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Cytotaxonomy and DNA taxonomy of lizards (Squamata, Sauria) from a tropical dry forest in the Chamela-Cuixmala Biosphere Reserve on the coast of Jalisco, Mexico

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Abstract

Tropical dry forests contribute to a substantial proportion of the herpetological diversity of Mexico. The south-western coast of Jalisco is one of the more important areas by number of endemics and the high presence of endangered and restricted species. In this paper we used a combined karyological and molecular genetic (sequences of mtDNA genes for NDH2, cyt*b* or 16S rDNA) approach to genetically characterize 13 lizard species belonging to seven families that inhabit the dry forests of the Chamela-Cuixmala Biosphere Reserve (Anguidae: *Gerrhonotus* cf. *liocephalus*; Eublepharidae: *Coleonyx elegans*; Phyllodactylidae: *Phyllodactylus lanei*; Gekkonidae: *Hemidactylus frenatus*; Phrynosomatidae: *Sceloporus melanorhinus, S. utiformis, Urosaurus bicarinatus*; Polychrotidae: *Norops nebulosus;* Scincidae: *Mabuya unimarginata*, *Plestiodon parvulus*; Teiidae: *Ameiva undulata, Aspidoscelis communis, A. lineattissima*). The karyotypes of six species were here described for the first time (*G. liocephalus*, 2n = 38, 14 macrochromosomes and 24 microcromosomes; *C. elegans*, 2n = 24 FN = 26; *N. nebulosus* 2n = 30, 13 macro- and 17 microchromosomes; *M. unimarginata* 2n = 32, 18 macro- and 14 microchromosomes; *P. parvulus* 2n = 26, 12 macro- and 14 microchromosomes; *A. undulata* 2n = 50, 26 macro- and 24 microchromosomes). Chromosomal heteromorphism was found in *C. elegans*, *N. nebulosus*, and *S. melanorhinus*. For *P. lanei* we found a karyotype different from that previously described in other localities. This variation matched with a high genetic divergence usually found in different species. The DNA typing of mtDNA genes allowed the identification of the taxonomic affinities of five Mexican endemic species, namely: *U. bicarinatus*, *A. nebulosus*, *P. parvulus*, *A. lineattissima* and *A. communis*. The specimen of *Gerrhonotus* from Chamela is very divergent by 16S rDNA and probably does not belong to the so far studied species of *Gerrhonotus*. High genetic divergence has been also observed between samples of *A. undulata* and *U. bicarinatus* from different regions. In these latter two cases, additional data are needed to understand the taxonomic status of these populations.

Key words: biodiversity hotspot, cytogenetic, dry forest, molecular systematic, NDH2, Reptilia, 16S rDNA

Resumen

El bosque tropical caducifolio contribuye con una proporción considerable a la diversidad herpetológica de México. La costa suroeste de Jalisco es una de las áreas más importantes por su elevado número de especies endémicas y la alta incidencia de especies en riesgo y de distribución geográfica restringida. En este trabajo utilizamos un enfoque cariológico y genético molecular (secuencias de genes ADNmt para NDH2, cyt*b* o 16S ADNr) para caracterizar genéticamente 13 especies de lagartijas pertenecientes a siete familias que habitan el bosque tropical caducifolio de la Reserva de la Biosfera Chamela-Cuixmala (Anguidae: *Gerrhonotus* cf. *liocephalus*; Eublepharidae: *Coleonyx elegans*; Phyllodactylidae: *Phyllodactylus lanei*; Gekkonidae: *Hemidactylus frenatus*; Phrynosomatidae: *Sceloporus melanorhinus*, *S. utiformis*, *Urosaurus bicarinatus*; Polychrotidae: *Norops nebulosus*; Scincidae: *Mabuya unimarginata*, *Plestiodon parvulus*; Teiidae: *Ameiva undulata*, *Aspidoscelis communis*, *A. lineattissima*). Aquí se describen por primera vez el cariotipo de seis especies (*G. liocephalus*, 2n = 38, 14 macrocromosomas y 24 microcromosomas; *C. elegans*, 2n = 24 FN = 26; *N. nebulosus* 2n = 30, 13 macro- y 17 microcromosomas; *M. unimarginata* 2n = 32, 18 macro- y 14 microcromosomas; *P. parvulus* 2n = 26, 12 macro- y 14 microcromosomas; *A. undulata* 2n = 50, 26 macro- y 24 microcromosomas). Se encontró heteromorfismo cromosómico en *C. elegans*, *N. nebulosus*, y *S. melanorhinus*. En *P. lanei* encontramos un cariotipo distinto al descrito en otras localidades. Esta variación es similar a la que generalmente se encuentra entre especies con divergencia genética alta. La tipificación del ADN de los genes del ADNmt permitió la identificación de las afinidades taxonómicas de cinco especies endémicas de México, que son: *U. bicarinatus*, *A. nebulosus*, *P. parvulus*, *A. lineattissima* y *A. communis*. El espécimen de *Gerrhonotus* de Chamela es muy divergente por 16S ADNr y probablemente no pertenece a las especies hasta ahora estudiadas de *Gerrhonotus*. También se observó alta divergencia genética entre las muestras de *A. undulata y U. bicarinatus* de diferentes regiones. Para estos dos últimos casos se requiere de datos adicionales para entender el estado taxonómico de estas poblaciones.

Introduction

Seasonally dry tropical forests contain approximately one third of the total endemic terrestrial vertebrate species in Mexico (Flores-Villela 1993a; Ceballos & Garcia 1995). This diversity can be explained principally by contribution of Neartic and Neotropical faunal elements, by geological and ecological isolations, vicariant speciation processes, and climatic changes during the Pleistocene (Flores-Villela 1993b; Ramamoorthy *et al.* 1993; Ceballos 1995; Ceballos & Garcia 1995; Marshall & Liebherr 2000). In Mexico, the dry forests encompass seven different ecoregions determined by the World Wildlife Fund (Olson *et al.* 2001), which fit together as a biogeographic unit: the Pacific Coast Biogeographic Province (CONABIO 1997).

Tropical dry forests contribute to a substantial proportion of the herpetological diversity of Mexico (Flores-Villela & Goyenechea 2003; Garcia 2006); they encompass 34% and 23% circa of the total of endemic species of reptiles and amphibians, respectively. In this context, a recent study of Garcia (2006), using ecological niche modelling, identified the south-western coast of Jalisco as one of the more important areas by number of endemics and the high presence of endangerment and restricted species. One of the main steps in the conservation of the biodiversity in this area has been the institution of the Chamela-Cuixmala Biosphere Reserve, where the tropical dry forest dominates on the hilly topography (Garcia 2003) (Fig. 1).

Basic information concerning the herpetofauna of this region is relatively well known since two field guides have been published (Garcia & Ceballos 1994; Ramírez-Bautista 1994). Many species are well known with respect to their ecology and distribution (e.g. Beck & Lowe 1991; Casas-Andreu & Gurrola-Hidalgo 1993; Ramírez-Bautista & Benabib 2001; Noguera *et al.* 2002; Ramírez-Bautista & Pardo-De La Rosa 2002; García 2008; García & Cabrera-Reyes 2008; García-Navarro *et al.* 2008). However, the current taxonomic information is mainly based on morphological data (see below in the results section), which is likely due to the scarcity of studies dealing with a genetic characterization of the species in this region.

Here we genetically studied 13 lizard species that inhabit the dry forests of Chamela-Cuixmala Biosphere Reserve using a combined karyologial and molecular approach. Saurians are one of the more diverse groups in term of karyotypic diversification among reptiles (Olmo 2005), which can be informative as taxonomic tool (e.g. Grismer 1999; Arribas *et al.* 2006; dos Santos *et al.* 2007). As molecular markers we use different fragments of mitochondrial genes. In conjunction with karyotypic data, these molecular markers are useful to help identify new putative cryptic species and/or new evolutionarily significant units (ESU) (Funk & Fa 2006). Moreover, genetic data can also be useful to access the “phylogenetic diversity”, a measure of biodiversity which incorporates taxonomic difference among species. This assessment type has an important role for both the understanding and the management of conservation priority areas (Faith 1992; Rodrigues & Gaston 2002).

Material and methods

A total of 13 species belonging to seven families were studied (Fig. 2). The specimens were collected by hand or with use of pitfall traps placed along transects in tropical dry forest in the Chamela-Cuixmala Biosphere Reserve (19º 22' 03" – 19º 35' 11" N and 104º 56' 13" – 105º 03' 25" W) (Fig. 1). Details concerning to the number of specimens for each species, distribution, endangerment level, and type of performed analysis are shown in Table 1.

For chromosomal analysis, specimens were injected with a 1:1000 solution of Velbe (Lilly) for one hour. The femurs, vertebral column and testes were removed, crushed and left in hypotonic solution (0.075 M KCl) for 40 minutes at environmental temperature (about 37° C). Cells were collected after centrifugation and were fixed with a methanol-acetic acid solution (3:1). Metaphase plates were prepared by air-drying method and slides were stained with Giemsa (pH = 7). Pictures of metaphases were collected using the Photometrics Sensys 1600 digital camera (Roper Scientific Photometrics, Tucson, AZ). For each species, we identified the diploid number (2n), the number of macro- and microchromosomes, and the morphology of macrochromosomes. In some species the morphology of largest microchromosomes was also possible to assess.

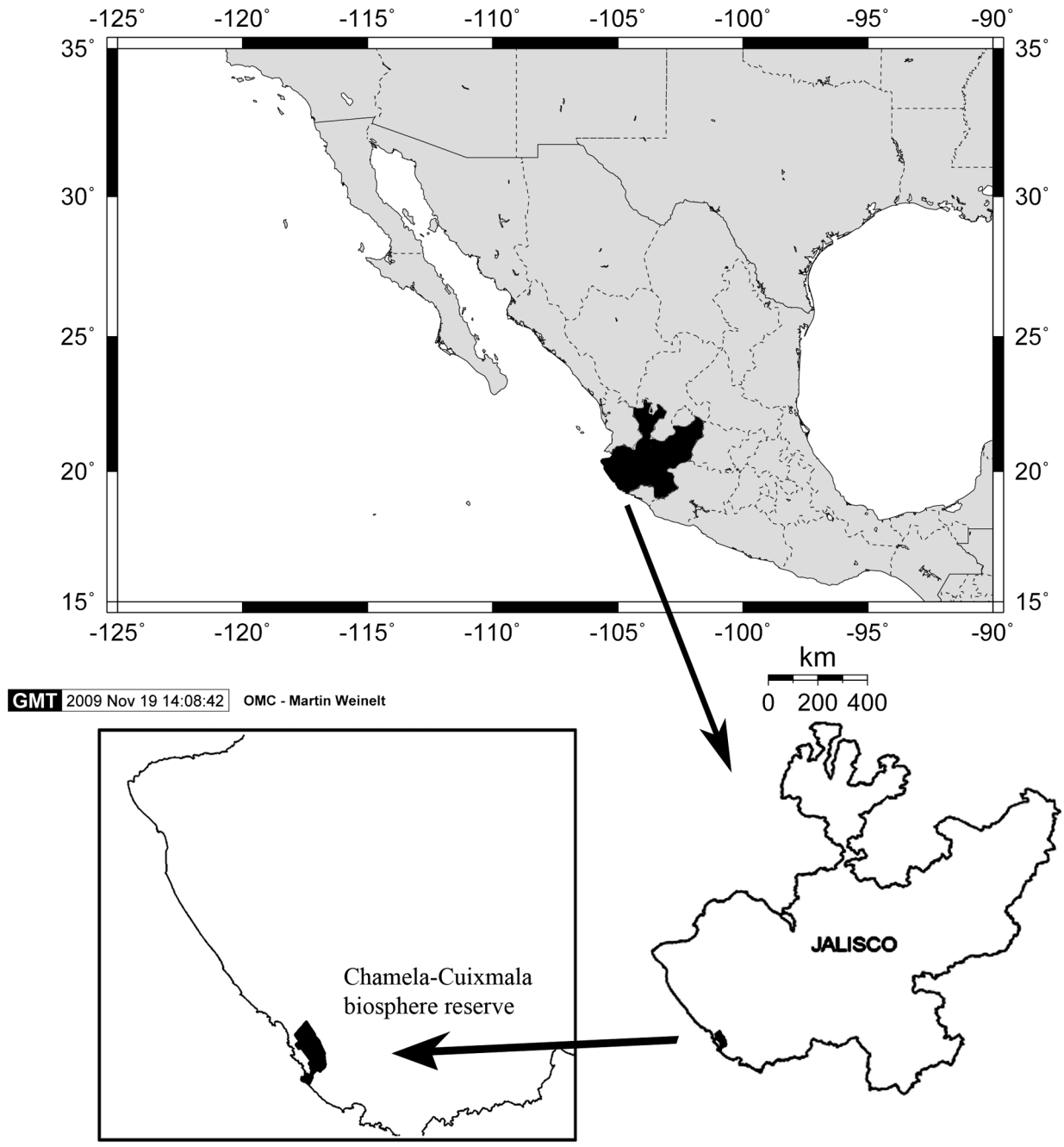

FIGURE 1. Map with the location of the Chamela-Cuixmala Biosphere Reserve.

For molecular typing, tissues were collected separately and preserved in 100% ethanol. For each species a fragment of the mtDNA was sequenced. The choice of the sequenced mitochondrial gene - NADH dehydrogenase 2 gene and flanking regions (NDH2), cytochrome b (cyt*b*) or 16S rDNA (16S)- has depended mainly on the possibility of comparison of the sequence with those of congeneric and/or conspecific specimens available in the GenBank. Almost all the species were typed for a fragment of 16S rDNA. This gene was proposed as standard DNA bar-coding marker for vertebrates (Vences *et al.* 2005).

DNA was extracted using the QIAmp tissue extraction kit (Qiagen). For 16S rRNA gene, sequences were obtained using the primers 16SA-L (light chain; 59-CGC CTG TTT ATC AAA AAC AT-39) and 16SB-H (heavy chain; 59-CCG GTC TGA ACT CAG ATC ACG T-39) (Palumbi *et al.* 1991). The PCR cycling procedure was performed as follows: 34 cycles of denaturation for 90 sec at 95° C, primer annealing for 60 sec at 50° C, and extension for 90 sec at 72° C. For the mtDNA gene NDH2 we used two pairs of primers:

L4160 ND1 5'-CGATTCCGATATGACCARCT-3' and H4980 ND2 5'-ATTTTTCGTAGTTGGGTTTGRTT-3'; L4437 tRNAMet 5'-AAGCTTTCGGGCCCATACC-3' and H5934a COI 5'-AGRGTGCCAATGTCTTTGTGRTT-3' designed by Macey *et al.* (1999). For cyt*b* we used the universal primers L14841 and H15149 (Kocher *et al.* 1989).

TABLE 1. Species studied, voucher specimens, GenBank accession numbers. Studied specimens belonging to localities other than the Chamela Cuixmala Biosphere Reserve are marked by an "*". K= karyotype; D = DNA-typing; und. = sex not determinated; End = endemic species to Mexico; PR = protected; T = threatned.

Species	End	Conservation status	Voucher specimens	16S	NDH2	cyt*b*
Sauria						
Anguidae						
Gerrhonotus cf. *liocephalus*		pr	CEAC7 male (K; D)		HM012704	
Eublepharidae						
Coleonyx elegans		t	CEAC8 female (K; D) UTA R 50283 und. (D)* UTA R 50286 und. (D)*	HM012686 HM012687 HM012688		
Gekkonidae						
Phyllodactylus lanei	X		CEAC3 male (D) CEAC4 female (D)	HM012689 HM012690		
Hemidactylus frenatus			CEAC9 female (K; D) CEAC10 male (K) CEAC11 male (K)	HM012691		
Phrynosomatidae						
Sceloporus utiformis	X		CEAC12 (K: D) CEAC13 (K) CEAC14 (K)	HM012692		
Sceloporus melanorhinus			CEAC15 male (D; K) CEAC16 male (K) CEAC17 female (K) CEAC18 female (K)	HM012693		
Urosaurus bicarinatus	X		CEAC19 male (D) AZR155 und. (D)* OFV445 und. (D)*	HM012694 HM012695 HM012696		
Polychrotidae						
Anolis nebulosus	X		CEAC20 male (K) CEAC21 male (K; D) CEAC22 female (K)		HM236483	
Scincidae						
Mabuya unimarginata			MZFC21804 female (K; D)			HM236484
Plestiodon parvulus	X		CEAC23 male (K; D) CEAC24 male (K)	HM012697		
Teiidae						
Ameiva undulata			CEAC25 female (K; D) ENS10011 und. (D)* UTA R 50344 und. (D)*	HM012698 HM012699 HM012700		
Aspidoscelis communis	X	pr	CEAC26 und. (D) CEAC30 und. (D)	HM012701 HM012702		
Aspidoscelis lineattissima	X	pr	CEAC27 und (D)	HM012703		

For a taxonomic evaluation of the DNA data, the obtained sequences were aligned with sequences belonging to the same species downloaded from GenBank to assess the level of intraspecific divergence. In general, a threshold value suggesting species distinction in reptiles was not studied. To evaluate threshold

values for the identification of candidate species among Neotropical frogs, Fouquet *et al.* (2007) proposed a pairwise genetic distance of 0.03 (3%) for 16S. In absence of comparable data for reptiles we compared the level of divergence with those found for sister species belonging to the same genus. Some species have never been studied before and therefore comparison with sequences in GenBank was impossible. In these cases we provided their phylogenetic position within their respective genus. Phylogenetic positions were evaluated with neighbour-joining (NJ), using the Kimura 2-parameter distances with MEGA 4 (Tamura *et al.* 2007), Maximum parsimony (MP) in PAUP 4.0b10 (Swofford 1998) and maximum likelihood (ML) with PHYML 3.0 (Guindon & Gascuel 2003). For ML the appropriate model of substitution was chosen using the Model Test 3.7 program (Posada & Crandall 1998). Models of evolution, which provide the best approximation of the data, were chosen for according to the Akaike information criterion (AIC). The robustness of the nodes was assessed by bootstrap with 1,000 replicates for NJ, MP and ML.

Higher taxonomic classification follows Benton (2005) while, for generic and specific levels, the current taxonomy is specified by each taxon section. Analyzed specimens are preserved in 70% ethanol and are housed in the herpetological collection of the Dipartimento di Biologia Animale e dell'Uomo, Università di Roma "La Sapienza" (CEAC). Voucher numbers are provided in Table 1.

Results and discussion

We obtained karyological preparations for a total of 11 species and molecular sequence data for all 13 analysed species. The account below describes the species of lizards studied, with comments on their distributions, karyotypes, systematics, and voucher specimens.

Order Squamata

Suborder Lacertilia

Family Anguidae

Gerrhonotus Wiegmann

The genus *Gerrhonotus* has a very problematic taxonomy, both at an intra- and interspecific levels. Good (1994) recognized three species, without subspecies, namely, *G. infernalis*, *G. liocephalus* and *G. ophiurus* (sometimes reported as subspecies of *G. liocephalus*). Recently *Elgaria parva* was included in a molecular phylogenetic analysis with other *Gerrhonotus* species, and it resulted in belonging to this genus (= *Gerrhonotus parvus*) (Conroy *et al.* 2005). The populations from Jalisco-Colima are reported as *G. liocephalus* (García & Ceballos 1994; Ramírez-Bautista 1994) but they are studied from two specimens only. Their morphological characters are intermediate among *G. liocephalus*, *G. infernalis* and *G. ophiurus* and therefore they remained of uncertain identity and referred to *G.* cf. *liocephalus* by Good (1994). Individuals possibly belonging to this taxon were also found in Colima, Durango and Sinaloa.

No species of this genus has been karyotyped. The karyotype is known only for three species of *Elgaria* and one species of *Mesaspis*, which also belongs to the subfamily Gerrhonotinae (Bury *et al.* 1969). These species show inter and intraspecific chromosomal variability. *Elgaria coerulea* has 2n = 38 (12 macro- and 26 microchromosomes); *Elgaria multicarinata* has 2n = 47–48 (21–22 macro- and 26 microchromosomes); *Elgaria paucicarinata* has 2n = 46 (20 macro- and 26 microchromosomes); *Mesaspis monticola* has 2n = 30 (18 macro- and 12 microchromosomes).

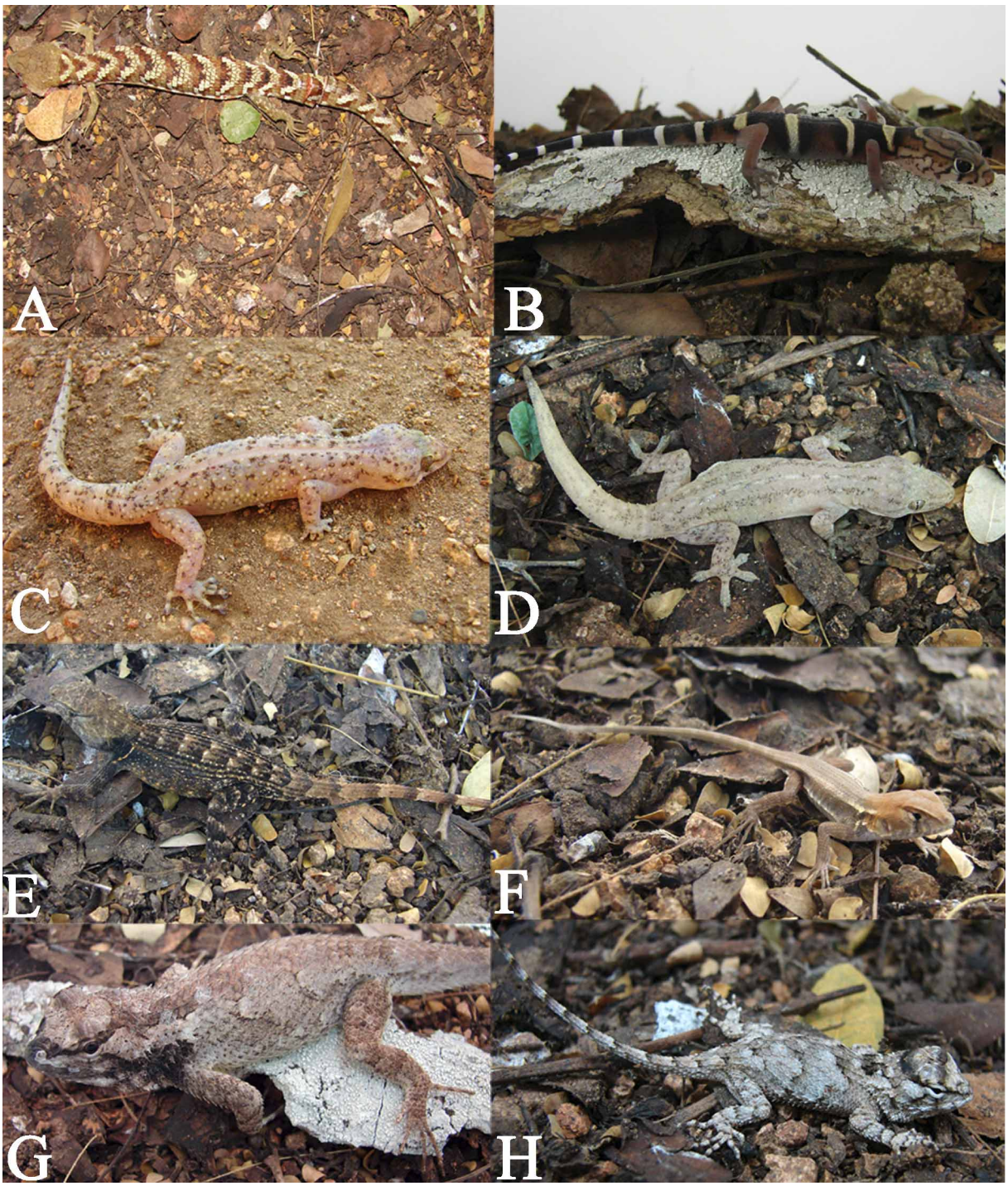

FIGURE 2. Photos of studied species from the study area. A, *Gerrhonotus* cf. *liocephalus*; B, *Coleonyx elegans*; C, *Phyllodactylus lanei*; D, *Hemidactylus frenatus*; E, *Sceloporus utiformis*; F, *Sceloporus utiformis* (young); G, *Sceloporus melanorhinus*; H, *Sceloporus melanorhinus* (young).

FIGURE 2 (continued) . I–J, *Anolis nebulosus*; K, *Mabuya unimarginata*; L. *Plestiodon parvulus*; M, *Ameiva undulata*; N, *Aspidoscelis communis* (young); O, *Aspidoscelis lineattissima* (young).

***Gerrhonotus* cf. *liocephalus* Wiegmann (Texas alligator lizard)**

Specimens analysed: one male (CEAC7).

Distribution: uncertain limits. Maybe limited to Jalisco and Colima.
Subspecies: Good (1994) did not recognize subspecies.

Good (1994) studied only two specimens belonging to coastal Jalisco. Here we report a morphological description of the male specimen that we studied. In particular, we report the characters that are significant for the morphological diagnosis of *Gerrhonotus* species following Good (1994). Canthal/loreal series: 3 canthals, 3 loreals; supralabial number: 28; preocular number: 1; number of transverse dorsal scales rows: 48; number of longitudinal dorsal scales: 16; number of dorsal crossbands: 9; ventral pattern: mottled; lateral fold bars: present; limb length: not measured; tail whorl number: tail incomplete. The pattern of coloration shows 9 evident "V" shaped cross-bands. Each of these bands has a width of 2–3 white scales, flanked by darker scales. The ventral pattern is immaculate. The morphological characters of this specimen collected by us are similar to the other three specimens from Jalisco and Colima reported by Good (1994).

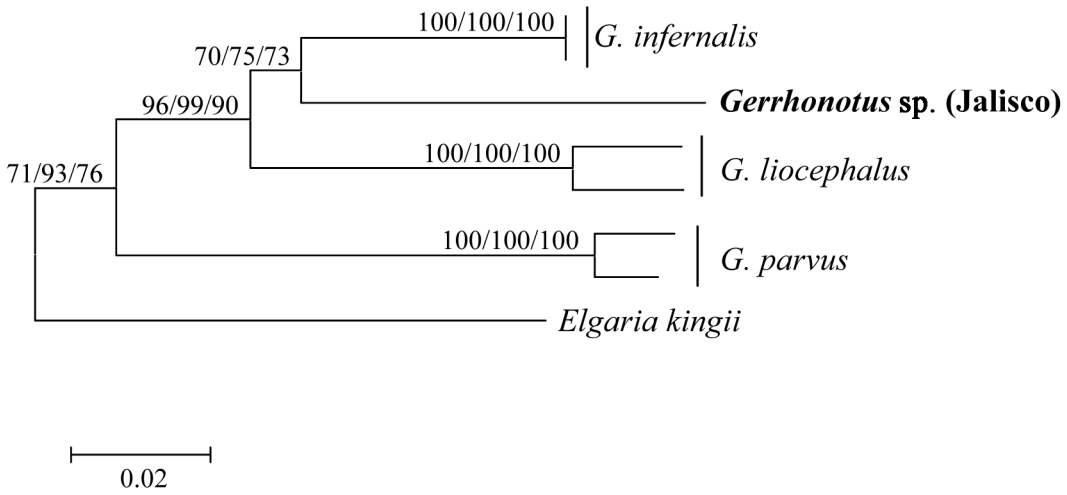

FIGURE 3. Neighbour-Joining tree of 16S rDNA haplotypes (511 bp) of *Gerrhonotus* species. Bootstrap values (%) obtained by the NJ, ML and MP are shown. The substitution model selected for ML was the Hasegawa, Kishino, Yano (HKY) model (Hasegawa *et al.* 1985) with rate variation among sites (+G), and a gamma distribution shape parameter of 0.2997.

Karyotype: *Gerrhonotus* cf. *liocephalus* showed 2n = 38 composed by 14 macrochromosomes and 24 microcromosomes (not shown). All macrochromosomes seem biarmed but, for the smallest ones, some doubt exists on their morphology. The karyotype of this species shares with *E. coerulea* the same diploid number but it has only 12 machrochromosomes.

DNA taxonomy: the phylogenetic position of species of *Gerrhonotus* was recently addressed by Conroy *et al.* (2005). A fragment of the NADH dehydrogenase 2 gene and flanking regions (511bp) was sequenced and aligned with published sequences of *G. liocephalus*, *G. infernalis* and *G. parvus*. The sequences of the specimen analysed here clustered with the two sequences belonging to *G. infernalis* (bootstrap values 70–75%) (Fig. 3). However, the sequence divergence with this species is high (9.6%). A similar divergence was found between *G. liocephalus* and *G. infernalis* (10%). These findings, together with the distinct morphological characteristics of the specimens in the area of Chamela (present work and Good 1994), support its identity as a taxon different from the two mentioned above (Nieto Montes de Oca, unpublished).

Family Eublepharidae

Coleonyx Gray

The genus *Coleonyx* includes seven species of terrestrial geckos commonly referred to as banded geckos. These species are found throughout the south-western United States of America and northern Mexico south into Central America to Costa Rica (Klauber 1945).

Phylogenetic relationships for four species within the genus were assessed by Jonniaux and Kumazawa (2008). Among Eublepharidae the karyotype is known for 4 species only: *C. switaki* (2n = 24; FN = 26) (Murphy 1974), *C. variegatus* (2n = 32; FN = 32) (Matthey 1933; Porter *et al.* 1994), *Eublepharis macularius* (2n = 38; FN = 38) (Gorman 1973), and *Goniurosaurus kuroiwae* (2n = 24) (Ota *et al.* 1987).

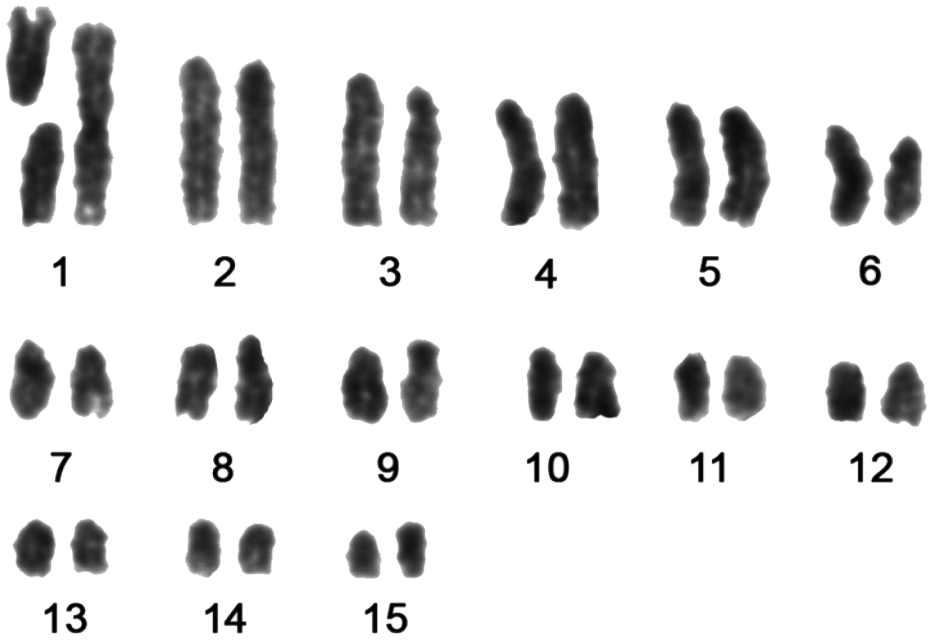

FIGURE 4. Karyotype of *Coleonyx elegans*, female (2n = 31 and FN = 32). Note the single large metacentric (no. 1) that it is tentatively paired with two medium sized acrocentric chromosomes.

***Coleonyx elegans* Gray (Yucatan Banded Gecko)**

Specimens analysed: one female (CEAC8), two specimens from Petén, Guatemala (UTA R 50283, UTA R 50286).

Distribution: Mexico, Belize, Guatemala, and El Salvador.

Subspecies: *C. e. elegans* Gray distributed from central Veracruz, Mexico to northern Guatemala and Belize and on the Pacific coast from eastern Chiapas, Guatemala to western El Salvador; *C. e. nemoralis* Klauber is localized along the Pacific coast of Mexico from Nayarit to southeast Oaxaca.

Following Klauber (1945), the diagnostic characters distinguishing the two subspecies of the Yucatan Banded Gecko are a non-triangular mental and the upper prenasals in contact in *C. elegans elegans*; the mental is usually triangular, the prenasals are usually not in contact, and there are fewer tubercular scales laterally in *C. e. nemoralis*. The studied specimen from Chamela is within the range of *C. e. nemoralis*, however, it represents intermediate morphological characters since the mental is clearly not triangular and since the upper prenasals are not in contact.

Karyotype: this is the first description of the karyotype for this species. It shows 2n = 31 and FN = 32 (Fig. 4). The karyotype is composed of one single unpaired metacentric (the largest chromosome) and 30 acrocentric chromosomes. The metacentric chromosome clearly represents a Robertsonian fusion of two acrocentric chromosomes. Among the species studied, this karyotype is most similar to that described for *C. variegatus*, with a 2n=32 all-acrocentric karyotype, but differs considerably from *C. switaki* (2n = 24, FN = 26). Therefore, it is the first instance of chromosomal heteromorphism reported for Eublepharidae. Few cases of heteromorphism due to Robertsonian fusion or fission have been reported in Gekkonidae, e.g., in *Gehyra australis* and *G. variegata* (King 1984), in *Gekko chinensis* Lau *et al.* (1997), in *Phyllodactylus lanei* (see

below) and in *Christinus marmoratus* (King & Rofe 1976). Clearly, additional data will be necessary to understand if this chromosomal heteromorphism represents a sex chromosome system, hybridization between chromosomal cytotypes or an intra-population autosomal polymorphism.

DNA taxonomy: only one rDNA 16S sequence from *C. elegans* is present in GenBank (Jonniaux & Kumazawa 2008) but the studied specimen belonged to a pet-shop (Yoshi Kumazawa, pers. comm.). For this reason we include two specimens from Petén (Guatemala) belonging to the other subspecies, *C. e. elegans*. The sequence of the specimen from Chamela differs by 4.5% with respect to the other haplotypes that are, conversely, very similar. This level of divergence is high but lower relative to that found between different species (*C. variegatus* vs *C. brevis*, 9.8%; *C. mitratus* vs *C. elegans*, 14–15.2%). In absence of additional data these results are in agreement with a subspecific status of the populations from Jalisco and Guatemala.

Family Phyllodactylidae

Phyllodactylus Gray

The genus *Phyllodactylus* was formerly included in a diverse group of leaf-toed geckos occurring all-over the world. Currently and on the basis of morphological and allozyme phylogenetic analyses, several lineages of Old World leaf-toed geckos are proposed as distinct genera, such as *Afrogecko* (southern Africa), *Christinus* (Australia), *Cryptactites* (southern Africa), *Goggia* (southern Africa), *Dixonius* (southeast Asia), *Euleptes* (Mediterranean), *Haemodracon* (Sokotra), and *Matoatoa* (Madagascar) (Bauer *et al.* 1997; Gamble *et al.* 2008). The species within the genus *Phyllodactylus sensu stricto* are now constrained to the New World. Nonetheless, although there are more than 50 species in the genus, molecular genetic and karyological data are very scant, with rDNA 16S sequences reported in less than ten species (Weiss & Hedges 2007; Blair *et al.* 2009).

Phyllodactylus lanei Smith (Lane's Leaf-toed Gecko)

Specimens analyzed: one male (CEAC3), one female (CEAC4).

Distribution: a Mexican endemic, with records from Nayarit, Guerrero, Jalisco, and Michoacán, and possibly Colima.

Subspecies: *P. l. lanei*: Guerrero; *P. l. rupinus*: Nayarit, coastal Jalisco, southern Michoacán; and two insular Subspecies: *P. l. lupitae* and *P. l. isabelae* (Castro-Franco & Uribe-Pena 1992).

Karyotype: karyological data in *P. lanei* were restricted to a report that described karyotypes of specimens from the state of Guerrero, that probably belong to *P. l. lanei*, 2n = 33–34 and FN = 40–41 (King 1981). The karyotype of specimens from Chamela region belonging to *P. l. rupinus* has been recently described (Castiglia *et al.* 2009). It shows 2n = 38 and FN = 38, composed of 19 pairs of acrocentric chromosomes. Thus the karyptypes belonging to the two subspecies differ by the presence of two pairs of large metacentric chromosomes in *P. l. lanei* that are absent in *P. l. rupinus*. The slight difference in the fundamental number found in the two samples is probably due to a different interpretation of the very small short arms (see Castiglia *et al.* 2009 for details). Moreover, in the karyotype from Guerrero, a pair of heteromorphic chromosomes was also observed. In females, one of the homologues of this pair was described as bi-armed (with tiny short arms) and this was considered, by the author, a possible ZW sex chromosome system. However, in the studied individuals from Chamela, no chromosome pairs showed a visible heteromorphic condition (Castiglia *et al.* 2009).

DNA taxonomy: a single sequence (rDNA 16S) of *P. lanei* from Guerrero is available in GeneBank (Blair *et al.* 2009). This sequence possibly belongs to *P. l. lanei*. The genetic divergence between the haplotypes from Chamela and those from Guerrero is relatively high, (8.4–8.7%; 449 bp). This divergence is similar to that found among three insular subspecies belong to *P. wirshingi*, which are considered full species by Weiss and Hedges (2007). Because of the high chromosomal and genetic differences found between the specimens

from Guerrero and Jalisco, is plausible the elevation of *P. l. rupinus* to a specific rank. However, molecular analysis from the type locality of *P. l. rupinus* (Lombardia, Michoacan, Mexico) are needed before any definitive taxonomic change can be made.

Family Gekkonidae

***Hemidactylus* Gray**

Hemidactylus, with at least 85 recognized species, is the second most specious genus of geckonid lizards. This genus is widely distributed throughout much of the Old World tropics and subtropics as well as in the Mediterranean region and in the American continents. Phylogenetic relationships within the genus have been addressed by Carranza and Arnold (2006). The ancestral lineage of the genus may have originated in Asia, which later spread to the Arabian-African region. Many species are associated with humans and are subject to passive transport as is the case with *H. brookii* (*sensu lato*), *H. mabouia*, *H. turcicus*, *H. garnotii*, and *H. frenatus*, which colonized the Mediterranean region, tropical Africa, much of the Americas and hundreds of islands in the Pacific, Indian, and Atlantic oceans.

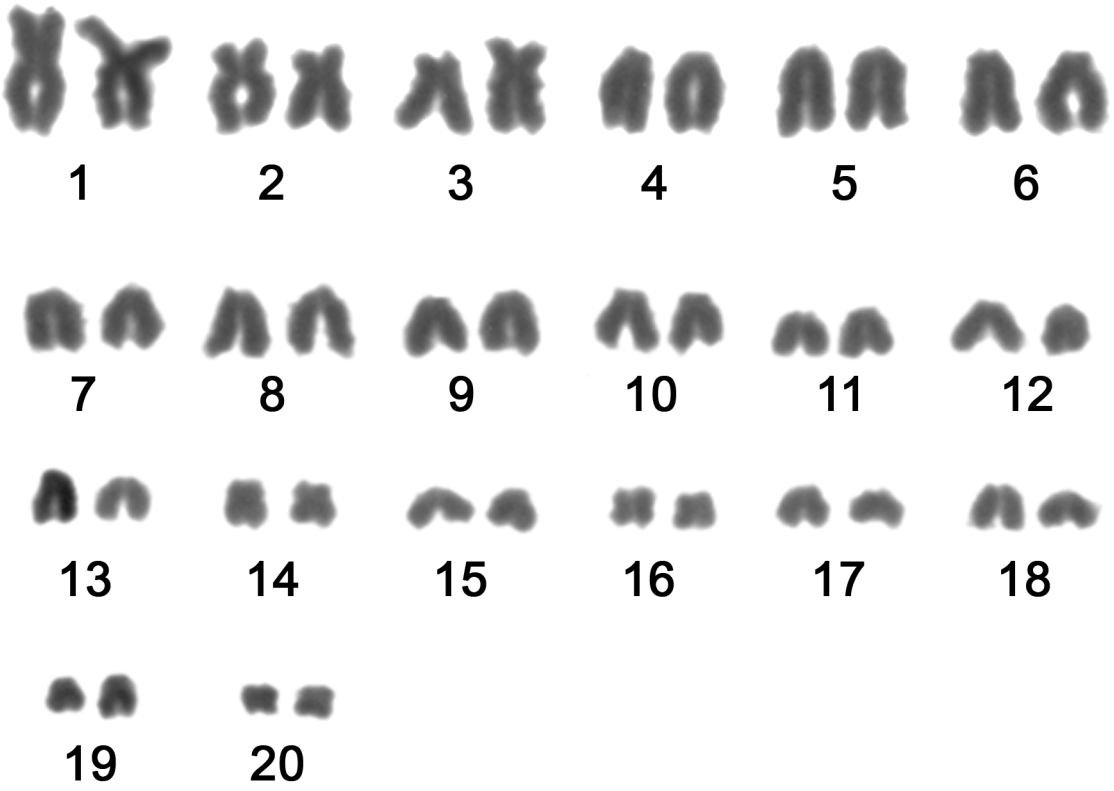

FIGURE 5. Karyotype of *Hemidactylus frenatus*, female (2n = 40 and FN = 54).

***Hemidactylus frenatus* Schlegel (Common house gecko)**

Specimens analysed: two males (CEAC10, CEAC11), one female (CEAC9).

Distribution: worldwide in tropical and subtropical regions. The species has been introduced into Mexico, where its presence was first reported in 1940 by Taylor and then by Burt and Myers (1942).

Subspecies: Not described. However the species is chromosomally polytypic (see below).

Karyotype: the chromosomal complement of this species is variable with 2n = 40 and 2n = 46. However the karyotype with 2n = 46 clearly belongs to *H. bowringii* (see Kupriyanova and Darevski 1989). Sporadic presence of triploid populations with 3n = 60 has been found in Vietnam (Darevsky *et al.* 1984). The specimens from Chamela conform to the most common karyotype with 2n = 40 (Fig. 5). This karyotype is composed of seven pairs of biarmed chromosomes (three large pairs and four pairs of small chromomomes). The remaining chromosomes are telocentrics. This is the first description of the karyotype of this species in the New World.

DNA taxonomy: the rDNA 16S has been studied in specimens from Madagascar by Vences *et al.* (2004) and in one single specimen from Papua New Guinea (Whiting *et al.* 2003). The sequence comparison shows that the specimen studied here is almost identical to the one from Oceania (sequence divergence: 0.2%) but differs more from those of Madagascar (sequence divergence: 0.8–3.1%). Oceania is believed to represent the centre of origin of the species from which it spreads worldwide due to human movements. The close relationships among the two haplotypes agree with a recent arrival of the species in Mexico. In fact, *H. frenatus* was probably introduced during the Spaniard dominium of Mexico. The importation likely dates to the time when Spanish galleons carried trade goods between Acapulco and the Philippines (Taylor 1940).

Family Phrynosomatidae

Sceloporus Wiegmann

The genus *Sceloporus* includes about 80 species of spiny lizards distributed from southern Canada south to Panama (Sites *et al.* 1992). In many areas, *Sceloporus* represents an abundant and conspicuous genus of terrestrial vertebrates. For this reason it has often been subject of researches in many field of biology. Recent phylogenies of the genus based on morphology, karyotypes, nuclear and mitochondrial DNA (Flores-Villela *et al.* 2000; Wiens and Reeder 1997) revealed the existence of different species groups and the inclusion of the genus "*Sator*" within *Sceloporus*. The karyotype of the genus is highly variable, with the diploid number ranging from 22 to 40 and the presence of sex chromosomes systems (with XY or X_1X_2Y males) (data from the ''chromorep'' database available at site http://www.scienze.univpm.it/professori/chromorep.pdf.).

Sceloporus melanorhinus Bocourt (Pastel Tree Lizard)

Specimens analysed: two females (CEAC18, CEAC17), one male (CEAC15).

Distribution: Pacific coast of Mexico, from Jalisco to central depression of Chiapas, and adjacent Guatemala.

Subspecies: *S. m. melanorhinus*, Pacific coast of Oaxaca; *S. m. calligaster*, from Nayarit, to Guerrero; *S. m. stuarti*, central depression of Chiapas, and adjacent Guatemala.

Karyotype and DNA taxonomy: intraspecific variation in karyotype has been reported in this species (Cole 1970; Hall 1973; 2009). Males have 2n = 39 (20 macrochromosomes, 19 microcromosomes) while females 2n = 40 (20 macrochromosomes, 20 microchromosomes). The odd chromosomal number in males is due to presence of a medium sized metacentric Y chromosomes probably generated by the centric fusion of one autosomal acrocentric and a true Y microchromosome. In fact, males show the presence of a trivalent formation in males diakinesis corresponding to an X_1X_2Y (Hall 1973; 2009).

Moreover, another chromosomal polymorphism was noted since the species is polymorphic for an enlarged microchromosome (Em). Of seven *S. melanorhinus* karyotyped by Cole (1970), two of three individuals from one locality near Acapulco (Guerrero) were heterozygous for the Em, while the third individual from that locality and the remaining four from Tuxtla Gutierrez (Chiapas) and in a female near Colima lacked it. Of the six *S. melanorhinus* karyotyped by Hall (1973), only one from Rio Maria Basio, western Manzanillo (Colima), was heterozygous Em; while all of the remaining specimens, representing a second locality near Manzanillo and two localities near San Bias (Nayarit), lacked the Em chromosome.

This chromosomal variation due to Em chromosome does not match with subspecies designation, because different karyotypes have been found even in the same population.

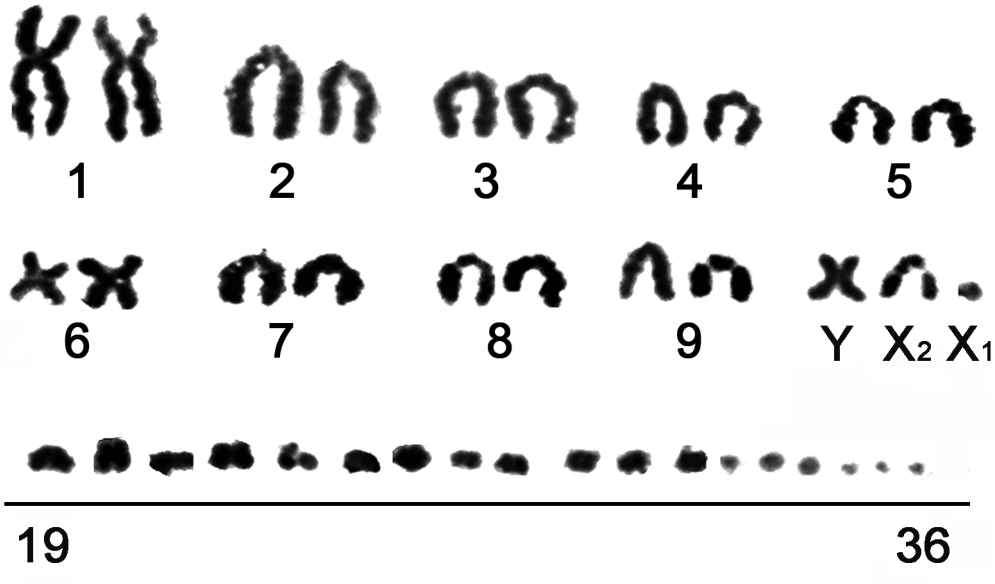

FIGURE 6. Karyotypes of *Sceloporus melanorhinus*; specimen CEAC15 male (2n = 39). Sex chromosomes are tentatively identified following Hall (1973, 2009).

The three specimens studied here shown two different karyotypes. The two females shows a karyotype with 20 macro- and 20 microchromosomes (not shown). In the male (CEAC15 - Fig. 6), the karyotype shows the additional medium-sized unpaired and biarmed chromosome identified by Hall (2009) as the Y chromosome. The enlarged microchromosome (Em) is lacking in the specimens here analyzed. The rDNA 16S has been studied for a single specimens from Guerrero, S of Chilpancingo (Wiens & Reeder 1997). The divergence between the specimen from Chamela and that from Guerrero is 3%, a value found commonly among populations of the same species in reptiles.

***Sceloporus utiformis* Cope (Cope's largescale spiny lizard)**

Specimens analysed: three males (CEAC 12, CEAC13, CAEC14)

Distribution: Mexican endemic. It is distributed along the Pacific slope from southern Sinaloa to western Guerrero.

Subspecies: no subspecies have been described.

Karyotype and DNA taxonomy: the karyotype for this species has been described from two specimens, one male and one female, both from Jalisco in a locality (northwest of Puerto Los Mazos) about 70 Km from Chamela (Cole 1971). The diploid number was 2n = 34 and the male carried a heteromorphic pair of microchromomes that were not present in the female. This polymorphism has been interpreted as a XY sex chromosomal system (Cole 1971). The specimens analysed in this study show a karyotype identical to the one previously reported (Fig. 7). It is composed of 12 biarmed chromosomes and 22 microchromosomes. In these male individuals one of the microchromosomes is very small. Therefore we confirm the presence of a XY sex chromosome system in this species.

The rDNA 16S has been studied for a single specimen from Jalisco (Boca de Iguanas), which is near the locality for specimens in the present study (Wiens & Reeder 1997; Flores-Villela *et al.* 2000). The from Chamela differ by 3% from the previous studied sample, which is a low value of divergence consistent with an intraspecific divergence.

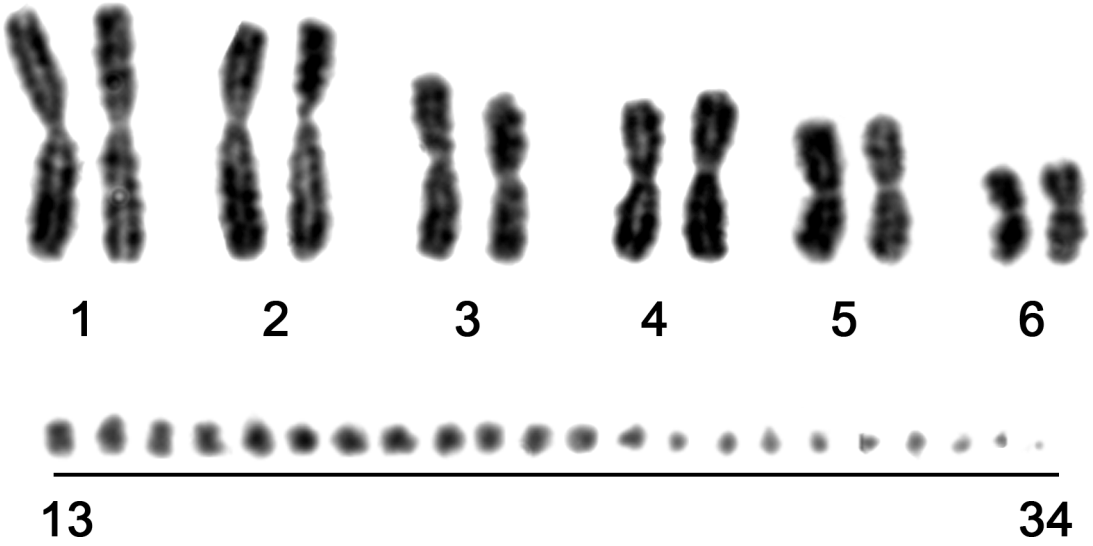

FIGURE 7. Karyotype of *Sceloporus utiformis*, male (2n = 34). The smaller microchromosome represents the Y chromosome. The X chromosome is another unidentified microchromosome.

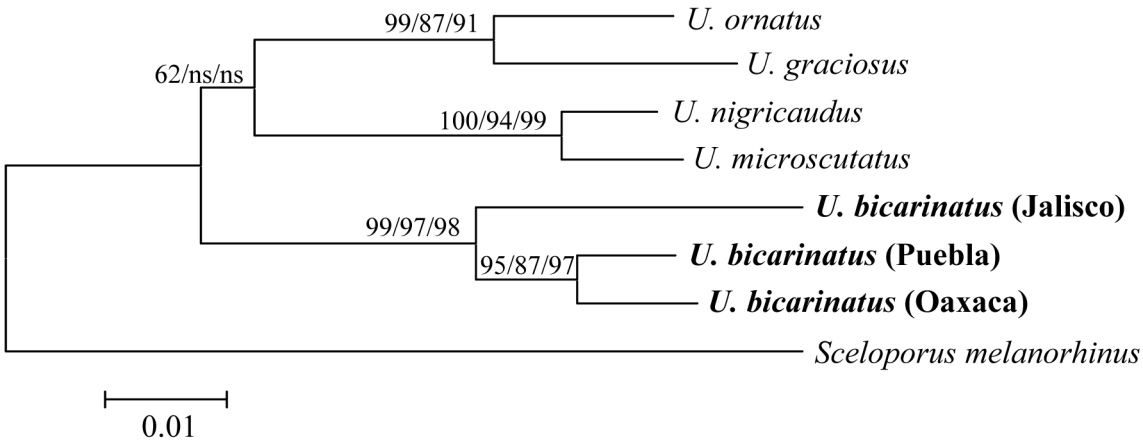

FIGURE 8. Neighbour-Joining tree of 16S rDNA haplotypes (455 bp) of *Urosaurus* species. Bootstrap values (%) obtained by the NJ, ML and MP are shown. The substitution model selected for ML was Tamura-Nei model (Tamura & Nei 1993) with rate variation among sites (+G), and a gamma distribution shape parameter of 0.0855.

Genus *Urosaurus* Hallowell

The genus includes nine species distributed in the southwestern United States of America and western Mexico, from southern Wyoming to northern Mexico along the border of USA and Mexico, and on the Pacific coast from Sonora, south entering the Balsas Basin to eastern Chiapas. Phylogenetic relationships between species have been assessed only on morphological grounds and with a single allozyme character by Wiens (1993). Only three species have been karyotyped: *U. graciosus* (Hall 1965; Gorman 1973), *U. nigricaudus* (Gorman *et al.* 1969), and *U. ornatus* (Porter *et al.* 1994). All these species have a similar karyotype, 2n = 34, with 12 macrochromosomes of metacentric morphology and 22 microchromosomes.

***Urosaurus bicarinatus* Duméril (Tropical tree lizard)**

Specimens analysed: one male from Chamela (CEAC19), one specimen from Rio Grande, Oaxaca (MZFC 12046), one specimen from Epatlan, Puebla (MZFC 6863).

Distribution: Mexican endemic. Pacific coast of Mexico, from Sonora to Chiapas.

Subspecies: *U. b. bicarinatus*, distributed from Michoacán to central Guerrero, and in the Río Balsas basin up to Morelos and southern Puebla; *U. b. anonymorphus*, found in east Guerrero, Oaxaca, and possibly western Chiapas; *U. b. nelsoni*, localized in northern Oaxaca; *U. b. tuberculatus*, distributed in Southern Sonora southward to Jalisco and Colima with isolated populations in Sinaloa; *U. b. spinosus*, from southwestern Chiapas. However, Wiens (1993) did not find morphological differences among the subspecies.

Karyotype: Unfortunately, we did not obtained good metaphases from this species.

DNA taxonomy: There is no sequence deposited in GenBank for this species. The available rDNA 16S sequences in GenBank are for *U. ornatus*, *U. nigricaudus*, *U. microscutatus*, and *U. graciosus* (Reeder 1995). We aligned these sequences with the sequence of *U. bicarinatus* from Chamela belonging to *U. b. tuberculatus* and with sequences from two additional individuals (Rio Grande, Oaxaca and Epatlan, Puebla) possibly belonging to *U. b. nelsoni* and performed a phylogenetic analysis using *Sceloporus utiformis* as the outgroup. The obtained tree is shown in Figure 8. Interestingly, the phylogenetic relationships among species are different from those identified using morphological characters by Wiens (1993) and are congruent with Reeder's (1995) results. Molecular analysis shows that *U. bicarinatus* has an external position with respect to the other species, which form a monophyletic group (supported only by NJ, 62%). Moreover in our tree *U. ornatus* is clearly the sister species of *U. graciosus* (supported by 87–99%). Conversely, phylogenetic relationships based on morphological characters show that *U. graciosus* was external to *U. bicarinatus*, *U. nigricaudus*, *U. ornatus* and *U. microscutatus* (Wiens 1993). The topology obtained with molecular data is congruent with the distribution of the species. *U. bicarinatus* is nested in the southern part of the range of the genus while the other species, which cluster together in the tree, are localized in the northern part.

The highest interspecific distance has been found between *U. bicarinatus* and the other species (8.4–9.4%), while lower values have been found between the other species (3.5–7.7%). A low divergence value (1.8%) was found between sequences of the Rio Grande (Oaxaca) and Epatlan (Puebla) populations of *U. bicarinatus*. Greater distance was found between these two localities and the sequences from Chamela (4.3–4.5%) belonging to a different subspecies. In the absence of additional data, it is very difficult to infer a conclusion regarding the taxonomic status of the Chamela population. These findings suggest that a complete intra and interspecific revision of the genus is needed using additional molecular markers.

Family Polychrotidae

***Anolis* (Daudin)**

Anolis (*sensu lato*) is the most specious genus among the reptiles, with circa 370 recognized species (Poe 2004). Within the genus two major groups of species called "alpha" and "beta" have been recognized (the latter composed of the subgenus *Norops*). Moreover, subgroups of species have also been defined within "alpha" and "beta" *Anolis* (Nicholson 2002). However, only few of these subgroups were supported by molecular analyses and many revealed ambiguous monophyletic status. For this reason, a well supported alternative classification is needed. A global phylogenetic analysis was assessed by Nicholson *et al.* (2005) in a molecular phylogenetic study including 189 species. Three geographically circumscribed clades were revealed [Cuba (Jamaica, and Mainland)]. The tree topology suggests a West Indian origin for mainland *Norops*. The typical karyotype of "beta" *Anolis* (*Norops*) consists of 14 macro- and 16 microchromosomes without obvious sex chromosome heteromorphism. Another frequently observed chromosome complement is 2n = 40 with 24 macro- and 16 microchromosomes. Presence of sex chromosomes has been reported in "alpha" as well as in "beta" *Anolis*. Among "beta" *Anolis* a XY system has been reported in *A. onca* (2n = 30) (Gorman 1969) and systems with two Xs and one Y (XXXX-XXY) have been reported in *A. biporcatus* and

A. sagrei (both with 2n = 29 for males and 2n = 30 for females) (Gorman & Atkins 1966, 1968; De Smet 1981).

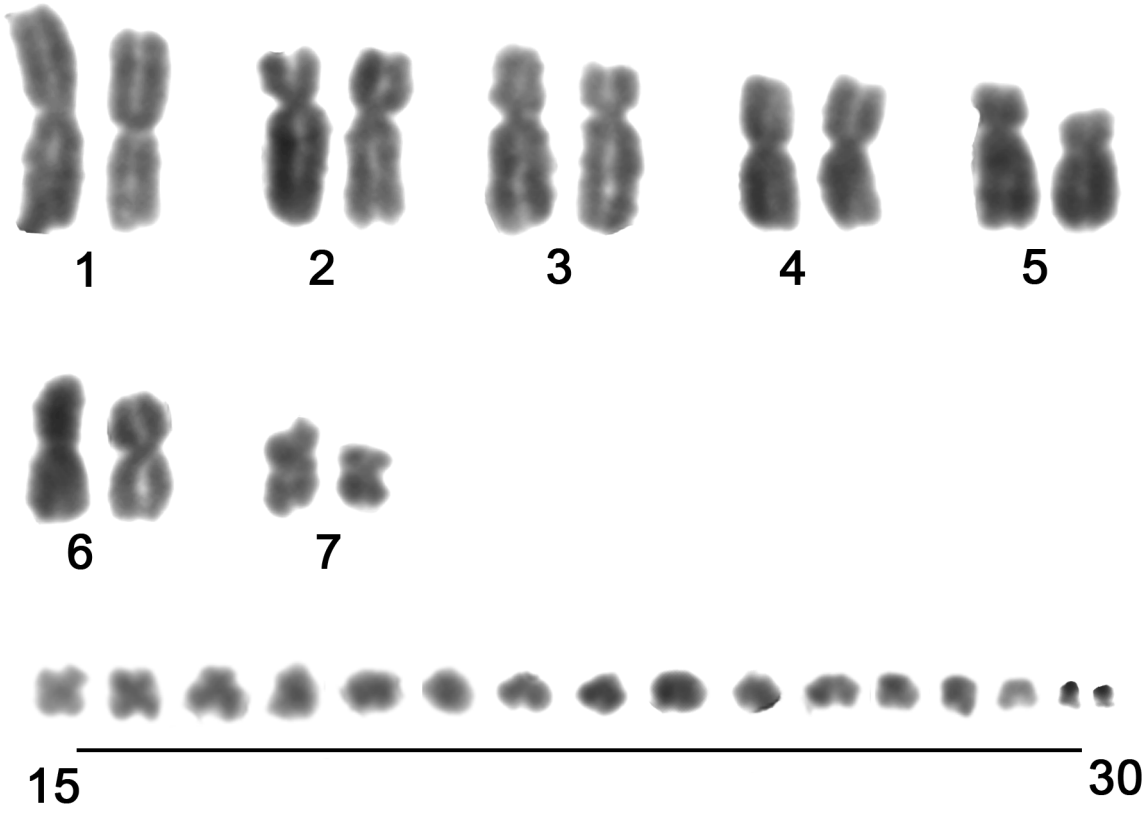

FIGURE 9. Karyotype of *Norops nebulosus*, male (2n=30). Note the three pairs of heteromorphic chromosomes (pairs 5, 6 and 7).

***Anolis* (*Norops*) *nebulosus* Wiegmann (Clouded anole)**

Specimens analysed: two males (CEAC20, CEAC21)

Distribution: Mexican endemic. Occurring from southern Sonora and northern Sinaloa, to western Guerrero, entering the Balsas Basin up to the southern State of Mexico.

Subspecies: not recognized.

The karyotype of *A. nebulosus* was briefly described by Gorman (1973) from an individual male that shows 2n = 30, with 13 macro- and 17 microchromosomes, and this karyotype has been reported as a possible case of X-Y heteromophism. However, Gorman (1973) did not show the karyotype. Lieb (1981) in his unpublished dissertation reported two different karyotypes for this species. Males from Sonora showed a karyotype with 2n = 36 chromosomes, 20 macro-chromosomes and 8 pairs of micro-chromosomes, including a pair of heteromorphic chromosomes. Males from Nayarit, Colima, Jalisco and Michoacán showed 2n = 30 chromosomes, of which 14 were macro-chromosomes, and the rest micro-chromosomes. A single pair of heterochromosomes was interpreted as XY sex chromosomes. Here we show for the first time the male karyotype of this species (Fig. 9). Diploid number is 2n = 30 with 14 macro- and 16 microchromosomes.

All the macrochromosomes are biarmed, metacentric or submetacentric, as well as the first two pairs of microchromosomes. Among the macrochromosomes, three pairs of heteromorphic chromosomes have been identified (tentatively pair numbers 5, 6 and 7, Fig. 9). These chromosomes differ in size and centromere position.

The karyotype described here is probably identical to the one described by Lieb (1981). However, we identified six unpaired chromosomes (rather than one). This is congruent with the complex system involving

multiple sex chromosomes already described in other species of the genus (data from the ''chromorep'' database: http://www.scienze.univpm.it/professori/chromorep.pdf). Additional data on male and female individuals from this species are required to understand the significance of this bizarre karyotype.

DNA taxonomy: neither gene sequence for this species is present in GenBank. We used the NDH2 gene and flanking tRNAs (596 bp) to assess its phylogenetic affinity. This sequence was aligned with all the other species of *Norops* present in GenBank (about 160 species). For ML the selected model was the Hasegawa, Kishino, Yano (HKY) model (Hasegawa *et al.* 1985) with a proportion of invariable sites I = 0.2664, rate variation among sites (+G), and a gamma distribution shape parameter of 0.7310.

The phylogenetic position of the species was not well supported probably due to the short sequence analysed (not shown). A relationship between *N. nebulosus* with *N. quercorum* and *N. nebuloides*, two other Mexican endemics, was supported with low bootstrap (50%) only by ML tree. These are the first data reporting the relationships of *N. nebulosus* with *N. quercorum* and *N. nebuloides.* In fact only *N. quercorum* was included in the same morphological species group with *N. nebulosus* while *N. nebuloides* belongs to a different group recognized on the basis of morphological characters (Etheridge 1960; Lieb 1981; Nicholson 2002).

Family Scincidae

Plestiodon Duméril and Bibron

The genus *Eumeces* has been recently split into four genera, namely *Pariocela*, *Eumeces*, *Eurylepis*, and *Mesoscincus* (Schmitz *et al.* 2004). Because of priority reasons, the name *Plestiodon* has been adopted instead of *Pariocela* for those American species formerly referred to as *Eumeces*, except for those placed in *Mesoscincus* (Smith 2005). The differences among the groups were based in part on analyses of chromosomes numbers. A large number of studies showed that all species of the American *Plestiodon* have 2n = 26 chromosomes (Deweese & Wright 1970; Wu 1983; Capriglione 1987; Guo & Dong, 1988; Kato *et al.* 1998), while all the African species of the genus *Eumeces* are unique in having a constant 2n = 32 chromosomes (Gorman 1973; Kupriyanova 1973; De Smet 1981; Kupriyanova 1986; Eremchenko *et al.* 1992; Caputo *et al.* 1993, 1994; Hassan 1996). The *Eurylepis taeniolatus* group can be also differentiated from other groups by uniquely having 2n = 28 chromosomes (Ivanov & Bogdanov 1975; Kupriyanova 1986; Eremchenko *et al.* 1992).

Molecular phylogenetic analysis by Schmitz *et al.* (2004), which included American species, identified four species groups in *Plestiodon*: a group comprised of *P. anthracinus, P. egregius* and, surprisingly, *Neoseps reynoldsi*; a "*laticeps*" species-group including *laticeps, inexpectatus, fasciatus, obsoletus, septentrionalis* and *obstusirostris*; a "*skiltonianus*" species-group with *skiltonianus, gilberti* and *rubricaudatus*; a clade composed of the two Mexican species *P. brevirostris* and *P. lynxe*.

Following the recent systematic revision of the genus, *Plestiodon* "*sensu stricto*" contains 41 species. Ten species have been karyotyped and all showed 2n = 26 (12 macro- and 14 microchromosomes) (Caputo *et al.* 1994). The karyotypes differ in the morphology of microchromosomes, however, this can be partly due to the interpretation of smaller chromosomes by different authors.

Plestiodon parvulus Taylor (Southern pigmy skink)

Specimens analysed: two males (CEAC23, CEAC24)

Distribution: Mexican endemic. The species occurs along Pacific coast from Sinaloa to Colima.

Subspecies: no subspecies have been described.

Karyotype: the karyotype is here described for the first time in this species. The karyotype shows 2n = 26, with 12 macro- and 14 microchromosomes (Fig. 10). All the macro-chromosomes are biarmed as are four

pairs of the microchromosomes. The other microchromosomes are telocentric. This karyotype differs in the morphology of the microchromosomes from other karyotypes of *Plestiodon* species. For example, the microchromosomes seem all biarmed in *P. inexpectatus* and *P. obsoletus* (Caputo *et al.* 1994).

DNA taxonomy: neither gene sequence of *P. parvulus* is present in GenBank. Therefore, the fragment of the 16S rRNA sequenced for this study was aligned with available sequences of other congeners (Schmitz *et al.* 2004) to assess the phylogenetic affinities and genetic distance of this species within the genus. The obtained tree is shown in Figure 11. The results suggest that *E. parvulus* is the sister species of another Mexican endemic species, *P. lynxe* (bootstrap values 71% with NJ and 58% with ML). The genetic distance between the two species is 4.5–5.0% and is among the lowest interspecific genetic distance of the analysed dataset. This is the first report of a relationship between these two species (Griffith *et al.* 2000; Schmitz *et al.* 2004).

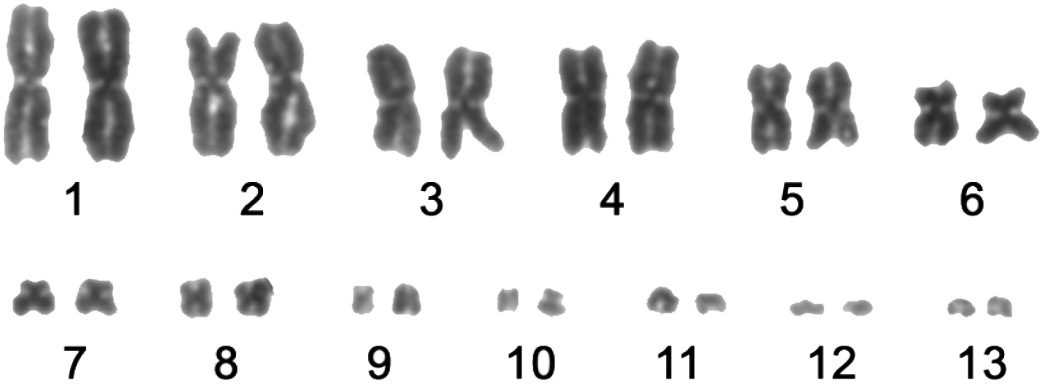

FIGURE 10. Karyotype of *Plestiodon parvulus* male (2n = 26).

Mabuya Fitzinger

The circumtropical genus *Mabuya* Fitzinger has recently been subjected to revision. Molecular analysis (Mausfeld *et al.* 2002) suggested that *Mabuya* consists of several long-separated evolutionary lineages, representing distinct and well supported monophyletic radiations. The South American species must retain the name *Mabuya* (Dunn 1935). The karyotype of the Neotropical species has been studied for only four species. *Mabuya caissara* and *Mabuya macrorhyncha* both have 2n = 32 (18 macrochromosomes and 14 microchromosomes) (Colus & Ferrari 1988). *Mabuya mabouya* showed 2n = 30 in the females and 2n = 31 in the males, indicating a XY sex chromosomal system (Beçak *et al.* 1972), whereas *M. frenata* showed 2n = 30 (Hernando & Alvarez 1990).

Mabuya unimarginata Cope (Central American mabuya)

Specimens analysed: one female from Chamela (MZFC 21804).

Distribution: from Jalisco on the Pacific coast and from Veracruz on the Gulf of Mexico south to Guatemala, Belize, Honduras, El Salvador, Nicaragua, Costa Rica, and Panama.

Subspecies: no subspecies have been described.

Karyotype: the karyotype of *M. unimarginata* is here described for the first time (Fig. 12). It has 2n = 32 with 18 macro- and 36 microchromosomes. Among the macrochromosomes it can be possible to identify two groups of chromosomes. The first group consists of four pairs of large biarmed chromosomes. The second group includes five pairs of smaller chromosomes arranged as three submetacentric pairs of and two acrocentric pairs. The karyotype here described is distinctive among the Neotropical species of *Mabuya*

already studied. In fact, the species that share the same chromosomal number (*M. caissara* and *M. macrorhyncha*) have the macrochromosomal complement constituted of all metacentric chromosomes while *M. unimarginata* have two pairs of acrocentrics macrochromosomes (pairs 8 and 9) (Colus & Ferrari 1988). The presence of acrocentric chromosomes in *M. unimarginata* could be a characteristic specific to this species.

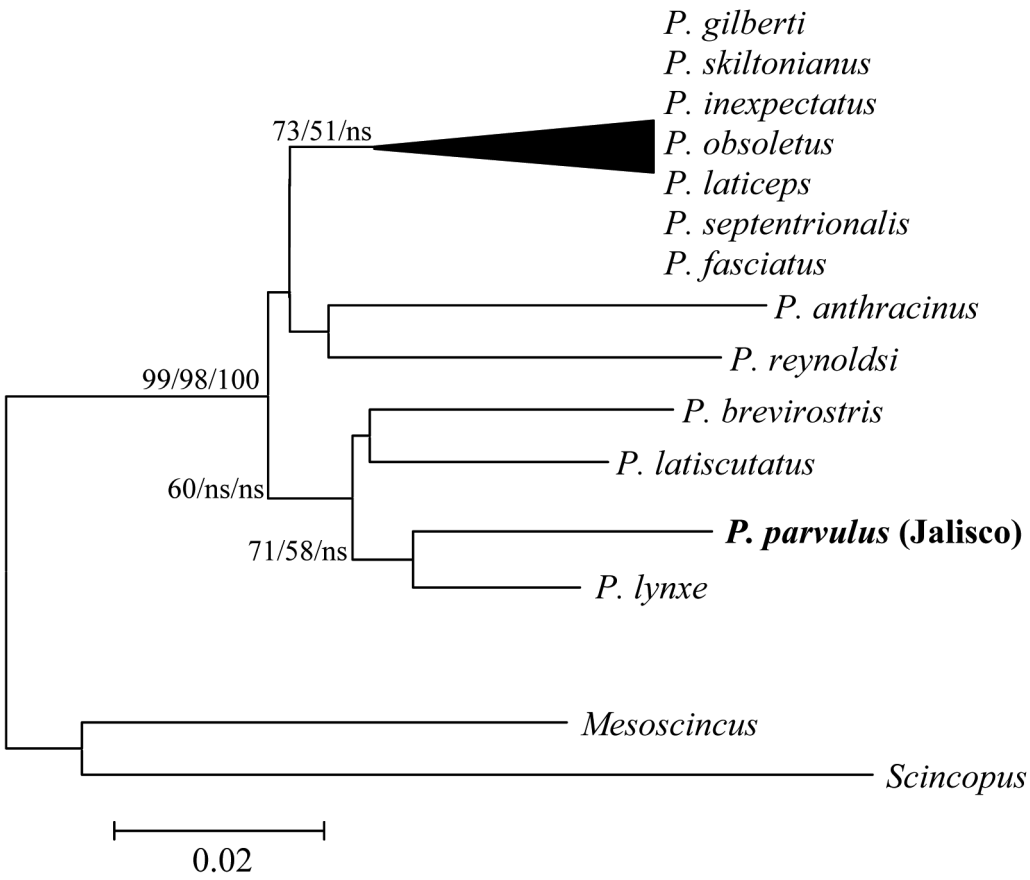

FIGURE 11. Neighbour-Joining tree of 16S rDNA haplotypes (518 bp) of *Plestiodon* species. Bootstrap values (%) obtained by the NJ, ML and MP are shown. The substitution model selected for ML was the Generalised time reversible (GTR) model with rate variation among sites (+G), a proportion of invariable sites I = 0.5020 and a gamma distribution shape parameter of 0.3681.

DNA taxonomy: a fragment of the cyt*b* sequence (350 bp) was aligned with the other sequences available in GenBank (belonging to Costa Rica, Guatemala, Honduras, Mexico, Guerrero, Mexico and Oaxaca) (Miralles *et al.* 2009). The specimens from Chamela represents the northernmost locality sampled for the species. The phylogenetic relationships among haplotypes do not reveal any geographic pattern. However, the genetic divergence observed within this species is high. The divergence ranges from 4% to 10%. The haplotype from Chamela is also divergent respect to all the others (6.3–9.5%). These values are of the same magnitude found among different species in *Mabuya* (min–max: 4.08–17.51, Miralles *et al.* 2009). These data suggest that *M. unimarginata* may constitute a species complex (Miralles *et al.* 2009). Alternatively, it can constitute a rare but not unique case of a species showing a high divergence in mtDNA (e.g Jesus *et al.* 2006). For this species we also sequenced a fragment of the 16S rDNA (502bp). A phylogenetic tree was built with other representative species of *Mabuya* from South America (dataset by Mausfeld *et al.* 2002.) including a sequence by *M. unimarginata* from Honduras (Honda *et al.* 2003). The results show a divergence of 3.2% between the samples of *M. unimarginata* from Chamela and Honduras. Interestingly these sequences are also very similar to the *Mabuya mabouya* sequence from Tobago, with 3.4% divergence. The three sequences cluster together but the two haplotypes belonging to *M. unimarginata* do not cluster together because of the

internal position of *Mabuya mabouya*. The simplest explanation for this pattern is that perhaps the haplotype from Tobago belongs to *M. unimarginata* and not to *M. mabouya*. These two species are very similar in morphology and cannot be easily recognized in the field. If this is the case, this finding represents the first report of *M. unimarginata* from Tobago.

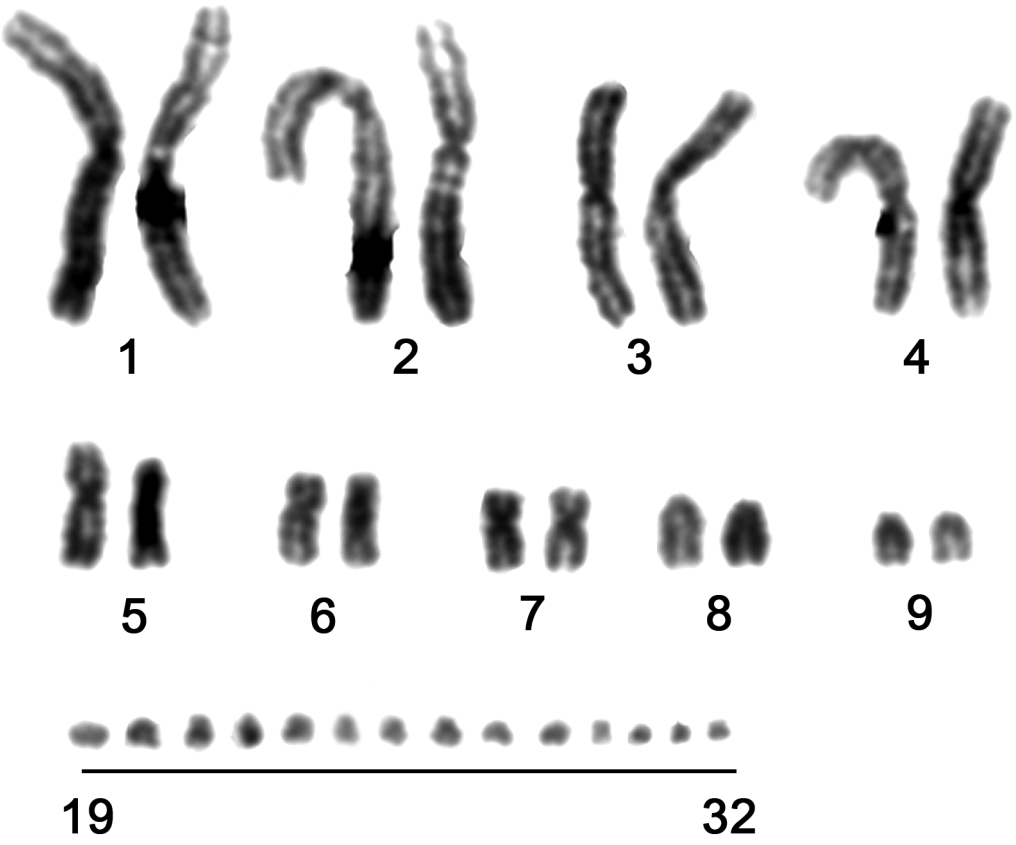

FIGURE 12. Karyotype of *Mabuya unimarginata* female (2n = 32).

Family Teiidae

***Ameiva* Duméril and Bibron**

Lizards of the genus *Ameiva* (Teiidae) include 34 species found throughout the West Indies and in Central and South America. Phylogenetic relationships and biogeography were investigated with sequences from portions of the 12S and 16S mitochondrial rRNA genes of sixteen West Indian species and three Central and South American species (Hower & Hedges 2003). The results evidenced that the West Indian species form a monophyletic group that diverged from the mainland species approximately 25–30 million years ago.

Currently, only six species of *Ameiva* have been karyotyped. The most common karyotype in the genus is characterized by having 2n = 50 with 26 macro- and 24 microchromosomes. The karyotypes of the previously studied species differ by the presence of biarmed chromosomes in the macrochromosomal complement (data from the ‘‘chromorep’’ database available at site http://www.scienze.univpm.it/professori/chromorep.pdf.). Thus, in *A. ameiva* and *A. exsul*, all the macrochromosomes are telocentrics. In *A. chrysolaema*, there are three pairs of biarmed chromosomes and in *A. dorsalis* and *A. maynardi* there are two pairs of biarmed chromosomes (Gorman 1970; Peccinini-Seale & Almeida 1986).

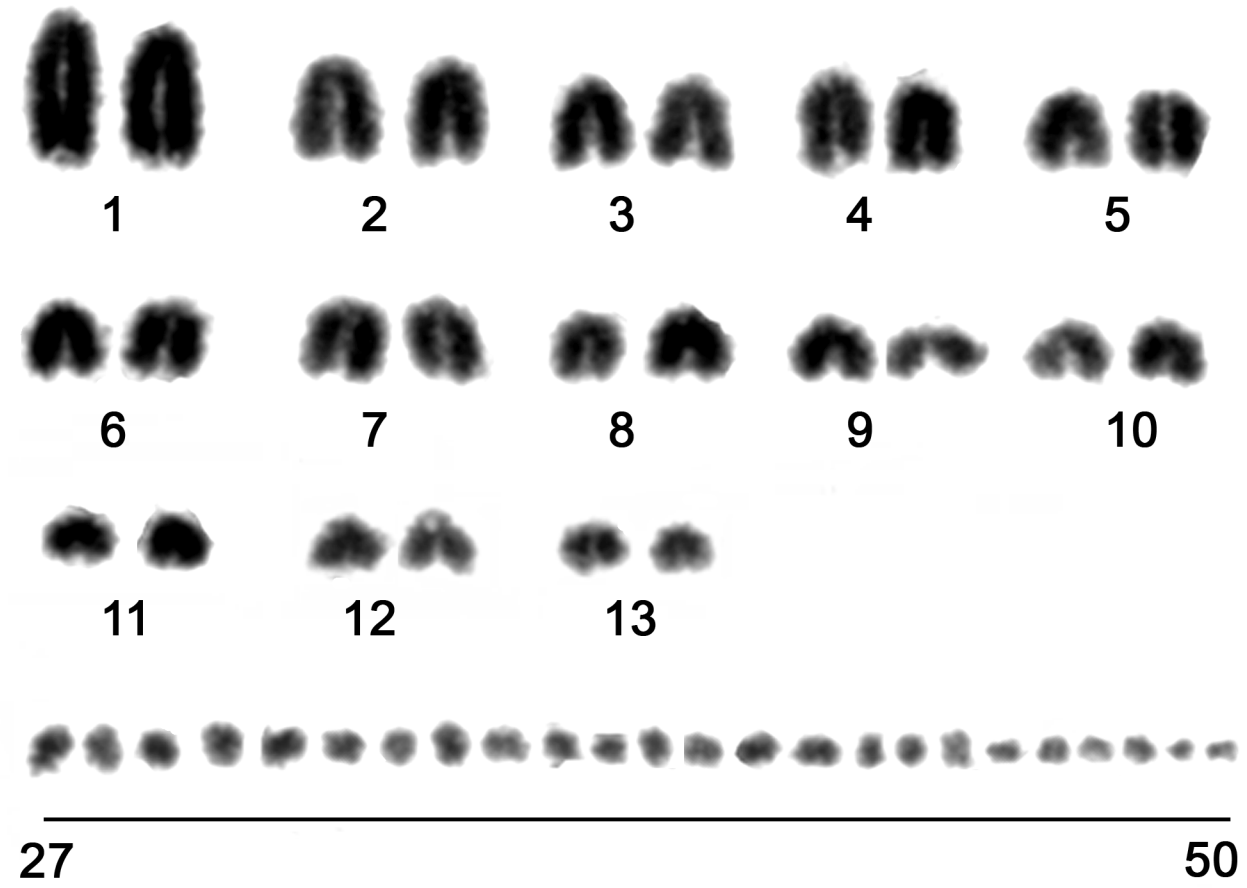

FIGURE 13. Karyotype of *Ameiva undulata* female (2n = 50).

***Ameiva undulata* Wiegmann (Rainbow Ameiva)**

Specimens analysed: One female (CEAC25), one specimen from Tuxtepec, Oaxaca (ENS10011) one specimen from Peten, Guatemala (UTA R 50334).
Distribution: From southern Tamaulipas and Jalisco Mexico to Costa Rica on both coasts.

Subspecies: geographical morphological variation is reported in this species. However the last taxonomic review of *Ameiva* in Central America does not recognize any subspecies (Echternacht 1971).

Karyotype: the karyotype of *A. undulata* has not been reported yet. All chromosomes were 2n = 50 and all were telocentric with 26 macro- and 24 microchromosomes (Fig. 13). This karyotype may represent the ancestral condition for the genus.

DNA taxonomy: the 16S sequence (490 bp) obtained from the specimens from Chamela was aligned with a dataset including other 18 *Ameiva* species including *A. undulata* from the Izabal Province, Guatemala (Hower & Hedges 2003). We also include sequences obtained from two additional samples of *A. undulata* (one from Tuxtepec, Oaxaca and another from Peten, Guatemala). Considering their geographic provenience, the studied specimens of *A. undulata* correspond to three different morphological forms found by in Echternacht (1971). The genetic divergence among the haplotypes from different locality is relatively high (3.7 – 5.9%). The most basal haplotype is the one from the Izabal Province, Guatemala. Its genetic divergence from the other haplotypes (5.4–5.9%) is even greater than those found between pairs of sister species in *Ameiva.* For example, the divergence between *A. exsul* and *A. wetmorei* is 3.9% while between *A. lineolata* and *A. maynardi* is 2.8%. However there is not a relationship among the genetic divergence and the

morphological forms revealed by Echternacht (1971) in Mexico. Therefore, the high genetic difference within *A. undulata* warrents additional study.

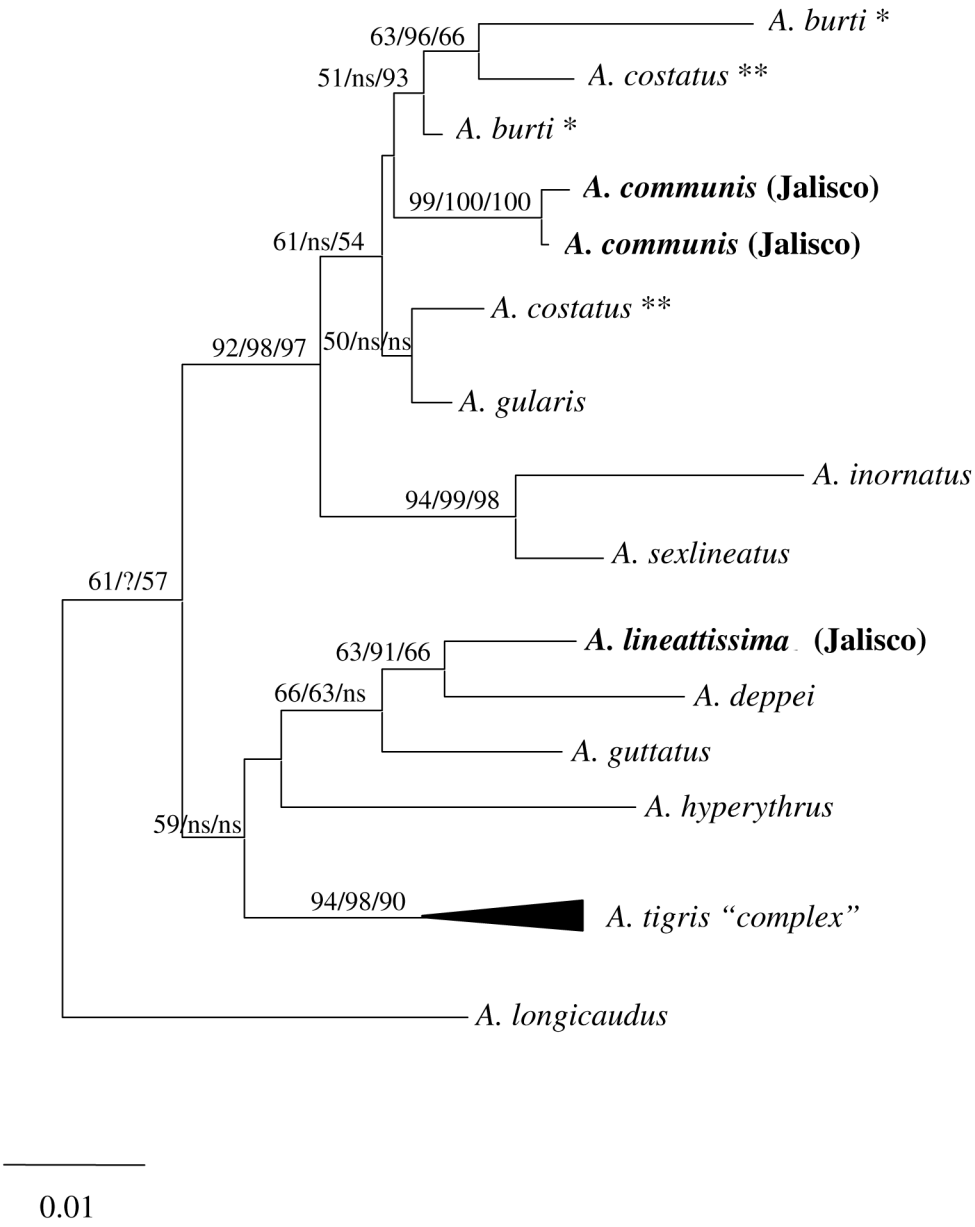

FIGURE 14. Neighbour-Joining tree of 16S rDNA haplotypes (462 bp) of *Aspidoscelis* species. Bootstrap values (%) obtained by the NJ, ML and MP are shown. The substitution model selected for ML was the GTR model with rate variation among sites (+G), a proportion of invariable sites I = 0.4604 and a gamma distribution shape parameter of 0.4453. Asterisks indicate taxon with paraphyletic haplotypes.

Genus *Aspidoscelis* Fitzinger

Species of the genus *Aspidoscelis* were previously included in *Cnemidophorus*, but based upon divergent morphological, molecular and enzymatic characters the two genera were recognized as separate (Reeder *et al.* 2002). Thus, *Aspidoscelis* is resurrected for the North American ‘‘*Cnemidophorus*’’ clade containing 87 species included in the *deppei*, *sexlineata*, and *tigris* species groups (and the unisexual taxa associated with them). *Aspidoscelis* occurs throughout most of North America (except Canada), reaching the East and West

Coasts of the United States, and ranging south through all Mexico and into Central America. The species groups differ also for their karyotypes. A 2n = 52 is observed in the *deppei* group, a 2n = 46 in the *sexlineata* group and a 2n = 46 with XY sex chromosomal system in the *tigris* group. Lowe *et al.* (1970) suggested a chromosomal evolution pattern through a reduction of the diploid number. This view has been slightly modified by Reeder *et al.* (2002), who considered that the ancestor probably had a karyotype of 2n = 50.

Aspidoscelis communis Cope (Colima giant whiptail)

Specimen analysed: two specimens (CEAC 26, CEAC30).

Distribution: Mexican endemic, distributed along the Pacific coast from Jalisco to Michoacán.

Subspecies: *Aspidoscelis communis mariarum* (Günther) and *Aspidoscelis communis communis* (Cope).

Karyotype: the karyotype of this species was reported by Lowe *et al.* (1970) as 2n = 46 and it was not studied again in the present study.

DNA taxonomy: the phylogenetic position of this species has not previously been ascertained using molecular markers and sequences of this species are not present in GenBank. We aligned the rDNA 16S sequences with all the so far studied *Aspidoscelis* species obtained from GenBank (39 sequences). A partial tree is shown in Figure 14. The phylogenetic analysis indicate that *A. communis* belongs to the "*sexlineata*" group (bootstrap support 92–97%). This confirms the affinities found on morphological and chromosomal ground (Reeder *et al.* 2002). In particular, it is included in a clade with *A. burti*, *A. costatus* and *A. gularis* but the relationships within this clade are supported only by NJ (61%) and MP (54%). Genetic divergence within this group is relatively low, ranging from 1.1 to 5.6%.

Aspidoscelis lineattissima (Cope) (Many-lined whiptail)

Distribution: Mexican endemic. Widespread along the Pacific coast, from Nayarit to Michoacán.

Subspecies: *Aspidoscelis l. duodecemlineatus* (Lewis), *A. l. exoristus* (Duellman & Wellman), *A. l. lineattissima* (Cope), *A. l. lividis* (Duellman & Wellman).

Karyotype: The karyotype of this species has been studied by Lowe *et al.* (1970), who reported a 2n = 52 to be typical of the "*deppei*" group. It was not studied again in this study.

DNA taxonomy: The phylogenetic position of this species has not been studied with molecular markers. The phylogenetic tree confirms the placement of *A. lineattissima* within the "*deppei*" group (Reeder *et al.* 2002) (Fig. 14). In fact, *A. lineattissima* results as sister species of *A. deppei* (bootstrap values 63–91%) within a clade that also includes *A. guttatus* (66% with NJ and 63% with ML). This is the first report of a relationship between *A. lineattissima* and *A. deppei*.

Conclusion

In this paper we present chromosomal and molecular sequence data for 13 lizard species from the Chamela-Cuixmala biosphere reserve in Jalisco, Mexico. The karyotype of 10 lizard species is reported. Among these, the karyotypes of six species are described here for the first time. These descriptions allowed the identification of distinct chromosomal markers characterizing each species. In three species (*Coleonyx elegans*, *Norops nebulosus*, and *Sceloporus melanhorinus*) we identified instances of chromosomal heteromorphism. In these cases, additional data from other populations are needed to understand the significance of such polymorphisms (presence of sex chromosomes, hybridization between chromosomal races or intrapopulation autosomal polymorphism). In this context, the case of *N. nebulosus* is notable with the presence of three pairs of heteromorphic chromosomes that probably represent a complex sex chromosome system. Regarding the species for which the karyotype has already been described from local populations, *Phyllodactylus lanei* we found a karyotype different from that previously described for the same species revealing a cryptic diversity.

These different chromosomal forms are interpreted here as having taxonomic significance. In fact, they correspond to two morphological populations that also show a high genetic divergence and may suggest a specific rank recognition for the two populations.

Mitochondrial (mtDNA) gene sequences were effective in delineating the taxonomic affinities of five Mexican endemic species, namely: *Urosaurus bicarinatus*, *A. nebulosus*, *Plestiodon parvulus*, *Aspidoscelis lineattissima* and *A. communis*. However, the gene fragment used in this study is short and these results should be considered as only provisional. In some cases, the taxonomic position of the species supports those based on morphology (for example, the species groups in *A. communis* and *A. lineattissima*). However, for *U. bicarinatus*, its taxonomic position does not match with that obtained with morphological data, but rather seems to agree with the species distribution by having a basal northern clade and a southern clade.

Finally, in two other cases we found an unexpected divergence revealed by comparison of mtDNA genes from different populations. The specimen of *Gerrhonotus* from Chamela is very divergent from the others so far analysed. Moreover, this population shows distinctive morphological characters and may likely belong to an undescribed taxon (Nieto Montes de Oca, pers comm). Genetic divergence between samples of *Ameiva undulata* from Chamela and Guatemala are also very high. The level of divergence is even greater than that observed for sister species in *Ameiva*. Similar consideration can also be made for *U. bicarinatus*, which shows a high genetic divergence between subspecies (Gonzalez Bernal and OFV unpublished data). In these cases additional data are needed to understand the taxonomic status of these populations.

The results obtained in this study were developed using specimens from a region subjected to a relatively high number of ecological studies (e.g. Noguera *et al.* 2002). This underscores the paucity of studies focusing on taxonomic questions of the herpetofauna along the Pacific coast of Mexico. Thus, the south-western coast of Jalisco represents an area of high reptile species richness (Flores-Villela & Goyenechea 2003; García 2006) and this study suggests that the species diversity and the level of endemism is underestimated in this region.

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