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Pericentric inversion events in karyotypic distinction of Brazilian lizards of genus *Phyllopezus* (Squamata, Gekkonidae) detected by chromosomal banding patterns

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Cytogenetic investigations based on conventional and differential staining analysis (C- and replication R-banding and Ag-staining) were carried out on eight specimens of *Phyllopezus periosus*, 17 of *P. pollicaris pollicaris*, and one of *P. pollicaris przewalskii* collected from different localities of Brazil. *P. periosus* and *P. p. pollicaris* share the same diploid number of $2n = 40$ chromosomes, and their karyotypes are very distinctive regarding to the number of biarmed and uniarmed chromosomes. After careful side-by-side comparison of R-banded chromosomes in both taxa, pronounced homology between, at least, eight pairs was revealed. The R-banding patterns allowed us to postulate that karyotype differentiation could be due to pericentric inversion events. *P. p. przewalskii* ($2n = 38$) exhibited a very similar karyotype to that found in *P. p. pollicaris*, except for the presence of one metacentric pair, which probably resulted from a Robertsonian rearrangement. Single and multiple pairs of NOR-bearing chromosomes, showing variation in number and location, were detected among the three forms of *Phyllopezus*. Similar C-banding patterns were found in *P. periosus* and *P. p. pollicaris*. Sex chromosomes were not positively identified.

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The lizards of the genus *Phyllopezus* are widespread along the large diagonal belt of open formations in South America ranging from Northeastern Brazil to Argentina. To this time, two species have been recognized: *P. pollicaris* and *P. periosus*. *P. pollicaris* encompasses an enormous range, occurring from the semiarid Caatingas of Northeastern Brazil to the Chaco in central Argentina. According to the last systematic revision (VANZOLINI 1953), two subspecies of *P. pollicaris* are recognized: the nominal form in the Caatingas of Northeastern Brazil and *przewalskii* in the Chaco, Pantanal, and part of central Brazilian Cerrados. RODRIGUES (1986) described *P. periosus*, one of the largest South American gekkos, from Cabaceiras in the State of Paraíba, Northeastern Brazil. This species occurs in a geographically restricted area and was only found in a few localities in the Caatinga, where it is syntopic with *P. pollicaris pollicaris*.

Gekkota comprises approximately 950 species presently arranged in about 100 genera, and about 200 of these species are restricted to the Neotropical region (KLUGE 1991). Fewer than 20 Neotropical species have been karyotyped. Most reports of gekkonid karyotypes refer to Gekkoninae, with about 74 species investigated (KING 1990). Only a few of these descriptions have been based on differential staining techniques (OLMO 1986),

Several cases of intraspecific variability in chromosome number and morphology have been reported for Australian species belonging to the genera *Gehyra* and *Heteronotia* (OLMO 1986). In most of these investigations, the application of banding techniques has allowed a better comprehension of the mechanisms responsible for the high level of chromosomal variability.

Herein, we describe, for the first time, the karyotypes of *Phyllopezus periosus*, *P. pollicaris pollicaris* and *P. pollicaris przewalskii*, after applying conventional and banding techniques. These data contribute to clarify the chromosomal differentiation of the three forms of the genus *Phyllopezus* and bring more information concerning the systematic status of the allopatric populations of *P. pollicaris*.

MATERIAL AND METHODS

Five males and three females of *Phyllopezus periosus*; nine males, five females and three embryos of *P. pollicaris pollicaris*, and one male of *P. pollicaris*

Table 1. *Variable number and location of Ag-NORs in Phyllopezus pollicaris pollicaris (2n = 40) from five localities of Northeastern Brazil*

Specimen number	Sex	Maximum number of Ag-NOR	NOR-bearing chromosomes	Locality
L 151	M	2	6q 6q	Cabaceiras (PB)
L 152	M			07°29′S, 36°17′W
L 154	F			
LG 527	M			
LG 528	M			
L 110	M	4	6q 6q aq aq	Manga (BA)
				11°28′S, 44°00′W
L 155	F			Alagoado (BA)
				09°29′S, 41°21′W
LG 922	M			Xingó (AL)
				09°36′S, 37°49′W
L 149	F	4	aq aq aq aq	Cabaceiras (PB)
LG 189	M			Cabaceiras (PB)
LG 1010	M			Xingó (AL)
LG 1008	F	5	Aq 6q 6q aq aq	Xingó (AL)
LG 291	E	6	6p 6p 6q 6q aq aq	Santo Inácio (BA)
LG 259	E			11°06′S, 42°44′W
LG 577	M	6	Aq Aq Aq 6p 6q 6q	Alagoado (BA)
LG 290	E	8	Aq Aq 6p 6p 6q 6q aq aq	Santo Inácio (BA)
*LG 181	F	–	–	Cabaceiras (PB)

(M) = male, (F) = female, (E) = embryo
p = short arm; q = long arm
6p = Ag-NOR on the short arm of pair 6
6q = Ag-NOR on the long arm of pair 6
Aq = Ag-NOR on the long arm of a large acrocentric chromosome
aq = Ag-NOR on the long arm of a small acrocentric chromosome
*Ag-NOR not available for this specimen

przewalskii were cytogenetically analyzed. All specimens of *P. periosus* were collected from Cabaceiras (07°29′S, 36°17′W), State of Paraíba, and *P. p. przewalskii* was collected at Rio Negro (19°27′S, 54°58′W), State of Mato Grosso do Sul. *P. p. pollicaris* was obtained in five localities of Northeastern Brazil (Table 1). The voucher specimens were deposited in the Museu de Zoologia, Universidade de São Paulo (MZUSP), State of São Paulo, Brazil.

Standard procedures were used for preparing chromosomes from bone marrow, spleen, liver cells, and testis (KASAHARA et al. 1987). Chromosome preparations from fibroblast cultures were also used as described in YONENAGA-YASSUDA et al. (1988). Chromosomes were analyzed after conventional and differential staining (CBG-banding and Ag-staining) according to routine techniques. Replication R-banding, after in vitro treatment with 5-bromodeoxyuridine (BrdU) was carried out following FPG staining (DUTRILLAUX and COUTURIER 1981).

RESULTS

Phyllopezus presents a typical gekkonid karyotype with chromosomes decreasing in size, without distinction between macro- and microchromosomes. Heteromorphic sex chromosomes were not detected in any of the three forms.

Phyllopezus periosus (2n = 40)

The karyotype is formed by 18 subtelocentrics (pairs 2 to 19) with small short arms, one large submetacentric pair 1, and by the smallest biarmed pair 20 (Fig. 1a). In 80 metaphases, the NORs were located on the short arm of a medium-sized pair, which is not morphologically identified (Fig. 2a). C-banding revealed a small amount of constitutive heterochromatin in the telomeric regions of most of the larger chromosomes. The small-sized chromosomes seemed to lack heterochromatin (Fig. 3a). In testis spreads, diplotene cells with 20 bivalents or metaphases II with 20 chromosomes were found (Fig. 4a, 4b).

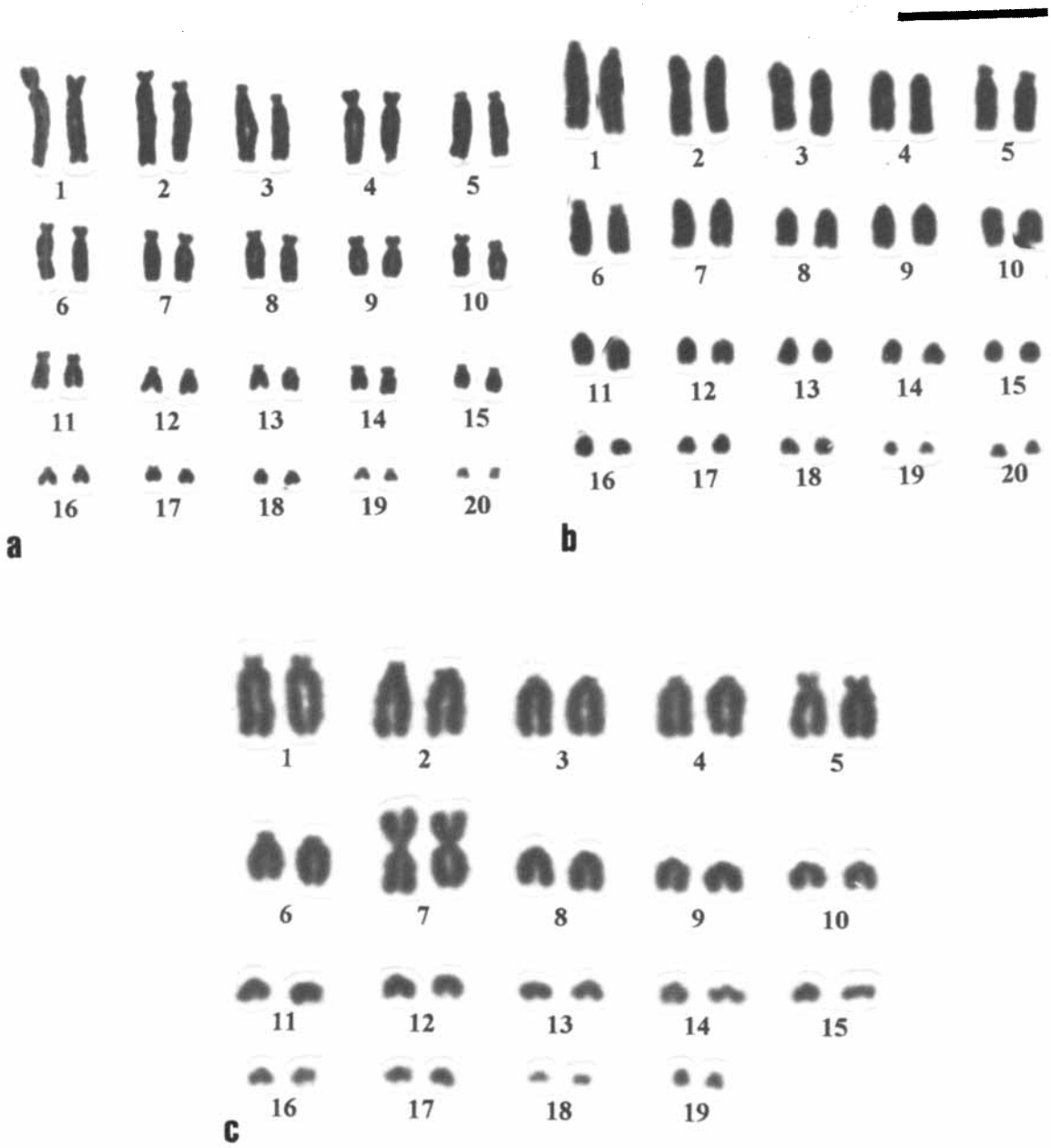

Fig. 1a–c. Conventional Giemsa-staining karyotypes. **a** Male of *Phyllopezus periosus*. **b** Embryo of *P. pollicaris pollicaris*. **c** Male of *P. pollicaris przewalskii*. Bar = 10 μm.

Phyllopezus pollicaris pollicaris (2n = 40)

The karyotype consists of 16 acrocentrics (pairs 2 to 4, and 7 to 19), three subtelocentrics (pairs 1, 5, and 6), and one small biarmed pair 20 (Fig. 1b). One specimen from Xingó showed heteromorphic pair 1, with one acrocentric and one subtelocentric chromosomes. In meiosis of male specimens, 20 bivalents in diplotene and 20 chromosomes at metaphase II were observed.

Interindividual variability in number and position of Ag-NOR was detected in *P. p. pollicaris*. Ag-NORs ranged from 2 to 8 on telomeric regions of some chromosomes in 273 metaphases in 16 specimens examined (Table 1). The only recognizable NOR-bearing chromosomes were those of subtelocentric pair 6, which harboured NORs on the telomere of one or both arms. Three large and four small acrocentric NOR-bearing chromosomes were also observed (Fig. 5). Faintly stained heterochromatin oc-

curred predominantly in the telomeric regions of most chromosomes, and some of them also showed faint C-bands at the centromeric regions (Fig. 3b).

Comparative R-banding patterns

A comparison between the R-banding patterns of *P. periosus* and *P. p. pollicaris* revealed a high level of chromosome homology. Pairs 5, 6, 13, and 16 to 20 of *P. periosus* are identical to the same pairs of *P. p. pollicaris*. The difference between the two karyotypes is due to small pericentric inversions that have changed the morphology of chromosome pairs 1 to 4, 7 to 12, 14 and 15 (Fig. 6).

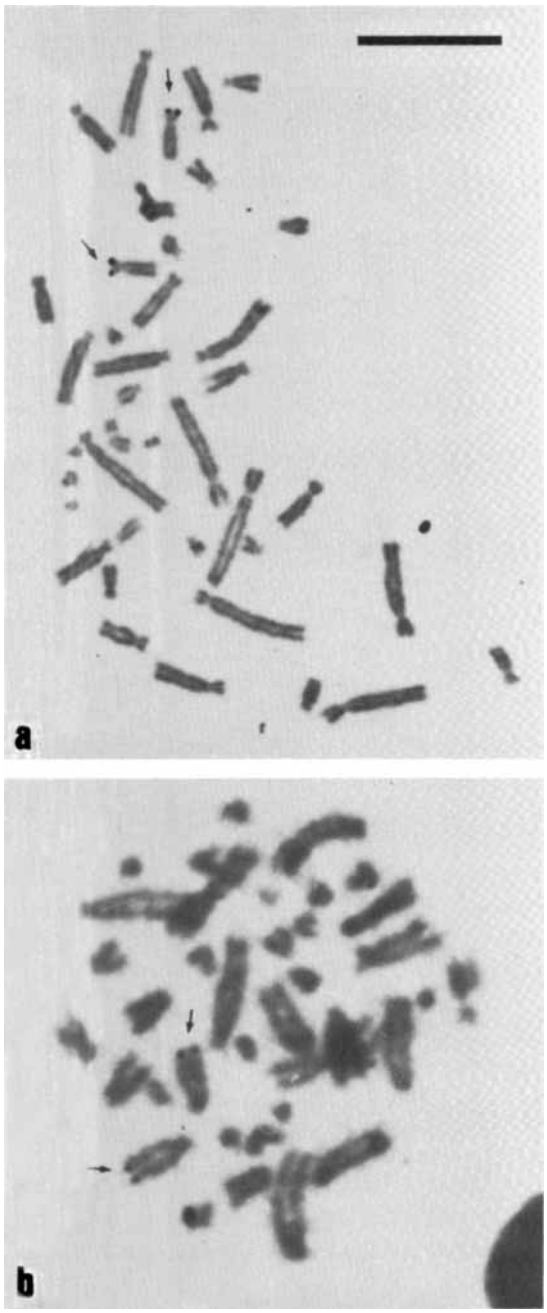

Fig. 2a and b. Metaphases after silver staining. **a** *Phyllopezus periosus* with Ag-NORs on the short arm of one medium-sized subtelocentric pair (arrows). **b** *P. pollicaris przewalskii* with Ag-NORs on the long arm of one medium-sized acrocentric pair (arrows). Bar = 10 μm.

Fig. 3a and b. C-banding patterns showing slightly stained heterochromatin. **a** *Phyllopezus periosus*. **b** *P. pollicaris pollicaris*. Bar = 10 μm.

Phyllopezus pollicaris przewalskii (2n = 38)

The *P. p. przewalskii* karyotype comprises acrocentric pairs 2 to 4, 8 to 18, subtelocentric pairs 1, 5, and 6, and metacentric pair 7. The characteristic metacentric 7 is probably the result of a Robertsonian rearrangement involving two medium-sized acrocentrics. The small pair 19 is biarmed (Fig. 1c). The analysis of ten Ag-NOR stained metaphases revealed NORs on the telomeric region of the long arm of an unidentified medium-sized acrocentric pair (Fig. 2b). Meiotic analysis detected 19 bivalents in diplotene cells (Fig. 4c).

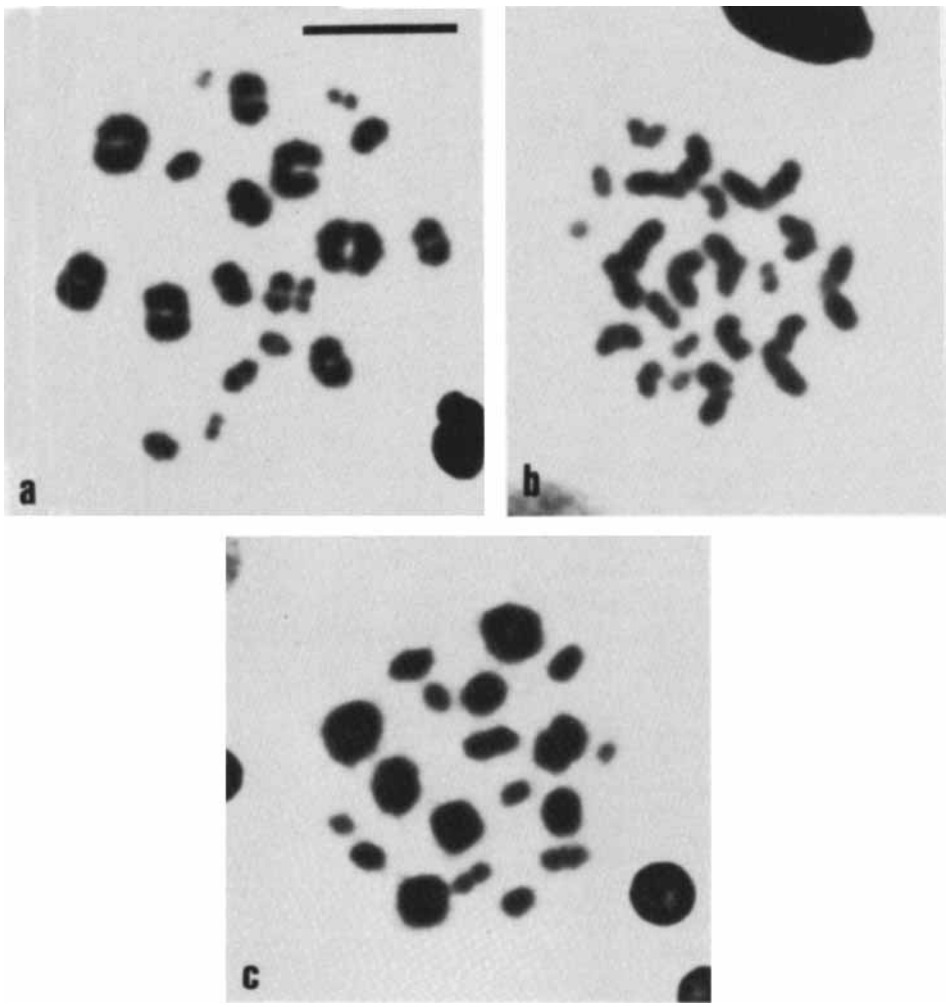

Fig. 4a–c. Meiotic cells of *Phyllopezus* male specimens. **a and b** Diplotene and metaphase II of *P. pollicaris pollicaris* with 20 bivalents and 20 chromosomes, respectively. **c** Diplotene cell of *P. pollicaris przewalskii* with 19 bivalents. Bar = 10 μm.

DISCUSSION

The three gekkos of the genus *Phyllopezus*, *P. periosus*, *P. p. pollicaris*, and *P. p. przewalskii*, exhibited distinctive karyotypes. Conventional staining as well as the analysis of banding patterns, suggested that pericentric inversions and Robertsonian mechanisms have been the events responsible for the differentiation among these karyotypes.

A detailed comparison between the 2n = 40 karyotypes of *P. periosus* and *P. p. pollicaris* shows that at least eight chromosome pairs (5, 6, 13, and 16 to 20) are morphologically indistinguishable in both forms. A comparative analysis of R-banded pairs revealed differences in the number of biarmed and uniarmed chromosomes due to the occurrence of twelve small pericentric inversions.

Although *P. periosus* and *P. p. pollicaris* have very similar C-banding patterns, characterized by faintly stained heterochromatin, a discrepancy with respect to the NORs was detected. While *P. periosus* has a single pair of NORs, *P. p. pollicaris* displays extensive variability regarding the number and locations of NORs. The most constant pattern in *P. p. pollicaris* was the presence of NORs in one or both arms of pair 6 (Table 1). The NOR-bearing chromosomes between *P. periosus* and *P. p. pollicaris* are dissimilar, reinforcing the distinctiveness of both karyotypes.

Contrary to mammalian species, NOR variability among squamates is limited (PORTER et al. 1991). Nevertheless, multiple NORs as well as variation in their location have been reported in lizard species (MORITZ 1986; SITES et al. 1990; YONENAGA-YASSUDA et al. 1995).

Sex chromosomes were not detected in *Phyllopezus*. The examination of meiotic cells confirmed the absence of heteromorphic bivalents related to sex. Reports on sexual mechanism with heteromorphic sex

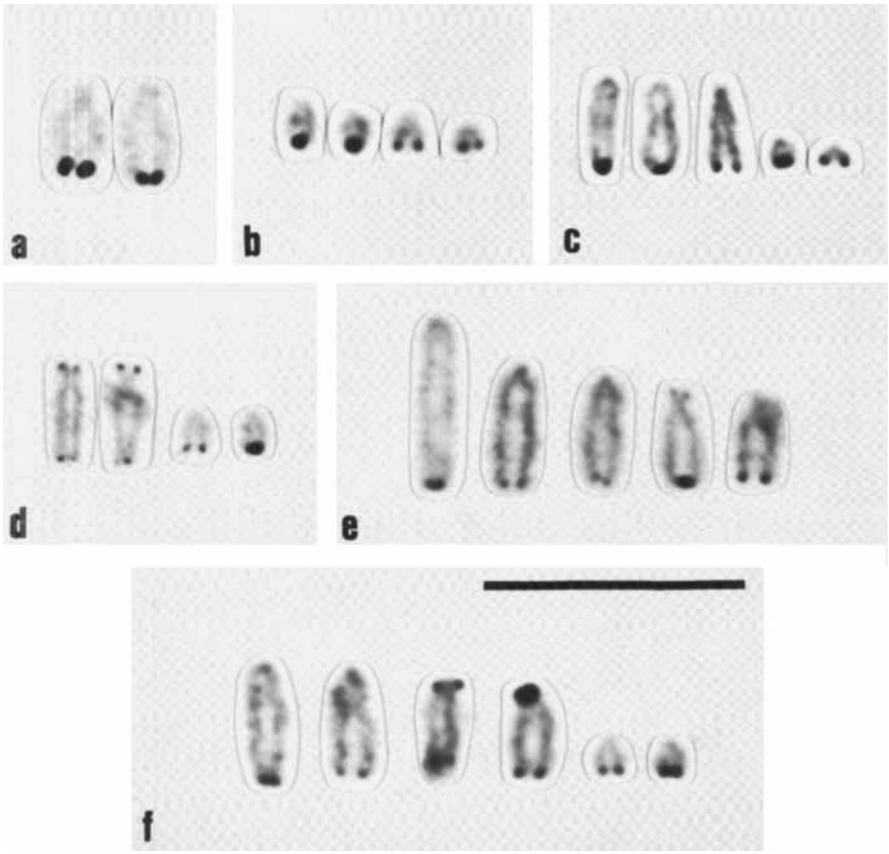

Fig. 5a–f. Ag-NORs variability in *Phyllopezus pollicaris pollicaris*. **a** 2 NORs: on the long arm of pair 6. **b** 4 NORs: on the long arm of four small acrocentrics. **c** 5 NORs: on the long arm of two large acrocentrics, on the long arm of one homologous of pair 6, on the long arm of two small acrocentrics. **d and e** 6 NORs: on both arms of pair 6, on the long arm of two small acrocentrics, and on the long arm of three large acrocentrics, on the short arm of one homologous of pair 6, on the long arm of pair 6, respectively. **f** 8 NORs: on the long arm of two large acrocentrics, on both arms of pair 6, on the long arm of two small acrocentrics. Bar = 10 μm.

chromosomes are scarce among gekkos with only few references of ZZ:ZW and XX:XY sexual mechanisms (MORITZ 1990).

The subspecies of *P. pollicaris* have different diploid numbers: *P. p. pollicaris* has 2n = 40 chromosomes and *P. p. przewalskii* has 2n = 38 chromosomes. The karyotype of *P. p. przewalskii* revealed a large metacentric pair not observed in any of the 17 specimens of *P. p. pollicaris* analyzed. The metacentric pair of *P. p. przewalskii* is probably the result of a Robertsonian fusion involving pairs 9, 10, or 11 of the *P. p. pollicaris* karyotype. This hypothesis is in agreement with the suggestion of OLMO (1986) that the main trend in chromosomal evolution within gekkos is towards the progressive reduction of the diploid number by centric fusion. The remaining chromosome pairs seem to be invariable in the karyotypes of *P. p. pollicaris* and *P. p. przewalskii*. Pairs 5, 6, and 20 of *P. periosus* and *P. p. pollicaris* are present in the karyotype of *P. p. przewalskii*, corresponding to pairs 5, 6, and 19, respectively. This is consistent with the conservation of at least three chromosome pairs in the three forms of *Phyllopezus*.

It is likely that *P. p. pollicaris* and *P. p. przewalskii* are closely related with *P. periosus* as their sister group. On this basis we postulate that the 2n = 40 karyotype is the ancestral condition for *Phyllopezus*. Reinforcing this assumption, *Bogertia*, the monotypic South American genus most closely related to *Phyllopezus*, has also a 2n = 40 karyotype which is very similar to that of *P. p. pollicaris* (PELLEGRINO unpublished data). The findings suggest that the *P. p. przewalskii* karyotype is the most derived condition for *Phyllopezus*.

While *P. p. pollicaris* showed a peculiar NOR variation in a sample of 16 specimens, only one specimen of *P. p. przewalskii* exhibited a single NOR-bearing pair. Based on morphology and size, it is

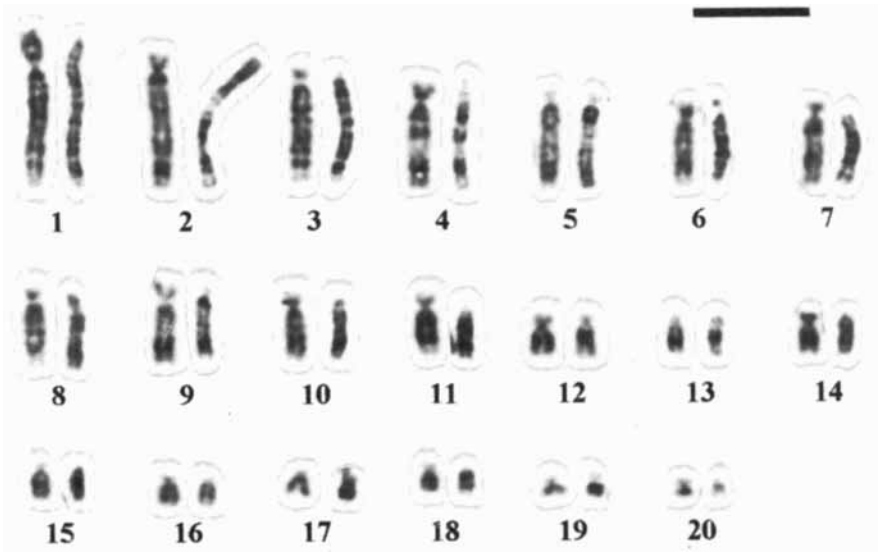

Fig. 6. Haploid sets of chromosomes, after replication R-banding, belonging to *Phyllopezus periosus* (at left) and *P. pollicaris pollicaris* (at right) arranged side-by-side. Bar = 10 μm.

possible that this *P. p. przewalskii* pair corresponds to pair 6 observed in the karyotype of *P. p. pollicaris*. A similar variability in the NOR number and distribution might be found in an enlarged sample of *P. p. przewalskii*.

P. p. pollicaris and *P. p. przewalskii* are morphologically recognized subspecies which can also be distinguished by their karyotype constitution. This may suggest that the Robertsonian fusion in *P. p. przewalskii* is responsible for the geographical karyotype differentiation between the two subspecies. Because just one specimen of *P. p. przewalskii* was karyotyped, it cannot be excluded that the Rio Negro population of *P. p. przewalskii* is polymorphic with respect to this character. The cytogenetic study of more specimens of *P. p. przewalskii* and a detailed systematic revision of the different forms of *P. pollicaris* might provide additional support to the hypothesis that we are, in fact, dealing with two different species.

It is evident that, if we want to use cytogenetic data in a phylogenetic context, the karyotypes have to be "decomposed" into separate characters (KLUGE 1994), that is, only homologous bands representing different characters should be compared. In this way, a frequent application of differential staining techniques is crucial to elucidate relationships among lizard species.

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