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ORIGINAL ARTICLE

Evolutionary dynamics of two satellite DNA families in rock lizards of the genus *Iberolacerta* (Squamata, Lacertidae): different histories but common traits

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Abstract Satellite DNAs compose a large portion of all higher eukaryotic genomes. The turnover of these highly repetitive sequences is an important element in genome organization and evolution. However, information about the structure and dynamics of reptilian satellite DNA is still scarce. Two satellite DNA families, HindIII and TaqI, have been previously characterized in four species of the genus *Iberolacerta*. These families showed different chromosomal locations, abundances, and evolutionary rates. Here, we extend the study of both satellite DNAs (satDNAs) to the remaining *Iberolacerta* species, with the aim to investigate the patterns of variability and factors influencing the evolution of these repetitive sequences. Our results revealed disparate patterns but also common traits in the evolutionary histories of these satellite families: (i) each satellite DNA is made up of a library of monomer variants or subfamilies shared by related species; (ii) species-specific profiles of satellite repeats are shaped by expansions and/or contractions of different variants from the library; (iii) different turnover rates, even among closely related species, result in great differences in overall sequence homogeneity and in concerted or non-concerted evolution patterns, which may not reflect the phylogenetic relationships among taxa. Contrasting turnover rates are possibly related to genomic constraints such as karyotype architecture and the interspersed organization of diverging repeat variants in satellite arrays. Moreover, rapid changes in copy number, especially in the centromeric HindIII satDNA, may have been associated with chromosomal rearrangements and even contributed to speciation within *Iberolacerta*.

Keywords Concerted evolution · FISH · *Iberolacerta* · Library model · Satellite DNA · Squamate reptiles

Abbreviations

Cy3	Cyanine 3
dNTP	Deoxyribonucleotide triphosphate

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FCA	Factorial correspondence analysis
FISH	Fluorescence in situ hybridization
FITC	Fluorescein iso-thyocianate
Mya	Million years ago
π	Nucleotide diversity
satDNA	Satellite DNA

Introduction

Satellite DNAs (satDNAs) represent one of the major classes of repetitive sequences in almost all eukaryotic genomes. They consist of tandemly repeated non-coding DNA sequences, typically arranged in large clusters of hundreds or thousands of copies usually located in the heterochromatic regions of chromosomes, close to the centromeres and telomeres (Charlesworth et al. 1994). Several satDNA families of independent origin are commonly found in the genome of a species or group of species, and they usually differ in nucleotide sequence, monomer length, and complexity, as well as in evolutionary history (Ugarković and Plohl 2002; Kuhn et al. 2008, 2010). The biological function of these sequences is not yet fully understood, although numerous reports point out the role of certain satellites in centromeric condensation, chromosome organization, or chromosome pairing (see Plohl et al. 2008). A growing field of research is also addressing the role of satDNA transcripts in the formation and maintenance of heterochromatin and even in regulation of gene expression (Ugarković 2009; Pezer et al. 2012). In addition, several examples support the hypothesis that the rapid evolution of satDNAs can act as a driver of population and species divergence (Ugarković and Plohl 2002; Feliciello et al. 2015).

Despite their biological significance, satDNAs are still the least understood genomic component, underrepresented in outputs of most genome projects (Plohl et al. 2012). A common feature of many of them is that, even though monomers can be present in many thousand copies per genome, sequence divergence between repeats of the same family is often very low, usually less than 15 % (Plohl et al. 2008). The non-independent or concerted evolution of repeat units is postulated to be a consequence of a two-step process called molecular drive, consisting of the gradual spread of a sequence variant (1) through a genome (homogenization) and (2) through a species (fixation) (Dover 1982). Sequence homogenization is due to diverse molecular mechanisms of nonreciprocal transfer, such as unequal crossing-over, gene conversion, rolling circle replication and reinsertion, and transposon-mediated exchange (Stephan 1986; Dover 2002), while fixation results from random chromosomal assortment in sexual reproduction, depending thus on population factors. This process results in rapid divergence of satellite sequences in reproductively isolated groups of organisms, and in this case, satDNAs can be used as phylogenetically informative markers (Plohl et al. 2012).

Accumulation of mutations in satellite families is not the only way to alter specific profiles of satellite repeats in short evolutionary periods. In addition to sequence changes, satDNAs are permanently altered in copy number by expanding and contracting arrays of satellite monomers (Ugarković and Plohl 2002; Plohl et al. 2012). Because usually more than one satellite family exists in a genome, fluctuations in their copy numbers can change very efficiently and rapidly any profile of genomic satDNA. The library model of satDNA evolution explains the occurrence of species-specific satellite profiles as a result of differential amplifications and/or contractions within a collection, or library, of satellite sequences shared by related species (Fry and Salser 1977; Meštrović et al. 1998; Ugarković and Plohl 2002). Not only distinct satDNAs but also monomer variants or subfamilies from a single family can be distributed in genomes in the form of a library (Cesari et al. 2003).

SatDNAs have been extensively studied in insects (Palomeque and Lorite 2008) and mammals (Enukashvily and Ponomartsev 2013), and less so in other taxa, although there are several exceptions. Squamata, by far the largest reptile order, is one of them (see, for example, Giovannotti et al. 2009, 2013; Chaiprasertsri et al. 2013). It includes the Lacertidae, a widespread species-rich group restricted to the Palearctic region, formed by two subfamilies, Gallotiinae and Lacertinae (Arnold et al. 2007; Sindaco and Jeremčenko 2008). So far, five satDNA families have been described in Lacertinae, with different taxonomic distributions. Three satellite families are genus-specific, namely, pLHS in *Podarcis* (Capriglione et al. 1994; Capriglione 2000), CLsat in *Darevskia* (Ciobanu et al. 2003; Grechko et al. 2006), and Agi160 in *Lacerta* (Ciobanu et al. 2004; Grechko et al. 2005). The other two families, on the contrary, are broadly distributed in Lacertinae: pLCS, shared by *Algyroides*, *Teira*, *Lacerta*, and *Podarcis*

(Capriglione et al. 1989, 1991; Capriglione 2000), and pGPS, present in *Podarcis*, *Archaeolacerta*, *Algyroides*, *Lacerta*, and *Zootoca* (Capriglione et al. 1998).

In a previous work (Giovannotti et al. 2014) we isolated two new satDNA families in the lacertid genus *Iberolacerta*, a monophyletic group of rock lizards mainly distributed in highland areas of Western Europe. This genus comprises eight species, which can be subdivided into three main units: (1) *I. horvathi*, occurring in the Eastern Alps and the north of the Dinaric Chains; (2) the subgenus *Pyrenesaura*, which includes the three species found in the Pyrenees, (*I. aranica*, *I. aurelioi*, and *I. bonnali*); and (3) the four species included in the "Iberian group" (*I. cyreni*, *I. martinezricai*, *I. galani*, and *I. monticola*), with disjunct distributions in central and northern mountain ranges of the Iberian Peninsula. Previous cytogenetic surveys of the *Iberolacerta* species (Capula et al. 1989; Odierna et al. 1996; Arribas and Odierna 2004; Arribas et al. 2006; Rojo et al. 2014) showed them to possess a diploid number of 2n=36, and a similar karyotypic macrostructure, with all chromosomes acrocentric. Only the karyotypes of the three Pyrenean species differ from this formula, with reduced diploid numbers that range from 2n=24 to 26 in males and from 2n=23 to 26 in females, and many biarmed chromosomes that probably evolved from the ancestral acrocentric complement through a series of Robertsonian fusions (Odierna et al. 1996).

According to the most recently published phylogeny (Arribas et al. 2014), speciation within *Iberolacerta* started *ca.* 13.5 million years ago (Mya; 95 % credibility interval 11.6–15.6), with the split between the clades formed by *I. horvathi* and the Iberian group, on one side, and by the Pyrenean species, on the other. This event was most likely quickly followed by the separation of *I. horvathi*, which took place approximately 11.5 Mya (9.6–13.7). Within the Iberian group, *I. cyreni* split earlier (7.3–8.5 Mya), while the speciation events within the clade formed by *I. martinezricai*, *I. galani*, and *I. monticola* occurred considerably later, at the beginning of the Pleistocene, 2.1–2.9 Mya. The three Pyrenean species probably originated in rapid succession *ca.* 3.8 Mya (2.7–4.9), although this phylogenetic analysis suggests that *I. bonnali* split first, shortly before the separation between *I. aranica* and *I. aurelioi*, 3.3 Mya (2.3–4.3). Notwithstanding minor uncertainties still remaining, the mapping of satDNA differences on that species tree is likely to provide valuable information about the time and mode of evolution of these repetitive sequences. In our previous work (Giovannotti et al. 2014), we analyzed two unrelated satDNA arrays in the Iberian clade of *Iberolacerta*: (1) the centromeric HindIII family, which comprises two subfamilies (I and II) and represents 5–10 % of the genome and (2) the TaqI family, which shows only interstitial loci and represents 2.5–5 % of the genome. The nucleotide sequences of the two families were presumably evolving at different rates, almost tenfold higher for centromeric than for instertitial repeats, after comparing *I. cyreni* vs. the other, relatively closer, species of the Iberian clade. In agreement with this conclusion, the HindIII family seems to be specific to the genus *Iberolacerta* (Capriglione et al. 1989, 1991, 1998; Capriglione 2000), whereas the TaqI satDNA has also been detected in representatives of three other genera of the subfamily Lacertinae (*Lacerta*, *Podarcis*, and *Timon*).

Here, we extend the study of both satDNAs to the remaining *Iberolacerta* species, and increase our dataset for HindIII satDNA, to further investigate the occurrence of two divergent subfamilies in the genomes of all these taxa. The results obtained offer a more complete portrait of the intra- and interspecific variability of these highly repetitive sequences and their genomic organization and chromosomal distribution, with the ultimate objective of contributing to assess the relative strength of the processes that determine their structure and mode of evolution.

Material and methods

Animals

Genomic DNA was isolated from a total of 20 specimens, representing all eight *Iberolacerta* species. The number of specimens per species and their geographical origin are given in Supplementary Table 1. In addition, one male and one female of *I. horvathi* and one female of *I. bonnali* were used to make metaphase chromosomes.

DNA extraction, PCR, cloning and sequencing

Genomic DNA was extracted from ethanol preserved tissues using standard protocols with proteinase K digestion followed by phenol/chloroform extraction (see Sambrook et al. 1989). Two primer pairs designed in our previous work (HindIII-F: 5′-

TGAGTGTTTTACAGTTGAAAAGCT-3′; HindIII-R: 5′-CATTGTGTTATTTGAGCGCAA-3′; TaqI-F: 5′-ATTCTGACCCTGGGGGTTAG-3′; TaqI-R: 5′-CATATTTAAAGAAATCAGGCCTCG-3′) were used for isolation of both satellite families from the genomes of *I. horvathi*, *I. bonnali*, *I. aranica*, and *I. aurelioi*. An additional primer pair was designed to specifically amplify HindIII-subfamily II in all eight *Iberolacerta* species (Hind_sfII-F: 5′-CTCTTGCTTATTTCGCTCCAAATGA-3′; Hind_sfII-R: 5′-ATTTCTGTGTGCAGCATGCATTGG-3′). PCR reactions were performed in a final volume of 25 μl containing ~25 ng of genomic DNA, 0.625 U of *Taq* DNA polymerase and 1× PCR buffer (Roche Diagnostics), 5 nmol of each dNTP (Roche Diagnostics), and 20 pmol of each primer. The general reaction conditions were as follows: initial denaturation at 94 °C for 5 min; 35 cycles of denaturation at 94 °C for 30 s, annealing at the following temperatures (HindIII-F/HindIII-R, 55 °C; TaqI-F/TaqI-R, 47 °C; Hind_sfII-F/Hind_sfII-R, 58 °C) for 30 s, extension at 72 °C for 30–60 s, and a final extension at 72 °C for 7 min. The obtained PCR products were run on 1.5 % agarose gels; DNA in bands of interest was eluted using Pure Link Quick Gel Extraction Kit (Invitrogen) and cloned in the T&A cloning vector with T&A cloning kit (Yeastern Biotech) following manufacturer's recommendations. Positive clones were selected through PCR amplification using the M13 forward and M13 reverse primers. Bidirectional sequencing with the M13 primers was performed on an ABI PRISM 3730XL (Applied Biosystems) automatic sequencer.

Sequence analysis

The newly sequenced repeats were analyzed together with the previously reported sequences of the HindIII and TaqI satDNA families from *I. cyreni*, *I. monticola*, *I. galani*, and *I. martinezricai* (DDBJ/EMBL/GenBank accession numbers for HindIII: from KF453637 to KF453681; accession numbers for TaqI: from KF453682 to KF453723) (Giovannotti et al. 2014). Multiple sequence alignment was performed with MUSCLE (Edgar 2004), using default parameters, as implemented in Geneious version 8.0.5 (Kearse et al. 2012). After visual inspection of alignments, sequences were classified into different sets according to shared nucleotide changes and indels.

Intraspecific nucleotide diversity (π) was estimated using DnaSP v. 5 (Librado and Rozas 2009). Net average genetic distances between groups were calculated using the Maximum Composite Likelihood model (Tamura et al. 2004) in MEGA v. 6.0 (Tamura et al. 2013). Sequence variability among satellite repeats was further investigated by performing a factorial correspondence analysis (FCA), carried out with Genetix v. 4.05.2 (Belkhir et al. 2004). For this analysis, we constructed a matrix with all the sequences, where the nucleotide present at each diagnostic position was coded with a unique integer (100, 120, 140, or 160).

For the subsequent phylogenetic analysis, a consensus sequence was obtained for each sequence set by choosing the most frequent nucleotide at each position, except when a combination of dinucleotides of the three pairs CpG, CpA, and TpG was present at the same doublet position. In that case, the CpG dinucleotide was chosen as the consensus unless the T or A nucleotides were present in >70 % of the sequences. A phylogenetic network of the consensus sequences was constructed with TCS v. 1.21 (Clement et al. 2000) using the statistical parsimony algorithm under the 95 % parsimony criterion (Templeton et al. 1992).

Chromosome analysis

Metaphase chromosome spreads were prepared as described previously (Giovannotti et al. 2014). As for *I. horvathi*, individuals of this species were induced to autotomize their tail tips, the tissues were collected in the field following the protocol by Waters et al. (2008) and transferred to the laboratory for the establishment of primary cell cultures. For fluorescence in situ hybridization (FISH) experiments, we developed species-specific probes obtained by PCR amplification of HindIII and TaqI satDNA clones. The probes were labeled either with Cy3, using a PCR labeling kit (Jena Bioscience), or with FITC, using the Platinum Bright 495 labeling kit (KREATECH Biotechnology). Slide pretreatment, denaturation, hybridization, post-hybridization washes, and detection were performed according to Schwarzacher and Heslop-Harrison (2000). Images were captured using the epifluorescence microscopes (Nikon Microphot-FXA; Leica Leitz

DMRBE) equipped with monochrome cameras (Nikon DS-Qi1Mc; JAI CV-M4+CL). The NIS-Elements D 3.10 (Nikon Instruments) and Leica CytoVision version 7.2 (Leica Microsystems) softwares were used to process the images and reconstruct the karyotypes.

Results

Isolation and characterization of satellite DNAs

PCR amplification using primers specific for HindIII and TaqI satDNA was successful in all tested species and produced a ladder-like banding pattern, which is typical for satellite DNA. PCR products included complete monomers and multimers (from dimers up to hexamers), flanked by partial monomer sequences. Only clones with complete repeat units were sequenced and, for further analyses, multimers were separated into individual monomers. A total of 187 new sequences were obtained for HindIII, whereas 109 clones were sequenced for TaqI. Comparison of these new sequences with the HindIII and TaqI monomers isolated from *I. cyreni*, *I. monticola*, *I. galani*, and *I. martinezricai* in our previous study (Giovannotti et al. 2014) indicated that all of them belong to the same satDNA families. Altogether, our dataset comprises 232 HindIII and 151 TaqI monomers from all eight *Iberolacerta* species, which are likely to reflect the overall variability of the two satellite families in the genus.

Both HindIII and TaqI satDNAs are characterized by an AT bias (average AT content of 58.9 and 59.1 %, respectively) and by the occurrence of short repeat motifs such as A and T stretches, dinucleotide TG and CA, and trinucleotide CAA and TTC (Supplementary Figs. 1a, b). The size of HindIII repeats ranged between 169 and 172 bp, with the exception of two monomers with lengths of 151 bp (IAR_99b) and 161 bp (ICY_209c) (Table 1). TaqI repeats showed a broader range of length variation, from 155 to 191 bp (Table 1). Several indels varying in size from 1 to 31 bp are the causes of the repeat length variation in this satDNA family.

After alignment, monomers within each satDNA family were classified into subfamilies, according to the state of diagnostic positions, characterized by nucleotide substitutions or indels shared by at least 90 % of all the members grouped in the same subfamily. The subfamilies were designated with Roman numerals following the nomenclature previously used in Giovannotti et al. (2014) for HindIII subfamilies I and II. Additional diagnostic positions further divided each subfamily into several sequence groups and subgroups, denoted by a Latin letter and a numeral, respectively, after the subfamily name (Table 2).

Sequence variability within HindIII satDNA

Within HindIII satDNA, we found a total of 30 diagnostic positions, which identified three subfamilies—namely HI, HII, and HIII—and 27 sequence groups (Table 2a and Supplementary Fig. 1a). Their abundances ranged from 1.3 to 17 % (3–39 representatives) of the examined sequences. Figure 1a overlies data on the abundance and distribution of HindIII sequence groups onto a phylogenetic tree for *Iberolacerta* derived from mitochondrial markers (Arribas et al. 2014). As evidenced in this figure, sequence groups were not equally represented in the different species. The Pyrenean species (*I. aurelioi*, *I. aranica*, and *I. bonnali*) harbor a wide diversity of HindIII repeats, mainly belonging to subfamilies HI and HII. Only 12 monomers were retrieved from *I. horvathi*, and they are all members of subfamily HI. Similarly, subfamily HI is also the most abundant variant of the HindIII family in the Iberian species *I. martinezricai*, *I. monticola*, and *I. galani*. A strikingly different profile of HindIII repeats was found in *I. cyreni*, also an Iberian species, which is characterized by the presence of several private sequence groups belonging to subfamily HIII and one exclusive sequence group within subfamily HI.

The coexistence of more than one subfamily explains the higher nucleotide diversity values (π) in species such as *I. bonnali* (4.91%) or *I. aurelioi* (3.96%), in comparison with the values obtained for those species in which all their HindIII repeats belonged to a single subfamily, i.e., *I. horvathi* (1.16%) and *I. martinezricai* (1.51%) (Table 1). Interestingly, despite their different abundances, mean π values for each subfamily were roughly similar (from 2.30 % in subfamily HII to 2.54 % in subfamily HIII).

The factorial correspondence analysis (FCA) based on diagnostic positions highlighted the differentiation among the three HindIII subfamilies, lending further support to our classification. Altogether, the three main axes of variation explain 96.53 % of the observed variation (Fig. 2a). The most informative is axis 1 (69.70 %), which identifies two main clusters,

Table 1 Summary of repeat features of HindIII and TaqI satDNA

Species	HindIII				TaqI			
	Subfamily	n	Repeat length	Nucleotide diversity (π)	Subfamily	n	Repeat length	Nucleotide diversity (π)
I. monticola	All combined	34		0.0151±0.0018	All combined	10		0.0600±0.0089
	HI	30	171	0.0142±0.0023	TI	10	171–188	0.0600±0.0089
	HII	4	170	0.0177±0.0060				
I. galani	All combined	31		0.0331±0.0040	All combined	16		0.0489±0.0001
	HI	23	171	0.0148±0.0019	TI	16	186–188	0.0489±0.0001
	HII	8	169–170	0.0211±0.0082				
I. martinezricai	All combined	33		0.0151±0.0018	All combined	7		0.0541±0.0103
	HI	33	171–172	0.0151±0.0018	TI	7	187–188	0.0541±0.0103
I. cyreni	All combined	40		0.0356±0.0037	All combined	9		0.0406±0.0001
	HI	7		0.0180±0.0030	TI	9	186–187	0.0406±0.0001
	HIII	33	161–171	0.0240±0.0029				
I. horvathi	All combined	12		0.0116±0.0028	All combined	33		0.1218±0.0079
	HI	12	171	0.0116±0.0028	TI	31	167–191	0.1184±0.0083
					TII	2	189 - 191	0.0699±0.0349
I. aurelioi	All combined	25		0.0396±0.0034	All combined	20		0.0976±0.0086
	HI	14	171	0.0290±0.0048	TI	1	187	
	HII	11	170	0.0262±0.0026	TII	19	177–188	0.0908±0.0074
I. aranica	All combined	22		0.0355±0.0043	All combined	34		0.1209±0.0070
	HI	7	151–171	0.0265±0.0055	TI	14	175–190	0.1082±0.0126
	HII	15	170	0.0164±0.0028	TII	20	177–190	0.0960±0.0059
I. bonnali	All combined	35		0.0491±0.0050	All combined	22		0.1204±0.0096
	HI	17	171	0.0257±0.0027	TI	17	155–188	0.1060±0.0102
	HII	15	169–170	0.0230±0.0076	TII	5	177–190	0.0983±0.0156
	HIII	3	171	0.0195±0.0033				
All species combined	HI	143		0.0241±0.0015	TI	105		0.1342±0.0060
	HII	53		0.0230±0.0018	TII	46		0.0961±0.0044
	HIII	36		0.0254±0.0029				
	TOTAL	232		0.0539±0.0020	TOTAL	151		0.1567±0.0038

Number of monomeric repeats sequenced (n), length of repeats (expressed in base pairs), and nucleotide diversities (π)±S.E. for both satDNAs for each *Iberolacerta* species investigated

corresponding to subfamily HIII repeats of *I. cyreni* and *I. bonnali* on one side, and to subfamilies HI and HII on the other. Axis 2, which accounts for 24.60 % of the observed variation, separates subfamilies HI and HII. Finally, axis 3, with 2.23 % of the observed variation, probably corresponds to sequence heterogeneity within each subfamily. The clustering of HindIII repeats revealed by the FCA matches the estimates of interspecies and inter-subfamilies net genetic distances, shown in Table 3a. Monomers of subfamily HIII are the most divergent, with average genetic distances of 7.50 and 9.90 % from subfamily HI and HII, respectively. These values are substantially higher than the average distance between subfamilies HI and HII (around 4.0 %). When *I. cyreni* is excluded from the analysis, pairwise interspecies genetic distances within each subfamily are all very low and uncorrelated with relative divergence times between species, with average values of 1.0 % within subfamily HIII, 0.34 % within subfamily HII, and 0.33 % within subfamily HI. Net genetic distances

Table 2 Nucleotide differences among the consensus sequences of the different groups of (a) HindIII subfamilies HI, HII, and HIII, and (b) TaqI subfamilies TI and TII. The second row refers to base positions relative to the alignment shown in Supplementary Fig. 1a (HindIII) and 1b (TaqI). The general consensus sequence of each satDNA was used as reference. Dots indicate identity with this reference sequence

a)

	1	2	3	4	6	7	8	10	11	12	13	14	15	17	18	19	20
Positions	14	15	21	27	38	39	56	73	83	84	85	86	87	95	98	99	101
Consensus	T	C	A	T	T	T	C	A	A	A	T	T	T	C	T	G	A
HI_A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
HI_B	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.
HI_C	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.
HI_D1	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.
HI_D2	.	.	G	.	.	.	T	.	.	.	C	.	.	.	.	.	.
HI_E	.	.	.	.	.	.	.	.	.	C	C	.	.	.	.	.	.
HI_F	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.
HI_G	.	.	.	C	.	.	.	.	.	.	C	.	.	.	.	.	.
HI_H	.	.	.	C	.	.	.	.	.	C	C	.	.	.	.	.	.
HI_I	.	G	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.
HI_J	.	G	.	.	.	.	.	.	.	C	C	.	.	.	.	.	.
HI_K	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.
HI_L	.	.	.	C	.	.	T	.	.	.	.	.	.	.	.	.	.
HI_M	.	.	.	C	.	G	T	.	.	.	.	.	G	.	.	.	.
HII_A	.	.	.	.	.	.	T	.	C	.	.	.	.	.	.	.	.
HII_B	.	.	.	C	.	G	T	.	C	.	.	.	.	.	.	.	G
HII_C	.	.	.	.	.	.	T	.	C	.	.	.	.	.	.	.	.
HII_D	.	.	G	.	C	.	.	.	C	.	.	.	.	.	.	.	.
HII_E	A	.	G	.	C	.	.	.	C	.	.	.	.	.	.	.	.
HII_F	.	.	G	.	C	.	.	.	C	.	.	.	.	.	.	.	.
HII_G	.	.	G	.	C	.	T	.	C	.	.	.	.	.	.	.	G
HII_H	.	.	G	.	C	.	.	.	C	.	.	.	.	.	.	.	G
HIII_A	.	.	.	C	.	G	T	G	C	.	.	.	G	A	C	A	.
HIII_B	.	.	.	C	.	G	T	G	C	.	.	.	G	A	C	A	.
HIII_C	.	.	.	C	.	G	T	G	C	.	.	.	G	A	C	A	.
HIII_D	.	.	.	C	.	G	T	G	C	.	.	G	G	A	C	A	.
HIII_E	.	.	.	C	.	G	T	G	C	.	.	G	G	A	C	A	.

b)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Positions	1	5	6	9	14	16	17	18	19	20	21	22	31	33	43	54	56	58	59	60	61	66	71	72
Consensus	C	G	C	G	C	T	A	A	C	C	T	A	A	C	C	T	T	C	C	A	G	G	C	G
TI_A1	.	.	.	.	.	.	C	.	G	G	.	.	.	.	T	.	C	.	.	G	C	.	T	.
TI_A2	.	.	.	.	.	.	C	.	G	G	.	.	.	.	T	.	C	.	.	G	C	.	T	.
TI_B1	.	.	.	.	.	.	C	.	G	G	.	.	.	.	T	.	C	.	.	.	C	.	T	.
TI_B2	.	.	.	.	.	.	C	.	G	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TI_C1	G	.	T	T	.	.	C	.	T	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.

Table 2 (continued)

TI_C2	.	.	.	.	.	.	C	.	T	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.
TI_D	.	.	.	.	.	.	.	.	.	T	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.
TI_E	.	.	.	.	.	.	G	.	.	G	.	T	G	.	.	.	.	.	.	.	.	.	.	.	.
TI_F1	.	.	.	.	.	.	G	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.
TI_F2	.	.	.	.	.	.	G	.	.	.	C	.	G	.	.	.	.	.	.	.	.	.	.	.	.
TI_G1	.	.	.	.	.	.	G	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TI_G2	.	.	.	C	.	.	G	T	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.
TI_H1	.	.	.	.	.	.	.	.	A	A	.	.	G	.	.	.	.	.	.	.	.	C	.	C	
TI_H2	.	.	.	.	.	.	T	.	A	A	.	.	G	.	T	.	.	.	.	.	.	C	.	C	
TI_I1	.	.	.	.	.	.	.	.	A	A	.	.	G	.	.	.	.	.	T	.	T	C	.	A	
TI_I2	.	.	.	.	.	.	T	.	A	A	.	.	G	.	.	.	.	.	T	.	T	C	.	A	
TI_J1	.	.	.	.	.	.	.	.	A	A	.	.	G	.	.	.	.	.	.	.	.	C	.	A	
TI_J2	.	.	.	.	.	.	T	.	A	A	.	.	G	.	.	.	.	.	.	.	.	C	.	A	
TI_K	.	.	.	.	.	.	.	.	A	A	.	.	G	.	.	.	.	.	.	.	.	C	.	C	
TI_L1	.	.	.	.	.	.	.	.	A	A	.	.	G	.	.	.	.	.	.	.	.	C	.	C	
TI_L2	.	.	.	.	.	.	.	.	A	A	.	.	G	.	.	.	.	.	.	.	.	C	.	A	
TI_L3	.	.	.	.	.	.	.	.	A	A	.	.	G	.	.	.	.	.	.	.	.	.	.	A	
TII_A	.	.	.	.	T	C	.	T	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	
TII_B1	.	.	.	.	T	C	.	T	.	.	.	.	.	G	.	C	.	T	.	.	.	.	T	.	
TII_B2	.	.	.	.	T	C	.	T	.	.	.	.	G	.	.	C	.	T	.	.	.	.	T	.	
TII_C1	.	.	.	.	T	T	.	T	.	.	.	.	.	G	.	C	.	T	.	.	.	.	T	.	
TII_C2	.	.	.	.	T	C	.	T	.	.	.	.	.	G	.	C	.	T	.	.	.	.	T	.	
TII_D	.	.	.	.	T	C	.	T	.	G	.	.	.	G	.	C	A	T	.	.	.	.	T	.	
TII_E1a	.	.	.	.	T	C	.	T	.	.	.	G	.	.	.	C	.	T	.	.	.	.	T	.	
TII_E1b	.	.	.	.	T	C	.	T	.	.	.	G	.	.	.	C	.	T	.	.	.	.	T	.	
TII_E2	T	.	.	.	T	C	.	T	.	.	.	G	.	.	.	C	.	T	.	.	.	.	T	.	
TII_F	.	.	.	.	T	C	.	T	.	.	.	.	.	.	.	C	.	T	.	.	.	.	T	.	
TII_G1	.	.	.	.	T	C	.	T	.	.	.	.	.	G	.	C	.	T	.	.	.	.	T	.	
TII_G2a	T	.	T	.	T	C	.	T	.	.	.	.	.	A	.	C	.	T	.	.	.	.	T	.	
TII_G2b	T	.	T	.	T	C	T	T	.	.	.	.	.	.	.	C	.	T	.	.	.	.	T	.	
TII_G3	.	T	.	.	T	C	.	T	G	.	.	.	.	G	.	C	.	T	.	.	.	.	T	.	
TII_G4	T	A	.	.	T	C	.	T	.	.	.	.	.	.	.	C	.	T	.	.	.	T	T	.	

a)

	21	22	23	24	26	27	28	29	30	31	32	33	35
Positions	113	114	117	119	129	140	144	145	147	149	150	151	165
Consensus	G	A	T	A	T	G	C	A	A	G	A	G	-
HI_A	.	.	.	.	.	.	.	.	.	.	.	.	-
HI_B	.	.	.	.	.	.	.	.	.	.	.	.	-
HI_C	.	.	.	.	.	.	.	.	.	.	.	.	-
HI_D1	.	.	.	.	.	.	.	.	.	.	.	.	-

Table 2 (continued)

HI_D2	.		.		.		.		.		.		.		.		.		.		.		.		-	
HI_E	.		.		.		.		.		.		.		.		.		.		.		.		-	
HI_F	.		.		C		.		.		.		.		.		.		.		.		.		-	
HI_G	.		.		.		.		.		.		.		.		.		.		.		.		-	
HI_H	.		.		.		.		.		.		.		.		.		.		.		.		-	
HI_I	.		.		.		.		.		.		.		.		.		.		.		.		-	
HI_J	.		.		.		.		.		.		.		.		.		.		.		.		-	
HI_K	.		.		C		.		.		A		.		.		.		.		.		.		-	
HI_L	.		.		.		.		.		A		.		.		.		.		.		.		-	
HI_M	.		.		.		.		.		.		.		.		.		.		.		.		-	
HII_A	.		.		C		.		.		A		-		-		T		T		G		.		A	
HII_B	.		.		G		.		.		A		-		-		T		T		G		.		A	
HII_C	.		.		G		.		.		A		-		-		T		T		G		C		A	
HII_D	.		.		G		.		.		A		-		-		T		T		G		C		A	
HII_E	.		.		G		.		.		A		-		-		T		T		G		.		A	
HII_F	.		.		G		.		.		A		-		-		T		T		G		.		A	
HII_G	.		.		G		.		.		A		-		-		T		T		G		.		A	
HII_H	.		.		G		.		.		A		-		-		T		T		G		.		A	
HIII_A	A		T		.		.		.		.		.		.		.		.		.		.		-	
HIII_B	A		T		.		.		C		A		.		.		.		.		.		.		-	
HIII_C	A		G		.		.		C		A		.		.		.		.		.		.		-	
HIII_D	A		G		.		.		C		A		.		.		.		.		.		.		-	
HIII_E	A		G		.		C		C		A		.		.		.		.		.		.		-	

b)

	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50
Positions	83	93	101	110	114	117	120	121	123	125	126	127	139	142	150	157	163	167	168	169	170	175	181	184	187	188
Consensus	G	T	T	G	G	T	T	G	T	A	A	A	C	G	T	C	A	A	G	T	T	G	T	G	G	T
TI_A1	.	.	.	.	.	.	.	.	.	.	.	C	G	.	.	.	.	T	.	.	.	T	G	.	.	.
TI_A2	.	.	.	.	.	.	.	.	.	.	.	C	G	.	.	.	.	T	.	.	.	T	G	.	C	.
TI_B1	A	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	T	G	.	.	.
TI_B2	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	T	.	.	.	.	G	.	.	.
TI_C1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.
TI_C2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.
TI_D	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.

Table 2 (continued)

TI_E	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	T	.	.	.	A	A	T	.	.	.	.
TI_F1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TI_F2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TI_G1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TI_G2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TI_H1	.	.	.	.	A	.	C	A	G	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.
TI_H2	.	.	.	.	A	.	C	A	G	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.
TI_I1	.	.	.	.	A	.	C	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.
TI_I2	.	.	.	.	A	.	C	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.
TI_J1	.	.	.	.	A	.	C	A	.	.	.	.	.	.	A	.	C	.	.	.	.	T	.	.	.	.
TI_J2	.	.	.	.	A	.	C	A	.	.	.	.	.	.	A	.	C	.	.	.	.	T	.	.	.	.
TI_K	.	.	.	.	A	.	C	A	.	.	.	.	.	C	.	.	.	.	.	.	.	T	G	.	.	.
TI_L1	.	.	.	.	A	.	C	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.
TI_L2	.	.	.	.	A	.	C	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.
TI_L3	.	.	.	.	A	.	C	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.
TII_A	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	-	-	-	.	.	.	.	C	.	G
TII_B1	.	G	.	.	.	.	.	.	.	.	.	.	G	.	.	.	-	-	-	.	.	.	A	.	.	G
TII_B2	.	G	.	.	.	.	.	.	.	.	.	.	G	.	.	.	-	-	-	.	.	.	A	.	.	G
TII_C1	.	G	.	.	.	.	.	.	.	.	.	.	G	.	.	.	-	-	-	.	.	.	.	C	.	G
TII_C2	.	G	.	.	.	.	.	.	.	.	.	.	G	.	.	.	-	-	-	.	.	.	.	C	.	G
TII_D	.	G	.	.	.	.	.	.	.	.	.	.	G	.	.	.	-	-	-	.	.	.	.	C	.	G
TII_E1a	.	G	.	.	.	C	.	.	.	G	G	.	.	.	.	.	.	T	C	.	.	.	.	.	.	G
TII_E1b	.	.	C	.	.	C	.	.	.	G	G	.	.	.	.	.	.	T	C	.	.	.	.	.	.	G
TII_E2	.	.	C	C	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	G
TII_F	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	G
TII_G1	.	G	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	A	.	.	.
TII_G2a	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	A	.	.	G
TII_G2b	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	A	.	.	G
TII_G3	.	G	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	A	.	.	G
TII_G4	.	G	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	T	.	.	.	.	A	.	.	G

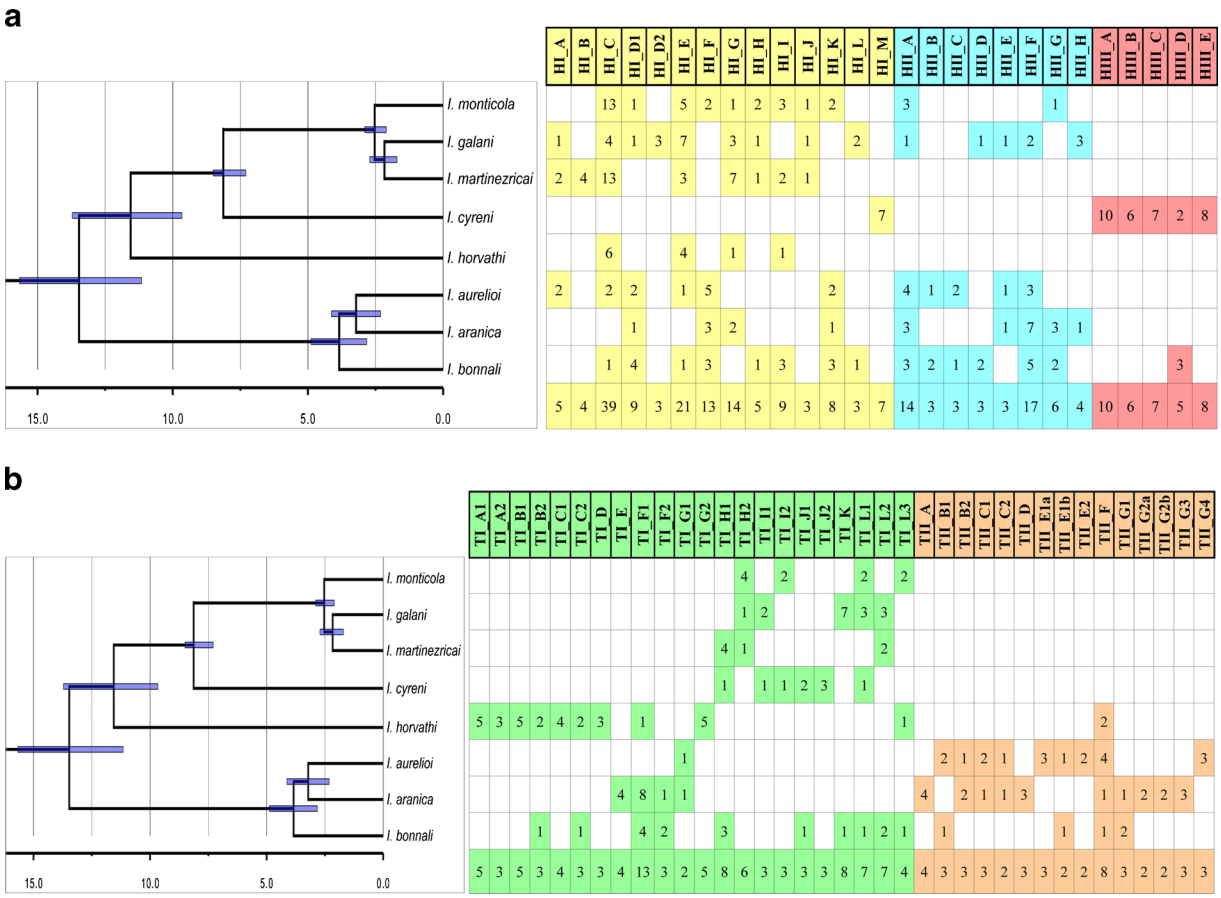

Fig. 1 Distribution and abundance of HindIII (**a**) and TaqI (**b**) subfamilies in *Iberolacerta* coupled to a Bayesian tree obtained from two mitochondrial loci (Cyt *b*, cytochrome *b*; CR, control region) (adapted from Arribas et al. 2014). *Node bars* indicate 95 % credibility intervals (regions of highest posterior density) for the corresponding divergence time (in million years). *Numbers in the table* indicate the number of repeats of each subfamily retrieved from each species. Colors identify different subfamilies

between HI repeats involving *I. cyreni* are always considerably higher (from 2.0 % between *I. cyreni* and *I. aranica* to 3.40 % between *I. cyreni* and *I. horvathi*).

Sequence variability within TaqI satDNA

From the alignment of TaqI sequences, we identified a total of 50 diagnostic positions, which defined two main subfamilies—namely TI and TII—and 37 sequence groups, whose abundances ranged from 1.3 to 8.5 % (2–13 representatives) of the examined sequences (Table 2b and Supplementary Fig. 1b).

In general, the species of the Iberian clade were characterized by the presence of TaqI repeats belonging only to subfamily TI (Fig. 1b), with a substantial proportion of private sequence groups (four groups, comprising 15 out of 42 sequences). Conversely, subfamily TII is essentially characteristic of the subgenus *Pyrenesaura*, although it has been residually observed also in *I. horvathi*. This subfamily appears to be the most abundant variant in the genomes of *I. aranica* and, above all, *I. aurelioi*, which show both species-specific and shared sequence groups. The sampled loci from *I. bonnali* and *I. horvathi* contain mostly T1 repeats. However, the clustering pattern of TI repeats differs markedly between the two species: while all the monomers retrieved from *I. bonnali* were grouped together with monomers from other species, *I. horvathi* shows the highest proportion of species-specific repeats (25 out of 33), allocated to six private sequence groups.

As expected from the distribution of subfamilies TI and TII in the genomes of the *Iberolacerta* species, intraspecific nucleotide diversity values

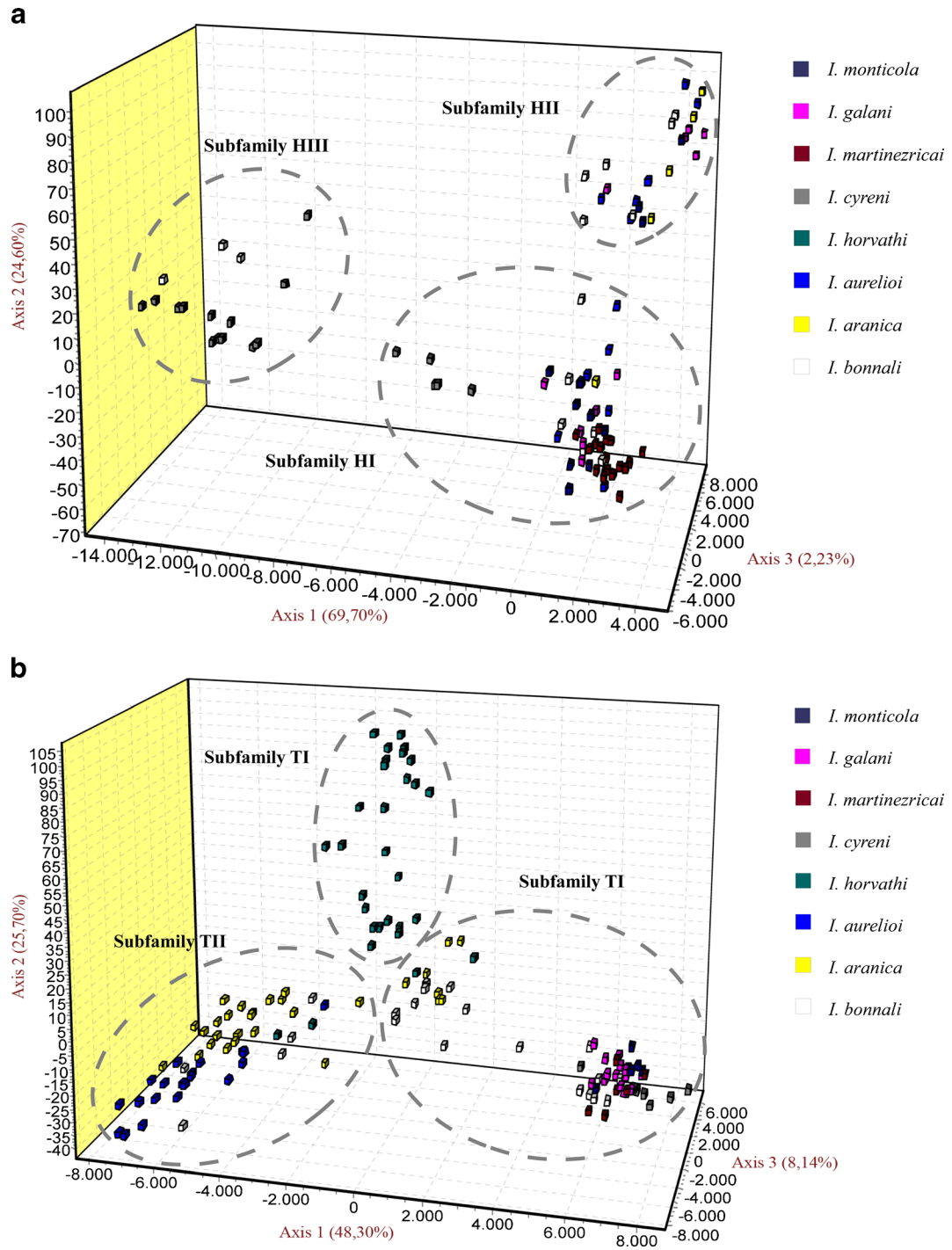

Fig. 2 Three-dimensional representation of a factorial correspondence analysis based on monomeric sequences of HindIII (**a**) and TaqI (**b**) satDNAs

are higher for *I. horvathi* and the Pyrenean species, which harbor both types of TaqI repeats in their genomes (Table 1). When each subfamily is analyzed separately, π values within subfamily TI are two- to threefold greater in these species than in the species of the Iberian clade (from 4.06 % in *I. cyreni* to 11.84 % in *I. horvathi*). High

Table 3 Interspecific and inter-subfamily net genetic distances for HindIII (a) and TaqI (b) repeats. Standard error estimates are shown above the diagonal. Color codes represent the different types of HindIII and TaqI subfamilies. Asterisks in b indicate those values obtained in comparisons involving IAU_TI, represented by only one sequence

a

	IGA HI	IMR HI	IAU HI	IHO HI	IMO HI	IBN HI	IAR HI	ICY HI	IGA HII	IBN HII	IAU HII	IAR HII	IMO HII	ICY HIII	IBN HIII
IGA_HI		0.001	0.003	0.001	0.000	0.002	0.003	0.011	0.017	0.016	0.016	0.017	0.016	0.023	0.026
IMR_HI	0.001		0.004	0.001	0.000	0.003	0.004	0.013	0.018	0.017	0.017	0.017	0.017	0.024	0.026
IAU_HI	0.006	0.008		0.005	0.003	0.000	0.001	0.011	0.015	0.013	0.013	0.014	0.012	0.023	0.025
IHO_HI	0.001	0.001	0.009		0.000	0.003	0.005	0.015	0.019	0.018	0.017	0.018	0.018	0.026	0.028
IMO_HI	0.000	0.001	0.005	0.000		0.002	0.003	0.012	0.017	0.016	0.016	0.017	0.016	0.024	0.026
IBN_HI	0.003	0.005	0.000	0.006	0.003		0.001	0.010	0.016	0.014	0.013	0.015	0.012	0.022	0.025
IAR_HI	0.005	0.006	0.001	0.008	0.004	0.001		0.010	0.016	0.014	0.013	0.015	0.013	0.021	0.024
ICY_HI	0.026	0.028	0.023	0.034	0.029	0.022	0.020		0.022	0.018	0.019	0.021	0.018	0.016	0.020
IGA_HII	0.047	0.048	0.039	0.050	0.046	0.042	0.041	0.065		0.001	0.004	0.001	0.007	0.032	0.030
IBN_HII	0.041	0.044	0.031	0.047	0.042	0.034	0.032	0.051	0.002		0.000	0.001	0.002	0.029	0.030
IAU_HII	0.038	0.042	0.026	0.044	0.038	0.029	0.028	0.051	0.007	0.000		0.002	0.001	0.029	0.030
IAR_HII	0.044	0.046	0.033	0.048	0.043	0.036	0.035	0.061	0.001	0.000	0.002		0.003	0.031	0.031
IMO_HII	0.039	0.043	0.024	0.045	0.038	0.027	0.026	0.049	0.012	0.004	0.000	0.010		0.028	0.030
ICY_HIII	0.077	0.080	0.074	0.086	0.080	0.073	0.068	0.043	0.115	0.102	0.101	0.112	0.100		0.005
IBN_HIII	0.088	0.090	0.086	0.097	0.091	0.085	0.080	0.063	0.104	0.103	0.105	0.106	0.108	0.011	

b

	IHO_TI	IBN_TI	IAR_TI	IAU_TI	IMR_TI	ICY_TI	IGA_TI	IMO_TI	IAR_TII	IAU_TII	IBN_TII	IHO_TII
IHO_TI		0.009	0.022	0.025	0.018	0.018	0.025	0.019	0.022	0.019	0.021	0.029
IBN_TI	0.033		0.004	0.027	0.006	0.006	0.004	0.008	0.020	0.021	0.015	0.019
IAR_TI	0.024	0.011		0.024	0.015	0.015	0.015	0.015	0.017	0.017	0.012	0.017
IAU_TI	0.100*	0.107*	0.084*		0.033	0.033	0.032	0.032	0.028	0.030	0.026	0.031
IMR_TI	0.066	0.014	0.050	0.152*		0.002	0.002	0.002	0.026	0.027	0.023	0.024
ICY_TI	0.070	0.016	0.051	0.154*	0.004		0.003	0.004	0.026	0.027	0.023	0.024
IGA_TI	0.064	0.014	0.049	0.147*	0.005	0.008		0.005	0.025	0.027	0.023	0.024
IMO_TI	0.061	0.016	0.047	0.146*	0.003	0.007	0.007		0.026	0.028	0.024	0.024
IAR_TII	0.062	0.075	0.056	0.122*	0.112	0.116	0.110	0.115		0.003	0.004	0.006
IAU_TII	0.066	0.075	0.056	0.128*	0.117	0.121	0.113	0.119	0.007		0.005	0.005
IBN_TII	0.044	0.054	0.036	0.104*	0.095	0.097	0.091	0.097	0.001	0.000		0.004
IHO_TII	0.055	0.057	0.046	0.120*	0.089	0.090	0.084	0.089	0.013	0.008	0.002	

π values were also obtained for subfamily TII in those species with a large number of monomers examined (9.08 % in *I. aurelioi* and 9.60 % in *I. aranica*).

The factorial analysis of TaqI monomers identified a main axis of variation (axis 1 at Fig. 2b, explaining 48.30 % of the observed variation), corresponding to the separation between three groups of repeats: (1) subfamily TII (i.e., essentially Pyrenesaura); (2) a subset of subfamily TI, including all the monomers of Iberian species and a few monomers of *I. bonnali*; and (3) a subset of subfamily TI, made up of monomers from *I. horvathi*, *I. aranica*, and *I. bonnali*. Axis 2 in the FCA, which accounts for 25.70 % of the total variation, separates a fourth group of repeats, comprising the remaining TI monomers of *I. horvathi*. Net genetic distances between repeats from the different species (Table 3b) give additional support to the FCA results. Leaving aside the comparisons involving the single monomer of TI in *I. aurelioi*, larger distances between T1 repeats correspond to pairs of the Iberian species with both *I. aranica* (4.70–5.10 %) and, above all, *I. horvathi* (6.10–7.0 %). As for the TII repeats, all the pairwise comparisons, involving the subgenus *Pyrenesaura* and *I. horvathi*, produce rather low values (0.0–1.30 %).

Organization of consecutive monomeric units

The cloning and sequencing of multimeric products allowed us to characterize the organization of consecutive monomeric repeats. In both satDNA families, and in all the species analyzed, we observed that adjacent monomers in a satellite array usually belong to different sequence groups and even to different subfamilies (for a list of all HindIII and TaqI composite arrays sampled in the *Iberolacerta* species, see Supplementary Tables 2 and 3, respectively).

Phylogenetic analysis

The statistical parsimony network obtained for HindIII satDNA showed a high degree of reticulation among the

members of subfamily HI (Fig. 3a). This pattern suggests that rearrangements due to recombination events are an important force generating new monomers in this subfamily —the most widespread among *Iberolacerta* species—, which occupies the central position of the parsimony network. Two sequence groups within this subfamily, HI_K and HI_M, branched into two separate lineages, corresponding to subfamilies HII and HIII, respectively. In contrast to subfamily HI, no evidence for recombination events has been found within subfamilies HII and HIII.

In the network of TaqI satDNA, all sequence groups converge on a group belonging to subfamily T1 (T1_ FI, Fig. 3b). The network shows a major separation of four clusters, connected to group TI_F1 by a few mutational steps. Three of them (T1_F2, T1_C2, and T1_G1, together with their related variants) include sequences only found in *I. horvathi* and in the subgenus *Pyrenesaura*. All sequence groups belonging to subfamily TII occupy a peripheral position within cluster G1. The extensive diversification within subfamily TII has been promoted, in some cases, by recombination events that created new monomer variants (e.g., TII_E1b or TII_G2a). Within the fourth cluster, the prolific lineage TI_L3 includes closely related sequence groups (separated by just one or two nucleotide changes), specific to the Iberian clade.

Chromosomal location of HindIII and TaqI satDNA families

FISH with HindIII satDNA probe on metaphase chromosomes of *I. monticola* and *I. galani* revealed that this repetitive element is present at centromeres of all the 36 chromosomes of the diploid complement (Fig. 4; Giovannotti et al. 2014). FISH on female metaphases

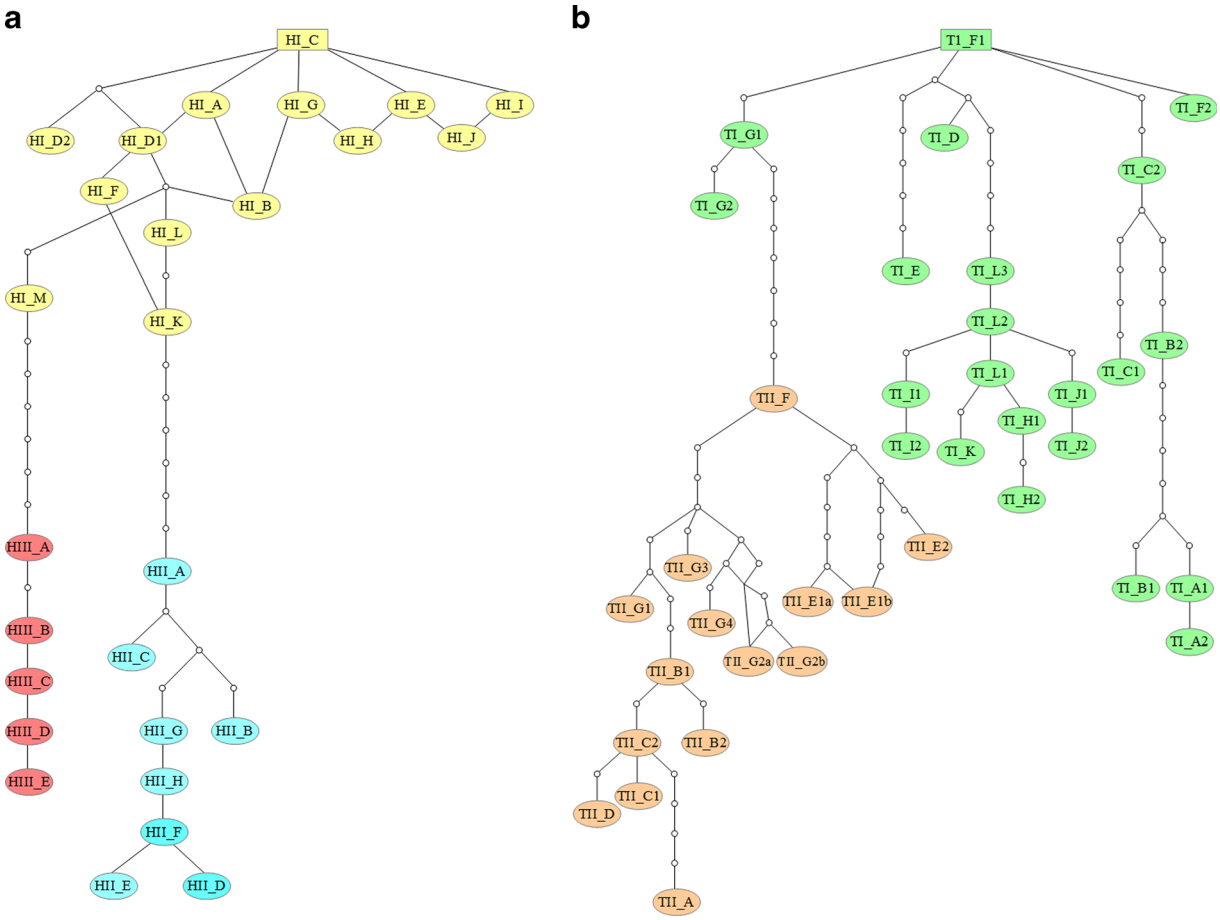

Fig. 3 Statistical parsimony network constructed from the consensus sequences of the different sequence groups of **a** HindIII satDNA and **b** TaqI satDNA

of *I. bonnali,* carried out in this work, showed hybridization signals in the centromeric regions of all the 23 chromosomes of the karyotype, although with variable signal strength in different chromosome pairs (Fig. 4). Moreover, the overall intensity of HindIII signals in *I. bonnali* was noticeably lower than in *I. monticola* and *I. galani*. No hybridization signals were observed in the chromosomes of *I. horvathi*.

FISH with TaqI satDNA probe in *I. monticola* and *I. galani* produced bright signals in interstitial position in a subset of 20 and 18 chromosomes, respectively (Fig. 5). In *I. bonnali*, similarly intense signals were detected interstitially on both arms of 10 meta-/submetacentric chromosomes. In some metaphases, an additional faint signal could be observed in a medium-sized chromosome pair (Fig. 5). In *I. horvathi*, strong hybridization signals were also observed in interstitial position but just in six chromosomes. However, after increased exposure times, 10 additional chromosomes appeared weakly labeled (Fig. 5).

Discussion

The turnover rate of a satDNA family is a complex feature that depends on many parameters, such as interchromosomal and intrachromosomal recombination rates, copy number and long-range organization of repeat units, genome location and distribution, putative functional interactions, reproductive mode, and population factors (Strachan et al. 1985; Dover 2002; Luchetti et al. 2003; Robles et al. 2004; Meštrović et al. 2006; Kuhn et al. 2008; Navajas-Pérez et al. 2009; Giovannotti et al. 2013). In consequence, sequence dynamics of satDNA families may differ not only among families but also, for a given family, among genomic regions (Kuhn et al. 2011), populations (Wei et al. 2014), species, or higher taxonomic groups (e.g., Macas et al. 2006; Kuhn et al. 2008; Martinsen et al. 2009; Plohl et al. 2010).

In agreement with Giovannotti et al. (2014), the results of the present work show that overall variability

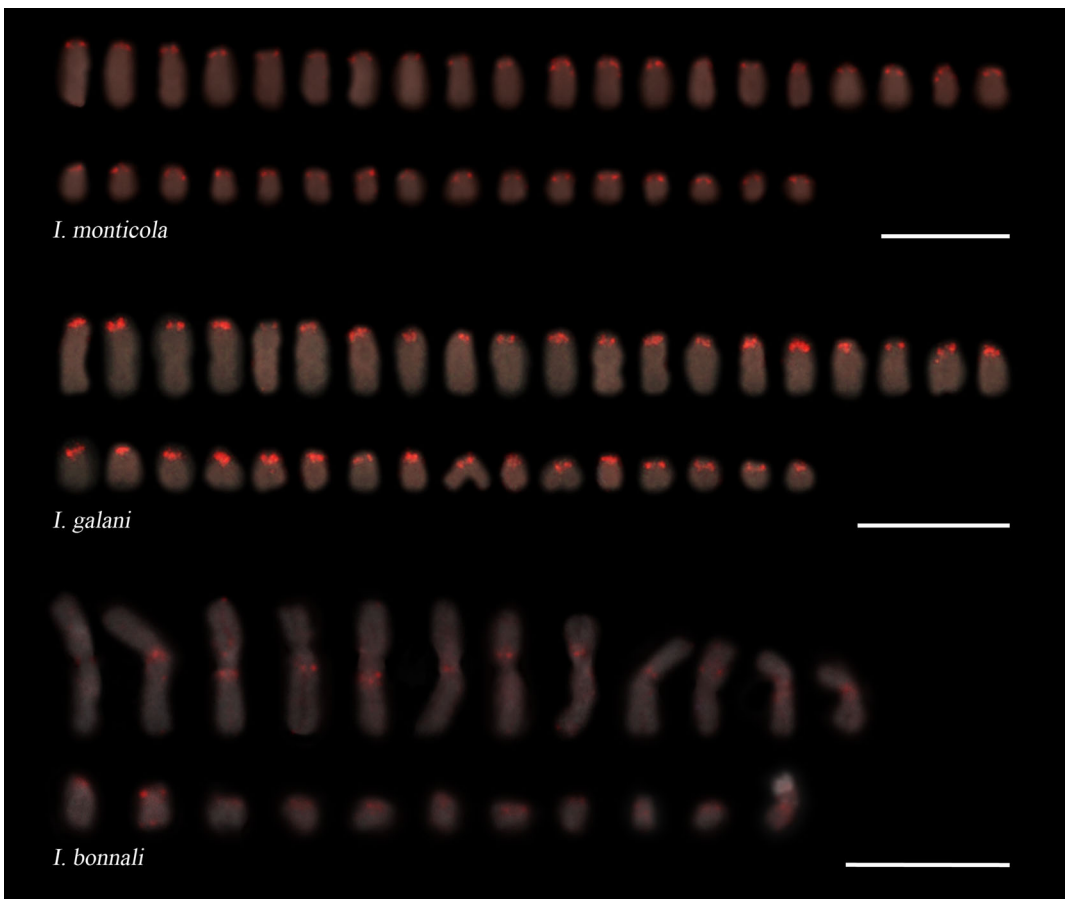

Fig. 4 Hybridization pattern of the HindIII probe in the karyotypes of *Iberolacerta monticola*, *I. galani* and *I. bonnali. Scale bar*=10 μm

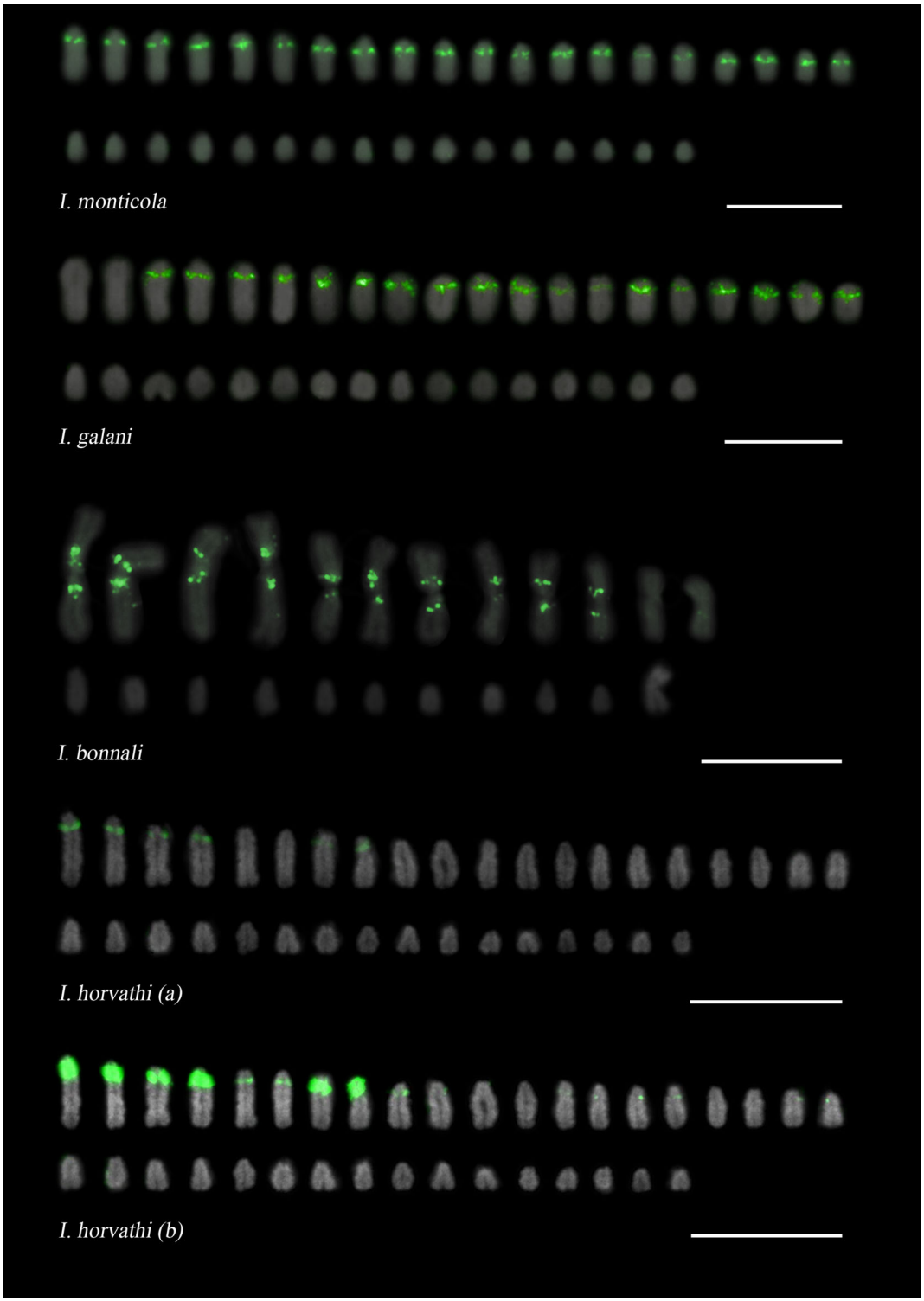

Fig. 5 Hybridization pattern of the TaqI probe in the karyotypes of *Iberolacerta monticola*, *I. galani*, *I. bonnali*, and *I. horvathi*. FISH signals on *I. horvathi* chromosomes are shown at standard (*a*) and increased (*b*) exposure times. *Scale bar*=10 μm

of TaqI repeats in the whole genus *Iberolacerta* is on average three times higher than the variability of HindIII repeats, which suggests a faster homogenization/fixation rate for the latter satDNA family. However, the detailed characterization of both satDNA families in all eight *Iberolacerta* species reveals that their evolutionary patterns are more complex than previously anticipated. The presence of HindIII_HI in all the species, and its central position in the phylogenetic network, suggests that this is the most ancestral variant of HindIII satDNA, from which subfamilies HII and HIII were derived. Interestingly, with the exception of *I. cyreni*, no intraspecific homogenization for any particular subfamily was detected in our study, and most different sequence groups of subfamilies HI and HII are widespread and shared by even distantly related species. Indeed, interspecific genetic distances within each subfamily are substantially lower than intraspecific genetic distances between repeats belonging to different subfamilies. On the contrary, *I. cyreni* shows a high proportion of private sequence groups belonging to subfamily HIII, and a well-differentiated subset of HI repeats, which explains the evidence of concerted evolution found for this species in our previous study. However, the finding of HIII repeats also in *I. bonnali* indicates that this subfamily is not exclusive of *I. cyreni*, but was already present in the common ancestral library of HindIII variants. Combining these data with the results of FISH experiments, the most parsimonious interpretation of HindIII satDNA evolution is that the diversification of HindIII repeats—which generated most of the extant variants—took place in the common ancestor of *Iberolacerta*, before species radiation, i.e., from 11.6 to 15.6 Mya (Arribas et al. 2014). In the ancestral species, HindIII satDNA might have been widely distributed in the centromeres of all chromosome pairs, with a subsequent decrease in copy number in *I. horvathi* and, at least, in the Pyrenean *I. bonnali*. In the latter species, and maybe also in the other two Pyrenean taxa, the reduced amounts of HindIII satDNA might obey to the possible involvement of this centromeric element in the Robertsonian fusions that originated the biarmed chromosomes characteristic of *Pyrenesaura* from the ancestral acrocentric karyotype, as has been suggested for other centromeric repeats in marsupials (Bulazel et al. 2007). Alternatively, HindIII could represent a minor satDNA family in the centromeres of the ancestral species, which was differentially amplified in the Iberian clade. In either case, the turnover of HindIII repeats in the different lineages mainly involved the same pool of "old" repeat variants. Long-term conservation of ancestral repeats could be a consequence of selective constraints imposed on functional motifs or structural features of satellite monomers (see, for example, Meštrović et al. 2006; Plohl et al. 2012), involved in any of the roles ascribed to satDNAs (reviewed in Ugarković 2009). Thus, even if we did not find any evidence of function in HindIII satDNA, selection may have favored the maintenance of some repeat variants and/or limited the diversification of this repetitive element. Nevertheless, the loss of HindIII repeats in *I. horvathi* and *I. bonnali* (or, alternatively, the amplification in the Iberian species) suggests that even if functional, a satellite family may be replaced by another in a relatively short evolutionary time.

Actually, and in contrast to the highly conserved function of the centromeres, the rapid evolution and extensive changes in copy number of satDNAs is a general characteristic of centromeric regions (Henikoff et al. 2001). The detection of recombinant sequences within subfamily HI suggests that mechanisms such as unequal crossovers between sister chromatids and gene conversion may have been an important source of new sequence variants in HindIII satDNA (e.g. Smith 1976; Talbert and Henikoff 2010). Moreover, unequal crossover occurring between highly homogeneous arrays can induce copy number alterations of satDNA repeats, such as those observed in the *Iberolacerta* species (Stephan 1986). This fast evolution of centromeric satDNAs can be linked to reproductive isolation and speciation (Bachmann et al. 1989; Bachmann and Sperlich 1993). For example, divergence of centromeric satDNA in *Drosophila* species can inhibit chromosome segregation in hybrids and thus directly cause hybrid incompatibilies and postzygotic isolation (Ferree and Barbash 2009). Likewise, the high copy number polymorphisms and rapid shifts in centromere sequence composition could have contributed and even triggered species radiation within *Iberolacerta*.

The TaqI satDNA family appears to have a very different evolutionary history from the HindIII family, and to evolve much faster in the lineage that leads to *I. horvathi*. According to the parsimony network, TaqI_TI, the most widespread subfamily among the analyzed species, would also be the most ancestral variant, from which subfamily TII was derived. Moreover, the phylogenetic distribution of the different sequence sets suggests that both subfamilies were

present in the common ancestor of *Iberolacerta.* Subsequently, subfamily TII spread in the Pyrenean species, whereas it was progressively lost in *I. horvathi* and maybe even completely removed from the genomes of the Iberian species. Altogether, TI repeats retrieved from *I. horvathi* show a general pattern of concerted evolution, with high interspecific distance values in all pairwise comparisons and a large subset of species-specific sequence groups. The allocation of these private groups (e.g., TI_A2 or TI_C1) in terminal clades of the statistical parsimony network indicates that they probably arose after the early separation of *I. horvathi* from the remaining species, about 11.5 Mya (9.6–13.7) (Arribas et al. 2014). The evolution of TaqI satDNA in *I. horvathi* was probably accompanied by a reduction in the abundance and chromosomal distribution, as inferred from the results of FISH experiments. TaqI satDNA also seems to evolve in concert in the Iberian clade but with a distinct pattern from that found in *I. horvathi*. In this case, the profile of TI repeats and the low levels of nucleotide diversity indicate that concerted evolution in the Iberian clade involved the preferential homogenization of a reduced subset of TaqI variants, all of which evolved from a single sequence lineage, TI_L3. After cladogenesis, however, the rate at which TI repeats evolved within the Iberian clade is presumably low, since TaqI sequences are poorly differentiated between the four taxa and we found almost no species-specific sequence sets.

In contrast with *I. horvathi* and the Iberian species, the turnover of TaqI satDNA seems to be remarkably slow in the Pyrenean *I. bonnali*. TaqI repeats from this species belong mainly to "old" sequence sets of subfamily TI, and lack species-specific diagnostic positions, which indicates that most of the variability found in *I. bonnali* obeys to synapomorphisms, and that TaqI repeats have been evolving with a low rate of sequence change after speciation. Conversely, the evolution of TaqI satDNA in the other two Pyrenean species, *I. aranica* and *I. aurelioi*, is characterized by the amplification of subfamily TII. Phylogenetic studies suggest that the three species of the Pyrenean clade originated in rapid succession, though *I. bonnali* probably split first, roughly 3.8 Mya (2.7–4.9) (Arribas et al. 2006, 2014). According to this phylogenetic reconstruction, the amplification of subfamily TII in the genomes of *I. aranica* and *I. aurelioi* may have occurred in a short time, after the separation of *I bonnali* and before the divergence of both species, *ca*. 3.3 Mya (2.3–4.3). A rapid expansion of subfamily TII agrees well with the high levels of intraspecific nucleotide diversity and interspecific sequence conservation observed for this subfamily in both species.

The different turnover rates of TaqI repeats among the Pyrenean species, *I. horvathi* and the Iberian species, could be related to differences in their karyotypes. It is possible that interchromosomal exchange and homogenization between the asymmetric meta-/submetacentric chromosomes of the Pyrenean species is more limited than in the species with all acrocentric chromosomes, more homogeneous in shape and size. Similar considerations have been proposed to explain the lower evolutionary rate of satDNAs in sturgeons as compared to sparids (de la Herrán et al. 2001). Limited interchromosomal exchange would lead to a progressive compartmentalization of satellite repeats, followed by a reduction in their interactions and, eventually, by a lack of homogenization of different sequence variants. However, this hypothesis is at least partially contradicted by our analysis of consecutive monomeric units, which revealed that, in both HindIII and TaqI satDNA families, adjacent repeats are not necessarily more similar than are repeats selected at random and that members of different sequence groups or even subfamilies can be interspersed in the same array.

In fact, this pattern of composite repeats may be a key factor explaining the disparate turnover rates of each satDNA family in different species. In eukaryotes, homologous recombination within or between chromosomes can be inhibited by only one mutation per 200 bp (Nijman and Lenstra 2001 and references therein). Likewise, mutations in new monomer variants would inhibit the interactions of repeat units, leading to sequence diversification, divergent evolution, and the formation of satDNA subfamilies. Accordingly, our estimates of intraspecific genetic distances between repeats belonging to different subfamilies suggest that each subfamily within HindIII and TaqI satDNAs is evolving independently. In this context, the intermixing between subfamilies HI and HII within HindIII arrays in most of the species analyzed, and between TaqI subfamilies TI and TII in the Pyrenean taxa, would strongly reduce recombination and homogenization within each subfamily, resulting in the pattern of non-concerted evolution observed

in our study. Conversely, the amplification of subfamily HIII in *I. cyreni*, and the preponderance of subfamily TI in *I. horvathi* and the Iberian species, allows a more efficient homogenization of HindIII and TaqI repeats, respectively, which translates into the overall patterns of concerted evolution observed for these satDNA families in the species mentioned above.

Taken together, our results on the dynamics of HindIII and TaqI satDNAs in *Iberolacerta* are congruent with proposed models of satDNA evolution and life history, intended to explain the considerable fluctuations in copy number and variability of satDNAs shared by related species (Nijman and Lenstra 2001; Plohl et al. 2010). They also support the idea that the "library model" may be extended to monomer variants of the same satDNA family, which were already present in a common ancestor and are currently distributed in related species in variant copy numbers (Cesari et al. 2003). As observed in *Iberolacerta*, this particular evolutionary pattern may result in species-specific profiles of satDNAs which do not reflect the phylogenetic relationships among taxa.

In conclusion, an in-depth analysis of intragenomic variability of HindIII and TaqI satDNAs in *Iberolacerta* revealed two disparate evolutionary histories which, nevertheless, showed some common traits: (i) each satDNA family is made up of a library of monomer variants or subfamilies shared by related species; (ii) species-specific profiles of satellite repeats are shaped by expansions and/or contractions of different variants from the library; (iii) different turnover rates, even among closely related species, result in great differences in overall sequence homogeneity and in concerted or non-concerted evolution patterns. Contrasting turnover rates are possibly related to genomic constraints such as karyotype architecture and the interspersed organization of diverging repeat variants in satellite arrays and maybe also to functional interactions. On the whole, these satDNA families constitute highly dynamic systems, which may have a critical role on the evolution of genome and species. Further studies aimed at investigating the genome-wide variability and organization of reptilian satDNAs may not only be useful to test current hypothesis and identify mechanisms influencing the evolution of this genomic component but also to improve its application as a molecular marker in phylogenetic studies.

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Ethical standards Permissions for field work and experimental procedures were issued by the competent authorities: Xunta de Galicia (for *I. monticola* and *I. galani*), Junta de Castilla y León (for *I. cyreni* and *I. martinezricai*), Gobierno de Aragón (for *I. bonnali*), and Italian Environment Ministry (for *I. horvathi*). All institutional and national guidelines for the care and use of laboratory animals were followed.

References

Arnold EN, Arribas O, Carranza S (2007) Systematics of the palaearctic and oriental lizard tribe Lacertini (Squamata: Lacertidae: Lacertinae), with descriptions of eight new genera. Zootaxa 1430:1–86

Arribas O, Odierna G (2004) Karyological and osteological data supporting the specific status of *Iberolacerta* (*cyreni*) *martinezricai* (Arribas, 1996). Amphibia-Reptilia 25: 359–367

Arribas O, Carranza S, Odierna G (2006) Description of a new endemic species of mountain lizard from Northwestern Spain: *Iberolacerta galani* sp. nov. (Squamata: Lacertidae). Zootaxa 1240:1–55

Arribas O, Galán P, Remón N, Naveira H (2014) A new mountain lizard from Montes de León (NW Iberian Peninsula): *Iberolacerta monticola astur* ssp. nov. (Squamata: Lacertidae). Zootaxa 3796:201–236

Bachmann L, Sperlich D (1993) Gradual evolution of a specific satellite DNA family in *Drosophila ambigua*, *D. tristis*, and *D. obscura*. Mol Biol Evol 10:647–659

Bachmann L, Raab M, Sperlich D (1989) Satellite DNA and speciation: a species specific satellite DNA of *Drosophila guanchel*. J Zool Syst Evol Res 27:84–93

Belkhir K, Borsa P, Chikhi L, Raufaste N, Bonhomme F (2004) GENETIX 4.05, logiciel sous Windows TM pour la génétique des populations. Retrieved from: www.genetix.univ-montp2.fr/genetix/genetix.htm. 18/3/2015

Bulazel KV, Ferreri GC, Eldridge MDB, O'Neill RJ (2007) Species-specific shifts in centromere sequence composition are coincident with breakpoint reuse in karyotypically divergent lineages. Genome Biol 8:R170

Capriglione T (2000) Repetitive DNA as a tool to study the phylogeny of cold-blooded vertebrates. In: Olmo E, Redi CA (eds) Chromosomes today. Birkhäuser Verlag, Basel, pp 183–194

Capriglione T, Olmo E, Odierna G, Smith DI, Miller OJ (1989) Genome composition and tandemly repetitive sequence at some centromeres in the lizard *Podarcis s. sicula* Raf. Genetica 79:85–91

Capriglione T, Cardone A, Odierna G, Olmo E (1991) Evolution of a centromeric satellite DNA and phylogeny of lacertid lizards. Comp Biochem Physiol B 100:641–645

Capriglione T, Cardone A, Odierna G, Olmo E (1994) Further data on the occurrence and evolution of satellite DNA families in the lacertid genome. Chromosom Res 2:327–330

Capriglione T, de Santo MG, Odierna G, Olmo E (1998) An alphoid-like satellite DNA sequence is present in the genome of a lacertid lizard. J Mol Evol 46:240–244

Capula M, Lapini L, Capanna E (1989) The karyotype of *Lacerta horvathi* (Reptilia, Sauria, Lacertidae). Genetica 79:11–16

Cesari M, Luchetti A, Passamonti M, Scali V, Mantovani B (2003) Polymerase chain reaction amplification of the Bag320 satellite family reveals the ancestral library and past gene conversion events in *Bacillus rossius* (Insecta Phasmatodea). Gene 312:289–295

Chaiprasertsri N, Uno Y, Peyachokinagui S et al (2013) Highly species-specific centromeric repetitive DNA sequences in lizards: molecular cytogenetic characterization of a novel family of satellite DNA sequences isolated from the water monitor lizard (*Varanus salvator macromaculatus*, Platynota). J Hered 104:798–806

Charlesworth B, Sniegowski P, Stephan W (1994) The evolutionary dynamics of repetitive DNA in eukaryotes. Nature 371: 215–220

Ciobanu D, Grechko VV, Darevsky IS (2003) Molecular evolution of satellite DNA CLsat in lizards from the Genus *Darevskia* (Sauria: Lacertidae): correlation with species diversity. Russ J Genet 39:1527–1541

Ciobanu D, Grechko VV, Darevsky IS, Kramerov DA (2004) New satellite DNA in *Lacerta* s. str. lizards (Sauria: Lacertidae): evolutionary pathways and phylogenetic impact. J Exp Zool B Mol Dev Evol 302:505–516

Clement M, Posada D, Crandall KA (2000) TCS: a computer program to estimate gene genealogies. Mol Ecol 9: 1657–1659

de la Herrán R, Fontana F, Lanfredi M et al (2001) Slow rates of evolution and sequence homogenization in an ancient satellite DNA family of sturgeons. Mol Biol Evol 18:432–436

Dover GA (1982) Molecular drive: a cohesive mode of species evolution. Nature 299:111–117

Dover GA (2002) Molecular drive. Trends Genet 18:587–589

Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 32: 1792–1797

Enukashvily NI, Ponomartsev NV (2013) Mammalian satellite DNA: a speaking dumb. Adv Protein Chem Struct Biol 90: 31–65

Feliciello I, Akrap I, Brajković J, Zlatar I, Ugarković Đ (2015) Satellite DNA as a driver of population divergence in the red flour beetle *Tribolium castaneum*. Genome Biol Evol 7:228–239

Ferree PM, Barbash DA (2009) Species-specific heterochromatin prevents mitotic chromosome segregation to cause hybrid lethality in *Drosophila*. PLoS Biol 7, e1000234

Fry K, Salser W (1977) Nucleotide sequences of HS-A satellite DNA from kangaroo rat *Dipodomys ordii* and characterization of similar sequences in other rodents. Cell 12:1069–1084

Giovannotti M, Nisi Cerioni P, Caputo V, Olmo E (2009) Characterisation of a GC-rich telomeric satellite DNA in *Eumeces schneideri* Daudin (Reptilia, Scincidae). Cytogenet Genome Res 125:272–278

Giovannotti M, Nisi Cerioni P, Splendiani A, Ruggeri P, Olmo E, Caputo Barucchi V (2013) Slow evolving satellite DNAs: the case of a centromeric satellite in *Chalcides ocellatus* (Forskål, 1775) (Reptilia, Scincidae). Amphibia-Reptilia 34: 401–411

Giovannotti M, Rojo V, Nisi Cerioni P et al (2014) Isolation and characterization of two satellite DNAs in some Iberian rock lizards (Squamata, Lacertidae). J Exp Zool B Mol Dev Evol 322:13–26

Grechko VV, Ciobanu DG, Darevsky IS, Kramerov DA (2005) Satellite DNA of lizards of the genus *Lacerta* s. str. (the group *L. agilis*), the family Lacertidae. Dokl Biochem Biophys 400: 44–47

Grechko VV, Ciobanu DG, Darevsky IS, Kosushkin SA, Kramerov DA (2006) Molecular evolution of satellite DNA repeats and speciation of lizards of the genus *Darevskia* (Sauria: Lacertidae). Genome 49:1297–1307

Henikoff S, Ahmad K, Malik HS (2001) The centromere paradox: stable inheritance with rapidly evolving DNA. Science 293: 1098–1102

Kearse M, Moir R, Wilson A et al (2012) Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28:1647–1649

Kuhn GCS, Sene FM, Moreira-Filho O, Schwarzacher T, Heslop-Harrison JS (2008) Sequence analysis, chromosomal distribution and long-range organization show that rapid turnover of new and old pBuM satellite DNA repeats leads to different patterns of variation in seven species of the *Drosophila buzzatii* cluster. Chromosom Res 16:307–324

Kuhn GCS, Schwarzacher T, Heslop-Harrison JS (2010) The non-regular orbit: three satellite DNAs in *Drosophila martensis* (*buzzatii* complex, *repleta* group) followed three different evolutionary pathways. Mol Genet Genomics 284:251–262

Kuhn GCS, Küttler H, Moreira-Filho O, Heslop-Harrison JS (2011) The 1.688 repetitive DNA of *Drosophila*: concerted evolution at different genomic scales and association with genes. Mol Biol Evol msr173

Librado P, Rozas J (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25: 1451–1452

Luchetti A, Cesari M, Carrara G et al (2003) Unisexuality and molecular drive: Bag320 sequence diversity in *Bacillus* taxa (Insecta Phasmatodea). J Mol Evol 56:587–589

Macas J, Navrátilová A, Koblížková A (2006) Sequence homogenization and chromosomal localization of VicTR-B satellites differ between closely related *Vicia* species. Chromosoma 115:437–447

Martinsen L, Venanzetti F, Johnsen A, Sbordoni V, Bachman L (2009) Molecular evolution of the *pDo500* satellite DNA family in *Dolichopoda* cave crickets (Rhaphidophoridae). BMC Evol Biol 9:1–14

Meštrović N, Plohl M, Mravinac B, Ugarković D (1998) Evolution of satellite DNAs from the genus palorus—

experimental evidence for the 'library' hypothesis. Mol Biol Evol 15:1062–1068

Meštrović N, Castagnone-Sereno P, Plohl M (2006) Interplay of selective pressure and stochastic events directs evolution of the MEL172 satellite DNA library in root-knot nematodes. Mol Biol Evol 23:2316–2325

Navajas-Pérez R, Quesada del Bosque ME, Garrido-Ramos MA (2009) Effect of location, organization, and repeat-copy number in satellite-DNA evolution. Mol Genet Genomics 282: 395–406

Nijman IJ, Lenstra JA (2001) Mutation and recombination in cattle satellite DNA: a feedback model for the evolution of satellite repeats. J Mol Evol 52:361–371

Odierna G, Aprea G, Arribas O, Capriglione T, Caputo V, Olmo E (1996) The karyology of the Iberian rock lizards. Herpetologica 52:542–550

Palomeque T, Lorite P (2008) Satellite DNA in insects: a review. Heredity 100:564–573

Pezer Ž, Brajković J, Feliciello I, Ugarković D (2012) Satellite DNA-mediated effects on genome regulation. In: Garrido-Ramos MA (ed) Repetitive DNA. Genome dynamics vol 7. Karger, Basel, pp 153–169

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

Plohl M, Petrovic V, Luchetti A et al (2010) Long-term conservation vs high sequence divergence: the case of an extraordinarily old satellite DNA in bivalve mollusks. Heredity 104: 543–551

Plohl M, Meštrović N, Mravinac B (2012) Satellite DNA evolution. In: Garrido-Ramos MA (ed) Repetitive DNA, vol 7, Genome dynamics vol. Karger, Basel, pp 126–152

Robles F, de la Herrán R, Ludwig A, Rejón CR, Rejón MR, Garrido-Ramos MA (2004) Evolution of ancient satellite DNAs in sturgeon genomes. Gene 338:133–142

Rojo V, Giovannotti M, Naveira H et al (2014) Karyological characterization of the endemic Iberian rock lizard, *Iberolacerta monticola* (Squamata, Lacertidae): insights into sex chromosome evolution. Cytogenet Genome Res 142:28–39

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York

Schwarzacher T, Heslop-Harrison P (2000) Practical in situ hybridization. BIOS Scientific Publishers Ltd., Oxford

Sindaco R, Jeremčenko VK (2008) The reptiles of the western paleartic, volume 1: annotated checklist and distributional atlas of the turtles, crocodiles, amphisbaenians and lizards of Europe, North Africa, Middle East and Central Asia. Latina: Edizioni Belvedere

Smith GP (1976) Evolution of repeated DNA sequences by unequal crossover. Science 191:528–535

Stephan W (1986) Recombination and the evolution of satellite DNA. Genet Res 47:167–174

Strachan T, Webb D, Dover G (1985) Transition stages of molecular drive in multiple-copy DNA families in *Drosophila*. EMBO J 4:1701–1708

Talbert PB, Henikoff S (2010) Centromeres convert but don't cross. PLoS Biol 8, e1000326

Tamura K, Nei M, Kumar S (2004) Prospects for inferring very large phylogenies by using the neighbor-joining method. Proc Natl Acad Sci U S A 101:11030–11035

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 30:2725–2729

Templeton AR, Crandall KA, Sing CF (1992) A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. Genetics 132:619–633

Ugarković D (2009) Centromere-competent DNA: structure and evolution. Prog Mol Subcell Biol 48:53–76

Ugarković D, Plohl M (2002) Variation in satellite DNA profiles—causes and effects. EMBO J 21:5955–5959

Waters PD, Marshall Graves JA, Thompson K, Sankovic N, Ezaz T (2008) Identification of cryptic sex chromosomes and isolation of X- and Y-borne genes. Methods Mol Biol 422: 239–251

Wei KH-C, Grenier JK, Barbash DA, Clark AG (2014) Correlated variation and population differentiation in satellite DNA abundance among lines of *Drosophila melanogaster*. PNAS 111:18793–18798