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Comparative cytogenetics of two species of ground skinks: *Scincella assata* and *S. cherriei* (Squamata: Scincidae: Lygosominae) from Chiapas, Mexico

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Abstract. Standard karyotypes of two species of the genus *Scincella, S. assata* and *S. cherriei,* both from Chiapas State, Mexico, were described for the first time. The diploid chromosome number was 28 in *S. assata*, whereas 30 in *S. cherriei*. The karyotypes of the two species, while differing in the number of microchromosomes, 14-15 in *S. assata* and 16-17 in *S. cherriei*, share four pairs of large metacentric, two pairs of medium-sized metacentric, and one particular pair (number 7) of chromosomes. Female *S. assata* carries chromosome pair 7 composed of two identical medium-sized subtelocentric chromosomes. This chromosome pair is heteromorphic in males of both species, i.e., one component of the pair is similar to the homomorphic chromosomes 7 of the *S. assata* female, while the other is nearly one-half the size of its counterpart and resembles a microchromosome. The homology of such externally different elements is deducted from the presence of an asymmetric bivalent in spermatocytes at diplotene-diakinesis. Female *S. cherriei* was not available. We suspect that the two *Scincella* species possess an XY sex determination system, as previously reported for the North American congeneric species, *S. lateralis.*

Key words. Mexican reptiles, sex chromosomes, *Sphenomorphus*.

Lizards of the scincid genus *Scincella* are small semi-fossorial ground skinks. The genus was originally established to accommodate species occurring almost exclusively in Central, East, and Southeast Asia (Greer, 1974; Ouboter, 1986), but a number of species are currently recognized in the New World (García-Vázquez and Feria-Ortiz, 2006; García-Vázquez et al., 2010; Uetz, 2012), which were thought to have resulted from a single dispersal event across the Bering land bridge during the Miocene (Honda et al., 2003; Macey et al., 2006). At present, Mexican herpetofauna includes seven *Scincella* species, which had formerly been regarded as the New World members of *Sphenomorphus*, but reassigned to *Scincella* on the basis of molecular phylogenetic analyses (Honda et al., 2003; Linkem et al., 2011). The two sister species *Scincella assata* (Cope, 1864) and *S. cherriei* (Cope, 1893) belong to this group.

Chromosomal data are known for only one of the total of 35 currently recognized species of the genus *Scincella* - the North-American *S. lateralis* (Say, 1823), in which a 2n = 30/29 karyotype and a male heterogamety with intraspecific variation were reported (Wright, 1973;

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Hedin et al., 1990). Karyological data are also available for four species of *Sphenomorphus* "*sensu lato*", a paraphyletic assemblage, which currently includes 122 species widely distributed in Asia and Australia (Uetz, 2012). In this latter grouping, the three karyotyped species, the Asian *Sph. indicus* (Gray, 1853) and *Sph. incognitus* (Thompson, 1912) (Ota and Lue, 1994) and the Australian *Eulamprus* (*Sphenomorphus*) *tympanum* (Lönnberg and Andersson, 1915) (Wright, 1973), have mainly 2n = 30 karyotype, but, in *Sph. indicus,* 2n = 28 (Makino and Momma, 1949; Yang et al., 1989) and 2n = 26 (Guo and Dong, 1988) karyotypes were also described in the earlier reports. In these species, no sex chromosome heteromorphisms have been asserted.

Here, we describe karyotypes of *Scincella assata* and *S. cherriei* and provide comparative cytogenetic analysis of these and related taxa. Specimens were identified on the basis of cephalic lepidosis and body coloration (Cope, 1864; Stuart, 1940; Kohler, 2008). One male (RCMX 85) and two females (RCMX 86, RCMX 92) identified as *S. assata* were collected in the leaf litter along a dry small stream in a dry tropical forest of the "La Sepultura" Biosphere Reserve, Chiapas State, Mexico (16°20'42" N, 93°50'26" W), and two males (RCMX 219, RCMX 235) identified as *S. cherriei*, were collected in the evergreen tropical forest of the "Montes Azules" Biosphere reserve, Selva Lacandona, Chiapas State, Mexico (16°06'44"N, 90°56'26"W) (Figure 1). Vouchers are deposited at the Herpetological Collection of the Comparative Anatomy Laboratory, "Charles Darwin" Department of Biology and Biotechnologies, "La Sapienza" University of Rome, Italy, and in the Museo de Zoologia "Alfonso L. Herrera", Universidad Nacional Autónoma de México, Mexico City.

For chromosomal analysis, femurs and vertebral column were removed from each animal one hour after an intra-peritoneal injection of a 0.1% solution of Vinblastine sulphate (Lilly); also, testes were taken from the males. The samples were processed following the procedure described in Castiglia et al. (2010). For each specimen, about 10 metaphases were photographed after conventional Giemsa staining.

The diploid numbers of chromosomes of *S. assata* and *S. cherriei* were found to be, respectively, 2n = 28 and 2n = 30. The karyotypes of both species are composed of four pairs of large and two pairs of medium-sized metacentric chromosomes (pairs 1-6), as well as one or two small to medium-sized subtelocentric chromosomes (pair 7), and a series of microchromosomes (Figure 2). In *S. assata*, females possess two apparently identical subtelocentric chromosomes for pair 7 and 14 microchromosomes (Figure 2a), while the male shows only one chro-

Figure 1. Above, *Scincella assata* from "La Sepultura" Biosphere Reserve (Chiapas, Mexico) (photo A.M.R. Bezerra). Below, *S. cherriei* from "Montes Azules" Biosphere reserve, Selva Lacandona (Chiapas, Mexico) (photo R. Castiglia).

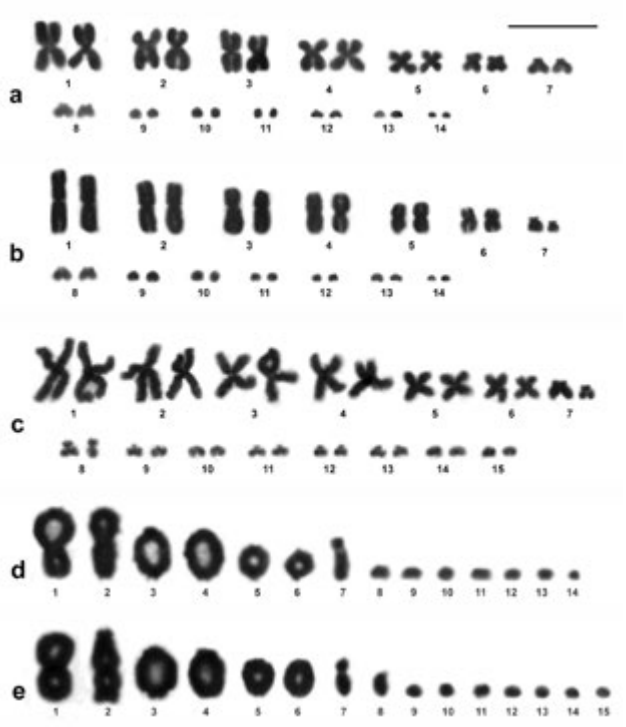

Figure 2. Karyotypes of (**a**) female (RCMX 92) and (**b**) male (RCMX 85) of *Scincella assata* (2n = 28) and of (**c**) male (RCMX 235) of *S. cherriei* (2n = 30). Diplotene-diakinesis bivalents of the males of (**d**) *S. assata* (RCMX 85) and (**e**) *S. cherriei* (RCMX 219). Note heteromorphic chromosomes 7 and corresponding asymmetric bivalents. Bar shows 10 μm.

mosome 7 and 15 microchromosomes (Figure 2b). Of these microchromosomes of the male *S. assata*, the largest one does not pair with either of the remainder. This feature of male karyotype of *S. assata* is shared with that of *S. cherriei*, although they differ from each other by the total number of microchromosomes, 15 in the former and 17 in the latter (Figure 2c). The unpaired microchromosome in each male karyotype may be the counterpart of the single subtelocentric chromosome 7, although the former is nearly one-half in size of the latter. In the analyses of male meiosis at diplotene-diakinesis for both species, we identified four large and two medium-sized bivalents plus one small asymmetric bivalent, as well as seven (*S. assata*) or eight (*S. cherriei*) micro-bivalents (Figure 2d and e).

Generally, karyotypes of the scincid lizards are composed of 2n = 26, 28, 30, or 32 chromosomes that form three distinct size-groups (large and small biarmed macrochromosomes, and a series of microchromosomes), with the exception of the *Eutropis* [formerly *Mabuya*] *macularia* species complex, which possesses 2n = 34 or 38 chromosomes forming a continuous series (Ota et al., 1996; 2001). Most scincid karyotypes so far studied can be assigned to one of the two main groups: the first group, represented by most species of the subfamily Lygogominae and a number of species of the subfamily Scincinae (*sensu* Greer, 1970), is distinguished by four pairs of distinctly large biarmed chromosomes, while the second group, accommodating some other scincine species, is characterized by no more than two pairs of distinctly large metacentric chromosomes (Giovannotti et al., 2009). For instance, two Asian lygosomine species, *Sphenomorphus indicus* and *Sph. incognitus,* possess four pairs of large and three pairs of distinctly smaller metacentric macrochromosomes and 8 pairs of microchromosomes (Ota and Lue, 1994).

The presently studied karyotypes of *S. assata* and *S. cherriei* both conform to the arrangement typical of scincid karyotype with four pairs of large biarmed chromosomes. The difference in diploid numbers between the two sister species is undoubtedly due to a lack of one pair of microchromosomes in *S. assata*. Reduction in the number of microchromosome pairs seems to have frequently occurred in reptiles. Microchromosomes may fuse together, or be converted into macrochromosomes by addition of heterochromatin, or be even translocated onto a macrochromosomal pair, as was reported for the Australian skink *Lampropholis delicata* (Donnellan, 1991).

It was not possible to specify the pair of microchromosomes to which the difference in chromosome numbers between *S. assata* and *S. cherriei* could be attributed, since the morphology of microchromosomes was resolved, to some extent, only in the metaphase chromosomes of *S. cherriei*. Actually, there are a few data reporting morphology of microchromosomes, e.g., Castiglia et al. (2006) and the recent analyses of slightly extended DAPI stained chromosomes carried out in several skink species including two lygosomines, *Lepidothyris fernandi* (2n = 30) and *Trachylepis quinquetaeniata* (2n = 32) (Giovannotti et al. 2009). However, in the congeneric species *Scincella lateralis,* which is the closest relative to the clade of the presently studied species, this task has been computed by synaptonemal complex (SC) karyotype analysis that revealed the exact position of all centromeres, thus recognizing one metacentric and seven acrocentric small microchromosomal bivalents, in addition to four large and two medium-sized metacentric bivalents (Hedin et al., 1990). This pattern corresponds very well with the one presently observed in *S. cherriei*.

Scincella assata diverges for the diploid number of chromosomes not only from the congeneric species, *S. cherriei* and *S. lateralis*, but also from the other species of the *Sphenomorphus* group (Greer, 1979; Honda et al., 2000; Reeder, 2003), matching only one karyotype reported for a population of *Sph. indicus* from Sichuan, China (Guo and Dong, 1988; Yang et al., 1989) and for one of *Tropidophorus* (Ota et al., 1991), a genus generally assigned to the *Sphenomorphus* group (e.g., Honda et al., 2000). If 2n = 30 is considered the ancestral chromosome number (Honda et al., 2000) in the *Sphenomorphus* group, reduction of the diploid chromosome number observed in *S. assata* and *Sph. indicus* most probably has independent origin, as the two species are phylogenetically distant (Linkem et al., 2011).

Finally, males of both species studied here have remarkably heteromorphic chromosome pair 7, which is unequivocally homomorphic in females of *S. assata* (females of *S. cherriei* were not available to us: see above), suggesting the presence of an XY sex chromosome system as in another New World congener, *S. lateralis* (Wright, 1973). The present hypothesis is worth investigating, considering that the male represents the heterogametic sex in the skinks where heteromorphic sex chromosomes occur (Olmo and Signorino, 2005). Further studies, examining more specimens of the two species, female *S. cherriei* in particular, are desirable to verify this idea.

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