



Conserved sex chromosomes and karyotype evolution in monitor lizards (Varanidae)

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Abstract

Despite their long history with the basal split dating back to the Eocene, all species of monitor lizards (family Varanidae) studied so far share the same chromosome number of $2n = 40$. However, there are differences in the morphology of the macrochromosome pairs 5–8. Further, sex determination, which revealed ZZ/ZW sex microchromosomes, was studied only in a few varanid species and only with techniques that did not test their homology. The aim of this study was to (i) test if cryptic interchromosomal rearrangements of larger chromosomal blocks occurred during the karyotype evolution of this group, (ii) contribute to the reconstruction of the varanid ancestral karyotype, and (iii) test homology of sex chromosomes among varanids. We investigated these issues by hybridizing flow sorted chromosome paints from *Varanus komodoensis* to metaphases of nine species of monitor lizards. The results show that differences in the morphology of the chromosome pairs 5–8 can be attributed to intrachromosomal rearrangements, which led to transitions between acrocentric and metacentric chromosomes in both directions. We also documented the first case of spontaneous triploidy among varanids in *Varanus albigularis*. The triploid individual was fully grown, which demonstrates that polyploidization is compatible with life in this lineage. We found that the W chromosome differs between species in size and heterochromatin content. The varanid Z chromosome is clearly conserved in all the analyzed species. Varanids, in addition to iguanas, caenophidian snakes, and lacertid lizards, are another squamate group with highly conserved sex chromosomes over a long evolutionary time.

Introduction

Varanids, also known as monitor lizards, are squamate reptiles belonging to the suborder Anguimorpha, which

together with iguanians and snakes forms the clade Toxicofera (Vidal and Hedges 2005). The family Varanidae comprises a single extant genus, *Varanus*, which split from the sister group, the family Lanthanotidae, ~70 MYA (Lin and Wiens 2017). Currently, 80 extant species of varanids are described (Uetz and Hošek 2017) and karyotypes have been reported for 24 *Varanus* species (Table 1). All these species have a diploid chromosome number of $2n = 40$, consisting of 16 macrochromosomes and 24 microchromosomes. This consistency in chromosome number

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Table 1 Basic cytogenetic data in Varanidae

Clade	Species	Subspecies	Reference	2n	Karyotype	Sex chromosomes	
niloticus clade	<i>V. albigularis</i>		King and King (1975) ^{*1}	40	40: 16(8,8) 24	ZW	
		<i>albigularis</i>	Present study	3n = 60 [‡]	60: 24(12,12) 36		
	<i>V. exanthematicus</i>		De Smet (1981) [†]	40	40: 16(8,8) 24		
			Srikulnath et al. (2013)	40	40: 16(10,6) 24		
			Present study	40	40: 16(10,6) 24		
		<i>griseus</i>	<i>griseus</i>	King and King (1975)	40	40: 16(16,0) 24	
	<i>V. niloticus</i>		De Smet (1981) [†]	40	40: 16(14,2) 24		
		<i>niloticus</i>	<i>niloticus</i>	King and King (1975)	40	40: 16(8,8) 24	ZW
			Porter et al. (1994)	40	40: 16(8,8) 24		
	indicus clade	<i>V. indicus</i>	<i>indicus</i>	King and King (1975)	40	40: 16(14,2) 24	
<i>V. beccarii</i>			Present study	40	40: 16(14,2) 24	ZW	
<i>V. boehmei</i>			Present study	40	40: 16(14,2) 24		
<i>V. macraei</i>			Present study	40	40: 16(14,2) 24	ZW	
<i>V. prasinus</i>			Present study	40	40: 16(14,2) 24	ZW	
salvator clade	<i>V. bengalensis</i>		Dutt (1968) [†]	40	40: 16(8,8) 24		
		<i>bengalensis</i>	Singh et al. (1970), Singh (1974)	40	40: 16(14,2) 24		
			De Smet (1981) [†]	40	40: 16(16,0) 24		
			Patawang et al. (2017a, 2017b)	40	40: 16(14,2) 24	ZW	
	<i>V. flavescens</i>		Singh et al. (1970), Singh (1974)	40	40: 16(14,2) 24		
	<i>V. nebulosus</i>		King and King (1975) ^{*2}	40	40: 16(14,2) 24		
	<i>V. rudicollis</i>		Gorman and Gress (1970)	40	40: 16(14,2) 24		
			Present study	40	40: 16(14,2) 24		
	<i>V. salvator</i>	<i>salvator</i>	King and King (1975)	40	40: 16(14,2) 24		
			De Smet (1981) [†]	40	40: 16(16,0) 24		
	<i>macromaculatus</i>	Srikulnath et al. (2013)	40	40: 16(14,2) 24			
		Present study	40	40: 16(14,2) 24	ZW		
varius clade	<i>V. varius</i>		King and King (1975)	40	40: 16(14,2) 24	ZW	
	<i>V. komodoensis</i>		Johnson Pokorná et al. (2016)	40	40: 16(14,2) 24	ZW	
gouldii clade	<i>V. giganteus</i>		King and King (1975)	40	40: 16(10,6) 24		
			Matthey (1931) [†]	40	40: 16(8,8) 24		
	<i>V. gouldii</i>	<i>gouldii</i>	King and King (1975)	40	40: 16(10,6) 24		
		<i>flavirufus</i>	King and King (1975)	40	40: 16(10,6) 24		
			Matsubara et al. (2014)	40	40: 16(10,6) 24	ZW	
	<i>V. mertensi</i>		King and King (1975)	40	40: 16(10,6) 24		
			Present study	40	40: 16(10,6) 24	ZW	
	<i>V. panoptes</i>	<i>horni</i>	Present study	40	40: 16(10,6) 24		
<i>V. rosenbergi</i>		King and King (1975) ^{*3}	40	40: 16(10,6) 24			
		Matsubara et al. (2014)	40	40: 16(10,6) 24	ZW		
tristis clade	<i>V. spenceri</i>		King and King (1975)	40	40: 16(10,6) 24		
	<i>V. glauerti</i>		Present study	40	40: 16(14,2) 24	ZW	
	<i>V. scalaris</i>		King and King (1975)	40	40: 16(14,2) 24		
	<i>V. semiremex</i>		King and King (1975)	40	40: 16(14,2) 24		
	<i>V. timorensis</i>	<i>similis</i>	King and King (1975)	40	40: 16(14,2) 24		
acanthurus clade	<i>V. tristis</i>	<i>orientalis</i>	King and King (1975)	40	40: 16(14,2) 24		
			King et al. (1982)	40	40: 16(14,2) 24	ZW	
	<i>V. acanthurus</i>		Matsubara et al. (2014)	40	40: 16(14,2) 24	ZW	
			Present study	40	40: 16(14,2) 24	ZW	

Table 1 (continued)

Clade	Species	Subspecies	Reference	2n	Karyotype	Sex chromosomes
	<i>V. gilleni</i>		King and King (1975)	40	40: 16(14,2) 24	
	<i>V. storri</i>		King and King (1975)	40	40: 16(14,2) 24	

Karyotypes are described as follows: diploid chromosome number: number of macrochromosomes (number of bi-armed macrochromosomes, number of uniarmed macrochromosomes) number of microchromosomes

*¹ Referred as *V. exanthematus albigularis* therein

*² Referred as *V. bengalensis nebulosus* therein

*³ Referred as *V. gouldii rosenbergi* therein

† Karyotypes described in these references were not considered in the ancestral state reconstruction

‡ Likely a case of spontaneous triploidy

and type suggests that the genome organisation might be highly conserved across the whole family. However, the morphology of certain chromosomes shows some variability among varanid species. As microchromosomes are hardly distinguishable by morphology, they were not included in these comparisons. The largest two pairs of macrochromosomes are metacentric and the pairs 3 and 4 are medium-sized bi-armed chromosomes in all species studied so far. Some variability exists in the morphology of the macrochromosomal pairs 5–8. In most species (Table 1), pair 5 is acrocentric and pairs 6, 7, and 8 are bi-armed chromosomes. Although this karyotype was considered to be ancestral for varanids (King and King 1975; Chaiprasertsri et al. 2013; Srikulnath et al. 2013), as yet there has been no robust phylogenetic reconstruction of the ancestral morphology of the varanid macrochromosomes.

Squamate reptiles do have, however, well-known variability in sex determination systems, from environmental to genotypic sex determination. Genotypic sex determination involves sex chromosomes at various stage of differentiation, comprising male (XX/XY) or female (ZZ/ZW) heterogamety (Valenzuela et al. 2003; Janzen and Krenz 2004; Sarre et al. 2004; Valenzuela and Lance 2004; Pokorná and Kratochvíl 2009; Johnson Pokorná and Kratochvíl 2016). Among varanids, sex chromosomes have been reported for only eight species (Table 1). All these species have female heterogamety with differentiated ZZ/ZW sex chromosomes, where the W is distinguishable by its highly heterochromatic region. In some species the W chromosome is distinctively larger than the other microchromosomes (King and King 1975; Matsubara et al. 2014). Differences in accumulations of microsatellite motifs on the W chromosome were reported among three species studied by Matsubara et al. (2014). Chromosome Z was not yet identified in any varanid species and data on homology of sex chromosomes among varanids are lacking.

Recently, Iannucci et al. (2019) produced a set of chromosome painting probes from a female *V. komodoensis* by flow sorting. In this study, we hybridized this set of probes

Table 2 List of individuals analyzed

Clade	Species	Number and sex of individuals
<i>niloticus</i>	<i>V. albigularis albigularis</i>	1 unknown sex
	<i>V. exanthematus</i>	1 ♂
<i>indicus</i>	<i>V. beccarii</i>	1 ♂, 1 ♀
	<i>V. boehmei</i>	1 ♂
	<i>V. macraei</i>	1 ♂, 1 ♀
	<i>V. prasinus</i>	1 ♂, 1 ♀
<i>salvator</i>	<i>V. rudicollis</i>	1 ♂
	<i>V. salvator</i>	1 ♂, 2 ♀
<i>gouldii</i>	<i>V. mertensi</i>	1 ♂, 1 ♀
	<i>V. panoptes horni</i>	1 ♂
<i>tristis</i>	<i>V. glauerti</i>	1 ♂, 1 ♀
<i>acanthurus</i>	<i>V. acanthurus</i>	1 ♂, 4 ♀

to a number of varanid species to (i) test if cryptic inter-chromosomal rearrangements of larger chromosomal blocks occurred during the karyotype evolution of this group, (ii) contribute to the reconstruction of the varanid ancestral karyotype, and (iii) test homology of sex chromosomes among varanids.

Material and methods

Studied species

The molecular phylogenetic study by Vidal et al. (2012) split the genus *Varanus* into seven distinct clades: *niloticus*, *indicus*, *salvator*, *varius*, *gouldii*, *tristis*, and *acanthurus*. This division is generally well supported also by more recent phylogenetic analyses (Zheng and Wiens 2016; Lin and Wiens 2017). We worked on 12 species of monitor lizards with a key phylogenetic position, representing all seven major varanid clades (Table 2). Males and females

were analyzed in seven of these species, whereas only males in the remaining species. The sex of the individuals was identified based on breeding history, sexual behavior, or morphology. Blood samples were obtained from animals originating from the pet trade, private breeders or Czech zoological gardens (Zoo Praha, Zoo Plzeň) under the supervision and with the approval of the Ethics Committee of the Faculty of Science, Charles University in Prague followed by the Committee for Animal Welfare of the Ministry of Agriculture of the Czech Republic (permission No. 35484/2015-14).

Chromosomal preparations, staining, and karyotyping

Metaphase chromosome spreads were prepared from whole blood cell cultures, following the protocol described in Pokorná et al. (2010) with slight modifications. Chromosomal preparations were stained with conventional Giemsa solution. C-banding staining was performed following Sumner (1972) as described in Pokorná et al. (2014). Chromosomes were arranged according to their size. When results from chromosome painting were available, karyotypes of the analyzed species were arranged based on homology with the karyotype of *V. komodoensis* (Iannucci et al. 2019). Karyotypes were arranged using the software Ikaros (MetaSystems).

Chromosome-specific probes

Preparation and labeling of *V. komodoensis* (VKO) chromosome-specific painting probes are described in detail in Iannucci et al. (2019). Briefly, chromosomes obtained by fibroblast cultivation were sorted using a Mo-Flo® (Beckman Coulter) cell sorter. Genetic material was then amplified and labeled by degenerate oligonucleotide primed PCR. Each *V. komodoensis* paint for macrochromosomes is specific for one single pair of chromosomes, except for VKO6/7 and VKO8/7, which contain one specific chromosome pair each (pair 6 and pair 8, respectively), plus a third pair that overlaps between the two of them (pair 7). The W and Z chromosomes are contained in probes VKO11/12/W and VKO17/18/Z, respectively, together with two pairs of other microchromosomes each (Fig. 1).

Cross-species chromosome painting

V. komodoensis paints for macrochromosomes and sex chromosomes were hybridized onto chromosomes of male individuals of *V. exanthematicus*, *V. prasinus*, *V. rudicollis*, *V. salvator*, *V. mertensi*, *V. panoptes horni*, *V. glauerti*, and *V. acanthurus* and of individual of unknown sex of *V. albigularis albigularis*. VKO11/12/W and

VKO17/18/Z were hybridized also to female individuals of *V. prasinus*, *V. salvator*, *V. mertensi*, *V. glauerti*, and *V. acanthurus*. Fluorescence in situ hybridization (FISH) experiments were performed as described in Iannucci et al. (2019). Images were captured using a Provis AX70 (Olympus) fluorescence microscope equipped with a DP30BW digital camera (Olympus). DP manager imaging software (Olympus) was used to capture gray scale images and to superimpose the source images with colors to visualize the results of FISH.

Ancestral state reconstruction

The phylogeny of cytogenetic characters, i.e., differences in the morphology of chromosome pairs 5–8, among varanids was reconstructed by maximum parsimony (Fitch 1971; Dobigny et al. 2004) using Mesquite v.3.51 (Maddison and Maddison 2018). The analyses were based on the phylogenetic tree of Lin and Wiens (2017). However, we took into account also the alternative topology of *V. griseus*, which is sister to other species of the *niloticus* clade in Lin and Wiens (2017), but sister to all species that do not belong to the *niloticus* clade in Zheng and Wiens (2016). In species where conflicting data were available, we included only the best data with respect to species identification, description of sampling locality, number of individuals studied, and quality of karyotype pictures (Table 1).

Results

Karyotypes and sex chromosomes

Karyotypes of *V. beccarii*, *V. boehmei*, *V. macraei*, *V. prasinus*, *V. panoptes horni*, and *V. glauerti* were described for the first time in this study. The cytogenetic analyses revealed that the 11 species analyzed all have a diploid number of $2n = 40$, consisting of 8 pairs of macrochromosomes and 12 pairs of microchromosomes (Fig. 2). The only exception is *V. albigularis albigularis*, which was triploid with $3n = 60$ chromosomes (Fig. 2a). Chromosome pairs (triplets in the case of *V. albigularis albigularis*) 1, 2, 3, and 4 have the same morphology in all species. Pairs 1 and 2 are large metacentric, whereas pairs 3 and 4 are medium-sized (sub)metacentric chromosomes. Pair 5 is acrocentric in all species except *V. exanthematicus*, where it is submetacentric. Pairs 6, 7, and 8 are (sub)metacentric in *V. beccarii*, *V. boehmei*, *V. macraei*, *V. prasinus*, *V. rudicollis*, *V. salvator*, *V. glauerti*, and *V. acanthurus*. Pairs 6 and 7 are acrocentric, whereas pair 8 is metacentric in *V. mertensi* and *V. panoptes horni*. *V. exanthematicus* has pairs 6, 7, and 8 acrocentric. *V. albigularis albigularis* triplets 6, 7, and 8 are acrocentric as well. The morphology of the

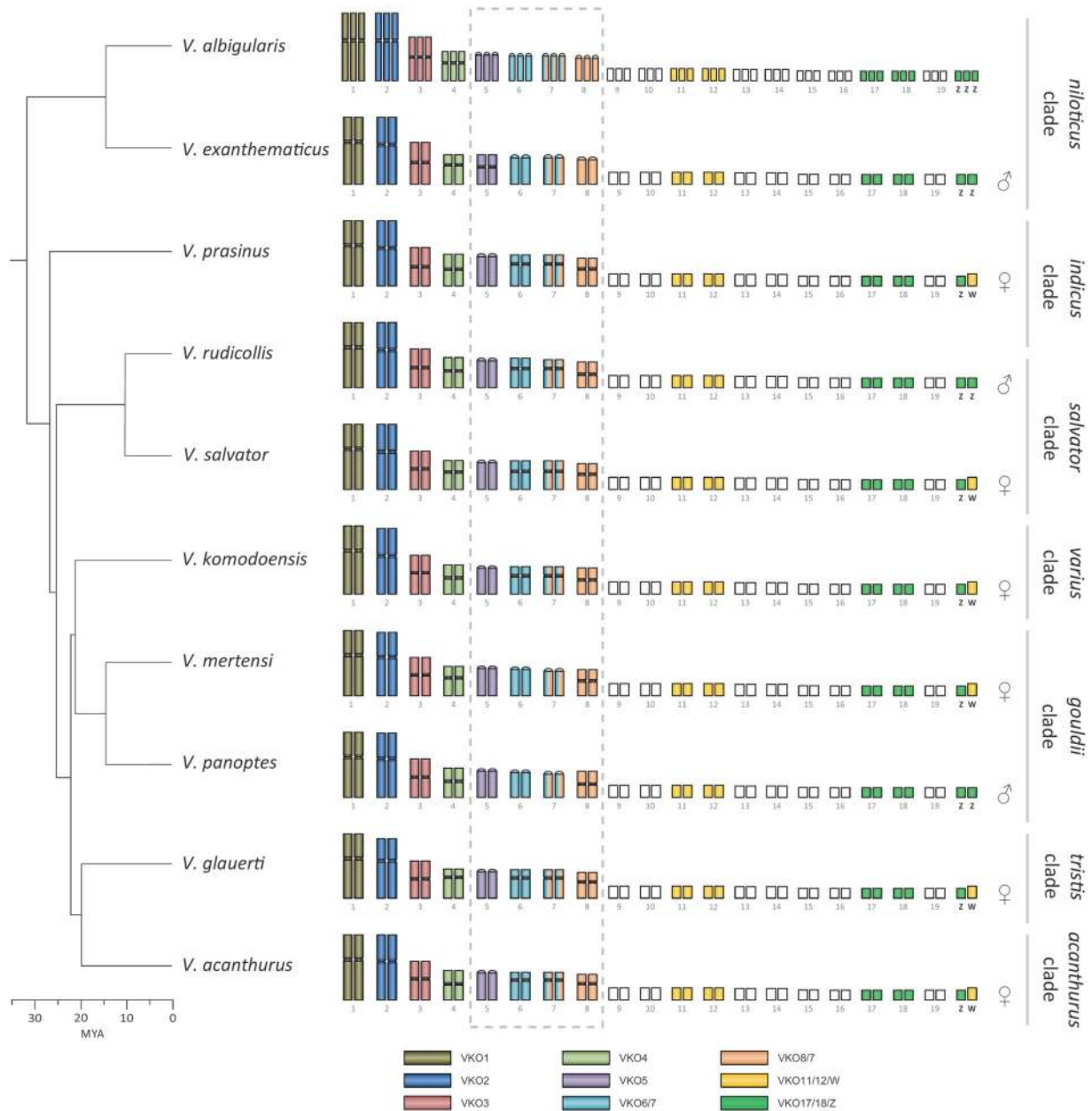


Fig. 1 Schematic representation of the results of the FISH experiments with *Varanus komodoensis* chromosome paints. Results of female individuals are shown only for those species in which both sexes were

studied. Phylogenetic relationships follow Lin and Wiens (2017). In *V. albigularis albigularis*, the studied individual was likely an exceptional spontaneous triploid

microchromosomes is not identifiable because of their small sizes (Fig. 2).

C-banding revealed variable heterochromatic patterns in the pericentromeric and telomeric regions of several macrochromosome and microchromosome pairs (Fig. 2). A strongly heterochromatic microchromosome was detected via C-banding in females of *V. beccarii*, *V. macraei*, *V. prasinus*, *V. mertensi*, *V. glauerti*, and *V. acanthurus*. This heterochromatic chromosome was absent in male metaphases, suggesting that it could represent the W chromosome (Fig. 2). C-banded karyotypes were similar in both

sexes of *V. salvator* (Fig. 2k, l). The larger unpaired microchromosome in male and female karyotype in *V. acanthurus* reported by Matsubara et al. (2014) and interpreted there as a likely polymorphism of an autosomal microchromosome was not observed in our study.

Chromosome painting

Chromosome painting demonstrates a strong conservation of macrochromosomes (Fig. 1; Suppl. Material 1). *V. komodoensis* macrochromosome pairs 1, 2, 3, and 4 are

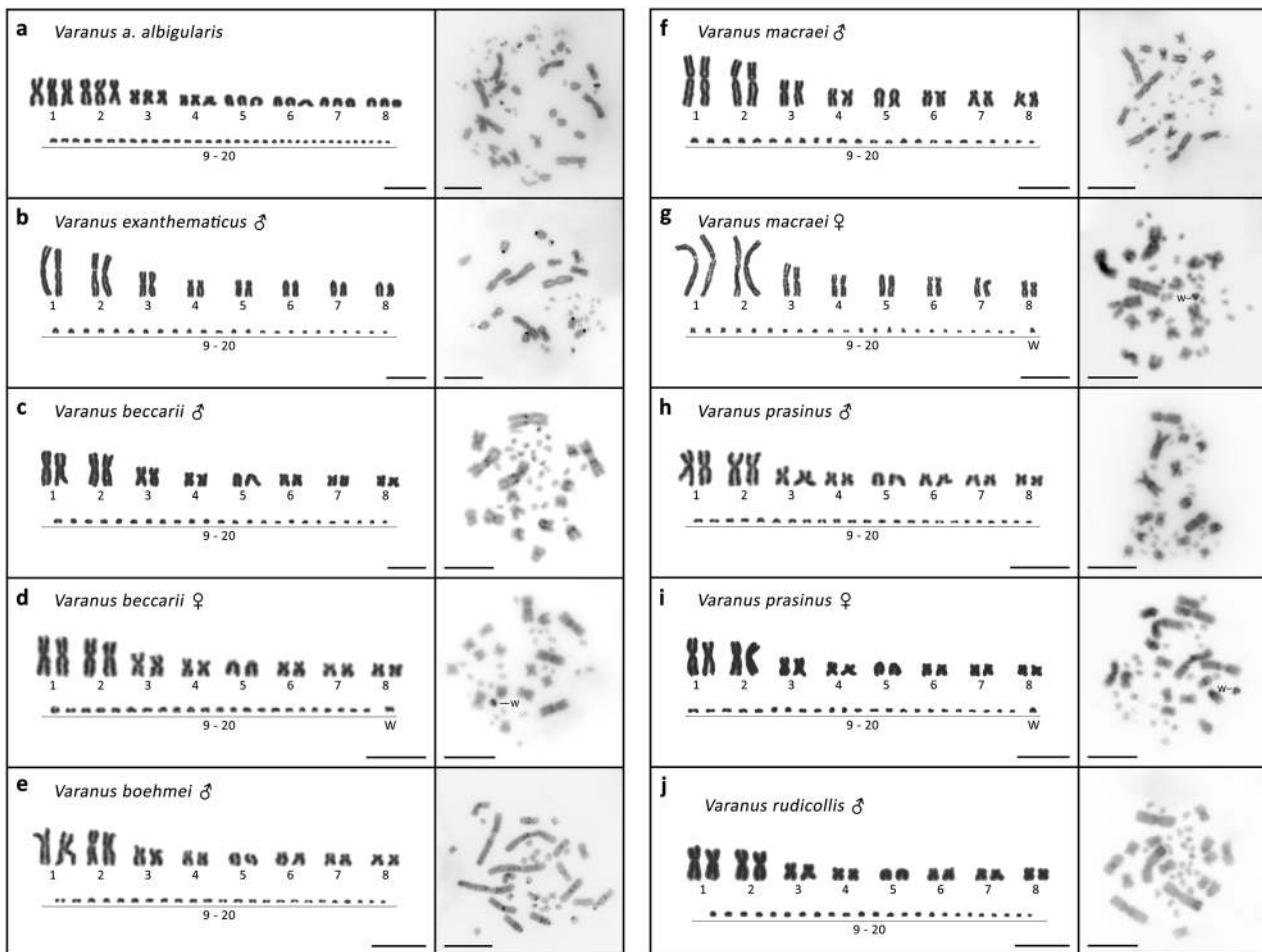


Fig. 2 Giemsa stained karyotype (left) and C-banded metaphases (right) of analyzed species. The W chromosomes in female karyotype were identified by sequential C-banding. The Z chromosomes are not assigned due to their similarity to other microchromosomes. Scale bars = 10 μ m

conserved in both morphology and painting pattern in other monitor lizards (e.g., Fig. 3a–c). Painting with VKO5, VKO6/7, and VKO8/7 gave the same results obtained for *V. komodoensis*, i.e., VKO5 painted chromosome 5, whereas VKO6/7 and VKO8/7 painted chromosomes 6 and 8, respectively, and pair 7, confirming the conservation in synteny for these four pairs of chromosomes despite their morphological diversity (e.g., Fig. 3d–f). Triploidy of *V. albigularis* was confirmed as all the paints painted triplets instead of pairs of chromosomes (e.g., Fig. 3g, h).

Hybridization of VKO11/12/W and VKO17/18/Z to male individuals resulted in painting four and six microchromosomes, respectively (e.g., Fig. 3i, k). VKO11/12/W hybridized to female metaphases painted five microchromosomes including the W (e.g., Fig. 3j). VKO17/18/Z probe painted five microchromosomes, one of which is probably the Z (e.g., Fig. 3l). Identical results were obtained for *V. komodoensis* by Iannucci et al. (2019). A comparison of hybridization pattern of VKO11/12/W and VKO17/18/Z in the same individual revealed that W chromosome is

usually larger than Z chromosome in varanids (e.g., Fig. 3i–l).

Ancestral state reconstruction

The ancestral state reconstruction did not unequivocally identify the ancestral pattern of the morphology for chromosomes 5–8 in varanids (Fig. 4). The ancestral state of chromosome pair 5, could be either submetacentric as in *V. griseus* or acrocentric. In any case, the situation in *V. exanthematicus* seems to be an apomorphy of this species. Our analysis suggests that chromosome pair 5 was subject to rearrangement from an acrocentric to a metacentric shape in the ancestor of *V. exanthematicus*. The ancestral state of chromosomes 6–8 for varanids could be submetacentric as in *V. griseus*, acrocentric, or metacentric. It appears that chromosomal rearrangements transformed chromosome pair 6 and 7 from the ancestral metacentrics in the common ancestor of the *gouldii* group to acrocentrics. The alternative topology of *V. griseus* suggested by Zheng and Wiens

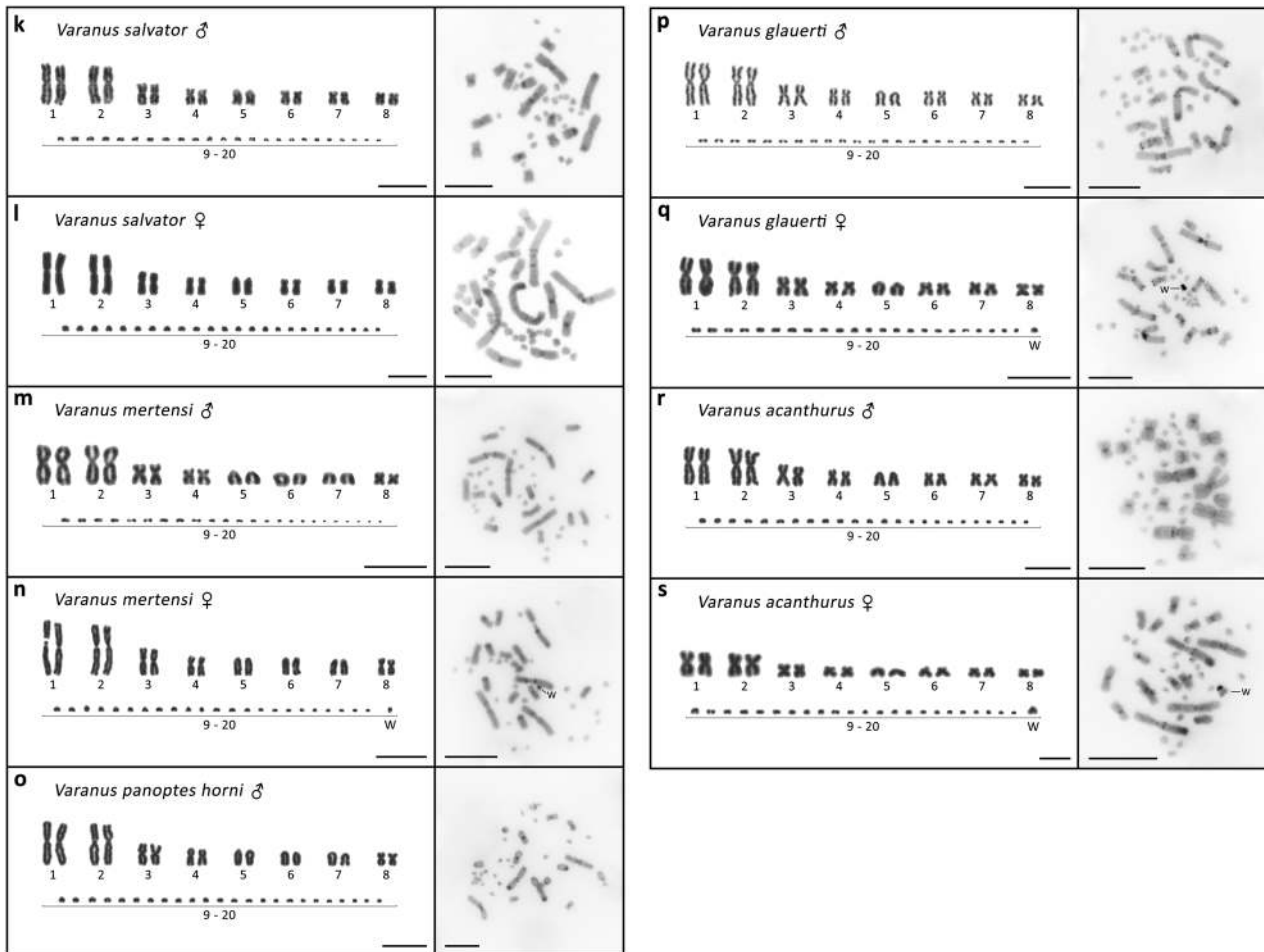


Fig. 2 (continued)

(2016) causes only a single change in interpretation of the phylogenetic pattern. In this case, the acrocentric shape of chromosome 5 is considered as ancestral for all varanids and the submetacentric chromosome of *V. griseus* is an apomorphy of this species.

Discussion

Karyotype evolution in monitor lizards

Our results strongly suggest that the chromosome number of $2n = 40$ is ancestral for varanids (Fig. 4). However, the lack of cytogenetic information on the accepted closest varanid outgroup, the sister family Lanthanotidae (Pyron et al. 2013; Zheng and Wiens 2016; Lin and Wiens 2017), prevents us from arriving at final conclusions on the composition of the varanid ancestral karyotype. Recently, the karyotype of the Gila monster, *Heloderma suspectum*, was described by Johnson Pokorná et al. (2014). The karyotype of this anguimorph species has $2n = 36$ chromosomes (14

macro- and 22 microchromosomes) and it is similar to karyotypes found in more distant varanid outgroups such as Iguania and snakes (Singh 1972; Altmanová et al. 2016; Olmo and Signorino 2016). Some rearrangements must have occurred along the evolutionary pathway from the last common ancestor of the varanids and the Gila monster, most likely in the lineage leading to the varanids. These rearrangements probably involved microchromosomes (Srikulnath et al. 2013).

The situation concerning the morphology of macrochromosomes 5–8 in the ancestral varanid karyotype is more complicated. In their pioneering work, King and King (1975) assumed that during evolution pericentric inversions tended to change these chromosomes from predominantly bi-armed to acrocentric. Therefore, they viewed the karyotype of the *salvator* clade as ancestral for varanids. However, this hypothesis does not correspond to the conclusions from current phylogenetic reconstructions (Pianka and King 2004; Lin and Wiens 2017). The results of the ancestral state reconstruction show that the ancestral situation for all varanids is not yet resolved (Fig. 4). It largely

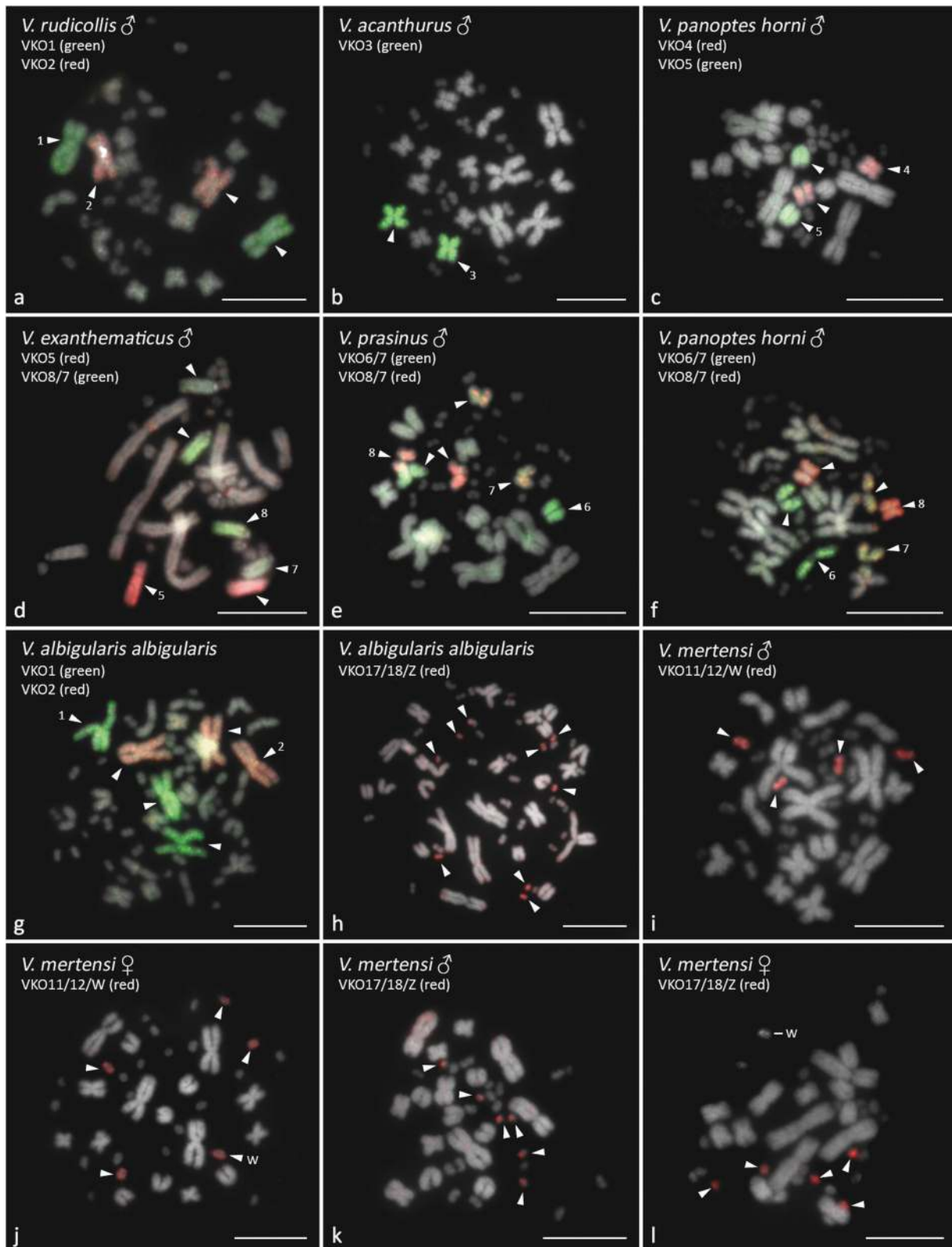


Fig. 3 Fluorescence in situ hybridization of *Varanus komodoensis* chromosomes paints. Arrowheads point to hybridization signals. Numbers are not given to microchromosomes as pairs are not distinguishable from each other by morphology. Scale bars = 10 μ m

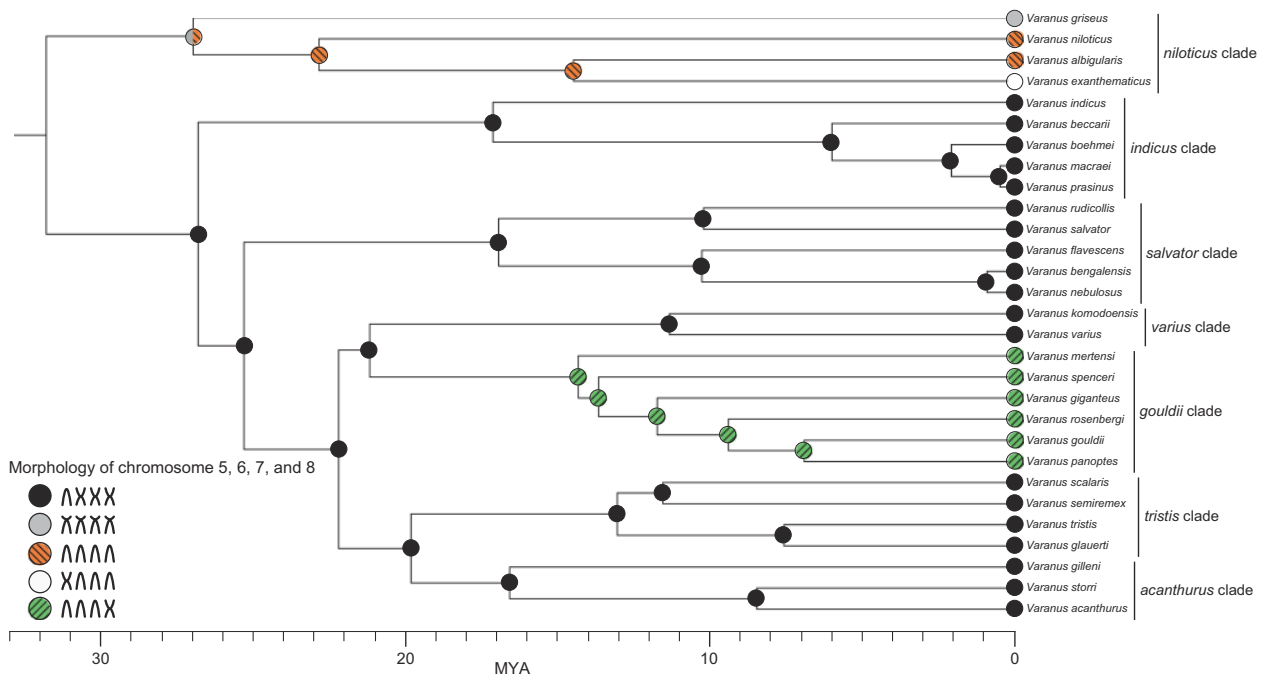


Fig. 4 Ancestral state reconstruction of morphology of chromosome pairs 5–8 using maximum parsimony. The phylogenetic relationships follow Lin and Wiens (2017)

depends on the homology of the morphology of chromosome pairs 5–8 between *V. griseus* and the majority of varanid species. Chromosome pairs 5–8 in *V. griseus* were considered to be submetacentric with remarkably shorter p-arms or even “subacrocentric” (King and King 1975), whereas chromosomes 6–8 are metacentric and the chromosome pair 5 is acrocentric in most of the other varanids. One hypothesis is that the morphology of chromosomes 5–8 may be apomorphic in *V. griseus*. However, a detailed comparison of gene order in *V. griseus* and other varanids might be needed to test this hypothesis. Regardless of the ancestral morphology of chromosomes 5–8, the patterns of chromosome morphology observed across species suggest that changes in macrochromosomes from acrocentrics to metacentrics went in both directions (Fig. 4). Chromosome painting data show that these changes between metacentric and acrocentric chromosomes can be attributed solely to intrachromosomal rearrangements involving pericentromeric inversion or centromere repositioning, and not to interchromosomal translocations.

Our results using chromosome painting do not support some of the conclusions from the study by Srikulnath et al. (2013) based on physical mapping of 17 functional genes in *V. salvator* and *V. exanthematicus*. These authors suggested that acrocentric chromosome pairs 6 and 8 of *V. exanthematicus* were homologous to two metacentric pairs of *V. salvator* and that a metacentric pair assigned as pair 7 in Srikulnath et al. (2013) of *V. exanthematicus* was homologous to the submetacentric pair 7 of *V. salvator*. Instead,

our chromosome painting results showed that metacentric chromosome pairs 6, 7, and 8 of *V. salvator* are homologous to three acrocentric pairs of *V. exanthematicus*. Further, the acrocentric chromosome pair 5 of *V. salvator* is homologous to a submetacentric pair of *V. exanthematicus*. These results suggest that the acrocentric morphology of chromosome pairs 6, 7, and 8 is a conserved feature in the *niloticus* clade (Fig. 1).

In summary, unlike mammals, where high rates of interchromosomal rearrangements were found (Ferguson-Smith and Trifonov 2007), chromosome painting data on varanids strongly support that similar to birds, squamate reptiles have a rather high rate of intrachromosomal and a low rate of interchromosomal rearrangements. This characteristic is probably common for the whole sauropsid group (Alföldi et al. 2011; Pokorná et al. 2011a, 2012; Skinner and Griffin 2012; Lithgow et al. 2014; Rovatsos et al. 2014a; Johnson Pokorná et al. 2015). The same karyotypic stability found in varanids was also described for other squamate lineages such as oplurids (Altmanová et al. 2016), skinks (Giovannotti et al. 2010) and geckos (Shibaike et al. 2009; Trifonov et al. 2011; Johnson Pokorná et al. 2015), even if Robertsonian rearrangements have been documented in some of these groups (Pokorná et al. 2010). On the other hand, a high karyotypic variability has been registered for chameleons (Rovatsos et al. 2017). A broader study involving comparative mapping or genome sequencing of representative species of these groups is necessary to determine whether any cryptic inter- or intrachromosomal

rearrangements occurred in the lineages with conserved karyotypes. It is not yet clear why some squamate lineages have more variable karyotypes than others, which is a more important and general question.

Sex chromosome evolution

Hybridization of probes containing Z chromosome of *V. komodoensis* onto metaphases of male and female individuals in other species revealed that the Z chromosome is conserved among all the species tested, supporting strong homology of sex chromosomes among the varanid clades *indicus*, *salvator*, *gouldii*, *tristis*, and *acanthurus* (Suppl. Material 1). Thus, the origin of the sex chromosomes in monitor lizards can be dated back at least to 27–31 MYA, which is the estimated age of the split between the *indicus* clade and the group containing the other mentioned varanid clades (Zheng and Wiens 2016; Lin and Wiens 2017). We did not have any female of a species from the *niloticus* clade available for a direct test for sex chromosome homology, however, earlier detection of ZZ/ZW sex chromosomes in species from this clade (Table 1) suggests that varanid sex chromosomes can be even older than the basal split of this group. The presence of a ZZ/ZW system in *H. suspectum* (Johnson Pokorná et al. 2014) suggests that these sex chromosomes might be ancestral for Anguimorpha. A test of homology of varanid and helodermatid ZZ/ZW sex chromosomes needs to be performed in future studies to clarify this point.

The high conservation of sex chromosomes described here in monitor lizards has also been reported for iguanas (XY, Rovatsos et al. 2014b, 2014c; Altmanová et al. 2016, 2018), caenophidian snakes (ZW, Matsubara et al. 2006; Vicoso et al. 2013; Rovatsos et al. 2015) and lacertid lizards (ZW, Rovatsos et al. 2016). This stability is comparable to viviparous mammals (XY, Graves 2006; Waters et al. 2007) and birds (ZW, Shetty et al. 1999; Zhou et al. 2014).

The W chromosome in *V. beccarii*, *V. macraei*, *V. prasinus*, *V. mertensi*, *V. glauerti*, and *V. acanthurus* analyzed in this study was strongly heterochromatic as detected via C-banding (Fig. 2d, g, i, n, q, s). No heterochromatic microchromosome was detected in the female of *V. salvator* (Fig. 2l). A sex ratio consistent with temperature-dependent sex determination was reported in this species by Hairston and Burchfield (1992). However, our chromosome painting results revealed ZZ/ZW sex chromosomes with extensive differences in genetic content between Z and W in *V. salvator*. Therefore, heterochromatinization may not be a universal feature of differentiated sex chromosomes. The lack of heterochromatin detectable by C-banding in *V. salvator* can be attributed to extensive variability in non-coding DNA in the W sex chromosomes of varanids. Unpaired chromosomes (Y and W) of vertebrates often

undergo heterochromatinization and accumulation of repetitive sequences during sex chromosome differentiation (e.g., Acosta et al. 2009; Pokorná et al. 2011b, 2014; Matsubara et al. 2016; Augstenová et al. 2018). Evidence for a likely secondary elimination of a heterochromatic block on differentiated sex chromosomes has also been reported, for instance, in oplurid iguanas (Altmanová et al. 2016). The nested phylogenetic position of *V. salvator* suggests that secondary loss of heterochromatin from W chromosome might be plausible also for this species.

Until now, the dynamics of the genetic content of the W chromosome among varanids has only been partially investigated. Matsubara et al. (2014) performed a test among *V. gouldii*, *V. rosenbergii*, and *V. acanthurus* by hybridization of microsatellite motifs and the W chromosome probe obtained from *V. acanthurus*. Most sequences were apparently not conserved between W chromosomes because the chromosome W probe from *V. acanthurus* showed only weak hybridization signals on the pericentromeric region of the W chromosomes in *V. rosenbergii* and *V. gouldii* females. FISH with microsatellite motifs also revealed a different repetitive sequence composition between the W chromosomes of the three species. Johnson Pokorná et al. (2016) obtained similar results. These authors did not find any accumulation of the microsatellites accumulated in the W chromosome of *V. acanthurus* on the W chromosome of *V. komodoensis*. However, in our case, hybridization results of VKO11/12/W onto metaphases of all female individuals revealed a strong conservation of the W chromosomes among varanids. This discrepancy could be explained by methodological differences. The part of the W labeled by the probe derived from flow sorting can be rich in euchromatic regions containing coding genes, which are more conserved across species than repetitive sequences such as microsatellites diverging rapidly during evolution (Matsubara et al. 2006; Pokorná et al. 2011b; Rutkowska et al. 2012; Altmanová et al. 2016). These dynamics would explain size differences between W chromosomes among varanids despite sex chromosome homology.

Triploidy in *V. albigularis*

The fully grown individual of *V. albigularis* was found to have a chromosome number of $3n = 60$. King and King (1975) described the typical varanid chromosome number of $2n = 40$ for this species (referred there as *V. exanthematicus albigularis*). They also reported ZZ/ZW sex chromosomes for this species. Based on the results of the chromosome painting it seems that our individual possesses a ZZZ sex chromosome constitution. Although further investigations are still needed to clarify the origin of triploidy in *V. albigularis*, we tentatively assume that it emerged spontaneously in this individual. Spontaneous

triploidy has been reported in sauropsids, including birds, iguanas, and snakes (Tiersch and Figiel 1991; Lamborot and Vásquez 1998 and citations therein; Stenberg and Saura 2013; Rovatsos et al. 2018). In amniotes it mostly leads to early mortality (Bonaminio and Fechheimer 1993; Baumer et al. 2000; Trukhina and Smirnov 2014). Triploidy is mainly attributed to dispermic fertilization of an egg or abnormal meiosis. However, in squamates it can be also the result of mating between a female of a diploid obligatory asexual hybrid and a male derived from one of the sexual ancestors (Moritz et al. 1989; Neaves and Baumann 2011; Trifonov et al. 2015; Ryskov et al. 2017). Facultative parthenogenetic births have been documented for several monitor lizards, such as *V. panoptes*, *V. komodoensis*, *V. ornatus*, *V. glauerti*, and *V. rainierguentheri* (Watts et al. 2006; Hennessy 2010; Wiechmann 2011, 2012; Hörenberg 2013; Grabbe and Koch 2014). The triploidy status of our individual *V. albigularis albigularis* could be the result of a parthenogenetic diploid egg followed by fertilization. Several parthenogenetic male individuals of *V. komodoensis* have been karyotyped by Johnson Pokorná et al. (2016) and all of them were diploids, however, the mother of these males did not have access to males at the time of clutch formation.

In conclusion, our study shows that genome organisation in monitor lizards is generally well conserved. Interchromosomal rearrangements were not observed, but we uncovered intrachromosomal rearrangements in four pairs of macrochromosomes during the evolution of the group. Our ancestral state reconstruction suggests that intrachromosomal rearrangements led to transitions from acrocentric to metacentric chromosomes and vice versa. We also documented the first case of triploidy among varanids. Sex chromosomes are clearly conserved across varanids during their long evolutionary history. Although homologous, the W chromosomes differ among varanid species in size, morphology, content of DNA repeats, and heterochromatinization. Future studies exploring and expanding our findings should investigate karyotype evolution in varanids at all molecular genomic levels. Sequencing and chromosome anchoring of *V. komodoensis* genome in the ongoing project will provide an important base for further research particularly in varanids and their anguimorph outgroups where partial cytogenetic maps or genomic data are already available (Srikulnath et al. 2013; Gao et al. 2017).

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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