

Karyotypes of a Cryptic Diploid Form of the Unisexual *Leposoma percarinatum* (Squamata, Gymnophthalmidae) and the Bisexual *Leposoma ferreirai* from the Lower Rio Negro, Amazonian Brazil

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ABSTRACT.—Karyotypes of *Leposoma* show a clear differentiation between species of the *scincoides* group from Brazilian Atlantic Forest ($2n = 52$, without distinctive size groups of chromosomes) and those of the *parietale* group from the Amazon ($2n = 44$, with $20M + 24m$). In a previous study, we found that in the *parietale* group the parthenoform *Leposoma percarinatum* from the state of Mato Grosso, Brazil, exhibited a triploid karyotype ($3n = 66$) with 30 macrochromosomes and 36 microchromosomes. It was suggested that this karyotype arose after hybridization between a bisexual species with $N = 22$ ($10M + 12m$) and a hypothetical unisexual cryptic diploid form of the *L. percarinatum* complex. Herein, we describe the karyotypes for two species of the *parietale* group occurring sympatrically in the Arquipélago das Anavilhanas, lower Rio Negro, in Amazonian Brazil. The first represents a distinctive diploid parthenogenetic clone of the *L. percarinatum* complex, and the other is the recently described *Leposoma ferreirai*. Both species have 44 biamred chromosomes clearly represented by 20 macrochromosomes and 24 microchromosomes and present Ag-NORs in one pair of the smallest sized microchromosomes; heteromorphism of size for these regions was detected in *L. percarinatum*. C-banding revealed blocks of constitutive heterochromatin on the telomeric and pericentromeric regions of macrochromosomes and some microchromosomes. The description of a diploid karyotype ($2n = 44$, $20M + 24m$) for the *L. percarinatum* complex and its sympatric congener *L. ferreirai* provides new insight for a better understanding of the origin of parthenogenesis in the *L. percarinatum* complex.

Gymnophthalmidae is a very diverse monophyletic assemblage of exclusively Neotropical lizards that currently encompasses about 180 species and 44 recognized genera (Rodrigues et al., 2009; Kok, 2009). Cytogenetically, it is still a poorly known family with data restricted to 40 species assigned to 20 genera (for an overview see Pellegrino et al., 2001; Yonenaga-Yassuda et al., 2005). Although only a few species have been analyzed thus far, a conspicuous karyotypic diversity has been found in the family. Karyotypes show diploid numbers ranging from $2n = 32$ ($14M + 18m$) in *Bachia dorbignyi* to $2n = 62$ – 64 in *Nothobachia ablephara* (Pellegrino et al., 1999b, 2001), heteromorphic sex chromosomes (including multiple mechanisms of sex determination), supernumerary chromosomes, and two parthenogenetic species in the genera *Gymnophthalmus* and *Leposoma* (for examples see Cole et al., 1990; Yonenaga-Yassuda and Rodrigues, 1999; Pellegrino et al., 2001; Yonenaga-Yassuda et al., 2005).

Despite the relatively small body size (snout-vent length less than 50 mm) of the gymnophthalmid lizards of the genus *Leposoma*, they represent a conspicuous component of the leaf litter of tropical rain forests from Costa Rica throughout the Amazon to the Atlantic slopes of eastern Brazil (Rodrigues,

1997; Rodrigues and Borges, 1997; Rodrigues et al., 2002; Rodrigues and Ávila-Pires, 2005). Traditionally, species of *Leposoma* have been assembled into two groups on the basis of morphological similarity (Ruibal, 1952): *parietale* and *scincoides*. The *parietale* group presently includes 10 bisexual species (*Leposoma parietale*, *Leposoma hexalepis*, *Leposoma ionna*, *Leposoma rugiceps*, *Leposoma southi*, *Leposoma snethlagae*, *Leposoma osvaldoi*, *Leposoma guianense*, and the recently described *Leposoma caparensis* and *Leposoma ferreirai*; Esqueda, 2005; Rodrigues and Ávila-Pires, 2005) and one parthenogenetic species (*Leposoma percarinatum*) and ranges throughout the Amazon to Costa Rica (Uzzell and Barry, 1971; Rodrigues and Ávila-Pires, 2005). The *scincoides* group has five formally recognized species (*Leposoma scincoides*, *Leposoma annectans*, *Leposoma nanodactylus*, *Leposoma puk*, and *Leposoma baturitensis*) that, except for *L. baturitensis* that is endemic to the relic forest in the Caatingas of state of Ceará, are all restricted to the Atlantic forest of eastern Brazil (Rodrigues, 1997; Rodrigues and Borges, 1997; Rodrigues et al., 2002).

The first cytogenetic report on *Leposoma* included data for two species of the *parietale* group (*L. osvaldoi* and *L. guianense*; $2n = 44$, $20M + 24m$) and one of the *scincoides* group (*L. scincoides*, $2n = 52$) (Pellegrino et al., 1999a). After conventional staining, the karyotype of *L. scincoides* with 52 acrocentric/subtelocentric chromosomes of decreasing size was easily distin-

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TABLE 1. Specimen identification (ID), number of metaphases with $2n = 44$ of the total metaphases analyzed in conventional staining (CS) and the number of cells after Ag-NOR staining in two species of *Leposoma*. The percentage (%) of heteromorphic NORs (Rr) and homomorphic NORs (rr) is presented.

Species	ID	CS	Total Ag-NOR	Rr (%)	rr (%)
<i>Leposoma percarinatum</i> ($2n = 44, 20M + 24m$)	MTR 9939	12/57	22	19 (86,4)	3 (13,6)
	MTR 9940	13/52	10	9 (90)	1 (10)
	MTR 9941	18/68	14	13 (92,9)	1 (7,1)
<i>Leposoma ferreirai</i> ($2n = 44, 20M + 24m$)	MTR 9948	3/40	9	1 (11,1)	8 (88,9)
	MTR 9949	26/87	6	0 (0)	6 (100)
	MTR 9950	4/33	4	0 (0)	4 (100)

guishable from those found in species of the *parietale* group with $2n = 44$ represented by 10 pairs of macrochromosomes and 12 pairs of microchromosomes, most of them biarmed. That study revealed a conspicuous karyotypic differentiation between both groups of *Leposoma*, coincident with their strict association with the two different regions of tropical rainforest. Later, Pellegrino et al. (2003) described a triploid karyotype with $3n = 66$ ($30M + 36m$) for three females of the parthenoform *L. percarinatum* from Vila Rica, state of Mato Grosso, Brazil, and hypothesized that the triploid karyotype was the result of a hybridization event between a bisexual species with a *L. guianense/osvaldoi*-like karyotype ($N = 22, 10M + 12m$) and an as-yet undiscovered unisexual diploid cryptic form of the *L. percarinatum* complex. Uzzell and Barry (1971) had already suggested that parthenogenesis in the genus probably originated through hybridization between closely related species, and based on geographic distribution, they suggested *L. guianense* and *L. parietale* as putative candidates for the parental species of *L. percarinatum*.

A field survey conducted in 2005 in the Arquipélago das Anavilhanas, state of Amazonas, in the lower Rio Negro (Brazil), revealed the occurrence of three sympatric (but apparently not syntopic) species of the *parietale* group in that locality: *L. percarinatum*, *L. guianense*, and a recently described bisexual species (*L. ferreirai*), that was suggested to be a possible parental species of the unisexual *L. percarinatum* (Rodrigues and Ávila-Pires, 2005). According to those authors, one possible origin for the diploid form of the *L. percarinatum* complex could be the hybridization between *L. guianense* and *L. ferreirai*, whereas the triploid form found in Mato Grosso (Pellegrino et al., 2003) would represent a further hybridization of this unisexual diploid form and *L. osvaldoi*, because *L. guianense* does not occurs that far south.

Of the three species found in the Anavilhanas area, we obtained chromosomal data for *L. percarinatum* and *L. ferreirai*. The new information, supplemented by additional chromosomal data on *Leposoma* species reported herein, provides new evidence concerning the origin of parthenogenesis in the genus.

MATERIALS AND METHODS

Three females of *L. percarinatum* (MTR 9939, 9940, 9941), collected at the Terra Firme station, Estação Ecológica de Anavilhanas, left bank of Rio Negro ($02^{\circ}29'19''S, 60^{\circ}52'35''W$), and two females and one male of *L. ferreirai* (MTR 9948, 9949, 9950) from Ilha do

Açaí, Rio Negro ($02^{\circ}38'42.4''S, 60^{\circ}45'49.5''W$), both localities in state of Amazonas, Brazil, received provisional ID numbers (MTR). Specimens were deposited at the Herpetological collection at Museu de Zoologia, Universidade de São Paulo (MZUSP), state of São Paulo, Brazil.

Chromosome spreads were obtained from intestine according to Schmid (1978), and chromosomes were analyzed after conventional staining, silver staining of nucleolus organizer regions (Ag-NORs), and CBG banding (King, 1980), following routine techniques. Only metaphases that allowed counting chromosomes with confidence were considered in the analyses.

RESULTS AND DISCUSSION

Cytogenetic analyses of 43 of 177 metaphases of *L. percarinatum* and 33 of 163 metaphases of *L. ferreirai* (Table 1) revealed a diploid number of 44, with 10 pairs of biarmed macrochromosomes and 12 pairs of microchromosomes (Fig. 1A, B). Despite the difficulty in obtaining good quality chromosome spreads in lizards, especially from intestinal epithelial cells, some metaphases clearly revealed the presence of 12 biarmed microchromosome pairs.

Both species of *Leposoma* showed the Ag-NOR regions located on the telomeres of the long arm of one small-sized microchromosome pair (Fig. 1C), and it was possible to identify an intrapopulation heteromorphism in size of Ag-NORs in more than 85% of the cells analyzed for the three specimens of *L. percarinatum* (Table 1; Fig. 1C). The unequal-sized homologous Ag-NORs may be caused by differences either in activity between both NORs or in the number of ribosomal sequence copies between homologues, which may arise by duplication in one of the two NORs (Schmid, 1982) or unequal crossing-over among repeated sequences (Amaro-Ghilardi et al., 2008; Garcia and Moreira-Filho, 2008). With the data available thus far, we cannot reject any of these possibilities to explain the heteromorphism observed in the size of Ag-NORs in the diploid form of *L. percarinatum*. C-banding patterns obtained here for two species of *Leposoma* revealed heterochromatic blocks on the telomeric and pericentromeric regions of macrochromosomes and on telomeric regions of some microchromosomes (Fig. 2A, B). In *L. ferreirai* it was possible to identify centromeric C-bands in some microchromosomes (Fig. 2B).

We are aware that we had access to only three specimens of *L. percarinatum*, all females. Although this sample size is small, all specimens were identical

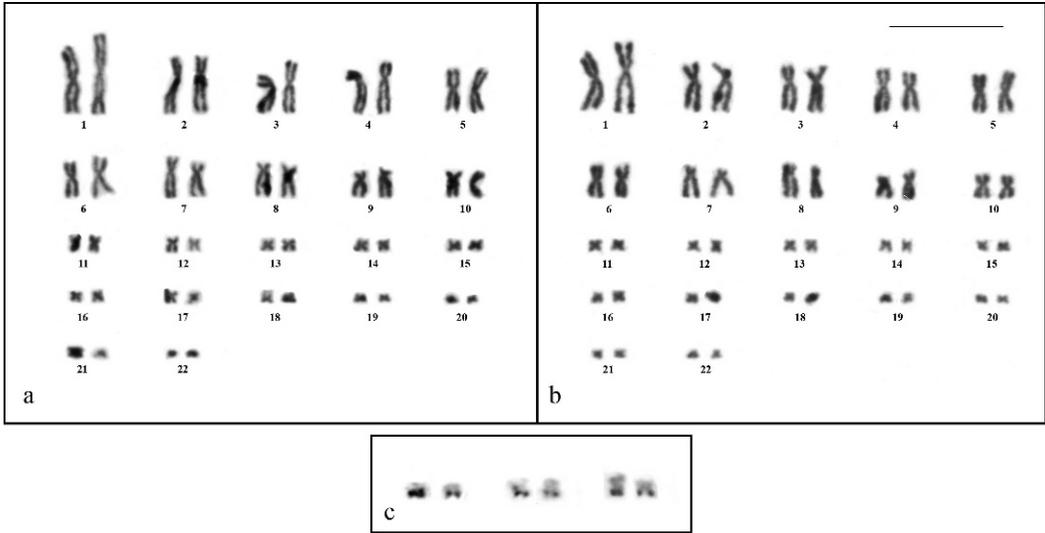


FIG. 1. Karyotypes of *Leposoma* species from Estação Ecológica de Anavilhanas, Amazonas, Brazil. (a) *Leposoma percarinatum*, female, $2n = 44$ (20M + 24m) and (b) *Leposoma ferreirai*, male, $2n = 44$ (20M + 24m), after conventional staining. (c) Ag-NORs on the telomere region of a small-sized microchromosome pair in *L. percarinatum*, showing heteromorphic (left) and homomorphic NORs (middle), and in *Leposoma ferreirai* (right). Bar = 10 μ m.

to those attributed to *L. percarinatum*, from which only females were obtained in all populations studied thus far. Based on this, we assume that the population from Anavilhanas investigated here is also parthenogenetic. The description of a very similar diploid karyotype of $2n = 44$ (20M + 24m) in conventional and differential staining for *L. percarinatum* and its bisexual relative *L. ferreirai*, both sympatric in the lower Rio Negro area (Anavilhanas), provides new insight into the origin of parthenogenesis in *L. percarinatum* and determination

of its putative parental species. We know now that within the unisexual *L. percarinatum* complex, there are both a diploid form found in Anavilhanas area, which is sympatric to *L. ferreirai* and *L. guianense*, and a triploid lineage previously observed in specimens from Vila Rica, state of Mato Grosso, Brazil (Pellegrino et al., 2003). The present data support the hypothesis proposed by Pellegrino et al. (2003) for the existence of a diploid unisexual of the *L. percarinatum* complex and its contribution to the origin of the triploid clone.

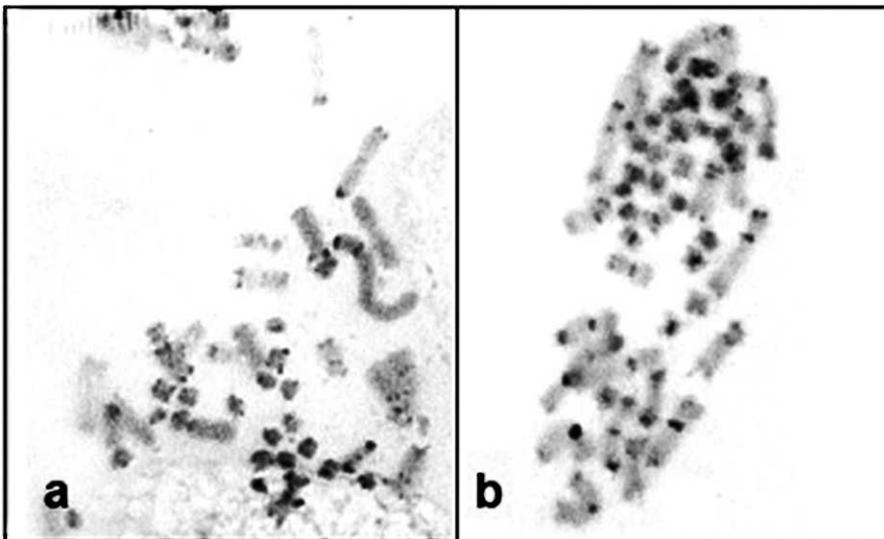


FIG. 2. C-banding patterns in *Leposoma* species. (a) *Leposoma percarinatum*. (b) *Leposoma ferreirai*.

Based on geographic distribution, Rodrigues and Ávila-Pires (2005) proposed a scenario for the evolution of the unisexual diploid clone of the *L. percarinatum* complex through hybridization between *L. ferreirai* and *L. guianense*, which may receive some support from the cytogenetic data reported here. For instance, *L. ferreirai*, *L. guianense*, and the diploid form of *L. percarinatum* share a very similar $2n = 44$ (20M + 24m) karyotype, with banded chromosomes, a single microchromosome pair with Ag-NORs, and also similar C-banding patterns.

Forty cases of natural parthenogenesis are known to date within eight lizard families, and almost all well-studied cases are considered to have had a hybrid origin (Kearney et al., 2009). In the Teiidae, which is the sister family of the Gymnophthalmidae, there are reports on several unisexual species, and all parthenogens have a clonal pattern of inheritance and a hybrid origin (Reeder et al., 2002). Similar to what was suggested for *Leposoma* here, some species of *Cnemidophorus* and *Aspidoscelis* have triploid clonal parthenogens, which were produced by hybridization between a diploid parthenogen of hybrid origin and closely related bisexual ancestors (Cole and Dessauer, 1993; Reeder et al., 2002; Woolley et al., 2004).

Currently, all known specimens (more than a hundred) attributed to *L. percarinatum* are females. Considering that the type locality of the species is Peixe Boi, in the state of Pará, Brazil, from which karyotype data are unknown, further taxonomic work, supported by robust information on geographic distribution of the parthenoforms, is still needed to accurately resolve the taxonomy of this species complex.

Although the present cytogenetic data contribute to the understanding of the origin of parthenogenesis in *Leposoma*, additional chromosomal studies based on differential staining techniques for triploid specimens and other species of *Leposoma* are still needed in order to allow homologies to be inferred with confidence and expand our knowledge about karyotypic evolution in the genus. Along with chromosome data, those results generated on the basis of phylogenetic studies (KCM Pellegrino, MT Rodrigues, JD Harris, Y Yonenaga-Yassuda, and JW Sites Jr., unpubl. data) should improve the search for the parental lineages of the parthenogenetic species in the *L. percarinatum* complex.

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LITERATURE CITED

- AMARO-GHILARDI, R. C., M. J. J. SILVA, M. T. RODRIGUES, AND Y. YONENAGA-YASSUDA. 2008. Chromosomal studies in four species of genus *Chaunus* (Bufonidae, Anura): localization of telomeric and ribosomal sequences after fluorescence *in situ* hybridization (FISH). *Genetica* 134:159–168.
- COLE, C. J., AND H. C. DESSAUER. 1993. Unisexual and bisexual whiptail lizards of the *Cnemidophorus lemniscatus* complex (Squamata: Teiidae) of the Guiana region, South America, with descriptions of new species. *American Museum Novitates* 3081:1–30.
- COLE, C. J., H. C. DESSAUER, C. R. TOWNSEND, AND M. G. ARNOLD. 1990. Unisexual lizards of the genus *Gymnophthalmus* (Reptilia, Teiidae) in the Neotropics: genetics, origin, and systematics. *American Museum Novitates* 2994:1–29.
- ESQUEDA, L. F. 2005. Un nuevo *Leposoma* (Squamata: Gymnophthalmidae) de la reserva forestal de Caparo, Estado Barinas, Venezuela. *Herpetotrópicos* 2:33–42.
- GARCIA, C., AND O. MOREIRA-FILHO. 2008. Localization of ribosomal genes in three *Pimelodus* species (Siluriformes, Pimelodidae) of the São Francisco River: 5S genes as species markers and conservation of the 18S rDNA sites. *Genetics and Molecular Biology* 31:261–264.
- KEARNEY, M., M. K. FUJITA, AND J. RIDENOUR. 2009. Lost sex in reptiles: constraints and correlations. In I. Schön, K. Martens, and P. van Dijk (eds.), *Lost Sex*. Springer Scientific, Dordrecht, Netherlands.
- KING, M. 1980. C-banding studies on Australian hylid frogs: secondary constriction structure and the concept of euchromatin transformation. *Chromosoma* 80:191–217.
- KOK, P. J. R. 2009. Lizard in the clouds: a new highland genus and species of Gymnophthalmidae (Reptilia: Squamata) from Maringma tepui, western Guyana. *Zootaxa* 1992:53–67.
- PELLEGRINO, K. C. M., M. T. RODRIGUES, AND Y. YONENAGA-YASSUDA. 1999a. Chromosomal evolution in Brazilian lizards of genus *Leposoma* (Squamata, Gymnophthalmidae) from Amazon and Atlantic forests: banding patterns and FISH of telomeric sequences. *Hereditas* 131:15–21.
- . 1999b. Chromosomal polymorphisms due to supernumerary chromosomes and pericentric inversions in the eyelidless microteiid lizard *Nothobachia ablephara* (Squamata, Gymnophthalmidae). *Chromosome Research* 7:247–254.
- PELLEGRINO, K. C. M., M. T. RODRIGUES, Y. YONENAGA-YASSUDA, AND J. W. SITES JR. 2001. A molecular perspective on the evolution of microteiid lizards (Squamata, Gymnophthalmidae), and a new classification for the family. *Biological Journal of Linnean Society* 74:317–340.
- PELLEGRINO, K. C. M., M. T. RODRIGUES, AND Y. YONENAGA-YASSUDA. 2003. Triploid karyotype of *Leposoma percarinatum* (Squamata, Gymnophthalmidae). *Journal of Herpetology* 37:197–199.
- REEDER, T. W., C. H. COLE, AND H. C. DESSAUER. 2002. Phylogenetic relationships of whiptail lizards of the genus *Cnemidophorus* (Squamata: Teiidae): a test of monophyly, reevaluation of karyotypic evolution, and review of hybrid origins. *American Museum Novitates* 3365:1–61.
- RODRIGUES, M. T. 1997. A new species of *Leposoma* (Squamata, Gymnophthalmidae) from the Atlantic forest of Brazil. *Herpetologica* 53:383–389.
- RODRIGUES, M. T., AND T. C. ÁVILA-PIRES. 2005. New lizard of the genus *Leposoma* (Squamata, Gymnophthalmidae) from the lower Rio Negro, Ama-

- zonas, Brazil. *Journal of Herpetology* 39:541–546.
- RODRIGUES, M. T., AND D. M. BORGES. 1997. A new species of *Leposoma* (Squamata, Gymnophthalmidae) from a relictual forest in semiarid northeastern Brazil. *Herpetologica* 53:1–6.
- RODRIGUES, M. T., M. DIXO, AND G. M. D. ACCACIO. 2002. A large sample of *Leposoma* (Squamata, Gymnophthalmidae) from the Atlantic forests of Bahia, the status of *Leposoma annectans* Ruibal, 1952, and notes on conservation. *Papéis Avulsos de Zoologia* 42:103–117.
- RODRIGUES, M. T., D. PAVAN, F. CURCIO, V. K. VERDADE, J. CASSIMIRO, AND K. C. M. PELLEGRINO. 2009. A new genus of microteiid lizard from the Caparaó Mountains, southeastern Brazil, with a discussion of relationships among Gymnophthalminae (Squamata). *American Museum Novitates* 3673:1–27.
- RUIBAL, R. 1952. Revisionary studies of some South American Teiidae. *Bulletin of the Museum of Comparative Zoology* 106:407–529.
- SCHMID, M. 1978. Chromosome banding in Amphibia. I. Constitutive heterochromatin and nucleolus organizer regions in *Bufo* and *Hyla*. *Chromosoma* 66:361–388.
- . 1982. Chromosome banding in Amphibia. VII. Analysis of the structure and variability of NORs in Anura. *Chromosoma* 87:327–344.
- UZZELL, T., AND J. C. BARRY. 1971. *Leposoma percarinatum*, a unisexual species related to *L. guianense*, and *Leposoma ioanna*, a new species from Pacific coastal Colômbia (Sauria, Teiidae). *Postilla, Peabody Museum* 154:1–39.
- WOOLLEY, S. C., J. T. SAKATA, AND D. CREWS. 2004. Tracing the evolution of brain and behavior using two related species of whiptail lizards: *Cnemidophorus uniparens* and *Cnemidophorus inornatus*. *ILAR Journal* 45:46–53.
- YONENAGA-YASSUDA, Y., AND M. T. RODRIGUES. 1999. Supernumerary chromosome variation, heteromorphic sex chromosomes and banding patterns in microteiid lizards of the genus *Micrablepharus* (Squamata, Gymnophthalmidae). *Chromosome Research* 7:21–29.
- YONENAGA-YASSUDA, Y., M. T. RODRIGUES, AND K. C. M. PELLEGRINO. 2005. Chromosomal banding patterns in the eyelid-less microteiid radiation: the $X_1X_2Y:X_1X_1X_2X_2$ sex chromosome system in *Calypotommatus*, and the karyotypes of *Psilophthalmus* and *Tretioscincus* (Squamata, Gymnophthalmidae). *Genetics and Molecular Biology* 28:700–709.

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