Cicadinea and Heteroptera by Kluge (2020: 582–585). Unfortunately, those characters were studied on few exemplary species only and accepted with the different exclusions and stipulations, so, they, in my mind, cannot also be used for the characterization of such huge taxonomic groups as a whole.

On the other hand, the presence of the fields of wax glands, well-studied on large material in all archaic groups of Homoptera as well as in many younger groups of this order (see, for example, Šulc 1929; Emeljanov 2009; Danzig and Gavrilov-Zimin 2014, 2015; Gavrilov-Zimin 2018 and others), is undoubtedly an example of a good synapomorphic character, which is being not in direct connection with the adaptation to feeding on plant sup. The reliable proof of such phylogenetic assumption is the total absence of the homologous character (i.e., the fields of wax glands) in the numerous families of sap-sucking true bugs (Heteroptera) and thrips (Thysanoptera).

Another phylogenetically important question is the origin of the filter chamber in the digestive tract of Homoptera (see the review in Emeljanov 1987: 67), which is still remaining under discussion. The chamber was reported in most groups of Homoptera, but has not been found in any Heteroptera and Thysanoptera. That would be considered as an argument for the apomorphic origin of the chamber in Homoptera. On the other hand, the large variation of the fine structure of the filter chamber in different families may allude to the parallel homologous origin of this organ in accordance to Vavilov's Law.

Homologous characters in the suborders

Phylogenetic relationships among suborders of Homoptera are more or less understood now and the differences between synapomorphies and parallelisms are rather clear. It seems that all modern specialists (irrespective of their general theoretical views) agree with the close relationships in the following combination: Coccinea + Aphidinea, Psyllinea + Aleyrodinea and separately Cicadinea. The sequence of the evolutionary origin of these groups as well as the possible origin of one group within another still remain debatable among taxonomists (see, for example, discussion in Gavrilov-Zimin et al. 2015).

Several examples of Vavilov's Law at this taxonomic level could be mentioned: cytogenetic and ontogenetic parallelisms as a larval meiosis (known in scale insects, aphids and whiteflies, but unknown in psyllids) (Fig. 2), the appearance of the immovable and arostrate instars in the ontogenesis of whiteflies, aphids from the families Phylloxeridae and Pemphigidae, some achaeococcids and also in thrips, modal numbers of chromosomes are comparatively low in scale insects and aphids, etc. (see for more detail information: Gavrilov-Zimin et al. 2015; Gavrilov-Zimin 2018).

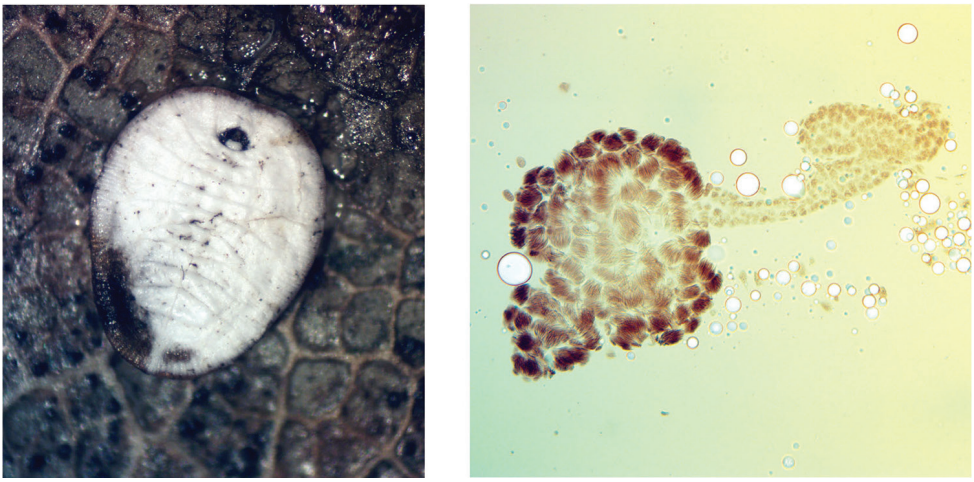

Figure 2. *Aleurochiton aceris* (Modeer, 1778) (Aleyrodinea), Russia (Moscow Prov.), male forth instar larva (pseudopuparium) and its testis with numerous bundles of sperm, produced in course of the larval meiosis.

Homologous characters in the superfamilies

In accordance with Vavilov's law, the number of homologous series increases significantly at lower taxonomic ranks. That is why, only examples primarily based on biology of scale insects, the group which is more familiar to the author, will be provided. The evolutionary advanced scale insect superfamily Coccoidea (so-called "neococcids") is characterized by a peculiar heterochromatinization of paternal haploid set of chromosomes in males (Fig. 3). This heterochromatinization is usually considered as a synapomorphy of all neococcids (Danzig and Gavrilov-Zimin 2014), although this character is occasionally missing in some neococcid groups (for example, *Puto* Signoret, 1876 and *Stictococcus* Cockerell, 1903). This leaves a possibility of hypothetical parallel origin of heterochromatinization in different neococcid families. Moreover, very similar, but undoubtedly separately originated, heterochromatinization was found in some aphids of the family Lachnidae from the "advaced" aphid superfamily Aphidoidea (Blackman 1980) as well as in some Psocoptera (Hodson et al. 2017). Together, neococcids (Coccoidea) and both aphid superfamilies (Phylloxeroidea and Aphidoidea) demonstrate such rare parallelisms as physiological sex determination and formation of only two sperms (instead of four) from each primarily spermatocyte, whereas the basal scale insect superfamily Orthezioidea (archaeococcids) demonstrates XX-X0 sex-determination system with a normal producing of four sperms from each spermatocyte, usual for the most of insects (Gavrilov-Zimin et al. 2015).

Homologous characters in the families

We (Danzig and Gavrilov-Zimin 2014; Gavrilov-Zimin 2018) accept 19 families of the scale insects in the world fauna. Almost all of these families show at least several

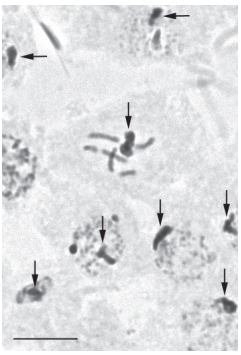

Figure 3. Heterochromatinization of paternal haploid set of chromosomes in the male embryonal cells of *Nipaeococcus delassusi* (Balachowsky, 1925) (Coccinea: Pseudococcidae), K 1276, Morocco (vicinity of Tangier); 2n = 12, heterochromatinized chromosomal sets are arrowed. Scale bar: 10 μm.

unique morpho-anatomical and physiological peculiarities, which are unknown in any other animal group. Several characters in scale insects lead to external similarity in related, but clearly not sister groups of insects. Thus, a very unusual "dizygotic development", which is similar in general appearance to the double fertilization of the flowering plants, is known for the members of the most archaic family of neococcids, Pseudococcidae, and also in the most evolutionary advanced family Diaspididae, but unknown in all other scale insect families. The presence of a paired symmetrical bacteriome is characteristic for some archaeococcids and for whiteflies (Aleyrodinea), whereas unpaired bacteriomes are characteristic for neococcids, aphids and psyllids (Buchner 1965). Most representatives of the family Pseudococcidae have so-called ostioles (one or two pairs of symmetrical openings on anterior and posterior segments of dorsum), which are probably homologous to siphunculi of aphids; the ventral abominal openings of some Pseudoccoidae (circuli) are probably homologous to marsupial opening in representatives of some genera of the archaeococcid family Margarodidae s.l. The cerarii, symmetrical groups of conical setae and wax glands along body margin of many Pseudococcidae, are clearly homologous with marginal tubercles of aphids, etc.

In many scale insect families, the whole "cycles of homologous variability" are to be observed in the accordance to the predictive modeling, based on Vavilov's Law (1920). Thus, for example, the originally oviparous scale insects, evolved into marsupial or pseudomarsupial groups, already inside of the family Margarodidae s.l., which, in turn, gave the rise to the groups with a complete obligate ovoviviparity and then with an incomplete facultative ovoviviparity, when the oviposition of the slightly developed eggs occurs in the external wax ovisacs; in such a case, the secondary oviposition is almost restored in the course of evolution (Gavrilov-Zimin 2018). Similar evolutionary cycle is also repeated in different nocococcid families. The aphids from the archaic superfamily Phylloxeroidea, are characterized by the normal oviparity, whereas the members of the more evolutionary advanced superfamily Aphidoidea, the reproductive mode evolves into the ovoviviparity and in the placental viviparity.

It is impossible to include all other homologous characters representing the evolution of wax glands, morphology of the anal apparatus, chaetotaxy, etc. in various scale insect families and genera; such comparison will require monographic treatment of numerous taxa. Many additional illustrations could be found, for example, in bi-volume book "Palaearctic mealybugs…" (Danzig and Garilov-Zimin 2014, 2015) and in the book on archaeococcids (Gavrilov-Zimin 2018).

To conclude, even this very brief review demonstrates difficulty in distinguishing Vavilov's homologies from the cladistic synapomorphies in the order Homoptera. In some cases, the differences are clear and easily arguable, whereas in other examples, the hypothetical assumptions, basing on the current, often very limited knowledge of the subject, could only be provided. Taking this into consideration, Vavilov's Law becomes a very uncomfortable factor in the practical work of the phylogeneticists and taxonomists, especially those who work in the field of the cladistic paradigm. Ideally, the researcher, when introducing a new character in phylogenetic analysis, should demonstrate not only the apomophic condition of this character in the putative sister taxa, but also he or she should prove that the character evolved only once in the hypothetical common ancestor, but not as a result of homologous variation in the related taxa. Such an approach would need long-time comprehensive study of each potential phylogenetic character in the numerous (ideally in all) species and genera of the analyzed higher taxon, which is, unfortunately, impossible in the frame of short-time projects, dominant now in modern day biology.

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References

Blackman RL (1980) Chromosome numbers in the Aphididae and their taxonomic significance. Systematic Entomology 5: 7–25. https://doi.org/10.1111/j.1365-3113.1980.tb00393.x

Buchner P (1965) Endosymbiosis of Animals with Plant-Like Microorganisms. New York, 909 pp.

Bulatova N (2020a) Archives: the law of homologous series in variation (N.I. Vavilov). Comparative Cytogenetics 14(3): 328–338. https://doi.org/10.3897/CompCytogen.v14i3.54511

Bulatova N (2020b) Notable homologous variation in chromosomal races of the common shrew. Comparative Cytogenetics 14(3): 313–318. https://doi.org/10.3897/CompCytogen.v14i3.54526

D'Urso V (2002) The wing-coupling apparatus of Hemiptera Auchenorrhyncha: structure, function, and systematic value. Denisia 4: 401–410.

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]

Danzig EM, Gavrilov-Zimin IA (2015) Palaearctic mealybugs (Homoptera: Coccinea: Pseudococcidae). Part 2. Subfamily Pseudococcidae. St. Petersburg, 619 pp. [Fauna of Russia and neighbouring countries. New series, № 149. Insecta: Hemiptera: Arthroidignatha]

Emeljanov AF (1987) Phylogeny of cicads (Homoptera, Cicadina) based on comparative morphological studies. Trudy Vsesoyuznogo Entomologicheskogo Obschestva 69: 19–109. [In Russian]

Emeljanov AF (2009) Evolutionary transformations of the abdominal wax-plates in the larvae of the Fulgoroidea (Homoptera, Cicadina). Entomologicheskoe Obozrenie 88(4): 745–768. [In Russian]

Gavrilov-Zimin IA (2018) Ontogenesis, morphology and higher classification of archaecococcids (Homoptera: Coccinea: Orthezioidea). Zoosystematica Rossica (Supplementum 2): 1–260. https://doi.org/10.31610/zsr/2018.supl.2.1

Gavrilov-Zimin IA (2020) N. Vavilov's law of homologous series in the evolution of Homoptera insects. Istoriko-Biologicheskie Issledova niya 12(4), in press. [In Russian]

Gavrilov-Zimin IA, Stekolshikov AV, Gautam DC (2015) General trends of chromosomal evolution in Aphidococca (Insecta, Homoptera, Aphidinea + Coccinea). Comparative Cytogenetics 9(3): 335–422. https://doi.org/10.3897/CompCytogen.v9i3.4930

Goncharov NP (2014) Nikolai Ivanovich Vavilov. Novosibirsk, 292 pp. [In Russian]

Hodson ChN, Hamilton PhT, Dilwoth D, Nelson J, Curtis CI, Pelman SJ (2017) Paternal genome elimination in *Liposcelis* booklice (Insecta: Psocodea). Genetics 206(2): 1091–1100. https://doi.org/10.1534/genetics.117.199786

Kluge NYu (2020) Insect Systematics and Principles of Cladoendesis. Moscow, 1037 pp. [In Russian]

Kolchinsky EI (2017) Nikolai I. Vavilov in the realm of historical and scientific discussions. Almagest 8(1): 4–37. https://doi.org/10.1484/J.ALMAGEST.5.113696

Markov AV (2020) Parallelizms during major evolutionary transitions. https://old.evolbiol.ru/aro2010.htm [Retrieved October 29, 2020]

Rozhnov SV (2006) N. Vavilov's law of homologous series and archaic diversity accrording to the paleontological data. In: Rozhnov SV (Ed.) Evoliutsiia biosfery i bioraznobraziia. K 70-letiiu A.Yu. Rozanova. Moscow, 134–146. [In Russian]

Trapezov OV (2007) Homologous series of fur color variability in American mink (*Mustela vison* Schreber, 1777) detected under domestication. Vestnik VOGiS 11(3/4): 547–560. [In Russian]

Shcherbakov DE, Popov YuA (2002) Superorder Cimicidea. In: Rasnitsyn AP, Quicke DLJ (Eds) History of insects. New York, Moscow, 143–157.

Šulc K (1929) Voskové žlázy a jich výrobky u imag sf. Cixiinae (Homoptera). Biologické Spisy 8(1): 1–53.

Vavilov NI (1920) The law of homologous series in hereditary variation. Presentation on III all-Russian selection meeting in Saratov, June 4, 1920. Saratov, 16 pp. [In Russian]

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RESEARCH ARTICLE

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Comparative karyotype study of three Cyprinids (Cyprinidae, Cyprininae) in Thailand by classical cytogenetic and FISH techniques

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Abstract

Three species of ornamental fishes in the subfamily Cyprininae (family Cyprinidae) namely, *Epalzeorhynchos frenatum* (Fowler, 1934), *Puntigrus partipentazona* (Fowler, 1934), *Scaphognathops bandanensis* Boonyaratpalin et Srirungroj, 1971 were studied by classical cytogenetic and fluorescent in situ hybridization (FISH) techniques. Chromosomes were directly prepared from kidney tissues and stained by using conventional and Ag-NOR banding techniques. Microsatellite $d(CA)_{15}$ and $d(CGG)_{10}$ probes were hybridized to the chromosomes of three cyprinids. The results show that the three cyprinid species share the same diploid number as 2n=50 but there are differences in the fundamental number (NF) and karyotypes i.e. *E. frenatum*: NF = 78, 18m+10sm+10st+12a; *P. partipentazona*: NF = 80, 6m+24sm+14st+6a; *S. bandanensis:* NF = 66, 4m+12sm+34a. NOR positive masks were observed at the regions adjacent to the telomere of the short arm of the chromosome pairs 10 (submetacentric) and 1 (metacentric) in *E. frenatum* and *P. partipentazona*, respectively whereas those were revealed at telomeric regions of the long arm of the chromosome pair 9 (acrocentric) in *S. bandanensis*. The mapping of $d(CA)_{15}$ and $d(CGG)_{10}$

microsatellites shown that hybridization signals are abundantly distributed in telomeric regions of several pairs except d$(CA)_{15}$ repeats in *S. bandanensis*, which are distributed throughout all chromosomes and d$(CGG)_{10}$ repeats in *P. partipentazona* display the high accumulation only in the first chromosome pair.

Keywords
Chromosome, *Epalzeorhynchos frenatum*, FISH, *Puntigrus partipentazona*, *Scaphognathops bandanensis*

Introduction

There are about 200 species of freshwater fish used as ornamentals in Thailand. More than half of all ornamental fishes in Thailand belong to the family Cyprinidae. The most popular species include *Betta splendens* Regan, 1910, *Gyrinocheilus aymonieri* (Tirant, 1883), *Epalzeorhynchos bicolor* (Smith, 1931), *E. frenatum* (Fowler, 1934), *Puntigrus tetrazona* (Bleeker, 1855), *Channa micropeltes* (Cuvier, 1831), *Barbonymus alter* Bleeker, 1853, *Bar. schwanenfeldii* (Bleeker, 1854) and *Balantiocheilos melanopterus* (Bleeker, 1850) (Sermwatanakul 2005).

Family Cyprinidae is the most abundant and globally widespread family of freshwater fish, comprising 3,000 extant and extinct species in about 370 genera (Eschmeyer et al. 2015). The subfamily Cyprininae is one of the largest groups of this family. The essential large tribes such as Labeonini, Poropuntiini and Smiliogastrini have many species that are economically important ornamental fish of Thailand, namely *Epalzeorhynchos frenatum* (Fowler, 1934), *Puntigrus partipentazona* (Fowler, 1934), *Scaphognathops bandanensis* Boonyaratpalin et Srirungroj, 1971 (Fig. 1A, D, G). However, there are few studies of cytogenetics of these ornamental fishes. To date, most reports are of conventional technique studies to determine chromosome number and karyotype composition and only a few ionclude NOR banding analysis. The 2n ranges from 48–50 in the tribes Labeonini and Smiliogastrini while the tribe Poropuntiini is more conserved as 2n = 50 (Arai 2011) (Table 1). Understanding of the basic information on cytogenetics can be applied to the development of potentially commercial stains/species in the future. The studies on the karyotypes help to investigate the genetic structure of aquatic animal species in each habitat, thus it can determine what species are related to each other in an accurate manner. This may help to facilitate the hybridization between them in the future for strain improvement (Sofy et al. 2008), breeding practices of organisms by using chromosome set management (Na-Nakorn et al. 1980), brood stock selection (Mengampan et al. 2004).

For some species, the simple characterization of the karyotype may be sufficient to identify intra- and inter-specific variants. However, in most cases, just the karyotype description appears to be inconclusive when not coupled with other methods capable of generating more accurate chromosomal markers. In this sense, the use of molecular cytogenetic analyses has played an important role in the precise characterization of the structure of genomes (Cioffi and Bertollo 2012). Multiple DNA copies or repetitive DNAs are a large substantial portion of the genome of eukaryotes that can be generally classified into two main classes: tandem repeats, such as the multigene families and the

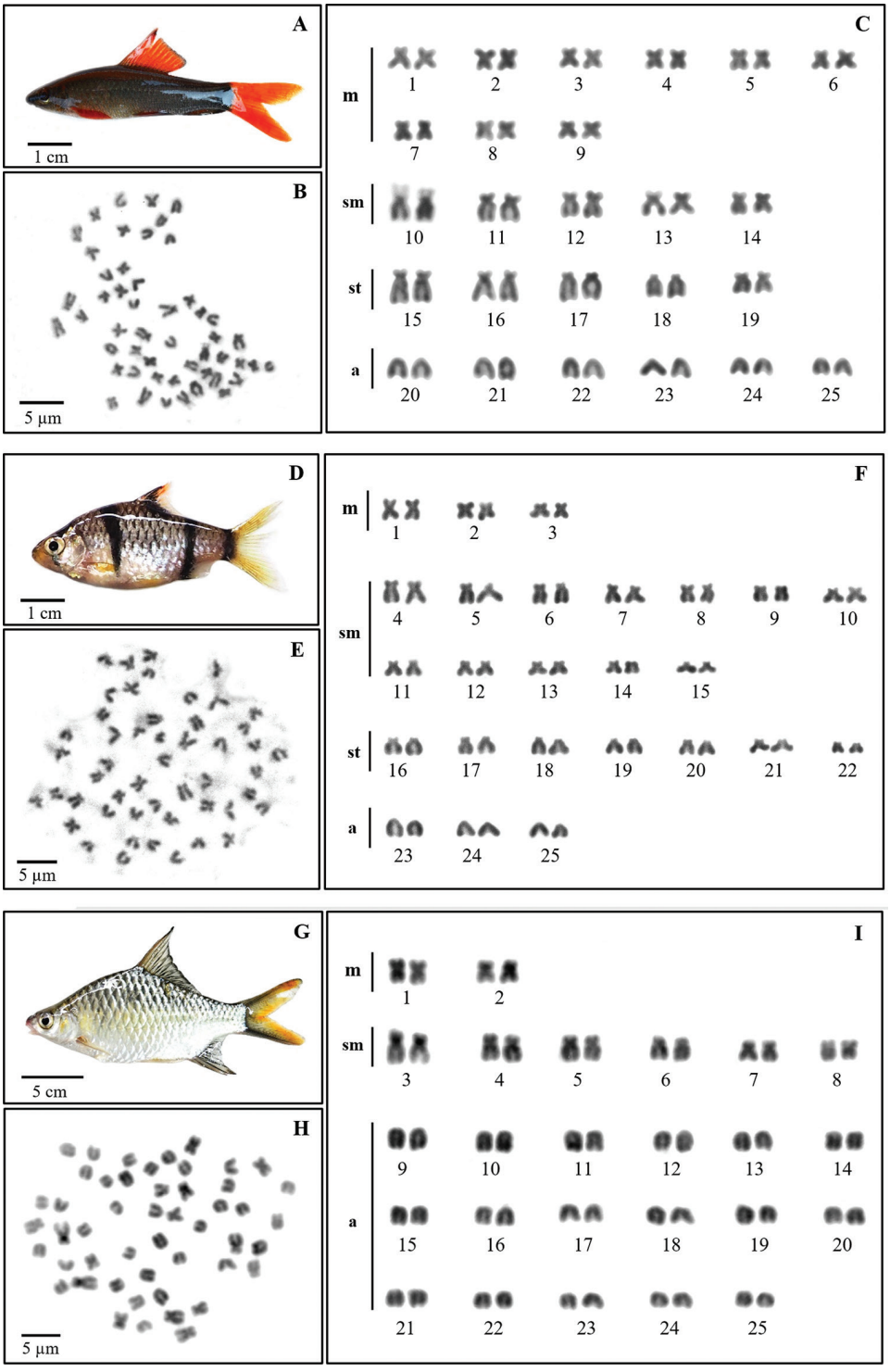

Figure 1. Specimens, metaphase chromosome plates and karyotypes of *Epalzeorhynchos frenatum* (**A–C**), *Puntigrus partipentazona* (**D–F**), *Scaphognathops bandanensis* (**G–I**) by conventional technique.

Table 1. Reviews of cytogenetic reports in the tribes Labeonini, Poropuntiini, and Smiliogastrini. (2n = diploid number, m = metacentric, sm = submetacentric, st = subtelocentric, a = acrocentric and NORs = nucleolar organizer regions, NF = fundamental number, – = not available).

Tribe / Genus / Species	2n	NF	Formula	NORs	Reference
Tribe Labeonini					
Barbichthys laevis (Valenciennes, 1842)	50	76	20m+6sm+4st+20a	–	Donsakul et al. (2006)
Bangana devdevi (Hora, 1936)	50	86	20m+16sm+14a	–	Donsakul et al. (2011)
Cirrhinus julleini	50	90	26m+14sm+4st+6a	–	Magtoon and Arai (1993)
(Valenciennes, 1844)	50	92	36m+6sm+2st+6a	–	Donsakul (1997)
C. microlepis Sauvage, 1878	50	88	22m+8sm+8st+12a	–	Donsakul and Magtoon (1997)
	50	72	12m+10sm+2st+26a	–	Donsakul et al. (2007)
Epalzeorhynchos frenatum (Fowler, 1934)	48	72	14m+10sm+8st+16a	–	Donsakul and Magtoon (1993)
	50	**78**	**18m+10sm+10st+12a**	2	**Present study**
E. bicolor (Smith, 1931)	50	74	20m+4sm+2st+24a	–	Donsakul and Magtoon (1993)
E. munensis (Smith, 1934)	50	84	22m+12sm+2st+14a	–	Donsakul et al. (2012)
Garra cambodgiensis (Tirant, 1883)	50	82	20m+12sm+4st+14t	–	Donsakul et al. (2016)
G. fasciacauda Fowler, 1937	50	84	18m+14sm+2st+16t	–	Donsakul et al. (2016)
G. notata (Blyth, 1860)	50	80	20m+10sm+20t	–	Donsakul et al. (2016)
Incisilabeo behri (Fowler, 1937)	50	78	12m+16sm+4st+18t	–	Donsakul and Magtoon (2003)
Labeo chrysophekadian (Bleeker, 1850)	50	78	4m+10sm+14st+22a	–	Seetapan (2007)
Labiobarbus lineatus (Sauvage, 1878)	50	80	20m+10sm+20a	–	Magtoon and Arai (1990)
L. spiropleura (Sauvage, 1881)	50	90	34m+4sm+2st+10a	–	Donsakul and Magtoon (1997)
Mekongina erythrospila Fowler, 1937	50	74	10m+14sm+26a(t)	–	Donsakul and Magtoon (2003)
Osteochilus melanopleura (Bleeker, 1852)	50	96	36m+10sm+2st+2a	–	Donsakul and Magtoon (1995)
O. microcephalus (Valenciennes, 1842)	50	86	26m+10sm+14st	–	Donsakul et al. (2001)
O. vittatus (Valenciennes, 1842)	50	96	16m+30sm+4st	–	Magtoon and Arai (1990)
	50	86	26m+10sm+14st	–	Donsakul (1997)
O. waandersi (Bleeker, 1853)	50	92	18m+24sm+4st+4a	2	Magtoon and Arai (1993)
Puntioplites falcifer Smith, 1929	50	80	14m+16sm+2st+18a	–	Donsakul et al. (2007)
	50	92	16m+10sm+16a+8t	–	Sophawanus et al. (2017)
Tribe Smiliogastrini					
Osteobrama alfrediana (Valenciennes, 1844)	50	96	24m+22sm+4a	–	Donsakul et al. (2011)
Hampala disper Smith, 1934	50	70	5m+5sm+3st+12a	–	Donsakul and Poopitayasathaporn (2002)
H. macrolepidota Kuhl & Van Hasselt, 1823	50	72	10m+12sm+8st+20a	–	Donsakul and Poopitayasathaporn (2002)
Puntigrus partipentazona (Fowler, 1934)	50	90	6m+34sm+10a	–	Taki et al. (1977)
	50	**80**	**6m+24sm+14st+6a**	**2**	**Present study**
P. tetrazona (Bleeker, 1855)	50	84	34m+6st+10a	–	Ohno et al. (1967)
	50	84	6m+28sm+16a	–	Hinegardner and Rosen (1972), Taki et al. (1977), Suzuki et al. (1995)
	50	–	–	–	Krishnaja and Rege (1980) Vinogradov (1998)
P. tetrazona partipentazona (Fowler, 1937)	50	90	6m+34sm+10a	–	Taki et al. (1977)
Puntius arulius (Jerdon, 1849)	50	82	6m+26sm+18a	–	Taki and Suzuki (1977)
	50	90	10m+18sm+12st+10t	–	Arunachalan and Murugan (2007)
P. binotatus (Valenciennes, 1842)	50	92	8m+34sm+8a	–	Taki et al. (1977)
P. brevis (Bleeker, 1850)	50	70	6m+14sm+8st+22a	–	Khuda-Bukhsh (1975)
	50	54	2m+2sm+2st+22a	–	Donsakul and Poopitayasathaporn (2002)
	48	56	2m+6st+40a	–	Seetapan (2007)
	50	62	4m+4sm+4a+38t	2	Nitikulworawong and Khrueanet (2014)
P. chola (Hamilton, 1822)	50	56	2m+4sm+44a	–	Taki and Suzuki (1977)
	50	54	2m+2sm+4st+42a	–	Tripathi and Sharma (1987)
	50	54	2m+2sm+46a	–	Sahoo et al. (2007)
P. conchonius (Hamilton, 1822)	50	94	6m+38sm+6a	–	Hinegardner and Rosen (1972), Taki and Suzuki (1977)
	48	78	10m+20sm+10st+8a	–	Sharma and Agarwal (1981)
	50	–	–	–	Vasiliev (1985)
	50	90	16m+24sm+2st+8a	–	Khuda et al. (1986), Ojima and Yamamoto (1990)

Tribe / Genus / Species	2n	NF	Formula	NORs	Reference
P. conchonius (Hamilton, 1822)	50	94	4m+40sm+6a	–	Takai and Ojima (1988)
P. cumingi (Günther, 1868)	50	94	18m+26sm+6a	–	Taki and Suzuki (1977)
P. daruphani Smith, 1934	50	70	12m+8sm+6st+24a	–	Magtoon and Arai (1989)
P. denisonii (Day, 1865)	50	74	4m+20sm+18st+8a	8	Nagpure et al. (2004)
P. everetti (Boulenger, 1894)	50	86	6m+30sm+14a	–	Hinegardner and Rosen (1972), Taki et al. (1977), Vinogradov (1998)
P. fasciatus (Jerdon, 1849)	50	80	30m+4st+16a	–	Ohno et al. (1967)
	50	82	6m+26sm+18a	–	Taki et al. (1977)
P. filamentosus (Valenciennes, 1844)	50	84	8m+26sm+16a	–	Taki and Suzuki (1977)
	50	78	12m+16sm+12st+10a	8	Nagpure et al. (2003)
P. lateristriga (Valenciennes, 1842)	50	88	6m+32sm+12a	–	Taki et al. (1977)
	50	86	22m+14sm+6st+8a	–	Sobita et al. (2004)
P. melanampyx Day, 1865	50	74	12m+12sm+14st+12a	–	Khuda et al. (1986)
P. nigrofasciatus (Günther, 1868)	50	100	16m+34sm	–	Taki and Suzuki (1977)
P. oligolepis (Bleeker, 1853)	50	88	8m+30sm+12a	–	Taki et al. (1977)
	50	80	14m+16sm+4st+16a	–	Arai and Magtoon (1991)
	50	92	6m+36sm+8a	–	Taki et al. (1977)
P. pentazona (Boulenger, 1894)	50	98	22m+26sm+2a	–	Taki et al. (1977)
P. sarana (Hamilton, 1822)	50	76	12m+14sm+12st+12a	–	Rishi (1981)
P. sarana subnasutus (Valenciennes, 1842)	50	88	12m+26sm+8st+4a	–	Nagpure et al. (2004)
P. semifasciolatus (Günther, 1868)	50	76	12m+14sm+14st+10a	–	Gui et al. (1986), Yu et al. (1989)
	50	76	12m+14sm+14st+10a	8	Nagpure et al. (2004)
	50	76	8m+18sm+24a	–	Suzuki (1991)
P. sophore (Hamilton, 1822)	48	52	2m+2sm+44a	–	Rishi (1973)
	48	54	2m+4sm+42a	–	Rishi et al. (1977)
	48	52	4m+2st+42a	–	Rishi and Rishi (1981)
	50	56	2m+4sm+44a	–	Khuda et al. (1986)
	48	52	4m+6st+38a	–	Tripathi and Sharma (1987)
P. sophoroides (Günther, 1868)	50	54	2m+2sm+46a	–	Magtoon and Arai (1989)
P. stoliczkanus (Day, 1871)	50	94	22m+22sm+4st+2a	–	Magtoon and Arai (1989)
P. tambraparniei Silas, 1954	50	94	12m+16sm+16a+6t	–	Arunachalan and Murugan (2007)
P. ticto (Hamilton, 1822)	50	82	20m+12sm+10st+8a	–	Sharma et al. (1995), Vinogradov (1998)
	50	100	28m+22sm	–	Taki and Suzuki (1977)
	50	94	28m+16sm+6st	–	Sahoo et al. (2007)
P. titteya (Deraniyagala, 1929)	50	98	20m+28sm+2a	–	Hinegardner and Rosen (1972), Taki and Suzuki (1977)
	48	52	4m+2sm+42a	–	Khuda-Bukhsh and Barat (1987)
Systomus sp.1	50	82	12m+20sm+6st+12a	–	Donsakul et al. (2006)
S. binotatus (Valenciennes, 1842)	50	88	24m+14sm+12a	–	Donsakul and Magtoon (2002)
S. orphoides (Valenciennes, 1842)	50	82	12m+20sm+4st+14a	–	Piyapong (1999)
	50	74	8m+16sm+10st+16a	–	Donsakul and Poopitayasathaporn (2002)
S. stoliczkanus (Day, 1871)	50	94	24m+20sm+6a	–	Donsakul et al. (2011)
Tribe Poropuntiini					
Amblyrhynchichthys truncatus (Bleeker, 1851)	50	78	16m+12sm+22a	–	Donsakul et al. (2006)
Balantiocheilos melanopterus (Bleeker, 1850)	50	72	10m+12sm+28a	–	Ojima and Yamamoto (1990)
	50	70	14m+6sm+10st+20a	–	Donsakul and Poopitayasathaporn (2002)
Barbonymus gonionotus (Bleeker, 1850)	50	72	2m+20sm+4st+24a	–	Magtoon and Arai (1989)
	50	74	16m+8sm+26a	–	Donsakul and Magtoon (1997)
	50	72	6m+16sm+6st+22a	–	Piyapong (1999)
	50	66	2m+4sm+10st+34a	–	Seetapan (2007)
	50	74	6m+18sm+16st+10a	2	Khuda-Bukhsh and Das (2007)
Cosmochilus harmandi Sauvage, 1878	50	82	22m+10sm+10st+8a	–	Donsakul et al. (2005)
Cyclocheilichthys apogon (Valenciennes, 1842)	50	70	12m+8sm+6st+24a	–	Magtoon and Arai (1989)
	50	76	18m+8sm+4st+20a	–	Donsakul and Poopitayasathaporn (2002)
	50	86	10m+16sm+10a+14t	6	Chantapan (2015)
C. lagleri Sontirat, 1989	50	86	12m+6sm+1st+6a	–	Donsakul et al. (2006)
C. repasson (Bleeker, 1851)	50	78	12m+16sm+6st+16a	–	Donsakul et al. (2005)
	50	84	6m+6sm+22st+16a	–	Seetapan (2007)

Tribe / Genus / Species	2n	NF	Formula	NORs	Reference
Cyclocheilos enoplos (Bleeker, 1849)	50	90	10m+30sm+4st+6a	4	Magtoon and Arai (1993)
	50	72	14m+8sm+10st+18a	–	Donsakul and Magtoon (1995a)
	50	78	16m+12sm+6st+16a	–	Donsakul and Poopitayasathaporn (2002)
Hypsibarbus lagleri Rainboth, 1996	50	74	4m+20sm+26a	–	Donsakul and Magtoon (2001)
H. malcolmi (Smith, 1945)	50	64	10m+4sm+36a	–	Donsakul et al. (2007)
H. vernayi (Norman, 1925)	50	58	6m+2sm+4st+38a	–	Donsakul and Magtoon (2002)
H. wetmorei (Smith, 1931)	50	70	12m+8sm+6st+24a	–	Magtoon and Arai (1989)
	50	74	12m+12sm+4st+22a	2	Piyapong (1999)
	50	74	12m+12sm+2st+24a	–	Donsakul and Magtoon (2002)
	50	82	10m+14sm+8a+18t	6	Chantapan (2015)
Mystacoleucus argenteus (Day, 1888)	50	76	6m+20sm+2st+22a	–	Donsakul et al. (2006)
M. marginatus (Valenciennes, 1842)	50	76	16m+10sm+24a	–	Arai and Magtoon (1991)
	50	68	14m+4sm+2st+30a	–	Donsakul and Poopitayasathaporn (2002)
Poropuntius deauratus (Valenciennes, 1842)	50	74	14m+10sm+26t	–	Donsakul et al. (2005)
P. sinensis (Bleeker, 1871)	50	82	10m+22sm+18st	–	Zen et al. (1984)
P. laoensis (Günther, 1868)	50	74	14m+10sm+10st+16a	–	Donsakul and Magtoon (2008)
P. normani Smith, 1931	50	72	10m+12sm+28a	–	Donsakul et al. (2007)
P. chonglingchungi (Tchang, 1938)	50	80	12m+18sm+20st	–	Zen et al. (1986)
Scaphognathops bandanensis Boonyaratpalin & Srirungroj, 1971	50	66	10m+6sm+34a	–	Donsakul et al. (2007)
	50	**66**	**10m+6sm+34a**	**2**	**Present study**
Sikukia gudgeri (Smith, 1934)	50	68	10m+8sm+4st+28a	–	Donsakul et al. (2005)

satellite DNAs; and the dispersed elements, such as transposons and retrotransposons, known as Transposable elements (TEs) (Jurka et al. 2005). Among the tandem repeats we can find the highly-repeated satellite DNAs and "moderate repeats", like mini- and microsatellite DNA (Charlesworth et al. 1994). These non-coding DNA sequences are organized as long arrays of head-to-tail linked repeats (Plohl et al. 2008).

Recently, the molecular cytogenetic studies using fluorescence *in situ* hybridization (FISH) for mapping repetitive DNA sequences have provided important contributions to the characterization of the biodiversity and the evolution of divergent fish groups (Cioffi and Bertollo 2012). Moreover, some microsatellite repeats are species-specific characters among some fish group (Cioffi et al. 2015). Most molecular cytogenetic studies in cypinid fishes were performed by FISH technique using rDNA probes (Inafuku et al. 2000; Kikuma et al. 2000; Ocalewicz et al. 2004; Zhu et al. 2006; Singh et al. 2009; Rossi et al. 2012; Nabais et al. 2013; Kirtiklis et al. 2014; Spoz et al. 2014; Han et al. 2015; Kumar et al. 2016; Han et al. 2017). However, NOR banding including fluorescence *in situ* hybridization (FISH) techniques to investigate chromosomal distribution of repetitive DNA sequences on the chromosomes of *E. frenatum*, *P. partipentazona*, *S. bandanensis* have not been performed.

In present study, we carried out an analysis of chromosomal structures and genetic markers on *E. frenatum*, *P. partipentazona*, and *S. bandanensis* using cytogenetics, and molecular cytogenetics techniques. The knowledge revealed will provide a powerful tool for the next generation of genome research in Thai freshwater fishes and discovering biodiversity, with useful applications in fish breeding for conservation and commercials of ornamental species. Moreover, it is useful applications in

evolution, systematics, phylogenetics, fish fauna management and suitable conservation of river basin.

Material and methods

Ten males and ten females of each species including *E. frenatum*, *P. partipentazona*, *S. bandanensis*, were collected from the Song Khram, Chi and Mekong Basins, respectively. Preparation of fish chromosomes was from kidney cells (Pinthong et al. 2015; Supiwong et al. 2015). The chromosomes were stained with Giemsa's solution for 10 min. Ag-NOR banding was performed by applying two drops of 2% gelatin on the slides, followed with four drops of 50% silver nitrate (Howell and Black 1980). Metaphase figures were analyzed according to the chromosome classification of Levan et al. (1964). Chromosomes were classified as metacentric (m), submetacentric (sm), subtelocentric (st) or acrocentric (a). Fundamental number, NF (number of chromosome arm) is obtained by assigning a value of two to metacentric and submetacentric chromosomes and one to subtelocentric and acrocentric chromosomes.

The use of microsatellite d$(CA)_{15}$ and d$(CGG)_{10}$ probes described by Kubat et al. (2008) was followed here with slight modifications. These sequences were directly labeled with Cy3 at 5′ terminal during synthesis by Sigma (St. Louis, MO, USA). FISH was performed under high stringency conditions on mitotic chromosome spreads (Pinkel et al. 1986). After denaturation of chromosomal DNA in 70% formamide/ 2×SSC at 70 °C, spreads were incubated in 2×SSC for 4 min at 70 °C. The hybridization mixture (2.5 ng/μL probes, 2 μg/μL salmon sperm DNA, 50% deionized formamide, 10% dextran sulfate) was dropped on the slides, and the hybridization was performed overnight at 37 °C in a moist chamber containing 2×SSC. The post hybridization wash was carried out with 1×SSC for 5 min at 65 °C. A final wash was performed at room temperature in 4×SSCT for 5 min. Finally, the slides were counterstained with DAPI and mounted in an antifade solution (Vectashield from Vector laboratories) and analyzed in an epifluorescence microscope Olympus BX50 (Olympus Corporation, Ishikawa, Japan).

Results

Diploid number, fundamental number and karyotype of *Epalzeorhynchos frenatum*, *Puntigrus partipentazona* and *Scaphognathops bandanensis*

Results have shown that the three cyprinid species have the same diploid number of 2n = 50. Although the three species analyzed share the same 2n, there are differences in the fundamental number (NF) and karyotypes i.e. *E. frenatum*: NF = 78, 18 metacentric (m), 10 submetacentric (sm), 10 subtelocentric (st) and 12 acrocentric

(a) chromosomes; *P. partipentazona*: NF = 80, 6m, 24sm, 14st, and 6a chromosomes; *S. bandanensis:* NF = 66, 4m, 12sm, and 34a chromosomes (Fig. 1).

Chromosome marker of *Epalzeorhynchos frenatum*, *Puntigrus partipentazona* and *Scaphognathops bandanensis*

NOR positive masks were observed at the regions adjacent to the telomere of the short arm of the chromosome pairs 10 (submetacentric) and 1 (metacentric) in *E. frenatum* and *P. partipentazona*, respectively whereas they were revealed at telomeric regions of the long arm of the chromosome pair 9 (acrocentric) in *S. bandanensis* (Fig. 2A, D, G and Table 2).

Patterns of microsatellite repeats on the genome of *Epalzeorhynchos frenatum*, *Puntigrus partipentazona* and *Scaphognathops bandanensis*

The mapping of $d(CA)_{15}$ and $d(CGG)_{10}$ microsatellites shown that hybridization signals are abundantly distributed in telomeric regions of several pairs except $d(CA)_{15}$ repeats in *S. bandanensis*, which are distributed throughout all chromosomes and $d(CGG)_{10}$ repeats in *P. partipentazona* display the high accumulation only in the first chromosome pair. In addition, interstitial signals of $d(CA)_{15}$ and $d(CGG)_{10}$ repeats can be observed at the short arm of the chromosome pairs 3 and 4, respectively in *E. frenatum* (Fig. 2 and Table 2). Figure 3 shows the idiograms representing the patterns of $d(CA)_{15}$ and $d(CGG)_{10}$ microsatellites distributions on the chromosomes of three studied species. Microsatellite $d(CGG)_{10}$ sequences were detected disperse hybridization signals with high accumulation of them at telomeric regions of several chromosomes in *E. frenatum* and *S. bandanensis*. However, it is interesting that the microsatellite $d(CGG)_{10}$ repeats coincide with the NOR positions in *P. partipentazona.*

Discussion

Diploid number, fundamental number and karyotype of *Epalzeorhynchos frenatum*, *Puntigrus partipentazona* and *Scaphognathops bandanensis*

The diploid numbers (2n) are same as found in *P. partipentazona* (Taki et al. 1977) and *S. bandanensis* (Donsakul et al. 2007) but there is difference in *E. frenatum* (2n = 48) reported by Magtoon and Donsakul (1993). The 2n in three cypinids studied have the same 2n = 50 as in several species in the subfamily Cyprininae (Arai 2011, Table 1). It seems to be that this subfamily is highly conserved for the 2n. To compare with the previous studies, the NF of *S. bandanensis* is same as the study of Donsakul et al. (2007) whereas ones of *E. frenatum* and *P. partipentazona* differ from the reports of

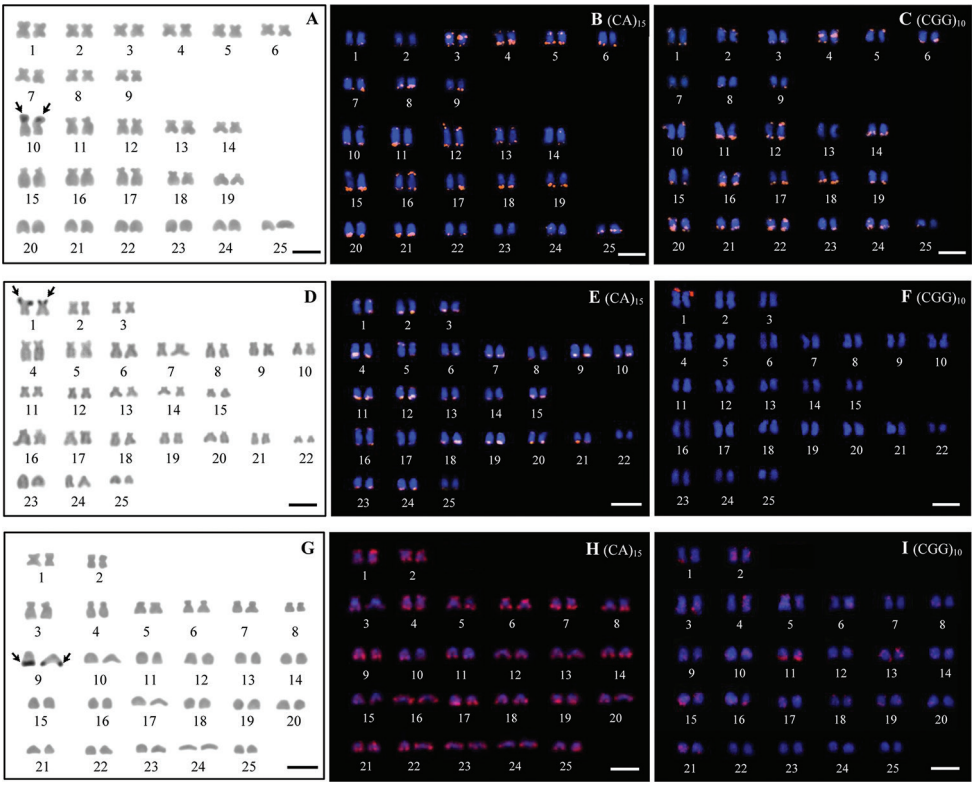

Figure 2. Karyotypes of *Epalzeorhynchos frenatum* (**A–C**), *Puntigrus partipentazona* (**D–F**), *Scaphognathops bandanensis* (**G–I**) by NOR banding and FISH techniques. Arrows indicate NOR-bearing chromosomes. Scale bars: 5 μm.

Table 2. Cytogenetic and FISH studies on three Cypinid fishes in Thailand. (2n = diploid chromosome number, NF = fundamental number (number of chromosome arm), m = metacentric, sm = submetacentric, a = acrocentric, st = subtelocentric chromosomes, NOR = nucleolar organizer region).

Species	2n	NF	Chromosome type				Ag-NOR pair (type)	CA_{15} pair	CGG_{10} pair
			m	sm	st	a			
E. frenatum	50	84	18	10	10	12	10(sm)	1–13,15–25	1–6,9–12,14–25
P. partipentazona	50	94	6	24	14	6	1(m)	1–16, 18–21, 23–25	1
S. bandanensis	50	66	4	12	-	34	9(a)	1–25	1, 3–5,9–11, 13, 15–16, 19–21

Magtoon and Donsakul (1993) and Taki et al. (1977), respectively. The differences of NFs have cause to differences of karyotypes among these fishes. These differences may be causes from the species-specific variations among populations, and/or misidentification of species or different species due to complex species. Three studied species cannot be observed heteromorphic sex chromosomes between male and female specimens. This phenomenon is same as many species in this family (Arai 2011).

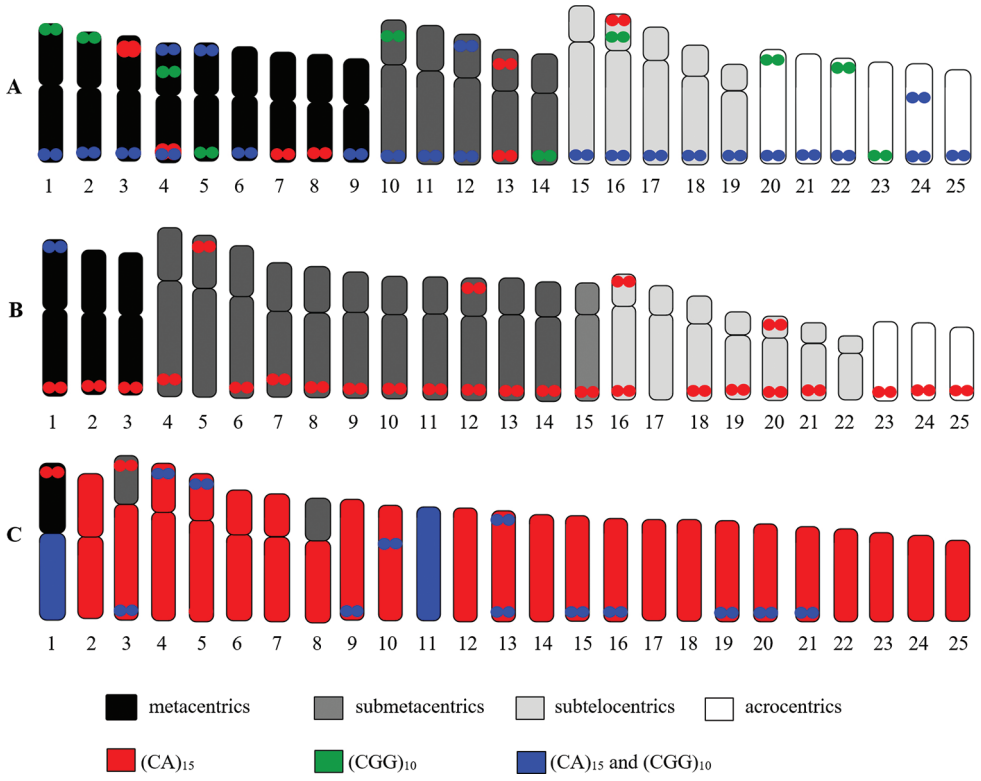

Figure 3. Idiograms represent the $(CA)_{15}$ and $(CGG)_{10}$ mapping on the chromosomes of *Epalzeorhynchos frenatum* **A** *Puntigrus partipentazona* **B** *Scaphognathops bandanensis* **C**.

Chromosome marker of *Epalzeorhynchos frenatum*, *Puntigrus partipentazona* and *Scaphognathops bandanensis*

The determination of nucleolar organizer regions (NORs) for these species was firstly proposed. If these loci are active during the interphase before to mitosis, they can be detected by silver nitrate staining (Howell and Black 1980) since they specifically stain a set of acidic proteins related to ribosomal synthesis process. The single NOR-bearing chromosome pair in the present result is consistent with results from *Barbonymus gonionotus* (Bleeker, 1849) (Khuda-Bukhsh and Das 2007), *Hypsibarbus wetmorei* (Smith, 1931) (Piyapong 1999), *Osteochilus waandersi* (Bleeker, 1853) (Magtoon and Arai 1993) and *Puntius brevis* (Bleeker, 1849) (Nitikulworawong and Khrueanet 2014). This character is common characteristic found in many fish groups as well as vertebrates (Supiwong et al. 2012, 2013). However, some species had two pairs (*Cyclocheilos enoplos* (Bleeker, 1849): Magtoon and Arai 1993), three pairs (*Cyclocheilichthys apogon* (Valenciennes, 1842): Chantapan 2015) and four pairs (*Puntius denisonii* (Day, 1865), *P. semifasciolatus* (Günther, 1868): Nagpure et al. 2004; *P. filamentosus* (Valenciennes, 1844): Nagpure et al. 2003). NORs are chromosomal

landmarks that consist of tandemly repeated sequences of ribosomal genes (rRNA). In eukaryotes, each unit is composed of three genes coding for 18S, 5.8S and 28S ribosomal RNA (Sharma et al. 2002). The number and position of the rDNA clusters have been widely used in systematics and phylogenetic reconstructions since these chromosomal characters are often species-specific (Britton-Davidian et al. 2012). Changes in chromosome number and structure can alter the number, and structure of NOR. Structure, number, and morphology of a NOR may be specific to populations, species, and subspecies. Robertsonian translocations (centric fusion) may cause losses of NOR. Studies on NOR variation in numerous organism groups have invariably described changes in the number and location of NORs even in closely related species, suggesting that rDNA clusters are highly mobile components of the genome (Britton-Davidian et al. 2012). Thus, species, which have limited gene exchange due to geographical isolation, have elevated karyotype varieties and NOR variations. The use of NORs in explaining phylogenetic relationships depends on a large extent on the uniformity of this characteristic and on the degree of variety within a taxon (Yüksel and Gaffaroğlu 2008). Normally, most fishes have only one pair of small NORs in a chromosome complement. If some fishes have more than two NORs, it may be caused by the translocation between NOR and another chromosome (Sharma et al. 2002).

Patterns of microsatellite repeats in the genome of *Epalzeorhynchos frenatum*, *Puntigrus partipentazona* and *Scaphognathops bandanensis*

The patterns of microsatellite $d(CA)_{15}$ in three species in the present study except in *S. bandanensis* are different from the nine species of the Bagridae family including *Hemibagrus filamentus* (Fang & Chaux, 1949), *H. spilopterus* Ng & Rainboth, 1999, *H. wyckii* (Bleeker, 1858), *H. wyckioides* Fang & Chaux, 1949, *Mystus atrifasciatus* Fowler, 1937, *M. multiradiatus* Roberts, 1992, *M. mysticetus* Roberts, 1992, *M. bocourti* (Bleeker, 1864), and *Pseudomystus siamensis* (Regan, 1913) (Supiwong et al. 2013, 2014), *Toxotes chatareus* (Hamilton, 1822) (Supiwong et al. 2017). From the previous and current studies, it may seem that all heterochromatins in fish genomes consist of microsatellites (Cioffi and Bertollo 2012). However, microsatellites have also been found in noncentromeric regions, many of them were located either near or within genes (Rao et al. 2010). This is the same as in the pattern of microsatellite $d(CGG)_{10}$ revealed in *S. bandanensis*.

Conclusions

The present research is the first report on the NOR -banding and FISH techniques in *E. frenatum*, *P. partipentazona*, *S. bandanensis*. Although all studied species have the same diploid chromosome number (2n = 50) and two NOR-bearing chromosomes, there are differences in the fundamental numbers, numbers of chromosomes

with equal sizes, pairs having NORs, and patterns of microsatellites distributions on chromosomes. The NORs can be observed at the regions adjacent to the telomeres of pairs 10, 1 and 9, respectively. The microsatellites are distributed throughout the chromosomes with high accumulations at some positions or all chromosomes which are species-specific characteristics. This result indicated that cytogenetic data can be used for classification in related fish species which have similar morphology.

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References

Almeida-Toledo LF, Ozouf-Costaz C, Foresti F, Bonillo C, Porto-Foresti F, Daniel-Silva MFZ (2002) Conservation of the 5S-bearing chromosome pair and co-localization with major rDNA clusters in five species of *Astyanax* (Pisces, Characidae). Cytogenet Genome Research 97: 229–233. https://doi.org/10.1159/000066609

Amemiya CT, Gold JR (1988) Chromosomal NORs as taxonomic and systematic characters in North American cyprinid fishes. Genetica 76: 81–90. https://doi.org/10.1007/BF00058806

Arai R (2011) Fish Karyotypes: A Check List. Springer Japan, Tokyo. https://doi.org/10.1007/978-4-431-53877-6

Arunachalan M, Murugan M (2007) Cytogenetics and cytotaxonomic consideration of two endangered ornalmental fishes *Puntius arulius* and *P. tambraparniei* (Cypriniformes: Cypeinidae) form Western Ghath, India. Zoo' Print journal 22(7): 2739–2741. https://doi.org/10.11609/JoTT.ZPJ.1552.2739-41

Britton-Davidian J, Cazaux B, Catalan J (2012) Chromosomal dynamics of nucleolar organizer regions (NORs) in the house mouse: micro-evolutionary insights. Heredity 108: 68–74. https://doi.org/10.1038/hdy.2011.105

Chantapan T (2015) Standardized karyotype and ideogram of cyprinid fishes (Subfamily: Cyprininae) inThailand. M.Sc. Thesis. Khon Kaen University. [In Thai]

Charlesworth B, Sniegowski P, Stephan W (1994) The evolutionary dynamics of repetitive DNA in eukaryotes. Nature 371: 215–220. https://doi.org/10.1038/371215a0

Cioffi MB, Bertollo LAC (2012) Distribution and evolution of repetitive DNAs in fish. In: Garrido- Ramos MA (Ed), Repetitive DNA. Karger, Genome Dyn. 2012, vol 7, Basel, 197–221. https://doi.org/10.1159/000337950

Cioffi MB, Bertollo LAC, Villa MA, Oliveira E A, Tanomtong A, Yano CF, Supiwong W, Chaveerach A (2015) Genomic organization of repetitive DNA elements and its implica-

tions for the chromosomal evolution of channid fishes (Actinopterygii, Perciformes). PLoS ONE 12: 1–13. https://doi.org/10.1371/journal.pone.0130199

Cioffi MB, Martins C, Bertollo LAC (2009) Comparative chromosome mapping of repetitive sequences. Implications for genomic evolution in the fish, *Hoplias malabaricus*. BMC Genetics 10: 1–34. https://doi.org/10.1186/1471-2156-10-34

Donsakul T, Magtoon W (1995a) Karyological studies of *Puntius brevis* (Bleeker, 1850) and *Cyclocheilichthys enoplos* in Thailand. Proceeding of the 21st Congress on Science and Technology of Thailand. Queen Sirikit National Convention Centre, Bangkok, 292–293.

Donsakul T, Magtoon W (1995b) Karyotypes of four cyprinid fishes, *Osteochilus melanopleura*, *Puntioplites proctozysron*, *Paralaubuca riveroi* and *Rasbora sumatrana*, from Thailand. Proceedings of the 33rd Kasetsart University Annual Conference: Fisheries. Kasetsart University, Bangkok, 128–138. [In Thai]

Donsakul T, Magtoon W (1997) A chromosome study of five species of cyprinid fishes, *Cirrhina microlepis*, *Leptobarbus hoevenii*, *Labiobarbus spiropleura*, *Barbodes schwanenfeldii* and *Barbodes gonionotus* from Thailand. Srinakharinwirot Science Journal 2: 30–42.

Donsakul T, Magtoon W (2001) Karyological studies of *Hypsibarbus lagler* and *H. vernay* in Thailand. Proceedings of the 27th National Science and Technology Development Agency, 434 pp.

Donsakul T, Magtoon W (2002) Karyotypes of two cyprinid fishes, *Hypsibarbus wetmorei* and *Morulius chrysophekadion*, from Thailand. Proceedings of the 28th Congress on Science and Technology of Thailand. Queen Sirikit National Convention Centre, Bangkok, 92 pp.

Donsakul T, Magtoon W (2008) Karyotypes of seven cyprinid fishes: *Systomus binotatus*, *Puntius brevis*, *Poropuntius laoensis*, *Labiobarbus siamensis*, *Catlocarpio siamensis*, *Tor tambroides* and *Probarbus jullieni* from Thailand. Srinakharinwirot Science Journal 24: 80–92.

Donsakul T, Magtoon W, Rangsiruji A (2005) Karyotypes of four Cyprinid fishes: *Cyclocheilichthys repasson*, *Cosmochilus harmandi*, *Poropuntius deauratus* and *Sikukia gudgeri* from Thailand. Proceedings of the 43rd Kasetsart University Annual Conference: Fisheries. Kasetsart University, Bangkok, 344–351. [In Thai]

Donsakul T, Magtoon W, Rangsiruji A (2006) Karyological studies of four Cyprinid fishes: *Barbichthys nitidus*, *Mystacoleucus argenteus*, *Cychocheilichthys lagleri* and *Systomus* sp.1 from Thailand. Proceedings of the 44rd Kasetsart University Annual Conference: Fisheries. Kasetsart University, Bangkok, 469–476. [In Thai]

Donsakul T, Poopitayasathaporn A (2002) Karyotype of Fifteen Species of Cyprinid Fishes (Family Cyprinidae) from Thailand. Research Project. Srinakharinwirot University, Bangkok.

Donsakul T, Rangsiruji A, Magtoon W (2007) Karyotypes of four cyprinid fishes: *Poropuntius normani*, *Hypsibarbus malcolmi*, *Scaphognathops bandanensis* and *Henicorhynchus caudiguttatus* from Thailand. The Proceedings of 45th Kasetsart University Annual Conference: Fisheries. Bangkok, 740–748. [In Thai]

Donsakul T, Rangsiruji A, Magtoon W (2011) Karyotypes of five cyprinid fishes (Family Cyprinidae): *Bangana devdevi*, *Osteobrama alfrediana*, *Systomus stoliczkanus*, *Oxygaster anomalura* and *Aspidoparia morar* in Thailand. Proceedings of the 49th Kasetsart University Annual Conference: Fisheries. Kasetsart University, Bangkok, 169–177. [In Thai]

Eschmeyer WN [Ed.] (2015) Catalog of Fishes: Genera, Species, References. http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp [Electronic version accessed 28 June 2016]

Gold JR, Amemiya CT, Ellison JR (1986) Chromosomal heterochromatin differentiation in North American cyprinid fishes. Cytologia 51: 557–566. https://doi.org/10.1508/cytologia.51.557

Gui JF, Li YC, Li K, Zhou T (1986) Studies on the karyotypes of Chinese cyprinid fishes. (VIII) Karyotypic analyses of fifteen species of Barbinae with consideration for their phyletic evolution. Transactions of the Chinese Ichthyological Society 5: 119–127.

Hatanaka T, Galetti Jr PM (2004) Mapping of the 18S and 5S ribosomal RNA genes in the fish *Prochilodus argenteus* Agassiz, 1829 (Characiformes, Prochilodontidae). Genetica 122: 239–244. https://doi.org/10.1007/s10709-004-2039-y

Hinegardner R, Rosen DE (1972) Cellular DNA content and the evolution of teleostean fishes. The American Naturalist 106: 621–644. https://doi.org/10.1086/282801

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

Hsu TC, Patak S, Chen TR (1975) The possibility of latent centromeres and a proposed nomenclature system for total chromosome and whole arm translocations. Cytogenetics Cell Genetics 15: 41–49. https://doi.org/10.1159/000130497

Jurka J, Kapitonov VV, Pavlicek A, Klonowski P, Kohany O, Walichiewicz J (2005) Repbase update, a database of eukaryotic repetitive elements. Cytogenet Genome Research 110: 462–467. https://doi.org/10.1159/000084979

Khuda-Bukhsh AR (1975) Somatic chromosomes of an exotic fish *Puntius japonicus*. The Journal of Cytology and Genetics (India) 118–120. https://doi.org/10.1007/BF00057565

Khuda-Bukhsh AR, Barat A (1987) Chromosomes in fifteen species of Indian teleosts (Pisces). Caryologia 40: 131–144. https://doi.org/10.1080/00087114.1987.10797817

Khuda-Bukhsh AR, Das JK (2007) Cytogenetic analyses in eight species of teleostean fishes (Pisces): karyotypes, multiple Ag-NORs, sex chromosomes. Research and Reviews in Biosciences (India) 1: 47–52.

Krishnaja AP, Rege MS (1980) Some observations on the chromosomes of certain teleosts using a simple method. Indian Journal of Experimental Biology 18: 268–270.

Kubat Z, Hobza R, Vyskot B, Kejnovsky E (2008) Microsatellite accumulation in the Y chromosome of *Silene latifolia*. Genome 51: 350–356. https://doi.org/10.1139/G08-024

Levan A, Fredga K, Sandberg AA (1964) Nomenclature for centromeric position on chromosomes. Hereditas 52: 201–220. https://doi.org/10.1111/j.1601-5223.1964.tb01953.x

Liehr T [Ed.] (2009) Fluorescence In Situ Hybridization (FISH)-Application Guide. Springer, Verlag, Berlin, Heidelberg. https://doi.org/10.1007/978-3-540-70581-9

Magtoon W, Arai R (1989) Karyotypes of five *Puntius* species and one *Cyclocheilichtltys* species (Pisces, Cyprinidae) from Thailand. Bulletin of the National Science Museum, Tokyo. Series A (Zoology) 15(3): 167–175.

Magtoon W, Arai R (1993) Karyotypes and distribution of nucleolus organizer regions in cyprinid fishes from Thailand. Japanese Journal of Ichthyology 40(1): 77–85. https://doi.org/10.11369/jji1950.40.77

Magtoon W, Donsakul T (1993) A chromosome study on three Cyprinid fishes, *Labeo erythrurus*, *L. bicolor* and *L. rohita*. Proceedings of 31st Kasetsart University Annual Conference: Animal, Fisheries and Veterinary Medicine. Kasetsart University, Bangkok, 543–551. [In Thai]

Maneechot N, Yano CF, Bertollo LAC, Getlekha N, Molina WF, Ditcharoen S, Tengjaroenkul B, Supiwong W, Tanomtong A, Cioffi MB (2016) Genomic organization of repetitive DNAs highlights chromosomal evolution in the genus *Clarias* (Clariidae, Siluriformes). Molecular Cytogenetics (2016) 9: 1–4. https://doi.org/10.1186/s13039-016-0215-2

Mantovani M, Abel LDS, Mestriner CA, Moreira-Filho O (2004) Evidence of the differentiated structural arrangement of constitutive heterochromatin between two populations of *Astyanax scabripinnis* (Pisces, Characidae). Genetics and Molecular Biology 27: 536–542. https://doi.org/10.1590/S1415-47572004000400012

Martins C (2007) Chromosomes and Repetitive DNAs: A Contribution to the Knowledge of the Fish Genome. In: Pisano E, Ozouf-Costaz C, Foresti F (Eds) Fish Cytogenetics. Science Publishers, 421–453. https://doi.org/10.1201/b10746-16

Mengampan K, Manosoy J, Meevetee A, Manosoy A (2004) Chromosomal Karyotyping from Peripheral Blood Lymphocytes of the Mekong Giant Catfish (*Pangasianodon gigas*, Chevey). Thai Fisheries Gazette 57(4): 349–351. [In Thai]

Nagpure NS, Kumar R, Srivastava SK, Gopalakrishnan A, Verma MS, Baseer VS (2004) Cytogenetic studies of fish species *Horabagrus nigricollaris*, *Puntius denisonii* and *Puntius sarana subnasutus* endemic to the Western Ghats. The Nucleus 47: 143–148.

Nagpure NS, Kushwaha B, Srivastava SK, Kumar R, Gopalakrishnan A, Baseer VS, Verma MS (2003) Characterization of three endemic fish species from Western Ghats using cytogenetic markers. The Nucleus 46: 110–114.

Na-Nakorn U, Tarnchalanukit W, Taptippawan P (1980) Induction of triploidy in *Clarias batrachus* by cold shock. Kasetsart Journal 22: 116–121. [In Thai]

Nelson JS (2006) Fishes of the World (4th ed.). John Wiley & Sons, New York.

Nitikulworawong N, Khrueanet W (2014) Karyotypic of golden little barb, *Puntius brevis* Bleeker, 1850 (Pisces: Cyprinidae) from Khon Kaen Province. KKU Science Journal 42(1): 106–118.

Ohno S, Muramoto J, Christian L, Atkin NB (1967) Diploid-tetraploid relationship among Old-World members of the fish family Cyprinidae. Chromosoma 23: 1–9. https://doi.org/10.1007/BF00293307

Ojima Y, Yamamoto K (1990) Cellular DNA contancts of fishes determined by flow cytometry. La Kromosomo, Series II 57: 1871–1888.

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

Plohl M, Luchetti A, Meštrović N, Mantovani B (2008) Satellite DNAs between selfishness and functionality: structure, genomics and evolution of tandem repeats in centromeric (hetero) chromatin. Gene 409: 72–82. https://doi.org/10.1016/j.gene.2007.11.013

Rishi KK (1973) Somatic karyotypes of three teleosts. Genen Phaenen 16(3): 101–107.

Rishi KK (1981) Chromosomal studies on four cyprinid fishes. International Journal of Academy of Ichthyology 2: 1–4.

Rishi KK, Rishi S (1981) Giemsa banding in fish chromosomes. Perspectives in Cytology and Genetics 3: 103–106.

Rishi KK, Sharma MP, Mankotia R (1977) Somatic chromosomes of three Indian teleosts. Matsya 3: 6–9.

Sahoo PK, Nanda P, Barat A (2007) Karyotypic analysis of *Neolissocheilus hexagonolepis* (McClelland), *Puntius ticto* (Ham.) and *P. chola* (Ham.) (family: Cyprinidae, Pisces). Cytologia 72: 409–413. https://doi.org/10.1508/cytologia.72.409

Seetapan K (2007) Karyotypes of sex fish species of the family Cyprinidae. Proceedings of the 45th Kasetsart University Annual Conference: Fisheries. Kasetsart University, Bangkok, 749–758. [In Thai]

Sermwatanakul A (2005) The Current State of Ornamental Fish Industry in Thailand. Extension Paper No. 2/2005. Ornamental Fish and Aquarium Plants Research Center, Department of Fisheries. [In Thai]

Sharma OP, Agarwal A (1981) The somatic and meiotic chromosomes of *Puntius conchonius* (Cyprinidae) from the Jammu and Kashmir state, India. Genetica 56: 235–237. https://doi.org/10.1007/BF00057565

Sharma OP, Tripathi NK, Sharma KK (2002) A review of chromosome banding in fishes. In: Sobti RC (Ed.) Some Aspects of Chromosome Structure and Functions. New Narosa Publishing House, Delhi, 109–122. https://doi.org/10.1007/978-94-010-0334-6_11

Sharma OP, Sharma KK, Tripathi NK, Gupta SC (1995) Localization of C-heterochromatin in two cyprinid fishes *Puntius ticto* and *Schizothorax niger*. Chromosome Information Service 58: 19–20.

Sobita N, Bhagirath TH, Dhananjoy CH (2004) Chromosome complements of a cyprinid fish *Puntius manipurensis*. The Nucleus 47: 59–63.

Sofy HI, Layla AM, Iman MKA (2008) Karyotypic diversity of some tilapia species. Nature and Science 6(1): 19–27.

Sophawanus M, Tanomthong A, Suphiwong W (2017) Standardized Karyotype and Idiogram of Sicklefin Barb (*Puntioplites falcifer*) in Thailand. Koch Cha Sarn Journal of Science 39(2): 75–84

Supiwong W, Liehr T, Cioffi MB, Chaveerach A, Kosyakova N, Pinthong K, Tanee T, Tanomtong A (2013) Karyotype and cytogenetic mapping of 9 classes of repetitive DNAs in the genome of the naked catfish *Mystus bocourti* (Siluriformes, Bagridae). Molecular Cytogenetics 6(1): 1–51. https://doi.org/10.1186/1755-8166-6-51

Supiwong W, Liehr T, Cioffi MB, Chaveerach A, Kosyakova N, Fan X, Tanee T, Tanomtong A (2014a) 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

Supiwong W, Liehr T, Cioffi MB, Chaveerach A, Kosyakova N, Pinthong K, Tanee T, Tanomtong A (2014b) Chromosomal evolution in naked catfishes (Bagridae, Siluriformes): A comparative chromosome mapping study. Zoologischer Anzeiger-A Journal of Comparative Zoology 253(4): 316–320. https://doi.org/10.1016/j.jcz.2014.02.004

Supiwong W, Tanomtong A, Jumrusthanasan S, Khakhong S, Leelarasamee K, Sanoamuang L (2012) A first karyotype analysis and chromosomal characteristic of nucleolar organizer regions (NORs) of common sheathfish, *Micronema apogon* (Siluriformes, Siluridae) in Thailand. Cytologia 77(1): 53–58. https://doi.org/10.1508/cytologia.77.53

Tanomtong A (2011) Cytogenetics. Khon Kaen University press, Khon Kaen. [in Thai]

Vidthayanon C (2005) Handbook of Freshwater Fish (2nd ed.). Sarakadee Press, Bangkok, Thailand. [In Thai]

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RESEARCH ARTICLE

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Detection of cryptic diversity in lizards (Squamata) from two Biosphere Reserves in Mesoamerica

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Abstract

A combined approach based on karyology and DNA taxonomy allowed us to characterize the taxonomic peculiarities in 10 Mesoamerican lizard species, belonging to six genera and five families, inhabiting two Biosphere Reserve in Chiapas, Mexico: La Sepultura Biosphere Reserve, and Montes Azules Biosphere. The karyotypes of four species, *Phyllodactylus* sp. 3 (*P. tuberculosus* species group) (2n = 38), *Holcosus festivus* (Lichtenstein et von Martens, 1856) (2n = 50), *Anolis lemurinus* Cope, 1861 (2n = 40), and *A. uniformis* Cope, 1885 (2n = 29–30) are described for the first time, the last one showing a particular $X_1X_1X_2X_2/X_1X_2Y$ condition. In *Aspidoscelis deppii* (Wiegmann, 1834) (2n = 50) and *Anolis capito* Peters, 1863 (2n = 42), we found a different karyotype from the ones previously reported for these species. Moreover, in *A. capito*, the cytogenetic observation is concurrent with a considerable genetic divergence (9%) at the studied mtDNA marker (MT-ND2), which is indicative of a putative new cryptic species. The skink *Scincella cherriei* (Cope, 1893), showed high values of genetic divergence (5.2% at 16S gene) between the specimens from Montes Azules and those from Costa Rica and Nicaragua, comparable to the values typical of sister species in skinks. A lower level of genetic divergence, compatible with an intraspecific phylogeographic structure, has been identified in *Lepidophyma flavimaculatum* Duméril, 1851. These new data identify taxa that urgently require more in-depth taxonomic studies especially in these areas where habitat alteration is proceeding at an alarming rate.

Keywords
Cytotaxomy, DNA, herpetofauna, taxonomy

Introduction

The Mesoamerican biota, with its number of endemics in different groups of taxa is one of the most diverse and interesting on the planet (for revision see Ríos-Muñoz 2013). The herpetofauna of this region is one of the richest in taxa groups in the continent (Savage 1982; Wilson and Johnson 2010). Part of this richness is managed and protected under the Biosphere Reserves (UNESCO 2018), which comprises terrestrial, marine, and coastal ecosystems and promote conservation of biodiversity along with its sustainable use. In Mexico, 42 Biosphere Reserves have been created since 1977, encompassing the majority of the environments found in the country (Udvardi 1984).

Saurians are one of the most representative group in terms of karyotypic diversification among reptiles (Olmo and Signorino 2005) and the study of chromosomal evolution in reptiles has received much attention thanks to advanced molecular cytogenetics tools (Deakin and Ezaz 2019; Rovatsos et al. 2019). However, even conventional karyotypes data can be informative in taxonomy (e.g. Santos et al. 2007; Matos et al. 2016; Hardy et al. 2017; Giovannotti et al. 2017).

Our previous studies aimed to genetically characterize the lizard community of a tropical dry forest in the Chamela-Cuixmala Biosphere Reserve (Jalisco state, Mexico) by means of DNA and chromosome analysis (Castiglia et al. 2009, 2010). Even if the herpetofauna of the area was previously quite well known, with two field guides already published (García and Ceballos 1994; Ramirez-Bautista 1994), several new karyotypes of unstudied species were described and species that showed high intraspecific genetic divergence were identified. Later, these findings were confirmed by more extended studies and led to the description of new species (García-Vázquez et al. 2018a, b; Ramírez-Reyes and Flores-Villela 2018).

This study aims to extend the genetic characterization of lizard species in two additional Biosphere Reserves in Mesoamerica: La Sepultura and Montes Azules Biosphere Reserves, both in Chiapas state, Mexico. From a biotic perspective, Chiapas is an area of transition between the herpetofauna of Mexico and that of Central America, along with the one of the Yucatan Peninsula (Lee 1996). Its herpetofauna, is the second largest among all the states in Mexico. The level of endemism is also high with 17.6% of species limited to Mexico. However, habitat alteration in Chiapas is proceeding at a rapid rate, as a result of rising human population growth and the damage that this creates to natural systems (Johnson et al. 2015).

In the present study, karyotypes of the sampled species have been characterized. Then, in conjunction with karyotype data, mtDNA genes for different species, sequenced here and available from GeneBank, were used as molecular markers to identify new putative cryptic species and/or new evolutionarily significant units (ESU) (Funk and Fa 2006).

Material and methods

Study area and sampling specimens

Lizard specimens here analyzed were sampled in two localities: La Sepultura Biosphere Reserve, during September 2009, and Montes Azules Biosphere Reserve during 2012, Chiapas state, Mexico (Fig. 1), hereafter La Sepultura and Montes Azules, respectively. The physiographic profile of Chiapas state consists of a set of layered regions oriented in a NW–SE direction. The sampled areas belong to two different physiographic regions, respectively: La Sepultura belongs to the Pacific Coastal Plain and is characterized mainly by dry tropical forest in its lower parts, while Montes Azules belongs to the Eastern Highlands with the evergreen tropical forest (García de Miranda and Falcón de Gyves 1986). Maps were generated in QGIS version 2.18.9 'Las Palmas' (QGIS 2017), using map shapes from North American Land Change Monitoring System (NALCMS 2020) for North American ecosystems and CONANP (2019) for protected and conservation areas of Mexico.

The specimens were captured by hand in active searching in random walks along the surveyed localities. Details on voucher numbers, genes sequenced, chromosome complements and sampled localities, for each species are shown in Table 1. Taxonomic classification and species distribution follow Uetz et al. (2020). All the tissues and chromosomal samples were labeled with RCMX (field numbers of Riccardo Castiglia) and housed in the herpetological collection of the Museum of Comparative Anatomy of Vertebrates "Battista Grassi" of the University "La Sapienza", Rome, Italy. The voucher specimens, preserved in 80% ethanol, were partly kept in the Museum of Zoology "Alfonso L. Herrera", Mexico City, D.F. (OFV field number of Oscar Flores-Villela), and the remaining specimens in the Museum of Comparative Anatomy of Vertebrates "Battista Grassi".

Karyotype and molecular analysis

For karyotyping, specimens were injected with a 1:1000 solution of Velbe (Lilly) for one hour. The femurs, vertebral column, and testes were removed, crushed and left in hypotonic solution (0.075 M KCl) for 40 minutes at room temperature. Cells were collected by centrifugation and fixed with a methanol-acetic acid solution (3:1). Metaphase plates were prepared by standard air-drying method and slides were stained with Giemsa (pH = 7). Metaphases images were captured with a Photometrics Sensys 1600 digital camera (Roper Scientific Photometrics, Tucson, AZ). For each species, we identified the diploid number (2n), the number of macro- and microchromosomes, and the morphology of macrochromosomes. In some species, it was also possible to assess the morphology of the largest microchromosomes.

For molecular analyses, tissues were extracted from liver and body muscle, and preserved in 100% ethanol. A fragment of the mtDNA genome was sequenced for each species, and the sequenced genes were either cytochrome b (MT-CYB), NADH-ubiquinone oxidoreductase core subunit 2 (MT-ND2) or mitochondrially encoded 16S rRNA

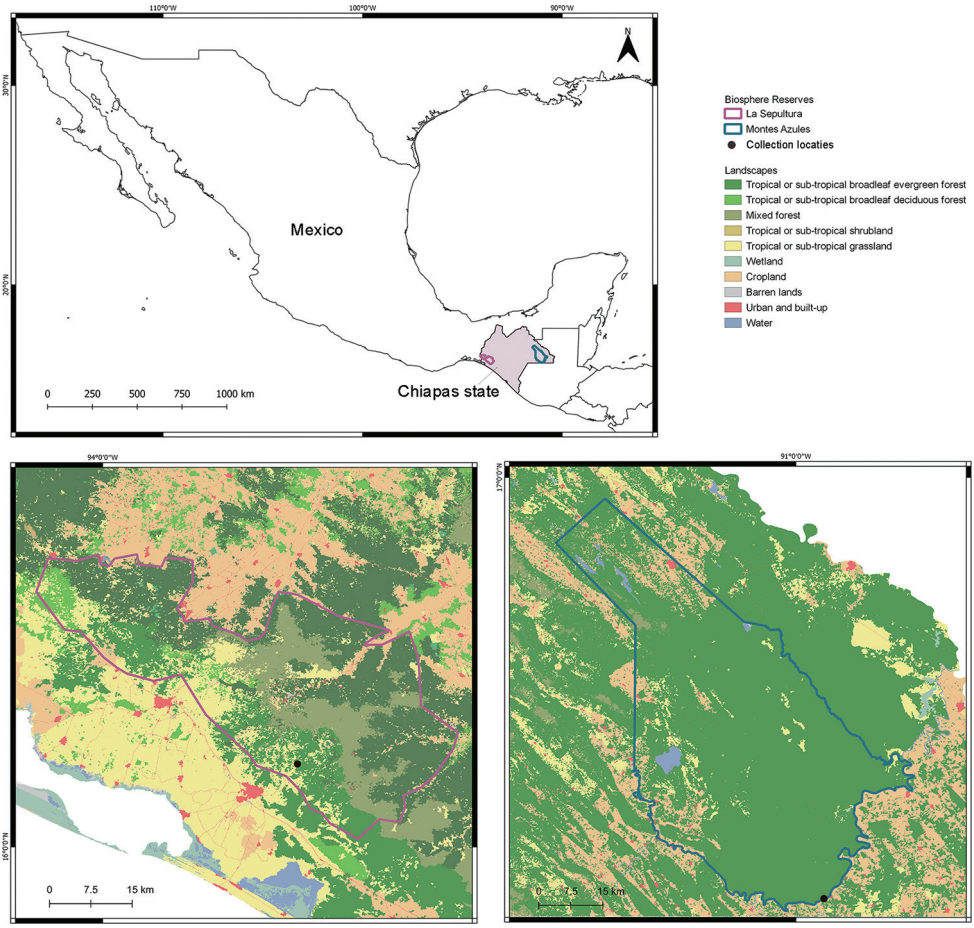

Figure 1. Map showing the collection localities of specimens used in this study, in La Sepultura and Montes Azules Biosphere Reserves, Chiapas state, Mexico.

(16S) (Table 1). The choice of molecular markers depended primarily on the availability of DNA sequences of congeneric and/or conspecific specimens in the GenBank (see results section for accession numbers of sequences downloaded from GenBank).

The QIAmp tissue extraction kit (Qiagen) was used for DNA extraction. We used the universal primers L14841 and H15149 (Kocher et al. 1989) for MT-CYB amplification and two pairs of primers, L4160-ND1 / H4980-ND2 and L4437 tRNAMet / H5934a COI, designed by Macey et al. (1999) for the MT-ND2 gene. Sequences of 16S gene were obtained using the primers 16SA-L and 16SB-H described in Palumbi et al. (1991). The standard PCR procedure was applied as detailed in Castiglia et al. (2010).

Molecular identification of the specimen was performed with the BLAST algorithm (https://blast.ncbi.nlm.nih.gov/Blast.cgi) using, the newly obtained sequences and searching for highly similar sequences (Mega BLAST) on the entire nucleotide

Table 1. Details of gene sequenced, chromosome complement and sampled localities, for each species studied in this work. In voucher numbers, OFV indicated those specimens held in the Museum of Zoology "Alfonso L. Herrera", Mexico City; every other specimen is held in the Museum of Comparative Anatomy of Vertebrates "Battista Grassi" of the Rome University "La Sapienza", Rome, Italy.

Taxon	Voucher	Gene sequenced	GenBank accession numbers	Karyotype	Locality
Squamata					
Scincidae					
Scincella assata	RCMX 85	16S	–	2n = 28 (7M + 14 m)	La Sepultura
	RCMX 86		–		Biosphere Reserve
	RCMX 92		MW265933		
Scincella cherriei	RCMX 219 (OFV 1197)	16S	MW265931	2n = 30 (7M + 16 m)	Montes Azules
	RCMX 235		MW265932		Biosphere Reserve
Phyllodactylidae					
Phyllodactylus sp.3	RCMX 67	MT-CYB	MW275909	2n = 38	La Sepultura
	RCMX 69		MW275910		Biosphere Reserve
	RCMX 93		MW275911		
Xantusiidae					
Lepidophyma	RCMX 207 (OFV 1177)	MT-CYB	–	2n = 38 (18M + 20m)	Montes Azules
flavimaculatum	RCMX 208 (OFV 1178)		–		Biosphere Reserve
	RCMX 212 (OFV 1179)		MW275912		
	RCMX 213 (OFV 1180)		MW275913		
	RCMX 232 (OFV 1255)		MW275914		
Teiidae					
Aspidoscelis deppii	RCMX 76	MT-CYB	MW275915	2n = 52 (28M + 24m)	La Sepultura
					Biosphere Reserve
Holcosus festivus	RCMX 223 (OFV 1213)	MT-ND2	MW275916	2n = 50 (26M + 24m)	Montes Azules
	RCMX 224 (OFV 1214)		–		Biosphere Reserve
	RCMX 233		MW275917		
Holcosus undulatus	RCMX 77	MT-ND2	MW275918	2n = 50 (26M + 24m)	La Sepultura
					Biosphere Reserve
Dactyloidae					
Anolis capito	RCMX 217 (OFV 1203)	MT-ND2	MW275927	2n = 42 (24M + 18m)	Montes Azules
	RCMX 218 (OFV 1204)		MW275928		Biosphere Reserve
Anolis lemurinus	RCMX 214 (OFV1186)	MT-ND2	MW275930	2n = 40 (24M + 16m)	Montes Azules
	RCMX 225 (OFV 1215)		MW275929		Biosphere Reserve
Anolis uniformis	RCMX 201 (OFV 1160)	MT-ND2	MW275919	2n = 29/30 (14M + 15/16m)	Montes Azules
	RCMX 203		MW275925		Biosphere Reserve
	RCMX 205 (OFV 1164)		MW275926		
	RCMX 206 (OFV 1176)		MW275920		
	RCMX 209 (OFV 1183)		MW275921		
	RCMX 210 (OFV 1173)		MW275922		
	RCMX 215 (OFV 1182)		MW275923		
	RCMX 226 (OFV 1211)		MW275924		

collection database. When sequence identity was below 98% the sequences were aligned with the sequences from the same species and/or same genus downloaded from GenBank. Phylogenetic relationships were evaluated with Bayesian inference (BI) and the BI tree was built with the software MrBayes v3.2.1 (Ronquist and Huelsenbeck 2003), under the assumption of a GTR + I + G (General Time Reversible) model of sequence evolution. The appropriate evolution model was chosen using the software jModeltest 2.1 (Darriba et al. 2012) following the Bayesian (BIC) and Akaike (AIC) information criteria. Two independent Markov Chain Monte Carlo (MCMC) analy-

ses were run with four chains and two million generations sampling the chains every 1,000 generations. A burn-in of 10% of generated trees was applied. The software Tracer 1.7 (Rambaut et al. 2018) was used to check parameters convergence. Only the values of posterior probabilities (p.p.) major than 50 are reported on the tree. All the twenty-five new sequences are submitted to GenBank (Table 1).

For some species a TCS Parsimony Network (Clement et al. 2002) connecting haplotypes was obtained with popART (Leigh and Bryant 2015) to visualize mutational steps among main lineages. Gene abbreviation follows HUGO Gene Nomenclature Committee at the European Bioinformatics Institute (HGNC 2019).

Results and discussion

We obtained karyological and molecular data for 10 species (Fig. 2, Table 1), belonging to six genera and five families. The accounts below describe the species of lizards studied, with comments on their distribution, karyotypes, systematics, and voucher specimens. Voucher specimens with an asterisk (*) were karyotyped.

Order Squamata
Family Scincidae
Genus *Scincella* Mittleman, 1950

The Mexican herpetofauna includes seven *Scincella* species that formerly belonged to the genus *Sphenomorphus* Fitzinger, 1843. They were reassigned to *Scincella* based on molecular phylogenetic analyses (Honda et al. 2003; Linkem et al. 2011). The two species, *Scincella assata* (Cope, 1864) and *S. cherriei* (Cope, 1893), belong to this group and are sister species following Linkem et al. (2011). Both of them have already been karyotyped in a recent study (Castiglia et al. 2013a, see comments below).

***Scincella assata* (Cope, 1864)**
Red forest skink

Distribution. This species is distributed from Colima state, Mexico, southwards to Chiapas state, on the Pacific coast, and towards the southwest to Guatemala and Honduras.

Samples. RCMX85 (male*), RCMX86 (female*) and RCMX92 (female*) from La Sepultura, Chiapas, Mexico.

DNA taxonomy. See below under *S. cherriei* (Cope, 1893) account.

Chromosomes. The karyotype, described in Castiglia et al. (2013a) shows a diploid number of 2n = 28 and heteromorphic sex chromosomes. The diploid complement present four pairs of large metacentrics, two pairs of medium sized metacentrics, and one pair of heteromorphic (XY) sex chromosomes (pair 7; one small subtelocentric and one microchromosome). The remaining chromosomes are microchromosomes.

Figure 2. Some lizard species analyzed in present study (Photos by Riccardo Castiglia) **A** *Anolis capito* **B** *Anolis lemurinus* **C** *Holcosus festivus* **D** *Lepidophyma flavimaculatum* **E** *Anolis uniformis*.

Scincella cherriei **(Cope, 1893)**
Brown forest skink

Distribution. This species inhabits Mexico, from central Veracruz to extreme southeastern Puebla, northern Oaxaca state, southwards to Central America on the Atlantic coast, including the Yucatan Peninsula in México, reaching the eastern Panama.

Samples. RCMX219 (male) and RCMX235 (male*) from Estación Chajul, Selva Lacandona, Montes Azules, Chiapas state, Mexico.

DNA taxonomy. The BI phylogenetic tree has been performed on 448-bp alignment of the 16S gene for four individuals of *Scincella cherriei* [RCMX219 and RCMX235 from the Montes Azules, one from Costa Rica (JF498076) and one from Nicaragua (AB057392)] and three individuals of *Scincella assata* [RCMX92 from La Sepultura, and two from El Salvador (JF498074 and JF498075)]. *Scincella lateralis* (Say, 1822) (AB057402 and JF498077) and *S. reevesii* (Gray, 1838) (JF498078) were used as outgroups. The tree (Fig. 3) shows *S. assata* as a monophyletic and well supported group (p.p.: 1.0), including the individual from La Sepultura. The two indi-

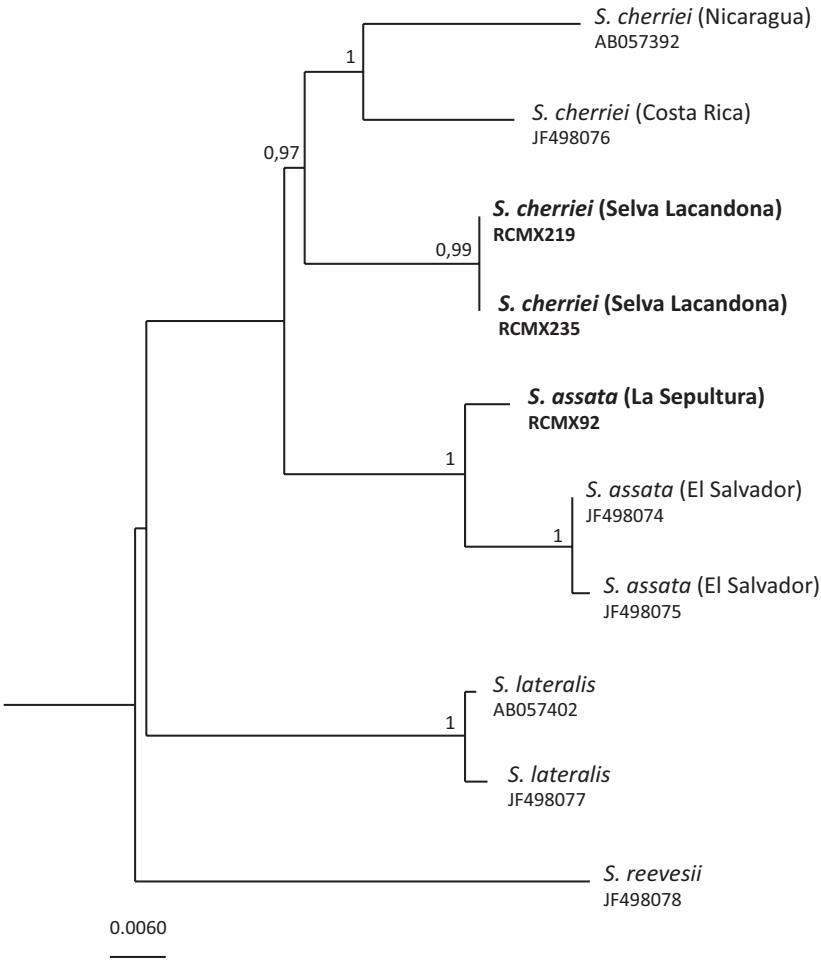

Figure 3. Bayesian phylogenetic tree (16S) of 16S haplotypes from Mexican *Scincella* species. In bold, the new individuals from this study; the geographic provenience of each individual is reported in brackets.

viduals of *S. cherriei* from the Montes Azules, southern Mexico, form a well-supported group separated from the other two individuals from Costa Rica and Nicaragua that fall in a well distinct clade (p.p.: 1.0).

The genetic divergence between the two specimens of *S. cherriei* from the Montes Azules and *S. cherriei* from other localities is high (5.2%), comparable to the divergence between *S. assata* and *S. cherriei* (6.6%-6.2%). The nominal subspecies *S. c. cherriei* (Cope, 1893), was described from Palmar, Costa Rica, which is far from from the Montes Azules. The lineage of *S. cherriei* from the Montes Azules may represent a different taxon worthy of additional detailed morphological and genetic studies.

Chromosomes. The karyotype, described in Castiglia et al. (2013a), shows a diploid number of 2n = 30 and in this case the presence of heteromorphic (XY) sex chromosomes. The diploid complement of *S. cherriei* differs from its sister species *S. assata* by the presence of an additional pair of microchromosomes.

Family Phyllodactylidae
Genus *Phyllodactylus* Gray, 1828

The genus *Phyllodactylus* is now constrained to the New World (Bauer et al. 1997; Gamble et al. 2008). Albeit there are more than 50 species in the genus, karyological data are very scant (Weiss and Hedges 2007; Blair et al. 2009; Nielsen et al. 2019). Recently, many species groups within the genus have been studied using molecular phylogenetic and species delimitation methods, and several additional cryptic species have been revealed (Blair et al. 2015; Koch et al. 2016; Ramírez-Reyes et al. 2017).

***Phyllodactylus* sp. 3 (*P. tuberculosus* species group, lineage A11 *sensu* Blair et al. 2015)**
Yellowbelly gecko

Distribution. provisional distribution of this lineage, probably representing an undescribed species, is restricted to Pacific coast of eastern Oaxaca and western Chiapas states, Mexico (Blair et al. 2015).

Samples. RCMX67 (female*), RCMX69 (male*) and RCMX93 (female*) from La Sepultura, Chiapas state, Mexico.

DNA taxonomy. Blair et al. (2015) reported the most complete phylogeny of the *Phyllodactylus tuberculosus* species group, defining the presence of 11 distinct lineages that represent separated species. We aligned the obtained 579-bp MT-CYB sequences from our samples to the 115 MT-CYB sequences of the 11 lineages reported by Blair et al. (2015) using *Tarentola mauritanica* (Linnaeus, 1758) (JQ425060) as the outgroup. The TCS network (Fig. 4) indicated that the haplotypes of our samples are similar those belonging to the lineage A11 (Blair et al. 2015), from Oaxaca and Chiapas states, and show a shallow genetic divergence (1.2%) compared to A11. Therefore, we provisionally assigned the samples from La Sepultura to this lineage.

Chromosomes. The first description of the karyotype of one species of the *P. tuberculosus* complex is reported here (Fig. 5A). The three specimens analyzed (two females and one male) showed a 2n = 38 with no distinction in macro- and microchromosomes. All chromosomes are telocentric with exception of two pairs of small metacentric chromosomes (pair 14). We found no evidence of heteromorphic sex chromosomes.

As previously reported, 2n = 38 is the most common karyotype found in species of the genus *Phyllodactylus* from the Pacific coast of Mexico (Castiglia et al. 2009; Murphy et al. 2009). Exceptions are constituted by *P. paucituberculatus* Dixon, 1960 and *P. lanei* Smith, 1935 (*sensu* Ramírex-Reyes and Flores-Villela 2018), which have 2n = 32 and 2n = 33–34, respectively (Castiglia et al. 2009). The 2n = 38 karyotype is normally all-acrocentric, except for some records in *P. bugastrolepis* Dixon, 1966 and *P. papenfussi* Murphy, Blair et Mendes de la Cruz, 2019 (Murphy et al. 2009). The ZW sex determination system has been found in *P. wirshingi* Kerster et Smith, 1955 (Nielsen et al. 2019) and, probably, in *P. lanei* (King, 1981). In all taxa, there is no distinct break between macro- and microchromosomes. The karyotype of the specimens from

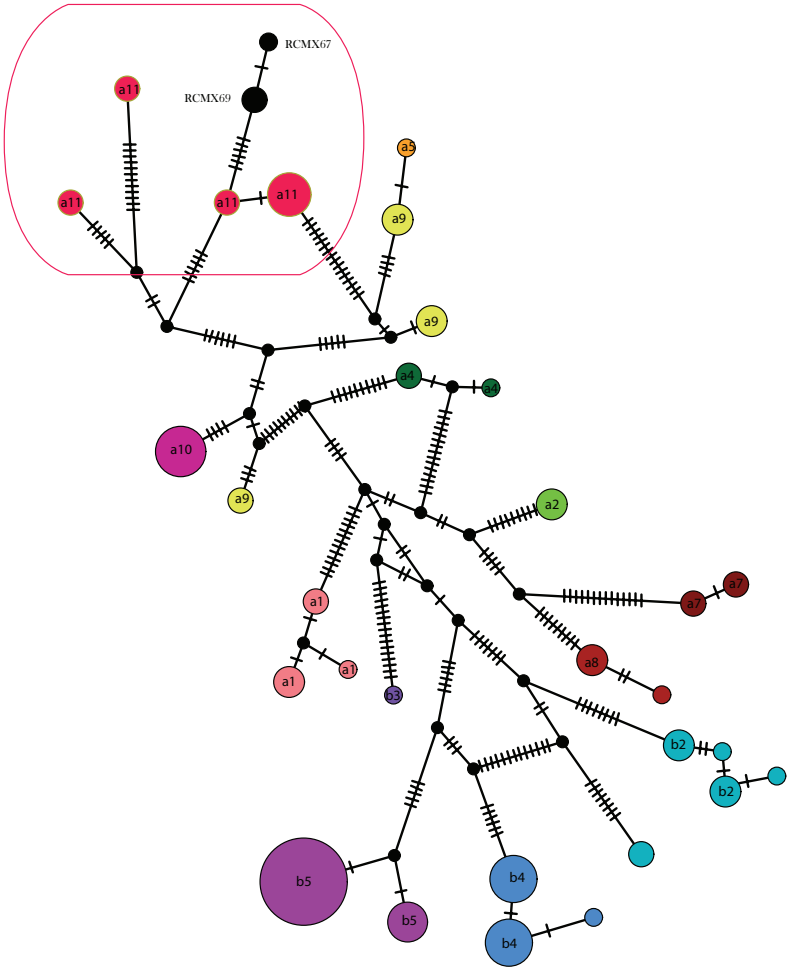

Figure 4. TCS network of MT-CYTB haplotypes of *Phyllodactylus tuberculosus* species group. The colors refer to the 11 lineages reported by Blair et al. (2015) for this species complex. The lineage "A11" and the new specimens here analysed are indicated (see text for further explanation).

La Sepultura described here, is similar to the gekkonid karyotype defined by Gorman (1973). In fact, the typical gekkonid karyotype is composed of a series of acrocentric chromosomes, gradually decreasing in size, with few or no bi-armed chromosomes and no distinct boundary between macrochromosomes and microchromosomes (Bickham 1984). The 2n = 38 acrocentric karyotype is considered to be the ancestral in the families Gekkonidae, Diplodactylidae, and Eublepharidae. In Phyllodactylidae the chromosomal number ranges from 2n = 32 to 2n = 44 (Pellegrino et al. 2009). While the karyotype of the genus *Phyllodactylus* seems rather conservative, the pair of metacentric chromosomes in the here studied specimens indicates presence of intrachromosomal rearrangements (Pokorná et al. 2015). Therefore, these chromosomes may represent chromosomal markers for further investigation in this genus characterized by multiple cryptic species (Blair et al. 2015).

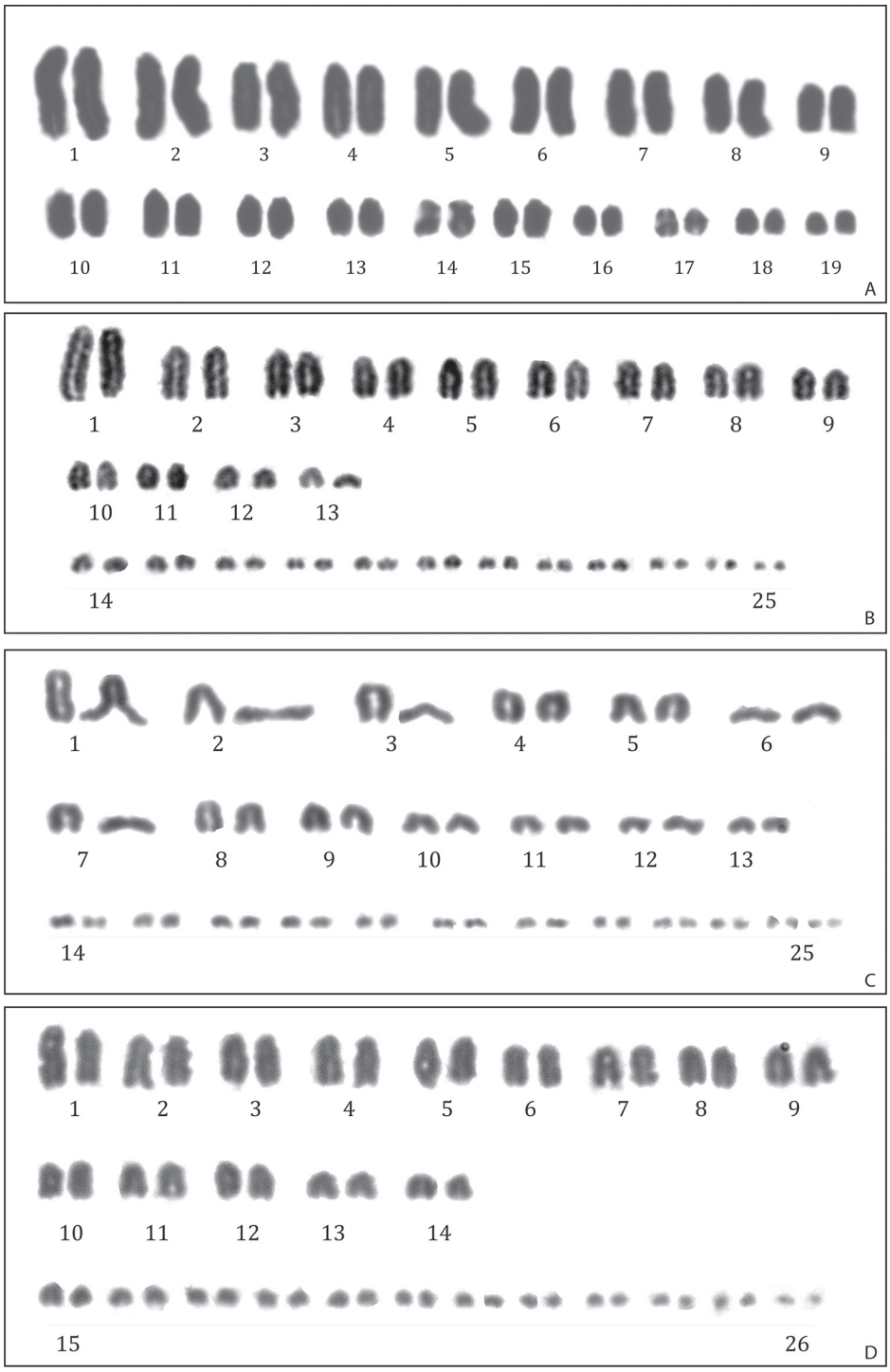

Figure 5. Karyotypes of **A** *Phyllodactylus* sp.3 (2n = 38, RCMX69 male) **B** *Holcosus festivus* (2n = 50, RCMX224 female) **C** *Holcosus undulatus parvus* (2n = 50, RCMX77 female) **D** *Aspidoscelis deppii* (2n = 52, RCMX76 female).

Family Xantusiidae
Genus *Lepidophyma* Duméril, 1851

The genus *Lepidophyma* comprises 20 recognized species and is particularly speciose in Mexico, where 15 species are endemic and, in some cases, restricted to a particular mountain landscape (Palacios-Aguilar et al. 2018). Only two species of this genus are widely distributed in Mexico and Central America: *L. smithii* Boucourt, 1876 and *L. flavimaculatum* Duméril, 1851. However, the former is paraphyletic with respect to *L. lineri* Smith, 1973 and the latter includes a previously unrecognized species from Chiapas state, Mexico (Noonan et al. 2013).

***Lepidophyma flavimaculatum* Duméril, 1851**
Yellow-spotted night lizard

Note. Bezy and Camarillo (2002) did not recognize subspecies, although they admitted that populations of this taxon form a complex, therefore representing more than one taxon. It is the only vertebrate species with unisexual parthenogenetic populations that are of non-hybrid origin (Sinclair et al. 2010).

Distribution. Found on the Gulf of Mexico coast from Veracruz and Oaxaca, crossing the base of the Yucatan peninsula, through Central America to Panama.

Samples. RCMX207 (female*), RCMX208 (male*), RCMX212 (female*), RCMX213 (male*), and RCMX232 (female*) from Montes Azules, Chiapas state, Mexico.

DNA taxonomy. Our samples have been identified on a morphological basis as *Lepidophyma flavimaculatum*, a species already reported for Chiapas. We aligned our 309 bp MT-CYB sequences to the 14 haplotypes of the same species published in Sinclair et al. (2010) from Honduras, Nicaragua and Belize, as well as the unisexual populations from Costa Rica and Panama; *L. reticulatum* Taylor, 1955 and *L. lipetzi* Smith et Del Toro, 1977 were used as outgroups. The phylogenetic trees (Fig. 6A) showed that our samples are sister to the *L. flavimaculatum* clade, but it forms a separate and well supported lineage (p.p. = 1) with 3.9% of genetic divergence. The TCS network (Fig. 6B) confirms that the samples from Chiapas are differentiated from all the other populations of *L. flavimaculatum* by 8 substitutions, whereas the other haplotypes differ from each other by not more than 3 substitutions. The shallow distinction of the Chiapas population may reflect the phylogeographic structure of the species, in accordance with its distant geographical location. Moreover, Bezy (1989) found that Chiapas specimens are morphologically distinct from other southern Mexican samples. Therefore, additional comparative studies at the northern edge of the species range are needed.

Chromosomes. Diploid chromosome complements vary from 2n = 24 to 2n = 40 in Xantusiidae (Olmo and Signorino 2005). Within *Xantusia* Baird, 1859 the karyotypic formula is highly conserved with all studied species displaying 2n = 40, while the genus *Lepidophyma* is much more variable with diploid number ranging from 2n = 32 to 2n = 38 (Olmo and Signorino 2005). There is no evidence of heteromorphic sex

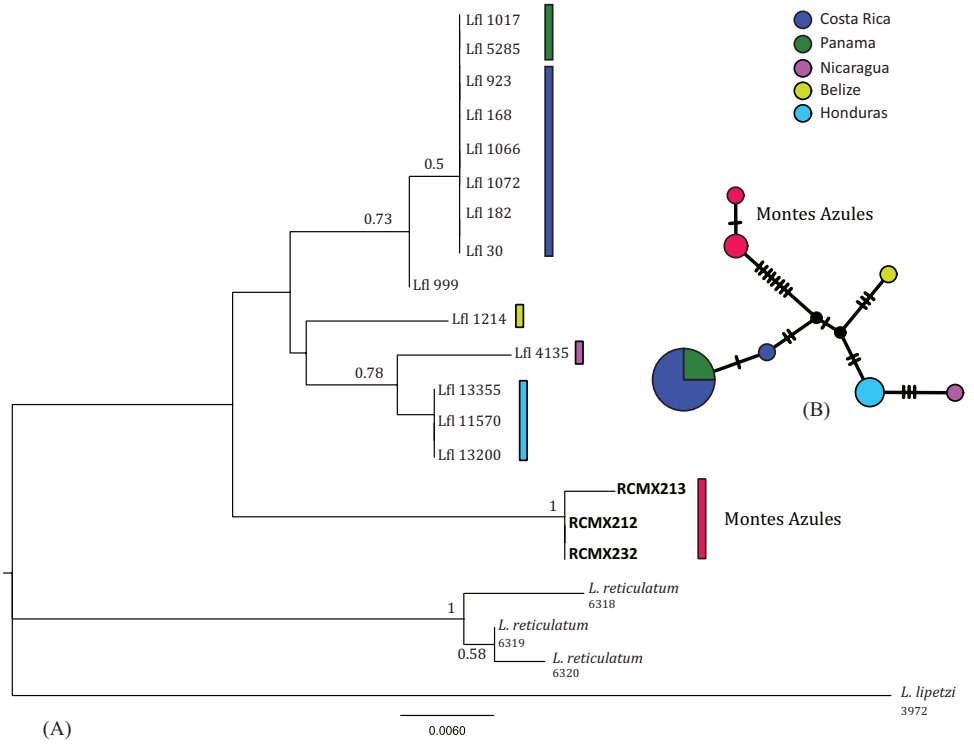

Figure 6. Bayesian phylogenetic tree (**A**) and TCS network (**B**) of 16S haplotypes belonging to *Lepidophyma flavimaculatum*. The colors refer to the geographic provenience of individuals. In bold, the new specimens from this study.

chromosomes within the family, but recently a ZZ/ZW sex chromosomes system was described in the *X. henshawi* Stejneger, 1893 (Nielsen et al. 2020). In *L. flavimaculatum* unisexual parthenogenetic populations are known from Panama and southeastern Costa Rica, whereas northern populations are bisexual. All unisexual populations so far studied are diploid (2n = 38), except one mosaic individual (2n/3n) (Bezy 1972). All individuals presently analysed (Fig. 7) showed 2n = 38 with 18 macrochromosomes and 20 microchromosomes, as previously reported by Bezy (1972).

Family Teiidae
Genus *Aspidoscelis* Fitzinger, 1843

Species of the genus *Aspidoscelis* were previously included in *Cnemidophorus* Wagler, 1830, but based upon divergent morphological, molecular, and enzymatic characters the two genera were separated (Reeder et al. 2002). Thus, *Aspidoscelis* was resurrected for the North American *Cnemidophorus* clade containing 87 species included in the *A. deppii*, *A. sexlineata* and *A. tigris* species groups (and the unisexual taxa associated with them). *Aspidoscelis* occurs throughout most of North America (except Canada and much

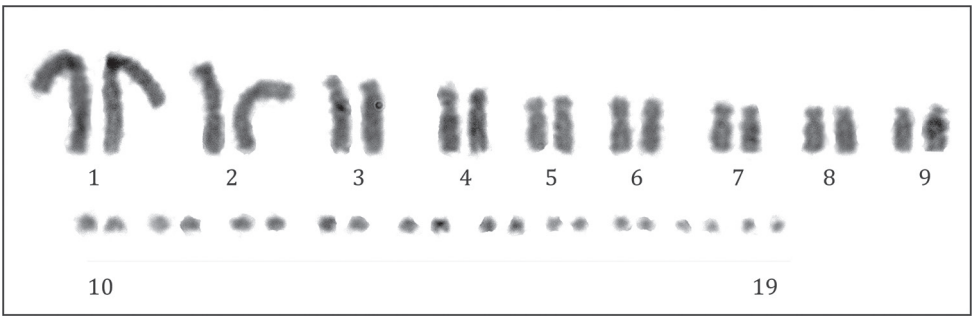

Figure 7. The karyotype of *Lepidophyma flavimaculatum* (2n = 38, RCMX208 male).

of northern United States), reaching the East and West Coasts of the United States, and ranging south through all Mexico and into Central America (Harvey et al. 2012).

The species groups differ also in their karyotypes. 2n = 52 is observed in the *deppii* group, 2n = 46 in the *sexlineata* group, and 2n = 46 with XY sex chromosomal system in the *tigris* group. Lowe et al. (1970) suggested a chromosomal evolution pattern through a reduction of the diploid number. This view has been slightly modified by Reeder et al. (2002), who considered that the ancestor probably had a karyotype of 2n = 50.

Aspidoscelis deppii (Wiegmann, 1834)

Blackbelly racerunner

Distribution. The species has a wide distribution from Morelos and Michoacan (Mexico) south to Guatemala, El Salvador, Honduras, Nicaragua and Costa Rica.

Samples. RCMX76 (female*) from La Sepultura, Chiapas, Mexico.

DNA taxonomy. The MT-CYB sequence (294-bp) is 4% divergent from GenBank sequences of *Aspidoscelis deppii* (KF555517-21) from Mexico (Playa Miramar, Tabasco). Despite the wide distribution, there are no studies on the intraspecific genetic variability of this species. It is a pity because this slight divergence in the MT-CYB could match with a different karyotype (see below).

Chromosomes. In the genus *Aspidoscelis* chromosomal number ranges from 2n = 44 to 2n = 56, with some species showing triploid numbers, such as *Aspidoscelis tesselatus* (Say, 1823), with 69 chromosomes (Walker et al. 1997). The 2n = 44 is the most common diploid number in this genus (Carvalho et al. 2015). Therefore, a low diploid number could represent an ancestral condition. All-acrocentric karyotypes with 2n = 52 (28M + 24m) (Lowe et al. 1970) and 2n = 50 (26M + 24m) (Manríquez-Morán et al. 2000) were reported in *Aspidoscelis deppii* from an unknown location and from Yucatan, respectively. Therefore, the two karyotypes differ in the number of macrochromosomes. Concurrently with Lowe et al. (1970), we found a 2n = 52 (28M + 24m) (Fig. 5D) all-acrocentric chromosome complement in our sample from Chiapas. This result is also consistent with phylogenetic relationships, since a diploid comple-

ment 2n = 52 (28M + 24m) was found in other two species so far analyzed, *A. guttatus* Wiegmann, 1834 and *A. lineattissimus* (Cope, 1878), which are closely related to *A. deppii* (Lowe et al. 1970; Carvalho et al. 2015).

Genus *Holcosus* Cope, 1862

Ten species formerly assigned to the genus *Ameiva* F. Meyer, 1795 have been reassigned to the genus *Holcosus* and reorganized in three species groups (Harvey et al. 2012). Both species analyzed here are included in the same *H. undulatus* species group, which contains a total of six species (Harvey et al. 2012): *H. chaitzami* Stuart, 1942, *H. festivus* (Lichtenstein et von Martens, 1856), *H. leptophrys* (Cope, 1893), *H. niceforoi* (Dunn, 1943), *H. quadrilineatus* (Hallowell, 1860), and *H. undulatus* (Wiegmann, 1834). The genus *Holcosus* has uncertain relationships within Teiidae (Harvey et al. 2012) and has been considered sister to the genus *Cnemidophorus* (Pyron et al. 2013).

Holcosus festivus (Lichtenstein et von Martens, 1856)

Middle American ameiva

Distribution. This species is found in the lowlands of Tabasco and Mexico down to Colombia; it does not enter in the Yucatan Peninsula.

Samples. RCMX223 (female*), RCMX224 (female*), and RCMX233 (female) from Estación Chajul, Selva Lacandona, Montes Azules, Chiapas, Mexico.

DNA taxonomy. The 600-bp PCR-amplified fragments of the MT-ND2 gene were identical in the two specimens (RCMX223 and RCMX233). The BLASTn search showed that this sequence belongs to *Holcosus festivus*, with 99.8% – 100% identity to *H. festivus* (KR058107, Montes Azules) and 96% identity to the other two *H. festivus* samples (KR058105 and KR058106, Costa Rica).

Chromosomes. Here we report the first karyotype description for *H. festivus* (Fig. 5B). We analyzed two female individuals, both with the diploid number 2n = 50. The karyotype is composed of a gradual series of acrocentric chromosomes: 26 macro- and 24 microchromosomes. The largest pair of chromosomes shows a secondary constriction at the distal end (see discussion below under the *H. undulatus* account).

Holcosus undulatus (Wiegmann, 1834)

Rainbow ameiva

Note. Meza-Lázaro and Nieto-Montes de Oca (2015), in a molecular phylogenetic study, proposed the elevation of 9 of the 12 *H. undulatus* subspecies to species rank. However, this change has not been widely accepted by other authors. Therefore, we formally use the previous classification, but we also take in account the results of the Meza-Lazaro and Nieto-Montes de Oca (2015) study.

Distribution. The species is distributed along both coasts of Mexico from southern Nayarit to northern Costa Rica Pacific coast) and from southern Tamaulipas to central Nicaragua (Atlantic coast) including the peninsula of Yucatan.

Samples. RCMX77 (female*) from La Sepultura, Chiapas, Mexico.

DNA taxonomy. The MT-ND2 sequence (556-bp) obtained from the individual from Chiapas has a 99% match to two GenBank sequences of *H. undulatus parvus* Barbour et Noble, 1915 (KR058051 and KR058063). According to Meza-Lazaro and Nieto-Montes de Oca (2015), this subspecies, distributed in the Pacific coast region of Southern Mexico and Northern Guatemala, should be elevated to species rank.

Chromosomes. The specimen analyzed shows a 2n = 50 chromosome number (Fig. 5C). The karyotype comprises a gradual series of acrocentric chromosomes (26M + 24m), as previously described in Castiglia et al. (2010) for *H. undulatus* from Chamela, Biological Station (Jalisco). In the genus *Holcosus*, only *H. festivus* (Chiapas, Castiglia et al. 2010) and *H. undulatus* (Jalisco, present data) have been karyotyped. In *Cnemidophorus*, a possible sister group of *Holcosus* (Pyron et al. 2013), 2n = 50 chromosome complement with one biarmed pair has been reported (Carvalho et al. 2015). Different species of *Kentropyx* Spix, 1825 and *Ameiva* show a 2n = 50 all-acrocentric karyotype, similar to the one found in *Holcosus* (Carvalho et al. 2015). Since these genera span the entire phylogenetic tree of Teiidae, we hypothesize that 2n = 50 all-acrocentric karyotype may represent an ancestral condition. However, to reveal more reliable pattern of chromosomal change, an ancestral state analysis combining karyotype and molecular phylogeny should be made (e.g. Castiglia et al. 2013a).

Family Dactyloidae
Genus *Anolis* Daudin, 1802

Anolis (*sensu lato*) is the most speciose genus among the reptiles, with about 380 recognized species that have been all enclosed in a complete molecular phylogenetic tree by Poe et al. (2017). Most of the mainland species belong to the clade *Norops* Wagler, 1830, a large monophyletic assemblage including nearly 170 species (Poe et al. 2017).

The ancestral karyotype of "beta" *Anolis* (*Norops*) consists of 2n = 28 or 2n = 30 chromosomes subdivided in 14 macro- and 14 or 16 microchromosomes without evident sex chromosome heteromorphism (Castiglia et al. 2013b). Another frequently observed chromosome complement in this group has 2n = 40 (24M+16m), which is considered to have been derived from the previous complement through fission events on macrochromosomes (Castiglia et al. 2013b). The presence of heteromorphic sex chromosomes has been repeatedly reported in *Norops*. Moreover, it might have occurred independently in different lineages (Castiglia et al. 2013b, Gamble et al. 2014). Among "beta" *Anolis*, heteromorphic XY chromosomes have been reported in eight species (Castiglia et al. 2013b; Giovannotti et al. 2016). Furthermore, a system with two X chromosomes and one Y ($X_1X_1X_2X_2/X_1X_2Y$) has been reported in *A. biporcatus* (Wiegmann, 1834) (2n = 29 for males and 2n = 30 for females) (De Smet 1981). This multiple sex-chromosome system also occurs also in other *Anolis*

species and it is believed to have been the result of a sex-autosome translocation event (Giovannotti et al. 2016; Kichigin et al. 2016).

Anolis capito Peters, 1863

Bighead anole

Distribution. *Anolis capito* has been found from Tabasco and northern Chiapas south to Central America on the Atlantic coast, to Costa Rica and Panama, where it is found on both coasts.

Samples. RCMX217 (female*), RCMX218 (female*) from Montes Azules, Chiapas, Mexico. The specimens were collected close to the northern part of species range and morphologically assigned to *Anolis capito*. Based on morphological studies from populations of almost all the species range, there is no evidence of cryptic species in *A. capito* (Köhler et al. 2005).

DNA taxonomy. We obtained a 685-bp MT-ND2 sequence showing 9% genetic divergence respect to an *A. capito* sequence collected in Costa Rica (GenBank AY909744). Such a high genetic divergence spurred us to perform a complete phylogenetic analysis with the MT-ND2 gene of *Anolis* species available in GenBank (not shown). The sequences from our samples cluster with the GenBank *A. capito* sequence, and together were sister to *A. tropidonotus* Peters, 1863. This tree topology has been already reported by Poe et al. (2017). Summarizing, the very high genetic divergence and discrepancies in diploid chromosome numbers (see below) of morphologically similar individuals recognized as *Anolis capito* indicate the possible existence of cryptic taxa. Further, it is worth noting that the specimens described here seem to have shorter limbs than other *A. capito* (O. Flores-Villela personal observation).

Chromosomes. Gorman (1973) described the karyotype of *Anolis capito*, under the name of *Norops capito*, as 2n = 40 (24M + 16m) with no evidence of heteromorphic sex chromosomes, but no details on the shape of the chromosomes were reported. Our specimens have a 2n = 42 chromosome complement, with 24 micro- and 18 microchromosomes, and no evidence of heteromorphic sex chromosomes but no males have been studied (Fig. 6A).

The specimens presently studied show, along with *Anolis nebuloides* Bocourt, 1973, the highest diploid number within the genus *Anolis*. The macrochromosomes include one pair of metacentric, six pairs of submetacentric, and five pairs of subtelocentric/acrocentric chromosomes. The chromosome shape of two pairs of microchromosomes appears to be biarmed. No heteromorphic sex chromosomes are discernible (unfortunately, no males have been analyzed).

The lack of description of chromosome morphology in Gorman's study (Gorman 1973) did not allow detailed comparison among the 2n = 40 chromosomal complements. Thus, *Anolis capito* occurs within a group of species with 2n = 40 (Castiglia et al. 2013b) and its additional chromosomal pair is probably due to a fission event. It has already been hypothesized that chromosomal fission is a characteristic trait of *Norops* chromosome evolution (Castiglia et al 2013b; Gamble et al. 2014).

***Anolis lemurinus* Cope, 1861**
Ghost anole

Distribution. Occurs on the Atlantic coast from central Veracruz to central Panama, and on the Pacific coast from Costa Rica to central Panama.

Samples. RCMX214 (male*), RCMX225 (male*) Estación Chajul, Selva Lacandona, Montes Azules, Chiapas, Mexico.

DNA taxonomy. BLAST analysis of the 630-bp MT-ND2 gene sequences from both individuals show 99.5% – 100% of identity with a sequence of *A. lemurinus* from Oaxaca (GenBank KT724761).

Chromosomes. No previous chromosomal data are available for *A. lemurinus* and its karyotype is here described for the first time. Both male specimens from Montes Azules have a 2n = 40 (24M + 16m) karyotype (Fig. 8B). The 12 pairs of macrochromosomes include eight pairs of submetacentric and four pairs of subtelocentric/acrocentric chromosomes. The metacentric chromosomes of pair 10 are of different size and may represent heteromorphic sex chromosomes of the XY type.

This karyotype has the same composition in micro- and macrochromosomes as all *Anolis* species with 2n = 40 so far described. Molecular phylogenetics (Poe et al. 2017) place *A. lemurinus* nested within a clade in which all the species so far karyotyped show 2n = 40 (Castiglia et al. 2013b). Ancestral state analysis (Castiglia et al. 2013b) indicates that the 2n = 40 karyotype is derived from by five centric fissions of macrochromosomes from an ancestral 2n = 30. What that should be further investigated are the chromosomal rearrangements occurring within macrochromosomes in the 2n = 40 karyotype.

***Anolis uniformis* Cope, 1885**
Lesser scaly anole

Distribution. Occurs from southern Tamaulipas to north-central Honduras on the Atlantic coast.

Samples. RCMX201 (male), RCMX203 (male), RCMX205 (male*), RCMX206 (female*), RCMX209 (female), RCMX210 (male*), RCMX215 (male*) and RCMX226 (female*) from Estación Chajul, Selva Lacandona, Montes Azules, Chiapas, Mexico.

DNA taxonomy. The species was formerly included in the *A. humilis* group, but it is now included in the *Draconura* clade (Poe et al. 2017). Over the 780-bp of the MT-ND2 fragment, the GenBank BLAST reports a 99% identity with *A. uniformis* from Belize (KJ954096 and KJ954099).

Chromosomes. We report here the first description of the karyotype of this species (Fig. 8C). The species is characterized by $X_1X_1X_2X_2/X_1X_2Y$ sex chromosome system. In fact, male individuals have a chromosome number 2n = 29 (14M + 15m) and females show 2n = 30 (14M + 16m). The macrochromosomes can be morphologically divided in two pairs of large metacentrics, three pairs of medium sized metacentrics, one pair of small metacentric and one pair of small acrocentric chromosomes. The X_1 was identi-

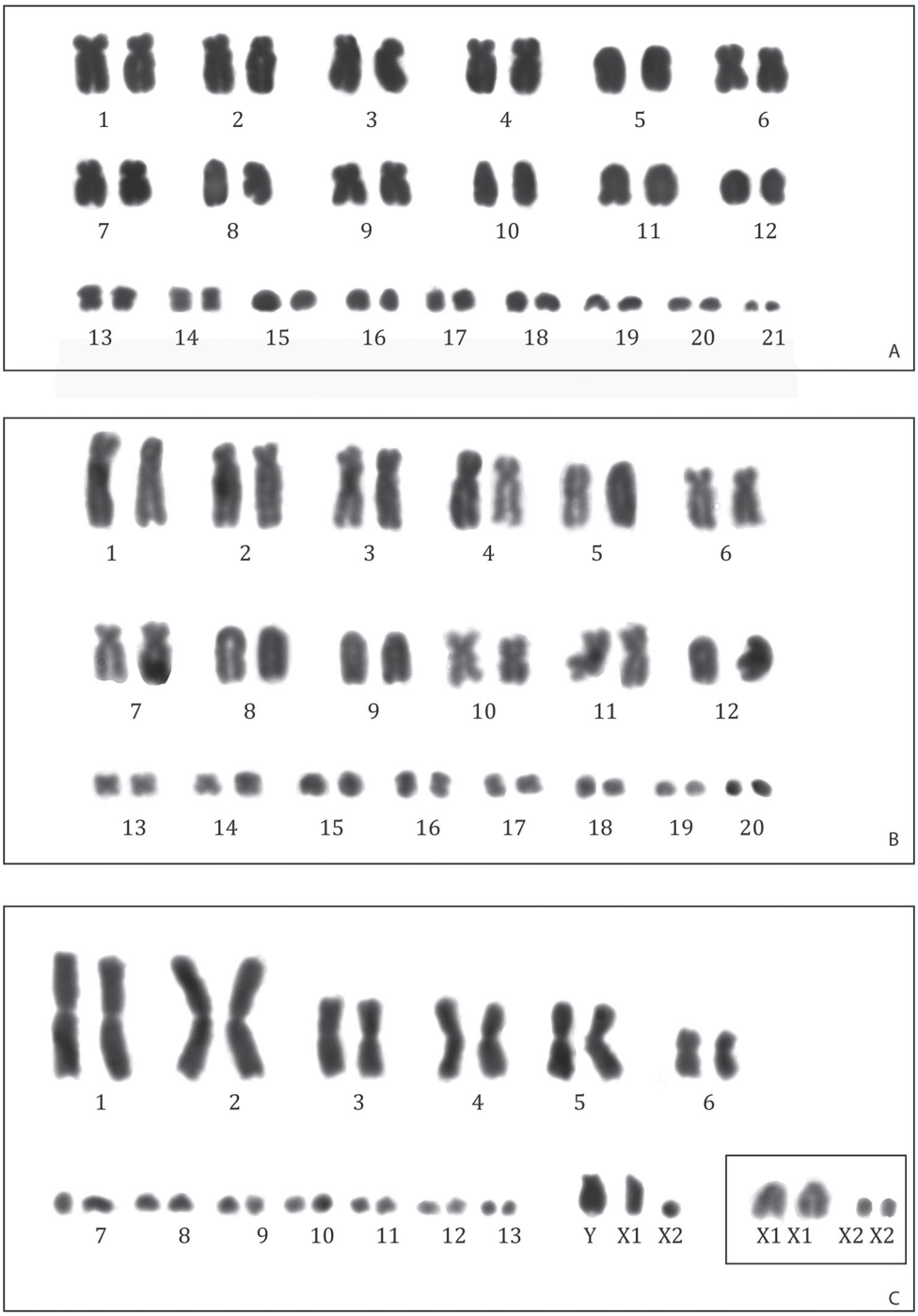

Figure 8. Karyotypes of **A** *Anolis capito* (2n = 40, RCMX218 female) **B** *Anolis lemurinus* (2n = 40, RCMX214 male) and **C** *Anolis uniformis* (2n = 50, RCMX210 male) with YX_1X_2 sex chromosomes; in the box the $X_1X_1X_2X_2$ (RCMX206 female) sex chromosomes.

fied as an acrocentric chromosome and X_2 as a microchromosome. The Y chromosome is an acrocentric one similar in size to X_1.

Among the species of the genus *Anolis* with a known karyotype, this species is phylogenetically close to *A. aquaticus* Taylor, 1956 and *A. biporcatus*. Furthermore, *A. biporcatus* has also a similar composition of the sex chromosomes system, even if the morphology of sex chromosomes is different. In fact, the so-called 2n = 30 karyotype is one of the most common karyotypes in *Anolis*. However, three variants of this karyotype, based on the number and shape of macro- and microchromosomes, have been described. Among them, two types of 2n = 29–30 are present, type-A and type-B (Castiglia et al. 2010).

The type-A, typical of *A. biporcatus*, presents a multiple sex chromosomes system where X_1 is an acrocentric chromosome, X_2 is a microchromosome, and Y is metacentric similar in size to X_1.

In our case, the Y is a small acrocentric chromosome, which might have been derived from a pericentric inversion in the submetacentric Y chromosome of the 2n = 29–30 type-A karyotype.Thus, although it is believed that the onset of multiple sex chromosomes in *Anolis* occurs independently (Castiglia et al. 2013b; Gamble et al. 2014), present data suggest that this condition may represent a trait derived from the common ancestor of the two species.

Conclusions

Combined karyological and DNA taxonomic approaches have allowed us to highlight some interesting taxonomic peculiarities in 10 Mesoamerican lizard species belonging to six genera and five families. The karyotypes of four species, *Phyllodactylus* sp. 3 (*P. tuberculosus* species group), *Holcosus festivus*, *Anolis lemurinus*, and *A. uniformis* are here described for the first time. In *Aspidoscelis deppii* and *Anolis capito*, we found different karyotypes from those previously reported for these species. Moreover, in *A. capito*, the cytogenetic observation is consistent with the considerable genetic divergence at the studied mtDNA marker (MT-ND2), which is indicative of a putative new cryptic species. The anole species here studied exhibited different sex chromosomes configurations including a $X_1X_1X_2X_2/X_1X_2Y$ condition in *A. uniformis* that should be in future studied by molecular cytogenetic techniques.

Another species that may include cryptic taxa is the skink *Scincella cherriei*, for which we found high values of genetic divergence among the specimens from Montes Azules and those from Costa Rica and Nicaragua, comparable to the divergence typical of sister species in skinks. A lower level of genetic divergence, compatible with an intraspecific phylogeographic structure, has been identified for *L. flavimaculatum*. In fact, the studied specimens belong to a mtDNA lineage that is sister with respect to the remaining haplotypes from other populations. However, it should be noted that the novel data represent only the first step in the identification of cryptic species and more efforts are necessary to investigate our assumptions. Both taxonomic revision and the notions related to the chromosome evolution in this hyper-diversified group of reptiles will be worthy of note.

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References

Bauer AM, Good DA, Branch WR (1997) The taxonomy of the southern African 'leaf-toed' geckos (Squamata: Gekkonidae), with a review of Old World '*Phyllodactylus*' and the description of five new genera. Proceedings of the California Academy of Sciences 49: 447–497.

Bezy RL (1972) Karyotypic variation and evolution of the lizards in the family Xantusiidae. Contributions in Sciences 227: 1–29.

Bezy RL (1989) Morphological differentiation in unisexual and bisexual Xantusiid lizards of the genus *Lepidophyma* in Central America. Herpetological Monographs 3: 61–80. https://doi.org/10.2307/1466986

Bezy RL, Camarillo JL (2002) Systematics of xantusiid lizards of the genus *Lepidophyma*. Contributions in Sciences 493: 1–41.

Bickham JW (1984) Patterns and models of chromosomal evolution in reptiles. In: Sharma AK, Sharma A (Eds) Chromosome in Evolution of Eukaryotic Groups (Vol. 2). CRC Press, Florida, 13–40.

Blair C, Méndez de La Cruz FR, Ngo A, Lindell J, Lathrop A, Murphy RW (2009) Molecular phylogenetics and taxonomy of leaf-toed geckos (Phyllodactylidae: *Phyllodactylus*) inhabiting the peninsula of Baja California. Zootaxa 2027(1): 28–42. https://doi.org/10.11646/zootaxa.2027.1.2

Blair C, Méndez-de la Cruz FR, Law C, Murphy R (2015) Molecular phylogenetics and species delimitation of leaf-toed geckos (Phyllodactylidae: *Phyllodactylus*) throughout the Mexican tropical dry forest. Molecular Phylogenetics and Evolution 84: 254–265. https://doi.org/10.1016/j.ympev.2015.01.003

Carvalho NDM, Arias FJ, da Silva FA, Schneider CH, Gross MC (2015) Cytogenetic analyses of five amazon lizard species of the subfamilies Teiinae and Tupinambinae and review of

karyotyped diversity the family Teiidae. Comparative Cytogenetics 9(4): 625–644. https://doi.org/10.3897/CompCytogen.v9i4.5371

Castiglia R, Annesi F, Bezerra AMR, García A, Flores-Villela O (2010) Cytotaxonomy band DNA taxonomy of lizards (Squamata, Sauria) from a tropical dry forest in the Chamela-Cuixmala Biosphere Reserve on the coast of Jalisco, Mexico. Zootaxa 2508: 1–29. https://doi.org/10.11646/zootaxa.2508.1.1

Castiglia R, Bezerra AMR, Flores-Villela O, Annesi F, Muñoz A, Gornung E (2013a) Comparative cytogenetics of two species of ground skinks: *Scincella assata* and *S. cherriei* (Squamata: Scincidae: Lygosominae) from Chiapas, Mexico. Acta Herpetologica 8: 69–73. https://doi.org/10.13128/Acta_Herpetol-11315

Castiglia R, Flores-Villela O, Bezerra AMR, Muñoz A, Gornung E (2013b) Pattern of chromosomal changes in 'beta' *Anolis* (*Norops* group) (Squamata: Polychrotidae) depicted by an ancestral state analysis. Zoological Studies 52: 1–60. https://doi.org/10.1186/1810-522X-52-60

Castiglia R, García A, Bezerra AMR, Flores-Villela O, Gornung E (2009) Karyotypic diversification due to Robertsonian rearrangements in *Phyllodactylus lanei* Smith, 1935 (Squamata, Gekkonidae) from Mexico. Rendiconti Lincei 20: 77–82. https://doi.org/10.1007/s12210-009-0005-4

Clement M, Snell Q, Walker P, Posada D, Crandall K (2002) TCS: estimating gene genealogies. Proceedings of the 16th International Parallel and Distributed Processing Symposium 2: e184. https://doi.org/10.1109/IPDPS.2002.1016585 [Accessed 06 October 2020]

CONANP (2019) CONANP – Comisíon Nacional de Áreas Naturales Protegidas. Áreas Naturales Protegidas. Información espacial de las Áreas Naturales Protegidas, Gobierno de Mexico. http://sig.conanp.gob.mx/website/pagsig/info_shape.htm [Accessed 24 June 2020]

Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. Nature Methods 9: e772. https://doi.org/10.1038/nmeth.2109

De Smet, WHO (1981) Description of the orcein stained karyotypes of 27 lizard species (Lacertilia Reptilia) belonging to the families Iguanidae, Agamidae, Chamaeleontidae and Gekkonidae (Ascalabota). Acta Zoologica Pathologica Antverpiensia 76: 35–72.

Deakin JE, Ezaz T (2019) Understanding the evolution of reptile chromosomes through applications of combined cytogenetics and genomics approaches. Cytogenetic and Genome Research 157: 7–20. https://doi.org/10.1159/000495974

Funk SM, Fa JE (2006) Phylogeography of the endemic St. Lucia whiptail lizard *Cnemidophorus vanzoi*: Conservation genetics at the species boundary. Conservation Genetics 7: 651–663. https://doi.org/10.1007/s10592-005-9068-7

Gamble T, Bauer AM, Greenbaum E, Jackman TR (2008) Out of the blue: a novel, trans-Atlantic clade of geckos (Gekkota, Squamata). Zoologica Scripta 37(4): 355–366. https://doi.org/10.1111/j.1463-6409.2008.00330.x

Gamble T, Geneva AJ, Glor RE, Zarkower D (2014) *Anolis* sex chromosomes are derived from a single ancestral pair. Evolution 68(4): 1027–1041. https://doi.org/10.1111/evo.12328

García A, Ceballos G (1994) Guía de campo de los reptiles y anfibios de la costa de Jalisco, México. Fundación Ecológica de Cuixmala, AC.

García de Miranda E, Falcón de Gyves Z (1986) Nuevo Atlas Porrúa de la Republica Mexicana. (7a. edición). Porrua, 219 pp.

García-Vázquez UO, Contreras-Arquieta A, Trujano-Ortega M, Nieto-Montes de Oca A (2018a) A new species of *Gerrhonotus* (Squamata: Anguidae) from the Cuatro Ciénegas Basin, Coahuila, Mexico. Herpetologica 74: 269–278. https://doi.org/10.1655/HERPETOLOGICA-D-17-00013

García-Vázquez UO, Nieto-Montes de Oca A, Bryson Jr RW, Schmidt-Ballardo W, Pavón-Vázquez CJ (2018b) Molecular systematics and historical biogeography of the genus *Gerrhonotus* (Squamata: Anguidae). Journal of Biogeography 45: 1640–1652. https://doi.org/10.1111/jbi.13241

Giovannotti M, Cerioni PN, Slimani T, Splendiani A, Paoletti A, Fawzi A, Barucchi VC (2017) Cytogenetic Characterization of a population of *Acanthodactylus lineomaculatus* Duméril and Bibron, 1839 (Reptilia, Lacertidae), from Southwestern Morocco and insights into sex chromosome evolution. Cytogenetic and Genome Research 153: 86–95. https://doi.org/10.1159/000484533

Giovannotti M, Trifonov V, Paoletti A (2017) New insights into sex chromosome evolution in anole lizards (Reptilia, Dactyloidae). Chromosoma 126: 245–260. https://doi.org/10.1007/s00412-016-0585-6

Gorman GC (1973) The chromosomes of reptiles, a cytotaxonomic interpretation. In: Chiarelli B, Capanna E (Eds) Cytotaxonomy and Vertebrate Evolution. Academic Press, London, 349–424.

Hardy LM, Raymond LR, Harris S (2017) The karyotype of *Plestiodon anthracinus* (Baird, 1850) (Sauria: Scincidae): a step toward solving an enigma. Southeastern Naturalist 16(3): 326–331. https://doi.org/10.1656/058.016.0318

Harvey MB, Ugueto GN, Gutberlet Jr RL (2012) Review of teiid morphology with a revised taxonomy and phylogeny of the Teiidae (Lepidosauria: Squamata). Zootaxa 3459: 1–156. https://doi.org/10.11646/zootaxa.3459.1.1

HGNC (2019) HUGO Gene Nomenclature Committee. https://www.genenames.org [accessed 07 May 2020]

Honda M, Ota H, Kohler G, Ineich I, Chirio L, Chen SL, Hikida T (2003) Phylogeny of the lizard subfamily Lygosominae (Reptilia: Scincidae), with special reference to the origin of the New World taxa, Genes and Genetic Systems 78: 71–80. https://doi.org/10.1266/ggs.78.71

Johnson JD, Mata-Silva V, García-Padilla E, Wilson LD (2015) The herpetofauna of Chiapas, Mexico: composition, physiographic distribution, and conservation status. Mesoamerican Herpetology 2: 272–329.

Kichigin IG, Giovannotti M, Makunin AI, Kabilov MR, Tupikin AE, Barucchi VC, Splendiani A, Ruggeri P, Rens W, O'Brien PC (2016) Evolutionary dynamics of *Anolis* sex chromosomes revealed by sequencing of flow sorting-derived microchromosome-specific DNA. Molecular Genetics and Genomics 291(5): 1955–1966. https://doi.org/10.1007/s00438-016-1230-z

King M (1981) Chromosome change and speciation in lizards. In: Atchley WR, Woodruff DS (Eds) Evolution and Speciation. Cambridge University Press, 262–265.

Koch C, Flecks M, Venegas PJ, Bialke P, Valverde S, Roedder D (2016) Applying n-dimensional hypervolumes for species delimitation: unexpected molecular, morphological, and ecological diversity in the Leaf-Toed Gecko *Phyllodactylus reissii* Peters, 1862 (Squamata: Phyllodactylidae) from northern Peru. Zootaxa 4161(1): 41–80. https://doi.org/10.11646/zootaxa.4161.1.2

Kocher TD, Thomas WK, Meyer A, Edwards SV, Pääbo S, Villablanca FX, Wilson AC (1989) Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proceedings of the National Academy of Sciences 86(16): 6196–6200. https://doi.org/10.1073/pnas.86.16.6196

Köhler G, Schulze A, Vesely M (2005) Morphological variation in *Norops capito* (Peters, 1863), a wide-spread species in southeastern Mexico and Central America. Salamandra 41: 129–136.

Lee JC (1996) The Amphibians and Reptiles of the Yucatán Peninsula. Comstock Publishing Associates, Cornell University Press, Ithaca, 500 pp.

Leigh JW, Bryant D (2015) PopART: Full-feature software for haplotype network construction. Methods in Ecology and Evolution 6(9): 1110–1116. https://doi.org/10.1111/2041-210X.12410

Linkem CW, Diesmos AC, Brown RM (2011) Molecular systematics of the Philippine forest skinks (Squamata: Scincidae: *Sphenomorphus*): testing morphological hypotheses of interspecific relationships. Zoological Journal of the Linnean Society 163(4): 1217–1243. https://doi.org/10.1111/j.1096-3642.2011.00747.x

Lowe CH, Wright JW, Cole CJ, Bezy RL (1970) Chromosomes and evolution of the species groups of *Cnemidophorus* (Reptilia: Teiidae). Systematic Zoology 19: 128–141. https://doi.org/10.2307/2412450

Macey JR, Schulte II JA, Larson A, Tuniyev BS, Orlov N, Papenfuss TJ (1999) Molecular phylogenetics, tRNA evolution, and historical biogeography in anguid lizards and related taxonomic families. Molecular Phylogenetics and Evolution 12(3): 250–272. https://doi.org/10.1006/mpev.1999.0615

Manríquez-Morán NL, Villagrán-Santa Cruz M, Méndez de la Cruz F-R (2000) Origin and evolution of the parthenogenetic lizards, *Cnemidophorus maslini* and *C. cozumelae*. Journal of Herpetology 34: 634–637. https://doi.org/10.2307/1565287

Matos NB, Ferreira M, Jesus Silva F, Rodrigues MT, Santos da Silva E, Garcia C (2016) Taxonomy and evolution of *Tropidurus* (Iguania, Tropiduridae) based on chromosomal and DNA barcoding analysis. Journal of Herpetology 50: 316–326. https://doi.org/10.1670/13-221

Meza-Lázaro RN, Nieto-Montes de Oca A (2015) Long forsaken species diversity in the Middle American lizard *Holcosus undulatus* (Teiidae). Zoological Journal of the Linnean Society 175: 189–210. https://doi.org/10.1111/zoj.12264

Murphy RW, Blair C, Méndez-de la Cruz FR (2009) A new species of leaf-toed gecko, genus *Phyllodactylus* (Squamata: Gekkota: Phyllodactylidae) from Guerrero, Mexico. South American Journal of Herpetology 4(1): 17–24. https://doi.org/10.2994/057.004.0103

NALCMS – North American land Change Monitoring System (2020) North American land cover. http://www.cec.org/tools-and-resources/north-american-environmental-atlas/mapfiles [Accessed 24 June 2020]

Nielsen SV, Daza JD, Pinto BJ, Gamble T (2019) ZZ/ZW sex chromosomes in the endemic Puerto Rican leaf-toed gecko (*Phyllodactylus wirshingi*). Cytogenetic and Genome Research 157(1–2): 89–97. https://doi.org/10.1159/000496379

Nielsen SV, Pinto BJ, Guzmán-Méndez IA, Gamble T (2020) First report of sex chromosomes in night lizards (Scincoidea: Xantusiidae). Journal of Heredity 111(3): 307–313. https://doi.org/10.1093/jhered/esaa007

Noonan BP, Pramuk JB, Bezy RL, Sinclaire EA, de Queiroz K, Sites Jr JW (2013) Phylogenetic relationships within the lizard clade Xantusiidae: Using trees and divergence times to address evolutionary questions at multiple levels. Molecular Phylogenetics and Evolution 69: 109–122. https://doi.org/10.1016/j.ympev.2013.05.017

Olmo E, Signorino GG (2005) Chromorep: a reptiles chromosomes database. http://chromorep.univpm.it/ [accessed 06 October 2020]

Palacios-Aguilar R, Santos-Bibiano R, Flores-Villela O (2018) A New Species of *Lepidophyma* (Squamata: Xantusiidae) from the Pacific Lowlands of Guerrero, Mexico. Journal of Herpetology 52(3): 327–331. https://doi.org/10.1670/17-061

Palumbi SR, Martin A, Romano S, McMillan WO, Stice L, Grabowski G (1991) The simple fool's guide to PCR, version 2.0. University of Hawaii, Honolulu, 45 pp.

Pellegrino KCM, Santos RML, Rodrigues MT, Laguna MM, Amaro RC, Yonenaga-Yassuda Y (2009) Chromosomal evolution in the Brazilian geckos of the genus *Gymnodactylus* (Squamata, Phyllodactylidae) from the biomes of Cerrado, Caatinga and Atlantic rain forest: evidence of Robertsonian fusion events and supernumerary chromosomes. Cytogenetic and Genome Research 127: 191–203. https://doi.org/10.1159/000295175

Poe S, Nieto-Montes de Oca A, Torres-Carvajal O, De Queiroz K, Velasco JA, Truett B, Gray LN, Ryan MJ, Köhler G, Ayala-Varela F, Latella I (2017) A phylogenetic, biogeographic, and taxonomic study of all extant species of *Anolis* (Squamata; Iguanidae). Systematic Biology 66(5): 663–697. https://doi.org/10.1093/sysbio/syx029

Pokorná MJ, Trifonov VA, Rens W, Ferguson-Smith MA, Kratochvíl L (2015) Low rate of interchromosomal rearrangements during old radiation of gekkotan lizards (Squamata: Gekkota). Chromosome Research 23(2): 299–309. https://doi.org/10.1007/s10577-015-9468-6

Pyron RA, Burbrink FT, Wiens JJ (2013) A phylogeny and revised classification of Squamata, including 4161 species of lizards and snakes. BMC Evolutionary Biology 13: 1–93. https://doi.org/10.1186/1471-2148-13-93

QGIS (2017) QGIS 2.18.9 'Las Palmas'. Free Software Foundation, Inc., Boston. http://www.qgis.org/en/site/forusers/download.html [Accessed 24 June 2020]

Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA (2018) Posterior summarization in Bayesian phylogenetics using Tracer 1.7. Systematic Biology 67(5): e901. https://doi.org/10.1093/sysbio/syy032

Ramirez-Bautista A (1994) Manual y claves ilustradas de los anfibios y reptiles de la region de Chamela, Jalisco, Mexico. Cuadernos del Instituto de Biologıa, UNAM, 127 pp.

Ramírez-Reyes T, Flores-Villela O (2018) Taxonomic changes and description of two new species for the *Phyllodactylus lanei* complex (Gekkota: Phyllodactylidae) in Mexico. Zootaxa 4407(2): 151–190. https://doi.org/10.11646/zootaxa.4407.2.1

Ramírez-Reyes T, Piñero D, Flores-Villela O, Vázquez-Domínguez E (2017) Molecular systematics, species delimitation and diversification patterns of the *Phyllodactylus lanei* complex (Gekkota: Phyllodactylidae) in Mexico. Molecular Phylogenetics and Evolution 115: 82–94. https://doi.org/10.1016/j.ympev.2017.07.008

Reeder TW, Cole CJ, Dessauer HC (2002) Phylogenetic relationships of whiptail lizards of the genus *Cnemidophorus* (Squamata: Teiidae): a test of monophyly, reevaluation of karyotypic evolution, and review of hybrid origins. American Museum Novitates 3365: 1–61. https://doi.org/10.1206/0003-0082(2002)365<0001:PROWLO>2.0.CO;2

Ríos-Muñoz CA (2013) ¿Es posible reconocer una unidad biótica entre América del Norte y del Sur? Revista Mexicana de Biodiversidad 84: 1022–1030. https://doi.org/10.7550/rmb.34170

Ronquist F, Huelsenbeck JP (2003) MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572–1574. https://doi.org/10.1093/bioinformatics/btg180

Rovatsos M, Farkačová K, Altmanová M, Pokorná MJ, Kratochvíl L (2019) The rise and fall of differentiated sex chromosomes in geckos. Molecular Ecology 28: 3042–3052. https://doi.org/10.1111/mec.15126

Santos RML, Pellegrino KCM, Rodrigues MT, Yonenaga-Yassuda Y (2007) Banding patterns and chromosomal evolution in five species of Neotropical Teiinae lizards (Squamata: Teiidae). Genetica 131: 231–240. https://doi.org/10.1007/s10709-006-9133-2

Savage JM (1982) The enigma of the Central America Herpetofauna: dispersals or vicarance? Annals of the Missouri Botanical Garden 69: 464–547. https://doi.org/10.2307/2399082

Sinclair EA, Pramuk JB, Bezy RL, Crandall KA, Sites Jr JW (2010) DNA evidence for non-hybrid origins of parthenogenesis in vertebrates. Evolution 64: 1346–1357. https://doi.org/10.1111/j.1558-5646.2009.00893.x

Udvardi M (1984) The IUCN/UNESCO system of biogeographic provinces in relation to the Biosphere Reserves. In: Unesco-UNEP (Ed.) Conservation science and society. Contributions to the First International Biosphere Reserve Congress, Minsk, Byelorussia/USSR. USSR, 16–19.

Uetz P, Freed P, Hošek J (2020) The Reptile Database. http://www.reptile-database.org [accessed on May 2020]

UNESCO (2018) UNESCO's commitment to biodiversity, Connecting people and nature for an inspiring future. UNESCO, Paris, 52 pp. https://unesdoc.unesco.org/ark:/48223/pf0000265200 [accessed 06, October 2020]

Walker JM, Cordes JE, Taylor HL (1997) Parthenogenetic *Cnemidophorus tesselatus* complex (Sauria: Teiidae): a neotype for diploid *C. tesselatus* (Say, 1823), redescription of the taxon, and description of a new triploid species. Herpetologica 53: 233–259.

Weiss AJ, Hedges SB (2007) Molecular phylogeny and biogeography of the Antillean geckos *Phyllodactylus wirshingi*, *Tarentola americana*, and *Hemidactylus haitianus* (Reptilia, Squamata). Molecular Phylogenetics and Evolution 45(1): 409–416. https://doi.org/10.1016/j.ympev.2007.01.006

Wilson LD, Johnson JD (2010) Distributional patterns of the herpetofauna of Mesoamerica, a biodiversity hotspot. Conservation of Mesoamerican Amphibians and Reptiles 30: e235.

CompCytogen 14(4): 639–643 (2020)
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CORRIGENDA

COMPARATIVE Cytogenetics
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Corrigenda: Cytogenetic markers as a tool for characterization of hybrids of *Astyanax* Baird & Girard, 1854 and *Hyphessobrycon* Eigenmann, 1907. Comparative Cytogenetics 14(2): 231–242. https://doi.org/10.3897/CompCytogen.v14i2.49513

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After the publication of our article, we detected some inconsistencies in figures and figure captions. The nominal species *Astyanax altiparanae* was recently recognized as a new junior synonym of *Astyanax lacustris* Lütken, 1875. Thus, we corrected this issue in all figures. Captions of figure 3, 5 and 6 were also incorrect. Corrected figures and captions are as follows:

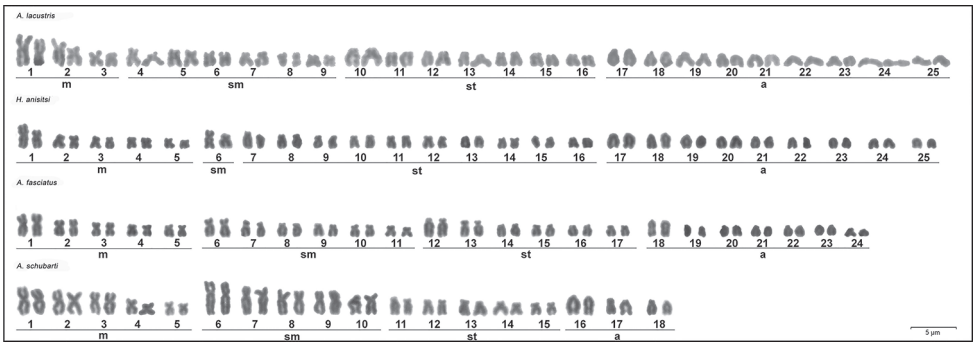

Figure 1. Karyotypes of parental individuals analyzed: *A. lacustris* (3m+6sm+7st+9a), *H. anisitsi* (5m+1sm+10st+9a), *A. fasciatus* (5m+6sm+6st+7a) and *A. schubarti* (5m+5sm+5st+3a). Scale bar: 5µm.

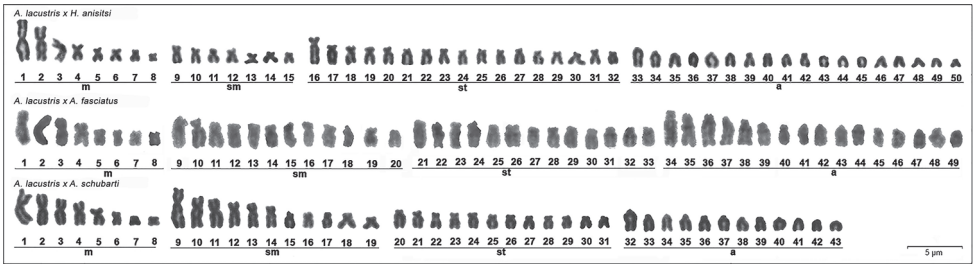

Figure 2. Karyotypes of hybrid products of the genus *Astyanax*: *A. lacustris* × *H. anisitsi* (8m+7sm+17st+18a), *A. lacustris* × *A. fasciatus* (8m+7sm+17st+18a) and *A. lacustris* × *A. schubarti* (8m+11sm+12st+12a), respectively. Scale bar: 5µm.

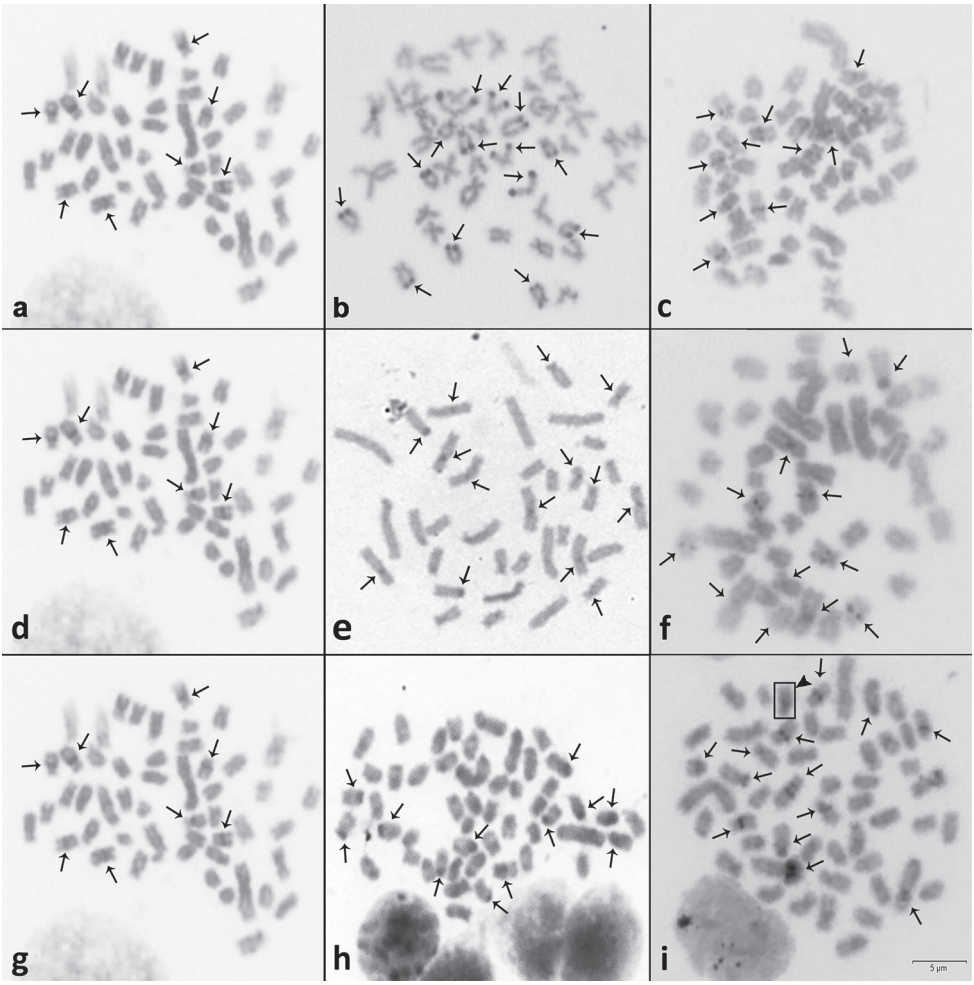

Figure 3. Heterochromatic markers obtained by C-banding on metaphase plates of *A. lacustris* (**a, d, g**), *A. fasciatus* (**b**), *A. schubarti* (**e**), *H. anisitsi* (**h**) and hybrids *A. lacustris* × *A. fasciatus* (**c**), *A. lacustris x A. schubarti* (**f**) and *A. lacustris* × *H. anisitsi* (**i**) after C-banding. The arrows indicate heterochromatic blocks. The chromosomes number of *A. lacustris* and *H. anisitsi* hybrid is 2N = 51, the metaphase plate of *A. lacustris* and *H. anisitsi* hybrid (i) contains a heterochromatic chromosome (arrowhead). Scale bar: 5µm.

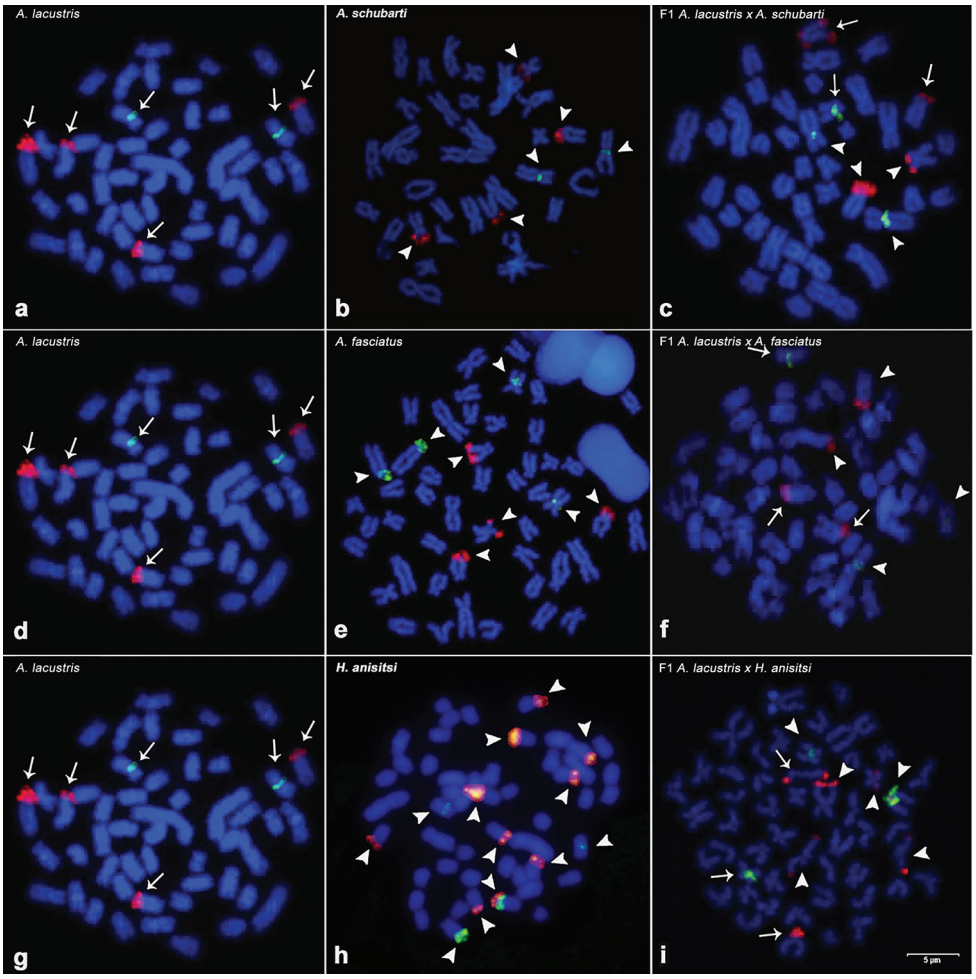

Figure 4. *FISH* with probes 5S (green) and 18S (red). The results are labeled as: *A. lacustris* (**a, d, g**), *A. schubarti* (**b**), hybrid *A. lacustris* × *A. schubarti* (**c**), *A. fasciatus* (**e**), hybrid *A. lacustris* × *A. fasciatus* (**f**), *H. anisitsi* (**h**), and hybrid *A. lacustris* × *H. anisitsi* (**i**). The arrows indicate chromosomes inherited from *A. lacustris*, and the arrowheads indicate chromosomes inherited from the other respective parents. Scale bar: 5µm.

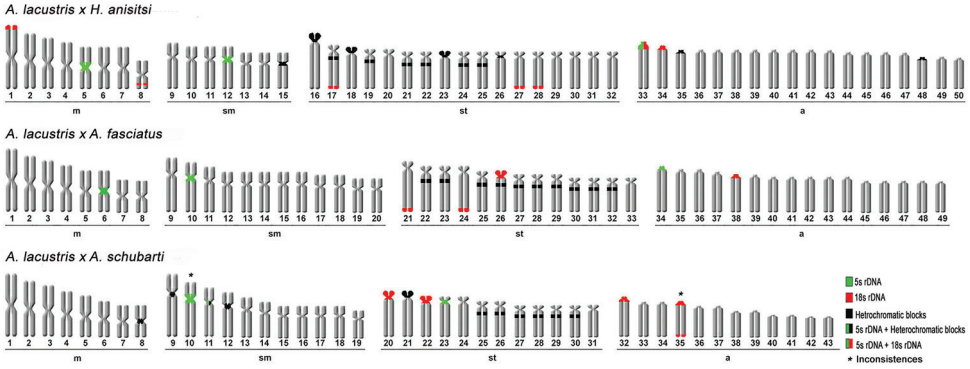

Figure 5. Ideogram of hybrids strains.

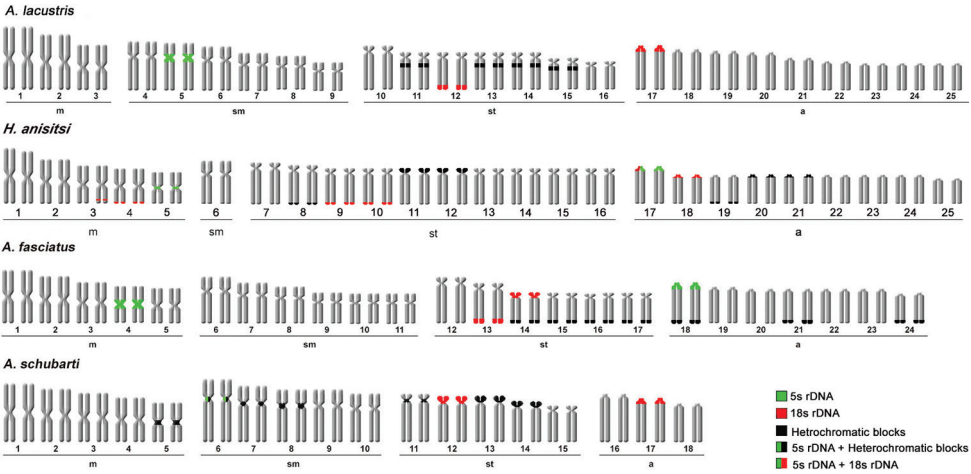

Figure 6. Ideogram of parental strains.