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Multiple Sex Chromosome System and Robertsonian Rearrangements Involved in the Chromosome Evolution of the *Phymaturus palluma* group (Iguania: Liolaemidae)

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ABSTRACT.—The Liolaemid genus *Phymaturus* is a clade of saxicolous lizards with 44 species recognized, grouped in the *Phymaturus palluma* and the *Phymaturus patagonicus* groups. The chromosome data about this genus are extremely scarce; however, unpublished evidence suggests a great karyotypic diversity, mainly in the *P. palluma* group. In this work, we describe the karyotypes of six species of the *P. palluma* group (one of them unnamed) and report a multiple chromosome sex determination system with heterogametic males ($X_1X_1X_2X_2 / X_1X_2Y$). This sex-system represents a putative synapomorphy for the group. In accordance with the published literature and data obtained in this study, we report a wide variability for the diploid number of the *P. palluma* group ($2N = 26$ to 36) but same autosomic fundamental number in all the species of the clade ($FNa = 32$). Such variation is a consequence of different numbers of telocentric macroautosome pairs among karyotypes (2 to 10), suggesting chromosomal evolution of the group, driven mainly by successive Robertsonian rearrangements.

RESUMEN.—Los liolémidos del género *Phymaturus* son un clado de lagartos saxícolas con 44 especies reconocidas, agrupadas en los grupos de *Phymaturus palluma* y *Phymaturus patagonicus*. Los datos cromosómicos acerca de este género son extremadamente escasos; sin embargo, la evidencia no publicada sugiere una gran diversificación cariotípica, principalmente en el grupo *P. palluma*. En este trabajo describimos los cariotipos de seis especies del clado *P. palluma* (una de ellas innominada) con un sistema múltiple de determinación cromosómico del sexo con heterogamia masculina ($X_1X_1X_2X_2 / X_1X_2Y$). Este sistema sexual representa una probable sinapomorfía para el grupo. De acuerdo con la bibliografía y los datos obtenidos en este trabajo, se describe una amplia variabilidad en el número diploide para el grupo *P. palluma* ($2N = 26$ a $2N = 36$), pero el mismo número autosómico fundamental para todas las especies del clado ($FNa = 32$). Tal variación es consecuencia del diferente número de pares de macroautosomas telocéntricos entre los cariotipos (2 a 10), lo cual sugiere que la evolución cromosómica del grupo ha sido predominantemente consecuencia de sucesivos rearrreglos Robertsonianos.

Phymaturus is a genus of saxicolous, herbivorous, and viviparous lizards endemic to Argentina and Chile with Andean-Patagonian distribution (Díaz Gómez, 2009; Debandi et al., 2012; Lobo et al., 2012). A flat body, prominent fat-filled lateral nuchal skin folds, and a spiny tail are distinctive morphological characters of this group (Etheridge, 1995; Lobo and Quinteros, 2005; Lobo et al., 2012).

The taxonomic review performed by Etheridge (1995) grouped the species of *Phymaturus* in two major groups (*Phymaturus palluma* and *Phymaturus patagonicus*), both recovered later as monophyletic in cladistic, Bayesian, and maximum-likelihood analyses with morphological and molecular characters (Espinoza et al., 2004; Lobo et al., 2012, 2016; Morando et al., 2013). The *P. patagonicus* group, composed of 26 species, is distributed from Mendoza to Chubut Provinces in Argentina, and the *P. palluma* group inhabits the northern region of Argentina and Chile, including 18 recognized species and several unnamed ones.

The cytogenetic information for *Phymaturus* is limited to a detailed description of the karyotype of *Phymaturus maulense* (as *P. palluma*, Lamborot and Navarro-Suárez, 1984) and comments about the diploid numbers for another five species (Ceï and Videla, 2003; Corbalán et al., 2009). In addition, karyotypic information from other *Phymaturus* species are available from two unpublished theses, describing (using only conventional staining techniques) a great diversity in number and morphology between both recognized groups (Pereyra, 1991; Morando, 2004), suggesting that the group would be of great interest to evaluate the mechanisms of chromosomal evolution in lizards.

Lizards show an astonishing variety of sex determining mechanisms (e.g. XY, XXY, ZW, ZZW, temperature-dependent sex determination, or genetic-environment interactions) and of nearly 1,000 karyotyped species, almost 200 have heteromorphic sex chromosomes; yet they display a remarkable diversity in size and morphology (reviewed by Ezaz et al., 2009). Although the majority of these have XY or ZW type systems, ~23% (nearly 40 species) have multiple sex chromosomes. Except for a group of lacertids with ZZW system (Olmo and Signorino, 2005), the remaining species with multiple sex chromosome systems share heterogametic males (XXY), and most (90%) of them occur in Iguanidae (reviewed by Ezaz et al., 2009). In *Phymaturus*, two different sex chromosome systems (i.e., XY and XXY) were described (Lamborot and Navarro-Suárez, 1984; Ceï and Videla, 2003; Corbalán et al., 2009), but almost nothing is known about its origin and phylogenetic distribution.

The aim of this work is to improve the cytogenetic knowledge for the *P. palluma* species group by analyzing the autosome and the sex chromosome determination system evolution in a robust phylogenetic context of *Phymaturus*. For this purpose, we described for the first time the chromosome number and morphology, sex determination system, nucleolar organization region localization (NORs), and patterns of DNA base-specific fluorochromes in six species (one of them not formally described yet) assigned to the *P. palluma* clade.

MATERIALS AND METHODS

In total, we karyotyped 28 adult specimens from both sexes, for five nominal species of *Phymaturus* (*Phymaturus denotatus*, *Phymaturus laurenti*, *P. palluma*, *Phymaturus roigorum*, and *Phymaturus williamsi*) plus 10 individuals from one population

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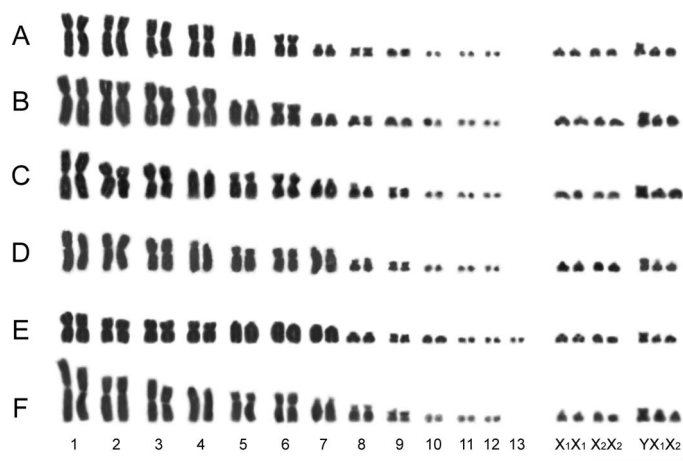


FIG. 1. Giemsa stained karyotypes. The sex chromosomes are shown separately. (A) *Phymaturus denotatus*. (B) *Phymaturus laurenti*. (C) *Phymaturus palluma*. (D) *Phymaturus roigorum*. (E) *Phymaturus williamsi*. (F) *Phymaturus* sp. usp. Bar = 10 μ m.

not formally described yet, named by Lobo et al. (2016) as *Phymaturus* sp. usp. (for detailed reference, sex, and voucher number, see Appendix 1). The specimens analyzed are housed at the collection of Museo de Ciencias Naturales (MCN), Universidad Nacional de Salta, Argentina.

Based on Lobo et al. (2012, 2016), we assigned the specimens from Cordón del Portillo, Laguna Diamante (type locality of *Phymaturus gynechloinus*) and Valle Hermoso (Mendoza Province, Argentina) as *P. palluma*, and those from Uspallata (Mendoza Province, Argentina) and El Portezuelo (San Juan Province, Argentina) to *P. sp. usp.* The species assignment, figures, and discussion follows the phylogenetic hypothesis of Lobo et al. (2016).

We obtained the chromosome spreads from intestinal epithelium, or bone marrow, and testis according to Schmid et al. (2010). We stained with Giemsa-PBS solution (pH 6.8) the mitotic and meiotic chromosome preparations. We also performed the silver staining of nucleolar organizer regions (Ag-NORs) based on Howell and Black (1980) technique, fluorochrome staining GC-specific chromomycin A₃ (CMA₃), and AT-specific 4, 6-diamidino-2-phenylindole (DAPI) follows Schweizer (1980).

We measured and calculated the centromeric ratio of each chromosome pair based on 10 metaphase plates (in males) with Micromesure v3.3 software (Reeves and Tear, 2000). We arranged the mitotic chromosomes in decreasing size following the nomenclature of Green and Sessions (2007). We follow Fillon (1998) for the assessment of microchromosomes (elements without morphological differentiation). For each species, we also also reported the number of the chromosomal arms per autosomic chromosome set (the fundamental number, FNa). We use the term “homeology” to designate the homology present between chromosomes of different species that have descended from a common ancestral chromosome (Huskins, 1932).

RESULTS

In all taxa analyzed, the males always present three sexual chromosomes: two unpaired telocentric chromosomes (X₁ and X₂) and one small metacentric (Y-chromosome), whereas the females present four sexual chromosomes: two small telocentric pairs (pairs of X₁ and X₂) that evidence a multiple sexual chromosome system, with heterogametic males (Fig. 1). The

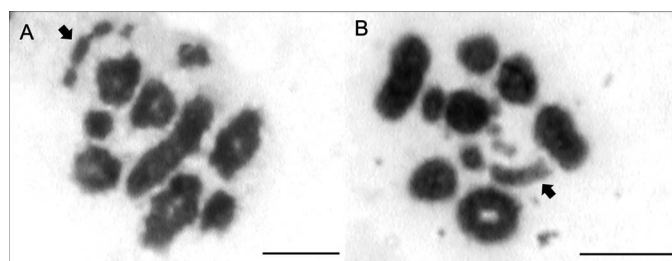


FIG. 2. *Phymaturus* male diakinesis with Giemsa stain. (A) *Phymaturus palluma*. (B) *Phymaturus* sp. usp. Arrows point to linear trivalent X₁X₂Y. Bar = 10 μ m.

presence of sexual chromosomes was confirmed by the analysis of 40 diakinesis plates (in males of *P. palluma* and *P. sp. usp.*) with 12 ring-shaped bivalents and one linear trivalent that involve the sexual chromosomes (Fig. 2). Additionally, all species analyzed share three pairs of microchromosomes (Fig. 1).

The karyotypes of *P. palluma*, *P. roigorum*, and *P. sp. usp.* share 2N = 27/28 and FNa = 32 (Figs. 1C–D, F and 3; Table 1). The macroautosomes are composed of three pairs of large biarmed chromosomes (1–3 metacentric), four medium-sized (4, 7 telocentric, 5 submetacentric, and 6 metacentric), and two small macrochromosome pairs (8 subtelo-centric and 9 metacentric).

Phymaturus denotatus and *Phymaturus laurenti* share 2N = 27/28 and FNa = 32 (Figs. 1A–B and 3; Table 1). The macroautosomes are composed by four pairs of large metacentric macrochromosomes (pairs 1–4), two medium-sized (5 telocentric, 6 metacentric), and three small macrochromosomes (pair 7 subtelo-centric, 8 metacentric, and 9 telocentric).

Phymaturus williamsi presents 2N = 29/30 and FNa = 32, with seven macrochromosome pairs decreasing gradually in size (Figs. 1E and 3; Table 1). Pairs 1–3 are metacentric, 4 submetacentric, 5–7 telocentric, and three small pairs (8 submetacentric, 9 metacentric, and 10 telocentric).

In all taxa analyzed, the Ag-NORs are located pericentromerically at the X₁ chromosome (arbitrarily named) coincident with the usually evident secondary constrictions: in females, it is present in both homologues in a small telocentric chromosome, and in males it is present in only one small telocentric chromosome (Figs. 3 and 4A). In addition, *P. palluma*, and *P. sp. usp.*, share an additional pericentromeric Ag-NORs on pair 4 (Fig. 4).

The fluorescent base-specific staining shows bands CMA₃ (+) / DAPI (–) in almost all centromeres of chromosome complement, except in pairs 6 and 9 of *P. denotatus*, 7 and 9 of *P. laurenti*, 7, 8 and Y chromosome of *P. palluma* and *P. sp. usp.*, pair 4 and Y chromosome of *P. roigorum*, and pairs 4–10 of *P. williamsi*. We additionally observed CMA₃ (+) / DAPI (–) bands located in coincidence with the NORs position described above (Fig. 4B, C).

DISCUSSION

Type Species P. palluma.—*Phymaturus palluma*, traditionally considered a transandean species (Argentina-Chile), was first collected by Darwin during the Beagle voyage, but its type locality had been a topic of debate in the last decade (e.g., Cei and Videla, 2003; Scolaro, 2010). Recently, Lobo and Etheridge (2013), by the examination of the specimen plus the analysis of Darwin’s field notes, suggested the type to be at Cordón del Portillo, Mendoza, Argentina. We analyzed specimens from this locality, and the karyotype is similar to the karyotype reported for *P.*

TABLE 1. Morphometric data of the karyotypes of species included in the present study. Relative length (RL), braquial relation (BR), standard deviation (SD), chromosome type (CT): *m* = metacentric; *sm* = submetacentric; *st* = subtelo-centric; *t* = telocentric; * = microchromosomes.

Pair	<i>P. denotatus</i>			<i>P. laurenti</i>			<i>P. palluma</i>			<i>P. roigorum</i>			<i>P. williamsi</i>			<i>P. sp. usp.</i>		
	RL	BR ±SD	CT	RL	BR ±SD	CT	RL	BR ±SD	CT	RL	BR ±SD	CT	RL	BR ±SD	CT	RL	BR ±SD	CT
1	15.40	1.53 ± 0.02	<i>m</i>	15.70	1.44 ± 0.08	<i>m</i>	16.82	1.11 ± 0.11	<i>m</i>	13.85	1.36 ± 0.05	<i>m</i>	12.62	1.22 ± 0.01	<i>m</i>	17.21	1.25 ± 0.05	<i>m</i>
2	14.78	1.68 ± 0.07	<i>m</i>	14.44	1.51 ± 0.10	<i>m</i>	12.62	1.69 ± 0.29	<i>m</i>	13.21	1.47 ± 0.37	<i>m</i>	10.49	1.28 ± 0.11	<i>m</i>	13.05	1.65 ± 0.10	<i>m</i>
3	13.21	1.14 ± 0.14	<i>m</i>	13.37	1.29 ± 0.21	<i>m</i>	11.77	1.10 ± 0.01	<i>m</i>	11.69	1.17 ± 0.04	<i>m</i>	10.33	1.28 ± 0.08	<i>m</i>	11.31	1.14 ± 0.09	<i>m</i>
4	11.53	1.03 ± 0.01	<i>m</i>	12.56	1.09 ± 0.11	<i>m</i>	9.96	1.32 ± 1.47	<i>t</i>	10.75	1.21 ± 0.64	<i>t</i>	9.41	1.36 ± 0.29	<i>sm</i>	9.95	15.26 ± 3.67	<i>t</i>
5	8.65	0.80 ± 0.14	<i>t</i>	8.63	0.75 ± 0.44	<i>t</i>	8.80	2.35 ± 0.37	<i>sm</i>	9.06	2.83 ± 0.15	<i>sm</i>	9.39	1.97 ± 1.07	<i>t</i>	8.72	2.68 ± 0.19	<i>sm</i>
6	8.09	1.53 ± 0.17	<i>m</i>	7.51	1.43 ± 0.14	<i>m</i>	8.32	1.40 ± 0.04	<i>m</i>	8.38	1.46 ± 0.01	<i>m</i>	9.14	11.86 ± 1.47	<i>t</i>	8.54	1.62 ± 0.02	<i>m</i>
7	4.49	5.06 ± 1.02	<i>st</i>	4.22	4.16 ± 0.35	<i>st</i>	6.62	8.01 ± 0.16	<i>t</i>	9.02	8.16 ± 2.12	<i>t</i>	8.39	9.02 ± 1.59	<i>t</i>	6.92	7.94 ± 0.67	<i>t</i>
8	3.71	1.39 ± 0.10	<i>m</i>	3.34	1.45 ± 0.11	<i>m</i>	5.19	3.13 ± 0.24	<i>st</i>	4.62	3.11 ± 0.10	<i>st</i>	4.78	3.40 ± 0.55	<i>sm</i>	4.78	3.14 ± 0.02	<i>st</i>
9	2.77	-	<i>t</i>	3.32	-	<i>t</i>	4.02	1.64 ± 0.08	<i>m</i>	3.24	1.49 ± 0.02	<i>m</i>	4.05	1.45 ± 0.19	<i>m</i>	3.31	1.28 ± 0.22	<i>m</i>
10	2.05	-	<i>*</i>	2.64	-	<i>*</i>	1.97	-	<i>*</i>	2.19	-	<i>*</i>	3.40	-	<i>t</i>	2.23	-	<i>*</i>
11	2.02	-	<i>*</i>	2.36	-	<i>*</i>	1.93	-	<i>*</i>	1.83	-	<i>*</i>	2.33	-	<i>*</i>	2.00	-	<i>*</i>
12	1.89	-	<i>*</i>	1.80	-	<i>*</i>	1.77	-	<i>*</i>	1.63	-	<i>*</i>	2.29	-	<i>*</i>	1.72	-	<i>*</i>
13													1.92	-	<i>*</i>			
X1	3.25	-	<i>t</i>	3.60	-	<i>t</i>	2.93	-	<i>t</i>	3.12	-	<i>t</i>	3.60	-	<i>t</i>	3.23	-	<i>t</i>
X2	3.05	-	<i>t</i>	3.40	-	<i>t</i>	2.90	-	<i>t</i>	3.00	-	<i>t</i>	3.17	-	<i>t</i>	2.90	-	<i>t</i>
Y	5.11	1.40 ± 0.24	<i>m</i>	4.65	1.41 ± 0.03	<i>m</i>	4.38	1.19 ± 0.16	<i>m</i>	4.41	1.14 ± 0.09	<i>m</i>	4.68	1.19 ± 0.15	<i>m</i>	4.13	1.36 ± 0.37	<i>m</i>

roigorum clade with 2N = 27/28, with almost all biarmed chromosomes. Previous authors also described karyotypes assigned to *P. palluma* (Lambrot and Navarro-Suarez 1984; Pereyra, 1991 unpubl. data; Morando, 2004 unpubl. data); however, these specimens were collected in Chile (Laguna de Maule). These karyotypes share 2N = 35/36, with almost all telocentric chromosomes, differing those in the sample from Mendoza. Therefore, the Chilean karyotypes could be considered conspecific—probably *P. maulense*, Nuñez et al. (2010)—although not assignable to *P. palluma*.

Chromosomal Evolution mainly by Robertsonian Rearrangements in Phymaturus.—The ancestral karyotype proposed for Iguanians has 2N = 36, involving 12 biarmed macrochromosomes—the first six chromosomes pairs with relative size notoriously larger than the rest of the chromosome complement—plus 24 microchromosomes (Matthey, 1949; Gorman et al., 1967; Webster et al., 1972; Paull et al., 1976). For Liolaemidae, there is no cytogenetic data available for *Ctenoblepharys*, but in *Liolaemus*, the most widespread karyotype is similar to the ancestral karyotype proposed for Iguanians. In contrast, for *Phymaturus*, a wide variation in the diploid numbers (2N = 26 to 42) is reported, as well as in the number of telocentric pairs (2–10 pairs); however, the number of autosomic macrochromosome arms is the same for all the species of the genus (FNa = 32). The three karyotypes described for the *P. patagonicus* group, present the highest diploid number of the genus (2N = 42), being composed by almost all telocentric macrochromosomes plus nine pairs of microchromosomes (Lambrot and Navarro-Suárez 1984; Pereyra, 1991 unpubl. data; Morando, 2004 unpubl. data; Morando et al., 2013). Meanwhile in the *P. palluma* group, the diploid number varies from 2N = 26 to 36, with variation in the telocentric macrochromosomes ranging between 2 and 10 pairs, plus three pairs of microchromosomes in all the species (Fig. 3, Table 2).

The analysis of the chromosome data available for the *P. palluma* group in the framework of the phylogenetic hypotheses (Lobo et al., 2016) is a first approach for understanding its chromosome evolution. The species of the *Phymaturus vociferator* clade present 2N = 36 (in females), with chromosome morphology clearly not homologous to the ancestral karyotype for iguanids, because the set of 13 pairs of large-sized macrochromosomes are almost all telocentric with gradual size-declination. The species grouped in the *P. roigorum* clade share 2N = 27/28, except for *Phymaturus verdugo* males with 2N = 26 (Cei and Videla, 2003); the known karyotypes present similar chromosome morphology, composed by seven biarmed macrochromosomes, plus two medium-sized telocentric macrochromosomes pairs, observed only in this clade. In addition, the species assigned to the *Phymaturus antofagastensis* clade also share 2N = 27/28, but only one medium-sized, and one small telocentric chromosome pairs are present. On the other hand, the species assigned to the *Phymaturus punae* clade share karyotypes with 2N = 29/30, with three medium-sized telocentric chromosomes pairs, and one small telocentric chromosome pair that correspond to a putative synapomorphy for the more inclusive *Phymaturus mallimacci* clade.

Regarding the differential staining, the location of the Ag-NORs in the autosomic complement in *P. palluma* and *P. sp. usp.* (pericentromeric over the pair 4), differ from the data previously reported for other iguanians, which usually share NORs in the pair 2 (e.g., Paull et al., 1976; Cole, 1978; Kasahara et al., 1996; Bertolotto et al., 2002). The different NORs pattern observed in the species included in the *P. palluma* group could be a consequence of the rearrangements that led to the distinctive chromosome

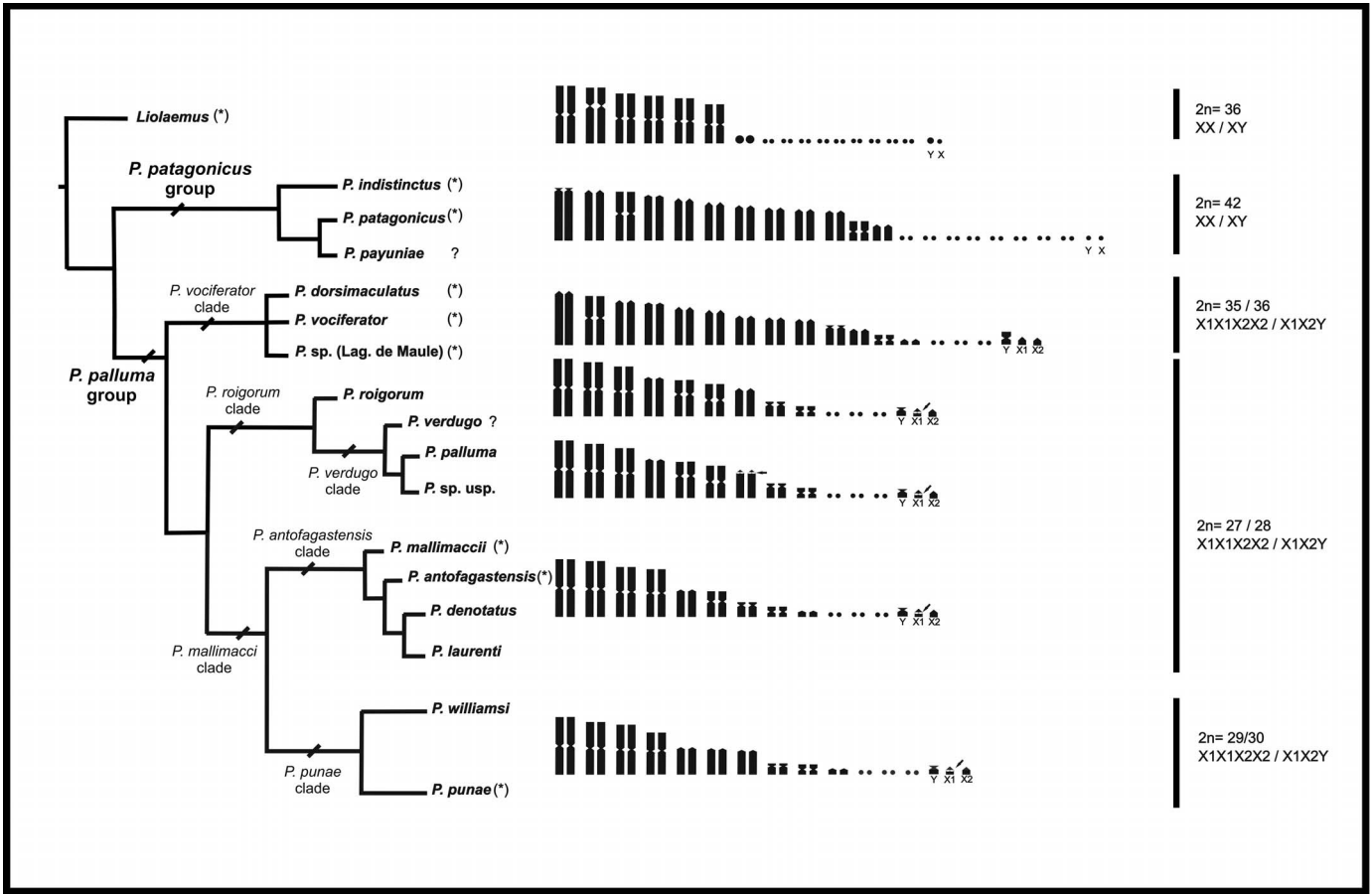


FIG. 3. Species of Lioalemidae with cytogenetic data available in the framework of the phylogenetic hypotheses of Lobo et al. (2012, 2016). Idiogram of *Phymaturus palluma* species analyzed in this study with diploid number, chromosomal sex determination system, and NORs position (arrows). Additionally, we added the idiogram of the karyotypes commonly found in *Phymaturus patagonicus* group and *Liolaemus* genus. Asterisk denotes karyological data from literature. Questions marks point to references with different karyotype (see text for discussion).

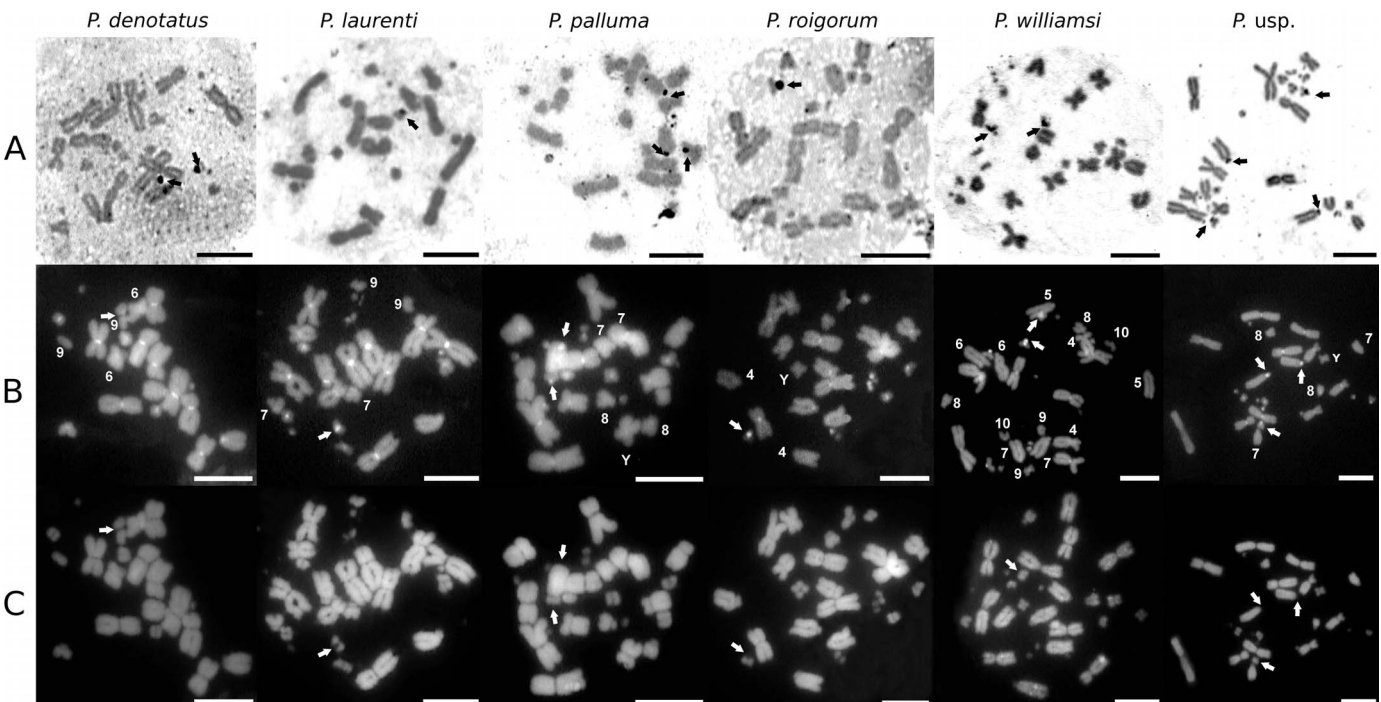


FIG. 4. Differential staining for six species of *Phymaturus*. (A) Ag-NOR; (B) CMA₃; (C) DAPI. Arrows show the nucleolar organization region with the different stains. The numbers indicate chromosomes without CMA₃ (+) bands. Bar = 10 μm.

TABLE 2. *Phymaturus* species with cytogenetic data available. 2N = diploid number, GSD = genetic sex determination, * = unpublished data.

Species	2N		GSD	Source
	Males	Females		
<i>Phymaturus palluma</i> group				
<i>P. antofagastensis</i>		28	X ₁ X ₁ X ₂ X ₂ / X ₁ X ₂ Y	E. A. Pereyra, 1991*
<i>P. denotatus</i>	27	28	X ₁ X ₁ X ₂ X ₂ / X ₁ X ₂ Y	This study
<i>P. dorsimaculatus</i>	35	36	X ₁ X ₁ X ₂ X ₂ / X ₁ X ₂ Y	M. Morando, A. Guerreiro, L. J. Avila, 2001*
<i>P. laurenti</i>	27	28	X ₁ X ₁ X ₂ X ₂ / X ₁ X ₂ Y	This study
<i>P. malimacci</i>	27	28	X ₁ X ₁ X ₂ X ₂ / X ₁ X ₂ Y	E. A. Pereyra, 1991*; M. Morando, 2004*
<i>P. maulense</i>	35	36	X ₁ X ₁ X ₂ X ₂ / X ₁ X ₂ Y	M. Lamborot and M. Navarro-Suarez, 1984; E. A. Pereyra, 1991*; M. Morando, 2004*; Morando et al., 2013
<i>P. palluma</i>	27	28	X ₁ X ₁ X ₂ X ₂ / X ₁ X ₂ Y	This study
<i>P. roigorom</i>	27	28	X ₁ X ₁ X ₂ X ₂ / X ₁ X ₂ Y	This study
<i>P. punae</i>	29	30	X ₁ X ₁ X ₂ X ₂ / X ₁ X ₂ Y	M. Morando, 2004*
<i>P. verdugo</i>	26			Cei and Videla, 2003
<i>P. vociferator</i>		36		V. Corbalán, A. Scolaro, and G. Debandi, 2009
<i>P. williamsi</i>	29	30	X ₁ X ₁ X ₂ X ₂ / X ₁ X ₂ Y	This study
<i>P. sp. usp.</i>	27	28	X ₁ X ₁ X ₂ X ₂ / X ₁ X ₂ Y	This study
<i>Phymaturus patagonicus</i> group				
<i>P. indistinctus</i>	42		XY	M. Morando, 2004*
<i>P. patagonicus</i>		42	XX	M. Morando, 2004*
<i>P. payuniaie</i>		42	X ₁ X ₁ X ₂ X ₂	E. A. Pereyra, 1991*

morphology for this clade and should be evaluated in a proper context, because no data exist for NORs locations in the *P. patagonicus* group. In addition, multiple NORs (in X₁ and pair 4) in the *P. verdugo* clade correspond to a putative synapomorphy of the group or even of a less inclusive group (*P. palluma* + *P. sp. usp.*), despite having no data about NORs locations in *P. verdugo* and *P. sp. 10* of Lobo et al. (2016). The distribution of rich sequences of AT and/or CG is not available for any Liolaemidae karyotypes. In this sense, the data obtained in the present work, with CMA₃ positive bands present in almost all centromeres and sparse marks in the chromosome complement, do not allow a correct assessment of chromosome homeology. A denser sampling is needed to evaluate the utility of these marks to establish homeology between chromosome pairs in related taxa.

The techniques performed in the present work (standard and differential staining and morphometric measurements) do not allow a proper establishment of chromosome homeology by the karyotype diversity exhibited by the *P. palluma* clade. In concordance with the previously proposed hypothesis by Cei and Videla (2003), however, our data support the chromosome evolution of the *P. palluma* group mainly by successive Robertsonian rearrangements. The same fundamental number in macroautosomes (shared with the *P. patagonicus* group), and the presence/absence of telocentric chromosome pairs, as well as their size (like the small chromosome pair present only in the species assigned to the *P. malimacci* clade), point to fusion and fission of entire macroautosome arms and the small telocentric ones. The lack of other sources of data, however, does not allow us to discard other types of chromosome rearrangements (as paracentric inversions) which could be involved in the chromosome evolution of the group.

Sexual Determination System in Phymaturus.—Reptiles present a high variability of modes of sexual determination (Ezaz et al., 2009; Wang et al., 2015), from temperature-dependent to different types of genotypic systems for sex determination. This variability implies multiple and independent origins of sex chromosomes and suggests an extremely labile mechanism in lizards (Ezaz et al., 2009). For *Phymaturus*, at least two different sexual determination systems are described, involving both micro- and macrochromo-

somes. In the *Phymaturus patagonicus* group for *Phymaturus indistinctus* and *P. patagonicus*, a simple sex chromosome system with heterogametic males (XX / XY) was described, being the X- and Y-chromosome dot-like microchromosomes (Morando 2004, unpubl. data); additionally, Pereyra (1991) reported for *P. payuniaie* a multiple sex chromosome system involving microchromosomes (X₁X₁X₂X₂) based on one female. We were unable to examine the vouchers used by Pereyra for *P. payuniaie*, but the distinction between a simple (XX / XY) and a multiple (X₁X₁X₂X₂ / X₁X₂Y) chromosome system involving microchromosomes with conventional stain, and based on only one female is insufficient evidence to confirm this statement. In the *Phymaturus palluma* group, the multiple sex chromosome system with heterogametic males (X₁X₂Y / X₁X₁X₂X₂) is shared by all species with karyotypic data available (Lamborot and Navaro-Suárez, 1984; Pereyra, 1991; Morando, 2004; this study). Chromosomes involved in the origin of this mechanism are still unknown; however, the different microchromosome numbers between the *P. palluma* (three pairs) and the *P. patagonicus* groups (eight pairs and one pair involved in sex determination system XX / XY) suggest that such elements could be involved in the origin of the multiple sex chromosome system. The centric fusion and tandem fusion could lead from a “single” to a “multiple” system, as was previously proposed for several taxa (White, 1940, 1941).

Given the phylogenetic distribution of the simple sex chromosome system (XX / XY) widespread in *Liolaemus* and in other pleurodontid iguanians and reported for the members of *P. patagonicus* group, the simple sex chromosome system XX / XY represents the plesiomorphic state for Liolaemidae (character state unknown in the basal monotypic genera *Ctenoblepharys*). Therefore, the events leading to a multiple sex chromosome system that occurred in the common ancestor of the *P. palluma* group correspond to a synapomorphy for this clade. Finally, a multiple sex chromosome system with one X-chromosome carrying the NORs, as observed for species of the *P. palluma* group, was previously described in the phylogenetically distant species *Tropidurus itambere* and *Tropidurus psammonastes* (Kasahara et al., 1996) that points to independent origins.

The karyotype of the basal monotypic genus of Liolaemidae, *Ctenoblepharys adpersa*, and further cytogenetic studies for species of the *P. patagonicus* and the *P. palluma* groups, with the implementation of molecular cytogenetic techniques, will allow corroboration of the correct chromosome homeology assessment between the chromosome pairs and the Robertsonians rearrangements implied in the karyotype evolution in *Phymaturus* and related groups.

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APPENDIX 1

Specimens examined are stored in the herpetological collection MCN (Museo de Ciencias Naturales, Universidad Nacional de Salta, Argentina).

Phymaturus denotatus: Argentina: Catamarca Province, Belén, Laguna Blanca (26°34'S; 66°56'40"W, 3,440 masl), MCN 3159 (male), MCN 3160–3161 (females).

Phymaturus laurenti: Argentina: Catamarca Province, Departamento Antofagasta de la Sierra, around 10 km south of El Peñón, Dpto., (26°39'40.6"S; 67°13'26.3"W 3,815 masl), MCN 2838, 2841 (males); MCN 2839–2840 (females).

Phymaturus palluma: Argentina: Mendoza Province, Depto. Tunuyán, Camino al Portillo Argentino, Cordón del Portillo, MCN 2894, 2897, 3627, 3629, 3130 (males), MCN 3131 (female).

Phymaturus roigorum: Argentina: Mendoza Province, Depto. Malargüe, Volcán Payún Matrú, MCN 2876 (female), MCN 2877 (male).

Phymaturus williamsi: Argentina: San Juan Province, Depto. Calingasta, Quebrada Vallecito, 40 km al O de Calingasta, MCN 2808, 2811–2812 (males), MCN 2809–2810 (females).

Phymaturus *usp.*: Argentina: San Juan Province, Depto. Sarmiento, El Portezuelo MCN 2658–2659, 2662 (males), MCN 2660–2661 (females).