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Karyotypic similarity among *Barycholos ternetzi* and five species of the genus *Eleutherodactylus* from southeastern Brazil (Anura, Brachycephalidae)

J.R.C. Campos [a], Fernando Ananias [b], Célio Fernando Baptista Haddad [c], Sanae Kasahara [a,*]

[a] *Departamento de Biologia, Instituto de Biociências, Universidade Estadual Paulista (UNESP), Av. 24A, 1515, 13506-900 Rio Claro, SP, Brazil*
[b] *Universidade São Francisco, Bragança Paulista, SP, Brazil*
[c] *Departamento de Zoologia, Instituto de Biociências, Universidade Estadual Paulista (UNESP), Rio Claro, SP, Brazil*

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Abstract

Comparative cytogenetic analyses were carried out in six species of Brachycephalidae from southeastern Brazil. *Barycholos ternetzi*, *Eleutherodactylus binotatus*, *Eleutherodactylus guentheri*, *Eleutherodactylus juipoca*, *Eleutherodactylus parvus* and *Eleutherodactylus* sp. have $2n = 22$ karyotypes with a marked variation in the morphology of chromosome pairs 8, 10 and 11, which are of telocentric or metacentric types, resulting in FN = 38, 40 and 44. *Eleutherodactylus* have a single chromosome pair bearing Ag-NOR, i.e. pair 1 in *E. binotatus*, pair 6 in *E. guentheri* and *E. parvus*, and pair 11 in *E. juipoca* and *Eleutherodactylus* sp. In contrast, *B. ternetzi* showed Ag-positive sites in the chromosome pairs 1, 4, 5, 9 and 11, and only one to three labelings per metaphase in each individual. Nevertheless, the main chromosome pair with Ag-NOR in the species seems to be the 11th, like in *E. juipoca* and *Eleutherodactylus* sp. The NOR site was confirmed by fluorescence in situ hybridization (FISH) technique in *E. binotatus* and in *B. ternetzi*, bearing 1p1p and 9p11p11p Ag-NOR pattern, respectively. All the species exhibited predominantly centromeric C-banding pattern, but interstitial bands have also been observed in some cases. In *E. binotatus*, there is an indication of geographical difference in the distribution of the interstitial C-bands. The fluorochromes GC-specific chromomycin A_3 (CMA_3) and AT-specific 4′,6-diamidino-2-phenylindole (DAPI), with distamycin A (DA) counterstaining, provided the molecular content of some repetitive regions in the karyotypes of the species. One male of *E. binotatus* presented an extensive heteromorphism, involving at least five different pairs, probably as a consequence of multiple reciprocal translocations. Such rearrangements might be responsible for the multivalent chain seen in the meiosis of this specimen, as well as in another male, although not exhibiting chromosome heteromorphism. The remaining males and those belonging to the other species have always shown 11 bivalents in diplotene and metaphase I cells. In all male specimens, metaphases II presented 11 chromosomes. Despite the observed discrepancies, the five species of *Eleutherodactylus* have a great uniformity in the $2n = 22$ karyotypes, suggesting an assemblage of species from southeastern and southern Brazil, in contrast to northern and northeastern assemblage which is characterized by higher diploid numbers. Undoubtedly, *B. ternetzi* could be included in that proposed assemblage, due to its karyotypic similarity with the *Eleutherodactylus* species, as evidenced in the present study. This fact strongly supports the close relationships of both genera, previously inferred on the basis of several characters shared by their species.

Keywords: Cytogenetics; Chromosomes; C-banding; Fluorochromes; FISH; Ag-NORs

1. Introduction

Eleutherodactylus has been considered the most diversified genus among anurans and vertebrate, in general. The relationships of the species were never very clear, giving rise to some revisions (Lynch, 1976; Miyamoto, 1983; Heyer, 1984; Lynch and Duellman, 1997; Savage and Myers, 2002), the most recent one performed by Frost et al. (2006). The extensive study of these authors introduced great modifications in the whole class of amphibians, as the transferring of all leptodactylids belonging to the subfamily Eleutherodactylinae, including *Eleutherodactylus* and *Barycholos*, to the family Brachycephalidae. Moreover, a significative part of

* Corresponding author. Tel.: +55 19 3526 4150; fax: +55 19 3526 4136.
E-mail addresses: jrcc@rc.unesp.br (J.R.C. Campos), fernando.ananias@saofrancisco.edu.br (F. Ananias), haddad@rc.unesp.br (C.F.B. Haddad), kasahara@rc.unesp.br (S. Kasahara).

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Eleutherodactylus was distributed in the genera *Craugastor*, *Euhyas*, *Pelorius* and *Syrrophus*.

The genus *Barycholos* has only two described species, *Barycholos ternetzi* and *Barycholos pulcher*, with occurrence in Central Brazil and Pacific lowlands of Ecuador, respectively (Lynch, 1980; Frost, 2006). According to Heyer (1969), Lynch (1980) and Caramaschi and Pombal (2001), *Barycholos* is close related to *Eleutherodactylus*, presenting very similar anatomy, reproductive mode and external morphology. On the contrary to what is observed in *Barycholos*, the genus *Eleutherodactylus* currently includes about 650 species, which are distributed from the south of the United States of America, where it was introduced, until Argentina (Frost, 2006; Frost et al., 2006). According to Castanho and Haddad (2000), more than 30 species are known in Brazilian fauna, but this number was considered an underestimation by these authors, since several new species had not been surveyed yet, while others were deposited in museum collections, without a formal description or with misidentification. For this reason, the number of species in the genus *Eleutherodactylus*, certainly, exceeds 40 in Brazil.

The species of *Eleutherodactylus* show a considerable variation in the diploid numbers, $2n = 18–36$, as well as in the fundamental numbers, FN = 32–58 (see review in Campos and Kasahara, 2006). It has been observed that karyotypes with high diploid numbers have predominantly uni-armed chromosomes, whereas those with low diploid numbers have mainly bi-armed chromosomes, which suggests centric fusion or fission as the major chromosomal rearrangements during the karyotypic evolution of that group. Nevertheless, other rearrangements, like reciprocal translocations, inversions, addition or loss of heterochromatin, must have contributed to produce the exceptional karyotype differentiation in *Eleutherodactylus* (Bogart, 1981, 1991; Bogart and Hedges, 1995; Schmid et al., 2002a, 2003).

Taking into account that many taxonomic and systematic problems are showed by *Barycholos* and *Eleutherodactylus*, a comparative karyological analysis of *B. ternetzi*, *E. binotatus*, *E. guentheri*, *E. juipoca*, *E. parvus* and *Eleutherodactylus* sp. was carried out, in order to obtain cytogenetic data supporting a better understanding of their relationships. *Barycholos ternetzi*, *E. juipoca* and *Eleutherodactylus* sp. were karyotyped for the first time, whereas *E. binotatus*, *E. guentheri* and *E. parvus* were previously analyzed by Beçak (1968), Brum-Zorrilla and Saez (1968), Beçak and Beçak (1974) and Siqueira et al. (2004), these latter authors also using differential staining.

2. Material and methods

Cytogenetics analysis was performed in 16 males of *B. ternetzi*; 9 males, 3 females and 1 juvenile of *E. binotatus*; 2 males, 2 females and 1 juvenile of *E. guentheri*; 4 males, 3 females and 1 juvenile of *E. juipoca*; 1 male and 2 females of *E. parvus*; 1 female of *Eleutherodactylus* sp., totalling a sample of 46 specimens, collected in the states of Espírito Santo (ES), Minas Gerais (MG), Rio de Janeiro (RJ) and São Paulo (SP), in southeastern Brazil (Table 1). The voucher specimens were deposited in the Amphibian Collection (CFBH) of the Departamento de Zoologia, Instituto de Biociências, UNESP, Rio Claro, SP, Brazil.

Chromosome preparations were obtained directly from bone marrow, liver and testes, according to Baldissera et al. (1993), or from intestinal epithelium by the technique of Schmid (1978), with minor modifications. Conventional staining was performed with Giemsa diluted in phosphate buffer, pH 6.8. Ag-NOR and C-banding were obtained by the techniques of Howell and Black (1980) and Sumner (1972), respectively. Fluorescent staining was obtained with GC-specific chromomycin A_3 (CMA_3) and AT-specific 4′,6-diamidino-2-phenylindole (DAPI), both combined with the counterstain distamycin A (DA), by the method of Schweizer (1980). Fluorescence in situ hybridization (FISH) with the rDNA probe HM123 was perfomed according to Martins and Galetti (1999). The bi-armed chromosomes were classified as metacentric or submetacentric, and the uni-armed, as telocentric, by visual inspection.

3. Results

3.1. Karyotype description

All the analyzed species have $2n = 22$ chromosomes, distributed into six pairs of large or medium sizes, and five pairs of small size, with exception of *E. binotatus*, in which the seven first pairs are large or medium-sized (Figs. 1 and 2).

Table 1
Species, number and sex, voucher number and collection locality

Species	Voucher number	Collection locality
B. ternetzi (16M)	CFBH07735-50	Gurinhatã, MG (19°12′S and 49°47′W)
E. binotatus (5M, 2F)	CFBH10013-19	Aracruz, ES (19°49′S and 40°16′W)
E. binotatus (2M, 1F)	CFBH07876-78	Mogi das Cruzes, SP (23°41′S and 46°21′W)
E. binotatus (2M, 1J)	CFBH10012, 10020-21	Ubatuba, SP (23°26′S and 45°04′W)
E. guentheri (1M, 1J)	CFBH07489-90	Camanducaia, MG (23°13′S and 45°51′W)
E. guentheri (1M, 1F)	CFBH10031-32	Jundiaí, SP (23°10′S and 53°29′W)
E. guentheri (1F)	CFBH10030	Salesópolis, SP (23°33′S and 45°50′W)
E. juipoca (3M, 2F, 1J)	CFBH10022-27	Santa Branca, SP (23°23′S and 45°53′W)
E. juipoca (1M, 1F)	CFBH10028-29	Itatiba, SP (23°01′S and 46°50′W)
E. parvus (1M, 2F)	CFBH10169-70,10172	Petrópolis, RJ (29°22'S and 51°11'W)
Eleutherodactylus sp. (1F)	CFBH09526	Salesópolis, SP (23°33′S and 45°50′W)

M, male; F, female; J, juvenile

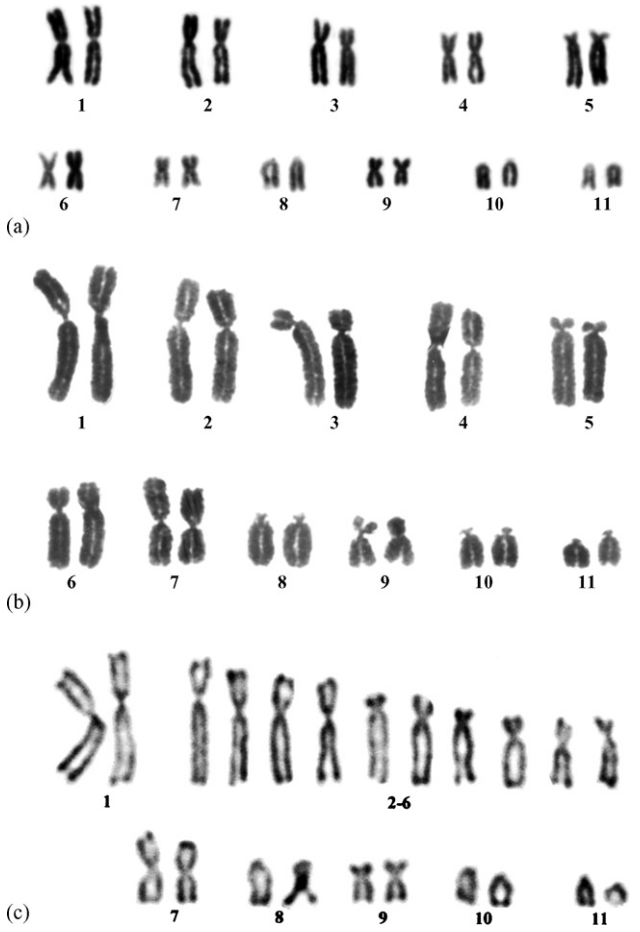

Fig. 1. Karyotypes with conventional staining of males with $2n = 22$ chromosomes. (a) *Barycholos ternetzi* and (b and c) *Eleutherodactylus binotatus*. Note the extensive heteromorphism in pairs 2–6 in (c).

Barycholos ternetzi have FN = 38 (Fig. 1a), with three metacentric pairs (1, 6 and 7), five submetacentric pairs (2, 3, 4, 5 and 9) and three telocentric pairs (8, 10 and 11). Similar karyotype with FN = 38 is presented by *E. binotatus* (Fig. 1b), but the chromosome pair 6 is submetacentric. In the male CFBH07878, from Mogi das Cruzes, SP, an extensive variation in the chromosome morphology, involving elements of pairs 2–6, was observed (Figs. 1c and 5a), but it was not possible to establish a modal karyotype.

Eleutherodactylus juipoca and *Eleutherodactylus* sp. presented indistinguishable karyotypes with FN = 40 (Fig. 2a and b). The chromosome pairs 1, 6 and 7 are metacentric, 2, 3, 4, 5, 9 and 10 are submetacentric and only pairs 8 and 11 are telocentric. Secondary constriction at proximal region of chromosomes 11 is visualized in both species, but not in all metaphases.

Eleutherodactylus guentheri and *E. parvus* have identical karyotypes with FN = 44 (Fig. 2c and d). Pairs 1 and 6 are metacentric and the remaining chromosome pairs are of submetacentric type. Occasionally, a large secondary constriction at the proximal long arms in the chromosomes 6 is visualized in both species.

Meiotic cells of male specimens of *B. ternetzi*, *E. guentheri*, *E. juipoca* and *E. parvus* showed 11 bivalents in diplotene and metaphase I cells, and 11 chromosomes in metaphase II. The

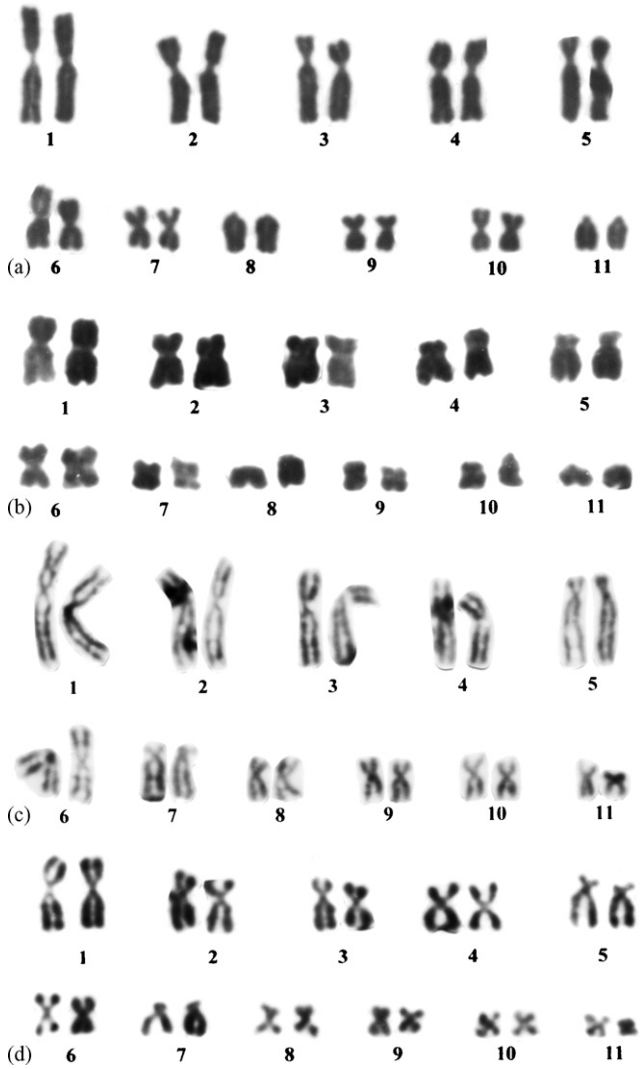

Fig. 2. Karyotypes with conventional staining with $2n = 22$ chromosomes. (a) Male of *Eleutherodactylus juipoca*, (b) female of *Eleutherodactylus* sp., (c) male of *E. guentheri* and (d) female of *E. parvus*.

same was observed for the majority of the males of *E. binotatus* (Fig. 3a and b), but in CFBH07878 and CFBH10021, multivalents were observed, besides a variable number of bivalents (Fig. 3c and d). A few number of metaphase II cells was analyzed in both males, showing 11 chromosomes, like in the other male specimens of the sample.

3.2. Differential staining

Among the 16 specimens of *B. ternetzi*, Ag-stained sites were found at the terminal region of chromosomes 1, 4, 5, 9 and 11, resulting in eight distinct patterns of presumed NOR location (Fig. 4). The total number of Ag-positive sites per metaphase varied between one and three, with at least one Ag-positive stained chromosome 11. In the specimen CFBH07742, one of the homologues of the pair 11 presented, simultaneously, Ag-stained site in the short and long arms. After FISH technique with an rDNA probe, the metaphases of the male

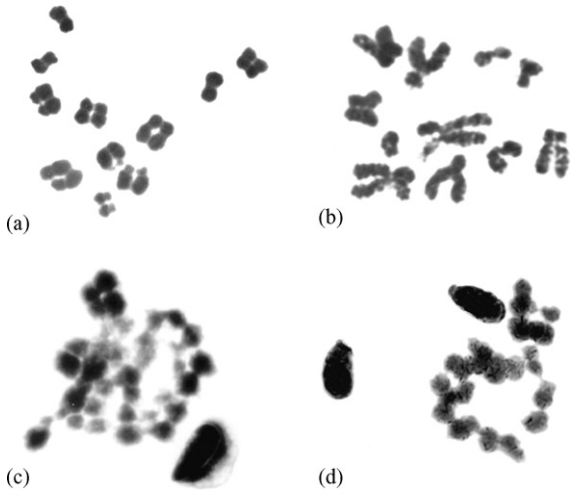

Fig. 3. Meiotic cells of males of *Eleutherodactylus binotatus*. (a) Metaphase I with 11 bivalents, (b) metaphase II with 11 chromosomes and (c and d) metaphase I in the specimens CFBH07878 and CFBH10021, respectively, showing bivalents plus multivalent chain.

CFBH07751, bearing 9p11p11p pattern, showed three hybridization signals (Fig. 6a), coincident with the Ag-positive sites.

In *E. binotatus*, Ag-NOR is at the proximal short arms of chromosome pair 1 (Fig. 5a). The nucleolar organizer region in this species was confirmed by FISH technique (Fig. 6b). In *E. juipoca* and *Eleutherodactylus* sp., the Ag-NOR is in the telocentric pair 11 at the proximal region (Fig. 5b and c), however, in the female CFBH10028 of the former species, from Itatiba, SP, only a single Ag-NOR per metaphase could be observed. *Eleutherodactylus guentheri* and *E. parvus* have Ag-NOR site located at the proximal region of the long arms of the chromosome pair 6 (Fig. 5d and e).

In all species, C-positive heterochromatin is predominantly distributed in the centromeric region, but in some cases, interstitial or telomeric bands are noticed (Figs. 7 and 8). Distinct interstitial C-banding patterns were observed in *E. binotatus* from the three localities (Fig. 7b and c). One of them refers to the C-band at the distal region of the telocentrics 10 and 11, very conspicuous in the samples from Ubatuba, SP, and Aracruz, ES, but subtle, almost inexistent, in the specimens from Mogi das Cruzes, SP; moreover, only in the specimens of the first two localities, telocentrics 8 have slight distal band. On the other hand, the interstitial C-band in both arms of chromosomes 7, seen in the specimens from all localities, were more prominent in the sample from Mogi das Cruzes, SP. In *Eleutherodactylus* sp., interstitial bands were observed in the short arms of chromosome pair 1, in the short and long arms of pair 10 and in the long arms of pair 11. *Eleutherodactylus parvus* showed interstitial C-positive staining in the long arms of one of the chromosomes 6, at the same site of the Ag-NOR (Fig. 8d).

CMA_3/DA/DAPI staining was carried out in the cytological preparations of the majority of the species, with exception of *Eleutherodactylus* sp. The specimens CFBH07748, CFBH07749 and CFBH07750 of *B. ternetzi*, bearing Ag-positive patterns 9p11p, 11p11p and 11p, respectively, had an unequivocal bright CMA_3 staining in the centromere of a unique chromosome 11;

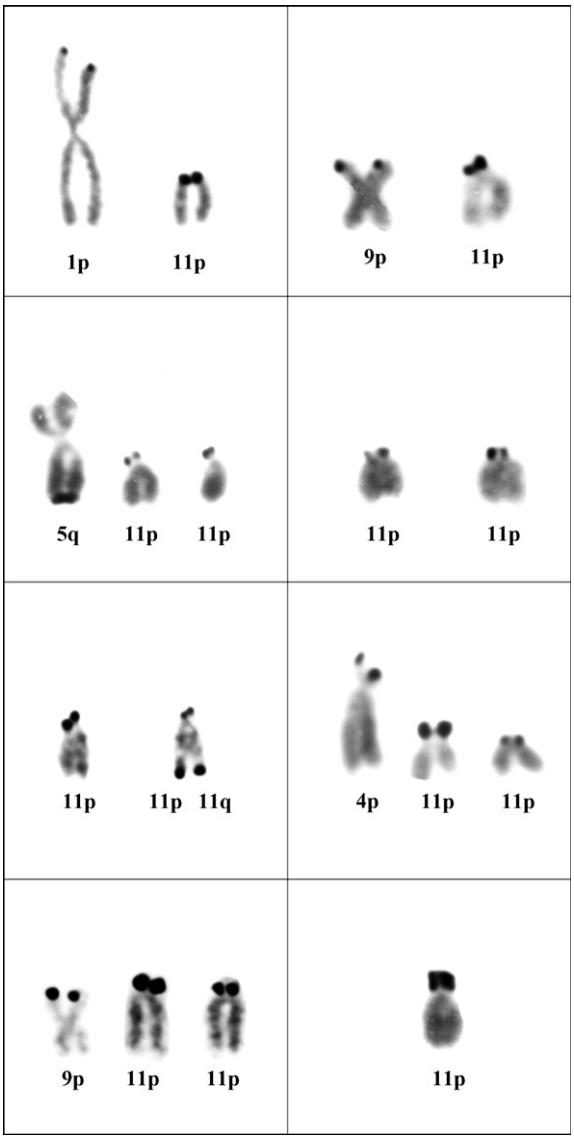

Fig. 4. Silver-stained chromosomes of *Barycholos ternetzi*, showing eight patterns of Ag-positive labelings.

only the specimen CFBH07751 with 9p11p11p pattern showed fluorescent signal in both the homologues, but not in all metaphases. With DAPI, no particular fluorescent region was observed.

In the specimen CFBH07877 of *E. binotatus* from Mogi das Cruzes, SP, a slight bright fluorescence was observed with CMA_3 in the proximal short arms of the chromosomes 1, coincident to the NOR; moreover, a conspicuous negative band was noticed in the centromere region of the telocentrics 8, and in the proximal and distal regions of the telocentrics 10 and 11 (Fig. 9a); with DAPI, these three chromosome pairs showed brilliant band, in the centromere of the chromosomes 8 and only in the distal region of the telocentrics 10 and 11 (Fig. 9b and c).

In the specimen CFBH10029 of *E. juipoca* from Itatiba, SP, and CFBH10026 from Santa Branca, SP, the centromere of the chromosomes is negatively marked after CMA_3 staining. In the latter specimen, the centromeric and the proximal regions on both chromosome arms eventually showed a tiny fluorescent

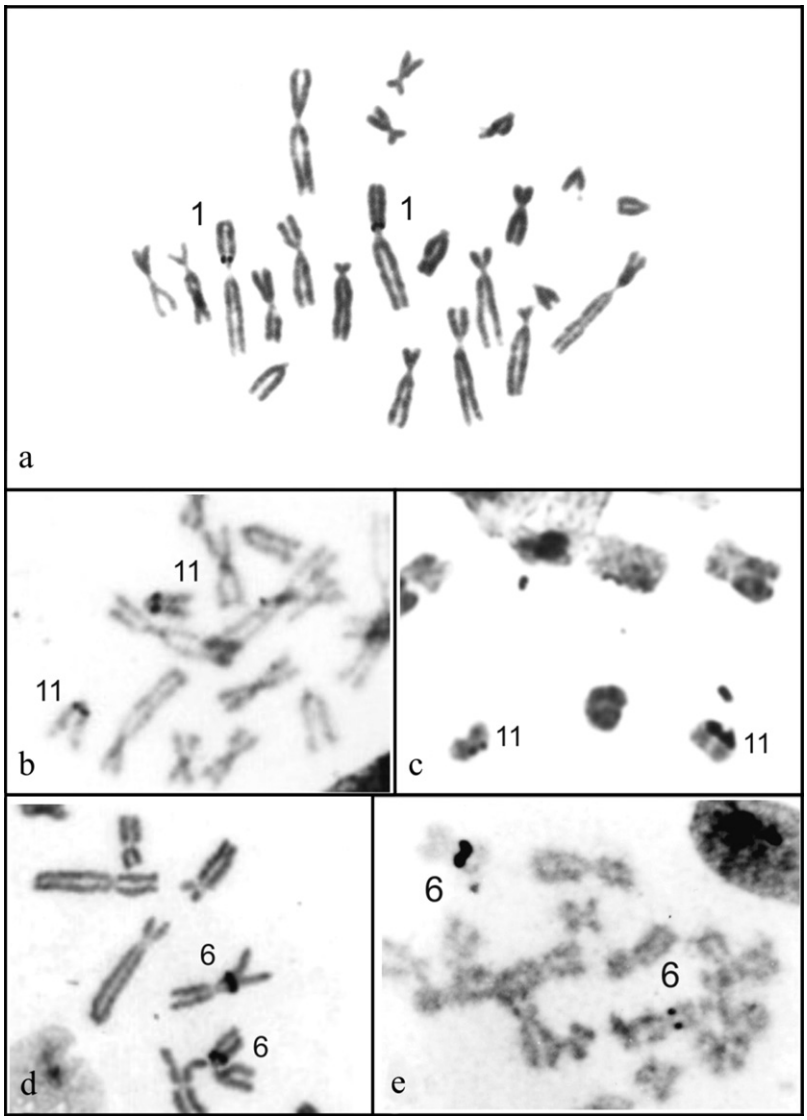

Fig. 5. Ag-NOR sites. (a) Metaphase of *Eleutherodcatylus binotatus* and (b–e) partial metaphases of *E. juipoca*, *Eleutherodactylus* sp., *E. guentheri* and *E. parvus*, respectively.

DAPI site. The specimen CFBH10030 of *E. guentheri* from Salesópolis, SP, and CFBH10170 of *E. parvus* from Petrópolis, RJ, showed no particular bright regions with neither of the fluorochromes, but in the former species the centromere of the chromosomes appeared negatively marked with CMA_3.

4. Discussion

All the species have $2n = 22$, but distinct FN, due to the presence of three telocentric pairs in *B. ternetzi* and *E. binotatus*, two in *E. juipoca* and *Eleutherodactylus* sp. and none in *E. guentheri* and *E. parvus*, both species with only bi-armed chromosomes. Considering that the six species must have homeologous chromosomes, the morphological difference in pairs 8, 10 and 11 is, probably, due to pericentric inversion. Unfortunately, BrdU treatment did not provide suitable chromosome replication banding to confirm that proposition. The occurrence of other chromosome rearrangements, such as the addition or loss of heterochromatin arms described for some *Eleutherodactylus* species, which have the same diploid number, but distinct fundamental numbers (Bogart, 1991; Schmid et al., 2002b, 2003), was discarded by the analysis of the C-banding.

Other minor karyotypic differences were noticed and they were more remarkable in *E. binotatus*. This species presented a relatively larger chromosome pair 7, most probably due to the presence of heterochromatin region, visualized by C-banding, in the interstitial region of both short and long arms; moreover, the chromosome pair 3 had centromere more distally located and pair 6 is clearly of the submetacentric type and not metacentric like in the other species. The pair 7 is, on the other hand, submetacentric in *E. guentheri* and *E. parvus* and metacentric in the remainder of the species.

Morphological variation in the homologues of the chromosome pairs 2 and 3 was already reported in *E. binotatus* (Siqueira et al., 2004), but the novelty in the present study is an heteromorphism much more extensive in the male

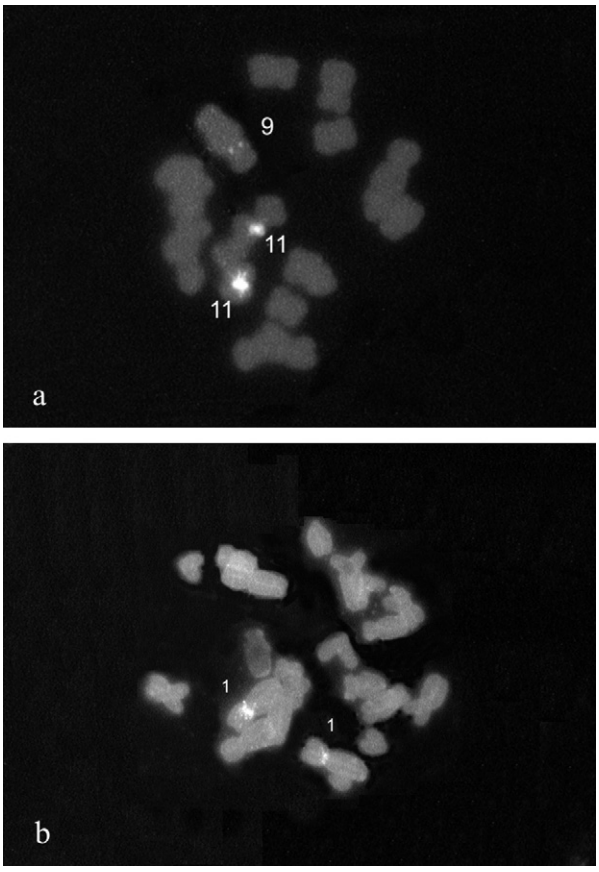

Fig. 6. FISH with an rDNA probe. (a) *Barycholos ternetzi* with 9p11p11p NOR pattern and (b) *Eleutherodactylus binotatus*, showing three and two hybridization signals, respectively.

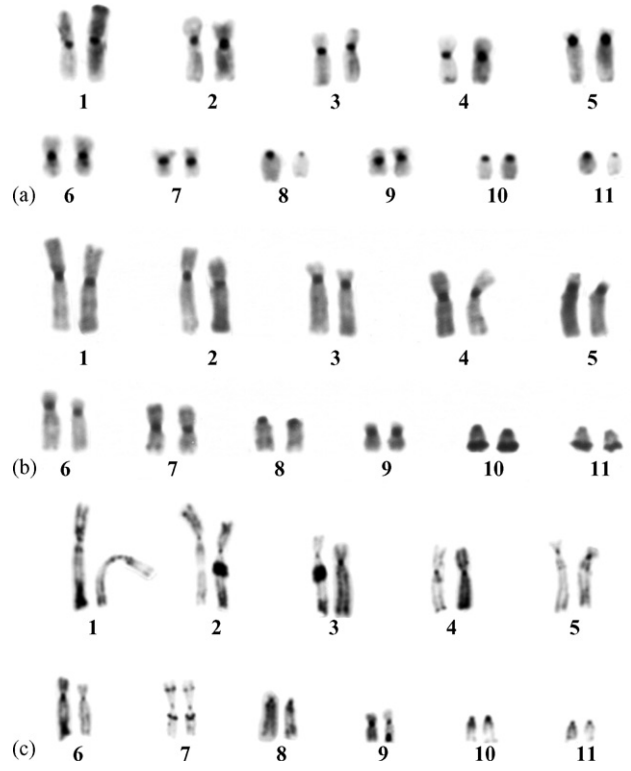

Fig. 7. C-banded karyotypes. (a) *Barycholos ternetzi* and (b and c) *Eleutherodactylus binotatus* from Aracruz, ES and Mogi das Cruzes, SP, respectively.

CFBH07878, since the chromosomes of the pairs 2–6 are probably rearranged in multiple reciprocal translocations. These rearrangements may be confirmed by the formation of meiotic multivalent, which is characteristic of translocation complexes, similarly to those showed by plants of the genera *Oenothera* and *Rhoeo* (Strickberger, 1976), termite (Martins, 1999), anuran (Lourenço et al., 2000) and mammal (Grützner et al., 2004). It is remarkable that multivalent chain was also observed in the male CFBH10021 of *E. binotatus*, but no chromosome heteromorphism was noticed in this individual. Nevertheless, the possibility of small reciprocal translocations, not altering the morphology of the chromosomes, is not completely ruled out. The presence of meiotic chromosome chain in *E. binotatus* is not limited to the specimens of the present study; it was also reported by Beçak and Beçak (1974) and by Siqueira et al. (2004), concerning all male specimens in the case of the latter report. Although few metaphase II cells were available for analysis in both specimens of our sample, the presence of 11 chromosomes in this meiotic phase probably indicates a normal chromosome segregation assuring balanced gametes, like in other male specimens not showing multivalent in meiosis.

No heteromorphic sex chromosome pair could be identified in any of the species, although in two of them only representatives of one sex were karyotyped. The possibility that the multivalent chain in *E. binotatus* is related to sex chromosomes cannot be completely discarded, since Grützner et al. (2004) demonstrated with FISH technique that the X and the Y chromosomes are associated in the meiotic chain in the platypus *Ornythorhynchus anatinus*. Although cytological differentiated sex chromosomes are relatively rare among anurans (Schmid et al., 1991), it is surprising that four species of Brachycephalidae, of which three are *Eleutherodactylus*, have already been described bearing $X_1X_1X_2X_2$:X_1X_2Y or ZZ:ZW mechanisms of sex determination (Schmid et al., 1992, 2002a,b, 2003).

In contrast to what is observed in anurans, usually with a unique pair of Ag-NOR, the data on *B. ternetzi* could be indicative of multiple nucleolar organizer regions. By comparing the Ag-stained patterns in the analyzed sample, one may conclude that the main Ag-NOR chromosome pair in the species is certainly the 11th. At least one homologue of this chromosome pair appeared labeled in all specimens, and 12 of them showed both homologues with Ag-NOR site. The FISH technique carried out in one specimen confirmed the pair 11 and one of the homologues of pair 9 as NOR-bearing chromosomes. For the remaining chromosomes with Ag-positive site, the possibility of argyrophilic non-histone proteins associated to telomeric regions cannot be discarded. As it was described previously in anurans (Kasahara et al., 1996; Silva et al., 2006) and among other vertebrates (Sánchez et al., 1995; Dobigny et al., 2002; Gromicho et al., 2005), some repetitive regions may be silver stained, but not representing true NORs.

The Ag-NOR data disclosed a karyotypic proximity between *E. guentheri* and *E. parvus*, both with nucleolar organizer regions on the chromosome pair 6. The same is true for *E. juipoca*,

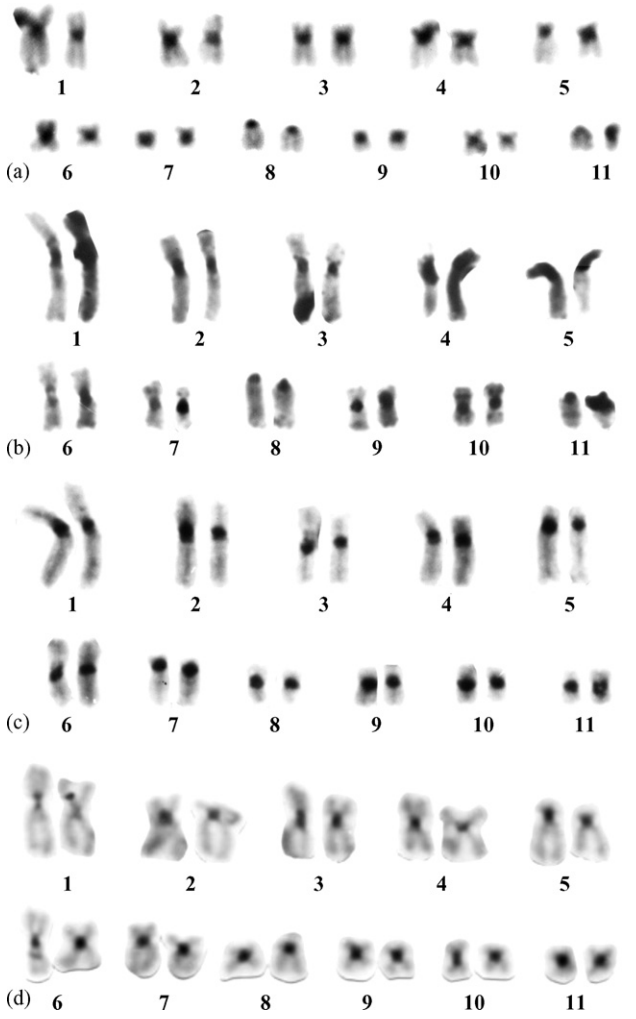

Fig. 8. C-banded karyotypes. (a) *Eleutherodactylus juipoca*, (b) *Eleutherodactylus* sp., (c) *E. guentheri* and (d) *E. parvus*.

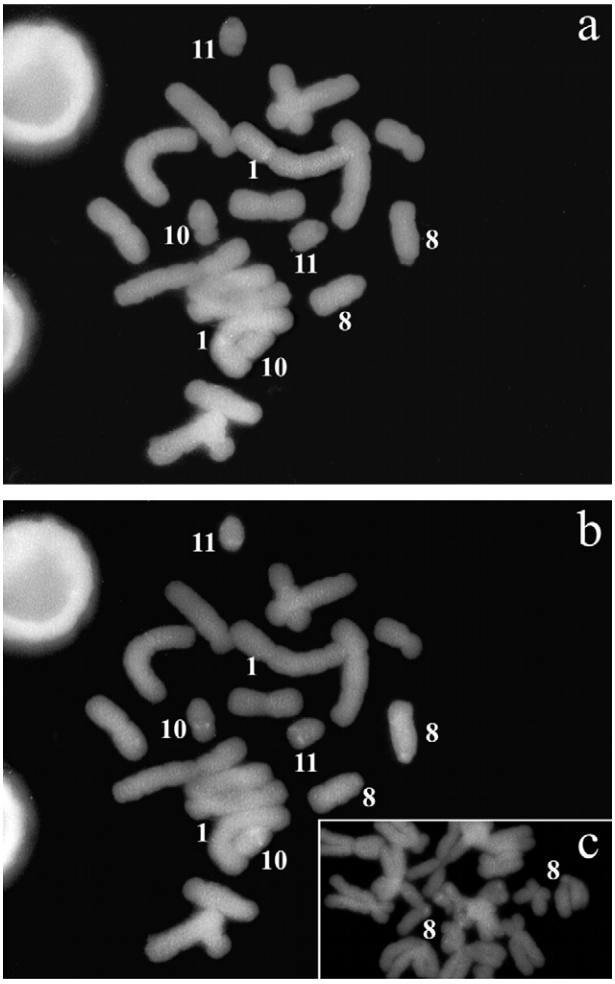

Fig. 9. Fluorochrome staining. (a) DA/CMA_3 and (b and c) DA/DAPI. (a and b) Sequential stained metaphases and (c) partial metaphase of CFBH07877 *Eleutherodactylus binotatus* from Mogi das Cruzes, SP.

Eleutherodactylus sp. and *B. ternetzi*, all of them with Ag-NORs on the chromosome pair 11, although in a non-coincident position in the case of the latter species, probably due to a small pericentric inversion. *Eleutherodactylus binotatus* is the unique species with Ag-NOR in the proximal short arms of chromosome pair 1, this NOR site also confirmed with FISH technique.

The six species showed positive C-bands in the centromeric regions, not roughly differing in the amount of heterochromatin. However, there are indications of geographical karyotypic differentiation related to the interstitial C-band distribution in *E. binotatus*, *E. guentheri* and *E. parvus* of the present study and in those previously karyotyped (Siqueira et al., 2004, present study). This is particularly evident for *E. binotatus*, in which the C-banding patterns are species-specific or even population-specific. The distinct C-band patterns are not fairly good to support the existence of a complex of species under the name *E. binotatus*. However, Haddad and Sazima (1992) indicated that *E. binotatus* might be a composed species and that this name would not be applicable to the southeastern Brazilian populations. Maybe the cytogenetic informations associated to morphological, bioacoustical and molecular data could define this taxonomic issue in the future.

The CMA_3/DA/DAPI staining carried out in five species, with exception of *Eleutherodactylus* sp., provided the molecular content of some repetitive regions in their karyotypes, which were either GC-rich or AT-rich. Particularly in *E. binotatus*, the fluorochrome staining associated with the C-banding data may be useful to elucidate the karyotype differences based on the presence of some interstitial C-bands. Certainly, other fluorochromes or the use of CMA_3 and DAPI, without DA or combined with other types of counterstains, might produce additional data on the karyotype differences.

Eleutherodactylus binotatus, *E. guentheri*, *E. juipoca* and *E. parvus* are unequivocally identified by morphology, bioacoustic, among other characters. The first three species share some characters that assign them in the same morphological group, that is *E. binotatus*, whereas *E. parvus* was allocated in a distinct group, that is *E. parvus* (Lynch, 1976; Lynch and Duellman, 1997). Since Lynch (1976), the genus *Eleutherodactylus* has been subjected to many revisions, but the relationships among the species are still not clear. Currently, Brazilian species of *Eleutherodactylus* are distributed among seven morphological groups proposed by Lynch and Duellman (1997), but some, as

Eleutherodactylus spanios, could not be included in any of them (Garcia, 1996).

The specimen of *Eleutherodactylus* sp. shows external morphology very similar to *E. spanios* from Salesópolis, SP (Heyer, 1985), but with much larger size. However, three other not described *Eleutherodactylus* species occur in this same locality (Heyer et al., 1990), so that *Eleutherodactylus* sp. could correspond to one of them, or even to a fourth species, indicating that the diversity of the genus in this region is not completely known.

In spite of the particularities of each karyotype, it is noticed that the five species of *Eleutherodactylus* share a similar pattern in the chromosome morphology, as well as *Eleutherodactylus holti* and *Eleutherodactylus lacteus*, although both presenting $2n = 20$ (Lucca and Jim, 1974; Lucca et al., 1974). This allows to join the species of *Eleutherodactylus* from the southeastern and southern Brazil in a same grouping characterized by low chromosome numbers, in contrast to a northern and northeastern grouping, with species showing in general $2n = 30$ and 34 (Bogart, 1973; DeWeese, 1975; Siqueira et al., 2005). This fact is in accordance to the previous suggestion of DeWeese (1975) of two distinct assemblages of species of *Eleutherodactylus*, on the basis of the chromosome data available so far.

Considering the karyotypic similarity of *B. ternetzi* and the five *Eleutherodactylus* species, the former certainly could be included in the same assemblage. It is important to emphasize that *Barycholos* shares other characters with *Eleutherodactylus*, such as subgular vocal sac and direct reproductive development, typical for all *Eleutherodactylus* (Lynch, 1980; Caramaschi and Pombal, 2001).

Recent molecular data also disclosed great proximity between the two genera, indicating that *E. juipoca* is most close related to *B. ternetzi* than to *E. binotatus*, which is an unexpected finding (Frost et al., 2006), considering the generic allocation of the species. The present cytogenetic data agree, to a certain extent, with these conclusions, since we can clearly separate the analyzed species into three different groups, one of them with *B. ternetzi*, *E. juipoca* and *Eleutherodactylus* sp., the second with *E. guentheri* and *E. parvus*, and the third including only *E. binotatus*.

All these facts strongly support the requirement of taxonomic and systematic reviews in *Barycholos* and *Eleutherodactylus* genera, to investigate the validity of the current morphological groups, mainly concerning the species from the southeastern and southern Brazil, and the monophyly of both genera. The genus *Barycholos* is probably non-monophyletic, as the unique two species assigned to it are separated by 3200 km (Frost et al., 2006). Undoubtedly, the use of cytogenetic data are highly recommended in these reviews, and karyotype analyses must be extended to related species, including *E. nigrovitatus* and *B. pulcher*, both species from north of South America.

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