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Comparative cytogenetics on *Zamenis lineatus* and *Elaphe quatuorlineata* (Serpentes: Colubridae)

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Abstract. Because of their peculiar genomic and chromosomal characteristics, reptiles are extraordinary model organisms to study karyotype and sex chromosome evolution, but despite the growing interest in their evolutionary cytogenetics, only a small fraction of species have a known karyotype. We performed a comparative cytogenetic analysis on *Elaphe quatuorlineata* and *Zamenis lineatus*, using classic and molecular techniques. We provide the karyotype of these two species and an assessment of their chromosomal features. Chromosome analysis was performed with standard karyotyping, C-banding, sequential C-banding + CMA_3 + DAPI and Ag-NOR staining. On *E. quatuorlineata*, we also performed CMA_3-methyl green staining and Fluorescence in situ Hybridization mapping NOR loci (NOR-FISH). *Elaphe quatuorlineata* and *Z. lineatus* show a very similar karyotype of 2n = 36, with 8 macro- and 10 microchromosome pairs, but differ in the morphology of the pair 8, which resulted submetacentric in the former and metacentric in the latter species. By comparing our data to those available from the literature on congeneric species, we analysed the occurrence of primitive and derivate chromosomal characters and provide cytotaxonomic insights, which further support the species status of *Z. lineatus.* In both species, the 4th pair was identified as the sex chromosome pair (ZZ/ZW) and NORs were localized on a microchromosome pair. We finally highlight in both genera *Elaphe* and *Zamenis* different stages of heterochromatinization of the W chromosome, in agreement with the progressive diversification model of sex chromosome as already shown in different reptile taxa.

Keywords. Chromosome, evolution, karyotype, NORs, squamates, snakes.

INTRODUCTION

Classic cytogenetic through differential staining and banding of chromosomes permits to describe and compare karyotypes, whereas the molecular cytogenetic approach, employing Fluorescence *in situ* Hybridization (FISH) with specific probes, allows the detection of particular sequences present in genomes (Matsuda et al., 2005; Dumas and Sineo, 2014), including repetitive DNA sequences (Scardino et al., 2020a). Among those repetitive DNA elements are the ribosomal DNA (rDNA), encoding rRNA. These elements have been successfully used as markers for comparative cytogenetic studies and phylogenetic analyses. The rDNA is organized into 2 families: 5S (minor) and 45S (major) rDNA. The latter comprises the genes for 18S, 5.8S, and 28S rRNA and is located in the so-called nucleolus organizer regions (NORs). The NORs can be identified either by silver staining, which detects only transcriptionally active loci, or more accurately, by FISH, which permits the identification of both active and inactive NORs. The location of the rDNA loci in the karyotype may show a species-specific pattern, so rDNA loci are often used for complex karyotype characterizations (Scardino et al., 2020a). Indeed, compara-

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tive chromosome analyses can be useful to identify plesiomorphic and apomorphic states and the occurrence of different evolutionary lineages (Deakin and Ezaz, 2014; Damas et al., 2018; Scardino et al., 2020b). Chromosome rearrangements may precede or follow molecular evolution, directly promoting cladogenesis or resulting from phylogenetic diversification (Noor et al., 2001; Rieseberg, 2001). In either case, they represent discrete evolutionary markers able to detect different evolutionary trends or apomorphisms in the taxa studied (Dobigny et al., 2004; Olmo, 2008; Dumas et al., 2015).

Squamate reptiles, due to their peculiar genomic and chromosomal characteristics, are exceptional model organisms in the study of karyotype evolution and sex chromosome diversification of vertebrates (Olmo, 2008; Alam et al., 2018). Squamates display a remarkable variability in chromosome number and morphology, number and location of different chromosome markers and the occurrence of environmental genetic sex determination, with the independent evolution of simple and multiple sex chromosome systems with either male or female heterogamety (Olmo, 2008; Pallotta et al., 2017; Deakin and Ezaz, 2019; Sidhom et al., 2020; Mezzasalma et al., 2021 a). The cytogenetic approach used for the study of chromosome rearrangements and different morphologies and/or levels of heterochromatinization of the heteromorphic sex chromosomes have been previously used in different phylogenetically closely-related European squamate taxa such as the snakes of the genus *Hierophis* (Fitzinger, 1843), *Anguis fragilis* Linnaeus, 1758, and *A. veronensis* Pollini, 1818, and geographically distinct populations of *Coronella austriaca* Laurenti, 1768 (Mezzasalma et al., 2013, 2015, 2018b; Mezzasalma and Odierna, 2021). However, despite the growing interest in the evolutionary cytogenetics of squamate reptiles, only a small fraction of the described squamate species have a known karyotype (Olmo and Signorino, 2006; Mezzasalma et al., 2021), leaving most of their chromosomal diversity still unexplored. This is also true for some peculiar Mediterranean reptile species such as the European four-lined snake *Elaphe quatuorlineata* (Bonnaterre, 1790) and the Italian Aesculapian snake *Zamenis lineatus* (Camerano, 1891).

In this work, we performed a comparative cytogenetic analysis on *E. quatuorlineata* and *Z. lineatus*, using a combination of standard staining and banding techniques. We provide the first karyotype description of these two species and an assessment of their chromosomal features. By comparing our data to those available from the literature on phylogenetically closely-related species, we evidence and discuss the occurrence and distribution of primitive and derivate chromosomal characters in the species studied and provide cytotaxonomic insights, which support the species status of *Z. lineatus*. We also highlight that both genera *Elaphe* and *Zamenis* show progressive evolutionary stages of the W chromosome, supporting the heterochromatinization model of sex chromosome diversification (see e.g., Mezzasalma et al., 2021).

MATERIAL AND METHODS

We analysed two samples (one male and one female) of *Z. lineatus* and one female of *E quatuorlineata* from Piedimonte Matese, Campania, Italy. Specimens were anesthetized on ice and, after taking a 0.5 ml of blood aliquot from the caudal vein, they were released in the capture site. Chromosomes were obtained from blood cultures following Odierna et al. (2004). Namely, blood aliquots were incubated for four days at 30 °C in 5 ml of lymphocyte medium culture (3.8 ml of DMEM, 0.5 ml sterile distilled water, 0.5 newborn calf serum, 0.1 ml antibiotics, 0.1 ml PHA). Chromosome harvesting was performed by adding 0.1 ml of Colcemid (10 μg/ml) and two hours later the cells were collected by centrifugation (1000 rpm/min), incubated for 30 min in 5 ml of hypotonic solution (KCl 0.075 M) and fixed in methanolacetic liquid (methyl alcohol + acetic acid, 3:1). Slides were prepared using the air-drying method, as described in Mezzasalma et al. (2019). The cytogenetic analysis was performed with traditional karyotyping (5% Giemsa solution at pH 7 for 10 min) and additional chromosome staining and banding techniques; in particular, C-banding was performed following Sumner (1972) and sequential C-banding + CMA_3 + DAPI according to Sidhom et al. (2020), which highligh CG and AT-rich regions, respectively. Nucleolus organizing regions (NORs) were identified following the Ag-NOR staining method described by Howell and Black (1980). Given quantity and quality of metaphase plates, on *E. quatuorlineata* we also performed Chromomycin A_3-methyl green staining (CMA/MG) (a staining method usefulf to hightlight CG-rich chromosome regions) as described by Sahar and Latt (1980) and Fluorescence *in situ* Hybridization (NOR-FISH) following Mezzasalma et al. (2018a), using as probe the PCR-amplified and biotinylated 18S rRNA gene of the gekkonid *Tarentola mauritanica* (Linnaeus, 1758). In brief, after denaturation in 70% formamide and 2x SSC for 2 min at 80 °C, slides were incubated overnight at 40 °C with the hybridization mixture (10 ng/ml biotinylated 16 dUTP probe 0.1 μm/ml *Escherichia coli* DNA in 50% formamide and 2x SSC). After washing in 2x SSC, cytochemical detection was performed using 5 μm/ml FITC-conjugated ExtrAvidin (Sigma) in 4x SSC + 1% BSA + 0.1%

Tween 20, pH 7. After washing three times in 4x SSC and 0.1% Tween 20 for 10 min at 42 °C, the detection of FISH signals was performed with ExtrAvidin FITC (Sigma Aldrich) counterstained with propidium iodide (PI) (200 ng/ml) in 2x SSC, pH 7, for 2 min at room temperature. Metaphase plates were scored and recorded with an optical and an epifluorescent microscope (Axioscope Zeiss) equipped with an image analysis system. Karyotype reconstruction was performed after scoring at least five metaphase plates from each sample studied and chromosomes were classified according to Levan et al. (1964).

RESULTS

The karyotypes of *E. quatuorlineata* and *Z. lineatus* are both composed of 2n = 36 chromosomes, with 8 macrochromosome pairs and 10 microchromosome pairs (Fig. 1A, 2A). The two species also show the same chromosome morphology with the exception of the chromosome pair 8, which resulted submetacentric in *E quatorlineata* and metacentric in *Z. lineatus* (Table 1, Fig. 2A). Arm number (AN) resulted = 50 in both colubrids. Morphometric parameters of each macrochromosome pair of both species studied are reported in Table 1.

C-banding and Ag-NOR revealed in both species the occurrence of NOR loci on a microchromosome pair, as confirmed by NOR-FISH in *E. quatuorlineata* (Fig. 1B-D). C-banding showed heterochromatin content on autosomes, mostly concerning telomeric and centromeric regions in both *E. quatuorlineata* (Fig. 1E-G) and *Z. lineatus* (Fig. 2B-D). Furthermore, in the female samples of both species, one element of the 4th macrochromosome pair resulted to be largely heterochromatic, allowing us to identify this pair as a homomorphic ZW sex chromosome pair (Fig. 1E-G, 2B-D). This W chromosome resulted highly positive with both CMA_3 and DAPI in *E. quatuorlineata* (Fig. 1E-G), whereas it was clearly evident with DAPI and less evident with CMA_3 in *Z. lineatus* (Fig. 2B-D).

Table 1. Chromosome morphometric parameters. Chr. = Chromosome number; RL = Relative length (Chromosome length/total karyotype length*100); CI = Centromeric index (short arm length/chromosome length*100); m = metacentric; sm = submetacentric; t = telocentric.

Chr.	*Elaphe quatuorlineata*		*Zamenis lineatus*	
	RL	CI	RL	CI
1	19.4 ± 0.8	44.9 ± 3.4 (m)	19.3 ± 1.0	48.4 ± 3.4 (m)
2	16.5 ± 0.5	34.8 ± 4.0 (sm)	16.1 ± 0.7	35.2 ± 4.0 (sm)
3	11.1 ± 0.5	48.4± 3.8 (m)	10.8 ± 0.4	43.2 ± 3,8 (m)
4(Z)	7.7 ± 0.6	45.9 ± 2.8 (m)	8.3 ± 0.6	45.4 ± 2.8 (m)
4(W)	7.6 ± 0.4	45.1 ± 3.4 (m)	8.4 ± 0,3	44.9 ± 3.4 (m)
5	7.5 ± 0.7	48.3 ± 4.3 (m)	7.9 ± 0.5	42.8 ± 4.3 (m)
6	6.0 ± 0.4	0.0 ± 3.0 (t)	7.1 ± 0.6	0.0 ± 3.0 (t)
7	5.6 ± 0.7	36.0 ± 3.1 (sm)	6.3 ± 0.5	36.3 ± 3.1(sm)
8	5.3 ± 0.4	36.9 ± 3.3 (sm)	4.4 ± 0.6	40.1 ± 3.3 (m)
9-18	20.6 ± 1.1		19.8 ± 1.3	

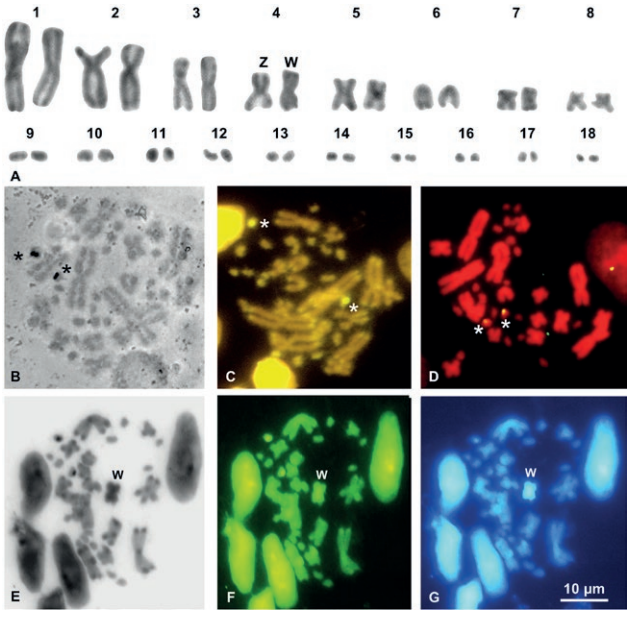

Fig. 1. Karyotype and metaphase plates of *E. quatuorlineata* stained with Giemsa (A), Ag-NOR (B), CMA_3/MG (C), NOR-FISH (D), C-banding + Giemsa (E), + CMA3 (F), + DAPI (G). * = loci of NORs.

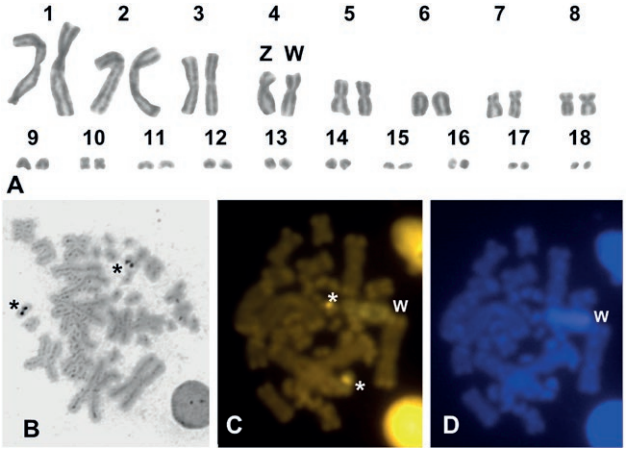

Fig. 2. Karyotype and metaphase plates of *Z. lineatus* stained with Giemsa (A), Ag-NOR (B), C-banding + CMA3 (C), + DAPI (D). * = loci of NORs.

DISCUSSION

Our results show that the karyotypes of *E. quatuorlineata* and *Z. lineatus* have the same diploid number (2n = 36) and a similar general structure, but a different morphology of chromosome pair 8.

In order to highlight the occurrence of simplesiomorphic, sinapomorphic and apomorphic states and add data for the reconstruction of the chromosomal evolution in the genera *Elaphe* and *Zamenis*, we compared the newly generated karyotypes to those available from the literature on congeneric species as well as with that of the hypothesized Ancestral Snake Karyotype (ASK) (see Kobel, 1967; Bianchi et al., 1969; Singh, 1972; Itoh et al., 1970; De Smet, 1978; Augstenová et al., 2017; Rovatsos et al., 2018; Cole and Hardy, 2019) (Fig. 3).

This comparison permits to show that *E. quatuorlineata* and *Z. lineatus*, as well as most congeneric species with a known karyotype, have different chromosomal characters which are considered simplesiomorphisms and found in the hypothesized ASK (see Kobel, 1967; Bianchi et al., 1969; Singh, 1972; Itoh et al., 1970; De Smet, 1978; Augstenová et al., 2017; Rovatsos et al., 2018; Cole and Hardy, 2019; this paper). These shared ancestral characters include: diploid number, number of macro- and microchromosome pairs, the general morphology of several macrochromosome pairs and the localization of NOR loci on a microchromosome pair (see also Cole and Hardy, 2019). All this evidence permits to confirm that *Elaphe* and *Zamenis* are karyologically very conservative, but for the morphology and sequence content of the W chromosome, which are variable among different taxa (see also Augstenová et al., 2017; Cole and Hardy, 2019; Mezzasalma and Odierna, 2021).

Nevertheless, the karyotypes of *E. quatuorlineata* and *Z. lineatus* also possess some peculiar derivate features, which characterize their respective karyotype from those of phylogenetically related species. In *Elaphe*, autosomal

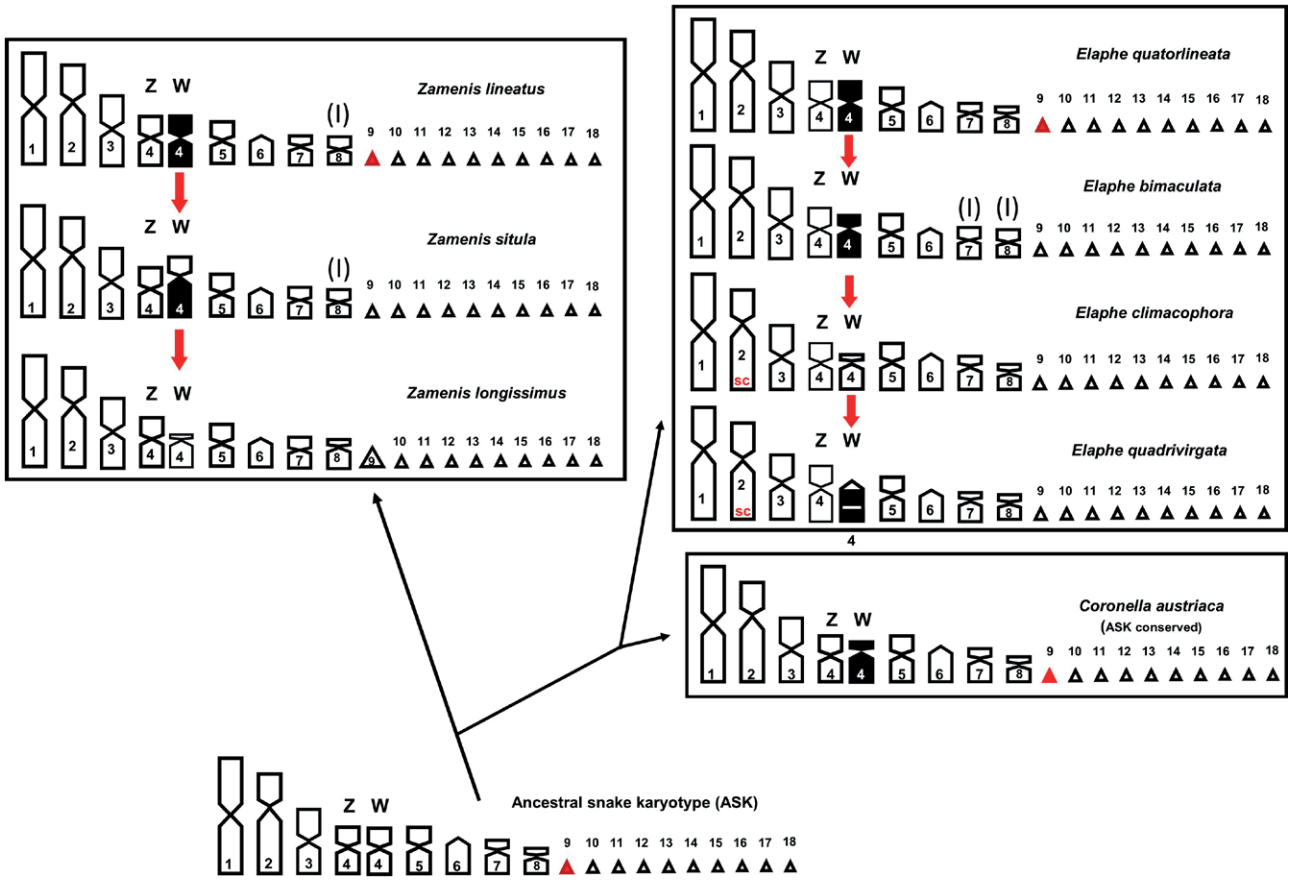

Fig. 3. Original karyograms of *Z. lineatus* and *E. quatuorlineata* compared with the Ancestral Snake Karyotype (ASK) and available literature data on congeneric species (Kobel, 1967; De Smet, 1978; Itoh et al., 1970; Augstenová et al., 2017; Rovatsos et al., 2018; Cole and Hardy, 2019; Mezzasalma and Odierna, 2021). sc = secondary constriction, (I) = chromosome inversion. Red chromosomes = NOR-bearing pair. Black regions/chromosomes = heterochromatin. Red arrows indicate progressive steps of sex chromosome diversification.

rearrangements from the hypothesized ancestral snake karyotype involve a putative inversion of the 8th pair in *E. quatuorlineata* and in *E. bimaculata* that can be considered a sinapomorphism, and in the second species, also an inversion of the 7th pair, as previously showed (see Fig. 3) (Itoh et al., 1970; Rovatsos et al., 2018), which can be considered an apomorphism.

Furthermore a translocation of loci of NORs on the 2nd chromosome pair in *E. climacophora* and *E. quadrivirgata*, evidenced by a secondary constriction (see Itoh et al., 1970), represent a further apomorphism, which is not present in the species here analyzed. *Zamenis lineatus* shows a metacentric 8th chromosome pair, which probably originated by means of a pericentromeric inversion as previously showed also in *Z. situla* (Augstenová et al. 2017), thus representing a sinapomorphism linking the two species.

It should also be noted that in the previously described karyotypes of *Z. longissimus* (Kobel 1967; De Smet, 1978), a different macrochromosome number (8 and 9, respectively) is reported, without any changes in the total chromosome count (2n = 36). The additional macrochromosome pair reported by De Smet (1978) is probably due to the amplification of NOR-linked heterocromatin of the NOR microchromosomes bearing pair, but more focused analyses are needed to confirm the occurrence of intraspecific chromosomal variability in *Z. longissimus*.

Progressive steps of the configuration of the heterogametic W chromosome are important events in reptiles and are clearly visible in many species, supporting the general heterochromatinization hypothesis of sex chromosome diversification (Augstenová et al., 2017; Alam et al., 2018; Cole and Hardy 2019; Mezzasalma et al., 2020). In fact, it is widely accepted that heteromorphic sex chromosome pairs begin their morphological and molecular diversification starting from a homomorphic state (Gamble et al., 2014; Mezzasalma et al., 2021). From this condition, two alternative pathways are known to potentially lead to a fully differentiated sex chromosome pair: a progressive heterochromatinization of the heterogametic chromosome or the insurgence of an inversion in the homomorphic proto-W chromosome (Wright et al., 2016; Natri et al., 2019; Mezzasalma et al. 2021). In either cases, the progressive diversification of the W element eventually leads to its evolutionary isolation (loss of recombination) and degeneration, finally reaching the size of a microchromosome (Marshall Graves, 2016; Mezzasalma et al., 2016; Wright et al., 2016).

Progressive steps of the configuration of the heterogametic W chromosome are visible in the species here analysed and in the phylogenetically closely-related taxa *Elaphe* and *Zamenis* (Fig. 3) (see also Kobel, 1967; De Smet, 1978; Itoh et al. 1970; Augstenová et al. 2017; Rovatsos et al., 2018; Mezzasalma and Odierna 2021). In particular, the W chromosome appears at a relatively earlier stage of diversification in *E. quatuorlineata*, in which it resulted largely heterochromatic, but homomorphic to the Z (this paper). More advanced diversification stages are represented by the W elements of *E. bimaculata* and *E. climacophora*, in which the morphology of the W chromosomes progressively diverged from the Z, reaching a telocentric configuration in *E. quadrivirgata* (Fig. 3) (see also Itoh et al., 1970; Rovatsos et al., 2018).

The W chromosome is homomorphic but largely heterochomatic in *Z. lineatus*, representing an initial diversification step from the Z element (this paper). A progressive addition of heterochromatin may produce a heterogametic chromosome, which appears sensibily larger than the Z: a condition similar to that reported in *Z. situla* (Augstenová et al., 2017) (Fig. 3).

Furthermore, it is possible to highlight that the differences in the morphology of the W chromosome and of the 8th and 9th chromosome pairs found between *Z. lineatus* and *Z. longissimus* (Kobel 1967; De Smet, 1978; this paper) are in agreement with their species status, originally proposed using a combination of morphological and molecular data (Lenk and Wüster, 1999; Utiger et al., 2002).

This evidence underlines that in squamate reptiles the cytotaxonomic approach is a useful tool for characterizing closely-related lineages as already shown in other squamate taxa (Mezzasalma et al., 2013, 2015, 2018b; Mezzasalma and Odierna, 2021).

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REFERENCES

Alam, S.M., Sarre, S.D., Gleeson, D., Georges, A., Ezaz, T. (2018): Did lizards follow unique pathways in sex chromosome evolution? Genes **9**: 239.

Augstenová, B, Mazzoleni, S, Kratochvíl, L, Rovatsos, M. (2017): Evolutionary dynamics of the W chromosome in caenophidian snakes. Genes **9**: 5.

Bianchi, N.O., Becak, W., de Bianchi, M.S.A., Becak, M.L., Rabello. M.N. (1969): Chromosome replication in four species of snakes. Chromosoma **26**: 188-200.

Cole, C.J., Hardy, L.M. (2019): Karyotypes of six species of colubrid snakes from the Western Hemisphere, and the 140-million-year-old ancestral karyotype of Serpentes. Am. Mus. Novit. **3926**: 1-14.

Damas, J., Kim, J., Farré, M., Griffin, D.K., Larkin, D.M. (2018): Reconstruction of avian ancestral karyotypes reveals differences in the evolutionary history of macro- and microchromosomes. Genome Biol. **19**: 155.

Deakin, J.E., Ezaz, T. (2014): Tracing the evolution of amniote chromosomes. Chromosoma **123**: 201-216.

Deakin, J.E., Ezaz, T. (2019): Understanding the evolution of reptile chromosomes through applications of combined cytogenetics and genomics approaches. Cytogenet. Genome Res. **157**: 7-20.

De Smet, W.H.O. (1978): The chromosomes of 23 species of snakes. Acta Zool. Pathol. Antverp. **70**: 85-118.

Dobigny, G., Ducroz, J.F., Robinson, T.J., Volobouev, V. (2004): Cytogenetics and cladistics. Syst. Biol. **53**: 470-484.

Dumas, F., Sineo L (2014): The evolution of human synteny 4 by mapping sub-chromosomal specific probes in Primates. Caryologia **67:** 281-291.

Dumas, F., Sineo, L., Ishida, T. (2015): Taxonomic identification of *Aotus* (Platyrrhinae) through cytogenetics. J. Biol. Res. **88**: 65-66.

Gamble, T., Geneva, A.J., Glor, R.E., Zarkower, D. (2014): *Anolis* sex chromosomes are derived from a single ancestral pair. Evolution **68**: 1027-1041.

Howell, W.M., Black, D.A. (1980): Controlled silver staining of nucleolus organizer regions with a protective colloidal developer: 1-step method. Experientia **36**: 1014-1015.

Itoh, M., Sasaki, M., Makino S. (1970): The chromosomes of some Japanese snakes, with special regard to sexual dimorphism. Japan. J. Genetics **45**: 121-128.

Kobel, H.R. (1967): Morphometrische Karyotyp analyse einiger Schlangenarten. Genetica **38**: 1-31.

Lenk, P., Wüster, W. (1999): A Multivariate approach to the systematics of Italian rat snakes of the *Elaphe longissima* complex (Reptilia, Colubridae): revalidation of Camerano's *Callopeltis longissimus* var. *lineata*. Herpetol. J. **9**: 153-162.

Levan, A., Fredga, K., Sandberg, A.A. (1964): Nomenclature for centromeric position on chromosomes. Hereditas **52**: 201-220.

Marshall Graves, J.A. (2016): Evolution of vertebrate sex chromosomes and dosage compensation. Nat. Rev. Genet. **17**: 33-46.

Matsuda, Y., Nishida-Umehara, C., Tarui, H., Kuroiwa, A., Yamada, K., Isobe, T., Ando, J., Fujiwara, A., Hirao, Y., Nishimura, O., Ishijima, J., Hayashi, A., Saito, T., Murakami, T., Murakami, Y., Kuratani, S., Agata, K. (2005): Highly conserved linkage homology between birds and turtles: bird and turtle chromosomes are precise counterparts of each other. Chromosome Res. **13**: 601-615.

Mezzasalma, M., Andreone, F., Glaw, F., Guarino, F.M., Odierna, G., Petraccioli, A., Picariello, O. (2019): Changes in heterochromatin content and ancient chromosome fusion in the endemic Malagasy boids *Sanzinia* and *Acrantophis*. Salamandra **55**: 140-144.

Mezzasalma, M., Andreone, F., Glaw, F., Odierna, G., Petraccioli, A., Guarino, F.M. (2018a): Chromosome aneupolyploidy in an endemic Malagasy gecko (Gekkonidae: *Geckolepis*). Salamandra **54**: 56-62.

Mezzasalma, M., Dall'Asta, A., Cheylan, M., Loy, A., Zuffi, M.A.L., Lymberakis, P., Tomovìc, L., Odierna, G., Guarino, F.M. (2015): A sisters' story: Comparative phylogeography and taxonomy of *Hierophis viridiflavus* and *H. gemonensis* (Serpentes, Colubridae). Zool. Scr. **44**: 495-508.

Mezzasalma, M., Di Febbraro, M., Guarino, F.M., Odierna, G., Russo, D. (2018b): Cold-blooded in the Ice Age: "refugia within refugia", inter-and intraspecific biogeographic diversification of European whipsnakes (Squamata, Colubridae, *Hierophis*). Zoology **127**: 84-94.

Mezzasalma, M., Guarino, F.M., Aprea, G., Petraccioli, A., Crottini, A., Odierna G. (2013): Karyological evidence for diversification of Italian slow worm populations (Squamata, Anguidae). Comp. Cytogenet. **7**: 217-227.

Mezzasalma, M., Guarino, F.M., Odierna, G. (2021): Lizards as model organisms of sex chromosome evolution: what we really know from a systematic distribution of available data? Genes **12**: 1341.

Mezzasalma, M., Odierna, G. (2021): Sex chromosome diversification in the smooth snake *Coronella austriaca* (Reptilia, Serpentes). Acta Herpetol. **16**: 37-44.

Mezzasalma, M., Visone, V., Petraccioli, A., Odierna, G., Capriglione, T., Guarino, F.M. (2016): Non-random accumulation of LINE1-like sequences on differentiated snake W chromosomes. J. Zool. **300**: 67-75.

Natri, H.M., Merilä, J., Shikano, T. (2019): The evolution of sex determination associated with a chromosomal inversion. Nat. Commun. **10**: 145.

Noor, M.A., Grams, K.L., Bertucci, L.A., Reiland, J. (2001): Chromosomal inversions and the reproductive isolation of species. Proc. Natl. Acad. Sci. U.S.A. **98**: 12084-12088.

Odierna, G., Aprea, G., Capriglione, T., Castellano, S., Balletto, E. (2004): Evidence for chromosome and Pst I satellite DNA family evolutionary stasis in the *Bufo viridis* group (Amphibia, Anura). Chromosome Res. **12**: 671-681.

Olmo, E. (2008): Trends in the evolution of reptilian chromosomes. Integr. Comp. Biol. **48**: 486-493.

Olmo, E., Signorino, G. (2006): Chromorep: a reptile chromosomes database. Internet references: http://193.206.118. 100/professori/chromorep.pdf, 15.09.06. [accessed on 22 July 2022]

Pallotta, M.M., Turano, M., Ronca, R., Mezzasalma, M., Petraccioli, A., Odierna, G., Capriglione, T. (2017): Brain gene expression is influenced by incubation temperature during leopard gecko (*Eublepharis macularius*) development. J. Exp. Zool. B Mol. Dev. Evol. **328**: 360-370.

Rieseberg, L.H. (2001): Chromosomal rearrangements and speciation. Trends Ecol. Evol. **16**: 351-358.

Rovatsos, M., Augstenová, B., Altmanová, M., Sloboda, M., Kodym, P., Kratochvíl, L. (2018): Triploid colubrid snake provides insight into the mechanism of sex determination in advanced snakes. Sex. Dev. **12**: 251-255.

Sahar, E., Latt, S.A. (1980): Energy transfer and binding competition between dyes used to enhance staining differentiation in metaphase chromosomes. Chromosoma **79**: 1-28.

Scardino, R., Mazzoleni, S., Rovatsos, M., Vecchioni, L., Dumas, F. (2020a): Molecular cytogenetic characterization of the Sicilian endemic pond turtle *Emys trinacris* and the yellow-bellied slider *Trachemys scripta scripta* (Testudines, Emydidae). Genes **11**: 702.

Scardino, R, Milioto, V, Proskuryakova, A.A., Serdyukova, N.A., Perelman, P.L., Dumas, F. (2020 b). Evolution of the human chromosome 13 synteny: Evolutionary rearrangements, plasticity, human disease genes and cancer breakpoints. Genes **11**: 383:

Sidhom, M., Said, K., Chatti, N., Guarino, F.M., Odierna, G., Petraccioli, A., Picariello, O., Mezzasalma, M. (2020): Karyological characterization of the common chameleon (*Chamaeleo chamaeleon*) provides insights on the evolution and diversification of sex chromosomes in Chamaeleonidae. Zoology **141**: 125738.

Singh, L. (1972): Evolution of Karyotypes in Snakes. Chromosoma **38**: 185-236.

Sumner, A.T. (1972): A simple technique for demonstrating centromeric heterochromatin. Exp. Cell Res. **75**: 304-306.

Utiger, U., Helfenberger, N., Schätti, B., Schmidt, C., Ruf, M., Ziswiler, V. (2002). Molecular systematics and phylogeny of Old World and New World ratsnakes, *Elaphe* Auct., and related genera (Reptilia, Squamata, Colubridae). Rus. J. Herpetol. **9**: 105-124.

Wright, A.E., Dean, R., Zimmer, F., Mank, J.E. (2016): How to make a sex chromosome. Nat. Commun. **7**: 12087.