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Cranial morphology and karyotypic analysis of *Ceratophrys joazeirensis* (Anura: Ceratophryidae, Ceratophrynae): taxonomic considerations

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Abstract

The frog species *Ceratophrys joazeirensis* Mercadal, 1986 is only known from the Caatinga (semi-arid savanna) and, until recently, from the type locality of Juazeiro, State of Bahia, Brazil. It has now also been found in the States of Paraíba and Rio Grande do Norte, in environments of hypoxerophylic Caatinga. Cytogenetic and comparative analysis of the cranial morphology of *C. joazeirensis* and *C. aurita* (Raddi, 1823) were undertaken. The karyotype of the species is also shown for the first time. The results show important differences in the cranial morphology between *C. joazeirensis* (8n) and *C. aurita* (8n), species whose areas of distribution (Caatinga and Northeast Atlantic forest, respectively) are contiguous. The chromosomes of *C. joazeirensis* show similar size and morphology to the ones of *C. aurita* (*C. dorsata*), *C. ornata* Bell, 1843 (8n) and *C. cranwelli* Barrio, 1980 (2n). Such data would be in agreement with the opinion of some authors who consider these species to constitute a monophyletic group.

Key words: *Ceratophrys joazeirensis*, *Ceratophrys aurita*, cranial morphology, karyotype, osteology, taxonomy

Introduction

The genus *Ceratophrys* Wied-Neuwied, 1824 is currently represented by eight species distributed throughout South America. These are *Ceratophrys cornuta* (Linnaeus, 1758), *C. aurita* (Raddi, 1823), *C. ornata* (Bell, 1843), *C. stolzmanni* Steindachner, 1882, *C.*

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calcarata Boulenger, 1890, *C. testudo* Andersson, 1945, *C. cranwelli* Barrio, 1980 and *C. joazeirensis* Mercadal, 1986 (Frost 2004; Frost *et al.* 2006). Lynch (1971) analysed about 50 characters, including bone and external morphology in his definition of the genus. Subsequently, Lynch (1982) redefined the genus *Ceratophrys* based on three synapomorphic character states: supratemporal fenestrae, posterior margin of the dermocranium indented, and fusion of most skull bones into an akinetic unit, except the pre-maxillae, the septomaxilla and the plectrum.

The species *C. joazeirensis* was first registered and described by Mercadal (1986), and is the only representative of the genus to occur in the biogeographic province of the Caatinga (scrub savanna), a typical semi-arid morphoclimatic domain in North-East Brazil (Ab'Saber 1974 and 2003). For diagnosing *C. joazeirensis*, Mercadal (1981) carried out a cytometric analysis that indicated an octaploid karyotype. However, no karyotype analysis on this species has so far been carried out. The author also analyzed characteristics of the upper eyelids and spot patterns, mainly those of the cephalic region, as well as morphometric data.

The species *C. joazeirensis* is quite similar to *C. cranwelli*, chiefly with regard to the pattern of body spots and also for occurring in a semi-arid environment, since *C. cranwelli* is distributed in the region of the Argentine Chaco, Bolivia and Paraguay (Mercadal 1986). The greatest difference between the two species is found at the ploidy level, since *C. cranwelli* is a 2n species and *C. joazeirensis* probably an 8n species (Mercadal 1981 and 1986; Mercadal de Barrio and Barrio 2002). The species *C. aurita* has a similar distribution to that of *C. joazeirensis*, both species occurring in contiguous areas (Atlantic forest and Caatinga of North-East Brazil) and may have a recent related biogeographic history (Lynch 1982; Mercadal de Barrio and Barrio 2002). *Ceratophrys joazeirensis, C. cranwelli,* and *C. aurita* occur in relatively plain areas (Caatinga, Chaco and Atlantic forest, respectively).

Goin *et al.* (1968) postulated the existence of a correlation between nuclear DNA content, developmental speed and ecological preference, in which individuals with slow embryonic development generally possess high DNA content and are adapted to humid environments. *Ceratophrys aurita* fits this profile as it is an 8n species distributed in the brazilian Atlantic forest (Heyer *et al.* 1990). *Ceratophrys joazeirensis*, however, has an 8n ploidy level, but unlike *C. aurita*, does not correspond to the relationship suggested by Goin *et al.* (1968).

Since Mercadal (1986) used only characters related to the external morphology to describe *C. joazeirensis*, the objective of this paper is to present new information, related to the karyotype and osteology, comparing cranial morphology data with that of *C. aurita*. Some osteological characters are also described and compared with *C. ornata* and *C. cranwelli,* as they were considered to be a monophyletic group together with *C. joazeirensis* (Lynch 1982; Mercadal 1986).

Materials and methods

The individuals of *C. joazeirensis* were collected at Parque Estadual Pedra da Boca (6°26'8"S and 35°38'36"W), a nature reserve area located in the municipality of Araruna, Curimataú Oriental Valley, geographic mesoregion of Borborema, in the north of the State of Paraíba, Brazil (Fig. 1). The Park is located at an altitude of about 200 m, with an area of 157.26 hectares (SUDEMA 2004) and has a differentiated kind of vegetation, with dense and relatively humid forest, typical of mound caatinga (IDEME 2004), restricted to small rocky enclaves. The climate in the region is tropical hot and humid, differentiating it from the drier climate of the surrounding areas. The mean annual temperature is 25.6°C and the mean annual humidity is 72%. The rainfall is 800mm/year.

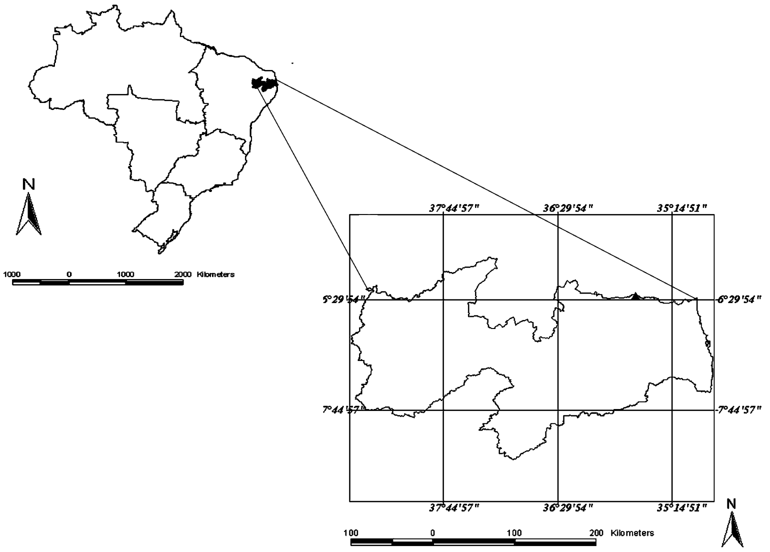

FIGURE 1. The triangle marks the occurrence area of *Ceratophrys joazeirensis*, situated at Parque Estadual Pedra da Boca, in Araruna town, State of Paraíba, Brazil.

Individuals of *C. joazeirensis* were collected by streams near the municipality of Passa e Fica, State of Rio Grande do Norte, Brazil, about 119km from Natal. Most of the captures were nocturnal, although some were carried out in the morning and afternoon. The sampled areas were covered randomly. Individuals were hand captured by exploring leaf litter, according to Heyer *et al.* (1994).

Seven individuals of *C. joazeirensis* were collected and fixed in formalin 4% and later preserved in alcohol 70%. The specimens were permanently deposited in the Coleção Herpetológica do Departamento de Sistemática e Ecologia/Centro de Ciências Exatas e da Natureza of the Universidade Federal da Paraíba, Campus I (DSE/CCEN/UFPB), João Pessoa, Paraíba, Brazil (UFPB 4303–4307) and the Célio F. B. Haddad Collection (CFBH), Departamento de Zoologia, Instituto de Biociências, UNESP, Rio Claro, São

Paulo, Brazil (CFBH 07411; CFBH 07412).

Two cranial preparations belonging to adult specimens of the genus *Ceratophrys* were analyzed, one of *C. joazeirensis* (UFPB 4303) obtained at Parque Estadual Pedra da Boca, and the other of *C. aurita* (UFPB 4308), housed in the Coleção Herpetológica do Departamento de Sistemática e Ecologia/CCEN/UFPB, collected in the Estação Ecológica de Pau Brasil, 15km NW from Porto Seguro, State of Bahia, Brazil, were analyzed. The pectoral girdle of *C. joazeirensis* was also observed. The osteological information obtained from the mentioned specimens was compared with the descriptions of Lynch (1971 and 1982) and Cei (1987).

The animals were dissected and their cephalic portions removed. The skull was cleared in a KOH solution (Reagen—Quimibrás Indústrias Químicas AS., Rio de Janeiro, Brasil) and then incubated for two days. A stereomicroscope coupled to a light chamber was used to make drawings of the dorsal, palatal and lateral view of the material, according to the methodology used by Izecksohn *et al.* (2005).

Cytogenetic analysis was carried out on two specimens (CFBH 07411, CFBH 07412) and mitotic chromosomes were obtained from directed preparations of bone marrow treated with 0.01% colchicine (Merck S.A. Indústrias Químicas, Rio de Janeiro, Brasil) at a proportion of 0.1 ml/10g body weight, as described in Baldissera Jr. *et al.* (1993). The voucher specimens are deposited in the Célio F. B. Haddad collection (CFBH), Departamento de Zoologia, Instituto de Biociências, UNESP, Rio Claro, São Paulo, Brazil. The following techniques were used: standard staining with 2% Giemsa (Merck S.A. Indústrias Químicas, Rio de Janeiro, Brasil) in phosphate-buffered saline, pH 6.8, and Ag-NOR staining (Howell and Black 1980).

Results

When compared, the skulls of both *C. aurita* and *C. joazeirensis* (Figs. 2 and 3) displayed bone shields, made up of small slots and conical nodules, resembling an anastomotic disposition. The pars facialis in the individual of *C. joazeirensis* was expanded in the extremity close to the orbits, the area between the canthus postero-lateralis and the posterior processus in the dorsal portion of the frontoparietals was convex, in contrast with *C. aurita*. The orbital branch of the bone shield, referring to the portion of the lamella alaris and to the ramus zygomaticus of the squamosal presented depressions, a character that is absent in the specimen of *C. joazeirensis*. In dorsal view, the torus terminalis of the prootic-exoccipital was wide and its extremities elliptical in *C. joazeirensis*, and more slender in *C. aurita*.

The pterygoids were much more evident in the specimen of *C. joazeirensis*, in which the ramus maxillaris and ramus interior were clearly delimited by sutures. In *C. aurita*, the pterygoids were fused to the maxillae and palatines and sutures were absent. The palatine teeth were clearer in *C. aurita*, in contrast to that observed for *C. joazeirensis*.

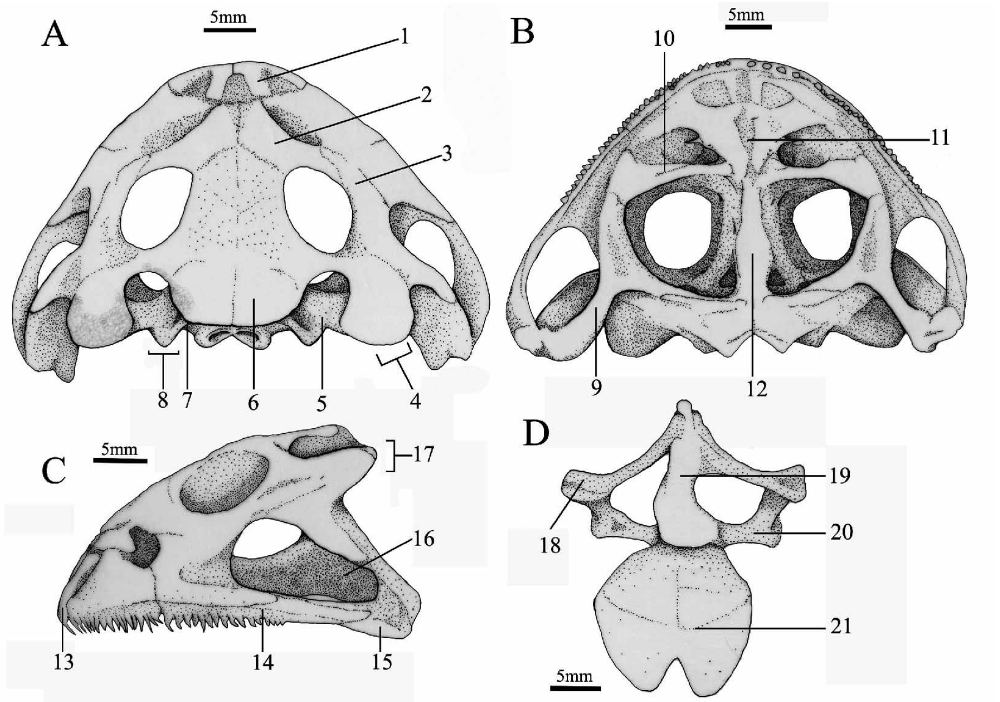

FIGURE 2. Skull of *Ceratophrys joazeirensis* (UFPB 4303) collected at Parque Estadual Pedra da Boca. A: dorsal view; B: palatal view; C: lateral view and D: pectoral girdle. 1—pars facialis; 2—absence of pre-orbital depression; 3—absence of depression in orbital arch of squamosal; 4—rounded extremity of the caudal portion (lamella alaris) of the squamosal; 5—prooticum; 6—convex dorsal posterior portion of the frontoparietals; 7—rounded extremity of the processus posterior in frontoparietals; 8—large and elliptical torus terminalis of the prootic-exoccipitals in dorsal view; 9—delimited pterygoids in the ramus maxillaries and ramus interior; 10—palatine teeth; 11—depression dividing the vomers in the margo medialis; 12—parasphenoid; 13—pré-maxillae; 14—maxillae; 15—quadratojugal; 16—pterygoid; 17—short caudal projection of the squamosal, shorter than quadratojugal in length; 18—clavicle; 19—epicoracoid; 20—coracoid; 21—sternum. Except for the pre-maxillae, the clear areas in A and C, inferior portions of the maxillae and quadratojugal concern to dorsal bone shields. Author Kleber da S. Vieira.

In *C. aurita*, the posterior areas referring to the lamella alaris of the squamosal, adjacent to the prootic-exoccipital, are not rounded as in *C. joazeirensis*. The vomers are clearly separated from the palatines in the torus dentigerus, and differentiated from the sphenethmoid in *C. joazeirensis*, but fused and indistinct in *C. aurita*.

The extremities of the shield, related to the processus posterior of the frontoparietals, are slightly rounded in *C. joazeirensis*, but concave in *C. aurita*. The pre-orbital depressions, equivalent to the processus parachoanalis, margo maxillaries and to the processus paraorbitalis, were observed in *C. aurita* but are absent in *C. joazeirensis*.

The shape of the sternum in *C. joazeirensis*, as well as the epicoracoid (Fig. 2D), was similar to that of *C. cranwelli* (Cei 1987), and was close to that observed for *C. ornata*.

The species *C. joazeirensis* has an octaploid karyotype (Fig. 4) constituted by 104 chromosomes arranged in 13 octets. The octets 1, 4 and 6 include metacentric chromosomes, while the octets 2, 3 and 5, submetacentric chromosomes. The octets 7 to 13 comprehend small chromosomes, which are distributed in two morphologic types, prevailing however, the submetacentric type. A clear separation is noticed between the six first octets and the others, as they include the largest chromosomes of the karyotype. The Ag-NORs are present in the chromosomes of octet 6, in the proximal region of the long arms. However, markings in six of the eight chromosomes were observed in all the 15 metaphases analyzed. Size heteromorphism was not verified in the Ag-NOR stains.

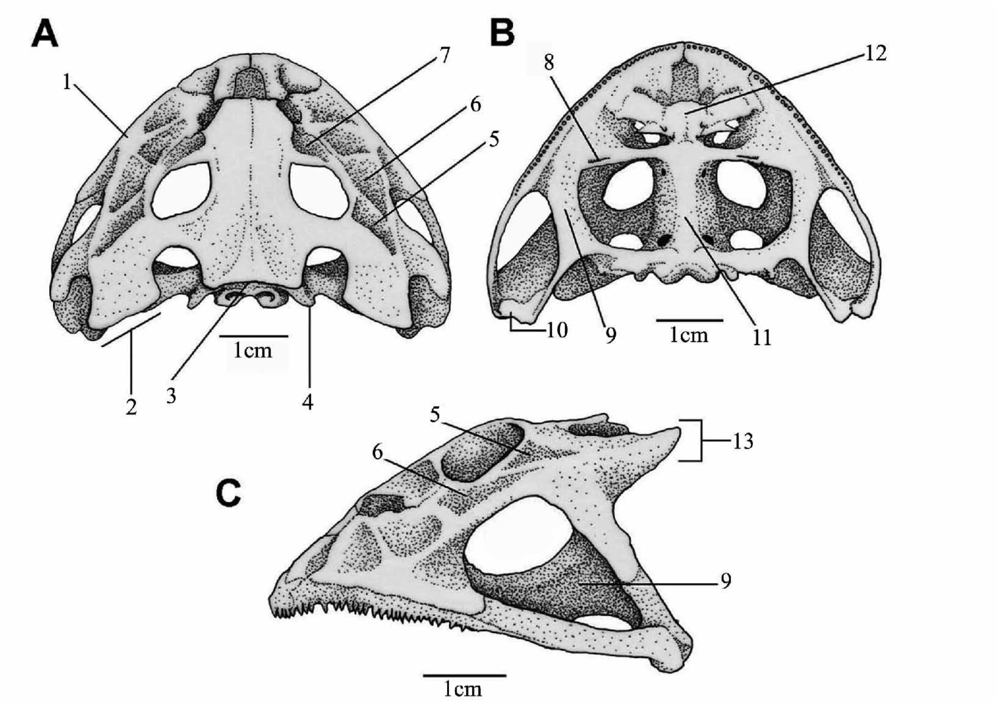

FIGURE 3. Skull of *Ceratophrys aurita* (UFPB 4308). A: dorsal view; B; palatal view and C: lateral view. 1—Except for the pre-maxillae, the clear areas in A and C, inferior portions of the maxillae and quadratojugal concern to dorsal bone shields; 2—non-rounded caudal projection of the squamosal (lamella alaris); 3—concave extremity of the processus posterior in frontoparietals; 4—narrow torus terminalis; 5 and 6—depressions in the orbital arch of the squamosal; 7—pre-orbital depression; 8—palatine teeth; 9—pterygoid; 10—quadratojugal; 11—parasphenoid; 12—absent depression between the vomers; 13—long caudal projection of the squamosal, slightly shorter than quadratojugal in length. Author Kleber da S. Vieira.

Discussion

Polyploidy is present in about 40 amphibian species (Kuramoto 1990; Tymowska 1991; Frost 2004). It consists of a significant phenomenon in the evolution process of the Anura, which becomes even more evident when the high number of naturally polyploid species in some groups occurring in the Neotropical region is considered. Although the octaploid status of the species *C. joazeirensis* has already been described by Mercadal (1986), based upon cytometric analysis, we present for the first time its karyotype, which is very similar to the one described for the octaploids *C. aurita*, *C. ornata* and the diploid *C. cranwelli* (Bogart 1967; Barrio and De Chieri 1970; Beçak *et al.* 1970; Schmid *et al.* 1985; Soares-Scott *et al*. 1988). The more evident differences concern the morphology of pairs 4 and 6 described in *C. ornata* by Bogart (1967) and Schmid *et al.* (1985), as submetacentrics and metacentrics respectively, and as metacentrics in *C. ornata* (studied by Barrio and De Chieri, 1970), *C. aurita* (Soares-Scott *et al.* 1988), and in *C. joazeirensis* (present study). The other chromosomes in the four species have a similar size and morphology, indicating that *C. joazeirensis*, *C. aurita*, *C. ornata and C. cranwelli* have a conservative karyotype. However, the positions of the secondary constrictions and the Ag-NORs are not coincident in these species. Schmid *et al.* (1985) observed Ag-NORs in all chromosomes of octets 7 and 8 in *C. ornata*, and Soares-Scott *et al*. (1988) observed Ag-NORs in three choromosomes of octect 6 and in six chromosomes of octect 11 in *C. aurita*. In the present study only six elements of octet 6 displayed Ag-NOR markings. The possibility of diploidization could explain the fact that the number of Ag-NORs found in *C. joazeirensis* does not correspond to the octaploid status of the species, since there has been a loss in the nucleolar organizing condition. Although two elements of the octets did not show genetic activity, the presence of NORs cannot be discarded since hybridization *in situ* was not undertaken.

It is likely that the octaploid species *C. joazeirensis*, *C. aurita*, and *C. ornata* derived from ancestors with diploid karyotypes; nevertheless, it has been difficult to determine the origin of polyploidization so far. Certain intra- and interspecific cytogenetic polymorphism detected in secondary constrictions or banding- technique of the nucleolus organizer regions (NOR) or C-banding in *C. ornata* and in *C. aurita* (Beçak and Beçak 1974; Ruiz *et al*. 1980, 1881; Schmid *et al*. 1985; Almeida *et al*. 1986; Soares-Scott *et al*. 1988), have been interpreted by Schmid et al. (1985) as post-polyploid alteration (Beçak and Kobashi 2004). The use of other banding techniques, as well as the increase in the number of species and populations of *C. joazeirensis* to be analyzed, are necessary for the understanding of the polyploidization process in this group of frogs.

Lynch (1982) considered *C. ornata* and *C. cranwelli* phylogenetically close to each other for possessing dorsal shields constituted of three or more elements and for possessing cornification of the inner metatarsal tubercle. Mercadal (1986) stated that *C. ornata*, *C. cranwelli* and *C. joazeirensis* form a monophyletic group, in which the inclusion of *C. aurita* is questionable. Cei (1987), related *C. cranwelli* to *C. ornata* because the females of both species had correlative skull sizes and similar diameters of the foramen magnum.

The shape of the sternum and the epicoracoid of *C. joazeirensis*, are similar to that of *C. cranwelli* (Cei 1987). Nonetheless, the dividing furrow of the sternum is similar to the pattern found in *C. ornata* (Cei 1987), as it is more opened, reinforcing the position of Mercadal (1986), who states that *C. joazeirensis* possesses characteristics found in both *C. ornata* and *C. cranwelli*.

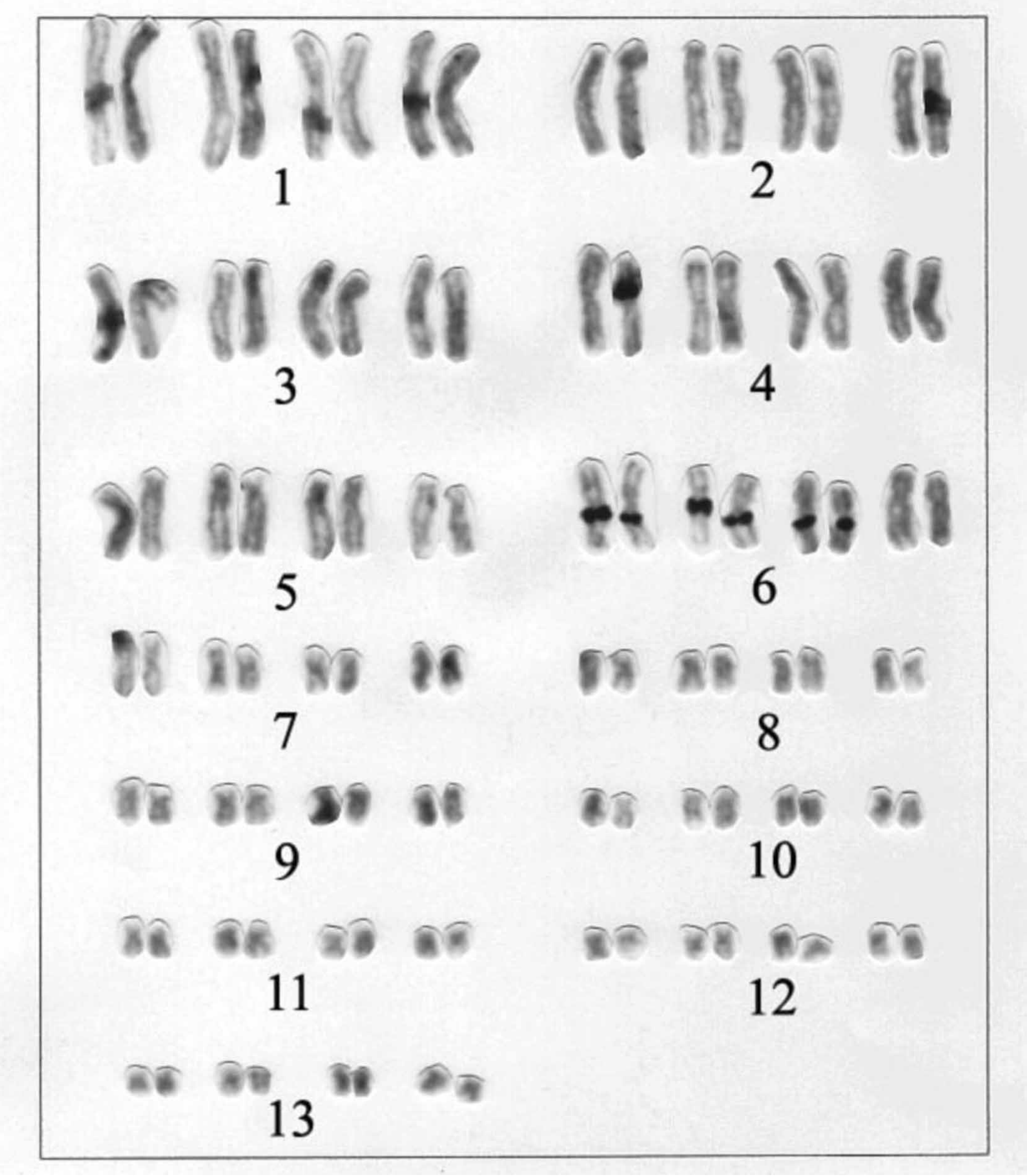

FIGURE 4. Octaploid karyotype of *Ceratophrys joazeirensis* from Parque Estadual Pedra da Boca (2n=8x=104) arranged in 13 octets: 1 to 4 –metacentric chromosomes; 2 and 3—submetacentrics; 5 and 6—chromosomes of medium size, submetacentrics and metacentrics; the octets 7 to 13 are constituted by small metacentric and submetacentric chromosomes.

The link between the species *C. ornata*, *C. joazeirensis* and *C. cranwelli*, in spite of the octaploidy of *C. joazeirensis* and *C. ornata*, appears to be quite narrow, also in terms of their geographic occurrence. Barrio and De Chieri (1970) registered diploid populations of *C. ornata* in soggy central areas of Argentina, Mercadal (1981) and Klappenbach and Langone (1992) found octaploid populations living in sympatry with *C. cranwelli*.

C. aurita differs from *C. joazeirensis*, according to Mercadal (1986), exclusively for possessing a prominent upper eyelid, inner metatarsal tubercle without cornification and pointed finger extremities, in contrast to the rounded fingers of *C. joazeirensis*. Moreover, *C. joazeirensis* differs from *C. aurita* in terms of its distribution (the latter is distributed along Brazilian coastal regions (Heyer *et al*. 1990)), and in all cranial characters aforementioned, sharing characteristics such as octaploid number of chromosomes, presence of a dorsal shield and interdigital membranes in the toes. Except for the octaploidy, the characters regarded as shared were considered plesiomorphies for the genus, according to Lynch (1982). Mercadal (1986 and 1988) accepts that position by stating that *C. joazeirensis* does not share any exclusive characteristic with *C. aurita*, referring to the octaploid number of chromosomes as homoplastic.

The presence of an octaploid number of chromosomes may be an argument to place *C. aurita*, *C. joazeirensis* and *C. ornata* together in a single group, made up of species from humid environments, except for *C. joazeirensis* that is a species from a semiarid environment, and excluding *C. cranwelli* for possessing a diploid number. However, the sharing of a few character states between *C. joazeirensis*, *C. ornata* and *C. cranwelli*, reinforces a past biogeographic relationship. This is probably why Mercadal (1986 and 1988) suggested that the octaploid number in *C. aurita* is homoplastic, although it is not clear as there were environments of humid forest in the last climatic Holocene (Vivo and Carmignotto 2004) in the region where the species of *C. joazeirensis* and *C. aurita* are currently found. It is likely that *C. joazeirensis* and *C. aurita* shared a more recent biogeographic history than the species aforementioned**.** The dorsal shield in *C aurita* strengthens the hypothesis of a possible origin in an arid or semi-arid morphoclimatic domain, as it is a characteristic related to this type of environment (Fabrezi 2006), as observed in the species *C. cranwelli*, *C. joazeirensis* and diploid forms of *C. ornata*. Furthermore, the characters presented by Lynch (1982) and Mercadal (1986) cannot be considered enough to elucidate the phylogenetic position of the species. Lynch (1982) also unites *C. cranwelli*, *C. ornata* and *C. aurita* into the same group due to the presence of a dorsal shield made up of three or more elements. Mercadal (1986) considers valid the group *C. joazeirensis*, *C. cranwelli* and *C. ornata*, only for possessing cornificate inner metatarsal tubercles. Yet, it is not evident whether such characters are plesiomorphic or not, or even homoplastic among the mentioned species.

Diploid *C. joazeirensis* have not been analyzed yet, in addition to samples of *C. aurita* that occur in sympatry with *C. joazeirensis*. Such an analysis would be of great value to the cytogenetic study, and may bring new information to enlighten the phylogenetic and biogeographic history of the genus, since the data available so far are still insufficient, as pointed out by Mercadal (2002).

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