



Short Communication

The karyotype of *Adenomera diptyx* (Boettger 1885) (Anura, Leptodactylidae) from northeastern Argentina

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Abstract

In this work we analyzed the karyotype of five populations of *Adenomera diptyx* from Argentina after conventional staining, Ag-NOR and C-banding. All specimens presented $2n = 26$ and $FN = 34$. The karyotype was formed by three submetacentric, one metacentric and nine telocentric pairs. Silver staining revealed that the NOR was located on a secondary constriction in pair 7. C-banding evidenced constitutive heterochromatin at the pericentromeric region of all chromosomes. The karyotype of *A. diptyx* was similar to that of *A. hylaedactyla* ($2n = 26$, $FN = 34$) and different from that of *A. andreae* ($2n = 26$, $FN = 40$) in the fundamental number and secondary constriction position. It also differed from the karyotypes of *A. marmorata* ($2n = 24$, $FN = 34$ and 36) and of *A. aff. bokermanni* ($2n = 23$, $FN = 34$) in diploid number. Until a comprehensive cytogenetic analysis of all the species of the genus is performed, their chromosome evolution will remain poorly understood.

Key words: *Adenomera diptyx*, Argentina, standard karyotype, Ag-NOR, C-bands.

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The genus *Adenomera* Fitzinger in Steindachner, 1867 was revalidated by Heyer (1974) to include the species of *Leptodactylus* belonging to the *marmoratus* group (Heyer, 1969). At present, the relationships between *Adenomera* and *Leptodactylus* are subject to debate and several authors advanced different taxonomic proposals. Frost *et al.* (2006) considered *Adenomera* as a synonym of *Leptodactylus*, suggesting the creation of the subgenus *Leptodactylus* (*Lithodytes*), which would include the species of the former genera *Lithodytes* and *Adenomera*. Some authors refer to the species of *Adenomera* as the *Leptodactylus marmoratus* species group *sensu* Heyer (1973) (Almeida and Angulo, 2006; Angulo and Reichle, 2008; Campos *et al.*, 2009), whereas others recognize *Adenomera* as a valid genus (Kwet, 2007; Kwet *et al.*, 2009; Ponssa and Heyer, 2007). In this paper, we adopted the latter systematic approach because there is morphological, ethological, and bioacoustic evidence suggesting that all species of *Adenomera* may form a natural group with a single ancestral lineage (Kwet *et al.*, 2009).

Adenomera occurs east of the Andes, from northern South America to northeastern Argentina and southern Brazil. Currently, 15 species of *Adenomera* are recognized,

most of them described or revalidated in recent years: *A. andreae*, *A. hylaedactyla*, *A. diptyx*, *A. thomei*, *A. heyeri*, *A. araucaria*, *A. lutzi*, *A. martinezi*, *A. bokermanni*, *A. marmorata*, *A. nana*, *A. ajurauna*, *A. coca*, *A. engelsi*, and *A. simonstuarti* (Angulo and Icochea, 2010).

Only five species had their karyotype described: *A. andreae* ($2n = 26$, $FN = 40$), *A. hylaedactyla* ($2n = 26$, $FN = 34$), *A. lutzi* ($2n = 26$), *A. bokermanni* ($2n = 23$, $FN = 34$), and *A. marmorata* ($2n = 24$, $FN = 34$ and 36) (Bogart, 1974; Kuramoto, 1990; Campos *et al.*, 2009).

The karyotypes of *A. aff. bokermanni*, *A. hylaedactyla* and of taxa belonging to the *A. marmorata* species-complex were recently analyzed after silver staining of the NORs, C-banding, fluorochrome staining and FISH (Campos *et al.*, 2009). The telocentric pairs 6, 7 and 11 were reported as NOR-bearing chromosomes and all chromosomes presented pericentromeric C-bands (Campos *et al.*, 2009).

The species identity in the genus *Adenomera* is hard to resolve because of the intra- and inter-populational morphological variation and due to the existence of cryptic species (Heyer, 1984; de la Riva, 1996; Angulo *et al.*, 2003). To clarify the systematics of the genus, it is necessary to use non-morphological characters, such as advertisement calls, cytogenetic and molecular data (Heyer, 1984).

In this work we describe the karyotype, Ag-NOR location and C-banding patterns of five *Adenomera* populations from northeastern Argentina currently recognized as

A. diptyx. This taxon was revalidated by de la Riva (1996), including the populations of *Adenomera* from the oriental region of Paraguay, southeastern Bolivia, Mato Grosso (Brazil), and northern Argentina, but the identity of these populations remains poorly investigated (Lavilla and Cei, 2001).

Cytogenetic analyses were carried out on 19 specimens of *A. diptyx* (fifteen males and four females) from localities of northeastern Argentina: two males and two females (UNNEC 9719, 9725-9727) from Laguna Naick Neck (25°12' S, 58°08' W); one male (UNNEC 8800) from Comandante Fontana (25°20' S, 59°41' W), both in the Formosa province; one male (UNNEC 8531) from Paraje Las Tablas, Chaco province (26°11' S, 59°38' W); three males and one female (UNNEC 9002, 9003, 9075, 9551) from Paso de la Patria (27°19' S, 58°34' W); eight males and one female (UNNEC 8974, 8994, 9704, 8294, 8295, 8354, 8367, 8505, 8293) from Corrientes (27°29' S, 58°46' W), both in the Corrientes province. Voucher specimens were deposited in the Colección Herpetológica de la Universidad Nacional del Nordeste (UNNEC).

Chromosome spreads were obtained from intestinal epithelium and testes following Schmid (1978). Conventional staining was performed with Giemsa diluted in phosphate-buffered saline solution, pH 6.8. Silver staining of the NORs (Ag-NOR) and C-banding were obtained following Howell and Black (1980) and Sumner (1972), respectively.

Adenomera diptyx presented a karyotype with $2n = 26$ chromosomes and $FN = 34$. The karyotype is composed of three submetacentric pairs (pairs 1-3), one metacentric pair (pair 5), and nine telocentric pairs (pairs 4 and 6-13) (Figure 1a, Table 1). Pair 7 showed a conspicuous proximal secondary constriction. The diploid number was confirmed

in meiotic preparations from testes, in which 13 bivalents were observed (not shown).

After silver staining, the Ag-NORs were located in the proximal region of both homologues of telocentric pair 7, at the same site of the secondary constriction, in seven specimens from Corrientes (Figure 1a). Three specimens (UNNEC 8294, 8354, 9704) showed a stronger silver impregnation on both homologues due to tandem duplications (Figure 1a). C-banding revealed the presence of constitutive heterochromatin at the pericentromeric region of all chromosomes (Figure 1b).

Current cytogenetic data, available for less than 50% of the recognized species of *Adenomera*, revealed five different karyotypes (Table 2), suggesting that *Adenomera*

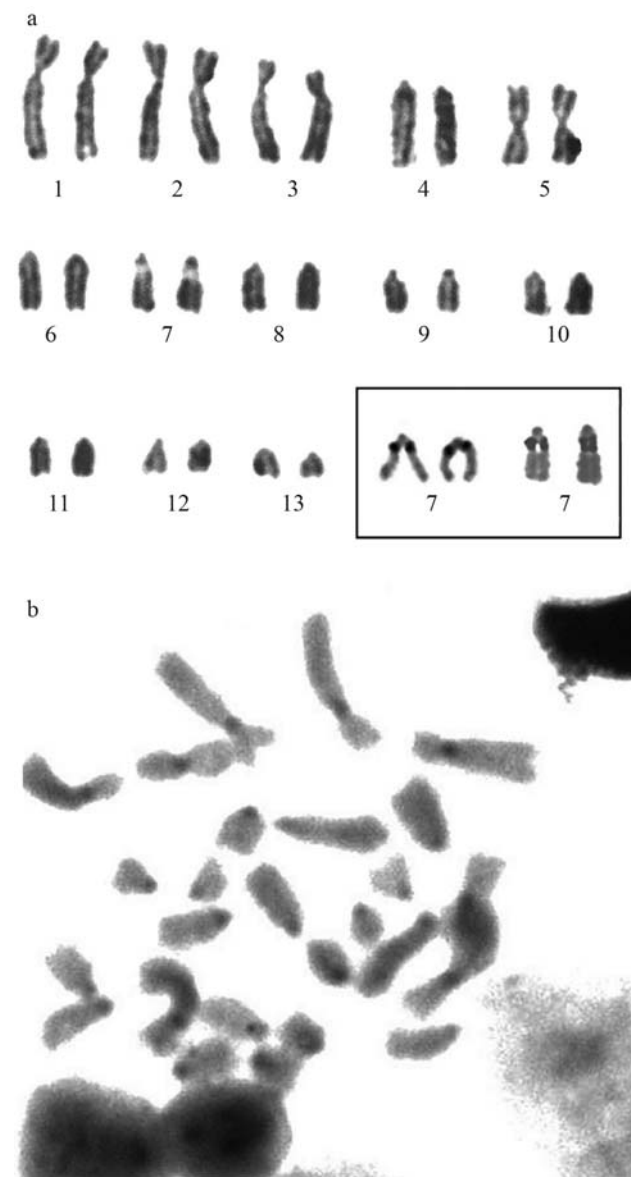


Table 1 - Quantitative characteristics (mean \pm SD) and morphology of *A. diptyx* chromosomes.

Pairs	Relative length	Centromeric index	Chromosome morphology
1	14.42 \pm 0.55	0.32 \pm 0.02	Submetacentric
2	12.79 \pm 0.32	0.33 \pm 0.03	Submetacentric
3	11.22 \pm 0.29	0.30 \pm 0.03	Submetacentric
4	9.53 \pm 0.34	0.00 \pm 0.01	Telocentric
5	9.25 \pm 0.33	0.47 \pm 0.02	Metacentric
6	7.37 \pm 0.32	0.00 \pm 0.00	Telocentric
7	6.34 \pm 0.42	0.00 \pm 0.00	Telocentric
8	6.26 \pm 0.15	0.00 \pm 0.00	Telocentric
9	5.47 \pm 0.17	0.00 \pm 0.01	Telocentric
10	4.99 \pm 0.11	0.00 \pm 0.01	Telocentric
11	4.64 \pm 0.10	0.01 \pm 0.01	Telocentric
12	4.17 \pm 0.16	0.01 \pm 0.02	Telocentric
13	3.58 \pm 0.15	0.01 \pm 0.02	Telocentric

Figure 1 - Karyotype of *Adenomera diptyx* ($2n = 26$, $FN = 34$) from northeastern Argentina after (a) Conventional Giemsa staining, note the presence of secondary constrictions on pair 7. Inset: NOR-bearing chromosome pair after silver staining; (b) metaphase after C-banding.

presents variable diploid numbers and chromosome morphologies, in contrast to the close related genera *Leptodactylus*, *Paratelmatobius* and *Scythrophrys* (Bogart, 1974; Silva *et al.*, 2000; Amaro-Ghilardi *et al.*, 2006; Lourenço *et al.*, 2007).

The specimens of *A. diptyx* from five localities of northeastern Argentina presented the same karyotype previously reported for *Adenomera* with $2n = 26$ and $FN = 34$. The similarities in $2n$, FN and NOR distribution between *A. diptyx* and *A. hylaedactyla* are shown in Table 2, as well as the differences in relation to the other species.

The presence of a single Ag- NOR-bearing chromosome pair, as observed in *A. diptyx*, and of tandem duplication involving the ribosomal DNA in one homologue are common in anurans (Schmid *et al.*, 1990). Nevertheless, some of our specimens of *A. diptyx* exhibited duplications in both homologues.

The C-banding pattern observed in *A. diptyx*, with heterochromatin at the pericentromeric regions of all chromosomes, is similar to that of other species of *Adenomera* already analyzed and is the most common pattern found among anurans (Campos *et al.*, 2009).

Two alternative hypotheses have been proposed to explain the chromosome evolution of *Adenomera*. According to Bogart (1974), Heyer and Diment (1974) and Campos *et al.* (2009), the karyotype evolution in *Adenomera*

could have involved centric fusions and pericentric inversions of uni-armed chromosomes from an ancestral karyotype with $2n = 26$ and a large number of telocentrics. Under this hypothesis, the primitive condition would be represented by *A. hylaedactyla* and *A. diptyx* and the karyotypes of *A. andreae*, of the *A. marmorata* species-complex and of *A. aff. bokermanni* would be derived. The typical large metacentric pair 1 and the lower diploid numbers of the *A. marmorata* species-complex ($2n = 24$) and of *A. aff. bokermanni* ($2n = 23$) may be a result of centric fusions (Bogart, 1974; Campos *et al.*, 2009). Pericentric inversions involving one or three pairs of telocentric chromosomes could explain the two different karyotypes reported for the *A. marmorata* species-complex ($2n = 24$, $FN = 34$ and $2n = 24$, $FN = 36$) and the distinctive fundamental number of *A. andreae* ($2n = 26$, $FN = 40$), respectively (Bogart, 1974, Campos *et al.*, 2009).

An alternative hypothesis assumes that a diploid number of $2n = 24$, also found in *Leptodactylus silvanimbus* and in the *Leptodactylus* sister-clade formed by *Paratelmatobius-Scythrophrys*, would be the ancestral condition. In this case, the karyotypes of most *Leptodactylus* ($2n = 22$), *Lithodytes* ($2n = 18$) and *Adenomera* species ($2n = 26$) would represent derived conditions (Amaro-Ghilardi *et al.*, 2006).

Table 2 - Available information on the karyotypes of *Adenomera*.

Species	2n	FN	Chromosome morphology	Site of SC	Site of Ag-NOR	Localities	Reference
<i>A. hylaedactyla</i>	26	34	1M, 1SM, 2ST, 9T	8	-	Provincia de Huanuco (Peru)	Bogart, 1974
<i>A. hylaedactyla</i>	26	34	1M, 3SM 9T	Small telocentric chromosomes	7	Amapá, Macapá and Porto Velho Rondonia (Brazil)	Campos <i>et al.</i> , 2009
<i>A. diptyx</i>	26	34	1M, 3SM, 9T	7	7	Laguna Naick Neck, Comandante Fontana, Las Tablas, Paso de la Patria, Corrientes (Argentina)	Present study
<i>A. andreae</i>	26	40	1M, 4SM, 2ST, 6T	-	-	Provincia de Huanuco (Peru)	Bogart, 1974
<i>A. lutzi</i>	26	-	-	-	-	-	Bogart, 1970 in Kuramoto, 1990
<i>A. marmorata</i>	24	34	2M, 1SM, 2ST, 7T	4	-	State of São Paulo (Brazil)	Bogart, 1974
<i>A. cf. marmorata</i> Karyotype A	24	34	2M, 3SM, 7T	6	6	Santa Branca and Ilha dos Alcatrazes (Brazil)	Campos <i>et al.</i> , 2009
<i>A. cf. marmorata</i> Karyotype B	24	36	3M, 3SM, 6T	6	6	Salesópolis, São Luís do Paraitinga and Ubatuba (Brazil)	Campos <i>et al.</i> , 2009
<i>A. aff. bokermanni</i>	23	34	2M, 3SM, 1 ST, 4T, 3NP (1M+2T)	-	11	Santa Branca (Brazil)	Campos <i>et al.</i> , 2009

2n- diploid number; FN – fundamental number; M – metacentric; SM - submetacentric; ST - subtelocentric; T - telocentric; NP - unpaired; SC - secondary constriction.

Adenomera seems to be a monophyletic clade (Heyer, 1974; de Sa *et al.*, 2005) without karyotypic uniformity, therefore representing an interesting group for the study of chromosome evolution. Until a comprehensive cytogenetic survey of all the species of the genus is performed, any hypothesis of chromosome evolution will remain poorly supported.

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