Genetica (2016) 144:37–46
DOI 10.1007/s10709-015-9876-8

# Intra-generic and interspecific karyotype patterns of *Leptodactylus* and *Adenomera* (Anura, Leptodactylidae) with inclusion of five species from Central Amazonia

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Received: 4 February 2014 / Accepted: 24 November 2015 / Published online: 9 December 2015


**Abstract** The genera *Leptodactylus* and *Adenomera* comprise 92 species distributed throughout the Neotropical region. These species have a modal diploid chromosome number 2n = 22. However, chromosome rearrangements are evident in the differentiation of five intra-generic groups in the genus *Leptodactylus* (*L. fuscus*, *L. latrans*, *L. marmoratus* (formally composed by the species of the genus *Adenomera*), *L. melanonotus*, *L. pentadactylus*), yet it is not clear if there is a karyotype pattern for each group. Aiming to understand the intra-generic and interspecific karyotype patterns of *Leptodactylus* and *Adenomera*, cytogenetic analyses were performed in *A. andreae*, *L. macrosternum*, *L. pentadactylus*, *L. petersii*, and *L. riveroi* using conventional staining, C-banding, nucleolus organizer region (NOR) and hybridization in situ fluorescent (FISH). The karyotype of *Leptodactylus riveroi* was described for the first time. *Adenomera andreae* had 2n = 26, while the remaining species 2n = 22. The NOR was found on pair No. 8 of *A. andreae*, *L. macrosternum*, *L. pentadactylus*, and *L. riveroi*, whereas *L. petersii* had it on pairs Nos. 6 and 10. These locations were confirmed by the FISH with 18S rDNA probe, except for pair No. 10 of *L. petersii*. The C-banding pattern was evident at the centromeres of chromosomes of all species and some interspecific variations were also observed. 2n = 22 was observed in the species of the *L. latrans* group, as well as in the intra-generic groups *L. fuscus* and *L. pentadactylus*; in the *L. melanonotus* group there were three diploid chromosome numbers 2n = 20, 22 and 24; and a larger variation in 2n was also evident in the *L. marmoratus* group.

**Keywords** Leptodactylid diversity · Anuran cytotaxonomy · Chromosome banding · rDNA FISH · NOR phenotypes

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## Introduction

The family Leptodactylidae is composed of 199 species, viz. 74 assigned to the genus *Leptodactylus* and 18 to the genus *Adenomera* (Frost 2015). Representatives of the genus *Leptodactylus* have a broad geographical distribution, ranging from the south of North America to South America, including the Caribbean, Antilles and the Bahamas, while *Adenomera* occurs in South America east of the Andes (Frost 2015). In Central Amazonia, species of these genera are found in open areas, as well as inside forests and along forest edges (Lima et al. 2012). The taxonomy of the genus *Leptodactylus*, as well as of the family Leptodactylidae, remains contradictory. Conventionally, the genus *Leptodactylus* was divided into five intra-generic groups [*Leptodactylus fuscus*, *L. marmoratus*, *L. melanonotus*, *L. ocellatus* (=*L. latrans*), and *L. pentadactylus*], based on ecological characters (Heyer 1969). However, in 1974 Heyer resurrected the genus *Adenomera* for the *L. marmoratus* group. Many revisions based on their morphological or genetic characteristics have been proposed over the last 10 years (Frost et al. 2006; Pyron and Wiens 2011;

de Sá et al. 2014), including the synonymy of *Adenomera* to *Leptodactylus* (Frost et al. 2006) and the new recent resurrection of the genus *Adenomera* (Pyron and Wiens 2011; de Sá et al. 2014). Following these recent changes, below we refer to the four species groups of *Leptodactylus* according to Heyer (1969) [*L. fuscus*, *L. melanonotus*, *L. latrans*, and *L. pentadactylus*], and to the genus *Adenomera.*

Reproductive mode is the most distinctive feature of the four *Leptodactylus* intra-generic groups (Heyer 1969) and *Adenomera*. The reproductive mode of species of the *L. latrans* group is basal, because the foam nests are deposited on the water surface and the tadpoles are exclusively aquatic (Haddad and Prado 2005; de Sá et al. 2014). Eggs in foam nests, as well as larvae in initial stages, found in underground chambers, are characteristics of species from the *L. fuscus* group (Haddad and Prado 2005). Species from the groups *L. melanonotus* and *L. pentadactylus* deposit the foam nests on the water accumulated in basins constructed by males. This mode is characterized as intermediate between an aquatic and a terrestrial environment (Heyer 1969; Haddad and Prado 2005). The majority of the *Adenomera* species deposit the nests in underground chambers constructed by males and the tadpoles of many species complete their development inside these chambers (Haddad and Prado 2005).

As to the cytogenetics of these frogs, around 43 % of species from the genus *Leptodactylus* and 22 % of species from the genus *Adenomera* have been karyotyped (Beçak 1968; Denaro 1972; Bogart 1974; De Lucca and Jim 1974; Silva et al. 2000; Amaro-Ghilardi et al. 2004; Silva et al. 2004; Amaro-Ghilardi et al. 2006; Silva et al. 2006; Arruda and Morielle-Versute 2008; Campos et al. 2009; Oliveira et al. 2010; Campos 2010; Suárez 2010; Gazoni et al. 2012; Coelho 2013). Among the *Leptodactylus* species, 94 % have diploid chromosome number 2n = 22 chromosomes, with variation in the composition of the karyotype and in the nucleolus organizer region (NOR) phenotypes, while in the genus *Adenomera*, the 2n = 23, 24 or 26. Moreover, karyotypes all of the species possess blocks of constitutive heterochromatin located in pericentromeric/centromeric position in the majority of the chromosomes, although additional heterochromatic marks were also found in some taxa.

This study deals with identification of karyotype and other chromosomal markers using conventional and molecular cytogenetic protocols among the intra-genetic groups of *Leptodactylus* and the genus *Adenomera*, including five species of anurans from Central Amazonia and inter-population differences and description of the karyotype of *Leptodactylus riveroi* for the first time.

## Materials and methods

### Species and collection localities

Chromosome analyses were performed using 15 individuals of five species: *Adenomera andreae* (3?), *Leptodactylus macrosternum* (1♂ and 1♀), *Leptodactylus pentadactylus* (1♂, 2♀, and 4?), *Leptodactylus petersii* (1♀), and *Leptodactylus riveroi* (1♂ and 1♀). All individuals were collected in the state of Amazonas, Brazil (Table 1). Acoustic and visual searches were carried out throughout the night, guided by the animals' vocalization, and the capture was active. The animals were anesthetized by lethal doses of gel xylocaine 5 %. The specimens were fixed in formaldehyde 10 % for 24 h, and kept in alcohol (70 %). The collection was permitted by Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) (# 35424-1, 3678-1, 11323) and by the Ethic Committee for Animal Experimentation (CEEA) at the Universidade Federal do Amazonas (# 076/2012).

### Conventional cytogenetics

Fresh baking yeast in the proportion of 0.1 ml/10 g body weight was used to stimulate mitotic cell division following the method of Cole and Leavens (1971). After 48 h, mitotic chromosomes were prepared from liver and femur bone marrow, according to the protocols of Ford and Hamerton (1956), and Baldissera et al. (1993). The detection of the NOR was performed according to Howell and Black (1980). Constitutive heterochromatin was detected using the method of Sumner (1972). A minimum of 30 metaphases were analyzed in each individual and the chromosomes were classified according to Green and Sessions (1991, 2007).

### Molecular cytogenetics

Total genomic DNA was extracted from the muscles of *L. fuscus* and *L. riveroi*, following the phenol–chloroform protocol detailed by Sambrook and Russell (2001). Amplification by polymerase chain reaction (Saiki et al. 1988) of the 18S rRNA gene was conducted using the primers DNAr 18S (IpF 5′-CCGCTTTGGTGACTCTTGAT-3′ and IpR 5′-CCGAGGACCTCACTAAACCA-3′) (Gross et al. 2010). The PCR products were labelled with *DIG-Nick$^{TM}$ Translation Mix Kit* (Roche) or *BioNick$^{TM}$ Labeling System Kit* (Invitrogen) and used as a probe in the in situ fluorescent hybridization technique (FISH). FISH using homologous probes was carried out based on the protocols described by Pinkel et al. (1986), with modifications. Signals 18 S rDNA probe were detected by the

**Table 1** Analyzed individuals of *Leptodactylus* and *Adenomera* indicating the species, sampling sites, sex, and identification number of the Paulo Bührnheim Zoological Collection (CZPB-UFAM) and Herpetological Collection of the Instituto Nacional de Pesquisa da Amazônia (INPA-H)

| Genus/group | Species | Collection localities | No. of specimens/sex | Number in the scientific collections |
|---|---|---|---|---|
| *Adenomera* | *Adenomera andreae* (Müller, 1923) | b | 2? | CZPB-UFAM 164–345, 169–366 |
| | | f | 1? | INPA-H 31449 |
| *Leptodactylus latrans* | *Leptodactylus macrosternum* Miranda-Ribeiro, 1926 | d | 1♂ and 1♀ | CZPB-UFAM 163–343, 163–344 |
| *Leptodactylus pentadactylus* | *Leptodactylus pentadactylus* (Laurenti, 1768) | a | 1♂ and 1♀ | CZPB-UFAM 160–333, 162–341 |
| | | e | 1♀ | INPA-H 31455 |
| | | g | 4? | INPA-H 31451–31454 |
| *Leptodactylus melanonotus* | *Leptodactylus petersii* (Steindachner, 1864) | c | 1♀ | CZPB-UFAM 161–335 |
| | *Leptodactylus riveroi* Heyer and Pyburn, 1983 | c | 1♂ and 1♀ | CZPB-UFAM 161–334, 161–336 |

As described: ♂ = male; ♀ = female; ? = undetermined sex. The sampling sites are: a—UFAM Campus, Manaus (03°06′4.3″S, 59°58′32″W); b—INPA, Manaus (03°5′21″S, 59°59′21″W); c—Adolpho Ducke Florestal Reserve, Manaus (02°55′37″S, 59°53′31″W); d—Catalão Lake, Iranduba (03°09′47″S, 059°54′29″W); e—Jatapu River, São Sebastião do Uatumã (0°53′36″W, 58°51′W); f—Darahá River, Santa Isabel do Rio Negro (0°24′24″N, 65°1′1″W); g—Jacinto Stream (Purus River), Tapauá (04°50′20″S, 62°53′28″W)

antidigoxygenin antibody. FISH was performed with high stringency (2.5 ng/µl of DNA, 50 % deionized formamide, 10 % dextran sulfate, and 2× SSC at 37 °C for 18 h). The slides were counter-stained with DAPI. Chromosomes were organized by decreasing size, and the morphology was determined based on the centromere position (Green and Sessions 1991).

## Results

*Adenomera andreae* had 2n = 26 chromosomes and fundamental number (FN) = 34 and karyotype composed of 2 m + 2sm + 4st + 18t (Fig. 1a). *Leptodactylus macrosternum*, *L. pentadactylus*, *L. petersii* and *L. riveroi* had 2n = 22 chromosomes and FN = 42 (Fig. 1d, g, j, m) and karyotypes composed of 8 m + 8sm + 6st, 12 m + 6sm + 4st, 8 m + 2sm + 10st + 2t and 10 m + 8sm + 4st, respectively. Karyotypes of all species did not exhibited differentiated sex chromosomes.

In the karyotypes of *A. andreae*, *L. macrosternum*, and *L. pentadactylus* the NORs were located at the terminal region of the p arms of chromosome pair No. 8. In the karyotype of *L. riveroi* the NORs were located on the proximal region of the q arms of chromosome pair No. 8 (Fig. 1c, e, h, n). Multiple NORs were observed in the karyotype of *L. petersii*, located on the interstitial region of the p arms of pair No. 6 and the proximal region of the p arms of pair No. 10 (Fig. 1k). The sites of 18 rDNA corresponded to the NOR sites for all of the species, except for *L. petersii*, whose pair No. 10 (impregnated by silver nitrate) did not present positive 18 rDNA site.

Regarding constitutive heterochromatin distribution, the karyotypes of five analyzed species had centromeric blocks in all of the chromosomes. Additionally, that of *A. andreae* had conspicuous blocks on pairs 1–3, and 4; and the other marks were weak when compared to those of the other species (Fig. 1a). In *L. macrosternum* heterochromatic blocks were pericentromeric (marks invading the p arms) on pairs 7 and 8, and terminal blocks were evident on pairs 1, 2, 6 (tenuous marks), 3, and 4 (conspicuous marks) (Fig. 1d). Karyotype of *L. pentadactylus* showed entirely heterochromatic chromosome arms on pairs Nos. 8 (q arm) and 10 (p arm); terminal heterochromatic blocks on the q arm of pairs Nos. 2, 3, and 5 and on the p arm of pair No. 1 (Fig. 1g). Karyotype of *L. petersii* had terminal heterochromatic blocks on pairs Nos. 5 and 10 and weak centromeric heterochromatic blocks and pericentromeric conspicuous marks on pairs Nos. 3 and 4 (Fig. 1j). In the karyotype of *L. riveroi*, terminal heterochromatic blocks were observed on the q and p arms of pairs Nos. 1–3, 5, 7–9 and 10, as well as on the short arm of pairs Nos. 4 and 6 (Fig. 1m).

## Discussion

Since the beginning of the 1970s it has been accepted that species of the family Leptodactylidae and from more derived families, such as Dendrobatidae, Hylidae and Ranidae, could have originated from a common ancestor with 2n = 26 chromosomes (Bogart 1973), with subsequent centric

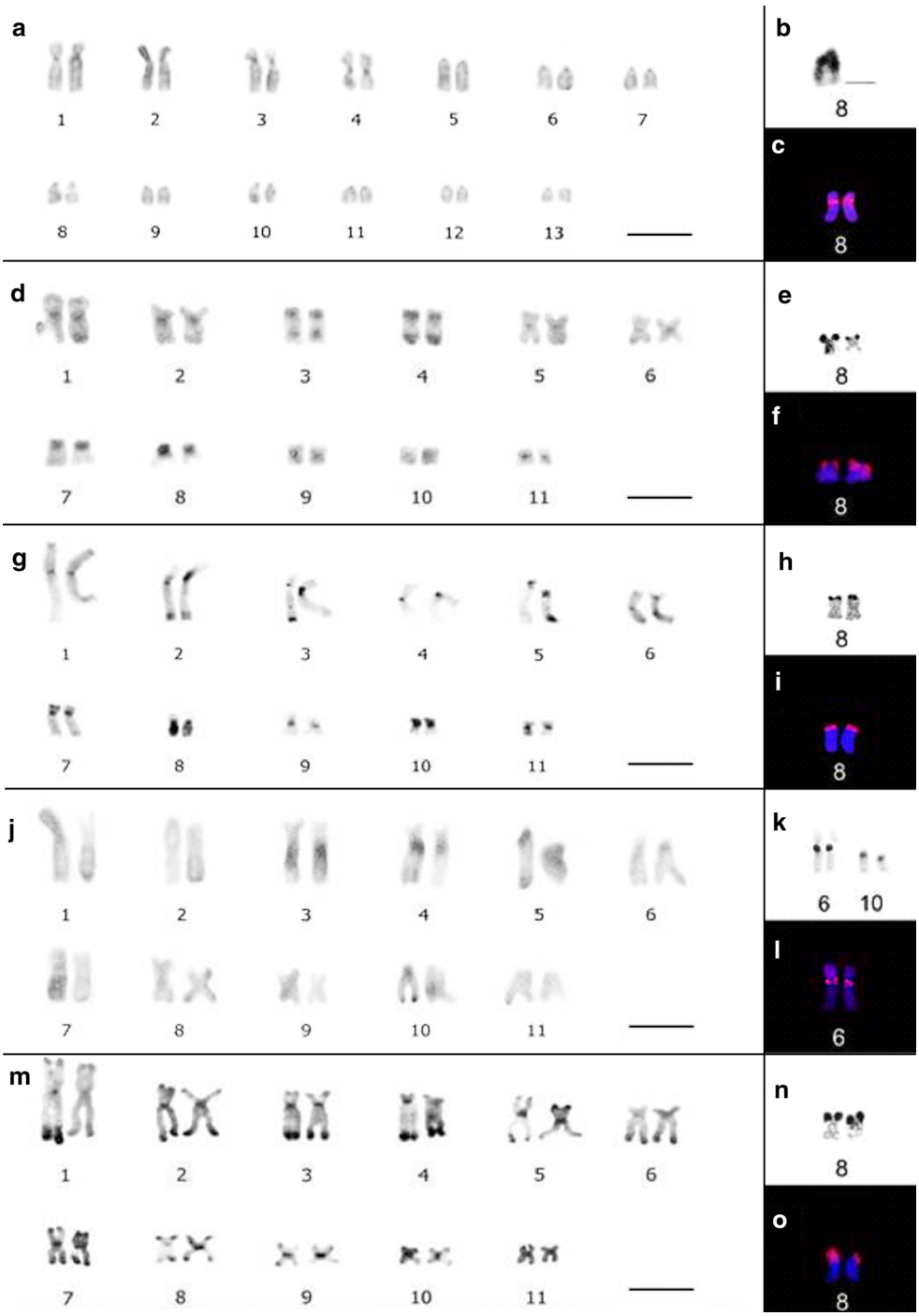


**Fig. 1** Karyotypes of *A. andreae* (*a–c*); *L. macrosternum* (*d–f*); *L. pentadactylus* (*g–i*); *L. petersii* (*j–l*); and *L. riveroi* (*m–o*). The karyotypes showing the distribution patterns of the constitutive heterochromatin (*a*, *d*, *g*, *j*, *m*), Ag-NORs (*b*, *e*, *h*, *k*, *n*) and 18S rDNA sites (*c*, *f*, *i*, *l*, *o*): *Bar* 10 μm

fusions reducing their 2n (Beçak 1968). A reduction of the 2n was considered an evolutionary tendency in anurans (Morescalchi 1973), given that a high number of telocentric chromosomes were found among basal anurans. However, there is no consensus about the basal 2n for the genus *Leptodactylus*.

All species in the group *Leptodactylus latrans* possess 2n = 22, and this same 2n has also been reported for species in the groups *L. fuscus* and *L. pentadactylus.* Yet in the group *L. melanonotus*, besides the 2n = 22, 2n = 20 and 24 have also been observed (Amaro-Ghilardi et al. 2006; Gazoni et al. 2012). For the genus *Adenomera*, none of the species had 2n = 22, but rather 23, 24, or 26 (Bogart 1974; Silva et al. 2000; Amaro-Ghilardi et al. 2004; Silva et al. 2004; Amaro-Ghilardi et al. 2006; Silva et al. 2006; Arruda and Morielle-Versute 2008; Campos et al. 2009;

Campos 2010; Oliveira et al. 2010; Suárez 2010; Zaracho and Hernando 2011; Gazoni et al. 2012). If we consider that the group *L. latrans* exhibits reproduction dependent on water, and that this is a plesiomorphic characteristic (Heyer 1969), we can also assume that 2n = 22, simple NOR phenotype and 18S rDNA sites located on only one chromosomal pair would be basal pattern for the genus because all the species from this intra-generic group share these characteristics (Table 2).

The chromosomal data available for *Adenomera* suggest that this is an advanced genus due to variation in the 2n, the NOR phenotypes, different karyotypes and the presence of interstitial telomeric sequences (ITS) (Bogart 1974; Campos et al. 2009; Suárez 2010; Zaracho and Hernando 2011; Gazoni et al. 2012; present study), corroborating the derivation (Heyer 1969). ITSs are repetitive sequences, which can be derived from chromosomal rearrangements (centric fusion, tandem fusion, or inversion) during vertebrate karyotype evolution, representing the remaining sequences in newly formed chromosomes (Wiley et al. 1992). Alternatively, ITSs can also result from the amplification of telomeric sequences, can be the result of unequal crossing-over and transposition, sequences introduced by a telomerase error, or be the result of integration between transposons and telomeric sequences (Meyne et al. 1990; Andrades-Miranda et al. 2002; Mattos et al. 2014). Within this group, species with elevated 2n and presence of telocentric chromosomes have been found (Bogart 1974; Campos et al. 2009; Suárez 2010; Zaracho and Hernando 2011), reinforcing the hypothesis that such chromosomal groups could have originated from fissions, leading to an increase in the 2n (Kuramoto and Allison 1989; Miura et al. 1995; Busin et al. 2001). However, different karyotypes were found in individuals from different populations of the genus *Adenomera*, such as in *A. andreae* (present study), corroborating the existence of a species complex previously described using vocalization patterns and morphological differences (Angulo et al. 2003; Angulo and Icochea 2010).

In the *L. melanonotus* group, NF values and karyotypes are variable among the species (Bogart 1974; Silva et al. 2000; Amaro-Ghilardi et al. 2006; Campos 2010; Suárez 2010; Gazoni et al. 2012), and the NOR phenotypes in this group is usually simple and coincident with the 18S ribosomal sites (Bogart 1974; Silva et al. 2000; Amaro-Ghilardi et al. 2006; Campos 2010; Suárez 2010; Gazoni et al. 2012). However, *L. petersii* had multiple sites positive after Ag nitrate impregnation and sites of 18S rDNA were found in one pair only. This is most likely caused by the presence of heterochromatic acid blocks that have affinity for silver (Sumner 1990).

Moreover, different patterns of constitutive heterochromatic distribution were observed for individuals from different populations in *L. petersii*. In many cases the variations in the quantity and distribution of blocks of constitutive heterochromatin arise due to the regulation mechanism of gene expression, performing an important role in adaptation (King 1991). However, the heterochromatin is rich in repetitive sequences and thus favors the accumulation of differences during the evolutionary process, due to it being less susceptible to selective pressures (Bohne et al. 2008; López-Flores and Garrido-Ramos 2012). The cytogenetic analyses performed on Leptodactylidae and Hylodidae species showed that the satellite DNA sequences are the most abundant components of heterochromatin, and that the number of repeats of a specific satellite family can vary even between the genomes of related species or populations (Vittorazzi et al. 2014). However, the localization on the chromosome is always coincident with regions of centromeric/pericentromeric heterochromatin (Vittorazzi et al. 2014).

Species from the group *L. pentadactylus* had a conserved karyotype as to 2n and NF values. Furthermore, karyotypes of all species have only bi-armed chromosomes, simple NOR phenotypes and 18S ribosomal sites. However, the pattern of the constitutive heterochromatin distribution can vary according to the species (Bogart 1974; Silva et al. 2000; Amaro-Ghilardi et al. 2004, 2006; Arruda and Morielle-Versute 2008; Campos 2010; Oliveira et al. 2010; Suárez 2010; Gazoni et al. 2012; present study). Intra-specific population differences are present in the group, as well as different karyotypes for *L. knudseni*, *L. labyrinthicus*, *L. pentadactylus*, and *L. rhodomystax* (Table 2). Part of the variation in the karyotypes can be related to the quality of the analyzed chromosomal preparation, to the DNA's compacting pattern, as well as to the chromosomal arm sizes, which leads to disagreements regarding karyotype interpretations. However, recent studies have shown that the centromere is rich in DNA repetitive sequences and, because of its repetitive nature, it can be considered a dynamic chromosomal part, able to relocate itself due to epigenetic responses, which would lead to different karyotypes for the same species (Rocchi et al. 2012). Nevertheless, chromosomal rearrangements cannot be disregarded because, according to Gazoni et al. (2012), *L. pentadactylus* has a complex chromosomal composition that involves multiple translocations. The absence of the ITSs in *Leptodactylus* species does not necessarily indicate that rearrangements do not occur, because chromosomes derived from Robertsonian and non-Robertsonian events may have small telomeric sites that cannot be easily detected by FISH, or the telomeres could be lost before the fusion or eroded by molecular processes (Mattos et al. 2014).

The group *L. fuscus* also has a conserved 2n and NF equal to 44 for the majority of the species (Bogart 1974;

**Table 2** Cytogenetic data available for the species *Leptodactylus* according to intra-generic groups and *Adenomera*

| Group/genus | Species | Locality | 2n | K | NF | Band C | NORs | 18S rDNA | ITS | References |
|---|---|---|---|---|---|---|---|---|---|---|
| *L. fuscus* | *L. albilabris* | Barrio Rio Grande, Puerto Rico | 22 | 14 m + 6sm + 2st | 44 | – | – | – | – | 1 |
| | *L. bufonius* | Córdoba, Corrientes and Santiago del Estero, Argentina | 22 | 14 m + 4sm + 4st<br>16 m + 4sm + 2st | 44 | Centromeric 1(i),8(i),q2,q9 | q8(i) | 8 | – | 1;11 |
| | *L. elenae* | Misiones and Santa Fé, Argentina | 22 | 14 m + 8sm | 44 | Centromeric 1,4,7,8(pe),q3,p11 | q8(i) | – | – | 11 |
| | *L. furnarius* | SP, Brazil Misiones, Argentina | 22 | 6 m + 6sm + 8 m/sm + 2st<br>14 m + 6sm + 2st | 44 | Centromeric p1,3,4(t) | p8(i),q8(i) | – | – | 9;11 |
| | *L. fuscus* | ES, GO, SP and RS, Brazil | 22 | 6 m + 8sm + 8 m/sm<br>14 m + 6sm + 2st<br>6 m + 6sm + 8 m/sm + 2st<br>10 m + 12sm | 44 | Centromeric, telomeric and interstitial<br>p5(i),p4,7,8(t) | p8(t) | 8 | – | 1;2;7;9;10;11 |
| | *L. gracilis* | Córdoba, Corrientes and Missiones, Argentina<br>SC and SP, Brazil | 22 | 10 m + 10sm + 2st<br>6 m + 16sm<br>12 m + 10sm<br>16 m + 4sm + 2st | 44 | Centromeric 1pt,8ppr,9qpr | p,q8(i) | 8 | – | 1;4;5;11 |
| | *L. latinasus* | Buenos Aires, Córdoba, Corrientes, Entre Ríos, Jujuy and Tucumán, Argentina | 22 | 10 m + 6sm + 4st + 2t<br>8 m + 8sm + 4st + 2t | 42 | Centromeric | 8(pe) | – | – | 1;11 |
| | *L. mystaceus* | PA, Brazil Caqueta, Colombia | 22 | 10 m + 6sm + 6st<br>6 m + 6sm + 8 m/sm + 2st<br>12 m + 8sm + 2st | 44 | Centromeric | p,q8(i) | – | – | 1;9;11 |
| | *L. mystacinus* | Cundinamarca, Colômbia<br>MT and SP, Brazil | 22 | 14 m + 2sm + 6st<br>10 m + 12sm<br>6 m + 8sm + 8 m/sm | 44 | Centromeric telomeric | q4(pr),q8 p1,8(i),q8(i),p11,p4(t) | 4,8,p4(t) | – | 1 (=*L. labialis*);5;6;11 |
| | *L. notoaktites* | SP, Brazil | 22 | 6 m + 8sm + 8 m/sm | 44 | Centromeric | p8 | – | – | 2 |
| | *L. plaumanni* | SC, Brazil<br>Misiones, Argentina | 22 | 6 m + 16sm<br>16 m + 4sm + 2st | 44 | Centromeric p1(t),p8(pr),q9(pr) | p8(i),q8 | 8 | – | 4;11 |
| | *L. troglodytes* | PI, Brazil | 22 | 14 m + 6sm + 2st | 44 | – | – | – | – | 11 |
| *L. melanonotus* | *L. griseigularis* | Huánuco, Peru | 22 | 4 m + 8sm + 2st + 8t | 36 | – | – | – | – | 1 |
| | *L. leptodactyloides* | PA, Brazil | 22 | 8 m + 2sm + 4st + 8t | 36 | Centromeric telomeric | 8q | 8 | Absent | 11 |
| | *L. melanonotus* | Vera Cruz, Mexico | 22 | 12 m + 6sm + 4st | 44 | – | – | – | – | 1 |
| | *L. natalensis* | RJ, Brazil | 22 | 6 m + 4sm + 6st + 6t | 38 | – | – | – | – | 1 |
| | ***L. petersii*** | AP, MT and **AM, Brazil** | 22 | 12 m + 10sm<br>16 m + 4sm + 2st<br>12 m + 8sm + 2st<br>**8 m + 2sm + 10st + 2t** | 44,**42** | **Centromeric**<br>q1(t),p7(t)<br>**q5(t),q10(t)**<br>**Pericentromeric (3,4)** | q4(pr),q8(i)<br>**p6(i),p10(pr)** | **6** | **Absent*** | 5;11;13;**14** |

**Table 2** continued

| Group/genus | Species | Locality | 2n | K | NF | Band C | NORs | 18S rDNA | ITS | References |
|---|---|---|---|---|---|---|---|---|---|---|
| | *L. podicipinus* | MG, MT and SP, Brazil Corrientes and Misiones, Argentina | 22 | 6 m + 4sm + 4st + 8t<br>6 m + 6sm + 8 m/t + 2t<br>6 m + 4sm + 2 m/sm + 2st + 8t<br>8 m + 2sm + 4st + 8t<br>6 m + 6sm + 2st + 8t | 34,36,42 | Centromeric | p8(t),q8(i) | 8 | Present | 1;2;9;11;13 |
| | *L.* sp. (aff. *podicipinus*) | MT, Brazil | 20 | 12 m4sm4st | 40 | Centromeric | q4(pr) | 4 | Present | 13 |
| | *L. pustulatus* | PA, PI and TO, Brazil | 22 | 12m + 10sm 14 m + 4sm + 2st | 44 | Centromeric telomeric | p8(t) | 8 | – | 5;11 |
| | *L. silvanimbus* | Belén Gualcho, Honduras | 24 | 12 m + 12sm | 48 | – | p8(t) | – | – | 5 |
| | ***L. riveroi*** | **AM, Brazil** | **22** | **10 m + 8sm + 4st** | **42** | **Centromeric p and q 1,2,3,5,7,8,9,10(t)**<br>**q4,6(t)** | **q8(pr)** | **8** | **Absent*** | **14** |
| *L. latrans* | *L. bolivianus* | Cundinamarca, Colombia | 22 | 16 m + 4sm + 2st | 44 | – | – | – | – | 1 (=*L. wagneri*) |
| | *L. chaquensis* | Chaco and Corrientes, Argentina MS and MT, Brazil | 22 | 12 m + 8sm + 2st | 44 | Centromeric<br>Telomeric p3,4,7,9,11(i),q8(i) | p3,5,p8(i) | 8 | Absent | 11;13 |
| | ***L. macrosternum*** | PA,TO and **AM, Brazil** | 22 | 14 m + 8sm 10 m + 10sm + 2st<br>**8 m + 8sm + 6st** | 44,**42** | **Centromeric**<br>Telomeric p4,5,6,11(i),q8,10(i)<br>**Pericentromeric (7,8)**<br>**Terminal (1,2,3,4,6)** | **p8(t)** | **8** | **Absent*** | 5;11;**14** |
| | *L. latrans* | Salto, Uruguay Córdoba, Argentina BA, GO, MG, MS, MT, PE, PI, PR, RJ, RS, SP and TO, Brazil | 22 | 12 m + 6sm + 4st 6 m + 8sm + 8 m/sm 12 m + 10sm<br>6 m + 6sm + 2st + 8 m/sm<br>14 m + 6sm + 2st | 44 | Centromeric q4(pr),p3,5,6,9,10(i) p4,7,8,9,q1(i),<br>Telomeric p8(pr),q3,4,7p1 | p8(t) | 8 | Absent | 1;2;3;5;7;9;10;11 (=*L. ocellatus*) |
| *L. pentadactylus* | *L. knudseni* | MT and PA, Brazil | 22 | 12 m + 10sm 10 m + 8sm + 2st | 44 | Telomeric (i),(pr),(pe), | p8 | – | Absent | 3;11 |
| | *L. labyrinthicus* | GO, PI and SP, Brazil Misiones, Argentina | 22 | 6 m + 8sm + 8 m/sm 12 m + 10sm<br>14 m + 4sm + 4st<br>10 m + 8sm + 4st<br>12 m + 8sm + 2st | 44 | Centromeric telomeric q2(i),(pe) | p8(t) | 8 | – | 2;5;7;10;11;13 |
| | *L. laticeps* | Chaco, Formosa and Salta, Argentina | 22 | 14 m + 4sm + 4st | 44 | Centromeric q1,4,6,7,10(t) p2,5,6,8,11(t) p1,3,5(i) | p2(i) | 2 | – | 11 |
| | *L. paraensis* | PA, Brazil | 22 | 12 m + 4sm + 6st | 44 | – | p8(t) | – | – | 11 |
| | ***L. pentadactylus*** | Huánuco, Perú MT and SP and **AM**,**Brazil** | 22 | 14 m + 6sm + 2st 12 m + 10sm (4up)<br>**12 m + 6sm + 4st** | 44,**42** | **Centromeric,** telomeric and Interstitial<br>**q2,3,5(t)**<br>**p1(t), q8, p10** | **p8(t)** | **8** | **Absent*** | 1;3;11;13;**14** |
| | *L. rhodomystax* | MT and PA, Brazil | 22 | 12 m + 6sm + 4st 12 m + 8sm + 2st | 44 | Centromeric q1,p8(t) | q3(t) | 3 | – | 11;13 |
| | *L. rhodonotus* | Huánuco, Peru | 22 | 14 m + 6sm + 2st | 44 | – | – | – | – | 1 |
| | *L. syphax* | GO and PA, Brazil | 22 | 6 m + 6sm + 8 m/sm + 2st<br>16 m + 2sm + 4st | 44 | Centromeric, telomeric and interstitial | p2,3(i) | – | Absent | 9;11 |

**Table 2** continued

| Group/genus | Species | Locality | 2n | K | NF | Band C | NORs | 18S rDNA | ITS | References |
|---|---|---|---|---|---|---|---|---|---|---|
| *Adenomera* | ***A. andreae*** | Huánuco, Peru, **AM, Brazil** | 26 | 2 m + 8sm + 4st + 12t<br>**2 m + 2sm + 4st + 18t** | 40,**34** | **Centromeric** | **p8(t)** | **8** | **Absent*** | **1;14** |
| | *A. diptyx* | Chaco, Corrientes, Formosa, Paso de la Prata, Argentina | 26 | 2 m + 4sm + 2st + 18t<br>2 m + 6sm + 18t | 34 | Centromeric pericentromeric | 7,8(pr) | – | – | 11;12 |
| | *A. hylaedactyla* | AP and RO, Brazil Huánuco, Peru | 26 | 2 m + 4sm + 2st + 18t<br>2 m + 6sm + 18t | 34 | Centromeric | 7(pr) | – | – | 1;8 |
| | *A. marmorata* | SP, Brazil | 24 | 4 m + 2sm + 4st + 14t<br>4 m + 4sm + 2st + 14t | 34 | Centromeric | 6(pr) | 6,8(pr) | Present | 1;13 |
| | *A.* cf. *marmorata* | SP, Brazil | 24 | 4 m + 6sm + 14t 24 m | 34,48 | Centromeric | 6,8(pr) | 6 | – | 8 |
| | *A.* sp. (aff. *bokermanni*) | SP, Brazil | 23 | 4 m + 6sm + 2st + 8t<br>1 m(up) + 2t(up) | 36 | Centromeric<br>7(pe) | 11(t) | – | – | 8 |

Collecting locations, diploid number (2n), karyotypes (K), fundamental number (NF), Band C, nucleolus organizer region (NORs), interstitial telomeric sites (ITS), and references are indicated. In order to calculate the fundamental number, two arms were considered: metacentric (m), submetacentric (sm) and subtelocentric (st). One single arm: the telocentric chromosomes (t). Short arm (p). Long arm (q). Terminal (t). Interstitial (i). Proximal (pr). Pericentromeric (pe). Unpaired (up). Amazonas (AM). Amapá (AP). Bahia (BA). Espírito Santo (ES). Goiânia (GO). Minas Gerais (MG). Mato Grosso do Sul (MS). Mato Grosso (MT). Pará (PA). Pernambuco (PE). Piauí (PI). Paraná (PR). Rondônia (RO). Rio Grande do Sul (RS). Santa Catarina (SC). São Paulo (SP). Tocantins (TO). – related to data that were not indicated in the original publication. The data of the present study are indicated in boldface

Absent* = Coelho (2013); [1]Bogart (1974); [2]Silva et al. (2000); [3]Amaro-Ghilardi et al. (2004); [4]Silva et al. (2004); [5]Amaro-Ghilardi et al. (2006); [6]Silva et al. (2006); [7]Arruda and Morielle-Versute (2008); [8]Campos et al. (2009); [9]Campos (2010); [10]Oliveira et al. (2010); [11]Suárez (2010); [12]Zaracho and Hernando (2011); [13]Gazoni et al. (2012); [14]Present study

Silva et al. 2000, 2004; Campos 2010; Suárez 2010). Centromeric heterochromatic blocks are found in all species. However, the presence/absence of distal and interstitial blocks can served as diagnostic character for species identification (Bogart 1974; Silva et al. 2000, 2004; Amaro-Ghilardi et al. 2006; Arruda and Morielle-Versute 2008; Campos 2010; Oliveira et al. 2010; Suárez 2010).

Due the persisting disagreements about the phylogenetic relationships between the leptodactylid genera, discussion about the precise evolutionary patterns among them without examining all available set of markers (or data-sets) is still controversial. The identification of basal and advanced lineages using cytogenetic data, i.e. 2n and NF values and other chromosomal characteristics within intra-generic groups thus can significantly contribute to this hotly debated topic (Frost et al. 2006; Grant et al. 2006; Ponssa 2008; Pyron and Wiens 2011). Recent studies have shown that inclusion of molecular markers, natural history, and reproductive traits have a strong impact on the topology of phylogenetic trees (Frost et al. 2006; Grant et al. 2006; Ponssa 2008; Pyron and Wiens 2011; de Sá et al. 2014). Therefore, the future inclusion of these cytogenetic patterns in phylogenetic studies has potential for generating novel and interesting results.

**Acknowledgments** We thank Anne Baldisseri for the English review; Alexandre Almeida and Karla da Silva for granting use of facilities at the INPA and UFAM Zoological Collection; the Herpetology group from the SISBIOTA project for collection and identification of some specimens. Financial support was provided by Fundação de Amparo à Pesquisa do Estado do Amazonas (FAPEAM) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (CNPq # 573976/2008-2, 558318/2009-6, 563348/2010-0; FAPEAM # 062.00383/2013; FAPEAM # 20/2013; FAPEAM/CNPq 062.02317/2011). The current study was supported by graduate fellowships from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) to ACC and TLM and a Research Productivity grant from CNPq to MM and FAPEAM to MCG.

**Compliance with ethical standards**

**Conflict of interest** The authors declare that there is no conflict of interest.

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